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Contents

PD-L1 Expression in triple-negative breast cancer and its prognostic significance

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Objective Triple-negative breast cancer (TNBC) patients generally have a poor outcome; there is a pressing need for more effective therapeutic strategies. Clinical trials targeting programmed death 1/programmed death ligand 1 (PD1/PDL1) in melanoma and non-small-cell lung cancer have achieved remarkable results, PDL1 expression in tumour cells has been suggested as a potential biomarker to enrich treatment strategies for patients. So far, there are only very limited data have reported PDL1 expression in TNBC. **Methods** 1114 cases of TNBC were collected from the Fourth Hospital of Hebei Medical University from January 2008 to June 2018, and the surgical excision from patients without chemotherapy and radiotherapy were included. Histopathologic analysis of tumor infiltrating lymphocyte (TIL) was performed on HE sections. Two kinds of PD-L1 (Dako 22C3/Ventana SP263) expression in TNBC were detected by tissue microarrays. **Results** PD-L1 (Dako 22C3/Ventana SP263) expression was 42% and 37% in TNBC respectively. There was no significant difference between the two antibodies (P=0.212). The expression of PD-L1(Dako 22C3/Ventana SP263) is related to grade and Ki67. PD-L1 (Dako 22C3) expression was associated with a poor overall survival(OS, HR=0.282, P=0.007). TIL scorce was positively relevated with OS. **Conclusion** PD-L1(Dako 22C3/Ventana SP263) expression and tumor infiltrating lymphocyte (TIL) are common in TNBC. These data provide further impetus for assessing immunotherapy in TNBC, in view of the clinical significance of the expression of PD-L1 and TIL in TNBC.

Research progress of PD1/pd-l1 in triple-negative breast cancer

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The three negative breast cancer (TNBC) especially refers to the estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 all negative breast cancer patients, because of its endocrine and target chimney hypertherapy is ineffective, make its clinical prognosis is very poor.
Therefore, it is particularly important to explore new treatments. Research on the clinical efficacy of anti-pd1 / pd-1 monoclonal antibody has brought a breakthrough in the treatment of TNEC. In this paper, PD1/ pd-l1 inhibitors and their research progress in the clinical trial of TNBC treatment are described, providing new ideas for the treatment of TNBC patients.

Category: Breast pathology
1628884

Tumor-educated B cells selectively promote breast cancer lymph node metastasis by HSPA4-targeting IgG

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Primary tumors may create the pre-metastatic niche in secondary organs for subsequent metastasis. Humoral immunity contributes to the progression of certain cancers, however, the roles of B cells and their antibodies in pre-metastatic niche formation are poorly defined. Using a mouse model of spontaneous lymph node (LN) metastasis of breast cancer, weshow that primary tumorinducesB cell accumulationin draining LNs. These B cells selectively promote LN metastasis by producing pathogenic IgG that targets glycosylated HSPA4 on tumor cell surface, and the ligation activatesHSPA4-binding protein ITGB5 and downstream Src/NF-κB pathway in tumor cells forCXCR4/SDF1α axis-mediated metastasis.High serum anti-HSPA4 IgG is correlated with high tumor HSPA4expression and poor prognosis of breast cancer patients.Our findings identify a key role for tumor-educated B cells and their derived antibodies in LN pre-metastatic niche formation， providing potentialtargets for cancer intervention.

Proliferative fasciitis of the Breast: A case report.

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PRESENTATION OF CASE:
We present a case of proliferative fasciitis occurring in the medial of
83-year-old, otherwise healthy, woman. The patient discovered the right upper quadrant
mass accidentally and then came to our hospital for treatment after 2 weeks. The tumour
was deep and close to the pectoralis major muscle. It was firm, not smooth, and indolent
with limitied mobility. Fine-needle aspiration was inconclusive, and the molybdenum
target showed that the right upper quadrant showed a uniform dense mass with unclear
margin and implicit calcification. A local excision was performed, and the
histopathological findings were consistent with proliferative fasciitis.

DISCUSSION:
Although rare, proliferative fasciitis is the most common preudosarcome of soft
tissues. Local excision is recommended; however, the tumour often regresses
spontaneosly, and recurrence is exceedingly rare.

CONCLUSION:
Awareness of proliferative fasciitis and its begign nature is essential to avoid
misdiagnosis and subsequent inappropriate aggressive of the patient.

Ductal carcinoma in situ of the breast: perspectives on tumor subtype and treatment

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Abstract

Objective: To evaluate ductal carcinoma in situ (DCIS) characteristics and the effect of different treatment strategies.

Patients and methods: Using data with known hormone receptor (HoR) and human epidermal growth factor receptor 2 (HER2) status obtained by the Surveillance, Epidemiology, and End Results (SEER) program from 2010-2014, the study was conducted to investigate tumor subtype-specific differences in various characteristics, overall survival (OS) and breast cancer-specific mortality (BCSM).

Results: A total of 3415 patients with DCIS were eligible. Compared with HoR+/HER-subgroup, patients with triple-negative (TN) and HoR-/HER+ were commonly higher in grade, larger in size and tended to receive mastectomy ($P < 0.05$). The multivariate analysis revealed patients with TN were more likely to have a poorer OS and a higher breast cancer-specific mortality were showed compared with the HoR+/HER- subgroup ($P < 0.05$). Multivariate analysis on history of local treatment and surgery showed patients receiving breast-conserving surgery (BCS) plus radiotherapy (R) and BCS plus sentinel lymph node biopsy (SLNB) was likely to improve OS without affecting breast cancer-specific mortality ($P < 0.05$).

Conclusion:

The results demonstrate that DCIS associated with TN subtype portends poor prognosis. Meanwhile, BCS plus R was a preferable option and resulted in survival rates better than those achieved with mastectomy, and SLNB should be considered as an appropriate assessment of axillary staging in patients with DCIS.

Co-translocation of myeloid-derived suppressor cells with Helicobacter hepaticus initiates and promotes breast carcinogenesis

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Objectives: Intestinal dysbiosis plays an important role in intestinal tract diseases. Recent studies suggest a tightening link between intestinal bacterium and mammary cancer. However, the underlying molecular mechanisms are largely unknown. Histidine decarboxylase (Hdc) marks a population of myeloid-biased hematopoietic stem cells (MB-HSCs) and granulocytic myeloid-derived immune cells (PMN-MDSCs). Thus, this study aimed to provide more insights into the crosstalk between dysbiosis, Hdc-expressing MDSCs, and breast cancer.

Materials and Methods: Female Hdc-eGFP; Apc$^{min/+}$ mice were used to establish an intestinal dysbiosis animal model by orogastric gavage with *Helicobacter hepaticus*. Breast tissues obtained from infected and uninfected mice were evaluated after 4 months. Fluorescence in situ hybridization (FISH) with specific bacterial DNA probe was employed to explore the translocation of *Helicobacter hepaticus* from small intestine to breast. The number and percentage of myeloid-derived suppressor cells (MDSCs) were counted by FACS. Two-photon microscope traced the migration of MDSCs in *H. hepaticus*-infected Hdc-eGFP; Apc$^{min/+}$ mouse. Mast cells (MCs) can secret several chemokines such as IL-17α and IL-23α to support regulatory T cell and MDSCs and contribute to carcinogenesis. Immunofluorescence and qRT-PCR were used to investigate levels of IL-17α and IL-23α and the existence of MCs in breast and small intestine. To further unveil the molecular mechanisms underlying the involvement of MDSCs and dysbiosis in breast cancer, the expression and location of Wnt/β-catenin in MDSCs and mammary ductular cells was observed using immunohistochemistry and qRT-PCR.

Result: Female Apc$^{min/+}$ mice infected with *Helicobacter hepaticus* exhibited increased mammary and small intestine tumor burden compared with uninfected littermates. Bacterial DNA was detected in small intestine, mesenteric lymph nodes, mammary cancer, and adjacent lymph nodes, indicating the migration pathways. Meanwhile, large amounts of CD11b$^+$Gr1$^+$ myeloid-derived suppressor cells (MDSCs) infiltrated these sites and secreted high levels of Wnts to promote tumorigenesis through aberrant activation of Wnt/β-catenin pathway. Cytokines and chemokines secreted by IL-17-expressing mast cells and tumor tissues promoted Hdc$^+$MDSCs expansion and trafficking towards mammary. Adoptive transfer of MDSCs isolated from *Helicobacter hepaticus*-infected mice increased recipient`s MDSCs frequencies in peripheral blood, mesenteric lymph nodes,

and mammary gland and lymph nodes. The transplantation also increased mammary cancer size and number.

Conclusion:This study provides preliminary evidence to demonstrate that dysbiosis-inducing bacterium can translocate from intestinal tract to mammary and recruit MDSCs to drive tumorigenesis process. Targeting translocated bacterium and MDSCs may be useful for cancer prevention and therapy, especially in patients with intestinal dysbiosis.

Category: Breast pathology
1640008

Pathological analysis of malignant phylloid tumor of breast with myxoid liposarcoma differentiation

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[Abstract] Objective: To investigate the pathological features, immunohistochemical characteristics and molecular phenotypes of malignant lobulated breast tumors with myxoliposarcoma differentiation. Methods:Pathological morphological observation, immunohistochemical labeling and molecular detection of FUS-DDIT3 gene status in 3 cases of malignant lobulated breast tumors with myxoliposarcoma were performed. Results: malignant lobulated breast tumors had long history of slow and painless growth. The general structure showed expansive lobulated growth with or without complete envelope. The glandular epithelium and myoepithelium lined by the slit under the microscope are well differentiated, and the mesenchymal cells are composed of spindle mesenchymal cells. The cell atypia is not large, and fat mother cells are visible or rarely seen. In the background, mucous matrix and branching capillary system are seen. Hematic and lymphatic metastases are rare. Immunohistochemical stromal tumor cell expression :Vim:3/3, AE1/AE3:0/3, EMA:0/3, CD117:2/3, CD34:1/3, S-100 :3/3, SMA:1/3, p53:0/3, CDK4 :2/3, MDM2 :1/3, P16 :2/3, ki-67: < 10%, ER:0/3, PR:1/3. Molecular detection: the FUS-DDIT3 fusion gene was found in 3 tumor cells. Conclusion: the pathological diagnosis of malignant lobular tumor with mucinous liposarcoma differentiation of breast was mainly based on histological observation. The prognosis was mainly determined by the scope of surgery, tumor size and histological grade of   liposarcoma.

Clinicopathological Analysis and Literature Review of Sclerosing Lymphocytic Lobulitis

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Sclerosing lymphocytic lobulitis (SLL), a rare benign lesion first reported by Soler in 1984 , is believed to be associated with autoimmune diseases, such as type I diabetes and thyroiditis, but there are still a small number of patients without autoimmune diseases.Sclerosing lymphocytic lobulitis is characterized by a single or bilateral hard, irregular, motile, painless mass of the breast, which is often considered by clinicians as a malignant tumor.Ultrasound is usually irregular and hypoechoic mass with obvious posterior acoustic shadow.X - ray appearance is unclear, uneven density of the mass, accompanied by calcification.Its imaging findings are easily confused with breast cancer, but biopsy showed sclerosing lymphocytic lobulitis.Pathological findings showed that lymphocytic lobulitis and ductitis were associated with glandular atrophy, lymphocytic mononuclear perivascular inflammation, lymphocytic and perivascular lymphocyte aggregation, mainly B cells, extensive interstitial fibrosis was scarring, with multiple microcalcification, and plasma cells and hypertrophic epithelioid fibroblasts were occasionally seen. We present a case of sclerosing lymphocytic lobulitis involving the left breast of a non-diabetic adult female who presented with complaints of a painless, hard palpable lumpin her left breast for 8 months. MRI examination showed structural disorder in the upper quadrant of the left breast with mass, and bilateral breast cystic hyperplasia was initially diagnosed as breast tumor by surgical excision. The tumor was pale yellowish white tissue, about 9.0cm×6.0cm×2.5cm in size, with pale yellowish white intersections, and qualitative pathological results showed sclerosing lymphocytic lobulitis.

Autophagic breast cancer cells enhance the stemness of TNBC by activating TLR4

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Abstract

Purpose: To illuminate the impact of autophagy of cancer stem cells(CSCs) of Triple negative breast cancer(TNBC) and the underlying mechanism.

Experimental Design: Specimens from 430 breast cancer cases with follow-up information, were used to analyze the association between autophagy protein LC3II and patients' prognosis by Kaplan-Meier and multivariate Cox regression analyses. TNBC cell lines (MDA-MB-231) were cultured with serum free media to induce autophagy. The culture media from autophagic breast cancer cells were used to stimulate breast cancer cells which were then exanimated the stemness by flow cytometry, RT-PCR, Western blot and sphere-formation. The putative factors released by autophagic MDA-MB-231 were analyzed both *in vitro* and *in vivo* experiments. IHC was used to detect the correlation between autophagic MDA-MB-231 and the progression of human breast cancer.

Results: In human TNBC specimens, the presence of breast cancer cells containing high levels of autophagy protein LC3II were associated with more progressive breast cancer. MDA-MB-231with high autophagy activity enriched breast cancer stem cells (BCSCs) in TNBC with increased tumourigenicity in immunocompromised mice. Mechanistically, autophagic MDA-MB-231activated its receptor TLR4 expressed by breast cancer cells to enhance their stemness and tumourigenicity.

Conclusion: Our findings demonstrated that autophagic breast cancer cells play a critical role in promoting the progression of TNBC by activating TLR4.

Expression patterns between Ezrin and AJAP1 and clinical significance in breast cancer

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Background: Ezrin and Adherent junctions associated protein 1(AJAP1) are structural proteins which are involved in numerous human malignancies. However, little is known about the relationship between them in breast cancer. This study was set out to investigate the relationship between them and to further explore the mechanism of AJAP1 mediating cytoskeleton in the breast cancer progression.

Methods: Ezrin and AJAP1 expression were detected in 377 samples of breast cancer by immunohistochemistry and different expression patterns between AJAP1 and Ezrin with clinicopathological parameters were analyzed. Besides, univariate and multivariate cox model were used to evaluate the prognostic potential of them. ROC curves and under the curve (AUC) were used to assess the accuracy of AJAP1 and Ezrin expression as biomarkers for breast cancer diagnosis. What's more, Enzyme-linked immunosorbent assay, western blot, qRT-PCR and Phalloidine staining of F-actin were used to explore the relationship and the mechanism between AJAP1 and Ezrin in cytoskeleton arrangement.

Results: 377 cases of breast cancer results showed that AJAP1 expression was negatively related with histological grade, lymph node involvement and could be an independent prognosis marker of breast cancer. AJAP1 expression tended to be higher in Ezrin-negative expression case. Patients with AJAP1[negative] and Ezrin[positive] expression had a worse prognosis($p<0.0001$) and shorter DFS ($p=0.015$). ROC curves' results demonstrated that AJAP1's AUC was 0.777 and the optimal cut-off value was 0.528. Meanwhile, Ezrin's AUC was 0.610 with the optical cut-off value was 0.329. AJAP1 expression specificity (0.561) was lower than Ezrin (0.9) but sensitivity (0.767) was higher than Ezrin (0.628). More importantly, AJAP1-depletion increased cell ability of F-actin formation through promoting Ezrin expression.

Conclusions: AJAP1 depletion might mediate breast cancer malignancy potential through promoting Ezrin expression and cytoskeleton formation.

Aberrantly expressed Nek9 is involved in carcinogenesis and enhances tumor progression by promoting invasion and metastasis in breast cancers

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Purpose: As a new member of Neks family, Nek9 regulates spindle assembly and controls chromosome alignment and centrosome separation. In current study we aimed to investigate the roles of Nek9 in tumor initiation and progression in breast cancer.
Methods: The expression of Nek9 was investigated in 316 breast cancer samples as well as normal breast tissues and benign breast lesions using immunohistochemistry, and its roles in breast carcinogenesis and progression were studied through assessing cell proliferation, migration and invasion, apoptosis and cell cycle in breast cancer cell lines. In vivo experiments served to identify the effect of Nek9 on tumor growth and invasion.
Results: Our data demonstrated that Nek9 expression was reduced or even lost in invasive ductal carcinomas. A decrease in Nek9 expression was associated with HER-2 positivity, triple negative breast cancer, p53 mutation, high ki67 index as well as larger tumor size and high grade. When the tumors proceeded from ductal carcinoma in situ to invasive carcinoma and to metastatic cancer in hormone receptor (HR) positive tumors, Nek9 expression was gradually down-regulated. Then the effects of Nek9 on proliferation, migration and invasion were confirmed in vitro and in vivo.
Conclusions: We conclude that aberrantly expressed Nek9 may contribute to tumor initiation and progression in breast cancers and help to identify a unique subtype of breast cancer.

Thymidylate synthase (TS) immunostaining in the diagnosis of the myoepithelial cell, basal cell, stratified epithelium cell, and associated tumor

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Abstract

Aim: The present study aimed to investigate the clinical relevance of TS as a reliable biomarker for identification and diagnosis of MEC, BC, TEC, SEC, and associated tumor using immunohistochemical (IHC) staining.

Methods and Results: Formalin-fixed, paraffin-embedded specimens from 186 cases of tumors were immunostained for TS and p63 expression. The diagnostic capability of TS as a reliable diagnostic marker was evaluated and compared with the expression of p63. the results showed that TS exhibited a strong specific and stable nuclear immunoreactivity in all of the specimens including MEC, BC, TEC, and SEC compared with p63. Notably, a variable degree of TS cytoplasmic positive immunoreactivity was also observed in 58.3% of squamous-cell carcinoma (SQCC), 37.5% of basal cell carcinoma (BCC), 44.4% transitional-cell carcinomas (TCC), 41.7% mixed tumor (MT) and 56.5% of adenocarcinoma (ADC) specimens.

Conclusions: Besides functioning as a strong prognostic factor for 5-FU resistance, TS also can serve as a promising putative diagnostic marker for identifying MEC, BC, SEC, and TEC from GEC, and for distinguishing SQCC, BCC, TCC, and MT from ADC. Moreover, these diagnostic and prognostic results can be obtained in a single immunostaining procedure.

The influence factors of neoadjuvant chemotherapy for invasive micropapillary carcinoma of breast

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Objective: To investigate the therapeutic effect of immunohistochemical phenotype and tumor biological behavior on Miller-Payne grade of neoadjuvant chemotherapy in patients with invasive micropapillary carcinoma (IMPC) of breast.

Materials and Methods: Sixteen patients with IMPC who received neoadjuvant chemotherapy in Renmin Hospital of Wuhan University from January 2017 to July 2019 were selected as subjects. The Miller-Payne grade was used to evaluate the therapeutic effect. The correlation between Miller-Payne grade of chemotherapy efficacy and the expression of Ki67, WHO grade of the tumor, proportion of micropapillary carcinoma and tumor diameter was analyzed by Spearman rank correlation coefficient. The relationship between the positive rate of ER, PR and Her-2 and the lymphatic metastasis, vascular invasion before chemotherapy and the efficacy of chemotherapy were analyzed by Fisher's exact text.

Result: The expression of Ki67 and WHO grade of the tumor had positive correlation with the Miller-Payne grade of neoadjuvant chemotherapy (r_s = 0.508, P = 0.045 for Ki67, r_s = 0.614, P = 0.011 for WHO grade). The proportion of micropapillary carcinoma and tumor diameter revealed no correlation with the Miller-Payne grade of neoadjuvant chemotherapy (r_s = 0.271, P = 0.310 for proportion of micropapillary carcinoma; r_s = 0.111, P = 0.683 for tumor diameter). There were no significant differences in the positive rate of ER, PR and Her-2 and the lymphatic metastasis, vascular invasion between the effective and ineffective group (P > 0.05).

Conclusion: The therapeutic effect of neoadjuvant chemotherapy in IMPC breast patients is positively correlated with the expression level of Ki67 and WHO grade of tumor, which should be recognized in clinical treatment.

Combined high expression of CD47 and CD68 is a novel prognostic factor for breast cancer patients

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Objective: Avoiding the phagocytosis by tumor-associated macrophages (TAMs) is necessary for the growth and metastasis of solid tumors. CD47 binds to the receptor signal-regulatory protein-α (SIRP-α) on the macrophages to avoid normal phagocytosis. In this study, we evaluated the expression and prognostic significance of CD47 and CD68-labeled TAMs in breast cancer solid tumors.

Material and Methods: Two hundred seventeen cases of breast cancer tissues and forty cases of benign breast lesions were collected for immunohistochemical staining of CD47 and CD68.

Result: Both of the CD47 and CD68 expression were significantly higher in breast cancer tissues ($P < 0.001$), and associated with multiple clinicopathological parameters in breast cancer($P < 0.05$). However, CD47 or CD68 expression alone was not an independent predictor of poor DFS in multivariate survival analysis ($P > 0.05$). Interestingly, combined high expression of CD47 and CD68 (CD47^{high}CD68^{high}) not only had a significant association with advanced TNM stage, histological grade, LNM, ER status, PR status and recurrence ($P < 0.05$), but also displayed a poorer 5-DFS ($P = 0.011$). Strikingly, CD47^{high}CD68^{high} served as a novel independent prognostic factor for poor DFS compared to the expression of CD47 or CD68 alone ($P = 0.045$). Furthermore, our study also showed for the first time that the prognostic significance of CD47^{high}CD68^{high} not only in breast cancer in general, but also in hormone receptor-negative breast cancer in particular.

Conclusions: Combined detection of CD47 and CD68 may provide guidance for the prognosis of breast cancer, especially hormone receptor-negative breast cancer.

The genomic profiling of breast fibroepithelial tumor

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objiective
Fibroepithelial lesions of the breast from benign fibroadenomas to malignant phyllodes tumors comprise a morphologically and biologically heterogeneous group of biphasic tumors with epithelial and stromal components that demosteate widely variable clinical behavior. Although some genes mutation has been found in fibroepithelial tumor, the genomic drivers of these tumors are still being elucidated. We performed whole-exome and partial intron sequencing of 425 genes to identify genomic alteratins in fibroepithelial tumor.

Materials and Methods
DNA was extracted from formalin-fixed, paraffin-embbed samples from 25 patient of fibroepithelial tumor. Extracted DNA was purified and qualified employing the Nanodrop 2000 and then quantified by Qubit3.0 with a dsDNA HS Assay Kit. We sequenced all to a meam coverage of 582x, and, on average, 78% of bases were covered by at least 20 reads. Orignal image data acquired from HiSeq 4000 sequencing platform were transferred by base calling analysis into raw sequence data, which contained sequence information and corresponding sequencing qualigy information. Single nucleotide variants (SNVs) and short insertions/deletions (indels) were identified by VarScan2 within minimum variant allele frequency threshole set at 0.01, and p-value threshold for calling variants set at 0.05 to generate Variant Call Format (VCF) files. All SNVs/indels were annotated with ANNOVAR, and each SNV/indel was manually checked on the Integrative Genomics Viewer (IGV). Copy number variantions(CNVs) were detected using in-house-developed software.

Result
The 25 cases of fibrepithelial tumors included 4 fibroadenoma, 5 juvenile fibroadenoma,5 benign phyllodes tumor, 6 borderline benign phyllodes tumor and 5 malignant phyllodes tumor. The most commonly mutated genes were MED12 (60.9%), TERT (34.8%), RARA (21.7%) in all the fibroepithelial tumors. And in different tumors, the mutated genes were e somewhat different. In fibroadenoma, the most mutated genes were MED12 (75%) In the five cases of juvenile fibroadenoma, there were 2 cases no mutation, and no recurrent mutations were detected in the other 3 cases. In benign phyllodes tumor, the most commonly mutated genes were MED12 (80%), TERT (40%), RARA (40%). In borderline phyllodes tumor, the most commonly mutated genes were MED12 (66.7%), TERT (66.7%). In malignant phyllodes tumor, the most commonly mutated genes were MED12 (60%), TERT (40%), and also the amplification of EGFR (40%) were recurrent. All the cases were microsatellite stable. The tumor mutation burden (TMB) increased as the tumor grade increased. The median TMB were 1.2, 1.7,3.6, 3.7 and 6.7 in the juvenile fibroadenoma, fibroadenoma, benign phyllodes tumor, borderline phyllodes tumor and malignant phyllodes tumor respectively.

Conclusion

This study identifies clinically relevant genomic alterations in fibroepithelial adenoma that provides insights into the molecular pathogenesis of breast fibroepithelial tumors and may provides new therapy target for malignant PT.

Solid Papillary Carcinoma Of The Breast: A Clinicopathologic Analysis of 49 Cases

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Abstract
Objectives: Solid papillary carcinoma (SPC), a newly-defined entity, is a rare subset of breast carcinoma accounting for less than 1% of all patients with breast cancer. It has distinctive histological pattern and frequently demonstrates extracellular mucin and neuroendocrine differentiation. Here, we analyze the clinicopathologic characteristics and immunophenotype of SPC to know more about this uncommon tumour.
Materials and Methods: A total of 49 patients with SPC of breast from the Guangdong Women and Children Hospital between 2012 and 2019 were enrolled in this study. Retrospective analysis of   the clinicopathologic characteristics and immunophenotype was performed.
Results and Conclusion: Among these cases, 22 cases were diagnosed SPC in situ, 27 cases were confirmed SPC with invasion by immunohistochemistry. Median age for all patients was 58 years (range 20-78). The sentinel lymph node biopsy (SLNB) was performed in 28 (57.1%). Only one of them was with isolated tumour cells and no metastasis was found in other cases. Immunohistochemical staining was strongly and diffusely positive for ER and negative for HER2 in all of the cases. 46 (93.8%) cases were strongly and diffusely positive for PR. Interestingly, The Ki-67 expression in young patients (< 50 years) was significantly higher than the old patients ($\geqslant$ 50 years, P = 0.001). However, no differences were observed between SPC in situ and SPC with invasion for Ki-67 expression (P = 0.215). Chromogranin A and /or synaptophysin were positive in 48 cases.
Conclusion: SPC represents a distinct entity of breast carcinoma. The tumour cells are diffusely hormone-receptor-positive and HER2-negative. Lymph node and distant metastases are uncommon. Most of SPC occur in older women with low proliferation index and have an indolent behavior. However, proliferation index of tumour cells is high in younger women with SPC and it maybe implys a poor prognosis.

Category: Breast pathology
1652043

ELF5 promotes chemosensitivity in triple negative breast cancers through inhibiting of pSTAT3

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Background: ELF5 has been proved to have high expression level in several normal tissues, such as salivary gland, breast, and bladder. It has a vital role in the development of breast tissue. It is a biomarker of normal luminal cells and promotes alveolar genesis. However, ELF5 has an adverse role in breast cancers compared with it in normal breast tissues. It is seldom expressed in luminal breast cancers but highly expressed in basal-like breast cancers. It has been reported that ELF5 suppresses estrogen sensitivity and induces antiestrogen resistance in luminal breast cancers. In our previous studies, ELF5 showed to be related with to chemosensitivity in breast cancers. But there is little known about the mechanism of ELF5 in chemosensitivity.
Material and Method: Four published microarray datasets were used for differential gene expression analysis to test ELF5 expression level in different subtypes of breast cancers. We used lentivirus-mediated overexpression and siRNA-mediated knockdown of ELF5 in different breast cancer cell limes. Chemotherapy sensitivity assay was conducted in cells incubated with cytotoxic agents. Pretreated MDA-MB-231 with different ELF5 expression level was implanted into the fat pad of female nude mice. The mice were treated with saline or cytotoxic agents once a week. The tumor volume and mice weight were measured twice a week. The mice were then sacrificed, and the tumor tissues were collected for the following study. Cell cycle-related genes and vessel formation related genes were tested by western blot and RT-qPCR in cell lines with different ELF5 expression level. Agonist and inhibitor of pSTAT3 were used in cell lines. The influence about the different expression level of ELF5 and pSTAT3 to downstream genes were also tested by western blot and RT-qPCR.
Result: In published microarray datasets, ELF5 had a higher expression level in triple-negative breast cancers than non-triple negative breast cancers. It showed to be related to chemosensitivity in triple-negative breast cancers. High ELF5 expression can promote chemosensitivity to epirubicin in triple-negative cell lines (MDA-MB-231 and HCC1937). Overexpressed ELF5 cells also showed higher efficacy of epirubicin in a xenograft model. In vitro, ELF5 inhibited cell proliferation and induced G1 arrest by suppressing CCND2 expression. We further found high ELF5 expression repressed VEGFA production and antiangiogenesis function in vivo and in vitro. ELF5 also suppressed pSTAT3 expression significantly. When applying pSTAT3 agonist to ELF5 overexpressed cells, the suppression of CCND2 and VEGFA was relieved. In the ELF5 low expressed cells, pSTAT3 inhibitor also repressed CCND2 and VEGFA expression. After incubating ELF5 low expression cells with pSTAT3 inhibitor, the sensitivity to epirubicin was significantly improved.
Conclusion: High ELF5 expression promote chemosensitivity through inhibiting pSTAT3 expression in triple-negative breast cancers.

Are the clinicopathologic characteristics different among subgroups of reclassified fluorescence in situ hybridization (FISH)?

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Background: With updates of the human epidermal growth factor receptor-2 (HER2) guidelines, an analysis of their influences on the HER2 amplification rate and differences in clinicopathological features among reclassified fluorescence *in situ* hybridization (FISH) results is needed. The aim of this retrospective study was to compare clinicopathologic features among the reclassified FISH subgroups with updates of 2018 guidelines in a large cohort of breast carcinoma cases. We also divided HER2 amplified cases (HER2/CEP17 ratio $\geq$ 2.0, HER2 copy number $\geq$ 4.0) into a high-amplified group (HER2 copy number $\geq$ 6.0) and a low-amplified group (HER2 copy number $\geq$ 4.0 and $<$ 6.0) to determine whether there were any differences between these two groups.
Methods: According to different *HER2/CEP17* ratios and average *HER2* copy numbers, the samples the FISH results of 3795 breast cancers were classified into six groups: group 1a, ratio $\geq$ 2.0 and numbers $\geq$ 6.0; group 1b, ratio $\geq$ 2.0 and numbers $\geq$ 4.0 and $<$ 6.0; group 2, ratio $\geq$ 2.0 and numbers $<$ 4.0; group 3, ratio $<$ 2.0 and numbers $\geq$ 6.0; group 4, ratio $<$ 2.0 and numbers $\geq$ 4.0 and $<$ 6.0; group 5, ratio $<$ 2.0 and numbers $<$ 4.0. Clinical features were collected and compared among different FISH groups.
Results: Compare to group 5, the cases in groups 1a and 1b had higher histological grade, higher chance of estrogen receptor (ER) and progesterone receptor (PR) negative status and a higher Ki67 index than did the samples in group 5 (p $<$ 0.05). The features in groups 2 and 3 were similar to those in groups 1a and 1b (p $>$ 0.05). The samples in group 4 showed a higher histological grade and higher Ki67 index (p $<$ 0.05) than did the samples in group 5 but had a lower histological grade and lower Ki67 index (p $<$ 0.05) than did the samples in group 1a. After re-evaluating these cases according to 2018 guidelines, the cases that converted to a *HER2*-amplified status had features similar to samples in group 1 that showed a classic FISH-amplified status (p $>$ 0.05). When comparing HER2 positive rate in IHC-2+ cases between 2013 and 2018 guidelines, there were no differences about HER2 positive rate between these two editions (21.9% in 2013 guidelines, 21.0% in 2018 guidelines, p = 0.518).
Conclusion: The updated HER2 guidelines have no impact on the *HER2* amplification ratio. Different categories of *HER2* FISH test results have significant differences in clinicopathological features. The updated HER2 guidelines make a clear division of HER2 status which is helpful for clinical decision making for target therapy.

Category: Breast pathology
1652140

Effect of the biological features by knockdown of CD44 in breast cancer cells MDA-MB-435

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Purpose   Breast cancer stem cells (BCSCs) are the source of breast tumors. Compared with other cancer cells, cancer stem cells show high resistance to both chemotherapy and radiotherapy. Targeting of BCSCs is thus a potentially promising and effective strategy for breast cancer treatment. The breast cancer cells of characterised by CD44 expression and low or absent CD24 expression (the $CD44^+/CD24^{-/low}$ phenotype) possess specific characteristics, such as anti-tumor-drug and radiation resistance with increased capacity for self-renewal, invasion, drug resistance and tumor progression. CD44 is commonly used as a cell surface marker of cancer stem-like cells in epithelial tumours, and more and more studies showed that CD44 is an important surface marker of breast cancer stem cells. But it is unclear that CD44 is only a molecular marker of BCSCs or stem cell-related genes. In this study we aimed to investigate the effects of CD44 knockdown on the stemness and differentiation of BCSCs in MDA-MB-435 cells in terms of gene expression, cell cycle, and tumorigenesis, in comparison with BCSCs. The results will facilitate the development of BCSC-targeting differentiating gene therapy for breast cancer treatment.

Methods   We isolated a breast cancer cell population ($CD44^+/CD24^{-/low}$ cells) from breast cancer cells MDA-MB-435 by flow cytometry. These cells were sorted into four sub-populations based on their expression of CD44 and CD24 surface markers. CD44 knockdown in the BCSC population was achieved using small hairpin RNA with recombinant plasmid. The differentiated status of CD44 knock-down BCSCs was evaluated on the basis of changes in $CD44^+/CD24^{-/low}$ phenotype, tumorigenesis in NOD/SCID mice, and gene expression in relation to renewal status, metastasis, and cell cycle in comparison with BCSCs and non-BCSCs.

Results   Flow cytometric data showed that 91.2% of cell surface expressed CD44 and absenced CD24 in breast cancer cell line MDA-MB-435. qRT-PCR showed that the highest expression is CD44 transcript variant 4(CD44s). Then we used shRNA to knockdown of CD44 in MDA-MB-435. Knockdown of CD44 caused BCSCs to differentiate into non-BCSCs with lower tumorigenic potential, and altered the cell cycle and expression profiles of some stem cell-related genes, reduced the proliferation and migration of cells, making them more similar to those seen in non-BCSCs.

Conclusions   Knockdown of CD44 is an effective strategy for attacking the stemness of BCSCs, resulting in a loss of stemness. The results of this study highlight a potential new strategy for breast cancer treatment through the targeting of CD44 in BCSCs.

Clinicopathological features of metaplastic carcinoma combined with apocrine carcinoma of breast

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Objective: To investigate the clinicopathological features, immunophenotypic characteristics, diagnosis and differential diagnosis of special subtype breast cancer containing metaplastic carcinoma and apocrine carcinoma.
Material and Methods: A case of metaplastic carcinoma combined with apocrine carcinoma of breast was collected. The clinical manifestations, Color Doppler Ultrasound, histopathology, immunophenotype and prognosis characteristics were analyzed, and the relevant literatures were reviewed for discussion.
Result: A female patient, 62 years old, the clinical manifestations were progressive enlargement of bilateral breast masses. Color Doppler Ultrasound showed local glandular structure disorder and echo reduction in the left breast, and solid mass in the right breast with calcified plaque. Microscopically, the luminal nucleus of the left breast tissue was pleomorphic , large chromatin and obvious nucleoli. Nuclear fission was common, and the normal structure of the right breast was destroyed. The inner wall of the cyst was lined with squamous cells with different degrees of nuclear abnormity and polymorphism. Some tumor cells were composed of cancer cells differentiated from apocrine glands. Microscopically, the cytoplasm was rich and had basophilic granules, and tumor cells had large nuclei and obvious nucleoli. The results of immunohistochemistry showed that P120 (membrane), E-cadherin, ER, PR and HER-2 were positively expressed, and CK5/6 was negatively expressed in the left breast tumor cells; in the right breast tissue, squamous cell carcinoma was negative for ER, PR and HER-2, but positive for CK5/6 and P63. The proliferation index of Ki67 was about 40%. Apocrine carcinoma of breast was negative for ER and PR, but positive for AR, GCDFP-15 and HER-2. The proliferation index of Ki67 was about 40%. Combined with the clinical data, breast Color Doppler Ultrasound, histopathological features and immunohistochemistry results of the patient, metaplastic carcinoma combined with apocrine carcinoma of breast was diagnosed.
Conclusion: Metaplastic carcinoma of the breast is a group of cancers with heterogeneous components. The diagnosis depends on histopathological features and immunophenotype. The main treatment is surgical resection.

Category: Breast pathology
1653200

Clinicopathological features of tumors of haematopoietic and lymphoid tissue in the breast

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Purpose To study the clinicopathologic features, diagnosis and differential diagnosis of tumors of haematopoietic and lymphoid tissue of the breast. **Methods** Fifty-five cases of tumors of haematopoietic and lymphoid tissue were selected from Peking University People's Hospital from Febrary 2005 to August 2019. According to WHO classification of tumours of haematopoietic and lymphoid tissues (2008) and updated classification (2016), the cases were studied by microscopy, immunohistochemistry, in situ hybridization and FISH. **Results** In 55 cases of tumors of haematopoietic and lymphoid tissue, the mean and median age was 42.4 and 44 years old (range:13-82 years). Thirteen cases presented with B symptoms. LDH was elevated in 14 cases (28.6%,14/49), and elavated percentage of lymphocytes in peripheral blood (PB) can be seen in 19 cases(37.3%, 19/51). Twenty-three cases of lesion invoved the left breast, and 28 invoved the right, 3 invoved bilateral. In Ann Arbor staging, 8 cases were categorized as stage I, 9 as II, 1 as III, and 37 as IV. In pathological classification, 16 cases were diffuse large B cell lymphoma （DLBCL）, 3 were high-grade B cell lymphoma, 2 were MALToma，1 was follicular lymphoma (FL), 7 were myeloid sarcoma,1 was chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL),17 were B lymphoblastic leukemia/lymphoma,5 were T lymphoblastic leukemia/lymphoma, and 1 was anaplastic large cell lymphoma,ALK negative.The tumor cells of myeloid sarcoma were primitive, expressing MPO、CD43、CD117, et al. Twenty-five (45.5%) cases were treated with haematopoietic stem cell transplantation. Fifty-five cases were followed up, and the median survival period was 14.0 months (range:1-130 months). The overall survival rate was 59% for 3 years, 50% for 5 years and 32% for 10 yesrs. The prognosis of those with elevated percentage of lymphocytes in PB, high clincal staging,B symptome, Ki67 index >60%, and older than 50 year old is obviously worse. Multivariate analysis revealed that age (P=0.017), LDH (P=0.030), and Ki67 (P=0.014) were prognostic predictors for survival in tumors of haematopoietic and lymphoid tissue of the breast. **Conclusions** The clinical history, pathological morphology, mmunophenotype and molecular studies are very important for diagnosing tumors of haematopoietic and lymphoid tissue of the breast. The most common pathological types are DLBCL, and both lymphoblastic leukemia/lymphoma and myeloid sarcoma also can involve the breast. Resection with chemotherapy are recommended in treatment.

Adenoid cystic carcinoma of the male breast: clinicopathologic analysis and literature review

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〔Abstract〕

Objective To explore the clinicopathological features and diagnosis of adenoid cystic carcinoma of the male breast (ACC).

Materials and methods The clinical features, histopathology and immunohistochemical characteristics were analyzed in one case of ACC. The relevant literatures were reviewed.

Result A 23-year old male patient complained a solid lump in the left side breast for more than ten years, and companied with pain for five years. Microscopically, the tumor has clear border with periphery normal tissues, mainly composed of cribriform pattern, part of solid growth pattern. A biphasic cellular pattern comprised of basal-like cells and ductal luminal epithelial cells，interstitial mucoid or hyaline degeneration. Basal-like cells composed major part of the tumor, the nuclei are round or angulated, scantly cytoplasm, the boundary of the cells are not clear, interstitial invagination form a pseudo-glandular structure. Squeezed ductal luminal epithelial cells are among cribriform pattern, cells show cubic, acidophilic cytoplasm. Immunohistochemistry of basal-like cells showed positive of P63, CK5/6; Ductal cells showed positive of E-cadherin, P120, CD117, negative of ER, PR, Her-2,Ki67 index about 20%.

Conclusion ACC was a very rare variant of the breast carcinoma, occurring in female patients were extremely rare. The diagnosis can be made based on clinical and histopathological features combined with immunohistochemical results.

Combined Therapy with Cytokine-Induced Killer Cells and Oncolytic Adenovirus Expressing anti-p21Ras scFv Induce Enhanced Antitumor Activity in Breast Cancer

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Background: Ras mutations and overexpression of the p21Ras protein is the main causes of cancer development and progression, which has made the Ras gene and p21Ras essential targets for therapy of Ras-driven cancers. In this study, we constructed a dual-promoter-regulated recombinant adenovirus KGHV500 that carried anti-p21Ras scFv and explore whether the combination of cytokine-induced killer (CIK) adoptive immunotherapy with the oncolytic adenovirus-mediated transfer of anti-p21Ras scFv gene induces the enhanced antitumor potency. **Method:** Genetic engineering technology was used to construct KGHV500 and cesium chloride density gradient centrifugation was selected to purify KGHV500. In vitro, the human breast cancer cell line MDA-MB-231 was employed to investigate the anti-tumor activity of KGHV500 harboring the anti-p21Ras scFv gene using MTT, wound healing, and transwell invasion assays in vitro. In vivo, MDA-MB-231-transplanted tumors in nude mice were constructed and utilized to evaluate the treatment effect of the combination of CIK cells with KGHV500. Groups of PBS, KGHV500, CIK, and the combination of CIK cells with KGHV400 were selected as control. **Results:** KGHV500 was successfully constructed and purified. In vitro, KGHV500 has significant antitumor activity compared to KGHV400. In vivo, CIK combined with KGHV500 not only enhanced the anti-tumor effect on MDA-MB-231 cell transplanted tumor but also reduced the toxic side effects of KGHV500 on various organs. Conclusion: CIK combined with KGHV500 could enhance the anti-tumor effect and safety, and can be considered for the treatment of ras-driven tumors in the future.

Suppression of KIF3A inhibits triple negative breast cancer growth and metastasis by repressing Rb-E2F signaling and EMT

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Objectives: Triple negative breast cancer (TNBC) displays higher heterogeneity, stronger invasiveness, higher risk of metastasis and poorer prognosis. KIF3A, a member of the kinesin family of motor proteins, serves as a microtubule-directed motor subunit and has been found to regulate early development, ciliogenesis, and tumorigenesis. Materials and Methods: To explore the expression, regulation and mechanism of KIF3A in TNBC, 3 TNBC cell lines, 98 cases of primary TNBC and paired adjacent tissues were examined. Immunohistochemistry, Real Time Polymerase Chain Reaction (RT-PCR), Western blot, flow cytometry, short hairpin RNA (shRNA) interference, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), colony formation techniques, transwell assay, scratch test and xenograft mice model were used. Result: We found that KIF3A was over-expressed in TNBC and such high KIF3A expression was also associated with tumor recurrence and lymph node metastasis. Silencing of KIF3A suppressed TNBC cell proliferation by repressing the Rb-E2F signaling pathway and inhibited migration and invasion by repressing epithelial-mesenchymal transition (EMT). The tumor size was smaller and the number of lung metastatic nodules was less in KIF3A depletion MDA-MB-231 cell xenograft mice than in negative control group. Conclusion: These results suggested that high expression of KIF3A in TNBC may be associated with the tumor progression and metastasis.

Category: Breast pathology
1654601

Prognostic Significance of Lymph Node Macrometastasis in Breast Cancer Patients with One or Two Positive Sentinel Lymph Nodes Following Breast- conserving Surgery and Radiotherapy

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Background: The American College of Surgeons Oncology Group (ACOSOG) Z0011 trial suggested that complete axillary node dissection (ALND) may be omitted in breast cancer patients with low tumor burden who are treating with breast-conserving surgery and whole-breast irradiation. However, it is still in controversy whether it is applicable to such patients with high tumor burden in LNs.

Purpose: The aim of this study was to evaluate the prognostic value of lymph node macrometastasis (LNMac) in early stage breast cancer patients who had one or two positive sentinel lymph nodes.

Materials and methods: We identified primary invasive breast cancers (T1-2 breast cancer with 1-2 positive sentinel lymph nodes following breast-conserving surgery and whole-breast irradiation) who met the ACOSOG Z0011 eligibility criteria in the Surveillance Epidemiology and End Results (SEER) database (n=1477) and an independent cohort from Fudan university Shanghai Cancer Center (n=180). Patients were classified to micrometastasis and macrometastasis groups based on the size of sentinel lymph node metastasis. The association between LNMac and disease-specific survival (DSS), disease-free survival (DFS) and overall survival (OS) was evaluated.

Results: Kaplan Meier curves and log-rank test showed that patients with macrometastasis had similar DSS (P = 0.055), and poorer OS (P = 0.020) compared with those with micrometastasis in the SEER dataset population, and patients with macrometastasis had similar DFS (P = 0.238) and OS (P = 0.644) compared with those with micrometastasis in the validation cohort. Cox proportional hazards regression analyses showed that LNMac was not an independent prognostic factor for DSS (HR 1.19, 95% CI 0.87-2.64, P = 0.082), and OS (HR 1.38, 95%CI 0.99-1.93, P = 0.055) in the SEER dataset. Similarly, LNMac was also not an independent predictor of DFS (HR 3.16, 95%CI 0.42-13.96, P = 0.266) and OS (HR 0.59, 95%CI 0.06-5.68, P = 0.648) in the validation group.

Conclusion: Our results indicated that LNMac was not correlated with reduced DSS, DFS and OS in patients with one or two positive sentinel lymph nodes. ALND may be omitted for those patients treated with breast-conserving surgery and whole breast irradiation regardless of the extent of LN metastasis.

Intense basolateral membrane staining indicates HER2 positivity in invasive micropapillary breast carcinoma

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Background

Invasive micropapillary carcinoma (IMPC) is characterized by the inside-out growth of tumor clusters in a pseudopapillary arrangement. IMPC usually displays incomplete (basolateral or U-shaped) basolateral membrane staining of human epidermal growth factor receptor (HER2) protein. According to the 2018 American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) HER2 testing recommendation, HER2 IHC (immunohistochemical) 3+ requires that >10% of invasive tumor cells exhibit intense and complete membrane staining. IMPC tumors with moderate to intense but incomplete staining should be reported as IHC2+, requiring an additional ISH (in situ hybridization) test. And the criteria of HER2 IHC3+ for IMPC are not mentioned in the guideline.

Materials and methods

One hundred and forty seven cases with varied proportion (10%–100%) of IMPC and moderate to intense HER2 imunostaining in more than 10% of tumor cells of IMPC were enrolled. IMPC components of all cases were scored as HER2 IHC2+ based on the 2018 ASCO/CAP recommendation. Immunohistochemical characteristics including estrogen receptor (ER), progesterone receptor (PR), HER2, Ki67 and epithelial membrane antigen (EMA) were reviewed. The immunohistochemical surrogate subtype of all included tumors was determined using the definitions adopted by the 2013 St Gallen Consensus Panel. Fluorescence in situ hybridization (FISH) was performed on all the cases.

Results

More than half of the cases displayed lymph-vascular invasion (64/147, 43.5%) and lymph node metastasis (90/147, 61.2%). And most of them were grade 2 (106/147, 72.1%) histologically. The IMPC component varied from 10% to 100%, with mean and median of 80%, 40%, respectively. All IMPC components exhibited reversed/peripheral polarity. The majority of the cases were of the luminal subtype (122/147, luminal A, 36/147, 24.5%; luminal B, 86/147, 58.5%), and the HER2 overexpression subtype and triple negative subtype accounted for 15.6% (23/147) and 1.4% (2/147), respectively. IMPC components of all 147 tumors exhibited incomplete basolateral HER2 membrane staining. One hundred and sixteen of the tumors (116/147, 78.9%) had moderate staining, and 36 (36/116, 31%) showed HER2 amplification by FISH, and the HER2 gene was amplified in all the remaining 31 tumors (31/147, 21.1%) that exhibited intense basolateral membrane staining. Altogether, HER2 gene amplification was identified in 67 (67/147, 45.6%) cases, including all tumors with intense membrane staining and 36 with moderate staining. Besides, mean HER2 signals per cell and ratio of HER2/CEP17 were significantly higher

in the intense staining tumors, in contrast with the moderate staining tumors (p<0.0001). One hundred and ten available patients were staged according to the eighth edition of the American Joint Committee on Cancer (AJCC) staging system, with 18 patients (18/147, 12.2%) of stage I, 45 patients (45/147, 30.6%) of stage II and 47 patients (47/147, 40%) of stage III. Follow-up data were available for 119 patients. None of the patients were died. The follow time ranged from 1 month to 99 months, with mean and median postsurgical intervals of 38 and 57 months, respectively. Thirteen (13/119, 10.9%) patients exhibited disease progression (recurrence or metastasis). HER2 gene amplification essentially was correlated inversely with ER or PR expression (p<0.05) and positively with histological grade and disease progression (p<0.05).

Conclusions

HER2 overexpression or gene amplification was positively correlated with disease progression. IMPC with intense basolateral membrane immunostaining indicates HER2 positivity, even if the staining is incomplete, it should be classified as IHC3+ rather than IHC2+, which would avoid further FISH testing and related time and financial costs. Ultimately, ensuring all patients with HER2 gene amplification can obtain effective targeted therapy in time.

Category: Breast pathology
1655746

Limited clinical impact of the extent of retraction clefts in invasive breast cancer patients from Central China

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Aims Previous studies reported that retraction clefts (RCs) may correlate with the clinicopathological features and prognosis in invasive breast carcinoma. Therefore, we examined the correlation between the extent of RCs and the clinicopathological features and prognosis of breast cancer patients from Central China as well as the association between RCs and *PIK3CA* mutational status in these patients.

Methods The extent of RCs was estimated in 541 invasive breast carcinoma samples and its relationships with patients' clinicopathological characteristics, *PIK3CA* mutations and prognosis were analyzed using the Pearson $x2$ tests and univariate Cox proportional hazards regression assays.

Results Contradictory results were obtained among different patient groups and different stratification methods. No convincing association was detected between the extent of RCs and the clinicopathological features, *PIK3CA* mutational status or prognosis.

Conclusions The extent of RCs does not correlate with clinicopathological characteristics or *PIK3CA* mutational status, nor can it act as a prognostic predictor in invasive breast carcinoma patients from Central China.

Significance of ER, PR, HER2 as biomarkers in predicting pathologic response after neoadjuvant chemotherapy for breast cancer

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Purpose   The aim of this study is to evaluate significance of ER, PR, HER2, Ki-67 as a biomarker in predicting pathologic response after neoadjuvant chemotherapy for breast cancer.

Methods   Formalin fixed and paraffin embedded tissues from 49 cases with breast cancer after neoadjuvant chemotherapy were studied by immunohistochemical method for expression of ER, PR, HER2, Ki-67. And the correlations of the biomarkers with pathologic response after neoadjuvant chemotherapy were investigated by univariate and multivariate analysis.

Results   According to the pathologic response after neoadjuvant chemotherapy, the patients with breast cancer were divided into highly effective group and mildly effective group. Statistical difference was found in average age of two groups (highly effective group 51 years old vs mildly effective group 44 years old, P=0.042), ER negative (highly effective group 19 cases vs mildly effective group 3 cases, P=0.02), PR negative (highly effective group 25 vs mildly effective group 4 cases, P=0.002), HER2 overexpression of molecular classification (highly effective group 11 vs mildly effective group 0 cases, P=0.015). Multivariate analysis identified PR as the independent pathologic response factor after neoadjuvant chemotherapy for breast cancer (OR: 0.131, 95%CI: 0.033-0.518, P=0.004).

Conclusion   The breast cancer patients with older age, ER negative, PR negative or HER2 overexpression is more effective receiving neoadjuvant chemotherapy. And PR was the independent pathologic response factor after neoadjuvant chemotherapy for breast cancer.

E2F1 induces KIF26A transcription and promotes cell cycle progression via the CDK-RB-E2Fs feedback loop in breast cancer

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Objectives: The development of novel targeted therapies for the treatment of breast cancer (BC) has been fueled by investigations into the molecular mechanisms. The aim of this study was to investigate the functional role of KIF26A in BC.

Materials and Methods: qRT-PCR and immunohistochemistry were conducted to explore KIF26A expression and functional contribution to BC development. MTS, EDU, colony formation assays, and flow cytometry analysis were conducted to assess cell proliferation characteristics and cell cycle progression. A series of 5'-flanking region deletion plasmids and mutating the binding site, with the luciferase reporter assay, were used to identify the core promotor region of KIF26A. The prediction by software and construction of the transcriptional factor plasmids were used to identify the transcriptional factor. Chromatin immunoprecipitation (CHIP) assay could demonstrated transcriptional factor directly binding to the KIF26A promoter. Human Genome Oligo Microarray Assay, gene ontology (GO) and pathway analyses, and the following qRT-PCR and Western Blot were used to predict and validate the downstream pathway.

Results: Our results showed that in BC tissues, elevated KIF26A expression was significantly correlated with lymph node metastasis. KIF26A could promote proliferation and G0/G1 phase cell cycle progression in BC cells. The core promoter region of the human KIF26A gene was located upstream of the transcription start site at position -395 to -385. The transcriptional factor E2F1 was shown to directly binding to the KIF26A promoter and activate KIF26A expression. Furthermore, KIF26A was shown to inhibit the expression of p21, then activate CDK-RB-E2Fs pathway. The elevated E2F1 can activate the cell cycle progression and also activate the KIF26A expression, forming feedback loop.

Conclusion: The present study demonstrated that KIF26A, directly upregulated by E2F1, promoted cell proliferation and cell cycle progression via CDK-RB-E2Fs feedback loop in BC.

Prognostic significance of tumor-stromal ratio in invasive breast cancer and proposal of a new Ts-TNM staging system

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Objective: Previous studies have demonstrated that tumor-stromal ratio (TSR) was an independent prognostic factor in several kinds of carcinomas. This study aims at exploring prognostic significance of TSR in invasive breast cancer using immunohistochemistry (IHC) stained tissue microarrays (TMAs), and integrating TSR into traditional tumor-node-metastasis (TNM) staging system.

Materials and Methods: The prepared 7 TMAs containing 240 patients with 480 invasive BC specimens were stained with cytokeratin (CK) by IHC staining method previously. Ratio of tumor cells and stromal cells were visually assessed. TSR > 1 and TSR $\leq$ 1 were categorized as high TSR group and low TSR group respectively, and the prognostic value of TSR on 5-year disease free survival (5-DFS) was analyzed. A new Ts-TNM (tumor stroma-tumor-node-metastasis) staging system was established and assessed.

Result: IHC staining of CK could specifically label tumor cells with clear contrast, making it easy to manually assess TSR. High TSR and low TSR were observed in 52.5% (n = 126) and 47.5 (n = 114) of the cases according to dividing value 1. Kaplan-Meier analysis showed that patients in low TSR group had a worse 5-DFS compared to patients in high TSR group (P = 0.022). Multivariate analysis indicated that T stage (P = 0.014), N status (P < 0.001), histological grade (P < 0.001), estrogen receptor status (P = 0.015) and TSR (P = 0.011) were independent prognostic factors of invasive BC patients. The new Ts-TNM staging system combining TSR, tumor staging, lymph node status, and metastasis staging was established. Receiver Operating Characteristic curve analysis demonstrated that the ability of Ts-TNM staging system to predict recurrence was not lower than TNM staging system.

Conclusion: This study confirms TSR as a prognostic indicator for invasive breast cancer. Ts-TNM staging system containing stromal and tumor information may optimize risk stratification for invasive breast cancer.

A case report of breast cancer metastasis to nasal cavity and literature review

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Abstract

Background: breast cancer is one of the most common malignant tumors in women, and its incidence rate has been increasing gradually in recent years, accounting for 7% ~ 10% of all kinds of malignant tumors in the whole body in China. The common metastatic pathways of breast cancer are local spread, lymphatic metastasis and hematogenous metastasis, and the most common distant metastasis are bone, lung and liver in sequence. The incidence of nasal metastasis of breast cancer is low and rare in clinic. **Objective:** to report a case of misdiagnosis in our hospital. **Methods:** a case of nasal metastasis of breast cancer diagnosed and treated in our hospital after surgical resection and pathological confirmation was reported as follows. **Conclusion:** nasal metastasis of breast cancer is rare and easily misdiagnosed in clinical practice. When the clinical work encountered space occupying lesions, in time to ask the history, exclude the possibility of metastatic tumor. At the same time, pathologists can pay attention to the microscopic characteristics of these biologically active metastatic tumors in their work and research, and conduct further research, which can play a role in suggesting and guiding clinical diagnosis and treatment.

Category: Breast pathology
1656069

TOPK: a new predictor for the therapeutic response of neoadjuvant chemotherapy and prognosis in triple negative breast cancer

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Abstract

Background: Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in Chinese females. Triple negative breast cancer (TNBC), characterized by lack of expression of α-estrogen, progesterone, and HER2 receptors, has a significantly higher probability of relapse and poorer overall survival compared with other breast cancer subtypes. At present neoadjuvant chemotherapy (NCT) is a routine therapy for TNBC, but there are still some patients who don't respond well to NCT. PDZ-binding kinase/T-LAK cell-originated protein kinase (PBK/TOPK), a member of mitogen-activated protein kinase kinase (MAPKK), is highly expressed in a variety of tumors including breast cancer and contributes to cancer cell proliferation and survival. However, the role of TOPK in TNBC NCT is still unrevealed.

Materials and Methods: In this study, we collected 67 cases of puncture specimens of TNBC before NCT and surgical specimens after NCT and investigated the relationship between TOPK expression and NCT treatment response and prognosis. Docetaxel and pirarubicin are the main neoadjuvant chemotherapy drugs. Immunohistochemistry analysis was used to detect the expression of TOPK and ki-67on formalin-fixed paraffin-embedded (FFPE) specimens of pre-and post-NCT. The Miller-Payne system was used to assess the therapeutic response of NCT of TNBC patients: Grade 1-3 represents non-responders and Grade 4-5 represents responders.

Results: Immunohistochemistry analysis showed that patients with Miller-Payne classification Grade 1-3 has a significantly higher TOPK expression level after NCT treatment than before (p=0.000, Figure 1A and 2A). On the contrary, patients with Miller-Payne classification Grade 4-5 has a significantly lower TOPK expression level after NCT treatment than before (p=0.039, Figure 1B and 2B). The expression of ki-67 with Grade 4-5 also decreased after NCT (p=0.000, Figure 2C). The TOPK positive rate before NCT of patients in Grade 1-3 (31/45, 68.9%) was higher than those in Grade 4-5 (10/21, 47.6%) (p=0.034, Figure 2D). Survival analysis revealed that TNBC cases with TOPK elevation after NCT treatment showed a worse progression-free survival (PFS) than those with TOPK reduction (p=0.006, Figure 3A). The prognosis of patients with higher TOPK level before NCT was poorer than those with lower TOPK level (p=0.04, Figure 3B).

Conclusions: In conclusion, our results suggest that patients with high TOPK level are resistant to NCT treatment and have a poor prognosis in TNBC. TOPK may present as a novel predictor for the therapeutic response of NCT and prognosis in TNBC.

Category: Breast pathology
1656344

Higher SMARCA4 (BRG1) expression may predict better prognosis in triple-negative breast cancer

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The chromatin remodeling complex SWI/SNF subunit BRG1 plays an important role in multiple tumors. However, few studies have examined BRG1 expression in triple negative breast cancer (TNBC). To investigate its role in disease development, we examined the expression of BRG1 in TNBC tissues and analyzed its association with clinicopathological variables and patient survival. Tissue microarray and immunohistochemistry analyses were used to initially evaluate BRG1 expression in 206 cases of TNBC. Finally, 197 cases were available for analysis. Of these, low and high expression were observed in 18.3% and 81.7% of cases, respectively. Our data showed that BRG1 expression was significantly associated with distant metastases and histological type (P < 0.05). Specifically, lower expression predicted distant metastases (P = 0.032). However, BRG1 expression was not associated with age, histological grade, tumor size, P53 status, TNM stage, lymph node metastasis, and the basal-like subtype. Kaplan–Meier survival curves showed that high BRG1 expression was associated with a favorable outcome (overall survival [OS], P = 0.018; progression free survival [PFS], P = 0.031). Univariate analysis showed that BRG1 expression, TNM stage, lymph node metastasis, and distant metastases were significantly associated with both OS and PFS in TNBC patients. Furthermore, multivariate analysis confirmed that BRG1 could be an independent prognostic marker for OS (HR＝0.479, 95% CI: 0.247–0.930, P = 0.030). Meanwhile, distant metastasis was an independent predictor of worse OS and PFS (P < 0.001). In conclusion, our data indicated that BRG1 could be an important prognostic and protective biomarker for TNBC.

Category: Breast pathology
1656391

Apelin is associated with lymph vessel density, nodal status, and prognosis in breast cancer patients

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Abstract

Apelin has been shown to be a novel angiogenic factor in various cancers. However, there is limited information regarding the role of apelin in breast cancer. In this study, we began by investigating the apelin expression in breast cancer with long-term follow-up (142 cases of invasive ductal and other breast cancers) using immunohistochemistry. We then analyzed the relationship between apelin expression and microvessel density (MVD), lymphatic vessel density (LVD), lymph node status as well as other established clinicopathological parameters. The relationship between apelin expression and prognosis was also studied. In addition, we compared the apelin expression between 30 breast cancer samples and normal breast tissues adjacent to the breast tumors using western blot and RT-PCR. Apelin protein expression was detected in the cytoplasm of the carcinoma cells at various intensities. Apelin expression was positive in 59.2% (84/142) of the breast cancer patients and apelin expression was significantly correlated with tumor size (p=0.030), stage (p=0.000), pathologic type (p=0.009), MVD (p=0.000), LVD (p=0.000), and lymph node metastasis (p=0.041). Survival curves determined by the Kaplan-Meier method and univariate analysis demonstrated that high expression of apelin was associated with both worse disease free survival (p<0.001) and overall survival (p<0.001). Interestingly, significant difference in apelin expression by WB as well as RT-PCR is observed between normal breast tissues adjacent to the breast tumors and breast cancer. Our results showed apelin is associated with lymph vessel density, nodal status, and poor prognosis in breast cancer patients. The presence of apelin is a new prognostic factor and potential therapeutic target for breast cancer.

The sensitization effect of Chk1 inhibition on ADR varies with ER/PR status

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Objectives: In previous studies, we confirmed that checkpoint kinase 1 (Chk1) is associated with acquired resistance to neoadjuvant chemotherapy for breast cancer. Recently, more and more research into tumor therapy has focused on Chk1-targeted inhibitors in combination with chemotherapy. However, application of Chk1-targeted inhibitors is limited by tumor heterogeneity in breast cancer, mainly in terms of how to choose an effective application method when the tumor has heterogeneous molecular characteristics. In response to this problem, Here, we selected the hallmarks in luminal typing to explore the relationship between application of Chk1-targeted inhibition and tumor heterogeneity in combination with adriamycin (ADR).

Materials and Methods: We analyzed Chk1 expression in cases of breast cancer with different ER, PR and HER2 statuses and their co-expressed genes using TCGA and GTEx databases. MDA-MB-231/ADR (ADR-resistant) cells induced with progressive concentrations of ADR. siRNA targeting the Chk1 transfected into MCF-7, T47D, MDA-MB-231, MDA-MB-468 and MDA-MB-231/ADR to measure ADR chemotherapy including drug sensitivity assay and cell apoptosis analysis. Next, with MCF-7, T47D, MDA-MB-231, MDA-MB-468 and MDA-MB-231/ADR cells exposed to ADR, we measured the changes in levels of mRNA, protein and chemical modifications for Chk1 using RT-qPCR and western blot. To explore the mechanism by which Chk1 regulates ADR chemosensitivity, we pioneered a cross-linking analysis of gene data sets and phenotype data sets including genes co-expressed with Chk1 from published TCGA data, RNA sequencing (RNAseq) data of the si–Chk1 and si–control groups for MDA-MB-231 cells, GSE24460 and GSE116441 from GEO database. Based on that, the potential targets were verified by western blot. In the study for the regulation of Chk1 by ADR, we analyzed GSE763 data, performed screening process for histone methylation and deacetylation and measured the molecules that mediate the regulation of Chk1 by ADR.

Results: The published data from TCGA and GTEx databases showed that Chk1, highly expressed in breast cancer, correlated with patient survival and ER/PR status. And interestingly, Chk1 knockdown enhanced chemosensitivity to ADR in ER-/PR-/HER2- cells instead of ER+/PR+/HER2-cells. In ER-/PR-/HER2- breast cancer, CHK1 can be induced by ADR, which in turn affected chemosensitivity by regulating cell cycle arrest mediated by the mitotic checkpoint complex (MCC)–anaphase-promoting complex/cyclosome (APC/C)–cyclin B1 axis and apoptosis induced by Msh homeobox 1 (MSX) and Bcl-2–like protein 11 (BIM). However, in ER+/PR+/HER2- breast cancer, due to significant suppression of centromere protein F (CENPF)–mediated transcriptional activation of

Chk1, induced by ADR itself, Chk1 inhibition failed to sensitize ADR toxicity in ER+/PR+/HER2- breast cancer.

Conclusion: In summary, our study showed that the sensitization effect of Chk1 inhibition on ADR toxicity varies with ER/PR status, which provided a basis for the effective application of CHK1-targeted inhibition. And we suggest that the molecular characterization of breast cancer and the interaction with the combined drug deserves more attention in targeted therapy for breast cancer.

An integrative bioinformatics analysis identified miR-375 as a candidate key regulator of malignant breast cancer.

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MicroRNAs(miRNAs) are key regulators that play important biological roles in carcinogenesis and are promising biomarkers for cancer diagnosis and therapy. hsa-miR-375-3p (miR-375) has been suggested to serve as a tumor suppressor or oncogene in various tumor types; however, its specific expression and potential regulatory role in malignant breast cancer remain unclear. In this study, the results from noncoding RNA microarray analysis indicated that the miR-375 expression level is significantly decreased in malignant basal-like breast cancer compared with luminal-like breast cancer. A total of 1895 co-downregulated and 1645 co-upregulated genes were identified in miR-375 mimic-transfected basal-like breast cancer cell lines. Predicted miR-375 targets were obtained from the online databases TargetScan and DIANA-microT-CDS. Combined KEGG enrichment analysis for coregulated genes and predicted miR-375 targets provided information and revealed differences in potential dynamic signaling pathways regulated by miR-375 and also indicated specific regulatory pathways , such as RNA transport and processing, inbasal-like breast cancer. Additionally, gene expression microarray analysis accompanied by UALCAN analysis was performed to screen upregulated genes in the basal-like subtype. Four potential key genes, including LDHB, CPNE8, QKI, and EIF5A2, were identified as candidate target genes of miR-375. Therefore, the present study demonstrated that miR-375 may be a potential key regulator and provide a promising direction for diagnostic and therapeutic developments for malignant breast cancer.

AJCC 8th Edition Prognostic Staging provides no better discriminatory ability in prognosis than Anatomical Staging in triple negative breast cancers

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Background　　The American Joint Committee on Cancer (AJCC) eighth edition cancer staging manual has updated with a prognostic stage group for breast cancer by incorporating traditional TNM anatomic parameters and biomarkers (ER, PR, HER-2 status and tumor grade, etc.). In this study, we retrospectively compared the prognostic value between the AJCC 8th Edition anatomic and prognostic staging system for triple negative breast cancer (TNBC) in a cohort from two involved institutions and a large population database.

Design　　Clinicopathological data of patients who underwent breast surgery as the initial intervention and with TNBC were identified in Sun Yat-sen University Cancer Center (SYSUCC) during 2005-2013 and Prince of Wales Hospital (PWH) during 2002-2008. Data from surveillance, epidemiology, and end results (SEER) database during 2010-2015 was also accessed. We restaged all cases into anatomic stage (AS) and prognostic stage (PS) group according to the AJCC 8th Edition staging system. The Kaplan-Meier analysis with log-rank test was used to compare differences in disease-specific survival (DSS), overall survival (OS) and progression-free survival (PFS) between groups. The Harrell concordance index (C index) was calculated to measure predictive performance for the AS and PS models. The relationship of AS and PS with DSS, PFS or OS was examined using a Cox proportional hazards regression model.

Results　　A total of 611 patients with stage I to IIIC TNBC were identified in the SYSUCC-PWH cohort with a median follow-up of 53.5 months. Compared with the AS, PS upstaged 46.1% of patients. No patient was downstaged by PS in this cohort. No significant difference was observed in C index between AS and PS models for either DSS (0.84 vs. 0.83, p=0.943) or PFS (0.82 vs. 0.80, p=0.887), demonstrating that PS do not reflect a more accurate predictive model than AS. A total of 31941 patients with stage I to IIIC TNBC were identified in the SEER cohort with a median follow-up of 27 months. PS upstaged 62.4% of patients and downstaged eight patients (from IIIC to IIIB) in this cohort. Similarly, PS model did not perform better than the AS in either DSS (C index, 0.85 vs. 0.86, p=0.95) or OS (C index, 0.90 vs. 0.90, p=0.98). $x2$ statistic and Hazard Ratio for PFS, DSS and OS showed better discrimination between IA and IB, IIB and IIIA, IIIA and IIIB in AS model than PS model, while PS model showed better discrimination between IB and IIA as well as IIIB and IIIC than AS model. Compared those with stage

changed to other without, in SYSUCC-PWH cohort, patients with IIIC unchanged stage showed worse PFS compared to patients with AS IIIA or IIIB upstaged to PS IIIC (p=0.049). Similarly, in SEER cohort, patients with IIIC unchanged stage showed worse OS and DSS compared to patients with AS IIIA or IIIB upstaged to PS IIIC (p<0.001).

Conclusion　　Our findings demonstrated that PS did not provide better discriminatory ability in predicting TNBCs prognosis than AS. Further update with additional morphological and genomic information regarding the prognostic significance should be incorporated into prognosis staging model for TNBCs.

Micropapillary pattern in pure mucinous carcinoma of breast is associated with unfavorable prognosis

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Background

Micropapillary pattern (MP) can be seen in different breast carcinomas and its prognostic significance is uncertain. While invasive micropapillary carcinoma possesses aggressive behavior and poor prognosis, a similar pattern may occur in pure mucinous carcinoma (PMC) named micropapillary variant of mucinous carcinoma (MPMC) and its prognostic significance remains controversial.

Design

A retrospective review of 161 cases of PMC diagnosed in SYSUCC during 2005-2015 by four pathologists was conducted. Clinico-pathologic features including age, tumor size, nuclear grade, lymphovascular invasion (LVI), lymph node metastasis (LNM), MP percentage (MP%), AJCC TNM stage and ER, PR, HER-2, Ki67 expression were evaluated. Intraclass correlation coefficient (ICC) was used to estimate the agreement among four pathologists for MP%. The patients were followed up for 12-192 months (median 63 months). 21-gene Recurrence Score Assay was applied in selected conventional PMC (cPMC, n=13) and MPMC (n=14) cases that were pT1-2 N0 M0 ER+ HER2-.

Results

MPMC was identified in 32 (19.9 %) of the 161 PMC. Compared to cPMC, MPMC occurred in younger age (median age, 42 vs. 46 years; p=0.003), exhibited higher nuclear grade (grade 1, 3.1 vs. 75.2%; grade 2, 71.9 vs. 24%; grade 3, 25 vs. 0.8%; p<0.001), more frequent LVI (50% vs. 9.3%; p<0.001), LNM (46.9% vs. 23.2%; p<0.001), higher HER2 overexpression (25.0% vs. 2.3 %; p<0.001). Higher risk of local recurrence (9.4 vs. 0%; p<0.001) was observed in MPMC. There was no difference in tumor size, ER, PR expression, distant metastasis and death of tumor when compared to cPMC. Kaplan–Meier analysis showed that MPMC patients had a decreased progression-free survival (PFS)(p<0.001) and distant disease-free survival(DDFS)(p=0.024) than cPMC patients. In univariate analysis, MPMC morphology was a prognostic indicator for PFS (HR=12.0, p<0.001) and DDFS (HR=5.0, p=0.041), but not OS. Nuclear grade was the only independent factor for PFS or DDFS in multivariate analysis. The optimal cut-off value of MP% was 17.5% (AUC=0.705; sensitivity, 56.3%; specificity, 84.8%). The ICC for agreement among pathologists was 0.92 (95 % CI 0.901–0.940; p<0.001). Compared to cPMC, a higher Recurrence Score was also observed in MPMC patients (41.71±5.54 vs. 23.21±3.29, p=0.009).

Conclusion

MP in breast cancers may contribute to aggressive behavior and indicate unfavorable prognosis in PMC. Moderate to high nuclear grade and the exact MP% should be assessed in the diagnosis of MPMC, which may represent an aggressive subtype of PMC.

Category: Breast pathology
1656930

miR-4472 promotes tumor proliferation and aggressiveness in breast cancer by targeting RGMA and inducing EMT

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Objective Breast cancer is the most common cause of cancer-related death in women worldwide. MicroRNA (miRNA) ectopic expression is reported to be involved in the regulation of gene expression in breast cancer. Here we screened several differentially-expressed miRNAs associated with breast cancer chemoresistance, growth, and metastasis using a miRNA microarray. Specifically, increased expression of miR-4472 has been associated with larger breast tumors and chemoresistance. However, the biological function of miR-4472 and its molecular mechanisms in cancer progression have not been reported.

Materials and Methods Real-time quantitative PCRwas used to measure the expression of miR-4472 in breast cancer tissues and cell lines. The biological functions of miR-4472 and its target gene were explored using Transwell, cell proliferation and flow cytometry assays. Bioinformatics tools, dual-luciferase reporter assays and western blot were employed to identify the target genes of miR-4472. Western blot was used to explain the participation of miR-4472 and target gene in epithelial-to-mesenchymal transition.

Results miR-4472 was significantly upregulated in highly metastatic breast cancer tissues, and its expression was positively associated with larger tumor size, and advanced pTNM stage. miR-4472 promoted breast cancer cell metastasis and growth. Repulsive guidance molecule A (RGMA) was a direct target gene of miR-4472. RGMA was identified as suppressor in cancer metastasis. miR-4472 downregulated expression of RGMA and promoted EMT by supressing E-cadherin and initiating vimentin, β-catenin and slug.

Conclusions miR-4472 contributes to the progression of breast cancer by regulating RGMA expression and inducing EMT, which indicates that miR-4472/RGMA might serve as a therapeutic target for breast cancer.

The circRNA circHIPK3 acts as a sponge of miR-326 to promote triple-negative breast cancer progression through regulating FGF1 expression

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BACKGROUND:
In recent years, circular RNAs (circRNAs), a new star of non-coding RNA, have been emerged as vital regulators and gained much attention for involvement of initiation and progression of diverse kinds of human diseases, especially cancer. However, the clinical significance and function of circRNAs in triple-negative breast cancer (TNBC) are remain unknown.
METHODS:
Here, the expression of the top upregulated circRNA, circHIPK3, was confirmed by qRT-PCR in breast cancer cell lines and tissues. Kaplan-Meier survival analysis was conducted to analyze the clinical impact of circHIPK3 on TNBC. Then, functional experiments in vitro and in vivo were performed to investigate the effects of circHIPK3 on tumor growth and metastasis in TNBC. Mechanistically, fluorescent in situ hybridization, dual luciferase reporter assay, RNA pull-down and RNA immunoprecipitation experiments were performed to confirm the interaction between circHIPK3 and miR-326 in TNBC.
RESULTS:
qRT-PCR analyses verified that circHIPK3 was significantly upregulated in TNBC, and its level was correlated with pathological grade and poor prognosis of patients with TNBC. The inhibition of circHIPK3 suppressed cell proliferation and migration in TNBC. Luciferase reporter assay and RNA immunoprecipitation assay revealed that circHIPK3 and FGF1 could bind to miR-326 and that circHIPK3 regulated the expression of FGF1 via sponging miR-326.
CONCLUSIONS:
Our findings suggest that circHIPK3 promotes TNBC progression through circHIPK3/miR-326/FGF1 axis and it may serve as a new diagnostic marker or target for treatment of TNBC patients.

LncRNA AC093818 regulate breast cancer proliferation, migration and its mechanism

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Abstract: Breast cancer is a major worldwide health problem due to its high prevalence and mortality rate. Hypoxia is a common phenomenon in solid tumors and a basic feature of microenvironment. LncRNA AC093818 has been induced by HIF-1 α in breast cancer cells. But, how AC093818 regulates breast cancer development remains to be elucidated. In this study, results showed that AC093818 promotes proliferation, colony formation, migration, invasion of breast cancer cells in vitro and proliferation and lung metastasis in mice by regulating its neighboring gene PDK1. In summary, this study highlights an oncogenic role for AC093818 by regulating PDK1 in breast cancer and suggests that AC093818 may be a novel molecular therapeutic target for breast cancer.

OR2T6 is involved in breast cancer progression via initiating epithelial-mesenchymal transition and MAPK/ERK pathway

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Purpose Breast cancer is the most common female malignancy in the world, however its molecular pathogenesis still needs in-depth investigation. Here, we first investigated the role of OR2T6, a subtype of the olfactory receptor (OR) family, in the progression of breast carcinoma.

Materials and Methods Real-time PCR and immunohistochemistry staining were used to assess the expression level of OR2T6 in tissue samples. Cell proliferation, apoptosis, invasion and migration were measured by EdU labeling, flow cytometry and transwell chamberseparately. The genes potentially regulated by OR2T6 were screened by human gene expression microarray and further validated by real-time PCR and Western blot analysis.

Results OR2T6 was over-expressed in breast cancer tissues compared with normal breast tissues. OR2T6 expression was tightly correlated with higher TNM staging and lymphnode metastasis, and associated with worse patients' overall and disease-free survival. In the *in vitro* level, OR2T6 enhanced the proliferation, invasion and migration ability of breast cancer cells. Moreover, OR2T6 plays a role in the regulation of epithelial-mesenchymal transition (EMT) process and MAPK/ERK pathway.

Conclusions In the present study, we have preliminarily confirmed that OR2T6 is a novel oncogene, and it contributed to the progression of breast carcinoma by the initiation of EMT and MAPK/ERK pathway.

Molecular typing and treatment in triple negative breast cancer

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Triple negative breast cancer (TNBC) is defined that Estrogen receptor (ER), Progesterone receptor (PR) and Human epidermal growth factor receptor-2 (her-2) are all negative subtypes of breast cancer, with clinical characteristics of high aggressiveness, easy metastasis, easy recurrence and poor prognosis. Due to the lack of expression of ER, PR and her-2 in TNBC patients, endocrine therapy and her-2 therapy are not sensitive. Standardized TNBC treatment regimens are still lacking. Therefore, it is urgent to seek new TNBC treatment. Previous researchers conducted cluster analysis of gene expression profiles in tumor samples of patients with TNBC to conduct molecular typing of TNBC. Through the analysis of gene expression profile in tumor samples of TNBC patients with different subtypes mentioned above, it was found that the expression of genes related to cell cycle, cell proliferation and DNA repair in the BL1 subtype was abnormal, and possible therapeutic drugs included PARP inhibitors and genotoxic drugs. In the BL2 subtype, abnormal activation of growth factor signal EGFR, MET, NGF, Wnt/ beta-catenin, igf-1r and other pathways occurs, and its potential targeted therapeutic drugs include mTOR inhibitors and growth factor inhibitors. In the M subtype, cell motion-related signaling pathways and protein components (regulated by actin), ECM receptor interaction pathways and differentiation pathways are highly activated, also known as chemoplastic breast cancer. It is characterized by sarcomatoid or squamous epithelioid tissue that is prone to tolerance to chemotherapeutic agents. Therefore, patients with this type can be treated with mTOR inhibitors or targeted drugs for EMT. Compared with the M subtype, the MSL subtype expresses low levels of cell proliferation-related genes and high expression of dry-related genes. It is speculated that patients with this type may be treated with PI3K inhibitors, Scr antagonists and anti-angiogenic drugs. In the IM subtype, immune cell-related genes and signal transduction pathways, such as TH1 / TH2 pathway, NK cell pathway, B cell receptor pathway, BCR signaling pathway, DC pathway, T cell receptor signal, and cytokine il-12 pathway and il-7 pathway, were significantly enriched, which was highly similar to myeloid carcinoma of breast. Therefore, for patients with this type, it is recommended to use immunodetection point inhibitors such as PD1, PDL1 and ctla-4 for treatment. The gene expression profile of the LAR subtype is quite different from that of other TNBC subtypes. Although the ER receptor expression of this subtype is negative, the pathway genes related to hormone regulation are still highly expressed. It is worth noting that the male hormone receptor (Androgen receptor AR) in the high expression of LAR subtypes of breast cancer, the mRNA level 9 times that of other TNBC subtypes， IHC and high

expression of AR protein was detected, but also detect a large number of AR downstream metabolic marker and its auxiliary activated factor, therefore, for this type of patients, suggest that choose to adopt AR treatment. In this paper, we summarize the existing TNBC typing treatment schemes, hoping to provide new ideas for finding TNBC treatment schemes.

Overexpression of ABCB4 contributes to acquired doxorubicin resistance in breast cancer cells in vitro

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Globally, breast cancer is the most common life-threatening malignant disease among women with over 1 million new cases diagnosed every year. Doxorubicin is one of the most active agents in the first-line therapy for women with metastatic breast cancer. However, after a variable period, about 30% of patients develop drug resistance with a poor prognosis. Although the mechanisms are complex and multifactorial in general, it is likely that the ATP-binding cassette (ABC) transporters play a pivotal role in the development of doxorubicin resistance in the clinical setting. To date, several ABC transporters, such as ABCB1, ABCG2, and ABCCs, have been implicated in doxorubicin resistance, and their interaction with doxorubicin has been thoroughly investigated and reviewed. It has been confirmed that ABCB4 can also act as an efflux transporter to limiting the intracellular accumulation of drugs such as digoxin, paclitaxel, daunorubicin, vinblastine, and ivermectin. Recently, accumulating evidence indicates that ABCB4 is overexpressed in cancer cells after exposure to some chemotherapeutic drugs including doxorubicin, which implicate the involvement of ABCB4 in the acquired resistance to these anticancer agents. However, the exact role of ABCB4 in doxorubicin resistance as well as the potential mechanisms still remains unclear. In this study, we provide the evidence that ABCB4 is overexpressed in breast cancer cells with acquired doxorubicin resistance, which can be attributed, at least partially, to the epigenetic modifications of ABCB4 gene. ABCB4 mediates the efflux transport of doxorubicin in vitro, which can decrease the intracellular accumulation and antitumor efficacy of doxorubicin in breast cancer cells.

Preliminary study on genomic characteristics of lung adenosquamous carcinoma

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Objective: Lung adenosquamous carcinoma was detected to observe its clinicopathological features, genomics characteristics. Provide genomics research data for clinical diagnosis and treatment of patients with LASC.

Methods: Surgical resection specimens of 21 cases of LASC without preoperative chemoradiotherapy, which the squamous cell carcinoma components and adenocarcinoma components accounted for more than 10% based on WHO, which were identified by two experienced pathologists. HE and immunohistochemical P63, P40, NapsinA, TTF1 for auxiliary diagnosis. To use microdissection to separate squamouscell carcinoma and adenocarcinoma components from lung adenosquamous carcinoma, using a large Panel containing more than 1000 lung cancer variant genes, of 21 cases LASC gene mutations were detected, and the tumor phylogenetic tree of LASC were delineated. Preliminary exploration of its genomics characteristics.

Result: (1) Clinicopathological features of lung adenosquamous carcinoma, of the 21 cases, 7 were males, 14 were females. 3 (14.3%) were smokers, 18 (85.7%) were nonsmokers. Stages T1-T2, T3-T4 were 17 (81%), 4 (19%) cases. The overall lymph node metastasis rate was 57% (12/21), mainly were adenocarcinoma, of the 12 cases, adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma were discovered in 6 (50%), 2 (16.7%), 4 (33.3%) cases, respectively. (2) Analysis of neoplastic tree, delineating the tumor phylogenetic tree of 21 cases with lung adenosquamous carcinoma, 20 were trunk mutations, 1 case were no trunk mutation, confirmed that lung Adenosquamous carcinoma is mostly of monoclonal origin. (3) Among the 21 lung adenosquamous carcinoma samples, the highest frequency gene mutation, including 17 (81%) with EGFR mutations, 15 (71%) with TP53 mutations, 4 (19%) with RB1 mutations, 4 (19%) with TERT mutations, 4 (19%) with SMARTA4 mutations, and 2 (14%) with MAP3K1 mutations. (4) Comparison of gene mutation profiles between adenocarcinoma component (ACC) and squamous cell carcinoma component (SCCC) in 21 cases of lung adenosquamous carcinoma. Although TP53 appears to account for a higher proportion of SCCC, it does not reach statistical significance (P>0.05). In addition, no statistical differences were found, probably because of the small sample size. (5) The difference in the mutational spectrum between ACC and the LAC database of Gene+-Beijing (n=170), three differential genes were found, the mutation rate of EGFR in ACC was significantly higher than LAC (81% vs 56% ; P=0.035) and the incidenceof mutations in EZH2 and MAP3K1 was also significantly higher than LAC (P=0.032).

In addition, no statistical differences were found. (6)ACC was compared with LAC database of Gene+-Beijing. EGFR in SCCC was significantly higher than LSCC (76%vs 11%; $P<0.001$). In addition, no statistical differences were found.

Conclusion: Lung adenosquamous carcinoma is mostly of monoclonal origin. It has its unique genomic and clinical pathological features, especially with high EGFR mutations. EGFR mutation detection and targeted therapy may play an important role in the treatment of LASC patients.

The expression of Bmi-1 in beast cancers

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Abstract: Objective   To investigate the expression of Bmi-1 in breast cancer and its clinical significance. **Methods** The expression of Bmi-1, p53, HER-2, ER and PR were detected by immunohistochemistry using tissue microarrays which contained 64 breast cancers and 26 cases of normal breast tissues. **Results** The positive rates of Bmi-1, p53, HER-2, ER and PR expression were 75.0%, 29.7% , 22.8%, 56.1% and 54.4% in breast cancers, respectively. The positive expression of Bmi-1, p53 and HER-2 in breast cancers was higher compared to the normal breast tissues ($P<0.05$). ER and PR were respectively negatively correlated with histological grading in breast cancers($P<0.01$), and p53 was contrary($P=0.036$). The positive correlation was also observed between expression of ER and PR in breast cancers (P <0.01). Kaplan‑Meier survival curves showed ER, PR and clinical stage were respectively correlated with survival. **Conclusion** Bmi-1 may play a role in tumorigenesis. ER, PR and p53 were all correlated with the differentiation of breast cancers and may also involve in disease pathogenesis of breast cancers. PR was also negative correlated with patients' age($P=0.006$).

DNA mismatch repair deficiency in triple negative breast cancer

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Recent studies have suggested microsatellite instability was related to anti-PD-L1 treatment efficiency. Anti-PD-L1 was an important new therapy for triple negative breast cancer (TNBC). However, the frequency and significance of microsatellite instability in TNBC of Chinese people is unclear. In this study, we investigate a series of 447 TNBCs from 2002-2014 in Peking Union Medical College Hospital, utilizing DNA MMR protein immunohistochemistry, among them 195 cases followed by PCR microsatellite instability testing. By immunohistochemistry, we identified only 1 TNBC (1/447, 0.2%) showing loss of MSH2 protein; Further PCR microsatellite instability testing of the 195 cases identified 13 tumors showing low-frequency microsatellite instability. None high-frequency microsatellite instability was found. MMR protein-deficient carcinoma were not concordance with microsatellite instability. The MMR protein-deficient carcinomas were ductal type with high histologic grade, but had no recurrence for 59 months of follow-up time. The tumors showing low-frequency microsatellite instability were also largely high grade ductal type carcinoma at least in II stage with few tumor infiltrate lymphocytes (TILs). They have no correlation with recurrence and overall survival time (P=0.446 and 0.664). Univariate analysis showed they are not significant related to PDL-1 expression of tumor or TILs, nor related to amount of TILs, age, tumour size, lymph node involvement, stage, histological type, P53 expression or Ki-67 index were associated with either OS or DFS All 4 Prominent lymphocytic infiltration was noted in 2 tumors. In conclusion, our results suggest that DNA MMR deficiency is especially rare in Chinese TNBC. And as such, it not recommended to test DNA MMR deficiency for estimating PD-L1 efficacy in TNBC.

A literature review about LncRNAs as molecular markers of prognosis in triple-negative breast cancer

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Objectives Triple-negative breast cancer (TNBC) is a tumor with strong aggressiveness and poor prognosis. Recently, studies have found that long non-coding RNA (LncRNA) plays an important role in the development of tumors, but their role in the prognosis of triple-negative breast cancer is still unclear. We systematically explore the prognostic value of LncRNA in TNBC through statistical analysis.

Materials and Methods We searched the PubMed, Web of Science, and Embase to retrieve all eligible studies for statistical analysis. The association between LncRNA expression levels and overall survival (OS) 、disease-free survival (DFS) and progression-free survival (PFS) in TNBC patients was explored by assessing risk ratio (HR) and 95% confidence interval (CI).

Result 1. This study retrieved 178 articles and screened them for exclusion criteria. The statistical analysis finally included 11 articles, and a total of 1139 TNBC patients were evaluated. The median sample size was 104 (The range of 37-238 cases) reported the prognostic value of 9 different LncRNAs, of which only one (MALAT1) had two or more studies; 2. The inverted funnel plot was constructed using Stata 13.0 software and the analysis showed that the distribution of each point was basic. Symmetry, indicating no significant publication bias; 3. No significant inter-study heterogeneity was observed after analysis ($I2 = 34.5\%$, $P = 0.217$), using a fixed-effects model. And the funnel plot results indicate that there is no significant publication bias. The forest map shows that down-regulation of the expression level of LncRNA MALAT1 is associated with shorter OS (HR: 0.60, 95% CI: 0.44-0.83).

Conclusion Statistical analysis showed that LncRNA MALAT1 may be a potential prognostic molecular marker for TNBC. Since circulating biomarkers can be measured not only before surgery but also for life monitoring, they have more clinical value than tissue biomarkers. Therefore, more research is needed to investigate the prognostic value of LncRNA expression levels in serum of TNBC patients.

The prognosis of the lymphocytes amount, distribution and cell type in triple negative breast cancer

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Background: Recent studies showed that tumor infiltrate inflammatory cytes especially lymphocytes (TILs) had significant influence on prognosis. There were many inflammatory cell types in stroma. Their distribution, amount and the function are not clear.
Objective: Our research tried to investigate different lymphocytes, their amount, distribution and their relationship with prognosis.
Methods: 10 pairs of tumors were selected to experiment according to recurrence and non-recurrence. Multicolor fluorescence immunohistochemistry was utilized to analyze CD3, CD4, CD8, CD19, CD163 expression in tumors. The stained slides were scanned using the PerkinElmer Vectra (Vectra 3.0.5; PerkinElmer, Massachusetts, USA). A selection of 15 representative original multispectral images was used to train the inform software and obtain the final image data.
Results: 1. There was no significant difference in the amount of all 5 marker in tumor, stroma and overall fields (limma, p>0.05). The CD4 positive cells were more in stroma and total fields in non-recurrence group comparing to recurrence(limma, stroma, p=0.059; Total, p=0.079). 2. CD163 low expression group in any area including tumor, stroma and total field all had a significant longer disease free survival time(DFS) than higher expression group(Log-rank, Tumor, p=0.0033; Stroma, p=0.0406; Total, p=0.0033). 3. The DFS was significantly longer in stroma CD4 high expression group comparing to low expression group. 4. The amount of CD3, CD8 and CD19 positive cells had no significant influence both on DFS (Log-rank, p>0.05) . 5. The amount of all five markers had no significant influence on overall survival time (OS).
Conclusion: Different amounts of different type of lymphocytes in different areas had different influence on prognosis. All areas histocytes and stroma CD4 positive lymphocytes showed the most significant effect on DFS. This information might give new insight on immunotherapy.

The long intergenic noncoding RNA MAFG-AS1 promotes breast cancer cell proliferation and migration by regulating TUBA1B

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Objectives

Breast cancer (BC) is the most common malignant tumor in Chinese women. A large number of recent studies have shown that lncRNAs play a key regulatory role in the invasion and metastasis of a variety of malignant tumors. However, the regulatory mechanisms of lncRNAs in breast cancer remain to be further studied. This topic focuses on LncRNA MAFG-AS1 to explore its role in the malignant progression of breast cancer.

Materials and Methods

1. We analyzed the expression of LncRNA MAFG-AS1 in BC tissues by bioinformatics tool "TANRIC" downloaded from TCGA database. Moreover, qRT-PCR analysis was used to examine LncRNA MAFG-AS1 expression in BC tissues and the adjacent noncancerous tissues. The relationship between LncRNA MAFG-AS1 expression and clinicopathological indexes was evaluated.

2. Function assays in vitro: cell proliferation was tested by CCK8 and colony formation experiments; transwell analysis was performed to determine cell migration; Flow-cytometric assay was carried out to describe the cell cycle and apoptosis; qRT-PCR experiment characterized the expression of cell cycle-related genes.

3. Study in vivo: nude mouse transplantation tumor model was established to explore the effect of LncRNA MAFG-AS1 on tumorigenesis of BC cells

4. Differently expressed genes after LncRNA MAFG-AS1 knockdown were screened out by means of high-solution microarray and identified by qRT-PCR.

5. Function assays were implemented to further assess the target gene, which was investigated whether the gene was involved in the oncogenesis of LncRNA MAFG-AS1 via rescue assay.

Result

1. According to the results from TCGA database and qRT-PCR, the expression of LncRNA MAFG-AS1 was relatively high in BC tissues comparing with the adjacent noncancerous tissues. High level of LncRNA MAFG-AS1 expression was obviously related to advanced TNM stage, larger tumor size, lymghtic metastasis and grave prognosis in BC.

2. Function assays in vitro showed that the cell proliferation and migration was significantly obstructed with LncRNA MAFG-AS1 knockdown. On the contrary, LncRNA MAFG-AS1 overexpression promotes cell growth and migration.

3. It is smaller tumors that mice in sh-LncRNA MAFG-AS1 group developed than those in the control group.

4. 2237 differetially expressed genes (fold change>2, $p < 0.05$) in T-47D cells

transfected with si-LncRNA MAFG-AS1　or scrambled were screened out by high solution microarray, 1387　of them was upregulated while the other was downregulated. We identified TUBA1B　as a downstream target of LncRNA MAFG-AS1　via further GO analysis and qRT-PCR　test.

5. Si-TUBA1B　was designed to apply to function assays and rescue experiments which demonstrated that si-TUBA1B　compromise the oncogenesis of pcDNA-LncRNA MAFG-AS1.

Conclusion

Our study revealed that LncRNA MAFG-AS1　play an oncogenic role in BC　on the basis of regulating the expression of TUBA1B, suggesting that it might be a latent indicator and therapeutical target in　BC.

The basal-HER2 positive breast carcinoma with distinctive clinicopathological features

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Objective Retrospective cohort study of basal-HER2+ breast carcinoma focused on clinicopathological features and effect of neoadjuvant chemotherapy.

Materials and methods The breast cancer database at Xijing Hospital was queried to identify patients diagnosed with co-expression of CK5/6 and HER2-overexpressing primary breast cancer between 2013 to 2019.Thirty patients were identified with sequential core needle biopsy, adjuvant chemotherapy and mastectomy. These cases were diagnosed as basal-HER2 + breast carcinoma, namely ER-,PR-,HER-2 +,CK5/6+. Low expression of CK5/6 was scored as 1+，1-10% tumor cells were positive for CK5/6; high expression of CK5/6 was scored as 2+，$\geqslant$10% tumor cells were positive.

Results The median age of thirty patients with basal-HER2 positive breast carcinoma was 50 years (range 25-71). All cases were high grade non-specific type of invasive breast carcinoma with eosinophilic cytoplasm, which were similar to ER/PR/HER2 triple negative basal-like carcinoma, including poor differentiation, high grade, geographic necrosis, pushing margins of invasion,
syncytial arrangement of tumor cells, stromal lymphocytic infiltrates, high mitotic index. 16 cases were histological grade 2 and 14 cases were grade 3. The 30 cases of control group of HER2 positive breast carcinoma without expression of CK5/6 were scored into three grades (11 cases were grade 1, 15 cases were grade 2, 4 cases were grade 3). Comparing with the control group, basal-HER2 positive breast carcinoma showed higher histological grades and basal like tumor features.
Immunophenotypically, basal-HER2 positive breast carcinomas were consistently negative for ER and PR, positive for HER2 andCK5/6. The percentage of CK5/6 positivity ranged from 1% to 95% in basal-HER2 positive breast carcinoma. Five cases showed lower expression (1+) and 25 cases showed higher expression (2+) of CK5/6, and higher expression of CK5/6 positively related with higher histological grade.
The percentage of histologically grade 3 among CK5/6 2+ HER2 positive breast carcinoma and without or with low expression of CK5/6 were 52%（13/25）and 14.3%（5/35），respectively（$p<0.05$）. Basal-HER2 positive breast carcinoma showed higher Ki67 proliferate index, but there is no significant difference concerning patients age, tumor size and TNM stage . Furthermore, the percentage of P53 diffusely positivity in basal-HER2 positive breast carcinoma and the control group were 90.5%（19/21）and 37.5%（9/24），respectively, suggesting basal-HER2 positive breast carcinoma probably harbored higher frequency of p53 gene mutation. Additionally, 30%（9/30）basal-HER2 positive BC expressed P63, while only one case of the control was positive for p63.
Thirty patients with the basal-HER2 positive breast carcinoma accepted neoadjuvant chemotherapy and six patients were PCR, 16 patients were scored as grade 1, and 8

patients were grade as grade 2. Four patients of the control group achieved PCR, and there was no significant difference concerning the effect of adjuvant chemotherapy.
Conclusion　　Basal-HER2 positive BC is special subtype of HER2 positive breast carcinoma with distinctive clinicopathological features. There is urgent need to recognize basal-HER2 positive breast carcinoma. It is necessary to further study the prognosis and effect of anti-HER2 targeted therapy of basal-HER2 positive breast carcinoma.

21-Gene Recurrence Score and Clinicopathological Features of Low-amplified HER2 Breast Cancer

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Background: Around 80% breast cancers are human epidermal growth factor receptor 2 (HER2) negative regardless of hormone receptors (HR) status, of those about 80% are HR positive and HER2 negative. Although patients with HR positive and HER2 negative tumors are considered to have superior prognosis than those were HER2 positive tumors and triple negative tumors, heterogeneity regarding clinicopathological features and prognosis exit within HR positive and HER2 negative tumors. Previous studies reported that CEP17 is an independent poor prognostic factor in HER2 negative and HR positive breast cancers. Here we sought to investigate whether molecular testing regarding HER2 status and 21-gene could provide additional information for subgrouping these tumors.
Methods: 1716 invasive breast cancers diagnosed between 2014 and 2018 and previously tested for HER2 fluorescence in-situ hybridization (FISH) assay were selected for this study. All the tumors had average HER2 copy number < 4/cell and HER2/CEP17 ratio < 2. One hundred and sixty-four fixed paraffin-embedded tumors that were HR positive and had lymph node metastasis no more than two nodes were previously tested for 21-gene recurrence score (RS) by the Oncotype DX assay. The RS is presented on a scale from 0 to 100, and the risk groups for distant recurrence were prospectively defined as low (RS <18), intermediate (RS 18-30), and high (RS ≥ 31). Clinicopathological features including patient age, tumor size, tumor grade, histological type, IHC results of estrogen receptor (ER), progestogen receptor (PR), HER2, Ki67 expression were retrieved. A cut-off of ≤ 1.5 was used to define HER2 loss, and a cut-off of ≥ 3 was used to define CEP17 polysomy. A cut-off of $\geq 20\%$ was used to define high Ki67 expression.
Results: The median age at diagnosis was 79 years (range 23-95 years), and the median tumor size was 2 cm (range 0.4-12 cm). HR were negative in 13.5% (231/1716) tumors and were positive in 86.5% (1485/1716) tumors. All the included tumors suggested for HER2 FISH assay were HER2 IHC 2+. According to the immunohistological staining of ER, PR and Ki67 expression, tumors were further classified into luminal A (33%, 566/1716) and luminal B (54%,921/1716), and triple negative (13%, 229/1716) tumors. The average HER2 copy number of these tumors was 2.4/cell (range 0.9-3.98), and of CEP17 signals was 1.9/cell (range 0.8-5.9). Luminal B had significantly higher average HER2 signals than those of luminal A (p<0.0001) and triple negative tumors (p=0.027). Whilst, luminal B had significantly higher average CEP17 signals than luminal A (p<0.0001), but similar to that of triple negative tumors. Although HER2/CEP17 ratios of all tumors were <2, luminal B and triple negative tumors were observed to have significant higher HER2/CEP17 ratios than those of luminal A tumors (p<0.05). Clinicopathological features and 21-gene RS between tumors with average HER2 signals ≤ 1.5 and tumors with HER2

signals >1.5, as well as tumors with average CEP17 signals ≥3 and tumors with average CEP17 signals <3 within each subgroup were performed. Luminal B with average CEP17 signals ≥3 had significantly higher Ki67 expression than those with average CEP17 signals <3, but not for the other two subtypes. Results of 21-gene RS were significantly associated with immunohistological staining of ER, PR and Ki67 expression. No significant difference regarding tumor grade, tumor size, Ki67 expression, 21-gene RS and lymph node metastasis between tumors with average HER2 copy number ≤1.5 and those with average HER2 copy number >1.5, neither between tumors with average CEP17 signals ≥3 and tumors with average CEP17 signals <3 within each subtype.

Conclusion: Upon grouping by the breast cancer subtypes defined by ER, PR, HER2 and Ki67 expression, luminal B tumors with average CEP17 signals ≥3 had significantly higher Ki67 expression than those with average CEP17 signals <3, no additional difference regarding to clinicopathological features and 21-gene RS were observed.

Two challenging cases of endometrial carcinoma (in Japan-IAP Special)

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Case 1: A 76-year-old woman referred to our hospital because of genital bleeding. MRI study revealed 6.5 cm mass in uterine cavity with >1/2 myometrial invasion. Total abdominal hysterectomy and bilateral salpingooophorectomy was performed. The tumor showed sheet-like or trabecular proliferation of carcinoma cells with eosinophilic cytoplasm. Immunohistochemistry showed that the tumor cells were positive for AFP, glypican 3, HNF-1beta. Overexpression of p53 was also demonstrated. The diagnosis of hepatoid carcinoma was made. After the diagnosis, her preoperative serum AFP was measured, and it was 3,691 ng/ml. Endometrium surrounding the tumor showed proliferation of epithelial cells with marked atypia and these cells also showed overexpression of p53 and considered as serous intraepithelial carcinoma. Although hepatoid carcinoma does not listed in recent WHO classification of epithelial endometrial tumors, several cases have been reported. The postoperative course has been uneventful for 3 years after operation.

Case 2: A 38-year-old woman visited clinic because of genital bleeding. Endometrial curettage revealed atypical endometrial hyperplasia. She underwent total laparoscopic hysterectomy (TLH) and bilateral salpingooophorectomy. Endometrioid carcinoma (grade 1) was found in the endometrium. Thorough histological examination failed to find myometrial invasion of carcinoma. However, there were some clusters of epithelium within lymphovascular vessels. Lymph node biopsy was not performed. There were some studies about association between TLH and lymphovascular invasion (LVSI). Authors of one article thought that it was a result of mechanical artifactual displacement of endometrium in vascular channel and referred as 'pseudoinvasion'. In contrast, some pathologists suggested that LVSI in TLH specimen is a grossing artifact. Clinical significance of this phenomenon needs further studies.

Stathmin expression as a complement to p16 helps diagnosis of cervical squamous intraepithelial lesions

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Objective The distinction of High-grade squamous intraepithelial lesions(CIN2/3) from low-grade squamous intraepithelial lesions(CIN1) is clinically significant with treatment recommendations being linked specifically to risk for cancer or CIN3 outcome. As we all know diffuse p16 staining is traditionally used as a surrogate marker for high-risk human papilloma virus (HPV) infection, it has been shown to stain a significant number of squamous intraepithelial lesions(SILs), including low-grade squamous intraepithelial lesions, which often contain carcinogenic

HPV infection. Therefore, looking for a more specific marker combined with p16 to grade SILs is very important. Stathmin, as a microtubule-destabilizing protein, is overexpressed in a variety of malignant tumors including those from the Müllerian tract and may be associated with poor outcome. However, the use of stathmin as a diagnostic marker in cervical SILs has not been deeply explored. The aim of our research is to analyze the role of stathmin and p16 in grading of cervical SILs, and to provide information for clinical treatment. **Methods** 898 cervical samples were obtained from the Fourth Hospital of Hebei Medical University. H&E staining biopsies were reviewed, and pathologic diagnosis was confirmed. All cervical biopsies were independently scored by 3 pathologists. Ultimately, all the biopsies were classified as 250 cases of non-neoplastic benign or reactive cervix, 202 cases of CINI, 293 cases of CINII, and 153 cases of CINIII. All cases were evaluated for stathmin, p16, and Ki67 expression by immunohistochemistry. Positive stathmin staining in SILs biopsies was defined as cytoplasmic immunoreactivity in at least two thirds of the epithelial thickness for squamous epithelia. p16 was considered positive when continuous stretches (block staining) of nuclei with or without cytoplasmic reactivity, and positive cells were in at least one third of the epithelial thickness. Scattered individual cells and noncontinuous stretches of epithelium that were immunoreactive with p16 were scored as negative. Ki67 was considered positive when neoplastic nuclei showing moderate to strong intensity of staining. The sensitivities and specificities of p16 and stathmin were calculated for CIN2-3 (HSIL) as well as CIN3 only, using all cervical biopsies showing noninvasive SILs or a benign/reactive cervix. **Results** Stathmin was positive in 273 cases of SILs, that with differential expression based on the grade of the lesion as follows: 9/202 (4%) CIN1, 116/293 (40%) CIN2, and 148/153(97%) CIN3. p16 staining in these same cases was positive in 489 cases of SILs, including 48/202 (24%) CIN1, 289/289(99%) CIN2, and 152/153 (99%) CIN3 lesions. The specificity of Stathmin for both CIN2-3 was 98% and for CIN3 was 83%, which is higher than that of p16 (87% and 53%, respectively). The sensitivity of p16 and Stathmin for CIN3 was similar, but it showed dropping off in CIN2-3 for Stathmin (59% for Stathmin

vs. 99% for p16). **Conclusions** Increased Stathmin expression is present in high-grade squamous intraepithelial lesions. Stathmin overexpression has potential to be useful diagnostically in identifying High-grade squamous intraepithelial lesions. Stathmin can be used in addition to p16 and Ki67 staining by routine immunohistochemical procedures when differentiation between cervix low-grade squamous intraepithelial lesions and high-grade squamous intraepithelial lesions is difficult.

IDO expression is associated with P53 mutation in Epithelial ovarian cancer

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Objectives:
We investigated IDO expression in epithelial ovarian cancer (EOC) and its relationship to P53 mutation.
Materials and Methods:
The protein expressions of IDO and P53 in 111 cases of EOC were detected by immunohistochemistry. A surrogate marker for TP53 mutation in ovarian epithelial carcinoma can be based on immunohistochemical staining pattern of P53. On-line software Kaplan-Meier Plotter performs survival analysis on IDO for TCGA database.
Result:
IDO expression rate is associated with EOC tissue subtypes. The expression of IDO showed no statistical correlation with age, clinical stage, peritoneal metastasis in EOC. The expression of IDO showed statistical correlation with TP53 mutation in EOC. The overall survival of the IDO high expression group was higher than that of the low expression group, but it was not statistically significant.
Conclusion:
IDO expression is associated with TP53 mutation. Anti-IDO immunotherapy may be beneficial for EOC with P53 mutation.

Category: Gynaecologic pathology
1624093

Intravenous leiomyomatosis: molecular analysis of 17 cases

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Intravenous leiomyomatosis (IVL) is a rare smooth muscle tumor with a benign histology but with a quasi-malignant intravascular growth. In this study, we investigated the molecular alterations in 17 IVL cases composed of concurrent uterine leiomyomas (n=12), uterine IVLs (n=17) and extra-uterine IVLs (n=12). We found that 8 tumors had a somatic *MED12* mutation (c.130G>A, p.G44S, n=7; c.131G>C, p.G44A, n=1). The frequency of *MED12* mutations was significantly higher in the concurrent uterine leiomyomas (6/12, 50%) than in the uterine (0/17, 0%) and extra-uterine IVLs (2/12, 16.7%). The frequency of HMGA2 over-expression or MED12 low-expression was not significantly different among uterine leiomyomas, IVLs and extra-uterine IVLs ($p>0.05$). Short tandem repeat (STR) analysis indicated that microsatellite instability positive (MSI+) was present in 1 uterine and 2 extra-uterine IVL tumors from 3 patients whereas loss of heterozygosity (LOH) was found in 1 uterine leiomyoma, 3 uterine and 3 extra-uterine IVL tumors from 5 patients. LOH was more frequently seen in uterine/extra-uterine IVL tumors (6/20, 30%) than in the concurrent leiomyomas (1/7, 14.3%) ($p<0.05$). *MED12* mutation, MSI and LOH were discordant between uterine and extra-uterine IVLs in all patients. These findings suggest that IVL harbors distinct molecular pathogenesis from common uterine leiomyomas. Uterine IVL and extra-uterine tumors may represent an independent origin rather than uniclonal dissemination from a single tumor. Further investigations are warranted to explore the underlying key molecular events in the pathogenesis of IVL.

Case series of placental mesenchymal dysplasia with live birth

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Objective: To report the clinical and pathologic characteristics of three cases of placental mesenchymal dysplasia. Method: pathologicdata were retrieved from the electronic medical record. Review of three cases of placental mesenchymal dysplasiain clinical and pathological data. Results: Maternal age was respective36 years, 31 years and 33 years. Cesarean section was performed at 39 weeks, 37 weeks and 38 weeks, respectively. Placenta for pathological examination were performed macroscopically thick-walled vessels. Microscopic observation showed that the part of the stem villus was saclikely dilated, the blood vessels were thickened in the villi, the blood vessel wall was thickened, the villus interstitial was loose, and the mucus was changed. Part of the villus edema and degeneration, no trophoblastic hyperplasia, the final villus structure is normal. Conclusion: The size of the lesions in the Placental mesenchymal dysplasiaand the location of the lesion are closely related to the birth weight of the newborn. Detailed histology is necessary for diagnosis and differential diagnosis.

Clinicopathological Features and Immunohistochemical Phenotypes of Adenoid Cystic Carcinoma of the Uterine Cervix

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Objectives　To investigate the clinicopathological and immunohistochemical features of adenoid cystic carcinoma (ACC) of the uterine cervix. **Methods**　One case who was diagnosed with ACC of the uterine cervix was collected from PLA General Hospital. To investigate the clinicopathological and immunohistochemical features of adenoid cystic carcinoma (ACC) of the uterine cervix. **Results**　This postmenopausal female is 68 years old. Her chief complaint was vaginal bleeding without any obvious causes. The growth pattern of ACC is cribriform composed of luminal adenoepithelial and abluminal myoepithelial cells. Immunohistochemical findings suggest ACC have 2 components: positive expression of CK8/18 in luminal adenoepithelial cells，and positive expressions of P63,Calponin and p63 in abluminal myoepithelial cells. We found that CD117，a ACC-specific marker，is positive expression. P16 present diffuse strong positive.**Conclusions**　ACC of the uterine cervix is a rare special type of adenocarcinoma. The immunohistochemical findings in ACC of the uterine cervix are similar with ACC in other organs，but associated with poorer prognosis. Radiotherapy and chemotherapy after sugery is recommended.

Clinicopathological and prognostic features of clear cell carcinoma of the female reproductive system

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OBJECTIVE: Clear cell carcinoma is a relatively rare histological subtype of female reproductive system malignancies. It is not sensitive to traditional chemotherapeutic drugs, and the prognosis of the disease is relatively poor. However, few specific prognostic data have been reported in China. The aim of the study was to evaluate the clinicopathological and prognostic features of different sites (cervical, uterus and ovary) and different forms of clear cell carcinoma (solid, glandular, papillary, cystic and other pathological types) and to evaluate the incidence of endometriosis-associated clear cell carcinoma and compare clinicopathological characteristics and overall survival (OS) between patients with endometriosis and those who were not associated with endometriosis. METHODS: The clinical and pathological data and prognostic data of patients with clear cell carcinoma of the female reproductive system from 2005 to 2015 of the Cancer Hospital/National Cancer Center of the Chinese Academy of Medical Sciences were collected, such as age, lesion location, tumor maximum diameter, vascular tumor thrombus, and Ki67 proliferation. Index, related immunological markers (ER, PR, P16, P53, WT-1, NapsinA, etc.) expression and pathological T stage and related overall survival (OS) and whether the concomitant presence of endometriosis affects its prognosis were compared, and the relevant clinical pathological prognostic factors will also be analyzed. The differences between pathological parameters of clear cell carcinoma in different sites were compared, and survival analysis was performed using Kaplan-Meier model and COX regression risk model. RESULTS: The morphology of clear cell carcinoma in different sites had different characteristics, and different immunohistochemical differential expressions appeared. The incidence of endometriosis appears to be associated with an earlier stage and confers a better OS. However, stratifying by Multivariate prognostic analysis, the advantage in survival disappears.

Category: Gynaecologic pathology
1631448

Primary non gestational pure choriocarcinoma arising in the ovary: A case report and literature review

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Abstract: Ovarian choricarcinoma is very rare and accounts for less than 1% of malignant germ cell tumors. It can arise from gestational tissue or pure germ cells of the ovary. Gestational choriocarcinoma (GCO) usually metastasized from uterine or tubal choriocarcinoma. Non-gestational choriocarcinoma (NGCO) usually seen along with other germ cell tumors, pure NGCO extremely rare, the estimate incidence of NGCO correspond to less than 0.6% of ovarian germ cell tumors. Here we report a case of a 22-years old unmarried woman who never had sexual intercourse with a primary pure NGCO in her right ovary. Her serum level of human chorionic gonadotropin (hCG) was elevated to 77928 mIU/ml. Pathological examination revealed that the tumor was composed of cytotrophoblast and syncytiotrophoblast cells, and there were no other germ cell tumor components, which was conformed NGCO. Immunohistochemical staining showed cancer cells were positive for β-hCG, Pan Cytokeratin, human placental lactogen (hPL), SALL4, Ki-67 (more than 80%) and negative for EMA, OCT4, AFP, P63. Her karyotype of chromosome in peripheral blood was 46XX. She was treated with surgery followed by six courses of chemotherapy. The patient was followed up of 24 months, and she is in good condition now. NGCO of the ovary is a rare and highly malignant tumor, accurate pathological diagnosis and timely chemotherapy can improve the prognosis of patients.

Extraovarian Brenner tumor in the uterus: a case report and review of literature

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Background
Extraovarian Brenner tumors (EOBTs) are extremely rare and can be observed incidentally in both female and male patients, raising concerns regarding the origin of Brenner tumors.

Case presentation
A 53-year-old postmenopausal woman presented with a nodular lesion in the left side of the corpus uteri, which was found at a routine health check. Macroscopically, the lesion appeared as a solid nodule with a yellowish-gray cut surface, approximately 6 cm in greatest diameter. Microscopically, the lesion consisted of well-defined epithelial nests and spindled stromal cells. Parenchymal cells expressed CK7, GATA3, CK5/6, 34βE12, and p63. A single layer of cavity-lined cells with umbrella-like shape showed apical Uroplakin Ⅲ positivity. Stromal cells were positive for SMA, ER, and PR. The final diagnosis was EOBT and the patient was followed for 2 months with no recurrence.

Conclusions
We report here the third case of EOBTs in the uterus. The combination of morphologic and immunohistochemical results supported the involvement of urothelial metaplasia in the development of EOBTs. The similarities between EOBTs and Walthard nests made Müllerian epithelium an attractive candidate as the cellular origin. Changes of tissue structure or sex hormones balance may lead to the translocation of Müllerian remnants to distant organs, resulting in the pathogenesis of EOBTs.

The clinicopathologic features of Ovariansmall-cell carcinoma hypercalcemictype of one case and literature review

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Smallcell carcinoma of the ovary, hypercalcemictype (SCCOHT) is an extremely rare and highly aggressive cancer of unknown histogenesis that primarily occurs in children and young females. We describe herein a 20-year-old woman with SCCOHT who was previously diagnosed as ovary granulosa cell tumour(GCTO).Symptom was mainly abdominal distension.The FIGO stage of the Patient was Ⅲc, and the serum calcium was 2.99 mmol/L.Histologically, the tumor was consist of large numbers of small round or ovoid cells with diffuse distribution accompanied by the presence of follicle-like spaces. In addition,the tumor cells were arranged in trabeculae-like or cable-like in some areas.The boundary of the tumor was not clear.Necrosis and mucinous epithelial were existed. Some cells have the characteristics of rhabdoid tumor and pathological fission was easy to be seen. The Immunohistochemical staining of CK-pan,Calretinin,INI,P53,WT-1,CD99 was positive.However,the Immunohistochemical staining of α-inhibin,TTF1,Desmin,S-100,BRG1 was negative.There was no genetic disruption on SS-18 gene or EWSR1 gene .Taken together OSCCHT is a rare and highly malignant tumor occurring in young women.Comprehensive analysis of clinical data, histological morphology and immunophenotype is required in the pathological diagnosis to avoid misdiagnosis.Relevant molecular detection need to be carried out when it is necessary .Immunohistochemical staining of BRG1 and P53 protein is important for the diagnosis of OSCCHT.

Endometriosis associated ovarian carcinomas: clinicopathological analysis of 2 cases

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[Abjective] Objective To investigate the cliciopathologic features and immunophenotype of Endometriosis associated ovarian carcinomas(EAOC). Methods The clinical data, pathological features and immunohistochemitry of ovarian endometrioid carcinoma, ovarian clear cell carcinoma were retrospectively analyzed and the related literature was reviewed. Results Case 1, a 55-year-old woman underwent total double-adnexal hysterectomy for endometrial cancer . Cysts 0.8-1.8 cm in diameter on the left ovary were found with chocolate-like fluid. Microscopically, the tumors showed fused glands and ethmoid structures, which invaded the superficial muscular layer. Left ovary showed endometriosis with fused glands and ethmoid structures (maximum diameter < 0.5mm). Immunohischemically, the uterus and the ovarian lesions expressed ER、PR and vimentin but not PAX8、p53 and WT1 . Pathological diagnosis is high-grade endometrioid carcinoma of the uterus with high-grade endometrioid carcinoma of the left ovary. Case 2, a 66-year-old woman, underwent total uterine adnexal excision for right ovarian tumors. The right ovarian cystic mass was 9.5 cm in diameter, with caffeine-like fluid in the cyst. Several nodules were seen on the cyst wall, with a diameter of 0.5-2.2 cm. Microscopically, the right ovary shows endometriosis with focal cystic wall tubular and papillary structures. Tumor cells vary from polygonal, cubic to flat, with clear or eosinophilic cytoplasm. Immunohischemically, Tumor cells expressed ER、PAX8 and P504s but not PR、vimentin and WT1. Pathological diagnosis of right ovarian is clear cell carcinoma . Conclusion Ovarian endometriosis is one of the most common benign lesions in gynecology, but it is closely related to the occurrence of ovarian cancer. Ovarian cancer deteriorated from ovarian endometriosis is called endometriosis-related ovarian cancer, of which endometrioid cancer and clear cell cancer are the most common types.

Diagnostic value of Human Papillomavirus detection and Thinprep cytologic test in clinical screening for cervical intraepithelial neoplasia: A meta-analysis

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Objective: The present meta-analysis was conducted to evaluate the diagnostic value of Human Papillomavirus DNA detection (HPV-DNA) and Thinprep cytologic test (TCT) in early clinical screening for cervical intraepithelial neoplasia (CIN). **Materials and Methods:** Relevant studies were identified according to inclusion criteria and collected for meta-analysis and SROC curve.
Results: A total of seventeen studies were included in our meta-analysis. For HPV-DNA detection, the combined sensitivity was 0.75, the combined specificity was 0.74, and the area under the SROC curve (AUC) was 0.826; and the Q value was 0.759. For TCT, the combined sensitivity was
0.63, the combined specificity was 0.74, and the area under the SROC curve (AUC) was 0.748; and the Q value was 0.692. For the group of HPV-DNA detection combined with TCT, the combined sensitivity was 0.79, the combined specificity was 0.82, and the area under the SROC curve (AUC) was 0.911 and the Q value was 0.842. The combined sensitivity, specificity and AUC in HPV-DNA detection combined with TCT group were higher than that in individual measurement group.
Conclusion: HPV-DNA detection combined with TCT can increase the detection rate of CIN have important clinical significance for early detection, diagnosis and treatment of CIN.

Clear cell borderline tumor of the ovary: a case report and literature review

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Objective: To investigate the clinicopathological features of clear cell borderline tumor of the ovary.

Material and Methods: The clinical manifestations, pathological features, immunophenotypic characteristics of this case of clear cell borderline tumor of the ovary were retrospectively reviewed, and related literatures were reviewed.

Result: We present a case of clear cell borderline tumor of the ovary in a 65-year-old postmenopausal woman with a pelvic mass. Magnetic resonance imaging showed 6.2 × 4.8 × 3.8cm mixed equal long T1 and short T2 signals on the upper edge of the uterus. On gross examination, the left ovary was a 6 × 5 × 5cm gray-white solid mass with grayish yellow, jelly-like, and honeycomb-shaped cut face. Histopathological examination showed scattered or crowded glands in dense spindle cells with a hyalinestroma background. Most of the glandular cavities were covered with monolayer cells. The cells were flat, cuboidal and hobnail-like with clear eosinophilic cytoplasm and mild to moderate nuclear atypia. No stromal invasion was found. Immunostaining showed that the neoplastic cells were positive for CK7 and Napsin A, negative for WT-1, PR, p53 and CK20. ER was expressed in individual cells, and the Ki67 index was about 5%. The diagnosis of borderline clear cell tumor of the left ovary was made based on these pathological findings. No recurrence has been seen since the operation.

Conclusions: Clear cell borderline tumor is extremely rare, and is considered as a precursor lesion of clear cell carcinoma. Currently, surgical resection is the main treatment. The scope of surgery depends on the fertility request of patient. Moreover, long-term follow-ups are inevitable.

Clinicopathological analysis of ovarian composite neuroendocrine carcinoma and literature review

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Objective To investigate the clinicopathologic features, diagnosis, treatment and prognosis of ovarian composite neuroendocrine carcinoma.
Material and Methods The clinical and pathological data were collected from 2 cases of ovarian composite neuroendocrine carcinoma and the clinical pathological features, immunophenotype were observed, and the literatures were reviewed.
Results 2 cases were females, aged 48 years and 40 years respectively, The mass showed the maximum diameter of 13 cm and 14 cm, respectively. Microscopically, 2 cases were composed of two tumour components with necrosis, one component was neuroendocrine carcinoma, another was malignant ovarian epithelial tumour (endometrioid carcinoma in case 1 and mucinous carcinoma in case 2). Immunohistochemically, CgA, Syn and CD56 were positive in the neuroendocrine carcinoma component in 2 cases. CK7, ER, PR and Vimentin were positive in the endometrioid carcinoma in case 1, CK7waspositiveinthemucinous carcinoma in case 2   The final pathological diagnosis showed 1 case of composite large cell neuroendocrine carcinoma and mucinous carcinoma and 1 case of composite small cell carcinoma of pulmonary type and endometrioid carcinoma.
Conclusion Ovarian composite neuroendocrine carcinoma is a rare disease with a poor prognosis, and the characteristic of the tissue morphology and immunohistochemical phenotype is helpful for diagnosis and differential diagnosis.

Intraplacental Choriocarinoma: a Case Report

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Abstract: Objectives Placental tumors are rare, and the most common placental tumor is chorioangioma. Choriocarcinoma is a highly malignant gestational trophoblastic tumor that occurs in the uterus, but it is very rare in the placenta，called intraplacental choriocarinoma. There were approximately 30 cases reported in the English literature. We describe a case of an intraplacental choriocarcinoma to study the clinical pathologic features, diagnosis and differential diagnosis. **Materials and Methods** Clinical History: A 25-year-old woman, gravida 4, para 2, had a lower uterus cesarean section at 37^{+6} weeks' gestation because of fetal distress and delivered a male infant. The placenta was sent for examination, histomorphological characteristics and immunophenotypes were analyzed. **Result** Grossly, there was an ill-defined soft gray nodule measuring $2 \times 2cm$ that resembled an infarction in the placenta. Microscopically, the tumor showed sheet-like growth of both cytotrophoblast and syncytiotrophoblast in the intervillous space with marked pleomorphism and frequent mitoses including abnormal mitotic figures, it is characterized by forming a biphasic pattern. The tumor showed extensive central necrosis in which the ghost-like outlines of necrotic villi could be discerned. Many villis surrounded by collars of neoplastic trophoblast. Immunohistochemically, CK-L, HCG, hPL, Inhibin, P53, cyclin E were positive, PLAP was focal positive, Ki67 was 95% position, P63 was negative. Therefore, a diagnosis of intraplacental choriocarcinoma was made. **Conclusion** Intraplacental choriocarcinoma is a rarely reported malignant tumor that arises from chorionic villous trophoblast. In the majority of these cases, the placenta was sent to pathological examination due to other pregnancy complications .The real prevalence of the disease may actually be higher than documented since it is not routine practice to analyze all the placentas following each delivery. The pathologist should do a thorough pathological examination of the placenta, The clinicians should have an increased awareness of the disease and its manifestations, so as to make early diagnosis and provide optimal treatments.

Category: Gynaecologic pathology
1654200

The expression of PD-L1 and CD8+Tils in cervical squamous cell carcinoma tissue and its influence on prognosis of cervical squamous cell carcinoma

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Objective:
To analyze the expression of PD-L1 and CD8$^+$Tils density and clinical significance in cervical squamous cell carcinoma, and to explore the indicators that may affect the prognosis of cervical squamous cell carcinoma, and to provide new ideas for better treatment of cervical squamous cell carcinoma.

Methods:
131 cases of cervical squamous cell carcinoma were surgically removed or biopsy from January 2009 to January 2018 in the First Hospital of Shanxi Medical University. The expression of PD-L1 and CD8$^+$Tils was observed by immunohistochemistry. The correlation between PD-L1 and CD8$^+$Til density in cervical squamous cell carcinoma was analyzed by chi-square test or fisher test. The relationship between expression of PD-L1 and CD8$^+$Tils clinical pathology was analyzed by single factor and multivariate Cox regression analysis of expression of PD-L1 and the relationship between clinicopathological features and prognosis. Survival data were analyzed by Kaplan-Meier method and Log-Rank test and the survival curves were drawn.

Results:
Among the 131 patients with cervical squamous cell carcinoma, the positive rate of PD-L1 was 64.89% ($P<0.05$). The expression of PD-L1 was not significantly different from the age, differentiation and lymphatic vascular invasion of patients with cervical squamous cell carcinoma ($P>0.05$), but the tumor size, interstitial infiltration depth, FIGO stage, lymph node metastasis and parametrial infiltration were statistically significant ($P<0.05$).
In 131 cases of cervical squamous cell carcinoma, the median CD8$^+$Tils was 21%/HPF (2-30). CD8$^+$Tils had a low density was 60 cases (45.80%) and CD8$^+$Tils a high density was 71 cases (54.20%). In low-density CD8$^+$Tils cases, 85.00% (51/60) of patients had high PD-L1 expression; in CD8$^+$Tils high-density cases, 47.89% (34/71) of patients had high PD-L1 expression. The expression of PD-L1 was significantly correlated with the density of CD8$^+$Tils ($P<0.05$).
PD-L1/CD8$^+$Tils density group was associated with tumor size, FIGO stage and lymphatic vascular invasion ($P<0.05$). There was no significant difference in depth, degree of tissue differentiation, lymph node metastasis and parametrial infiltration in cervical squamous cell carcinoma patients($P>0.05$).
Of the 131 patients with cervical squamous cell carcinoma, 8 were lost to follow-up because of no regular follow-up or telephone number changes. During the follow-up period,

32 patients died and the 5-year survival rate was 69.9%. Kaplan-Meier method and Log-Rank test showed that patients with cervical squamous cell carcinoma without lymph node metastasis had better prognosis than patients with lymph node metastasis ($P<0.05$); patients with cervical squamous cell carcinoma of stage I-IIa had higher than stage IIb-IV. The prognosis of patients with good prognosis was good ($P<0.05$). The prognosis of patients with moderately differentiated cervical squamous cell carcinoma was better than that of poorly differentiated patients ($P<0.05$). The prognosis of patients with PD-L1 negative expression was better than that of PD-L1 positive patients ($P<0.05$). Patients with high-density CD8$^+$Tils had a better prognosis than patients with low-density CD8$^+$Tils ($P<0.05$); the prognosis of PD-L1$^-$/CD8$^+$Tils high-density group was the best, and the prognosis of PD-L1$^+$/CD8$^+$Tils low-density group Worst ($P<0.05$). Multivariate analysis showed that the degree of differentiation, lymphatic vascular invasion, lymph node metastasis and FIGO stage were independent factors affecting OS in patients with cervical squamous cell carcinoma ($P<0.05$).

Conclusion：

PD-L1 was highly expressed in cervical squamous cell carcinoma. The expression of PD-L1 in cervical squamous cell carcinoma was negatively correlated with the density of CD8$^+$Tils cells. The prognosis of PD-L1 positive cervical squamous cell carcinoma was lower than that of negative expression. PD-L1 combined with CD8$^+$Tils density can assess the prognosis of patients with cervical squamous cell carcinoma and and provide new ideas for better treatment of cervical squamous cell carcinoma

The role of NDRG1 in the process of endometrial-mesenchymal transition in endometrioid carcinoma

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Objective:

To study the expression of human N-MYC down-stream-regulated-gene 1 (NDRG1) in endometrioid carcinoma, and to investigate the role and related mechanisms of NDRG1 in the development of epithelial-mesenchymal transition (EMT).

Methods:

1. Immunohistochemistry (En Vision method) was used to detect the expression level of NDRG1 in tumor tissues and matched paracancerous tissues of 57 patients with endometrioid carcinoma (collected from the First Hospital of Shanxi Medical University from November 2016 to November 2018). Its relationship with clinical pathological parameters;

2. Immunohistochemistry (En Vision method) was used to detect the expression levels of EMT-related proteins (E-cadherin and β-catenin) in the tumor tissues and matched para-carcinoma tissues of the above specimens and analyze their relationship with clinicopathological parameters;

3. Western blot analysis of RAS, phosphatidylinositol 3-kinase (PI3K), TGF-β protein expression levels during NDRG1 and signal transduction; ELISA assay for local tumor tissue culture supernatant (TTCS) and non-tumor tissue culture TGF-β secretion levels in clear (NTCS).

Results:

1. Compared with adjacent tissues, immunohistochemistry results showed that the positive rate of NDRG1 protein in tumor tissues was lower, and the difference was statistically significant ($P<0.05$); the positive rate of NDRG1 protein increased with the formation of FIGO And decreased ($P=0.006$), which was significantly associated with tissue grade (degree of differentiation), pelvic lymph node metastasis, and vascular invasion ($P_1=0.005$, $P_2=0.011$, $P_3=0.009$), whereas NDRG1 protein expression and sexual age, There was no significant correlation between the maximum diameter of the tumor ($P>0.05$).

2. The positive rate of E-cadherin in tumor tissues was higher than that in matched para-carcinoma tissues ($P<0.05$). The positive rate of β-catenin in tumor tissues was higher than that in matched para-carcinoma tissues. There was a statistical difference ($P<0.05$). The positive rates of E-cadherin and β-catenin increased with the increase of FIGO stage ($P=0.013$), which was significantly correlated with tissue grade (degree of differentiation), pelvic lymph node metastasis and vascular invasion. ($P_1=0.016$ $P_2=0.008$, $P_3=0.012$), and the expression of E-cadherin and β-catenin protein was not

significantly correlated with age and tumor maximum diameter (P>0.05).

3. Western blot results showed that the expression level of NDRG1 protein was down-regulated in tumor tissues compared with matched para-carcinoma tissues (P<0.05). Compared with matched para-carcinoma tissues, RAS, PI3K, The expression level of TGF-β protein was up-regulated, and the difference in expression was (P_1= 0.046, P_2 = 0.017, P_3 = 0.009). Compared with TTCS, the secretion level of TGF-β in NTCS was significantly lower, and the difference was statistically significant (P <0.05).

Conclusion：

NDRG1 can inhibit the occurrence of EMT in endometrioid carcinoma and reduce the expression of E-cadherin and β-catenin in endometrioid carcinoma cells through TGF-β signaling pathway.

Cervical Gastric-type Adenocarcinomas: a clinicopathological analysis of 7 cases

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Objective To investegate the clinicopathological and immunological phenotypes of cervical gastric-type adenocarcinomas(G-ECA). **Methods** Clinicopathological features and the immunophenotypes of 7 G-ECAs patients were retrospectively analyzed. **Results** All the 7 patients showed vaginal contacted bleeding and/or irregular bleeding, the sizes of tumor were from 0.6 to 6 cm. The age of onset was 34 to 66 years old, and the average age was (43.57±10.31). The histology of G-ECA was various, it can be expressed as a highly differentiated or moderately differentiated adenocarcinoma with invasive growth, also it can be expressed as a secretory mucinous gland, or well-differentiated glands with irregularly arranged, various sizes, eosinophilic cytoplasm which expressed as transparent, foamy or pale, pathological mitotic figures can be existence, and in some cases small nucleoli can be found. The characteristic of Immunohistochemical staining was the diffuse staining of Muc-6, the focal or diffuse staining of CDX2, Pax8 and CEA in part of cases, CK20 and ER were always negetive, or point positive. **Conclusions** G-ECA is a rare subtype of cervical adenocarcinoma, which always occurs in middle-aged women, it is lack of specificity in clinical, and various in histologically, Immunohistochemistry is helpful for diagnosis and differential diagnosis.

Clinical and pathological observation of tubular-villous adenoma of vagina

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Objective To explore the clinicopathologic features, immunophenotype, differential diagnosis, treatment, and prognosis of vaginal tubulo-villous adenoma (TVA). **Materials and Methods** The clinicopathological features and immunophenotypes of one case of TVA were analyzed by light microscopy and immunohistochemistry, and the related literatures were summarized.

Result Femle, 40 years old. The clinical manifestations were vaginal bleeding after a sexual intercourse and vaginal exogenic mass under the colonoscopy. The detection of HPV16 was positive. Microscopically, we can see abundant of tubulo-villous architectures containing the central fibrovascular axis which consists of stroma cells and many inflammatory cells. The epithelium is multilamellar columnar epithelium. Tumor cell nuclears were oval or oblong. Villin, CDX2, CEA, CK7 and CK20 were positive in tumor cells. P16 was diffuse and strongly positive in tumor cells. P53 showed wild type. While SATB-2 was negative in tumor cells. Ki67 index was about 90%.

Conclusion TVA is a rare adenocarcinoma of the vagina, which is not much difficult to differentiate it from other vaginal messes by clinical manifestations. TVA usually has a favorable prognosis. However, the incidence of TVA is still low, and more epidemiological data investigations are still needed to develop the standard diagnostic and therapeutic methods.

Villoglandular adenocarcinoma of cervix: clinicopathologic features, immunophenotype and management

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Objective To explore the clinicopathologic features, immunophenotype, management of villoglandular adenocarcinoma (VGA) of the uterine cervix.

Materials and Methods 10 cases of VGA were analyzed by light microscopy and immunohistochemistry. The clinical characteristics, management and prognosis were retrospectively reviewed.

Result 10 cases of VGA were 39-62 years old, with an average age of 50.9 years, five of whom have a history of pregnancies and abortions both over twice. The presenting symptoms were mostly abnormal vaginal bleeding, with 8, 1 and 1 patients staging IB, IIA and IIIB, respectively. The follow-up ranged from 9 to 52 months. Seven patients are alive with no evidence of recurrence, and 2 patients were lost to follow-up. Pathologically, the tumor macroscopically showed an polypoid pattern in 7 patients and an erosion pattern in the remaining 3 patients. Microscopically, we can see many villoglandular architectures with vascular axis, which consists of stroma cells and many inflammatory cells. Tumor cells represent a shape of low column and a round nuclear, with mild to moderate cytologic atypia. Of the 10 cases of VGA, only one case caused an invasion of endometrium, and the remaining 9 cases did not invade the nerve, vessel or surrounding organs. Immunophenotype showed that CEA, p16 were positive in tumor cells, while Vimentin, estrogen receptor (ER) and progesterone receptor (PR) were mostly negative. Ki67 index was about 10% - 90%.

Conclusion VGA is a rare subtype of the adenocarcinoma of the uterine cervix, which is difficult to be differentiated from other cervical adenocarcinomas just depending on clinical symptoms. The diagnosis should be based on its histopathological features. VGA usually has a favorable prognosis, thus a conservative surgical procedure like conization may be recommended. So it matters to achieve individualized treatment according to the patient characteristics to avoid the excess of the treatment.

A single positive core prostate cancer(Gleason≤6 and Maximum extent cancer per core≤10% and serum PSA <10ng/ml) at mpMRI/TRUS fusion-guided biopsy can be considered a low-risk disease-a single Chinese centre retrospective cohort study.

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PURPOSE:
The extensive use of multiparametric magnetic resonance imaging (mpMRI) has led to an even more widespread use of different targeted biopsy techniques and approaches including mpMRI/TRUS fusion-guided biopsy. Prostate cancer (PCa) may be a multifocal or bilateral disease. A single positive biopsy core is usually associated with indolent PCa, and may choose to perform active surveillance(AS). To identify who benefits from mpMRI/TRUS fusion-guided biopsy to identify the specific subset(s) of patients for whom AS would be appropriate. We investigated the correlation between finding a single positive biopsy core and the pathological outcome after radical prostatectomy (RP).

METHODS:
We carried out a retrospective analysis for 2296 consecutive men with PC suspicion and scheduled for who underwent both 12-core transrectal ultrasound (TRUS) biopsy and mpMRI targeted biopsy in an academic hospital in 2016 and 2019. Among them,157 patients with a single positive core PCa were followed up, and the clinical and pathological parameters influencing prognosis were analyzed after radical prostatectomy. All of the guidelines contain serum PSA-based criteria and biopsy Gleason score for eligibility for AS. The patients were divided into dirrerent groups with or without serum PSA <10ng/ml,Gleason≤6 and/or Maximum extent cancer per core≤10%.

RESULTS:
Of 157 men, 65 (41%) had Gleason 3+4 or 4+3 and 92 (59%) had 3+3 on initial biopsy. Among 157 patients, 118 (75%) had one core positive in targeted biopsy location and 78 (51%) had a reclassification Gleason Score (GS) or grade group based on their RP findings. And 52(66%) patients experienced Gleason score upgrading from 3+3 to 3+4 or 4+3, 26(34%) patients experienced Gleason score downgrading, especially, 10 (8.4%) patients upgrading from 4+3 or 3+4 who had one core positive in targeted biopsy location. The risk category upgrade results was lower in combined fusion than systematic biopsy group (2% vs 18%, p = 0.001). On multivariate analysis, independent risk factors for upgrade or stage upgrading were Gleason > =7, maximum extent cancer per core>10% and serum PSA >10ng/ml. Among 157 patients, 34(21%) were met AS criteria including Gleason 3 + 3=6,<10% adenocarcinoma at maximum one biopsy

cores, prostate-specific antigen (PSA) <10 ng/mL.

CONCLUSIONS:

Upgrading and upstaging at RP are quite common among Chinese men with
a single positive core PCa. A single positive core prostate cancer(Gleason≤6 and
Maximum extent cancer per core≤10% and serum PSA <10ng/ml) at mpMRI/TRUS fusion-guided
biopsy can be considered a low-risk disease who are candidates for AS.

A rare primary endometrial gastric type mucinous carcinoma with synchronous mucinous metaplasia and neoplasia of the female genital tract

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Objective To investigate the clinicopathological features of primary gastric type mucinous carcinoma of the uterus and discuss how to recognize the synchronous mucinous metaplasia and neoplasia of the female genital tract (SMMN-FGT) .
Methods The detailed clinical and pathological data of this rare case were collected. The HE staining sections were used to observe the histomorphology. Immunohistochemical staining and next-generation sequencing were adopted to confirm the primary site of the tumor and the related literature were reviewed. **Results** A 60-year-old woman presented at our institution because of irregular vaginal bleeding for 5 months. No matter imaging examination, observed in operation, gross specimens checking or microscopic examination, all supported the primary site was uterus(Figure1). Histomorphology and immunohistochemistry (MUC6) confirmed the differentiation character of gastric type mucinous carcinoma. Endometrial lesions including the morphological lineage of simple gastric mucinous metaplasia, atypical gastric mucinous metaplasia and invasive gastric type mucinous carcinoma; gastric mucinous metaplasia and atypical gastric mucinous metaplasia can also be seen in the surrounding adenomyosis(Figure2). After totally examination of the cervix, the lobular endocervical glandular hyperplasia(LEGH) and atypical gastric mucinous metaplasia were found. Bilateral fallopian tubes were sectioned by SEE-FIM, there were discontinuous gastric mucinous metaplasia, atypical gastric mucinous metaplasia and intraepithelial carcinoma in both sites(Figure3). Immunohistochemical staining for ER/PR were both negative in dysplasia and invasive carcinoma area,   and p53 was mutation-type expression; but p53 was wild-type expression in simple gastric mucinous metaplasia and LEGH hyperplasia. Molecular testing revealed no *STK11* and *KRAS* gene mutations. RTQ-PCR was used to detect the tumor gene separately in the endometrial site and the fallopian tube and compared with the tumor gene expression profile database. The results further confirmed that the tumors were occurred in multiple centers of endometrial and fallopian tube (non-endometrial cancer metastasized to the fallopian tube). **Conclusion** The primary gastric type mucinous carcinoma of the uterus has not been reported in the literature. It is a very rare type of endometrial carcinoma. It is advantageous to identify the primary site of tumor if we can find the coexistence of benign, atypical and malignant component. Immunohistochemistry can help confirm gastric mucinous metaplasia. In the case of

SMMN-FGT, carefully judging of primary site or metastasis is important. If necessary, genetic test can be used to assist the diagnosis. At present, the biological behavior and prognosis of primary gastric type mucinous carcinoma of the uterus are still unclear, and it is necessary to cause the attention of gynecologist and pathologist.

A rare case of papillary thyroid carcinoma in ovarian mixed with ovarian carcinoid

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Ovarian carcinoid is an very rare germ cell tumor, ovarian carcinoid complicated by papillary thyroid carcinoma are even rarer, while both tumors originate from ovarian teratoma remain evern more rare. The diagnosis id so difficult as to require pathological diagnosis and IHC analysis during and after operation.

Here, we present a 56 year old woman with cystadenoma due to a cystic mass at the right uterine adnexum, which was diagnosed as an ovarian carcinoid complicated by thyroid papillary carcinoma. For interventions, combining patients's requirements with the needs of treatment, the tumor was removed subtotally through laparoscopic assisted hysterectomy, as well as bilateral ovariectomy and pelvic lymph node dissection.

Category: Gynaecologic pathology
1656844

Liposomal Curcumol Nanocomposite Improved the Anti-tumor Effect of Cisplatin on Ovarian Cancer Cells

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Ovarian cancer is one of the most common tumors among women and also the most fatal gynecologic tumor. Cytoreductive surgery followed by postoperative systemic combination chemotherapy with platinum drugs and paclitaxel have become the standard therapeutic methods for advanced ovarian cancer. However, allergic reactions to chemotherapy drugs, chemotherapy resistance caused by long-term chemotherapy and high recurrence rate of patients have made it increasingly urgent to find new therapeutic drugs and methods. In this experiment, it was explored whether Liposomal Curcumol Nanocomposite could enhance the anti-tumor effect of cisplatin.

Objectives:
To study the effects of Liposomal Curcumol Nanocomposite (LC) combined with cisplatin, on the proliferation and apoptosis of two different human ovarian cancer cell lines: HO8910 and SKOV3, and to explore changes in cell signaling pathways after drug combination so as to provide new ideas for developing new drugs to treat ovarian cancer.

Methods:
1. Preparing LC by using thin-film dispersion method;
2. Using MTT assay to detect the effects of different concentrations of liposome, LC, using cisplatin alone or in combination with LC on the proliferation inhibition of human ovarian cancer cells,
3. Using Flow Cytometry assay to detect the changes of cell apoptosis after the treatment;
4. Using Western-Blot to detect the changes in protein expression of Cleaved-Caspase8, Cleaved-Caspase9 and Cleaved-Caspase3.

Result:
1. MTT results showed that compared with the control group, there was no significant change in the proliferation of ovarian cancer cells in the liposome and the low-dose LC experimental group ($P > 0.05$). With the rise in LC concentration, the rate of cell proliferation was significantly decreased ($P < 0.01$). The proliferation rate of ovarian cancer cells in the experimental group using cisplatin alone decreased with the increase of cisplatin concentration ($P < 0.01$), and the proliferation rate of ovarian cancer cells treated with cisplatin combined with low-dose liposome LC treatment also occurred. Decreased, and the difference was significant compared with the cisplatin group alone ($P < 0.05$);
2. Flow cytometry revealed that there was no significant difference in apoptosis between

the low-dose liposome and the control group. The percentage of apoptotic cells in cisplatin group was significantly increased (P <0.01), and percentage of apoptotic cells combination group was significantly higher than cisplatin alone (P <0.01);
3. Western Blot results showed that the expression levels of Cleaved-Caspase8, Cleaved-Caspase9, and Cleaved-Caspase3 protein were significantly increased in the cisplatin group and the combination group, and the protein expression level increased more in the combination group than that in the cisplatin alone group. This difference was statistically significant (P < 0.01).

Conclusion:
1. Low-dose LC can enhance the inhibition of cisplatin on the proliferation of human ovarian cancer cells;
2. Low-dose LC can increase the ability of cisplatin to induce apoptosis in human ovarian cancer cells;
3. Changes in the expression levels of Cleaved-Caspase8, Cleaved-Caspase9, and Cleaved-Caspase3 proteins after low-dose LC combined with cisplatin, suggesting that LC improved the anti-tumor effect of cisplatin on ovarian cancer cell may be related to mitochondria apoptosis pathway.

Target diagnosis against ovarian serous cancer with a DNA aptamer generated by primary cancer cell-SELEX

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Histological types of ovarian cancer are initially diagnosed by rapid intraoperative pathological examination using frozen tissue section, which is sometimes quite challenge based on hematoxylin and eosin staining only. Therefore, an accurate rapid assistant technique for intraoperative pathological diagnosis is highly required. In order to obtain target aptamers against human ovarian serous cancer (OSC), freshly cultured cancer cells from 5 patients diagnosed with OSC, which highly expressed human epididymis protein 4, were used as positive cells in systematic evolution of ligands by exponential enrichment (cell-SELEX) process. After 11 cycles of cell-SELEX, a series of DNA aptamers including Apoc46 were generated. The truncated aptamer mApoc46 was verified to strongly bind primary OSC cells from different patients, and the equilibrium dissociation constants (Kd) of mApoc46 was 152.43 ± 48.37 nM. In addition, mApoc46 showed its specificity to bind OSC cells in frozen tissue sections in 15 min. Moreover, this aptamer showed no affinity with normal ovarian tissue, other types of primary and metastatic ovarian neoplasms in frozen tissue sections. This aptamer was also verified to target the xenograft OSC in patient-derived tumor xenograft models. In conclusion, the aptamer mApoc46, may serve as a novel and promising probe against OSC.

A comparative study of clinicopathological features between adenoid basal cell carcinoma and adenoid cystic carcinoma of the cervix

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objective   To investigate the clinical and histopathological characteristics of adenoid basal cell carcinoma and adenoid cystic carcinoma of the cervix, and to improve the understanding of these lesions by clinicians and pathologists.

Method   Nine cases of adenoid basal cell carcinoma of cervix and three cases of adenoid cystic carcinoma of cervix were retrospectively analyzed. Clinical data were reviewed in detail. All pathological sections were reviewed. Relevant immunohistochemical results were counted. Analyse the clinicopathological characteristics , then follow up and consult relevant literature.

Result   Both are common in postmenopausal women (the age of onset is 43-74years). Adenoid basal cell carcinoma is often asymptomatic in clinic. Most of them are abnormal smears of the cervix during physical examination, and there is no definite mass in colposcopy. Adenoid cystic carcinoma is mostly treated with abnormal vaginal bleeding. Colposcopy often has a mass. Histologically, the two lesions are characterized by nest-like growth of the tumors, consisting of basal-like tumor cells, often surrounded by palisade structures. The two lesions may coexist or may be mixed with squamous cell carcinoma or high-grade squamous intraepithelial lesions. Most of the clinical HPV tests were positive, and p16 was positive. The follow-up period ranged from 1 month to 37 months. 9 cases of adenoid basal cell carcinoma recovered well, 7 cases had no recurrence and metastasis, and 1 case had ground glass nodules in the lung, 1 case of stump recurrence. 1 case of adenoid cystic carcinoma developed pulmonary metastasis 8 months after operation, and died 2 years after operation. 1 case was followed up for 1 month, and there is no recurrence or metastasis now. 1 case lost follow-up.

Conclusion   Both adenoid cystic carcinoma and adenoid basal cell carcinoma of the cervix are tumors originating from cervical reserve cells and are associated with high-risk HPV infection. Adenoid basal cell carcinoma has a lower malignancy. Surgical removal of the whole uterus is enough without radiotherapy and chemotherapy, and the prognosis is good. Adenoid cystic carcinoma is highly invasive. Surgical excision plus radiotherapy and chemotherapy are adopted according to clinical stages. The prognosis of adenoid cystic carcinoma is much poorer.

Prognostic significance of the pretreatment serum gamma-glutamyltransferase levels in Chinese patients with non-metastatic cervical cancer

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Objective: This study was performed to evaluate the prognostic significance of the pretreatment serum gamma-glutamyltransferase (GGT) levels in a Chinese cohort of patients with early-stage or locally advanced cervical cancer.

Methods: The pretreatment serum GGT levels were examined in 290 cervical cancer patients with stage I-III disease and 230 healthy controls selected from a cancer-free population in the same region. Patients were assigned to normal or high-risk GGT groups, as previously described, and the GGT levels were correlated to clinicopathologic parameters and survival data.

Results: The GGT levels in cervical cancer patients were significantly higher than those in healthy controls (35.6 $\pm$ 29.1 vs. 24.1 $\pm$ 14.7 U/L, $P<0.001$). In addition, the pretreatment serum GGT levels were associated with the histology type ($P=0.023$), lymph node involvement ($P=0.040$), stage ($P=0.029$), recurrence ($P=0.015$) and death ($P=0.005$), but not with age ($P=0.432$), tumor size ($P=0.067$) or degree of differentiation ($P=0.901$). Moreover, univariate survival analysis revealed that patients with high GGT levels tended to have poorer disease-free survival (DFS) [hazard ratio (HR), 1.721; 95% confidence interval (CI), 1.189-2.491; $P=0.004$] and overall survival (OS) (HR, 1.929; 95% CI, 1.294-2.876; $P=0.001$) compared to those with normal GGT levels.

However, a multivariate Cox-regression model did not support these data (HR, 1.373; 95% CI, 0.925-2.039; $P=0.116$ for DFS and HR, 1.357; 95% CI, 0.887-2.078; $P=0.160$ for OS, respectively) after adjusting for other confounding variables.

Conclusions: High pretreatment serum GGT was associated with more advanced tumor behavior, but could not serve as an independent prognostic indicator in patients with early-stage or locally advanced cervical cancer.

Primary cervical clear cell adenocarcinoma complicated with cervical adenomyosis:: A Clinicopathologic Study of Two Cases and Literature Review

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Objective To investigate the clinical pathological features, diagnosis, treatment and prognosis of primary clear cell adenocarcinoma of the cervix (PCCAC) combined with cervical adenomyosis.

Materials and Methods HE staining and immunohistochemicalstaining method were used to analyze the pathology and immunohistochemical phenotypes of 2 patients with PCCAC and cervical adenomyosis.

Result Patientin Case 1 was a 62 years old womanwith post-menopausal vaginal bleeding. Patientin Case 2 was a 51 years old womanwhose physical examination revealed a mass in the cervix. Microscopically, the tumor cells were large clear cells or spike-like cells. The tumor cells were arranged in a sheet-like solid and papillary. In both cases, patients were combined with cervical adenomyosis. The immunohistochemicalstaining of Case 1 was Napsin A (+), CEA (−), CK7 (+), ER (−), CD10 (−), Ki67 (li40%), p16 (−), p53 (focal +, wild type), PR (−), Vimentin (−), WT-1 (−), CD 15 (−). According to the FIGO staging criteria, both cases were I B. The case was followed up until September 2019, and no recurrence was found in either case.

Conclusion PCCAC is a rare type of cervical cancer. The diagnosis depends mainly on pathological examination and immunophenotype. The treat strategy was surgery combined with radiotherapy and chemotherapy.

Immunohistochemical staining of podoplanin is helpful to determine the microinvasion of cervical squamous cell carcinoma

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Objectives: To assess the expression patterns of podoplanin in cervical tissues and evaluate its role in the diagnosis of early infiltration in cervical cancer.
Materials and Methods：The paraffin specimens, including 96 cases of normal cervix squamous epithelium (NS), 91 cases of normal cervix columnar epithelium (NC), 72 cases of micro-invasive squamous cell carcinoma (MISCC) and 118 cases of invasive squamous cell carcinoma (ISCC), were enrolled in the study, and immunohistochemistry staining was used to explore the expression of podoplanin in these specimens. Patients were followed-up and the correlation between their clinicopathological features and podoplanin positivity were analyzed by SPSS 17.0 software (SPSS Inc., Chicago, Illinois).
Results：Podoplanin was specifically expressed in the basal layer cells of normal cervical squamous epithelium (NS) (100%, 96/96) and HSILs (81%, 57/70) in a continuous linear or discontinuous linear pattern. However, its expression was completely absent in micro-invasive lesions (0%, 72/72), and the location of podoplanin expression loss wasconsistent with that of microinvasive lesions. Furthermore, podoplanin was expressed in 3.4% (4/118) of ISCC, and its expression was not correlated with patients' age, tumor size, differentiation, FIGO stage, depth of invasion, lymph node and distant metastasis. The prognosis of patients with positive podoplanin was slightly better than those without it ($p > 0.05$).
Conclusions：Podoplanin was a new specific marker for the basal layer cells of cervical squamous epithelium. For HSILs with positive podoplanin expression, the sudden loss of podoplanin represents the occurrence of early invasion. Thus, podoplanin can assist the diagnosis of microinvasion in cervical squamous cell carcinoma, and its specific staining pattern provides the possibility of clinical application in the future.

A novel orally active proteasome inhibitor Oprozomib increase sensitivity of cervical cancer cells to cisplatin-induced apoptosis

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Objective: The activity of proteasome is very important for the maintenance of cellular functions. Proteasome inhibitors can inhibit the growth of tumor cells by inhibiting the activity of proteasome and interfering with the normal function of cells.Oprozomib is a novel orally bioactive .The application of oral oprozomib will improve the flexibility of clinical dosage and facilitate the use of patients.The aim of this study was to investigate the activity of oprozomib in cevical cancer cells. **Methods:** A diverse of cervical cancer cell lines including Hella-DDP cells were used to explore the anti-tumor activity of oprozomib.

Results: oprozomib had obviously anti-proliferative activity in Hella, Helle-DDP, Siha, C33a and Caski cell lines, after 96 hour with IC50 values exposure from <0.009μM to 0.064 μM. Western blot analysis of oprozomib treated all the cervical cancer cells showed cleavage of poly ADP ribose polymerase (PARP) and caspase-3, and marked synergistic anti-tumor efficacy was observed for oprozomib + cisplatin (CDDP).
 Conclusions:oprozomib induced cell apoptosis in cervical cancer cells.Moreover, oprozomib enhanced the cytotoxic effect of DDP on cervical cancer cells and DDP-induced JNK phosphorylation. In addition, oprozomib inhibited DDP-induced NF-κB activation by stabilizing the protein level of IκBα. Taken together, our study provides the rationale for clinical protocols evaluating oprozomib, either alone or in combination with DDP,to reduced tumor progression and prolonged survival.

Clinicopathological features of ovarian sclerosing stromal Ttumor: a report of 13 cases with literature review

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Objective: Ovarian sclerosing stromal sumor (SST) is a rare neoplasm. This study aims to investigate the clinicopathological features of SST. **Method:** The clinicopathological data of 13 patients with were collected and analyzed. **Results:** The patients were aged 12-51 years, (average age:27.7, median age:22 years). Major patients presented menstrual disorders, most of the menstrual cycle were shortened. Ultrasound showed mixed low echo in most patients, CDF1 showed that solid area, the periphery of the mass and cystic were seen rich blood flow signal. The majority of patients (9/10) presented varying degrees of abdominopelvic cavity effusion. Gross were mostly cystic and solid mass(90%, 9/10). Immunohistochemistry: SMA and Vim were positive. a-inhibin, CR, CD99 and CD10 had varying degrees of positive (respectively 63.6%, 37.5%, 71.4%, 33.3%), PR (+), EMA and S-100 were negative. The Ki-67 index was less than 10.0%. Histologic findings: the tumors are lobulated, with alternating pattern of hypercellular and hypocellular areas. Solid area is usually located the periphery of the mass, with rich blood sinus .The abundant blood sinuses are surrounded by tumor cells. The nuclei were ovoid or fusiform. Nuclear chromatin was fine with vesicular nuclei. The small nucleoli was seen in some cells. Scattered collagen was distributed between cells. **Conclusion** all patients prognosis is good after tumor resection.

Special transfer system of ovarian neoplasm metastasis from cervical squamous cell carcinoma at FIGO IA stage: a case report and literature review

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We present the case of a 58-year-old postmenopausal woman who presented for vaginal bleeding for more than 4 months, never received hormonal treatment and had no family history of malignant diseases. Routine exams revealed a 12cm×10cm×8cm right ovarian mass, CEA 64ng/ml, and HPV 16(+). TCT could find atypical squamous cells, but with no significance. Colposcopy showed cervical high-grade intraepithelial neoplasia and cervical biopsy showed (cervical 6 degrees, cervical canal) high-grade squamous intraepithelial lesion, HSIL/CIN3. The bilateral appendectomy and hysterectomy were performed on schedule. However, the intraoperative pathological outcome revealed that the neoplasm of   the right ovary was borderline Brenner tumor with highly suspected canceration. Then omentectomy was performed. Meanwhile,the same malignant cells could be seen in mucosa of right fallopian tube, the endometrium and uterine cervix. With Immunohistochemical and RNAscope in-situ hybridization outcomes,all the neoplasm arised from the cervical squamous cell carcinoma. Our conclusion was that ECSCCs transfered to right ovary for ovarian lesions ,with lymphovascular and vascular invasion and trans-tubal migration.

PD-L1 and Tumor Infiltrating Lymphocytes Status in Patients with cervical cancer

jingjing chen
PD-L1 and tumor infiltrating lymphocytes status in patients with cervical cancer

Abstract

Objectives-This study investigates the association of PD-L1 expression and immune cell infiltrates in the occurrence and development of cervical cancer

Methods-PD-L1, CD8 and FOXP3 immunohistochemical digital analysis was performed on cervicectomy specimens of 110 cervical cancer and 52 cervical intraepithelial neoplasia (CIN) patients. Clinical characteristics of the population were compared between cervical cancer and CIN. Overall survival was estimated and was compared between PD-L1 - positive and PD-L1 - negative groups as well as tumor infiltrating lymphocytes (TIL) - high and TIL - low groups.

Results-The positive expression rate of PD-L1 and FOXP3 in cervical cancer was significantly higher than that of CIN (P<0.05); Compared with the normal cervix, there were significant differences. Among them, the positive expression rate of PD-L1 in cervical cancer cells was 41%(45/110), The positive rate of FOXP3 in the cervical carcinoma group was 85.9%, and did not express in normal cervix group, the difference was statistically significant (P < 0.05); The positive index of CD8 in advanced stage cervical cancer decreased with the increase of FOXP3 positive index, FOXP3/CD8 ratio increased significantly in cervical squamous cell carcinoma; there was a positive correlation between expression of FOXP3/CD8 and PD-L1 (r=0.493, and P< 0.05). The expression of PD-L1, FOXP3 and CD8 proteins was correlated with tissue differentiation and FIGO stage (P < 0.05), but not with age, tumor diameter, HPV infection or typing (P > 0.05).

Conclusions-cervical cancer showed higher PD-L1 expression and higher FOXP3 and CD8 - positive cell density than CIN. Our results indicate a notable immunosuppressive environment in cervical cancer. Our results suggest PD-1/PD-L1 signaling pathway plays an important role in the occurrence and development of cervical cancer.

Category: Digestive pathology
1370950

RAC1-GTP promotes epithelial-mesenchymal transition and invasion of colorectal cancer by activation of STAT3

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Background: Epithelial-mesenchymal transition (EMT) plays a critical role in initiating tumor invasion and metastasis of colorectal cancer (CRC), although the underlying mechanisms remain to be clarified. We previously demonstrated that the active form of RAC1 (RAC1-GTP) is involved in maintenance of stemness in CRC stem cells. In this study, we aimed to investigate the role of RAC1-GTP in promoting the EMT and invasion of CRC and the underlying mechanism, to provide potential target-therapeutics for CRC treatment.

Methods: uman CRC cell lines HCT116, SW480, and SW620 were maintained as monolayer culture in Dulbecco's modified Eagle medium as CRC models. A total of 147 formalin-fixed and paraffin-embedded (FFPE) surgical cancerous tissues and the corresponding adjacent normal tissues were collected from CRC patients from Southwest Hospital. All the specimens were diagnosed by at least two senior pathologists according to the World Health Organization classification. A patient cohort containing 380 CRC cases and 50 adjacent normal cases from The Cancer Genome Atlas (TCGA) database (https:// tcga-data.nci.nih.gov/tcga) was applied to analyze the gene expression of RAC1. The lentiviral construct expressing Flag-tagged constitutively activated STAT3 (STAT3-C) was constructed by cloning STAT3-Flag into a pCDH-MCS-T2a-Puro-MSCV vector. Cell proliferation was measured using Cell Counting Kit-8. Quantitative real-time PCR and western blot analysis was conducted to determine the target expression or the activation of STAT3 signaling. Co-immunoprecipitation (Co-IP) was performed using the Thermo Scientific Pierce Co-IP kit following the manufacturer's protocol. Cell migration and invasion assay were performed using transwell assays. Immunohistochemical staining was performed using DAKO REAL EnVision Detection System. Immunohistochemical scores of RAC1-GTP, E-cadherin, and Slug were independently semi-quantified by two pathologists according to the staining intensity and percentage of positive tumor cells. Xenograft experiments were performed on six-week-old male nude mice. Subcutaneous tumor volume was calculated using the following formula: volume=length $\times$ width^2 $\times$ 1/2. All statistical analyses were performed using SPSS 18.0 software. The unpaired Student's t-test was used to determine the statistical difference between two groups. The correlation between RAC1-GTP expression and pathological features of patients were analyzed by Pearson's $\times$ 2 test. The Kaplan–Meier method with log-rank test was used for patient survival analysis.

Results: We demonstrate that the active form of Rac family small GTPase 1 (RAC1-GTP)

is overexpressed in CRCs and promotes the EMT-mediated invasion of CRC cells through activation of the signal transducers and activators of transcription 3 (STAT3) pathway. RAC1-GTP was highly expressed in CRC cancerous tissues and its expression level was significantly correlated with TNM stages, lymph node spread, and distant metastasis as well as patient survival. Targeting RAC1-GTP activity by its specific inhibitor NSC23766 markedly suppressed the migration and invasion of CRC cells. Mechanistically, RAC1-GTP directly interacted with STAT3 to promote STAT3 phosphorylation, thus promoted EMT of CRC cells. Enforced expression of constitutively activated STAT3 (STAT3-C) abrogated the suppressive effect of RAC1-GTP disruption on the migration and invasion of CRC cells. Importantly, targeting RAC1-GTP by NSC23766 disrupted EMT in CRC cells and significantly diminished growth of CRC xenografts.

Conclusion: Our data indicate that RAC1-GTP is an important regulator in invasion of CRC by directly activating STAT3 pathway to induce EMT. RAC1-GTP may serve as a new prognostic indicator and a potential therapeutic target for CRC.

PREVALANCE OF HCV GENOTYPES IN THE POPULATION OF KARACHI, USING GENE FLOW HYBRIDIZATION

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Background: Genotypic analysis of hepatitis C virus (HCV) strains showed substantial variability leading to a classification into several genotypes and subtypes. The data correlating HCV genotypes and subtypes with hepatitis C viremia levels, demographic characteristics of patients (age, mode of transmission, duration of infection), and severity of liver disease are variable.

Objective: The objectives of the present study were the knowledge of the HCV genotypes in Pakistan, and determining their prevalence.

Material & Methods: A prospective cross-sectional study was carried out at Pathological and molecular Laboratories, Karachi. During the course of this study, initially, PCR was used to determine the positive HCV RNA samples. Positive sera samples from 200 chronically infected patients were assessed by genotyping, using flow-through hybridization technology. Genotyping assay utilized type-specific primers for amplification of the core region.

Results: During our study we came across that genotype 3a was the most prevalent (36%) followed with 3b (26%), 3c (3.5%), 3d (4%), 1a (2.5%), 1b (1%), 1c (2.5%), 1d (0.5%), 2a (12%), 2b (0.5%), 2c (0.5%), 4 (0.5%) and 4a (1.5%). Genotypes 3d, 1c, 1d and 2c were reported for the first time from Pakistan

Conclusion: Advances in the field of molecular biology have provided rapid diagnostic tools that have reduced the turnaround times for detecting HCV genotype by using "Flow-through" hybridization in Pakistan. Furthermore, the clinical presentation and severity of infection varies with each genotype.

Detection of circulating tumour cells in colorectal carcinoma

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Objectives: Circulating tumour cells (CTCs) could account for tumour recurrence and distant metastases.   The aim of this study is to study the properties of the CTCs in colorectal carcinoma in correlations with the clinical pathological features.

Materials and Methods: Peripheral blood from twenty-five patients with colorectal carcinoma underwent surgical resection and six healthy individuals were recruited prospectively to exam for CTCs by Negative selection technology.

Results: Sixty-eight per cent (17 out of 25) of the patients with colorectal carcinoma had more than 2 CTCs detected in the peripheral blood.   More CTCs occurred in carcinoma from the rectum, in conventional adenocarcinoma and patents without hereditary nonpolyposis colorectal cancer (HNPCC) ($p < 0.05$).   CTCs could occur in the early stage cancers without metastases.

Conclusion: To conclude, the detection of CTCs and the association with clinical pathological features indicate their potential roles as biomarkers in patients with colorectal carcinomas.

Enrichment of receptor tyrosine kinase fusions in sessile serrated adenoma/polyps defines an alternative route of colorectal serrated neoplasia pathway

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Objectives
The serrated polyps are clinically and molecularly heterogeneous lesions that contribute to the development of colorectal cancers. To have a more comprehensive understanding of molecular mechanisms underlying the development of serrated lesions, we conducted targeted capture sequencing on SSA/P, TSA and compared the genetic alterations with conventional adenoma, the precursor of the classical adenoma-carcinoma pathway.

Materials and Methods
The molecular alterations of 86 colorectal adenomas including 35 sessile serrated adenomas/polyps, 15 traditional adenomas and 36 conventional adenomatous polyps were investigated by multiple technologies including target capture sequencing, immunohistochemistry and fluorescent in situ hybridizations. *In vitro* and *in vivo* studies were performed to investigate the oncogenic properties of a novel NCOA4-RET fusion gene.

Result
Molecular profiling revealed distinct clinocopathological and molecular features in sessile serrated adenoma/polyps and traditional serrated adenomas. Importantly, receptor tyrosine kinase translocations were identified exclusively in 17% (6/35) of sessile serrated adenoma/polyps but not found in traditional serrated adenomas and conventional adenomatous polyps. Kinase fusions, *BRAF* and *KRAS* mutations were mutually exclusive of each other. The alternative occurrence of oncogenic mutation in the *RTK/RAS/RAF* axis underscored the importance of MAPK signaling activation in the serrated pathway of colorectal tumorigenesis. We further demonstrated a continuum of serrated tumorigenesis from sessile serrated adenoma/polyps to carcinoma in which the kinase fusion occurred in precursor lesions. The subsequent loss of TP53 drove the transformation to carcinoma. The oncogenic properties and the sensitivity to targeted therapeutics of a novel NCOA4-RET fusion was evaluated by *in vitro* and *in vivo* studies.

Conclusion

Enrichment of kinase fusions in sessile serrated adenoma/polyps defines a novel route of serrated neoplasia pathway of colorectal tumorigenesis. The presence of kinase fusions may serve as biomarkers for the identification of precursor lesions with malignant potential and enable personalized therapeutic options to emerge.

Clinicopathological analysis of 6 cases with special type of solid pseudopapilloma of the pancrea

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solid-pseudopapillary neoplasm of the pancreas (SPTP) is a kind of cystic solid tumor of primary pancreatic. It has been encoded in 3 in the new version of WHO, that is malignant tumors. The incidence of this disease is low, about accounting for 0.9%-2.7% in all exocrine tumor of pancreas, and 5% in cystic tumors. The disease mainly occurred in the young women, only about 10% in men. Although it is classified as malignant tumors, but by operation, the curative effect is good, and the recurrence and metastasis rates are low. The data show that the transfer rate is only about 10%-15%. In view of the report of the solid pseudopapillary tumors of the pancreas and with metastases are rarely in domestic and foreign magazines, so that we know seldom about these two kinds of cases with lower incidence. So, we collected the clinicopathological data of 3 male cases and 3 cases with metastasis from a total of 39 cases from 2009 to 2018 in the past ten years. The purpose of this study is to analyze and compare the clinical date of rare cases and to research the clinical pathology and its impact on prognosis.

Category: Digestive pathology
1630437

SIRT1 antagonizes liver fibrosis by intercepting hepatic stellate cell activation

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Background and aims: Hepatic stellate cells (HSCs) are a major source of fibrogenesis in the liver contributing to cirrhosis. When activated, HSC undergoes profound functional alterations paralleling an overhaul of its transcriptome. HSCs can swiftly transition from a quiescent state primary responsible for fat storage to an activated state with a re-wired transcriptional program characterized by augmented expression of ECM genes (e.g., type I and type III collagens) and contractile genes (e.g., a-SMA). These changes, at the cellular levels, are dictated by rather conserved signaling pathways and transcription factors (TFs), the mechanism of which remains largely undefined. SIRT1 is a class III lysine deacetylase relying on intracellular NAD for activity. Previous studies, relying on mostly on systemic administration of SIRT1 agonists have demonstrated that SIRT1 activation attenuates liver fibrosis in mice. The conclusiveness of these data, while abundant, is eclipsed by the delivery method and the potential off-target effects of the chemicals. We investigated the involvement of the class III deacetylase SIRT1 in HSC activation.

Methods: Hepatic stellate cell-specific conditional SIRT1 knockout mice were obtained by crossing the *Sirt1*[f/f] line to the GFAP-cre/ERT2 strain. To induce acute liver injury, 6-8 week-old male mice were given four i.p. administrations of CC14 dissolved in olive oil (1.0 mL/kg body weight as 50%, vol/vol) within a week with a one-day interval between each injections. Chronic liver injury was induced by injecting mice with CC14 (1.0 mL/kg body weight) once a week for six weeks. Alternatively, liver fibrosis was induced by bile duct ligation (BDL). Expression of mRNA and protein was measured by real-time PCR, and Western blotting. Protein binding to DNA was assayed by chromatin immunoprecipitation (ChIP).

Results: SIRT1 levels were down-regulated in the livers of mice after seven days of CC14 injection at both mRNA and protein levels. Similarly, chronic CC14 injection also induced a-SMA expression in mice while simultaneously repressing SIRT1 expression. In a third model in which BDL was performed to induced liver fibrosis, we again found that a decrease of SIRT1 expression paralleled an increase of a-SMA expression. More importantly, there was a significant reduction of hepatic SIRT1 proteins and a simultaneous induction of a-SMA expression in patients with cirrhosis compared to healthy individuals. Finally, qPCR and Western blotting analyses revealed that there was a progressive down-regulation of SIRT1 expression mirroring up-regulation of a-SMA expression when primary mouse HSCs underwent spontaneous activation to

become myofibroblasts. ChIP assays showed that when HSCs were activated *in vitro*, acetylation of histones H3 and H4 was suppressed surrounding the proximal, but not the distal, SIRT1 promoter region in keeping with decreased SIRT1 expression levels. Histone deacetylase 4 (HDAC4) was recruited the SIRT1 promoter to mediate its trans-repression during HSC activation. Over-expression of HDAC4, but not HDAC5 or HDAC7, in cultured HSCs directly repressed SIRT1 promoter activity in dose-dependent manner. In contrast, depletion of endogenous HDAC4 by siRNA restored SIRT1 expression in primary HSCs. SIRT1 activation halted whereas SIRT1 inhibition promoted HSC trans-differentiation into myofibroblast. Liver fibrosis was exacerbated in mice with HSC-specific deletion of SIRT1 (cKO) compared to wild type (WT) littermates as illustrated by elevated levels of collagen type I, type III, and a-SMA. These observations were further corroborated by picrosirius red staining and Masson's trichrome staining, both of which showed more extensive fibrogenesis in cKO mice compared to WT mice. SIRT1 regulated PPARg transcription by deacetylating EZH2 in quiescent HSCs. Finally, EZH2 inhibition or PPARg activation ameliorated fibrogenesis in cKO mice.

Conclusion: Our data reveal a SIRT1-EZH2-PPARg axis that coordinately regulates HSC activation and suggest that combined usage of EZH2 inhibitors and SIRT1/PPARg activators may effectively combat liver fibrosis. One of the most devastating consequences for liver fibrosis is the development of hepatocellular carcinoma (HCC), in which SIRT1 plays an ambiguous role being known to both promote and oppose HCC pathogenesis. Clearly, more studies are warranted to delineate the long-term effects of SIRT1 deficiency in HSCs to yield novel therapeutic strategies.

Genetic alterations and expression of PTEN and its relationship with cancer stem cell markers to investigate pathogenesis and to evaluate prognosis in hepatocellular carcinoma

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Aims: To investigate molecular alteration and expression of gene PTEN in hepatocellular carcinoma (HCC) and compare their concordance, to study PIK3CA changes in HCC and its relation with PTEN, to evaluate correlation between PTEN and CSC markers and the prognostic value of these markers.

Materials and Methods: We evaluated changes of PTEN and cancer stem cell markers (CD133, EpCAM and CK19) in 183 resection specimens by immunohistochemistry and detected PTEN and PIK3CA gene by FISH in some specimens.

Results: PTEN expression was reduced in 91 HCC tissues (49.7%). PTEN expression in non-tumor tissues were significantly higher than that in HCC tissues (P<0.001). There were 16 HCCs with PTEN deletion (16/31, 51.6%). Comparison between PTEN IHC and FISH showed that the analysis was highly concordant (54/59, 91.5 %). There were 19 (19/31, 61.3%) HCCs with PIK3CA amplification. Deletion of PTEN was positively correlated with amplification of PIK3CA (r = 0.556, P = 0.001). There were 4 of 16 (25%) HGDN foci with PTEN loss. Expression of EpCAM, CD133, and CK19 were significantly higher in HCC tissues than those in non-tumor tissues (CD133, 168/183 [91.8%] vs. 86/183 [47.0%], P<0.001; EpCAM, 69/183 [37.7%] vs 2/183 [1.1%], P<0.001; CK19, 47/183 [25.7%] vs. 0/183 [0.0%]). Reduced expression of PTEN was more frequent in older patients (≥50 years, P = 0.025) and in tumors with moderate to poor differentiation (P = 0.015). Positive expression of CD133 was correlated with higher glutamyltransferase levle (P = 0.026). Positive expression of EpCAM was related to steatosis (P=0.024), higher alanine aminotransferase level (P = 0.002) and alkaline phosphatase level (P = 0.06). Positive expression of CK19 was correlated with steatosis (P=0.020) and moderate to poor differentiation (P = 0.001). Reduction of PTEN expression was negatively correlated with positive expression of CD133, EpCAM and CK19 (r =-0.181, P = 0.014; r =-0.165, P = 0.026; r=-0.159, P=0.031). Reduced expression of PTEN (P=0.028) were independent predictors for HCC recurrence and overall survival in HCC. the PTEN-/CD133+ group had shorter OS time (P=0.036) and RFS time (P=0.005), compared with that of the other group. The PTEN-/EpCAM+ and PTEN-/ CK19+ groups had shorter OS time (P = 0.031, P = 0.013), but not for RFS time (P=0.000), compared with that of the other groups.

Conclusions: PTEN plays a key role in hepatocarcinogenesis and reduction of PTEN expression is related to increased expression of CD133, EpCAM and CK19, which is a useful tool to evaluate HCC prognosis and recurrence. Detection of PTEN reduction by IHC can be applied as a screening method in clinical practice.

Multiple adenomatoid tumors in the liver and omentum

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Xijing Hospital of Airforce Medical University

Objectives： Adenomatoid tumors are uncommon benign tumors of mesothelial origin. They have rarely been reported in the liver. We report multiple adenomatoid tumors involving the liver and omentum.

Materials and Methods： The immunohistochemical staining method and the FISH method were used to detect antibody expression and gene mutation.

Result and Conclusion: A 26-year-old man had multiple mass in the liver and the omentum, and underwent a partial of liver and large omentum resection. Grossly, there were multiple solid masses, and all with a well-circumscribed border. Histologically, the tumors showed low-columnar, cuboidal or flattened epithelioid cells in lined，tubular，or glandular structures. The epitheliod cells showed small vacuoles, clear or eosinophilic cytoplasm with round to oval nuclei. The cystic and angiomatoid spaces were also observed. But there were no mitotic figures, no cellular atypia or invasive growth pattern. The tumour cells were diffusely positive for vimentin, cytokeratin7, EMA, calretinin, cytokeratin5/6 and MC. There was no p16 gene mutation detected by FISH. The patient has now been free of disease for 3 years postsurgery.The differential diagnosis of adenomatoid tumor mainly includes metastatic carcinoma and other mesothelioma. It is suitable to complete the integrity of the tumour.

Jejunal myoglandular hamartoma combined gastrointestinal stromal tumor: a case report

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Myoglandular hamartoma (MH) is a rare benign tumor characterized by tubular or gland-like structures embedded within smooth muscle cells. We report a case of a 31-year-old man with gastrointestinal stromal tumors (GIST) admitted to our hospital for abdominal pain. Although not found during endoscopic examination and operation, a MH under the jejunum mucosa was found by chance during macroscopic examination by a pathologist. Histologic examination of the MH specimen showed the submucosal nodule with irregularly arranged tubular or gland-like structures, surrounded by interlacing smooth muscle cells. Although MH is extremely rare in jejunum, it should be always considered in the differential diagnosis of other gastric tumor. Pathologist should distinguished carefully to avoid misdiagnose.

ALDH1A1 maintains the cancer stem-like cells properties of esophageal squamous cell carcinoma by activating the AKT signal pathway and interacting with β-catenin

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Objectives: Aldehyde dehydrogenase 1A1 (ALDH1A1) has been used as a marker of cancer stem-like cells (CSCs) in most studies, but little is known about the molecular mechanism of ALDH1A1 in maintaining the properties of CSCs. Therefore, we performed the study to further investigate the role and biological function of ALDH1A1 in the development of esophageal cancer as well as the signaling pathway that maintains cancer CSCs properties in ESCC tumor cells.

Materials and Methods: Three human ESCC cell lines (KYSE-150, KYSE-510 and TE-1) were obtained from the German Resource Center for Biological Material (DSMZ). A cohort of 610 subjects with ulcerative ESCC was recruited between 2008 and 2014 from the Department of thoracic surgery, Affiliated Hospital of Jining Medical University (Shandong, PR China). We collected relevant clinical data and prognostic information of patients. Tissue microarray were made for clinicopathological analysis. Immunohistochemical staining of the ALDH1A1 protein was performed on the TMAs slides using the streptavidin-peroxidase (S-P) method. Cell lines stably expressing ALDH1A1 or negative control (NC) vector were constructed by lentivirus infection. RT-qPCR and Western blot was used to determine the level of ALDH1A1. Protein-protein interaction studies were performed by Co-Ip analysis. For immunofluorescence, the images were captured with a confocal microscope (Zeiss). Colony formation assays, Sphere-formation assay and CCK8 were performed to assess biological behavior and cell viability. Statistical analyses were performed using SPSS software (SPSS Standard version 17.0, SPSS Inc) and GraphPad Prism 8 Software (GraphPad Software, San Diego, CA, USA. Survival curves were plotted using the Kaplan-Meier method and compared with log-rank tests. P values < 0.05 were considered significant.

Result: The expression of ALDH1A1 was correlated with the differentiation level of patients (Medium-High compare Medium, P=0.002; Medium-High compare Medium-Low, P=0.04), but not with age (P=0.234), sex (P=0.5), tumor size (P=0.132), clinical stage (P=0.728) and lymph node metastasis (P=0.129). Survival analysis showed that high expression of ALDH1A1 was closely related to the overall survival (P=0.0065) of ESCC patients. ALDH1A1 promotes spherogenesis and chemical resistance of ESCC cells. Transplanted tumor models indicated that ALDH1A1 promotes tumor initiation of ESCC

cells. RT-qPCR assay showed that ALDH1A1 expression increased the mRNA expression levels of β-catenin and the downstream target genes (Slug, c-Myc and Vimentin), but did not affect the level of AKT1.

Conclusion: In this study, we found that ALDH1A1 expression maintained the CSCs properties of esophageal squamous cell carcinoma (ESCC) cells: enhanced the ability of chemo-resistance, clonogenicity and spherogenesis *in vitro* and tumorigenicity in nude mice *in vivo*, and ALDH1A1 high expression is an adverse prognostic factor of ESCC patients. We also found that small-molecule inhibitor NCT-501 down-regulates ALDH1A1 expression and inhibits the AKT-β-catenin signaling pathway; overexpression of ALDH1A1 activates the AKT signaling pathway, and ALDH1A1 interacts with β-catenin, co-localization in the cytoplasm of adherent KYS-510 cells and in the nucleus of cancer stem cell spheres. Taken together, our findings illustrated a new function of ALDH1A1 that maintains cancer CSCs properties in ESCC tumor cells by positively regulates the expression of upstream molecules AKT1 and β-catenin.

Diverse Breakpoints and Fusion Modes of FGFR2 Fusion should Determine the Response to FGFR Inhibition in FGFR2 Fusion Intrahepatic Cholangiocarcinoma

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Fibroblast growth factor receptor 2 (FGFR2) gene translocation has emerged as a promising and druggable target in intrahepatic cholangiocarcinoma (ICC), and several FGFR2 fusion partners have been found. However, the efficacy of targeted drugs is different, and it is unclear how to predict which translocation is sensitive to FGFR inhibitors. In this study, we identified novel FGFR2 fusions and explored the therapeutic efficacy of inhibiting FGFR kinase in ICC with these fusions.

Using the break-apart probe, we found nine samples (5.2%) with FGFR2 translocation in 173 ICC tumors by fluorescence in situ hybridization (FISH). All ICCs with FGFR2 translocation were of the mass-forming (MF) type (p=0.038). Screened by next-generation sequencing and proved by Sanger sequencing, eight fusion chimeras with novel two breakpoints(FGFR2 p767 and p430) and four fusion partners were found (BICC1,MCU,AFF4,PIBF1). Based on the breakpoints and functional regions of the FGFR2 gene, we firstly classified the fusion chimeras into three subtypes as 1) classical fusions, which retained the tyrosine kinase (TK) and the Immunoglobulin (Ig)-like domain, 2) sub-classical fusions, which only retained TK but missed the Ig domain, and 3) non-classical fusions, which missed both of Ig and TK domains.

Using ICC cell lines, we showed that both classical and sub-classical fusions responded to FGFR-selective small molecule kinase inhibitors (SMKIs) through suppressing the activation of MAPK/ERK and AKT/PI3K signaling pathways. None of the fusions responded to non-selective SMKIs.

Our study implied FGFR2 translocation only occur in MF type ICC and found three novel partners of FGFR2 fusion. More importantly, we firstly identified that the efficacy of FGFR inhibitors was determined by the breakpoints and fusion modes, but not only fusion partners of FGFR2. Therefore, it is essential to detect the breakpoints and fusion modes of FGFR2 in specific gross subtype of ICC before targeted treatment.

C-Met in Intrahepatic cholangiocarcinoma: high-frequency amplification predicts protein expression and predicts a unique molecular subtype

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With more and more gene alterations been discovered in ICC, molecular targets are promising in distinct subpopulations carrying the unique molecular signature. *C-MET* amplification is associated in several tumors including ICC, however, the characteristics of this type of alteration have not been assessed in ICC. By determining the ratios of *C-MET*/Chromosome Enumeration Probe (CEP) 7 double-color probes, we evaluated a cohort of 133 ICC tumors for the presence of *C-MET* amplification by fluorescence in situ hybridization (FISH). We further determined levels of MET protein expression by immunohistochemistry (IHC) and analyzed the clincopathologic records. Out of these samples, 21 (15.8%) were found with high-frequency, 41(30.8%) were with low-frequency C-Met genetic amplification and 71(53.4%) were C-Met gene normal. There were significant differences in the etiology of gross classification (p=0.045), Microscopic chilangitis (p=0.030), mucus level in tumors (p=0.012) and T stage (p=0.007) in these three groups. When we combined high-frequency and low-frequency amplification of *C-MET* into one group, only the etiology of microscopic chilangitis (p=0.010) and stage (p=0.016) showed significant differences compared to *C-MET* gene normal. However, When we combined low-frequency amplification of *C-MET* into *C-MET* gene normal, high-frequency amplification of *C-MET* group preferred younger patients (p=0.047), tended to more Non-mass-forming (MF) type in gross classification (p=0.015), secreted more mucus (p=0.002) and appeared to be higher T stage (p=0.031). For IHC results, although only cluster of *C-MET* amplification predicts protein overexpression, high-frequency amplification preferred more protein expression compared with other genetic status (p=0.000). As C-Met low-frequency amplification exhibits similar biology to the gene normal, we regarded high-frequency amplification of *C-MET* as a unique molecular subtype. It may play important roles in tumor progression and may be used as welcoming prognostic marker for targeted therapy.

Category: Digestive pathology
1644751

Exosomal transfer of miR-501 confers doxorubicin resistance and tumorigenesis via targeting of BLID in gastric cancer

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Objectives: We aim to investigate whether miR-501 can be transmitted by exosome and play a role in the doxorubicin resistance and tumorigenesis in gastric cancer (GC).
Materials and Methods: Exosomes were isolated and confirmed. Colony formation, migration and invasion, and apoptosis assays were performed. The xenografts of BALB/c nude mice were to determine the roles of exosomal miR-501 in vivo.
Results: miR-501 was overexpressed in exosomes isolated from doxorubicin-resistant SGC7901/ADR cells (ADR Exo) than from SGC7901 cells (7901 Exo). ADR Exo could be internalized by SGC7901, and a Cy3-miR-501 mimic was transferred from SGC7901/ADR to SGC7901 via exosomes. ADR Exo conferred doxorubicin resistance, proliferation, migration and invasion abilities to negative control miRNA inhibitor-expressing GC cells, whereas it inhibited apoptosis. miR-501 knockdown or BLID overexpression could reverse the effects of ADR Exo on recipient cells. The intratumoral injection of ADR Exo into negative control miRNA inhibitor-expressing SGC7901 cells induced rapid subcutaneous tumor growth and resistance to doxorubicin compared to that of miR-501 knockdown or BLID-overexpressing cells. This effect is possibly achieved by exosomal miR-501-induced downregulation of BLID, subsequent inactivation of caspase-9/-3 and phosphorylation of Akt.
Conclusions: Exosomal miR-501 might be a potential therapeutic target for GC.

Identification of ALK Expression in GIST Harboring PDGFRA Exon 18 D842V Mutation

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Gastrointestinal stromal tumors (GIST) are the most common type of adult mesenchymal neoplasms and their oncogenic driver events are majorly *KIT* or *PDGFRA* mutations, which lead to the susceptibility of these tumors to small-molecule tyrosine kinase inhibitors such as imatinib and sunitinib. However, previous studies have shown that patients with D842V-mutant GIST have a very low rate of response to imatinib treatment. Therefore, novelly developed tyrosine kinase inhibitors (TKI) are currently being evaluated in clinical trials to treat D842V mutant GIST. Anaplastic lymphoma kinase (ALK) overexpression was not supposed to appear in GIST and was used as a biomarker to distinguish GIST from other types of mesenchymal tumors. Here we report a 37-year-old male patient who suffered from a large mass in the right upper abdomen and was subsequently diagnosed as GIST harboring a *PDGFRA* D842V mutation. We unexpectedly found that the GIST in this patient had a simultaneous ALK expression. This is the first GIST case ever reported with ALK expression. This rare phenomenon suggests that GIST cannot be excluded absolutely if a tumor shows ALK expression. Besides, ALK may be a potential therapeutic target for patients with imatinib-resistant stromal tumor.

WTX-RRBP1 regulates Ras/Raf/Mek/Eek signaling and colon cancer progression

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Wilms tumor gene on the X chromosome (WTX) is a putative tumor suppressor gene in colorectal cancer(CRC) and lost in CRC which was highly correlated with cell proliferation, tumor invasion and metastasis. Currently, ribosome-binding protein 1 (RRBP1) is considered to be a novel oncogene that is overexpressed in colorectal cancer. Mechanistically, WTX loss enhanced the expression of RRBP1 and activated Ras/Raf/MEK/ERK signaling ,which promotes CRC development and liver metastasis. These study defined the mechanism how WTX-mediated RRBP1 loss regulates CRC progression and metastasis, and provided a potential therapeutic target for preventing CRC progression.

Establishment and genomic characterization of an esophageal squamous cell carcinoma cell line from the Chaoshan Littoral, China

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Objectives: In China, esophageal cancer(EC) ranks third in incidence and fourth in mortality. Esophageal squamous cell cancer(ESCC) is the dominant subtype of EC in China. The incidence of ESCC varies geographically, the Chaoshan littoral in southeast China prossessing one of the highest incidence rate domestically. With such epidemiologic actuality, however, few ESCC cell lines have been established from high incidence areas. The purpose of this study was to established well characterized ESCC cell lines from from Chaoshan litoral.

Materials and Methods: Tumor tissues were taken by endoscopic biopsy from ESCC patient from Chaoshan litoral with no chemo-radiotherapy. The specimens were minced into small fragments and seeded in T-25 flask coated with collagen for primary culture. Immunoflourescence (IF) staining was performed to identified the origination of the cells and the proliferation activity. The in vitro migration and invasion abilities was tested by transwell assay. DNA STR profiling was performed for authorization. The karyotype was demonstrated by spectrum karyotyping (SKY). Whole genome squencing (WGS) were utilized to screen genomic variations.

Results: CSEC216 was originated from a 45-year old male ESCC patient from Chaoshan litoral, China. CSEC216 cells has been authorized as unique and uncontaminated cell line, exhibit epithelial cell features with polygonal morphology and adherent growth as monolayer. IF staining demonstrated its epithelial origination. CSEC216 possess high proliferation rate with Ki-67 labeling index of 67.1% and doubling time of 29.7h. The karyotype demonstrated tumor cell patterns with aneuploidy and complex chromosomal aberrations. Deletion, translocation, isochromosome and derivation chromosome were frequently observed. Derivation chromosome of 2, 3, 13 composed by the most sophisticated translocations was observed,
der(2)t(2;8)(q2?;?)t(2;8)(?;?)t(2;8)(?;?)t(8;12)(?;p11)、
der(3)t(3;?7)t(p11;?)t(?7;?10)(?;?)t(?3;?10)(?;?)、
der(13)t(?8;13)(?;p11)t(?8;?10)(?;?)t(?10;?13)(?;?)t(?8;?13)(?;?). Chromosomal break points at p1 or q1 was common in chromosome 1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 16, 17, 18, 21, X. Genomic SNV and CNA has been profiled by WGS. A total of 5870 SNVs located in gene coding region were identified, corresponding to 1.93 mutations per Mb, 3571 of which were in exonic region. C:G>T:A transitions were predominant substitutions which was consistent with our previous work and other published data, accounted for 21.6% (1270/5870) of all SNVs. Mutations in TP53, NOTCH2, NOTCH3, PTEN, NFE2L2, FAT1,

CREBBP coding regions were identified. 9 mutated genes were significantly in association with 12 anti-cancer drug responses. Tumor related genes like MYC, CDKN2A, PIK3CA, etc. were found amplified in gene copy number.

Conclusions: ESCC cell line CSEC216 from high ESCC incidence region in China has been established. Biological features were studied. Karyotypic and genomic features were characterized which provided thorough cytogenetic background for furture use. This novel ESCC cell line would serve as preferable model to comprehend the tumorigenesis of ESCC and for anti-cancer drug testing.

Primary gastric choriocarcinoma: clinicopathological study of three cases from one institution

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Background
Primary gastric choriocarcinoma (PGC) is a rare and highly malignant neoplasm, accounting for 0.08% of all gastric malignancies. It is critical to recognize PGC as it is treated differently from other types of gastric carcinomas, though the optimal chemotherapy regimen for PGC remains controversial.

Materials and Methods
Three cases of PGC who underwent primary surgery in our hospital were retrieved. Their clinicopathological features and clinic outcomes were analyzed. Immunohistochemical staining using a panel of antibodies was performed to differentiate PGC from its mimics.

Results
Two of these three PGCs are pure and the third one is admixed with hepatoid adenocarcinoma. Their clinicopathological characteristics and clinical outcomes are summarized in Table 1. None of them was diagnosed as PGC in the biopsy specimens. Post-operative serum beta-HCG was available in two cases and it remained remarkably high (Case 1, 106.1 mIU/ml, six days after surgery; Case 3, 348.4 mIU/ml, seven days after surgery). Two patients (case 1 and case 3) received neoadjuvant chemotherapy with adenocarcinoma regimen and both showed progressive disease. All three patients died with a follow-up period of 6 weeks to 10 months after surgery. Histologically all 3 PGCs are composed of syncytiotrophoblastic and cytotrophoblastic cells admixed in a plexiform pattern, with marked hemorrhage and necrosis. Syncytiotrophoblastic cells are immunohistochemically positive for beta-HCG and CK7, whereas cytotrophoblastic cells are diffusely positive for CD10 and GATA3. SALL4 is diffusely positive in the cytotrophoblastic cells. Inhibin-alpha is negative in all these three PGCs. In case 2, AFP and SALL4 are positive in the hepatoid adenocarcinoma cells which show negative staining for CD10 and GATA3.

Conclusions
PGC is a highly lethal malignancy. Immunohistochemical staining using a panel of antibodies, including beta-HCG, CD10, GATA3, AFP, and SALL4, is helpful to recognize this entity and differentiate it from its mimics. PGCs responded poorly to the chemotherapies using adenocarcinoma regimens, suggesting that gestational choriocarcinoma chemotherapy regimens might be more suitable for PGCs.

A case of gastric neuroendocrine neoplasm(NEN) in Japan-IAP Special "Topic: Challenging Cases in Surgical Pathology" :A case of MiNEN

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A 68 year old Japanese man was found to have a large gastric mass by endoscopy. His biopsy showed a few mucosal fragments which contained focal well differentiated tubular adenocarcinoma and separate foci of poorly differentiated neuroendocrine carcinoma(NEC), small cell type. Immunohistochemically, the former showed positive membranous staining for HER2(3+) and the latter revealed positive staining for synaptophysin, chromogranin A and CD56. In addition, therapeutic surrogate marker SSTR2 and SSTR5 were also positive in the NEC. So the biopsy was diagnosed as Mixed neuroendocrine non-neuroendocrine neoplasm(MiNEN)(WHO 2017, 2019). This tumor is the mixture of adenocarcinoma and NEC, small cell type. This was previously called as Mixed adeno- and neuroendocrine carcinoma(MANEC)(2010).

In WHO classification published in 2017(Endocrine organs) and 2019(Digestive system), the tumor is composed of epithelial component(adenocarcinoma, acinar cell carcinoma, ductal carcinoma) and neuroendocrine component(neuroendocrine tumor;NET, neuroendocrine carcinoma:NEC). In 2017, in the pancreatic neuroendocrine neoplasms(PanNEN), the lesion is divided into two different categories, well differentiated neuroendocrine tumor:NET and poorly differentiated neuroendocrine carcinoma;NEC. According to the proliferative activities, mitoses or Ki-67 labeling index, NET is graded into NET G1, G2 and G3. Ki-67 in NET G3 is higher than 20%. In contrast, NEC is poorly differentiated carcinoma, small and large cell type, Ki-67 labeling higher than 20%. In the literature, it has been shown that NET and NEC show different genomic typing but share the monoclonal origin. Apparently, NET can be a component of MiNEN.

The patient received mixed therapies of chemotherapy, Trastuzumab and Somatostatin analogue-LAR, but followed rapid downhill course and died after seven months of clinical course.
Autopsy was performed and the stomach disclosed a large tumor mass, 9x8cm. in the tumor and massive metastasis in the liver. The other metastatic sites included pancreas and para-aortic lymph nodes. The primary tumor was predominantly composed of NEC and about 10% of adenocarcinoma. Metastatic site, particularly in the liver, was composed of only NEC, small cell type. No adenocarcinoma component was observed in the metastases. The patient died of liver failure.
This is a case of typical highly malignant MiNEN and in addition a challenging case in both pathological diagnosis and appropriate therapy.

Peripheral infusion of human umbilical cord mesenchymal stem cells improves acute liver failure in monkeys: a preclinical study

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Introduction: Acute liver failure (ALF) is associated with high mortality, and liver transplantation is the only treatment option. We have previously reported the pathophysiological mechanisms of ALF in a non-human primate model challenged by α-amatoxin. Our data highlighted the critical role of circulating monocyte-derived IL-6 in the initiation and acceleration of cytokine storm and ALF. We also reported that early peripheral infusion of human umbilical cord mesenchymal stem cells (hUC-MSCs) disrupts the development of cytokine storm by inhibiting the activation of circulating monocytes, offering a promising therapeutic strategy to this lethal syndrome. However, it is unknown whether this treatment is still effective when the cytokine storm is fully developed. This study was designed to evaluate the efficacy of hUC-MSC treatment in rhesus monkeys with fully developed ALF.

Methods: Sixty healthy male rhesus monkeys, aged at 4-6 years, were randomly and averagely divided into two groups and the monkeys in each group were intraperitoneally administered α-amatoxin at either 20 or 40 μg/kg, respectively. Forty-eight hours after toxin injection, monkeys in each group were further averagely divided into 3 groups and were treated either $1x10^7$hUC-MSCs, $2x10^7$hUC-MSCs, or equal value of saline. Blood and liver specimens were harvested and imaging examination was performed at indicated time points.

Results: 48 hours after toxin exposure, the liver indices of the monkeys increased significantly. All the monkeys received 20 μg/kg toxin survived and their sera indices returned to normal levels at 312 h, 84 h or 60 h following infusion of saline or 1U cells or 2U cells, respectively. However, in animals received 40 μg/kg toxin, 1, 2, or 3 animals survived when they were treated with saline, 1U cells or 2U cells, respectively. Despite the high mortality, hUC-MSCtherapy has also shown potential to protect liver histology and improve system aberrance.

Discussionand conclusion: Although systemic inflammatory response has been thoroughly activated, delayed hUC-MSCtreatment can decrease cytokine storm and improve liver tissue and systemic inflammatory reaction in a dose-dependent manner. This study provides a promising strategy for the treatment of ALF.

Downregulation of G Protein-Coupled Estrogen Receptor (GPER) is Associated with Reduced Prognosis in Patients with Gastric Cancer

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Objective: This study is aimed to investigate the prognostic significance of the expression of G protein-coupled estrogen receptor (GPER) in gastric cancer tissue using bioinformatics data and immunohistochemistry.
Materials and Methods: Expression of GPER mRNA in gastric cancer tissues and normal adjacent tissues was investigated using data from The Cancer Genome Atlas (TCGA), the Gene Expression Omnibus (GEO), and Oncomine database. Kaplan- Meier Plotter identified the association between GPER mRNA and prognosis. Correlation between GPER mRNA and DNA methylation used the cBioPortal for Cancer Genomics and the MethHC website. Genes co-expressed with GPER were identified from The Cancer Genome Atlas Stomach Adenocarcinoma (TCGA-STAD) underwent FunRich analysis. Immunohistochemistry and Western blot evaluated GPER protein expression in tissue micro- arrays (TMAs) and gastric cancer cell lines.
Result: GPER mRNA and protein levels were significantly lower in gastric cancer tissue and cells lined when compared with normal tissues and cells. The results from GSE15459 showed that patients with low levels of GPER mRNA had a reduced overall survival (OS) (P=0.013) and disease-free survival (DFS) (P=0.019). A negative correla- tion (r=-0.611) between GPER mRNA and DNA methylation was found using the cBioPortal and MethHC. Co- expressed epithelial-mesenchymal transformation (EMT) genes were enriched with GPER (P<0.0001). Cox regression analysis showed that GPER protein expression was an independent prognostic factor (P=0.035)
Conclusion: Downregulation of GPER predicts poor prognosis in gastric cancer. GPER may act as a tumor suppressor through the regulation of EMT in gastric cancer.

Category: Digestive pathology
1651388

A Unique Lesion of the Esophageal Mucosal Epithelium: Basal-layer-type Squamous Cell Carcinoma

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Objective: Esophageal low-grade intraepithelial neoplasia is associated with ultrastructural and cytological abnormalities confined to the lower half of the epithelium, and squamous cell carcinoma is defined as tumor invasion of the lamina propria or penetration into deeper tissue layers. There has beencontroversial about the authorized criteria of basal layer-type squamous cell carcinoma in the esophagus between Western and Japanese pathologists. Since low-grade intraepithelial neoplasia has more favorable prognosis than squamous cell carcinoma, differences in diagnostic will result different choices of patient management.

Material and Methods: 200 cases of esophageal intraepithelial neoplasia from 2014 to 2017 were collected. Retrospective screening gastroscopy study was performed according to the Japanese Classification of Esophageal Cancer, 11th Edition.

Result: Among the 200 cases, 57 cases were initially diagnosed as the low-grade intraepithelial neoplasia. But reviewed again by the experienced gastrointestinal pathologist according to the Japanese Classification of Esophageal Cancer, 11th Edition, it determined a prevalence of low-grade intraepithelial neoplasia of 5% and basal layer-type squamous cell carcinoma of 3% (total intraepithelial squamous cell carcinoma prevalence 13%). The lesion detection rate ofbasal layer-type squamous cell carcinoma reached up to 37.5% through endoscopic submucosal dissection. In addition, several clinical features differed between basal layer-type squamous cell carcinoma and low-grade intraepithelial neoplasia, especially for patients' smoking state, drinking state, and the location of the lesion. Interestingly, 100% (6/6) of the basal layer-type squamous cell carcinoma located inlower esophagus. The mean size of basal layer-type squamous cell carcinoma was 0.175 cm (0.1-0.25cm).

Conclusion: Basal layer-type squamous cell carcinoma with a high lesion detection rate in a screening population should be attached importance to diagnose distinctly compared to low-grade intraepithelial neoplasia.

Category: Digestive pathology
1651402

Chemokine receptor CXCR3 correlates with M2 macrophage infiltration, MVD and favorable prognosis in gastric cancer

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Objective: The aim of this study was to explore the correlation of chemokine receptor CXCR3 with M2 macrophage infiltration, microvascular density (MVD), various clinicopathological features and prognosis in patients diagnosed with gastric cancer (GC). **Material and Methods:** Expression of CXCR3protein, M2 macrophage and MVD was conducted in 156 GC patients and corresponding paracancerous tissues by immunohistochemical (IHC) analysis.

Results: In our study, 59 (37.82%) showed high expression of CXCR3 protein in 156 GC tissues. CXCR3 protein expression was significantly increased in tumor tissues compared with corresponding noncancerous GC tissues ($P< 0.001$). Overexpression of CXCR3 protein correlated with decreased M2 macrophage infiltration ($P = 0.001$) and negative associated with MVD ($P=0.049$). By analyzing the correlation between clinicopathological factors of patients and expression of CXCR3 protein, it showed that high level of CXCR3 protein expression was significantly associated with a more poorly differentiation ($P =0.017$) , III/IV TNM stage ($P = 0.02$), and deeper invasion depth ($P = 0.003$). Kaplan-Meier analysis and log-rank test indicated that patients with high CXCR3 protein expression, low M2 macrophage infiltrationand low MVD had better survival and low mortality rate ($P< 0.001$, $P = 0.024$and, $P = 0.047$ respectively). The multivariate Cox proportional hazard model demonstrated that CXCR3 protein expression was an independent prognosis parameter in GC patients [hazard ratio (HR): 0.342 (0.204-0.571); $P< 0.001$].

Conclusion: Our study indicates that CXCR3 expression and MVD were negative correlation. Furthermore, overexpression of CXCR3 protein in GC is associated with decreased M2 macrophage infiltration and improved OS, and thus can be further exploited as a biomarker and therapeutic target in GC.

PD-L1 protein expression prompted a poor prognosis

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Objective　To investigate the relationship of PD-L1 protein expression and gene amplification in gastric cancer and their correlation to the biological behavior.　**Methods**　A total of 247 gastric cancer specimens with the follow-up data and clinicopathological data were selected from Shanxi Cancer Hospital. PD-L1 was detected by the immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH).　**Results**　(1) PD-L1 protein expression: the positive rate of PD-L1 protein expression on tumor cells (TC) was 25.9% (64/247), and the positive rate on tumor infiltrating immune cells (IC) was 26.7% (66/247). The expression of PD-L1 on TC was correlated with the degree of differentiation and tumor diameter. The PD-L1 expression on IC was correlated with vascular cancer thrombi. (2) The amplification rate of PD-L1 gene detected by FISH was 19.0% (47/247). PD-L1 gene amplification was associated with age, large/small curvature of the stomach, tumor location, tumor diameter, and lymph node metastasis. (3) Positive coincidence rate of PD-L1 protein expression and PD-L1 gene amplification was 25.0%(16/64), and negative coincidence rate was 83.0% (152/183), total coincidence rate was 68.0% (168/247). The results suggested that both of the detection of PD-L1 by IHC and FISH was disaccord. (4) Univariate survival analysis showed that there was a negative correlation between PD-L1 protein expression on TC and prognosis in gastric cancer. Vascular tumor thrombi, tumor diameter, depth of invasion, and lymph node metastasis are all poor prognostic factors of gastric cancer. (5) Multivariate COX regression analysis showed that PD-L1 protein expression, depth of invasion and lymph node metastasis were all independent risk factors for prognosis of gastric cancer.　**Conclusion**　Consistency between PD-L1 protein expression and gene amplification was poor. PD-L1 protein expression prompted a poor prognosis. There was no significant correlation between PD-L1 gene amplification and prognosis.

Exosomes Derived from Human Umbilical Cord Mesenchymal Stem Cells Relieve Inflammation of the Liver through miR-455-3p

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In the previous study, we established an amanita-induced acute liver failure (ALF) model in monkeys and demonstrated that the activation of early circulating monocytes and increasing IL-6 secreted by them led to cascade inflammation reaction. The cascade of inflammatory signals is an important cause of local liver damage, multiple organ dysfunction syndrome and even animal death. hUC-MSCs transfused into early ALF monkey models could strongly inhibit circulating monocyte activation and IL-6 secretion, which significantly improved ALF progression and prognosis. However, the mechanism remains unclear. MSCs play a therapeutic role in different environments by producing specific active factors adaptively. We hypothesize that high level of IL-6 in peripheral blood is the most prominent microenvironmental stress when hUC-MSCs enter the body. However, treatment with transplanted MSCs may inhibit circulating monocyte activation by releasing exosomes. In this study, we found that hUC-MSCs produced a large number of miR-455-3p enriched-exosomes after stimulation with IL-6 *in vitro*. Exosomes inhibited macrophage from producing IL-6 by targeting PI3Kr1, which significantly inhibited the secretion of inflammatory factors by macrophages after LPS stimulation. In chemical liver injury and endotoxin mouse model, overexpression of miR-455-3p can attenuate liver tissue damage, macrophage infiltration and reduce the level of inflammatory factors in serum. CONCLUSION: By secreting exosomes rich in miR-455-3p, hUC-MSCs significantly inhibit monocyte-macrophage activation and improve liver damage and systemic inflammatory response.

LncRNA SEMA3B-AS1-SEMA3B-NRP1 signal axis inhibited the proliferation and metastasis of colorectal cancer by inhibiting tumor angiogenesis

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Objectives   Long noncoding RNAs (lncRNA), important new members of the ncRNA family, have crucial functional importance in various biological processes, ranging from epigenetic gene regulation and transcriptional control to posttranscriptional regulation. Increasing evidence has also established that the deregulation of lncRNAs expression may be important in cancer biology, typically resulting in the aberrant expression of gene products that contribute to the progression of human tumors. Howerver, lncRNAs with abnormal function in colorectal cancer(CRC) remain largely unknown.
Materials and Methods LncRNA expression profiling of CRC tissues and normal mucosal tissues was performed to identify unexplored lncRNA in CRC. Then, lncRNA *SEMA3B-AS1* was focused and its correlation with clinical outcome of CRC patients was analyzed. The effects of *SEMA3B-AS1* on CRC cells were determined using *in vitro* and *in vivo* assays. The aberrant expression of downstream genes regulated by *SEMA3B-AS1* and the underlying mechanisms were explored using RNA pull-down, RIP, ChIP and western blotting.
Result We found that lncRNA *SEMA3B-AS1* expression was significantly downregulated in CRC, and the down-regulation of *SEMA3B-AS1* was associated with high TNM staging and poor prognosis of CRC patients. Function experiments revealed that up-regulation of *SEMA3B-AS1* significantly inhibit the proliferation, invasion and metastasis of colorectal cancer cells *in vitro and in vivo*. Further studies showed that *SEMA3B-AS1* suppressed CRC progression by serving as a scaffold to recruit p300, whose acetylation of H3K9 at the SEMA3B promoter upregulated expression of SEMA3B. As an important regulator of angiogenesis, SEMA3B inhibits the VEGF signaling pathway and tumor angiogenesis by binding to NRP1 receptor, thereby inhibiting the metastasis of CRC.
Conclusion Our study revealed the molecular mechanism of *SEMA3B-AS1*-SEMA3B-NRP1-VEGF signaling axis inhibiting CRC metastasis, and providing important theoretical and experimental basis for the treatment of CRC.

Expression and significance of programmed death ligand 1 and programmed death ligand 2 in esophageal squamous cell carcinoma

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Objective: To detect the expression of programmed death receptor 1 (PD-1) and programmed death receptor 2 (PD-L2) in esophageal squamous cell carcinoma and to explore the relationship between the level of PD-L1/L2 expression and the clinicopathological features of patients with esophageal squamous cell carcinoma。

Methods: The paraffin specimens and clinicopathological data of 50 patients with radical esophageal squamous cell carcinoma were analyzed retrospectively. The expression of PD-L1 and PD-L2 both in esophageal squamous cell carcinoma and normal esophageal mucosa adjacent to esophageal carcinoma were observed and compared, and the relationship between the expression level and clinicopathological features of esophageal squamous cell carcinoma was analyzed.

Results: The positive expression rates of PD-L1 and PD-L2 in esophageal squamous cell carcinoma were 50% (25/50) and 48.0% (24/50), respectively, which were higher than those in normal esophageal mucosa adjacent to carcinoma (20%, 10/50) and 16% (8/50), respectively, the difference was statistically significant (P < 0.05). The expression of PD-L1 is related to the depth of tumor invasion and TNM stage. The expression of PD-L2 is associated with tumor invasion depth and lymph node metastasis.

Conclusion: PD-L1/L2 is highly expressed in esophageal squamous cell carcinoma and is closely related to the disease progression of esophageal squamous cell carcinoma. Blocking PD-L1/L2 signaling pathway may provide a new target for immunotherapy in patients with esophageal squamous cell carcinoma.

Long non-coding RNA KRT19P3 suppresses proliferation and metastasis through COPS7A-mediated NF-κB pathway in gastric cancer

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Long non-coding RNAs (lncRNAs) have emerged as critical regulators in gastric cancer (GC). LncRNA expression microarray data indicates that KRT19P3 (Keratin 19 Pseudogene 3) is downregulated in GC samples. However, the expression pattern and molecular mechanism of KRT19P3 in GC have not been characterized. The present study confirmed the downregulation of KRT19P3 in GC tissues and cells. Decreased expression of KRT19P3 was correlated with larger tumor size, advanced TNM stage, Lauren's classification, positive lymph node metastasis and poor prognosis. Enforced expression of KRT19P3 significantly inhibited cell proliferation, migration, and invasion *in vitro*, as well as tumorigenesis and metastasis *in vivo*. Conversely, KRT19P3 knockdown had opposite effects. Mechanistically, RNA pull-down and RIP assay revealed that KRT19P3 could directly bind COPS7A. KRT19P3 enhanced COPS7A protein stability in GC cells, and KRT19P3 suppressed GC cell proliferation and metastasis partly through regulation of COPS7A expression. COPS7A could promote deubiquitinylation of IκBα which was executed by CSN-associated deubiquitinylase USP15, and then KRT19P3 inactivated NF-κB signaling pathway in a COPS7A-dependent manner. For the first time, we revealed that KRT19P3 could suppress tumor growth and metastasis through COPS7A-mediated NF-κB pathway which may serve as potential targets for treatment of GC in the future.

Category: Digestive pathology
1655750

KNK437 restricts the growth and metastasis of colorectal cancer via targeting DNAJA1/CDC45 axis.

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As an inhibitor of heat shock proteins (HSPs), KNK437 has been reported to play an anti-tumor role in several cancers. But its therapeutic effect and mechanisms in colorectal cancer (CRC) remain unclear. Here, KNK437 sharply inhibited the level of DnaJ heat shock protein family (Hsp40) member A1 (DNAJA1), followed by DNAJB1, but had little effect on the levels of HSP27, HSP105, HSP90 and HSP70 in CRC cells. DNAJA1 promoted CRC cell proliferation in vitro and tumor growth and metastasis in vivo. Mechanistically, DNAJA1 was activated by E2F transcription factor 1 (E2F1) and then promoted cell cycle by stabilizing Cell division cycle protein 45 (CDC45), which could be reversed by KNK437. DNAJA1 was significantly up-regulated in CRC tissues and positively correlated with serosa invasion, lymph node metastasis. High level of DNAJA1 predicted poor prognosis for CRC patients. Its expression was highly linked with E2F1 and CDC45 in CRC tissues. More importantly, KNK437 significantly suppressed the growth of DNAJA1 expressing tumor in vivo. The combined treatment of KNK437 with 5-FU/L-OHP chemotherapy reduced liver metastasis of CRC. These data reveal a newly mechanism of KNK437 in anti-tumor therapy of CRC and provides a newly therapeutic strategy with potential translation to the CRC patients.

MDSCs activate dormant cells by secreting CCL7 to promote liver metastasis in colorectal cancer

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The alterations and roles of infiltrating myeloid-derived suppressor cells (MDSC) in colorectal cancer (CRC) during micro-metastasis to macro-metastases are poorly understood. Here, we showed that MDSCs accumulated constantly in liver metastases during CRC progression. In human peripheral blood samples, the number of MDSCs in CRC patients with metastasis was more than that in those without metastasis. Compared with tumor cells in macrometastases, tumor cells in micrometastases showed higher expressions of stemness related genes such as CD44, CD133, lower expressions of proliferation related genes like Ki67, c-myc and epithelial to mesenchymal transition (EMT) related gene vimentin, which presented similar characteristics with tumor dormant cells. MDSCs could increase the protein levels of Ki67, cyclin D1 and c-Myc and decreas the expression of CD44, Sox2, CD133 and Lgr 5. MDSCs also promoted the proliferation of micrometastatic cells and dormant cells. MDSCs shortened the time of micrometastasis to macrometastasis. CCL7 secreted by MDSCs could combine with membrane protein CCR2 of micrometastatic cells or dormant cells and stimulate JAK/STAT3 pathway and increase the expression of JAK2、p-STAT3，c-Myc and cyclin D1 to promote the proliferative ability and up regulates cell cycle of of micrometastatic cells or dormant cells. CCL7 inhibitor Bindarit sharply reversed the effect of CCL7 screated by MDSCs on activating dormancy cells and suppressed the proliferation and liver metastasis of CRC cells. Together these data provide direct evidence that MDSCs can promote the dormant cells' activation and proliferation, which might be a new therapeutic target to prevent distant metastasis and prolong the survive of CRC patients.

OLFM4 is a novel predictor for prognosis and a potential therapeutic target for colorectal cancer

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Objectives
Olfactomedin 4 (OLFM4) is a secreted glycoprotein predominantly expressed in bone
marrow and gastrointestinal tissues. Aberrant expression of OLFM4 has been shown in
several cancers. However, the clinical significance hereof is currently controversial.
The present study investigates the expression and clinical significance of OLFM4 in
colorectal cancer.
Methods
Real-time polymerase chain reaction and Western blotting were used to evaluate OLFM4
mRNA and protein expression in 15 pairs of fresh-frozen colorectal cancer samples, which
were compared with adjacent normal mucosa. The OLFM4 protein was evaluated by
immunohistochemical techniques using colorectal tissue microarrays. The sample
included 309 cancer specimens and corresponding normal colorectal mucosa. The effect
of OLFM4 knockdown on colorectal cancer cell proliferation was investigated using Cell
Counting Kit-8 assays and colony-formation assays.
Results
Both the mRNA and protein level expression of OLFM4 gene was found to be upregulated
in colorectal cancer tissues. Furthermore, the upregulated expression of OLFM4 was
significantly correlated with the results of AJCC stage, invasion depth, nodal
involvement and distant metastasis. OLFM4 was also shown to be an independent prognostic
indicator of disease-free survival and overall survival. Knockdown of OLFM4 expression
inhibited the proliferation of colorectal cancer cell lines through G1/S transition
regulation.
Conclusion
The results indicate that OLFM4 might play an important role in colorectal cancer
progression and function as a novel prognostic indicator and a potential therapeutic
target.

Category: Digestive pathology
1655810

The Expression and Clinicopathological Significance of BRAF V600E and Mucin 6 in Intrahepatic Cholangiocarcinoma

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Aim: To explore the expression the expression and clinicopathological significance of BRAF V600E and Mucin 6 in intrahepatic cholangiocarcinoma.
Methods: Immunohistochemistry for BRAF V600E and Mucin 6 was performed in 110 cases of intrahepatic cholangiocarcinoma. Medical records were reviewed and clinicopathological analysis was performed.
Results: BRAF V600E expression was detected in 11 cases (10.0%); Mucin 6 expression was observed in 19 ICC specimens (17.3%). Meanwhile, BRAF V600E expression was significantly in positive correlation with Mucin 6 expression. Thereafter, cox regression models indicated that positive expression of either BRAF V600E (Hazard Ratio (HR):0.13, 95% confidence interval (CI):0.30-0.67) or Mucin 6 (HR:0.07, 95% confidence interval (CI):0.02-0.32) was significantly linked with longer overall survival for ICC patients.
Conclusion: BRAF V600E positive expression and Mucin 6 positive expression implied significant survival benefits for ICC patients.

Category: Digestive pathology
1655874

Iron gastropathy: a case report and literature review

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Background and Objective   Iron gastropathy is the accumulation and deposition of exogenous or endogenous iron in gastric mucosa. The current study was aimed at assessing the clinicopathological features, diagnosis and prognosis of Iron pill gastritis and enhancing the understanding of the disease.

Materials and Methods   A case of iron pill gastritis was analyzed by clinical pathology, and the expression of iron was detected by Prussian blue stainning. The histological features were reviewed in combination with literature.

Results   Female patient, 65 years old. "Hemolytic anemia" was diagnosed in another hospital 4 months ago , and treated with ferrous succinate tablets, folic acid tablets and prednisone tablets orally. The general morphology of specimen was shown a grey-red polyp tissue with pedicle, the size of which was 1.9cm×1.2cm×0.3cm, the surface was congested and erosive, dark red, with thick and short pedicles attached, the length of 0.4cm,the width of 0.8cm .Microscopically, gastric mucosal erosion and necrosis, cystic dilation of some glands, hyperplasia of interstitial inflammatory granulation tissue, and deposition of the lamina with polynuclear giant cell phagocytosis.The yellowish brown material is coarsely granular or lumpy. Special staining shows Prussian blue stain (+).

Conclusion According to histological manifestations, there are 3 patterns of iron accumulation: Accumulation in macrophages, endothelial and stromal cells、Epithelial accumulation、Extracellular deposition. Patients should be used the liquid iron supplements that are less toxic or drugs that are less corrosive. The diagnosis of the disease should be combined with a clinical history and Prussian blue stain should be routinely performed.

High expression of C9orf86 promotes cell proliferation and predicts poor prognosis in esophageal squamous cell carcinoma

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Background: chromosome 9 open reading frame 86 (C9orf86) has been reported an important player in several cancers. Nevertheless, its role in esophageal squamous cell carcinoma (ESCC) is unclear.

Methods: C9orf86 mRNA expression was detected in 93 frozen ESCC samples by qRT-PCR. C9orf86 expression in tissue microarray containing 171 ESCC tumors was detected by immunohistochemistry, and its correlation with clinicopathological characteristics was evaluated. The impact of C9orf86 knockdown on cell proliferation, apoptosis, migration and epithelial-mesenchymal transition (EMT) of ESCC cells was investigated by MTT, Focus formation, flow cytometry, Transwell assays, qRT-PCR and western blot, respectively.

Results: C9orf86 was highly expressed in ESCC. The high-level expression of C9orf86 associated with worse prognosis. Silencing of C9orf86 inhibited proliferation, invasion and migration of ESCC cells. Knockdown of C9orf86 inhibited the malignant behaviors by inhibiting EMT in ESCC cells.

Conclusion: C9orf86 displays tumor-oncogenic functions in ESCC. It would be a potential prognostic biomarker and a novelty therapeutic target for ESCC.

IL-36s in the colorectal cancer: is interleukin 36 good or bad for the development of colorectal cancer?

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Background & Aims: Colorectal cancer (CRC) is still a major killer, despite decades of extensive development. Host immunity plays an important role in tumorigenesis. Direct comparison among IL-36α, IL-36β and IL-36γ in prognosis of CRC remains to be explored, despite two studies of IL-36α and IL-36γ in CRC are reported.
Methods: CRC tissue arrays were generated from the colorectostomy samples with different TNM stages and invasion depths in The Department of Pathology, Tongren Hospital, Shanghai Jiaotong University School of Medicine. Demography of these patients was collected. Using immunohistochemistry and histopathology, IL-36α, IL-36β and IL-36γ were determined in the tissue array of CRC with different TNM and depth of invasion (T1-4) of CRC patients (n=185), comparing to non-cancer tissues with demographic information.
Results: It was detected that significant association of colonic IL-36α, IL-36β or IL-36γ between non-cancer and cancer (with all P<0.0001), using Wilcoxon signed-rank test. Using ROC curve analysis specificity and sensitivity of IL-36α, IL-36β or IL-36γ were confirmed with area under curve (AUC) of 0.68, 0.73 or 0.65, respectively. Using log-rank test, there were significant differences in between IL-36α ^{high} and IL-36α ^{low} (P=0.003) or IL-36γ ^{high} and IL-36γ ^{low} (P=0.03). There was significant difference of survival curves among IL-36α plus IL-36β group (P=0.01), or IL-36α plus IL-36γ group (P=0.002). In particularly in the combination of IL-36α ^{high} plus IL-36β ^{high}, or IL-36α ^{high} plus IL-36γ ^{low} groups it was observed the longest survival time post-surgery among CRC patients. In contrast, the group of IL-36α ^{low} plus IL-36β ^{high} or IL-36α ^{low} plus IL-36γ ^{high} had the shortest overall survival. Using log-rank test, there were significant differences in the sub-group of IL-36α ^{high} and IL-36α ^{low} i.e. invasion depth of T4 (P<0.0001), lymph node metastasis (P=0.04), TNM III-IV (P=0.03) and right-sided colon (P=0.02). There were significant differences in the sub-group of IL-36γ ^{high} and IL-36γ ^{low}, i.e. no lymph node metastasis (P=0.008), TNM I-II (P=0.03) and left-sided colon (P=0.05). Multivariate analysis demonstrated that among IL-36α, IL-36β and IL-36γ, only IL-36α (HR, 0.37; 95%CI, 0.16-0.87; P=0.02) was independent factors in survival, using Cox proportional hazards regression analysis. IL-36β and IL-36γ were not significant in multivariate analysis among these CRC patients.
Conclusions: IL-36α or IL-36γ seems to be reliable biomarkers in predicting the prognosis of CRC at the later or early stage, respectively. Combining IL-36α plus IL-36γ appears be more accurately in determine the postoperative prognosis of CRC patients. Our data may be useful in development of in the management of CRC.

Interleukin-38 in colorectal cancer: a potential role in precision medicine

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Colorectal cancer (CRC) is the third leading cause of cancer-related death around the world, partly due to a lack of reliable biomarkers for early diagnosis. To improve the outcome of CRC, it is critical to provide diagnosis at an early stage with promising sensitive/specific marker(s). Using immunohistochemistry and histopathology, IL 38 expression was determined in the tissue array of CRC with different TNM and depth of invasion of CRC patients, comparing to non-cancer tissues and correlated with demographic information, including survival.

A substantial reduction of IL-38 was detected in the CRC compared to adjacent non-cancer colonic tissue. IL-38 correlated with differentiated tumours ($P<0.0001$); CRC located in the left side of the colon ($P<0.05$), and smaller tumour size (≤ 5 cm; $P<0.05$). ROC curve analysis demonstrated both the high specificity and high sensitivity of IL-38 for the diagnosis of CRC (AUC=0.89). By sub-group analysis, AUC of IL-38 for the diagnosis of CRC was higher in poorly-differentiated, right-sided CRC or tumour size >5 cm (AUC=0.98, 0.94 or 0.97, respectively). Significantly longer survival was observed for the IL-38high versus the IL-38low groups in CRC patients ($P=0.04$). By sub-group analysis, survival was longer for IL-38high patients with lymph node metastasis ($P=0.01$) and TNM stage III-IV ($P=0.02$). Multivariate analysis demonstrated that IL-38 (HR, 0.43; 95%CI, 0.19-0.98; $P=0.05$) and tumour invasion depth (HR, 2.30; 95%CI, 1.03-5.13; $P=0.04$) were independent factors in survival. High IL-38 in CRC is an independent prognostic factor for the longer survival of CRC patients. IL-38 signalling may constitute a therapeutic target in CRC.

Late stage gastric cancer patients with extra gained HER2 positivity by dual block assessment do not show compromised efficacy to trastuzumab treatment

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Objective: Dual block HER2 assessment can effectively increase the HER2 positive rate in resected specimens of gastric cancer (GC). The aim of this study is to explore whether GC patients with extra gained HER2 positivity by dual block assessment can benefit from trastuzumab therapy.
Materials and Methods: Twenty-eight GC patients receiving gastrectomy prior to trastuzumab treatment were retrospectively analyzed. All the cases routinely accepted dual block HER2 assessment. The cases were divided into 2 cohorts based on HER2 status: cohort A with concordant HER2 results and cohort B with discordant HER2 results between the two blocks (cases with extra gained HER2 positivity). Response rate (RR), progress free survival (PFS) and overall survival (OS) were compared between the two cohorts.
Results: The results showed that no significant differences were found between the two cohorts in main clinicopathologic characteristics. No statistical difference was found in response rate (47.6% vs 57.1%) (P=1.0). The two cohorts did not demonstrated statistical difference in the PFS (10.5 months (95%CI 6.4-14.6) vs 8.0 months (95%CI 3.2-12.8), P=0.686) and OS (23.3 months (95%CI 12.1-34.5) vs 20.0 months (95%CI 10.1-29.9), P=0.776).
Conclusion: In conclusion, patients with extra gained HER2 positivity does not show compromised efficacy to trastuzumab treatment.

Pathologic upgrade rates after endoscopic forceps biopsy compare with endoscopic submucosal dissection for type 0-II superficial gastric lesions.

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Endoscopic submucosal dissection (ESD) has been widely implemented for the treatment of gastric superficial neoplasia. However, the final pathologic diagnosis after ESD may be different from that indicated by the results of endoscopic forceps biopsy (EFB) especially for ulcerative lesions. This study identified risk factors for gastric epithelial lesions so that early gastric cancer (EGC) could be diagnosed after ESD. From December 2018 to January 2019, 270 type 0-II superficial gastric lesions (408 patients) diagnosed by endoscopic forceps biopsy were enrolled. Among 270 cases, one third (91 cases) had the final diagnosis following the ESD specimens. The diagnosis was upgraded (from low-grade dysplasia to high-grade dysplasia or adenocarcinoma, or from high-grade dysplasia to adenocarcinoma,-tub1 or -tub2) in 31 cases (34.1%), concordant in 60 (65.9%), and downgraded (from high-grade dysplasia to low-grade dysplasia or non-neoplasia, or from low-grade dysplasia to non-neoplasia) in 2(2.1%). Endoscopic findings for size, nodularity, erosion, irregular microsurface pattern and fine network pattern were significantly associated with EGC and lesions indefinite for neoplasia during the initial endoscopic forceps biopsy. Lesion size $\geq$2.0 cm and irregular microsurface pattern at initial EFB were independent risk factors for pathologic upgrade in ESD. For type 0-II superficial gastric lesions, endoscopic biopsy in the diagnosis of low-grade intraepithelial neoplasia, may exist or progression to high-grade intraepithelial neoplasia or EGC, should take more active treatment. Therefore, these endoscopic characteristics should be considered together to reduce under-diagnosis of gastric intraepithelial neoplasia and early gastric carcinoma in endoscopic forceps biopsy.

Loss of RDM1 enhances hepatocellular carcinoma progression via p53 and Ras/Raf/ERK pathways

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Hepatocellular carcinoma (HCC), with ineffective therapeutic options and poor prognosis, represents a global threat to human lives. In the present study, we show that RAD52 motif 1 (RDM1), a key regulator of DNA double-strand break repair and recombination, is downregulated in HCC tissues and suppresses tumor growth. In clinical HCC samples, low expression of RDM1 correlates with larger tumor size, poor tumor differentiation and unfavorable survivals. *In vitro* and *in vivo* data demonstrate that knockdown of RDM1 increases HCC cell proliferation, colony formation and cell population at G2/M phase, whereas RDM1 overexpression results in the opposing phenotypes. Mechanistically, RDM1 binds to the tumor suppressor p53 and induces its expression by enhancing its protein stability. In the presence of p53, RDM1 suppresses the phosphorylation of Raf and ERK. Overexpression of p53 or treatment of ERK inhibitor significantly abolishes the cell proliferation induced by the depletion of RDM1. In addition, overexpression of methyltransferase-like 3 (METTL3) markedly induces N6-methyladenosine (m^6A) modification of RDM1 mRNA and represses its expression. Taken together, our study indicates that RDM1 functions as a tumor suppressor and serves as a potential prognostic and therapeutic factor in HCC.

Pharmacological PP2A inhibition reverses nab-PTX resistance in ESCC by downregulating OXPHOS in CSCs through MCL1

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Advanced esophageal squamous cell carcinoma (ESCC) is commonly treated with taxane and has shown better response to nano albumin bound paclitaxel (nab-PTX) as initial therapy. However, tumor response is generally short-lived due to acquisition of drug resistance by molecular mechanism that are poorly understood. Herein, we demonstrated that elevated protein phosphatase 2A (PP2A) activity contributed to nab-PTX resistance and is a novel target for overcoming nab-PTX resistance. Pharmacological inhibition of PP2A by LB-100 increased mitotic catastrophe, polyploidy and apoptosis in all three nab-PTX resistant ESCC cell lines *in vitro* and inhibited nab-PTX resistant ESCC xenograft growth *in vivo* but not in parental models. Elevated PP2A inhibited degradation of MCL1, which is a biomarker of anti-tubulin chemotherapeutics resistance. Through proteomics analysis, we found that nab-PTX resistant cells rely on oxidative phosphorylation (OXPHOS) for their survival. Dependence on OXPHOS is particularly increased in the cancer stem cells (CSCs) of nab-PTX resistant ESCC cells. Genetic inhibition of MCL1 and pharmacological inhibition of PP2A OXPHOS especially in CSCs and was associated with re-sensitization of nab-PTX resistant cells to nab-PTX. In nab-PTX resistant xenograft models, LB-100 pretreatment restored nab-PTX sensitivity. Combination of nab-PTX with LB-100 synergistically inhibited resistant xenograft tumor growth after LB-100 pretreatment. In summary, our findings demonstrated that PP2A is a novel therapeutic target in reversing nab-PTX resistance in patients with advanced ESCC.

The expression and significance of SMYD5 and H4K20me3 in colon cancer

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Abstract(#br)Objective(#br)To detect the expression patterns and positive rates of SMYD5 and H4K20me3 in colon cancer, and to analyze the correlation between the expression patterns and positive rates of SMYD5 and H4K20me3 and different pathological characteristics and prognosis of colon cancer, and to explore the correlation between SMYD5 and H4K20me3 in the occurrence and development of colon cancer, so as to provide molecular markers and targets for the diagnosis, treatment and prognosis of colon cancer.(#br)Methods(#br)Paraffin specimens of colon cancer tissues (100 cases) adenoma tissues (30 cases) and paracancer normal tissues (30 cases) with complete clinicopathological data collected by the pathology department of the first hospital of shanxi medical university were selected. Immunohistochemistry EnVision method was adopted to detect the protein expression of SMYD5 and H4K20me3 in different tissues. TNM staging was performed in combination with postoperative pathology. Chi-square test was used to analyze the differences in the expressions of SMYD5 and H4K20me3 in the adenoma tissues and adjacent normal tissues of colon cancer tissues, and the relationship between the expressions of SMYD5 and H4K20me3 and the clinicopathological parameters after colon cancer surgery was analyzed. The relationship of SMYD5 and H4K20me3 proteins with disease-free survival (DFS) and total survival (OS) of colon cancer patients after radical resection was analyzed by kaplan-meier log-rank test, and the correlation between the expression of SMYD5 and H4K20me3 proteins in colon cancer tissues was analyzed by Pearson correlation analysis.(#br)Result(#br)The positive rates of SMYD5 and H4K20me3 proteins in different tissues: > adenoma tissues > cancer tissues in paracancer normal tissues, the difference was statistically significant (P<0.05), indicating that the expression of both proteins decreased in the development of colorectal cancer. The expression of SMYD5 and H4K20me3 protein was significantly correlated with the presence or absence of lymph node metastasis and ki-67 index in TNM stage of the tumor (P<0.05), suggesting that the absence of these two proteins may be related to the invasion and metastasis of colon cancer. The expression of SMYD5 and H4K20me3 protein was independent of the tumor site and microsatellite stability of patients of age and gender (P>0.05). Patients with colon cancer with low expression of H4K20me3 protein had shorter DFS after surgery, suggesting that the lack of H4K20me3 expression may promote the development of colon cancer. In colon cancer tissues, the expression of SMYD5 and H4K20me3 proteins was positively correlated, indicating a synergistic effect between the two proteins during the occurrence and development of colon cancer.(#br)Conclusions(#br)Lack of protein expression of SMYD5 and H4K20me3 may promote the development of colon cancer. SMYD5 and H4K20me3 play synergistic roles in the development of colon cancer. SMYD5 and H4K20me3 may be molecular markers for early

detection and prognosis prediction of colon cancer, and may also be new therapeutic targets.

Solitary fibrous tumors of the esophagus: clinicopathological observation and literature review

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Objective To investigate the clinicopathological features, diagnosis, differential diagnosis, treatment and prognosis of solitary fibrous tumors (SFT) of the esophagus.
Material and Methods The pathological and immunophenotypic features of a SFT of the esophagus were analyzed and the related literatures were reviewed.
Result Male, 48 years old. Tumor is a pedicled mass with clear boundary under the esophageal mucosa. Spindle-shaped and oval tumor cells were arranged in short bundles, sheets and disordered structures, among which collagen fibers were found. Abundant interstitial blood vessels and hyaline degeneration of vascular wall were also observed. Immunohistochemistry showed that the tumor cells were positive for CD34 and STAT6, partly positive for H-Caldesmon, while negetive for CD117, DOG1, SMA, desmin and S-100. The proliferation index of Ki67 was less than 5%.
Conclusion SFT of the esophagus is very rare. Their diagnosis mainly depends on pathological morphology and immunohistochemistry. They need to be differentiated from other spindle cell tumors of the esophagus.

SOX2 amplification and chromosome 3 gain significantly impact prognosis in esophageal squamous cell carcinoma

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Objectives: We aimed to investigate prevalence and prognostic role of *SOX2* amplification and expression in surgically resected esophageal squamous cell carcinoma (ESCC).

Methods: 474 ESCC were assessed by fluorescence in situ hybridization and immunohistochemistry for *SOX2* amplification and protein expression, respectively. The relationship of gene status with various clinicopathological characteristics and survival of patients was statistically analyzed.

Results: 4.2% ESCCs were found with *SOX2* amplifications, and 12.4% cases with *chromosome* 3 gain. *SOX2* amplification was associated with later clinical stage ($P=0.039$), *chromosome* 3 gain was associated with earlier clinical stage. Low and high SOX2 expression were found in 33.3% and 19.8% cases. SOX2 expression was significantly associated with gene copy number variation ($P=0.003$). *SOX2* amplification was associated with a significantly shorter disease free survival (DFS) or overall survival (OS), however, *chromosome 3* gain was associated with a significantly longer DFS or OS ($P<0.001$). Multivariate analysis using the cox proportional hazard model indicated that *SOX2* amplification was an independent poorer prognostic factor (DFS, $P=0.021$, HR=1.833, 95%CI=1.095-3.069) (OS, $P=0.034$, HR=1.745, 95%CI=1.043-2.921), along with pTNM stage, *chromosome 3* gain was an independent better prognostic factor (DFS, $P=0.001$, HR=0.459, 95%CI=0.286-0.735) (OS, $P=0.001$, HR=0.450, 95%CI=0.277-0.730).

Conclusions: This is the first study assessing *SOX2* amplification and *chromosome 3* gain in a large cohort of ESCC. *SOX2* amplification is an independent poorer prognostic factor, but *chromosome 3* gain is an independent favorable prognostic factor. Our results suggest that *SOX2* amplification and *chromosome 3* gain may be potential biomarkers related to tumor progression, and risk stratification in ESCC.

YY-57 is a novel double-target kinase inhibitor of ribosomal S6 kinase 4 (RSK4) and epidermal growth factor receptor (EGFR) in esophageal squamous cell carcinoma

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Objectives: The EGFR signaling pathway regulates diverse cellular processes, including growing, survival, and differentiation, and is aberrantly activated in human cancers. As such, numerous compounds targeting the EGFR pathway are currently being clinically evaluated for the treatment of cancer including esophageal squamous cell carcinoma (ESCC). However, some ESCC patients still have therapeutic resistance to existing EGFR inhibitors. We previously found that ribosomal S6 kinase 4 (RSK4) promotes survival, metastasis and cancer stemness of ESCC cells, which partly explains the resistance mechanism of EGFR inhibitors. In this study, we screened out the double-target kinase inhibitors that have inhibitory effects on both RSK4 and EGFR from our EGFR inhibitors library which contains 140 small molecule compounds.
Method: *In vitro* kinase assay was used to screen out small molecules compounds with strong inhibitory effects on both RSK4 and EGFR from our EGFR inhibitors library. Transwell method was used to verify the inhibitory effect of agents on tumor cell invasion and migration in vitro. CCK-8 assay was used to detect the effect of drugs on cell proliferation. Flow cytometry was used to analyze the interference of inhibitors on cell cycle. Western blot was used to analyze the effect of inhibitor on phosphorylation levels of GSK3β and RPS6 in ESCC cell lines.
Result: Three small molecules (E21, zw-025, zw-012) with strong inhibitory effects on RSK4 were screened out from our EGFR inhibitors library and modified to obtain new compounds (YY-41, YY-43, YY-57). Western blot results showed that YY-57 most significantly inhibited the phosphorylation of RPS6 and GSK3β in cells at 10 μM in these three compounds (Figure 1). Transwell assay showed that YY-57 could inhibit ESCC invasion and migration *in vitro* (IC50=2.613 μM, Figure 2). CCK-8 assay showed that YY-57 could obviously inhibit ESCC proliferation when YY-57 concentration is greater than 10μM (Figure 3). Flow cytometry assay showed that YY-57 could lead to G2/M phase blockade of ESCC cells (Figure 4).
Conclusion: Our results provide a strong rationale for the use of YY-57 as a novel double-target inhibitor of RSK4 and EGFR in ESCC patients. This double-target inhibitor will help the treatment of ESCC patients and improve their prognosis.

Vascularized and functional bioengineered hepatic tissue generated from heparinized decellularized liver scaffold alleviates liver injury

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Background: Currently, liver transplantation is the only proven treatment for a wide variety of refractory liver diseases; however, it is limited by the shortage of available organs. The challenge of using bioengineered liver lies in sustaining the quantity of high-quality hepatocytes and the vasculature for blood perfusion. We characterized the heparinization of a porcine decellularized liver scaffold (DLS) as a carrier to support hepatocyte angiogenesis, thereby developing functional and vascularized hepatic tissue useful to treat chronic liver injury.

Method: The porcine DLS was obtained by the removal of cellular components and then subjected to heparinization by the end-point attachment technique. The heparinized DLSs were recellularized with rat hepatocyte spheroids to construct engineered hepatic tissue. The hepatic tissue was heterotopically implanted in the omentum majus of a rat model with chronic liver injury induced by carbon tetrachloride (CCl_4).

Results:

1. Expansion of the hepatocyte spheroids in bioengineered hepatic tissue

Hepatocyte spheroids in the heparinized DLS remained viable for at least 10 weeks *in vivo*. HE stain analysis of implanted hepatic tissue showed earlier degradation of the biomaterial in the implanted area. Larger hepatic grafts were observed and measured in the heparinized DLS group at peak growth after chronic liver injury, with more regular arrangement and less lymphocyte infiltration due to heparinization. Interestingly, the hepatocytes in the expanded hepatic tissue were organized into dense aggregates and arranged into hepatic-cord-like structures. The abdominal ultrasound and morphometric analysis of the histologic sections confirmed that the heparinized group had significantly more graft surface area than the native group. It was assumed that the grafts were spherical, and the graft volume was calculated based on the surface area measurements. The results showed an average 461-fold graft expansion in the heparinized DLS group between weeks 2 and 10, compared to a 46-fold expansion found in the DLS group. The implanted hepatic tissue can spontaneously grow in the liver-injured microenvironment, especially the heparinized microenvironment.

2. Vascularization and functionality of the bioengineered hepatic tissue

We then identified glycogen storage in the hepatocyte grafts by PAS staining, drug metabolism by immunostaining for CYP450, tight junction protein ZO-1 and functional

protein HPSE. The cell characterization examination revealed that the grafts contained densely packed polyhedral cells resembling hepatocytes, some of which were binucleated. The grafts from heparinized DLS hepatic tissue expressed higher levels of positive cells or areas than the native hepatic tissue, suggesting activity of their biological function. They also showed a higher proportion of cells expressing albumin-ZsGreen and a lower number of TUNEL-positive apoptotic cells, indicating that the expanded hepatocytes had originated from the cells that had been seeded on the bioengineered hepatic tissue and that most of the cells in the hepatic tissue were alive.

To assay for the active proliferation in hepatic tissue at 10 week after implantation, we double-stained sections using ALB and Ki-67 antibodies. We identified numerous ALB-positive cells with the round nuclei characteristic of hepatocytes aggregated in the internal tissue. The rare Ki-67-positive cells that actively undergo proliferation were dispersed in the marginal tissue. Compared to the native DLS hepatic tissue, more ALB-positive and fewer Ki-67-positive cells were observed in grafts of the heparinized DLS hepatic tissue.

We then used immunostaining against CD31, a biomarker of endothelial cells, in the hepatic tissue sections. Increased numbers of vessels were identified throughout the grafts from the heparinized DLS group at 10 week after implantation. Furthermore, after the intravenous injection of FITC-dextran into the rats, the live images revealed greater numbers and larger sizes of vessels with rapid blood flow. The presence of red blood cells in the expanded hepatic tissue suggested that vascular networks had developed in the grafts.

To explore the potential utility of the heparinized bioengineered hepatic tissue for studies of functionality, completed gene arrays of the heparinized bioengineered hepatic tissue were compared to those of the rat native liver and the hepatocyte spheroids using hierarchical clustering analysis. The results showed that hepatocyte spheroids lost some specific characteristics of the native liver *in vivo*. In contrast, the expanded heparinized hepatic tissue reconstructed the specific native functionality in metabolism, and the genes associated with synthesis and metabolism were obviously upregulated. The gene arrays revealed high levels of expression of hepatic-enriched transcription factors, conjugating enzymes and metabolic transcription factors in the bioengineered hepatic tissue ($p < 0.05$).

The heparinized bioengineered hepatic tissues were collected for key gene expression analyses. The liver-specific genes *alb* and *aat*; key genes in urea cycle, *ass-1*, *asl*, *cps-1* and *otc*; and genes related to drug metabolism, *cyp1a2* and *sult1c3*, were highly expressed in the heparinized bioengineered hepatic tissue ($p < 0.05$). These results demonstrated that the implanted heparinized hepatic tissue was actively functional.

3. Treatment effects on chronic liver injury

Since the bioengineered hepatic tissue demonstrated liver-specific functions, we hypothesized that the implants played a role instead of the native livers to help the animals recover from chronic liver injury. The HE and Masson staining showed that the livers developed extensive necrosis with obvious expansion in the blood sinus at 4 week

after CCl$_4$ administration. Liver damage was still prominently observed, and liver-generated fibrosis among the lobules in the control group was observed through 8 week, and slight fibrosis existed at 10 week. The implantation of the bioengineered hepatic tissue derived either from the DLS or heparinized DLS notably alleviated the tissue injury. Especially in the heparinized DLS group, necrosis and fibrosis were reversed in the native liver 6 weeks after implantation.

Conclusion: Primary hepatocyte spheroids survived for an extended time on the heparinized DLS and expanded to generate vascularized and functional bioengineered hepatic tissue that can alleviate chronic liver injury in rats.

Diffuse intestinal ganglioneuromatosis: two cases report and literature review

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Objective: Diffuse ganglioneuromatosis (DG) is a rare condition characterized by an abnormal disseminated, intramural, or transmural proliferation of ganglion cells, nerve fibers and Schwann cells. It is typically associated with multiple endocrine neoplasia IIb (MEN2b) or Neurofibromatosis 1 (NF1). Thus, we summarized the two cases of DG and explore the strategy of diagnosis.

Materials and Methods: Clinical, pathologic, radiological, treatment and follow-up information of two cases of DG treated at the the Affiliated Drum Tower Hospital of Nanjing University Medical School were retrospectively collected. All tumors were routinely fixed and processed, embedded in paraffin, and stained with hematoxylin and eosin. Immunohistochemical stains were also performed in all the cases using S100, synaptophysin, neuron specific enolase (NSE) and glial fibrillary acid protein (GFAP) etc.

Results: The patient 1 was a 48 -year-old man who presented with a four-month history of intermittent abdominal pain with diarrhea. A enteroscopy was requested revealing diffuse polypoid lesion. Gross examination of the resected specimen revealed many irregular nodular polyps with diameters ranging from 3cm to 5.5 cm. The patient 2 is a 45 -year-old woman presented to the emergency department with acute bdominal pain and CT scan revealed a soft tissue mass of pelvic cavity, which was partially connected with the intestine. Grossly, the specimen showed a diffuse thickening of the intestinal wall including cecum, appendix and ileocaecal and produced stricture-like thickenings of segments of the bowel. Histologic examination revealed a diffuse infiltration of the muscularis propria by a proliferation of nerve fibers and mature ganglion cells in the two cases. Immunohistochemical staining also showed that the lesional cells stained positively for S100 and synaptophysin and were negative for glial fibrillary acid protein (GFAP). Final pathologic diagnosis of diffuse intestinal ganglioneuromatosis was made. Genetic testing revealed a novel frameshift mutation in the NF1 gene in the patient 1and was negative in patient 2. To date these two patients has not shown any other clinical features suggesting a tumour syndrome.

Conclusions: We showed two cases of DG and the final diagnosis was made according to the pathological examination of the resected specimen, which are composed of mature neural fibres, ganglion cells. Although the prevalence of ganglioneuromatosis is low, it is important to recognize these lesions so that an opportunity to identify patients and families with tumour syndromes is not missed.

Tumor budding determine prognosis in resected pancreatic ductal adenocarcinoma

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Abstract

Background The prognostic effect of tumour budding was retrospectively analysed in a cohort of 203 patients with resected pancreatic ductal adenocarcinomas (PDACs) in Zhongshan Hospital Fudan university.

Methods Haematoxylin and eosin (H&E)-stained whole tissue slides were evaluated. The mean number of tumour buds was analysed according to the consensus criteria in colorectal cancer, in one 0.785 mm2 field of view.

Results Tumour budding was significantly associated with a higher tumour grade (p < 0.001) and vascular invasion (p=0.002) but not with distant or lymph node metastasis. An increased number of tumour buds was significantly associated with reduced overall survival (OS) and disease-free survival (DFS) (consensus method OS: HR = 1.94 (95% CI 1.29–2.89), p < 0.001;DFS: HR = 2.22 (95% CI 1.46–3.28), p < 0.001).

Conclusions The presence of tumor budding is an independent adverse prognostic factor in pancreatic ductal carcinoma. The assessment of budding with H&E could be used to better risk stratify patients with pancreatic ductal adenocarcinoma.Further standardisation and validation in additional clinical cohorts are necessary.

CDH6 is an indicator of poor prognosis in gastric cancer

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The expression of CDH6 in gastric cancer (GC) remains not clear. The Cancer Genome Atlas (TCGA) showed that the level of CDH6 expression was higher in GC tissues than in adjacent normal tissues (P < 0.05). And 11% of GC patients showed CDH6 genetic alterations, including missense mutation, amplification, and deep deletion. In addition, elevated mRNA level of CDH6 predicts poor outcome of gastric cancer patients and high CDH6 expression was correlated with worse disease-free survival (DFS) and overall survival (OS) by bioinformatic analysis. And the co-expression of TRPC6, PLXDC1, TBX2 and CDH6 suggest that they may act synergistically in development and progression in GC. Then, CDH6 was recognized as a potential marker of the prognosis for GC patients by integrative analysis of the constructed PPI network. The PPI network showed that ERBB2, CTNND2,CSNK2A1 and FAT3were closely connected with CDH6.Overall, these results reveal that CDH6 may potentially be a novel prognostic biomarker in GC.

LTBP1 promotes esophageal squamous cell carcinoma progression through epithelial-mesenchymal transition and cancer-associated fibroblasts transformation

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Objectives:
Esophageal squamous cell carcinoma (ESCC) is one of the most prevalent cancers worldwide. Due to its high morbidity and mortality rates, it is urgent to find a molecular target that contributes to esophageal carcinogenesis and progression. In this research, we aimed to investigate the functions of Latent transforming growth factor β binding protein 1 (LTBP1) in ESCC progression and elucidate the underlying mechanisms.

Materials and Methods:
The tandem mass tag(TMT)-based quantitative proteomic approach was applied to screen the differentially expressed proteins (DEPs) between 3 cases of ESCC tumor samples and paired normal tissues. Then the DEPs were validated in human ESCC tissues using western blot assays and GEPIA database respectively. The expression level of LTBP1 was detected in 64 cases of ESCC tissues and paired normal tissues and the relationships between LTBP1 and clinicopathological parameters were further analyzed. Then loss-of-function assays were performed to detect the function of LTBP1 in vivo and in vitro. Immunofluorescence and Western blot assays were used to detect the expression of apoptosis, EMT and CAFs markers.

Results:
A total of 39 proteins were screened to be up-regulated (ratio > 2.0) in all three ESCC tissues. Then we chose 5 candidate proteins and identified their expression by western blot and GEPIA database. The results of immunohistochemistry assays indicated that the expression level of LTBP1 was higher in ESCC tissues than that in paired normal tissues (P<0.05). Overexpression of LTBP1 was positively associated with lymphatic metastasis in ESCC. Down-regulation of LTBP1 inhibited the invasion and migration as well as metastatic abilities in vitro and in vivo. It was also observed the down-regulation of LTBP1 not only decreased the mesenchymal phenotypes but also inhibited TGFβ-induced epithelial–mesenchymal transition (EMT) in ESCC cell lines. We further found that down-regulation of LTBP1 enhanced ESCC cells' sensitivity to 5-FU treatment. Inhibition of LTBP1 expression could also attenuate induction of cancer-associated fibroblasts (CAFs) transformation and restrain fibroblast express fibronectin 1 (FN1) in ESCC cell lines.

Conclusion:
We screened differentially expressed proteins and validated 5 of them in ESCC

tissues in the current study. It was found that overexpression of LTBP1 in ESCC was associated with lymph node metastasis. Our results indicated that LTBP1 not only increased the malignant behaviors of ESCC cells but also induced EMT and CAFs transformation. Our studies suggested an oncogenic role of LTBP1 in ESCC initiation and progression and it may also serve as a potential therapeutic target for ESCC patients.

TUSC3 induces chemotherapy resistance and cellular stemness via Hedgehog signaling pathway in colorectal cancer

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Background: Cancer stem cells (CSCs) and chemotherapy resistance are the major causes that induce recurrence and metastasis of colorectal cancer (CRC), and lead to poor prognosis of advanced CRC patients. Tumor suppressor candidate 3 (TUSC3/N33) gene plays an oncogenic role in CRC, however, the role of TUSC3 in regulating CRC cell stemness and chemotherapy resistance are still unclear.
Methods: Western blot, qRT-PCR and Immunohistochemistry/immunofluorescence were performed to detected the expression of TUSC3 in CRC cell lines and tissues. A series of in vivo and in vitro assays were performed to reveal the function of TUSC3 and the possible mechanisms underlying its role in CRC chemotherapy resistance and cellular stemness.
Results: We found that upregulation of TUSC3 in CRC cells and tissues is positively correlated to tumor stage, while negatively associated with overall survival and disease-free survival of CRC patients. Subsequently, overexpression of TUSC3 promotes the formation of CSCs phenotypes and effectively induces the chemotherapy resistance to 5-fluorouracil (5-FU) and cisplatin in CRC cell lines, while the opposite effects are observed in TUSC3 shRNA CRC cell lines. The effects of TUSC3 on CSC phenotype and chemotherapy resistant are reversible after treatment with Hedgehog signaling pathway agonist or inhibitor. Increased SMO, PTCH1, Gli1 and ABCC1 abundances are detected in TUSC3 overexpressed CRC cells by Western Blot assays. The tissue microarray assay and bioinformatic analysis indicated that TUSC3 activate the hedgehog signaling pathway and promote the expression of CD133 and ABCC1 proteins, respectively. Furthermore, a tight relationship between TUSC3 and SMO protein was obtained by co-immunoprecipitation and immunofluorescence assays.
Conclusion: TUSC3 promotes the formation of cancer stem cell phenotypes and induce chemotherapy resistance by directly regulating Hedgehog signaling pathway in human CRC. Therefore, targeting TUSC3 may be a potential therapy target for inhibiting the recurrence and metastasis of CRC.

LncRNA-HNF1A-AS1 functions as a competing endogenous RNA (ceRNA) to activate PI3K/AKT signaling pathway by sponging miR-30b-3p in gastric cancer

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Abstract

Background: Accumulating evidence demonstrated that long noncoding RNAs (lncRNAs) played important regulatory roles in many cancers. However, the role of lncRNAs in gastric cancer (GC) progression remains unclear.

Methods: RT-qPCR assay was performed to detect the expression of HNF1A-AS1 in gastric cancer tissues and non-tumorous gastric mucosa. Overexpression and RNA interference approaches were used to investigate the effects of HNF1A-AS1 on GC cells. Insight into competitive endogenous RNA (ceRNAs) mechanisms was gained via bioinformatics analysis and luciferase assays, combined with an RNA Binding Protein Immunoprecipitation (RIP) assay.

Results: The present study displayed that increased expression of HNF1A-AS1 was associated with positive lymph node metastasis in GC. Moreover, HNF1A-AS1 significantly promoted gastric cancer invasion, metastasis, angiogenesis and lymphangiogenesis *in vitro* and *in vivo*. In addition, by using bioinformatics analysis and luciferase assays, combined with RIP assay, HNF1A-AS1 was demonstrated to function as a ceRNA for miR-30b-3p. HNF1A-AS1 abolished the function of the miRNA-30b-3p and resulted in de-repression of their target, PIK3CD, which is a core oncogene involved in progression of GC.

Conclusions: These present studies demonstrated that HNF1A-AS1 worked as a ceRNA and promoted PI3K/AKT signaling pathway-mediated GC metastasis by sponging miR-30b-3p, offering a novel insights of metastasis mechanism in GC.

HDAC3 inhibits hepatocellular carcinoma through Foxa1/a2 in female mice

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Hepatocellular carcinoma(HCC) is one of the most high malignant tumors and the fourth most common cause of cancer-related death around the world. Our previous studies have shown that in HDAC3 deficient liver, the overall content of heterochromatin is significantly reduced, H3K9/K14ac and H4K5ac accumulate largely, the stability of the genome is damaged seriously, and HCC formed spontaneously in all mice. We also noticed that there are significant gender differences in the occurrence and development of HCC, Female HDAC3$^{-/-}$ mice at 9 months developed HCC earlier (64%, 7/11) than male mice of the same age, which had no obvious tumor nodules (0%, 0/9). In addition, gender differences in HCC are associated with abnormalities in immune function. Gender differences in HCC due to sex differences in cytokine interleukin-6 (IL-6) production in mice. Estrogen inhibits the secretion of IL-6 by Kupffer cells and inhibits the activity of IL-6-STAT3 signaling pathway, which reduces the incidence of HCC in female mice. Deletion of the *IL-6* gene did not eliminate the gender difference in HCC of HDAC3$^{-/-}$ IL-6$^{-/-}$ mice, and female HDAC3$^{-/-}$ IL-6$^{-/-}$ mice developed tumors earlier. Foxa1,Foxa2 combine ERα to inhibit HCC in Foxa1/a2 deficient liver mice. The expression of Foxa1 and Foxa2 in the liver of female and male HDAC3$^{-/-}$ mice decreased significantly, and ERα and Foxa1 and Foxa2 did not bind to each other in the liver of female HDAC3$^{-/-}$ mice. Conclusion: HDAC3 inhibit HCC through regulating the transcription of Foxa1/a2, which eliminates the inhibitory effect of estrogen on HCC.

The clinicopathological characteristics and prognosis of DNA mismatch repair, Epstein–Barr virus, Her-2, P53, KI67 in gastric cancer patients after D2 gastrectomy

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Objectives MSI, EBV, and p53 might be markers for prognosis and precise treatment according to genomic classifications of gastric cancer. For conforming this   suggestion, it is necessary to figure out their clinicopathological characteristics and prognosis value in China.

Materials and Methods The level of MMR(MLH1, MSH2, MSH6, PMS2), EBV, HER-2, P53 and Ki67 in 467 patients with D2 gastrectomy were detected by immunohistochemistry and the relations with prognosis were excavated from cohort study.

Result 94 of the 467 stomach cancers (20.1%) had dMMR. MLH1/PMS2/ MSH2/ MSH6 were absent in 90(19.3%)/82(17.6%)/23(4.9%)/55 (11.8%) of 467 cases. dMMR tumors related with older age (p=0.015), non-diffuse histologic subtype (p=0.023), T3-4 stage (p=0.032), III stage (p=0.048), Her-2 IHC (0) (p=0.006), diameter≥5cm for primary tumor (p=0.001) and less commonly had signet-ring cell carcinoma (p=0.001). dMMR status was independently negative prognostic factor for OS and DFS, but others were not.

Conclusion This study   has shown the molecular characteristics of GC in China, especially MMR status, which has advantage for estimation of therapy and prognosis.

Clinicopathological features of schwannoma in pancreas

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Abstract: **Purpose** To investigate the clinicopathological features and diagnosis of pancreatic schwannoma. **Methods** Six cases of pancreatic schwannomas were described, and the pathologic features and immunophenotypic profile were studied. **Reasults** There were 5 women and 1 man with the average age of 44 years. Pancreatic Schwannoma grew on the head of pancreatic (1 cases) and 5 in body and tail of pancreatic, respectively. There were no clinical symptoms. Histologically, there were two different features including type A and type B. Immunohistochemically, the spindle cells were positive for s100 , but negative for CK and EMA. All the patients were well during the following up after operation. **Conclusion** Pancreatic schwannoma is a rare benign lesion on pancreatic. The diagnosis can be made based on histopathology of the tumor.

Nucleus-accumulated RASSF8 promote colorectal carcinoma progression via activating Wnt signaling through facilitating β-catenin nuclear importing

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Objectives Colorectal carcinoma (CRC) is the third most commonly diagnosed cancer in males and the second in females in the world, with approximately 1.3 million new cancer cases and over 0.6 million deaths reported each year. Thus, a better understanding of the molecular mechanisms underlying CRC carcinogenesis and progression is essential for the development of CRC-specific diagnostic markers and novel effective therapies for CRC patients. The Ras-association domain family (RASSF) is a group of Ras-effector molecules that contain C-terminal or N-terminal Ras-association domain. There is some evidence suggesting that RASSF8 may act as a tumor suppressor gene in many cancer. However, the expression levels, cellular localization, precise function and mechanism of RASSF8 in CRC are largely unknown.

Materials and Methods Real-time PCR, Western blot and immunofluorescence assay were used to detect the expression and localization of RASSF8 in CRC tissue samples and cell lines. The GEO public database was used to analyze the expression difference of RASSF8 transcript in colorectal cancer tissues and normal intestinal mucosa epithelium and its effect on overall survival rate and metastasis-free survival rate of colorectal cancer patients. Gain-and loss-of-function experiments were performed to investigate the biological roles of RASSF8 in CRC. The in vitro proliferation ability of colorectal cancer cells was detected by CCK8 assay and plate cloning assay. The ability of colorectal cancer cells to move and migrate in vitro was detected by scratch healing test and transwell migration assay. The effects of RASSF8 on the tumorigenesis and metastasis ability of colorectal cancer cells in nude mice were observed by subcutaneous tumor formation experiment and spleen subcapsular tumor experiment in nude mice. Protein immunoprecipitation, duel-luciferase reporter assay and fluorescent bleaching recovery assays were conducted to investigate the mechanisms underlying the functions of RASSF8 in CRC.

Result   We observed that nuclear RASSF8 was significantly up-regulated in human CRC tissues and cell lines in comparison with normal controls and high RASSF8 expression in nucleus of CRC cells was associated with a shorter overall survival and poorer prognosis of CRC patients. Nucleus-accumulated RASSF8 promoted cell proliferation, migration and invasion in CRC in vitro. We found that RASSF8 and β-catenin have obvious co-localization in the nucleus of each colorectal cancer cell. After silencing RASSF8, the transcription of AXIN2 and MYC genes regulated by β-catenin was significantly decreased. We then confirmed that RASSF8 can bind to the major members of the degradation complex in the Wnt signaling pathway, Axin1, GSK3β, and can bind to β-Trcp, one of

the members of the E3 ubiquitin ligase family. Furthermore, Wnt3a inhibits RASSF8 protein degradation and promotes nuclear RASSF8 protein accumulation. Notably, the nuclear location of RASSF8 was required for nuclear accumulation of β-catenin during Wnt activation.

Conclusion Taken together, we established a previously unknown function for nuclear RASSF8 in CRC. The effects of RASSF8 on cell proliferation, migration and invasion suggest that RASSF8 enhances the tumourigenesis and progression of CRC. We also provide evidence that RASSF8, as a regulator of the Wnt pathway, promoted CRC progression by facilitating β-catenin nuclear accumulation. The development of RASSF8-based therapeutic strategies may provide a novel therapeutic approach for CRC treatment.

TRAF6 promotes hepatocarcinogenesis by enhancing c-Myc gene expression and protein stability

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Liver cancer is one of the most common cancer in the world and the fourth leading cause of worldwide, hepatitis b virus (HBV) and hepatitis c virus (HCV) infection, and alcoholic liver disease are considered to be   major risk factors for HCC initialization and progress. Traditional treatments consist of surgery, radiotherapy, chemotherapy, commonly methods of traditional Chinese medicine, so far, considering the cancer of the liver in the clinical treatment of less related to . The molecular pathogenesis of hepatocellular carcinoma is extremely complex and heterogeneous. Therefore, it is of great significance to reveal the molecular mechanism in the occurrence and development of hepatocellular carcinoma for its treatment and prognosis. The tumor necrosis factor receptor-associated factor (TRAF6) is a member of the TRAF family;Previous studies have demonstrated that TRAF6 plays a key role in inflammation and immune processes;New research suggests that TRAF6 may also play an important role in cancer. Elevated TRAF6 expression has been detected in hepatocellular carcinoma, however, the pro-cancer mechanism of TRAF6 in hepatocellular carcinoma is still unclear, and there have been no relevant reports on TRAF6 in animal experiments during liver tumorigenesis.

C-myc is one of the most common oncogenes, and its genetic changes are found in more than 70% of human cancers, including hepatocellular carcinoma. For example, increased expression of c-myc can promote the growth of liver cells, promote the occurrence and development of hepatocellular carcinoma, and is closely related to its poor prognosis. In this study, we found that TRAF6 can significantly up-regulate the expression of c-myc during the development of liver cancer. In tissue samples from patients with clinical hepatocellular carcinoma, high expression of TRAF6 is associated with poor prognosis: compared with wild-type mice ($TRA6F^{+/+}$), TRAF6 heterozygote gene deletion mice ($TRA6F^{+/-}$) significantly affects the formation of liver tumors in mice. Further mechanism studies have found that: first, TRAF6 can interact with histone deacetylase 3 (HDA3) and promote its ubiquitination at K63, leading to the separation of HDA3 and c-myc promoter binding region and the acetylation at K9 of histone H3, thereby enhancing the expression of c-myc gene mRNA. Secondly, ubiquitination of HDA3 protein K63 weakens its interaction with c-myc and promotes the acetylation of c-myc protein, thereby enhancing the stability of c-myc protein. In clinical tissue samples, TRAF6 is positively correlated with the mRNA and protein levels of c-myc gene, and the high expression of TRAF6 and c-myc is closely related to poor prognosis, suggesting that TRAF6 and c-myc synergism promotes the occurrence of human liver cancer. Similarly, inhibition of c-myc expression

by inhibiting TRAF6 activity or silencing c-myc expression by siRNA significantly inhibited the formation of mouse tumors.

Our study shows that TRAF6 plays a pro-cancer role by regulating TRAF6/HDAC3/ c-myc signaling pathway during the occurrence of liver cancer, which is of great significance for the discovery of a new molecular targeted therapy for hepatocellular carcinoma.

Colitis cystica profunda with solitary Peutz-Jeghers polyps-associated dysplasia: case report and literature review

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Background: Colitis cystica profunda is an uncommon lesion, which appears cancerous and is defined by the presence of mucin‑filled cysts below the muscularis mucosa. While its etiology is not elucidated, unknown post-traumatic, infectious, and congenital factors have been considered responsible. **Case presentation:** We describe two patients with colitis cystica profunda with low grade dysplastic changes involving the small intestine, associated with solitary Peutz-Jeghers polyps on the colonic epithelium. The lesions occurred in the small intestine in both cases. One was a 48-year-old female while the other was a 66-year-old male patient. They were both treated with surgery and remained asymptomatic during follow-up. The extracted tissue was subjected to histological examination after hematoxylin and eosin staining, which revealed coexistence of colitis cystica profunda and a solitary Peutz-Jeghers-type polyp, with the epithelium showing low-grade dysplasia. The lesions did not express P53 and β-catenin on immunohistochemistry and were negative for both *KRAS* and *BRAF* mutations tested using an amplification refractory mutation system. We also reviewed recently published reports and summarized clinicopathological features of colitis cystica profunda. **Conclusions:** Colitis cystica profunda usually presents like a malignant tumor, particularly when atypical hyperplasia involves the enterocytes or when it occasionally relapses after surgical extraction. It can occur concurrently with inflammatory bowel disease and even colorectal cancer. A comprehensive review of the literature suggests a possible relation between colitis cystica profunda and inflammatory bowel disease or colorectal cancer. However, the reasons for this association and whether colitis cystica profunda is absolutely benign are unclear.

Category: Digestive pathology
1657264

Histological analysis of immune-mediated hepatitis

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Immune checkpoint inhibitors (ICIs) has been approved for treatment in many cancers because of their antitumor effects.   ICIs can cause mmune-mediated hepatitis (IMH).Inhibitors of multiple receptor tyrosine kinases (TKIs) have also been used in cancers. The rate of IMH have not been clarified in the combination treatment of ICIs and TKIs.
Methods. We collected 5 cases   treated with monotherapy ICIs, and another 5 cases were treated with ICIs combined with TKIs. We compared the histological features.
Results. Patients treated with   ICIs presented different levels of lobular hepatitis and portal inflammation. There also present cholangitis, endothelialitis, activation of Kupffer cell and peliosisihepatitis. The extent of IMH were much more severe in   the group treated with ICIs and TKIs . There often appeared moderate-severe portal inflammation, interface hepatitis, confluent or bridging necrosis. Among them, 1 patient   developed acute severe hepatitis with massive hepatocyte necrosis.
Conclusions. Combination of ICIs and TKIs may cause overlapping hepatocyte injury, which is much worse than the IMH caused by monotherapy ICIs.

Oesophageal squamous cell carcinoma or high-grade dysplasia overlying leiomyoma, rare but not to be neglected

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Background Oesophageal squamous cell carcinoma (ESCC) and leiomyoma are common tumours. The coexistence of these two tumours can be classified into two types: the overlying type and the separate type. The overlying type is rare.
Methods Here, we report 12 cases of the overlying type of coexisting ESCC and leiomyoma treated by endoscopic submucosal dissection (ESD). The 12 patients underwent pre-ESD endoscopic examination with white-light imaging, iodine staining, narrow-band imaging (NBI), endoscopic ultrasound (EUS), and biopsy. Haematoxylin and eosin (H&E)-stained slides of ESD specimens were reviewed by two experienced pathologists. The clinical, endoscopic and pathologic characteristics were reviewed.
Results Among the 12 patients, 3 were female and 9 were male, and the age range was 49-76 years. The 12 cases accounted for 4.0% of 300 cases of oesophageal leiomyoma and 0.9% of 1206 cases of superficial SCC and high-grade dysplasia treated by endoscopic resection. After endoscopic examination and biopsy, ESCC or its precursor lesion, high-grade dysplasia, combined with leiomyoma was considered in four cases; leiomyoma was considered but without the squamous cell lesion (underdiagnosis) in one case; and leiomyoma was mistaken for submucosal ESCC (overdiagnosis) in the other seven cases. Histologically, seven cases were intramucosal ESCC overlying leiomyoma originating from the muscularis mucosa, three cases were high-grade dysplasia overlying leiomyoma originating from the muscularis mucosa, and two cases were high-grade dysplasia overlying leiomyoma originating from the muscularis propria. The twelve cases were successfully treated by ESD, with no recurrence during follow-up.
Conclusions We must keep in mind that ESCC or high-grade dysplasia can occur overlying leiomyoma. These cases are rare but should not be neglected, especially in high-risk areas for ESCC or high-grade dysplasia. These patients can receive appropriate treatment if overdiagnosis or underdiagnosis can be avoided.

Expression profiling of metabolic rearrangement related genes pave the way for a better understanding of gastric cancer

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Backgroud: Metabolic rearrangement has been shown to be an important characteristic for stomach adenocarcinomas. Here we aimed to improve our understanding of the tumorigenesis of gastric cancer by means of gene and protein expression profile analysis with a focus on metabolic genes and pathways.
Design: Array-based gene expression profiling of fresh frozen cancer tissues and adjacent normal tissues were obtained from 8 patients with gastric cancer at early stage by using Affymetrix oligonucleotide microarray. Assays targeted 179 unique genes related to cancer metabolism. The raw expression data were normalized using nSolver Analysis Software 3.0 and a dataset of gene expression ratios for GC vs. controls was generated. The p values were calculated using a paired t-test, and the threshold for up- and down-regulated genes was set at p value < 0.05. Hierarchical clustering was performed based on differentially expressed gene using Multi Experiment Viewer (Dana-Farber Cancer Institute, MA). The protein expression of the dysregualted genes were detected in the THPA database (http://www.proteinatlas.org/).
Results: Our results showed increased expression of 20 metabolic genes (AKT2, SLC2A3, AKT2, MAP2K1, PRPS1, OAZ2, TPI1, HRAS, EGLN1, PGK1, HIF1A, ARNT, NUBP1, PLCG1, G6PD, SLC2A3, H6PD, PRPS1, PGAM1, NOX4, TPI1, HK2, SF3A3, HIF1A and GLS) and decreased expression of 6 metabolic genes (CC2D1B, RET, NTRK3, SUCLG2, EGLN3 and RPS6KB1) in all cases of gastric cancer at early stage. Besides of the undetected NOX4 (no available antibody), the protein levels of all the others 19 upregualted genes are higher in gastric cancer tissues than normal gastric mucous, while the protein levels of all 6 downregualted genes are lower in gastric cancer at early stage tissues. Half of all dysregulated genes (AKT2, EGLN3, G6PD, GLS, HIF1A, HK2, HRAS, MAP2K1, NTRK3, PGK1, PLCG1, RET and RPS6KB1) in our gastric cancer population are implicated in Carbon Metabolism, a pivotal metabolic approach involving in nucleic acid biosynthesis. Five of all dysregulated genes (ARNT, EGLN1, EGLN3, HIF1A and NOX4) in our gastric cancer population are molecular implicated in hypoxia signaling, which suggested that hypoxia is a pivotal the oncogenesis of gastric cancer. inducement for the oncogenesis and development of gastric cancer. Among these 26 altered genes, the upregulated HIF1A is a cancer metabolism driver, which means that HIF1A may induce the oncogenesis of gastric cancer.
Conclusions: The gastric mucosa in gastric cancer at early stage is characterized by dysregulated expression of a limited repertoire of metabolic genes. The nature of the corresponding metabolic rearrangement and pathways may help guide further investigations into its etiology.

Upregulation of OSBPL3 by HIF1A promotes colorectal cancer progression through activation of RAS signaling pathway

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Oxysterol-binding protein like protein 3 (OSBPL3) has been shown involving in the development of several human cancers. However, the relationship between OSBPL3 and colorectal cancer (CRC), particularly the role of OSBPL3 in the proliferation, invasion and metastasis of CRC remains unclear. In this study, we investigated the role of OSBPL3 in CRC and found that its expression was significantly higher in CRC tissues than that in normal tissues. In addition, high expression of OSBPL3 was closely related to poor differentiation, advanced TNM stage and poor prognosis of CRC. Further experiments showed that over-expression of OSBPL3 promoted the proliferation, invasion and metastasis of CRC cell. Moreover, we revealed that OSBPL3 promoted CRC progression through activation of RAS signaling pathway. Furthermore, we demonstrated that the expression of OSBPL3 could be regulated by hypoxia induced factor 1A (HIF1A). In conclusion, our study suggested that OSBPL3 not only plays an important role in the regulation of cell progression in CRC, but also may serve as a valuable clinical prognostic marker for this disease.

TRIB2 maintains the stemness of esophageal cancer stem cells by regulating SOX2

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Objectives: Cancer stem cells (CSCs) are identified as a sub-population of cancer cells with self-renewal and differentiation potential, which can generate heterogeneous cancer cells. CSCs are responsible for tumorigenesis, metastasis, drug resistance and recurrence. However, the characteristics, molecular mechanism and the role in carcinogenesis of esophageal cancer stem cells are still unclear. TRIB2 is an important serine/threonine pseudokinase of eukaryotic cells, which plays an important role in immune function, lipoprotein metabolism, cell differentiation and proliferation. This study aimed to reveal the isolation, gene expression of esophageal cancer stem cells and the role of TRIB2 in esophageal squamous cell carcinoma (ESCC).
Materials and Methods: Esophageal cancer stem cells were enriched by sphere formation assay. The stemness was verified by measuring the expression of stem cell markers through qRT-PCR and CD133 staining. Then, we analyzed the differentially expressed genes of esophageal cancer stem cells and esophageal cancer cells by RNA-seq, and found that TRIB2, with significantly differential expression, may be a potential regulator of the characteristics of esophageal cancer stem cells. The differential expression of TRIB2 in esophageal cancer stem cells and ESCC tissues was confirmed using qRT-PCR and bioanalysis. TRIB2 in ESCC cells was knockdown using lentiviral transfection. Then, the role of TRIB2 in self-renewal, proliferation, cell cycle and migration were respectively examined by sphere formation assay and subcutaneous xenograft model, CCK-8 and colony formation assay, PI staining and Transwell assay. Furthermore, the molecular mechanism of TRIB2 in ESCC cells was examined by qRT-PCR and Western blot.
Results: Stem cell markers were high expressed in cells enriched from sphere formation assay, indicating that these cells possessed stemness. TRIB2 was high expressed in ESCC cells and ESCC tissues, while the expression of TRIB2 in esophageal cancer stem cells was higher than in ESCC cells. TRIB2 knockdown significantly inhibited the self-renewal ability in vitro and in vivo, induced cell cycle arrested in G0/G1 phase, inhibited cell proliferation and migration in ESCC cells. Moreover, TRIB2 knockdown inhibited the expression of SOX2.
Conclusion: TRIB2 is an important gene regulating the stemness of esophageal cancer stem cells, and its effect may be achieved by regulating the expression of SOX2.

Category: Digestive pathology
1657457

Glypican-3: A New Target for Diagnosis and Treatment of Hepatocellular Carcinoma

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Liver cancer is the second leading cause of cancer-related deaths worldwide, andhepatocellular carcinomais the most common type. The pathogenesis of hepatocellular carcinoma is concealed, its progress is rapid, its prognosis is poor, and the mortality rate is high. Therefore, novel molecular targets for hepatocellular carcinomaearly diagnosis and development of targeted therapy are critically needed. Glypican-3, a cell-surface glycoproteins in which heparan sulfateglycosaminoglycan chains are covalently linked to a protein core, is overexpressed in HCC tissues but not in the healthy adult liver. Thus, Glypican-3 is becoming a promising candidate for liver cancerdiagnosis andimmunotherapy. Up to now, Glypican-3 has been a reliable immunohistochemical marker for hepatocellular carcinomadiagnosis, and solubleGlypican-3 in serum has becoming a promising marker for liquid biopsy. Moreover, various immunotherapies targeting Glypican-3 have been developed, including Glypican-3vaccines, anti- Glypican-3 immunotoxin and chimeric-antigen-receptor modified cells. In this review, we summarize and analyze the structure and physicochemical properties of Glypican-3 molecules, then review their biological functions and applications in clinical diagnosis, and explore the diagnosis and treatment strategies based on Glypican-3.

Progress in Animal Models of Pancreatic Ductal Adenocarcinoma

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As a common gastrointestinal tumor, the incidence of pancreatic cancer has been increasing in recent years. The disease shows multi-gene, multi-step complex evolution from occurrence to dissemination. Furthermore , pancreatic cancer has an insidious onset and an extremely poor prognosis, so it is difficult to obtain cinical specimens at different stages of the disease, and it is, therefore, difficult to observe tumorigenesis and tumor development in patients with pancreatic cancer. At present, no standard protocols stipulate clinical treatment of pancreatic cancer, and the benefit rate of new targeted therapies is low. For this reason, a well-established preclinical model of pancreatic cancer must be established to allow further exploration of the occurrence, development, invasion, and metastasis mechanism of pancreatic cancer, as well as to facilitate research into new therapeutic targets. A large number of animal models of pancreatic cancer are currently available, including a cancer cell line-based xenograft, a patient-derived xenograft, several mouse models (including transgenic mice), and organoid models. These models have their own characteristics, but they still cannot perfectly predict the clinical outcome of the new treatment. In this paper, we present the distinctive features of the currently popular pancreatic cancer models, and discuss their preparation methods, clinical relations, scientific purposes and limitations.

E2F1-activated SPIN1, a potential therapy target, promotes tumor growth via MDM2-p21-E2F1 feedback loop in gastric cancer

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Objectives: To clarify the role of SPIN1 in the development of gastric cancer (GC), investigate the mechanism of high expression of SPIN1 and explore downstream signaling pathway regulated by SPIN1.

Materials and Methods: 1. Immunohistochemistry was used to detect SPIN1 expression in GC and analyze the correlation between SPIN1 and clinicopathological parameters. 2. Cell migration and invasion assays, MTS, EdU, colony formation assay and flow cytometry analysis were performed to test the effects of SPIN1 on cell biological behaviors. 3. Identify the proximal promoter region and transcriptional factors. 4. Genome-wide expression profiling microarray was used to make signaling pathway analysis. 5. Tumor xenografts model was conducted and ShRNA-SPIN1 recombinant lentiviral vector was treated *in vivo.*

Results: 1. SPIN1 was upregulated in GC tissues, increased expression of SPIN1 was closely associated with poorer prognosis. 2. SPIN1 enhanced GC cells migration, invasion, proliferation and promoted cell-cycle progression *in vitro*. 3. E2F1 could bind directly to the SPIN1 promoter region and activate its transcription. 4. SPIN1 promotes GC cell proliferation via activating the MDM2-p21-E2F1 signaling pathway through binding with the H3K4ME3 of MDM2 promoter region, establishing a positive feedback loop. 5. Lentiviral packaged ShRNA-SPIN1 inhibited GC tumor growth.

Conclusion: SPIN1 plays an important role in the development of GC and may serve as a potential therapeutic target for the treatment of GC.

Clinicopathological significance of PD-L1 expression in classification of poorly differentiated gastric adenocarcinoma

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Objectives The aim of the present study was to evaluate the expression pattern of PD-L1 defined by clone SP142 antibody in poorly differentiated gastric adenocarcinoma (PDGA) and investigate the clinical significance of SP142-PD-L1 expression in tumor classification. **Materials and Methods** Formalin-fixed and paraffin-embedded tumor tissues from 163 PDGA patients treated with surgically resection were included in the study, which were analyzed for PD-L1 expression in tumor cells (TCs) and tumor associated immune cells (ICs) by immunohistochemistry with the use of SP142 antibody. Tumor infiltrating lymphocytes (TILs) were evaluated by morphological and immunohistochemical detection. PDGA was sub-classified into various subsets based on PD-L1 expression or PD-L1 expression in combination with characteristics of TILs. **Result** PD-L1 expression was observed in TCs as well as ICs of PDGA, with positive rate of 36.81% and 99.39%, respectively. High expression of PD-L1 in TCs was closely correlated with histological subtype of tumor, status of lymphovascular and perineural involvement. High expression of PD-L1 in ICs was significantly associated with several clinicopathological characteristics, including tumor size, histological subtype, pTNM stage, status of lymphovascular and perineural involvement, tumor budding as well as CD3+ TILs. PDGA could be classified into four subsets defined by either PD-L1 expression or PD-L1 expression in combination with CD3+ TILs. There have significant associations of different PDGA subsets with different clinicopathological characteristics respectively. **Conclusion** The clinicopathological significance of PD-L1 expression in TC is different from IC in PDGA. The classification of PDGA according to PD-L1 expression as well as the features of TILs might provide additional information regarding prognosis of PDGA.

Category: Digestive pathology
1657569

Aberrant Infiltrition Pattern of Macrophages and Dendritic Cell Subsets in Ulcerative Colitis

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Objective Ulcerative colitis(UC) is an important type of inflammatory bowel disease(IBD) worldwide. Although anti-TNF treatment are well effective in more than 70% of patients, this leaves one third of patients with limited therapeutic options. Rremoval of peripheral monocytes and dendritic cells(DCs) by leukocyte adsorption apheresis may provide the beneficial effects in patients with UC. However,the infiltration pattern of the reactive macrophages and dendritic cell subsets in UC by immunohistochemistry are still not clearly described. The present study was to evaluate the relationship between macrophages and dendritic cell subsets infiltration and the severity of acute and chronic inflammations in UC and provide the potential evidence for the therapeutic strategy of UC. **Methods** Formalin-fixed and paraffin-embedded tissue blocks provided by department of pathology, general hospital,Ningxia Medical University.The biopsy tissue specimens were obtained from the patients with UC during colonic endoscopy(n=14).Resected colonic tissues were obtained during operations on patients with UC(n=5). Non-cancerous colonic mucosa specimens were as control. The surgical and biopsy specimens were fixed and embedded in paraffin, and cut into 4-μm-thick sections for immunohistochemistry.Primary antibodies were D68(macrophage),DEC205(DCs),CD21and CD23(FDC), CD123(pDCs), respectively. The immunostaining was indepen- dently evaluated by two pathologists.**Results** The ratio of male to female patients was 2.8：1.The average age was 49.1years old(from 34 to 83). Numerous DEC205+ DCs were scattered beside the lymphocytes and plasma cells,and the number of the CD68+ macrophages were less than that of the DEC205+ DCs. Numerous CD68+ macrophages were clustered in the crypt abscesses of a case with active inflammation of UC. In mucosal lymphoid follicle,numerous CD21 and CD23+ FDCs were scattered in the germinal center showing a dense meshwork and CD123+ pDCs were mainly scattered in paracortex. In the surface mucosa and mucosal lamina propria, CD21+ or CD23+ cells(FDCs) and CD123+ pDCs were scattered in the area.There was a significantly higher for the number of CD68+ macrophages in crypt abscesses of UC compaired with that of the lymphoid follicle and chronic inflammation(p<0.001). DEC205+ DCs were significantly lower in crypt abscesses of UC than that of the lymphoid follicle and chronic inflammation(p<0.001). The number of the DEC205+ DCs were significantly lower in control tissues than that of the patiences with UC(p<0.05, p<0.001 and p<0.001,respectively).In crypt abscesses of UC,the number of macrophages was significantly higher than DCs(p<0.001).In both lymphoid follicle and chronic inflammation of the patients with UC, ,the number of DCs were significantly higher than macrophages(p<0.001 and p<0.05). There was a significantly higher for the number of CD21,CD23 and CD123+ cells in lymphoid follicle than that of the crypt abscesses and

chronic inflammation of UC(p<0.001).**Conclusion** Abberant elevation of reactive macrophages and DCs in the the mucosal lamina propria and lymphoid follicle of the patients with UC..Elevation of DCs in the lymphoid follicle and chronic inflammation tissues of the patients with UC.Our present study provided the hard evidence for the treatment of UC by monocytes and dendritic cells adsorption apheresis. The present study indicated that DCs may play more important role in the chronic inflammation of the UC than that of the macrophages.

Relationship between Epstein-Barr virus infection and clinicopathological features of gastric cancer

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Objective To investigate the relationship between Epstein-Barr virus (EBV) infection and clinicopathological features of gastric cancer. **Methods** From March 2016 to May 2019, 397 paraffin-embedded specimens of gastric cancer underwent radical resection in Anhui Cancer Hospital were collected. The expression of EBER was detected by automatic in situ hybridization (AISH),corresponding analysis was made according to the clinical and pathological features. **Results** EBER was localized in the nucleus, showing blue signal and diffuse or scattered dot staining, which was in sharp contrast to the background of red contrast staining. EBER was detected in 21 of 397 cases of gastric cancer, the positive rate was 5.3%. In the analysis of clinical and pathological features,there was significant difference in EBER expression between different tumor sizes(x^2=5.450,p<0.05). The positive rate in the smaller group was higher than that in the larger group.However, there was no significant difference in the positive rate of EBER between different gender, age, TNM stage, lymph node metastasis and tumor location(p>0.05). Only 2 cases of lymphoepithelioid carcinoma were collected and both of them were EBER positive. There was no significant difference in the positive rate of EBER among the histological subtypes because of the small number of lymphatic epithelioid carcinomas collected(p>0.05). Even in different tumor subtypes, EBER is almost expressed in intestinal type glandular epithelium. Except in the tumor tissue region, EBER is also found in dysplasia adjacent to the tumor, and the stroma is often accompanied by a large number of lymphocyte infiltration. **Conclusion** The occurrence and development of gastric cancer may be related to EBV infection. The pathogenesis of EBV in gastric cancer should be further explored.

Immune milieu in the microvascular invasive lesion and primary tumor in HBV-related hepatocellular carcinoma

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Metastasis and recurrence of HBV-related hepatocellular carcinoma (HCC) are the major cause of cancer-related death in patients. The immune milieu in tumor regulates multiple behaviors of tumor cells, whereas the interactions of tumor cells and the immune milieu in HBV-related HCC are challenging to analyze because of HBV complication. However, the interaction of tumor cells and the immune milieu are a critical indicator of the efficiency of immunotherapy or outcome of patients. Here, we analyzed the immune milieu, in both microvascular invasive (MVI) lesion and primary tumor in HCC patients with whole slide immunostaining and tested the correlation of immune milieu by a general synthesis of all clinical-pathological parameters. Our data showed that MVI predicts a poorer prognosis and earlier relapse of HBV-related HCC patients, while higher expression of CD3+ and CD8 + cells infiltrating in MVI lesions predict a later relapse and a better survival. We also found that PD-1 + cell infiltrates in the reactive stroma and noncancerous liver highly associated with an earlier relapse of HCC after surgery. Contrary to PD-L1 negative tumors, PD-L1+ tumor cells exerted PD-1+ immune cells accumulating in the surrounding regions. Collectively, these data indicate that CD3+ and CD8+ cells might have attenuated the invasion and metastasis of HCC, and the liver within enriched PD-1+ immune cell population might be easy to create an immunosuppressive milieu that favour tumor invasion or metastasis; tumor cells in primary HCC tumor might express PD-L1 to escape immune cells surveillance. These observations support the rationale of immune-based therapy for selected HBV-related HCC patients.

Role of biopsy in the diagnosis, management and prognotification of liver tumor

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Liver biopsy plays very important role in the diagnosis and prognosis of HCC, but with technical advancements and progression in the field of imaging, clinical guidelines have restricted the role of biopsy to very limited situations. Biopsy also has its own problems of needle tract seeding of tumor, small risk of complications, technical and sampling errors along with interpretative errors. Despite this, tissue analysis is often required because imaging is not always specific, limited expertise and lack of advanced imaging in many centers and limitations of imaging in the diagnosis of small, mixed and other variant forms of liver cancer. In addition, biopsy confirmation is often required for clinical trials of new drugs and targeted therapies. Tissue biomarkers along with certain morphological features, phenotypes and immune-phenotypes that serve as important prognostic and outcome predictors and as decisive factors for therapy decisions, add to the continuing role of histopathology. Advancements in cancer biology and development of molecular classification of liver cancer with clinic pathological correlation, lead to discovery of liver tumor phenotypic surrogates of prognostic and therapeutically significant molecular signatures. Thus tissue characteristics and morphology based correlates of molecular subtypes provide invaluable information for management and prognosis. This review thus focuses on the importance of histopathology and resurgence of role of biopsy in the diagnosis, management and prognostication of HCC.

The expression and clinical significance of 5-hmC and TETs in hepatocellular carcinoma

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Objective   To investigate the levels of 5-hydroxymethylcytosine (5-hmC) and ten-eleven-translocation proteins (TET1/2/3) in diagnosis and prognosis prediction of hepatocellular carcinoma (HCC). **Methods**   The expression of 5-hmC in 130 cases of HCC tissues were detected by immunohistochemical staining. Kaplan-Meier test was used for survival analysis. TET family plays critical role in the conversion of 5-methylcytosine (5-mC) to 5-hmC. The TET levels were detected by using immunohistochemical staining and RT-PCR, the correlation between 5-hmC and TET was analyzed. **Results**   The level of 5-hmC decreased in HCC tissues, as compared with non-tumor tissues, the expression of TET1 was downregulated in HCC. There was significant difference in the expression between low and high grades of HCC tissues ($x2=10.611$, $P=0.001$). Kaplan-Meier survival analysis showed that there was significant difference between the 5-hmC expression level and the survival rate of HCC patients ($x2=4.412$, $P=0.036$). **Conclusions** In HCC tissues the expression of 5-hmC was specifically downregulated. Low 5-hmC level is significantly correlated with poor differentiation of the tumor and worse overall survival. Decreased expression of TET1 is likely one of the mechanisms underlying 5-hmC loss in HCC.

Significant role and research progress of biomarkers in gastric cancer

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Abstract

Gastric cancer is one of the most common gastrointestinal tumors, both the incidences and mortality rates are higher in men than women. Various studies have revealed that gastric cancer is a spectrum of tumors, which has biological and genetic diversity. It has proven difficult to improve the overall survival and disease-free survival of patients t by performing traditional surgery and chemoradiation, as gastric cancers are usually identified at an advanced stage. In consequence, the outcome is frequently poor. Thus, novel biomarkers and anticancer targets are required to improve the outcome. As the identification of biomarkers has increased due to advances and availability of bioinformatics and functional genomics, the potential therapeutic regimens available has also increased concurrently. These advances have also improved the ability to predict responses to chemotherapy, targeted therapy and immunotherapy, meanwhile, others predict the post-treatment survival and recurrence based on the expression of various markers. This review pays close attention to the significant role of biomarkers in the timely diagnosis and treatment of gastric cancer, as well as the advances in the study of some new markers in gastric cancer.

Category: Digestive pathology
1658147

Genetic analysis using next-generation sequencing in 45 patients with suspected Lynch syndrome

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Objective To detect gene mutations in patients with suspected Lynch syndrome (LS) using next-generation sequencing (NGS). **Methods** 45 cases of suspected LS patients with colorectal cancer (34 cases), gastric cancer (6 cases) or endometrial cancer (5 cases) were selected to extract genomic DNA from paraffin tissue samples without cancer. Gene variants for 12 genes were analyzed using NGS and bioinformatics. And then Sanger sequencing was performed to verify the gene variants detected by NGS. **Results** Among 28 cases of colorectal cancer, there were 4 cases of pathogenic *MLH1* mutation, 1 case of suspected *MLH1* pathogenic mutation, 2 cases of pathogenic *MSH2* mutation and 2 cases of suspected *MSH2* pathogenic mutation. Mutation of mismatch repair (MMR) gene was not found in 6 cases of gastric cancer. In 5 cases of endometrial cancer, 2 cases had pathogenic *MLH1* or *MSH2* mutations, respectively. 1 case had suspected *MLH1* mutations. Meanwhile, NGS also detected many gene mutation sites which had not been reported yet. Pathogenic and suspected pathogenic *MLH1* and *MSH2* mutations were verified by Sanger sequencing. The results detected using both sequencing methods were consistent.
Conclusion High-throughput NGS is a quick, accurate and reliable technique to identify gene variants in suspected LS patients. It has a wide application prospect for detecting gene aberrations in tumors, such as colorectal cancer.

Expression and prognosis of PD-L1 in pancreatic neuroendocrine tumors

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Research Background

Pancreatic neuroendocrine neoplasms (pNENs) are derived from pancreatic ducts and acinar pluripotent stem cells. They are a general term for a large number of heterogeneous tumors with diverse clinical features and large differences in prognosis. Both pNENs have malignant potential and have a series of biological behaviors from inert slow growth, low malignancy, and high invasiveness. In histopathology, pancreatic neuroendocrine tumors are based on the WHO (2010) grading system. Divided into three levels of G1, G2 and G3. In terms of treatment, the formulation of the program needs to be based on accurate tumor staging. Surgical resection is the only curable method, but many patients have already discovered metastasis when pNENs have been used. Therefore, in addition to traditional surgery, radiotherapy and chemotherapy, some new Therapeutic targets and therapeutic strategies, including targeted therapies and immunotherapy, are gaining increasing attention.

Programmed death molecule 1 / programmed death molecule 1 ligand pathway is an important pathway of immune regulation, the immune system can be used as a tumor initiation factor to promote tumor cell transformation, tumor growth and tumor cell antigen formation, on the other hand As a foreign tumor suppressor to prevent the development and infiltration of tumors, the immune system affects the development of tumors through the dual effects on tumor cells.

The expression of PD-L1 can be used as a predictor of clinical anti-PD-1/PD-L1 response. There is evidence that anti-PD-1/PD-L1 treatment is effective in many malignant tumors. of.

In this study, PD-L1 was detected in pancreatic neuroendocrine tumors, and its differential expression in various grades and its relationship with prognosis were compared, which provided a basis for immunotherapy of pulmonary neuroendocrine tumors.

Materials and Method

1. Research object

A total of 120 patients with neuroendocrine tumors of the pancreas who underwent pancreatic tumor surgery and confirmed by pathology from January 2011 to December 2017 in Zhongshan Hospital of Fudan University were collected. The clinical features of pancreatic neuroendocrine tumors were retrospectively analyzed. On the basis of this, the tissue of each case was selected to embed the wax block, and the tumor area and the non-tumor area (normal lung control tissue) were confirmed by microscopy in each case, and immunohistochemical staining was performed.

2. Research methods

On the basis of the complete tissue chip, the tissue chip was subjected to immunohistochemical staining of PD-L1 (SP142). PD-L1 stained the cell membrane of the tumor tissue as a positive staining site, and the membrane positive range was ≥5% positive.

3. Statistical methods

Statistics were performed using SPSS 23.0 statistical software.

Result

1. The expression of PD-L1 in pancreatic neuroendocrine tumors is correlated with pathological grade.

2. The effect of PD-L1 expression on the survival rate of pancreatic neuroendocrine tumors. The expression of PD-L1 was significantly correlated with G2 overall survival (P=0.019).

Conclusion:

1. The positive expression of PD-L1 was significantly correlated with pathological grade, and the expression in low-grade (G1+G2) pancreatic neuroendocrine tumors was significantly higher than that of high-grade pancreatic neuroendocrine tumors.

2. In patients with pancreatic neuroendocrine tumor G2, patients with PD-L1 negative expression had significantly longer progression-free survival (DFS) than those with PD-L1 positive expression (P=0.019).

Up-regulation of LINC00467 promotes the tumourigenesis in colorectal cancer

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Abstract：
Objectives: Recent studies have reported that long non-coding RNAs (lncRNAs) are associated with the tumourigenesis of colorectal cancer (CRC); however, several of these are yet to be identified and characterised. In this study, we report a novel lncRNA, LINC00467, which was significantly up-regulated in CRC; we investigated its function and mechanism in CRC.
Material and Methods: Our study demonstrated that LINC00467 levels in 45 pairs of CRC tissues were higher than those in the corresponding normal colon mucosal tissues. We used the Gene Expression Omnibus (GEO) and Gene Expression Profiling Interactive Analysis (GEPIA) databases for the analysis and measurement of clinical samples, and following the short interfering RNA (siRNA) knockdown of LINC00467 in the CRC cell line.
Results: The results shown that the CRC patients with high LINC00467 expression levels showed poor overall survival (OS) and recurrent-free survival (RFS) rates. Furthermore, LINC00467 suppresses the proliferation, invasion and metastasis of CRC cells in vitro. Moreover, its molecular mechanism of LINC00467 decreased the expression of Cyclin D1, Cyclin A1, CDK2, CDK4 and Twist1 as well as enhanced the expression of E-cadherin.
Conclusion: Collectively, these findings suggest that LINC00467 may be crucial in the progression and development of CRC, and may serve as a potential therapeutic target for CRC patients.

Expression of miR-320a, RAD51 and XRCC4 in esophageal squamous cell carcinoma

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Abstract OBJECTIVE: By examining the expression of miRNA-320a in esophageal squamous carcinoma cells and the expression of RAD51 and XRCC4 proteins in esophageal squamous carcinoma cells and tissues, the present study elucidated effects of mirna-320a, RAD51 and XRCC on the occurrence and development of esophageal squamous cell carcinoma. **METHODS:** qRT-PCR was used to detect the expression level of miRNA-320a in esophageal cancer cell line TE-1 cells, ECA-109 cells and human esophageal epithelial cell line Heep cells; target genes for miRNA-320a are predicted by using the softwares (TargetScan, miRecord and Pictar bioinformatics online sites); Immunocytochemistry was used to detect the expression of RAD51 and XRCC4 proteins in esophageal cancer cell line TE-1 cells and normal esophageal epithelial cell line HEEp and to detect the expression of RAD51 and XRCC4 proteins in esophageal squamous cell carcinoma. **RESULTS:** The results of QRT-PCR showed that miRNA-320a was up-regulated in the esophageal cancer cell lines TE-1 and CEA109 compared with the normal esophageal epithelial cell line, and the difference was statistically significant (p<0.05). The predicted target genes of miR-320a include RAD51 and XRCC4. The results of immunocytochemistry showed that RAD51 and XRCC4 proteins were positively expressed in esophageal cancer cell line TE-1 cells, while negative expression was observed in normal esophageal epithelial cell line HEEp cells. The results of immunohistochemistry showed that RAD51 and XRCC4 proteins were up-regulated in esophageal squamous cell carcinoma, and the difference was statistically significant compared with adjacent tissues and matched metastatic lymph nodes (p<0.05). The positive rate of RAD51 protein expression in women was higher than that in men (P <0.05). In other clinicopathological features (such as tumor diameter, depth of invasion, degree of differentiation, P-TNM staging), the positive expression rates of the two were not statistically significant (P>0.05). **Conclusion:** miRNA-320a, RAD51 and XRCC4 are highly expressed in esophageal squamous cell carcinoma, suggesting that Mir-320a may be involved in RAD51 and XRCC4 involved in DNA double-strand break (DBS), thus participating in the development of esophageal squamous cell carcinoma. However, the specific mechanism of action remains to be further studied.
Key words: Esophageal squamous cell carcinomas; miR-320a; RAD51; XRCC4

Neoadjuvant Therapy does not Alter the Microsatellite Status of Gastric Cancer but Reduce the MSH6 Expression

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Background: With the approval of anti-PD-1 antibody in the dMMR/MSI advanced gastric cancer (GC), evaluation of the MMR/MSI status of GC become crucial. Neoadjuvant chemoradiation therapy was reported to induce the heterogeneous staining of MMR in colorectal carcinoma, especially loss of MSH6. Thus, evaluating the effect of neoadjuvant therapy with MMR expression provide important clinical value to GC treatment.

Materials and Methods: We studied the immunohistochemical expression of MLH1, PMS2, MSH2, MSH6, and Ki67 in gastric cancer with (n=29) or without (n=35) preoperative neoadjuvant therapy.

Results: Two of the 29 GC showed deficient mismatch repair (dMMR) both in biopsy before neoadjuvant therapy and radical specimen. One was MSH2-MSH6-, and the other was MLH1-PMS2-. Twenty-seven cases possess MSS before and after neoadjuvant therapy, while 4 of them (14.8%) demonstrated reduced MSH6 expression to varying degrees, with the loss ranging from 20% to 90% of the tumor cells. And the Ki67 showed the same degree of reduction. The control group without neoadjuvant therapy barely showed the reduction of MSH6 expression.

Conclusions: Neoadjuvant therapy do not alter the MMR expression of GC but may reduce the stain of MSH6. In a sense, biopsy before the neoadjuvant therapy may provide more accurate information for immunotherapy.

Challenging Cases in Surgical Pathology: Bone Tumors

Prof. Yoshinao Oda
Kyushu University

In this session, I will demonstrate two cases of peculiar bone tumors with long clinical course and discuss their clinicopathological and molecular differential diagnoses.
Case 1: 26 years old male presented with 3 months history of redness and a gradually growing mass of his right hip joint. When he was 12 years old boy, he received curettage and bone graft for iliac bone cyst. The 1st and 2nd recurrences occurred at 18 and 21 years old, respectively. Histologically, the initial lesion shows thin fibrous cyst wall, which is mainly composed of fibroblastic spindle cells with aggregates of multinucleated giant cells. Adjacent to fibrous cyst wall, thick fibrous component made up of blunt spindle cells and abundant collagen fibers, mimicking the features of desmoid tumor, is recognized. The 2nd recurrent lesion shows increased cellularity of atypical spindle cells with enlarged hyperchromatic and irregular nuclei. The currently recurrent tumor reveals cellular proliferation of atypical spindle cells with neoplastic tumor osteoid and frequent mitotic figures. Molecular genetic analysis demonstrates MDM2 gene amplification by FISH. Our final diagnosis of this case is dedifferentiated low-grade central osteosarcoma with extensive cystic change.
Case 2: A 16 year old girl complained 3 months history of left knee pain. Radiographic examination revealed osteolytic lesions in proximal and distal tibia. After open biopsy, en bloc resection for proximal tibia and surgical curettage for distal tibia were performed. When she was 32 years old, 16 years later, osteolytic lesion was pointed out in the left acetabulum and surgical curettage was carried out. 26 years after en bloc resection for primary site at knee, the local recurrence occurred, which was also treated by surgical curettage. Initial lesion of proximal tibia shows sharply demarcated cartilaginous tissue and giant cell containing lesion at the periphery. Cartilaginous tissue shows no cellular atypia. Giant cell lesion is made up of uniformly distributed osteoclast-like giant cells with intermingling oval stromal cells. No IDH1/2 mutations were detected in either cartilaginous or giant cell lesions. At the distal tibia, the lesion is composed of osteoclast-like giant cells and stromal cells with reactive osteoid. Partially, florid fibrohistiocytic proliferation with scanty giant cells is recognized. Acetabular lesion shows a proliferation of mononuclear cells admixed with mumerous osteoclast-like multinucleated giant cells, which is compatible with the feature of typical giant cell tumor of bone. The recurrent lesion at the knee reveals mononuclear stromal cells and intermingling multinucleated giant cells, accompanied by secondary aneurysmal bone cyst. The primary proximal tibia and distal tibia lesions, acetabular lesion, and recurrent knee lesion were positive for G34W antibody in stromal cells. Our final diagnosis of this case is multicentric giant cell tumors with extensive cartilaginous metaplasia.

Category: Osteopathology
1629142

Pathological spectrum of denosumab-treated giant cell tumor of bone: study of 13 cases

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Objectives: Giant cell tumor of bone (GCTB) is an osteolytic, locally aggressive, rarely metastasizing primary bone neoplasm. It is characterized by an abundance of osteoclastic giant cells that are induced by the neoplastic mononuclear cells expressing high levels of RANK ligand which is a mediator of osteoclast activation.   Denosumab,   a human monoclonal antibody inhibiting the action of RANKL, has been used to treat selected cases of GCTB.   We herein evaluate the histopathological features of denosumab-treated GCTB in 13 cases.

Materials and Methods: Thirteen biopsy-confirmed patients with GCTB, who underwent curettage after neoadjuvant denosumab therapy, were collected. Clinicopathological data were reviewed. Immunohistochemistry for histone H3.3 G34W (G34W) was performed in all cases.   H3F3A mutation status was verified by direct Sanger sequencing in one case.

Results: There were 9 females and 4 males with age ranging from 16 to 54 years (median, 27 years). The histopathological features of the post-denosumab-therapy GCTB included marked depletion of giant cells, different degrees of ossification, fibrosis, and proliferation of mononuclear cells. With these changes, denosumab-treated GCTB may mimic other lesions such as fibrous dysplasia, juvenile ossifying fibroma, non-ossifying fibroma, and osteoblastoma. A less frequent but more relevant finding is the presence of cellular atypia and/or patterns of ossification that resemble a conventional osteosarcoma. One of the cases was initially misdiagnosed as osteosarcoma. Further testing for H3F3A mutation by direct sequencing was positive and immunohistochemical staining for G34W was positive in both of the pre- and post-therapy specimens, confirming the diagnosis of GCTB . In all cases, post-therapy tumors still maintain abundant G34W positive cells.

Conclusion: Post-denosumab-therapy GCTB can have a spectrum of morphologic appearances. The treated tumors showing marked giant cells depletion and massive intralesional bone deposition may share little resemblance with their pre-treatment counterpart.   Special attention needs to be paid to the clinical history of denosumab administration to avoid a misdiagnosis.

Extrskeletal mesenchymal chondrosarcoma : a clinicopathological analysis of three cases and review of the literature

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Abstract: Objective: To explore the clinicopathological and immunohistochemical features, diagnosis and differential diagnosis of extrskeletal mesenchymal chondrosarcoma (EMC). **Methods**: The clinicopathological information of three EMC specimens including demographics, clinical information, relevant images, surgical treatment, the follow-up and representative pathological data of tumors were available for analysis. In addition, the immmohistochemical staining related to the tumor as well as HEY1-NCOA2 fusion transcript were analysed. **Results**: The group consisted of 1 female and 2 male patients with age as 45, 49 and 23 years and the tumors located at mid and upper pole of the right kidney, left arm triceps and the right frontal-parietal lobes, respectively. Symptoms including swelling, pain and compression were noticed. Radiologically, the lesion revealed soft tissue masses with ''ring and arc calcifications''. The tumor was cytologically featured as small cells with round shape and bare nuclei. Histologically, the lesions were typically characterized by biphasic pattern. It is noted that the well differentiated cartilage was alternated with stromal component, comprising small sized, oval to spindle shaped cells with moderate nuclear pleomorphism, hyperchromatic and inconspicuous nuclei, and moderate cytoplasm. The small spindle shaped cells were arranged as a hemangiopericytoma like pattern. Large islands of mature cartilage with foci of calcification were observed. These two components were juxtaposed sharply to each other or blended gradually. Immunohistochemically, tumor cells were strongly positive for Sox9, CD99, vimentin, and Bcl-2 , the cartilage islets were positive for S-100 and D2-40. CD34 expression was only observed in the vessel wall. STAT6 and Desmin were negative. Notably, HEY1-NCOA2 fusion transcript was detected in all cases of mesenchymal chondrosarcoma. After 18 to 36 months of follow-up, there was no evidence of local recurrence, only the lung nodules were founded in the male patient with left arm triceps mass, which were suspected to be metastatic lesions. Conclusions: EMC is a rare malignant neoplasm. The correct diagnosis mainly relies on the typical histopathological features and immunohistochemistry. The identification of HEY1-NCOA2 fusion transcript could be used as an auxiliary diagnostic marker.

Bone case #2 in Japan-IAP Special "Topic: Challenging Cases in Surgical Pathology"

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The patient was a 7th decade lady with a consistent pain of her calf. MRI revealed a STIR-low and T1-low mass in the sacrum. Chordoma was suspected and a bone needle biopsy was carried out. Histologically hematopoietic cells in the marrow were hypocellular but normal-looking population and no significant blastic proliferation. In the marrow, scattered foam cell accumulations were found and the nuclei of the foam cells were small and round without any atypia. Histiocytic lesion was suspected and immunohistochemistry was performed. Unexpectedly, histiocytic markers, CD68 and CD163 were negative, but S100 was positive, and CD1a was negative.

Problem; What the diagnosis?

Category: Osteopathology
1656545

Coexistence of lung carcinoma metastasis and enchondroma in the femur of a patient with Ollier's disease: a case report and review of literature

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Objectives: Tumor-to-tumor metastasis is a rare occurrence, and has been described as tumor metastasis into another histologically different tumor. It is extremely rare in the bone. We report a case of lung squamous cell carcinoma metastasized to an enchondroma in the femur of a patient with Ollier's disease.

Materials and Methods: We analyzed the clinical, radiological, and histological features of the case and did a literature review.

Results: A 61-year-old female had a history of a poorly differentiated squamous cell carcinoma of the lung. She underwent a video-assisted thoracoscopic lobectomy and the follow-up MRI scan showed tumors in the left distal femur and the left proximal tibia which were radiologically interpreted as metastasis. The tumor resection biopsy was performed. The tumor from the left proximal tibia was consistent with enchondroma without metastatic component. Resection from the left distal femur revealed metastatic poorly differentiated carcinoma with foci of hyaline cartilage, which was most consistent with a metastatic carcinoma in a preexisting enchondroma. MRI film was re-reviewed, and the lesion in the left proximal tibia was a typical benign cartilage tumor, while the multiple lesions in the left distal femur were consistent with malignant tumor with extensive edema in surrounding soft tissue and some cartilage component in the tumor, which confirmed the presence of a tumor-to-tumor metastasis. The final diagnosis was metastatic poorly differentiated carcinoma of the lung into a co-existent enchondroma.

Conclusion: The diagnosis of metastatic carcinoma to a primary bone tumor can be challenging and can be easily overlooked both radiologically and histologically. Additional clinical and radiological information is important for the diagnosis and awareness of the tumor-to-tumor metastasis phenomenon can avoid an inaccurate diagnosis by pathologist, therefore preventing inappropriate clinical intervention.

Cortistatin antagonizes intervertebral disc degeneration by modulating the NF-κB and Wnt/β-catenin signalling pathways

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Objective: To investigate the potential role of cortistatin (CST) in intervertebral disc degeneration (IDD) .

Method: CST expression levels in the degenerative group and control group were assessed and compared in human and mice IVD tissue. CST knockout ($CST^{-/-}$) mice were established, and the ageing phenotype of IVD was compared between WT and $CST^{-/-}$ mice. The murine IVD tissues were isolated for *ex vivo* analysis to validate the role of CST in IL-1β-induced IVD degeneration. Human NP cells were isolated and cultured with IL-1β or CST treatment. Lithium chloride (LiCl) and SN50 were used for *ex vivo* and *in vitro* experiments to explore the underlying mechanisms of CST in IDD.

Results: The CST expression level was reduced during aging process. Furthermore, exaggerated degeneration was evident in the IVD tissues of the $CST^{-/-}$ mice. Moreover, exogenous CST inhibited the IL-1β induction of inflammation, catabolism and apoptosis *ex vivo* and *in vitro*. Additionally, the protective role of CST in IDD might be associated with the NF-κB and Wnt/β-catenin pathways.

Conclusion: This study suggests a protective role and demonstrates the underlying mechanism of CST in IDD.

Desmoplastic Trichilemmoma of the Vulva Region Mimicking Invasive Carcinoma

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Objectives: The trichilemmoma is a benign solid tumor that originates from external sheath cells of pilosebaceous follicles and presents as a lobular formation like growth of glycogen rich clear cells with palisading. Desmoplastic trichilemmoma is a histologically rare benign variant, which can mimic an invasive carcinoma. The initial lesion of desmoplastic trichilemmoma can be misleading clinically and confuse with other cutaneous lesions, such as squamous cell carcinoma, basal-cell carcinoma and viral lesions.
Materials and Methods: We report a case of 74-year-old female who presented with a 0.4 cm x 0.4 cm nodular lesion in the region above clitoris for 3 months, with progressive increase in size. The lesion was clinically suggestive of a vulva nodule. A biopsy was performed. Histology, immunochemistry and special stain were used to diagnose.
Result: It revealed a well-demarcated circumscription and characteristic tumor cell population through histology. At the periphery, typical lobules of conventional trichilemmoma were displayed. Several "cystic structures" resembling cross-sections of the infundibular portion of hair follicles were also present. Towards the center, however, narrow irregular cords of epithelialcells from hair roots were distributed in a desmoplastic stroma. This eosinophilic hypocellular stroma contained Alcian blue-positive material. Immunohistochemistry was consistent with a diagnosis of desmoplastic trichilemmoma, showing positivity for CD34, partial positivity for Ber-EP4, low cell proliferation rate of Ki-67 and negativity for GCDFP-15.
Conclusion: Desmoplastic trichilemmoma is of particular importance because its interdigitation of islands of epithelial cells wrapped in fibromyxoid connective tissue results in overdiagnosing as squamous cell carcinoma and metatypical basal-cell carcinoma. However, some pathological characteristics like good overall lesional circumscription lack of squamous differentiation or significant cytologic atypia, and immunohistochemical methods are key aspects for differentiating desmoplastic trichilemmoma from other two carcinomas. Since it was a benign cutaneous neoplasm, the initial healing was satisfactory. Patient is still under follow-up after 17 months, with good clinical evolution and free from recurrence.

Category: Dermatopathology
1647667

A Case of Primary Langerhans Cell Histiocytosis Restricted to the Vulva

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Objectives: Langerhans cell histiocytosis (LCH), formerly known as histiocytosis X or Langerhans cell granulomatosis, is characterized by a neoplastic clonal proliferation of bone marrow-derived Langerhans cells with variable clinical presentations. Although cutaneous involvement is relatively common, LCH confined to the vulvar region is a rare phenomenon.
Materials and Methods: We report a case of 40-year-old female from Dermatology department with a 13-month history of pruritic lesion on her vulva. Physical examination revealed scattered tiny hemispheric papules on the labia majora and labia minora, with progressive increase in numbers. Patient had consulted to gynecologist before but previous treatment was not effective. A vulvar biopsy was performed. Histology and immunochemistry were used to diagnose.
Result: It illustrated characteristic tumor cell population through histopathology. Tumor cells had a relatively uniform appearance and were distributed in clusters and sheets beneath the epidermis. They possessed large ovoid cells with abundant lightly eosinophilic cytoplasm, and a nucleus that was usually indented or reniform with occasional mitotic figures. There were admixture of other inflammatory cells, including lymphocytes, neutrophils and variable eosinophils in the background.
Immunohistochemistry confirmed a diagnosis of LCH, showing positivity for CD1a and S-100, focally positive for CD68 and low cell proliferation rate of Ki-67.
Conclusion: Primary vulvar LCH was a rare phenomenon. Occasionally, vulvar LCH heralds subsequent multisystem involvement with an aggressive clinical process. Moreover, LCH limited to the vulvar area can occur with local relapse despite of complete excision, chemotherapy and radiation. According to clinical history and our further workup, there was no evidence of disseminated disease involving other organs till now. Patient is still under follow-up after 20 months, with good clinical evolution and free from recurrence. Although primary vulvar LCH is uncommonly seen, it is of particular importance of recognizing this condition and assuring long-term follow-up to rule out a systemic involvement.

Clinicopathological analysis of cutaneous atypical Spitz tumor

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Objective To explore the clinicopathologic features, immunohistochemical features of atypical Spitz tumor (AST), with emphasis on diagnosis and differential diagnosis. **Methods** The clinicopathologic features were analyzed in a case of AST with review of the literature. **Results** The patient was a 25-year-old man presented with pigmented skin lesions on his right shoulder three months ago. Grossly, a 0.4cm×0.3cm dark brown nodular protuberance without surface ulceration were observed in excised skin tissue. Microscopically, the tumor was composed of epithelioid and spindled melanocytes, located at the epidermal-dermal junction and the dermis. Local dumbbell-shaped infiltration into the deep dermis were detected. The tumor cells contain abundant eosinophilic cytoplasm with conspicuous small nucleoli, mitotics were easily seen downward, with scattered atypical mitoses. Immunohistochemically, the tumor cells were positive for MelanA, S-100 and p16, but negative for CK, HMB45, ALK, BRAF^{V600E}. Ki-67 proliferative index was 5%. **Conclusion** AST is a very rare melanocytic tumor with uncertain malignant potential. *It has distinct histological and genetic characteristics, and has a good prognosis after surgical resection.*

A self-regressing cutaneous squamous cell carcinoma mouse model driven by BRaf V600E activation

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Objectives: To establish a new mouse model for addressing the underlying mechanism of the initiation and self-regression of the cutaneous squamous cell carcinoma (cSCC), and informing the therapeutic strategies of squamous malignancies and beyond.
Materials and Methods: Through crossing the $BRaf^{V600E/V600E}$ transgenic mouse with a $Rosa26^{LacZ/LacZ};RIP-Cre$ transgenic mouse, we generated a mouse model with well-differentiated cSCCs. The tumors' phenotypes were recorded during mouse growing and their histological morphologies were analyzed. X-gal staining, PCR assays, phosphorylated Erk immunohistochemical staining as well as $BRaf$ V600E inhibitor – vemurafenib treatment were employed to explore the relationship between $BRaf$ V600E activation and cSCCs progression.
Results: We established a new mouse model that could develop cSCCs in the midline of the dorsal skin in 2 weeks. More interestingly, some of these cSCCs spontaneously regressed. The induction, maintenance and spontaneously regressing of cSCC in our model was dependent on the oncogenic activation status of $BRaf$ and associated with Ras-Raf-MEK-ERK signaling pathway.
Conclusion: The oncogenic activation of $BRaf$ in mice by Cre recombinase driven via the rat insulin 2 promoter led to cSCCs with a very short latency and exhibited self-regressing phenomenon. This mouse model provides a useful research subject to address the physiological mechanisms that drive the initiation and regression of cSCC.

Strong Th2-polarization of autoreactive CD4+ T cells in inflamed Scurfy skin and lung with Thymic Stromal Lymphopoietin (TSLP) as potential differentiation factor

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Scurfy mice have a deletion in the Foxp3 gene, which results in the lack of functional Foxp3+ regulatory T cells, and they subsequently develop severe autoimmune multiorgan inflammation including skin and lung. It is known that autoreactive CD4+ effector T cells mediate the disease since isolated Scurfy CD4+ T cells transfer the same disease in RAG-/- recipients after i.v. injection.
TSLP is secreted primarily by epithelial cells and characterized as a lymphocyte growth factor. Recent studies have shown that TSLP, acting on CD4+ T cells and dendritic cells, can promote Th2 cell differentiation and Th2 cytokine-associated immune response.
In this study, we analyzed the inflammatory infiltrate of Scurfy skin and lung and the cytokine profile of skin-infiltrating autoreactive CD4+ T cells especially in regard to TSLP as potential differentiation factor. CD4+ T cells and granulocytes are the predominant cell types in inflamed skin by FACS-analysis. When we used intracellular FACS-analysis after in vivo restimulation with PMA/Ionomycin, We observed CD4+ T cells isolated from inflamed Scurfy skin expressed high levels of Th2-cytokines (IL-4 and IL-5), but low levels of the Th1-cytokine interferon-c. Since TSLP is known to mediate Th2-differenciation of CD4+ T cells, we analyzed the expression of TSLP. We found that Scurfy mice presented high TSLP-serum levels compared to WT mice as measured by ELISA. To identify TSLP gene expression in Scurfy skin and lung, we performed RT-PCR analysis and found upregulated expression of TSLP RNA in Scurfy but not WT lung and skin epithelial cells. We also analyzed TSLP expression by immunohistochemistry in inflamed Scurfy skin and lung and demonstrated high TSLP expression in Scurfy epidermis and lung but low expression in WT mouse tissues. Finally, to determine if skin-infiltrating CD4+ T cells can respond to TSLP, we analyzed TSLP-receptor expression by FACS-analysis. The result demonstrated that CD4+ T cells in Scurfy but not in WT skin showed upregulated TSLP-receptor expression.
Taken together, these data indicate that Scurfy autoreactive CD4+ T cells spontaneously develop a Th2-phenotype with high expression of the Th2-differentiation factor TSLP in Scurfy skin and lung and serum as potential driving factor for this Th2-polarization.

New entities of soft tissue tumors, which would be appear in upcoming WHO classification

Prof. Yoshinao Oda
Kyushu University

After the publication of 2013 WHO soft tissue blue book, several new entities of soft tissue tumor has been documented in the literature. Some of these new entities would be incorporated in new WHO blue book. In this session, I will explain about clinicopathological and molecular aspects of the following new soft tissue tumor entities.

1. Atypical spindle cell/ pleomorphic lipomatous tumor: Benign adult adipocytic tumor with atypical spindle cells, pleomorphic cells, adipocytes, lipoblasts, and myxoid to collagenous stroma. Significant cases show loss of RB1 expression. this tumor lacks *MDM2* and *CDK4* amplification.
2. Myxoid pleomorphic liposarcoma: Mainly located in the mediastinum of children and adolescents. Myxoid liposarcoma-like features with scattered pleomorphic spindle or ovoid cells. *FUS/EWSR1-DDIT3* fusion gene and *MDM2* gene amplification are not detetcted.
3. Angiofibroma of soft tissue: Benign fibroblastic tumor typically occur in the lower extremity of middle-aged female. Tumor is composed of uniform short spindle cells with thin walled branching vessels and myxoid or collagenous stroma. *NCOA2* gene rearrangement is detected in majority of the cases.
4. Superficial CD34+ fibroblastic tumor: Low-grade cutaneous or subcutaneous tumor with cellular proliferation of CD34 positive spindle or epithelioid cells with abundant eosinophilic cytoplasm. Nuclear pleomorphism is usually noted in neoplastic cells.
5. EBV-associated smooth muscle tumor: Smooth muscle tumor with EBV infection, mainly occurring in immunosuppressed patients. Most tumors do not metastasize.
6. Inflammatory leiomyosarcoma: Rare adult malignant tumor with smooth muscle differentiation with prominent diffuse inflammatory infiltrate. Xanthomatous appearance is occasionally associated.
7. *NTRK* rearranged spindle cell neoplasm: Pediatric spindle cell tumors with wide range of morphologies and histologic grade. Tumor cells show *NTRK* rearrangement or pan-NTRK immunoreactivity.
8. *CIC* rearranged sarcoma: Undifferentiated round cell sarcoma with *CIC* gene rearrangement. Occasionally, short spindle or epithelioid cells and myxoid stroma are associated. Bone involvement is rare. Most of the cases have *CIC-DUX4* fusion gene. The prognosis is worse than classical Ewing sarcoma.
9. Sarcomas with *BCOR* genetic alteration: Undifferentiated round and spindle cell sarcomas with *BCOR* genetic alterations, most of them are *BCOR-CCNB3* fusion or BCOR-internal tandem duplications (ITD). This sarcoma more often affects bone, compared with soft tissue. The prognosis is essentially similar to those of Ewing sarcoma.

Ossifying fibromyxoid tumor of soft tissue: report of 3 cases and literature review

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Abstract: objective to investigate the clinicopathological, immunophenotypic characteristics, diagnosis and differential diagnosis of ossifying fibromyoid tumor of soft tissue (OFMT) in soft tissue. Methods: the clinical pathological data of 3 cases OFMT were analyzed, observed by light microscope and immunohistochemical markers, and the related literatures were reviewed. Results all the 3 patients presented subcutaneous slow-growing masses. The mass was completely resected with a thick fibrous pseudocapsule covering the surface of the gross specimen. Postoperative histologic examination tumor border and clear, coated in a thin layer of matured by nature of discontinuity of lamellar bone trabecular bone, tumor substance consisting of one fiber separating lobule, see within short spindle, ovoid or round tumor cells, and thin grid in nests, short beam or lace pattern, cytoplasmic light dye, nuclear chromatin meticulous, not seen obvious fission, focal cartilage, intercellular matrix fiber myxoid. Immunophenotypes: vimentin, S-100 and CD10 proteins (+), CK(-). Conclusion OFMT is a rare tumor whose classification has not been determined, and it is an intermediate tumor. Its characteristic bone and shell structure, unique cell morphology and arrangement, and immunophenotype have diagnostic significance.

Malignant Melanotic Xp11 Neoplasm Exhibit a Clinicopathological Spectrum and Gene Expression Profiling Akin to Alveolar Soft Part Sarcoma, With a Proposal for the Reclassification

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Recently, a group of distinct mesenchymal neoplasms corresponding to Xp11 translocation renal cell carcinomas (RCCs) have attracted increasingly attention. The classification of these distinctive neoplasms, first described as "Xp11 translocation perivascular epithelioid cell tumor (PEComa)" and for which recently the term "melanotic Xp11 neoplasm" or "Xp11 neoplasm with melanocytic differentiation" has been proposed, remains challenging and controversial. It is limited by overlapping histopathologic features, inconsistent terminology, and uncertain clinical behavior. To further define the true biological behavior and investigate their relationship to other closest mimics, we collected 27 cases of melanotic Xp11 neoplasm, the largest series to date, for a comprehensive evaluation. Fourteen of the cases, along with 8 alveolar soft part sarcomas (ASPS), 9 conventional PEComas, and a control group of 7 normal renal tissues were submitted to RNA sequencing. The gene expression profile data were used for subsequent clustering analysis, combined with previously obtained data from Xp11 translocation RCCs (12 cases) and *PRCC-MITF* RCC (1 case). The distinctive clinical (younger patients and female predominance), morphology (alveolar or nested architecture, purely epithelioid clear cell morphology, and melanin pigment) and immunophenotype (TFE3, cathepsin K and HMB45/Melan-A positive, PAX8 negative) were still the clues to the diagnosis of melanotic Xp11 neoplasms. Follow-up available in 22 patients showed 5-year overall survival (OS) and 5-year disease-free survival (DFS) of 47.6% and 35.7% respectively, which was similar to ASPS (57.2%, $P = 0.580$; 30.7%, $P = 0.667$), and significantly worse than conventional PEComa (100%, $P = 0.000$; 92.5%, $P = 0.000$). Local recurrence and distant metastatic rates were 31.8% and 31.8%, respectively. Univariate analysis of location (occurring in the kidney vs not kidney), infiltrative growth pattern, nuclear pleomorphism, mitotic activity $\geqslant$ 2/50 HPF, necrosis, and lymphovascular invasion were found to be associated with OS and/or DFS. Multivariate analysis identified that location (occurring in the kidney vs not kidney, $P=0.025$) was the only factor found to independently correlate with DFS. More importantly, RNA sequencing-based unsupervised clustering analysis segregated melanotic Xp11 neoplasm and ASPS, clearly from other tumor entities including conventional PEComa and Xp11 translocation RCC, and formed a compact cluster representative of the largely similar expression signature. In this study, we first clearly define the true biologic nature that melanotic Xp11 neoplasms are distinctive malignant mesenchymal tumor, rather than a simply PEComa variant with occasionally unpredictable behavior. Meanwhile,

melanotic Xp11 neoplasm and ASPS more likely represent phenotypic variants of the same entity, which is distinct from conventional PEComa and Xp11 translocation RCC. Based on these important findings, melanotic Xp11 neoplasm can be reclassified into a distinctive entity together with ASPS, independent from PEComa, in the future revisions of the current World Health Organization categories of tumors of soft tissue and bone for the improved reclassification.

Category: Soft tissue pathology
1651350

A new case of muscular tuberculosis and review of literature

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Objective: To investigate the clinical manifestations, underlying diseases, pathological features and therapeutic responses in patients with muscular tuberculosis (MT), which is rare and often misdiagnosed in clinical practice.

Materials and Methods: This study describes a rare MT case that was recently diagnosed in our department. Additionally, eighteen other MT cases retrieved from the PubMed database from 2000 to date are included in this study. The clinical manifestations, areas of residence, underlying diseases, laboratory test results, pathology results and outcomes of the patients were recorded and analysed.

Result: The MT patients in this study included 13 males and 6 females with anaverage ageof 34.58 years old. Eight patients were from Asia, and 6 patients were from Africa, accounting for the majority of patients, at 73.68%. Underlying diseases included flu-like illness in one patient; Chronic Kidney Disease (CKD) in one patient; Sjögren's syndrome in one patient; systemic lupus erythematosus (SLE), lupus nephropathy and deep vein thrombosis in one patient; and Alzheimer's disease (AD) and Paget's disease in one patient. Two patients had a previous history of tuberculosis, and two patients had contact with suspected tuberculosis patients. All patients presented chronic occult onset, with an average of 3 (1.75, 5) months. Six cases had local masses, and 13 cases had swelling as the main clinical manifestations. Twelve patients (63.2%) presented manifestations at single sites, and 7 patients presented manifestations at multiple sites, including the thigh, calf, arm, chest wall, dorsal, psoas, gluteal and forehead muscles. Of the 19 total patients, 13 (68.4%) reported pain, andonly eight (42.1%) patients presented tuberculosis symptoms. All patients received laboratory results associated with Mycobacterium tuberculosis infection. Fourteen (73.7%) of the 19 patients underwent skeletal muscle biopsy, where granulomatous inflammation was observed. Eighteen patients were treated with anti-tuberculosis therapies. Sixteen patients improved or recovered after anti-tuberculosis treatment, and unfortunately, two patients died.

Conclusion: As a kind of systemic disease, MT is mainly characterized by painful or painless muscle masses and swelling at a single site or at multiple sites. Patients with a history of tuberculosis and immunesystem disease are susceptible to MT. A diagnosis is mainly made on the basis of the results of pathological biopsy and bacteriological culture. Early diagnosis and timely standardized anti-tuberculosis treatment can improve the prognosis.

Integrated bioinformatics analyses identify a novel HDGF/ALCAM/GTPases axis in metastasis of Ewing sarcoma

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Metastatic spread is the most powerful predictor of poor outcome in Ewing sarcoma (ES), but specific molecular mechanisms underlying ES metastasis have not been fully elucidated. We previously showed that the transcription factor Hepatoma-derived growth factor (HDGF) regulates ES growth and its expression in tumor predicts poor prognosis of ES patients. However, whether and how HDGF is involved in ES metastasis remains largely unknown. Here, through integrated analyses of HDGF ChIP-seq data and gene expression profiling data in ES cells, we revealed that HDGF regulated a cohort of metastasis-associated genes involved in cell motility, cell adhesion and actin cytoskeleton remodeling. Functionally, HDGF induced *in vitro* mesenchymal features and *in vivo* metastasis of ES cells, whereas depletion of endogenous HDGF attenuated these effects. Mechanistically, HDGF directly targeted and suppressed Activated leukocyte cell adhesion molecule (ALCAM), which led to activation of the downstream effectors Rac1 and Cdc42. HDGF exerted the pro-metastatic functions partially through repression of ALCAM, which emerged as a metastasis suppressor in ES. In human patients, high HDGF expression in primary ES correlated significantly with low ALCAM expression and reduced metastasis-free survival. These findings suggest that the HDGF/ALCAM/GTPases axis represents a promising therapeutic target for limiting ES metastasis.

Clinicopathological Analysis of 6 Cases of Composite Pheochromocytoma

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Abstract

Objective: To study the clinical and pathological features, immunophenotype, differential diagnosis and prognosis of composite pheochromocytoma.

Method: Six cases of composite pheochromocytoma diagnosed in the Department of Pathology, First Medical Center, the General Hospital of the Chinese People's Liberation Army from 2013 to 2019 were retrospectively analyzed. Their clinical features, imaging findings and pathological features were summarized.

Results: The patients ranged in age from 37 to 68 years, with a median age of 48 years. The ratio of men to women is 5:1. The diameter of the tumors ranged from 3 cm to 9 cm, with an average diameter of 4.6 cm. CT showed solid tumors. Pheochromocytoma was considered in 3 cases and cortical adenoma in 3 cases. Microscopically, pheochromocytoma was mixed with ganglioneuroblastoma in 1 case and pheochromocytoma with ganglioneuroma in 5 cases. Immunohistochemistry showed that syn, cgA and CD56 were expressed in components of pheochromocytoma, S-100 was expressed in varying degrees, and Ki67 proliferation index was less than 3%. In ganglioneuroma, NSE, NF and syn were expressed in ganglion cells, S-100 was expressed in mesenchyme, and Ki67 proliferation index was less than 3%. In ganglioneuroblastoma, neuroblastoma expressed NSE, NF, syn, weakly positive CD99, and Ki67 proliferation index was less than 3%. Follow-up data were obtained in all 6 cases, where all patients were alive without recurrence or metastasis with the survival time being 2 months to 76 months after operation.

Conclusion: Composite pheochromocytoma is a rare neoplasm with ganglioneuroma being a relatively common mixed component of it, which can not be distinguished from pheochromocytoma in imaging and clinic, so the clinical treatment and prognosis should be based on the different mixed components.

Aortic intimal sarcoma metastatic to small intestine mimicking primary soft tissue sarcoma: case report and literature review

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Objectives: Aortic intimal sarcoma is a very rare tumor with a poor prognosis. We report an unusual metastatic aortic intimal sarcoma that presented as a small intestine tumor initially misdiagnosed as a primary intestinal poorly differentiated sarcoma.
Materials and Methods: We summarized the clinical,
radiological, and histological features of the case and did a literature review.
Results: A 70-year-old woman with a long history of hypertension and diabetes presented with acute abdominal pain. An exploratory laparotomy was performed. Multifocal nodules were noted during the procedure. An intestinal perforation was identified, and segmental resection of the intestine was conducted. Gross examination of the submitted specimen revealed a 3x2 cm ulcer in the mucosa of the small intestine with transmural perforation and an underlying white-tan nodule measuring 0.8 cm in diameter. Microscopically, the tumor was composed of atypical spindle cells with a mucinous stroma. There are frequent mitotic figures. Immunohistochemically, the tumor cells expressed vimentin and smooth muscle actin (SMA). The diagnosis was primary intestinal poorly differentiated sarcoma, favoring leiomyosarcoma. Subsequent CT and MRI scan revealed a tumor significantly narrowing the descending thoracic aorta. Histological review of the intestinal tumor identified a micronodule beyond the tumor which may be interpreted
as metastasis. It is noted that four months prior to the surgery the patient had undergone a thoracic aortic stent implantation. The overall findings were most consistent with a primary intimal sarcoma of the thoracic aorta metastasized to the small intestine. Further testing for MDM2 amplification by FISH was negative. The patient was treated with anlotinib but expired 6 months after the surgery.
Conclusion: To our knowledge, this is the first report of intestinal metastatic aortic intimal sarcoma. The diagnosis can be challenging, and clinical and radiological correlation is critical for the correct diagnosis.

Clinicopathological features of Merkel cell carcinoma: a report of three cases with literature review

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Objective: To explore the clinicopathology of Merkel cell carcinoma (MCC).
Methods: The clinicopathological data of three MCC patients were retrospectively analyzed, and the relevant literature was reviewed.
Results: There were two men and one woman aged 78–84 years (mean: 80.3 years; median: 79 years), with lesions in the limbs. The tumor cells were small and uniform in size, with scant cytoplasm and nude nuclei. They also had hyperchromatic nuclei and fine salt-and-pepper chromatin; the nucleoli were not obvious, and there were many mitotic and apoptotic cells. Tumors were located in the cutaneous or subcutaneous tissues, and in one case it invaded the epidermis. A tumor capsule was present in two cases but was not obvious in the other. Immunohistochemistry was positive for Syn, CgA, neuron-specific enolase, CD56; paranuclear dot-like immunostaining was positive for CK20, CK and epithelial membrane antigen.
Conclusions: MCC is more common in old men, with the limbs being the most frequently affected site, in addition to the head and neck. However, MCC is rare in Asian populations. Neuroendocrine markers and CK20 are specific for MCC.

Category: Soft tissue pathology
1657425

Clinicopathological analysis of 81 cases of soft tissue granular cell tumor

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Objective: To investigate the epidemiology, pathological features and diagnostic criteria of benign and malignant granular cell tumors.
Methods: The clinical data, pathological morphology and immunohistochemical expression of 81 granular cell tumors in our hospital were retrospectively reviewed, and related literatures were reviewed.
RESULTS: Of the 81 patients, 36 were male and 45 were female. The oldest person to visit was 81 years old, the youngest was 8 years old, with an average of 43.7 years old. Tumor location: 13 cases of head and neck, 40 cases of digestive tract, 21 cases of limbs and trunk soft tissue, 7 cases of breast. 2 cases were diagnosed as malignant granular cell tumor, 1 case was atypical granular cell tumor, and 78 cases were benign granular cell tumor. One case of malignant morphological does not meet the diagnostic criteria for malignancy, which is characterized by metastasis. One case is vacuolar nucleus and large nucleoli, tumor cell fusiform, pleomorphic, high nuclear to cytoplasmic ratio and necrosis. Immunohistochemical staining: S-100 positive (75/75), CD68 positive (29/44), NSE positive (14/14), TFE-3 positive (9/9), syn positive (19/29), SOX -10 positive (3/3). Special dye: PAS positive (8/8). Conclusion: Granulosa cell tumor is a rare soft tissue tumor. The pathological morphology is not difficult to diagnose by immunohistochemistry and special staining. The judgment of benign and malignant tumor is important, and there are cases of good metamorphosis.

Uncommon histopathologic variants and potential diagnostic traps of classical follicular dendritic cell sarcoma

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Objective　To investigate the clinicopathologic features, special morphologic variants and potential diagnostic traps of classical follicular dendritic cell sarcoma (FDCS).　**Methods**　Twenty-five cases of classical FDCS diagnosed in the First Hospital Affiliated to Army Medical University from 2006 to 2018 were examined by hematoxylin-eosin staining, immunohistochemistry and in situ hybridization of Epstein-Barr virus-encoded mRNA (EBER). Meanwhile, the types and characteristics of the special variants of FDCS were summarized along with those reported in the literature.　**Results**　The age of patients ranged from 23 to 77 years (mean 52 years), the ratio of male to female was 1.5:1, and the maximum diameter of tumor was 1.5 to 20 cm (mean 7.4 cm). 48% (12/25 cases) of the cases were misdiagnosed at the initial evaluation. Follow-up information was available for 17 patients, and the follow-up time was 5 to 96 months. The rates of recurrence, metastasis and mortality were 17.6%, 29.4% and 11.8%, respectively. Microscopically, besides the typical morphology, 10 cases of FDCS showed special histomorphologies and / or structures, including those mimicking lymphoepithelioma-like carcinoma, desmoplastic infiltrated carcinoma, classical Hodgkin's lymphoma (CHL), anaplastic large cell lymphoma (ALCL) and hemangiopericytoma, etc. The above morphologic variants were potential diagnostic pitfalls which should be paid high attention to. Immunohistochemistry showed that more than two markers of follicular dendritic cells (such as CD21, CD23, CD35, etc.) could be expressed in both typical morphology and special variants. 25 cases were all negative for EBER by in situ hybridization.　**Conclusions**　Classical FDCS is rare, besides the typical morphologic features, there are many special variants. Particularly, it deserves more attention for the variants similar to the lymphoepithelioma-like carcinoma in the nasopharynx, CHL or ALCL in the mediastinum / lymph node. It is very helpful to strengthen the understanding of these scarce variants and raise the diagnostic vigilance.

Category: Neuropathology
1446457

Long non-coding RNAs expression patterns in glioblastoma and their association with overall survival

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Objectives: The prognosis of glioblastoma (GBM), the most frequent primary brain tumor in adults, remains very poor with the median overall survival (OS) being between 12 and 16 months from diagnosis despite early use of conventional medical therapy. Therefore, identifying new therapeutic targets, as well as prognostic and predictive biomarkers for accurate stratification of patients is of utmost importance. Long non-coding RNAs (lncRNAs) are regulators of gene expression having critical impact on both physiological processes and the molecular pathology of GBM, indicating their potential as biomarkers and therapeutic targets.

Materials and Methods: Our study included 222 GBM patients and 29 patients with non-dominant anterior temporal cortexes resected during surgery for intractable epilepsy. Informed consent approved by the local Ethical Commission was obtained from each patient. RNA (RIN > 8) from 77 specimens was used for next-generation RNA sequencing (RNAseq). rRNA depletion and cDNA library preparation were done with RiboCop rRNA Depletion Kit V1.2 (Lexogen) and NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB), respectively. RNAseq was performed using NextSeq 500 High Output Kit and NextSeq 500 instrument (both Illumina). 8,414 lncRNAs and their sequential variants with non-zero RPKM at least in one sample were statistically evaluated. The alignment and target counts were performed with CLC genomic workbench. Selected significantly dysregulated lncRNAs between GBM and non-tumor controls were analyzed in a new cohort of 188 specimens by qRT-PCR and the expression data normalized to PPIA was then evaluated by Mann-Whitney analysis. GBMs from the same cohort were also divided according to IDH mutational status and expression was analyzed by the same statistical test in the two subgroups.

Result: Statistical analysis revealed 538 (P < 0.001) dysregulated lncRNAs in GBMs compared to non-tumor brain tissue samples. The expression of top 10 downregulated lncRNAs (SNAI3-AS1, LINC00882, RFPL1S, MIR137HG, TTLL7-IT1, PWAR6, LINC00634, LINC00632, DGCR5, LINC00982; logFC ≤ -2; P < 0.001) and 1 upregulated lncRNA (BTN2A3P; logFC ≥ 2; P < 0.001) in GBM and non-tumor controls was succesfully validated by qRT-PCR (P < 0.0001). Statistical analysis of the expression of these 11 lncRNAs in IDH mutated

and IDH wild-type subgroups revealed two significantly dysregulated lncRNAs (SNAI3-AS1, LINC00632; both P < 0.001). Moreover, the statistical analysis revealed 22 lncRNAs significantly dysregulated between patients with OS less than 12 months and those with OS equal or more than 12 months.

Conclusion: We observed significant dysregulation of lncRNAs in GBM tissues compared to non-tumor controls based on the results of both RNASeq and qRT-PCR. We also found 2 lncRNAs to be dysregulated in relation to IDH mutation status and 22 lncRNAs dysregulated in relation to overall survival. Our study indicates that lncRNAs could serve as promising diagnostic and prognostic biomarkers in GBM.

Category: Neuropathology
1446477

MicroRNA profiles in cerebrospinal fluid of patients with primary brain tumors and metastases

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Objectives: Primary brain tumors and brain metastases counts yearly around 40 patients per 100 000 persons in the world and their incidence rate is still growing and accurate and early diagnosis might improve survival and life quality of patients. Cerebrospinal fluid (CSF) has many important functions and baths extracellular environment of the central nervous system, and thus is supposed to reflect all pathological conditions. From this perspective, CSF may represent an ideal source of diagnostic biomarkers. MicroRNAs (miRNAs), short non-coding RNAs involved in the pathogenesis of many cancers including brain tumors, might represent group of new biomarkers. In addition, dysregulated levels of brain tumor specific miRNAs have been already observed also in CSF. Following these facts, analysis of CSF miRNAs in brain tumor patients might help to develop additional diagnostic platform.

Materials and methods: Next-Generation sequencing (NGS) was performed for analysis of small RNAs in 89 CSF samples taken from 32 patients with glioblastomas (GBM), 14 low-grade gliomas (LGG), 11 meningiomas, 13 brain metastases and 19 non-tumor donors. CleanTag Small RNA Library Prep Kit (TriLink BioTechnologies) were used for cDNA library preparation. NextSeq 500 instrument together with Next 500/550 High Output v2 Kit – 75 cycles (both Illumina) were used for final sequencing analysis. Subsequently, according to NGS results levels of 10 miRNAs were measured in independent set of CSF samples (41 GBM, 44 meningiomas, 12 brain metastases and 20 non-tumor donors) using TaqMan Advanced miRNA Assays (ThermoFisher Scientific).

Result: NGS analysis revealed 22, 12 and 35 CSF miRNAs with significantly different levels in GBM, meningiomas, and brain metastases (adj.p < 0.0005, adj.p < 0.01, and adj.p < 0.005) respectively, in comparison with non-tumor CSF samples. Subsequent validation of selected CSF miRNAs has confirmed different levels of 7 miRNAs in GBM, 2 in meningiomas, and 2 in brain metastases compared to non-tumors. Based on the results we chose specific panels of selected miRNAs and showed their ability to classify patients with brain metastases. Panel of miR-30e-5p and miR-140-5p was able to distinguish brain metastases with 65% sensitivity and 100% specificity compared to non-tumor samples (AUC = 0.8167); further panel of miR-21-3p and miR-196-5p classified metastatic patients with 78% sensitivity and 92 % specificity in comparison to GBM (AUC

= 0.90854) and with 75% sensitivity and 83% specificity compared to meningiomas (AUC = 0.84848).

Conclusion: We have observed that CSFs from patients with various primary brain tumors and metastases are characterized by specific miRNA signatures. This work was supported by Ministry of Health of the Czech Republic grant nr. NV18-03-00398 and by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601).

Dynein Improved Cognitive Function In App/Ps1 Double Transgenic Mice By Regulating Autophagic Retrograde Axonal Transport Related Proteins

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Objectives: Autophagy is a conserved lysosomal degradation pathway in cells that is essential for maintaining cell homeostasis and adapting to external stimuli. However, due to the polarization of neuron cells, the formation and degradation of autophages have the characteristics of spatial separation, resulting in axons easy to accumulate and degrade proteins and damaged organelles. This can easily lead to many neurodegenerative diseases such as Alzheimer's disease (AD). In the early stage of AD, autophagosomes can be observed to accumulate at the axon terminals, resulting in autophagy stress. In neurons, Dynein drives macromolecular substances to move retrograde along microtubules, which plays a key role in the transport of substances. The expression of the Dynein intermediate chain DIC reduced in the brains of AD patients, Dynein-mediated transport was impaired in the brains of AD mice, and autophagosomes accumulated in large numbers along dystrophic neurites. However, the mechanism of autophagosome transport in AD brain is not yet clear, and the specific role of Dynein in axonal transport of autophagosomes is poorly understood. Therefore, the purpose of this study was to dynamically observe the changes of cognitive function, A β deposition, autophagy and axonal transport related proteins in APP/PS1 double transgenic mice. The effects of DIC on cognitive function, Aβ deposition, autophagy and axonal transport related proteins in AD model mice were further analyzed by overexpression and interference with DIC.
Methods: APP/PS1 double transgenic mice were propagated and divided into Non Tg (C57BL/6J mice-6 months old), Tg-3M (APPswe/ PSEN1dE9 mice-3 months old), Tg-6M (APPswe/PSEN1dE9 mice-6 months old), Tg-9M (APPswe/PSEN1dE9 mice-9 months old), Tg-12M (APPswe/PSEN1dE9 mice-12 months old) carried out the first part of the experiment. Firstly, the water maze test was used to detect the cognitive level of mice. Immunohistochemistry was used to detect the deposition of insoluble A β in hippocampus of mice. Transmission electron microscopy was used observe autophagy in hippocampal region, and Western Blot was carried to detecte the protein expression levels of LC3 and P62. The protein expression levels of LAMP2 and cathepsin D were measured, too. Furthermore, the protein expression of Kinesin, Dynein protein and axonal transport-related proteins P150, P50, Rab7 and ORP1L were also detected. On this basis, DIC interference / overexpression adeno-associated virus was used to inject Tg mice, and then the second part of the experiment was carried out. After verifying the efficiency of virus interference, the cognitive level of mice was detected by water maze test, the insoluble A β deposition in hippocampus of mice was detected by

immunohistochemistry, and the protein expression of LC3, P62, LAMP2 and cathepsin D was detected by Western Blot. Furthermore, the protein expression of Kinesin, Dynein protein and axonal transport-associated proteins P150, P50 and Rab7 were detected. Result: The average escape latency of APP/PS1 mice was higher than that of Non Tg mice, and increased with age. The number of mice crossing the platform in space exploration experiments showed a decreasing trend in APP/PS1 mice; Immunohistochemistry showed that there was Aβ deposition in the hippocampus of Non Tg mice, but began to deposit in the hippocampus of Tg-3M mice and increased gradually with the increase of age. There was no obvious autophagy in hippocampal region of Non Tg mice and APP/PS1-3M mice observed by electron microscope. With the increase of age, the autophagy in the hippocampus of APP/PS1 mice showed a stacking state, and the protein levels of LC3Ⅱ and p62 in APP/PS1 mice were higher than those in Non Tg mice. The levels of DIC and KIF5B in APP/PS1 mice were higher than those in Non Tg mice, but decreased with age. The expressions of P150, P50, Rab7, ORP1L and LAMP2 in hippocampus of APP/PS1 mice were higher than those of Non-Tg, but with the increase of age showed a first increase and then downward trend; The expression of cathepsin D gradually decreased in Tg group, indicating that lysosomal function was impaired. Then, AD mice were subjected to DIC interference or overexpression, and the Part Ⅱ experiment was carried out. It was found that the interference of DIC expression aggravated the cognitive impairment of AD mice and promoted the deposition of Aβ. Overexpression of DIC improved the recognition of AD mice. Western Blot showed that the change of LC3 was not statistically significant, but the increase of p62 after interfering with DIC indicated that autophagy degradation was hindered, and p62 decreased after overexpression of DIC, indicating that overexpression of DIC promoted autophagy degradation. The protein levels of DIC, P150, P50, LAMP2 and CTSD decreased and the expression of driving protein KIF5B increased. after overexpression of DIC, the protein levels of DIC, P150, P50, LAMP2 and CTSD increased significantly, while the expression of driving protein KIF5B decreased, while the expression of Rab7 increased after interfering with DIC, after interfering with DIC. The increase of Rab7 level was more obvious after overexpression of DIC. Conclusion: The learning and cognitive impairment and insoluble Aβ deposition in AD transgenic mice increased gradually with the increase of age. And autophagic accumulation increased in the hippocampus of AD mice, the expression of axonal transport related proteins decreased, and lysosome function was impaired. DIC can promote the expression of axonal transport related proteins, improve lysosome function, reduce the accumulation of autophages, and reduce the deposition of insoluble Aβ, thus improving the cognitive impairment of AD mice. It can be seen that, Dynein plays an important role in alleviating autophagy accumulation in AD, and is expected to provide a new idea for the treatment of AD.

Valinomycin reduces amyloid β-peptide (Aβ) by activating pink1/parkin in N2a/695swe cells

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Objectives: Mitochondria are the main places for energy metabolism in eukaryotic cells, providing the necessary energy for various cellular activities. Mitophagy is a mechanism by which cells selectively remove damaged or redundant mitochondria through autophagy. Normally, mitophagy can remove damaged mitochondria, but in neurons of patients with Alzheimer's disease (AD), mitochondria are not only damaged, mitophagy is also impaired. It can lead to the accumulation of impaired mitochondria, causing oxidative damage and lack of cellular energy, then leading to aggravation of Aβ, the characteristic lesion of AD. Valinomycin is a respiratory chain ionophore inhibitor that gently induces a decrease in mitochondrial membrane potential and activates mitophagy in which Pink1/Parkin is involved.

In our study, we observed whether the mitophagy pathway involved in Pink1/Parkin could attenuate Aβ by using Valinomycin at different times in N2a/APP695swe cells. To explore the effect of Pink1/Parkin in the cellular model of AD , we also detect the changes of mitochondrial membrane potential, ATP , ROS, as well as the level of mitophagy.

Methods: First of all, the cells were divided into 2 groups: WT group (N2a/WT cells), Control group (N2a/APP695swe cells). Western Blot was used to detect the expression of LC3 II, Pink1, Parkin, APP, and β-actin proteins. The morphological changes of mitophagosomes in each group were observed by transmission electron microscopy. Then, the cells were divided into 6 groups: Control group (N2a/APP695swe cells), Sham group (N2a/APP695swe cells treated with DMSO at 5uM for 24h), Valinomycin group (N2a/APP695swe cells treated with Valinomycin at 1uM for 3h), Valinomycin group (N2a/APP695swe cells treated with Valinomycin at 1uM for 6h), Valinomycin group (N2a/APP695swe cells treated with Valinomycin at 1uM for 12h). Flow cytometry was used to detect the differences of cell apoptosis and mitochondrial membrane potential in each group. Using a microplate reader to detect the variation of ATP and ROS levels. Elisa assay was performed to analyze the expression of Aβ in the cell culture mediums. The level changes of mitophagy in each group were observed by immunofluorescence microscope. At the same time, the expression of LC3II, Tom20, Pink1, Parkin, APP, and Tubulin proteins were detected by Western Blot.

Results: Compared with WT group, the expression of LC3 II, Parkin and Pink1 in APP group decreased, suggesting that mitophagy was damaged. Immunofluorescence and WesternBlot showed that APP was expressed in APP group, but not in WT group. Western blot demonstrated that the expression of APP, Tom20, Pink1, Parkin and LC3 II decreased when Valinomycin was added to N2a/APP695swe cells with 3h, 6h, 12h. Elisa showed that the expression of Aβ(1-42) was decreased gradually in the groups which the cells were

treated with Valinomycin for 3h, 6h, 12h. Immunofluorescence showed that the co-localization of LC3 and TOM20 decreased with the prolongation of drug treatment time. At the same time, compared with the APP group, the expression of ATP increased gradually at 3h, 6h and 12h, while the expression of ROS decreased gradually. However, compared with APP group, apoptosis did not change significantly at 3 h and 6 h, but increased notably at 12 h.

Conclusion: With the prolongation of valinomycin in N2a/APP695swe cells, the mitochondrial membrane potential increased, ATP increased, ROS decreased, and Aβ decreased with the enhancement of mitophagy. However, the over-activated mitophagy will disorder the energy metabolism in N2a/APP695swe cells and may induce apoptosis, which needs to be further confirmed. In conclusion, proper activation of mitophagy is beneficial for attenuating Aβ in AD.

Molecular stratification of IDH-wildtype glioblastoma with distinct prognoses based on gene expression profiles

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A handful of recent studies have assessed the molecular spectrum of isocitrate dehydrogenase (IDH)-mutant glioblastoma (GBM) on the basis of genome-wide DNA methylation analysis, copy-number profiling and gene expression profiling, respectively. Nevertheless, the systematical investigation of IDH-wildtype GBM (88%), which is overwhelmingly more than IDH-mutant GBM and also heterogeneous with respect to clinical outcomes, remains to be further discussed. The aim of this study was to determine candidate genes that might function as biomarkers to further distinguish patients with IDH-wildtype GBM, which were heterogeneous with respect to clinical outcomes. In this study, RNA sequencing expression data from 105 patieants with IDH-wildtype GBM was obtained from the Chinese Glioma Genome Atlas (CGGA) database as a training set, while another 525 IDH-wildtype GBM patients in The Cancer Genome Atlas (TCGA) datasets consisting of RNA sequencing and microarray data were used to validate. Firstly, we screened for critical genes associated with over survival by univariate Cox regression through gene expression from IDH-wildtype GBM patients in the CGGA and TCGA RNAseq cohorts, respectively. And finally, 41 candidate genes including 34 risky genes and 7 protective genes were selected. Then, we established a seven-gene-based signature by LASSO regression algorithm, which allocated each patient to a risk group (low or high), and our results showed that patients in high-risk group had dramatically shorter overall survival than low-risk counterparts in three independent cohorts. Then, univariate and multivariate analysis showed that the seven-gene signature was independently associated with OS by adjusting for clinicopathological factors (age, gender, subtype, radiotherapy, chemotherapy, MGMT promoter status and TERT status; $P < 0.001$). Moreover, the seven-gene risk signature exhibited striking prognostic validity, with AUC of 78.4% and 73.9%, which were higher that for traditional "age" (53.7%, 62.4%) and "subtype" (57.7%, 52.9%) in CGGA- and TCGA-RNAseq database, respectively, underscoring the superiority of a gene expression profile-based signature. Meanwhile, we investigated the association between the seven-gene signature and subtype, and found that patients with mesenchymal GBM had a higher risk score than those with classical GBM in the CGGA ($P < 0.0001$) and TCGA cohorts ($P < 0.05$). Subsequently, GO enrichment showed that the top five genes-upregulated involved biological processes in the high-risk group, were "inflammatory response", "immune response", "cell adhesion", "innate immune response" and "apoptotic process". In addition, GSEA analyses were performed for validation, showing that the high-risk groups were positively associated with inflammatory response and TNFα signaling via NFκB, IL2-STAT5 signaling, K-ras signaling and apoptosis. More importantly, we discovered the prognostic value of the seven-gene signature could be extended to lower

grade glioma by calculating a risk score using the same formula in the CGGA RNA-seq database. Through deeper understanding of the molecular basis of IDH-wildtype GBM, we expect to improve the stratification of these tumors according to the risk signature and supply an additional therapeutic target for IDH-wildtype GBM. In conclusion, our seven-gene-based signature refined the current classification system of IDH-wildtype GBM, and these results indicated that the seven-gene signature could be a potential prognostic biomarker and might provide a new perspective for the research and individual therapy of IDH-wildtype GBM.

Downregulation of Nurr1 is associated with PARP1 nuclear translocation in neuron injury upon ischemia/reperfusion

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Objectives: Nurr1 belongs to the nuclear receptor 4 family of orphan nuclear receptors. our previous study has demonstrated that lower expression of Nurr1 decrease TNF-a transrepression in microglia in ischemia/reperfusion (I/R). **Materials and Methods:** Herein, we further explore the expressions and interaction of Nurr1 and PARP1 systematically by isolating the nucleic and cytoplasmic lysate from brain tissues or neurons following ischemia in each group. By using computer predictions, we identified and confirmed that Nurr1 showed decreasing interaction with PARP1 after hypoxia induction by Co-IP analysis and immunofluorescence staining. **Result**: Nucleic PARP1 was inhibited by Nurr1 overexpression, and the process of PARP1 translocation to the nucleus was regulated by Nurr1. Nurr1 overexpression, synergistic with PJ-34 (a PARP1 inhibitor), improved neurological outcome of rats by decreasing the infarct volume, brain water content and foot fault post I/R. The protective effect of Nurr1 results from its ability to inhibit PARP1 translocation to nucleus, therefore attenuating the generation of PAR which is crucial in the process of PAR-driven AIF and its accumulation in the nucleus. **Conclusion**: It might be an effective therapeutic strategy to relieve neurons injury upon I/R through interrupting Nurr1-mediated signaling pathway in acute phase in rats.

Category: Neuropathology
1639830

Bio-banking Platform for Precision Cancer Medicine

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Background: The "Precision Medicine Initiative" is considered to be a new era of medical care and has been listed as key projects by many countries. Cancer research, due to its heterogeneity and complexity, is one of the best fields for the practice of precision medicine. The successful development of this research requires a variety of omics-based big data, which is based on the collection and management of a large number of patient samples and complete clinical information. To this end, Southwest Hospital (The first hospital affiliated to Army Medical University), one of the best hospitals in southwestern of China, established a clinical biobank in 2014. **Methods:** Biological specimens were collected from the tumor patients at Southwest Hospital in accordance with the ethical rules of Southwest Hospital. Based on the preoperative diagnoses, surgeons determined the specimens including fresh human pathologic and normal tissues, blood and urine for sample collection. Samples were collected, treated, stored and registered by experienced residents following our standard operating procedures. Further processes such as paraffin section, frozen section, HE staining, DNA, RNA and tissue microarray will be done by inquiry. **Results:** In total, 36 tumor specimens including glioma, breast carcinoma, rectal cancer, hypophysoma, lung cancer, etc. were collected with the total number of cases reached 5,797 and the total sample copies reached 132,161 (41,657 frozen tissues, 6,025 paraffin embedded tissues, 32,789 blood specimens, 729 patient derived primary cells and others) from 2014 to June, 2019. Among them, nearly half of the samples (62,307) are gliomas, which greatly promoted the clinical and translational medicine research in the field. For example, the biobank fully assisted the research of 70 projects and 8 domestic and international awards of the Institute of Pathology (one of the pioneers in glioma research), Southwest Hospital. In addition, we also supported 43 articles published in well-known magazines such as Nature, Nature Neuroscience, Science Translational Medicine, Cell Research, etc. **Conclusion:** We have successfully established a set of mechanisms for the collection, processing, storage and use of clinical samples, and promoted the scientific research of cancer biomarkers, molecular mechanisms of tumor initiation and development, laying a foundation for clinical medical transformation. This will greatly promote the development of precision cancer medicine and benefit patients.

Kir2.1 promotes the Invasion and Metastatic of Group 3/4 Human Medulloblastoma via activating Notch2 pathway

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The transcriptionally discrete subgroups of medulloblastoma are WNT, SHH, Group 3 and Group 4. However unlike WNT and SHH subgroups, Group 3/4 tumors do not have targetable molecular drivers identified as of yet, and contains the inconsistent disease outcomes. These lead to a paucity of therapeutic targets for these two subgroups. This study established a previously unrecognized molecular subgroup from Group 3/4 medulloblastoma, in which the key genetic driver is Kir2.1-Notch axis. This novel subgroup of medulloblastoma displays the poorest clinical outcome. Our results provide a fundament for improvement of the clinical diagnosis and may assist ultimately in the selection of patients for future clinical trials of molecular targeted therapies for the most aggressive and malignant subgroup of medulloblastoma.

Fluorescence in situ hybridization (FISH) is an useful method for BRAF fusion detection in pilocytic astrocytomas

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Objectives: Pilocytic astrocytomas represent the most common glioma subtype in young patients. They are characterized by alterations in the RAS-MAP kinase pathway. The most common mechanism is a tandem duplication on chromosome 7q34, resulting in a transforming fusion protein, consisting of the KIAA1549 and BRAF. Unfortunately, the fusions between KIAA1549 and BRAF, can be derived from at least nine different fusion site combinations. This makes simple assays, such as PCR, difficult to identify or exclude all variants of the fusion gene. Therefore, we aimed to analyse the application value using interphase FISH with a dual color, BRAF(7q34) break-apart probe and BRAF/KIAA1549 fusion probe to predict KIAA1549-BRAF fusion in pilocytic astrocytomas.

Materials and Methods: In this study a prospective cohort of 43 cases, including 29 pilocytic astrocytomas, 8 diffuse gliomas and 5 focal cortical dysplasias (non-tumor cases) were analyzed for BRAF gene fusions using FISH with dual color, BRAF(7q34) break-apart probe and BRAF/KIAA1549 fusion probe. 7 BRAF fusion positive cases were further verified by Next generation sequencing (NGS).

Result: Pilocytic astrocytomas were aged from 1 to 47 years (median = 6 years, 21 pediatric and 8 adult). Tumors were mainly located in the sellar region and cerebellum. The BRAF gene fusion was detected in 19/29 (65.5%) pilocytic astrocytomas by dual color, BRAF(7q34) break-apart probe. While BRAF-KIAA1549 gene fusion was detected in 20/29 (69%) pilocytic astrocytomas by dual color, BRAF/KIAA1549 fusion probe.   The results detected both by BRAF break-apart probe and BRAF/KIAA1549 fusion probe are highly consistent. BRAF gene fusion was not detected in 13 control cases (8 diffuse gliomas and 5 focal cortical dysplasias cases) neither by BRAF(7q34) break-apart probe nor BRAF/KIAA1549 fusion probe. It is worth noting that, according to the BRAF fusion pattern, the BRAF-KIAA1549 fused tumor cell nuclei showed two red and green signals, in addition a yellow signal when using dual color BRAF/KIAA1549 fusion probe.   While BRAF fusion positive tumor cell nuclei showed two yellow signals and in addition a green signal, or one yellow signal and one separated red and green signal when using dual color, BRAF(7q34) break-apart probe. These positive signals are different from routine break-apart signals in other gene fusions.

Conclusion: We could demonstrate the usefulness and high accuracy of FISH, including both BRAF(7q34) break-apart probe and BRAF/KIAA1549 fusion probe, to assess

the BRAF fusion in pilocytic astrocytomas. We should pay more attention to interpretation, since positive signals are often neglected.

Intranasal Delivery of Immunotherapeutic Nanoformulations for Treatment of Glioma through In Situ Activation of Immune Response

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Glioma, the most common primary malignancy in the brain, has extremely low overall survival rates, and its treatment potions are very limited. Recent breakthroughs in immunotherapy for cancer have caused immune-related treatment strategies for glioma to become subjects of active investigation. Some chemotherapeutic and immunostimulatory drugs are documented to induce both the release of DAMPs (Damage-associated molecular patterns) and type I interferon (IFN-I) production, leading to immunogenic cell death (ICD). However, as standard chemotherapy drug for glioma, temozolomide (TMZ) alone can't induce ICD of glioma cells due to lack of ability to activate IFN-I signalling. What more, the immunotherapy drugs are difficult to cross the blood-brain barrier (BBB), resulting in suboptimal therapeutic outcome.

Herein, a new oligonucleotide nanoformulation that could stimulate the local immune response in the brain and simultaneously serve as the adjuvant with co-administered TMZ to induce ICD in glioma is reported. The nanoformulations (Au@PP/poly(I:C)) are fabricated by coating gold nanoparticles (AuNPs) with polyetherimide (PEI) modified polyethylene glycol (PEG), followed by electrostatic interaction with polyinosinic-polycytidylic acid (poly(I:C)). To bypass the BBB, the nose-to-brain route represents a direct gateway for administering therapeutics to the brain. This route permits therapeutics to efficiently pass through the olfactory epithelium into the olfactory bulb via the olfactory nerve and then enter the hindbrain. Our results demonstrated that 13nm AuNPs could efficiently enter the brain by intranasal (IN) administration. Besides, after the Au@PP/poly(I:C) entered the brain, AuNPs could promote endocytosis to carry poly(I:C) into tumour cells and the IFN-I production was induced by combination of poly(I:C) to the receptor.

To explore the therapeutic outcome of the Au@PP/poly(I:C) and TMZ combination therapy in vivo, GL261 intracranial tumour-bearing mice were treated with various formulations. The survival and body weight changes of mice were observed and recorded daily, the log-rank test was used to analyse the differences in the overall survival rates of mice under the different modes of administration. There was no significant difference in survival between the control and Au@PP/poly(I:C) group (p=0.336), indicating that Au@PP/poly(I:C) alone could not directly inhibit glioma growth. Meanwhile, combination treatment (Au@PP/poly(I:C) + TMZ) and TMZ alone treatment extended the median survival span compared to the control regimen (31.00 and 35.50 days vs 21.00 days, respectively; both p <0.0001). Furthermore, combination therapy (Au@PP/poly(I:C) + TMZ) significantly improved survival time compared to TMZ alone (p

= 0.0120). In addition, TMZ treatment significantly reduced mouse body weight, whereas Au@PP/poly(I:C) did not obviously affect body weight, implying that Au@PP/poly(I:C) was no toxicity to the mice. The tumour sizes on the 13th, 21st and 28th days after various treatment were monitored by MRI T2-weighted imaging (T2WI), and the RadiAnt DICOM Viewer was used to process the MRI images for calculation of the maximum tumour area. The tumour size in the control group at day 13 was much larger than that in all of the treatment groups, and the difference in the tumour lesions was statistically obvious (maximum tumour cross-sectional areas, control group: 16.80 $\pm$ 1.625 mm^2, Au@PP/poly(I:C) treatment group: 10.80 $\pm$ 1.594 mm^2, TMZ treatment group: 5.200 $\pm$ 2.131 mm^2, and combination treatment group: 3.800 $\pm$ 1.562 mm^2; n=5 mice/group). Only TMZ and combination therapy group treated mice survived to days 21 and 28, and the tumour sizes of TMZ treatment group were lager than combination therapy group (day 21: 12.63 $\pm$ 2.146 mm^2 vs 6.500 $\pm$ 1.134 mm^2, and day 28: 29.50 $\pm$ 4.213 mm^2 vs 13.20 $\pm$ 2.417 mm^2). These results proved that concurrent IN administration of Au@PP/poly(I:C) can increase the therapeutic effect of TMZ on intracranial glioma and prolong the survival time of mice.

To investigate the mechanism of the combination therapy, we performed transcriptome sequencing to examine the expression changes in the tumour tissues of the various treatment group. The results indicated that combination therapy of TMZ with Au@PP/poly(I:C) could result in IFN-I induction followed by immune response activation, consistent with the effects of other ICD activators. Furthermore, the IHC was used to assess lymphocyte infiltration into the intracranial GL261 gliomas. The average number of CD3+ and CD8+ cells was significantly higher for the combination therapy group than TMZ group, suggesting that combination therapy could active the T cell immune response. Taken together, these findings demonstrated that the reduction in tumour size and prolongation of survival time in the combination treatment group might root in the activation of anti-tumour immune responses. In addition, the results of RNA-seq analyses and IHC showed the higher expression of CTLA-4 and PD-L1 in tumour cells, which suggest that though the mice with combination treatment performed with the longest survival time among all the treatments, still not too long compared to the mice receiving TMZ alone, possibly due to the activation of immunosuppressive pathway followed with administration of Au@PP/poly(I:C). Therefore, the therapeutic effects of intranasally administered AuNPs conjugated with PD-L1 antibodies and poly(I:C) on intracranial glioma are worth investigating in the future.

In brief, this study demonstrates that intranasal administration of Au@PP/poly(I:C) combined with TMZ can stimulate in situ immune response activation to inhibit glioma growth by inducing ICD.

Identification of Suitable Reference Genes for Gene Expression Study in Glioma Stem Cells

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Glioma is the most prevalent adult brain tumor, and the presence of glioma stem cells (GSC) or glioma initiating cells, a small subpopulation of glioma cells, has been known to be associated with worse prognoses for glioma patients. Considering the importance of gene expression studies to understand the biology of GSC, we herein aimed to identify the reliable and stable reference genes in the GSCs-derived from the glioma cell lines T98G, LN229, 090116 and 091214.

An extensive literature review was performed to identify validated reference genes currently used to normalize RT-qPCR data in glioma cell lines. Then, the cell lines were cultured under the condition for regular monolayer or tumor sphere formation to yield GSCs. RT-qPCR was employed using 11 reference genes that were identified through the literature search, and assessment of the stability by Normfinder, Bestkeeper and DCq methods was performed to validate the selected genes as suitable reference genes.

RPL13A is the gene with the lowest variability and represented as the most recommended reference gene in T98G and 090116 cells grown as monolayer or GSC, while HPRT1 is strongly recommended as the most suitable reference gene for LN229 cells grown as monolayer or GSC, and B2M is the most recommended reference gene for 091214 cells grown as monolayer or GSC. We then applied the selected reference gene(s) to analyze the expression of stemness-related genes and found that CD133 was highly expressed by GSCs than their parent cell lines-derived monolayer. However, CD9 expression was lower in the GSCs than the monolayer derived from 090116 and 091214 cells, but was higher in the GSCs than the monolayer yielded by T98G and LN229 cells. SOX2 was expressed more by 090116 cells-derived GSC, while its expression was not different between the monolayer and the GSCs derived by 091214, LN229 and T98G cells.

Our data indicate that it is necessary to select suitable reference genes based on the tissue and to further identify novel reference genes with greater expression stability for application of gene expression studies in GSCs.

Long-term drinking induces tau hyperphosphorylation and cognitive deficit

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Objective: To investigate the effects of alcohol on Tau protein phosphorylation as well as learning and memory and its possible mechanism.

Materials and Methods: The male Sprague-Dawley rats were intragastric treatment with alcohol(4 ml/kg/day), or saline with the same volume, or simultaneous intragastric treatment, and intragastric treatment with Melatonin(40 μg/day). After the intragastric treatment for five weeks, rats were put to find a hidden platform in water maze. The animals were killed 24 h after the final intragastric followed by the measurement of spatial memory.

Results: In the present study, employing alcohol-induced oxidative stress injury in brain tissues of rats, we found long-term drinking leads to a decrease of PP2A activity, an increase of GSK-3β activity, and abnormal hyperphosphorylation of Tau at Ser 199, Ser 396 and Thr 404. Morris water maze behavioral test revealed that alcohol treated rats induced spatial reference memory and memory consolidation deficits. Treatment of melatonin, an inhibitor of oxidative stress, attenuates the decrease of PP2A activity, increase of GSK-3β activity, and Tau hyperphosphorylation.

Conclusion: These findings suggest that long-term alcohol induced excessive abnormal phosphorylation of Tau in rats and cognitive dysfunction. The antioxidant melatonin may reverse the long-term alcohol consumption in rats caused by aberrant Tau phosphorylation and cognitive dysfunction

Toxoplasmic encephalitis of basal ganglia with tumor-like features proven by pathogen-specific polymerase chain reaction and direct DNA sequencing

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Objectives   We report a case of a young female patient who developed progressive neurological dysfunction with a ringenhancing tumor-like nodule on brain magnetic resonance imaging.
Materials and Methods   Urgent surgery was performed to remove the mass in the left basal ganglia. Pathological findings showed that the necrotic brain areas were accompanied by congestion, edema, discrete hemorrhage, and intestinal and perivascular lymphohistiocytic infiltration. Immunohistochemical staining results showed that Toxoplasma gondii (T. gondii) immunoreactivity was detected in both cysts and tachyzoites in these areas. The glycerol-3-phosphate dehydrogenase gene (B1) of T. gondii was amplified by sequence-specific polymerase chain reaction (PCR) and the PCR products were bi-directional Sanger sequenced. A 195 bp consensus sequence of the gene B1 was found to be 98% identical to a reference T. gondii sequence (GenBank accession No. kx270373).
Result   The final diagnosis was toxoplasmic encephalitis in the left basalganglia.
Conclusion   This report suggests that PCR and bi-directional DNA sequencing of T. gondii gene might be the most convenient and rapid tools for accurate diagnosis of toxoplasmic encephalitis .

CD24 and PRAME are novel grading and prognostic indicators for pineal parenchymal tumors of intermediate differentiation

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The pineal parenchymal tumors of intermediate differentiation (PPTIDs) are extremely rare tumor entities. They exhibit low risk (grade Ⅱ) and high risk (grade Ⅲ) malignancies, which may lead to different therapies and prognosis. However, the histological grading criteria remains elusive, and novel biomarkers may be helpful to differentiate the grade of PPTIDs. Immunohistochemical staining for CD24, PRAME, POU4F2, HOXD13 and their clinicopathologic analyses were performed in Pineal parenchymal tumors and other tumors in the pineal region. CD24 and PRAME were expressed in 9/11 (81.8%) and 8/11 (72.7%) cases of PPTIDs grade Ⅲ, compared to 6/18 (33.3%) and 5/18 (27.8%) cases of PPTIDs grade Ⅱ. The levels of CD24 and PRAME were significantly higher in PPTIDs grade Ⅲ than grade Ⅱ. However, there were no differences of HOXD13 and POU4F2 expression levels in PPTIDs grade Ⅱ and grade Ⅲ. Interestingly, high expression of CD24 and PRAME were prevalently found in high-grade tumors of central nervous system. In addition, PPTIDs patients with high expression levels of CD24 and PRAME exhibited a significant shorter survival time. The results of PPTIDs grading by CD24 and PRAME were mostly consistent with WHO criteria, except for two cases. According to the prognostic information of patients, we found that the combination of CD24 and PRAME expression for grading PPTIDs might be more valuable than WHO criteria only. CD24 and PRAME are novel markers for grading and prognostic evaluation of PPTIDs that may be helpful to determine therapeutic decision for PPTIDs patients.

New development in molecular diagnostics in pituitary neuroendocrine neoplasms(PitNEN)

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Pituitary neuroendocrine neoplasms(PitNENs) are the neoplasms derived from the anterior lobe cells and are unique and rare intracranial neoplasms. The anterior pituitary cells produce hormones and share the morphological characteristics of neuroendocrine cells such as the presence of secretory granules of various size. They are usually benign without intracranial or extracnranial metastases. But frequently they grow aggressively by invading to the adjacent tissues such as maxillary or sphenoid sinuses. The anterior pituitary cells are designated as somatotroph, lactotroph, thyrotroph, corticotroph, gonadotroph according to the hormones they produce growth hormone(GH), prolactin(PRL), thyroid stimulating hormone(TSH), adrenocorticotropic hormone(ACTH) and gonadotropin(FSH and LH). The Rathke's pouch derived cells differentiate into these cell types by the effects of transcription factors, i.e. Pit-1 for GH, PRL and TSH, SF-1 for FSH and FSH, Tpit for ACTH. The intermediate cells usually differentiated into ACTH production. The pituitary adenomas now can be classified according to the hormones they produce and the transcription factors. The nomenclature includes somatotroph adenoma, lactotroph adenomas, thyrotroph adenomas, corticotroph adenomas and gonadotroph adenomas. Null cell adenomas which are negative for hormones and transcription factors are rare.

Recent studies show that pituitary adenomas share the insulioma-associated protein1(INSM1) with the other neuroendocrine neoplasms(NENs). Thus, the pituitary adenomas have been designated as pituitary neoplasms(PitNENs). Aggressive behavior with invasion into the adjacent tissue is often associated with certain histological types, sparsely granulated somatotroph adenomas with keratin inclusions(fibrous bodies) and Crooke cell adenomas with ring-like keratin deposition. Pituitary carcinomas with metastases are very rare, but if they occur often associated with PRL or ACTH production as well as p53 mutations. Expression of SSTR2 in somatotroph and thyrotroph adenomas suggests the effects of somatostatin analogue, octoreotide, therapy. The expression of SSTR5 is associated with pasireotide therapy in somatotrph and corticotroph adenomas.

Recent genomic studies disclosed several mutations of particular genes .
Recent genomic analysis has disclosed that 40-60% of somatotroph adenoma reveals the G-protein alpha subunit(GNAS) mutation. And 40-60% of corticotroph adenomas show somatic mutation of ubiquitin-specific protease 8(USP8). These mutations are related to oncogenesis or hyperfunctioning.
Another topics in genomics in pituitary is the genes related hereditary pituitary neoplasms.

Multiple endocrine neoplasia(MEN) type 1 is the inheritable disease with autosomal dominant. The patients can have pituitary adenoma, parathyroid adenoma and pancreatic neuroendocrine neoplasms(PanNENs). Detection of MEN1 gene mutation is diagnostic and can be done by blood and tissue FFPE.

The other neuropathological neoplasms such as ganglioneuromas, paragangliomas with sometimes clinically obvious hormone production, can occur in the sella, but are extremely rare.

This talk will overview the pituitary neoplasms and update our knowledge on the genomics related to PitNEN.

Interhemispheric Cistern Lipoma Associated with Malformations of Cortical Development, Hypogenesis of the Corpus Callosum and Abnormal Vasculature

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Objectives: We present an unusual case of interhemispheric lipoma, which associated with malformations of cortical development (MCD), hypogenesis of the corpus callosum and abnormal vasculaturein a 5-year-old patient with epilepsy.
Materials and Methods: A 5-year-old male patient was admitted to our hospital with a prior diagnosis of epilepsy for two years. CT and MR imaging showed an interhemispheric lipoma as well as agenesis of the corpus callosum. HE staining, IHC and next generation sequencing were used to study the pathologic features.
Result: The pathological finding showed the diagnosis of interhemispheric lipoma. The normal structure of the cerebral cortex and the white matter was disrupted by the proliferation of vasculature and fibrous tissue. Some vascular walls were thickened and showed hyaloid degeneration. The neurons in the cerebral cortex are arranged in a disorderly pattern. However, none of the known pathogenic molecular alterations was found using next generation sequencing.
Conclusions: We present a rare case of an interhemispheric lipoma associated with corpus callosum hypoplasia, the malformation of cortical development and abnormal vasculature in a patient with epilepsy.

Preliminary study on the effect of lncRNA HULC on the growth of glioblastoma

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Abstract
Objective: The effect of long non-coding RNA (lncRNA) HULC on growth of glioblastoma cells was investigated.
Methods: Real-time PCR (qRT-PCR) was used to detect the expression level of HULC in glioblastoma U87 cell which include overexpression group (HULC-over) and its control group (VEC), knockdown expression group (HULC-siRNA) and its control group (NC). Proliferation ability of the four groups was analyzed by CCK8 and colony formation assay. The apoptosis ability of U87 cells was detected by flow cytometry. Orthotopic xenotransplantation model was established by stereotactic injection, and observe the survival of these four groups. magnetic resonance perfusion weighted imaging (MR-PWI) was used to observe the tumor volume and blood flow of nude mouse. The expression of Ki67, GFAP and Oligo2 was detected by immunohistochemistry.
Results: The qRT-PCR results showed that the HULC expression level in the HULC-over group was higher than that of the VEC group; The HULC expression level in the HULC-siRNA group was lower than that of the NC group (P<0.01). Compared with VEC group, the cell proliferation ability of the HULC-over group was significantly increased and the cell proliferation ability of the HULC-siRNA group was significantly decreased than that of the NC group (P<0.01). The clone formation experiment showed that the colony formation rate of HULC-over group was significantly higher than that of VEC group; the formation rate of HULC-siRNA group was significantly lower than that of NC group (P<0.01). Apoptosis experiments showed that the early apoptotic rate of HULC-over group was significantly higher than that of VEC group; the early apoptosis rate of HULC-siRNA group was significantly lower than that of NC group (P<0.01). The survival curve indicated that the HULC-over group had a shorter survival time than the VEC group, while the HULC-siRNA group had a longer survival time than the NC group (P<0.05). The results of MR-PWI showed that the blood flow and blood volume of tumor tissues were significantly higher than those of normal tissues, and the mean transit time and peak time were significantly lower than normal tissues. Compared with the VEC group, Ki67, GFAP and Oligo2 proteins were up-regulated in the HULC-over group; compared with the NC group, these proteins were down-regulated in the HULC-siRNA group (P<0.05).
Conclusion: LncRNA HULC can promote the growth of glioblastoma.

Primary histiocytic sarcoma of central nervous system: a clinicopathologic study of four cases and literature view

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【Abstract】 Objective: To study the clinicopathological features, differential diagnosis and prognosis of four Primary HS of CNS. Methods: Four cases of CNS HS were analyzed by clinical pathological characteristics, and related literature was reviewed. Results: In the four cases, three female and 1 male were included, ages 24 to 58, the median age was 40. MRI showed heterogeneously enhancing lesion which was considered meningioma, high-grade glioma or metastatic carcinoma respectively. Histopathology showed moderately pleomorphic, mitotically active tumor cells with a loose arrangement, effacing the normal brain tissue, with abundant eosinophilic cytoplasm, highly atypical nuclei, predominant nucleoli, and Bloodthirsty phenomenon, Occasional multinucleated or spindled forms are also common, as is background reactive inflammation. In two of the four cases, spindle cell areas were found locally, with xanthomatoid cells and eosinophilic bodies. The tumor cells are typically positive for CD68, CD163, Vimentin and lysozyme, S-100, two of four cases are positive for Braf V600E, one of three cases is partly positive for CD45、CD45RO、CD4 CD34 and negative for GFAP、Olig-2、CK、EMA、SSTR2、CD99、CD117、MPO、CD1a、langerin、CD21、CD23、CD35、CD15、CD30、CD38、CD138 and so on. The index of ki67 was 30%~75%. Rich reticular fiber were seen in all cases. *BRAF V600E* mutation was present in two cases. Conclusion: A total of 37 cases of CNS HS were reported worldwide (In the past, 33 cases and 4 cases were reported), CNS histiocytic sarcoma is rare malignant tumor, histopathology and immunohistiochemistry is necessary to make the diagnosis and to exclude other primary CNS and lymphohematopoietic tumors. Primary CNS histiocytic sarcoma should be treated intensively with surgery, chemotherapy and radiation therapy. However, the prognosis is poor. Complete resection combined with high dose focused radiotherapy can improve the prognosis.

MiR-21 protects ischemic neuronal apoptosis by inhibiting p53 signaling pathway

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Backgroud: Focal cerebral ischemia leads to a large number of neuronal apoptosis and secondary neuronal death is the main cause of cerebral infarction. MicroRNA-21 (miR-21) has been shown to be a strong anti-apoptosis and pro-survival factor, which could down regulate the expression of apoptosis related proteins. P53 is a key transcriptional regulator in various cell death models, controlling transcriptional-dependent apoptosis and necrosis. Our previous studies have shown that miR-21 was downregulated, while p53 was upregulated following ischemia. However, whether miR-21 is associated with p53 signaling pathway in ischemic neuronal apoptosis has not been reported.

Methods: Oxygen glucose deprivation (OGD) and middle cerebral artery occlusion (MCAO) were established for *in vitro* and *in vivo* experiments, respectively. Quantitative real time PCR (qRT-PCR) and western blot were used to detect the expression of microRNAs and proteins. Apoptosis was detected by TUNEL staining.

Results: After OGD treatment, miR-21 expression was down-regulated in neuron ($P<0.05$), while p53 expression was up-regulated ($P<0.05$). Overexpression of miR-21 in neuron inhibited the expression of p53, Bax and cleaved-caspase 3, while up-regulated the expression of Bcl-2. and decreased the apoptosis of neural cells induced by OGD. Overexpression of miR-21 and treatment with different concentrations of p53 (20nM, 40nM, 60nM, 80nM) was applied in neuron to detect the expression of p53, Bax, Bcl-2 and cleaved-caspase-3. We found that when the concentration of p53 was 60 nM, the inhibitory effect of miR-21 on p53 signaling pathway was blocked, including the up-regulation of p53, Bax, cleaved-caspase 3, and the down-regulation of Bcl-2. In addition, TUNEL staining showed that overexpression of miR-21 significantly abolished OGD-induced neuronal apoptosis. More importantly, over-expression of miR-21 *in vivo* could also inhibit p53 signaling pathway and reduced neuronal apoptosis after MCAO in rats, thus protecting the brain from ischemic injury.

Conclusions: Overexpression of miR-21 protects against ischemic neuronal apoptosis by inhibiting p53 signaling pathway. These novel findings suggest that miR-21-p53 may be attractive therapeutic molecules for treament of ischemic stroke.

LncRNA HULC Promotes VM and EMT of Glioblastoma Cell SHG44

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【Objective】 To explore the effects of long-chain non-coding RNA highly up-regulated in liver cancer (lncRNA HULC) on vasculogenic mimicry and epithelial-mesenchymal transition of human glioblastoma cell line SHG44. 【Methods】 qRT-PCR was used to verify the levels of lncRNA HULC in lncRNA HULC overexpression group (HULC group) and vector group (vec group). Vasculogenic mimicry (VM) was used to detect the VM formation of glioblastoma cells. qRT-PCR was used to detect the levels of mRNA associated with EMT (snail and slug). Western blot was used to detect the levels of proteins associated with EMT (snail and slug). 【Results】 qRT-PCR confirmed that HULC group has higher level of lncRNA HULC than vec group (P=0.013). VM showed that the tube node counts of vec and HULC groups are (430.7±81.84) and (841.7±26.56), it suggested that lncRNA HULC overexpression promotes VM formation of glioblastoma cells(P=0.028). qRT-PCR showed that HULC group has higher levels of mRNA snail (P=0.001) and mRNA slug (P=0.006) compared with vec group, suggesting a more obvious trend of EMT. Western blot showed that HULC group has higher levels of protein snail (P<0.001) and protein slug (P=0.009) compared with vec group, suggesting a more obvious trend of EMT. 【Conclusion】 LncRNA HULC promotes VM and EMT of glioblastoma cells.

Expression of thyroid transcription factor-1 distinguishes solitary fibrous tumor/haemangiopericytoma of the meninges from extra-central nervous system and meningiomas

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Aims Meningeal solitary fibrous tumor (SFT) and hemangiopericytoma (HPC) were categorized as the same entity in the World Health Organization (WHO) 2016 classification of tumors of the central nervous system (CNS), and assigned three grades within this entity. *NAB2-STAT6* gene fusions has been identified in most of SFT/HPC, therefore the STAT6 protein can be detected in nucleus by immunohistochemistry to distinguish these tumors from other sarcomas. However, marker that can separate meningeal SFT/HPC from extra-CNS have not been found.

Methods We encountered a meningeal 'papillary' SFT/HPC aberrant expression of TTF-1 in our routine work, and we infer that TTF-1 may be expressed in classical meningeal SFT/HPC. Then we evaluated TTF-1 expression using two different clones of antibodies (8G7G3/1 from Abcam, SPT24 from Novocastra) in 67 meningeal SFT/HPC, 62 extra-CNS SFT/HPC and 201 cases meningiomas in all grades. The copy number variation and mRNA expression of TTF-1 were measured by real-time quantitative PCR (qPCR) in meningeal SFT/HPC.

Results Our results showed that SPT24 was positive in 23 of 67 (34.3%) meningeal SFT/HPC, 3 peritoneal SFT/HPC and none of meningiomas, but 8G7G3/1was only positive in two intracranial SFT/HPC. qPCR demonstrated that the SPT24 expression was significant correlated with TTF-1 mRNA expression level independent of gene copy number variants.

Conclusions Our results suggest that aberrant expression TTF-1 monoclonal antibody SPT24 in SFT/HPC not only distinguish meningeal SFT/HPC from various meningiomas, but also the meninges and extra-CNS, and TTF-1 can be used as auxiliary diagnostic marker in meningeal SFT/HPC.

The mechanisms of autophagy in regulating PDGFA/PDGFRA and PDGFB/PDGFRA pathways and its effect in glioblastoma

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Human glioblastoma is the most malignant glioma in astrocytic tumors, which seriously harms human health, and autophagy plays an important role in it. Our group found that autophagy significantly influences the expression of PDGFRA, a key molecule that mediates the main features of proneural-like glioblastoma, and this effect is significantly different in PDGFA/PDGFRA and PDGFB/PDGFRA pathways. In this project, based on this study, the downstream binding protein complexes of PDGFRA stimulated by PDGFRA/PDGFB were analyzed, and the key acetylated regulatory proteins regulated by PDGFRA were screened to reveal the effect of acetylated regulatory proteins on autophagy status and autophagy related genes. The effects of autophagy on PDGFRA downstream binding protein complexes, signal transduction, and eventual glioblastoma cell growth and invasion were determined. This study first investigated the differential regulation of PDGFRA and PDGFB by PDGFRA and its interaction with autophagy, providing a theoretical basis for the discovery of new drugs and new strategies for the diagnosis and treatment of glioblastoma.

Category: Neuropathology
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Marine natural product A was a potent inhibitor for glioblastoma via targeting RHOA

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Glioblastoma multiforme (GBM) is the most malignant form of glioma and the overall survival time of patients with GBM is usually less than 14 months. Although Temozolomide has been widely used as chemo drug to treat GBM in clinic, disease relapse is almost inevitable, which contributes the most to patient death. Therefore, it is urgent to find new and effective medicine for GBM. In this study, we investigated the inhibitory effect of the marine natural products A on GBM cells. Moreover, A could effectively target glioma stem cells (GSCs) in GBM through blocking the formation of tumor spheres and decreasing several molecular markers of GSCs. Further, we performed gene expression microarray and revealed that RHOA played critical roles for A-mediated inhibitory effect on GSCs. Altogether, our results highlighted A as a promising inhibitor against GBM potentially via targeting GSCs of GBM.

The relationship between Hedgehog signaling pathway and CD133 in glioma neural stem cells and its significance in the diagnosis and treatment of tumor

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Objective: To explore the relationship between genes related to Hedgehog signaling pathway and CD133 and its significance in the diagnosis and treatment of tumor. Methods: He staining and immunohistochemistry were used to detect the contents of SHH and CD133 in brain tissues of patients with glioma diagnosed pathologically by the affiliated hospital of guizhou medical university from 2014 to 2016. Results: Both SHH and CD133 were expressed in the above research objects, which was positively correlated with the positive rate. Conclusion: Hedgehog signaling pathway may be involved in the occurrence of glioma stem cells and regulate the pathogenesis of glioma, and the positive rate is positively correlated with the positive rate of CD133. In this study, the glioma sections were tested by He staining and immunohistochemical staining. We found that all the 6 glioma patients studied expressed SHH protein and located in the cell membrane; all the glioma patients studied expressed CD133 protein and mainly located in the cytoplasm; and the positive rate of SHH protein was positively correlated with that of CD133 protein. We speculated that SHH protein might regulate the growth of glioma stem cells and enhance their tumorigenicity, and its related Hodghog signaling pathway might be involved in the occurrence and development of GSCs. The positive expression rate of CD133 was positively correlated with the positive expression rate of SHH, suggesting that CD133 may be one of the indicators for the proliferation of glioma stem cells, providing a theoretical basis for clinical judgment of the therapeutic effect and prognosis of glioma.

Tumor suppressive miR-637 is associated with cellular migration, invasion and glioma diagnosis

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Objectives: Abnormal expression of miRNAs occurs in many human tumors, and the normal regulation of cells can be disrupted by tumor-suppressive or oncogenic miRNAs. The purpose of this study is to investigate the roles of miR-637 in glioma.

Materials and methods: 98 glioma tissues and 16 nontumoral brain tissues (Control) were assessed for miR-637 expression by in situ hybridisation (ISH). ROC curves be calculated to determine the specificity and sensitivity of miR-637 biomarkers. Then, the effects of miR-637 on glioma cell migration and invasion were determined using transwell assay. Furthermore, candidate target genes were analysed by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment.

Result: Results showed that miR-637 was significantly downregulated in glioma tissues. And it had potential as a diagnostic biomarker for glioma. In addition, miR-637 suppressed migration and invasion of glioma cells.

Conclusion: The results suggest that miR-637 can inhibit the invasion and migration of glioma and may be a potential diagnostic marker of glioma. We will further study the potential mechanism of miR-637 as a diagnostic marker and therapeutic target of glioma.

Clinicopathological analysis of 250 cases of pituitary adenoma under the new WHO classification

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Objective: To analyse the clinical and pathological features of different types of pituitary adenomas (PAs) according to the WHO (2017) Endocrine Organ Tumor Classification guidelines. Methods: The clinical data of 250 patients with PAs were collected and analysed. Differences in the incidence of invasion, recurrence and apoplexy in patients between high-risk PAs and non-high-risk PAs were compared, as were differences in the Ki-67 index between invasive PAs and non-invasive PAs and between recurrent PAs and non-recurrent PAs. Results: Of the 250 cases in our series, 45 caseswere diagnosed as somatotroph adenomas,26 cases were diagnosed as lactotroph adenomas,1 case was diagnosed as thyrotroph adenoma,61 cases were diagnosed as corticotroph adenomas,93 cases were diagnosed as gonadotropin adenomas,15 cases were diagnosed as null cell adenomas, and 9 cases were diagnosed as plurihormonal adenomas. Five types of high-risk pituitary adenoma were identified: 17 cases of sparsely granulated somatotroph adenoma,11 cases of lactotroph adenoma in men,3 cases of plurihormonal PIT-1 positive adenoma, and 42 cases of silent corticotroph adenoma. Crooke's cell adenoma was not identified. High-risk PAs had higher rates of invasion, recurrence and apoplexy than did non-high-risk types, and the difference was statistically significant (P<0.01). Invasive PAs had a higher Ki-67 index than non-invasive PAs(3.5±1.8 vs 2.8±1.3), and the difference was statistically significant (P<0.01).Recurrent PAs had a higher Ki-67 index than non-recurrent PAs(3.9±1.9 vs 2.8±1.3),and the difference was statistically significant (P<0.01).Conclusions: According to the new classification criteria, patients most frequently had gonadotrophin cell adenomas, followed by corticotroph adenomas, and the proportion of null cell adenomas was reduced. Differences were noted in the proliferation, recurrence and apoplexy characteristics of high-risk PAs and non-high-risk PAs. The invasion and recurrence of PAs were found to be related to the Ki-67 index.

Category: Neuropathology
1656999

A new small molecule with anti-inflammatory properties for the treatment of autoimmune and inflammatory diseases

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Objectives:
Immune and inflammatory response is a crucial physiological response for human to clean invasive pathogens, however, this process can be detrimental if dysregulated. Many signaling pathways have been implicated the pathogenesis of human autoimmune and inflammatory diseases. We screened a new small molecule from a 100K compound library, named LA1, with potent anti-inflammatory properties. Here we tested its efficacy in DSS-induced mouse colitis and experimental autoimmune encephalomyelitis (EAE) and evaluated its capability of normalizing immune and inflammatory response.

Methods:
To test the efficacy of LA1 in autoimmune and inflammatory diseases, we first evaluated the potency of LA1 in vitro with RAW264.7 cell line and mouse primary peritoneal macrophage with inflammasome and JAK-STAT pathway activation assays. In vivo LPS challenge was employed to estimate LA1 preliminary ability in suppressing inflammatory reaction.   Next we explored whetherLA1 could balance and normalize immune and inflammatory response employing T cell proliferation assay. Finally, we analyzed its capacity of restraining the overreacting immune and inflammatory response in experimental autoimmune encephalomyelitis (EAE) and dextran sulfate sodium (DSS) induced colitis.

Results: In vitro we surprisingly found LA1 not only almost totally abolished Caspase-1 activation under different stimuli but also attenuated and prolonged JAK-STAT signaling pathway in RAW 264.7 cell line and primary mouse peritoneal macrophage. In vivo LPS challenge LA1 significantly reduced the production of proinflammatory cytokines. To further investigation, we tested the effect of LA1 in autoimmune and inflammatory signaling pathways. In vivo study showed LA1 has ameliorated experimental autoimmune encephalomyelitis (EAE) and dextran sulfate sodium (DSS) induced colitis and normalized immune and inflammatory response.

Conclusion: Our data demonstrated that LA1 ameliorated the excessive autoimmune and inflammatory response via inhibiting JAK-STAT signal and Caspase-1 activation. This makes it a potential therapeutic candidate for the treatment of human inflammatory diseases.

Key words: Inflammatory diseases; Drug discovery

Clinicopathological Features of Fifteen Cases of Diffuse Midline Gliomas with Histone H3 K27M Mutation

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Objective To investigate the immunohistochemical and clinicopathological features of diffuse midline gliomas with histone H3 K27M mutation.

Methods and Materials Fifteen cases were collected. The clinical manifestations, radiological appearances, pathological and immunohistochemical findings and sequencing results were analyzed.

Results There were 9 males and 6 females , the age ranged from 8 to 55 years (median=33, mean=31.9). The clinical features included headache, blurred vision, nausea and vomiting. Histologically, these tumors may manifest as glioblastoma-like, astrocytoma-like and oligodendroglioma-like. Tumor cells showed immunoreactivity for H3 K27M, other biomarkers like ATRX, p53 and Ki-67 were variably expressed, but none was positive for IDH1R132H. Sequencing showed the mutation of AAG to ATG in all cases.

Conclusion The diffuse midline gliomas with histone H3 K27M mutation is a high-grade glioma mostly occurred in children and young adults. They are more aggressive and have worse prognosis. Integrative analysis of radiology, histomorphology, immunohistochemistry and sequencing will be helpful for accuracy diagnosis.

A Case Report of Intracranial Haemangiopericytoma with Liver and Pancreatic Metastasis

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Objective Intracranial solitary fibrous tumour / haemangiopericytoma (SFT/HPC) is a rare mesenchymal tumour, accounts for less than 1% of all primary central nervous system (CNS) tumors. It was previously classified as meningeal solitary fibrous tumour and haemangiopericytoma separately. Nowadays, the neuropathologists adopt a combined diagnosis in the CNS: solitary fibrotic tumor / haemangiopericytoma (WHO grades I to III). The CNS HPC was originally considered as a variation of meningioma, and called "angioblastic meningioma", but due to its special clinical behavior, immunophenotype, and genetic characteristics, it was distinguished from meningioma in the 1993 WHO of CNS classification, and classified into the group of "mesenchymal, nonmeningothelialtumors". It is easy to misdiagnose HPCs as meningiomas when considering the similarity of morphology. Differential diagnosis of HPCs and meningiomas is important because of the higher recurrence rate and a long-term risk of systematic metastasis of the former.

Materials and Methods Herein, we report a case of a 69-year-old male with liver and pancreatic HPC in 2019, who suffered two surgeries of brain tumor outside (the postoperative pathological diagnosis were "meningioma") in 1999 and 2009, respectively, and got a surgical resection of liver tumor 2 years ago in our hospital.

Results The patient came to our hospital for hepatectomy in 2017 for the first time. We found that the tumor looks like a HPC. Together with the history of "meningioma", we reviewed the slides of brain tumor of 2009. The morphology of the brain tumor and the immunohistochemistry results were the same as those of liver tumor. The two tumors were highly cellular, with thin-walled branching "staghorn" vessels. Plenty of ovoid to spindle-shaped cells arranged closely in a haphazard pattern, with limited intervening stroma. Almost 3-4 mitoses /10 HPF can be observed in the brain tumor, but the mitosis index is more in the liver tumor. The tumors were all showed diffusely positive for CD34 and nuclear relocalization of STAT6, and negative for EMA and SSTR2. So, the final diagnosis was intracranial HPC with metastasis to the liver. The morphology and immunophenotypes of liver and pancreatic tumors resected in 2019 were similar with the above tumors, and were diagnosed as metastatic HPCs.

Conclusion Intracranial SFT/HPC is rare and easy to be misdiagnosed as meningioma, due to the similarity of morphology. It is thus important to distinguish HPCs from meningiomas, owing to the different clinical behavior, such as the higher recurrence and distant metastasis rate. Furthermore, the primary SFT/HPC of digestive system is even rare. We are required to pay more attention to the patient's medical history, and identify the tumor from other tumors. Immunophenotype is useful for differential diagnosis. Positive expression of STAT6 can be detected in nucleus with high specificity and sensitivity by immunohistochemistry.

Pathological Features and Preliminary Study of Etiology of Paravertebral muscles in Adolescent Idiopathic Scoliosis

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BACKGROUND: Adolescent idiopathic scoliosis (AIS) is a type of disease that causes unexplained spinal deformity in the first two decades, but the etiology is currently unclear. Paravertebral muscles abnormalities were seen in some patients underwent surgery. In recent years, the pathological examination methods of muscle biopsy have become more and more advanced and powerful, and more etiological hinds have been provided for clinical suspension. This study aims to explore the pathological and possible pathogenesis of AIS's paravertebral muscle's morphological changes through a variety of pathological examinations.
METHODS: Patients with scoliosis and excluded muscular dystrophy (such as Duchenne and Becker muscle dystrophy) in clinical diagnosis were enrolled in this study. Routine muscle biopsy was performed on the concave side during corrective surgery, and H&E, enzyme chemical staining, immunohistochemical staining and transmission electron microscopy were performed.
RESULTS: A total of 16 patients with AIS had an average age of 17 years and a male/female ratio of 3/13. In the biopsy muscle tissues, some muscle fiber's volume was small and arranged in a large bundle. There are varying degrees of muscle fiber degeneration, occasionally necrosis, accompany with variable internal nuclei (16/16, 100%) and ring fibers (6/16, 37.5%). Focal interstitial edema of muscle tissue were presented (10/16, 62.5%). In each case, the expression of Dystrophin protein in some muscle fibers was abnormal. Most of the sarcolemmal immunostaining pattern of atrophic muscle fibers was banded rather than normal linear, furthermore discontinuous or even absent. The non-atrophic muscle fibers also had focal weak expression of Dystrophin protein, or loss, in which Dystrophin-N terminal fragment, followed by Dystrophin-C terminal fragment expression decreased.
Almost all degrees of perivascular inflammatory cells infiltration was demonstrated in muscle fascicle and tendon tissue, mainly CD4 positive T lymphocytes (15/16, 93.75%), and a small number of CD8 positive T lymphocytes (9/16, 56.25%) and macrophages (6/16, 37.5%). In most cases, the sarcolemma expressed MHC-1 (15/16, 93.75%), which showed a focal distribution, and both atrophic or non-atrophic muscle fibers were involved. The ultrastructure of some biopsies showed that the plasma membranes of the muscle fibers were different thickness, the subsarcolemmal space increased with the mild increase of glycogen deposits, and some of the myofibrils showed abnormality of the sarcomere structure, which was destroyed in the myofilament irregularly and loosened or even disappeared in the A-band structure.

Conclusion: The pathological features of the Paravertebral muscles in patients with AIS are characterized by both neurogenic muscle atrophy and myogenic damage, suggesting that the muscle lesions may be caused by multiple causes: neurogenic muscle atrophy may be associated with long-term scoliosis and peripheral nerve tissue dysfunction. Due to the influence of myogenic lesions, the abnormal expression of Dystrophin suggests that there may be abnormalities related to Dystrophin gene or protein level, and last but not least, inflammation are also one of the pathogenic factor.

Relationship between expression of programmed death ligand 1(PD-L1) and common molecular characterization of 500 adult Glioblastoma (GBM) tumors by immunohistochemical methods.

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Objectives PD-L1 is highly expressed in many cancers. PD-L1 in tumor cells is known to promote immune escape of cancer by interacting with programmed cell death 1 (PD-1) in tumor infiltrating immune cells. Immunotherapy targeting PD-L1 is emerging as a new strategy for the treatment of GBM. Understanding the relationship between the PD-L1 and molecular characterization in GBM patients may be helpful to predict the effects of immunotherapy. **Materials and Method** We investigated the expression of PD-L1 and its relationship with, isocitrate dehydrogenase 1 (IDH1) R132H mutant, ATRX-negative, EGFR variant III (EGFRvIII), Ki-67 and mutant P53 expression by immunohistochemical methods in 500 patients with GBM. **Result** The expression rate of PD-L1 in GBM was 45.6%. PD-L1 levels correlated with IDH1R132H ($p < 0.01$), EGFRvIII ($p < 0.01$) and Ki-67 ($p < 0.01$) status. The positive IDH1R132H and EGFRvIII expression were more frequently observed in the PD-L1 negative group. The high Ki-67 expression were more frequently observed in the PD-L1 positive group. Expression of PD-L1 was a negative prognosticator for GBM outcome. **Conclusion** These results indicate that EGFRvIII and IDH1R132H may not be involved in immune escape of GBM. And it was shown a direct association between the proliferation and the expression of PD-L1 in GBM patients. Positive expression of PD-L1 in GBM was a prognostic factor for poor OS. Further investigation of the relationship between PD-L1 and other common molecular features will be necessary, and will contribute to the development of such therapies for GBM.

Objective pathological grading markers of solitary fibrous tumour/hemangiopericytoma occurred in the central nervous system: Evidence from a small series

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[Objective] To explore the objecive pathological grading markers of   solitary fibrous tumor/hemangiocarcinoma(SFT/HPC)   occurred in the central nervous system and further to provide the precision diagnosis and grading for clinical management of the disease.
[Materials andMethods] Retrospectively analysed diagnosis of patients suffering from SFT/HPC originated from the department   at Zhongnan Hospital of Wuhan university between january 2016 and june 2019, and followed up the survival of patients. Immunohistochemistry and reticular fiber staining were assessed, relevant literature was reviewed.
[Results] In total, 18 patients were retrospectively and followed up. Follow up time ranges from 2 months to 43 months and 1/18(5.56%) patients deid of developed local disease recurrence, 8/18(44.4%) patients were recurrent.   Nuclear staining for STAT6 was present in 100% (18/18).   CD34 negative   expression   rate is   3/18(16.7%) , among which 2 cases were recurrent.   And 2 of the 3 CD34 negative   cases CK shows diffuse or scattered punctate positive and the other one shows P53 positive (mutation type) .   Reticular fiber staining showed 3 patterns   each shows: much yellow collagen and only scattered in the distribution of sparse black reticular fiber(2/18, 11.1%), a small mount of yellow collagen and black reticular fiber arranges in parellel with no nets or with wide nets(10/18, 55.6%), and a largr number of black reticular fiber which arranges in a dense fine network(6/18, 33.3%). The only death case and 3/8(37.5%) relapsed cases were showed dense black reticular fiber. Combined with reticular fiber staining, immunohistochemistry, mitotic count and the date of followe up, we degraded 2 cases previous Grade 3 according to WHO creteria to Grade 2, 5 WHO Grade 2 cases to Grade 1, and 1 Grade 2 case upgraded to Grade 3.
[Conclusion]: SFT/HPC originating from the CNS is rare and WHO grading is the main prognostic factor in CNS SFT/HPC. STAT6 has high sensitivity and specificity for the diagnosis of this disease. CD34 negative expression and a largr number of black reticular fiber in a form of dense fine network were significantly associated with high grade of SFT/HPC and a poorer disease-free interval, which could be a objecive pathological grading markers of SFT/HPC of CNS.

Prognostic value of desmoplastic fiber ratio in nodular/desmoplastic medulloblastoma : a retrospective clinical analysis

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Medulloblastoma (MB) consists of at least four distinct molecular subgroups on the basis of traditional histopathologic classification; New insight that desmoplastic fiber facilitates tumor cell metastases. The aim of this study is to investigate the molecular subgroups in desmoplastic/nodular medulloblastoma (DNMB) and reidentify their clinical and prognostic implications in a large, single-institution cohort. We retrospectively identified a discovery cohort of 38 DNMBs. Reticulocyte dyeing was used to identify desmoplastic fiber and ratio of fiber was assessed using a computer-based image analysis. One single molecular variant of DNMB was identified: the SHH subgroup, p53 wild-type. The ratio of desmoplastic fiber, overall survival (OS) and progression free survival (PFS) were assessed in a dependent manner. Desmoplastic fiber ratio (threshold: 50%) was identified as independent significant prognostic factor via multivariate Cox proportional hazards model. In conclusion, desmoplastic fiber ratio can be considered as a valuable prognostic morphological marker for SHH subgroup of DNMB.

Sestrin2 promotes angiogenesis to alleviate brain injury by activating Nrf2 through regulating the interaction between p62 and Keap1 following photothrombotic stroke in rats

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Background and Purpose: The lack of effective treatments for improving quality of life or enhancing the survival rate after ischemic stroke is concerning. Here, we aimed to examine the protective effects of sestrin2 in ischemic stroke and determine the mechanism by which sestrin2 attenuates cerebral injuries.
Methods: Ischemic stroke was induced in Sprague-Dawley rats using a photothrombotic ischemia (PTI) model. After sestrin2 was overexpressed or silenced, neurological deficits and brain infarction were evaluated. Cerebral angiogenesis and the expression of related proteins were examined by Western blotting and immunofluorescence. The interaction between p62 and Keap1 was measured by coimmunoprecipitation (CoIP) and an in situ proximity ligation assay (PLA).
Results: The overexpression of sestrin2 was found to improve the neurological function of rats 10 days after photothrombotic ischemia (PTI) and to reduce the infarct volume in rats 10 days after PTI. It was shown that upregulating sestrin2 also enhanced the relative immunofluorescence intensity of CD31, CD34 and DCX and increased the expression of nuclear and total Nrf2, HO-1, VEGF and p62. However, downregulating sestrin2 induced almost the opposite results. Furthermore, we demonstrated that sestrin2 increased the interaction between p62 and Keap1.
Conclusion: Based on our data, sestrin2 may promote angiogenesis by activating the Nrf2 pathway through increasing the interaction between p62 and Keap1 via upregulating p62 expression. It may represent a potentially promising therapeutic candidate for the treatment of ischemic stroke.

Enhancing the retrograde axonal transport by curcumin promotes autophagic flux in N2a/APP695swe cells

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The accumulation of autophagosomes and dysfunction at the axonal terminal of neurons play crucial roles in the genesis and development of Alzheimer's disease (AD). Abnormalities in neuron axonal transport-related proteins prevent autophagosome maturation in AD. Curcumin, a polyphenol plant compound, has been shown to exert neuroprotective effects by increasing autophagy in AD, but the underlying mechanism of its effect on autophagy axon transport remains elusive. This study investigated the effects of curcumin on autophagosome formation and axonal transport in N2a/APP695swe cells (AD cell model) as well as the mechanism underlying those effects. Curcumin treatment significantly increased the expression of Beclin1, Atg5, and Atg16L1, induced the formation of autophagosomes, and promoted autophagosome–lysosome fusion in N2a/APP695swe cells. At the same time, curcumin promoted the expression of dynein, dynactin, and BICD2 as well as their binding to form the retrograde axonal transport molecular motor complex. Moreover, curcumin also increased the expression of the scaffolding proteins Rab7- interacting lysosomal protein (RILP) and huntingtin in N2a/APP695swe cells. Taken together, our findings indicate that curcumin increases autophagic flux by promoting interactions among autophagic axonal transport-related proteins and inducing lysosome–autophagosome fusion. This study provides evidence suggesting the potential use of curcumin as a novel treatment for AD.

A case of Embryonal tumour with multilayered rosettes, C19MC-altered

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【Abstract】 Objective: To investigate the clinicopathological, immunophenotype and molecular genetic features of one case of Embryonal tumour with multilayered rosettes, C19MC-altered. Methods: A 2-year-old boy mainly presented intermittent headache for half a month and found intracranial space for more than 10 days. Head MRI displayed large abnormal mixed signal mass on the left parietal lobe, considering Atypical teratoid/rhabdoid tumour. The patient underwent operation, and the lesion was totally removed. The tumor tissue was red, soft, rich blood supply, clear boundary and no capsule. Morphology showed the tumor composed of biphasic architecture featuring dense clusters of small cells with round or polygonal nuclei, scanty cytoplasm, and indistinct cell bodies, as well as large, paucicellular, fibrillar/neuropil-like areas, infrequently containing neoplastic neurocytic and ganglion cells. In addition, the neuropil has a fascicular quality. Hypercellular areas contain numerous mitoses and apoptotic bodies. In the densely packed area, the primitive neuroepithelial-like cells are radially distributed around the central cavity with the inner limiting membrane to form a true multilayered rosettes. Homer-Wright rosettes and perivascular pseudo-purple clusters is also seen. The cell sparse area is uniform with rich cytoplasm, perinuclear nucleus, nuclear staining, oligodendrocyte-like. The tumor recurred five times after the operation. The first tumor recurrence also showed that the primitive neuroepithelial cells formed a tubular or papillary structure around the fibrovascular axis, that is, the morphology of the myeloid tumor. The fourth tumor recurrence was also seen ganglioglioma. Immunohistochemistry showed that the dense cell area: lin28A, Nestin, CD99 cytoplasm positive, CD56 and Syn local cytoplasm positive, INI-1 nucleus positive, p53+20%, ki67+90%; the sparse cell area : CD56 and Syn cell sparse cytoplasm and neuropilin positive, NeuN individual neuron nuclear positive, GFAP scattered cell cytoplasm positive, olig-2, S-100 scattered cell nucleus positive, p53 negative, ki67+1%. FISH detection: >30% of tumor cell nucleus 19q13.42 locus cluster distribution, C19MC gene amplification positive. Chemotherapy, autologous stem cell transplantation, and radiotherapy were performed postoperatively. Currently followed for 2 years and 9 months, the child is still alive. Conclusion: ETMR is a new embryonic tumor entity of the 2016 central nervous system WHO, with special clinicopathological features and 19q13.42 specific genetic changes. Immunohistochemical detection of lin28A is a more effective diagnostic method. The prognosis is poor and complete surgical resection combined with chemotherapy and autologous stem cell transplantation is effective and can prolong the survival time of patients.

SIRT1 Reduces Oxdative stress and Neurocyte Apoptosis After Cerebral Ischemia Reperfusion Injury in Rats via PGC-1α/ PPARγ/Nrf2 Pathway

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Background
Cerebrovascular disease is one of the three deadly diseases and is the first cause of disability. Ischemic cerebrovascular disease accounted for the vast majority of the cerebrovascular disease. However, oxidative stress is likely to be a key factor in causing cerebral ischemia / reperfusion injury. Oxidative stress may cause lipid peroxidation, inhibition of protein, DNA damage and a series of pathological damage. Silent information regulator 1 (SIRT1), an endogenous antioxidant protein, involved in the cell's oxidation / reduction equilibrium and can resist oxidative stress. However antioxidant protective effect of Srxn1 and its possible regulatory mechanism is not clear.
Objective
To explore the protective effect of SIRT1 on Cerebral Ischemia Reperfusion Injury in Rats and its potential mechanism.
Methods
(1) Building one siRNA of SIRT1, siRNA was injected intracerebroventricularly 24h before MCAO. After SIRT1 knockdown, neurological deficit scores, brain water content, morphological alterations, SOD, MDA were evaluated. And we use Western Blot to detect the expression of SIRT1, PPARγ, PGC-1α, Nrf2, antioxidant stress-related proteins. Immunoprecipitation (IP) was used to examine the combination of PPARγ and PGC-1α.
(2) Building one adeno-associated virus (AVV) of SIRT1 was injected intracerebroventricularly 3 weeks before MCAO to over-expression of SIRT1. And building one siRNA of PGC-1α, siRNA was injected intracerebroventricularly 24h before MCAO. After over-expression of SIRT1 and knockdown of PGC-1α, Western Blot was used to detect the expression of SIRT1, PGC-1α, Nrf2, antioxidant stress-related proteins. Immunoprecipitation (IP) was used to examine the combination of PPARγ and PGC-1α.
(3) Building one adeno-associated virus (AVV) of SIRT1 was injected intracerebroventricularly 3 weeks before MCAO to over-expression of SIRT1. And building one siRNA of PPARγ, siRNA was injected intracerebroventricularly 24h before MCAO. After over-expression of SIRT1 and knockdown of PPARγ, Western Blot was used to detect the expression of SIRT1, PPARγ, Nrf2, antioxidant stress-related proteins. Immunoprecipitation (IP) was used to examine the combination of PPARγ and PGC-1α.
(4) Building one adeno-associated virus (AVV) of SIRT1 was injected intracerebroventricularly 3 weeks before MCAO to over-expression of SIRT1. And building one siRNA of Nrf2, siRNA was injected intracerebroventricularly 24h before MCAO. After

over-expression of SIRT1 and knockdown of Nrf2, Western Blot was used to detect the expression of SIRT1, Nrf2, antioxidant stress-related proteins a.

Results

(1) Knockdown of SIRT1 promotes neurological deficit after cerebral ischemia / reperfusion injury, increases brain water content, increases neuronal damage and promotes oxidative stress injury in rats. At the same time, SIRT1 protein and mRNA expression levels were reduced, and the expression of PGC-1a, PPARγ, Nrf2 and downstream antioxidant stress-related proteins.

(2) Interference with PGC-1a inhibited the expression of PGC-1a, Nrf2 and downstream antioxidant stress-related proteins induced by over-expression of SIRT1, and also inhibited the binding of PGC-1a to PPARγ.

(3) Interference with PPARγ inhibited the increase of PPARγ, Nrf2 and downstream antioxidant stress-related proteins induced by over-expression of SIRT1. It also inhibited the binding of PGC-1a to PPAR gamma.

(4) Interference with Nrf2 inhibited the increase of Nrf2 and downstream antioxidant stress-related proteins induced by over-expression of SIRT1.

A Case Of Primary Melanocytoma In C3-6 Spinal Canal: Case Report And Literature Review

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BACKGROUND: Melanocytoma is a primary melanoma with CNS, which originates from the melanocytes of the pia mater. It accounts for about 0.06% -0.1% of brain tumors, which is rare and difficult to distinguish from other nervous system tumors with melanosis in clinical and pathological diagnosis. Therefore, this article will report a case of misdiagnosed melanocytoma, and then explore the clinical and pathological features of this tumor, in order to improve the understanding of the tumor and the level of pathological diagnosis.
CASE DESCRIPTION: The 54-year-old male patient had repeated right shoulder and neck pain for half a year, aggravated with weakness of the right upper limb for 1 month. Admission MRI revealed C3-6 intraspinal and extraspinal space occupying lesions. The tumor was hyperintense on T1-weighted image and hypointense on T2-weighted image with a homogeneous enhancement. The clinical features, radiologic presentations, treatment choice, and pathologic characteristic were illustrated. Puncture biopsy of intraspinal tumor was performed and observed under optical microscope. It was found that the tumor cells grew in the shape of nest and cord, the shape of the cells was oval, the size was the same, and the cytoplasm was eosinophilic. The nucleoli of the cells were enlarged and eosinophilic, and the mitotic images were extremely rare. No pathological mitotic images and singular nuclear large cells were found, and no necrosis and pigmentation were found. Immunohistochemistry revealed that the tumor cells were positive for HMB-45, S-100 protein, SOX10, MUM-1 and Nestin. Also, the immunohistochemistry revealed that the tumor cells were positive for negative for MelanA, EMA, CK, CD138, CD38, Vimentin, Olig-2 and Fli-1. The positive index of Ki-67 was 8% -10%.
CONCLUSIONS: Melanocytoma is rare and atypical in clinical and imaging manifestations. It is difficult to diagnose and often misdiagnosed as schwannoma or meningioma, but the diagnosis can be confirmed by histopathology and immunohistochemistry. HMB-45 has been suggested as a significant marker for the diagnosis of meningeal melanocytoma. Most melanocytes have melanin granules deposited in the cytoplasm, and a very small number of melanocytomas do not contain melanin. At this time, it should be diagnosed by immunohistochemical staining to avoid omissions and misdiagnoses.

DJ-1 exerts anti-inflammatory effects and regulates NLRX1-TRAF6 via SHP-1 in stroke

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The acute inflammation developed by reactive astrocytes after cerebral ischemia/reperfusion injury that is an important in protecting the lesion.   Our previous study demonstrated that DJ-1 is abundantly expressed in reactive astrocyte after cerebral ischemia/reperfusion (I/R) injury. Here we show that DJ-1 negatively regulates inflammatory response by facilitating the interactions of SHP-1 with TRAF6, thereby inducing NLRX1 dissociates with TRAF6. We used primary cultures astrocytes under oxygen and glucose deprivation/reoxygenation in vitro and transient middle cerebral artery occlusion/reperfusion in vivo to mimic ischemic reperfusion insult. Inhibiting DJ-1 expression led to increases inflammatory cytokines TNF-α, IL-1β, IL-6. DJ-1 knockdown   facilitated the interactions of NLRX1 with TRAF6. However, loss of DJ-1 attenuated the interactions of SHP-1 with TRAF6. Subsequent experiments showed that SHP-1 inhibitor altered its interaction with TRAF6, and facilitated the interactions of NLRX1 with TRAF6 in DJ-1 overexpression astrocytes. This suggests that DJ-1 exerts anti-inflammatory actions on inducing NLRX1 the dissociations with TRAF6 in cerebral ischemia/reperfusion (I/R) injury may dependent on SHP-1. Thus, DJ-1 may be a efficacious therapeutic target for treating ischemia/reperfusion injury.

MGMT promoter methylation in diffuse gliomas

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Aims: Studies have demonstrated that high O-6-methylguanine-DNA-methyltransferase (MGMT) expression in the tumor cells may cause tumor tolerance to chemotherapy in glioblastoma patients. However, the studies on MGMT promoter methylation in diffuse gliomas are relatively rare. The aim of the study is to determine the pathologial features, as well as clinical courses to clarify in more detail the pathological and genetic characteristics of diffuse gliomas that carry the MGMT promoter methylation.
Methods: 181 adult patients with diffuse gliomas were collected for this retrospective observational study. The status of MGMT promoter methylation were evaluated by pyrosequencing. IDH1/2 gene and TERT promoter mutations were evaluated using sanger sequencing. 1p/19q co-deletion was detected using fluorescence in situ hybridization. Progression-free survival and Overall survival were calculated by Kaplan-Meier estimation, and group comparisons were assessed by the log-rank test.
Results: 69.7% (126/181) were detected with MGMT promoter methylation in all diffuse gliomas. In the 126 cases with MGMT promoter methylation, 70 cases (57.1%) harbored IDH1/2 mutation, 44 cases (39.4%) harbored 1p/19q. 66 cases (52.4%) occurred in the frontal lobe. Most cases showed oligodendroglioma features on morphology. The frequency of MGMT promoter methylation was 36.5% (n=46) in grade II gliomas, 26.2% (n=33) in grade III gliomas, and 37.3%(n=47) in grade IV gliomas. MGMT promoter methylation were frequently associated with TERT promoter mutations. Patients with MGMT promoter methylation were associated with longer PFS and OS (p<0.05).
Conclusion:MGMT promoter methylation are more frequently in adults diffuse gliomas, which are associated with better prognosis. MGMT promoter methylation are recommend routine detection.

Duty of HER2-PI3K-AKT signaling pathway in proliferation and migration of malignant meningioma cells

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As the second-most common adult intracranial tumor, most meningiomas are usually benign. However, a fraction of meningioma, belong to WHO grade III, tend to be more aggressive, high propensity for recurrence, and in rare cases of intracranial metastasis, posing a challenge to current meningioma therapy that combines surgery with radiation therapy. HER-2, as an oncogene target, has been therapy target for some solid tumors, also showed important value for meningioma cells progression and poor prognosis. Because of limited knowledge about the exact mechanism of HER-2-mediated signaling pathway in HER-2-positive meningioma. The current goal is to survey the changes of cell proliferation, migration and cell cycle, protein synthesis and PI3K/AKT signaling pathway in HER-2-overexpression human malignant meningioma, treated with LY294002 and KU-0063794, further to explore the possible inhibition mechanism via PI3K/AKT/mTOR signaling pathway used by q-PCR analysis, western blot analysis, CCK8 assay and transwell migration assay. At first, the results showed that when the expression of HER-2 is down regulated in human meningioma cells, it inhibits cell proliferation and migration, resulting in the arrest of G0/G1 cell cycle and the expression of PI3K/AKT protein was also blocked. By contrast, the HER-2-overexpression group had the opposite effect on cell proliferation, migration and cell cycle. Then, HER-2-overexpression meningioma cells, treated with LY294002 and KU-0063794, resulted in a standstill of the G0/G1 phase because of its blatant suppression of cell proliferation and migration resistance. PI3K/AKT protein expression was substantially decreased. These discoveries indicated that the proliferation and migration of human meningioma cells can be influenced by the HER-2 gene and HER-2/PI3K/AKT signaling way may be beneficial to carcinogenesis and progression of HER-2-overexpression in human meningiomas.

Twist2 promotes glioma cell lines invasion and migration through an epithelial-mesenchymal- transition (EMT) pathway

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Background/Aim: Twist2 is a member of the highly conserved basic helix-loop-helix transcription factor family that regulates epithelial-mesenchymal transition (EMT) and promotes progress and metastasis in tumors. However, limited evidence indicates the function of Twist2 in glioma. Here, we investigated the expression of Twist2 in human gliomas and non-cancerous brain tissues. Then, we studied the role of Twist2 in the proliferation, invasion, migration and EMT of Glioblastoma cell lines U87, T98G and human SHG44 glioma cells. *Materials and Methods:* Used for Immunohistochemistry to detect the expression of Twist2 in tissues. Over-expressed U87, T98G, at the same time, silenced SHG44 to observe the effects of Twist2 on proliferation, invasion, migration and EMT in each group. *Results:* The positive rate of Twist2 expression in gliomas is 90% while in non-tumor brain tissue is 30%. Twist2 has no effect on the proliferation but promotes its invasion and migration ability of U87, T98G and SHG44 through EMT. *Conclusion:* The possible mechanism by which Twist2 promotes the invasion and migration of U87, T98G and SHG44 is an EMT.

Pauci-immune crescentic glomerulonephritis: 5 years experience from a large renal centre in India

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Objectives

Pauci-immune crescentic glomerulonephritis (PICN) is characterized clinically by rapidly progressive renal failure developing over a period of days to weeks and untreated cases can be potentially life threatening. On serology 80-90% patients have ANCA positivity however ANCA negative serology has be seen in 30-40% cases in various studies. These patients usually have systemic involvement but renal limited vasculitis can be seen. In majority of patients crescent are seen in >50% of the glomeruli with only a minority of patients having focal crescentic lesions. Present study was undertaken to
1.Determine incidence of pauci-immune crescentic glomerulonephritis
2.To study the clinical, histopathological and serological features of pauci-immune crescentic glomerulonephritis

Materials and Methods

Renal biopsy electronic records were retrospectively analyzed from January 2014 to December 2018. Sixty four cases of ANCA associated pauci-immune crescentic glomerulonephritis were retrieved. Renal biopsy slides comprising of hematoxylin and eosin stain (HE), periodic acid Schiff (PAS) and periodic acid silver methanamine stain (PSM) were reviewed. Immunofluorescence findings, biochemical findings, ANCA serology findings by both indirect immunofluoroscence (IIF) and ELISA were retrieved from hospital records.

Results

Mean age of the patients was 48 years with a M:F ratio of 2.3:1. There were 5 pediatric patients. PICN accounted for 3% of the total 2123 renal biopsies performed during study duration. The mean serum creatinine at presentation was 6.57 ± 4.31. ANCA positivity was seen in 41 patients (64%) of which 23 were positive for C-ANCA and 18 were positive for P-ANCA. All the positive cases also tested positive on ELISA. 23 patients (36%) had ANCA negative serology on repeated testing. On histopathology, 49 patients had cellular to fibrocellular crescents whereas 15 patients had predominantly fibrous crescents. All but 5 patients had diffuse crescents (>50% glomeruli with crescents). Periglomerular or interstitial granulomas were identified in 3 patients and 13 patients had fibrinoid necrosis accompanying crescents. None of the cases should vasculitic lesion on renal biopsy. 27 patients had interstitial fibrosis and tubular atrophy with mild atrophy in 20 patients and moderate changes in 7 cases. Fourteen patients had lower respiratory tract involvement, 1 patient had upper respiratory tract involvement and

1 patient had vasculitic skin lesions. All the patients with crescents received initially 3 doses of 500 mg intravenous methyprednisolone followed by oral steroids (1mg/kg body weight) and cyclophosphamide (2mg/kg body weight) as maintenance therapy for 6 months. Follow up was available in forty patients (follow up duration 6-60 months) of which 4 patients died and 4 developed end stage renal disease (ESRD).

Conclusion

ANCA associated PICN is the most common cause of rapidly progressive glomerulonephritis. ANCA negative PICN was seen in one third patients however no difference was seen with respect to number of glomeruli with crescents or type of crescents as compared to patients with ANCA positive PICN. Granuloma and vasculitis are infrequently seen in patients with PICN. Renal biopsy along with immunofluorescence plays an essential and important diagnostic role and is helpful in guiding treatment and accessing prognosis.

Detrusor muscle change of interstitial cystitis in cystectomy specimen

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Background & objectives

Interstitial cystitis is a chronic inflammatory disease of unknown etiology. Recently, bladder augmentation with partial cystectomy is highlighted as an alternative treatment option if conservative management has failed. Common histopathologic findings on biopsy include urothelial denudation, ulceration, fibrosis of muscularis propria, and infiltration of mast cells. However, no previous studies have been documented on detrusor muscle change in cystectomy specimens.

Methods

We retrospectively analyzed cystectomy specimens (11 partial cystectomy and 1 radical cystectomy) from 12 patients with clinically defined interstitial cystitis and 4 controls from 2014 to 2017. The histopathologic features were reviewed and distribution of mast cells in lamina propria, detrusor muscle and adventitia were quantitatively evaluated using immunohistochemistry with CD117. Masson trichrome staining was performed to semiquantitatively evaluate the extent of fibrosis in detrusor muscle. The difference in mast cell infiltration and degree of fibrosis in lamina propria, detrusor muscle, and adventitia were analyzed by student t-test.

Results

The mean age of the patients (male 2, female 10) was 69 years and the mean disease duration was 4.7 years. All 12 cases showed urothelial denudation, fibrosis of detrusor muscle, and ulceration. The mast cell count was significantly elevated in patients with interstitial cystitis not only in lamina propria, but also in detrusor muscle: mean 97.3 ± 38.1 cells/mm2 in lamina propria, 75.9 ± 19.8 cells/mm2 in detrusor muscle, and 27.7 ± 10.1 cells/mm2 in adventitia compared to the control: 45 ± 14.6 cell/mm2, 24.3 ± 6.2 cells/mm2, and 13.3 ± 3.4 cells/mm2 (p-value <0.01). The mean area percentage of fibrosis in detrusor muscle was also significantly increased in patients with interstitial cystitis ($46.7 \pm 19.3\%$) compared to the control group ($20 \pm 12.2\%$, p-value <0.01). Degree of mast cell infiltration and fibrosis are not significantly correlated with gender, age, and duration of symptoms.

Conclusion

The extent of mast cell infiltration and fibrosis were significantly increased in detrusor muscle as well as in lamina propria in cystectomized bladder of interstitial cystitis patients.

Pathological analysis and literature review of MiTF family translocation renal cell carcinoma

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Renal cell carcinoma (RCC) is a group of heterogeneous diseases characterized by genetic abnormalities. Microphthalmia-associated transcription factor (MiTF) family translocation RCC is a rare renal malignant tumor, which mainly includes TFE3 translocation RCC and TFEB translocation RCC. The 2016 WHO Classification of Tumors of the Urinary System recognizes microphthalmia transcription factor (MiTF) family translocation carcinomas as a separate entity among renal cell carcinomas. This paper mainly discusses the clinicopathological characteristics, immunophenotype, molecular characteristics, differential diagnosis and prognosis of MiTF family translocated renal cell carcinoma. The clinicopathological data of 4 cases of MiTF family translocated renal cell carcinoma were analyzed. Tumor specimens were examined by histopathology, immunohistochemistry and FISH. and then relevant literature was reviewed. Results: 2 cases were female and 2 cases were male. The patients were between 16 and 65 years old, with an average age of 39 years. The tumor cells were arranged in nests and papillary structures, and the cytoplasm was clear or abundant in eosinophilic staining. One case of TFEB translocated RCC shows the characteristic biphasic histologic morphology. The cells are clustered in the center of the acini and arranged in a wreath around the pink transparent stroma. TFE3 and TFEB were strongly expressed in the nuclei of 3 cases and 1 case MiTF family translocation RCC respectively. Abnormal separation signals were detected in 3 cases of TFE3 translocation RCC and 1 case of TFEB translocation RCC by fluorescence in situ hybridization. MiTF family translocation RCC is a rare tumor, and TFEB translocation RCC is even rarer. The TFE3 and TFEB FISH tests help identify the types of MiTF family translocation renal cell carcinoma, so the detection of genetic translocation by TISH is the gold standard for the diagnosis of this tumor.

Clinicopathologic characteristics and prognosis of patients under 40 years old suffering from renal tumor: a retrospective analysis of 123 cases

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Objective: To explore the clinicopathological characteristics and prognosis of patients under 40 years old suffering from renal tumor and improve diagnosis and treatment of these patients. **Methods：**We retrospectively analyzed the clinical and pathological data of 123 patients under 40 years old who received radical and partial nephrectomy for renal tumor in our hospital from January 1 st , 2009 to December 31st , 2018. The follow-up information was obtained by telephone and reexamination. Results: A total of 123 patients, 64 male and 59 female, with a mean age of 30.8 years were included in the study. Of these, 26 (21.1%) had benign tumors and 97(78.9%) had malignant tumors. 62.6% (77/123) patients were found in conventional medical examination without symptoms, the others complained of hematuria, abdominal pain, abdominal distention and weight loss. The most common renal tumors are clear cell renal cell carcinoma (CCRCC), angiomyolipoma (AML) and Xp11 translocation renal cell carcinoma. 4 of these patients died from tumor, survival time from 4 to 36 months. The pathology subtypes were CCRCC (2/4), Xp11 translocation carcinoma (1/4) and unclassified renal cell carcinoma (1/4), all with high Fuhrman nuclear grade. All these 4 patients had lung or bone metastasis in disease progression. **Conclusion:** At the pathological diagnosis of young patients with renal tumor, we should pay more attention on Xp11 translocation carcinoma which is the third common subtype in young patients. This kind of renal cell carcinoma can resemble other renal neoplasm, strong nuclear TFE3 immunoreactivity and TFE3 break-apart FISH assays have proven to be more useful.

Genome-wide analysis identifies NR4A1 as a key mediator of T cell dysfunction

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T cells become dysfunctional when they encounter self antigens or are exposed to chronic infection or to the tumour microenvironment1. The function of T cells is tightly regulated by a combinational co-stimulatory signal, and dominance of negative co-stimulation results in T cell dysfunction2. However, the molecular mechanisms that underlie this dysfunction remain unclear. Here, using an in vitro T cell tolerance induction system in mice, we characterize genome-wide epigenetic and gene expression features in tolerant T cells, and show that they are distinct from effector and regulatory T cells. Notably, the transcription factor NR4A1 is stably expressed at high levels in tolerant T cells. Overexpression of NR4A1 inhibits effector T cell differentiation, whereas deletion of NR4A1 overcomes T cell tolerance and exaggerates effector function, as well as enhancing immunity against tumour and chronic virus. Mechanistically, NR4A1 is preferentially recruited to binding sites of the transcription factor AP-1, where it represses effectorgene expression by inhibiting AP-1 function. NR4A1 binding also promotes acetylation of histone 3 at lysine 27 (H3K27ac), leading to activation of tolerance-related genes. This study thus identifies NR4A1 as a key general regulator in the induction of T cell dysfunction, and a potential target for tumour immunotherapy

Targeted Next-generation Sequencing Revealed Distinct Clinicopathologic and Molecular Features of VCL-ALK RCC: A Unique Case from An Older Patient without Sickle Cell Trait

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Anaplastic lymphoma kinase (*ALK*)-rearranged renal cell carcinoma (RCC) is a novel entity of rare tumors with only 10 cases reported in the literature. Three RCC cases bearing *VCL-ALK* gene fusion were all young African American patients and associated with sickle cell trait notably. In contrast to the 3 reported cases, this neoplasm occurred in a middle-age woman (57 years old) without any evidence of sickle cell trait and demonstrated an infiltrating growth pattern with tubular, tubulopapillary, and tubulocystic structures, overlapping with collecting duct carcinoma and renal medullary carcinoma. Abundant intraluminal mucin was also noted significantly in the histologic sections. Immunostaining showed strong membranous labeling for ALK protein. We applied a large panel-targeted next-generation sequencing to explore the molecular alterations in the current case, revealing a driver oncogene *VCL-ALK* gene fusion co-occurring with pathogenic mutations in *EP300* and *TRRAP* genes. Thereafter, fluorescence in situ hybridization assay was used to detect the *ALK* gene rearrangement. Reverse transcription polymerase chain reaction confirmed the presence of a *VCL-ALK* gene fusion, a fusion of *VCL* exon 16 to *ALK* exon 20. Our report draws the attention to the possibility that *VCL-ALK* genotype can be involved in older patients unassociated with sickle cell trait, also expanding the spectrum to *ALK*-rearranged RCC.

Clinicopathological and molecular analysis of the TFEB fusion variant reveals new members of TFEB translocation renal cell carcinomas (RCCs): Expanding the genomic spectrum

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Xp11 renal cell carcinoma (RCC) with different gene fusions may have different clinicopathologic features. We hypothesized that *TFEB* translocation RCC could exhibit similar results. A total of 31 cases of *TFEB* RCCs were selected for the current study; *MALAT1-TFEB* fusion was identified in 25 cases (81%, 25/31) using fusion probes. The remaining 6 cases (19%, 6/31) were further analyzed by RNA sequencing and 5 of them were detected with *TFEB*-associated gene fusions, including 2 *ACTB-TFEB*, 1 *EWSR1-TFEB*, 1 *CLTC-TFEB*, and 1 *PPP1R10-TFEB* (a paracentric inversion of *TFEB* gene, consistent with "negative" *TFEB* split FISH result, and advising a potential diagnostic pitfall in detecting *TFEB* gene rearrangement). Four of the 5 fusion transcripts were successfully validated by RT-PCR and Sanger sequencing. Morphologically, approximately one-third (29%, 9/31) of *TFEB* RCCs showed typical biphasic morphology. The remaining two-thirds of cases (71%, 22/31) exhibited nonspecific morphology, with nested, sheet-like or papillary architecture, resembling other types of renal neoplasms, such as clear cell RCC, Xp11 RCC, perivascular epithelioid cell tumor (PEComa) or papillary RCC. Although cases bearing a *MALAT1-TFEB* fusion demonstrated variable morphologies, all 9 cases featuring typical biphasic morphology were associated with *MALAT1-TFEB* genotype. Accordingly, typical biphasic morphology suggests *MALAT1-TFEB* fusion, while atypical morphology did not suggest fusion type. Isolated or clustered eosinophilic cells were a common feature in *TFEB* RCCs, which may be a useful morphology diagnostic clue for *TFEB* RCCs. Clinicopathologic variables assessment showed necrosis was the only morphologic feature that correlated with aggressive behavior of *TFEB* RCC (P=0.004). In summary, our study expands the genomic spectrum and the clinicopathologic features of *TFEB* RCCs, highlights the diagnosis challenges and the importance of subtyping of this tumor by combining morphology and multiple molecular techniques.

Hemangioblastoma: clinicopathologic study of 42 cases with emphasis on TFE3 expression

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Hemangioblastomas (HBs) histologically overlap with *TFE3* rearrangement-associated tumors, which represent as alveolar architecture and clear or eosinophilic granular cytoplasm. However, TFE3 expression and its significance in HBs remains unexplored. Herein, we aimed to determine TFE3 expression and its clinicopathological significance in HBs. A total of 42 HBs were evaluated for TFE3 expression by immunohistochemisty staining. Thirty eight cases were sporadic and 4 were regarded as a part of von Hippel-Lindau (VHL) syndrome according to clinical presentation. Nineteen patients were male and 23 were female. Patient age ranged from 17 to 70 years (mean, 44 years; median, 43 years). Eight cases were multiple tumors and 34 solitary. Thirty-three tumors arose in the cerebellum, 6 in the medulla oblongata, 6 in the spinal canal, and 1 in the cerebellopontine angle. Tumor size ranged from 0.4 to 4.8 cm (mean 2.2 cm). Follow-up ranged from 1 to 60 months. Six patients had recurrence, 2 had unresectable local lesion and the others had no evidence of recurrence or metastasis at last follow-up. TFE3 were mainly expressed in nucleus or cytoplasm of tumor cells. Thirty-six (85.7%) and 26 (61.9%) out of 42 cases showed nuclear and cytoplasmic TFE3 expression, respectively. Besides, nuclear TFE3 expression could be detected in the granulosa cells within the normal cerebellar tissues or the vascular endothelial cells. High nuclear TFE3 levels were significantly associated with older ages (p=0.018) and larger tumor size (p=0.001). Also, TFE3 cytoplasm expression also occurred preferentially in older adults (p=0.048). Nineteen HB cases including 17 sporadic and 2 *VHL*-related HBs with high TFE3 nuclear or cytoplasm expression were negative for *TFE3* break-apart fluorescence in situ hybridization analysis. However, we first identified polyploid X-chromosome with *TFE3* gene amplification in 3 HBs including 2 sporadic and 1 *VHL*-related HB. In conclusion, our results first show that TFE3 expression is found in HBs and might be of potential diagnostic, differential diagnostic, and therapeutic relevance.

Value of Human Papillomavirus (HPV) DNA and p16ink4a in a Chinese Penile Carcinoma Cohort of 226 Cases

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Objectives: There is evidence that one third to one half of of penile cancer is caused by infection with human papillomavirus (HPV). However, little work has been done on the prevalence of HPV infection in Chinese cohort and its correlation with clinical outcome of penile cancer. Hence, we evaluated the prevalence of HPV DNA and in a large series of Chinese penile cancer, correlating the results with the histologic subtype, p16^{INK4a} expression, and prognosis.

Materials and Methods: We pathologically classified 226 invasive penile carcinomas and assessed HPV genotyping by real-time PCR and p16^{INK4a} immunohistochemistry. The results were correlated with histopathological and clinical parameters and disease-specific survival (DSS).

Results: HPV DNA was detected in 74 of 226 (32.7%) penile cancer cases. The most frequent genotype was HPV 16 (64/74, 86.5%), followed by HPV 18 (6/74, 8.1%). 59 (26.1%) cases were positive for the p16^{INK4a} expression, and p16INK4a expression had a sensitivity of 56.8% (95% CI: 45.2%–68.3%) and a specificity of 88.8% (95% CI: 83.8%–93.9%) for defining HPV status. HPV prevalence varied by the histologic subtypes with the highest prevalence in basaloid (2/3, 66.7%) and warty carcinomas (6/14, 42.9%). HPV DNA (p=0.019), p16^{INK4a} (p=0.038), the status of age (p=0.018), the grade of differentiation (p=0.001), lymph nodes (p<0.001), T-stage (p<0.001), M-stage (p<0.001), and lymphovascular invasion (LVI, p=0.0014) were prognostic for DSS. HPV DNA (HR 0.334, 95%CI: 0.158–0.705, p=0.004) was still a significant prognostic factor for DSS, after adjustment for age, grade of differentiation, lymph nodes status, T-stage group, M-stage and LVI.

Conclusions: HPV DNA was observed in one-third of Chinese penile cancer cases. The p16^{INK4a} expression can be used as a marker of HR-HPV infection. HPV-positive penile tumors confer a survival benefit over HPV-negative tumors.

Melanotic Xp11 Translocation Tumor with TFE3-ASPSCR1 Fusion of Liver

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Objectives: Melanotic Xp11 translocation tumor is first described by Argani et al in 2009, which represent the cases with TFE3 gene fusions coexist with melanin production. The entity previously called PEComa with TFE3 translocation with a potential of metastasis. The main genetic changes of these tumors were TFE3 gene fusions, and the reported fusion candidates of TFE3 in this entity included SFPQ(PSF), NONO, RPM10, and ARID1B. So far, all of the reported tumors occurred in kidney, and no other site was documented. This study is firstly to report a case with melanotic Xp11 translocation in liver, and harbor a novel TFE3-ASPSCR1 fusion, which expand the site and fusion spectrum of this entity.

Materials and Methods: A 33-years old women was presented with a complaint of abdominal pain. B ultrasonic found that there was a well-defined mass sized14.9*12.5*12.6cm in the right posterior lobe with abundant blood flow signal. The patient had a past history of retroperitoneal mass, but the pathologic data was not available. The patient underwent partial hepatectomy. The histopathological observation, immunohistochemistry, RNA sequence, fluorescence in situ hybridization (FISH) and molecular biology experiments were used in this study.

Result: The mass was well-defined and measured 15cm in greatest diameter, with hemorrhage and necrosis in the central of tumor. Microscopically, the tumor cells were pleomorphic with an eosinophilic large nucleolus, arranging in a radial pattern around the vessel lumen. There were scattered giant tumor cells and calcification. Hemorrhage and necrosis were found in some areas. For immunophenotype, the tumor cells did not express epithelial markers such as AE1/AE3、Cam5.2、EMA as well as hepatic marker. Instead, the tumor cells were positive for SMA, cathepsisn K, HMB-45, but negative for S-100. Based on the morphology and immunophenotype, it was considered as a perivascular epithelioid cell tumor (PEComa). However, this case showed more atypical morphology and aggressive features compared with common PEComa, and it rendered a question whether it was a special PEComa or other tumor entity? Further work found all of tumor cells showed a diffuse strong positiveity of TFE3. RNA-sequence revealed this case indeed harbored TFE3 rearrangement and support the diagnosis of Xp11 translocation tumor of liver. Different from the previous reports, there was a new fusion of TFE3-ASPSCR1 in Xp11 translocation tumor, and the results were comfirmed by further RT-PCR and fluorescence in situ hybridization (FISH).

Conclusion: Combined the morphology, immunophenotype and genetic changes, the case was finally diagnosed as hepatic melanotic Xp11 translocation tumor harboring *TFE3-ASPSCR1* fusion, belongs to the TFE3 translocation related tumor. And this is previously called

PEComa with TFE3 translocation with a potential of metastasis. This is the first report of melanotic Xp11 translocation tumor in liver, and we also firstly found a novel target *ASPSCR1* fusion with TFE3 in this tumor, which could expand the TFE3 fusion spectrum of melanotic Xp11 translocation tumor. It is important to discriminate the melanotic Xp11 translocation tumor from common PEComa, because the former is more aggressive and the prognosis of patient is poor.

Category: Urological Pathology
1657256

Basal cell carcinoma of the prostate: A case report and review of the literature

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Basal cell carcinoma (BCC) of the prostate is a rare tumor exhibiting various morphological characteristics, and its progression varies from an indolent to an aggressive type, with local recurrence or distant metastasis. Because of its rarity, the clinicopathologic features and theprognosis of BCC of the prostatehave not been well characterized, we herein report one case and review of the literature. An 84 year-old-man visited our hospital and complained of lower limb edema for 3 months. His serum prostate-specific-antigen (PSA) level was measured to be1.31ng/ml and the clinical diagnosis was benign prostatic hyperplasia, the patient
underwent transurethral resection of prostate (TURP). Histological examination revealed BCC of the prostate, with immunostaining examination of tumor cells showing positive results for p63, HCK and Bcl-2, but negative results for PSA, P504S, CK7 and CK20. Simultaneously，break- apart fluorescence in situ hybridization for MYB was performed, and the MYB rearrangement was not identified. Imaging examination showed no metastasis. No further surgery was performed, but adjuvant endocrine therapy was recommended. Histopathologically, the tumors had various growth patterns, including basaloid components or adenoid cystic-like tumors with cribriform appearance. There was no extracapsular infiltration or lymph node metastasis. The patient remained alive and recurrence-free with the follow-up up to now. We also retrospectively analyzed additional reported cases fromthe literature.

SIRT1 suppresses oxidative stress in diabetic nephropathy by regulating p66shc.

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Oxidative stress plays a crucial role in the development and progression of diabetic nephropathy (DN). We previously demonstrated that SRT1720, a SIRT1 activator, retards diabetes-induced renal fibrosis. This study investigated whether SIRT1 prevents diabetic-induced oxidative injury and whether p66Shc, a major inducer of oxidative metabolism and apoptosis, is involved in the protective effects of SIRT1 *in vivo* and *in vitro*. We found that SIRT1 expression and activity were downregulated in diabetic kidneys. SRT1720 administration alleviated proteinuria, ameliorated pathological manifestations, decreased oxidative damage and inhibited apoptosis, as well as restored SIRT1 expression and activity in diabetic mice. In HK-2 cells, SRT1720 exerted a prohibitive effect on HG-induced apoptosis. Moreover, SRT1720 decreased NADPH oxidase 4 (NOX4) and heme oxygenase-1 (HO-1) expression, and increased manganese superoxide dismutase (MnSOD) expression both *in vitro* and *in vivo*. Mechanistically, SRT1720 suppressed HG or diabetes-induced p66Shc expression and phosphorylation, as well as histone H3 acetylation. Furthermore, p66Shc short interfering RNA increased MnSOD expression, decreased NOX4 and HO-1 expressions, inhibited mitochondrial ROS generation and apoptosis in HG-induced HK-2 cells, which mimicked antioxidant Tempol or N-acetylcysteine treatment. These results suggest that SIRT1 alleviates diabetes-induced apoptosis via SIRT1/p66Shc-mediated attenuation of oxidative stress.

Case report of a t (6; 11) renal cell carcinoma in a young woman composed of almost totally of larger cells showing cystic and tubular patterns

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Objectives
T(6;11) renal cell carcinoma(RCC) is rather rare and it sometimes showed different patterns which made it confusing to diagnose. Here we reported a solid-cystic mass of a young lady to present some unique features of this tumor.

Materials and Methods
The clinical feature, imaging manifestation, histopathological morphology, immunohistochemistry feature and molecular pathology results of a t(6;11) RCC case was retrospectively analyzed and we reviewed several literatures.

Result
A mass was found on the lower right kidney of a 24 year old young woman. The solid-cystic tumor was confined to kidney and was 3.5cm in diameter. Tumor cells were composed almost completely of larger cells with obvious cell membranes
and amphophilic to eosinophilic cytoplasma. Nuclei were low grade and no mitosis was discovered. Cystic, tubule-like or papillary patterns were presented. Some cysts were lined with a layer of large tumor cells like hobnails. Blood vessels branched in the tumor, most were composed of thin walls and some were thickened and hyalinized. There was also some calcification. The tumor was strongly positive for TFEB, PAX8, PAX2, P504S, Vimentin, Desmin, WT1 and Melan-A, and was focally positive for HMB45, RCC, CK, CD10 and SDHB. TFE3, S100, CK7, CA9, CD117 and SMA were negative. The t(6;11) RCC diagnosis was further demonstrated by TFEB break-apart fluorescence in situ hybridization(FISH) test.

Conclusion
T(6;11) RCC is similar to several RCCs and other tumors like AML, the uncommon patterns reported here make it more confusing in differential diagnosis which is worth of attention. In this case, the tumor was solid-cystic and was composed of several special patterns, which were uncommon in other cases. The t(6;11) RCCs sometimes resemble several different kinds of tumors, of witch mainly are some RCCs originate from the kidney, incluing clear cell RCC or papillary RCC. These tumors are negative for TFEB immunohistochemistry or split FISH examination. TFE3/Xp11 translocation carcinoma is more progressive than t(6;11) RCC and usually occurs in the elderly. Besides, it has unique molecular character. Epithelioid angiomyolipoma sometimes overlap the feature of t(6;11) RCC, but it is positive for MelanA, HMB45 or Cathepsin K and is negative for renal markers.

Loss of KDM6A is associated with high histologic grade and poor prognosis in upper tract urothelial carcinoma of Chinese patients

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Objective   Upper tract urothelial carcinoma (UTUC) in China which is closely   related to aristolochic acid (AA) in Chinese herbs, exhibits more aggressive behavior and worse prognosis than the counterparts of this tumor in Western countries. Whole-genome and exome sequencing of AA-associated UTUCs revealed that the most frequently mutated gene in this tumor is KDM6A which is a histone H3K27me3 demethylase. We therefore explored the clinicopathologic significance and prognostic value of KDM6A expression in Chinese UTUC patients.

Methods   In this study 108 patients with pathologically confirmed UTUC at Peking University Shougang Hospital and Peking University Third Hospital between 2007 and 2017 were retrospectively reviewed. Expression of KDM6A in tumor specimens was quantified with immunohistochemistry, and the clinicopathologic significance of KDM6A expression and its prognostic values in patients with UTUC was evaluated.

Results   Loss of KDM6A expression was detected in 64.8% of UTUC patients and was associated with high tumor grade (P=0.007), advanced stage (P=0.016), poor cancer-specific survival (CSS, P=0.023), and poor disease-free survival (DFS, P=0.033). KDM6A expression was also positively correlated with H3K27me3 expression (P=0.002) by immunohistochemistry analysis, However, KDM6A was not found to be significantly correlated with EZH2, which is a histone methyltransferase that generates H3K27me3.

Conclusions   Our results strongly suggest KDM6A could be used as a prognostic marker or a potential therapeutic target in Chinese UTUC patients.

Analysis of clinicopathological features of 414 cases of renal angiomyolipoma in a single institution

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Background: Renal angiomyolipoma (RAML) is relatively common mesenchymal tumors in kidney. Morphologically the vast majority of cases showed classic and epithelial type. The classic ones are always benign in clinic. And only rare cases with recurrence, lymph nodes or distant metastasis were reported in the literature, with a certain degree of malignancy. Although there are a lot of reports on RAML, most of them are case reports or small sample studies. Up to now, there are only 6 large sample studies of more than 100 cases in the literatures, and the conclusions are not consistent. Currently, there is still some controversy about the biological behavior and prognosis of RAML. At the same time there are only two articles compared the classic and epithelial RAML. The differences between the clinicopathological parameters are not yet clear. In addition morphologically epithelial type RAML and other renal tumors have a certain degree of overlap, which cause difficulties in differential diagnosis.

Design: We collected 414 cases of RAML diagnosed and treated the PLA General Hospital from 2004 to 2011. Based on the large sample size and the follow-up results, the clinicopathological features were retrospectively reviewed and statistically analyzed and summarized combining with follow-up data to further explore the differences between the classic and epithelial RAML samples, to clarify the relationships between clinicopathological features and biological behavior, and finally to provide the theoretical guidance for clinical treatment of RAML.

Results: 414 cases of RAML were diagnosed and treated in those 8 years, accounting for 8.11% of the renal parenchymal tumors in the same period. Of the 414 patients, 122 were male and 292 were female, with a ratio of 1:2.39 for male-to-female men, average age 44.02 (range: 15-74 years). 191 cases occurred in the left kidney (46.13%), 209 cases (50.48%) of the right kidney and 7 cases (1.69%) bilaterally. Clinically some cases were identified by hypochondrial pain，hematuria and palpable masses. Gross examination showed tumor sizes ranged from 0.3 to 31 cm, with an average of 6.2 cm. Histologically, 394 (95.16%) showed classic type, 20 (4.83%) with epithelioid RAML, and, 54, 23 and 7 cases with hemorrhage, necrosis and cystic degeneration. Peri-kidney fat invasion, atypical cells and the polymorphic / giant tumor cell were found in 5, 30 and 14 cases. Statistical analysis showed that there was a correlation between atypia cells and tumor type. Atypia cells were found in epithelioid type more often. The other clinicopathological parameters were not correlated with histological type. Follow-up data was obtained from 360 patients. Follow-up lasted from 3 to 99

months. One case died from other causes. The remaining cases remained physically normal. No tumor recurrence or metastasis was noted.

Conclusion: RAML is one of the common neoplasms of the renal parenchyma. Clinically, it is characterized as usually biologically benign. Histologically, the major histological features are the classic and epithelioid types. Due to similar morphology, the epithelioid type should be differentiated from the classic renal cell carcinoma, and translocation of MitF gene family associated with renal tumor and renal hemangioblastoma. The atypia cells can be found in epithelioid type more often, but do not affect the clinical prognosis of patients.

The clinical pathological features and grading diagnoses of bladder inverted urinary neoplasm

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Objective To investigate the clinical, pathological   of inverted bladder urothelial neoplasm(UN), and propose pathologic grading   criteria with verification.
Materials and Methods 156 patients of inverted bladder UN were searched during 2010.01-2016.12 of the General Hospital of PLA. The correlation between morphologic characteristics with infiltrative rate and survival rate were analyzed.
Results In 156 cases of inverted UNs selected in the study, 126 cases were non-invasive tumors, 30 invasive cases. The ratio of male and female was 6.4:1, the average age was 54 years old. 7 morphologic characteristics were analyzed, including the exogenous papillary, the structure of endogenous trabeculaes, central umbrella cells, tumor cell polarity, nuclear atypia, mitotic figures and pathological mitosis.   Between invasive and non-invasive tumors, there were statistical   significance in the structure of endogenous trabeculaes, tumor cell polarity, nuclear atypia, mitotic figures and pathological mitosis   ($P < 0.01$), but there was no significant difference in exogenous papillary and central umbrella cells ($P > 0.05$).   Follow-up results of 133 patients were obtained. Up to date of follow-up, 20.30% of the cases had recurrences and/or metastases. The tumor-free survival time was 1-30 months. And 7.52% died of bladder tumor. The overall survival time was 7-134 months. The structure of endogenous trabeculaes, central umbrella cells, tumor cell polarity, nuclear atypia, mitotic figures and pathological mitosis   were statistically correlated with tumor-free and overall survival ($P < 0.01$), but gender, age, exogenous papillary structure had no correlation with tumor-free and overall survival ($P > 0.05$). According to the results above we raised the grading standard of non-invasive inverted UN. Of the 156 cases, 33 cases were diagnosed with IP, 50 I-PUNLMP, 36 I-NIPUC, LG, and 7 cases were diagnosed to I-NIPUC, HG. The results of reverse analysis showed there were significant differences in tumor-free and overall survival ($P < 0.01$) among the inverted UNs with different pathologic grading. All the 7 pathologic characteristics were correlated with pathologic grading ($P < 0.01$), but gender and age were not significantly correlated ($P > 0.05$).
Conclusions In routine pathologic diagnostic work,   the structure of endogenous trabeculaes, tumor cell polarity, nuclear atypia, mitotic figures and pathological mitosis can be used as a pathologic grading reference index of non-invasive inverted UNs.   There was no significant difference in prognosis between the bladder UNs with different growth patterns. The grades of non-infiltrative inverted UNs were the influence factors of tumor infiltration and prognosis.

Overexpression of NGEF and CDKN2B are Associated with Malignancy of Papillary Thyroid Carcinoma

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Papillary thyroid carcinoma (PTC) is the most common type of cancer in the endocrine system. Owing to the strong links between phenotypes and genetic changes, identification of molecular biomarkers are of great importance in the diagnosis and treatment of cancer. In present study, we demonstrated that neuronal guanine nucleotide exchange factor (NGEF) and cyclin dependent kinase inhibitor 2B (CDKN2B) were differentially expressed between neoplastic and adjacent tissues. By comprehensively analyzing the transcriptome and corresponding clinical data from the Cancer Genome Atlas (TCGA), we further revealed that these two genes were upregulated in patients with higher stage, higher risk-group, higher MACIS score and the tall cell variant of PTC, which has an unfavorable prognosis. Moreover, the expression of these two genes were significantly associated with molecular characteristics of PTC, including TERT promoter mutation and $BRAF^{V600E}$-RAS score. Gene annotations suggested that the functions of NGEF and CDKN2B mainly involved cell motion, cell adhesion, proliferation and several tumor-related biological processes and pathways. Collectively, our observations identified NGEF and CDKN2B as oncogenes for tumor malignancy, as well as a monitoring and therapeutic biomarker for PTC patients.

Neuroendocrine neoplasms of the middle ear: report of 2 cases and review of the literature

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Objectives: Neuroendocrine adenoma of the middle ear is a rare tumor of the middle ear. And the origin as well as classification of the disease are still controversial. To better characterize this rare entity, we reviewed our case archive with respect to clinical characteristics, pathological features, and immunohistochemistry.
Materials and Methods: We collected relevant clinical data information of the two patients, including clinical manifestations, physical examination and imaging examination. Immunohistochemical staining of CK、CgA、Syn、CD56 and Ki-67 were performed using the streptavidin-peroxidase (S-P) method.
Result: In this article, we described 2 cases of neuroendocrine adenoma of the middle ear separately occurring in a 60-year-old male and 48-year-old patient who both presented with hearing loss. One of the case presents a growth mode in which the adenoid structure coexisting with trabecular, while another case predominantly show trabecular growth pattern composed of cuboidal-to-columnar cells with minimal pleomorphism. Both of them are positive for CK and Syn as well as a low proliferation index.
Conclusion: Neuroendocrine adenomas of the middle ear are rare tumors, at present, the classification, etiology and biological characteristics of these diseases are not clear. The existing research tends to believe that it is a kind of low-grade malignant lesion with mild cell morphology and local recurrence, but there is still a great controversy about the existence of distant metastasis. The average age of tumorigenesis was about 50 years old (14-80 years old). No significant gender difference was found. The most common and main symptoms were progressive hearing loss. Grossly, the cut surface is white gray or reddish brown, most tumors are less than 1cm in diameter. Under low magnification, with back-to-back glandular pattern, adenoid structure predominates in diversed growth pattern, and the cavity often contains amorphous mucin secretion. The lesions generally present as bilayer cells with eosinophilic cytoplasm in the inner cavity and columnar cubic cells in the surrounding basement. Other common growth patterns of tumors include solid, lamellar, trabecular, cystic, nested and sieve-shaped, pseudohemangioma-like, plasma cell-like, etc. Microscopically, cell pleomorphism is often seen, but mitotic phenomena rarely occur. Generally, there is no necrosis. CK7 is strongly and uniformly expressed in endoluminal cells of adenoid structure, and also expressed in other growth structures to varying degrees. Neuroendocrine markers are positive in basal cell layer of adenoid structure, which to some extent proves the double-layer cell morphology of adenoid structure. Middle ear neuroendocrine adenomas

need to be differentially diagnosed with a range of diseases, including cervical tympanic ganglionoma, adenoid cystic carcinoma, endolymphatic papillary tumor, meningioma with glandular structure, acoustic neuroma, rhabdomyosarcoma as well as other primary and metastatic adenocarcinoma of the ear.

Nanog and Gli1 positive feedback loop promotes stem-like traits of glioblastoma cells

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Objectives: To explore the effects of Nanog on stem cell-like properties and malignant biological behaviors of GBM cells and the possible positive feedback loop between Nanog and Hedgehog signaling pathway. Materials and Methods: NANOG overexpression (OE) GBM cells were established. Bioinformatics method, limit dilution assay, plate clone formation assay, transwell migration and invasion assay, qRT-PCR analysis, western blot were used to determine the effects of Nanog on stem cell-like properties and malignant biological behaviors of GBM cells. Result: NANOG is closely associated with stem-like traits of GBM patients　and forced-expression of NANOG markedly increased cancer stem cells markers and the capabilities of tumor spheres formation, clone formation, migration and invasion in GBM cells. Mechanically, overexpression of Nanog increased SHH signaling molecules indicating the possible positive feedback loop between Nanog and Hedgehog signaling pathway. Conclusion: Nanog acts as a crucial regulator of stem cell-like properties in GBM cells and it could be served as a novel therapeutic target in GBM.

The significance of the morphological and immunological characteristics in the diagnosis of parotid mammary analogue secretory carcinoma: five case reports and a review of the literature

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Abstract: Mammary analogue secretory carcinoma (MASC) of the salivary glands is rarely reported. In this article, the histopathological features of 5 cases of parotid MASC were retrospectively analyzed. AB/PAS and immunohisto-chemical staining of S-100, mammaglobin and P63 was performed, which were validated by the ETV6-fluorescent FISH detection of NTRK3 gene. The tumors were composed of two kinds of tumor cells. One kind of cells was rich in cytoplasms, transparent or vacuole-like, partial basophilic double tropism, but another type of cellular cytoplasm was acidophilic. The karyotype was consistent between two types of tumors in the vacuolar pattern. Although the tumor cells were arranged in different forms, the cystic (capsule or microcapsule) structures were constantly ob-served. Two kinds of tumor cells produced different secretions, which were distributed among the tumor cells. The tumor tissues were divided or wrapped by hardened interstitial collagen. The expression of mammaglobin and S-100 significantly differed between the two types of tumor cells. These characteristics contribute to the differential diagnosis of MASC. ETV6-NTRK3 gene detection can be applied in the diagnosis of atypical cases, but it should not be done routinely.
Figure 1. The tumor cells were divided and packed in collagen, even in the infiltrating tissues (A, B). Of the two types of tumor cells, one type was a cyto- plasm acidophilic and the other type was a cytoplasmic empty cell mass. The nuclei were round and vacuole and the cell abnormalities were not obvious (C, D). The tumor cells were arranged in sieve-like, cystic, follicular, papillary and solid flake patterns, but microcapsular structures could be observed (E, F).

Figure 2. Eosinophilic or basophilic sediments were irregularly distributed in the tumor cells (A, B). The special staining showed that the red dye (PAS staining) was located among the eosinophilic tumor cells, and blue dye was seen among the tumor cells with vacuolar cytoplasms (AB staining) (C). The cytoplasm was lightly stained and the

mammaglobin was highly expressed in the tumor cells, whereas the mammaglobin was lowly or not expressed in cytosolic eosinophilic tumor cells (D). S-100 was expressed in both types of tumor cells, but the expression level and location were inconsistent (E), ETV6 gene rearrangement was detected in MASC (F).

ERBB3, IGF1R, and TGFBR2 expression correlate with PDGFR expression in glioblastoma and participate in PDGFR inhibitor resistance of glioblastoma cells

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Glioma, the most prevalent malignancy in brain, is classifed into four grades (I, II, III, and IV), and grade IV glioma is also known as glioblastoma multiforme (GBM). Aberrant activation of receptor tyrosine kinases (RTKs), including platelet-derived growth factor receptor (PDGFR), are frequently observed in glioma. Accumulating evidence suggests that PDGFR plays critical roles during glioma development and progression and is a promising drug target for GBM therapy. However, PDGFR inhibitor (PDGFRi) has failed in clinical trials, at least partially, due to the activation of other RTKs, which compensates for PDGFR inhibition and renders tumor cells resistance to PDGFRi. Therefore, identifying the RTKs responsible for PDGFRi resistance might provide new therapeutic targets to synergetically enhance the effcacy of PDGFRi. In this study, we analyzed the TCGA glioma database and found that the mRNA expressions of three RTKs, i.e. ERBB3, IGF1R, and TGFBR2, were positively correlated with that of PDGFR. Co-immunoprecipitation assay indicated novel interactions between the three RTKs and PDGFR in GBM cells. Moreover, concurrent expression of PDGFR with ERBB3, IGF1R, or TGFBR2 in GBM cells attenuated the toxicity of PDGFRi and maintained the activation of PDGFR downstream targets under the existence of PDGFRi. Thus, ERBB3, IGF1R, and TGFBR2 might participate in PDGFRi resistance of GBM cells. Consistent with this notion, combination of PDGFRi with inhibitor targeting either ERBB3 or IGF1R more potently suppressed the growth of GBM cells than each inhibitor alone. The positive correlations of PDGFR with ERBB3, IGF1R, and TGFBR2 were further confrmed in 66 GBM patient samples. Intriguingly, survival analysis showed that ERBB3 predicted poor prognosis in GBM patients with high PDGFRA expression. Altogether, our work herein suggested that ERBB3, IGF1R, and TGFBR2 were responsible for PDGFRi resistance and revealed that ERBB3 acted as potential prognostic marker and therapeutic target for GBM with high PDGFRA expression.

Familial hereditary salivary gland cancer: presentation of six cases and pathological comparisons with salivary gland counterparts

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Objectives: The 2017 World Health Organization (WHO) classification divides salivary gland cancers into 20 different histological types. We described an unusual familial hereditary salivary gland cancer which differ from previous reported tumors. We aimed to study the detailed clinicopathologic features of this tumor and compare with salivary gland counterparts.
Materials and Methods: In our database, there were materials of 6 patients with familial hereditary salivary gland cancer from the same family. They were diagnosed and received treatment between 2009 and 2018 in our hospital, primary or recurrence
cases. All of the patients were female. The tumor affected three generations of the family. Family history was obtained. Using a wide panel of antibodies, we attempted to corroborate our observations on the immunohistochemical (IHC) level. These cases were immunohistochemically analyzed by a epithelial markers (CK7, CK8/18, CK19, EMA) , myoepithelial markers (S100 protein, p63, p40, SOX10, calponin, SMA) and other markers (Vimentin, E-cadherin, Mammaglobin, GCDFP15, MUC4, Dog1, CD117, SOX10, CEA, Synaptophysin, ChromograninA, Estrogen receptor, Progesterone receptor, Androgen receptor). A clinicopathologic review was conducted by at least 2 Head and Neck pathologists.
Result: The tumors involved major and minor salivary glands, size 2-10cm. Histologically, the tumor showed prominent papillary growth including true-papillae with fibrovascular cores and pseudopapillae. Another pattern always present was the tumor cells formed varying amounts of solid nests, cords, trabeculae structure separated by the eosinophilic hyaline or basement membrane－like material. High-grade tumor features such as an increased mitotic count or tumor necrosis were not identified. All tumor components were positive strongly and diffusely for CK8/18, Vimentin and E-cadherin, whereas negative for CK7, CK19, EMA, Mammaglobin, GCDFP15, MUC4, Dog1, CD117, SOX10, P63, P40, Calponin, SMA,CEA, Synaptophysin, ChromograninA, Estrogen receptor, Progesterone receptor, Androgen receptor, S100 protein, minority and scattered expression or completely negative. Four of 6 patients showed regional recurrences, none of the distant metastasis. Follow up for 1to10 years, all patients were alive.
Conclusion: According to our observations, this tumor may be a distinct entity of salivary gland based on its characteristic morphology and unique immunophenotype. It represents a family with initial and recurrent tumors in

the major and minor salivary glands. The tumor is a low-grade malignant neoplasms occurring predominantly in young women which have a potential to recur, but none of the distant metastasis. Our findings suggest that lack of CK7, but diffuse CK8/18, Vimentin and E-cadherin expression may be a clue of this tumor.

Clinical and pathological analysis of 53 cases of encapsulated papillary thyroid carcinoma

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Objective To investigate the clinicopathological features, lymph node metastasis rate, treatment and prognosis of encapsulated papillary thyroid carcinoma (EPTC).
Materials and Methods A retrospective analysis of 53 cases of EPTC was performed from September 2015 to December 2018 in the Pathology Dept, Renmin Hospital of Wuhan University.
Result 53 patients with EPTC were 20-73 years old (mean age was 44.2 years); the ratio of male to female was 1:2.5; tumor diameter was 0.1-3.2cm; unilateral tumor was more common; cervical lymph node metastasis rate was 13.2%; the cervical lymph node metastasis rate of common papillary carcinoma was significantly higher than that of papillary micro-carcinoma ($P<0.05$), and the prognostic survival rate was 100%.
Conclusion EPTC is a special subtype of papillary thyroid carcinoma. The rate of cervical lymph node metastasis is closely related to tumor size. The treatment is mainly surgical resection and the prognosis is relatively good.

Solitary Fibrous Tumor of Nasal Cavity: A Clinicopathologic Study of Four Cases and a Literature Review

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〔Abstract〕

Objective To explore the clinicopathological features and diagnosis of solitary fibrous tumor (SFT) of nasal cavity. SFT are rare neoplasms first described in 1973 by Klemperer and Rabin. They arise mostly from serous membranes, such as pleura, also occur in many other sites, mainly in mediastinal space, lungs, vulva, orbit, thyroid, nasopharyngeal region, larynx, salivary glands, while SFTs of the nasal cavity and paranasal sinuses are extremely rare, accounting for <0.1%. Adults are mainly affected, with no sex predilection.

Materials and methods: we have collected four cases primary SFTs of nasal cavity of Ren Min Hospital of Wuhan University between year 2017 to 2019. Hematoxylin and eosin stained slides from all cases were reviewed.

Result: Patients included two females and two males, ranging from 32 to 53 years old, average 44 years old. All patients presented with nasal obstruction and epiphora, no epitaxis. The duration of symptoms ranged from 0.5-3years. Abnormal signal of CT or MRI can be observed in nasal cavity and paranasal sinuses. Three of them on left side. The tumors size ranged from 1.5cm-6.5cm in greast dimension with an average size of 4.5cm. Two tumors involved the nasal cavity, two tumors were invasive of both nasal sinus and nasal cavity. Tumors are polypoid, firm, and white, cutting tan to pale. Microscopically all tumors show the same pathomorphism. Tumors are submucosal, psudoencapsulated, bland pulmp spindle cells dispersed within a collagenous stroma, haphazard arrangement, hypercellular and hypocellular sclerotic foci, and a prominent branching vasculature. Paucicellular areas of the tumor were dominated by sclerotic to hyalinized stroma with rare interspersed spindle cells, more cellular region, the spindle cells had a vaguely storiform or fascicular arrangement. Ovoid nuclei with fine chromatin, inconspicuous nucleoli and scant eosinophilic cytoplasm. Immunohistochemically, the cells show a specific reaction with STAT6 (nuclear) and CD34, also positive to BCL2, CD99, but are non-reactive with desmin, S100 protein, actins, and nuclear beta-catenin. For SFTs of nasal cavity endoscopic excision is the preferred surgical approach, 5 to 10% of all extra pleural SFTs have shown recurrence. Therefore, prognosismainly depends on the completeness of the surgical resection.

Conclusion: SFTs are rare cases in nasal cavity and paranasal sinus, the definitive diagnosis of SFTs rely on the characteristic histopathological features and specific immunohistochemical markers.

The value of BRAF V600E gene detection in thyroid cytological diagnosis via a large population

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Objective To study the diagnostic value of $BRAF^{V600E}$ gene detection combined with the thyroid fine needle aspiration cytology (FNAC) in thyroid nodule disease, and the clinical significance of $BRAF^{V600E}$ gene mutation detection combined with the thyroid fine needle aspiration cytology in the prognosis of papillary thyroid carcinoma (PTC). **Methods** A total of 6399 patients with thyroid nodule disease were collected from Affiliated Tumor Hospital of Zhengzhou University between February 2016 and September 2018. All patients underwent $BRAF^{V600E}$ gene mutation detection. There were 2447 patients with histopathological control results. The relationship between $BRAF^{V600E}$ gene mutation and the clinicopathologic features was retrospectively analyzed in 1535 cases of PTC patients with complete clinicopathologic data. **Results** The definite diagnosis rate of the thyroid FNAC in the diagnosis of thyroid nodule disease was 73.68% (1803/2447). The sensitivity, specificity and accuracy of $BRAF^{V600E}$ gene detection in the diagnosis of thyroid nodule disease was 90% (1756/1948), 97% (513/526) and 92.72% (2269/2447), respectively. The sensitivity, specificity and definite diagnosis rate of the thyroid FNAC combined with $BRAF^{V600E}$ gene detection in the diagnosis of thyroid nodule disease was 98% (2046/2080), 96% (195/204) and 93.34% (2284/2447), respectively. $BRAF^{V600E}$ gene detection can significantly improve the sensitivity and accuracy of the thyroid FNAC in the diagnosis of thyroid nodule disease ($P<0.05$). The presence of $BRAF^{V600E}$ gene mutation in PTC patients correlated with age at diagnosis, extrathyroidal extension, number of cervical lymph node metastasis and central region lymph node metastasis, type of calcification, hashimoto's thyroiditis and focal involvement of bilateral thyroid ($P<0.05$). **Conclusions** 1. $BRAF^{V600E}$ gene detection can improve the sensitivity and accuracy of the thyroid FNAC in the diagnosis of thyroid nodule disease. 2. The combination of thyroid FNAC and $BRAF^{V600E}$ gene detection can improve the preoperative definite diagnosis rate. 3. The presence of $BRAF^{V600E}$ gene mutation in PTC patients was correlated with multiple clinicopathologic features, which could be used as a correlation factor to predict PTC recurrence.

Clinicopathological features of sinonsal teratocarcinosarcoma

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Abstract: Objective To study the clinical and pathological characteristics of sinonsal teratocarcinosarcoma (SNTCS) and to analyze the points of diagnosis and differential diagnosis. **Methods** The cases were collected in Department of Pathology of the first medical center of PLA General Hospital from January 2005 to July 2019. The clinical data, pathological features，immunohistochemical staining results were analyzed with review of the related literature.　**Results** All the 6 patients diagnosed as SNTCS were male, aged from 26 to 61 years(mean 41 years). The clinical presentation was nasal obstruction, nose bleeding, decreased olfactory function and headache，1 patient presented with cervical lymph nodes enlargement. Histopathologically，all the 6 cases had primitive neuroectodermal and squamous epithelium components，ameloblastoma-like structure was seen in 1 case, adenoid structure was seen in 3 cases with obvious atypia, spindle cell sarcoma components were seen in 3 cases，and osteosarcoma components were seen in 1 case. The tumor involved bone tissue, and necrosis was seen in some areas. Immunohistochemical staining showed CKpan positive in squamous and glandular epithelium. The primitive neuroectoderm components were positive for NSE，Syn and CD99. Spindle cell components expressed Vimentin. Five of the six patients underwent surgical resection of the tumor and received postoperative radiotherapy, chemotherapy or combined radiotherapy and chemotherapy; the other one received chemotherapy combined with local radiotherapy after biopsy due to the huge tumor accompanied by cervical lymph nodes metastasis. The follow-up period was 6-98 months, and the mean survival time was 37.7 months. 2 patients died of tumor recurrence 6 months and 9 months after surgery, respectively. **Conclusions** Sinonsal teratocarcinosarcoma is a rare and highly malignant tumor，which is highly invasive e and prone to recurrence. However, some patients can still survive for a long time after operation combined with radiotherapy and chemotherapy. This disease needs to be differentiated from olfactory neuroblastoma, squamous cell carcinoma and teratoma，etc. At present, there is insufficient understanding of this disease and it is easy to be misdiagnosed，so the understanding of this disease needs to be strengthened.

MED12 down-regulates resistance to the treatment of papillary thyroid cancer 131I by TGFBR2

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Laboratory of Pathology

Objective: MED12 has been shown to be negatively associated with multiple drug resistance in a variety of tumors, but its function in the papillary thyroid has not been demonstrated. Therefore, the role of MED12 in the occurrence and development of papillary thyroid carcinoma was investigated by studying its function.
Methods: previous studies have reported that MED12 is associated with multiple drug resistance in tumors, but its role in papillary thyroid cancer has not been reported. Therefore, in this study, we constructed papillary thyroid cancer cell stable strains (tpc-1 and b-cpap) with MED12 knockdown (MED2^{KD}). The motility of MED2^{KD} cells was detected by transwell and scratch test. Cell sensitivity of ^{131}I in different groups was analyzed . TGFβ pathway inhibitors were used to restore some of the experimental results. The changes of downstream signal factors were analyzed by immunofluorescence technique and WB. Subcutaneous tumorigenesis model in mice was used to analyze the in vivo results of ^{131}I treatment.
Results: In our experiment, we proved that the down-regulated expression level of MED12 could promote the development of EMT in cells, enhance the exercise ability, and induce the resistance of ^{131}I treatment. Meanwhile, we found that the down-regulation of MED12 can activate the TGFβ pathway, and CO-IP showed that MED12 and TGFBR2 can interact directly. TGF beta pathway inhibitors (LY2157299) were used to restore the down-regulated phenotype of MED12. The expression levels of p-erk1/2 and p-smad2 were up-regulated in MED2KD cells. In vivo experiments demonstrated a reduced ^{131}I sensitivity to MED2^{KD} tumors in mice.
Conclusion: In this study, it was revealed that the down-regulation of MED12 promoted EMT in papillary thyroid cells, enhanced cellular motor ability, and decreased sensitivity to ^{131}I treatment. It provides a new target for the treatment of papillary thyroid carcinoma.

Category: Head & neck Pathology
1655987

MED16 regulates the sensitivity of 131I to papillary thyroid carcinoma

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Objective: To investigate the role of MED16 in the development of papillary thyroid carcinoma and its function.

Method: Using the online database MERAV (http://merav.wi.mit.edu/SearchByGenes.html) found that MED16 expressed in tumor tissues in low. Papillary thyroid cancer cells (tpc-1 and b-cpap) MED16 knockdown (MED16^{KD}) stable strains were constructed and verified by q-PCR and WB. Cell growth, migration and ^{131}I sensitivity (200mCi/ well, 96-well plate) were analyzed by using this cell line. Subcutaneous tumor formation model in mice was used to verify whether MED16^{KD} reduced the therapeutic sensitivity of ^{131}I in vivo tumors (1.5mci/mouse) and to detect changes in downstream signaling pathway factors.

Results: In the experiment, two shRNA targeted knockdown MED16 expression levels were constructed. The knockdown efficiency of sh1 and sh2 was 79% and 42% respectively at PCR level, and the WB level was also significantly knocked down. The migration ability of MED16KD cells was significantly enhanced (scratches and transwell test).The sensitivity of MED16^{KD} cells to ^{131}I treatment was significantly lower than that of the control group, and similar results were obtained in vivo.

Conclusion: In this study, we found that the expression level of MED16 in tumor tissues was relatively low, suggesting that it plays an important role in the development of tumor. At the same time, in the cell lines with low MED16 knockdown and in vivo mouse models, we found that its ^{131}I treatment sensitivity was lower than that of the control group, and the cellular motor ability was enhanced. Therefore, we proposed a new molecular mechanism of 131I resistance in papillary thyroid carcinoma and provided a new possible target for clinical treatment.

Ameloblastoma with mucous cell differentiation: a clinicopathological and BRAF V600E mutation study of five cases

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Context.—Ameloblastoma is one of the most common epithelial odontogenic neoplasm of the jaws. However, ameloblastoma with mucous cell differentiation is an extremely rare phenomenon.

Objective.—To investigate the clinicopathological features, *BARF V600E* mutation status and discuss the prognosis significance of the amelablastoma with mucous cell differentiation.

Design.—Five cases of ameloblastoma with mucous cell differentiation were retrieved from the document files between 2008 and 2018 and reviewed by two independent pathologists. Clinicopathological information including sex, age, tumor location, size and medical history was obtained from patients' medical records. All the cases were submitted for *BRAF V600E* mutation detection. Alcian blue (AB) staining is used to confirm the presence of the mucous cells. *MAML2* fluorescence in situ hybridization (FISH) was performed to eliminate the possibility of diagnose of primary intraosseous mucoepidermoid carcinoma of the jaw. Follow-up information was obtained after surgery.

Results.—Of five cases, two cases were male and three cases were female. The age ranged from 18 to 55 years, with a mean age of 39.8 years. Four cases located in mandible and one case located in maxilla. Histologically, two cases were diagnosed as unicystic ameloblastoma and three were conventional ameloblastoma. Two of those three conventional ameloblastom showed cystic degeneration. Three of five cases (60%) were presented with varying degrees of squamous metaplasia. The mucous cells were located in the epithelial islands or the luminal aspect of the cystic cavities. *BRAF V600E* mutation was found in three of five cases (60%). All the cases showed no *MAML2* rearrangement. The clinical follow-up information was available for all five patients (12-48 months, mean 26 months) and three cases were recurrent lesions or had local recurrence during the follow-up.

Conclusions.—Ameloblastoma with mucous cell differentiation is closely related to the squamous metaplasia, cystic features and shows a high prevalence of *BRAF V600E* mutation. Due to the relationship between mucous cell differentiation and ameloblastoma recurrence, once the presence of the mucous cells is confirmed in a particular case, much closer follow-up might be needed.

Category: Head & neck Pathology
1656443

The expression and significance of EpCAM and beta-catenin in mucoepidermoid carcinoma of salivary gland

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Abstract(#br)Objective(#br)To investigate the expression and clinicopathologic significance of EpCAM and beta-catenin proteins in mucoepidermoid carcinoma (MEC). (#br)Method(#br)The expression of EpCAM and beta-catenin in 57 cases of MEC and 20 cases of normal salivary gland tissues was detected by immunohistochemical staining (Envision method). (#br)Result(#br)The positive expression rate of EpCAM and beta-catenin in MEC was significantly higher than that in normal tissues (P=0.002, P=0.001). The positive expression rates of EpCAM in MEC with high, medium and low differentiation were 36.36%, 61.11% and 82.35%, respectively (P=0.015). The positive expression rate of beta-catenind was 45.45%, 72.22% and 88.24%, respectively (P=0.016). Both EpCAM and beta-catenin expression were correlated with patient age (P=0.004, P=0.004). EpCAM was correlated with lymph node metastasis (P=0.024). The expression of EpCAM and beta-catenin was independent of the gender of the patient and the location of the tumor. The expression of EpCAM and beta-catenin in MEC was positively correlated (R=0.48, $P < 0.001$). (#br)Conclusions(#br)EpCAM and beta-catenin are associated with the degree of malignancy of MEC, and in MEC, combined detection of EpCAM and beta-catenin is helpful for MEC classification and for prognosis prediction. It provides the basis for the use of EpCAM targeted therapy in MEC.

Clinicopathologic analysis of primary parotid mucinous epidermoid carcinoma

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Abstract: Objective Mucoepidermoid carcinoma of the head and neck was the most common primary malignant tumor of the salivary glands, but there were few related studies at home and abroad. This study mainly analyzed the clinical and pathological characteristics of 38 patients with mucoepidermoid carcinoma in anhui provincial hospital from July of 2007 to January of 2019, so as to provide better references for clinical and pathological studies.   **Methods** Relevant clinicopathological datas were retrospectively analyzed, and the clinicopathological associations of patients in early and late stage and different sites were analyzed with SPSS23 software.   **Results 1. Basic clinicopathological features:** the average age of the patients was 49 years old (24–93 years old), with 18 males (47%) and 20 females (53%). Twenty cases (53%) were located in the parotid gland, and 18 cases (47%) were not located in the parotid gland. All patients were admitted with corresponding site mass, and the course of the disease ranged from 1 week to more than 20 years. Early TNM stage was 29 cases (76%), with stage Ⅰ 15 cases and stage Ⅱ 14 cases; Late stage was 9 cases (24%) , with stage Ⅲ 4 cases and stage Ⅳ 5 cases. Six cases (16%) were cystic, 17 cases (45%) were solid, and 15 cases (39%) were solid. The mean maximum diameter of tumor was 2.5cm (0.8cm–9.3cm) : 15 cases were <2cm (39%), 15 cases (39%) were 2cm–4cm, 8 cases (22%) were > 4cm. Microscopically, mucus, intermediate and epidermal cells was identified, with low grade (17 cases, 45%), medium grade (14 cases, 37%) and high grade (7 cases, 18%). **2. The clinicopathological correlation between different sites and different stages of MEC:** compared with the non-parotid group, the parotid group presented significantly increased cystic and low-to-intermediate grade (P<0.05), but there were no significant differences in gender, age, left and right sides, maximum tumor diameter and survival. Compared with the early stage, the advanced stage MEC patients with older age, solid, maximum diameter of >4cm, high grade and disease death were significantly increased (all P <0.05). In addition, women with early stage were more than those with advanced stage (P=0.058). TNM stage was not related to the left and right sides and the site of occurrence. **Conclusion** In this region, the prognosis of MEC in parotid gland and female was good, and the prognosis of MEC in late stage , advanced age, solid, maximum diameter >4cm and high grade was poor.

Clinicopathologic features and prognostic factors of widely invasive carcinoma ex pleomorphic adenoma of parotid gland: a clinicopathologic analysis of 126 cases in a Chinese population

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Objectives: Salivary carcinoma ex pleomorphic adenoma (CXPA) can be divided into three subtypes: intracapsular CXPA (ICCXPA), minimally invasive CXPA (MICXPA) and widely invasive CXPA (WICXPA). The former two entities generally have favorable outcomes, whereas the latter frequently results in disease related death. The aim of this study was to analyze the clinicopathologic features and prognostic factors of WICXPA of parotid gland.
Materials and Methods: The clinicopathologic parameters of 126 patients with primary WICXPA of parotid gland, 63 patients with ICCXPA and 52 patients with MICXPA were retrospectively reviewed. The differences in the clinicopathologic features between IC/MI CXPA and WICXPA and the correlation between the clinicopathologic parameters and survival of patients with WICXPA were statistically analyzed.
Result: Patients with WICXPA were older than IC/MI CXPA (59.6 vs 51.4 years; $P<$ .001), and had larger tumor diameter (3.9 vs 3.3 cm; $P=$ .040). Proportion of histological high-grade ($P<$ .001) and proportion of carcinoma more than 50% ($P<$ .001) in WICXPA was higher than IC/MI CXPA. Of 118 (94%) patients with WICXPA the follow-up information was available, 36 (31%) died of the tumor. Using the Kaplan-Meier method, age, T stage, N stage, and proportion of carcinoma were found to be significantly associated with disease-specific survival (DSS) of WICXPA. Cox regression analysis indicated that age, T stage and N stage were independent prognostic factors of DSS of WICXPA.
Conclusion: Older age, later T stage, higher proportion of carcinoma and histological high-grade were associated with WICXPA. Age, T stage and N stage were the important independent factors for predicting prognosis in patients with WICXPA.

Evaluation of P16 as a surrogate marker for transcriptionally active HPV status of OPSCC in an eastern Chinese population

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Objectives: Transcriptionally active human papillomavirus (HPV) is proved to be an etiologic risk factor and independent prognosis predictor for oropharyngeal squamous cell carcinoma (OPSCC). Immuno-expression of protein P16 is widely used as a surrogate marker for HPV status detection. However, the prevalence of HPV in OPSCC and the diagnostic ability of P16 for Chinese OPSCC patients remain unclear. This study aimed to investigate prevalence, clinicopathological characterization for HPV-positive OPSCC patients and evaluate diagnostic validity of P16 for transcriptionally active HPV status in an eastern Chinese population.

Materials and Methods: A total of 257 paraffin-embedded primary OPSCC specimens were collected from 2014 to 2019 in this study. HPV RNA In Situ Hybridization (ISH) was conducted as the gold standard for the detection of transcriptionally active HPV status and HPV DNA PCR, P16 immunochemistry (IHC) were also performed and analyzed for comparison.

Result: The HPV infection rate in OPSCC patients was 18.29% (47/257), which showed an increasing trend from 5.71% to 19.05% between 2014 and 2019 in this eastern Chinese population. Among 66 P16-positive cases, only 66.67%% (44/66) of the cases were determined to be truly HPV-positive by using HPV RNA ISH. P16 IHC had the worst diagnostic ability with sensitivity of 93.48% and specificity of 89.1% when compared with HPV DNA PCR and combination of P16 IHC and HPV DNA PCR, and it could not serve as a prognostic predictor (disease-specific survival, DSS, p=0.405). Notably, the survival discrepancy was observed between P16 and HPV RNA double positive and P16 positive but HPV RNA negative OPSCC patients (DSS, p=0.03).

Conclusion: The HPV infection rate of OPSCC is increasing in this eastern Chinese population. Solitary P16 IHC is insufficient for HPV status detection, and additional HPV DNA specific testing may be necessary for accurate HPV status identification.

Category: Head & neck Pathology
1656656

Clinicopathologically Relevant Molecular Subtypes in 85 cases of pleomorphic adenoma, a review study.

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Abstract
Objectives
Pleomorphic adenoma is the most frequent salivary gland tumor with tendency to recur and ability of malignant transformation. Owing to its morphological overlap and recurrent translocations, pleomorphic adenoma shows a variety of biological features. Here we investigated the association between PLAG1 rearrangement and clinicopathologic characteristics, which could further be evaluated for clinical decision-making.

Methods
85 cases of pleomorphic adenomas tissues were collected in the period October,2015 to May,2016 and detected for PLAG1 rearrangements. All pathological descriptions were reviewed by two pathologists. Association of PLAG1 rearrangement with clinicopathologic parameters in patients with pleomorphic adenoma was evaluated.

Results
Forty-one cases (48.2%) were diagnosed as cell-rich PA and forty-four cases (51.8%) as mesenchymal-stromal subtype. Thirty-one (36.5%) cases were detected with PLAG1 rearrangements in all 85 patients. Among all patients, 2 cases were detected with PLAG1-CHCHD7 rearranged genotypes and 29 cases were PLAG1-CTNNB1 rearranged genotypes. Mesenchymal-stromal subtypes had higher level PLAG rearrangement (P=0.026). Pleomorphic adenomas with PLAG1 rearrangements were related to satellite nodules with myxoid predominant subtype. With PLAG1 rearrangement, pleomorphic adenomas showed preferable myoepithelial differentiation.

Conclusions
These results suggest that the detection of PLAG1 rearrangements may potentially predict the clinicopathologic features of pleomorphic adenomas. The present study is essential in the choice of the therapeutic strategy.

Clinicopathological analysis of Warthin-like variant of papillary thyroid carcinoma

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Objective To investigate the clinicopathological features, immunohistochemistry, diagnosis and differential diagnosis of Warthin-like variant of papillary thyroid carcinoma (WLPTC). **Methods** The clinical manifestations, histopathological features and immunohistochemical findings of a case of WLPTC was analyzed, and the related literatures were reviewed. **Results** The patient was a 34-year-old female. There was a mass of the left neck two months ago with no obvious incentive. B-ultrasound indicates very low echogenic nodules under the thyroid gland (TI-RADS classification-4b). Cytological smears showed that thyroid follicular epithelial cells were atypical. Histologically, the tumor tissues were arranged in broad papillary shape, and the nuclei of the cells were characterized by the nuclei of thyroid papillary carcinoma. A great number of lymphocytes and plasma cells infiltrated in the center of the papillary axes and scattered in multinucleated giant cells. Immunohistochemistry showed that CK19, Galectin-3 and HBME-1 were expressed in tumor cells, and CD16 3 and CD68 were expressed in some multinucleated giant cells. **Conclusion** WLPTC is a rare subtype of papillary thyroid carcinoma. The accuracy diagnosis depends mainly on histological morphology, immunohistochemistry and differentiation from other benign and malignant thyroid diseases.

Biological effects of USP22 on thyroid papillary cancer cells

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Objective To explore the role of USP22 in thyroid papillary cancer cell (TPC).
Method   USP22 was detected in TPC and normal thyroid epithelial cell lines, and its biological function was analyzed.
Result   After silencing the USP22 gene of TPC-1 cells, expression of USP22 gene and protein decreased significantly. Migration was less and the cells became smaller within 6 hours, compared with the control group($P<0.05$). The number of invasive cells at 24 hours was significantly lower than that of the control group($P<0.05$). The cell viability test showed that the difference increased from day 4 to 5 ($P<0.05$). The number of colony-forming cells also decreased significantly after 10 days ($P<0.05$).
After silencing of USP22 gene in normal HT-ori3 cells, expression of USP22 gene and protein decreased significantly ($P<0.05$). Cell viability test showed the difference was increased   ($P<0.01$), and the number of cloned cells decreased significantly ($P < 0.01$).
Conclution USP22 gene plays an important role in the growth, proliferation, invasion and migration of papillary thyroid cancer cells. USP22 also play an indispensable role in the growth of normal thyroid cells.

The rare histological features in a primary mucinous carcinoma of the thyroid gland: a case report and review of the literature

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Objectives: This study reports a case of primary mucinous carcinoma of the thyroid gland with lymph nodes metastasis and invasion of striated muscle, and reviews the literature to assess its incidence, fatality and the prognosis of these patients.
Materials and methods: A 64-year-old male patient presented with a 5×5×4-cm mass in the right lobe of thyroid gland and a 1-week history of progressively increased swelling in the thyroid gland. Computed tomography revealed a a solido-hypoechoic nodule with calcification in the right lobe of the thyroid gland, accompanied with lymphadenectasis. The TSH levels of blood was elevated at 4.45mL U/L. The patient have underwent the right thyroidectomy with right-sided modified radical neck dissection was performed. Postoperative pathological examination revealed the tumor was characterized by the tumor cells arranged in small nests or trabeculae with the presence of abundant mucus and agglomeration of large atypical cells. The tumor cells formed diffuse invasion among thyroid follicles. In the peripheral regions, myxoid cells extended into the extrathyroidal striated muscle and fat tissue. Tumor cells can be seen in the interstitial vessels, and which lead to 6 lymph nodes metastasis. Immunohistochemical studies showed staining of the tumor for TTF-1, AE1/3, Vimentin, NSE and CD34. Stains for S-100, P63, CD23, Calcitonin and SMA were negetive in the tumor cells. The patient remained alive more than 1 month after operation.
Results: On the basis of the histological features and immunohistochemical expressions, a diagnosis of primary mucinous carcinoma of the thyroid gland with lymph nodes metastasis and invasion of striated muscle was confirmed.
Conclusion: This case will cause high attention not only because of it's low incidence but also lie in the rare histological features of a mucinous carcinoma. We believe that the case report will play an important and beneficial role for diagnosing and distinguishing the cases of mucinous carcinoma of the thyroid in the future.

Sebaceous Carcinoma of the Parotid Gland: 5 cases of the clinicopathological features analysis

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Abstract:

Objective: to investigate the clinic-pathological features of sebaceous gland carcinoma of salivary gland.

Methods: to collect 5 cases of salivary gland sebaceous gland carcinoma diagnosed in the First Medical Center of People's Liberation Army General Hospital, from 2005 to 2019. And retrospectively analyzed the clinical pathological characteristics, and reviewed the literature.

Results: Among 5 cases, one was consultation case, and 4 cases were from our hospital surgical samples. There were 3 cases of female patients, 2 cases of male patients. Aged 53-72yr, with the median age of 55yr. Location: there were 3 cases in the left parotid gland, 1 case in right parotid gland, and 1 case in right submandibular gland. There were 1 case occurred in the left parotid gland had invasive of temporal bone and the plane for the first time to see the doctor. In 5 patients, there were 3 cases performed for painless mass, which was because of gradually increasing mass to attendance; other two cases showed the pain and gradual increasing masses. Macroscopic examination: there was gray mass in salivary gland tissue, the largest diameter were 2-5.5 cm, the cutting surface was plane hoar, soft, and there was no clear boundary between the surrounding tissue. Microscope examination: epithelioid tumors were arranged in nests or lobules with invasive growth pattern. The tumor cells were atypia, with rich cytoplasm; and some of the cells' cytoplasm were bright or containing small vacuoles. There were a little of basaloid cells around, and the gradual differentiation characteristics of the transparent vacuolated sebaceous gland cells could be seen. Nerve invasion and vascular tumor emboli were detected in 3 cases. The immunohistochemistry phenotype was still lack of special antibodies. Prognosis: there were 2 cases had postoperative recurrence in 1 month and 6 months, respectively, and a second surgery was carried out. There were 2 cases had lymph node metastasis. Besides operation, only 1 case accepted 3 courses of radiation and 2 courses of chemotherapy.
Conclusion: sebaceous gland carcinoma occurred in salivary gland is very rare, and aggressive. It is necessary to study the clinical pathological characteristics of this tumor to avoid misdiagnosis.

The study of molecular pathology and genetic characteristics in adenoid cystic carcinoma

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Objectives

Adenoid cystic carcinoma is one of the most common salivary gland malignancies, which has a known propensity for aggressive perineural invasion and high rates of metastasis, ultimately resulting in low survival rates. In order to explore the genetic alterations of Chinese cohort of ACC, we performed genomic analysis of these tumors and utilized PDX models to seek potential targets for therapy.

Materials and Methods

31 fresh tumors and matched-normal tissues from Chinese ACC patients were collected for WES and RNA-seq. Sample library construction and bioinformation analysis were applied. We established 9 PDXs from the primary tumors and screened up for assessment of the recapitulation of biology of the original tumors. H&E, immunohistochemistry and fluorescence in situ hybridization (FISH) were also exhibited to confirm the biological stability. Potential targets were explored using PDX models in vivo, according to clinical pathological and genetic information.

Result

We identified a mean of 31.75 somatic mutations per sample, which is low compared to most solid tumors. The range of mutation per tumor was from 122 to 293. The differences in the genetic landscape of tumors based on different pathological type. The solid type ACC tended to harbor more Notch1 mutations (2 out of 6) while non-solid type possessed 2 mutations in 28 cases. POU6F2 was the most frequently mutated gene, which reflected in 7 samples (32.3%). Somatic mutations, including KMT2C, Notch2, TP53, PIK3CA, SMARCA2 and KDM6A, were identified in at least two tumors in this group. The fusion gene of MYB/MYBL1-NFIB were presented in 57.1% samples, which were in consistent with the FISH results. NFIB-MAP3K5 was detected in 034-patient as a new fusion gene，which was also confirmed by direct DNA sequence. Comparing to 9 PDXs and their primary tumors, PDXs appeared to retain the heterogeneity or undergo a selection upon transplantation. PDXs showed similar genomic rearrangements, copy number alterations, mutation profiles and variant allele frequencies. In histological level, PDXs were virtually indistinguished from the original tumors, which included H&E staining and IHC for biomarkers such as MYB, Ki67, P63 and CK19 positivity and location. PI3K inhibitor, Alpelisib, was administered in 044-PDX *in vivo*, which harbored PIK3CA amplification. We observed a tumor growth inhibition (TGI) index of 130% when combined with cisplatin administration. IHC also showed a reduction of Ki67 and MYB after applying Alpelisib and cisplatin.

Western blot exhibited a lower expression of P110a and downstream pathway of PI3K, p-AKT and p-ERK.

Conclusion

Fusion gene MYB-NFIB and POU6F2, KMT2C, Notch1 and PIK3CA are the most frequently mutated genes in this group of ACC. Meanwhile, a majority of genes retain in PDXs. PDXs could also recapitulate the genetic and biological characteristics, including tumor heterogeneity, from the original patient tumors. Therapeutic inhibition Alpelisib inactivates PI3K and downstream pathway and causes tumor regression in combination with cisplatin through PDX models of adenoid cystic carcinoma.

Chromophobe Renal Cell Carcinoma-like Thyroid Carcinoma-Challenging Cases in Surgical Pathology in Japan-IAP special

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Chromophobe renal cell carcinoma (CRCC)-like thyroid carcinoma was reported in 2017 as a distinctive clinicopathological and histological entity of thyroid carcinoma. However, this type of thyroid carcinoma is extremely rare and therefore, the biological nature has not been clarified yet.

\<Case\>
We retrieved three cases with CRCC-like appearances from the files of Ito hospital in Tokyo. All three patients (case 1, 2, and 3) were male and 29, 42, and 74 years of age, respectively. They did not have any family history of genetic disorders including tuberous sclerosis complex. Although metastases and/or recurrence after surgery were not identified in two cases, one case (case 3) revealed the metastatic foci in lymph nodes at surgery and tumor recurrence in the lymph nodes and possible lung metastasis were detected 8 years after initial surgery.
\<Histology\>
They were not encapsulated and invading into surrounding thyroid tissue. All three tumors were very similar in histology. These tumors were composed of large polygonal or columnar cells with trabecular and/or alveolar pattern. The tumor cell nests were surrounded by thin vascular stroma. The cytoplasm of tumor cells was abundant, finely granular, and eosinophilic. Necrosis of tumor tissue was found in 1 case.
\<Note\>
CRCC-like thyroid carcinoma revealed to be distinctive in histology and could be misdiagnosed as oxyphilic cell thyroid carcinoma, malignant hyalinizing trabecular tumor, and metastatic carcinoma. Further characterization by immunohistochemistry and genetic analyses in this rare tumor were discussed in this report.

Category: Head & neck Pathology
1657703

Clinicopathological characteristics and prognosis analysis of 103 cases for thyroid carcinoma showing thymus-like elements (CASTLE): A Case Series Study

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Objective This study aims to summarize the clinicopathologic characteristics of thyroid carcinoma showing thymus-like elements (CASTLE), and to identify the factors affecting the treatment and prognosis of patients.

Methods Reported studies with CASTLE patients published from January 1991 to January 2019 were retrieved from a cross-database literature search of PubMed, Web of Science, CQVIP, CNKI and Wanfang databases. Keywords used were "CASTLE," "thyroid," "carcinoma," "thymic," and all their combinations. A systematic analysis was performed on the clinicopathologic features, treatment and prognosis of 102 cases reported in 41 literatures from a cross-database literature search and a CASTLE case treated in our hospital.

Results The most common clinical manifestations of CASTLE were neck masses (70.7%), tumors were mostly single, and 67.1% were located in the lower thyroid. The pathological and immunophenotype of CASTLE is similar to that of thymic carcinoma. Follow-up time ranged from 2 to 312 months, 20.4% of patients had local recurrence or distant metastasis after surgery. There was a significant correlation between extrathyroidal invasion and lymph node metastasis and progress-free survival (PFS) (P=0.040, 0.000, respectively). Total thyroidectomy with neck dissection were significantly prolonged PFS in patients with extrathyroid invasion and lymph node metastasis($P < 0.05$). And radiotherapy significantly improved PFS in patients with lymph node metastasis (P = 0.022).

Conclusion Extrathyroidal infiltration and nodal metastasis are important factors for CASTLE. Total thyroidectomy supplemented by neck dissection are recommended for patients with extrathyroidal extension and/or lymph node metastasis. Radiation therapy appears to be important for better outcomes in CASTLE patients with extrathyroidal extension.

Differential expression of CD56 in papillary thyroid carcinoma with normal thyroid background and papillary thyroid carcinoma with Hashimoto's thyroiditis background

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Background: Papillary thyroid carcinoma (PTC) which accounts for about 85% of thyroid cancer is the most common endocrine neoplasm with an increasing incidence. Hashimoto's thyroiditis is one of the most common autoimmune diseases, often associated with PTC, but the correlation between them is still unclear. CD56 is a membrane glycoprotein with similar affinity and is often highly expressed in neuroendocrine tumors. However, the differential expression of CD56 between PTC with normal thyroid background and PTC with Hashimoto's thyroiditis remains unclear.
Aim: To compare the expression of CD56 in PTC with normal thyroid background and PTC with Hashimoto's thyroiditis, and to study the role of CD56 in PTC with Hashimoto's thyroiditis.
Methods: This study reviewed the data of 109 patients with PTC and 103 patients with Hashimoto's PTC in Tumor Hospital of Chinese Academy of Medical Sciences, National cancer center from 2014 to 2016. Paraffin specimens of PTC tissues were collected from all patients. CD56 expression in the two groups of PTC patients was detected by immunohistochemical automatic staining instrument, and the results were evaluated. The data were analyzed by SPSS 23.0 statistical software to compare the expression of CD56 in PTC with normal thyroid background and PTC with Hashimoto's thyroiditis.
Results: In PTC with normal thyroid background group, CD56 was positive in 42 (44.2 %) cases, negative in 53 (55.8 %) cases in peripheral thyroid tissue and positive in 14 (14.6 %) cases, negative in 82 (85.4 %) cases in tumor tissue. Chi-square test showed that the expression of CD56 in tumor tissues was significantly lower than that in surrounding thyroid tissues (***$p < 0.001$). In Hashimoto's PTC group, CD56 was positive in 43 (50.6 %) cases, negative in 42 (49.4 %) cases in peripheral thyroid tissue and positive in 24 (28.2 %) cases, negative in 61 (71.8 %) cases in tumor tissue. Chi-square test showed that the expression of CD56 in Hashimoto's PTC tumors tissue was much lower than that in surrounding thyroid tissues (**$p < 0.01$). Compare the tumor tissues in PTC with normal thyroid background and PTC with Hashimoto's thyroiditis, the expression of CD56 in the latter was significantly higher than that in the former (*$p < 0.05$).
Conclusion: The expression of CD56 in PTC with normal thyroid background was significantly decreased, suggesting that CD56 protein may be involved in the occurrence and development of PTC, which is helpful for the diagnosis of PTC. Compared with PTC with normal thyroid background, the expression of CD56 in PTC with Hashimoto's thyroiditis was significantly increased, and it may be one of the potential directions to study the pathogenesis of PTC with Hashimoto's thyroiditis.

Preliminary establishment and analysis of differentiation of salivary malignant pleomorphic adenoma cell line

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Objective: To culture and develop a malignant pleomorphic adenoma (MPA) cell line and analyze its differential characterization.

Materials and Methods: To begin with, the postoperative malignant pleomorphic adenoma samples were obtained in the aseptic environment. After that, the single cell suspension was prepared from the MPA samples by using enzyme digestion method in vitro. Laterly, employing high glucose DMEM to culture the single cell suspension in order to passage and induce. Furthermore, the genetic analysis of the cell line was performed by using DNA short tandem repeat(STR) analysis. Lastly, using the immunocytochemistry to detect the biomarker of this cell line.

Results: The human MPA cell line was cultured and screened from primary cells in vitro. Up to now, the cell line has been cultured until the 43rd generation. STR has shown that there are no cell lines in the ATCC, DSMZ, JCRB and RIKEN cell banks that match to this MPA cell line. In addition to this, no alleles were found from this identification. And the immunocytochemistry has indicated that the cell expressed CK (++) , P63 (++) , S-100(+) and Vimentin(+++) , which has the characteristics of differentiation on myoepithelial cell.

Conclusion: The MPA cell line with the characteristics of myoepithelial differentiation were initially established. It will provide a useful biological model for further study on the occurrence, development, and treatment of malignant pleomorphic adenoma of salivary glands.

Category: Head & neck Pathology
1658122

LMP1 Up-regulates Calreticulin to Induce Epithelial-mesenchymal Transition via TGF-β/Smad3/NRP1 Pathway in Nasopharyngeal Carcinoma Cells

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Abstract

Background: Latent membrane protein 1 (LMP1) is known as an oncogenic protein encoded by the EBV genome. The purpose of this study was to investigate the mechanism of LMP1-induced cell epithelial-mesenchymal transition (EMT).

Methods: The NP69 cell line of nasopharyngeal epithelial cells with high expression of LMP1 was established to observe the effect of high expression of LMP1 on cell growth, proliferation, cycle, apoptosis, migration and invasion. We used proteomics to screen and identify differentially expressed proteins related to LMP1-mediated epithelial cell transformation. Then, we analyzed the expression and significance of differentially expressed calreticulin (CRT) in nasopharyngeal carcinoma (NPC), and observed the effect of CRT expression on EMT in CNE2 cells of NPC. Finally, the expression of neuropilin-1 (NRP1), which is a protein downstream of the EMT-related signaling pathway TGF-β (transforming growth factor β), was detected.

Results: LMP1 promoted NP69 cells proliferation, inhibited apoptosis and induced EMT. We identified 22 differentially expressed proteins associated with LMP1-induced EMT. Among them, CRT expression level was significantly increased in NPC compared with adjacent tissues, and was interrelated with TNM staging and lymph node metastasis of NPC. After knockdown of CRT expression, the cell EMT was reduced, and the ability of cell migration and invasion was weakened. CRT regulated NRP1 expression by affecting SMAD3 phosphorylation.

Conclusion: LMP1 induced cell EMT via TGF-β/Smad3/NRP1 pathway, which promoted migration and invasion of NPC cells.

Cytokeratin and EBERs: a cost-effective panel derived from a multicentered study for pathological diagnosis of nasopharyngeal carcinoma

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Objectives

Nasopharyngeal carcinoma (NPC), an epithelial malignancy originating from the nasopharynx, is remarkable for its distinct geographic distribution and Epstein-Barr Virus (EBV) infection. The treatment of the disease is based on pathological diagnosis, which is made by pathologists. Although NPC is the most frequently occurred malignancy in nasopharynx, differential diagnosis is still required as the morphology of NPC and other diseases like lymphoma could be very similar under microscope. Therefore, pathologists often resort to immunostaining or in-situ hybridization for diagnosis. Unfortunately, at present, there is no consensus of IHC/ISH panel selection among the pathologists worldwide, either undermining confidence of making diagnosis or causing unnecessary financial burden for patients. In this project, we aim to establish a cost-effective panel to assist NPC diagnosis

Materials and Methods

Totally, we reviewed 79154 cases diagnosed with nasopharyngeal disorders from 8 cancer hospitals in China. Cases diagnosed with HE staining only were excluded from further analysis. Then we retrospectively investigated the most frequently used panel in 5530 NPC patients who were diagnosed with IHC or ISH. Patients diagnosed with the most frequently used panel were enrolled as training cohort and optimization of the panel was based on logistic regression model and ROC analysis. The optimized panel was subsequently validated in two cohorts, which consisted of patients from Sun Yat-sen University Cancer Center and the rest 7 cancer hospitals respectively.

Results

The retrospective analysis of 5530 NPC cases diagnosed with IHC or ISH in Sun Yat-sen University Cancer Center indicated that CK(AE1/AE3)+CK5/6+p63+EBERs was the most frequently used panel. Subsequent logistic analysis for patients in training cohort indicated that only CK(AE1/AE3) and EBERs were significant predictors. The logistic model was then used to construct ROC curve to evaluate the diagnostic performance of the panel. The AUC of this panel was 0.989 (95%CI:0.983-0.994, Sensitivity:94.1%, Specificity:98.9%). The result was further validated: the sensitivity/specificity of the panel in validation cohort 1 and 2 were 98.0%/96.9% and 94.6%/94.3%, respectively.

Finally, we introduced this panel in the department of pathology, Sun Yat-sen University Cancer Center to prospectively observe whether financial burden of patients can be reduced by less prescribed diagnostic markers without sacrificing diagnostic quality. In a 5-months observation period, 520 NPC patients were diagnosed with IHC or ISH. The average number of IHC/ISH markers prescribed for each established NPC diagnosis dropped from 4.57 to 2.91, saving up to 36.3% of diagnostic expenses. Additionally, no pathology report was challenged during the period, indicating diagnostic quality was also guaranteed.

Conclusion
In summary, our study established a cost-effective diagnostic marker panel for differential diagnosis of NPC with a large cohort of participants. The application of this panel significantly decreased patients' expenses but kept diagnostic quality at the same time. For the pathologists, their selection of panel can be based on evidence from systematic evaluation rather than experience of their own.

Lung case in Japan-IAP Special "Topic: Challenging Cases in Surgical Pathology"

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A 73-year-old male was followed under the diagnosis of usual interstitial pneumonia with advanced stage. Chest CT examination revealed a nodule in the left lower lobe. Partial resection of the lung by video-assisted thoracoscopic surgery. Resected specimen was 5x5x3cm in size. Sections demonstrated marked honeycomb lesions and a well-demarcated, white, homogeneous, elastic hard, 2.5cm in maximal diameter tumor. Histologically, honeycomb lesions and lung fibrosis were advanced with bronchioloectasis and sqaumous metaplasia. The tumor consists of cuboidal epithelial cells with bland nuclei and eosinophilic cytoplasm which sometimes form a small duct-like structure surrounded by cells with clear cytoplasm. There are many foci where the tumor cells grow in a solid fashion, increased mitosis and several foci of necrosis. Our final diagnosis is epithelial-myoepithelial carcinoma. We demonstrate immnohistochemical examinations and discuss the clinic-pathological profile.

The expression of PD-L1 and Prognosis Study of Basaloid Squamous Cell Carcinoma of Lung

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Objectives Basaloid squamous cell carcinoma (BSCC) of the lung is highly invasive carcinoma. The consistency of the expression of PD-L1 was analyzed, and the clinicopathological features were analyzed to find out the factors affecting the prognosis of BSCC patients.

Materials and Methods A retrospective study has enrolled 33 cases of BSCC from the Fourth hospital of Hebei Medical University from January 2013 to January 2018. The expressions of PD-L1(E1L3N, 22C3, SP263, SP142) were detected by immunohistochemistry. The clinical and pathological data were analyzed, and the survival time was calculated by telephone follow-up. Kaplan-Meier survival curve was used for survival analysis, Log-rank test was used for comparison of survival curves, and multivariate Cox risk regression model was used for influencing factors of survival time.

Results The results showed that the 1-, 3-and 5-year survival rates were 80.5%, 53.9% and 39.3%. Immunohistochemistry showed the staining consistency of tumor cells was higher in E1L3N, 22C3, SP263. Kaplan-Meier univariate analysis showed that TNM stage, pleural invasion and the expression of PD-L1 (cut off 50% of E1L3N, 22C3, SP263) were significantly associated with the median survival time of BSCC patients ($P < 0.05$). Multivariate analysis of Cox proportional hazard model showed that TNM stage was an independent prognostic factor affecting the survival time of BSCC patients ($P < 0.05$).

Conclusion Cutoff of PD-L1 50% is more effective in predicting the prognosis of BSCC patients. TNM staging is an independent prognostic factor affecting the survival time of BSCC. Early diagnosis and treatment should be pursued.

Category: Mediastinal and thoracic pathology
1453939

A case showing uncommon diagnostic feature of AA amyloid deposit in alveolar macrophages

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Introduction
Amyloidosis is a condition in which extracellular deposits of fibrillar proteins result tissue damage and organ dysfunction. There are several types of amyloid fibrils. Some of the most common forms are AL (amyloid light chain) protein and AA (amyloid-associated) type of amyloid fibril protein. We presented a case of 78-year-old male with systemic AA amyloidosis complicating multiple myeloma, in which detection of amyloid deposit in alveolar macrophages is the only clue for diagnosis.

Case presentation
A 78-year-old male with history of multiple myeloma followed by systemic amyloidosis presented with abnormal chest CT showing diffuse interlobular thickening in the whole lung field with bilateral pleural effusion. Bronchoalveolar lavage and transbronchial biopsy were performed. Due to the patient's poor condition and hemorrhage, only one fragment was available from forceps biopsy. Histologically, there was no amyloid deposition in the lung parenchyma nor neoplastic infiltration. However, some macrophages showed eosinophilic granular contents which were positive with direct fast scarlet (DFS) staining and AA amyloid immunohistochemically. Similar macrophages with AA amyloid were also confirmed by bronchoalveolar fluid.

Discussion and conclusion
We experienced a case with AA amyloidosis affecting in the lung diagnosed by presence of intracytoplasmic amyloid in alveolar macrophages. The changes are so subtle that they may be overlooked. Recognition of amyloid deposition in alveolar macrophages may be an important clue to detect its affection to the lung.

Clinicopathologic features and genetic alterations in adenocarcinoma in situ and minimally invasive adenocarcinoma of the lung:long-term follow up study of 121 Asian patients

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Abstract: This study retrospectively analyzed and compared the clinicopathologic characteristics, genetic alterations, and outcomes of surgical resected and pathological diagnosed adenocarcinoma in situ (AIS) (n=59) and minimally invasive adenocarcinoma (MIA) (n=62) cases that presented as ground glass opacities (GGOs) on CT screening. Clinicopathologic features and genetic alterations of these tumors were analyzed. A long-term preoperative (ranged from 2 - 1967 days) and postoperative (ranged from 0 - 92 months) follow-up was conducted. Seventy-eight percent and 22.6% of AIS and MIA presented as pure GGOs. The tumor size and consolidation/tumor ratio were significantly larger in the MIA cohort than AIS both on CT scan and microscopy. Immunohistochemically, the expression of p53, Ki67, and cyclinD1 was higher in MIA than in AIS. *EGFR* mutation rate was significantly higher in MIA, while other genetic alterations including *KRAS* mutation and *ALK* translocation showed no differences. All the MIA patients with a predominant micropapillary invasive pattern showed recurrence. MIA cases with a predominant micropapillary invasion pattern were more likely to relapse. The differences in clinicopathologic features and prognosis between AIS and MIA indicate a more aggressive behavior of MIA. And the current CT measurements have limited utilities to predict the natural history of unchanged pure GGOs precisely.

Immunohistochemical evaluation of the immune microenvironment in non-small cell lung carcinoma based on PD-L1 expression and tumor-infiltrating lymphocytes

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Immunohistochemistry was applied to evaluate the expression of programmed cell death-ligand 1 (PD-L1) and the spatial distribution of programmed cell death protein 1 (PD-1) and CD8 tumor-infiltrating lymphocytes (TIL) on the specimens from 354 non-small cell lung carcinoma (NSCLC), and investigate whether the distinct tumor microenvironment immune types (TMIT) subtypes differentially affect clinical outcomes of patients. Our data reveal that PD-1 and CD8 TILs are more frequently distributed in squamous cell carcinoma (SCC) than those in lung adenocarcinoma (LAC). The density of TILs is a poor prognostic factor in LAC but a favorable one in SCC. PD-L1 levels and its clinical prognostic significance are differed in LAC versus SCC. TMIT stratification could be considered as an independent prognostic factor of SCC patients' survival. Our study indicates that different type of TMIT provide its specific microenvironment with diverse impact on the survival of NSCLC patients and highlights the importance of the integrative assessment of PD-L1 status and TILs' spatial distribution to predict patients' prognosis.

Primary endobronchial leiomyoma: a case report and review of literature

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Primary pulmonary leiomyoma is a rare condition. Symptoms of leiomyoma of the respiratory tract manifest differently according to the site of the tumor. If definitive diagnosis can be made with biopsy, then the lung surgery such as endobronchial removal or bronchoplasty can be used.

Case report:

A 63-year-old male was admitted to our hospital with complaints of chest distress and occasional cough for two months. He had a ten cigarettes per day smoking history who had quit 2 years prior to presenting. The findings of the physical examination was normal. Computed tomography thorax revealed a homogenous nodular lesion located in right middle segment bronchus. Endobronchial bronchoscopy showed a solid new neoplasm at the right middle segment bronchus almost completely occluding the lumen. Biopsies of the mass initially consider mesenchymal tumors. Immunohistochemical staining showed that the tumor cells were strongly positive for Desmin, SMA, H-caldesmon and Vimentin; but negative for S-100 and SOX10. Ki-67 expression is low (<5%). The diagnosis is consistent with leiomyoma according to immunohistochemical results. The patient subsequently underwent thoracoscopic sleeve resection of right middle and lower lobe. Postoperative pathological again confirm the diagnosis of leiomyoma. In addition, partial of the bronchial epithelium over the surface of the tumor occurred squamous metaplasia. Follow-up at 1, 3 and 6 months after surgery was satisfactory.

Discussion:

Primary pulmonary leiomyoma is an extremely rare benign tumor, it was first described by Forkel in 1909. These neoplasms can occur in parenchymal, endotracheal, or endobronchial sites. Endobronchial leiomyoma accounts for 33% of primary pulmonary leiomyoma. The mean patient age is the third and fourth decades of life without sex predilection. We report the case of an elderly male with primary endobronchial leiomyoma. There is general agreement that endobronchial leiomyomas derive from smooth muscle layer of the bronchial wall.

Intraluminal lesions cause a series of clinical symptoms due to partial or total obstruction of the affected airways, which mainly include secondary pneumonia and atelectasis such as fever, cough, and difficulty breathing. Missed diagnosis and misdiagnosis are not uncommon, because the disease is rare and lack of specific clinical characteristic. Ayperi et al. reported a case that tracheal leiomyoma misdiagnosed as asthma. In our case, The patient only presented with chest distress and cough.

The diagnosis of primary pulmonary leiomyoma is mainly based on imaging and pathological examination. Computed tomography is an excellent instrument to precisely depict tumor

location and obstructed extent. Bronchoscopy can detect tumor early, but it is difficult to determine the nature and type of tumors. Finally, the diagnosis must be confirmed by pathological examination. The microscope showed the tumor was composed of bundles of uniform, spindle cells with abundant eosinophilic cytoplasm and baculiform nuclei; without significant nuclear atypia, mitosis and necrosis. The morphology of tumor cells is similar to normal smooth muscle cells, which can rule out the diagnosis of leiomyosarcoma. Immunohistochemical stains are useful in ruling out other differential diagnosis such as such as fibroma, neurofibroma, and schwannoma. Tumor cells of leiomyoma are positive for smooth muscle markers (SMA, Desmin, H-caldesmon) but negative for S-100 and SOX10. In our case, morphological characteristics of this tumor accorded with the above description and immunohistochemistry strongly expressed smooth muscle markers (SMA, Desmin, H-caldesmon). The tumor was diagnosed as primary endobronchial leiomyoma. Furthermore, partial of this bronchial epithelium over the surface of the tumor occurred squamous metaplasia. Until now, this rare characteristic that leiomyoma and squamous metaplasia coexist in the respiratory tract has only one case. We consider that squamous metaplasia is related to long-term smoking in patients. Pulmonary leiomyoma is essentially treated with surgical or bronchoscopic resection, depending on airway location and size of the lesion. In our case, Due to concerns about the location and wide base of the tumor, the patient underwent a thoracoscopic sleeve resection of the right middle and lower lobe. Leiomyoma usually has favourable prognosis after complete resection and rare recurrence.

Primary mediastinal mixed germ cell tumor: a case of clinicopathologic analysis and literature review

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Primary mixed germ cell tumors are uncommon lesions in the mediastinum, and most of them are mixed with 2 kinds of non-spermatogonial germ cell tumors. This disease is mainly found in males aged 20-40 years, and it is a mixed tumor composed of different types of germ cell tumors, including spermatogonial germ cell tumor and non-spermatogonial germ cell tumor. Mediastinal non-spermatogonial germ cell tumor is often associated with significantly increased AFP, poor response to chemotherapy, frequent recurrence, and poor prognosis. Herein we describe a case in a 23-year-old male, with pathological diagnosis, mediastinal mixed germ cell tumor (GCT) consisting of 4 types of germ cell tumor. Mediastinal spermatoblastoma accounts for about 40%, and non-spermatogonial components account for about 60 % (mainly immature teratoma, and the remaining few are yolk sac tumor and embryonal carcinoma). Immunohistochemical: seminoma area AFP (+), CD117 (+), PLAP (+), OCT4 (+), SALL4 (+), yolk sac tumor area Glypican - 3 (+), OCT4 (-), SALL4 (+), CD30 embryonal carcinoma area (+), OCT4 (+), EMA (-), CD117 (+), PLAP (+), CK (+), SALL4 (+).This case is very rare. Through this case report and literature review, we hope to improve the understanding of the pathological diagnosis of primary mediastinal mixed germ cell tumor, and guide the clinical treatment and prognosis evaluation.

Two MicroRNA Panels for Lung Cancer Subtyping in Endobronchial Ultrasound Guided Biopsy Specimens

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Objectives: Lung cancer is classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), which mainly contains adenocarcinoma (AC) and squamous cell carcinoma (SQ). Lung cancer subtyping plays an important role in choosing therapeutic schemes. Endobronchial ultrasound (EBUS) guided biopsy is a routine examination for diagnosis and staging of lung cancer. Unfortunately, it is hard to classify subtype via classic cytology on EBUS biopsy specimens. MicroRNA is reported to be stable and sensitive biomarker of lung cancer. This study aimed to identify microRNAs which have the potential for lung cancer subtyping in EBUS biopsy specimens.

Materials and Methods: In this study, 229 EBUS biopsy specimens (115AC, 41 SQ, and 73SCLC) were investigated. Reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) was performed to evaluate expression of 7 candidate microRNAs discovered via microarrays previously. Two logistic regression models were constructed based on a training cohort (n = 124) and then validated in an independent cohort (n = 105). The area under the receiver operating characteristic curve (AUC) was used to assess the diagnostic accuracy of microRNA panels. The diagnostic performance was compared between microRNA panels and cytology.

Results: Panel A, consisting of miR-34a and miR-375, was built to discriminate SCLC from NSCLC. In training phase, the AUC value was 0.941 with sensitivity of 90% and specificity of 92%, and in validation phase, the corresponding AUC value was 0.928 with sensitivity of 85% and specificity of 94%. Similarly, panel B, consisting of miR-205 and miR-375, was used to discriminate SQ from AC. In training phase, the AUC value was 0.989 with sensitivity of 94% and specificity of 97%, and in validation phase, the corresponding AUC value was 0.810 with sensitivity of 83% and specificity of 88%. Compared with cytology, microRNA panels or the combination of microRNA panels and cytology were of higher sensitivity and specificity in diagnosis of AC, SQ and SCLC.

Conclusion: In EBUS biopsy specimens, we constructed two microRNA panels for lung cancer subtype discrimination with high sensitivity and specificity. Moreover, the combination of microRNA panels and cytology could improve the diagnostic accuracy in further. These findings could be helpful in therapy of lung cancer.

High expression of CMTM6 indicates a poor prognosis in NSCLC and may help guide treatment decisions

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Objective: This study sought to investigate the role of CMTM6 in the prognosis of non-small cell lung cancer (NSCLC) patients. **Methods**: The expression of CMTM6 was detected by immunohistochemistry staining in a series of 141 non-small cell lung cancer and 110 normal lung tissue. The results were quantitated using the HSCORE system, which consider both staining intensity and the percentage of cells stained at given intensities. Relationships of the expression level of CMTM6, patient clinicopathological parameters and overall survival (OS) were analyzed by using the univariate Kaplan-Meier method and studied with the multivariate COX regression model. **Results**: CMTM6 was expressed in non-small cell lung cancer tissues, meanwhile it was also presented in macrophages, lymphocytes, and bronchial epithelial cells in normal lung tissues. CMTM6 was expressed more highly in lung cancer tissues (161.04±86.60) than normal tissues (71.20±45.10) ($P < 0.001$). Patients were divided into two groups on the basis of their expression level of CMTM6: high CMTM6 expression group (n=49) and low expression group (n=40). Patients with tumors harbouring higher expression of CMTM6 showed a significantly worse prognosis with a hazard ratio (HR) of 2.191 (95% CI, 1.304 to 3.682; $P = 0.003$) for overall survival (OS). Multivariate analyses revealed that CMTM6 expression would serve as an independent predictor of overall survival ($P=0.009$). **Conclusion**: The expression of CMTM6 is upregulated in NSCLC, and a higher expression of CMTM6 might play a role as a negative prognostic marker in NSCLC patients.

Clinicopathological and gene mutation analysis between peripheral-type and central-type lung squamous cell carcinoma

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Background: Lung squamous cell carcinoma can be classified into peripheral-type squamous cell carcinoma(P-SQCC) and central-type squamous cell carcinoma(C-SQCC) based on primary tumor site. In this study, we investigated clinicopathological characteristics and gene expression profile both in P-SQCC and C-SQCC groups.
Materials and Methods: 68 P-SQCC and 62 C-SQCC samples underwent lobectomy in Jinling Hospital Affilitated to Medical College of Nanjing University were retrospectively reviewed from January 2013 to January 2018. C-SQCC was defined as tumor located at trachea to the segmental bronchi while P-SQCC was defined as tumor limited to sites more peripheral than the fifth bronchiole according to CT images. To analyze gene mutational status in exons 2 and 3 of KRAS gene, 9 and 20 of PIK3CA gene, 18-21 of EGFR gene, we carefully selected 30 representative samples of P-SQCC and C-SQCC respectively using PCR-based sanger sequencing by 2 experienced pathologists.
Results: Statistical analysis revealed that P-SQCC and C-SQCC patients share similar clinicopathological characteristics including smoking history, TNM stage, tumor size, angiolymphatic invasion and lymph node matastasis. One noteworthy point was that P-SQCC group was significantly older than C-SQCC group at the time of receiving tumor resection operation, with the average age of 67.3 and 62.8 respectively (p=0.032). As to gene mutation analysis, we found that L858R mutation of EGFR gene was just found only in one case of C-SQCC group. Meanwhile, neither P-SQCC nor C-SQCC group hold KRAS gene mutation in our study. However, PIK3CA gene proved significantly difference between the two groups for hot spot mutations including E545K mutation in exon 9 and H1047L mutation in exon 20 were exclusively detected in P-SQCC group, as opposed to, C-SQCC group was totally negative.
Conclusion: Our findings indicated that P-SQCC and C-SQCC patients might be different in gene expression profile, for PIK3CA hotspot mutations were exclusively detected in P-SQCC group. Therefore, future investigations that developing molecular events in lung squamous cell carcinoma are necessary to examine primary tumor location.

Primary pulmonary T cell lymphomas in adults: a clinicopathological analysis of 16 cases

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Objectives: Primary pulmonary T cell lymphoma (PPTCL) is a rare disease and difficult to diagnose, especially in small biopsies. We analyzed the clinicopathological features of PPTCL in 16 adults to help improve understanding of T cell lymphomas in lung as well as to establish the diagnosis and differential diagnoses in routine practice.
Methods: 16 cases with PPTCL were retrospectively reviewed in this study followed at the Affiliated Drum Tower Hospital of Nanjing University Medical School between January 2006 and July 2019, including clinical manifestations, baseline biochemical indices, histological findings and other available ancillary studiessuch as immunostaining, Epstein-Barr virus encoded RNA (EBER) in situ hybridization, Immumnoglobin/T-cell receptor (IG/TCR) rearrangement analysisand radiological results upon diagnosis.
Results: The cases consisted of 8 extranodal natural killer/T-cell lymphomas (ENKTCL), 7 peripheral T-cell lymphomas and 1 anaplastic large cell lymphoma. The median age of patients was 42.5 years (range: 18-62years) at diagnosis with a female predominance (68.75%). Most patients complained of fever, cough, wheezing, chest congestionor fatigue. Specific changes of blood biochemical indices were primarily elevated lactate dehydrogenase (LDH) and ferritin (FER). Computed tomography scans mainly revealed multiple ill-defined nodules/masses of consolidation with or without airbronchogram. Pleural effusion was common. Patchy opacities or nodules of ground-glass opacity with hilar or mediastinal lymphadenopathy were noticed in some cases. Microscopically, the lesions showed heterogeneous but non-specific features, such as extensive infiltration of atypical lymphocytes with vascular walls involved sometimes, conspicuous broadening alveolar septa without collagen fibers proliferation. Necrosis can be recognized in several cases. The cytological spectrum was broad. Cells may be small, medium-sized, large or anaplastic, and often expressed T-cell markers such as CD3, CD5, CD7, CD4 or CD8. Other B-cell-associated antigens including CD20, CD19 and CD79 α were usually negative. Cytotoxic molecules (e.g. granzyme B, TIA1, perforin) were positive in largely ENKTCL cases and individual PTCL, NOS cases. IG/TCR rearrangement analysis was available in 6 cases and only two of them were positive for TCR rearrangement. In situ hybridization stain for EBV-encoded RNAs was positive in 53.33% (8/15) cases.
Conclusions: PPTCL is extremely rare. In general, patients with PPTCL have poor outcomes. There are no specific morphological features and it can be much more difficult to diagnose in small biopsies especially in the context of abundant reactive inflammatory cells. In most such occasions, it is nearly impossible to diagnose merely basing on histology. Comprehensive analysis of immunophenotypic markers, CT scan images, severity of clinical course and some clues in histologic findings may alert pathologists of the diagnosis.

Aberrant expression of MDM2 and CDK4 in pulmonary angiomyolipoma: a case report

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Abstract: **Objectives** The tumor deriving from the perivascular epithelioid cell family and occurring in lung, are mostly clear cell "sugar" tumor (CCST) or lymphangioleiomyomatosis (LAM). The occurrence of Angiomyolipoma (AML) in lung is uncommon. **Materials and Methods** We reported a case with morphology and immunohistochemistry supporting the diagnosis of pulmonary fat-predominant AML, but expression of MDM2 and CDK4, which have been used to support a diagnosis of well-differentiated liposarcoma (WDLS). **Results** A 46-year-old woman presented with a 15 days history of chest pain and tightness. A computed tomography scan revealed a nodule with high density in the right lower pulmonary. The patient underwent local pulmonary resection. Gross examination showed a nodule with a diameter of 1.5cm. Microscopically, the tumor was predominantly composed of mature adipose tissue and scattered atypical cells resembling lipoblasts. The tumor cells were positive for HMB-45, Melan-A, TFE3, MDM2 and CDK4, but no MDM2 amplification was found by fluorescence in situ hybridization (FISH). A diagnosis of fat-predominant AML with aberrant MDM2 and CDK4 expression was made. **Conclusion** To our knowledge, this is the first case report of MDM2 and CDK4-positive pulmonary fat-predominant AML. Such aberrant expressions should be bear in mind in case misdiagnosis of WDLS, especially when AML occurs in rare sites.

Diagnosis of lung biopsies with the 2015 WHO criteria and detection of sensitizing mutations: a single-institution experience with 5032 cases

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Purpose   The 2015 WHO classification of lung tumors provided the first specialized classification for small biopsies. This article aims to apply the newest classification to reclassify a group of small lung biopsies and analyze their statuses of the main driver mutations.

Methods   5032 cases of small lung biopsies (bronchoscopic, needle, or core biopsies) were selected, which range from 2014 to 2018. We applied the newest classification to reclassify them and analyze their status of the main driver mutations.

Results   The numbers of small lung biopsies each year during 2015–2018 were respectively 1151, 1347, 1572 and 1259. There were 3480 men and 1849 women, ranging in age from 11 to 93 years (median 63 years). The most common diagnosis is primary malignant tumor (3166, 62.9%), followed by inflammatory lesion (1326, 26.4%), uncertain case (350, 7.0%), metastatic tumor (165, 3.3%), and benign tumor (25, 0.5%). Among the primary malignant tumors, the dominant type is adenocarcinoma (3130, 58.7%), followed by NSCC, favor adenocarcinoma (501, 10.0%), squamous cell carcinoma (368, 7.3%), and NSCC, favor squamous cell carcinoma (360, 7.2%). The tests of the main driver mutations using ARMS-PCR technology demonstrates that EGFR is positive in 49.7% (536/1079, in adenocarcinomas), ALK in 4.3% (9/211, in NSCC), and ROS1 in 0.5% (1/211, in NSCC). Moreover, 898 NSCC specimens went through a immunohistochemical (IHC) test for ALK (D5F3) and 38 of them are positive (4.2%).

Conclusion   The criteria for small lung biopsies proposed by the 2015 classification of lung tumors provided a detailed and dependable reference for pathologists to make a diagnosis. It can improve the diagnostic efficiency and quality of small lung biopsies and assist oncologists in accurately understanding the pathologic diagnosis. In this way, accurate treatment and improved prognosis are more available to the patients.

BATF acts as an oncogene in non-small cell lung cancer

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One of the main causes of cancer disease and death worldwide is lung cancer. This study focused on the function of basic leucine zipper ATF-like transcription factor (BATF) in non-small cell lung cancer (NSCLC). Using NSCLC patient data from the Cancer Genome Atlas (TCGA), we found that BATF expression in NSCLC tissue was significantly higher than that in the adjacent normal tissue (P=6.56E-06). Lentivirus-mediated short hairpin RNA (shRNA) was used to knockdown BATF expression in the human A549 NSCLC cell line and assessed by RT-qPCR and Western blot. Cell proliferation was evaluated by MTT assay and Celigo imaging cytometry. Apoptosis was detected by FACS and caspase 3/7 analysis. The results showed that knockdown of BATF inhibited the proliferation of A549 cells. Compared with that of the control group, the apoptosis rate of the BATF-shRNA group was significantly higher. In summary, knockdown of BATF inhibits proliferation of the A549 cell line and promotes apoptosis. These results provide important information to explain the pathogenesis of NSCLC.

Epididymal Protein 3A is Overexpressed and Promotes Cell Proliferation in Non-Small Cell Lung Cancer

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One of the main causes of cancer disease and death worldwide is lung cancer. This research investigated the function of family with Epididymal Protein 3A   (EDDM3A) in non-small cell lung cancer (NSCLC). NSCLC patients' data from the Cancer Genome Atlas (TCGA) was retrieved and analysed. Compared with adjacent non-tumor tissues, we found that EDDM3A expression was significantly increased (P=4.19E-02) in NSCLC tissues. In order to knockdown EDDM3A expression in the human NSCLC cell line, lentivirus-mediated short hairpin RNA (shRNA) was used. We assessed knockdown Eefficiency by RT-qPCR and Western blot. Cell proliferation was evaluated by MTT assay and Celigo imaging cytometry. Cell apoptosis were detected by Annexin V staining. The results showed that knockdown of EDDM3A inhibited the proliferation of A549 cells. Compared with that of the control group, the apoptosis rate of the EDDM3A -shRNA group was significantly higher. In conclusion, our study highlights the essential role of EDDM3A in NSCLC, suggesting that EDDM3A might be a potent therapeutic target for patients with NSCLC.

metaplastic thymoma: a clinicopathologic study of three cases

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Objective To study the clinicopathologic features, differential diagnosis and prognosis metaplastic thymoma. **Methods** Resection specimens of 3 cases of metaplastic thymoma were studied by microscopy and immunohistochemistry. **Results** All the 3 patients and aged 28, 42 and 77 years respectively. Pathological diagnosis of 2 cases of metaplastic thymoma, 1 case of metaplastic thymoma with sarcomatoid carcinoma. Histologically, the tumors showed a biphasic pattern with epithelial cells merging gradually with spindle cell component. The epithelial cells showed mild cytologic atypia, nuclear grooves and pseudonuclear cytoplasmic inclusions were variable. These cells were arranged in nests and anastomosing cords. Mitotic figures were rarely seen. Sarcomatoid carcinoma cells showed poorly differentiated, distinctly cytologic atypia, necrosis and brisk mitotic activity. On the other hand, the spindle cells were arranged in fascicles, and show bland-looking. Immunohistochemistry study showed that the epithelial cells strongly AE1/AE3 and CK19, but not for CD5. The proliferation index, as demonstrated by Ki-67 immunostaining was about 5%. Sarcomatoid carcinoma cells were focally reactive forCK, Ki-67 higher proliferation index. In contrast, the spindle cells were diffusely positive for vimentin and focally reactive for EMA, but not for CD5, CD20. The background lymphocytes were positive for CD3, CD5, CD20, not for TDT, CD99. **Conclusion** Metaplastic thymoma is a rarely encountered indolent or low-grade thymic tumor and its differential tumor and may represent a distinct clinicopathological entity. The accurate diagnosis play an important role in patient's therapy and prognosis by microscopy and immunohistochemistry. Complete resection has a good prognosis, but with sarcomatoid cancer changes, the prognosis is much worse than that of metaplastic thymoma.

Circ-PAX2 promotes proliferation and metastasis by absorbing miR-186 in lung cancer cells

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Circ-RNAs are a type of non-coding-protein RNAs. The current study is to detect the expression of circ-PAX2 in lung squamous cell carcinoma (LSCC) tissues and the physiological functions of circ-PAX2. Circ-PAX2 was distinguished in LSCC samples and matched non-tumor samples by human circRNA microarray analysis and was validated to be up-regulated in 86 specimens of LSCC tissues and lung cancer cell lines by qRT-PCR. Functional validation experiments showed that knockdown of circ-PAX2 promoted apoptosis of lung carcinoma cells, and then suppressed proliferation and migration of tumor cells. Small interfering RNA (siRNA) to circ-PAX2 inhibited growth in lung tumor cells. Bioinformatics prediction and rescue experiments showed that circ-PAX2 was a target of microRNA-186, confirmed by qRT-PCR and double luciferase reporter assay. On the whole, our findings reveal that circ-PAX2 was up-regulated and may be an oncogene in lung cancer; its function was reducing apoptosis, promoting cell proliferation and migration in lung carcinoma cells, which might be a novel therapeutic target gene in lung cancer.

Category: Mediastinal and thoracic pathology
1657058

Lymphocyte percentage as a valuable predictor of prognosis in lung cancer

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Objectives
The aims of this study were to investigate the relationship between LY%/NEUT% and the clinical characteristics of lung cancer patients, and to explore whether LY% and NEUT% could be used as independent factors in lung cancer prognosis.
Materials and Methods
A total of 1312 patients with lung cancer were included for analysis. LY% and NEUT% were defined as the percentage of lymphocyte count to white blood cell count, and the percentage of neutrophil count to white blood cell count, respectively, and they were classified based on their reference ranges. Survival curves were determined using the Kaplan-Meier method, and univariate and multivariate Cox regression analyses were performed to identify the independent predictors.
Results
The results of both the training and validation cohorts indicated that LY% was more correlated with clinical characteristics and the occurrence of metastasis in lung cancer patients, compared with NEUT%, and the correlation was most significant in adenocarcinoma (ADC) patients. Both LY% and NEUT% were closely associated with survival status (all $p < 0.0001$), and correlations were also observed in different histological subtypes, and in the advanced stage of lung cancer. Cox regression analysis showed that low LY% could serve as an independent predictor of poor prognosis in all lung cancer patients, as well as in ADC and squamous cell carcinoma (SCC) patients, while a high NEUT% level conferred an unfavorable outcome in small-cell lung cancer (SCLC) patients.
Conclusion
Pretreatment lymphocyte percentage, rather than pretreatment neutrophil percentage, independently predicted the prognosis of lung cancer patients, and it was a more accurate response indicator in ADC and advanced lung cancer patients.

Correlation between PD-L1 Expression and Clinicopathological and Molecular Characteristics of Non-small Cell Lung Cancer: A Large scale Multi-centric Real-world Study of Chinese Cohort

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Background Programmed cell death ligand-1 (PD-L1) is a potent predictive marker of anti-PD-1/PD-L1 therapies for non-small cell lung cancer (NSCLC). However, the definite relationship between PD-L1 expression and clinicopathological, molecular profiles of NSCLC in Chinese population remains unclear. Furthermore, there is still lack of a large scale data from multi-centric real-world study on Chinese NSCLC patients.

Methods and Materials A total of 6197 NSCLC specimen including 4525 surgically resected lung cancer speciman and 1672 biopsy specimens were enrolled from 6 centers in P.R. China. We analyzed PD-L1 (22C3) expression by immunohistochemistry on Dako Autostainer Link 48 platform with strict quality control. The status of *EGFR* was defined by RT-PCR or NGS in 2382 samples and *ALK* was tested by IHC, FISH or NGS in 1716 samples. Subsequently, the association of PD-L1 expression with clinicopathological features and major molecular profile was statistically analyzed.

Results High PD-L1 expression was observed in 11.9% of the recently surgically resected 4471 NSCLC samples, including in 9.0% of invasive adenocarcinoma (ADC, n=3507), and 25.0% of squamous cell carcinoma (SqCC, n=768). The preinvasive lesions (AAH/AIS and MIA) did not show expression of PD-L1. The prevalence of PD-L1 expression in surgically resected samples was lower than in biopsy samples. Clinically, PD-L1 high expression was tightly associated with smoking ($p = 0.001$), advanced stage, larger tumor size, pleural invasion, and lymphovascular invasion ($p < 0.001$). Histopatholoically, PD-L1 high expression was more prevalent in ADC samples with aggressive histologic subtypes included solid, micropapillary, and cribriform subtype ($p < 0.001$). PD-L1 high expression was more frequent in *EGFR*-wild type than in mutant type (12.9% vs 4.7%, $p < 0.001$). Furthermore, PD-L1 high expression was more prevalent in rare mutant types (G719X, 20insertion and T790M) than in common mutations (19 deletion and L858R) (42.1% vs 20.8%, $p = 0.031$). Besides, PD-L1 high expression was also more frequently identified in *ALK* fusion cases (14.6% vs 6.5%, $p = 0.001$).

Conclusions This study gave an essential panoramic view on PD-L1 expression and clinicopathological profiles based on the largest Chinese cohort. The interpretation of PD-L1 should be combined with genetic alterations. Relatively fresh status and appropriate specimen types are significant for the evaluation of PD-L1.

Category: Mediastinal and thoracic pathology
1657200

Analysis of the clinical pathological features of clear cell Lung tumor

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Abstract

Objective: to analysis the clinical pathological characteristics of clear cell lung tumor (CCLT).
Methods: 9 cases of CCLT were collected in the First Medical Center of the People's Liberation Army General Hospital, department of pathology, from 2005 to 2019. All cases were retrospectively analyzed of the clinical-pathological features, and the relevant literatures were reviewed.

Results: Clinical features: 6 cases of 9 cases were women, 3 cases were men. Aged from 28-70yr, with median age 49 years. 6 patients' tumors located in the right lung, and 3 cases occurred in the left lung. 3 cases occurred in upper lobe of the lung (left upper lobe in 1 case, 2 cases of the right upper lobe). 6 cases occurred in lower lobe of the lung (left lower lobe in 2 cases, 4 cases were located in right lower lobe). There were 8 cases of single nodule, while only 1 case of multiple nodules (left lower lobe, more than 8 nodules). Of 9 cases, 5 cases had no obvious symptoms; due to the regular physical examination found that lung nodules. In 4 cases, cough and expectoration, or chest pain was the chief complaint. There were 5 cases underwent surgery due to increasing growth of tumor. Macroscopic examination: the tumor was round or oval, with clear boundaries and smooth surface, and was easy to "jump out" from the surrounding lung tissue. The cutting surface were pale, solid, texture soft. And the maximum diameter were 0.5-5.5 cm. Microscopy examination: the size of tumor cells was consistent, with plate shaped tumor growth. The tumor cells were mild form, circular, ovoid or polygon. The cell boundaries were clear, with cytoplasm bright or eosinophilic. The nucleus size was consistent and middle, with visible nucleoli. There were lots of slender thin-walled blood vessels in the stroma. Local visible cell atypia could be seen in only 1 case. Immunohistochemical and histochemical staining: tumor cells positive/partly positive for: HMB-45, Melan-A, CD34, S-100, Syn, TFE3; did not express CK or EMA, PAX8, CD10, SMA; Ki-67 index was low. Histochemical staining PAS was positive in most of the cases. Differential diagnosis: primary clear cell carcinoma of the lung, lung clear cell carcinoid, granular cell tumor of the lung, metastatic clear cell tumors, paraganglioma, clear cell sarcoma or malignant melanoma were identified. The right diagnosis can be made by using immunohistochemical antibodies and histochemistry staining (PAS). Such as epithelial markers (CK and EMA), melanin markers (HMB45, Melan-A), neuroendocrine markers (Syn, CgA, NSE, CD56), some organizations source

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specific markers (PAX8, CD10, Hepatocyte, TTF1, NapsinA, etc.).

Conclusion: CCLT is a rare benign tumor, and generally single, accidentally multiple. The macroscopic inspection has certain characteristics. Sometimes microscopic performance can be easily misdiagnosed by other clear cell tumors. Clinical, pathological features, morphological and immunohistochemical and histochemical staining should be used to make the right diagnosis.

Category: Mediastinal and thoracic pathology
1657279

GPRC5A reduction contributes to poor prognosis of IIP patients

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ABSTRACT

Background: Idiopathic pulmonary fibrosis (IPF) is a chronic interstitial lung disease with unknown etiology. Many potential risk factors have been reported for many years to be related to the pathogenesis of IPF. Among the risk factors, smoking and pollutant exposure are responsible for the recently increased number of IPF patients.

Method and results: We focused on the first barrier epithelium in lung injury to summarize the reported environmental exposure-induced differentially expressed genes (DEGs) in epithelial cells and overlapped with our IPF patients' lung sequencing data, drawing an overlap map of the common DEGs (CO-DEGs). These CO-DEGs are identified as the potential biomarkers in IPF pathogenesis. To narrow these latent transcriptome data, we simply validated the CO-DEGs to finally focus on a top one dysregulated gene GPRC5A which showed an obvious time-dependent decrease in pollutant Benzo[a]pyrene (BaP) treated epithelial cell line. With the verification of human sequencing data, we performed IHC staining within the selected protein GPRC5A on human IPF, INSIP and control lung samples. The IHC array revealed that GPRC5A protein mainly expressed in normal lung epithelium and decreased in IPF/INSIP epithelial cells by automated image analysis, especially in abnormal hyperplastic epithelium. The low GPRC5A expressed score is dramatically associated with poor prognosis, high age and IPF disease type.

Conclusion: In conclude, GPRC5A deficiency contributes to poor prognosis of idiopathic interstitial pneumonia (IIP) patients.

LINC00520 Promotes EGFR-TKIs Acquired Resistance in Non-Small Cell Lung Carcinoma

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Objectives: *EGFR* tyrosine kinase inhibitors (TKIs) therapy is a validated approach in the treatment of *EGFR*-mutated non-small cell lung carcinoma (NSCLC), but resistance universally develops and it has become a major obstacle in prolonging the survival of patients. More novel molecular biomarkers are still urgently required to elucidate the underlying mechanisms of resistance. This study aimed to investigate the role of *LINC00520* in the acquired resistance of NSCLC to *EGFR*-TKIs.

Materials and Methods: The *EGFR*-mutated NSCLC cell line PC9 was cultured with gefitinib for more than 6 months to acquire gefitinib-resistance, which was designated as PC9R. The expression patterns of *LINC00520* were characterized using reverse transcription quantitative polymerase chain reaction (RT-qPCR), and lentiviral vectors were used to infect cells to regulate the expression. Cytotoxicity of *EGFR*-TKIs on infected cells was determined by cell counting kit-8 (CCK-8). Gene expression profile from GEO dataset and survival follow-up time of 948 NSCLC samples from TCGA dataset were enrolled in this study. In addition, Statistical analysis was mainly performed by R programming language and GraphPad Prism 6.0 (GraphPad).

Results: *LINC00520* is highly expressed in gefitinib-resistant cell line PC9R relative to PC9 (log2 fold change>3, p<0.05). Inhibiting *LINC00520* with lentivirus vectors induces apoptosis in PC9R. *LINC00520* could promote cell proliferation and induce resistance. Statistics from TCGA dataset demonstrate there is no significant difference in *LINC00520* expression between LUAD tissues (483) and normal tissues (347), but the expression level in LUSC tissues (486) is much higher than normal (338). More interestingly, higher *LINC00520* TPM implies a worse survival rate in LUAD (OS: HR=1.4, p =0.028, DFS: HR=1.4, p =0.043), while this phenomenon does not appear in LUSC (OS: HR=0.86, p =0.28, DFS: HR=1.1, p =0.7).

Conclusions: *LINC00520* is involved in acquired resistance of *EGFR*-TKIs in NSCLC. It may serve as a predictor and a potential therapeutic target for *EGFR*-TKIs resistance.

Category: Mediastinal and thoracic pathology
1657868

Degradation of stemness by SREBP1-mediated metabolic reprogramming after FRK knockout in NSCLC

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Aim
To explore the regulatory molecule between metabolic reprogramming and lung cancer cell stemness after knockout of non-receptor tyrosine kinase (FRK) in non-small cell lung cancer (NSCLC).

Methods
1. Detection of metabolic reprogramming (Warburg effect and energy phenotype) and stemness (cell marker CD133/CD44) in the FRK knockout H1299 cell line by CRISPR/Cas9
2. RNA-Sequence was used to detect differential genes in control and experimental cells. Sterol regulatory element binding protein 1 (SREBP1) was identify the possible regulatory factor between metabolic reprogramming and stemness by literature.
3. Western blot analyzed SREBP1 protein level between the two groups
4. Immunoprecipitation was used to detect the relationship between SREBP1 and CD44(stemness marker), statistical analyze the correlation between SREBP1 and CD44 of immunohistochemical results in lung cancer cases.

Results
1. Inhibition of metabolic reprogramming and NSCLC stemness after FRK knockout.
2. RNA-Sequence Wayne results showed the SREBP1 of knockout group reduced to 0.5 times of the control group, thus SREBP1 was initially identified to be regulator between the dry and metabolic reprogramming by relevant literature.
3. Western blot results showed that the level of SREBP 1 in the knockout group was significantly decreased, consistent with the trend of RNA-Sequence results.
4. IP results showed that SREBP1 interacted with CD44, and Spearman rank correlation test results indicated that SREBP1 and CD44 are correlated (r=0.45).

Conclusion
It is preliminarily shown that SREBP1 is an intermediate regulator that regulates metabolic reprogramming and stemness after FRK knockout, and ultimately mediates the driver role of in promoting cancer in lung cancer.

Category: Mediastinal and thoracic pathology
1657960

Mediastinal follicular dendritic cell sarcoma

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Objectives: Mediastinal follicular dendritic cell sarcoma (FDCS) is extremely rare. Due to potential under-recognition of this disease, it happens to be misdiagnosed, especially on core needle biopsy. We report 3 cases of mediastinal FDCS and provide a literature review to improve better understanding of the tumor and to reduce misdiagnosis. **Methods:** 3 cases of mediastinal FDCS in our clinic practice were studied, including their core needle biopsy and resected specimens, and those cases reported previously in English literature were retrieved and analyzed. **Results:** The core needle biopsy of case 1 showed a tumor reminiscent of classical Hodgkin's lymphoma (CHL), while the resected mass was finally diagnosed with FDCS combined with hyaline-vascular Castleman's disease. Both the biopsy and resected tissue of case 2 were constitutive of the clear epithelioid cells with marked atypia. In both cases, definitive diagnoses were not made on core needle biopsy. In case 3, there were some areas morphologically similar to CHL, and some areas contained ovoid to spindle-shaped tumor cells with fascicular pattern. The analysis of 43 cases of mediastinal FDCS showed the age of patients were from 16 to 76 years old, the male to female ratio was 1.5:1, the maximal tumor diameters were 3-17 cm. 18 cases were underwent preoperative biopsy, whereas 15 (83.3%) of which were misdiagnosed initially, often as lymphoma. 32 patients had available follow-up data, the rates of recurrence, metastasis, and mortality were 12.5%, 18.8% and 28.1%, respectively. Current limited data suggested no statistical differences between adverse prognosis and gender, age, tumor size, necrosis, or different therapeutics, respectively. **Conclusions:** Mediastinal FDCS is a rare malignancy that has yet not been fully understood and been often misdiagnosed, particularly when making a diagnosis on core needle biopsy. Increased awareness of this enigmatic tumor is crucial to avoid diagnostic pitfalls.

Category: Mediastinal and thoracic pathology
1657996

Clinicopathological observation and literature review of Multifocal Micronodular Pneumocyte Hyperplasia

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Objectives: To explore the clinical imaging, pathological features and differential diagnosis of Multifocal Micronodular Pneumocyte Hyperplasia (MMPH).
Materials and Methods: Retrospective study on a rare case of MMPH along with literature review.
Result: The patient was a 53-year-old man, who found extensive distribution of ground-glass nodules in both lungs on CT by routine physical examination. There were no effect of anti-infective treatment including against tuberculosis. PET/CT showed multiple subependymal calcified nodules, multiple low-density images in the liver, multiple nodular dense shadows in the skeletal, multiple cysts in the kidney. All the imaging findings suggested Tuberous Sclerosis with multiple organs involvement. For clear diagnosis, he received lung wedge resection with VATS. Grosslly, there were more than 10 scattered gray and yellow nodules in the right upper lung and the right lower lung, with a diameter of 0.2cm-0.7cm. Microscopically, type II alveolar epithelium was actively proliferated, some of which showing hobnailed pattern along with alveolar wall. There were abundant macrophages clustered in the alveolar space. In some nodules, the alveolar septa were expanded, with fibrous tissue hyperplasia and scattered lymphocytes infiltration. High power, the type II alveolar cells were bland with normal nuclear-to-plasma ratio. Immunohistochemistry showed alveolar epithelium TTF-1 (+), p53 (wild type), ki-67 (individual +), CD68 (macrophages +). Elastic fiber staining showed hyperplasia of elastic fiber in some nodules without involvement of pulmonary membrane. Gene mutational test showed that EGER, KRAS, NRAS, BRAF, PIK3CA, HER2, RET, EML4-ALK, ROS1 were all wild type. And there was no specific deletion or repeat variation in the exon region of Tsc1/2 gene. The patient was followed up for 16 months, and there was no any evidence of progress. The most important differential diagnoses is atypical adenomatoid hyperplasia (AAH) and adenocarcinoma in situ (AIS). Both of them can show ground glass nodules, but AAH/AIS often has solitary nodule, even if multiple foci, the number is often less than MMPH. Clinical history of TSC can support differential diagnosis. Pathologically, AAH and AIS emphasize the atypia, dark nuclear staining, overlapping crowding of the tumor cells, bulb or hobnail like projections, and also increased nuclear-to-plasma ratio. The background is relatively clean, the alveolar septum is not widened or only mild rigid, no active macrophages and inflammatory cell infiltration. In addition, metastatic carcinoma of the lung should be ruled out. Generally, metastatic carcinoma cells have significant malignant characteristics with obvious atypia, high nuclear-to-plasma ratio, and different nuclear sizes. Meantime, clinical history is very critical, and relative immunostaining markers may be helpful. Lung miliary tuberculosis is another differential diagnosis, imaging of which usually

show multiple cavernous nodules. Typical tuberculous granuloma with caseous necrosis can be seen under the microscope. Acid-fast dyeing can highlight *Mycobacterium tuberculosis*.

Conclusion: MMPH is a very rare tumor-like lesion with good prognosis. It is associated with Tuberous Sclerosis and often has no specific clinical symptoms. It is usually found in physical examination or accidental conditions. In the imaging examination, the multiple nodules of bilateral lungs were randomly distributed and easily misdiagnosed as various infections or metastatic cancers. Definite diagnosis depends on pathology.

MET exon 14 skipping mutation and amplification in a Chinese Non-small cell lung cancer cohort

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Background Somatic mutations of *MET* gene are emerging as important driver mutaions for lung cancers. In this study, we annotate the types and frequencies of *MET* exon 14 skipping mutations and gene amplification in non-small cell lung cancer (NSCLC) and describe their clinicopathologic associations.

Methods and Materials Next generation sequencing was performed on 1358 NSCLC cases collected between June 2017–June 2019. *MET* gene altered cases were reviewed and correlated with patient demographic data annotated from electronic medical records.

Results Overall, among 1358 NSCLC cases, *MET* exon 14 skipping mutation and gene amplification were observed in 14 (1.03%) and 16 (1.18%) cases, respectively. Histopathological data were available for review in all 30 cases, which included 28 adenocarcinoma (93.4%), one poorly differentiated NSCLC (3.33%), and one adenosquamous carcinoma (3.33%). *MET* exon 14 skipping mutations were identified from one minimally invasive adenocarcinoma and one poorly differentiated NSCLC, both of two patients did not carry other driver mutations. The remaining 12 cases were invasive adenocarcinoma, including 8 acinar subtype predominant, 2 solid subtype predominant, 1 papillary subtype predominant, and 1 micropapillary subtype. Of the *MET* exon 14 skipping mutation patients, the pathological stage of each tumor revealed 10 patients in stage I, 1 patients in II, 2 patients in III, and 1 patients in IV. *MET* gene amplification was identified from one adenosquamous carcinoma that harbored *KIF5B-RET* and *CCNYL2-RET* gene fusions, simultaneously, which was a rare molecular phenomenon. The remaining 15 *MET* gene amplified cases were all invasive adenocarcinoma. Among those abovepatients, pathologic stage was as defined as follows, 4 patients were in stage I, 1 in II, 7 in III, and 4 in IV.

Conclusions The two types of *MET* gene alterations are both present in low frequency (~1%) in the lung cancer population of Chinese. *MET* exon 14 skipping mutation was more commonly observed in early stage, whereas *MET* gene amplification was relative frequent in advanced stage patients. The presence of *MET* exon 14 skipping mutation and amplification in minor histological types of lung cancers urge to extend screening scope including rare mutations in lung cancer and treatment response evaluation in future clinical trials. These would be important next steps for the guidance of *MET* targeted therapy in clinical practice.

Clinicopathological observation of 15 cases of lung neoplasms with bronchial adenoma

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[Abstract] **Objectives** To explore the clinicopathological characteristics, diagnosis and differential diagnosis of bronchiolar adenoma (BA). **Materials and Methods** Clinicopathological data of 15 cases of lung neoplasms with BA features were collected from the First Affiliated Hospital of Nanjing Medical University (Jan 2016-Aug 2019). The clinical data, imaging examination, morphology, immunostaining and molecular changes were analyzed. **Results** Most of the patients were female (male: female = 3:12), mainly in middle-aged and elderly (range from 51 to 77 years), with only one case being a 26-year-old young woman. Three of them had a history of smoking. The patients usually had no clinical symptoms. The imaging findings indicated ground-glass and lobulated nodules. Grossly, the tumor was gray-whitish, taupe solid or cystic solid nodule with explicit boundary but no capsule, with a maximum diameter of 0.4-3.3 cm. Histologically, we observed glandular, papillary, or flat structures composed of basal cells, mucous cells, and ciliated cells, some tumors with basal cell proliferation and squamous cell metaplasia. However, there were some cases without mucous and/or ciliated cells. The glandular type was the most common, followed by the papillary type, The flat type could be seen adjacent the glandular type. The main diagnostic criteria for lung neoplasms with BA include: (1) double-layered cell structure of the glandular or papillary, showing a continuous basal cell layer; (2) papilla containing a large fibrous core; (3) most tumors showing cilia existence; (4) most tumors have abundant extracellular mucin and floating micropapillae in a mucin pool. In addition, the following histological manifestations can be observed: unclear cell wall, disordered or multi-layered arrangement, and mostly round or cylindrical, the cytoplasm seems to have turbidity; a large number of focal lymphocyte infiltration in the fibrous core or stroma; a thick-walled large vessel and bronchiole are usually seen inside or around the tumor. Immunophenotype, basal cells were positive for P63, P40 and CK5/6, TTF-1 was positive in basal cells, type II alveolar cells and Clara cells, but negative in mucous cells and ciliated cells. CK20 was not expressed in three tumor cells, NapsinA was scattered in cilia and mucus cells. Generally, the Ki-67 proliferation index was low(2-15%). BRAF-V600E mutation was the most frequently molecular change in these kind of tumors, however, its mechanism and significance were still unclear. All patients were followed up for 1-23 months without any recurrence or metastasis. **Conclusions** Up to date, it is known that BA is a benign neoplasm that develops in the peripheral lung with good prognosis. Definite diagnosis is very crucial for surgical treatment, especially in frozen consultation. Differential diagnosis mainly include invasive mucinous adenocarcinoma, colloid adenocarcinoma,

mucoepidermoid carcionma. Immunohistochemistry and molecular analysis will be helpful if necessary.

Comparison of ALK Fusion Protein Positive Rate between Small Biopsy Specimens and Surgical Resected Specimens of Lung Cancer

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Objective To investigate the consistency of ALK fusion protein positive rate between small biopsy specimens and surgical specimens of lung cancer. **Methods** The expression of ALK fusion protein in 468 paraffin-embedded specimens of primary lung cancer was detected by Ventana automatic immunohistochemistry. The difference of ALK fusion protein expression between small biopsy specimens and surgical specimens was analyzed retrospectively. **Results** Of the 468 lung cancer specimens, 214 cases were small biopsy specimens and 254 cases were resected specimens. The positive rates of ALK were 4.2% (9/214) and 3.5% (9/254), respectively. There was no significant difference between the two groups ($p > 0.05$). **Conclusion** Both small biopsy specimens and resected specimens of lung cancer can be used as ALK fusion protein detection specimens.

Category: Mediastinal and thoracic pathology
1658149

Clinicopathological Characteristics of SMAD4 Mutation in Non-Small Cell Lung Carcinoma

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Objectives: Research suggests that *SMAD4* is specifically inactivated in over half of pancreatic duct adenocarcinoma. Recently, we have noticed *SMAD4*-mutated non-small cell lung carcinoma (NSCLC) cases, and these rare cases will be summarized and analyzed to explore its clinicopathological characteristics in this study.

Materials and Methods: Next-generation sequencing (NGS) was performed on 1358 NSCLC cases collected from June 2017 to June 2019. *SMAD4*-mutated cases were reviewed and correlated with electronic medical records. Immunohistochemistry was performed to measure *SMAD4* expression. Selected NSCLC cases from TCGA dataset were enrolled in this study.

Results: Of 1358 NSCLC cases, 16 (1.18%) harbored *SMAD4* mutation, 9 women and 7 men. The mean age was 61 years old (range, 35-79), and the average age of women (66) was higher than men (55). Most of the patients were non-smokers (13/16). Pathology materials were available for review in 16 cases, which included 13 (81.25%) primary cancer patients without metastasis and 3 cases (18.75%) with distant metastasis. In terms of pathologic type, the great majority were adenocarcinomas (15/16) and only 1 was adenosquamous carcinoma (1/16). In these 16 cases, 13 (81.25%) were accompanied by *EGFR*-mutated gene. Immunohistochemistry results showed *SMAD4* protein expression was significantly heterogeneous. Statistics from TCGA dataset demonstrate the expression of *SMAD4* in NSCLC tissues (483) is lower than normal (347).

Conclusions: *SMAD4* mutation could occur in NSCLC, besides serving as a significant molecular biomarker for pancreatic cancer. *SMAD4* may be involved in the initiation and development of NSCLC and its protein expression is significantly heterogeneous.

Recent advances in pathologic research and targeted therapies of thymoma

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Thymoma is a rare tumor that was reclassified by the World Health Organization in 2015. Recent studies have made advances in molecular targeted therapies, The role of some molecular markers in the development of thymic epithelial tumors was discovered, such as c-KIT, EGFR, IGF-1R, PTEN, HDAC, VEGF and PD-L1. Additionally, new molecular markers such as CTV/CTS, GTF2I, Pax8 and DSG-3 have been used in the differential diagnosis of thymoma. This article reviews molecular pathogenesis of thymoma, application of molecular pathology in the differential diagnosis of thymoma and recent progress in targeted therapies for thymoma. In order to provide reference for the study of thymic tumors.

Analysis of clinicopathological features of 800 cases with cardiac tumors

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Objective: To observe the clinical and pathological data of 800 patients with primary and secondary cardiac tumors. To explore the morphological features, distribution of age, gender and sites of tumor as well as their correlation, providing data for pathological diagnosis and epidemiological studies of cardiac tumors in China. **Methods:** We have retrospectively reviewed 800 consecutive cases diagnosed with cardiac tumor in the Department of Pathology, Beijing Anzhen Hospital from Aug. 2007 to Jul. 2018, with all clinical data, HE results, immunohistochemical(IHC) results and molecular pathological results available. Pathological diagnosis and classification were completed according to the 4th edition of "WHO lung, pleural, thymus and cardiac tumor classification" criteria. Then, the results were summarized and analyzed using SPSS20. **Results:** The 800 cases (age range:23-81yrs; Mean age:52yrs; Peak incidence:40-59yrs; 258males&542 females), of which 792 cases was primary tumors (792/800, 99%), and 8 cases was secondary tumors (8/800, 1%). Benign tumors, more commonly, accounted for 95.1% (761/800), on the other hand, the malignant tumors accounted for 4.9%(39/800). Female have a higher prevalence rate than male no matter benign tumors, borderline or malignant cardiac tumors (513:239, 3:1, 30:14). Myxoma appeared most commonly (677/800, 84.6%). With regard to sites of tumor, 633 occurred in the left atrium, followed by the right atrium (83 cases), pericardium (8 cases), right ventricle (25 cases), left ventricle (33 cases), and 18 cases occurred in multiple hearts cavity or valve/artery. Benign tumors occurred more commonly in the left heart system (523/625, 76.3%), however, borderline tumors occurred more commonly in the right heart system (5/9, 55.6%), in addition, malignant tumors occurred more commonly in the right heart system (22/55, 40.0%). Both benign and malignant tumors occur in the left heart system (80%), and the incidence of borderline tumors in the left heart systems is almost the same as that of right heart systems(1:1), followed by pericardium(32.7%). Heart papillary elastic fibroma and rhabdomyoma performed unique histopathological features. However, other cardiac tumors showed similarly morphologically, leading to misdiagnose. **Conclusions:** Myxoma prevelanted in the primary cardiac benign tumors. Angiosarcoma and mesothelioma are dominant in primary cardiac malignancies. Rhabdomyomas and fibroids are predominant in children with cardiac tumors. As the largest samples of cardiac tumors in China, our study reflected the epidemiological and pathological features of various cardiac tumors in the Chinese population.

Mesothelial/monocytic Incidental Cardiac Excrescence (MICE) in a Patient with Suspected Acute Rheumatic Heart Disease: A Case Report

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Introduction
Rheumatic heart disease (RHD) is a pan-carditis resulting from an altered immune response to Group A β-hemolytic Streptococcal pharyngitis. The acute phase is seldom seen in the local surgical pathology settings. Rheumatic valvular disease is a long-term complication and the mitral valve is typically involved. Mesothelial/monocytic incidental cardiac excrescence (MICE) is a rare benign, incidental finding of histiocytic/mesothelial proliferation, encountered in cardiothoracic surgery. Here we present a case of MICE in a patient with suspected acute RHD.
Case presentation
A 17-year-old male was incidentally found to have pansystolic murmur during pre-enlistment medical examination. Transthoracic echocardiography revealed mitral valve prolapse with severe mitral regurgitation. He underwent mitral valve replacement. Intraoperatively, there was thickened mitral valve and features of pericarditis. A 2.5cm anterior mediastinal lymph node was identified and excised for histopathological examination together with the mitral valve.
The mitral valve showed acute-on-chronic endocarditis. Aggregates of activated macrophages were seen with focal peripheral palisading and surrounding central fibrinous material, reminiscent of the Aschoff nodules.
The "mediastinal lymph node" included two small reactive lymph nodes and a loose nodular fragment comprising sheets of histiocytes admixed with rare islands of mesothelial cells. There were also entrapped adipocytes, fibrin and mixed inflammatory infiltrate. The histiocytes were positive for CD68 and CD31, negative for S100 and CD1a. Diagnosis of MICE and possible acute RHD was made. Retrospectively, the patient confirmed a 2-3 days history of sore throat before his initial check-up.
Discussion
There are approximately 50 cases of MICE reported, with 15% associated with RHD. It is recently believed to be part of the entity "histiocytosis with raisinoid nuclei". A reactive or iatrogenic etiology has been proposed. Langerhans cell histiocytosis, Hodgkin lymphoma, and metastatic carcinoma are among the main differential diagnoses. Diagnosis is made through clinic-pathological correlation supported by typical morphological and immunohistochemical features.

Category: Cardiovascular pathology
1651654

Atresia of Common Pulmonary Vein (ACPV) of Fetus: An Autopsy Analysis

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Objective: Atresia of common pulmonary vein(ACPV) is a rare congenital anomaly. We reported 5 cases of fetal ACPV.

Patients and Methods: The autopsy records of fetus diagnosed with atresia of common pulmonary vein were studied in our study of 397 autopsied cases of congenital heart disease who had been found using echocardiography.

Results: Five fetus (four females and one male) had ACPV. Thses included three cases of total atresia of common pulmonary vein(TACPV) (females) and two cases of partial atresia of common pulmonary vein(PACPV) (one female and one male). One case of congenital pulmonary venous hypoplasia with total anomalous pulmonary venous drainage. There were 4 cases with single ventricle, 3 cases with single atrium, 3 cases with persistent left superior vena cava(PLSVC), 2 cases with persistent truncus arteriosus(PTA), 2 cases with right aortic arch, 2 cases with unroofed coronary sinus syndrome. There were 4 cases with asplenia, 3 cases with pulmonary hypoplasia and 1 csae with visceral heterotaxy. It is difference from infant that the fetus was not associated with pulmonary lymphangiectasis.

Conclusion: ACPV is an extremely rare congenital heart disease and it was not associated with pulmonary lymphangiectasis. It is very difficult to establish a diagnosis of ACPV for fetus.

Category: Cardiovascular pathology
1651678

Clinicopathological characteristic of cardiac myxoma with arterial embolism as the first symptom

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Objective：To investigate the clinical and pathological features as well as differential diagnosis of patients with cardiac myxoma diagnosed with arterial embolization as the first symptom.

Materials and Methods：7 cases of cardiac myxoma with arterial embolization as the first symptom were collected and all of them received surgical treatment, the clinical data were reviewed, and the clinical manifestations, imaging characteristics and pathological changes were analyzed.

Results：①7 patients were diagnosed with arterial embolism, including 3 patients with pulmonary embolism, 2 patients with abdominal aorta and iliac arteries embolism, 1 with femoral artery embolism, and 1 with popliteal and posterior tibial arteries embolism. The pathological examination confirmed myxoma embolus. ②Among the 7 patients, 5 were male and 2 were female, with an average age of 47.57 ± 10.39 years old. ③3 patients showed clinical manifestations of pulmonary embolism, 2 patients had constitutional symptoms, and 1 patient had only lower extremity arterial embolism symptoms. 1 patient had no symptoms and was admitted to hospital due to arterial embolism after trauma. ④Echocardiography suggested that there were 4 cases of left atrial myxoma, 2 cases of right atrial myxoma, and 1 case of right ventricular myxoma.

Conclusion：The clinical manifestations of embolism caused by cardiac myxoma are not specific, mainly depending on the embolization site, and it is difficult to identify with thromboembolic disease, which often leads to misdiagnosis and missed diagnosis. Therefore, when patients with arterial embolism are encountered, it is hoped that clinicians can exclude the possibility of embolism caused by cardiac myxoma, and make accurate and timely diagnosis based on imaging examnation.

Cardiac metastases from solid papillary carcinoma of the breast: analysis of clinicopathological characteristics

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Objective: To gain further understanding of the pathological features and biological behavior of solid papillary carcinoma (SPC) of the breast. **Methods:** We stained tissue sections of a right atrial tumor excised from a patient with SPC of the breast with metastasis to the right atrium, using hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC). **Results:** The solid area of the tumor showed an intermingled fibrovascular network with no apparent papillary structures on H&E staining, providing an auxiliary diagnosis for myocardial invasion. IHC staining confirmed cardiac metastasis of invasive SPC of the breast. **Conclusions:** Pathological examination of this case improves our understanding of the diagnosis and treatment of metastatic SPC.

Category: Cardiovascular pathology
1655767

Congenital isolation of the subclavian artery in fetus

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Objective:: Isolation of the subclavian artery is an uncommon aortic arch anomaly. We report 4 cases of isolation of the subclavian artery.

Patients and Methods: The autopsy records of fetus diagnosed with isolation of the subclavian artery were studied in our study of 397 autopsied cases of congenital heart disease.

Results: Four cases of congenital isolation of the subclavian artery in fetus are described. There is one case of congenital isolation of the left subclavian artery, two cases of congenital isolation of the innominate artery, one case of congenital isolation of the right subclavian artery. Congenital isolation of the left subclavian artery is associated with tetralogy of Fallot and right aortic arch. Congenital isolation of the subclavian artery has also been associated with other rare anomalies, such as innominate vein behind the aortic arch or cervical aortic arch. The expression of Cx43 in myocardial tissue was detected by immunohistochemical staining, and abnormal expression of Cx43 was detected in fetus with congenital isolation of the subclavian artery.

Conclusion: Isolation of the subclavian artery is an extremely rare congenital heart disease. it is abnormal expression of Cx43 in fetus with congenital isolation of the subclavian artery.

A single - center clinical pathology study of cardiac tumors

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Beijing anzhen hospital

Abstract

Background: cardiac tumors are rare compared to other cardiac diseases. The clinical symptoms ranged from lack to nonspecificity. This huge variation and a general lack of heart tumor-related symptoms often lead to delays in diagnosis and treatment. **Objective:** To provide data support for the epidemiological study of cardiac tumors in Chinese population, analyze the treatment and prognosis of various cardiac tumors, and provide reference for the pathological diagnosis and clinical treatment of cardiac tumors. **Methods:** clinical data and postoperative specimens of all patients who underwent cardiac surgery due to space-occupying lesions treated in Anzhen hospital from 2007 to 2019 were retrospectively analyzed. The clinicopathological features, imaging features and disease outcomes were analyzed and compared with existing literature. **Results:** in the past 12 years, about 1000 patients underwent cardiac mass resection in our hospital, and 600 patients were selected as study subjects. The proportion of primary heart tumor was 70.2% (n = 432). Were falls into 394 benign tumors and 38 malignant tumors. In 55 cases (9.6%) of malignant tumors, secondary cardiac tumors were metastasized from various parts of the body. Most cardiac tumors are benign, mainly cardiac myxoma. Papillary fibroelastoma is the second most common primary tumor. **Conclusions:** our data were compared with literature on the frequency and distribution of different cardiac tumors. There is little difference between the data obtained and the data in previous literatures. Patients with benign heart tumors can be cured by direct surgical resection, but there is still the possibility of recurrence and malignant transformation. In most cases, surgery is a palliative strategy for primary cardiac tumors and cardiac metastases. The prognosis and survival rate analysis of cardiac malignancy need further study.

Case series: acute myocardial infarction with atypical symptoms

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Introduction
Acute myocardial infarction (AMI) remains a leading cause of morbidity and mortality worldwide. Rapid and accurate diagnosis of AMI are critical for initiating effective treatment and achieving better prognosis.

Methods
A multi-center, retrospective study of patient died of AMI was from six judicial expertise centeres during 2010 to 2018. The cases were divided into two groups: one group with no obvious symptoms and sudden onset or death in sleep as asymptomatic group, the other group with prodromal symptoms but not typical symptoms of AMI, as atypical symptoms group. Data of clinical treatment, autopsy and histopathology were manually reviewed for adherence to diagnostic criteria of AMI. Cases with other cause of death were excluded.

Results
From 2010 to 2018, there were 202 patients identified with AMI with atypical symptoms. Of this, male accounted for 90.5%, the age was mainly 40 years old 50 years old, followed by over 60 years old. There were 70 cases were in the atypical symptom group and 132 cases in the asymptomatic group. Of the atypical symptom group, 39 cases were the time from symptoms to death was more than 24 hours, accounting for 55.7%. According to the results of autopsies and histology, 24 cases (61.5%) of the atypical symptom group, had AMI with attack time less than 12 hours. 100 cases (75.8%) had AMI within 12 hours, and 32 cases (24.2%) had AMI within 12 hours in the asymptomatic group.

Conclusion
In the asymptomatic group, 75.8% had acute myocardial infarction in less than 12 hours and 24.2% had AMI in more than 12 hours. The lack of symptoms failed to attract the attention of the patient himself or the doctor. Most asymptomatic cases are accompanied by hypertrophy or severe coronary atherosclerosis, and age is mainly concentrated in people aged 40-50 years or over 60 years of age. Physical examination and detailed evaluation of cardiovascular system should be strengthened in this group. In the atypical symptoms, 39 cases (55.7%) were more than 24 hoursfrom onset to death. Of 39 cases, 24 cases were less than 12 hours from onset of AMI. These 24 cases considered early angina pectoris or myocardial ischemia, and further developed into AMI because of atypical symptoms. These cases suggest that attention should be paid to the possibility of angina pectoris or myocardial ischemia in people over 40 years old, especially in men, even if they are treated with stomachache, upper sensation and other atypical symptoms.

Adult Cellular Rhabdomyoma of the Heart: A Case Report And Review of the Literature

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Cardiac rhabdomyomas are the most common cardiac tumor in infancy and childhood, and there is a strong association between cardiac rhabdomyoma and tuberous sclerosis. Cardiac rhabdomyomas are extremely uncommon in the adult patient, mainly occurs primarily in the head and neck region of men and women older than 40 years (extracardiac rhabdomyoma) . Adult cardiac cellular rhabdomyoma is extremely rare, at present, there are less than 10 cases reported. We present such a case and focus on the clinical and histologic features of an adult cardiac cellular rhabdomyoma.

[Abstract] Objective: The purpose of this report focus on the clinical and histologic features of an adult cardiac cellular rhabdomyoma, diagnosis, differential diagnosis and prognosis of adult cardiac cellular rhabdomyoma. Methods: We present the case of a 60-year-old man , the patient remains free of recurrence one year after excision. The mass was visible in the right ventricle, attached to the septum, the tumors was observed by light microscopy and immunohistochemical staining, and the literature was analyzed and discussed. Results:Histologically, the tumors were composed of round or polygonal acidophilia cell, with fine granular cytoplasm, occasional vacuolar degeneration, occasional spider cells, and necrosis and mitotic figures were absent. Immunohistochemical stains showed tumor cells to be positive for AE1/AE3, Vimentin, Myoglobin, SMA, Desmin, and negative for myogenin ,MoyD1, Calretinin, Fli-1, ERG, CD31, CD34, S-100 ,Two hundred nuclei were counted, and proliferative markers showed 1% of nuclei staining with Ki-67.Conclusion:Adult cardiac rhabdomyoma is extremly rare,the diagnosis depend on the comprehensive analysis of clinical features, histological morphology and immunohistochemical results. It is a proliferating neoplasm with an excellent short-term course,and considered to be a benign neoplasm,but uncertain long-term clinical behavior.

CD36 expression in coronary artery of postmortem computed tomography cases with and without contrast application: A preliminary report

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Introduction: Atherosclerosis is the underlying pathology behind cardiovascular disease, the leading cause of mortality worldwide. Postmortem diagnosis depends on gross and microscopic examination of the artery involved. In situation where only a limited autopsy is possible, postmortem computed tomography (PMCT) angiography is a valuable tool for its diagnosis. It is uncertain whether use of contrast media affects atherosclerotic lesion, especially the expression of the markers of inflammation. CD36 is a membrane glycoprotein; it plays a pivotal role in atherogenesis.

Aim: To investigate coronary artery endothelial CD36 expression in PMCT subjects with and without contrast application.

Methods: This study has been approved by the institutional ethics committee. Sudden death cases received at the National Forensic Medicine Institute, Kuala Lumpur from 2014 to 2016 that had underwent PMCT with and without contrast were chosen. During autopsy, the coronary arteries were sampled, fixed in 10% formalin, processed into paraffin-embedded blocks, sectioned and stained by immunohistochemistry method utilizing CD36 primary antibody. Briefly, after a deparaffinisation process, the sections were treated with hydrogen peroxide. Heat induced antigen retrieval process was then performed. The sections were cooled and subsequently incubated with primary antibody for CD36 overnight at 4℃. Then the sections were washed and incubated with a biotinylated secondary antibody. Subsequently DAB chromogen was applied, followed by washing steps and counter staining with haematoxylin. The stain was viewed under light microscope and scored using a semi-quantitative scoring scheme. Data was analysed as using chi square or Fisher's exact test.

Results: Thirty nine cases were included in this preliminary study. There is no difference in endothelial CD36 expression between contrast and no contrast groups (p>0.05). Overall, low expression of CD36 was seen in 61.5%, while high expression was seen in 38.5%. Endothelial CD36 expression in normal, early, established and complicated atherosclerosis were comparable (p>0.05).

Conclusion: PMCT with contrast application did not lead to significant alteration in coronary artery endothelial CD36 expression.

Category: Hematopathology
1469087

Crystal-storing histiocytosis associated with a primary gastric extranodal marginal zone lymphoma of mocosa-associated lymphoid tissue

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Objectives:

Crystal-storing histiocytosis (CSH) is a rare condition in which crystal accumulates in the histiocyte cytoplasm. The crystals are often of immunoglobulin origin and highly associated with an underlying B-cell lymphoproliferative or plasma cell neoplasm although non-immunoglobulin variants have been reported in the past. Despite this strong association with lymphoproliferative disorder, CSH remains an under-recognized entity in general pathology practice. The current case illustrates the diagnostic importance recognizing CSH.

Materials and Methods:

A 57-year-old male presented with progressive dyspepsia. Gastric biopsy was performed at an outside hospital 5 month ago and was positive for *H. pylori*. He was treated with antibiotics in order to eradicate the microorganism. He underwent a follow-up upper endoscopy and a mucosal "lesion" was identified in the greater curvature of the stomach. Stomach biopsies were performed and the samples were evaluted by histomorphology, immunohistochemistry and PCR analysis of immunoglobulin heavy chain (IgH) rearrangement. At the time of endoscopic examination, relevant laboratory studies were essentially unremarkable. CT study of abdomen showed no evidence of organomegaly or lymphadenopathy.

Results:

Histomorphology review of gastric biopsy showed no evidence of dysplasia or intestinal metaplasia in the mucoca. No Helicobacter pylori-like microorganisms was identified on H&E or immunohistochemical stain against *H. pylori*. The lamina propria is diffusely infiltrated by cells which have eosinophilic fibrillary crystalloid material in the cytoplasm. These cells demonstrated round to ovoid bland nuclei. Immunohistochemical stains showed these cells were positive for CD68 and CD163; negative for S100, consistent with histiocytes. The intracellular crystals were positive for IgA and kappa light chain; negative for lambda light chain, PAS, PASD, GMS, and AFB. In addition, there were scattered lymphoplasmacytic infilatrates and occasional lymphoid aggregates composed of centrocyte-like lymphoid cells in the laminal propria and submucosa. Occasional lymphepithelial lesions were identified. Immunohistochemical stains showed these aggregates were predominantly CD20+ B cells with scattered background CD3+ T cells.

The B lymphocytes were aberrantly positive for CD43 but negative for CD5, BCL-6, CD10, and cyclin D1. The plasma cells were positive for CD138 and IgA with kappa light chain restriction. CD3 shows scattered T-cells and highlights T-cells in the periphery of the lymphoid aggregates. Molecular study of IgH rearrangement showed reproducible clonal IgH gene PCR products of~ 334 bp, ~268 bp and ~119/127bp (bi-clonal) with three separate primer sets respectively. A diagnosis of extranodal marginal zone lymphoma of mocosa-associated lymphoid tissue was rendered. Additional laboratory study showed no evidence of monoclonal protein in the serum. The patient was followed up for two years without any emerging symptoms.

Conclusion

CSH is commonly associated with immunoglobin producing neoplasms in the gastrointestinal tract. Identification of CSH should trigger a thorough hematopathology workup to discover a probable underlying lymphoproliferative disorder.

The association between JAK/STAT signaling pathway and NF-κB signaling pathway in Natural killer/T-cell lymphoma, nasal type

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Abstract
Objective: To investigate whether there was a relationship between the JAK/STAT signaling pathway and NF-κB signaling pathway in ENKTCL.

Materials and Methods: In total, 109 patients with ENKTCL were included. Immunohistochemistry was used to detect the expression of P65, Rel-B, c-Rel and p-STAT3 in all samples.

Results: NF-κB signaling pathway activation was observed in 24.8% of the cases (27/109). JAK/STAT signaling pathway activation was presented in 56.9% of the cases (62/109). The activation of JAK/STAT signaling pathway was negatively correlated with the activation of NF-κB signaling pathway (P=0.016).

Conclusion: The activation of JAK/STAT signaling pathway and NF-κB signaling pathway were frequent in ENKTCL, and there is no synergistic relationship between the two in the pathogenesis of ENKTCL.

Tumor-forming plasmacytoid dendritic cells in acute myelomonocytic/monocytic leukemia

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Purpose: Plasmacytoid dendritic cells (PDCs) act as a link between innate and adaptive immunity, PDCs infiltration has been reported in infectious diseases, autoimmune diseases and neoplasms. PDCs infiltration in AML patients was urgent need.
Material and methods: we evaluated the frequency of PDCs in 80 acute myelomonocytic leukemia (AML-M4) and 83 acute monocytic leukemia (AML-M5) patients by flow cytometry, Two-step FCM analysis strategy was taken in all AML patients, first, using CD117, HLA-DR, CD34, CD123, we identified myeloid primordial cells and made a diagnosis of AML. Second, gating on the CD123 positive cells, by using CD304, CD56, CD11c, CD13, CD33, CD15, CD14, CD64, CD34, CD117, CD38, TF-PDC was identified as
Lin$^-$HLA-DR^{bri+}CD123^{bri+}CD304$^+$CD56$^+$CD11c$^-$CD13$^+$CD33$^+$CD15$^-$CD14$^-$CD64$^-$CD34$^-$CD117$^-$CD38$^+$.
Results: Results showed that 62 cases were positive for PDCs, which were closely associated with cycles of chemotherapy before achieved CR, patients' WBC count, Hb concentration, platelet count and bone marrow blasts, and the patients with PDC infiltration had a shorter survival time, meanwhile, allo-HSCT could abrogated the side effects of PDCs on AML patients.
Conclusion: We concluded that PDC infiltration has a possible role in risk stratification for patients AML-M4/M5, and our work emphasizes the significance of heterogeneity of AML-M4/M5.

The application of antigen receptor gene rearrangement of BIOMED-2 in the pathologic diagnosis of 348 cases with non-Hodgkin lymphoma in a single institution in Southwest of China

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Objective: To explore the clinical value of immunoglobulin (Ig) and T cell receptor (TCR) gene rearrangement in the diagnosis of non-Hodgkin lymphoma. *Methods*: Using the standardized BIOMED-2 multiplex PCR strategy to detect IgH, IgK and TCR in 272 cases of mature B-cell lymphoma, 55 cases of mature T-cell lymphoma, 21 cases of extranodal NK/ T-cell lymphoma, nasal type, and 20 cases of lymphoid tissue reactive hyperplasia. *Results*: Among all mature B-cell lymphomas, the sensitivity of Ig gene rearrangement was 91.18% (248/272), IgH and IgK gene rearrangement was 76.47% (208/272) and 75.00% (204/272), respectively, meanwhile the sensitivity of TCRγ rearrangement was 3.68% (10/272). In the 55 cases of mature T-cell lymphoma, the sensitivity of the detection of TCRγ was 76.36% (44/55), at the same time the sensitivity of Ig gene rearrangement was 14.55% (8/55), IgH and IgK gene rearrangement was 7.27% (4/55) and 12.73% (7/55), respectively. In 21 cases of extranodal NK/T cell lymphoma, nasal type, and 20 cases of reactive lymphoid hyperplasia, no gene rearrangement was found in the samples of IgH, IgK and TCR. The sensitivity of gene rearrangement in Ig/TCR in B and T-cell lymphoma was significantly different from that in the control group ($P < 0.05$). *Conclusion*: The Ig/TCR gene rearrangement of BIOMED-2 multiplex PCR strategy has important auxiliary value in the diagnosis of B/T-cell non-Hodgkin lymphoma respectively, however, a few B-cell lymphomas may company TCR gene rearrangement as well as a few T-cell lymphomas may accompany Ig gene rearrangement, it must be comprehensively judged with the combination of morphology, immunohistochemistry and clinical features.

Category: Hematopathology
1621496

Clinicopathological observation of idiopathic multicentrial center castleman disease (iMCD) with plasma cell tumor

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[abstract] objective: to explore the clinicopathological characteristics, immunohistochemistry, diagnosis and differential diagnosis of idiopathic multicentrial center castleman disease with plasma cell tumor . Methods: to observe the histological morphology and immunohistochemical characteristics of 1 case of multi-center castleman disease complicated with plasma cell tumor, and to review the relevant literature and comprehensively analyze its clinicopathological characteristics. Results the histological morphology of multi-center castleman disease with plasma cell tumor was different from typical single-center castleman disease, and there was no specificity. Immunohistochemistry showed that tumor cells CD38, CD138, CD79a, mum1 (+), k-chain (-), lambda chain (+), and the number of ki-67 positive cells is 10%, while HHV8, CD3, CD5, CD20, PAX5 are all (-). Conclusion: multi-center castleman disease with plasma cell tumor is a rare disease of lympho-hematopoietic system, which overlages with many diseases, and can only be diagnosed if some other diseases with similar changes are excluded. Therefore, careful observation of the morphology under the microscope, improvement of immunohistochemistry, and joint diagnosis and treatment of multi-disciplines are necessary.

Biochemical, haematological and histopathological studies of extract of Ageratum conyzoides L. in Sprague Dawley rats

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This study was conducted to evaluate the safety potential of the leaf extract of *Ageratum conyzoides* Linnaeus in Sprague Dawley (SD) rats using biochemical, haematological and histological indices of toxicity. Four groups of seven male SD rats per group were used for the study. To group A was administered 0.25% CMC-Na/ kg body weight and was used as the control group, while groups B, C and D were respectively administered with 500, 1000 and 1500 mg/kg body weight of the ethanolic leaf extract of *A. conyzoides* by gastric intubation for 14 days. Animals were subsequently anaesthetized, blood samples were collected for biochemical and haematological assays; organs were isolated and weighed, while the liver, kidney and spleen were processed for histopathological studies. Aspartate amino transferase, lactate dehydrogenase, creatine kinase and alkaline phosphatase were significantly ($p < 0.05$) reduced in the groups treated with 1000 and 1500 mg/kg body weight of the extract. Furthermore, there was a significant ($p < 0.05$) elevation in white blood cell count, mean platelet volume and % platelet distribution width. Histopathological studies indicated various degrees of hepatocellular necrosis in all the treated groups accompanied by significant increases in the weight of liver and spleen. The results showed that the ethanolic leaf extract of *A. conyzoides* significantly alters the biomarkers of cardiac and skeletal muscle disorders, and higher doses could induce liver cell injury.

Tumor Microenvironment Immune Cells' Impact On Prognosis of Primary Gastrointestinal Diffuse Large B Cell Lymphoma

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Objective: Diffuse Large B Cell Lymphoma (DLBCL) accounts for the most part of Non-Hodgkin Lymphoma. The most common type that develop from extra nodal sites is the primary gastrointestinal diffuse large B cell lymphoma (PGI-DLBCL),which has strong heterogenicity in clinical and pathological characteristics. Although the overall survival rate has been lifted to over 70% by the R-CHOP combined chemotherapy, there exists patients underwent extremely bad prognosis. Immune cells, including tumor-associated macrophages, (TAM) and tumor infiltrated lymphocytes (TILs) are reported to be related with prognosis of patients in many kinds of solid tumor. At the same time, the immune therapy has made great success in some tumors by PD1-PDL1, which reminds us that the research on TME's impact on tumor could be of vital importance to analyze characteristics of TME in DLBCL and find new immune therapy strategies. As a result, we collected cases with the diagnosis of PGI-DLBCL in our department, to analyze the clinical features, immunophenotype, distribution and quantity of macrophages and T cells in TME, and to explore their correlation.
Methods: The patients with PGI-DLBCL diagnosed by our department from January 2004 to December 2018 were collected and reviewed. The related clinical data (age, sex, location, tumor stage, IPI score) were consulted and all patients available were followed up by telephone. The overall survival rate (OS) and progression-free survival rate (PFS) were counted. CD4, CD8, CD68 and CD163 immunohistochemical staining were performed on the tumor wax blocks of qualified cases. We observed the outcomes of these tests with microscope and carefully counted the number of cells that express these antibodies. The percentage of positive cells in the center and periphery of the tumor to the total number of visual field cells was calculated. Stata was used to do T test to identify the numerical differences of different regions of the same antibody. Logistic regression along with ROC curve was used to find the appropriate cutoff number of data, and the patients were divided into two groups by using the data when analyze outcomes of different antibodies. Kaplan-Meire method along with Log-Rank test was used for survival analysis. At the same time, we analyze the relationship between clinical factors and results of immune cells by logistic model.
Results: Statistical analysis of immunohistochemical results showed that there was no significant difference in the number of CD68 positive cells between the edge and the center of the tumors (P=0.1305). The percentage of cells expressing CD4, CD8 and CD163 in total cells was different in the central and marginal regions of tumors (P = 0.0000).

The results of prognosis analysis by different group showed that the percentage of CD68 and CD4 positive cells in the total number of cells was not different between the higher group and the lower group(P=0.657; P=0.713). The prognosis of cases of more CD8 positive cells was better than that of cases of less CD8 positive cells (P = 0.0386; P = 0.0369). Multivariate regression analysis confirmed that it was an independent prognostic factor (central area: P = 0.010, margin area: P = 0.015). The prognosis of the group with higher ratio of CD163 cells to the total number of was worse than that of the group with lower ratio, but we only observed the difference when we analyzed the number of CD163 positive cells in the central region of the tumor (OS: P = 0.0382 PFS: P = 0.0336). COX multivariate analysis showed that CD8 had the highest risk function ratio.

Conclusion: The percentage of CD68 and CD4 positive cells in the total number of visual field cells detected by immunohistochemistry was not related to the prognosis of patients. The high percentage of CD8 positive cells in the peripheral zone or central area of tumors (central area > 3%, margin area>10%) suggests a better prognosis, which support it as an independent prognostic factor. The high percentage of CD163 positive cells in central area(> 3%) indicates poor prognosis.

Mononuclear sarcoma with MDS: a case report and literature review

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【Abstract】　Objective To investigate the clinicopathological features, diagnosis and differential diagnosis, treatment and prognosis of mononuclear sarcoma with myelodysplastic syndrome (MDS). Methods A retrospective study of clinical pathology　and follow-up were performed in a case of mononuclear sarcoma with MDS. The immunophenotype was detected by Envision method, and the morphology of bone marrow cells was observed by bone marrow aspiration. Results The male patient, 45 years old, with a course of 1 month, had multiple superficial lymph nodes with anemia and elevated white blood cells. Immunophenotype: lymph node monocyte-like cells express CD68 (PGM1), CD13, and CD45; do not express MPO, CD68 (KP1), Lysozyme, CD15, CD163, CD43, TdT, CD117, and T/B lymphocyte differentiation antigen, The Ki-67 index is 40% to 60%. Bone marrow aspiration: The proliferation of bone marrow cells is extremely active. The granulocyte, mononuclear and erythroid lines are all morbid hematopoiesis. The proportion of primordial cells accounts for 17%, considering MDS. After the diagnosis, two courses of DA　regimen (daunorubicin + cytarabine) chemotherapy were performed, and IV degree of bone marrow suppression occurred and the chemotherapy was terminated. Patients were followed up and died 4 months later. Conclusion Mononuclear sarcoma with MDS is rare and has a poor prognosis. It is morphologically difficult to distinguish from granulocyte sarcoma and other small cell tumors. It must be confirmed by immunophenotyping.

Tonsillar and gastric dissemination of angioimmunoblastic T-cell lymphoma

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Angioimmunoblastic T-cell lymphoma (AITL) is a systemic disease that often has evidence of extranodal involvement at presentation, but tonsillar and/or gastric involvement is rare. We describe an AITL case with both tonsillar and gastric dissemination. A 54-year-old male came to hospital with progressive enlarged cervical lymph node (LN) for over a year. One year ago, the patient suffered from hematochezia, and enlarged right cervical lymph node and right tonsil. His gastrointestinal endoscopy showed polyps in gastric antrum, terminal ileum and colon. Biopsies of stomach, intestine, LN and tonsil revealed chronic inflammation, tubular, atypical lymphoid hyperplasia(ALH) and ALH, respectively and follow-up was recommended. At the present time, the patient developed generalized lymphadenopathy, including massive mass around right side of neck, enlarged axillary and inguinal LN, with fever, night sweat, skin rash and hepatosplenomegaly. Lab examination showed anemia, elevated lactate dehydrogenase level and positive fecal occult blood. LN biopsy was conducted and showed classical AITL. Gastrointestinal endoscopy revealed ulcer in the greater curvature and normal intestine. Gastric ulcer biopsy illustrated atypical cells infiltrated in the inflammatory background, positivity in Follicular helper T cell marker and clonal T-cell receptor-gamma gene (TRG) rearrangement, which led to the diagnosis of AITL gastric involvement. The previous LN and tonsil specimens was reviewed which showed AITL pattern I on the first LN biopsy and tonsillar AITL involvement. Additionally, the four biopsies showed an identical band-size when polymerase chain reaction results for TRG rearrangement were directly compared. The clinical diagnosis was AITL, with tonsil and gastric dissemination, accompanied with hepatosplenomegaly and B symptoms. The patient had already 3 circles of chemotherapies which showed good response and more cycles of chemotherapy were planned. This new additional AITL with tonsillar and gastric dissemination would add better understanding of this disease.

The expression and clinical significance of MEF2B in mantle cell lymphoma

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【Abstract】 **Objective** To investigate the expression of cardiomyocyte enhancer 2B (Myocyte enhancer factor 2B, MEF2B) in mantle cell lymphomas, and to analyze the correlation between the expression of MEF2B and pathological subtypes, structural subtypes and SOX11 expression and its clinical significance.
Methods Paraffin-embedded tissue were stained with HE, immunohistochemistry (EnVision method) and fluorescence in situ hybridization (FISH) , and the clinical and pathological data of 60 cases of MCL were collected for analysis. **Results** The patients with typical MCL were more than 60 years old. The lesion sites of most of the pathological subtypes or structural subtypes of MCL often coexisted in nodal and extralnodal. SOX11 (+) MCL was common in classical MCL (P = 0.040) and tended to be complete-FDC meshwork type MCL (P=0.086). The expression rate of MEF2B in MCL was 60% (36/60). The expression rate of MEF2B in typical type, complete- FDC meshwork type and SOX11 (+) MCL was significantly higher than that in variant type, no complete- FDC meshwork type, SOX11 (-) MCL (P < 0.05) respectively. There was no difference in clinical characteristics of MCL between MEF2B positive and negative groups. Compared with SOX11 (-) MCL, the percentage of MEF2B expressed in tumor cells of SOX11 (+) MCL was significantly higher (P=0.027). The expression of MEF2B was not related to the proliferation of tumor cells (P=0.341). There was no significant difference in the survival rate between different expression groups of MEF2B and SOX11 (P=0.304 and P=0.819, respectively). Only the mortality of variant type (blastoid/pleomorphic) MCL within 2 years was significantly higher than that of classical type MCL (P<0.05).
Conclusions The expression of MEF2B in MCL is related to pathological subtypes, structural subtypes and the expression of SOX11, but not to the proliferation and prognosis. The high mortality rate within 2 years is only found in variant MCL. However, the role of MEF2B in MCL needs to be further studied.

Category: Hematopathology
1648373

Clinicopathological analysis of coexistence of T-lymphoblastic lymphoma and Langerhans cell histocytosis in the same lymph node

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Objective   To investigate the clinicopathologic features, immunophenotype, genetics, prognosis of T-lymphocyte lymphoma/myeloid sarcoma combined with Langerhans cell histocytyosis(coexistence of T-LBL/MS and LCH). **Methods** Clincial and pathological data of 6 patients with T-LBL/MS combined with LCH, The methods of hematoxylin and eosin stain, immunohitochemistry (EnVision)and in situ hybridization were used for analysis and literatures were reviewed. **Results**   Four patients were T-LBL combined with LCH, 1 was T-LBL/MS combined with LCH, and 1 was MS combined with LCH. The ratio of male to female is 2:4, the age was from 5 month to 77 years old and median age was 59 years old. Three cases showed multiple lymph node (LN) swelling only, the other 3 cases displayed both multiple LN swelling and skin/liver and spleen lesion. The structure of LN of 5 cases were destroyed, 3 cases showed several residual atrophy follicles. There were two types of tumor cells, One kind of abnormal lymphoid-cell exhibited small to medium-sized blast cells, typically showing a nested distribution. These cells were mainly distributed beside of residual follicle and paracortical areas. The other type of histiocytoid cell has large size in volume and abundant pale or dichromatic cytoplasm. The nuclei are irregularly shaped, showing folded appearance. And the sulcus can be found. These cells are mainly distributed in marginal sinus, medullary sinus and interstitial area between follicle. Eosinophilic infiltration in the background was not evident in all cases. The lymphoid-cells with medium size showed TdT+/CD99+/CD7+, with variable expression of CD34/MPO/CD2/CD3. Ki67 index was mostly 30-50%. However, histiocytoid cell presented phenotype of CD1a+/ S-100+/ Langerin$^{+/-}$, while CD163/CD68 were positive in some degree. These cells did not express T or B cell markers, the ki67 index mostly ranged between 10-20%. None of the cases and epstin-barr virus infection. Among 6 patients, 4 patients were followed up (6-63 moths, median time was 18.5 moths), of which 1 patient died of disease and 3 patients are alive with disease. **Conclusions**   T-LBL/MS combined with LCH is a rare mixed type of immature hematopoietic disease, mainly occurred in lymph node and skin. The clinical course is aggressive, with poor prognosis. Therefore, it is helpful to full understanding of the two lesion components in the same tissue   for accurate diagnosis and treatment.

Inactivation of FOXO1 induces T follicular cell polarization and involves angioimmunoblastic T cell lymphoma

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Objective: Angioimmunoblastic T cell lymphoma (AITL) is an aggressive form of non-Hodgkin lymphoma derived from mature T cells. However, the underlying pathogenesis of AITL remains unresolved. We aimed to explore the role of FOXO1-mediated signaling in the tumorigenesis and progression of AITL.

Methods: FOXO1 expression was assessed using immunohistochemistry on a total of 46 AITL tissue samples. Retroviruses encoding FOXO1 shRNA were used to knockdown FOXO1 expression in $CD4^+$ T cells. Flow cytometric assays analyzed the proliferation and survival of FOXO1 knockdown $CD4^+$ T cells. Furthermore, we performed adoptive T-cell transfer experiments to identify whether inactivation of FOXO1 induced neoplastic follicular-helper T (Tfh) cell polarization and function.

Results: Patients with low FOXO1 protein levels were prone to have an advanced tumor stage ($P = 0.049$), higher ECOG ps ($P = 0.024$), the presence of bone marrow invasion ($P = 0.000$), and higher IPI ($P = 0.035$). Additionally, the survival rates of patients in the FOXO1 high-expression group were significantly better than those in the FOXO1 low-expression group ($x2 = 5.346$, $P = 0.021$). We also observed that inactivation of FOXO1 increased $CD4^+$ T cell proliferation and altered the survival and cell-cycle progression of $CD4^+$ T cells. Finally, we confirmed that inactivation of FOXO1 induces Tfh cell programing and function.

Conclusions: Inactivation of FOXO1 in AITL plays a key role in the tumorigenesis and progression of AITL. We propose that FOXO1 expression could be a useful prognostic marker in AITL patients to predict poor survival, and to design appropriate therapeutic strategies.

Expression and significance of CDK6 and its related genes in peripheral T-cell lymphoma based on multi-database mining

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Objectives To explore the expression and significance of *CDK6* and its related genes in peripheral T cell lymphoma (PTCL) and further study the mechanism of *CDK6*'s involvement in the occurrence and development of lymphoma. **Materials and Methods** 1. Bioinformatics analysis: Due to the lack of PTCL-related data in multiple databases, Oncomine and the Cancer Genome Atlas (TCGA) were used for data mining to analyze the expression level of *CDK6* in diffuse large B-cell lymphoma (DLBCL). *CDK6* alterations in DLBCL and related functional networks were analyzed with c-BioPortal, and the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were analyzed with DAVID and FunRich software. In addition, LinkedOmics was used to screen and enrich differential gene expression with *CDK6*, and GeneMANIA was used to construct its functional network. 2. The expression of *CDK6* in 80 DLBCL tissues was verified with immunohistochemistry experiments. 30 reactive hyperplasia lymph nodes were taken as the control. 3. The expression of *CDK6* and related genes in 166 PTCL was detected. 30 reactive hyperplasia lymph nodes were taken as the control. **Result** 1. Bioinformatics analysis: CDK6 gene expression is closely related to the cell cycle and has a specific relationship with several related kinases (CDK2), miRNAs (miRNA-99a/100), and transcription factors (SRF). Meanwhile, FoxM1 was found in our functional network analysis. Like CDK6, FoxM1 is involved in cell cycle regulation. 2. The positive rate of CDK6 in DLBCL was 61.3%, and it was negative in 30 cases of reactive hyperplasia. 3. The positive rates of CDK6 and FoxM1 in PTCL tissues were 27.7% and 80.7%, respectively, and 0 and 27% in 30 reactive hyperplasia lymph nodes, respectively. The positive rate of CDK6 in peripheral T cell lymphoma (not otherwise specified) tissues was 16.2%, 48.1% in NK/T cell lymphoma tissues, 33.3% in angioimmunoblastic T-cell lymphoma (AITL) tissues, and 19.4% in anaplastic large cell lymphoma (ALCL) tissues. **Conclusion** We revealed and verified the overexpression of *CDK6* in DLBCL. We found that both CDK6 and FoxM1 were overexpressed in PTCL and at the same time, CDK6 expression was significantly different in different subtypes of PTCL. Our data mining results and immunohistochemical experiments provide multi-level evidence for the importance of CDK6 and its related gene, FoxM1, in PTCL, as well as its potential as a marker of lymphoma; thus, laying a foundation for further research on the role of CDK6 in the occurrence and development of lymphoma.

Primary and Secondary Extranodal NK/T cell Lymphoma of Breast: a consecutive 10-year case series

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Objectives: Extranodal NK/T-cell lymphoma of breast (ENKTL-B) is rare detected in clinical practice. The clinicopathological features of such disease remain unclear. This study aims to systematically review the clinicopathological features of ENKTL-B and summarize the difference between primary and secondary ENKTL-B.

Materials and Methods: A consecutive 10-year (2010-2019) case series of ENKTL-B was identified from the database of the Department of Pathology, West China Hospital, Sichuan University. The clinical data were collected from the electric medical records and/or telephone interviews. Pathological data were reviewed by 2 hematopathologists independently.

Eight cases of ENKTL-B were enrolled, including 3 of primary lesion (primary ENKTL-B), 3 with medical history of ENKTL (secondary ENKTL-B), and 2 with coexisting breast lesion and other organ involvement at the initial diagnosis (Undetermined cases). The median age was 43.5 (range, 26-63) with the male to female ratio of 1:7. Clinically, the lesion of right breast was detected in 5 cases while the lesion of left side was found in 2 cases. Only one case of bilateral lesion was detected. The mass was usually located in upper outer area of the breast (7/8) while one case showed areolar mass. Pathologically, the tumor cells were infiltrated in the subcutaneous area with septal necrosis and mammary glands with geographical/ patchy necrosis. The neoplastic cells were always positive for CD3 (8/8), CD56 (5/8), GrB (8/8) but negative for CD5 (5/5). The median Ki-67 was 80% (range, 40%-90%). The EBER-ISH positive rate ranged from 50% to 80% (median, 70%). For the 3 primary ENKTL-B cases, the tumor cells were large with obvious atypia and high CD30 expression (>50%). All the tested cases (2/3) showed monoclonal TCRγ rearrangement. For the 3 secondary ENKTL-B cases, the neoplastic cells were small to medium size with low CD30 expression (<10%) and polyclonal TCRγ rearrangement. The necrosis was absent in 2 cases. For the 2 undetermined cases, the coexisting of gastric involvement was found in 1 case, while the other one coexisted cutaneous involvement of contralateral breast. One case showed lymphoma cells in medium to large-size with low CD30 expression and polyclonal TCRγ rearrangement, while the other one showed tumor cells in large size with high CD30 expression and polyclonal TCRγ rearrangement.

Conclusion: Breast is a rare site of ENKTL. The lesion was usually located in the upper outer area of right breast and characterized of subcutaneous infiltration with septal necrosis and mammary glands infiltration with geographical/ patchy necrosis. The primary ENKTL-B always showed relatively larger cell size and higher CD30 expression than that of secondary ENKTL-B. Monoclonal TCRγ rearrangement was also frequently detected in primary ENKTL-B which indicates the T-cell origin.

The clinicopathologic analysis for MYC and BCL2 and/or BCL6 rearrangements in 127 cases of diffuse large B-cell lymphoma

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Objective   To investigate the frequency of double-hit lymphoma (DHL) in diffuse large B-cell lymphoma (DLBCL) and improve the diagnosis efficiency.   **Methods**   127 cases diagnosed with DLBCL from December 2016 to August 2019 were collected. The expression of C-MYC, BCL2 and BCL6, etc. was detected by immunohistochemistry. The genetic rearrangements of C-MYC, BCL2 and BCL6 were analyzed by fluorescence in situ hybridization (FISH).   **Results**   127 cases were included in this analysis (60 males, 67 females), and the median age was 61 years (range: 15-81 years). C-MYC and BCL2 protein were positive in 45 cases (35.43%, 45/127) and 69 cases (54.33%, 69/127) respectively. FISH analysis showed that there were 16 cases (12.60%, 16/127) with C-MYC gene rearrangement, 3 cases (2.36%, 3/127) with BCL2 gene rearrangement and 34 cases (26.77%, 34/127) with BCL6 gene rearrangement. For the cases with genetic alterations, 7 cases (5.51%, 7/127) harboring concurrent gene rearrangements of C-MYC as well as BCL2 or BCL6 (DHL), including 2 cases (1.57%, 2/127) for C-MYC/BCL2 rearrangements and 5 cases (3.94%, 5/127) for C-MYC/BCL6 rearrangements. In Hans classification, non-GCB subgroup accounted for 55.12% (70/127), and GCB subgroup 44.88% (57/127). Among the 7 cases with DHL, 3 cases were classified as non-GCB subtypes.   **Conclusions**   The majority of patients with DLBCL do not warrant FISH testing due to the low frequency of DHL (accounting for only 5.51%).   The key factors of he histomorphology and immunotype could decrease the detection of gene rearrangements.

The inferior effects of copy number amplification of E-protein on survival of non-GCB subtype diffuse large B cell lymphoma

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Objectives: Amplification on chromosome 18 frequently appears in non-Germinal Center B cell (non-GCB) like Diffuse Large B Cell Lymphoma (DLBCL). E-protein can determine B cells fate at several stages of differentiation, of which *TCF-4* (18q21.2) is a key target of chromosome18 copy number (CN) gain. Although, TCF-4 is a physiologically indispensable multifunctional transcription factor, so far, there are rare studies on its role during DLBCL development, and underlying mechanisms remain elusive. This study, first, aims to explore CN variations of genes located at chromosome 18. Since E-protein can potentially regulate B cell receptor (BCR) pathway, class switch recombination (CSR) and hundreds of downstream molecules, we next investigate the association between aberrations of E-protein members, particularly TCF-4, and prognosis. Also, we seek to figure out the involved molecular mechanisms.
Materials and Methods: 74 DLBCL cases including pairwise fresh frozen and formalin-fixed paraffin-embedded samples and associated clinical information were successfully collected. 5 DLBCL cell lines (2 are GCB subtype and 3 are active B cell like subtype) were cultured for in-vitro experiments. Whole genomic CN was examined using high resolution gene microarray to comprehensively demonstrate genomic variations of genes particularly comprised in chromosome 18. Subsequently, expression of E-protein members and BCR signaling pathway components were tested by gene expression profiling and immunohistochemistry staining on RNA and protein scale respectively. To evaluate effects of TCF-4 on tumor cell activity, cell counting kit-8 method was performed to examine living cells after treatment by BTK inhibitor. Gene translocations of *BCL-2*, *BCL-6* and *MYC* were inspected by fluorescent in situ hybridization. Additionally, we assessed hot spot mutations of *TCF-4* through whole exon sequencing. Kaplan-Meier method was applied to assess overall survival and 3-year progress free survival, and SPSS 13.0 software was utilized for all statistical analyses. Differences with p value<0.05 was considered as statistically significant.
Result: Totally, 99 genes encompassed in chromosome 18 with high frequency of CN variations were identified. Transcriptional expression of *TCF-4* was validated by both

DLBCL cases and cell lines. CN-amplification of *TCF-4* was observed with high occurrence in non-GCB rather than GCB subtype, in contrast to the other two members, *TCF-3* and *TCF-12*. Both *TCF-4* and *TCF-12* CN variations were significantly associated with inferior 3-year overall survival (p=0.039 and p=0.038 respectively). Unexpectedly, CN-amplification of *TCF-4* was additionally associated with that of *BCL-6* (p=0.031). Cases with co-occurrence of CN-amplification of *TCF-4* and *BCL-6* had worst survival in comparison to those without or with either of them. CN-deletion of *prdm1* and CN-amplification of *eaf2*, downstream genes of *TCF-4*, were also associated with poorer survival. It was noteworthy that, these effects were not found in GCB type. IgM was found with quite high expression in non-GCB subtypes compared with IgG. Moreover, immunohistochemistry staining showed that, TCF-4 did not express among GCB type tumor cells here. As expected, it could be observed with intensive expression in active B cell like subtype cell lines. Furthermore, majority mutations were identified at bHLH domain of TCF-4. To further validate this subtype preference, RNA expression is assessed, and we observed consistent trend to immunohistochemistry staining results. Of note, cells with positive expression of TCF-4 did not response to BTK inhibitor treatment.

Conclusion: Significant association between CN-amplification of *TCF-4* and *BCL-6* suggested a synergistic reaction in contributing to immunoglobulin isotype CSR. This might interpret the exclusively existence of immunoglobulin isotype expression in GCB and active B cell like subtypes to some extent, which could initiate different signaling pathways leading to B cell transformation eventually. Importantly, over activation of *TCF-4* is probably a risk factor to predict poor outcome for non-GCB subtype DLBCL. Also, it might drive inability to BTK inhibitor chemotherapy. Further functional explorations on targeting E-protein, especially how they are able to regulate CSR, will be essential to deepen the understanding of their roles in BCR signaling activation and lymphomagenesis.

Detailed molecular analysis of anaplastic variant of diffuse large B-cell lymphoma

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Objectives: Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma, showing high heterogeneity in clinical pathology and molecular genetics. Anaplastic diffuse large B-cell lymphoma (A-DLBCL) is a rare morphological subtype that accounts for approximately of 3.4% all DLBCL, characterized by the polygonal tumor cells with bizarre pleomorphic nuclei. Our previous research found that A-DLBCL displays clinicopathologic features that distinguish it from ordinary DLBCL. Most patients follow an aggressive clinical course and have a high frequency of *TP53* mutation and genetic abnormalities of *MYC, BCL2,* and *BCL6*. However, the detailed genetic features of this variant are largely unknown.

Materials and Methods: The clinicopathological features of 35 A-DLBCL cases were retrospectively analyzed, and the prognosis of these patients was followed up. Targeted next-generation sequencing (446 panel genes that are important for the pathogenesis of hematologic tumors) of 35 A-DLBCL cases was carried out, and bioinformatics analysis was performed to estimate the differential gene mutation spectrum between A-DLBCL and ordinary DLBCL in COSMIC database.

Result: Targeted next-generation sequencing analysis identified 304 mutations in 127 genes for 35 A-DLBCL cases. The most frequently mutated genes were *TP53* (19/35) followed by *PIM1* (12/35), *MYD88* (9/35), *BTG2* (7/35), *ETS1* (7/35), *KMT2D* (7/35), *ACTB* (5/35) and *CREBBP* (5/35). Comparing to the COSMIC database, 7 genetic alterations occurred more frequently in A-DLBCL cases than in ordinary DLBCL, namely *TP53, PIM1, MYD88, BTG2, ETS1, ACTB* and *TBL1XR1* ($P<0.05;$). On the contrary, *BCL2* is lower mutated in A-DLBCL ($P<0.05;$). Univariate analysis showed that patients with *PIM1* and *TBL1XR1* mutations had a significantly poorer overall survival (OS) than those who tested negative ($P<0.05;$). *TP53* and *MYD88* mutations were marginally associated with a shorter OS ($P=0.117$ and 0.072, respectively). Clinicopathological analysis showed that patients with *TP53* and *TBL1XR1* mutations more often had a high International Prognostic Index (IPI) score and that patients with *PIM1* and *TBL1XR1* mutations more frequently had an elevated serum lactate dehydrogenase (LDH) level when compared to those with wild type. Besides, the most frequent genes with copy number amplifications in A-DLBCL were CD274 (8/35), *PDCD1LG2* (7/35), *BCL2* (7/35), *MCL1* (6/35), *MALT1* (5/35), *MYC* (4/35) and *GNAS* (4/35).

Conclusion: In conclusion, our study suggests that A-DLBCL displays a distinct mutational landscape that distinguish it from ordinary DLBCL. Targeted next-generation sequencing identifies a higher level of *TP53, PIM1, MYD88, BTG2, ETS1, ACTB* and *TBL1XR1* mutations in A-DLBCL than in ordinary DLBCL. Our findings may provide a rationale for therapeutic strategies in patients with A-DLBCL.

Alpha fetoprotein (AFP) participates in the build up of hematopoietic cells in the early embryonic stage: An abortion case observation

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Abstract

Background: At the 3rd week of human embryo, some cell clumps are formed by the hyperplasia of mesenchymal cells at the germ layer of the yolk sac wall. These cell clumps are known as blood islands. The cells in the center of the blood islands further develop into primitive blood cells, such as hematopoietic stem cells. The blood island in the yolk sac further develops into the extramedullary hematopoietic tissue in one week at the 3rd to 4th week.

Case presentation: A 32-year-old pregnant woman who missed menstruation for 42 days discovered that her pregnancy required an abortion. The tissue collected after the abortion was a piece of gray-yellow and villus-like intrauterine tissue of a size of approximately 4 cm×3 cm×1.3 cm. The paraffin section stained with hematoxylin and eosin and observed under the light microscope showed a visible small embryo tissue in the early placental tissue. In the embryonic tissue, a large amount of extramedullary hematopoietic tissue was present, including myeloid, erythroid and megakaryocytic cells. The extramedullary hematopoietic cells were located in the blood vessels or naive liver sinus, were positive for alpha fetoprotein (AFP) and were without lymphocytes. The erythrocytes consisted of a large number of nucleated red blood cells. In addition, a neural tube and cystic structure were found. The final pathological diagnosis was as follows: Early embryonic tissue with a cystic structure formation in the embryo. After medical abortion the pregnant woman recovered well, without complications.

Conclusions: Our case illustrates that AFP is an important structural protein of nucleated erythrocytes and myeloid hematopoietic cells, suggesting that it may participate in the build up of nucleated erythrocytes and myeloid hematopoietic cells. Furthermore, our case suggests that nucleated red blood cells can be detected from the 42nd day of pregnancy by a peripheral blood sample from the mother.

Subcutaneous panniculitis-like T-cell lymphoma: An examination of the clinicopathological characteristics of two case studies and a literature review

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Background: Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a cutaneous T-cell lymphoma of cytotoxic T-cell type infiltrating subcutaneous tissue, and is frequently misdiagnosed because of its rarity.

Aim: To analyze and summarize the clinical and pathological features of subcutaneous panniculitis-like T-cell lymphoma.

Methods: We research the files of the Department of Pathology at Zhongshan Hospital in Shanghai, China for cases between May 1, 2014 and August 31, 2019. We analyze the macroscopic, histological and immunohistochemical features and review the previously reported   cases in the English literatures.

Results: The patients' age were 26 and 79 years, respectively. They both had a several months history of multiple subcutaneous nodules on the lower extremities. Before onset of the necrosis and ulceration, the 26-year-old boy had a fever for several days. The tumors were composed of atypical small, bland lymphocytes and histiocytes. There was rimming of some of the fat cells by atypical lymphoid cells in a lace-like. Some histiocytes contained one or more engulfed lymphocytes. Immunohistochemically, the atypical lymphoid cells are positive for CD3, CD8, Granzyme B, CD56 (one case) and negative for CD20 and EBER. The histiocytes expressed CD68+. Remarkably, the atypical lymphocytes invaded the upper dermis and showed angioinvasive and angiodestructive features in the CD56+ case. Two patients were transferred to the department of pediatrics, and currently receiving combination chemotherapy of cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP). One patient died of multiple organ failure after 9 months.

Conclusions: SPTCL is a rare disease, accounting for less than 1% of all cutaneous T-cell lymphomas. SPTCL is characterized by rimming of some of the fat cells by T cells showing a degree of atypia. Meticulous examinations are essential as the diagnosis of SPTCL, which needs to   be differentiated from other aggressive cutaneous T-cell lymphomas and panniculitis.

Clinicopathologic characteristics of ALK-positive large B-cell lymphoma:an analysis of four cases and review of literature

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Objective To investigate the clinical and histopathological characteristics, diagnosis and differential diagnosis and biological behavior of ALK-positive large B-cell lymphoma. **Methods** Four cases of ALK-positive large B-cell lymphoma were collected and their clinical features, histomorphology, immunophenotype and prognosis were observed. The relevant domestic and foreign literature was summarized and reviewed. **Results** Among the 4 tumors, 3 were male and 1 was female, with age ranging from 22 to 50 years; Biopsy site: lymph nodes (right supraclavicular fossae lymph nodes, left cervical lymph nodes) in 2 patients, submandibular gland in 1 patient, sigmoid colon and cervical lymph nodes in 1 patient. 2 of the 4 cases were clinically stage II and 2 cases for stage Ⅳ. Of the 4 cases, 3 were puncture specimens and 1 was radical resection specimens (sigmoid colon). Histologically, tumor cells were diffusely grown. The 3 tumor cells showed typical immunoblastic and plasmablastic appearance, with large cell volume, round and pale nuclei, thick and clear nuclear membranes, and large median nucleoli. In one case, the tumor cells were atypical, with large size, light staining of some cytoplasm, irregular nuclei, like kidney nucleation, some of them were binuclear, some of them were eosinophilic, with hyperchromatic, pyknotic and irregular nuclei. Immunohistochemistry showed that tumor cells did not express common B cell markers (CD20, CD79a, PAX5) and common T cell markers (CD3, CD2). Tumor cells express markers of terminal differentiation B cells/plasma cells, including CD38, CD138 and MUM1, and express B cell specific transcription factors BOB-1 and OCT-2. T cell marker CD4 was often abnormally expressed (2/4) and EMA was positive (4/4) . Among the 4 cases, 3 cases were negative in CD30, 1 case was focal diffuse strong positive in CD30, ALK protein was strongly positive, 4 cases were cytoplasmic granular staining, and EBER was negative(0/4). All 4 patients were followed up for 6-17 months, 1 died 10 months after treatment, and 3 survived (lymph node enlargement). **Conclusion** ALK-positive large B-cell lymphoma is a rare aggressive subtype of diffuse large B-cell lymphoma. It is highly aggressive and has a poor prognosis. The diagnosis of ALK-positive large B-cell lymphoma depends mainly on its characteristic histomorphology and immunophenotype. At the same time, the diagnosis should be differentiated from ALK-positive anaplastic large cell lymphoma, poorly differentiated cancer, malignant melanoma and a series of B-cell lymphomas with plasma/immunoblastic morphology, especially when CD30 is focally positive, it is easy to be misdiagnosed as anaplastic large cell lymphoma.

Category: Hematopathology
1657052

Clinical and pathological analysis of 7 cases of splenic sclerosing angiomatoid nodular transformation

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Objectives: To investigate the clinicopathological features, diagnosis and differential diagnosis of splenic sclerosing angiomatoid nodular transformation (SANT). Materials and Methods: The clinical manifestations, imaging features, pathological features and immunohistochemical phenotypes of 7 cases of splenic SANT were retrospectively analyzed, and the related literature was analyzed.
Result: There were 3 males and 4 females in 7 patients, aged 26-57 years. Except for one patient with abdominal pain, all other were found to have splenic space occupied by physical examination. Imaging manifestations were hypoechoic mass/solid mass lesions of the spleen with a maximum diameter of 3-8 cm. All patients survived after simple splenectomy (follow-up for 1-61 months). Macroscopic examination generally showed gray/gray-yellow reticular or scar-like tissues with moderate or hard texture. Under light microscopy, hemangioma-like nodules were seen, with different size of vascular lumen in the center, swelling of endothelial cells, distribution of red blood cells inside and outside the lumen, and dense fibroblasts surrounding the lumen. Immunohistochemistry showed 7 cases of CD31 (+), 5 cases of CD34 (+), and 2 cases of CD8 (+).
Conclusion: SANT is a rare benign tumor of the spleen with good prognosis. The definite diagnosis of SANT should be based on imaging combined with pathological histology and immunohistochemical phenotype to differentiate it from hemangioma, angiosarcoma, littoral cell angioma and inflammatory pseudotumor, so as to reduce misdiagnosis or missed diagnosis.

EZH2/ miR-26a-5p/ NSD2 pathway is suppressed by GSK126 deriving malignant phenotype in diffuse large B-cell lymphoma

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Objectives
Diffuse large B-cell lymphoma (DLBCL) is one of the most common malignant tumors among non-Hodgkin's lymphomas. EZH2 and NSD2 are the members of histone methyltransferases (HMTases). Accumulating experimental evidence has shown that aberrant expression of EZH2 is involved in the development and progression of Diffuse large B-cell lymphoma (DLBCL). It has been reported that EZH2-NSD2 HMTase axis is coordinated by a microRNA network. To date, DLBCL has not been fully elucidated the relationship among them. This study aimed to elucidate the relationship between EZH2, NSD2, and miRNA in DLBCL and further explore the potential regulatory mechanisms.
Materials and Methods
In this study, U2940 cells were treated with different concentrations of GSK126, a selective inhibitor of EZH2, and then cell proliferation was assessed by CCK8 assay and soft agar colony formation assay. Cell cycle and cell apoptosis were analyzed by flow cytometry. EZH2, NSD2, histone methylation levels and DNA repair proteins were detected by western blot analysis. MiRNA array in DLBCL, bioinformatic analysis and qPCR were performed to identify miRNAs by which EZH2-NSD2 axis is coordinated. EZH2-miRNA-NSD2 axis was verified in 293T cells transfected with EZH2-shRNA. Dual-luciferase reporter assay was performed to confirm whether NSD2 is the target gene of identified miRNA.
Results
GSK126 suppressed growth, promotes apoptosis and blocks cell cycle in U2940 cells. Suppressing EZH2 activity using GSK126 also inhibited NSD2 and DNA damage repair proteins. Besides, five miRNAs including miR-26a-5p, miR-30c-2-3p, miR-196a-5p, miR-200c-3p and miR-622, were downregulated in miRNA profiling of DLBCL and predicted as regulators of NSD2. GSK126 significantly increased the expression of miR-26a-5p in U2940 cells. Furthermore, the similar expression changes of these proteins and miR-26a-5p were observed in 293T cells transfected with EZH2-shRNA. MiR-26a-5p was demonstrated to bind to 3'-UTR of NSD2 gene and negatively regulate NSD2 expression.
Conclusion
In conclusion, GSK126 can inhibit proliferation, trigger apoptosis and induce cell cycle arrest via the EZH2/miR-26a-5p/NSD2 axis in the DLBCL cell line, which is might represent an attractive therapeutic target in DLBCL.

Clinicopathologic features and prognostic analysis of Hepatitis B virus-associated diffuse large B-cell lymphoma: A retrospective study in Southern China

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【Abstract】Diffuse large B-cell lymphoma (DLBCL), the most common type of non-Hodgkin's lymphoma (NHL) in adults, accounts for approximately 30-40% of newly diagnosed lymphomas worldwide. While the epidemiologic association between hepatitis B virus (HBV) infection and diffuse large B-cell lymphoma (DLBCL) is established, little is known more than this epidemiologic evidence. We studied a cohort of 420 Chinese patients with DLBCL for HBV infection status, clinicopathologic features, and prognostic factors. In our study, one hundred and twenty-seven (127/420, 30.2%) patients were HBsAg-positive. Compared with HBsAg-negative DLBCL, HBsAg-positive DLBCL displayed a younger median onset age (50 vs. 54 years), more frequent involvement of spleen (19.7% vs. 6.1% , $p < 0.001$) less frequent involvement of intestinal (2.3% vs 11.2%, $p = 0.003$), more advanced disease (stage III/IV: 56.7% vs 45.1%, $p = 0.028$), and high expression bcl-2 (68.5% vs. 56.3%, $p = 0.019$). The median follow-up time was 45.6 months. Survial analyses (Kaplan-Meier) showed that there was no significantly difference in overall survival (OS) between HBsAg-negative DLBCL and HBsAg-positive DLBCL ($p=0.577$). In the HBsAg-positive DLBCL subgroup, spleen involvement had significantly worse outcome ($p=0.034$), patients treated with R-CHOP have better prognosis. In conclusion, the incidence of HBsAg-positive DLBCL in South China is higher than that in Northern China and western countries, which may correlate with the differences in geographical and ethnic origin. Furthermore, our study indicates that HBV-associated DLBCLs has unique clinicopathologic features and poor prognostic factors and strongly suggests that HBV may be involved in the tumorigenesis of DLBCL in regions of high infectious.

Advances in diagnosis of primary cutaneous lymphoma

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The diagnosis of primary cutaneous lymphoma (PCL) is one of the difficulties in the diagnosis of lymphoma. The main features of WHO-EORTC's newly published 2018 update was illustrated on the diagnosis of PCL. Also there are recent reports focusing on the new findings in molecular genetics of PCL. The latest developments in the diagnosis of PCL, which are easy to be misdiagnosed and challenging could be summarized to seven aspects: the problem of new categories with new features; the problem of indolent or invasiveness in primary cutaneous lymphoma; the problem of rare sites in rare primary cutaneous lymphoma; the problem of clinical information and treatment related to diagnosis of PCL; the problem of differential diagnosis of lesions with similar features; inflammation related PCL; PCL remains challenging and controversial. Summarizing the latest diagnostic progress of PCL from different perspectives may enhance our in-depth understanding of the diagnosis, differential diagnosis and the latest progress of primary cutaneous lymphoma.

The clinicopathological and molecular features of sinusoidal large B cell lymphoma

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Background: Many lymphomas revealed a sinusoidal pattern of involvement focally or throughout the initial lymph node, such as anaplastic large cell lymphoma, which were often misdiagnosed as metastatic carcinoma, metastatic malignant melanoma, or malignant histiocytosis.
Methods: We reported 10 cases of sinusoidal large B cell lymphoma, and their clinical, morphologic, immunophenotypic and molecular features were detected and analyzed.
Results: The age of the patients ranged from 43 to 66 years (mean, 54.5 years) with a male to female ratio of 1:1.5. All tumors occurred in the lymph nodes, located in cervical lymph node for seven cases, and in supraclavicular, axillary, and inguinal lymph nodes for one case, respectively. All cases had an obvious sinusoidal growth pattern, often associated with residual atrophic lymphoid tissue. All tumors contained large pleomorphic lymphoid cells with polylobulated nuclei, vesicular chromatin, one or more prominent nucleoli, and abundant amphophilic cytoplasm. Scattered Reed-Sternberg - like cells were also seen in some cases. Nine cases were divided into ABC subtype, and one case was classified as GCB subtype. The neoplastic cells express pan-B-cell markers CD20 (10/10) and PAX-5 (5/5), but all lacked expression of CD10. 90% (9/10) of cases illustrated positive staining for MUM-1. The neoplastic cells expressed CD30 focally or totally in 90% of cases except one case with negative MUM-1 expression. Bcl-6 expression was detected in 9 of 10 cases. MYC staining showed positive expression of 20%~80% of neoplastic cells in 8 patients. Expression of p53 was seen in three cases (3/10), and negative expression of p53 was also observed in three cases (3/10). The neoplastic cells exhibited significant hyperproliferative activity with ≥80% of Ki-67 proliferation index for 9 cases and approximately 40% for one case. All six cases that detected Epstein Barr virus (EBV) RNA by in situ hybridization were negative for EBV infections. Gene rearrangement for *BCL6*, *BCL2* and *MYC* were performed by fluorescence *in situ* hybridization in ten cases. Break-out of *BCL6* and *BCL2* were detected in four cases and one case, respectively, and none was detected for *MYC* break-out. Interestingly, copy number gains for *BCL6*, *BCL2* and *MYC* were detected in 80%, 70% and 70% of cases, respectively, and five cases of neoplasias showed simultaneous copy number gains of three genes. The neoplastic cells showed different proportions of PD-L1 positive cells, from the weakly positive expression of a small number of neoplastic cells to the strong positive expression of almost all cells. Whole exome sequencing (six cases) and large panel sequencing (446 genes, one case) results revealed some genes had high mutation frequencies, such as *TP53* (4/7), *MYD88* (3/7), *KMT2D* (3/7), *CREBBP* (3/7), *PIM1* (3/7). Four cases with *TP53* mutation had mutant

immunohistochemistry results.

Conclusions: Sinusoidal large B cell lymphoma may be a specific diffuse large B-cell lymphoma occurred in middle-aged and elderly patients. It had characteristic morphological features with a sinusoidal growth pattern, and immunohistochemical results suggested the B-cell origin. The neoplastic cells typically express CD30, and usually lack the events of EBV infection. As many as 40% of cases showed *BCL6* rearrangement. Copy number gains of *BCL6*, *BCL2* and/or *MYC* were common molecular events, and the neoplastic cells had high genetic mutation frequency.

follicular dendritic cell sarcoma with four atypical histomorphologic features: an unusual case report

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Objectives: Follicular dendritic cell sarcoma (FDCS) is a rare malignancy. In addition to the classical histopathologic features, it has also some special morphological variants that can present a challenge in the diagnosis of this disease. **Methods and Results:** A 45-year-old male who presented with a left supraclavicular mass was given a final diagnosis of FDCS after lymph node biopsy. The specimen obtained during radical resection revealed five different morphologies, including the classical histological appearance and atypical areas resembling desmoplastic infiltrative carcinoma, anaplastic large cell lymphoma (ALCL), hemangiopericytoma and classical Hodgkin's lymphoma (CHL). Immunohistochemistry was notable for positive CD21 and CD23 expression across all morphologies. Given the atypical appearance and location, the specimen was initially misdiagnosed as a metastatic carcinoma based on histology alone at an outside institution. The patient eventually underwent surgical resection followed by adjuvant chemotherapy and radiation. Despite treatment, the disease progressed, and the patient passed away 36 months after surgery. **Conclusions:** This unusual case of FDCS contains four types of atypical histomorphologies within a single tumor specimen, including those resembling ALCL and hemangiopericytoma which are described here for the first time. Our report further expands the histopathologic spectrum of FDCS and may help assist in the diagnosis of other such challenging cases.

Five cases of indolent T-cell lymphoproliferative disease of gastrointestinal tract with unusual clinical outcomes

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Objective:
To explore the clinic-pathologic and endoscopic features and biological behaviors of 5 cases of indolent T-cell lymphoproliferative disease of gastrointestinal tract (GI indolent T-LPD), so as to discover the clinic and pathologic clues indicating the malignant transformation and the relationship with EBV infection.

Materials and Methods:
We selected accurately diagnosed 5ncases of GI indolent T-LPD, and analyze the 5 patients' clinic, pathologic and endoscopic data, and the information about treatment, response and follow up.

Results:
The 5 patients are all male, aged from 33 to 58 years old with recurrently abdominal pain and diarrhea for 4 to 13 years. They were all misdiagnosed as peripheral T cell lymphoma, not otherwise specified (PTCL, NOS), inflammatory bowel disease (IBD) or tuberculous enteritis before the correct diagnosis, and so received corresponding therapy. Endoscopically, superficial and multiple ulcer were observed in small intestine or colon. Multiple biopsies showed ulceration and effacement of intestine architecture by blue-stained lymphoid infiltrate, from mucosa to submucosa. Neither lymphoid follicles nor epitheliotropic feature was identified. The bland small lymphocytes all expressed CD3, CD2 and TIA-1, but not CD20, PAX-5 and CD56. Except one disease was CD4+/CD8-, the others were CD4-/CD8+. Another case is CD5 negative, the others were CD5 positive. Serum EBV-DNA copies number was higher than 1×10^4 copies/ml in 3 of 5 cases, but there were a few of EBER positive cells (2-10 EBER+ cells/HPF) in 2 cases of biopsy. One of the 3 cases negative for EBER in biopsy, the patient's condition sustained progressed with the high level of EBV-DNA copies in serum and then he received pancolectomy. In the surgical sample, we found the small T lymphocytes infiltrate from mucosa to muscular layer, even the subserosa, especially around the ulcer sites. In addition, we observed the tumor cells were CD30 positive diffusely with

a little scattered EBER positive cells (about 15cells/HPF), and the Ki-67 Labeling index (LI) is about 30% in hot spots. We diagnosed this case as low grade periphery T cell lymphoma transformed from classic GI indolent T-LPD. In another case, the biopsy indicated the tumor cells Ki-67 LI 20% with a few of EBER positive cells, and the other 3 cases Ki-67 LI < 10%. TCR gene rearrangement were observed in all of the 5 cases. Including the progressed patient, 4 of the 5 patients received prednisone, supportive and symptomatic treatments, and all of the 5 patients are survival after fellow-up from 4 months to 36 months. The only patient, who had been misdiagnosed as PTCL, NOS and correspondingly received many cycles of radiotherapy and chemotherapy, administered a shot of Gemcitabine in March 2019, after he got the diagnosis of GI indolent T-LPD.

Conclusions:

The patient accompanied with EBV infection, the GI indolent T-LPD tumor cells may proliferate actively, with a little of transformed large cells scattered among the small lymphocytes. These kind of T-LPD is prone to progress, and the molecular mechanism may be associated with the disbalance of the immune system and activation of JAK/STAT or NF-κB signal pathway, which will be confirmed by us in the near future.

Correlation between different lymphoma types and Epstein-Barr virus infection

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Objective To investigate the relationship between different lymphoma types and Epstein-Barr virus (EBV) infection. **Methods** From August 2015 to December 2018, 175 cases of lymphoma were collected from the Department of Pathology, Anhui Cancer Hospital. The correlation between the results of EBER in situ hybridization and different types of lymphoma was analyzed. **Results** There were 175 cases of lymphoma. The constituent ratios of mature B-cell lymphoma, mature T- and NK-cell lymphoma, and Hodgkin lymphoma were 42.3%, 42.3% and 15.4%, respectively. 72 cases (41.1%) were EBER positive. The positive rates of EBER in mature T- and NK-cell lymphoma and Hodgkin lymphoma were higher than those in mature B-cell lymphoma (66.2%, 59.3% and 9.5%, respectively)(P<0.05). Among them, 28 cases were NK/T-cell lymphoma, 27 cases were positive (96.4%). Among 16 cases of angioimmunoblastic T-cell lymphoma, 14 cases were positive (87.5%). Of 63 cases of diffuse large B cell lymphoma, 6 cases were positive (9.5%). Of the 6 Burkitt lymphomas, 1 was positive (16.7%). NK/T-cell lymphoma was diffusely strongly expressed. **Conclusion** The infection rate of EBV is different in different types of lymphoma, the infection rate of mature T- and NK-cell lymphoma is higher than that of other types of lymphoma, especially NK/T-cell lymphoma and angioimmunoblastic T-cell lymphoma. However, EBV may play an inconsistent role in the pathogenesis of them, and may play a more important role in the development of NK/T-cell lymphoma.

Comprehensive genomic profiling of EBV-positive diffuse large B-cell lymphoma and the expression and clinicopathological correlations of some related genes

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Abstract

Background: Epstein-Barr virus (EBV)-positive diffuse large B-cell lymphoma (EBV+ DLBCL) is a rare type of lymphoma with a high incidence in elderly patients, poor drug response, and unfavorable prognosis. Despite advances in genomic profiling and precision medicine in DLBCL, EBV+ DLBCL remain poorly characterized and understood. Material and Methods: We include 236 DLBCL patients for EBV-encoded mRNA (EBER) *in situ* hybridization detection and analyzed 9 EBV+ and 6 EBV negative cases by next-generation sequencing (NGS). We then performed fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) to analyze chromosome rearrangements and gene expressions in 22 EBV+ and 30 EBV negative cases.
Results: The EBER results showed a 9.3% (22/236) positive rate. The NGS results revealed recurrent alterations in *MYC* and *RHOA*, components of apoptosis and NF-κB pathways. The most frequently mutated genes in EBV+ DLBCL were *MYC* (3/9; 33.3%), *RHOA* (3/9; 33.3%), *PIM1* (2/9; 22.2%), *MEF2B* (2/9; 22.2%), *MYD88* (2/9; 22.2%) and *CD79B* (2/9; 22.2%) compared with *KMT2D* (4/6; 66.7%), *CREBBP* (3/6; 50.0%), *PIM1* (2/6; 33.3%), *TNFAIP3* (2/6; 33.3%) and *BCL2* (2/6; 33.3%) in EBV-negative DLBCL. *MYC* and *KMT2D* alterations stood out the most differently mutated genes between the two groups. FISH detection displayed a lower rearrangement rate in EBV+ cohort. Furthermore, KMT2D expression was highly expressed and associated with poor survival in both cohorts. MYC was only overexpressed and related to an inferior prognosis in the EBV+ DLBCL cohort.
Conclusion: In summary, we depicted a distinct mutation profile for EBV+ and EBV-negative DLBCL and validated the differential expression of KMT2D and MYC with potential prognostic influence, thereby providing new perspectives into the pathogenesis and precision medicine of DLBCL.

A case of the transformation of CLL/SLL into CHL

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ABSTRACT: **Objective** To analyze the morphology, immunophenotypic characteristics and molecular pathological results of lymph node and bone marrow biopsy specimens from a case of chronic lymphocytic leukemia/small lymphocytic lymphoma transforming into classical Hodgkin lymphoma, and to enhance the understanding of this rare lesion. **Methods** A case of chronic lymphocytic leukemia/small lymphocytic lymphoma transformed into classical Hodgkin's lymphoma was examined by histological, immunohistochemical and molecular pathological methods, and the related literature was reviewed. **Results** A 80-year-old male patient was admitted into hospital because of left cervical lymph node enlargement for more than 3 months and complaining of chest and abdominal pain for 2 weeks. PET-CT showed multiple lymph node enlargement with increased FDG metabolism and destroy of bone regions with increased FDG metabolism in the whole body. Macroscopic examination: left cervical lymph nodes: gray-white oval nodules, measured with 2.7*2*1.5 cm in size with a gray, solid and tender section. Microscopic examination: The structure of lymph nodes was destroyed, small lymphoid cells grew diffusely , some of them infiltrated into extracapsular adipose tissue of lymph node. The nuclei were hyperchromatic and the cytoplasm was few, the light-stained nodule-like areas were scattered in the dark small lymphoid cells. In the nodules some cells were medium or medium-large with small nucleoli and basophilic cytoplasm, and R-S-like cells were noticed in some nodules. Immunohistochemical stains showed that small lymphoid cells were positive for CD20, Pax-5, CD23, Bcl-2, and partly positive for Lambda,negative for Kappa, CD5, cyclinD1, CD10, Bcl-6, CD3, P53, CD38, H3K27M and ZAP-70. CD30, CD15, PD-L1, MUM1 were positive in R-S-like cells, Pax-5 was weakly positive, GATA3 was weakly positive, CD20, OCT2, BOB.1, LMP1, ALK, EMA, PD-1 were negative, EBER was negative. Bone marrow biopsy showed diffuse infiltration of samll lymphoid cells between trabeculae, and no infiltration of R-S-like cells and eosinophils. Immunohistochemical stains showed small lymphoid cells were positive for CD20, CD19, CD23 and Bcl-2, but negative for CD5 and Bcl-6. Bone marrow cytology showed that clonal lymphoid cells accounted for 60.5%. Flow cytometry showed that these cells were positive for CD20 and CD23 , while CD5 and CD10 were negative. Ig gene rearrangement in lymph nodes and bone marrow showed that the tumor cells were positive for IGH FR1-JH, FR2-JH and IGK Vk-Jk. The absolute value of peripheral blood lymphocyte did not increase, and the number of EBV-DNA copies in peripheral blood increased. **Conclusion** The transformation of CLL/SLL to DLBCL is more common than to CHL. The diagnosis of transformation from CLL/SLL to CHL is mainly depended on morphology and immunophenotype. Macroscopic examination shows alternate distribution of dark and light stained areas. Two kinds of tumor components including CLL/SLL and CHL can be identified by immunohistochemical stains.

Integrated analysis of the host and EBV miRNA expression profiling in Mature T/NK cell lymphomas

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Objective: Apart from Extranodal NK/T-cell lymphoma, nasal type (ENKTCL), Epstein-Ban-virus (EBV) are also commonly found in other mature T/NK cell lymphomas. However, the influence of which Epstein-Barr virus (EBV) contributes to the development of T/NK cell lymphoma are unclear, thus we investigated the miRNA prolifing of both virus and human in mature T/NK cell lymphomas.

Methods: we collected formalin fixed paraffin-embedded(FFPE)tissues and corresponding information of 97 cases of patients with mature T/NK cell lymphomas. at Cancer Hospital, Chinese Academy of Medical Sciences, and perform the miRNA expression profiling (both human and viral-derived miRNA) by the microarray chip, Human miRNA Microarray kit (V3), the miRNA expression profiling was analysed by using LVSmiRNA package from R (www.r-project.org).

Results: There were 4 types of mature T/NK cell lymphomas included into this study, including Angioimmunoblastic T cell Lymphoma (AITL) (35,36%), Peripheral T-cell lymphoma, Non-specific (PTCL, NOS) (27,28%), Anaplastic large cell lymphoma, ALK positive (ALCL, ALK+) (19, 28%), Anaplastic large cell lymphoma, ALK negative (ALCL, ALK-) (16, 16%). The results of miRNA expression profiling demonstratedd thatthat a total of 255 hsa-miRNAs and 26 EBV-miRNAs could be detected. Besides, all samples could be divided into three groups according to the relative expression of EBV-miRNA, named EBV-miRNA high expression group, EBV-miRNA medium expression group and EBV-miRNA low expression group, respectively. And the prognosis of the three groups was significantly different (Log-rank test, P＜0.05), with the EBV-miRNA low expression group had the best prognosis, followed by the EBV-miRNA high expression group, and then EBV-miRNA medium expression group had the worst prognosis. Thus theEBER intensity and the expression of EBV-miRs of Extranodal NK/T cell lymphoma (NKTCL) are much higher than other types, and we verified that a higher expression of miR-210, miR-664 and has-let-7g are related to a better prognosis; and a grouping based on the expression of EBV-miRNAs wais an importanndependent prognostic factor inof   other types of mature T/NK lymphoma. Compared with EBV-miRNA low expression group with the best prognosis,   mentioned except the NKTCL. tThere were 72 differential has-miRNAs with 11 up-regulated and 61 down-regulated miRNAs in the EBV-miRNA medium expression groupgroup. Through target gene prediction, Gene Ontology annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway analysis, it was found that the upregulated hsa-miRNAs regulate pathways related to MAPK signaling pathway, ErbB signaling pathway, Wnt signaling pathway and Focal adhesion signaling pathway, whereas the downregulated hsa-microRNA mainly target VEGF signaling pathway, the dysregulated miRNAs and aberrant activation of targeted

signaling pathways might play a role in the oncogenesis of EBV-related T/NK cells. Conclusion: the different expression patterns of EBV-miRNA reflect the different mechanisms of the initiation and progression of mature T/NK cell lymphomas, and the expression of EBV-miRNA is related to the clinical prognosis of mature T/NK cell lymphoma. after screening the differential miRs, and GO-enriched analysis demonstrated that these differential miRNAs and corresponding potential target mRNAs play a major role in multiple tumor-related signaling pathways, such as ErbB signaling pathway, MAPK signaling pathway, and several cellular signaling related to focal adhesion and cell metabolism. 、

Tumor Microenvironment and Checkpoint Molecules in Anaplastic Variant of Diffuse Large B-Cell Lymphoma

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Objectives: Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma, showing high heterogeneity in clinical pathology and molecular genetics.　Anaplastic diffuse large B-cell lymphoma (A-DLBCL) is a rare morphological subtype that accounts for approximately of 3.4% all DLBCL, characterized by the presence of polygonal, bizarre-shaped tumor cells. Our previous research found that A-DLBCL displays many genetic alterations and biological features that differ greatly from ordinary DLBCL. Most patients follow an aggressive clinical course and have a high frequency of *TP53* mutation and genetic abnormalities of *MYC, BCL2,* and *BCL6*. However, the status of the tumor microenvironment and checkpoint molecules in A-DLBCL remains unclear.

Materials and Methods: 30 A-DLBCL cases were enrolled to study tumor microenvironment and checkpoint molecules and its association with clinicopathological features and prognosis by immunohistochemical analysis. Besides, we compared these markers between the A-DLBCL group and ordinary DLBCL group (n = 50) from our institution. The tumor microenvironment was characterized by immunostainings for CD3 (tumor-infiltrating lymphocytes, TILs), CD8 (cytotoxic T lymphocytes), T-bet (Th1 cells), GATA3 (Th2 cells), FOXP3 (regulatory T cells), CD68 (tumor-associated macrophages, TAMs), CD163 (M2-TAMs), CD33 (myeloid-derived suppressor cells, MDSCs). PD-L1/PAX5 immunohistochemical double staining was used to evaluate PD-L1 expression in tumor cells (PD-L1$^+$) or in nonmalignant stromal cells (defined as microenvironmental PD-L1, mPD-L1$^+$). PD-1$^+$ TILs was also evaluated by immunohistochemical staining.

Results: Patients with A-DLBCL presented higher expression of PD-L1$^+$ (40% vs 10%, *P* = 0.001) and mPD-L1$^+$ (72.2% vs 33.3%, *P*= 0.005) when compared with patients with ordinary DLBCL. The numbers of PD-1$^+$ TILs (23.3±6.0/HPF vs 50.6±7.2/HPF, *P* = 0.010) and CD8$^+$ cells (34.7±5.7/HPF vs 58.6±7.5/HPF, *P* = 0.026) were significantly lower in A-DLBCL versus ordinary DLBCL. On the contrary, the numbers of GATA3$^+$ cells (30.5±7.1/HPF vs 15.1±3.8/HPF, *P* = 0.039), FOXP3$^+$ cells (17.5±6.0/HPF vs 6.6±2.1/HPF, *P* = 0.048) and CD33$^+$ cells (33.8±4.2/HPF vs 20.6±4.1/HPF, *P* = 0.039) were significantly higher in A-DLBCL compared to ordinary DLBCL. The associations between clinicopathological features and the numbers of tumor microenvironment cells were analyzed in A-DLBCL patients. Briefly, the numbers of PD-1$^+$TILs was lower (14.7±5.2/HPF vs 42.5±16.3/HPF, *P* = 0.049) but CD33$^+$ cells was higher (37.4±5.0/HPF vs 19.3±6.2/HPF, *P* = 0.044) in patients with mutational *TP53* compared with wild type. The number of FOXP3$^+$ cells (10.3±4.5/HPF vs 35±13.4/HPF, *P*= 0.032) was much lower in patients with non-complete response (CR) on chemotherapy. CD8$^+$ cells showed a trend of decreasing in number in patients with high International Prognostic Index (IPI) score (23.8±5.7/HPF vs 47.4±8.2/HPF, *P* = 0.075) and in those with concurrent *MYC* and *BCL2* and/or *BCL6*

abnormalities (26.8±5.4/HPF vs 50.0±9.8/HPF, P = 0.068). Univariate survival analysis showed that patients with PD-L1$^+$/mPD-L1$^+$ or with mPD-L1$^+$ had a significantly poorer overall survival (OS) than those with PD-L1$^-$ (P = 0.034 and P = 0.046, respectively). An increase in CD3$^+$ cell numbers, FOXP3$^+$ cells numbers and T-bet$^+$ cell numbers were significantly associated with prolonged OS in patients with A-DLBCL (P = 0.040, P = 0.000 and P = 0.046, respectively).

Conclusion: Our study suggests that A-DLBCL displays a distinct pattern of tumor microenvironment and checkpoint molecules that distinguish it from ordinary DLBCL. The analysis of tumor microenvironment and checkpoint molecules could help in predicting the prognosis of A-DLBCL patients and determining therapeutic strategies targeting tumor microenvironment.

The effect of section thickness on the differential diagnosis of indolent lymphoma and invasive lymphoma

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Objective: To compare and analyze the immunohistochemical staining of Ki-67 in Diffuse Large B cell lymphoma and Mucosa-associated lymphoid tissue lymphoma with different section thickness, so as to screen out the optimal section thickness in clinical immunohistochemical work. **Methods:** 28 cases of diffuse Diffuse large B cell lymphoma and 18 cases of Mucosa-associated lymphoid tissue lymphoma in Southwest hospital were collected，and the section thickness of 2, 4, 6 and 8 μm were settled to experiment. The results of the average positive staining area percentage (APSAP) of Ki-67 were analyzed with SPSS 22.0. **Result:** It was significantly effect on the positive rate of Ki-67 protein with different section thickness, and the data were statistically significant with the increase of section thickness, the staining of Ki-67 in nuclear and the average positive area percentage of Ki-67 (APSAP) were gradually increased. **Conclusion:** 1. when the section thickness of lymphoma tissue was 4 μm (± 1μm), a clearer cell structure, a sharper staining contrast, and a lower background value were obtained, which was more conducive to the cell count and accurate analysis of the staining results; 2. when the Ki-67 proliferation index at the critical value of 45% between inert lymphoma and invasive lymphoma, the thickness of 4-6μm was optical recommended to use. The error of APSAP in this range was minimum, the anastomosing rate with clinical diagnosis was the highest, therefore, relatively stable and reliable results can be obtained.

Category: Pediatric pathology
1652659

Clinicopathologic features of congenital leukemia in fetal autopsy: a case report

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Objectives
To summarize the clinicopathologic features of congenital leukemia with Down syndrome.
Materials and Methods
A 2875g female fetus occurred intrauterine death at gestation age of 34 weeks and 4 days. Down syndrome screening test at 16th gestation weeks showed a high risk. Chromosomal analysis showed an abnormal karyotype (47,XN,+21).Placenta examination and fetal autopsy had been carried out to identify the cause of death.
Results
  Fetal examination showed the typical facial features of Down syndrome, including wide eye spacing, flat nose, open mouth, extended tongue and webbed neck. The liver of fetus was significantly enlarged with the size13.5cmx9cm x3.5cm and the weight 210g. The lower edge of liver was below umbilical level.The spleen of fetus was also enlarged with the size 8.0cm x 4.0cm x 2.0 cm and the weight 39 g.The placenta was edematous and enlarged with weight 636g and size 17.5cm x 15.0cm x 2.5 cm .
Microscopic examination showed that abundant hematopoietic cells in the vasculatures of placenta, umbilical cord and fetal organs. Nucleated cells increased significantly with enlarged size, cytoplasmic eosinophilic granules, round nuclear and nucleoli. Immunohistochemistry staining MPO (+), CD34 (-),CD117 (-),PGM1(-). The final pathological diagnosis supported congenital acute myeloid leukemia.
Conclusion
Congenital leukemia is rare and often accompanied by Down syndrome.It can lead to intrauterine fetal death. For congenital leukemia cases in perinatal period, placenta examination, fetus autopsy and a panel of immunohistochemistry biomarkers are helpful for the diagnosis .

Analysis of Clinicopathological Features and prognosis of Pancreatoblastoma.

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Objective To investigate the clinicopathological features, immunohistochemical expression and prognosis of pancreatoblastoma (PB) in children and adult . **Methods** Six PB including two cases of kids and four cases of adult, diagnosed in the Department of Pathology of PLA General Hospital from 2009 to 2019 were collected for pathological and immunohistochemical observation, and clinical data and related literature were reviewed.

Results Two kids had a male to female ratio of 1:1, the girl was 4 years old and the boy 8 years old. Tumors were located at the body and tail of the pancreas, with maximum diameters of 8.5 cm and 4.5 cm, respectively. Four adult patients, male to female ratio 1:3, aged 26-52 years, mean age 42 years. 2 cases were located in the head of the pancreas, and 2 cases were located in the body and tail of the pancreas. The maximum diameter of the tumor was 6.5cm-11cm, with an average of 8.1cm. Microscopically, 6 cases of tumors were composed of a mixture of epithelial and mesenchymal components. The epithelial components are separated by a fibrous interstitial into a lobular, organ-like structure. Acinar areas and squamous cell nests (squamoid corpuscles) appeared in all cases. One adult case showed keratinized. Two adult cases showed ductal differentiation with mucus secretion. No ductal differentiation and mucus were found in children cases. Cartilage and bone tissue appeared in two adult cases. 2 cases of children and 2 adults cases showed obvious hemorrhagic and necrosis. Immunohistochemistry showed that epithelial components expressed CK7, CK8/18, CK19, etc., and Markers of neuroendocrine , such as SYN, CgA, CD56 were positive too. The area of ductal differentiation was positive for CEA. The squamous cell nest expresses CK5, P63, and the mesenchymal component expresses Vim. one case of children showed nuclear expression of β-catenin. Two children cases who received a complete resection, were disease free, the DFS was 14 months and 84 months respectively. Four adult cases received chemotherapy after surgery and the mean OS was 61.3 months (range 12-124 months).

Conclusion PB is a fairly rare pancreatic tumor that may originate from primitive multi-potential stem cells and has various differentiations. The acinar structure and squamoid corpuscles are histologically characteristic changes. PB in children and adult have different prognosis, and the presence of ductal differentiation and mucus secretion suggests a worse prognosis.

Intestinal metastases of infantile choriocarcinoma: a case report

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Choriocarcinoma presenting in an neonate or infant is a rare and highly malignant trophoblastic neoplasm. Intestinal metastasis in infantile choriocarcinoma is even rarer with only one case report available[1]. The diagnostic triad of choriocarcinoma includes anemia, hepatomegaly, and elevated βHCG. The natural disease course is rapidly fatal. As a chemosensitive tumor, it's treatable nowadays with multi-agent cisplatinum-based chemotherapy and delayed or primary tumor resection[2]. Herein, we reporte the case of a 7-hour-old boy infant with choriocarcinoma presented by severe anemia, hemotochezia and widespread multiple metastatic lesions in the liver, spleen and lungs with an elevated level of ßHCG. Subsequently, the infant's mother was diagnosed with intraplacental choriocarcinoma. Choriocarcinoma was not suspected in this case until pathologic examination revealed the diagnosis. In order to promote timely consideration for this malignancy as a differential consideration in the approach to hematochezia in infancy and add this significant presentation to the disease , we present the findings of this neoplasm in an infant. While imaging and clinical characteristics similar to Michael's diverticulum, pathology and further imaging investigations revealed multifocal metastatic choriocarcinoma.

Thyroid nodule FNA washout-DNA somatic copy number profiling identified high risk thyroid cancer

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Objective: Thyroid fine needle aspiration (FNA) is the most common preoperative diagnostic methods. However, it is difficult to differentiate high-risk from low-risk papillary thyroid carcinoma (PTC) cytologically. Here we investigated somatic copy number variations (CNVs) of thyroid FNA washout-DNA (wDNA) by whole genome sequencing (WGS) as a biomarker to differential diagnosis.

Methods: Seventeen thyroid FNA washouts were recruited from Shandong University Qilu Hospital. wDNA was sent to low coverage WGS with median genome coverage 1.86✖□ (range from 1.03✖□ to 3.17✖□) by Illumina ✖□10. Sequencing coverage across chromosomes was summarized by samtools, normalized, and followed by segmentation analysis as provided by R package 'DNACopy'. A significant changed segment was defined by segment size ≥10M and P value ≤0.01.

Results: All 17 thyroid FNAs were cytologically diagnosed as Bethesda VI, malignancy. Nine of 17 (52.9%) samples were found with chromosomal instability (CIN). Among them, 1q, 22del are the most frequent genomic changes identified with frequency 4/9(44.4%) and 3/9 (33.3%) respectively.

Sixteen cases were confirmed to be PTC, and 1 case was proved to be Hurthle cell tumor with uncertain malignant potential histologically. Taking together, significant wDNA CNVs were found in 50% of PTC patients. Further analysis identified frequent CNVs on chromosome 1q, 22 and 3 in the PTCs, which were detected in 4 (25.0%), 3 (18.8%) and 1 (6.3%) PTC patients, respectively. Multiple CNVs including 2,5,7,12,16, X gain and 8,11,15,18 loss were confirmed in the HTT, which supported it to be Hurthle cell carcinoma. The results indicated that wDNA CNVs analysis might be a useful tool to differentiated high-risk PTC from low-risk PTC.

Conclusions: It is feasible to use wDNA CNVs by low coverage WGS for preoperatively identifying high-risk PTC from low-risk PTC, and to identify Hurthle cell tumor from PTC.

Cytologic diversity of high grade urothelial carcinoma in urinary washing cytology: correlation with histopathologic variants

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Background & objectives

Urothelial carcinoma shows a wide diversity of histologic patterns, some that mimic nonurothelial malignancies.   In this study, we evaluated the morphologic diversity of high-grade urothelial carcinoma in bladder washing specimens and correlated the findings with the histopathologic features of radical cystectomy.

Methods

We collected 100 cases of radical cystectomy, diagnosed as high-grade urothelial carcinoma and retrospectively reviewed matched urine cytology (80 washed specimen, 1 voided urine) using liquid based preparation. Following cytological parameters were evaluated: conventional urothelial carcinoma features, squamous differentiation, micropapillary change, plasmacytoid/signet ring cell change, spindle cell/sarcomatoid change, clear cell/vacuolar change.   Histopathologic variables were reviewed, and correlated with cytologic variables.

Results

The patients included 71 men and 10 women (age 37-84 years). Among the total 81 cases, the following cytologic diversities were found in 28 cases (34.6%): squamous/squamoid features in 12 cases (14.8%), clear cell/vacuolar change in 5 cases (6.2%), micropapillary features in 4 cases (4.9%), spindle cell change in 4 cases (4.9%) and plasmacytoid/signet ring cell features in 3 cases (3.7%). Compared with their histologic findings in the surgical specimens, seven cases (25%) were histopathologically well correlated: squamous differentiation in 3 cases out of 12 cases (25%), micropapillary features in 2 cases out of 4 cases (50%), plasmacytoid features in 1 case out of 3 cases (33%), and spindle cell/sarcomatoid feature in 1 case out of 4 cases (25%).   Fifteen cases(18.5%) of radical cystectomy showed differentiation of specific variants ; squamous(6 cases), neuroendocrine(1 case), glandular(1 case), plasmacytoid(3 cases), micropapillary(3 cases), and sarcomatoid(1 case).

Conclusion

Diverse cytologic features were found in 34.6% of urine cytology of high-grade urothelial carcinoma. Among them, about 25% of cases were correlated with diverse variants of urothelial carcinoma, histopathologically.

Differences between Asian and Western Thyroid Practice (in Japan-IAP Special)

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Introduction

Western medical practice has played a significant role in establishing standardized diagnostic approaches to disease and optimal clinical management for patients. In the thyroid field, most pathologists in the world follow international diagnostic systems developed principally by Western authors, such as the Bethesda system for reporting thyroid fine-needle aspiration (FNA) cytopathology, the World Health Organization (WHO) classification of tumors of endocrine organs, and clinical guidelines published by the Western societies such as The American Thyroid Association. However, when these Western systems were implemented in Asian practice, Asian pathologists show results that differ from those in Western series.

Objectives

The Asian Working Group for Thyroid Cytology/Pathology (Asian WG) was established in 2017 to elucidate differences between Western & Asian thyroid pathology & cytology practice (1). This presentation will introduce recent achievements by the Asian WG. From a practical standpoint, Asian pathologists pay close attention to these differences when Western systems are introduced into Asian patient cohorts because Western systems were designed and are usually based on data derived from Western patients.

Materials and Methods

Forty Asian cytopathologists or pathologists from 13 countries were invited to participate in the Asian WG study using virtual data and Excel answer sheets. Data were collected and analyzed by each principal investigator who led the projects and was published in more than ten peers reviewed international journals.

Results

Prevalence of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) was an average 0.8 % (0-4.7%) of papillary thyroid carcinoma (PTC) in 9 Asian practices, which was significantly lower than that (15-25%) of Western practice (2).

Observer variation of nuclear features in the diagnosis of NIFTP was studied among nine members using 30 cases of morphologically diagnosed NIFTPs. Although a three-point nuclear scoring system for the diagnosis of NIFTP is widely used in Asian practice, inter-observer variation was considerable (3). It could not reliably separate NIFTP from an encapsulated follicular variant PTC with minimal lymph node metastasis or BRAFV600E mutation (3).

Impacts of NIFTP on risks of malignancy (ROMs) of the Bethesda cytological categories

were studied using a meta-analysis of 14 studies (4). This study confirmed the decreased ROMs due to NIFTP reclassification for most of Bethesda cytological categories, which was more significant in Western than in Asian practice.

Hurthle cell-rich lesion on cytology was studied among 42190 FNAs from 9 Asian institutes (5). This study confirmed that the ROM for all resected Hurthle cell-rich follicular neoplasm (FN) nodules was from 15.3% to 30.6%, and within the suggested range (10-40%) of ROM for the Bethesda category FN.

We conducted a survey among 18 pathologists in 10 Asian and Austrasia countries and found that there was no personal history of litigation in the thyroid field and defensive medicine practiced in North America is not common among pathologists practicing in other countries (6). Five Asian thyroid pathologists commented that they prefer under-diagnosis rather than over-diagnosis while malpractice fears push many pathologists in USA to diagnose malignancy in equivocal lesions.

Conclusion

We believe these differences in Asian thyroid practice further elucidate differences in clinical approaches and the social background of Asian practice different from those of Western countries (6, 7). In addition to the thyroid field, there should be significant differences in other organ systems. We believe that Asian pathologists have to pay more attention to those differences when Western systems are introduced to Asian practice because they were established usually based on data only from Western patient cohorts.

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Category: Cytopathology
1650024

MnO2 decorated GOx/MOFs nanocomposites for enhanced tumor starvation therapy

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Objective: Constructing a nanoplatform for efficient tumor starvation therapy based on glucose consumption mechanism. Herein, Manganese oxide (MnO2) modified glucose oxidase-metal organic frameworks (GOx/MOFs) composites were constructed, which could release the GOx and generate oxygen in tumor microenvironment, and thus enhance the consumption of glucose under GOx catalysis, inducing enhanced starvation therapy.
Methods: MnO2 nanoparticles were prepared by reacting polyallylamine hydrochloride (PAH) with potassium permanganate (KMnO4) and the GOx@MOFs nanomaterials were synthesized by encapsulating glucose oxidase (GOx) into the framework of ZIF-8 MOFs, and MnO2/GOx@MOFs nanocomposites (M/G@MOFs) were prepared by self-assembly of PAH-modified MnO2 and GOx@MOFs due to the electrostatic interaction. The pH, glucose and hydrogen peroxide (H2O2) conditions of the tumor microenvironment were simulated in vitro to investigate the drug release ability and catalytic performance of M/G@MOFs. Glioma cells (LN229) were adopted to investigate the effectiveness of M/G@MOFs in inhibiting tumor cell proliferation. Finally, the distribution of the nanomaterials in mice, the targeting ability of tumor sites and the tumor suppression effect were also performed.
Results: The synthesized M/G@MOFs had uniform particle size (~120 nm). After surface modification of polyethylene glycol (PEG), it had good dispersibility under physiological conditions. In vitro simulation experiments showed that M/G@MOFs could effectively release GOx and MnO2 under acidic conditions, and also exhibit good catalytic activity for glucose and H2O2; In vitro cell experiments showed that when the concentration of M/G@MOFs was 100 μg/mL, the survival rate of LN229 cells was less than 20%, exhibiting good cell proliferation inhibition ability; In vivo experiments showed M/G@MOFs nanocomposites had no obvious systemic toxicity, and could effectively accumulated in tumor tissues and strongly inhibit the tumor growth.
Conclusions: M/G@MOFs nanocomposites could achieve effective drug delivery, accumulate in the tumor site for a long time, reduce adverse drug reactions, and finally induce tumor cell apoptosis by consuming glucose in the tumor site. These research results can provide new solutions for improving the therapeutic effect of oncology drugs.

Tumor Tropic Delivery of Bi2Se3 Nanodots Using Mesenchymal Stem Cells for Targeted Cancer Radiotherapy

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Objectives: Targeted delivery of radiosensitive Bi2Se3 Nanodots to tumor sites using Mesenchymal Stem Cells for targeted radiotherapy.

Materials and methods: Bi2Se3 nanodots were synthesized using high-temperature decompsition method and modified with Polyethylene glycol 1000 vitamin E succinate (TPGS), and then co-cultured with Mesenchymal Stem Cells to obtain then MSCs-Bi2Se3 composites. Firstly, viability, differentiation or migration potentia of Bi2Se3 incorparated MSCs were elvaluated. Then, MSCs-Bi2Se3 composites as radiosensitizer for tumor radiotherapy were performed in vitro and in vivo.

Results: Incorporating Bi2Se3 nanodots in MSCs did not affect their viability, differentiation or migration potential. The nano-engineered MSCs (MSCs-Bi2Se3) induced dose dependent cytotoxicity in LN229 and G261 glioma cells under X-ray exposure in vitro. In a subcutaneous LN229 model to monitor the in vivo distribution, intravenous injection of Bi2Se3 nanodots resulted in non-specific biodistribution with significant accumulation in the liver and spleen, while nano-engineered MSCs largely improve accumulation and retention in the tumors. As for in vivo tumor-eradication studies, MSCs- Bi2Se3 exhibited the much higher therapeutic index as expected under X-ray irradiation.

Conclusions: Mesenchymal stem cells (MSCs) with inherent tumor-tropic property employed as carrier to deliver Bi2Se3 nanodots indicated the availability of MSCs delivery strategy for targeted cancer therapy.

The value of fine needle aspiration cytology combined with BRAF (V600E) molecular detection in early diagnosis of papillary thyroid carcinoma

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Objective: To explore the value of FNA cytology combined with BARF (V600E) in the early diagnosis of papillary thyroid carcinoma (PTC). **Methods**: 104 cases of thyroidectomy in our department in 2017 were retrospectively analyzed. The samples of FNA cytology and BRAF (V600E) molecular detection were screened before operation. The samples of FNA operation and molecular detection before operation were extracted and detected by ultrasound-guided FNA puncture cytology according to the standard operating procedure. The samples of each patient were divided into two parts. One sample was cytologically smeared and the other sample was placed in lysate for BRAF (V600E) molecular detection. Cytological diagnosis and molecular detection of these two samples were performed simultaneously. The final pathological diagnosis, preoperative FNA cytological diagnosis and preoperative BRAF (V600E) molecular diagnosis of these 104 cases were statistically analyzed. The sensitivity and specificity of preoperative FNA cytological diagnosis and preoperative BRAF (V600E) molecular diagnosis was compared. **Results**: Among 104 patients, 21 were males and 83 were females, the oldest was 77 years old and the youngest was 17 years old, with an average age of 43.8 years. 43 nodules were located in the left lobe, 55 in the right lobe, 4 in bilateral nodules and 2 in isthmus. After thyroidectomy, 70 out of 104 patients were diagnosed as PTC, 4 out of 104 cases were diagnosed as thyroid follicular carcinoma, 1 case was diagnosed as metastatic carcinoma, 29 out of 104 patients were diagnosed as benign thyroid diseases .But before thyroidectomy, 62 out of 104 patients were FNA cytologically diagnosed as PTC. The BRAF (V600E) mutation rate in all patients was 52.9% (55/104), and mutation rate in PTC patients was 88.6% (55/70) . All patients with BRAF (V600E) mutation were diagnosed as PTC after operation, and no BRAF (V600E) mutation was found in non-PTC patients after operation. The sensitivity and specificity of preoperative FNA for PTC diagnosis were 88.6% (62/70), 76.5% (26/34), and 78.6% (55/70), respectively. The sensitivity and specificity of FNA combined with BRAF (V600E) were 97.1% (68/70) and 100% (34/34) respectively. **Conclusion**: Preoperative FNA cytology or BRAF (V600E) test alone has high specificity and sensitivity in the diagnosis of PTC, but the combination of the two can further improve the sensitivity and specificity of early diagnosis of PTC. The combined detection method has wide application value.

Clinicopathological analysis of nested variant of urothelial carcinoma with tubule formation

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Objective To investigate the clinicopathological features, diagnosis, differential diagnosis and prognosis of nested variant of urothelial carcinoma with tubule formation.

Materials and Methods Conventional H&E staining and immunohistochemistry of Envision method were used to observe a case of nested variant of urothelial carcinoma with tubule formation under light microscope. The histopathological features were analyzed and related literatures were reviewed.

Result Male, 48 years old. The first symptom was gross hematuria. Under microscope, the circular or polygonal small tubular structure was diffusely distributed and irregularly arranged，and structure of small nests were seen occasionally; the tumor cells were mild in morphology, medium in size, round in shape, and rich in cytoplasm. The nuclear atypia was bland, the nuclear/cytoplasm ratio was slightly elevated, nuclear deep staining was rare, and pathological mitotic figure was not seen. The tumor cells had granular chromatin with inconspicuous nucleoli. Tumor cells infiltrated into the muscular layer, which made cell dysplasia increases, nucleus enlarge and nuclear chromatin thicken. Interstitial fibrosis was seen around the tumor. There was no invasion of blood vessels, lymphatic vessels and nerves. Immunohistochemical study exhibited that the tumor cells were focal positive for MOC31 and BreEp4, and strongly positive for CK7, CK5/6, p63, CK34beteE12 and p53. All tumors were negative for CK20、Villin、CDX-2、TTF-1、PSA、P504S and PAX-8. Ki67 index were 10% approximately.

Conclusion Nested variant of urothelial carcinoma with tubule formation was a rare variant of urothelial carcinoma and was difficult to distinguish from non-neoplastic lesions of the bladder. The diagnosis depended mainly on the biologically present pattern of irregular infiltration, and immunohistochemical staining can be used as an adjunct to diagnose such lesions.

Clinicopathologic analysis of two cases of adult mesoblastic nephroma

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Objective To explore the clinicopathological features and diagnosis of adult mesoblastic nephroma(AMN).

Materials and Methods By using histological and immunohistochemical technique, the clinicopathological data were analyzed in two cases of AMN and the related literatures were reviewed.

Result Pathological examination of renal space-occupying specimens of two female patients in our hospital showed thatthe tumor was composed of fibroblastic cells arranged in bundles with slim and sharp terminal nuclei, eosinophilic plasma, rare mitoses. There were abundant collagen deposits in the tumors.. The expansion growth pattern of the tumor caused renal parenchyma sink into tumors like islands. The immunohistochemistry showed that actin, desmin and vimentin were positive in spindle cells, while negative in remaining renal tubular epithelial cells. CK, CK7, CK34βE12 and CK19 were negative in spindle cells, while positive in remaining renal tubular epithelial cells. Both kinds of cells were negative for S-100 and CD34. The proliferation index of Ki67 was about 1%.

Conclusions Mesoblastic nephroma is an uncommon, distinctive renal tumor reported in infants. It is a special type of nephroblastoma that rarely occurs in adults. It should be identified from other tumor by immunohistochemical methods.

The correlation between the expression of pd-l1 in pleural effusion of lung adenocarcinoma and the clinicopathological features and molecular changes

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OBJECTIVE To evaluate the feasibility of immunocytochemistry (ICC) in non-small cell lung cancer to detect PD-L1 and to investigate the correlation between PD-L1 expression and molecular features. **METHODS** 60 cases of lung cancer pleural cytology were collected and PD-L1 sp263 reagent was used for immunocytochemical staining. High-throughput second-generation sequencing (NGS) was performed on pleural cytology specimens. **RESULTS** Of the 60 cases of lung cancer pleural effusion cell block ICC, 35 cases were positive for PD-L1 expression, the positive expression rate was 58.3%. The positive rate of PD-L1 expression in the specimens of surgical pathology was 33.3%, and there was no significant difference between two specimens (p>0.05). 26 were tested for NGS, and 15 (57.7%) were found to have EGFR mutations. No correlation was found between PD-L1 expression and EGFR mutation. **Conclusion** When no surgical specimens are available, pleural cytology cell block specimens can be used for immunocytochemical detection of PD-L1, and the results are feasible.

A Comparison of Small Biopsy and Cytologic Specimens: Subtyping of lung Adenocarcinoma

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Objective: Adenocarcinoma is a heterogeneous disease with different morphological subtypes, which were significantly correlated with different prognosis. Nearly 70% of the primary lung cancer patients only have access to obtain small biopsies or cytological samples. Here, we investigated whether cytologic samples can be used to diagnose subclassification of lung adenocarcinoma and analysis cytologic-histologic correlation diagnosis of lung adenocarcinoma subtype using small biopsy and cytology specimens.
Materials and Methods: Cytological characteristics of different subtypes of lung adenocarcinoma were summarized through relevant literature review. Based on the summarized cytological features, cytology samples from 115 patients with lung adenocarcinoma confirmed by small biopsies were performed subtyping classification. Diagnostic concordance of subtypes between small biopsy and cytology specimens was assessed.
Results: Among the 115 lung adenocarcinoma patients confirmed by small biopsy specimens, 62 cases(53.9%) were acinar predominant pattern, 16 cases(13.9%) were papillary predominant pattern, 29 cases(25.2%) were solid predominant pattern, 3cases (2.6%) were lepidic predominant pattern, and 5 (4.3%) were micropapillary predominant pattern. All corresponding cytologic samples were classified into 5 subtypes based on cytomorphology features and the concordance rate was 53.9% (62 patients)in acinar subtype, 56.3% (9 patients)in papillary subtype, 24.1%(7 patients)in solid subtype, 66.7% (2 patients)in lepidic subtype and 40% (2 patients) in micropapillary subtype .
Conclusions: Subtyping of lung adenocarcinoma based on cytologic specimens is challenging and the total diagnostic concordance rate of subtypes between small biopsy and cytology specimens was only 57.4%. The consistency rate varies according to different subtypes. A acinar -predominant tumor has an excellent cytologic-histologic correlation compared with tumors with a predominant solid or micropapillary pattern. Familiar with the cytomorphologic features of different subtypes of lung adenocarcinoma contributes to reduce the false negative rate of lung adenocarcinoma, especially micropapillay subtype which have mild atypical and improve the diagnostic accuracy.

Category: Cytopathology
1656800

Application of fine needle aspiration cell blocks in the diagnosis of salivary mucoepidermoid carcinoma

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Objective   Mucoepidermoid carcinoma(MEC) is the most common salivary gland malignancy in children and adults,which is challenging to diagnose by fine needle aspiration (FNA).Cell blocks were used for immunohistochemical and intracellular mucin staining to solidify the diagnosis.

Methods   A total of 34 FNA biopsies were collected using a Youyi aspirator. Some specimens were used to make cell smears for cytological diagnosis; the remaining specimens were used to make cell blocks according to protein-alcohol-coagulation method and then HE, PAS and immunohistochemical staining were performed. The sensitivity/final diagnostic rates were compared with those of simple smears and a combination of smears and CBs.

Results   All aspirates are hypocellular, containing rare flat sheets of epidermoid cells in a background of abundant thick mucoid material.Groups with a combination of epidemoid cells, intermediate cells, and admixed mucinous cells is the most diagnostic feature.Background lymphocytes are seen in 20.5% (7/34)of cases, and   14.7% (5/34)cases contain hemosiderin-laden macrophages and cholesterol crystals. Alcian Blue(AB) stain and immunohistochemical stains for keratin and CD68 were used to distinguish epithelial-containing mucus cells from histiocytes. Immunohistochemical stains for myoepithelial cells (calponin, smooth muscle actin,   S-100) were used for the differential diagnosis of cystic pleomorphic adenoma and MEC. The sensitivity and the final diagnostic rates were lowest with the simple cell smears (67.6%, 23/34 cases), moderate with the smears combined with CB sections (79.4%, 27/34 cases), and the highest with AB and IHC staining (97.1%, 33/34 cases).

Conclusion   Fine needle aspiration sampling technique and application of cell block preparation method can efficiently improve the diagnostic accuracy of MEC. Moreover, cell blocks   have high application value in MEC histological type and classification , which is close to the pathological diagnosis after operation.

Highly expressed IMP3 and GLUT-1 in combination with BAP1 loss, as detected by immunohistochemistry, is useful for differentiating malignant mesothelioma from reactive mesothelial hyperplasia

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Abstract

Objectives: Malignant mesothelioma is a highly invasive cancer which is usually diagnosed in the advanced stage. Therefore, high sensitivity and specificity of markers are necessary for its early diagnosis. Our study aims to evaluate the diagnostic utility of BAP1, IMP3, and GLUT-1 in malignant mesothelioma.
Materials and Methods: The expressions of BAP1, IMP3, and GLUT-1 were investigated by immunohistochemistry in 38 malignant mesotheliomas and 32 reactive mesothelial hyperplasias.
Result: BAP1 was loss in 20 (52.6%) of 38 malignant mesotheliomas. IMP3 and GLUT-1 were positive in 26(68.4%) and 25(65.8%) of 38 malignant mesotheliomas. All IHC of BAP1，IMP3 and GLUT-1 were characterized by a 100% specificity and their sensitivities were 52.63%, 68.42%, and 65.79%, respectively. Furthermore, the combination of BAP1, IMP3, and GLUT-1 gave the highest sensitivity (81.58%) and the specificity was 100%.
Conclusions: Overexpressing IMP3 and GLUT-1 in combination with BAP1 loss were highly specific to malignant mesothelioma in differentiating from reactive mesothelial hyperplasia and improved the diagnostic accuracy especially in malignant mesothelioma.

Fine needle aspiration cytology findings of Xp11 translocation renal cell carcinoma with supraclavicular lymph nodes metastases: A case report and mini-review

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BACKGROUND: Xp11.2 translocation/transcription factor E3 (TFE3) gene fusion renal cell carcinoma (Xp11.2 translocation RCC) was first recognized as a distinct type of renal cell carcinoma by the World Health Organization in 2004. Recently, it is placed under the "MiT family translocation RCC" at the last 2013 ISUP Vancouver classification of renal neoplasia. This tumor is predominantly found in children and young adults, although there have been cases described in older patients. Histologically, this tumor demonstrates variable proportions of clear cells arranged in papillary structures with dense hyaline cores which could easily mimic papillary RCC, clear cell type, and clear cell papillary RCC. To date, only seven case reports about the cytologic findings of Xp11.2 translocation RCC have been published in the literature.
CASE PRESENTATION: We describe a case of a 34-year-old woman with a history of prior nephrectomy who presented with solitary supraclavicular lymphadenopathy on imaging surveillance follow-up. Fine-needle aspiration of the lymph node presented tumor cells with predominantly voluminous clear to finely granular eosinophilic cytoplasm, well-defined cell borders and variably prominent nucleoli arranged in papillary architecture especially in cell blocks. On immunohistochemical staining of the tumor cells on cell block, there was tumor positivity for CD10, a-methylacyl coenzyme A racemase (AMACR), RCC, and transcription factor E3 (TFE3) supporting the diagnosis of Xp11 translocation RCC. TFE3 break-apart fluorescence in situ hybridization (FISH) assay was also performed on the tumor cells of the prior nephrectomy specimen and TFE3 gene translocation was identified.
CONCLUSION: Xp11.2 translocation RCC in adults is clinically more aggressive than in children and often presents at advanced stage with poor prognosis as seen in this case. The younger patient and unusual architectural arrangement of clear or eosinophilic cells may clue a cytopathologist into this entity. However, ancillary studies including immunohistochemical stains or cytogenetic test to determine the type of gene fusion will be extremely useful for correct diagnosis.

Category: Liquid biopsy
1651106

Serum Chemokine CXCL7 as a Diagnostic Biomarker for Colorectal Cancer

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Abstract

Objectives: Identification of effective biomarkers is crucial for monitoring the treatment and remission of colorectal cancer (CRC) and improving survival. It is particularly important to diagnose CRC before the tumor metastasizes (stage I – II disease) where possible, to provide the greatest opportunity for patient recovery. Materials and Methods: We evaluated the clinical value of serum chemokine (C-X-C) ligand 7 (CXCL7) concentration as a biomarker for CRC diagnosis. An enzyme-linked immunosorbent assay was used to measure CXCL7 concentration in 560 serum samples from patients with CRC and controls. Logistic regression and receiver operating characteristic (ROC) curve analysis was used to assess the diagnostic efficacy and build mathematical diagnostic models. Result: The concentration of CXCL7 in the CRC group was significantly higher than that in the control group ($P < 0.001$), with an area under the ROC curve (AUC) value of 0.862 (95% confidence interval (CI): 0.831 – 0.890). Further, the AUC of a regression model including the markers carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), and carbohydrate antigen 125 (CA125), along with CXCL7, was 0.933 (95% CI: 0.909 – 0.952). For stage I – II tumors, CXCL7 had the highest AUC (0.823, 95% CI: 0.783 – 0.858) among the four individual biomarkers. The AUC value for combination model analysis of samples from patients with stage I – II tumors was 0.904 (95% CI: 0.872 – 0.930), with a sensitivity of 82.76% and a specificity of 87.14%, and an optimal cut-off value of 2.66. AUC values for application of the regression model in subgroup analysis were 0.947 (0.917 – 0.968) and 0.919 (0.874 – 0.951) for males and females, respectively. Conclusion: These results suggest that CXCL7 has potential as a serum diagnostic biomarker for detection of CRC. Importantly, the combination of CXCL7, CEA, CA125, and CA19-9 may facilitate diagnosis of CRC with relatively high sensitivity and specificity.

The natural killer cells engineered with anti-GPC3 chimeric antigen receptors in immunotherapy for hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the second leading cause of cancer deaths in the world. The incidence cases and number of deaths of HCC both account for more than 50% of the total all over the world respectively, while the mortality rate is in the third place. Although the HCC treatment protocols tend to be diversified and combined, it is difficult to achieve further improvement in the long-term survival of HCC patients in the last 10 years. Therefore, it is urgent to find a new, effective and safe treatment protocol. In recent years, chimeric antigen receptor (CAR)-modified T cells is one of the tumor immunotherapies that attract the most attention. This technology allows T cells to specifically target tumor surface antigens by transfecting chimeric antigen receptors in T cells, while the intracellular domain provides the first and second signals to activate T cells, and it is a new generation of cell therapy with targeting ability, which overcomes the drawback of insufficient infiltrating cells and maintains the killing activity. This technique has shown excellent therapeutic effects in the treatment of various tumors, especially hematological tumors. At the same time, safety has become a major problem that affects the clinical applications of CAR-T therapy: cytokine storm, off-target effects, severe allergic reactions and neurotoxicity, and the potential safety hazards posed by viral vectors have severely limited further applications of CAR-T therapy.

NK cells are important effector cells of the innate immune system. NK cells do not require pre-sensitization by antigen to perform extremely high cell killing activity and are not MHC restricted; mature NK cells do not secrete IL-6, and the physiological cycle is short and does not cause GVHD response. Studies in bone marrow transplantation have shown that hematological tumors can be effectively recognized and killed by allogeneic NK cells, which can significantly increase disease control and reduce recurrence rate. However, many tumor cells often acquire immune evasion ability by expressing non-classical HLA class I molecules, expressing immunosuppressive ligands, or secreting immunosuppressive factors, etc., thus the clinical efficacy of NK cells adoptive therapy is poor. Therefore, CAR modified NK cells, which show target killing effect on tumor cells, has become an important strategy to improve the efficacy of NK cells adoptive therapy.

On this basis, in this study we have studied the TCGA big data, identified the HCC-specific antigen GPC3, and obtained high-affinity and high-specificity antibodies

of GPC3 by phage antibody display technology; and further optimized the CAR structure based on the activation signal of NK cells, and then the CAR structure was selected and used on NK cells. Finally, the key technologies in the clinical application of CAR-NK were explored, and a liposome system for increasing NK cell expansion and cytotoxic activity was established, and a PDX model based on circulating tumor cells of HCC patients was established.

Study on inhibition of islet allograft rejection by Qa-1/PD-L1 artificial liposome

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NKG2A and PD1 are important immune checkpoint molecules, when combined with their ligands, whichcan arrest activation of immune cells and inhibit the allograft rejection. However, extracellular domain of the NKG2A/PD1 ligandsQa-1 / PD-L1 is small and easily exhausted in peripheral. Loading Qa-1 / PD-L1extracellular domain to the artificial liposome couldeffectively overcome this shortcoming and develop novel immunosuppresses. **Object** To investigate the effects of Qa-1 and PD-L1 loaded artificial liposome treatment in allograft rejection and its outcomes.**Methods** The extracellular domain of Qa-1 and PD-L1 was loaded on liposome surface by streptavidin-biotin system. The loading efficiency was measured by fluorescence and half-life*in vivo* was measured by FACS. The mixed lymphocyte reaction was performed to measure Qa-1/PD-L1 liposome biological function. Then the liposome was co-transplanted with allo-islets via portal vein in TD1 mice. The blood glucose and C-peptide was detected every day after transplantation. The hepatic lymphocytes after transplantation were also isolated to determine proportion of activated cells and signaling pathway changes. **Results** Theloading efficiency with biotinylatedpeptide was more than 95%, and liposome diameter was between 50nm to 500nm. Qa-1/PD-L1 liposome could significantly inhibit lymphocyteproliferation, activation and secretion of IFN-γ in MLR by activation of SHP1/2 and inhibition of Syk pathway.Compared to the control group, Qa-1/PD-L1 liposomesprotect islet allografts from rejection and maintainnormal blood glucose levelof recipients more than 14days. **Conclusion** Qa-1/PD-L1 loadedliposome could effectively inhibit allograft rejection and improve the outcomes of islet transplantation.

Inhibition of Allogeneic Islet Graft Rejection by VISTA-conjugated Liposome

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The Ig superfamily member V-domain Ig-containing suppressor of T-cell activation (VISTA) is a negative regulator with broad-spectrum activities and has reported that blockade of VISTA or combination with other negative checkpoint receptors sufficiently break tumor tolerance. However, it remains unclear whether VISTA could induce allogeneic T-cell hyporesponsiveness and inhibit allograft rejection. Here we found VISTA treatment significantly inhibited lymphocyte proliferation and activation in allogeneic MLR assay through impairing SYK-VAV pathway. Interestingly, though neither VISTA protein nor VISTA-Fc fusion protein administration exerted satisfactory immunosuppressive effect on allograft survival due to their short half-life in circulation, this problem was solved by loading VISTA protein on liposome by biotin-streptavidinsystem, which markedly prolonged its circulating half-life to 60 hrs. With islet transplant model, administration of VISTA loaded liposome could markedly prolong allograft survival by inhibition of SYK-VAV pathway, thus maintained the normal blood glucose level of recipients during treatment period. The results indicate VISTA is a promising therapeutic target to treat allograft rejection of islet transplantation.

The overexpression of PD-L1, a potential biomarker correlates with EGFR-TKI resistance in NSCLC

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Objectives: Molecular detection guiding the treatment of non-small-cell lung cancer (NSCLC) followed by clinical practice has collected in major guidelines and widely recognized. Currently, molecular detections, such as epidermal growth factor receptor (EGFR)、 programmed death-ligand 1 (PD-L1), mesenchymal to epithelial transition factor (MET), V-Erb-B2 Avian Erythroblastic Leukemia Viral Oncogene Homolog 2 (ERBB2), have become a routine detection project of NSCLC as well as tyrosine kinase inhibitors (TKI) resistance detection, becoming one of the important factors in NSCLC. Besides, immuno-correlation detection and immune checkpoint inhibitors, notably antibodies targeting PD-1 and PD-L1, have modified the management environment of patients with locally advanced or metastatic NSCLC, significantly prolonging the poor prognosis caused by EGFR resistance. There was insufficient evidence whether the expression of PD-1/ PD-L1 is related to the mutation of EGFR or the resistance of EGFR-TKI.

Materials and Methods: 853 cases of NSCLC patients collected for EGFR, PD-L1(Ventana SP263), MET and ERBB2 detections simultaneously from the medical records of Fourth Hospital of Hebei Medical University between January 2017 and January 2019, 196 cases had a high expression of PD-L1 (> 50%) in positive EGFR mutations (445/853,52.2%). Real-time quantitative polymerase chain reaction (RT-qPCR) and Next-generation sequencing (NGS) was used for molecular detection , such as EGFR and MET. ERBB2 results were verified in Fluorescence in situ hybridization (FISH) while immunohistochemical staining were decided to be 2+. ALL the test results were confirmed by gold standard method. Statistical analyses were performed using IBM SPSS software. The chi-square test was used to analyze the objective response rates according to PD-L1 expression.

RESULTS: There were 24 cases (24/196, 12.24%) with high expression of PD-L1 as well as T790M mutation, which was significantly higher than that of 36 cases in low expression group (36/546, 6.59%) or 6 cases in moderate group (6/111,5.41%), P = 0.039, X2 = 6.478. The number of patients with high expression of PD-L1 and ERBB2 amplification was 5 (5/196, 2.55%; P = 0.008, X2 = 9.729), had positive correlation with PD-L1 overexpression. Among 91 EGFR-TKI resistance cases, the positive rate of high expression of PD-L1 in 17 cases of primary resistance to EGFR was higher than that of acquired resistance to EGFR-TKI (58.82% vs 29.73%, X2 = 5.132, P = 0.046). In addition, the primary drug resistance always accompany the high expression level of PD-L1(positive ratio≥50%). The patients with primary drug-resistant and high expression level of PD-L1 had a shorter progression-free survival (PFS) (P = 0.040).According to our statistics, the high

expression level of PD-L1 is related to poor clinical pathological feature, size, lymph node or distant metastasis rate were all include(P <0.000).In EGFR positive cases, the number of patients with high expression of PD-L1 was different in adenocarcinoma, squamous cell carcinoma, adenosquamous cell carcinoma and mucinous adenocarcinoma (X^2= 157.241, P = 0.000). The overexpression of PD-L1 happened in mucinous adenocarcinoma more often.

CONCLUSIONS: It can be inferred that the high expression of PD-L1 is related to poor prognosis and EGFR-TKI resistance. The high expression of PD-L1 may speculate the existence of drug resistance mutation, especially the mutation before medicated, and the resistance mechanism may be related to immunity.

From basic to advanced, does NGS have an edge in accurate lung cancer diagnosis?

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Objective: Next-generation sequencing (NGS) is a massively parallel sequencing technique that can be used to detect many forms of defense nuclear agency(DNA) variation, including point mutations, small fragment insertion deletions, gene recombination, and copy number variations. Based on the recommendations of the NCCN guidelines, 8 gene panels (EGFR, ALK, ROS1, BRAF, MET, RET, ERBB2, KRAS) on nonsmall-cell lung cancer(NSCLC) patients detected by Next-generation sequencing (NGS) which were highly correlated with individualized therapy to explore the applicable scope of NGS detection.
Method: Patients with NSCLC in the Fourth Hospital of Hebei Medical University were collected for the detection of genetic variation through NGS, RT-PCR and fluorescence in situ hybridization(FISH), and the positive rate of the detected sample types and detection methods were compared and analyzed.
Results: Firstly, a total of 307 NSCLC patients were detected, 230 cases of formalin fixed paraffin embedding (FFPE) tissues and 77 cases of humoral (including malignant exudate or blood) samples were found. The positive rate of MET in FFPE (11.74%) was higher than that in humoral samples (3.90%). However, the EGFR gene showed the opposite results, and in FFPE the positive rate was 46.09%, while in humoral samples was 62.34% (P<0.05). This may be related to the different depth of FFPE and humoral samples which was 1000× in FFPE, and 2000× in humoral samples. Meanwhile, The positive rate of MET was significantly lower than that of tissue samples. Whether this means that the content of MET genes in ctDNA is lower than that of MET genes in tumor tissues remains to be further studied. Secondly, the effect of different detection methods on the positive rate of gene detection were also compared. In FFPE samples, there were no significant difference in the positive rate of EGFR, ALK and ROS1 detected by RT-PCR and NGS (P>0.05). The positive rate of ALK detected by FISH (10.15%) was significantly higher than that by NGS(3.91%), while the positive rate of MET also showed different (the rate was 2.60% by FISH, and 11.74% by NGS)(P<0.05). Thus, classical methods such as RT-PCR and FISH still play an very important role in the detection of EGFR, ALK and ROS1 hot spot mutation, while MET amplification can improve the detection rate through NGS. Among the 8 gene test results based on the NCCN guidelines, we found that there were 31 cases with two or more mutations, among which EGFR missense mutation and MET amplification co-existed the most frequently (8/31). At the same time, many uncommon mutations were found, such as EGFR - L844V, EGFR - L858Q. EGFR-L747P mutation was also detected, which is resistant to TKI, but ARMS will recognize it as 19del wrongly. Therefore, NGS can help detect more genetic variation and solve the problem of drug resistance in clinical practice. However, the application scope of NGS still needs to be further studied in thr future.

Conclusion: The EGFR gene status of patients with advanced NSCLC can be well reflected by NGS detection of humoral samples, and the detection rate of MET amplification by NGS is higher than that of FISH.

MMP-2 contributes to tumor aggressive phenotypes, invasion and poor outcome in nasopharyngeal carcinoma

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Background: Though matrix metalloproteinase 2 (MMP-2) is well-known about being involvement in tumor aggressiveness and invasion, its prognostic impacts still remain largely controversial. Furthermore, the correlation between MMP-2 and epithelial-mesenchymal transition (EMT) have not been directly established in nasopharyngeal carcinoma (NPC).

Materials and methods: The purpose of this study was to investigate MMP-2 expression in NPC. Tissue microarrays containing 144 patients with NPC and 45 non-cancerous pharynx tissues were analyzed for MMP-2 expression by immunohistochemistry. MMP-2 expression in relation to clinicopathological characteristics and EMT were assessed in NPC. Tumor-invasive potential affected by exogenous expression of MMP-2 in NPC cells was also detected *in vitro*.

Results: Compared to normal nasopharyngeal epithelium, high expression of tumoral MMP-2 was detected in 47.9% of NPC samples, respectively. Significant associations was found between MMP-2 expression and various aggressive features including T classification, M classification and tumor stage ($P < 0.05$). Of note, high expression of MMP-2 was prominently observed at tumor invasive front, neoplastic spindle cells migrating into the stroma and vessel invasion. Importantly, high MMP-2 expression predicted worse survival in patients with stage III-IV ($P = 0.039$). Overexpression of MMP-2 could decrease cell-cell adhesion, promote tumor invasion and EMT including downregulation of E-cadherin and upregulation of N-cadherin, Fibronectin and Slug of NPC cells.

Conclusion: Our findings demonstrate that MMP-2 expression contributes strongly to tumor aggressiveness, EMT and poor prognosis in NPC.

Hes1 promotes the proliferation and cancer stem cell features in nasopharyngeal carcinoma

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Objective Cancer stem cells (CSCs), which has self-renewal and unlimited proliferation potential, is highly resistant to radiotherapy and chemotherapy and is the source of tumor recurrence and metastasis. Up to date, the regulation mechanism about stem cells characteristics of nasopharyngeal carcinoma (NPC) has not been fully understood. Therefore, it is of great significance to find new therapeutic targets for NPC to effectively eliminate CSCs.
Method Immunohistochemistry was used to detect the expression of Hes1 protein in 122 NPC tissues and to analyze the relationship between Hes1 expression and clinicalpathological factors of NPC patients. Cell cloning assay and flow cytometry were used to detect the effect of Hes1 on NPC proliferation in vitro. Side population and tumor sphere formation assay were used to examine the effects of Hes1 on the self-renewal and tumorigenic abilities of NPC. The changes of the expression of Hes1 on CSCs-related proteins were detected by Western blot analysis. Chromatin immunoprecipitation (ChIP) was used to detect whether Hes1 can bind to the EZH2 promoter.
Results The expression of Hes1 was significantly higher in NPC tissues, and it correlated closely with tumor stage, lymph node metastasis, distant metastasis, clinical stage and poor prognosis. Overexpression of Hes1 enhanced tumor proliferation in vitro. Meanwhile, Hes1 promoted the expression of these stemness-related genes in vitro. Moreover, FoxM1 conferred the self-renewal properties of cancer cells by increasing side populations (SP) cells and formed larger and more tumor spheres. On the contrary, down-regulation of Hes1 gene had the opposite results in NPC cell lines. Promoter activity assay showed that the expression of EZH2 was positively regulated by Hes1 in NPC.
Conclusions We demonstrate that Hes1 greatly induces cancer progression and cancer stem cell (CSC) features in NPC, which might be helpful to explore the potential target of CSCs therapy in NPC.

Category: Molecular pathology
1535348

Expression profile of epithelial-mesenchymal transition markers in nasopharyngeal carcinoma

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Objective A majority of nasopharyngeal carcinoma (NPC) patients diagnosed with advanced stages III or IV, will have a poor outcome. Nevertheless, most patients die because of the development of local relapse and metastasis after therapy. However, the precise mechanisms accounting for its high-aggressive behaviors remain largely unclear.

Method 25 cases of NPC tissues and 8 cases of chronic nasopharyngeal inflammation were detected by whole gene expression microarray, and the deferentially expressed genes related to EMT were found. The effects of candidate genes on invasion and proliferation of nasopharyngeal carcinoma cells were detected in vitro.

Result Bioinformatics analysis showed that 50 candidate genes were closely related to EMT, including FOXM1, FN1, MMP2 and POSTN. KEGG pathway analysis showed that these genes were mainly involved in Wnt, p53 and MAPK signaling pathways. Genes closely related to EMT could cluster 25 cases with NPC into two groups: Group A group and Group B. Interestingly, Group B group, which were more enriched NPC patients with in T3 and T3, N2 and M1 stages. The candidate gene FOXM1 was increased in nasopharyngeal carcinoma, especially in neoplastic spindle cells. In vitro experiments showed that FOXM1 promoted the proliferation of NPC cells in vitro and enhanced the invasion of nasopharyngeal carcinoma cells, and induced the EMT-related markers including E-cadherin and FN.

Conclusion EMT-related genes are closely related to the development of NPC and can be used to predict the risks of malignant progression in patients.

Influence of tissue decalcification procedure on immunohistochemistry and molecular targets in bone metastatic carcinomas.

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Biomarker analysis on bone metastatic carcinoma (BMC) is routinely recommended in clinical utilities and establishing a diagnosis of BMC often requires immunohistochemistry (IHC) and nuclear isolation for mutation assay of actionable targets. Decalcification procedures may negatively affect antigenicity and DNA quality. Present study investigated the effects of different decalcification agents on immunoreactivity, DNA quality and mutational profiles on breast cancer (BC), non-small cell lung cancer (NSCLC) and colorectal cancer (CRC). We performed immunohistochemistry, DNA integrity measurement and molecular analysis on non-decalcified tissue routinely processed for diagnostic purposes and in parallel tissue decalcified in hydrochloric acid (HCL) or ethylenediaminetetraacetic acid (EDTA). We found the percentage of immune-positive cells were on average lower in HCL-treated or EDTA-treated cases, compared with controls (without decalcification), and the decrease after HCL exposure was more obvious than that after EDTA treatment. DNA quality and quantity were seriously hampered in HCL-treated cases, compared with controls while DNA integrity in EDTA-treated group remained consistent. Moreover, missing dectection of oncogenic driver mutations such as *EGFR*, *KRAS*, and *PIK3CA* were identified in half HCL-treated cases but only in few EDTA-treated samples. 53.5% (38 out of 71) bone biopsies from advanced NSCLC patients were demonstrated to harbor *EGFR* mutations, while *EGFR* mutations were validated in 55.6% (5 out of 9) bone metastases from paired *EGFR*-mutant NSCLC patients. In brief, we conclude that EDTA-based decalcification could provide better conservation on immunoreactivity and nuclear integrity while EDTA-based decalcification is therefore to be preferred for clinical application.

TumourMutational Burden is Associated with Poor Outcomes which couldn'tbe Predicted by Pan-cancer Targeted Sequencing in Diffused Glioma.

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Purpose: Tumor mutation burden (TMB) is biomarker for immune checkpoint therapy. The impact of TMB on clinical outcomes and correlation coefficient between exosome sequencing and targeted sequencing in glioma has not been explored.

Experimental Design: Clinical and RNA-seq information for 654 patients were available for analyzing the clinicopathological characteristics, enrichment pathways and correlation of immune checkpoint therapy related genes. TMB based onpan-cancer panel was evaluated to compared correlation coefficient with TMB determined by exosome sequencing.

Results: TMB was higher in mutant group of genes which frequently were mutated in glioblastomas (GBM), the TMB was lower in mutant group of genes which frequently were mutated in lower grade gliomas (LGG). Patients with higher TMB had shorter overall survival (OS). TMB was associated with grade, age, subtype and genome structure mutation. The signaling pathways of cell cycle and immune effector process were enriched in TMB^{High} group.

Conclusions: TMB is associated with poor outcomes in diffused glioma. High proliferation activity and immune response in TMB^{High} group could be account for shorter survival.

Multiple origin and tumor heterogeneity of ductal adenocarcinoma of the prostate in the Han Chinese population

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Despite being discovered almost 50 years ago, little is known regarding the genetic profile of ductal adenocarcinoma of the prostate (DAC). In recent years, progress has been made in the understanding of the genetics of acinar adenocarcinomas. DAC is known to present at an advanced stage with a high rate of extraprostatic extension and seminal vesicle invasion, and a decreased interval to biochemical recurrence and the development of metastatic disease when compared with acinar adenocarcinoma. Our aim was to investigate the genetic profile of DAC to determine whether there is a genomic rationale for the aggressive behavior associated with this tumor type. FFPE samples from 11 cases of DAC with paired benign tissue was analyzed. After DNA extraction, copy-number alteration analysis was performed, as well as identification of mutations and indels. We compared the fraction of the DAC genome with copy-number alteration to previous results from primary acinar adenocarcinomas of TCGA. The alteration rate in DAC was comparable to that of acinar adenocarcinoma of high Gleason score. DAC harbored somatic changes seen in advanced and/or metastatic castration-resistant acinar adenocarcinoma, which likely accounts for its aggressive biological behavior.

High expression of EZH2 and NSD2 predict poor prognosis and accelerate tumor progression in triple-negative breast cancer

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Background and purpose Two histone methyltransferases (HMTases), Enhancer of zeste homolog 2 (EZH2) and Nuclear SET domain-containing 2 (NSD2), have been identified as vital genes in carcinogenesis. However, the relationship between EZH2 and NSD2 in breast cancer remains unclear, and the clinical significances and prognostic values of them in breast cancer have not been fully elucidated. In the present study, we aimed to investigate the expression and roles of EZH2 and NSD2 in breast cancer.
Methods The tissue microarrays (TMA) containing 146 cases of breast cancer and 24 cases of benign lesion were constructed and immunehistochemistry (IHC) was used to examine the expression of EZH2 and NSD2 in TMA sections. The differential mRNA expression of EZH2 and NSD2 in breast cancer and normal breast tissues were evaluated using the gene expression data from the Oncomine database and the UALCAN database. The correlation between their expression and clinicopathological characteristics were analyzed. The Kaplan-Meier Plotter database was utilized to assess the prognostic values of EZH2 and NSD2 in the relapse-free survival (RFS), overall survival (OS), distant metastasis-free survival (DMFS) and postprogression survival (PPS) of 3951 patients with breast cancer. Subsequently, we performed knockdown or overexpression of EZH2 in a triple-negative breast cancer (TNBC) cell line using lentiviral EZH2-shRNA vector or adenovirus EZH2-overexpressing vector, respectively. The effects of EZH2 on TNBC cell proliferation, migration and invasion were determined by CCK8, Wound healing and Transwell assays. EZH2, NSD2 and histone methylation marks, such as H3K27me3 and H3K36me2, were detected by qRT-PCR and Western blot. Furthermore, knockdown of NSD2 was performed in EZH2-overexpressing cells to investigate whether the oncogenic effects of EZH2 depended on NSD2 expression.
Results Both the protein and mRNA expression of EZH2 and NSD2 were significantly upregulated in breast cancer tissues compared to breast benign lesions or normal tissues. EZH2 expression was highly correlated with NSD2 expression in breast cancer. Notably, EZH2 and NSD2 expression were coordinately higher in TNBC than that in luminal A, luminal B and HER2-enriched breast cancer. Both high expression of EZH2 and NSD2 were significantly correlated with pathological grade of tumor and lymph node metastasis. Kaplan-Meier survival analysis showed that EZH2 expression was negatively correlated with RFS, OS, DMFS and PPS in patients with breast cancer, but NSD2 expression was only negatively correlated with RFS and DMFS. Knockdown of EZH2 in MDA-MB-231 cells inhibited cell proliferation, migration and invasion ability and downregulated NSD2 expression. Knockdown of EZH2 also reduced the levels of H3K27me3 and H3K36me2, which are histone

methylation marks catalyzed by EZH2 and NSD2, respectively. By contrast, overexpression of EZH2 displayed an inverse phenotype. In addition, knockdown of NSD2 in EZH2-overexpressing cells could dramatically attenuated EZH2-mediated oncogenic effects.

Conclusion EZH2 and NSD2 were coordinately overexpressed in breast cancer, particularly in TNBC. High expression of EZH2 and NSD2 predicted poor clinical prognosis in patients with breast cancer. EZH2 could promote TNBC cell proliferation and metastasis by upregulating NSD2 expression. EZH2 and NSD2 may be novel therapeutic targets for patients with TNBC.

PBRM1 mutation in colorectal cancer cells regulates chromosomal stability and promotes anti-PD1 therapy

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Objectives: Colorectal cancer (CRC) is one of the malignant tumors that poses a great threat to human survival and health. Immunotherapy has become an important part of clinical treatment of CRC following surgery, radiotherapy and chemotherapy. However, the main predictive target —microsatellite instability (MSI) is with low positive detection rate and insufficient benefit currently. It is urgent to seek new and accurate immunotherapy prediction targets. The chromosomal remodeling complex protein, PBRM1, encodes the protein BAF180, which is involved in the component coding of the chromatin remodeling complex-SWI/SNF, enabling specific genes to be read, activated and transcribed by opening tightly entangled DNA. It has been reported that in patients with melanoma and renal clear cell carcinoma, individuals with somatic PBRM1 mutations are more sensitive to anti-PD1 therapy, but their role in immunotherapy of colorectal cancer remains unknown. This study focused on PBRM1 and explored its mechanism of action on the sensitivity of colorectal cancer microenvironment and immunotherapy.

Methods: 1. Bioinformatics and immunohistochemistry were used to analyze the relationship between the expression of PBRM1 in CRC tissues and the sensitivity of immunotherapy; 2. Introduction of humanized mouse and immune cell knockout mouse models, CRISPR-Cas9 gene editing, flow cytometry and immunofluorescence was used to explore the chemotaxis and activation of immune cells such as CD8+ T cells/NK cells induced by somatic mutation; 3. Simulating the tumor microenvironment during PBRM1 mutation, screen for the inflammatory factor that activates immune cells.

Results: Bioinformatics' data analysis showed poor expression of PBRM1 in a variety of tumors including colorectal cancer, and the somatic mutation is proportional to the number of infiltration of tumor infiltrating lymphocytes such as CD8+ T cells, neutrophils and dendritic cells. In *vitro*, plate clones and CCK8 results showed that PBRM1 mutation alone did not affect the proliferation ability of tumor cells. Meanwhile target cell killing experiments showed that the killing ability is significantly enhanced when PBMCs were co-cultured with HCT116-gPbrm1-1 mutants compared with the control group. In *vivo*, mouse subcutaneous tumors were constructed by implanting CT26-NC cells and CT26-gPbrm1-1 mutant strains. Compared with the control group, the tumor volume of the mutant mice did not change significantly, but after 14 days of PD-1 antibody treatment (100ug/bw/ip) was given, the tumor volume of the mutant group was significantly reduced. The HE staining results showed that the number of immune cells in the tumor stroma was significantly increased in the mutant group. Flow cytometry showed that the spleen and tumor infiltrating lymphocytes of mice after 14 days of tumor implantation showed that CD8+T and NK cells were significantly increased in tumor

infiltrating lymphocytes and spleens in the mutant group compared with the control group. Similar results were obtained by immunofluorescence detection of mouse tumor tissues.

Conclusion: PBRM1 exhibits deletion mutation in colorectal cancer, which promotes CRC anti-PD1 treatment by chemotactic microenvironment immune cells such as CD8+ T cells and NK cell activation. In the following, we will analyze the tumor burden and chromosomal stability of CRC cells in PBRM1 mutation by whole exon sequencing and NGS sequencing, and explain the specific molecular mechanism of induction of immune cell activation and promotion of PD1 treatment sensitivity and provide scientific basis for the application of immunotherapy for clinical colorectal cancer.

Genome-wide DNA methylation profiling identifies epigenetic signatures of gastric cardiac intestinal metaplasia

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Objective Measuring the DNA methylome may offer the opportunity to identify novel disease biomarkers and insights into disease mechanisms. Although aberrant DNA methylation has been investigated in many human cancers and precancerous lesions, the DNA methylation landscape of gastric cardiac intestinal metaplasia (IM) remains unknown. Therefore, we aimed to investigate the genome-wide DNA methylation landscape and to search for potential epigenetic biomarkers of gastric cardiac IM.

Design Histopathologic profiling was performed in a total of 118 gastric cardiac biopsies from cancer-free individuals. Genome-wide DNA methylation analysis was performed in 11 gastric cardiac mucosal biopsies (IM = 7; normal = 4) using Illumina 850K microarray. Transcriptional relevance of the candidate epigenetic biomarker was validated by qRT-PCR.

Results The point prevalence of gastric cardiac IM was 23% (27/118) in cancer-free individuals. Genome-wide DNA methylation profiling showed a global decreased methylation in IM compared with normal tissues (median methylation = 0.64 and 0.70 for gastric cardiac IM and normal tissues, respectively). Differential methylation analysis between gastric cardiac IM and normal tissues identified 38,237 differentially methylated probes (DMPs) with a majority of sites showing hypermethylation in IM compared with normal tissues (56.3% vs. 43.7%). Subsequent analysis revealed a significant enrichment of hypermethylated DMPs in promoter and CpG islands ($p < 0.001$ for both, Pearson x^2 test). For DMPs located in promoter CpG islands showing extreme hypermethylation, the candidate gene with the largest number of DMPs ($n = 7$) was mapped to *HOXA5*. Accordingly, mRNA expression of HOXA5 was significantly reduced in IM compared to normal tissue.

Conclusion Our results suggest the implication of DNA methylation alterations in gastric cardiac IM and highlight *HOXA5* hypermethylation as a promising epigenetic biomarker, emphasizing the role of aberrant HOXA5 expression in the pathogenesis of gastric cardiac IM.

A novel DNA methylation-based signature can predict the responses of MGMT promoter unmethylated glioblastomas to temozolomide

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Obejectives: Glioblastoma (GBM) is the most malignant glioma, with a median overall survival (OS) of 14-16 months. Temozolomide (TMZ) is the first-line chemotherapy drug for glioma, but whether TMZ should be withheld from patients with GBMs that lack O6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation is still under debate. DNA methylation profiling holds great promise for further stratifying the responses of *MGMT* promoter unmethylated GBMs to TMZ.

Methods: In this study, we studied 147 TMZ-treated *MGMT* promoter unmethylated GBM, whose methylation information was obtained from the HumanMethylation27 (HM-27K) BeadChips (n=107) and the HumanMethylation450 (HM-450K) BeadChips (n=40) for training and validation, respectively. The least absolute shrinkage and selection operator (LASSO) Cox regression algorithm was conducted to develop a risk-signature. The Kaplan–Meier curves were used to study the prognostic value of the risk-signature.

Reuslts: In the training set, we performed univariate Cox regression and identified that 3565 CpGs were significantly associated with the OS of the TMZ-treated *MGMT* promoter unmethylated GBMs. Functional analysis indicated that the genes corresponding to these CpGs were enriched in the biological processes or pathways of mitochondrial translation, cell cycle and DNA repair. Based on these CpGs, we developed a 31-CpGs methylation signature utilizing the least absolute shrinkage and selection operator (LASSO) Cox regression algorithm. In both training and validation datasets, the signature identified the TMZ-sensitive GBMs in the *MGMT* promoter unmethylated GBMs, and only the patients in the low-risk group appear to benefit from the TMZ treatment. Furthermore, these identified TMZ-sensitive *MGMT* promoter unmethylated GBMs have a similar OS when compared with the *MGMT* promoter methylated GBMs after TMZ treatment in both two datasets. Multivariate Cox regression demonstrated the independent prognostic value of the signature in TMZ-treated *MGMT* promoter unmethylated GBMs. Moreover, we also noticed that the hallmark of epithelial-mesenchymal transition, ECM related biological processes and pathways were highly enriched in the MGMT unmethylated GBMs with the high-risk score, indicating that enhanced ECM activities could be involved in the TMZ-resistance of GBM.

Conclusion: Our findings promote our understanding of the roles of DNA methylation in *MGMT* umethylated GBMs and offer a very promising TMZ-sensitivity predictive signature for these GBMs that could be tested prospectively.

Category: Molecular pathology
1648460

Expression of RSK4, CD44 and MMP-9 in Metastasis Renal Cell Carcinoma and its Relationship to Prognosis

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Abstract:

Purpose – To investigate the relationship between the expression of RSK4, MMP-9 and CD44 in primary renal cell carcinoma (pRCC) and metastasis renal cell carcinoma (mRCC), as well as the clinicopathological features of patient.

Method– The expression of RSK4, CD44 and MMP-9 in 52 pRCC samples and 48 mRCC samples were detected by immunohistochemistry, and the relationship between RSK4, CD44 and MMP-9 expression and clinicopathological features as well as prognosis of mRCC patients was statistically analysed. Ectopic RSK4 expression in RCC cell lines was performed to determine its effect on cell cycle regulation, tumour invasiveness, and metastatic capability.

Results – The positive expression rates of RSK4, MMP-9 and CD44 in mRCC are 75%, 68.75% and 91.7%, respectively, while the rates in pRCC tissues are 44.2%, 34.6% and 69.2%. Thus, the positive rates in mRCC were higher than those in pRCC (P_{RSK4} = 0.002; P_{MMP-9} = 0.002; P_{CD44} = 0.001). However, the expression of RSK4, MMP-9 and CD44 is unrelated with ages, genders, Fuhrman grades or metastatic sites ($P>0.05$). In mRCC, expression among the three proteins showed positive correlation ($P=0.008$). Moreover, expression between RSK4 and CD44($P=0.019$), MMP-9 and CD44($P = 0.05$) also showed positive correlations, whereas RSK4 and MMP-9 showed no significant correlation ($P=1.00$).

Conclusions – The over-expression of RSK4, MMP-9 and CD44 is associated with the invasion and metastasis of RCC, indicating that they could be potential prognostic factors and serve as new potential therapeutic targets for RCC patients.

Hepatocyte-Specific Deletion of Mettl14 Promotes Non-alcoholic Steatohepatitis in Mice

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Abstract

N^6-Methyladenosine (m^6A) is the most prevalent internal modification of mRNAs in eukaryotes. Abnormal m^6A is closely related to the occurrence and development of many human diseases. Mettl14, a m^6A methyltransferases, is involved in the installation of m^6A, but there are few studies about Mettl14 at present. **Objectives:** To demonstrate the effects and mechanism of Mettl14 gene in nonalcoholic steatohepatitis (NASH) in mice. **Materials and Methods:** Cultivating liver-specific Mettl14 knockout mice, inducing NASH disease model in wild-type (WT) and knockout (KO) mice by feeding with methionine-choline-deficient (MCD) diet, comparing hepatic outcomes after 6 weeks of MCD feeding combined with histology, serum chemistries, quantitation of hepatic triglyceride and cholesterol, Western Blot and quantitative PCR. **Result:** Hepatocyte-specific knockout of Mettl14 had no significant effect on the development, structure, function and glucose and lipid metabolism of liver in mice. The degree of liver injury was comparable between the two groups, based on histologic, and serum ALT, AST. Liver/weight ratio, serum triglyceride (TG) and cholesterol (CHOL), liver triglyceride (TG) and cholesterol (CHOL), serum inflammatory cytokines (IL-6, IL-1β, TNFα) were higher than those in WT mice. Oil red O and HE staining showed that hepatic steatosis and inflammatory cell infiltration were more severe in KO mice than WT mice. It was consistent with greater hepatic induction of inflammatory (IL-6, IL-1β, TNFα) and lipogenic genes (Acc1, Fasn, Dagt2, Scd1) in KO mice. **Conclusion:** Hepatocyte-specific deletion of Mettl14 promotes non-alcoholic steatohepatitis in mice by promoting inflammation and fat synthesis.

Category: Molecular pathology
1649376

Microbiological evidence for the development of cardia inflammation caused by microflora dysbiosis in population with high incidence of gastric cardia cancer

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Abstract

Objective: Gastric cardia cancer (GCC) is one subtype of gastric cancer (GC), and has high incidence in Chaoshan district, the southeastern inshore of China. Nowadays, few studies focus on the relationship between cardia microbial infection, inflammation and GCC. This study aims to detect the microbial community in cardia and find inflammation-related microorganisms for preventing GCC.

Materials and Methods: 11 healthy and 89 individuals with different degree of cardia inflammation were recruited and performed by 16S rDNA sequencing for identifying the inflammation-related microbiota. Then, the method of immunohistochemical staining, culturing and sequencing were used for verifying the bacteria. PICRUSt was used for predicting the pathways involved by cardia microflora.

Result: We found that the composition and structure of cardia microflora changed in cardia inflammation individuals ($P<0.05$). We detected 12 inflammation-related species ($P<0.05$), especially *H. pylori*, *Acinetobacter ursingii* and *Streptococcus agalactiae*. There were 64 species parallel increased with cardia inflammation, included *H. pylori, Lactobacillus. Spp.* Among these bacteria, we found the colonization density of *H. pylori* positively correlated with cardia inflammation (*gamma coefficient*=0.678, $P<0.001$). These *H. pylori* strains all were high virulence with CagA+, EPIYA -A-B-D-type motifs. The microflora of cardia participated in DNA repair ($P<0.01$), and this pathway affected by the relative abundance of *H. pylori* ($P<0.0001$).

Conclusion: In conclusion, cardia microflora varied with cardia inflammation, and overgrowth of *H. pylori* may be an important reason for the high incidence of GCC in Chaoshan district. This microbiological evidence provides a rationale for the use of antimicrobials in the prevention of GCC.

Category: Molecular pathology
1649487

HOXB7 activates Wnt/β-Catenin signaling and promotes metastasis in colorectal cancer

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Objectives

Metastasis is the leading cause of death in colorectal cancer and the abnormal activation of Wnt/β-Catenin pathway plays an essential role in metastasis. Our previous study revealed the overexpression of HOXB7 predicts poor survival and promotes tumor growth and metastasis in CRC, but the underlying mechanism has not yet to be excavated.

Materials and Methods

Affinity purification and mass spectrometry (MS) were used to identify HOXB7-interacting proteins in SW480 CRC cells. The interaction between b-Catenin and HOXB7 was further confirmed by immune-precipitation (IP) analysis. Chromatin immunoprecipitation (ChIP) and Luciferase reporter assay were applied to analyze the interaction between HOXB7 and the promoter loci of downstream target genes. Migration assays, Sphere formation assays, Side population cells analysis, and Flow cytometry analysis were used to examine the effects of HOXB7 on cell migration and stem cell properties. Orthotopic mouse metastatic model and Irinotecan treatment experiment were performed to investigate the effect of HOXB7 on metastatic colonization in *vivo*. IHC was performed to investigate the clinical relevance of HOXB7 and Wnt/β-Catenin pathway.

Result

Ingenuity Pathway Analysis demonstrated a significant modulation of HOXB7 on WNT/β-Catenin pathway. Overexpression of HOXB7 significantly increased, while knocking down of HOXB7 decreased the levels of nuclear β-Catenin. Mass spectrometry analyses showed that β-Catenin was one of the potential HOXB7-interacting proteins. To further investigate the physical association between HOXB7 and β-Catenin, we constructed HOXB7 truncations and found that the homeobox domain of HOXB7 interacted with β-Catenin. In addition, HOXB7 could only interact with and the β-Catenin-TCF/LEF complex in the nuclear, while could not interact with the destruction complex in the cytoplasm. ChIP assays confirmed that HOXB7, β-Catenin, and TCF4 could respectively interact with the WRE region of LEF-1 promoter in SW480 cells. In addition, HOXB7 interacted with the LEF-1 promoter much less efficiently in HOXB7 or β-Catenin-knocking down cells, indicating that the association was specific.

Given that HOXB7 stimulates WNT signaling, we speculated that HOXB7 may also involve in the regulation of EMT and stem cell properties in CRC cells. Expectedly, overexpression of HOXB7 decreased the expression of E-cadherin and α-Catenin but increased the expression of vimentin, N-Cadherin, and Snail, while knockdown of HOXB7 increased the expression of E-cadherin and α-Catenin but decreased the expression of

Vimentin, N-cadherin and Snail. In addition, overexpression of HOXB7 strongly enhanced, but knockdown of HOXB7 reduced the invasive and migratory ability of CRC cells as analyzed by Transwell migration and invasion assays. Moreover, overexpression of HOXB7 led to a dramatic upregulation of stem cell-related markers, including CD133, CD44, OCT-4, SOX-2, and Bmi-1. Conversely, knockdown of HOXB7 significantly decreased the expression of CD133, CD44, OCT-4, SOX-2, and Bmi-1. Taken together, these results indicated that HOXB7 plays an important role in promoting EMT, stem cell sphere formation and stem cell-like phenotype in CRC.

To investigate the effect of HOXB7 on metastatic colonization in *vivo*, cells overexpressed or silenced with HOXB7 were orthotopically injected into the cecum of 6-week-old nude mice. Mice injected with overexpression of HOXB7 significantly increased the growth of primary tumors, metastasis along with intestine, liver and lung metastases, as compared with control cells. In addition, mice with HOXB7-overexpressed tumors have poorer survival than mice with control tumors. Conversely, knockdown of HOXB7 dramatically inhibited the growth of primary tumors, metastasis along the intestine, liver metastases and lung metastases. Knockdown of HOXB7 significantly increased the survival time of mice with tumors. IHC staining showed that overexpression of HOXB7 increased, while knockdown of HOXB7 decreased the nuclear localization of β-Catenin as well as the expression of downstream targets Cyclin D1 and CD44, indicating that HOXB7 activated Wnt/β-Catenin pathway in vivo.

Tumor sphere formation assays were performed to examine the effect of HOXB7 on self-renewal of spherogenic CRC cells. Strikingly, HOXB7-transduced cells formed approximately 2.5-fold more spheres compared with the spheres formed by control cells. Conversely, HOXB7-silenced cells formed fewer spheres compared with control cells. It has been reported that the side population (SP) is a subpopulation of cells that may exhibit stem cell-like features.

Tumors with stem cell-like properties are hypothesized to be a major reason for resistance to chemotherapy. Since HOXB7 was able to activate Wnt/β-Catenin and increase the stemness of CRC cells, we reason that overexpression of HOXB7 might contribute to the resistance to chemotherapy in CRC cells. Irinotecan, one of the most popularly used drugs for first-line therapy in metastatic CRC. Irinotecan treatment reduced the expression of β-Catenin, stemness markers CD133, c-Myc, Bmi-1, CD44, and cell proliferation marker Cyclin D1. In addition, irinotecan treatment decreased cell proliferation, tumorsphere formation, as well as the proportion of CD44+CD133+ cells. Moreover, irinotecan treatment inhibited tumor growth in nude mice. However, overexpression of HOXB7 abolished the effect of irinotecan on these effects. These data demonstrated that overexpression of HOXB7 promotes the resistance to chemotherapy in CRC cells.

To investigate whether the regulation of Wnt/β-Catenin pathway by HOXB7 could be reflected clinically, IHC was performed using anti-HOXB7 and anti-β-Catenin antibodies in 40 clinical CRC samples. Statistical analysis revealed that the expression of HOXB7 was positively associated with nuclear expression levels of β-Catenin. In addition, high expression of HOXB7 was closely associated with high expression of nuclear β-Catenin in the lymph node metastasis loci. Moreover, Gene Set Enrichment Analysis (GSEA) of the CRC datasets revealed that β-Catenin phosphorylation cascade and signaling by Wnt were among the top activated pathways in HOXB7-high CRC. Collectively, our data reveal that HOXB7 expression is positively correlated with

Wnt/b-Catenin pathway in CRC.

Conclusion

In this study, we demonstrated a novel role of HOXB7 in activating Wnt/β-Catenin signaling in CRC by directly interacted with β-Catenin in the nuclear and forming a complex with LEF1/TCF4 transcriptional factors. As a result, as a result of Wnt/β-Catenin activation, HOXB7 promoted EMT and increased the propagation of stem cells population in CRC, which in turn promoted invasion, metastasis, as well resistance to chemotherapy in CRC cells.

Epstein-Barr Virus microRNA BART2-5p promotes metastasis of nasopharyngeal carcinoma via suppressing RND3

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Objectives: Nasopharyngeal carcinoma (NPC) is an EBV related malignancy which is highly prevalent in South China. Recently, we found an EBV encoded microRNA *BART2-5p* increased in preclinical patients' circulation and the copy number of which was positively correlated with disease progression. However, its biological role remains unknown. In this study, we aimed to identify its exact role in NPC progression, explore underlying mechanisms and clinical significance.
Materials and Methods: We firstly examined clinical significance of *BART2-5p* by survival analysis and multivariate analysis. Next, a series of *in vitro* and *in vivo* assays were performed to investigate functions of this microRNA and underlying mechanisms. Furthermore, clinical correlation of this microRNA and its target was validated in patients from our institute.
Results: We demonstrated that *BART2-5p* was an independent unfavorable prognostic factor for progression free survival and its circulating abundance was positively associated with distant metastasis. Further investigation showed ectopic expression of *BART2-5p* could promote EBV (-) NPC cells migration and invasion, whereas knock down of *BART2-5p* in EBV (+) NPC cells showed undermined aggressiveness. Mechanistically, RND3, a negative regulator of Rho signaling, was predicted as the target of *BART2-5p* by bioinformatic analysis and validated by luciferase reporter and RIP assay. Knock down of RND3 could phenocopy the effect of *BART2-5p* and reconstitution of RND3 rescued the phenotype. By suppressing RND3, *BART2-5p* activated Rho signaling to enhance cell motility.
Conclusion: Our findings suggested a novel role of EBV-*BART2-5p* in promoting NPC metastasis and its potential value as a prognostic indicator or therapeutic target.

Transcriptional downregulation of AJAP1 by Zeb1 promotes the epithelial-mesenchymal-transition through upregulation of miR-3941 in breast cancer

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Background: Our previous study identified that adherent junctions associated protein 1 (AJAP1) was downregulated in breast cancer and prohibited malignant activity of breast cancer both *in vitro* and *in vivo*. However, the biological role of AJAP1 in epithelial-mesenchymal-transition and molecular mechanism are still unclear.
Methods: Western blot, immunofluorescence, RT-PCR and bright-filed microscope were utilized to observe the effect of downregulated and upregulated AJAP1 expression in MCF-7 and MDA-MB-231 cells. Then the effects of AJAP1 on TGF-β1 induced EMT were also explored by Western blot, RT-PCR, Transwell and wound- healing assays. Specific targeting binds between miRNAs and AJAP1 were determined through software prediction, luciferase reporter assay. The functions of miR-3941 on AJAP1 and breast cancer were accessed using gain- and loss-of-function approaches. Besides, chromatin immunoprecipitation (ChIP) and dual-luciferase reporter assays were used to further validate the regulation mechanism of AJAP1—zinc-finger E-box binding homeobox1(ZEB1)—miR-3941 axis in EMT process.
Results: Here, we demonstrated that AJAP1 depletion can promote the EMT occurrence of breast cancer and AJAP1 can also suppress TGF-β1 induced EMT and migration and invasion in breast cancer. Besides, miR-3941 directly regulated AJAP1 expression by binding its 3'-UTR and miR-3941 was proved to be related with poor prognosis in patients with breast cancer. In our study, we also identified that miR-3941 enhanced the ability of cells' invasion, proliferation and colony-formation. Further, miR-3941 upregulation increased Zeb1 expression but Zeb1 acted as a transcription repressor by targeting the E-box elements in the promoter region of AJAP1 gene, which process can obviously promote the malignant behavior of breast cancer.
Conclusions: Collectively, our research uncovered a new role of miR-3941 in breast cancer progression and miR-3941/ZEB1/AJAP1/TGF-β1 axis participated in EMT of breast cancer, which might become a feasible therapeutic option of breast cancer.

suppression of Numb promotes hepatocellular carcinoma through abnormal proliferation of stem cells

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Hepatocellular carcinoma is one of the most common malignant tumors. There is a kind of cancer stem cell (CSC) with infinite proliferation, self-renewal, invasive phenotype and high tumorigenicity in liver cancer, and invasion and metastasis, radiotherapy and chemotherapy. Currently, hepatic progenitor cells (HPC) are considered to be the main source of CSC in the liver. NUMB, as a key node regulating HPC differentiation, determines the differentiation direction of HPC by regulating Notch signal during normal proliferation of HPC. However, the role of Numb in the abnormal proliferation of HPC and even malignant lesions is rarely reported. METHODS: We used the Alb-Cre/Loxp system to establish hepatocyte-specific Numb knockout mice, and constructed a 70% hepatic cut model of the liver and fed 1,4-dihydro-2,4,6-three.
3,5-diethoxycarbonyl-1,4-dihydro-collidine (DDC) induces HPC abnormal proliferation model after hepatocyte injury to explore Numb in liver injury and malignant lesions The role. RESULTS: Hepatocyte-specific knockout of Numb gene had no significant effect on liver development, structure, function and glucose and lipid metabolism in mice. After 70% hepatectomy, there was no significant difference in hepatocyte proliferation compared with wild-type (WT) mice, which did not spontaneously form liver cancer. However, in the DDC model, liver Numb-specific deletion mice were more active than HPC proliferation in WT mice, and proliferating HPC spread from the portal area to the hepatic lobules. Interestingly, Numb deletion did not alter the expression of HPC markers (CK19, OV6, CD133, SOX9, HNF1b), indicating that HPC remains in a poorly differentiated state. After 10 months of DDC feeding, WT mice did not develop liver cancer, but 3 of the 9 liver-specific Numb-/- mice developed liver cancer, indicating that inhibition of Numb expression in the liver may lead to the formation of liver cancer. It was further found that Numb deletion significantly inhibited the formation of P53-HDM2-Numb complex, promoted HDM2-mediated P53 ubiquitination, and finally inhibited P53-dependent apoptosis, leading to the occurrence of liver cancer. Conclusion: When the liver is specifically deficient in Numb, when the liver is damaged, it will stimulate the massive activation and proliferation of HPC, and inhibit the apoptosis by enhancing the ubiquitination and degradation of P53, which may transform HPC into CSC, thereby promoting The occurrence of liver cancer.

The up-regulation of SOX9 by extracellular ATP contributes to breast cancer Invasion and chemoresistance

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Objectives

Our previous research demonstrated extracellular adenosine 5' -triphosphate (ATP) could promote breast cancer cell invasion and chemoresistance. However, mechanism remains unclear. Here we aimed to determine indicated molecules or signaling involved.

Materials and Methods

cDNA microarray and RNA-sequencing were performed to identify the differentially expressed genes. *In vitro* invasion and migration assays, would-healing assays, cell apoptosis assays, WST-1 assays, soft agar assays and trypan blue dye exclusion assays were applied to examine the role of sex-determining region Y-box 9 (SOX9) in breast cancer cell *in vitro*. Silver staining and mass spectrometry were carried to elucidate SOX9 partner proteins. qRT-PCR, western blotting and chromatin immunoprecipitation (ChIP) assays were utilized to determine indicated molecules involved in ATP-SOX9 signaling. Balb/c nude mice were used to illustrate tumor growth and metastasis *in vivo*.

Result

Based on cDNA microarray, SOX9 was elevated in ATP treatment in breast cancer cells. Knocking down SOX9 attenuated ATP-driven invasion abilities. SOX9 interacted with Janus kinase 1 (JAK1), which could also able to mediate ATP-driven SOX9 upregulation. Indeed, the whole IL-6-JAK1-STAT3 signaling could promote SOX9 expression and invasion in ATP treatment. Also, ATP-IL-6-SOX9 signaling played a vital role in breast cancer chemoresistance. SOX9 provoked amount of target genes transcription, among which carcinoembryonic antigen-related cell adhesion molecule 5/6 (CEACAM5/6) mediates ATP pro-invasive function, and ATP binding cassette subfamily B member 1 (ABCB1), ATP binding cassette subfamily G member 2 (ABCG2) mediates ATP-driven chemoresistance. In addition, SOX9-overexpressed MCF-7 cells injected into nude mice exhibited increased tumor growth and metastasis *in vivo*. And SOX9-knockout MDA-MB-231 cells injected into nude mice exhibited decreased metastasis ability and increased drug sensitivity. Moreover, molecules involved in ATP-SOX9 signaling were upregulated in human breast carcinoma specimens and were associated with poor prognosis.

Conclusion

Altogether, SOX9 is vital in ATP-driven invasion and chemoresistance progresses, which may serve as a potential target for breast cancer therapies.

Category: Molecular pathology
1652122

curcumin affect gastric cancer cell migration, invasion and cytoskeletal remodeling through Gli1- β-catenin

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The SonicHedgehog (Shh) and Wnt signaling pathway play an important role in embryonic development, adult tissue maintenance and oncogenesis. Gli1 and β-catenin, as important transcription factors in Shh and Wnt signaling pathways, their effect on gastric cancer cell line SGC-7901 is not clear. Whether curcumin can inhibit gastric cancer cells by inhibiting Gli1 and β-catenin is unclear. **OBJECTIVE** To investigate curcumin affects the biological behavior of gastric cancer SGC-7901 cells through Gli1-β-catenin. **METHODS** The curcumin effects on cell proliferation were observed by CCK-8 assay. The IC50 of curcumin was calculated for subsequent experiments. Curcumin inhibits Shh and Wnt signaling pathways by western blotting and qPCR. The invasion and migration ability of SGC-7901 cells were verified by transwell assays. The apoptosis rate and cell cycle arrest of SGC-7901 cells were detected by flow cytometry. Molecular changes of gastric cancer cells during epithelial mesenchymal transformation (EMT) were determined by western blotting. The cytoskeleton changes of SGC-7901 cells on Gli1 or β-catenin knockdown and curcumin stimulation were observed by Immunofluorescence assay. Co-immunoprecipitation assays were performed to investigate whether Gli1 and β-catenin interact in SGC-7901 cells. **RESULTS** ① Curcumin inhibits the proliferation of SGC-7901 cells and Shh and Wnt signaling pathways. ② Inhibition of Shh and Wnt signaling pathways and curcumin stimulation affect the migration and invasion of SGC-7901 cells. ③ Inhibition of Shh and Wnt signaling pathways and curcumin stimulation treatment regulate apoptosis and cell cycle arrest of SGC-7901 cells. ④ Inhibition of Shh and Wnt signaling pathways and curcumin stimulation affect the EMT process and cytoskeletal remodeling in SGC-7901 cells. ⑤ Gli1 interacts with β-catenin in SGC-7901 cells. **CONCLUSION**: This study demonstrates that curcumin affects the biological progression of gastric cancer cell line SGC-7901 by affecting Gli1-β-catenin and confirms the presence of Gli1 and β-catenin interactions in SGC-7901 cells, Which suggest that there is a crosstalk between Shh and Wnt signaling pathways. Taken together, this study provides novel insights into gastric cancer tumorigenesis and associated potential treatments.

Adenovirus KGHV500 containing anti-p21Ras scFv gene delivered by CIK cells for the treatment therapy of glioma

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Objectives Gene therapy delivered by an adenovirus that is a promising trend in the treatment of gliomas. Cell-based drug carrier system could solve the problem of systemic delivery that clearance and decrease the level of circulating particles by neutralizing antibodies. In this study, we use CIK cells as a secondary carrier to delivery adenovirus KGHV500 containing the anti-p21Ras scFv gene to test the anti-tumor effect of glioma cell line and U251 xenograft. **Materials and Methods** Human glioma U251 cells lines were infected with adenovirus KGHV500. In vitro, the anti-tumor ability was determined MTT assay, TUNEL, wound healing assay, transwell invasion assay. In vivo, CIK cells as a carrier to deliver adenovirus KGHV500 to U251 glioma by intravenous injection. Tumor volume, cell apoptosis, p21Ras scFv expression were tested to evaluate targeting ability and safety. **Result** Our study demonstrated that adenovirus KGHV500 turned up successfully anti-tumor ability to U251 glioma as compared with the control groups in vitro. Moreover, CIK cells combined adenovirus KGHV500 induce higher antitumor activity against glioma xenografts in vivo than that produced by KGHV500 alone. The anti-p21Ras scFv were observed in tumor tissue and showed relatively safety. **Conclusion** Our data suggest that adenovirus KGHV500 and CIK cells as a carrier deliver anti-p21 Ras scFv has the potential to be useful treatment against Ras-involved cancer.

Developing sensitive detection of IDH1 and TERT Promoter mutations with Droplet Digital PCR in diffuse gliomas

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PURPOSE: Mutations in isocitrate dehydrogenase (*IDH*) and telomerase reverse transcriptase promoter (*TERT*p) exert a far-reaching influence on clinical pathological diagnosis and prognosis of glioma. Traditional approaches, such as sanger sequencing and ARMS, lack the sensitivity due to tumor heterogeneity and low tumor purity of glioma samples. Therefore, we propose a highly sensitive detection method of *IDH1* and *TERTp* mutations based on ddPCR technology, named IDH1-TERT-mutation ddPCR (IT-ddPCR).
EXPERIMENTAL DESIGN: We determined the *IDH1* and *TERTp* mutations of 80 patients by sanger sequencing, ARMS and IT-ddPCR in parallel. Meanwhile, we detected the *TERTp* mutations of 8 patients with probes by IT-ddPCR and Bio-Rad.
RESULTS: *IDH1*-positive singles were detected in 43 cases by sanger sequencing, and in 44 and 56 cases by ARMS and IT-ddPCR, respectively. *TERT*p-positive singles were detected in 44 cases by sanger sequencing, and in 44 and 50 cases by ARMS and IT-ddPCR, respectively. There was a slight difference in the total events, occupancy events and C228T/C250T droplets between these two probes of IT-ddPCR and Bio-Rad. Regression analysis of the *TERTp* variant frequencies detected by two different probes produced a slope of 1.0425 and a coefficient (R^2) of 0.9231.
CONCLUSIONS: We found that IT-ddPCR showed a higher sensitivity compared with sanger sequencing and ARMS in the detection of *IDH1* and *TERTp* mutations. Meanwhile, there are no significant differences in variant frequencies of *TERTp* mutations between the two probes of IT-ddPCR and Bio-Rad. The IT-ddPCR can be used to detect the low mutation frequency of *IDH1* and *TERTp* in glioma patients.

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Category: Molecular pathology
1652627

Application of the long noncoding RNA LINC01614 as a diagnostic and prognostic biomarker in human cancers

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Abstract

Background: Recently, several studies have found that the long intergenic non-protein coding RNA 1614 (LINC01614) is highly expressed and may be acted as an oncogene and biomarker in several malignant tumors. However, until now studies on LINC01614 was rare, and the role of LINC01614 in malignant tumors remains unclear.

Materials and methods: We performed a comprehensive analysis, including data mining, receiver operating characteristic (ROC) curve analysis, survival analysis, meta-analyses and bioinformatics analyses, to evaluate the role of LINC01614 in human cancers through data collected from Gene Expression Omnibus (GEO) and the Cancer Genome Atlas (TCGA) databases.

Results: A total of 42 datasets involving 8292 patients were included in this comprehensive analysis. LINC01614 expression was validated to be up-regulated in various malignant tumors. The results of ROC curve analysis and summary receiver operating characteristic (SROC) curve analysis showed that LINC01614 was a potential diagnostic predictor in malignancies (pooled sensitivity = 0.80, pooled specificity = 0.85, pooled AUC of SROC = 0.89). Additionally, the combined hazard ratio (HR) and 95% confidence intervals (CI) of survival analysis revealed that LINC01614 up-regulation was associated with poor overall survival (OS) in human malignancies (HR=1.909, 95%CI: 1.577-2.312). As for the bioinformatics analyses, the overlapping GSEA enrichment plots of 8 datasets revealed that LINC01614 expression was strongly associated with epithelial-mesenchymal transition (EMT), extracellular matrix (ECM) receptor interaction, focal adhesion, gap junction, adherens junction and cell adhesion molecules (CAMS). Besides, 32 of 36 overlapping co-expression genes for LINC01614 were associated with EMT, suggesting that LINC01614 could be mainly involved in the EMT and its associated signaling pathways to influence the progression, metastasis and prognosis of human cancers.

Conclusion: This study validated that LINC01614 was up-regulated in various human cancers. More importantly, LINC01614 could be a potential diagnostic, prognostic and metastasis predictor for malignant tumors, which was mainly involved in the process of EMT to influence the progression of human cancers.

In silico Driven reveal the KGH-R1-ScFv affinity maturation and aggregation site

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Background: Our previous experiments have demonstrated that anti-p21Ras single-chain antibody (KGH-R1-ScFv) can bind to both H, N, and Kp21Ras proteins and have an excellent inhibitory effect on many types of tumors. However, KGH-R1-ScFv still needs to be further optimized before it becomes a market drug. *In silico* has been widely used in drug research and development.
Methods: In this study, homology modeling and molecular docking were used to study the direct interaction of KGH with p21Ras protein. Alanine scanning mutagenesis can predict the affinity maturation amino acid residues in KGH-R1-ScFv, and spatial aggregation propensity (SAP) analysis was selected to predict amino acid residues which can be optimized in KGH-R1-ScFv.
Results: We found that the mutation of wild-type *NRAS* and *KRAS* affect the binding site of KGH-R1-ScFv. Moreover, KGH-R1-ScFv compete with GTP sites in $KRAS^{mutantG13D}$, wild type *NRAS,* and $NRAS^{mutantG12C}$. Asp55, Ser58, Ser105, Gly106, Ser107, Glu198, Ser210 in KGH-R1-ScFv can be optimized, and these sites can enhance the targeting of specific types p21Ras protein. We found amid residues, Tyr59, Tyr61, Tyr104, Pro147, Leu150, Ala151, Leu154, Tyr173, Pro183, Leu197, Pro202, Ala203 and Leu237 were the potential aggregation sites. These amid residues could be engineered for enhanced antibody stability. However, there are still many amino acid residues with higher SAP values and red areas indicating exposed hydrophobic regions. These amid residues of high SAP values could be engineered for enhanced antibody stability.
Conclusions: In this study, we predicted the KGH-R1-ScFv affinity maturation and aggregation site. As all experiments are conducted by using *in silico*, further experiments studies are needed to be conducted.

Category: Molecular pathology
1653008

3D QSAR pharmacophore modeling and in silico screening of potential PFKFB3 inhibitors

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Background: 6-Phosphofructo-2-kinase/fructose-2, 6-bisphosphatase 3 (PFKFB3) is overexpressed in many tumor cells and plays a crucial role in the progression of cancer cells. PFKFB3 has been a promising target for expanding novel anticancer drugs. The purpose of this research is to screen potential PFKFB3 inhibitors based on *in silico* methods. **Methods**: In the present study, 3D QSAR pharmacophore models were established, and a best pharmacophore model (hypo 1) was obtained. Hypo 1 was confirmed by using the test set and Fischer's random test. Additionally, hypo 1 was employed as a 3D search query to screen the Enamine database, TCM (traditional Chinese medicine) database and ZINC natural database. The obtained compounds were then subjected to molecular docking and ADMET prediction studies. **Results**: four hits were identified as potential inhibitor candidates for the PFKFB3 protease.

Category: Molecular pathology
1654074

Using the patterned microarray culture to obtain multi-gene editing monoclonal cells

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Background: One of the key steps for using the CRISPR/Cas9 technology to obtain gene editing cells used in the study of disease mechanism, or generate gene editing animals combining the somatic cell nuclear transplantation, is to harvest pure monoclonal cells with genetic modification. The common method including limit dilution methodand mechanical pickmethod. However, the cells isolated by extreme dilution or flow cytometry often grow slowly or fail to proliferate normally in the independent environment of a single cellon account of the density dependence of cell growth. For using the mechanical method to obtain monoclonal cells, the damage caused by mechanical strength usually leading to theharvest cells with poor state, and the operation istime consuming, monoclonal harvest rate is low, which will greatly influence the experiment process for harvesting mass of monoclonal cell. What's more, this method to obtain monoclonal cells often mixed with other cells, can't promise cells differentiated from a single cell. It is also more difficult to obtain monoclonal cells with double or even multi-gene editing by traditional methods.

Objective: Here we aim to report a novel strategy to quickly obtain monoclonal cells with low damage by microarray, and to produce efficient multi-gene editing monoclonal cells in batches.

Methods: Polydimethylsiloxane (PDMS) seal was obtained by means of laser etching characteristic pattern silicon wafer as template, and then using PDMC micropattern printing technology, the fiber-junction pattern microarray was laid at the bottom of the noncellular attachment culture dish. The gene editing cells weregrown at a certain density in these special dishes. And a single cell is confined to a micropattern and grows into a sphere of monoclonal cells.

Results: Micropattern printing limited cell growth and adhesion space in cell culture substrate. After 48h culture, 3D cell microspheres 50 microns in diameter were formed. After 96h the cell sphere barely continued to grow, remaining about 100 microns in size. Due to the narrow contact surface between the microsphere and the bottom of the culture, the attachment is not firm, so when the cell reaches a certain size, shaking or using a pipette to gently blow the cell sphere, it will automatically fall off from the bottom. The detached cell spheres were diluted and visible to the naked eye, and the single cell spheres were extracted and cultured separately in the pore plate, so that the monoclonal cells with good growth condition could be rapidly obtained. Using this method, we have quickly obtained the *Fah/Rag2* double gene homozygous editing cells, laying a

foundation for the subsequent production of *Fah/Rag2* double knockout pigs as human hepatocyte bioreactor.

Conclusions： We firstly used the technology of patterned microarray culture to rapidly obtain monoclonal cell spheres with good growth condition. This method could improve the harvest rate of monoclonal cells, minimize the time of monoclonal selection, and avoid the problem of impurity of monoclonal selection, which could be widely used in the acquisition of multi-gene editing cells and cancer monoclonal cells .

Anti-tumor effect of CIK cells combined with recombinant oncolytic adenovirus KGHV500 expressing anti-p21Ras scFv in liver cancer

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Background: Oncolytic adenovirus-mediated gene therapy for abnormal changes in ras-driven genes is an emerging strategy in gene therapy. However, oncolytic adenovirus-mediated gene therapy is mainly local injection of tumors, and the infusion method is limited, which limits its application range. Intravenous administration is still a key problem that needs to be solved urgently.

Methods: We constructed a recombinant oncolytic adenovirus KGHV500 carrying anti-p21Ras single-chain fragment variable antibody (scFv), and tested the anti-liver cancer ability of KGHV500 in vitro by TUNEL, MTT, wound healing assay and Transwell invasion assay. In vivo animal experiments, Using CIK cells as a second vector for KGHV500, we observed whether CIK cells carrying KGHV500 could reach tumor tissue and perform anti-tumor effects, and observed their expression in normal tissues to assess their safety.

Results: In vitro experiments indicated that KGHV500 inhibited the migration, proliferation, invasion and apoptosis of liver cancer cells, showing obvious anti-tumor effects. In vivo animal experiments showed that CIK cells carrying KGHV500 can reach the tumor site and exert anti-tumor effect, which is better than the CIK cell treatment group and the KGHV500 treatment group. Morever, the distribution of KGHV500 and anti-p21Ras scFv in normal tissues of nude mice were detected. It was found that there were KGHV500 and anti-p21Ras scFv in the liver, kidney and spleen, and the other normal tissues were not expressed.

Conclusions: Recombinant oncolytic adenovirus KGHV500-mediated gene therapy for ras-driven gene mutation or overexpression is effective in vitro. Intravenous injection of CIK cells and KGHV500 in vivo significantly inhibits tumor growth and has little effect on normal tissues and is relatively safe.

FBX8 promotes metastatic dormancy of colorectal cancer in liver

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Background

Patients with colorectal cancer (CRC) often develop malignant regrowth of metastatic dormant tumor cells in liver years after primary treatment. Deciphering the causal basis of tumor dormancy therefore has obvious therapeutic significance. FBX8 is involved in suppressing tumor metastasis. In the current study, we aim to investigate the role and molecular mechanism of FBX8 in regulating the dormancy of liver metastases from CRC which can provide new potential therapeutic targets for tumor metastasis and also provide a new treatment strategy for the prevention and treatment of clinical tumor metastasis.

Methods

Short-term chemotherapy experiments and liver metastasis mice model of orthotopic injection into the cecum were performed to construct the dormant models. GST pull-down, Co-IP and immunofluorescence were used to confirm the bindings among FBX8 and its substrates.

Results

FBX8 up-regulated the expression of epithelial and stemness markers, while down-regulated the expression of mesenchymal and proliferative markers associated with tumor cell dormancy. FBX8 promotes the maintenance of metastatic dormancy of CRC cells. Mechanistically, FBX8 directly bound to HIF-1α, CDK4 and C-myc through its Sec-7 domain and led to the ubiquitin degradation of these proteins, thereby inhibiting cell cycle progression, proliferation, angiogenesis and metastasis. Clinically, FBX8 expression was negatively correlated with the HIF-1α, CDK4 and c-Myc in CRC tissues.

Conclusion

Our study revealed a novel mechanism of FBX8 in regulating tumor metastatic dormancy in liver. A wake-up strategy for disseminated tumor cells based on FBX8 inhibition might be of value in combination with conventional chemotherapy for the treatment of CRC metastasis.

Category: Molecular pathology
1654688

Liver-Specific Deletion of Mettl14 Promotes Non-alcoholic Steatohepatitis in mice

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Abstract

N^6-Methyladenosine (m^6A) is the most prevalent internal modification of mRNAs in eukaryotes. Abnormal m^6A is closely related to the occurrence and development of many human diseases. Mettl14, a m^6A methyltransferases, is involved in the installation of m^6A, but there are few studies about Mettl14 at present. **Objectives:** To demonstrate the effects and mechanism of Mettl14 gene in nonalcoholic steatohepatitis (NASH) in mice. **Materials and Methods:** Cultivating liver-specific Mettl14 knockout mice, inducing NASH disease model in wild-type (WT) and knockout (KO) mice by feeding with methionine-choline-deficient (MCD) diet, comparing hepatic outcomes after 6 weeks of MCD feeding combined with histology, serum chemistries, quantitation of hepatic triglyceride and cholesterol, Western Blot and quantitative PCR. **Result:** Hepatocyte-specific knockout of Mettl14 had no significant effect on the development, structure, function, glucose and lipid metabolism of the liver in mice. The degree of liver injury was comparable between the two groups, based on histologic, and serum ALT, AST. But liver/weight ratio, serum triglyceride (TG) and cholesterol (CHOL), liver triglyceride (TG) and cholesterol (CHOL), serum inflammatory cytokines (IL-6, IL-1β, TNFα) were higher than those in WT mice. Oil red O and HE staining showed that hepatic steatosis and inflammatory cell infiltration were more severe in KO mice than WT mice. It was consistent with greater hepatic induction of inflammatory (IL-6, IL-1β, TNFα) and lipogenic genes (Acc1, Fasn, Dagt2, Scd1) in KO mice. **Conclusion:** Hepatocyte-specific deletion of Mettl14 promotes non-alcoholic steatohepatitis in mice by promoting inflammation and fat synthesis.

Overexpression of chloride channel-3 is a poor prognostic biomarker for gastric cancer with increased transcriptional level regulated by X-Ray Repair Cross Complementing 5

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Background: Recently many potential prognostic biomarkers for gastric cancer (GC) have been identified, but the prognosis of advanced GC patients remains poor. Chloride channels are promising cancer biomarkers, and their family member chloride channel-3 (ClC-3) is involved in multiple biological behaviors. However, whether ClC-3 is a prognostic biomarker for GC patients is rarely reported. The molecular mechanisms by which ClC-3 is regulated in GC are unclear.
Methods: The expression of ClC-3 and XRCC5 in human specimens was analyzed using immunohistochemistry. The primary biological functions and pathways related to ClC-3 were enriched by RNA sequencing. A 5' -biotin-labeled DNA probe with a promoter region between −248 and +226 was synthesized to pull down ClC-3 promoter-binding proteins. Functional studies were detected by MTS, clone formation, wound scratch, transwell and xenograft mice model. Mechanistic studies were investigated by streptavidin-agarose-mediated DNA pull-down, mass spectrometry, ChIP, dual-luciferase reporter assay system, Co-IP and immunofluorescence.
Results: The results showed that ClC-3 was overexpressed in human GC tissues, and that overexpression of ClC-3 was a poor prognostic biomarker for GC patients ($P = 0.012$). Furthermore, higher expression of ClC-3 was correlated with deeper tumor invasion ($P = 0.006$) and increased lymph node metastasis ($P = 0.016$), and knockdown of ClC-3 inhibited cell proliferation and migration *in vitro*. In addition, X-ray repair cross complementing 5 (XRCC5) was identified as a ClC-3 promoter-binding protein, and both ClC-3 (HR 1.671, 95% CI 1.012-2.758, $P = 0.045$) and XRCC5 (HR 1.795, 95% CI 1.076-2.994, $P = 0.025$) were prognostic factors of overall survival in GC patients. The *in vitro* and *in vivo* results showed that the expression and function of ClC-3 were inhibited after XRCC5 knockdown, and the inhibition effects were rescued by ClC-3 overexpression. Meanwhile, the expression and function of ClC-3 were promoted after XRCC5 overexpression, and the promotion effects were reversed by ClC-3 knockdown. The mechanistic study revealed that knockdown of XRCC5 suppressed the binding of XRCC5 to the ClC-3 promoter and subsequent promoter activity, thus regulating ClC-3 expression at the transcriptional level by interacting with PARP1.
Conclusions: Our findings indicate that the overexpression of ClC-3 is a poor prognostic biomarker for GC with increased transcriptional level regulated by XRCC5. Double targeting ClC-3 and XRCC5 may provide promising therapeutic potential for GC treatment.

Detection of PIK3CA gene mutations association with PD-L1 expression levels and TILs in breast cancer

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Objectives: Breast cancer is one of the major causes of female morbidity and mortality, accounting for ~25% of the total cancer cases in women. Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic α subunit (PIK3CA) mutations serve a major role in downstream signaling of receptor tyrosine kinases. The present study aimed to investigate the relationship between PIK3CA mutation and clinicopathological features, PD-L1 expression and ITLs in invasive breast cancer.
Materials and Methods: Postoperative paraffin-embedded tissues from 191 Chinese breast cancer patients (180 invasive ductal carcinoma, 7 invasive lobular carcinoma, 4 invasive mucinous carcinoma) were analyzed with Amplification Refractory Mutation System PCR (ARMS-PCR) for PIK3CA mutations (exon 9 and exon 20), the PD-L1 expression (22C3) in corresponding tissues was detected by immunohistochemistry and TILs were independently counted by two pathologists.
Result: PIK3CA mutations were found in 52 (27.2%). exon 9 mutations (E545K, E542K) were found in 16 tumor samples, exon 20 mutations (H1047L, H1047R) in 34, where 2 tumor sample had two mutations (E542K/H1047R; E545K/H1047R). We found that PIK3CA mutation has no significant correlation with age, histological type, tumor stage and grade of invasive breast cancer patients, but it is significantly associated with molecular classification of invasive ductal carcinoma(Luminal A, 47.1%; Luminal B, 25.3%; HER2 type, 33.3%; Triple negative type , 6.3%), p=0.028. Moreover, we found PIK3CA mutation had no significant difference in PD-L1 expression and TILs content in breast cancer tissues, but in patients whose tumor tissue shows high PD-L1 expression (≥1.0%) , The PIK3CA mutation rate in tumor with low TILs (<50%, n=30) was significantly higher than those with high TILs (≥50%, n=10), p=0.003 (46.7% vs 0.0%).
Conclusion: The current study emphasized the potential of *PIK3CA* mutations as an important biomarker for breast cancer classification and the possible use of *PIK3CA* inhibitor as Immunotherapy for breast cancer.

BCOR-CCNB3 fusion and BCOR internal tandem duplication in undifferentiated round cell sarcomas: a pathologic and molecular study of 5 cases

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Undifferentiated round cell sarcoma (URCSs) usually remained unclassified due to lack of known genetic abnormalities. Herein, we retrospectively collected 5 cases of URCSs and sought to investigate their unique clinicopathologic and molecular features for providing more accurate classification. There were 2 males and 3 females with age ranged from 7 months to 17 years. The tumors were respectively located in the sacrum, fibula, neck, perineum or groin. Microscopically, all 5 tumors were composed of small-to-medium sized cells with primitive morphology and variable cellularity, distributed within loose myxoid or collagenized fibroid stroma. These tumors lacked specific immunophenotypes and known gene rearrangements. However, the expression levels of CD99 and cyclin D1 were variable. RNA-sequencing data identified one BCOR-CCNB3 gene fusion-positive sarcoma occurring in the sacrum of a 17-year-old male patient. Whole genome sequencing analysis detected BCOR exon 15-internal tandem duplication (BCOR-ITD) in the tumor arising in the groin of one 7-month-old female infant. No specific gene abnormalities were found in the other 3 cases. Interestingly, a morphological and immunohistochemical overlap existed between BCOR-rearrangement tumor and BCOR-ITD-positive tumor, including areas with hypercellularity alternating with hypocellularity, a mixture of round cells and focal spindle cells, pale nuclear chromatin, inconspicuous nucleoli and abundant myxoid matrix, diffuse strong cyclin D1 expression, relatively strong expression of CD99 but lower than that in Ewing sarcoma, and a low Ki-67 proliferation index of about 10%. Our findings demonstrated a significant link between genetic aberration and histopathologic appearances, thus supporting the crucial role of genetic characteristics in accurate clinicopathological classification.

Comparison of clinicopathological characteristics and molecular mutation spectrum between colorectal adenocarcinoma with and without mucinous component in a Chinese cohort

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Background: Colorectal cancer (CRC) is characterized by high heterogeneity and thus varied prognosis and outcomes. Based on histological subtypes, most CRCs belong to classical adenocarcinomas(AC), with several histological variants associated with specific molecular characteristics. Mucinous adenocarcinoma (MAC) and signet-ring cell carcinoma (SRCC) are two special entities in the WHO classification defined by over 50% of the lesion composed of extracellular or intracellular mucin, respectively. Previous research suggested that MAC and SRCC performed homogeneous clinicopathological features but behaved differently from AC, which highlights the importance of histological appearance. Some researchers also believed that there are clinicopathological differences between MAC and SRCC. However, the entity of adenocarcinoma with mucinous component <50% is rarely studied. Some publications have emphasized the existence of mucinous component rather than the amount. Besides, the mutation spectrum of colorectal adenocarcinoma with mucinous component (AWMC) as a whole entity is never explored, The aim of the study is to analyze the clinicopathological characteristics and molecular mutation spectrum of AWMC and comparing with AC in a Chinese cohort.
Methods: Medical records of AWMC and AC patients were reviewed. Targeted next-generation sequencing with a panel of colorectal cancer-related genes was performed on formalin-fixed paraffin-embedded tissues. AWMC was further classified into two groups: with or without signet ring cell component. Genetic alterations and clinicopathological features were analysed.
Results: 181 AC patients and 101 AWMC patients were included from July 2010 to September 2016. Patients with neoadjuvant chemotherapy were excluded. In AWMC group, patients were predominantly males (59 people, 58.4%). The average age was 57 years (range: 20-84 years). 13.9% of them were over 75 years old when surgery was performed, and 46.5% were 56-75 years old. 50.5% of the MAC were larger than 5cm. 44 cases (43.6%) were located in the proximal colon. 92.1% of the cases had no metastasis when presented. 10 cases (9.9%) were stage I, 32 (31.7%) were stage II, 49 (48.5%) were stage III, and 8 (7.9%) stage IV. Compared with AC, AWMC occurred at younger age, had predilection for proximal colon, had less frequent lymph node and distant metastasis, and tended to be at earlier stage. There was no significant difference in sex distribution or tumor size between AWMC and AC. Within AWMC group, 15 cases (14.9%) had signet-ring cell

component, with 9 males (60%) and 6 females (40%). The average age was 50 years (range: 28-71 years).   93.3% had no metastasis when presented. UICC tumor stages were stage I in 1 patient (7.1%), stage II in 5 patients (35.8%), stage III in 7 patients (50.0%), and stage IV in 1 patient (7.1%). Age, gender, tumor location and TNM staging were not significantly different between AWMC with and without signet-ring cell component (p=0.108, 0.893, 0.408, 0.978, respectively), whereas the latter prefers to affect younger patients. Tumor size was found to be distinct between AWMC with and without signet-ring cell compoennt (p=0.010): the latter had a smaller size when detected, while 55.8% of the former was larger than 5cm.

The two most commonly mutated and studied genes, KRAS and BRAF were detected in AWMC.   The mutation frequency of KRAS in AWMC was 45.5% (46/101), with the most common site G12D in exon 2 (26.1%), followed by G12V (23.9%), G13D (19.6%), and A146T (15.2%). 9 AWMC cases were BRAF-mutated, with the vast majority being the hotspot V600E. Three cases harbor BRAF and PIK3CA concomitant mutations. AWMC with and without signet-ring cell component also had different mutational status, especially for KRAS (mutation rate: 52.3% vs 6.7%). Overall, AWMC showed distinct mutation spectrum from AC in a subset of genes such as ERBB2 and TP53.

No obvious difference in clinicopathological features was observed between KRAS or BRAF-mutated and wild-type AWMC.
Conclusion: AWMC shows different clinicopathological features from AC, whereas the existence of signet ring cell component in AWMC doesn't show a peculiar characteristic, suggesting the possibility of classifying colorectal depending on the existence of rather than the amount of mucin. The mutation spectrum between AWMC and AC, and between AWMC with and without signet-ring cell component are both different, providing the different potential target for treatment. The most two commonly studied genes, KRAS and BRAF, are not associated with specific clinical features in AWMC.

Different TNM classification in lung cancer patients with and without type 2 diabetes

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Objective: Diabetes is reported to be associated with the risks of many cancers but not lung cancer. It is worthwhile to investigate the relation between diabetes and lung cancer from more perspectives. To characterize the tumor, node, and metastasis (TNM) classification in lung cancer patients with and without type 2 diabetes.

Materials and Methods: A cross-sectional survey of lung cancer patients. Information on TNM classification was collected from pathological reports. Information on type 2 diabetes was collected via face to face interview. All patients diagnosed with lung cancer (n=1825) at the Renmin Hospital and Zhongnan Hospital from April 1, 2017 to January 30, 2018.

Result: Compared to lung cancer patients without diabetes, lung cancer patients with diabetes were more likely to be small cell cancer (18.6% vs. 10.2%, $P = 0.01$), less likely to be adenocarcinoma (47.4% vs. 58.3%, $P = 0.03$). Lung cancer patients with diabetes were more likely to have neoplasms inright upper lung lobe (25.8% vs. 14.4%, $P= 0.002$), less likely in left upper lung lobe (12.4% vs. 22.5%, $P = 0.02$). Lymph node metastasis (36.8% vs. 15.5%, $P = 0.008$)was more likely to occur in lung cancer patients with diabetes. Distant metastasis was more likely to occur in lung cancer patients with diabetes (58.8% vs. 43.0%, $P=0.002$), particularly for liver metastasis (19.3% vs. 7.7%, $P = 0.006$), but with lower likelihood for brain metastasis (12.3% vs. 26.0%, $P=0.02$) and bone metastasis (22.8% vs. 37.7%, $P=0.03$).

Conclusion: The TNM classification of lung cancer is different between patient with and without diabetes. Diabetes is associated with higher possibility of small cell lung cancer and lower probability of lung adenocarcinoma, higher probability of right upper lung lobe and lower probability of left upper lung lobe; higher probability of lymph node metastasis and distant metastasis, particularly liver metastasis, but lower probability of brain and bone metastasis.

DNAJC12 promotes the resistance of doxorubicin in neoadjuvant chemotherapy for breast cancer

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Objective: Finding the gene that affects the efficacy of neoadjuvant chemotherapy for breast cancer is the key to improve the remission rate of neoadjuvant therapy. Heat shock proteins are highly conserved proteins in evolution, most of which have the function of molecular chaperones and are involved in protein folding, assembly, transportation, etc. DNAJC12 is a member of the heat shock protein family. The purpose of this study was to investigate the role of DNAJC12 in drug resistance to neoadjuvant chemotherapy.

Method: 160 female breast cancer patients receiving anthracyclines and taxanes based neoadjuvant chemotherapy in West China hospital of Sichuan University were included in this research, among which 32 cases reached pathological complete response (pCR) and 128 cases were non-pCR. The expression of DNAJC12 was detected by quantitative real-time PCR. siRNA was used to interfere the expression of DNAJC12 in breast cancer cell lines MCF-7, MDA-MB-231 and SKBR3. The effects of DNAJC12 on chemotherapy drug resistance and cell function were studied by drug sensitivity experiments of doxorubicin and docetaxel, and cell function experiments including proliferation, scratching and transwell migration, respectively.

Results: The expression of DNAJC12 in non-pCR patients was higher than that in pCR patients (p<0.05).The CCK8 test results of MCF-7, MDA-MB-231 and SKBR3 showed that the proliferation activity of the control group was higher than that of the interference group (p<0.05), and the control group showed obvious drug resistance. There was no significant difference in the proliferation activity between the experimental group and the control group under the action of docetaxel(p>0.05). The results of scratch test and transwell migration test showed that the migration rate of the interference group was lower than that of the control group (p<0.05).

Conclusion: Our study showed that high expression of DNAJC12 promoted cell migration and increased tolerance of breast cancer cell lines to doxorubicin, thereby affecting the efficacy of neoadjuvant chemotherapy in patients.

Category: Molecular pathology
1655934

SCUBE2 acts as a biomarker predicting the efficacy of neoadjuvant chemotherapy in breast cancer

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Objective: Neoadjuvant chemotherapy can reduce tumor stage to favor surgery, or change inoperable to operable; If pCR can be achieved, it indicates a better long-term effect. However, many patients are not sensitive to neoadjuvant chemotherapy and may even delay the operation, so it is very important to find molecular indicators that can predict the efficacy of neoadjuvant chemotherapy for breast cancer. This study aims to explore the role of SCUBE2 in predicting the efficacy of neoadjuvant chemotherapy in breast cancer.

Method: Firstly, find the genes with consistent expression difference in the four GEO data sets GSE41998, GSE25065, GSE20194 and GSE20271 through integrated analysis, and then choose SCUBE2 through literature reading. Then, quantitative real-time PCR was used to detect the SCUBE2 expression in 160 female breast cancer patients receiving anthracyclines and taxanes based neoadjuvant chemotherapy in west China hospital of Sichuan university, among which 32 cases reached pathological complete response (pCR) and 128 cases were non-pCR. The effect of SCUBE2 on chemotherapy drugs doxorubicin and docetaxel was verified in vitro by breast cancer cell lines.

Results: The expression of SCUBE2 in the four GEO data sets was higher in non-pCR patients (p<0.05), and was more than 2 fold change. The result of quantitative real-time pcr also showed that SCUBE2 expression in non-pCR patients was higher than that in pCR patients (p<0.05), which was consistent with the chip results. The drug sensitivity tests of breast cancer cell lines MCF-7, T47D, MDA-MB-231 and SKBR3 all showed that over expression of SCUBE2 promoted the resistance to anthracyclines and taxanes (p<0.05).

Conclusion: SCUBE2 can be used as a molecular indicator for the prediction of efficacy of neoadjuvant chemotherapy for breast cancer.

Mettl14 participates in liver regeneration by modulating hepatic endoplasmic reticulum stress in m6A-dependent manner

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N^6-methyladenosine(m^6A) is the most prevalent and dynamic post-transcriptional methylation modification in eukaryotic RNAs. Mettl14, the core component of m^6A methyltransferase complex, is related to embryonic development and cancer. Previous studys of m^6A function was limited to experiments in vitro, which can not truly reflect the biological function of Mettl14 in vivo. Therefore, in this study, we constructed hepatocyte-specific Mettl14 knockout mice by Cre/Loxp system to explore the role of Mettl14 in liver development and regeneration. Hepatocyte-specific Mettl14 knockout (Mettl14$^{-/-}$) mice were born in accordance with Mendel's genetic law and developed and reproduced normally. The disruption of Mettl14 did not affect the development of liver, as well as the histological and physiological functions, and all Mettl14$^{-/}$ mice did not develop tumor spontaneously. After 70% hepatectomy, the recovery of liver weight/body weight was slowed, the expression of key protein in G1 phase was significantly inhibited, and so did the number of BrdU and Ki67 positive hepatocytes. In particular, small patchy necrosis in the parenchyma occurred in regenerated liver. Specific depletion of Mettl14 in hepatocyte resulted in reduced m^6A level in mRNA of protein processing in endoplasmic reticulum (ER), with decreased expression of protein level, and the hepatocytes were in a state of sustained ER stress, which in turn triggers cell death.
Conclusion, Our findings provided basis for exploring the role of Mettl14-mediated m^6A in liver development, liver regeneration, and liver-related diseases. We have revealed that m^6A mediated ER stress plays an important regulatory role in liver regeneration, and provided a new research field for liver regeneration research.

Category: Molecular pathology
1656193

Survival prediction for patients with lung adenocarcinoma: a prognostic risk model based on gene mutations　　Running title: SMARCA4, GNAS and TSC2: a gene mutation signature plays a prognostic role

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Abstract Objective The aim was to identify a signature mutation genes for prognostic prediction of lung adenocarcinoma. **Methods** 462 lung adenocarcinoma cases were screened out and downloaded from TCGA database. Mutation data of 18 targeted genes were detected by MuTect. LASSO-COX model was used to screen gene loci, and then a prognosis model was established. The survival and ROC curves were drawn to study the efficiency of the prognosis model. Afterwards, 40 clinical patients of lung adenocarcinoma were collected to verify the mutation features and the predictive function of the above prognostic model. The mutations of above 18 genes were sequenced with targeted next generation sequencing (NGS) and analyzed with GATK and MuTect. **Results** *TP53* (282, 32.38%), *NF1* (82, 9.41%) and *EGFR* (80, 9.18%) were the top 3 most frequent mutation genes. A total of 7 variables were screened out after lasso-COX analysis (tumor stage, age, diagnostic type, SMARCA4, GNAS, PTCH2, TSC2). *SMARCA4*, *GNAS* and *TSC2* were a gene mutation signature to predict a poor prognosis. **Conclusion** We established a prognostic model for lung adenocarcinoma, and further concluded that *SMARCA4*, *GNAS* and *TSC2* were a gene signature which plays a prognostic role.

CLIP170 promotes the development of thyroid papillary carcinoma through regulating cell cycle

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Abstract:
Objectives: To investigate the effect of CLIP170 gene expression on proliferation, migration and cell division of thyroid cancer cells, and to explore the role of CLIP170 in the development of thyroid papillary cancer and potential therapeutic targets.
Materials and Methods：The recombinant CLIP170/shRNA lentiviral vector containing CLIP170 shRNA was transfected into TPC-1 cells of thyroid papillary carcinoma, to acquired stable inhibitor cell line. Then the cells were divided into control group and shRNA group. Later CCK8 and transwell were used to observe the cells proliferation and migration capacity. With flow cytometry technology contrast before and after transfection of TPC-1 cell mitosis. The expression of mitosis related protein cyclin B, securin, cdc2 and EMT related protein E-cadherin、N-cadherin、Vimentin were detected by Western blot.
Result: contrast with control, CLIP170 gene silencing cell proliferations decreased obvious (P<0.05).
The proportion of G2-M mitotic arrest cells increased. The expression levels of mitotic related proteins securin and cyclin B protein and mRNA increased significantly (P < 0.05), but there was no significant difference in the expression levels of cell migration related proteins.
Conclusion: Downregulation of CLIP170 gene expression does not affect the migration ability of thyroid papillary carcinoma cells, but it can inhibit the degradation of mitotic proteins such as securin and cyclin B, lead to cell mitotic disorder and genome disorder, resulting in growth inhibition of thyroid cancer cells.

Application of Ventana immunocytochemical analysis on ThinPrep cytology slides for detection of ALK rearrangement in patients with advanced non- small-cell lung cancer

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Objective To explore the feasibility of *ALK* (D5F3) immunocytochemistry on fine needle aspiration cytology (FNAC) conventional smears and liquid-based cytology of advanced lung adenocarcinoma. **Methods** 147 cases of specimens are collected in this study. The expression of ALK was detected by Roche Ventana ICC, compared with   FISH，RT-PCR. **Results** The positive rate of ALK
was 11.56% while in conventional smears and liquid-based cytology reached 10.64% and12.00% ,respectively. Among all 147 cases, there are 57 cases referred to corresponding molecular methods verified. The coincidence rate reached 96.49% , while sensitivity and specificity in ALK(D5F3) fusion protein expression are 94.12% ,97.5% respectively. The sensitivity and specificity in conventional smears both reached 100% while 91.67%(11/12) and 96.67%(29/30) respectively in liquid-based cytology slides. **Conclusion** Conventional smears and liquid-based cytology in FNAC samples can be used in the detection of ALK ICC analysis， especially for those who have no chance to surgery and difficulty in making cell block to perform gene analysis.

Category: Molecular pathology
1656220

TRIP13 promotes the proliferation and invasion of lung cancers via Wnt signaling pathway and EMT, and indicates poor prognosis

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Purpose: Thyroid hormone receptor interactor 13 (TRIP13) is a member of the ATPases associated with various cellular activities family, and was found overexpressed in many tumors. The aim of this study was to investigate the role of TRIP13 and its underlying mechanisms in lung cancers.
Methods: We examined the expression of TRIP13 in lung cancer tissues and corresponding normal lung tissues using western blot. The expression level of TRIP13 was enhanced or knocked-down using TRIP13 gene transfection or siRNA interference in lung cancer cells. The expression levels of the key proteins of Wnt signaling pathway and epithelial to mesenchymal transition (EMT) were examined. The interaction of TRIP13 and LRP6 was examined using co-immunoprecipitation and laser confocal immunofluorescence. We also used colony formation, cell proliferation and matrigel invasion assays to detect the cell proliferative and invasive ability.
Results: The expression of TRIP13 was higher in lung cancer tissues than in corresponding normal lung tissues, and was correlated with poor prognosis. Overexpression of TRIP13 enhanced the expression levels of β-catenin, and the target proteins of Wnt signaling pathways, such as MMP7, cyclin D1 and c-Myc. TRIP13 can colocalize and bind with LRP6. Furthermore, overexpression of TRIP13 up-regulated N-cadherin, Snail and Vimentin, and down-regulated the expression of E-cadherin. The results above were inversed after knocking-down TRIP13 expression.
Conclusion: TRIP13 is highly expressed in lung cancers and indicates poor prognosis. Overexpression of TRIP13 promotes the proliferative and invasive ability of lung cancer cells via activating Wnt signaling pathway and EMT process.

Category: Molecular pathology
1656233

FAM83A promotes the proliferation and invasion of lung cancers by regulating Wnt and Hippo signaling pathways, and indicates poor prognosis

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Background and objective: Family with sequence similarity 83, member A (FAM83A) is a potential tumor-specific gene. It has been found that FAM83A is highly expressed and involved in the occurrence and development of various human cancers. The purpose of this study was to reveal the role and mechanism of FAM83A in the proliferation and invasion of lung cancers.

Methods: We increased or decreased FAM83A expression in lung cancer cells by gene transfection or siRNA interference, respectively, and detected key proteins of Wnt signaling pathway, Hippo signaling pathway and epithelial mesenchymal transition (EMT). We also investigated the effects of FAM83A on the proliferation and invasion of lung cancer cells using cell proliferation, colony formation and matrigel invasion assays.

Results: Compared with normal lung tissues, the expression of FAM83A in lung cancer tissues was significantly increased, and was closely correlated with advanced TNM stage and poor prognosis. Overexpression of FAM83A enhanced the proliferation, colony formation, and invasion of lung cancer cells. Meanwhile, overexpression of FAM83A increased the expression of β-catenin and Wnt target genes, such as cyclin D1, c-myc and MMP7. The expression of Twist and Snail, the key proteins of EMT, were increased, while E-cadherin was decreased. Furthermore, the expression of LATS, MST and P-YAP, the key proteins of Hippo signaling pathway, were down-regulated. The expression of YAP and its downstream cyclin E and CTGF were up-regulated. The inhibitor of Wnt signaling pathway, XAV-939, can reversed the promoting effect of FAM83A on YAP, cyclin E and CTGF. When knocking-down the expression of FAM83A in lung cancer cells, we obtained the opposite results. But, the inhibitor of GSK3β, CHIR-99021, can restore the expression of YAP, cyclin E and CTGF after FAM83A was knocked-down.

Conclusion: FAM83A is highly expressed in lung cancers and correlated to advanced TNM stage and poor prognosis. FAM83A promotes the proliferation and invasion of lung cancer cells by regulating Wnt and Hippo signaling pathways and EMT process.

The role of SPI1 expression in the prognosis of triple negative breast cancer

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Objectives: Breast cancer is the most common malignancy in women, and triple negative breast cancer has the worst prognosis, accounting for 15% to 25% of all breast cancer types. Metastasis is the main cause of high mortality and poor prognosis of triple negative breast cancer. Further study on the molecular mechanism of recurrence and metastasis of triple negative breast cancer has important scientific and clinical value for the treatment of this type of breast cancer. **Materials and Methods:** Firstly, in the GEO dataset GSE8977, 15 cases of normal tissue/ductal carcinoma in situ and 7 cases of invasive ductal carcinoma were analyzed by limma R package. It was found that SPI1 was differentially expressed in the two type tissues. UALCAN was used to analyze the expression of SPI1 in TCGA breast database and in each subtype of triple negative breast cancer. Finally, Kaplan-Meier analysis of breast cancer in KM-plotter database further proved that SPI1 expression was related to survival and prognosis of breast cancer. **Result:** The results showed that 234 differentially expressed genes were identified according to | log2fold-change | 1 and FDR < 0.05. The expression of SPI1 in invasive ductal carcinoma was significantly higher than that in normal tissues (p < 0.05). In TCGA breast cancer data, the expression of SPI1 in various subtypes of breast cancer was higher than that in normal tissues (p < 0.05). The expression of SPI1 in the triple negative breast cancer was higher than that in the Luminal (p < 0.05). The expression of SPI1 in TNBC_IM was higher than that in other subtypes except TNBC-MSL in triple negative breast cancer (p < 0.05). However, Kaplan-Meier analysis of OS, RFS and DMFS based on SPI1 expression in KM-plotter database showed that low SPI1 expression was associated with poor prognosis of triple-negative breast cancer (p < 0.05). **Conclusion:** SPI1 may be an important prognostic molecular marker of triple negative breast cancer, and it has a scientific value for further research on the molecular mechanism of recurrence and metastasis of triple negative breast cancer.

Differential diagnosis of multiple primary and intra-lung metastasis of lung cancer by multiple gene detection

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Objective: To study the differential diagnosis of multi-focal lung cancer and lung cancer with pulmonary metastasis by detecting the different lesions of the same patient. To explore the differences in prognosis between MPLC and IM, and to explore the factors affecting the prognosis of multi-focal lung cancer and the tumor heterogeneity of multi-focal lung cancer in combination with histopathology and molecular biology.

Methods: Fifty patients with multi-focal lung cancer were screened, and the relevant clinical information was noted; the patients were diagnosed by ACCP standard. Mutations of the lesions were detected by ARMS-PCR, and the detected genes included EGFR, ALK, ROS1, MET, KRAS, RET, HER-2, BRAF, NRAS and PIK3CA. The results of genetic testing were compared with those of ACCP standard diagnosis.

Results: We analyzed a total of 101 tumors from 50 patients. Classification based on gene testing contradicted the clinicopathologic diagnosis in 10 (20%) of the comparisons, identifying independent primaries in 6 cases diagnosed as metastasis and metastases in 4 cases diagnosed as independent primaries. Another 7(14%) tumor pairings were assigned an "equivocal" result based on gene testing. The results of gene testing of the remaining 33(66%) tumor pairings were consistent with the clinicopathologic diagnosis. The mutant heat map indicated that IM patients have a higher rate of mutation consistency than MPLC patients. The difference of prognosis between patients with mutations and those with wild-type genes patients was statistically significant (P=0.002). The difference of prognosis between patients with lymph node metastasis and those with no metastasis of lymph nodes was statistically significant (P=0.006). The difference of prognosis between patients with MPLC and those with IM was statistically significant (P=0.038). The difference of prognosis between patients who had different condition was statistically significant (P=0.038).

Conclusion: Multi-gene detection of multi-focal lung cancer has a certain auxiliary effect on the differential diagnosis of multiple primary lung cancer and lung cancer with pulmonary metastasis, which can complement the clinical standards, but also has some limitations.

Increased nodal expression suggests colorectal tumor progression and might be used as a potential diagnostic marker for CMS4 subtype of colorectal cancer.

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Nodal is related with tumorigenicity and progression in various tumors including colorectal cancer (CRC). However, the difference of Nodal expression between CRC and colorectal polyps has not yet been investigated. Besides, whether Nodal can be used as a marker for consensus molecular subtype classification-4 (CMS4) of CRC is also worth studying. We analyzed Nodal expression in patients of CRC (161), high-grade intraepithelial neoplasia (HGIN, 28) and five types of colorectal polyps (116). The Mann–Whitney U-test was used for analyzing the Nodal expression difference among groups. The Pearson Chi square test was used to analyze the association between Nodal expression and clinicopathological features, two categories logistic regression model was used to predict the odds ratio (OR) of risk factors for high tumor-stroma percentage (TSP), and ROC curve was used to assess the diagnostic value of Nodal in predicting high TSP in CRC. We found that Nodal expression was significantly elevated in CRC and HGIN ($p < 0.01$). The increased expression of Nodal was related with high TSP, mismatch repair-proficient (pMMR) status, lymph node metastasis and advanced AJCC stage ($p < 0.05$). Besides, Nodal expression was the only risk factor for high TSP (OR= 6.94; $p < 0.001$), and ROC curve demonstrated that Nodal expression was able to efficiently distinguish high and low TSP. In conclusion, different expression of Nodal between CRC/HGIN and benign lesions is suggestive of a role for Nodal in colorectal tumor progression. Besides, Nodal might also be used as a marker for CMS4 subtype of CRC.

PIWIL2/piR-27398 promotes invasion and metastasis of esophageal squamous cell carcinoma and predicts poor outcomes of the patients

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Objectives

Invasion and metastasis are the main causes of death in patients with esophageal squamous cell carcinoma (ESCC), but the underlying molecular mechanisms remain unclear. We previously found for the first time that PIWIL2 is highly expressed in ESCC. The aim of this study was to explore the role and mechanisms of PIWIL2 in ESCC and clinical significance in ESCC.

Materials and Methods

We plan to implement a series of in vivo and in vitro experiments to confirm this hypothesis, such as RNAi, RNA-seq, which is expected to deepen our understanding of the invasion and metastasis mechanisms and provide new therapeutic targets for ESCC.

Result

In this study, we found that high levels of PIWIL2 expression were correlated with poor overall survival of ESCC patients. Cox regression analysis showed that the expression of PIWIL2 is an independent prognostic indictor in patients with ESCC. Silencing of PIWIL2 in human ESCC cells effectively suppressed the migration and invasion in vitro and metastasis capabilities in vivo of the cells. We observe that PIWIL2 could mediates epithelial–mesenchymal transition (EMT) in the in vitro ESCC cell models. We further elucidate that a PIWIL2 relative piRNA, piR-27398 is involved in those malignant process in ESCC cells.

Conclusion

Hence, we hypothesize that elevated PIWIL2 in ESCC may promote the production of piR-27398 to activate Wnt pathway, thereby promoting the invasion and metastasis of ESCC.

Category: Molecular pathology
1657386

Bioinformatics identification of potential biomarker miR-130b in lung adenocarcinoma

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Abstract. Non-small cell lung cancer (NSCLC) is reported to be a major public health issue worldwide and the overall prognosis of patients remains poor. The poor prognosis is mainly due to the lack of effective early diagnostic methods. MicroRNA (miRNA/miR), a type of non-coding RNA molecule, can regulate the expression of target gene in almost all aspects of tumor biology and function as molecular markers for early diagnosis of tumor. Objectives: In this study, we aimed to detect the expression and potential mechanisms of miR-130b in lung adenocarcinoma through bioinformatics analysis. Materials and Methods: Four microRNA expression profiles of lung cancer were downloaded from Gene Expression Omnibus and the data were analyzed using SPSS 20.0 software. Result: Compared to normal lung tissue, expression of miR-130b was significantly increased in lung adenocarcinoma tissue (GSE48414, GSE51853, GSE63805, GSE74190). Furthermore, miR-130b was verified to be elevated in six fresh lung adenocarcinoma tissue and three different lung cancer cell lines by Real-time PCR experiment. GO analysis of 85 predicted target genes of miR-130b showed that 24 of the obtained GO terms are highly correlated with specific cellular processes including transcription factor activity and binding. KEGG results indicated that the miR-130b targets are mainly involved in the FOXO signaling pathway, and Glucagon signaling pathway. Conclusion: In conclusion, miR-130b, as an oncogene, may play a significant role in the development and progression of lung cancer. miR-130b may be the potential molecular biomarker for the early diagnosis of lung adenocarcinoma.

Effect of FOXF2 on the invasion and metastasis of colorectal cancer by activating JAK-STAT signaling pathway on tumor microenvironment

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Colorectal cancer (CRC) is the third most common malignancy in the world. The incidence of CRC in China is increasing, especially in Guangdong Province, ranking the first in China. The key scientific problems of research to find specific molecular biomarkers play an important role in elucidating molecular mechanism of colorectal cancer metastasis. FOXF2 promotes cell metastasis and has a certain relationship with tumor progression and its poor prognosis. The precious results showed that FOXF2 expression may be involved in the development of colorectal cancer, but the role of FOXF2 in colorectal cancer, molecular mechanism and upstream regulation is not clear. In this study, we investigated the effects of FOXF2 on the regulation of tumor micreenvironment and the invasion and metastasis of colorectal cancer cells by building AOM/DSS mouse model、transwell invasion and migration experiments, cell adhesion experiments, angiogenesis experiments and subcutaneous tumor formation in nude mice. The GSEA gene enrichment analysis, immunoprecipitation、immunization Co-precipitation and protein mass spectrometry were used to study the molecular mechanism of FOXF2 in the development and progression of colorectal cancer. The aim of this study was to provide a theoretical basis for the individualized diagnosis and treatment of targeted colorectal cancer and the development of targeted drugs.

Assessment of SHOX2 and RASSF1A methylation in paraffin-embedded biopsy specimen improves accuracy in lung cancer diagnosis

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Objectives: The objective of this study was to determine whether the assessment of SHOX2 and RASSF1A methylation level in paraffin-embedded biopsy specimen improves the sensitivity and the NPV of lung cancer diagnosis.

Materials and Methods: To facilitate the use in a diagnostic setting, a RT-PCR-based in vitro diagnostic test kit (Lung-Me®) to quantify SHOX2 and RASSF1A DNA methylation in paraffin-embedded tissue specimen was developed and characterized. Firstly, to establish the methylation cut-off for patient stratification, left-over paraffin-embedded biopsy tissue samples from 116 patients (60 lung cancer cases, 56 benign controls comprised of 26 Inflammatory and 30 granulomatous) were used in order to transfer the clinical decision point to the test kit. Secondly, to evaluate the clinical performance, the individual SHOX2 and RASSF1A DNA methylation levels of the paired cancers and paracancerous tissues samples from 40 patients were compared. Thirdly, 20 biopsy specimen from lung cancer patients, in which were small mammary focus not detected by conventional pathologic evaluation, were analyzed for SHOX2 and RASSF1A DNA methylation levels.

Result: The clinical cut-off of the test kit Lung-Me® was established : samples with ΔCtSHOX2 values $\leqslant$9.0, or ΔCtRASSF1A values $\leqslant$13.0 are rated as test positive while samples with both ΔCtSHOX2 values >9.0 and ΔCtRASSF1A values >13.0 as test negative. The results describe the test as a robust and reliable diagnostic tools for identifying patients with lung cancer using paraffin-embedded specimens (AUC [95% IC] = 0.96 [0.92-0.99], sensitivity 87% [0.85-0.92] / specificity 94% [0.93-0.99]). The intended use of the test was validated in a clinical performance evaluation study comprised of 40 paired cancers and paracancerous tissues samples. In cancer samples, the postive rate of Lung-Me® was 93% (37/40), but also in paracancerous samples, Lung-Me® detected 15 positive samples. In the cases without metastases , a significate decrease of methylation levels was observed in paracancerous sample compared to its paired cancer tissue. Furthermore, in 20 lung cancer patients' previous biopsy specimen , which were evaluated as negative by conventional pathologic methods, Lung-Me® detected 19 positive from 20 samples.

Conclusion: Applying SHOX2 and RASSF1A DNA methylation measurement to pathologic evaluation will not only improved the diagnosis of biopsy specimen affected by malignant

diseases, but with its high specificity also supports benign results.

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Pre-metastatic niche triggers SDF-1/CXCR4 axis and promotes organ colonization by hepatocellular circulating tumor cells via downregulation of Prrx1

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Background: Circulating tumor cells (CTCs), especially mesenchymal CTCs, are important determinants of metastasis, which leads to most recurrence and mortality in hepatocellular carcinoma (HCC). However, little is known about the underlying mechanisms of CTC colonization in pre-metastatic niches.
Methods: Detection and classification of CTCs in patients were performed using the CanPatrol™ system. A lentiviral vector expressing Prrx1-targeting shRNA was constructed to generate a stable HCC cell line with low expression of Prrx1. The effect of Prrx1 knockdown on stemness, migration, and drug resistance of the cell line was assessed, including involvement of SDF-1/CXCR4 signaling. Promising clinical applications of an inhibitor of STAT3 tyrosine phosphorylation, C188-9, and specific blockade with CXCR4 antibody were explored.
Results: The number of mesenchymal CTCs in blood was closely associated with tumor recurrence or metastasis. Pre-metastatic niche-derived SDF-1 could downregulate Prrx1, which induced the stemness, drug resistance, and increased expression of CXCR4 in HCC cells through the STAT3 pathway in vitro. In vivo, mice bearing tumors of Prrx1 low-expressing cells had significantly shorter survival. In xenograft tumors and clinical samples, loss of Prrx1 was negatively correlated with increased expression of CXCR4 in lung metastatic sites compared with that in the primary foci.
Conclusions: These findings demonstrate that decreased expression of Prrx1 stimulates SDF-1/CXCR4 signaling and contributes to organ colonization with blood CTCs in HCC. STAT3 inhibition and specific blockade of CXCR4 have clinical potential as therapeutics for eliminating organ metastasis in advanced HCC.

Category: Molecular pathology
1657534

Systematic analysis of DCAF13 expression and clinical prognosis in various tumors

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DDB1 and CUL4 associated factor 13 (DCAF13), which is a hotspot amplified in various cancer types, is a protein coding gene located on chromosome 8q22.3. A number of previous reports showed evidences of prognostic value of DCAF13 in human cancers. In this study, we performed a systematic analysis to determine whether DCAF13 could be used as a biomarker for the prognosis in different tumors as well as the relationship with other functional genes. DCAF13 expression was assessed using Oncomine analysis, the relationship between expression of DCAF13 and survival probability of cancer patients were identified by Kaplan-Meier Plotter and Prognoscan. Copy number alterations and mutations in DCAF13 gene were further analyzed using cBioPortal online database, and the interactive protein analysis was performed by the STRING program. We found that DCAF13 was more frequently overexpressed in breast, ovarian, lung, liver, and skin cancer and its expression were negatively related to the prognosis. Also, DCAF13 was related to NOP58, NOP56, RRP9, TBL3 and WDR36 both in gene and protein levels. Those findings suggest that DCAF13 might serve as a diagnostic and therapeutic target for certain types of cancer. However, future research is required to validate our findings and thus promote the clinical utility of DCAF13 in prognostic evaluation.

Category: Molecular pathology
1657578

Mechanism of XPO1-mediated WTX nuclear localization abnormality and regulation of Paraspeckle structural stability to promote gastric cancer progression

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WTX encodes a tumor suppressor, frequently inactivated in Wilms tumor, with both plasma membrane and nuclear localization. WTX is present in distinct subnuclear structures and co-localizes with the paraspeckle marker p54NRB/NONO, however, the nuclear function of WTX still remains unclear. Here we show that WTX speckles accumulate dynamically in nuclear granules of Gastric carcinoma cells, with completely corresponding to paraspeckle maker p54NRB/NONO, SFPQ and PSPC1. The paraspeckle component Neat1 and its partner p54nrb are required for WTX's association with paraspeckles and for pro-apoptotic activity. Moreover, WTX also plays an important role in paraspeckle phase separation and organization. Chemical perturbation of the nuclear export receptor XPO1 (also known as CRM1), with a clinically available drug, revealed a robust synthetic-lethal interaction WTX nuclear hyperexpression both in vitro and in vivo. XPO1 inhibitors disrupt paraspeckle structural stability by enhancing WTX nuclear aggregation and attenuating acetylation modification of p53. In WTX-reconstitution experiments, cell-cycle arrest, apoptosis, and p53 target-gene expression are promoted after inhibition of XPO1. Our findings indicate that clinically available XPO1 inhibitor, which exert effect in a WTX-dependent way, is a promising therapeutic strategy for a considerable cohort of patients with gastric cancer when coupled to genomics-guided patient selection and observation.

Category: Molecular pathology
1657618

HSD17B4 regulates prostate cancer progression through its K669 acetylation-mediated degradation

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Objectives:
Steroidogenic enzymes are crucial in prostate cancer (PCa) progression.
17β-Hydroxysteroid dehydrogenase type 4 (HSD17B4), encoded by HSD17B4, lacks catalytic capacity in androgen metabolism. The detailed role and molecular mechanism of PCa development are largely unknown.

Methods:
The levels of HSD17B4 and SIRT3 were analyzed in prostate cancer samples using immunohistochemistry and western blotting. Cell proliferation and migration were measured by the cell counting kit 8 assay, wound healing assay, colony formation assay and transwell assay. The interaction between SIRT3 and HSD17B4 was determined by co-IP and western blotting.

Results:
Here, we showed that the expression of HSD17B4 was increased in PCa tissues when compared to paired paratumor tissues. HSD17B4 knockdown in PCa cells significantly suppressed its proliferation, migration, and invasion, while overexpressing HSD17B4 had opposite effects. Mechanistically, we found that the protein level of HSD17B4 was regulated by its K669 acetylation. Dihydroxytestosterone (DHT) treatment increased HSD17B4 K669 acetylation and then promoted its degradation via chaperone-mediated autophagy (CMA). SIRT3 directly interacted with HSD17B4 to inhibit its K669 acetylation and enhance its stability. In addition, we identified CREBBP as a regulator of the K669 acetylation and degradation of HSD17B4, affecting PC cell proliferation, migration and invasion. Notably, in PCa tissues and paired paratumor tissues, the level of HSD17B4 was negatively correlated with its K669 acetylation.

Conclusions:
Taken together, this study identified a novel role of HSD17B4 in PCa progression and suggested that HSD17B4 and its upstream regulators may be potential therapeutic targets for PCa intervention.

Identification of PTPRR and JAG1 as key genes in castration resistant prostate cancer by integrated bioinformatics methods

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To identify novel genes of the castration resistant prostate cancer (CRPC), we downloaded three microarray datasets contains CRPC and primary prostate cancer in Gene Expression Omnibus (GEO). R package affy and limma were performed to identify differentially expressed genes (DEGs) between primary prostate cancer and CRPC. After that, we did functional enrichment analysis including Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG). In addition, protein-protein interaction (PPI) analysis were used for searching hub genes. Finally, to validate the significance of these genes, we performed survival analysis. As a result, we identified 53 upregulated genes and 58 down regulated genes that changed in at least two datasets. Functional enrichment analysis showed significant changes in the positive regulation of osteoblast differentiation pathway and aldosterone-regulated sodium reabsorption pathway. PPI network identified hub genes like CTTNBP2, RND3, PTPRR, JAG1 and LUM. Combined with functional enrichment analysis, we identified two genes (PTPRR and JAG1) as key genes. Further survival analysis observed relationship between the high expression of the two genes and poor prognosis of prostate cancer. In conclusion, PTPRR and JAG1 are key genes in the CRPC, which may serve as a promising biomarker of diagnosis and prognosis of CRPC.

Neuroendocrine Differentiation Does Not Significantly Affect the Prognosis of Triple-negative Breast Cancer Patients

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Objectives: Triple-negative breast cancer (TNBC) is an aggressive subgroup of breast cancer which lacks effective endocrine therapy or targeted therapy. The prognosis of TNBC patients with neuroendocrine (NE) differentiation hasn't been revealed. The aim of this study was to investigate the incidence of NE differentiation and its association with major clinicopathological parameters, and to determine the prognostic significance of NE differentiation in patients with TNBC.

Materials and Methods: Immunohistochemical staining for NE markers, chromogranin A (CgA) and synaptophysin (Syn) was performed on 172 TNBC samples using tissue microarrays. The chi-square test was used to evaluate the correlations between NE differentiation and patient characteristics. The effects of NE differentiation and other parameters on overall survival (OS) and disease-free survival (DFS) were accessed using univariate and multivariate analyses. Survival curves were plotted using the Kaplan-Meier method.

Result: The data showed that NE differentiation was present in 19.2% of 172 TNBC cases. The presence of any one of two NE differentiation indicators was associated with higher stage (P=0.015), but had no significant influences on OS and DFS in univariate analysis (OS, P=0.685; DFS, P=0.319) and multivariate analysis (OS, P=0.178; DFS, P=0.083). Kaplan-Meier survival curves of NE differentiation for OS (P=0.684) and DFS (P=0.314) didn't reveal a significantly prognosis, either. In brief, by univariate analysis, age, tumour size, lymph node involvement, stage, histological type were associated with either OS or DFS. By multivariate analysis, tumour size, lymph node involvement, stage and histological type were independent variables associated with either OS or DFS.

Conclusion: This study suggests that NE differentiation does not significantly affect the prognosis of TNBC patients. More cases need to be included and further analyses should be conducted to draw a significant conclusion.

Rassf10 exhibits tumor suppressor potential involving tumor proliferation, metastasis and epithelial-mesenchymal transition in esophageal squamous cell carcinoma

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Objectives: Growing evidence indicates that Rassf10 is a novel tumor suppressor involved in inhibition of tumor progression and metastasis. However, the biological functions and molecular mechanisms of Rassf10 in esophageal squamous cell carcinoma (ESCC) have not yet been thoroughly elucidated.
Materials and Methods: The expression of Rassf10 in ESCC tissues and adjacent non-tumor tissues was investigated employing qRT-PCR and immunohistochemistry of tissue microarrays. The function of Rassf10 in ESCC cell growth, migration and invasion were determined by CCK8, Colony formation assay, Scratch wound healing assay and Transwell invasion assay, respectively. The correlation between Rassf10 and EMT-related markers was evaluated by TMA-IHC, Western blotting and Immunofluorescence staining.
Result: Rassf10 was highly downregulated in ESCC tissues compared with the adjacent non-tumor tissues, and closely correlated with tumor progression and patient prognosis. Moreover, functional studies demonstrated that Rassf10 overexpression not only resulted in reduced cell growth and colony formation but also inhibited migration and invasion in ESCC cells. Tumor Rassf10 expression was positively correlated with E-cadherin expression and negatively correlated with Vimentin. Furthermore, we found that the antineoplastic functions of Rassf10 may mediate the inactivation of the Wnt/β-catenin pathway in ESCC.
Conclusions:Our findings revealed that Rassf10 might constitute a prognostic factor for ESCC patients and a crucial candidate for target therapy of ESCC.

CLEC5A promotes the proliferation of gastric cancer cells by activating the PI3K/AKT/mTOR pathway

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Objectives: Gastric cancer, as one of the most prevalent malignancies, contributes to the high morbidity and mortality worldwide.

Materials and Methods: By analyzing the bioinformatics, qRT-PCR and IHC assays, we found that CLEC5A was overexpressed in GC and associated with poorer prognosis. Silencing of CLEC5A inhibited cell growth and DNA replication and induced cell cycle arrest and cell apoptosis.

Result: Furthermore we found that CLEC5A expression negatively correlated with Bax and positively correlated with Bcl-2. Bioinformatics analyses and Western blotting revealed that CLEC5A depletion led to dysregulation of the PI3K/AKT/mTOR pathway. CLEC5A-mediated GC proliferation and anti-apoptosis were impaired by blockage of PI3K/AKT pathway with LY294002.

Conclusions: We hypothesize that CLEC5A is of vital importance to GC initiation and progression via the PI3K/AKT/mTOR pathway and the results may represent promising therapeutic strategies for GC patients.

Functional Study of TbBDF5 Related Proteins from Trypanosoma brucei

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Objectives: *Trypanosoma brucei,* a eukaryotic unicellular organism, causes human African trypanosomiasis. Here, we indicated that the deficiency of TbBDF5 afffected the expression of many genes, which impies TbBDF5 has important functions in various cell events of *Trypanosoma brucei.*
Materials and Methods: ITC, NMR perturbation, affinity purification, GST pull down and immunofluorescence assay were used.
Result: The first bromo domain of TbBDF5 can bind to H4K10ac and H4K14ac. Six proteins (including TbP55, TbP59, TbP62, TbHAT2, TbBDF3 and TbENT) associated with TbBDF5 and the interaction relationship were identified. Furthermore, we demonstrated that TbENT, TbBDF5 and other proteins co-localize in the nucleus. The nuclear localization of TbBDF5 was significantly affected after the depletion of TbHAT2.
Conclusion: TbBDF3 was previously reported to colocalize with H4K10ac in the transcription initiation site. Thus we speculate that TbHAT2 may acetylate H4K10 at first and recruit TbBDF5 to H4K10ac. TbENT and TbBDF3 as well as other proteins are then recruited to transcription start site and take part in transcription regulation.

Up in the air: ALDEFLUOR activity and ALDH isoforms

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The aldehyde dehydrogenase (ALDH) family is the most important aldehyde metabolic enzyme system in the body. High ALDH activity is one of the metabolic features of adult hematopoietic cells, neural stem cells and various tumor stem cells. ALDH1A1 is the most important and most studied subtype of ALDH1 family, and is widely expressed in human normal tissues such as brain and thyroid. , lung, breast, liver, pancreas, kidney colon, bone, prostate, ovary and cervix. The ALDEFLUORTM system is currently the most commonly used method for assessing ALDH enzyme activity. The principle is that the uncharged ALDH-substrate BODIPY-aminoacetaldehyde (BAAA) is passively diffused into living cells, and BAAA is converted into LB by intracellular ALDH. The negatively charged reaction product BAA (BODIPY-glycine), BAA is retained in the cells, allowing cells with high expression of ALDH to exhibit bright fluorescence, which can be detected by flow cytometry. DEAB was added as an inhibitor of ALDH and used as a control. Only cells with intact cell membranes retain the reaction product of ALDEFLUOR, so ALDEFLUOR only identifies active ALDH cells (http://www.aldh.org/). This system is currently widely used to isolate normal stem cells and cancer stem cells from heterogeneous cell populations. Normal stem cells such as: neural stem cells, adipose-derived adult stem cells，myogenic precursor cells. Cancer stem cells such as: digestive tract CSCs, such as oral squamous cell carcinoma (OSCC), nasopharyngeal carcinoma (NPC), esophageal squamous cell carcinoma (ESCC), esophageal adenocarcinoma (esophageal adenocarcinoma, EAC), gastric cancer, and colon and rectum (CRC). Other solid tumors include head and neck squamous cell carcinoma (HNSCC), thyroid cancer, lung cancer, liver cancer, pancreatic cancer, osteosarcoma, prostate cancer, bladder cancer, Glioblastoma (GBM), melanoma, ovarian cancer, cervical cancer and so on. Tumor cells isolated from these tumor cells with ALDH+ have the characteristics of cancer stem cells, and ALDH can be used as a marker for cancer stem cells.
Despite the widespread use of Aldefluor detection or sorting systems, which has greatly advanced the field of stem cell research, there are still some unresolved issues. The most striking of these is that among the 19 ALDH subtypes, which one or which group of enzymes determines the positive rate of Aldefluor. Recent studies have found that other ALDH subtypes, including ALDH1A3, ALDH2, ALDH3A1, etc., can also significantly affect ALDH activity significantly.

Transcriptome-wide association study identifies PSMB9 as a susceptibility gene for coal workers' pneumoconiosis in a Chinese population

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Background: Coal workers' pneumoconiosis (CWP), as one of the most important occupational diseases, is characterized by diffuse lung fibrosis due to the long-term inhalation of coal mine dust. Under the same level of dust exposure, genetics determine the individual's susceptibility to pneumoconiosis. The aim of this study was to identify novel susceptibility genes associated with CWP.

Methods: Based on our previous GWAS data and the gene expression data both in lung and whole blood from GTEx, we performed a transcriptome-wide association study (TWAS) to identify CWP related genes and further explored the function of the genes in vitro. The effect of best GWAS SNP on the activity of PSMB9 promoter was investigated by luciferase report assays. Molecular mechanisms underlying PSMB9 polymorphisms that involved in CWP development were analyzed by qRT-PCR, Western blotting, Immunofluorescence and TUNEL assays.

Results: We identified PSMB9 (*Proteasome subunit beta 9*) as a novel candidate gene for CWP both in lung tissue and whole blood (Lung: $P_{\text{TWAS}}=4.22\times10^{-4}$; Whole blood: $P_{\text{TWAS}}=2.11\times10^{-4}$). Rs2071480 and rs1351383 are located in the promoter region and the first intron of the PSMB9 gene and are in high linkage disequilibrium (LD, $r^2=0.98$) with the best GWAS SNP rs4713600 (OR=0.55, 95% CI: 0.42-0.74, $P=6.86\times10^{-5}$). Our luciferase report assays revealed that both mutation of rs4713600 and rs1351383 significantly enhanced the transcription activation of PSMB9. Furthermore, we demonstrated that lung epithelial cells knocked down of PSMB9 exhibited significant apoptosis, which was similar to the cells treated with silica. On the contrary, up-regulation of PSMB9 significantly resisted the increase of silica-induced cell apoptosis.

Conclusion: Overall, we identified PSMB9 as a novel susceptibility gene for CWP and provided important insights into the deeper exploration of the CWP pathogenesis.

LIMK-1 and ERK Interacts Synergistically to Active EGFR signalling Pathway and Stimulate HCC Metastasis

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Lim Kinase I (LIMK-1) was confirmed as a metastasis-promoting factor of hepatocellular carcinoma (HCC) from a cDNA microarray data with lymphatic metastasis. Clinical data indicates that the nuclear translocation of LIMK-1 is highly related with HCC metastasis. In this study, we confirmed the vital role of nuclear LIMK-1 in promoting HCC cell proliferation, migration and metastasis both in vitro and in vivo using molecular and cell biological techniques. We have also verified the direct interaction between LIMK-1 and ERK. EGF could dramatically stimulate the nuclear translocation of LIMK-1. LIMK-1 and ERK could interact synergistically to active EGFR signaling and stimulate HCC metastasis. We also explore the mechanism of HCC metastasis induced by the nuclear translocation of LIMK-1, as well as its activation of EGFR signaling pathway. The nuclear translocation of LIMK-1 led a poor prognosis based on clinical data and pathological parameters. Our results will certainly improve our understanding on LIMK-1 induced HCC progression and also provide evidence to the development of LIMK-1 as a new therapeutic target.

Bioinformatic Analysis Revealing Mitotic Spindle Assembly regulated BUB1B and NDC80 as New Prognostic Biomarkers in Non-Small Cell Lung Cancer

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Abstract

Objectives Lung cancer has been a main killer among various cancer types with increasing morbidity, and 80%~85% of it is non-small cell lung cancer (NSCLC). Even with the rising of molecular targeted therapies, for example EGFR, ROS1, ALK, BRAF, MET and so on, the treatment of the disease is still challenging. The study is to identify credible responsible genes during the development of NSCLC using Gene Expression Omnibus (GEO) database and bioinformatic analysis, developing new prognostic biomarkers and potential gene targets to the disease. **Materials and Methods** Firstly, from GEO database, three genes expression profiles GSE44077, GSE18842 and GSE33532 containing a total of of 181 NSCLC samples and 131 normal lung samples were downloaded to analyze the genes with different expression level (GDEs) between NSCLC and normal lung tissues. And FunRich was then used to analyze the basic functions of the GDEs including the cellular location, molecular function and the biology pathways the GDEs enriched in. Not the least, the protein-protein interaction network (PPI) of the genes was constructed, and top 15 core genes that connect with most surrounding genes were picked based on PPI, followed by genes annotation using GeneCard, as well as overall survival, functioning modules analysis by GEPIA software and signaling pathways exploration by Oncomine and KEGG websites. **Results** Three GEO profiles shared a total of 664 GDEs, including 232 down-regulated genes and 432 up-regulated genes. The functiong analysis of the GDEs revealed that the 232 down-regulated genes were mostly enriched in the cell communication, cell adhesion and cell surface interaction with vascular walls pathways and the 432 up-regulated GDEs were mostly cell cycle and mitotic related.   Then the PPI network of the 664 GDEs was constructed and gene connectivity with each other was analyzed. Based on the PPI network, top 15 genes with high connectivity with each other were identified. Kaplan-Meier survival analysis results of the 15 genes showed that 14/15 genes were significantly correlate with NSCLC overall survival including TOP2A, CCNB1, CCKN3, NDC80, BUB1, CCNA2, MELK, CDC20, KIF23, PBK, KIAA0101, AURKA, CHEK1, MAD2L1. Genes annotation showed that 4/14 genes were spindle assembly checkpoints, namely NDC80, BUB1B, MAD2L1 and AURKA. GEPIA analysis was additionally performed to validate these genes' gaped expression level between NSCLC and normal lung tissues. Meanwhile, TCGA data including 574 lung adenocarcinoma and 555 lung squamous cell carcinoma was downloaded to test the independent prognostic indicators value of the 4 genes using COX regression analysis,

and the results supported NDC80 and BUB1B as independent prognostic indicators in NSCLC. **Conclusions** Using bioinformatic analysis, we revealed 14 core genes that potentially function as new prognostic biomarkers and feasible gene targets to NSCLC development, and 4/14 were cell mitotic spindle assembly regulated genes including NDC80, BUB1B, MAD2L1 and AURKA. TCGA data and COX regression analysis validated the independent prognostic indicators value of the NDC80 and BUB1B. All results supported NDC80 and BUB1B as potential new survival biomrkers in NSCLC. However, further clinical trials needed to be performed to verify the potential drug targets value of the genes.

CIP4 targeted to recruit Cdc42 involving in invadopodia formation promotes invasion and metastasis of colorectal cancer

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CIP4 (Cdc42 - interacting protein 4) is a protein encoding by TRIP10 gene. We confirmed that CIP4 expression was upregulated in colorectal cancer (CRC) tissues and cells, and closely related to migrate and invasive ability of CRC cells. Studies found that CIP4 and Cdc42 were closely associated with tumor invadopodia formation, and interacted each other. Thus, we propose that CIP4 targeted to recruit Cdc42 involving in invadopodia formation promotes invasion and metastasis of CRC. In order to confirm our hypothesis, we evaluate the correlation between CIP4 , Cdc42 expression and CRC metastasis by IHC and tissue samples analysis. Vitro and vivo biological function assays will be used to clear the effect of CIP4 on biological functions of CRC cells related to metastasis. Deletion mutant construction and GST-pull down assay will be adapted to explore the interaction between CIP4 and active Cdc42. Eventually, Using GTP-bound GTPase pull-down and immunofluorescence to confirm that CIP4 targeted to recruit Cdc42 involving in invadopodia formation promotes invasion and metastasis of CRC.

Category: Molecular pathology
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Phosphoproteomics analysis of latent membrane protein 1 for which induce epithelial-mesenchymal transition in nasopharyngeal carcinoma cells

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Abstract

Background: Post-translational regulation of protein phosphorylation plays an vital role in signal transduction and regulation of gene expression. Studies have shown that latent membrane protein 1 (LMP1) is an Epstein-Barr virus-encoding oncogene protein associated with various signaling pathways such as NF-kB, activator-1, P38/MAPK and JAK3/STAT leucine phosphorylation. However, many signaling molecules associated with LMP1 and downstream target proteins are unclear. Therefore, we analyzed the phosphorylated proteome of the LMP1 signaling pathway to explore the regulatory pathway. **Methods:** We used concentrated retrivirus (RV-pLNSX, RV-LMP1^{WT}, RV-LMP1^{TRADD} and RV-LMP1$^{\triangle 232-351}$) to infect the nasopharyngeal epithelium NP69 cells, then combined with two-dimensional electrophoresis, antiphosphotyrosine imumuoblotting and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). The expression of LMP1 and Vimentin in 30 cases nasopharyngeal epithelial tissue and 60 cases nasopharyngeal carcinoma (NPC) were detected by immunohistochemistry. To investigate the effect of vimentin expression on EMT in NPC cells. **Results:** We identified some differentially tyrosine-phosphorylated proteins among NP69-PLNSX, NP69-LMP1^{WT} and NP69-LMP1^{TRADD} cell lines. Proteins, including heat shock protein 70, cytoskeleton 7, phosphatidylethanolamine binding protein 1 and tubulin, were yet unknown functional signaling molecules or targets in LMP1 signaling. In comparison to LMP1 (-) group, Vimentin expression significantly increased in nasopharyngeal epithelial tissue and NPC within LMP1 (+). High expression of Vimentin up-regulates TGF-β expression, down-regulates E-cadherin expression, and promotes cell migration and invasion. **Conclusions:** Our study identified 23 differentially tyrosine-phosphorylated proteins, which was helpful to clarify the molecular mechanism of LMP1. LMP1-mediated vimentin induces EMT and promotes migration and invasion in NPC cells.

TXNDC9 Regulates Oxidative Stress-Induced Androgen Receptor Signaling to Promote Prostate Cancer Progression

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Objective: Reactive oxygen species (ROS) and ROS induced oxidative stress are associated with prostate cancer (PCa) development and castrate-resistant tumor progression. This is in part through the activation of the androgen receptor (AR) signaling. However, the molecular underpinning of ROS to activate AR remains poorly understood. Previously, we and others have reported that the TRX family members, thioredoxin domain-containing protein 5 (TXNDC5) and thioredoxin-1 (TRX1), contribute to PCa progression by activating AR or protecting against AR-induced redox stress. Most recently, our preliminary data suggest that thioredoxin domain-containing protein 9 (TXNDC9) may play an important role in maintaining redox balance in PCa cells.
Materials and methods: A total of 177 localized PCa and 26 cases with CRPC were included in the current study. Immunohistochemistry (IHC) was used to evaluate the expression of TXNDC9 and its clinico-pathological significance. Bioinformatics analysis was used to determine the correlation between TXNDC9 expression and prostate cancer progression using public databases. Cellular function was evaluated by CCK-8, flow cytometry assays, respectively. Real-time PCR and western blot were used to analysis AR activity and relevant signaling pathways. Animal models were utilized to examine the effect of TXNDC9 *in vivo*. Co-immunoprecipitation was used to test the binding between proteins. GST-pull down assays followed by Mass Spectrometry to profile the interactome of TXNDC9 in PCa cells were used to elucidate its molecular mechanism in PCa.
Results: TXNDC9 expression is upregulated by ROS inducer, and increased TXNDC9 expression in patient tumors is associated with advanced clinical stages. TXNDC9 promotes PCa cell survival and proliferation. It is required for AR protein expression and AR transcriptional activity under oxidative stress conditions. Mechanistically, ROS inducers promote TXNDC9 to dissociate from PRDX1, but enhance a protein association with MDM2. Concurrently, PRDX1 enhances its association with AR. These protein interaction exchanges result in not only MDM2 protein degradation, but also PRDX1 mediated AR protein stabilization, and subsequent elevation of AR signaling. Blocking PRDX1 by its inhibitor, Conoidin A (CoA), suppresses AR signaling, PCa cell proliferation, and xenograft tumor growth even under androgen-deprived conditions. These tumor suppressive effects of CoA were further strengthened when in combination with enzalutamide treatment.
Conclusion: TXNDC9 is an important regulator of ROS to trigger AR signaling. Our data

demonstrate that the TXNDC9-PRDX1 axis plays an important role for ROS to activate AR functions. It provides a proof-of-principle that co-targeting AR and PRDX1 may be more effective to control PCa growth.

CRISPR/Cas9 screen identifies KLF11 as a key reguialor and potential target of sarcoma cancer stem cells

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Sarcoma cancer stem cells (CSCs) play crucial roles in tumor initiation, relapse, and therapeutic resistance, and therefore are the attractive therapeutic target. Through reporter-based CRISPR/Cas9 library screen, we uncovered Kruppel-like factor 11 (KLF11) as a key negative regulator of sarcoma CSCs. Mechanistically, KLF11 interacts with YAP and recruits SIN3A/HDAC repressor complex to restrain the transcriptional output of YAP. In turn, YAP/TEAD promoted KLF11 transcription to form a negative feedback loop. Intriguingly, KLF11 is epigenetically silenced in CSCs and aggressive sarcomas, contributing to loss of response to YAP/TEAD, and facilitating sustained
YAP activation. Low KLF11 expression was significantly associated with poor prognosis and chemotherapy response in sarcoma patients. Nevertheless, the thiazolidinedione activated KLF11 by PPARγ-mediated promoter demethylation, thus effectively restoring chemotherapy response and eliminating CSCs. Collectively, our study identifies KLF11 as a negative regulator for YAP/TEADdependent stemness maintenance, thus revealing a therapeutic strategy for sarcoma treatment.

Digital pathology for primary diagnosis: one-year experience at Medicover Digital

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Objectives:
Implementation of whole slide imaging (WSI) for routine practice has been accomplished in many pathology laboratories worldwide. Medicover Digital is the first digital platform in Eastern Europe that connects the three laboratories within this company. Laboratories are located in Poland, Romania and Serbia. We report the transition to an effective digital pathology workflow in the pathology laboratory at Beolab in Belgrade, Serbia.
Methods:
Histopathology glass slides were digitized at x40 using Philips Ultra Fast scanner. We scanned entire range of surgical pathology cases: biopsies and resections, including hematoxylin and eosin, immunohistochemistry, and special stains. Five senior pathologists daily used WSI in routine diagnostics and compared results with microscopic diagnostics. Digital images were accessed through laboratory system on either 15" laptops or desktop computers with 23" displays for remote or on-site digital reporting.
Results:
During one year, 4526 cases (about 18% of our routine work) with 34582 glass slides have been digitized. Scan fail rate was less than 1%, only in the cases of samples smaller than 1mm. Drying glass slides for two hours before scanning minimized them sticking to scanner racks. Most of the digitized cases were from pulmonary pathology (1500), hematopathology (1362), gastrointestinal pathology (786) and skin pathology (662).
Conclusion:
We conclude that WSI is equal to microscopy for primary diagnosis in surgical pathology, including biopsies and resections stained with hematoxylin and eosin, mmunohistochemistry and special stains. This conclusion is valid across a wide variety of organ systems and specimen types. We found that the use of WSI is faster and more precise in the diagnostics of bronchial biopsies and bone marrow biopsies.

Application of digital imaging and AI technologies in molecular cytopathology.

Prof. Robert Y. Osamura
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Now **genomic analysis i**s done very often for the prediction of molecular target therapies which are dependent upon certain genomic changes. For example, EGFR mutation predicts the effects of gefitinib, ALK rearrangement for crizotinib in non-small cell carcinomas of the lung. Now for the lung non-small cell carcinomas, the panels of EGFR mutation, ALK rearrangement, ROS1 rearrangement, BRAF mutation, and PD-L1 expression are recommended to analyze for the detection of the appropriate therapies. The genomic analysis can be done by the method of allele specific detection of genomic changes or panel detection such as next generation sequencing(NGS). And it has been known, in the preanalytic process, that cytology materials are suitable to detection genomic changes including allele specific detection and NGS. It has been shown that the cytology materials without FFPE process do preserve DNA and RNA better.
Both conventional and LBS materials do also perform better for the preservation of genomic status.
Digital pathology is now popular tool, in the analytic process, for the histological diagnosis of tissues obtained by biopsies or surgical resection. Recently, cytology slides also frequently scanned for digital images. One big issue here is the uneven thickness of the cell clusters in cytology, particularly adenocarcinomas. So capturing the images with multiple layers for Z-stacks is essential for scanning the cytology materials. The **artificial intelligence (AI)** is now on its way to be applied on the pathology sections and cytology(including hematology). Combination with AI technologies and digital images can select specific cancer cells which can be annotated for the specific application of technologies, such as molecular (genomic) analysis. Therefore, even though validation of each step is very critical, one of the future directions of molecular cytopathology would be the fully automated process from scanning the images to microdissection of the particular annotated areas which undergo to the molecular analysis.

KI-67 automatic analysis in Breast Invasive Ductal Carcinoma based on Whole Slide Image

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The positive rate of KI-67 protein in breast invasive ductal carcinoma (IDC) has important guiding significance for clinical pathological manifestations, tumor molecular typing, prognosis and treatment evaluation. On one hand, the KI-67 manual counting method based on traditional microscope was not accurate, and the estimates between different levels of pathologists were poorly repeatable, while the accurate KI-67 count needed to identify positive cells in tens of thousands of cells which was time-consuming and labor-intensive, it was difficult to achieve in the daily clinical work of pathologists; on the other hand, some KI-67 automatic counting methodswas difficult to automatically identify the matching IDC area. Therefore, in this study, we explored the possibility of developing KI-67 automatic identification and counting method in IDC pathological images based on IMAGE J. The research process was divided into three stages: identification of IDC area from the HE slides, registration of HE and IHC slides from the same tissue, and counting the positive rate of IHC (ki67). At the first step, we included 1017 breast IDC diagnosed slides on which the IDC regions were marked, then we extracted the features of these labeled digital slices, and trained the classification network model by GoogleNet Concept V1, which could be uesd to identify IDC regions automatically. Secondly, we chose 100 cases with both HE slices and their corresponding Ki-67 stained slices which is made by the serial sectioning technique for registration. Finally, the pathologist labeled these KI-67 stained sections and trained a classifier which could identify KI-67 positive / negative cells by the optimized IMAGE J program according to the labeled information to achieve the goal of automatically counting the positive rate of KI-67 in IDC area. The results showed that the accuracy, sensitivity and specificity of using the artificial intelligence software to automatically identify breast IDC regions were 89.44%, 85.05% and 95.23%, respectively. The accuracy of automatic calculation of Ki-67 positive rate in IDC could reach over 98.9%. Using this system to participate in the human-machine challenge of identifying the positive rate of KI-67 in tumor tissues, the accuracy of the software system was over 9/10 doctors, and the average time of 1 slice was 2.3 minutes with 1 GPU. In conclusion, this study confirmed that the use of artificial intelligence technology to develop WSI-based IDC pathology image KI-67 automatic counting software was feasible, and its accuracy could reach more than 98.9%. This research sheds light not only greatly improved on the pathologist's estimation efficiency and accuracy, and the repeatability of counting between different pathologists, but also provide more

reliable reference data for clinical work. Future, thisresearchwould meet the needs of pathologists in actual clinical work.

Development of Pathological Super-resolution Images using Artificial Intelligence based on Whole Slide Image

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Pathology plays a very important role in the cancer diagnosis, as the gold standard for the identification of tumors. The rapid development of digital pathology (DP) which based on Whole Slide Image (WSI) has led to many improvements in telepathological consultation, digital management, and computer-assisted diagnosis by artificial intelligence (AI). In DP, the common digitization strategy is to scan the pathology slice with X20 or X40 objective, the data generated by scan is called WSI (or digital slide). Usually, X40's WSI is 4 times bigger than the X20's which from the same slice, and obviously, the storage space and transmission time of the data should be 4 times. These increased costs will be great negative factor in the popularization of DP. But at the same time, some cases have to use the high magnification WSI for reliable diagnosis. In this article, we present a novel super-resolution process which could be used for WSI through Deep Learning. This process powered by AI, have the ability to switch X20 WSI to X40 without loss of whole and locally features. Furthermore, we collect the examples of WSI data of patients with 100 uterine leiomyosarcoma and adult granulosa cell tumor (AGCT) of ovary respectively, which are used to test our super-resolution process. We applied our trained model to the testing dataset including 2000 images with benchmark. We test bilinear down-sampled images from $40\times$ images (HR), and images directly from the $20\times$ images in WSI (SR). The mean PSNR and SSIM values between the SR images is 43.92 and 0.99, and which of HR images is 42.09 and 0.99, we can conclude that our SR results are extremely similar to the HR images. We used another evaluation method named BRISQUE, which is a kind of no-reference image quality assessment, the score is between 0 and 100, and the smaller the score is, the better quality the image has. The mean BRISQUE scores of the HR images is 52.19 and the SR images is 49.22, from the results, we can see that the quality of our results are even better than the HR images. Then, we tested the subjective evaluation of our SR images from the pathologist's perspective, two pathologists (intermediate titles) evaluated the SR images' authenticity subjectively, the results indicate that the SR images matches the performance of the HR ones. In addition, we extracted 400 images provided HR images and our SR images respectively but without any tags or comments, and let the pathologist to determine which are the true images (HR images). The test results showed that the probability of the two pathologists could accurately select the real images was 51.75% and 54.25% respectively, which objectively proved that the pathologists could hardly distinguish the difference between these two kinds of images, and further

confirmed the consistency between our SR images and the real HR images. The testing results indicate that the X40 WSI synthesized by the super-resolution matches the performance of the one generated from the X40 objective in diagnosis of both tumors. We believe that this is a reliable method can be used in a variety of tumors' digital slides, and will be available for a large scale in clinical pathology as an innovative technique.

Summary of rapid pathological diagnosis and analysis in transurethral plasma prostatectomy

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Abstract: Objective: To summarize the experience and key points of frozen pathological diagnosis in transurethral plasma prostatectomy by rapid pathology and routine histopathological diagnosis in 79 cases of transurethral plasma resection of the prostate. **Methods:** Patients with suspected prostatic cancer and urinary retention in the hospital were enrolled in our hospital from January to September 2019. All patients underwent minimally invasive transurethral resection of the prostate. During the operation, the frozen section was diagnosed and the histopathological examination was performed in parallel. **Result:** The surgical treatment of 79 patients with advanced prostate cancer and urinary retention was satisfactory. The residual urine volume of the bladder was significantly improved 3 months after operation. Among them, 11 cases of intraoperative rapid freezing could not be diagnosed and delayed diagnosis. Histopathology and adjuvant immunohistochemistry were diagnosed as prostate cancer. 11 cases were not clearly diagnosed, including tissue sampling defects, poor frozen sections, high degree of differentiation of prostate cancer (such as foam cell-like cancer) and low degree of differentiation of cancer cells, and difficult to identify with other malignant tumors. **Conclusion:** Rapid pathological diagnosis during prostatectomy, due to the type of the sample and the degree of differentiation of the tumor itself, the rapid freezing of the prostate is different from the conventional histopathology. The intraoperative frozen diagnosis must be carefully observed. Characteristics and changes should be based on the definition of the nature of the lesion and the degree of malignancy.

Gastric Histopathological Assistant Diagnosis System Based on Deep Learning: The First In-House Practice

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Objectives: Pathology plays a critical role in modern clinical practice to make diagnoses from the micro-level of tissue morphology. However, until now, pathology departments generally still practice in a more traditional and labor-intensive manner. Moreover, many countries suffer from pathologist's shortage, which can delay cancer diagnosis and, more grievously, decrease diagnosis accuracy. In this research, we report the latest progress of digitalization and artificial intelligence (AI) assistance system of the Chinese PLA General Hospital, China.

Materials and Methods: We built a gastric cancer dataset of over 3000 pixel-level labeled digital slides, which includes a large variety of specimens with diverse subtypes. The labeling was performed on a self-developed annotation system based on iPad and Apple Pencil. We utilized a CNN of Deeplab v3 architecture for our binary image segmentation approach, which allows for pixel-level cancer detection, to build our AI assistance diagnosis system. The whole slide images and their corresponding annotations were split into over 30 million 320x320-pixel patches before feeding into the network for training. In the inference phase, each pixel was assigned by the trained model a probability of being malignant. Compared with the commonly adapted patch classification with the sliding window method, the semantic segmentation framework generally runs much faster in the inference phase and gives a more detail-rich prediction. To understand how it can help pathologists with the diagnosis process, we also put the AI system to the test and conducted an experiment to see the difference between pathologists working with and without the help of AI.

Result: We found that each junior pathologist achieved higher accuracy and completed in less time with the assistance of AI. Furthermore, we tested the AI assistance system on over 6,000 gastric slides from the hospital's data archive from a consecutive period, obtaining an area under the curve (AUC) of over 0.97. It showed promising results on the large test set thus indicates the potential application of the AI assistance system as an initial screening system to further decrease the workload of pathologists and to help prevent misdiagnosis. By analyzing the details of the test set, we found our AI assistance system could generalize to different specimen types and performed equally

well on different scanners. Furthermore, we found that our AI assistance system was able to make a diagnosis for difficult slides that may require an immunochemistry test in the current diagnosis procedure. We ran the H&E stained slides through our AI assistance system and have achieved excellent performance (AUC = 0.959).

Conclusion: We demonstrate the first long-period in-house evaluation of an AI assistance system for digital pathology, that can classify a wide range of cancer subtypes and specimen types. The system has also been shown to help increase pathologist's diagnosis accuracy and stability, and prevent misdiagnosis. Lastly, the long-period in-house trial run shows the potential of using the AI assistance system as a preliminary screening measure in clinical procedures.

Category: Digital pathology and artificial intelligence
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Automated Histological Diagnosis of Colorectal Adenoma in Colon Biopsy Using Deep Learning

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Objectives: Colorectal adenoma is the most common precancerous lesion of colorectal adenocarcinomas. It is estimated that more than 50% of western people may suffer from colorectal adenoma during their lifetime, among which 5%-16% develop to adenocarcinoma. The early detection and treatment of adenomas can help avoid further development of the disease and save lives. However, analyzing hematoxylin and eosin (H&E) staining slides of colorectal adenoma is repetitive work, it is labor-intensive and somewhat subjective even to the most experienced pathologists. The microscopic evaluation of slides has been gradually moving towards all-digital in recent years, leading to the possibility for computer-aided diagnosis. In this research, we applied deep learning and modeled the task by using semantic segmentation to detect adenoma.
Materials and Methods: To effectively train the deep CNN, we had collected a total of 411 histological colorectal slides from the Chinese PLA General Hospital, of which 232 were diagnosed as colorectal adenomas, and 179 were normal mucosa or chronic inflammation which were categorized as non-neoplasm. We selected 177 cases for the training set, 40 cases for validation, and 194 cases as test samples. To further test the generalization ability of our model, we had also collected 168 slides from two other hospitals, including China-Japan Friendship Hospital and Cancer Hospital, Chinese Academy of Medical Sciences, composing the generation testing group. The detailed labeling was performed on a self-developed annotation system based on iPad. We built our model based on DeepLab v2 with ResNet-34. The model performance was tested on the test samples and compared with five pathologists. Furthermore, the generalization ability of the learning model was tested by extra 168 slides (111 with adenoma) collected from two other hospitals.
Result: The deep learning model achieved an area under the curve (AUC) of 0.92 on 194 test samples. The performance was on par with the performance of experienced pathologists, exceeding the average pathologist. Without any fine-tuning on the original model, we obtained a slide-level accuracy of over 90% on slides from two other

hospitals. By investigating the feature maps and cases misdiagnosed by the model, we found the concordance of thinking process in diagnosis between the deep learning model and pathologists. We have built a system supporting multiple GPUs for the automatic diagnosis process, the system performance increases near linearly with the hardware configuration (i.e., number of GPUs).

Conclusion: As modern medical treatments becoming more affordable, colon biopsies are now accessible to a wider population. It is necessary to establish an automatic diagnosis system with high accuracy, efficiency, and scalability. We built a complete system to assist the pathologists with the diagnosis process for colorectal adenomas. It is designed to be distributed and scales almost linearly with the increase of hardware resources. The automated diagnosis of colorectal adenoma in colon biopsy would avoid the time-consuming manual diagnosis and eliminate the interference of subjective factors, achieving more objective and accurate results.

The experience of culturing independent practice of pathology doctor by one to one teaching in clinic pathology residency training

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With the development of clinic medicine, the needs of clinic medicine for pathology are increasing. However, the number of pathology doctors in our country is very short. Culturing independent practice of pathology resident rapidly becomes very urgent. Herein , we introduce one to one teaching experience in pathology residency training. Meanwhile, we organize systemic clinic pathology courses, teaching case presentation, difficult cases discussion, multi-disciplinary team (MDT) , and report of advance of clinic pathology by pathology residents. After 3- year training, our pathology residents have the capacity of independent practice as pathology doctor, such as making correct pathology diagnosis for common diseases and cytology specimen, and choosing the proper technique and markers for difficult cases' diagnosis and differentiation. In conclusion, one to one teaching is an effective mode for culturing independent practice for pathology resident.

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