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STAT6 acetylation potentiates anti-tumor immunity

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STAT6 is known to drive macrophage M2 polarization. However, how macrophage polarization is fine-tuned by STAT6 is poorly understood. We found that during macrophage M2 polarization, STAT6 was acetylated by the acetyltransferase CREB-binding protein (CBP), which in turn suppressed macrophage M2 polarization. Additionally, we identified Trim24, a CBP-associated E3 ligase, promoted STAT6 acetylation through directly catalyzing CBP ubiquitination, which facilitated its recruitment to STAT6. Therefore, loss of Trim24 dramatically inhibited STAT6 acetylation, and thus promoted M2 polarization in both mouse and human macrophages, which consequently compromised the anti-tumor immune responses of macrophages. Together, our findings established STAT6 acetylation as an essential negative mechanism to curtail macrophage M2 polarization and highlighted the first identified acetylation site in STAT6.

Human Pluripotent Stem Cell-Based Disease Modeling and Drug Screening

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Human pluripotent stem cells (hPSCs) provide unlimited starting material to generate differentiated cells that can be used to build a functional organ. Essential to this pursuit is an efficient way to differentiate hPSCs into specific types of mature cells. Cell-permeable small molecules that can modulate the function of specific proteins provide a convenient and efficient approach to controlling stem/progenitor cell fate. Our laboratory has an in house chemical library containing 6,000 chemicals, including kinase inhibitors, signaling pathway regulators, nature products and FDA-approved drugs, and protein library containing 400 growth factors. Using high content and high throughput screening approaches, we have identified a series of small molecules that control stem cell self-renewal, differentiation and reprogramming. In addition, we have identified small molecules that direct hPSC differentiation into certain cell types, including pancreatic endocrine cells, pancreatic ductal epithelial cells, cardiac SA nodal cells, trophoblast cells, and colonic organoids.

Ferroptosis, Mechanisms and Role in Disease

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Programmed cell death (PCD) plays important role in normal biology, and its deregulation contributes to the development of various diseases. In recent years, multiple forms of PCD that are distinctive from the canonical PCD mechanism – apoptosis – have been discovered. Among these non-apoptotic mechanisms, ferroptosis is an iron-dependent (thus the name) modality of PCD. Execution of ferroptosis involves an imbalance of cellular redox coupled with strong cellular metabolism, leading to a wave of iron-dependent cellular lipid peroxidation, and ultimate cell death. In this talk, our recent findings on the mechanisms of ferroptosis, particularly the intimate communication of cellular metabolism with ferroptosis, will be discussed. The role of ferroptosis in human disease, including cancer and ischemic heart disease, and the potential of targeting ferroptosis for disease treatment, will also be discussed.

Leveraging Chromatin Interactome Information in post-GWAS studies

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Chromatin spatial organization information revealed from chromosome conformation capture (3C) and 3C-derived technologies has been delivering on the promise of aiding post-GWAS studies to generate mechanistic hypotheses. I will first point out challenges in the analysis of data from 3C-derived technologies, presenting methods we have recently developed to address these challenges, particularly for detecting long-range chromatin interactions. I will then introduce several chromatin interactome data we generated using Hi-C, PLAC-seq and promoter capture Hi-C technologies, focusing on those generated in brain related tissues and primary cell types. Finally, I will showcase examples of how these data have been used in our recent endeavors to better understand genetic mechanisms underlying multiple brain related diseases and traits, including schizophrenia and Alzheimer's disease.

Mitochondrial factors regulate the formation and dissolution of protein aggregates

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Proteostasis defect and mitochondria dysfunctions are two major hallmarks of aging. How these two hallmarks talk to each other is a current topic of aging research. In this study, we use budding yeast to explore the cellular strategies called in response to the loss of proteostasis and focus on the mechanism regulating the formation and dissolution of protein aggregates. We found that mitochondrial factors play a key role in determining the formation and localization of protein aggregates under stress conditions. Together with our previous discoveries that mitochondria import misfolded proteins for degradation, these results strengthen the direct connection between proteostasis and mitochondria.

A high-throughput screen identifies a class-II histone deacetylase as a novel aging regulator

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Aging is a complex biological phenomenon featuring functional declines associated with accumulation of damages or abnormalities at all levels of an organism, from organs and tissues to macromolecules inside the cell. The single-celled budding yeast is an important model for cellular and organismal aging due to its powerful genetics, biochemistry and short life span. Several aging regulatory pathways were initially discovered in yeast and are evolutionarily conserved across species, offering valuable insights into our understanding of the aging process.

Yeast replicates by asymmetric cell division, which produces a larger mother and a smaller daughter cell. The traditional replicative lifespan (RLS) assay by manual microdissection is labor-intensive and low-throughput, limiting its usefulness as a broad genetic screening platform for aging research. Using the old mother cell sorting approach and the next generation sequencing, we have developed a novel high-throughput method capable of screening thousands of mutants from any existing yeast mutant libraries for aging phenotypes. We performed this screen using the yeast ORF deletion library and found a strong correlation with an existing lifespan dataset obtained by manual microdissection method.

Through this screen, we identified 285 long-lived deletion mutants. Among the dozens of previously unidentified long-lived mutants, we focused on those with mutations to the histone deacetylase complex HDA. We found that HDA regulated aging through its roles in repressing the stress response pathways, especially DNA damage stress response. Specifically, HDA deacetylated the promoters of genes regulating the metabolism of storage carbohydrate, trehalose and glycogen. Activation of these storage carbohydrate pathways offered protection for the cells against stresses, antagonizing aging and promoting longevity.

Novel function of RNA binding protein in metabolic syndrome

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The main pathologic changes of metabolic syndrome are systemic insulin resistance and massive production of proinflammatory cytokines. In adipose tissue, macrophages infiltration and proinflammatory cytokines release arise in the early stage of insulin resistance and always last for the complete progression of diabetes. It has been reported that inhibition of macrophage-mediated inflammation will improve the sensitivity of peripheral tissues to insulin in early stage of disease and definitely slow down the progression of diabetes.

p34A is a kind of RNA binding protein with multiple functions, but its role in macrophages and insulin resistance has not been reported. Owing to the amelioration of obesity and insulin resistance in macrophage-specific p34A knockout mice (p34A^{Δmφ}), this paper aims to reveal the function of p34A in macrophage-mediated inflammation and insulin resistance. Firstly, in vivo results showed that p34A deletion in macrophage significantly improved HFD-induced overweight, body fat ratio, hepatic steatosis. Serum total triglyceride and total cholesterol were also significantly reduced in p34A^{Δmφ} mice compared with control group. GTT/ITT test and insulin signaling pathway analysis indicated that HFD-induced insulin resistance was notably improved in p34A^{Δmφ} mice. Obesity-associated insulin resistance is a result of imbalance of energy metabolism. Results of metabolic cage showed that p34A knockout increased energy expenditure and respiratory exchange rate, while did not impact energy intake. It suggested that p34A of macrophage may play a more important role in the process of insulin resistance and metabolism syndrome. Then, we found that quantities of infiltrated M1 macrophages in adipose tissue were decreased in p34A^{Δmφ} mice with HFD treatment, while not changed in normal diet treatment. Furthermore, M1 markers were evidently decreased in adipose tissue of p34A^{Δmφ} mice, and M2 markers were not significantly changed except IL-10. Therefore, p34A regulates insulin resistance and metabolic syndrome through controlling the polarization phenotype of ATMs. Our results enriched the regulatory

network of macrophage polarization and provided a new potential treatment for insulin resistance and metabolic syndrome by intervention of macrophage-mediated inflammatory responses.

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Diet, Gut Microbiota, and Health

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Our research focuses on how diet impacts health through gut microorganisms. We aim to treat diet-associated diseases using gut microorganisms or microorganism-generated products. The followings described our findings that show how diet affects cognition as well as skin and liver health. Our data revealed that a Western diet (WD) that contains a moderate amount of fat (21%) and high sucrose content (34%) could induce synaptic impairment measured by long-term potentiation tests suggesting reduced memory and learning ability caused by WD intake. In addition, WD-fed mice also had reduced expression of hippocampal neuronal growth factor and activated ERK1/2, a hallmark of Alzheimer's Disease. Moreover, WD consumption reduced brain post-synaptic density protein 95 (PSD-95) and increased the inflammatory signaling in the isolated microglia. Thus, WD intake leads to cognitive dysfunction. Water-soluble fiber inulin via bacterial fermentation generates butyrate. Our data revealed that inulin supplementation of WD-fed mice had intact neuroplasticity like control diet (CD)-fed mice. To understand the effect of diet on gut microbes, we quantified the copy number of cecal butyrate (*bcoA* and *buk*) and secondary bile acid deoxycholic acid (DCA) (*baiJ*)-producing bacterial genes. WD reduced the copy number of *bcoA* but did not change *buk*. However, supplementation with inulin increased both butyrate-producing genes in WD-fed mice. Conversely, WD intake increased *baiJ*, and inulin reduced it to a level lower than that of CD-fed mice suggesting the benefits of inulin. Thus, there is an inverse relationship between the growth of butyrate and DCA-generating bacteria. Interestingly, such an inversed growth relationship was also noted in polyps and colon cancer specimens implicating the importance of gut microbes. Together, these findings suggested the benefits of inulin and butyrate as well as the potential negative impact of DCA in neuroplasticity. Although obesity is a well-known co-morbidity of psoriasis, the causative relationship, if any, between obesity and psoriatic inflammation, remains to be elucidated. Moreover, whether all obesity-inducing diets

predispose patients to psoriasis is unclear. To address this question, we placed mice on the same WD used in cognition study followed by application of topical imiquimod (IMQ) to induce psoriasiform dermatitis (PsD). In comparison, we used a high-fat diet (HFD), which had 35% of fat and standard sucrose content found in CD. Our data revealed that HFD-fed mice had more body weight gain than WD-fed mice, but WD-fed mice showed more prominent PsD than HFD-fed mice after a 5-day IMQ treatment course. Gene expression levels of NLRP3, interleukin-1 β , and interleukin-6 were higher in WD-fed mice than that of HFD-fed mice. In addition, WD induced higher expression of neutrophil-associated markers and showed more Munro's microabscess upon IMQ stimulation. Thus, a WD, but not HFD, predisposes mice to enhanced susceptibility to the development of PsD, suggesting obesity alone is not enough to promote psoriasiform inflammation in the skin. Bile acids converted from hepatic cholesterol not only serve as digestive surfactants; but also regulate inflammation as well as lipid and sugar metabolism. This paradigm shift was spurred by the identification of their receptors including farnesoid x receptor (FXR). Primary and secondary bile acids are produced by hepatic and microbial enzymes, respectively. Thus, the production of bile acids is influenced by diet, hepatic enzymes, and individual gut microbiota, and bile acids can be the intrinsic molecules underlying how nutrient-associated gut microbes affect health at the systemic level. In the brain as well as the digestive tract, WD-fed mice had reduced signaling regulated by retinoic acid and bile acids, whose receptors form heterodimers to control metabolism and inflammation. Furthermore, WD intake caused dysbiosis and dysregulated bile acid synthesis with reduced endogenous ligands for bile acid receptors. Like WD-fed mice, FXR knockout mice had dysbiosis and dysregulated bile acid synthesis with increased Proteobacteria, and develop spontaneous steatohepatitis and liver carcinogenesis. Thus, dysregulated bile acid synthesis is always accompanied by dysbiosis, which is an etiology of liver carcinogenesis. Using antibiotic treatment, fecal transplantation, and butyrate supplementation, our data revealed the importance of butyrate and butyrate-generating bacteria in steatohepatitis development and prevention. Butyrate alone could prevent the development of cancer-prone steatohepatitis in WD-fed FXR knockout mice. Thus, diet through microbiota affects the liver disease process. To further prove the concept that bacteria and prebiotics can be used to treat steatohepatitis, we studied the effect of prebiotic milk oligosaccharides (MO) in combination with probiotics *Bifidobacterium infantis* (*B. infantis*). MO are complex sugars that selectively enhance the growth of *B. infantis*. WD-fed FXR KO mice,

which had cancer-prone steatohepatitis and reduced *B. infantis*, were supplemented with *B. infantis*, MO, or a combination of both. Our data showed that *B. infantis* and/or MO improved insulin sensitivity, the risk for cancer. In addition, all three treatments reduced expression of pro-inflammatory genes in the liver and ileum. Consistently, long-term 7 months treatment reduced hepatic lymphocyte infiltration in WD-fed FXR KO mice. In addition, *B. infantis*, but not MO, decreased hepatic triglyceride and cholesterol. A combination of both further reduced hepatic cholesterol. Furthermore, all three treatments reduced DCA and increased chenodeoxycholic acid as well as ursodeoxycholic acid level. Moreover, MO alone could increase the abundance of butyrate-generating bacterium that had a beneficial effect on steatohepatitis treatment. Together, *B. infantis* and prebiotics MO have their unique and combined effects in reversing steatohepatitis in WD-fed FXR KO mice.

In summary, diet via gut microorganisms has a huge impact on health. Since most diseases, including cancer, occur due to environmental factors, there is an urgent need to develop precision medicine and personalize dietary supplementation to prevent and treat disease based on an individual's microbiome.

Nuclear receptors in hepatomegaly and liver regeneration

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Nuclear receptors (NRs) play important roles in xenobiotic and endobiotic metabolism, and some of them are known to promote liver size and liver regeneration. However, only a few studies have explored the role and the exact mechanism of NR in hepatomegaly and liver regeneration. In this presentation, the effects of activation of pregnane X receptor (PXR), peroxisome proliferator-activated receptor- α (PPAR α) and constitutive androstane receptor (CAR) on liver enlargement and regeneration were evaluated in several strains of genetically-modified mice and animal models. Mechanistically, the interactions between these nuclear receptors and yes-associated protein (YAP) were presented. These findings have implications for understanding the physiological functions of nuclear receptors and suggest the potential for manipulation of liver size and liver regeneration.

Role of tumor microenvironment in myeloma drug resistance

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Multiple myeloma (MM) is an incurable plasma cell cancer characterized by tumor cell accumulation in the bone marrow (BM). The nature of MM as a bone cancer poses additional difficulties in disease management. Not only does the BM microenvironment confer MM chemoresistance, but bone cancer also causes bone pain, pathologic fractures, and hypercalcemia that require treatment. MM cell homing to BM is an active process throughout the disease pathogenesis. MM progression involves BM homing in which tumor cells from primary BM site(s) enter the peripheral circulation and migrate to secondary BM sites in the axial skeleton. However, the mechanism of MM BM homing is still poorly understood. We identified macrophage migration inhibitory factor (MIF) as a novel regulator of MM BM retention. Our results showed that MM cells overexpressed MIF, which was associated with advanced disease stage and poorer patient survival. Although knockdown of MIF in MM cells did not affect MM cell growth and survival *in vitro*, MIF-KD MM cells exhibited reduced affinity for BM and formed mainly extramedullary tumor in a human MM xenograft SCID mouse model. We further showed that MIF regulated the expression of several adhesion molecules on MM cells, and MIF-KD MM exhibited decreased cell adhesion to BMSCs, explaining at least in part why MIF-KD MM generated more extramedullary tumors *in vivo*. MM BM confers MM drug resistance and provides a tumor-promoting microenvironment. Our data indicate that MM-derived MIF may play an important role in conditioning MM tumor microenvironment. Since normal BMSCs secreted low MIF and MM-derived MIF was necessary to upregulate MIF expression in BMSCs, it is reasonable to speculate that normal BMSCs, when interacting with MIF^{low} MM cells, could not produce more MIF to overcome the shortage of this protein. Therefore, normal BM stroma could not retain MIF-KD MM cells. Therefore, treating MM by inhibiting MIF may not have any substantial technical obstacle. We are conducting further studies to address the potential of MIF-targeted treatment in MM.

Key Words: SCBA, Hematological Malignancies, Cancers, Microenvironment

Systematic genetic dissection of the hematopoietic stem cell niche in the fetal liver

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The liver is the main hematopoietic organ and the site of hematopoietic stem cell (HSC) expansion and maturation in mammalian fetuses. However, little is known about the nature of the HSC niche in the developing liver *in vivo*. Here, we genetically dissected the cellular components of the developing liver niche by determining the cellular sources of a key HSC niche factor, stem cell factor (SCF). We found that *Scf* was primarily expressed by endothelial cells and hepatic stellate cells. Conditional deletion of *Scf* from hematopoietic cells, hepatocytes, *Ng2*⁺ cells or endothelial cells did not affect HSC number or function. Deletion of *Scf* from hepatic stellate cells depleted HSCs. Nearly all HSCs were lost when *Scf* was deleted from both endothelial cells and hepatic stellate cells in the developing liver. Hepatic stellate cells significantly downregulate SCF and other candidate HSC-supporting factors around birth, coinciding with HSC egress from the liver. Strikingly, the transcriptome of fetal stellate cells resembled that of adult bone marrow mesenchymal stromal cells, but not adult stellate cells, suggesting that the HSC-supporting activity of stellate cells is specific to fetal development. Thus, hepatic stellate and endothelial cells create perivascular HSC niche in developing livers *in vivo* by producing SCF.

**Deciphering new mechanisms of hematopoietic stem cell development
in zebrafish and mouse embryos**

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The hematopoietic system is a paradigm for stem cell research. Hematopoietic stem cells (HSCs) are a population of multipotent cells that can self-renew and differentiate into all blood lineages. The development of HSCs and their derivatives must be tightly controlled, which involves a complex of extrinsic signaling and intrinsic factors. Studying regulatory mechanisms of developmental hematopoiesis in vivo in zebrafish and mouse models has greatly facilitated our understanding of HSC biology in vertebrates. I will talk about our recent new findings on HSC development, which may help to design new strategies for the generation and/or expansion of transplantable and functional HSCs in vitro.

Stress and Obesity

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Obesity and its associated metabolic diseases impose a huge burden to both patients and the society, and the current obesity epidemic is accompanied by exposure to high-fat high-caloric diet (HFD) and increasingly socioeconomic stress; however, the neural basis underlying diet-induced obesity and its potential interaction with stress response remain elusive. Here we found that HFD feeding blunted responsiveness of paraventricular hypothalamic (PVH) corticotropin-releasing hormone (CRH) neurons, a key stress-responsive neurons in the brain, to stressful stimuli, which normally induces rapid activation of CRH neurons. To specifically examine the role of loss of CRH neuron responsiveness, we generated mouse models with PVH CRH neuron activity clamped at a high or low level and both models were confirmed to show disrupted responsiveness of CRH neurons to stressful stimuli. Despite contrasting behaviors and metabolism at baseline chow-fed conditions, both models developed rapid HFD-induced obesity. Thus, blunted responsiveness of stress-responsive neurons, but not their absolute activity levels, mediates HFD-induced obesity, and may represent a key neural mechanism underlying diet- and stress-induced obesity.

A novel mechanism of Frizzled regulation in vertebrate development and human pathogenesis

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Our main research focus covers Wnt signaling in vertebrate (*Xenopus* and mouse) development, mechanisms of pluripotency during embryogenesis, gastrointestinal stem cell regulation in homeostasis, regeneration, and tumorigenesis in the mouse and human organoid models. We aim to provide insights into embryonic development and disease/cancer pathogenesis and therapeutics.

In the presentation I will discuss a genome-wide CRISPR/Cas9 screen designed to identify novel gene products that regulate Wnt signaling. I will focus on characterization of an orphan transmembrane protein that controls Frizzled (Wnt receptor) protein levels through an ER-lysosomal degradation pathway and regulates early vertebrate embryogenesis. Importantly, its characterization provides novel insights into a common inflammation disease.

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Regulation of Hippo and TAZ signaling in breast cancer

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TAZ and YAP are transcriptional coactivators that can contribute to cancer by promoting proliferation, tumorigenesis, and cancer stem cell expansion. Hippo signaling activates the Lats family of kinases, which phosphorylate TAZ and YAP, resulting in cytoplasmic retention and degradation and inhibition of their transcriptional activity. Hippo signaling can be activated by a wide variety of upstream signals and is itself regulated by crosstalk with other intracellular signaling pathways. One such regulator is SnoN. SnoN regulates multiple signaling pathways, including TGF- β /Smad and p53, and displays both pro-oncogenic and anti-oncogenic activities in human cancer. We have observed previously that both its intracellular localization and expression levels are sensitive to cell density, suggesting that it may crosstalk with Hippo signaling. Indeed, SnoN interacts with multiple components of the Hippo pathway to inhibit the binding of Lats2 to TAZ and the subsequent phosphorylation of TAZ, leading to TAZ stabilization. Consistently, SnoN enhances the transcriptional and oncogenic activities of TAZ, and reducing SnoN decreases TAZ expression as well as malignant progression of breast cancer cells. Interestingly, SnoN itself is downregulated by Lats2 that is activated by the Scribble basolateral polarity protein. Thus, SnoN is a critical component of the Hippo regulatory network that receives signals from the tissue architecture and polarity to coordinate the activity of intracellular signaling pathways. New results from recent investigations will be discussed.

Restriction of HIV infection and viral countermeasures

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The T cell Ig and mucin domain (TIM) proteins inhibit release of HIV-1 and other enveloped viruses by interacting with cell- and virion-associated phosphatidylserine (PS). Here, we present evidence that the Nef proteins of HIV-1 and other lentiviruses antagonize TIM-mediated restriction. TIM-1 more potently inhibits the release of Nef-deficient relative to Nef-expressing HIV-1, and ectopic expression of Nef relieves restriction. HIV-1 Nef does not down-regulate the overall level of TIM-1 expression, but promotes its internalization from the plasma membrane and sequesters its expression in intracellular compartments. Notably, Nef mutants defective in modulating membrane protein endocytic trafficking are incapable of antagonizing TIM-mediated inhibition of HIV-1 release. Intriguingly, depletion of SERINC3 or SERINC5 proteins in human peripheral blood mononuclear cells (PBMCs) attenuates TIM-1 restriction of HIV-1 release, in particular that of Nef-deficient viruses. In contrast, coexpression of SERINC3 or SERINC5 increases the expression of TIM-1 on the plasma membrane and potentiates TIM-mediated inhibition of HIV-1 production. Pulse-chase metabolic labeling reveals that the half-life of TIM-1 is extended by SERINC5 from <2 to ~6 hours, suggesting that SERINC5 stabilizes the expression of TIM-1. Consistent with a role for SERINC protein in potentiating TIM-1 restriction, we find that MLV glycoGag and EIAV S2 proteins, which, like Nef, antagonize SERINC-mediated diminishment of HIV-1 infectivity, also effectively counteract TIM-mediated inhibition of HIV-1 release. Collectively, our work reveals a role of Nef in antagonizing TIM-1 and highlights the complex interplay between Nef and HIV-1 restriction by TIMs and SERINC.

Ubiquitination regulation of type-I interferon signaling and antiviral efficacy

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The outbreak of viral infections has been a global health burden. Despite advances in development of drugs for specific viruses, these drugs are far from sufficient for coping with frequent mutations of viruses. This fact warns us of the importance and urgency of developing broad-spectrum antiviral drugs. The interferon (IFN) family has broad-spectrum antiviral actions that are much more diverse than any of synthetic compounds. However, IFN antiviral efficacy in host cells is largely attenuated by either intracellular signaling or viral infection, which limits IFN application for the therapy of many types of viruses. Recently, protein ubiquitination and deubiquitination has been recognized to be a delicate mechanism for regulating cellular signaling pathway. Importantly, the agents targeting the ubiquitination and deubiquitination system have offered great hope for therapy of many diseases. Thus, we have been exploring the detailed mechanisms by which ubiquitination/deubiquitination controls the strength of type-I IFN signaling, aiming at providing new strategies for enhancing IFN antiviral efficacy. In this presentation, we will talk about several interesting findings about ubiquitination regulation and IFN signaling.

Epigenetic modifications in the development of intestinal stem cells

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Adult organ-specific stem cells are essential for organ homeostasis and tissue repair and regeneration, but the underlying mechanism for their development is unclear. Intestinal remodeling during frog metamorphosis offers a unique opportunity to study developmental formation of such stem cells. During metamorphosis, the intestine is completely remodeled with the larval epithelial cells undergo apoptosis and are replaced by adult epithelial cells formed de novo in a process controlled by thyroid hormone (T3). We have shown that the adult stem cells are induced by T3 through dedifferentiation of larval epithelial cells. T3 exerts its metamorphic effects through T3 receptors (TRs). TRs recruit, in a T3-dependent manner, cofactor complexes for chromatin remodeling/histone modifications. We have demonstrated that the expression of two histone methyltransferases, Dot1L and PRMT1, are activated by T3 during intestinal remodeling. Our studies further suggest that both are recruited by TR during metamorphosis to function as TR coactivators to promote gene regulation and intestinal stem cell formation and/or proliferation through histone methylation.

The metabolic regulation by hepatic thyroid hormone receptor: a lesson from the biological evaluation of its liver-targeted agonists

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Thyroid hormone (TH) plays pivotal roles in growth, differentiation, development and metabolic homeostasis. The physiological function of TH is mainly carried out by TH receptor (TR) α and β in nucleus, which are differentially expressed in adult tissues and regulate transcription in a ligand-dependent manner. It has been recognized for decades that TH has profound effects on energy metabolism, as well as lipid and carbohydrate homeostasis. Based on the findings from human subject research, the application TH mimetics, and TR isoform-specific knockout and knockin mouse models, it has been well-accepted that TR β 1, the predominant TR in the liver, is primarily responsible for the lipid-lowering and anti-obesity effect of TH. Growing evidence suggest that crosstalk between key metabolic tissues plays an important role in the regulation of the whole-body physiology. However, whether hepatic TR is able to regulate whole-body homeostasis through mechanisms of interorgan communication remains largely unexplored. In current study, in order to substantiate our understanding of TR isoform specific functions, especially hepatic TR β 1-mediated metabolic effects, we took advantage of a group of liver-selective compounds, which are cytochrome P450-activated prodrugs of phosphonate-containing TR agonists and undergo hepatic first-pass extraction and tissue-selective activation by enzymatic cleavage. In agreement with previously findings, we found that administration of the synthetic liver-selective compound could lead to selective activation of liver TRs without affecting TH responsive marker genes in other tissues, accompanied with a decrease in serum cholesterol levels. Interestingly, we also found that treatment of liver-selective compound favorably affects body adiposity, suggesting that crosstalk between the liver and adipose tissue might be involved. Importantly, we observed that the serum levels of FGF21 were increased after treatment, suggesting that FGF21 might contribute

to the beneficial effects. Thus, we established an approach to investigate the role of hepatic TR in the metabolic regulation of whole-body energy homeostasis. Our work will largely substantiate the understanding of TR isoform specific functions in energy metabolism.

The role of oncogenic lipid kinase in neuroendocrine prostate cancer progression

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Androgen deprivation therapy (ADT) is considered the most effective regimen to treat metastatic prostate cancer (PCa). However, almost all patients eventually develop castration resistant PCa (CRPC) that is associated with the majority of mortality of this disease. Although many new agents have been introduced for these patients, the outcome is still unsatisfied. Obviously, characterizing cell features of CRPC could provide new strategies of eradicating this disease. Recent clinical observations indicate the appearance of neuroendocrine markers in 20% of Enzalutamide or Abiraterone-resistant CRPC patients, which is classified as neuroendocrine progression prostate cancer (NePC) with neuronal markers expression and neuronal factors secretion in an endocrine fashion. NePC is a lethal disease subset, with a median overall survival of less than 1 year from the time of detection. NePC is commonly seen as a treatment-emergent phenomenon among patients with CRPC following treatment with ADT. Currently, there is no effective regimen for treating NEPC. Thus, developing new therapeutic strategy based on mechanisms leading to resistant phenotype is urgently needed for prolong the survival of CRPC patients.

PCa is known as lipid-rich tumor; elevated lipogenic enzymes have been observed in clinical specimens; PCa patients receiving long-term ADT had metabolic syndrome compared with control group. However, it is unclear the link of lipid metabolism with NePC onset in CRPC patients. By searching cBioPortal of PCa database for genetic alteration, we noticed that 22% NePC has Sphingosine kinase-1 (SphK1) gene amplification, which is significantly higher than primary PCa. SphK1 is the enzyme converting sphingosine into Sphingosine 1-phosphate (S1P) that is a lipid mediator playing a major regulatory role in tumor cell growth, survival, invasion, angiogenesis, and therapeutic resistance. SphK1 serves the dual function of producing the pro-growth, anti-apoptotic S1P, and decreasing intracellular levels of pro-apoptotic ceramide. We further

analyzed the relationship between SphK1 mRNA expression with several NE transcriptional factors (NETF: FOXA2, BRN2, SOX2, EZH2) and NE makers (NEMK: synaptophysin [Syp], chromogranin A [CgA]) from TCGA PCa dataset and results indicated a significant positive correlation between among the mRNA expression of these genes. Indeed, the elevated SphK1 mRNA expression level is associated with those highly elevated NETFs and NEMKs in NePC specimens. Using immunohistochemical (IHC) staining of a small set of PCa specimens, the representative result indicated highly significant elevation of SphK1 protein expression in NePC compared with that in benign or adenocarcinoma of prostate. Taken together, we believe that overexpression of SphK1 is associated NePC development.

ADT has generally been perceived to be a direct response of the androgen receptor-expressing PCa cells to an androgen-depleted environment, some studies of the PCa regression process suggest that it might instead be initiated by an indirect response of the prostatic parenchyma to a hypoxic environment caused by a drastic reduction of blood flow to the tissue that occurs when androgens are withdrawn. Also, the expression of hypoxia-inducible factor (HIF-1- α) increases the risk of CRPC and metastases in patients on ADT. We noticed that both LNCaP and 22RV1 under hypoxia condition underwent NED. Concurrently, a significant elevation of SphK1 protein was also detected in both cells plated in hypoxia Chamber (HypOxystation® H35, HypOxygen, Frederick, MD) compared with normoxia for 48 hrs. In addition, cells pre-treated with SphK1 inhibitor (5 μ M SKI-II, Sigma-Aldrich, St. Louis, MO) 30 mins prior to hypoxia treatment and results indicated that significant reduction of several NETFs and NEMKs mRNA expression were detected in both cells compared with those under hypoxia condition but without SKI-II treatment. These data indicate that the induction of SphK1 in PCa cells under hypoxia plays a key driver role in NePC development.

To evaluate SphK1 as a druggable target, we first tested two small molecule inhibitors (i.e., SKI-II and FTY720). By treating NePC cells with different concentrations of SKI-II (100 nM, 1 mM and 10mM), this inhibitor is able to suppress the growth of PC3 cells in a dose-dependent manner; IC50 is about 10 mM (48 hrs) or 1 mM (96 hrs), indicating that Sphk1 is responsible for NePC proliferation. However, this inhibition can be partially reversed by adding 10 mM sphingosine-1-phosphate (S1P as a SphK1 product), suggesting that the presence of alternative pathway(s) in addition to SphK1 canonical pathway (i.e., S1P-S1P receptor) might contribute to the

growth of NePC. These results of growth inhibition are similar with the expression of several NETFs; the expression of FOXA2, BRN2 and EZH2 protein was suppressed by 10 mM SKI-II and S1P treatment only recovered EZH2 but not FOXA2, BRN2. In addition, LNCaP-MDVR cells showed good sensitive to another SphK1 inhibitor (FTY720, 5 mM). Furthermore, combination of FTY720 and Enzalutamide exhibited a synergistic effect on LNCaP-MDVR. These data provide a potential new therapeutic strategy to NePC.

Regulation of the 26S Proteasome in Health and Disease

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The 26S proteasome is the major machinery for protein degradation in eukaryotic cells. Despite its critical importance for almost every cellular activity, how the proteasome itself is regulated remains largely unknown. Mounting evidence suggests that the proteasome is a highly dynamic protein complex whose biogenesis, enzymatic activity, substrate selectivity and subcellular localization can change under various physiological and pathological conditions. To understand the underlying mechanisms, we have been focusing on post-translational modifications of proteasome proteins, with an emphasis on reversible proteasome phosphorylation. Over 400 phosphosites have been identified on human 26S proteasome subunits by mass spectrometry, whereas ~99% of them have not been functionally characterized. Using phospho-specific antibodies, gene editing, kinome library screening, quantitative MS and mouse models, we have investigated the regulation and function of several proteasome phosphorylations and determined their kinases and phosphatases. The findings established new links between proteasome dynamics and normal development as well as cancer formation. More recently, we became interested in membrane-anchored proteasomes, whose localization and assembly are controlled by crosstalks among several types of proteasome modifications. I will describe our latest findings and discuss the potential importance of spatio-temporal regulation of the proteasome in health, disease and evolution.

A glucose-induced protein degradation axis in control of insulin secretion, obesity and diabetes

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The family of Cullin RING E3 Ligases (CRLs) are activated by neddylation and regulated by the deneddylase COP9 Signalosome (CSN), which sequesters yet protects CRL. Biochemical principles and physiological implications of such exquisite regulation in mammals are not clear. Through x-ray crystallography and cryo-EM map analysis, we show that the small metabolite inositol hexakisphosphate (IP6) is a CSN cofactor bridging CRL4-CSN interactions to promote CRL4 deneddylation. IP6 binds to a basic pocket formed by CSN subunit 2 (CSN2) and the RING domain protein Rbx1. The IP6 binding-deficient Csn2K70E/K70E knock-in mice are embryonic lethal. Heterozygous Csn2WT/K70E mice exhibits enhanced pancreatic Cul4 neddylation, CRL4COP1 assembly, and degradation of the obesity-associated transcription factor ETV5, which suppress insulin release in a fasting-inducible manner. Consequently, Csn2WT/K70E mice display congenital hyperinsulinemia, insulin resistance, and aggravated obesity and diabetes under high-fat diet (HFD). The CRL neddylation inhibitor Pevonedistat/MLN4924 prevents feeding- or HFD-induced ETV5 degradation, normalizes insulin levels and body weight of Csn2WT/K70E mice, and attenuates HFD- or leptin deficiency (ob/ob)-induced obesity and diabetes. These structural and functional data uncover IP6 regulation of a CSN-CRL4COP1-ETV5 proteolytic checkpoint safeguarding nutrient-induced insulin secretion, and support insulin hypersecretion inhibition as an unorthodox strategy to reduce obesity and diabetic risk.

A novel role of Ca²⁺ in genome protection

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DNA replication is essential for life, but it also presents a leading source of mutations and genomic instability in cancer. The progression of the replication fork can be impeded by many factors including insufficient nucleotides, DNA lesions and secondary structures, and collisions with the transcription machinery. These challenges necessitate mechanisms that preserve stalled fork structure so that replication can restart and complete after the removal of the stress. The ATR-Chk1 checkpoint and a number of fork-associated factors such as BRCA1, BRCA2, FANCD2, RAD51 and BOD1L have been shown to play a crucial role in fork protection after replication stress. We have recently identified a new fork protection signaling pathway, which acts to restrain the activity of the exonuclease Exo1 to prevent aberrant fork processing that otherwise could cause fork collapse and DNA damage. Our results suggest that replication stress elevates intracellular Ca²⁺ levels, leading to the activation of CaMKK2 and the downstream kinase AMPK. Following activation, AMPK directly phosphorylates Exo1 to prevent its association with stressed replication forks, thereby avoiding deleterious fork processing. Disruption of this signaling cascade results in excessive fork degradation, chromosomal instability and hypersensitivity to replication stress inducers. This finding reveals a novel link between calcium signaling and genome maintenance during replication stress, and may have implications for cancer formation and treatment.

Novel insights into GATA1 regulation at post-translational level in human erythropoiesis

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Erythropoiesis refers to the process of differentiation of pluripotent hematopoietic stem cells into mature red blood cells, which is necessary for the body to maintain normal hematopoietic function. Erythropoiesis is precisely regulated by transcription factors. Among them, GATA1 is one of the most important transcription factors. It has been found that disorder in GATA1 protein expression is closely related to β -thalassemia, MDS and other diseases, and thus it is worthy of further study. Previous studies have shown that GATA1 undergoes dynamic changes in expression during erythropoiesis and is subject to complex regulation at multiple levels from gene to protein expression. Studies on the new complex components of GATA1 still need to be further explored. In this study, we identified the novel interacting molecules of GATA1 and the new transcription factor regulating GATA1. Creatively clarify the new mechanisms of GATA1 expression regulation. Our study enriches the basic theory of erythroid development regulation and provide a new strategy for the diagnosis and treatment of GATA1-related blood diseases.

Diet, Metabolism and Cancer

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Lifestyle choices such as diet and physical activity significantly affect risk for cancer. However, since most of the epidemiological studies do not control for specific genetic backgrounds, a pathogenic link between diets and particular oncogenic mutations remains unknown. In addition, dietary advice and dietary supplement choice are typically not based on an individual's specific genetic background, thus the identity and action mechanisms are unclear of circulating diet-derived nutrients and dietary supplements that are defined as "blood chemicals" and may affect oncogenesis involving oncogenic mutations or carcinogens that "battery-charge" oncogenic mutations. Our metabolic signaling-based studies to date strongly support the overarching goal of this research proposal that mechanistic understanding of oncogene-specific metabolic requirements and their pathogenic consequences in cancer holds promise for defining the pathogenic links between diet and cancer. These studies are rooted in new discoveries made in my laboratory that dietary fat-fueled ketogenesis plays a pathogenic role in BRAF V600E tumor growth, where mechanistically ketone body acetoacetate selectively promotes BRAF V600E-MEK1 binding. Moreover, we found that chondroitin sulfate, a generally safe and widely used dietary supplement for osteoarthritis and joint pain, exhibits BRAF V600E-specific pro-tumor effect. Thus, our mechanistic and translational studies not only inform development of metabolism-targeted therapeutic strategies and provide mechanism-driven rationales for clinical and epidemiological studies, but also allow physicians or pharmacists to consider an individual's specific genetic background to provide reliable advice for diet or dietary supplement choices with low cancer risk, and educate people to seek advice from informed resources, since many people "self-prescribe" dietary supplements.

A Non-Canonical Metabolic Function of Circadian Clock Gene in

Obesity

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Hyperinsulinemia is a hallmark of pre-diabetes that exacerbates breast cancer (BC) outcomes. We sought to understand how hyperinsulinemia drives tumor progression and dysregulated cancer cell respiration. Circadian rhythms regulate a wide variety of pathophysiological events, including cellular metabolism and pathogenesis of cancer. Disrupted circadian signaling is likely to be a feature of cancer. We will discuss using two distinct BC models to investigate the impact of intrinsic BMAL1 dysfunction on triple-negative BC (TNBC) cell biology in the context of hyperinsulinemic obesity. We demonstrate a novel impact by tumor BMAL1 on shaping the mitochondrial fuel flexibility and anti-tumor landscape. Our observations provide insights into the mechanism underlying tumor intrinsic BMAL1-mediated TNBC suppression and evidence that BMAL1 dysfunction combined with extrinsic hyperinsulinemia exacerbate TNBC outcomes. We will discuss the therapeutic implications for TNBC patients with metabolic syndrome/pre-diabetes and whose tumors have decreased BMAL1 expression.

Targeting KDM8, an AR coactivator and metabolism regulator, in prostate cancer

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During the evolution into castration or therapy resistance, prostate cancer cells reprogram the androgen responses to cope with the diminishing level of androgens, and undergo metabolic adaption to the nutritionally deprived and hypoxia conditions. AR (androgen receptor) and PKM2 (pyruvate kinase M2) play key roles in these processes. We report in this study, KDM8/JMJD5, a histone lysine demethylase/dioxygenase, exhibits a novel property as a dual coactivator of AR and

PKM2 and as such, it is a potent inducer of castration and therapy resistance. Previously we showed that KDM8 is involved in the regulation of cell cycle and tumor metabolism in breast cancer cells. Its role in prostate cancer has not been explored. Here, we show that KDM8's oncogenic properties in prostate cancer come from its direct interaction 1) with AR to affect androgen response and 2) with PKM2 to regulate tumor metabolism. The interaction with AR leads to the elevated expression of androgen response genes in androgen-deprived conditions. They include ANCCA/ATAD2 and EZH2, which are directly targeted by KDM8 and involved in sustaining the survival of the cell under hormone-deprived conditions. Notably, in enzalutamide resistant cells, the expressions of both KDM8 and EZH2 are further elevated, so are neuroendocrine markers. Consequently, EZH2 inhibitors or KDM8 knockdown both resensitize the cells toward enzalutamide. In cytosol, KDM8 associates with PKM2, the gate keeper of pyruvate flux and translocates PKM2 into nucleus, where the KDM8/PKM2 complex serves as a coactivator of HIF-1 α to upregulate glycolytic genes. Using shRNA knockdown, we validate KDM8's functions as a regulator for both androgen-responsive and metabolic genes. KDM8 thus presents itself as an ideal therapeutic target for metabolic adaptation and castration resistance of prostate cancer cells.

BIK ubiquitination controls life-death fate of cellular stress responses and anti-tumor activity

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The BH3-only pro-apoptotic protein BIK is regulated by ubiquitin-proteasome system. However, the underlying mechanism of this regulation and its physiological functions remain elusive. Here, we identify the function of E3 ligase CRL5^{ASB11} in targeting BIK for ubiquitination and degradation. Under ER stress, ASB11 is activated by XBP1s during the adaptive phase of unfolded protein response, thereby stimulating BIK ubiquitination, interaction with p97/VCP, and proteolysis. This BIK degradation mechanism contributes to ER stress adaptation by promoting cell survival. Conversely, genotoxic agents downregulate IRE1a/XBP1s/ASB11 axis to stabilize BIK. This mechanism participates in part to the apoptotic response to DNA damage. We further show that blockage of this BIK degradation pathway by IRE1a inhibitor leads to stabilization of BIK active mutant and increase of its ant-tumor efficacy. Our study uncovers the opposite regulations of this BIK ubiquitination pathway by different cellular stresses for determining cell life-death decision, and develops an anti-cancer strategy involving the targeting of this BIK ubiquitination pathway.

Targeting DNA damage repair in cancer therapy

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DNA double strand breaks (DSBs) are repaired by nonhomologous end joining (NHEJ) and homologous recombination (HR) pathways in mammalian cells. It is speculated that which pathway to use for DSB repair is mainly controlled by end resection process. This repair pathway choice is important for tumor response to PARP inhibition, which is now accepted therapeutic strategy for cancer patients carrying BRCA mutations. While BRCA1 promotes end resection and therefore favors HR repair, 53BP1 inhibits end resection and engages NHEJ pathway for DSB repair. We and others showed previously that RIF1 is a major downstream effector of 53BP1 and participates in 53BP1-dependent inhibition of end resection. Interestingly, while RIF1 accumulation at DSBs is antagonized by BRCA1 in S and G2 phases, the translocation of BRCA1 to damage sites in G1 cells is inhibited by RIF1, indicating that 53BP1-dependent pathway and BRCA1 counteract each other in a cell cycle-dependent manner. We showed that this cell cycle-dependent regulation is in part regulated by BRCA1-dependent inhibition of 53BP1 phosphorylation in S/G2 phase cells, which requires the E3 ubiquitin ligase activity of BRCA1. Besides RIF1, another DNA damage repair protein PTIP could also act downstream of 53BP1 and counteract BRCA1 function in DNA repair. We discovered that a nuclease SNM1C/Artemis associates with PTIP and functions to prevent end resection and HR repair. In addition, we and others demonstrated that REV7/MAD2L2 acts downstream of RIF1 and inhibits HR repair. Therefore, it is believed that 53BP1 controls RIF1-REV7 and PTIP-Artemis to promote NHEJ and suppress HR repair. We and others recently uncovered another 53BP1-binding protein, NUDT16L1 (also called Tudor Interacting Repair Regulator, TIRR), which associates with 53BP1 and regulates 53BP1 localization to DNA damage sites. We are now further investigating the regulation of DSB repair pathways and damage-induced checkpoint control. In addition, we are performing genome wide CRISPR/Cas9 screens and have identified RNASEH2 deficiency as potential biomarker for ATR inhibitor (ATRi)-based therapy. Moreover, we showed that ATRi could potentiate radiation-induced anti-tumor immune response. Therefore, these studies reveal the interplays between DNA damage repair and multiple cellular processes, which will help improve therapeutic outcome for cancer patients.

Calcium-dependent mechanisms in neurodevelopment

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Calcium ions serve as critical signalling elements essential for neuronal development, maturation, and survival. An optimal intracellular calcium level is required for the cellular and subcellular processes, and is tightly regulated to ensure proper functions of neurons. The cytoplasmic calcium conducting channels permitting transient influx of extracellular calcium ions are one of the major mechanisms that regulate the intracellular calcium levels. The transient elevated free calcium can be amplified further by calcium released from the internal stores. Calcium-dependent signaling processes require calcium-binding proteins. In this talk, I will discuss the functional insight into regulatory mechanisms of calcium signaling during neurodevelopment and maturation, under physiological and pathophysiological conditions.

The neuronal protective effect of artemisinin and its implication in the treatment of Alzheimer's Disease

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Artemisinin is a clinically safe and potent anti-malarial drug that possess the most rapid action of all the currently available drugs against plasmodium falciparum malaria. At present, we found that artemisinin protected different neuronal cells such as PC12 cells, SH-SY5Y cells and primary cultured neurons from oxidative stress-induced cell damage. For instance, artemisinin pretreatment significantly attenuated H₂O₂/ SNP/A β -induced cell damage in PC12 cells, and this protective effect was attributed to reduced intracellular reactive oxygen species (ROS) production, LDH release, caspase 3/7 activities, cell apoptosis and the maintenance of mitochondrial membrane potential. Western blot analysis showed that artemisinin induced the phosphorylation of ERK, AMPK and CREB, but not AKT. Moreover, the protective effect of artemisinin was abolished by knocking down ERK and AMPK by siRNA or blocking these signaling pathways by specific inhibitors PD98059 and Compound C, whereas the PI3K inhibitor LY294002 had no effect. These results suggested that artemisinin confers neuroprotective effects by suppressing oxidative stress induced cell damage via the activation of ERK/AMPK signaling. In addition, our current data showed that artemether improved the recognition deficit of AD mice. These results support that artemether could be potentially used for preventing neuronal degenerative disorders like Alzheimer's disease.

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Circuit Mechanisms Underlying the Cortical Control of Innate Reflexes

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Innate reflexes, critical for an animal's survival, are mediated by hardwired subcortical structures such as the brainstem. Although innate, in mammals these behaviors can be plastically modified according to prevailing conditions or past experience, which greatly expands their behavioral repertoire. A prime example is the potentiation of the optokinetic reflex (OKR), an involuntary eye movement that stabilizes images on the retina while the animal moves. Previously, OKR plasticity was thought to be entirely mediated by the brainstem and cerebellum. However, we discovered a prominent role of the visual cortex in this plasticity. In particular, we showed that a plastic increase in the amplitude of the OKR, induced by impairing another image stabilization mechanism, was reversed upon silencing visual cortex. Furthermore, selective ablation of the descending projection from the visual cortex prevented the cortical contribution to the OKR potentiation, indicating that this specific corticofugal pathway is necessary for the visual cortex to adaptively regulate OKR. Finally, our preliminary data suggest that this descending projection originates mainly from specific visual cortical area and carry unique visual information. Our findings will provide critical insights into the role of corticofugal projections in adaptively regulating innate behaviors.

CCDC3: A NEW p63 TARGET INVOLVED IN REGULATION OF LIVER LIPID METABOLISM

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TAp63, a member of the p53 family, has been shown to regulate energy metabolism. Here, we report coiled coil domain-containing 3 (CCDC3) as a new TAp63 target. TAp63, but not Δ Np63, p53 or p73, upregulates CCDC3 expression by directly binding to its enhancer region. The CCDC3 expression is markedly reduced in TAp63-null mouse embryonic fibroblasts and brown adipose tissues and by tumor necrosis factor alpha that reduces p63 transcriptional activity, but induced by metformin, an anti-diabetic drug that activates p63. Also, the expression of CCDC3 is positively correlated with TAp63 levels, but conversely with Δ Np63 levels, during adipocyte differentiation. Interestingly, CCDC3, as a secreted protein, targets liver cancer cells and increases long chain polyunsaturated fatty acids, but decreases ceramide in the cells. CCDC3 alleviates glucose intolerance, insulin resistance and steatosis formation in transgenic CCDC3 mice on high-fat diet (HFD) by reducing the expression of hepatic PPAR γ and its target gene CIDEA as well as other genes involved in de novo lipogenesis. Similar results are reproduced by hepatic expression of ectopic CCDC3 in mice on HFD. Altogether, these results demonstrate that CCDC3 modulates liver lipid metabolism by inhibiting liver de novo lipogenesis as a downstream player of the p63 network.

Helping p53 to better protect stem cells and life

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p53 is the guardian of the genome and a potent tumor suppressor frequently mutated, lost or inactivated in human cancer. However, p53-dependent normal tissue injury such as in the intestinal epithelium leads to dose limiting side effects in patients receiving radiation and chemotherapy, currently with no FDA-approved treatment. The intestinal epithelium is the fastest renewing tissue in an adult mammal and maintained by intestinal stem cells (ISCs). It is responsible for nutrient absorption and hormone production, and serves as the vital barrier against microorganisms to prevent inflammation. Our recent work uncoupled the protective and destructive p53 arms in ISC-intrinsic DNA damage response. Selectively blocking p53- and PUMA-dependent apoptosis, not p53 or p21, potently and selectively protects against radiation- and chemotherapy-induced lethal GI injury through enhanced stem cell survival, regeneration and DNA damage repair and removal. This can be achieved using small molecular inhibitors of GSK3, CDK4/6 or PUMA targeting distinct regulatory mechanisms. p53- and PUMA-dependent loss of Lgr5+ CBC stem cells triggers niche perturbation, activation of upper quiescent stem cells, which is exacerbated upon repeated DNA damage and leads to persistent barrier dysfunction, inflammation and stem cell exhaustion. Interestingly, blocking PUMA-dependent apoptosis delays the kinetics of local inflammation and proliferation with enhanced ISC intrinsic immune signaling. On the contrary, p53 loss strongly sensitizes mice to DNA damage and lethal GI inflammation. Our going work aims to uncover mechanisms coupling p53-dependent DNA damage response to ISC regeneration to help extend ISC longevity. This approach is envisioned to be highly selectivity based on prevalent p53 pathway inactivation in human cancer, and might offer patient new hopes for better outcome and less suffering.

Regulation of gain-of-function mutant p53 in cancer progression and treatment

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Tumor suppressor p53 is the most frequently mutated gene in human tumors. Mutant p53 (mutp53) not only loses the tumor suppressive activity of wild type p53, but also often gains new oncogenic activities to promote tumorigenesis, which is defined as mutp53 gain of function (GOF). While the concept of mutp53 GOF is now established, its underlying mechanism is not well-understood. Further, mutp53 proteins often accumulate at high levels in human tumors, which is important for mutp53 to exert their GOFs. The mechanism underlying mutp53 proteins accumulation and stabilization in tumors is also not well-understood.

Employing an unbiased high throughput immunoprecipitation combined with mass spectrometry screening assay to examine tumors from tumor-associated hot-spot mutp53 transgenic mice, we identified several mutp53 specific binding proteins that regulate mutp53 levels and/or GOFs.

We found that BAG2 and BAG5, two Bcl-2 associated athanogene (BAG) family proteins, preferentially interact with mutp53 proteins through the BAG domain. This interaction inhibits mutp53 degradation to increase mutp53 protein stabilization and accumulation, which in turn promotes mutp53 GOF in tumorigenesis. Furthermore, BAG2 and BAG5 proteins exhibit a cooperative effect on promoting mutp53 protein accumulation and GOF in cancer cells. Both BAG2 and BAG5 proteins are overexpressed in many types of human cancers, including breast cancer, lung cancer, skin cancer and colorectal cancer.

We also found that mutp53 activates small GTPase Rac1 which is a critical mechanism for mutp53 GOF in promoting tumorigenesis. Mutp53 activates Rac1 through enhancing Rac1 SUMOylation, a modification that is critical to maintain the active Rac1 form and enhance its activity in cells. Mechanistically, mutp53 interacts with Rac1, and mutp53-Rac1 interaction inhibits Rac1 to interact with SUMO-specific protease 1 (SENP1), which in turn inhibits SENP1-mediated de-SUMOylation

of Rac1. Furthermore, mutp53 expression is associated with enhanced Rac1 activity in clinical tumor samples. Targeting Rac1 signaling by RNAi or the pharmacological Rac1 inhibitor can effectively block mutp53 GOF in promoting tumor growth and metastasis.

These results reveal previously unidentified and critical mechanisms for mutp53 accumulation in cancers and mutp53 GOF to promote tumorigenesis and metastasis. These results identify several potential therapeutic targets in cancers containing mutp53.

Towards making a good egg: Sculpting the transcriptome of mouse oocytes by MARF1

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An unique gene expression program built up within the oocyte orchestrates the development of the oocyte itself, as well as that of the early stage embryos before zygotic genome activation. Of particular, in mice, active transcription takes place in oocytes during their growth phase, and ceases when oocytes reach their final stage of growth and become fully-grown. The subsequent events of oocyte maturation and fertilization, as well as early stage embryo development, proceed in a transcriptionally quiescent state, and rely on the maternal transcripts that are synthesized and stored during oocyte growth. Maternal mRNAs in oocytes are remarkably stable during the growth phase, however, coincident with the selective translation of certain mRNAs during oocyte meiotic maturation, oocyte maturation (*i.e.*, resumption of meiosis) triggers a transition from mRNA stability to instability, in which many maternal mRNAs are extensively degraded during oocyte meiotic maturation. These fascinating changes in the maternal transcriptome content and transcript dosage are crucial for the transition of an immature oocyte into a totipotent embryo, thus are molecular defining feature for oocyte quality. More intriguingly, recent studies point to the existence of active degradation of maternal transcripts in growing oocytes before the reinitiation of meiosis. However, the mechanisms underlying this “premature” degradation of maternal mRNAs remains unclear. Here, we provide structural, molecular, and genetic evidences showing that meiosis regulator and mRNA stability factor 1 (MARF1), a recently identified oocyte preferentially-expressed master regulator of key oogenic processes essential for oocyte meiotic resumption and retrotransposon silencing, is an oocyte-specific executor for maternal RNA degradation process during oocyte growth. We demonstrated that MARF1 is a bona fide

ribonuclease, and its ribonuclease activity depends upon conserved aspartic residues in the catalytic NYN domain and the RNA-binding activity of the LOTUS domain. By functioning as an executor of RNA-degradation processes in oocytes, MARF1 controls the mRNA homeostasis and genome integrity of mammalian oocytes. MARF1 may coordinate with TUT4/7 in the same functional module in oocytes to specifically sculpt the maternal transcriptome for making a "good egg". These observations provided insight into the posttranscriptional control of oocyte gene expression.

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Regulation of translesion DNA synthesis and its implication in genotoxic therapy

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Translesion DNA synthesis (TLS) is one mode of DNA damage tolerance, which utilizes multiple specialized DNA polymerases to replicate damaged DNA to maintain genome integrity. DNA polymerase η (Pol η) facilitates TLS across UV irradiation- and cisplatin-induced DNA lesions implicated in skin carcinogenesis and chemoresistant phenotype formation, respectively. However, since Pol η replicates undamaged DNA with a high error rate of 10^{-2} - 10^{-3} , its recruitment and residence at replication forks has to be stringently regulated. It is known that in addition to protein-protein interactions, protein post-translational modifications fine-tune Pol η recruitment and bypass of CPD lesions after UV radiation. However, the basis for recruitment of Pol η to stalled replication forks is not completely understood. Here I will present some of our recent progress on this topic, including the roles of OGT-mediated Pol η O-GlcNAcylation and a pre-mRNA splicing factor SART3 in regulation of Pol η 's function. Furthermore, we also found that a compound from *Ganoderma boninense*, a traditional Chinese medicine, can impair cisplatin-induced Pol η focus formation, PCNA monoubiquitination as well as mutagenesis. These results not only provide mechanistic insights into how cells regulate TLS, but also suggest a potential neoadjuvant for platinum-based therapy.

Reprogramming the epigenome of tumors with oncohistones

H3.3K36M and H3.3K27M

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With the extension of deep-sequencing power in cancer genome, many chromatin-regulating genes are found mutated. The surprising finding that histone proteins, the basic structural component of human chromatin, are mutated in a variety of cancers. Specifically, the lysine 27 to methionine (K27M) mutation in histone H3.3 is found in around 60% of diffuse intrinsic pontine glioma (DIPG), a high-grade pediatric brain tumor with dismal prognosis. In addition, a somatic histone H3.3 lysine 36 to methionine (K36M) mutation is identified in over 90% of chondroblastomas. In human genome, there are 15 genes encoding canonical and non-canonical histone H3. H3K27 and H3K36 are conserved among all these histone proteins. Therefore, it is unknown how mutations at one allele of 15 histone H3 genes are linked to tumorigenesis. We have shown that the H3.3K27M mutation dominantly reprograms H3K27 tri-methylation (H3K27me₃) in DIPG cells. In addition to a global loss, H3K27me₃ is retained at hundreds of genomic loci. In the chondroblastomas studies, we observed that the levels of H3K36 di- and tri-methylation (H3K36me₂/me₃) are reduced dramatically. Mechanistically, we show that H3.3K36M mutant proteins inhibit enzymatic activity of MMSET and Setd2. In addition to the histone methylation changes, the H3.3K27M and H3.3K36M mutations also alter the expression of genes associated with tumorigenesis. Based on these studies, we propose that different histone mutations reprogram the epigenome of different progenitor cells, which in turn alters gene expression and induces carcinogenesis.

Mammalian Sex Chromosome Structure, Gene Content and Function in Male Fertility

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Sexual reproduction begins with the male and female gametes. In mammals, males produce X-bearing and Y-bearing sperm while females produce X-bearing eggs only. So, the difference between male and female gametes is the sex chromosome. During evolution, sex chromosomes have become the driving force for sexual development and differentiation, brain development and reproduction. Mammalian sex chromosomes evolved from an ordinary pair of autosomes. The X chromosome is highly conserved (Ohno's law), while the Y chromosome varies among species in size, structure, and gene content. Unlike autosomes that contain randomly mixed collections of genes with extremely heterogeneous patterns of developmentally regulated expression in different tissues, the sex chromosomes are enriched for sex-biased genes related to sex development and reproduction, particularly in spermatogenesis and male fertility. In this presentation, I will focus on how sex chromosome dosage compensation takes place and why meiotic sex chromosome inactivation occurs during spermatogenesis. I will also emphasize on those genes that are exceptional to Ohno's law and how testis-biased genes are enriched on the X and Y chromosomes via an "autosome-to-sex chromosome" transposition/retroposition mechanism. Furthermore, I will discuss the future research on the multicopy nature of the testis gene families on the sex chromosomes and their potential functions in male fertility. I believe that the more we study the sex chromosomes, the more knowledge we would gain to understand evolution, speciation, and reproduction in mammals.

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MAVS-suppressing activity of influenza A (H7N9) virus PB1-F2 protein

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Since 2013, avian influenza A (H7N9) virus has emerged in human population as epidemics with over 1500 laboratory-confirmed cases and up to 40% case fatality rate. Fatal cases are presented with serious symptoms including pneumonia, acute respiratory distress syndrome and multi-organ failure. Although there is no evidence of human-to-human transmission, H7N9 virus possesses significant pandemic potential. Exactly why H7N9 virus is highly pathogenic in humans remains to be understood. PB1-F2 protein is a small non-structural viral protein of influenza A virus (IAV) and a virulence factor that downregulates the production of type I interferon (IFN). Mechanistically, PB1-F2 protein sequesters and inhibits MAVS protein, which is an essential adaptor of RIG-like receptor (RLR) antiviral signaling. The resulting impairment in type I IFN production liberates viral propagation leading to more severe outcome. Ascribed to an N66S mutation, PB1-F2 proteins from some pathogenic IAV strains exhibited enhanced ability to inhibit MAVS. Most H7N9 viruses express full-length PB1-F2 that potently suppresses MAVS, but N66S mutation is not found. How PB1-F2 protein of H7N9 virus suppresses MAVS more efficiently remains to be elucidated. In this study, the type I IFN-suppressive effect of H7N9 PB1-F2 was characterized. Recombinant IAVs with or without H7N9 PB1-F2 expression were rescued from 8-plasmid transfection system (respectively H7-WT or H7- Δ F). We found that infection of THP-1 cells with H7- Δ F virus elicited type I IFN response much more robustly than infection with H7-WT virus, suggesting that H7N9 PB1-F2 suppresses type I IFN production during IAV infection. To evaluate the potency of type I IFN-suppressive effect of H7N9 PB1-F2, type I IFN reporter assay was performed in HEK293T cells. By overexpressing PB1-F2 protein in Sendai virus-infected cells, we found that H7N9 PB1-F2 exerted potent type I IFN-suppressive effect when comparing to PB1-F2s of laboratory IAV strain WSN (H1N1) and H5N1 virus. Although H7N9 PB1-F2 also inhibited MAVS, we found that H7N9

PB1-F2 did not bind to MAVS protein. Instead, H7N9 PB1-F2 enhanced MAVS protein degradation when MAVS was activated to form protein aggregate that recruited downstream effector proteins for type I IFN transcription. The MAVS signalosome was strikingly perturbed by H7N9 PB1-F2 that binding of TRAF6, TBK1 and IKK ϵ to activated MAVS protein was abolished. Altogether, H7N9 PB1-F2 was found to be a potent type I IFN inhibitor against RLR antiviral signaling by targeting MAVS protein for degradation. Our findings shed new light on the pathogenesis of H7N9 virus.

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Kaposi's Sarcoma Herpesvirus Is Associated with Osteosarcoma

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Osteosarcoma is the most common malignant tumor of bone predominately affecting adolescents and young adults with a major peak between 10 and 14 years old. Viral etiology of osteosarcoma has been proposed for more than a half century when Finkel isolated viruses from mice that produced similar tumors when injected into new-born mice in 1966. There was also evidence of a bone tumor virus in the human disease as injection of cell-free extracts of human bone cancer into newborn Syrian hamsters induced a variety of mesenchymal tumors including osteosarcomas. However, the viral etiology has never been proven by identification of any virus that is authentically associated with human osteosarcoma. The Uyghur ethnic population in Xinjiang China has an extremely high prevalence of Kaposi's sarcoma-associated herpesvirus (KSHV) infection and high incidence of osteosarcoma. Taking advantage of the special ethnic population, we explored the possible association of KSHV infection and osteosarcoma development. A seroepidemiological study was performed for KSHV prevalence in osteosarcoma patients versus normal population in Xinjiang Uyghur population. Result provided strong epidemiological evidence that KSHV infection is a risk factor for osteosarcoma (OR, 10.23; 95%CI, 4.25, 18.89). The KSHV genome was detected in the majority of osteosarcoma tumors of KSHV-positive patients and viral latent nuclear antigen LANA was also detected in the nucleus of osteosarcoma cells by immunohistochemical analysis. Gene expression profiling analysis showed that KSHV infection regulates the genes and signaling pathways that are known to be important for osteosarcoma development. It also revealed remarkable similarity among KSHV-positive osteosarcomas and diversity to KSHV-negative tumors, suggesting the need for them to be classified into distinct categories. In conclusion, KSHV infection is a risk factor for osteosarcoma. Some osteosarcomas may be caused by KSHV infection, which is more frequently seen in the populations that have high prevalence of KSHV infection.

Translation of Papillomavirus Studies to Living Biobanks, Disease

Modeling, and Precision Medicine

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The E6/E7 oncogenes of the high-risk HPVs are both necessary and sufficient to immortalize HFKs and their presence and expression is required for the continued proliferation of HPV-positive cervical cancer cells. We and others have shown previously that hTERT induced by E6 and cytoskeleton alteration by E7 are critical. Both E6 and feeder cells activate telomerase, while both E7 and Rock inhibitor (Y-27632) disrupt the actin cytoskeleton and inactivate Rho. Feeders and Y-27632 to induce unlimited cell proliferation of human keratinocytes. Unexpectedly, we observed that feeders and Y-27632 could be used to establish both normal and tumor cell cultures from non-keratinocyte tissues. This culture has been termed as “conditional reprogramming”, since CR cultures stop proliferating or terminally differentiate after its removal, depending on culture conditions. The Combination of CR and Organoids ((Matrigel, air-liquid interface (ALI)) cultures represents next generation human cancer models and functional diagnostics for cancer precision medicine as described in August 2015 in the NCI precision medicine initiative and three nature review articles (Nat Rev Cancer. 2015 Dec; Nat Rev Genet. 2015 Jul; Nat Rev Clin Oncol. 2014 Nov.). The technique is relatively simple and has been reproduced in more than 50 laboratories. Importantly, the CR technology can generate 2×10^6 cells in a week from small biopsies, and can generate cultures from cryopreserved tissue and from fewer than four viable cells. We therefore initiated studies to examine whether CR cultures reflected the biology and genotype of the original tumor and whether cultures might be used to predict clinical responses. Moreover, the epithelial cells can be propagated indefinitely in vitro, yet retain the capacity to become fully differentiated when placed into conditions that mimic their natural environment. Thus, the CR method significantly advances applications in disease modeling, regenerative and precision medicine.

Circular RNAs in exercise induced physiological hypertrophy

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In our previous study, we found that miR-222 and miR-17-3p contributed to exercise induced physiological hypertrophy. In this report, we will present our most recent studies about circular RNAs in exercise induced physiological hypertrophy. Besides, cardiac ischemia/reperfusion (I/R) injury causes cardiomyocytes loss and dysfunction, which may lead to cardiac remodeling and heart failure. Promoting myocardial survival and repair is an essential way to reduce cardiac I/R injury and heart failure. Circular RNAs (circRNAs) are a group of non-coding RNAs with structural stability and high conservation, which involved in regulating cardiac physiology and pathophysiology. Here, based on cardiac I/R injury murine model and OGDR-induced neonatal rat cardiomyocytes (NRCMs) apoptosis model, we found that expression of circRNA MIRAC (Myocardial Ischemia/Reperfusion injury Associated circRNA, MIRAC) was significantly increased. Using divergent primers, we identified MIRAC was evolutionarily highly conserved among human, mouse and rat. Next, we showed that inhibition of MIRAC can significantly alleviate the apoptosis of cardiomyocytes in the OGDR-induced apoptosis model, and further promote the proliferation of cardiomyocytes. Conversely, overexpression of MIRAC can aggravate the apoptosis of cardiomyocytes as determined by TUNEL staining and Western blot. Further, activation of Akt pathways is involved in the protective effect of MIRAC suppression in reducing cardiomyocyte apoptosis. Moreover, we demonstrated that knockdown MIRAC significantly reduced the myocardium infarct size of I/R mice and decreased the ratio of myocardial apoptosis. Inhibition of circular RNA MIRAC might be a novel therapy for cardiac ischemic injury.

UPS-mediated Regulation of the Stability of Stem Cell Factor Nanog

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Nanog is an essential transcriptional factor for the maintenance of embryonic stem cells (ESCs) and cancer stem like cells (CSCs). However, it remains unclear how Nanog protein is regulated to promote cancer progression. Here, we report that Nanog is degraded by SPOP, a frequently mutated tumor suppressor of prostate cancer (PCa). Cancer-associated mutations of SPOP or Nanog at S68Y abrogated the SPOP-mediated Nanog degradation and exhibited evaluated ability to promote the stemness of CSCs and PCa progression. Moreover, SPOP-mediated Nanog degradation is controlled by AMPK-BRAF signal axis through phosphorylation of Nanog at Ser68, which blocked the interaction between SPOP and Nanog. Clinically, Nanog phosphorylation at Ser68 is positively correlated with its protein levels in human PCa tissues. Thus, our findings uncover a mechanism by which prostate cancer stemness is regulated by the Nanog phosphorylation and stability and provide potential therapeutic targets of prostate CSCs.

Furthermore, we also found that the deubiquitinase USP21 stabilizes Nanog, USP21 is a transcriptional target of the LIF/STAT3 pathway and is downregulated upon differentiation. Moreover, differentiation cues promote ERK-mediated phosphorylation and dissociation of USP21 from Nanog, thus leading to Nanog degradation. Together, our findings provide a regulatory mechanism by which extrinsic signals regulate mESC fate via deubiquitinating Nanog.

Our studies suggest that the protein stability of Nanog might be controlled by at least a pair of specific DUB and ubiquitin E3 ligase. More and more studies show that stem cell transcriptional factors (SCTFs), such as Nanog, Sox2, c-Myc and Oct4, play an important role in the maintenance of self-renewal of cancer stem cells. Therefore, dissecting this paradigm of reciprocal post-translational control, especially ubiquitination and deubiquitination, in stem cell regulatory networks not only advances stem cell biology but also promotes our understanding of cancer stem cells.

Regulation of a positive feedback loop between inflammation and stemness in cancer cells by ubiquitination and deubiquitination

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Cancer stemness confer resistance to different type of therapies and is responsible for cancer relapse. Inflammation is a hallmark of cancers and nuclear factor kappa B (NF- κ B) is a master regulator of inflammation. Accumulating evidence indicates that activation of NF- κ B promotes tumor development. The activation of NF- κ B in cancer cells results in a number of downstream events, including the suppression of apoptosis, induction of anti-apoptotic genes, and induction of the regulators of epithelial–mesenchymal transition (EMT). The EMT phenotype has been suggested to initiate metastasis and development of stemness. Tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and intrinsic Toll-like receptor (TLR) ligands are the major pro-inflammatory stimuli in tumor microenvironment. Activation of these receptors triggers cellular signalings leading to activation of NF- κ B. The inflammatory signaling pathways initiated by TNF- α , IL-1 β , and TLR activations are controlled by ubiquitination and deubiquitination. RelA, also known as p65 is a subunit of NF- κ B. Expression of RelA is positively associated with multiple types of cancer. Copper metabolism MURR1 domain-containing 1 (COMMD1) is the primary member of the COMM domain family that functions as a scaffold protein to promote ubiquitin-mediated degradation of the interaction partners. We found that the expression of COMMD1 was down regulated in different types of tumor by a miR205. This microRNA is upregulated by NF- κ B activation. In contrast, COMMD1 controlled NF- κ B activation by promoting ubiquitination and proteolytic degradation of RelA. Down regulation of COMMD1 by up regulation of miR205 in cancer cells enhanced TNF- α , IL-1 β , and TLR ligands induced inflammatory responses and promoted tumor growth. Tumors derived from the COMMD1 knockdown cells showed a higher activation of RelA and an increased expression of inflammation cytokines compared to the tumors derived from the parental cancer cells. These results indicated that COMMD1 is a regulator of a positive feedback loop between inflammation and stemness in cancer cells by regulation of ubiquitination.

Drug repurposing of cancer stem cell inhibitors: from big data to therapeutics

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Of 71 anti-cancer drugs for solid tumors approved by the FDA between 2002 and 2014, the median gains in progression-free and overall survival are ~2 months. These observations suggest two potential unmet clinical needs: (1) the existence of drug resistance (either intrinsic or acquired), and (2) the importance of patient stratification. In other words, it is imperative to implement a companion diagnostic biomarker along with drug discovery pipeline. To address these two questions, we have accessed several big data to identify compounds (old drugs) for anti-cancer drug discovery. Firstly, resistance to chemotherapy or targeted therapy is a major problem for systemic lung cancer treatment. Such resistance may be explained by cancer stem-like cell (CSC) theory. By using the Connectivity Map dataset, we have identified phenothiazine-like antipsychotic drugs which may reverse the CSC-associated gene expression, followed by a phase 1 clinical trial. This study demonstrated a novel platform for screening potential anti-CSC drugs, which may overcome the drug resistance. Secondly, synthetic lethality (SL) has emerged as a novel anti-cancer strategy. SL is an interaction between two genes such that simultaneous perturbations of the two genes result in cell death or a decrease of cell viability. The successful application of SL concept in the drug development is the approval of olaparib (a PARP inhibitor) by FDA in 2014 for the treatment of advanced ovarian cancer with *BRCA1/2* mutations. We have first built a big data approach to simulate this clinical trial results. Then, we evaluated several old drugs, which have been used in oncology, and mapped their corresponding SL pairs. The predicted results were further validated via *in vitro* biochemical assay and retrospectively sequencing of patient specimens. In conclusion, this systematic analysis strategy could rapidly place old drugs with biomarkers for clinical study.

RET-aberrant cancers: targeted therapy and resistance mechanisms

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Gene alterations resulting in active RET protein tyrosine kinase (PTK) are found in many types of human cancer, especially in thyroid and non-small cell lung cancer (NSCLC). A number of protein tyrosine kinase inhibitors (TKIs) with anti-RET activity have either been approved or are in clinical trials to treat RET-aberrant thyroid cancer or NSCLC. A common issue in PTK-targeted cancer therapy is acquired resistance due to secondary mutations that sterically block drug binding. Identifying TKI-resistant mutants and finding new TKIs effective against these mutants are critical for controlling tumor progression. We generated KIF5B-RET transgenic mice that developed KIF5B-RET-dependent lung tumors and KIF5B-RET-dependent cell lines for studying oncogenic properties of the RET fusion oncogene and profiling TKI-resistant mutants. We identified fourteen KIF5B-RET mutants resistant to cabozantinib, lenvatinib, vandetanib, or nintedanib, and cross-profiled their TKI sensitivities. Most of the mutated residues are located along the drug-binding pocket. Interestingly, the cabozantinib-, lenvatinib-, and vandetanib-resistant mutants (V871I, F998V) are located at distance sites outside the drug binding area. RET(V871I) and RET(F998V) remained sensitive to nintedanib. To understand the structural basis of TKI binding to the RET kinase and mechanism of TKI-resistant mutations, we have determined a nintedanib-RET co-crystal structure and a solvent-front RET(G810A) mutant, which is resistant to vandetanib but sensitive to nintedanib. These protein structures give novel insights into the mechanisms of TKI-resistant mutations.

Translational regulation during mouse spermiogenesis

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Male gametes play important functions during fertilization, development and transmission of genetic and epigenetic information from generation to generation. In mouse, as well as in human, spermatogenesis encompasses three consecutive stages: mitosis, meiosis and spermiogenesis, during which spermatogonial stem cells (SSCs) differentiate into mature spermatozoa. Abnormalities that occur in any of these events can cause not only fertility problems but also genetic or chronological disorders in human. Much have been learned from the past research about how SSCs undertake the long journey toward generating functional spermatozoa, however, little is known about how cellular morphogenesis occurs during spermiogenesis, the post-meiotic developmental process that transform round haploid spermatids into mature sperm with the unique shape. Combining biochemical, cell biological and genetic approaches, we analyzed the roles of RNA-binding proteins (RBPs) and cAMP-dependent protein kinase (PKA) during mouse spermiogenesis. I will report here our current understanding of the potential functions of RBPs during the regulation of post-meiotic messenger RNAs and the direct involvement of PKA signaling during translation of sperm components that are required for the morphogenesis of developing spermatids into mature and functional spermatozoa.

The p53-MDM2 Axis and Therapeutic Implication in Cancer

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This year marks the 40th anniversary of the discovery of p53– a discovery that changed our understanding of cancer biology and cancer diagnosis and treatment! This presentation will briefly discuss the role of p53 and its major interactive pathways in cancer medicine. It is generally accepted that malignant transformation of human somatic cells cell is attributed to a series of genetic and epigenetic events involving tumor-suppressor genes and oncogenes and that genomic instability resulting from the accumulation of multiple lesions may lead to changes in cell signaling, gene expression, cell growth, and cell cycle progression culminating in the malignant phenotype characterized by sustained proliferation, evasion of growth suppressors, resistance to cell death, increased angiogenesis, activation of invasion, and metastasis. Oncogene activation and tumor suppressor gene inactivation are the most widely studied mechanisms for cancer onset, development, and progression and regarded as viable therapeutic targets. The p53 tumor suppressor, the mostly studied molecule thus far, is a key transcription factor regulating various cellular pathways such as DNA repair, cell cycle, apoptosis, angiogenesis, and senescence and often acts as an important defense mechanism against cancer onset and progression. In human cancers, the *TP53* gene is frequently mutated or deleted, leading to downregulation of its tumor suppressive pathways. The *mdm2* gene was first identified as the gene responsible for the spontaneous transformation of an immortalized murine cell line, BALB/c 3T3, and has been subsequently confirmed as an oncogene, whose overexpression has been confirmed in many human cancers through various mechanisms, including gene amplification, single nucleotide polymorphism in its gene promoter, and increased transcription and translation. There is a p53-MDM2 feedback regulatory loop that is crucial for restricting p53 levels and activity during normal cell physiology, and is tightly regulated by many factors. These co-factors alter MDM2 or p53 conformation, binding, localization, expression, and modulate the E3 ligase activity of MDM2 towards itself, p53, and other substrates; consequently,

regulating a variety of different cellular processes. Therefore, the inhibition of MDM2-p53 interaction presents an appealing therapeutic strategy for the treatment of cancer. There is a huge ongoing research effort in this field. Recent studies from our laboratory and others have revealed the MDM2-p53 interaction to be more complex than previously thought. Furthermore, MDM2 has extensive p53-independent activities. The p53-MDM2 interactions provide a focal point to improve cancer therapy. The prime goal of p53 based cancer therapy has been to increase levels of functional p53 and/or inhibit MDM2 levels to prevent further p53 degradation. Several transcription factors such as NFAT1 are known to upregulate MDM2 transcription; inhibition of these transcription factors may provide a novel strategy to inhibit MDM2 and increase p53 levels. Though a number of the MDM2 inhibitors have entered clinical trials, and have shown sufficient cancer selectivity, the ultimate proof of concept is yet to come.

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Thyroid hormone receptor β blocks the tumor-initiating capacity of cancer stem-like cells in anaplastic thyroid cancer

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Compelling evidence has indicated that thyroid hormone receptor β (TR β) could function as a tumor suppressor in many types of cancer. However, it is not known whether TR β is involved in suppressing thyroid cancer initiating cancer stem-like cells (CSC). We therefore analyzed the suppressor functions of TR β in CSC using human anaplastic thyroid cancer cells (ATC: THJ-11T and THJ-16T) stably expressing TR β . The expression of TR β decreased tumor cell proliferation by 40% and 50% in THJ-11T and THJ-16T cells, respectively. The mRNA expression of stem cell markers ALDH1A1, SOX2, NANOG, EZH2, and BMI1 was suppressed by 40-95% in ATC cells expressing TR β . Consistent with the suppression of mRNA levels, protein levels of SOX2, NANO and ALDH1 were decreased 60-95% in ATC cells expressing TR β . Further, we found that TR β expression inhibited the capacity of tumor sphere formation in ATC cells. Taken together, these data strongly suggest that TR β functions to block the tumor-initiating capacity of stem-like cells in ATC. Thus the present studies for the first time, have uncovered a novel function of TR β , which could be targeted for a new treatment modality for ATC.

Vitamin K2 Promotes Glycolysis in Bladder Carcinoma Cells that Leads to AMPK-dependent Autophagic Cell Death

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Cancer cells exhibit a high rate of glycolysis, compared to normal cells, to meet their fast growth and requirement for metastasis. Targeting glycolysis against cancer cells appears to be intelligent strategies. Here, we show that Vitamin K2, an anticancer agent, promotes the glycolysis in bladder cancer cells, while inhibiting the tricarboxylic acid (TCA) cycle. Activation of PI3K/AKT and HIF-1 α is crucial for Vitamin K2-induced glycolysis upregulation that results in metabolic stress and subsequent AMPK-dependent autophagy and apoptosis. Intriguingly, glucose supplementation abrogates AMPK activation and attenuates autophagic cell death in Vitamin K2-treated cells. Both PI3K/AKT inactivation and HIF-1 α blockade counteract Vitamin K2-induced AMPK-dependent autophagic cell death. Besides, 2-DG, DCA and 3-BP (three typical glycolytic inhibitors) respectively abolish AMPK-dependent autophagic cell death triggered by Vitamin K2 through glycolysis inhibition. Collectively, these findings reveal that Vitamin K2 could trigger AMPK-dependent autophagic cell death in bladder cancer cells by elevating the glycolytic process.

Isolation and identification of lactic acid bacteria from Xinjiang small reed (*Phragmites australias*) silage

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Lactic acid bacteria (LAB) play important roles in preserving silage quality. Using 16S rDNA gene sequence analysis, nine LAB strains were identified from 90-day silage of Xinjiang small reed. These strains belonged to four genera and six species, including 1 strain of *Pediococcus pentosaceus*, 1 strain of *Enterococcus faecium*, 3 strains of *Enterococcus faecium*, 1 strain of *Lactococcus garvieae*, 1 strain of *Weissella thailandensis* and 2 strains of *Lactococcus lactis*. Biochemical analysis revealed that these strains were all Gram-positive and catalase-negative cocci. All strains were able to grow between 5°C and 40°C, and in the pH range of 4 to 8. All strains produced lactic acid, and *Weissella thailandensis* had the strongest ability to produce acid. To the best of our knowledge, this is the first report of isolation and identification of LAB strains from Xinjiang small reed. These strains may be used as additives in the production of silage of Xinjiang small reed.

ROS damage induced RNA modification in Cancer

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Reactive oxygen species (ROS) generated by cellular metabolism and radiation are a major source of DNA damage. The efficient repair of ROS-induced DNA double-strand breaks (DSBs) in transcribed regions relies on transcription-coupled homologous recombination (TC-HR), a newly emerged DNA repair pathway. We found that ROS-generated DSBs and single-strand breaks (SSBs) induce DNA:RNA hybrids in transcribed regions, eliciting the RNA modification in a transcription-dependent manner. Our findings suggest that RNA modification has an important regulatory function in TC-HR, linking an RNA modification to the repair of ROS-induced DNA damage and suppression of ROS-associated genomic instability.

A lysosome to nucleus signaling pathway in Alzheimer's disease

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Accumulating evidence implicates that hyperphosphorylated and misfolded Tau (pTau), the main components of neurofibrillary tangles (NFTs) in Alzheimer's disease (AD), are subject to degradation by the autophagy-lysosomal pathway. TFEB was discovered as a master regulator of cellular clearance through coordinated expression of autophagy and lysosomal target genes whose activity is mediated by its phosphorylation and nuclear translocation. We reported that AAV-mediated TFEB expression is highly efficacious in ameliorating the pTau/NFT pathology in tauopathy mouse models whereas it has no adverse effect in wild-type mice. This is because TFEB targets only the aberrant pTau species while leaving the normal Tau intact, indicating that pTau serves as an upstream activator of TFEB. Supporting this assessment, our RNAseq analysis of human brains and Tau transgenic mouse models revealed significant upregulation of TFEB and lysosomal genes controlled by TFEB in AD subjects and tauopathy mice compared to their corresponding controls, documenting a conserved TFEB-mediated lysosomal response to disease pathology between mice and humans. Interestingly, the TFEB target genes include multiple subunits of the vacuolar-type H⁺-ATPase (vATPase) that plays critical roles in lysosomal acidification and function. Our in vitro results demonstrate that pTau directly induces TFEB activation and that this effect is blunted by vATPase inhibition. We conclude that pTau acts as a stress signal to activate TFEB nuclear signaling and vATPase activity; this TFEB-vATPase mediated nucleus-to-lysosome coordination is critical for lysosomal homeostasis and cellular clearance.

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Targeting Inflammation and Oncogene for Cancer Therapy

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The MDM2 oncogene is amplified and/or overexpressed in human cancers, promoting tumor growth and metastasis and resistance to therapy. The role of uncontrolled inflammation in carcinogenesis and cancer development and progression has been well documented. More interestingly, the MDM2 oncogene and inflammatory responses also have pivotal roles in cancer immunotherapy and resistance development. Our laboratory has long been interested in developing dual inhibitors of inflammatory and oncogene pathways for the prevention and treatment of human cancers. We have recently discovered that the transcription factor NFAT1, an inflammatory factor, activates the MDM2 oncogene, independent of p53. This presentation will focus on several recently discovered inhibitors in our lab, including several synthetic and naturally-occurring NFAT1-MDM2 inhibitors, namely MA242, Jap A and InuA. Their anticancer activities have been confirmed in vitro and in vivo in various models of human cancers, including breast, pancreatic, and prostate cancers. The molecular mechanisms of action have been associated with the down-regulation of expression and stability of NFAT1, MDM2, and MDMX. In conclusion, these compounds represent novel first-in-classes of dual inhibitors of inflammation and oncogene pathways, providing a promising strategy for effective and safe cancer therapy.

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Tracking hematopoietic precursor division ex vivo in real time

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Deciphering molecular mechanisms underlying division of hematopoietic stem cells (HSCs) and malignant precursors would improve our understanding of basis of stem cell-fate decisions and oncogenic transformation. Using a novel reporter of hematopoietic precursor Evi1-GFP, we track the division of hematopoietic precursors in culture in real time. First, we confirmed that Evi1-GFP is a faithful reporter of HSC activity and identified three dividing patterns of HSCs: symmetric renewal, symmetric differentiation, and asymmetric division. Moreover, we found that the cytokines and growth factors combination (STIF) promotes symmetric renewal, whereas OP9 stromal cells balance symmetric renewal and differentiation of HSCs ex vivo. Interestingly, we found that *Tet2* knockout HSCs underwent more symmetric differentiation in culture compared to wild type control. Furthermore, we demonstrated that *Tet2*^{-/-}; FLT3-ITD acute myeloid leukemia (AML) precursors primarily underwent symmetric renewal divisions in culture. Our recent studies showed that GFP intensity strongly correlates with “stemness” state of hematopoietic precursors in Evi1-GFP mouse. Specifically, the GFP-high population is true functional long-term HSCs. Our study establishes a new system to explore the molecular mechanisms of the regulation of benign and malignant hematopoietic precursor division ex vivo. The knowledge learned from these studies will provide new insights into the molecular mechanisms of HSC fate decision and leukemogenesis.

A New Combinatorial Immunotherapeutic Strategy for HCC by Boosting Innate and Adaptive Immunity Simultaneously

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Immunotherapy by blocking inhibitory pathways in T lymphocytes, such as the PD-L1/PD-1 axis, is being widely tested in various solid tumors. Notably, this emerging therapeutic approach is already in clinical trials for advanced hepatocellular carcinoma (HCC), although the outcomes can be compounded by the unique immunotolerant microenvironment in the liver. Many combinations of immune checkpoint inhibitors or with other drugs are ongoing in clinical trials, without support by preclinical data in animal models. In previous experiments, we identified unexpectedly a robust liver tumor-preventive effect of a synthetic double-stranded RNA (dsRNA) polyinosinic-polycytidylic acid (polyIC) in mice. In this study, we tested a combination of polyIC with anti-PD-L1 Ab in treatment of HCC in mouse models.

In this study, we have used hepatocyte-specific gene deletion mouse lines, mouse HCC models driven by hydrodynamic tail vein injection (HTVi) of oncogenes, such as NRas, c-Myc, c-MET and b-catenin, and metastatic liver tumor models by splenic injection of tumor cells. polyIC, anti-PD-L1 or anti-CTLA4 antibodies were injected intraperitoneally into tumor-bearing mice. Tumor burdens were evaluated by the ratios of liver/body weights, numbers and maximal sizes of tumor nodules. Liver tumors and non-tumor liver tissues were dissected out for molecular and cellular analyses. polyIC given at the pre-cancer stage effectively prevented liver tumorigenesis by activation of NK cells and macrophages, with no inhibition on tumor progression if injected after tumor initiation. Nevertheless, polyIC administration potently induced PD-L1 expression in liver sinusoid endothelial cells, which prompted us to test a combination treatment of polyIC and PD-L1 antibody. Although injecting PD-L1 antibody alone did not show significant therapeutic effect, polyIC sensitized hepatic response to PD-L1 blockade, resulting in sustained accumulation of active CD8

cytotoxic T cells, robust tumor suppression and survival advantage. Similar results have been obtained for other combinations. Therefore, a powerful combinatorial immunotherapy may shift the paradigm in liver cancer treatment by boosting multiple innate and adaptive immune functions simultaneously. In conclusion, it is feasible to develop efficacious combination immunotherapies for primary and metastatic liver tumors by coordinated activation of innate and adaptive immunity.

Oct4 phosphorylation determines ESC differentiation

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OCT4 is required to maintain the pluripotency of embryonic stem cells (ESCs); yet, overdose-expression of OCT4 induces ESC differentiation toward primitive endoderm. The molecular mechanism underlying this differentiation switch is not fully understood. Here, we found that substitution of threonine³⁴³ by alanine (T343A), but not aspartic acid (T343D), caused a significant loss of OCT4-phosphorylation signal in ESCs. Loss of such OCT4-phosphorylation compromises its interaction with SOX2 but promotes interaction with SOX17. We therefore propose that threonine³⁴³-based OCT4-phosphorylation is crucial for the maintenance of ESC pluripotency. This OCT4-phosphorylation-based mechanism may provide insight into the regulation of lineage specification during early embryonic development.

**miR-223 inhibits the tumor suppressor gene FBXW7 through Notch
and NF- κ B pathways affecting the proliferation and apoptosis of
colorectal cancer cells**

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The tumor suppressor gene *FBXW7* plays important roles in human cancers, which regulates cell division, growth, and differentiation. MicroRNAs (miRNAs) are also involved in various biological processes, such as cell death and proliferation. Recent studies have revealed a correlation between miR-223 expression and *FBXW7* expression. However, the precise regulatory mechanisms of colorectal cancer (CRC) cells regulating miR-223 are still unknown. The aim of this study was to investigate the effect of miR-223 inhibiting *FBXW7* on the proliferation and apoptosis of CRC cells. MiR-223 binding to the *FBXW7* gene was confirmed. After overexpression of miR-223, the mRNA level of *FBXW7* was downregulated. Simultaneously, the protein level decreased, the activity of HCT 116 cells increased and the amounts of apoptotic cells decreased. After complement expression of *FBXW7*, the activity and number of apoptotic cells were reversed. In addition, miR-223 expression was suppressed by Notch and NF- κ B pathway inhibitors. In summary, this study demonstrated that the expression of miR-223 was upregulated in HCT 116 cells by the Notch and NF- κ B pathway, and miR-223 promotes the cell proliferation and inhibits apoptosis of HCT 116 cells by inhibiting the expression of *FBXW7*.

Imaging of the immune cell immunological synapse and its clinical application

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The glass-supported planar lipid bilayer (SLB) system has been utilized in a variety of disciplines. One of the most useful applications of this technique has been in the study of immunological synapse formation due to the ability of the SLBs to mimic the surface of a target cell while forming a horizontal interface. Recent advances in super-resolution microscopic imaging have further allowed scientists to better view the fine details of synapse structure. In this study, one of these advanced techniques, stimulated emission depletion (STED), is utilized to study the structure of Natural Killer (NK) and Chimeric Antigen Receptor (CAR) cell synapses on the SLB and vertical cell pairing (VCP) system. We imaged human NK cells on this bilayer using STED super-resolution microscopy, with a focus on distribution of perforin positive lytic granules and filamentous actin at NK synapses, as well as CAR-modified T cells. We thus demonstrate the feasibility and application of this combined technique, as well as intracellular structures at immunological synapse with super-resolution and its dynamics.

ADVANCES IN THE STUDY OF BIOLOGICAL SYMBIOTICS

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There have been many new disciplines established through intersecting and integrating among different subjects since the beginning of the 21st century. Biological symbiotics is established on the basis of biology, which is not only a branch of biology, but also a new, comprehensive and important interdisciplinary. The definition, nature, derivation source, research object, research method, research content, research purpose and research significance of biological symbiotics have been much developed and advanced in recent 10 years in the world, especially her theoretical system and special research methods. The worldwide research institutions, academic teams, teaching units, academic groups, academic activities and publications of this discipline have been established, and more and more developed to be able to form a new intersecting and integrating discipline. This should be beneficial to enrich the discipline system, knowledge, theory and technology of biology, with promoting the growth and development of biology. It is concluded that the subject of Biological Symbiotics has a subject attribute that is completely consistent with the traditional subject, and it is suggested that it can be included in the national recommended subject list as a second-level discipline under “Biology”, a first-level discipline belonging to natural sciences.

Structural assembly and reaction chemistry of the DNA replisome

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DNA replication is essential for cell proliferation. In this presentation, I will first present a prototypical structure of a simple DNA replication fork, where leading and lagging strand DNA syntheses are coupled to downstream DNA unwinding and primer synthesis. Replication fork arrangement indicates how lesions may be detected by DNA polymerase and helicase. Secondly, by combining in crystal catalysis with time-resolved X-ray diffraction analysis, we have observed reaction intermediates of DNA synthesis at unprecedented atomic detail. Contrary to the conventional view that DNA synthesis occurs by a two-Mg²⁺-ion mechanism, we have discovered that two Mg²⁺ ions bound to the polymerase active site are insufficient for product formation. A third Mg²⁺ ion must be captured by the enzyme-substrate complex en route to product formation. This third metal ion is free of enzyme coordination and appears to drive phosphoryl-transfer by breaking the existing phosphodiester bond in dNTP. Lastly, we find that cation trafficking in the DNA synthesis reaction is not an exception to the rule of the transition state theory, but it also drives RNA hydrolysis by nucleases.

The Interplay of Tyrosine Kinases and Innate Nucleic Acids Sensing in Antiviral and Antitumor Immunity

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Cytosolic nucleic acid sensing warns host cells both microbe invasion and cellular damage and initiates counter measures, by igniting cascades through MAVS/STING adaptors, TBK1/IKK ϵ kinases and IRF3 transcription factor. Yet, it remains largely unknown for how the magnitude of this critical type of innate immune sensing is controlled. Through functional screens of proteomics and analyses of interactome, we defined a few interesting tyrosine kinases, the classical regulators of many biological events, in governing of cytosolic nucleic acid sensing. We identified the direct tyrosine modification and inhibition of TBK1 by LCK/HCK/FGR, whose expression is induced by IRF3, thereby constituting a negative feedback to restrict the cytosolic nucleic acid sensing. We also revealed a classical oncogenic pathway that interplays and suppresses cytosolic DNA sensing, and thus dominates both antiviral immunity and antitumor immunity. Accordingly, genetic or pharmacological targeting of these tyrosine kinases determines both antiviral and antitumor immunity in cells, mice, and/or zebrafish. These findings provide mechanistic insights as well as new strategies to against pathogen infection and prevent tumorigenesis.

Key Words: innate immune sensing; tyrosine kinase; antiviral immunity; antitumor immunity; senescence; cGAS-STING

Andrographolide derivative as anti-inflammation agent

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Andrographolide derivatives exhibit potent anti-inflammatory effects. Our structure-activity-relationship study revealed that in addition to the importance of the good pharmacophore of 4-nitro-2-methoxyphenoxy, the derivative's inhibitory potency and selectivity of a derivative are determined by 14-stereochemistry, 13-monoacetylation, 3,19-diacetylation, 3-alcohol, or 3-ketone. Among these andrographolide derivatives, compound **3b** is a potent and specific NF- κ B inhibitor that prevents the phosphorylation of the NF- κ B p65 subunit without affecting the endogenous expression of NF- κ B family members. Compound **3b** reduced disease activity index and shortened colon length, suppressed elevated activities of myeloperoxidase, and suppressed histologic evidence of inflammation in dextran sulfate sodium-induced colitis in a dose-dependent manner. Treatment with **3b** suppressed both serum and transcription levels of pro-inflammatory cytokines. In addition, **3b** increased PCNA-positive cells in intestinal crypt, and mRNA levels of β -catenin target genes in the intestine. Regulation of β -catenin level affect the anti-inflammation and anti-apoptotic activities of **3b**. Determination of the expression level and mutation of β -catenin in patients could help to predict the outcome of immunosuppressive agents in treating colitis. This study provided a novel andrographolide derivative that suppressed inflammation and maintained integrity of colon epithelium through regulating the crosstalk of β -catenin and NF- κ B signaling.

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Nannocystin ax induces G1 cell cycle arrest and apoptosis in colon cancer cells

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OBJECTIVE Colon cancer (CRC) is one of the most common causes of cancer related death in the worldwide. Nannocystin ax (NAN), a 21-membered cyclodepsipeptide initially isolated from myxobacteria of the *Nannocystis* genus, was found to target the eukaryotic translation elongation factor 1 α (EF-1 α). This study aimed to evaluate the anticancer effect and underlying mechanisms of NAN in CRC. **METHODS** Human colon cancer (HCC) cells HCT116 and HT29 were used. The effects of NAN on cell proliferation were evaluated using the MTT assay and colony formation assay. HCC cells were stained with propidium iodide (PI) to analyze the cell cycle progression. For apoptosis assay, cells were tested with an Annexin V-PE/7AAD apoptosis detection kit. Levels of proteins related to cell cycle and apoptosis were examined by western blotting. The mRNA expression of Cyclin d1 were tested by Real-time PCR. **RESULTS** NAN treatment resulted in growth inhibition of HCT116 and HT29 cells in concentration- and time-dependent manners. NAN arrested cells in G1 phase in both cell lines and downregulated the protein expression of Cyclin d1 and CDK4/CDK6. The mRNA expression of cyclin d1 was not affected by NAN. Furthermore, NAN triggered Cyclin D1 downregulation could be significantly reversed by MG132, while the ubiquitin level of Cyclin d1 was not increased, indicating a non-ubiquitin dependent proteasome degradation of Cyclin d1. In addition, NAN induced caspase-independent apoptotic cell death in both line cells. **CONCLUSION** NAN suppressed HCC proliferation through inducing G1 cell cycle arrest and caspase-independent apoptosis mediating by regulating cyclin D1.

Key words: nannocystin ax; G1 cell cycle arrest; cyclin d1; apoptosis

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En Masse Discovery of Anti-Cancer Human Monoclonal Antibodies

by De novo Assembly of Immunoglobulin Sequences

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Generation of specific antibodies for cancer therapy is a major endeavor. As a radical departure from the conventional approach, we hereby describe rapid and potentially *en masse* identification of cancer-specific antibodies directly from human cancer tissues. A computational framework was developed and successfully tested for antibody discovery by mining RNA-sequencing (RNA-seq) data of 1945 solid tumor samples from The Cancer Genome Atlas (TCGA). Surprisingly, synthetic antibodies based on high-abundance complementarity-determining region 3 (CDR3) sequences from lung adenocarcinoma (LUAD) patients bound all lung cancer samples tested and cross-reactive to other cancer types but rarely to normal tissues. The targeted DNA sequencing of the B cell receptor (BCR-seq) from 5 lung tumor tissues also allowed us to identify variable region sequences with somatic mutations. Antibodies based on predominant variable region sequences showed specific binding to autologous lung cancer samples. Our platform dramatically reduces the barrier in developing human anti-cancer antibodies and paved the way for cancer treatment using patient-derived tumor-reactive monoclonal antibodies.

BRCA1 Deficiency Impairs Mitophagy and Promotes Inflammasome Activation and Mammary Tumor Metastasis

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Breast cancer susceptibility gene 1 (*BRCA1*) is a major tumor suppressor gene and most frequently mutated in hereditary breast cancer. *BRCA1* plays a critical role in many biological processes, especially in maintaining genomic stability in the nucleus, yet its role in the cytoplasm remains elusive. Here we reveal that *BRCA1* maintains a healthy mitochondrial network through regulating mitochondrial dynamics including fission and fusion. *BRCA1* deficiency causes dysfunctional mitochondrial dynamics through increasing expression of mitofusin1/2. Upon mitochondrial stress, *BRCA1* is recruited to mitochondrial outer membrane, where it plays an essential role in maintaining health mitochondrial network. *BRCA1* deficiency, consequently, impairs stress-induced mitophagy through blocking ATM-AMPK-DRP1 mediated mitochondrial fission, and triggers NLRP3 inflammasome activation, which creates tumor-associated microenvironment facilitating tumor proliferation and metastasis. We further show that inflammasome inhibition could block tumor recurrence and metastasis. This study uncovers an important role of *BRCA1* in regulating mitophagy and suggests a therapeutic approach for fighting with this deadly disease.

**Qushi Huayu decoction inhibits de novo lipogenesis initiated by
IRE1 α -XBP1s pathway in mouse of non-alcoholic fatty liver disease
induced by high-fructose diet**

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Background and Aim: Qushi Huayu decoction (QHD) is a traditional Chinese medicine used in clinic for non-alcoholic fatty liver disease (NAFLD) treatment, previously verified in many kinds of NAFLD models. *De novo* lipogenesis (DNL), controlled by spliced x-box binding protein 1 (XBP1s) during hepatic insulin resistance bypass of carbohydrate response element-binding protein (ChREBP) and sterol regulatory element-binding protein (SREBP) 1, is the important source of fatty acid (FA) in NAFLD. In the present study, the effect of QHD on DNL initiated by inositol-requiring enzyme (IRE) 1 α - XBP1s was evaluated to disclose the mechanism of QHD inhibiting steatosis in NAFLD.

Methods: 1) DNL was induced by high-fructose diet (HFD) for 2 weeks in mice. Mice in QHD group were administrated by gavage simultaneously. Hepatic histology, triglyceride (TG), FA and protein levels of key enzymes of DNL and esterification of TG including acetyl-coenzyme A carboxylase (ACC), fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD)1, glycerol-3-phosphate acyl transferase (GPAT)1, acylglycerol-3-phosphate acyltransferase (AGPAT), phosphatidic acid phosphatase (PAP), diacylglycerol O-acyltransferase (DGAT) 2 were observed. IRE1 α , phospho-IRE1 α and XBP1s in total hepatic protein, and nuclear ChREBP, SREBP1 and XBP1s was evaluated. 2) XBP1s activation was induced by tunicamycin for 24h in HepG2 cell. Seven components of QHD were identified in serum of mouse, which were added into the medium of HepG2 cells respectively. Cellular content of TG and FA, protein levels of IRE1 α , phospho-IRE1 α , XBP1s and the targets of XBP1s, ACC2 and SCD1, was evaluated.

Results: Obvious hepatic steatosis was visualized by hematoxylin-eosin and oil red staining in

HFD-fed mice, as well as increased hepatic TG, FA and protein of the key enzymes, IRE1 α , phospho-IRE1 α , XBP1s and nuclear ChREBP, SREBP1 and XBP1s. QHD ameliorated the pathological changes mentioned above except nuclear SREBP1. In HepG2 cells, geniposide, chlorogenic acid and polydatin respectively inhibited protein expression of IRE1 α , phospho-IRE1 α , XBP1s, ACC2, SCD1, cellular content of TG and FA induced by tunicamycin.

Conclusion: QHD inhibited hepatic DNL associated with IRE1 α – XBP1s pathway closely, in which, geniposide, chlorogenic acid and polydatin was the effective component.

Treatment of Glioblastoma by Targeting Heat Shock Protein 90

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Glioblastoma (GBM) is the most malignant type of brain tumors. Treatment of GBM includes surgical removal of the tumors, radiotherapy, and chemotherapy. However, the efficacy of the treatment did not last long and often ended up with resistance. Thus, an alternative strategy is needed. In the present study, we studied the effects of NVP-AUY922 (AUY922), a novel heat shock protein 90 (HSP90) inhibitor, on GBM cells. AUY922 decreased cell viability of U87MG, T98G, P5 and P5 temozolomide (TMZ) resistant cells in a concentration-dependent manner with IC₅₀ of 30 nM, 35 nM, 9.68 nM, and 8.22 nM respectively. Colony formation assay showed that AUY922 significantly reduced colony number. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay revealed that AUY922 significantly increased TUNEL positive cells. We found that AUY922 increased levels of cleaved caspase-3, cleaved caspase-9 and poly (ADP-ribose) polymerase (PARP) after 48 h treatment. These results suggest that AUY922-induced cell death is associated with apoptosis.

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Hippocampal 5-HT_{1B} receptor modulates impulsive aggression in post-weaning social isolation mice

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Impulsive aggression is characterized by outbursts of rage with violence when facing stressors. The present study aims to investigate the role of 5-HT_{1B}R within the ventral hippocampus in impulsive aggression. We established post-weaning social isolation mice model to mimic child neglect in human. Mice under social isolation (SI) showed increased attack number after receiving footshocks compared to group housing (GH) mice. SI mice showed decrease in attack number after bilateral hippocampal microinjection with anpirtoline or CP-93129, the 5-HT_{1B}R agonists. Pre-treatment with SB-224289, a 5-HT_{1B}R antagonist, blocked the response of SI mice to CP-93129. To examine the role of PKA in impulsive aggression, we used H89, a PKA inhibitor. After intraperitoneal injection of H89, SI mice showed reduction in response to anti-aggressive effect of CP-93129. Our results suggest that 5-HT_{1B}R regulates the impulsive aggression in post-weaning social isolation mice and targeting 5-HT_{1B}R could be a new strategy for the treatment of impulsive aggression.

**Development and Application of Kinase-Focused Library: Design and
Synthesis of Pyrimidine Derivatives Bearing Amino Substituents
Utilizing High Throughput Parallel Synthesis**

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Since imatinib (Gleevec[®]) was first launched in 2001, the search for inhibitors of protein kinase has become a tremendous interest area of drug discovery. Therefore, researchers continually seek methodologies and technologies to discover drugs more efficiently. In order to accelerate drug discovery, the application of high throughput parallel synthesis (HTPS) has been flourishing in recent decades. On the other hand, pyrimidine derivatives bearing amino substituents have been of multifold biological and pharmacological interest. Moreover, S_NAr reaction has been well utilized as a practical and efficient synthetic protocol in medicinal chemistry. Herein, a kinase-focused library of pyrimidine derivatives was rapidly synthesized in parallel reactor via S_NAr displacement and the progress of all reactions was monitored by UPLC and LCMS. All library compounds were further screened for kinase activities leading to the identification of interest.

Characterization of a Novel Botulinum Neurotoxin-like Toxin PMP1

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Botulinum neurotoxins are family of potent bacterial toxins. They target vertebrate motor nerve terminals and block neurotransmission by cleaving SNARE proteins required for synaptic vesicle exocytosis. A novel BoNT-like toxin PMP1 was recently identified in *paraclostridium bifermentans malaysia*. It was shown to be toxic for mosquitoes by cleaving mosquito SNARE protein syntaxin 1, but whether its protease domain is capable of cleaving mammalian SNARE proteins remains to be characterized. Here we screened all major mammalian SNARE proteins for their susceptibility to the protease domain of PMP1. We found that PMP1 not only cleaved *Drosophila* and *C. elegans* syntaxin 1, but also multiple mammalian SNARE proteins VAMP1, 2, 3, 5, 7, 8, as well as syntaxin 1, 2, and 3. Notably, PMP1 is the only toxin known to date that is capable of cleaving VAMP7 and VAMP8. These findings establish a unique substrate profile for a new member of the emerging BoNT-like toxin family, and reveal the potential of utilizing this new toxin for modulating membrane trafficking events in mammalian cells.

Proteomic Dissection of the Planar Cell Polarity (PCP) Signaling

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Planar cell polarity (PCP) refers to the coordinated alignment of cell polarity across the tissue plane and is of critical importance to tissue development and health. Establishment of the asymmetry distribution of core PCP components, such as Vangl/Prickle and Fzd/Dvl, represents the key molecular events in PCP signaling. For example, Dvl recruits Smurf2 E3 ubiquitin ligase to mediate degradation of Prickle, a process of critical importance in controlling cytoskeleton networks and morphological dynamics of migrating cells. However, many questions remain outstanding with regards to the molecular mechanisms that regulate the interplay among PCP components and the establishment of asymmetry.

To explore the functional organization of PCP signaling, we carried out affinity purification-mass spectrometry (AP-MS) analysis of the protein-protein interaction (PPI) networks of Vangl, Prickle, Fzd and Dvl. We identified >600 novel protein partners that assemble around the core PCP components through different degrees of interactions. Interestingly, network analysis revealed that a significant number of proteins from the ubiquitin-proteasome system (UPS) play a key role in mediating the mutual regulation between core PCP components. For example, we found that deubiquitinating enzymes (DUB) USP7/USP9X mediate the deubiquitination and stabilization of Prickle1, counteracting its negative regulation by Dvl2/Smurf2. Moreover, we observed that Vangl2 interacts with Nedd4, an E3 ubiquitin ligase. Interestingly, Nedd4 is activated in the presence of Dvl2, leading to significant degradation of Vangl2. Taken together, our work will provide insights into the molecular aspects of how the UPS system participates in the establishment of PCP asymmetry.

**Investigation of the molecular mechanisms underlying the
pronounced high gene targeting frequency at the Myh9 gene locus in
mouse embryonic stem cells**

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The production of genetically modified mouse models derived from gene targeting (GT) in mouse embryonic stem (ES) cells has greatly advanced both basic and clinical researches. Our previous finding that gene targeting at the Myh9 exon2 site in mouse ES cells has a pronounced high homologous recombination (HR) efficiency (>90%) has facilitated the generation of a series of nonmuscle myosin II (NM II) related mouse models. Furthermore, this finding has also been expanded for site-specific insertion of other exogenous genes. In the current study, we intended to investigate the molecular biology underlying for this unprecedented high HR efficiency from various aspects. Our results confirmed some previously characterized and revealed some unreported observations: 1) In regard to a HR hotspot, the analysis of the Myh9 gene locus failed to identify a distinct sequence element of this locus. Moreover, the sequences surrounding the exon2 region used for creating the homologous arms even had a low sequence similarity (14-28%) among multiple species including mouse, rabbit, rat, chimpanzee, pig and human; 2) the detected expression of the Myh9 gene was similar, while the GT efficiency was markedly different in mouse ES cells (mESCs), induced pluripotent stem cells (iPSCs) and mouse embryonic fibroblasts (MEFs), in the order of mESCs (91.7%) > iPSCs (58.4) >> MEFs (0%), suggesting a cell-type dependent mechanism and for the first time revealing a potential difference between the former two in term of GT; 3) Despite maintaining the similar length of the homologous arms, shifting the targeting site from the Myh9 exon2, to intron2-exon3 led to a gradually reduced GT frequency (91.7, 71.8 and 50.0%, respectively). This finding provides the first evidence that the HR frequency may also be related to the exact targeting site in the same locus, as opposite to previous report; 4) the high HR efficiency at

the Myh9 exon2 site appears to be associated with the length of homologous arms since truncating the arms also resulted in an obvious decrease of GT efficiency, while the effect of the reduced homologous arm length on HR frequency was not as high as reported before. Additionally, the better performance of an optimal ratio of 2:1 for the length of the 5' and 3' homologous arms was observed. Based on these facts, we speculate that the high HR efficiency at the Myh9 exon2 site may be ascribed to a unique but uncharacterized sequences/structure of this locus or unidentified HR-associated factors in mouse ES cells. Though further investigation is warranted, the Myh9 gene locus appears to be an ideal location for identifying those factors which will benefit the practical application of gene editing.

DNA approaches to Novel antibody discovery

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Antibodies are of the most rapidly growing fields within medicinal research that play an important role in precision diagnostics and therapeutics. In this study we developed a novel antibody discovery and development platform using DNA reading, designing and writing technologies. First, deep sequencing provided a promising DNA reading technology in the antibody discovery workflow. Our proprietary primer design algorithm and sequencing library can recover most information of the antibody repertoire. This had been proved an effective antibody discovery approach for determining a neutralization antibody from a pathogen infected individual. a convolutional neural network (CNN) can be used to assist in the prediction of antibody heavy and light chain pairing within an NGS sequencing library. The preliminary result of the trained CNN model showed that a discriminating accuracy of 67.4% was achieved. Second, a candidate antibody library can be designed by two methods. One is to extract germline information from antibody sequencing results. Another is to use a de novo antibody design strategy which constructs antibody sequence and structure libraries based on the heavy and light chain databases and the CDRs databases, followed by modelling the interaction between antigen and antibody library to screen possible antibodies. Then, the full-length candidate antibody sequences can be written in DNA through codon optimization and our proprietary Syno® Synthesis Platform. Using this approach we have de novo designed and synthesized an anti-PDL1 antibody that showed an in vitro affinity with KD value of 10^{-11} scale. In summary, a combination of an in-silico algorithm (AI designing, structure-based antibody modeling) and novel DNA engineering provides a novel approach for both time and cost-effective antibody discovery.

Fine mapping and conservation analysis of linear B-cell epitopes of peste des petits ruminants virus hemagglutinin protein

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Peste des petits ruminants (PPR) is a highly infectious disease of domestic and wild small ruminants that is caused by PPR virus (PPRV), a member of the genus Morbillivirus that includes rinderpest virus (RPV), measles virus (MeV), canine distemper virus (CDV), phocine distemper virus (PDV), cetacean morbillivirus (CeMV), and feline morbillivirus (FeMV). Hemagglutinin protein (H), one of the two glycoproteins of peste des petits ruminants virus (PPRV), binds to its receptor on the host cell and acts as a major antigen that induces and confers highly protective immunity in the host. In order to delineate the epitopes on H protein, fine epitope mapping and conservation analysis of linear B-cell epitopes (BCEs) on PPRV H has been undertaken using biosynthetic peptides and rabbit anti-PPRV H sera. Thirteen linear BCEs were identified and their corresponding minimal motifs were located on the H protein of PPRV China/Tibet/Geg/07-30. Conservation analysis indicated that two of the 13 minimal motifs were conserved among 52 PPRV strains. Nine of the 13 peptides containing the minimal motifs were recognized using anti-PPRV serum from a goat immunized with PPRV vaccine strain Nigeria 75/1. Identified epitopes and their motifs improve our understanding of the antigenic characteristics of PPRV H and provide a basis for the development of epitope-based diagnostic assays and multiple epitopes vaccine.

Notch signaling in tumor-associated macrophages: implications for innate immunity-targeted cancer therapy

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Tumor-associated macrophages (TAMs) play pivotal roles in tumor microenvironment to facilitate tumor growth and metastasis. TAMs strongly inhibit anti-tumor immunity by recruiting myeloid-derived suppressor cells (MDSCs) and regulatory T-cells (Tregs), and by reducing the number and activity of CD8⁺ cytotoxic T-cells. At the molecular level, TAMs are characterized by a molecular signature coinciding with the alternatively activated (M2) macrophages, which express higher levels of immunosuppressive cytokines such as transforming growth factor (TGF)- β and IL-10, together with arginase-1 (Arg-1), mannose receptor (MR) and other molecules involved in anti-inflammatory and/or pro-tumor. In contrast to M2-like TAMs or M2 macrophages, M1 macrophages upregulate the expression of interleukin (IL)-12, inducible nitric oxide synthase (iNOS), and tumor necrosis factor (TNF)- α , accompanied by increased antigen presentation capacity. Macrophages with the M1 phenotype repress tumor growth through phagocytosis and enhancement of anti-tumor immunity. Therefore, it is possible to re-educate TAMs to elicit anti-tumor activities, given that the regulation and mechanisms of macrophage polarization are established.

The Notch-RBP-J (recombination signal-binding protein J κ) pathway plays critical roles in cell fate specification during embryonic development and cell plasticity in adults. Previous reports including our study have shown that Notch signaling is involved in macrophages activation and polarization through several downstream molecules, such as SOCS3, CYLD and IRF8. However, the exact role of Notch signal in TAM function remains to be elucidated.

Accumulating evidence has indicated that microRNAs (miRNAs) participate in myeloid differentiation and macrophage activation. Therefore, we activated BMDMs from the RBP-J^{icKO}

and control mice with LPS, and compared their miRNA profiles. Thirteen miRNAs exhibited differential expression between RBP-J^{icKO} and the control macrophages. We chose three microRNAs, such as miR125a, miR148a and miR99b, to investigate their role in macrophages activation and tumor development. After a series of experiments, we demonstrated that miR125a/miR99b/miR148a was downstream molecular of Notch signaling on regulating macrophage M1 polarization. Delivery of miR125a/miR99b into TAMs could reduce tumor growth significantly through re-educating M2-like TAMs. Taken together, our studies show that Notch signaling regulates TAM function through microRNAs, which might be a promising target in cancer therapy.

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**Personalized Drosophila Models for Congenital Heart Disease,
Kidney Diseases, and Leukemia**

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The Drosophila heart, kidney and blood originate from the same mesodermal progenitors, just like humans. These three tissue types share remarkable cellular, structural and molecular similarities with their human counterparts. Research in my lab, as well as others, showed that approximately 75 - 85% of known disease genes associated with heart and kidney diseases are evolutionarily conserved from flies to humans. With the powerful genetic tools and rich genetic resources, Drosophila can be used to provide functional data for novel genetic variants and chromosome fusions efficiently. My lab has developed a novel disease modeling approach called "Drosophila Gene Replacement System". Using this system, we have generated many personalized Drosophila disease models for novel genetic variants identified from exome sequencing of heart and kidney disease patients. We also made a series of leukemia models in flies with novel mutations or chromosome fusions identified from leukemia patients. I will present examples in which we use these personalized Drosophila disease models to confirm causal mutations for Congenital Heart Disease, to identify therapeutic treatment for a specific kidney disease, and to discover new therapeutic target for oncogenic KRAS induced leukemia.

Neuronal Epac1 mediates retinal neurodegeneration in mouse models of glaucoma

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Glaucoma is characterized by progressive loss of retinal ganglion cells (RGCs), resulting in irreversible visual deficits. Cyclic AMP (cAMP) is a universal second messenger that regulates many pathophysiological processes. Yet the role of cAMP and its downstream mediators in neurodegeneration in glaucoma are largely unknown. Here, we found the level of cAMP and the activity and expression of its newly identified mediator Epac1 were increased in retinas of two mouse models of glaucoma. Genetic depletion of Epac1 or pharmacologic inhibition of Epac activity significantly attenuated ocular hypertension-induced detrimental effects in the retina, including vascular inflammation, neuronal apoptosis and necroptosis, thinning of ganglion cell complex layer, RGC loss and retinal neuronal dysfunction. With bone marrow transplantation and various Epac1 conditional knockout (KO) mice, we further demonstrated that Epac1 in retinal neuronal cells (especially RGCs) was responsible for their death. Moreover, in vitro study on primary RGCs showed that Epac1 activation was sufficient to induce RGC death, which was mechanistically mediated by CaMKII activation. Taken together, these findings indicate neuronal Epac1 plays a critical role in retinal neurodegeneration and suggest that Epac1 could be considered a target for neuroprotection in glaucoma.

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Zika virus infection induces retinal neuronal and vascular defects during development

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Purpose: Zika virus (ZIKV), a mosquito-borne flavivirus, can cause severe eye disease characterized by chorioretinal atrophy, optic neuritis, and blindness in newborns. However, the retinal lesions have not been described explicitly. Here, we developed a mouse model of ZIKV infection to evaluate its impact on retinal structure.

Methods: ZIKV (20 PFU) was injected into the neonatal C57BL/6 mice at Postnatal day (P) 0 subcutaneously. Retinas of ZIKV-infected mice and age-matched controls were collected at P21. Neuronal injury, vascularization, inflammation and gliosis were analyzed by immunostaining with anti-Tuj-1, isolectin B4, anti-CD45 and anti-GFAP in retinal flatmounts. Retinal structural alteration was assessed in retinal sections by DAPI staining and immunohistochemistry with antibodies against GFAP, glutamine synthetase, PKC α , calbindin, Dab1, cone-arrestin and rhodopsin.

Results: Compared to control mice, the retinal surface area was much less in ZIKV-treated mice, with dramatically decreased retinal ganglion cells, abnormal vasculature, increased leukocytes, and induced activation of astrocytes and Müller cells. ZIKV-infected retina also displayed evident lack of cells in different retinal layers, including the ganglion cell layer (GCL), inner nuclear layer (INL) and outer nuclear layer (ONL), resulting in reduced thickness of the whole retina. Moreover, the expression of PKC α , calbindin, Dab1, cone-arrestin and rhodopsin decreased significantly in ZIKV-infected retina, indicating the loss of bipolar cells, horizontal cells, amacrine cells and photoreceptor cells.

Conclusions: We established a novel mouse model of retinal abnormalities associated with ZIKV. These data provide a direct causative link between ZIKV and retinal lesion in vivo. The massive

neuronal death, abnormal vasculature and structural disorder in the retina indicate that ZIKV infection can lead to a severe impact on the eye.

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The E3 ligase HECTD3 promotes cancer metastasis through upregulating expression of adhesion molecules in endothelial cells

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Tumor metastasis, which commonly occurs at the late stage, is responsible for the majority of cancer deaths. Tumor microenvironment plays a critical role in metastasis. Many types of stromal cells can affect the growth and development of tumor, including tumor infiltrating lymphocytes (TILs), tumor-associated macrophages (TAMs), tumor-associated fibroblasts and endothelial cells, while the colonization of circulating tumor cells (CTCs) in distant organs depends on the adhesion mediated by vascular endothelium. The initial arrest of CTCs can be determined by specific adhesion events between vascular endothelium and cancer cells through adhesion molecules and their ligands. Inflammatory stimulation can induce the expression of adhesion molecules in endothelium and promote tumor metastasis. Here we demonstrated that Hectd3 KO mice significantly reduced lung metastasis of breast cancer cells in the MMTV-Neu mouse model and the experimental metastasis model by injecting tumor cells through tail vein. We found that less PyMT-GFP tumor cells resided in the lung of Hectd3 KO mice in the presence of LPS, which meant that Hectd3 KO inhibited the lung colonization of PyMT-GFP tumor cells in vivo. Hectd3 KO downregulated the expression of adhesion molecules, including VCAM-1, ICAM-1 and E-selectin, in the mouse primary endothelial cells (mEC) isolated from pulmonary vessels, when the mEC were treated with LPS or TNF α . When HECTD3 was knocked down in HUVEC (human umbilical vein endothelial cell), the expression of adhesion molecules (VCAM-1, ICAM-1 and E-selectin) induced by inflammation factors, including LPS, TNF α and IL-1 β , were inhibited. Consistently, significantly less human breast cancer cells, including HCC1937, MDA-MB-231 and MDA-MB468, adhered to HUVEC. Subsequently, we found that HECTD3 can interact with and stabilize IKK α . On the other hand, HECTD3 promoted the nuclear translocation of IKK α through

non-degradative ubiquitin modification. In the nucleus, IKK α can phosphorylate Histone3 Ser10 and upregulate the expression of adhesion genes. In summary, HECTD3 interacts with IKK α and ubiquitinates IKK α with non-degradative poly-ubiquitin chains, which stabilize IKK α and promote expression of adhesion molecules and cancer metastasis. HECTD3 may be a safety drug target, because the Hectd3 KO mice were born at expected Mendelian ratios and had comparable survival rate as the WT mice. Small molecule inhibitors of HECTD3-IKK α axis may provide a novel strategy for tumor metastasis prevention and treatment.

**Molecular characterization of the NK-lysin in a teleost fish,
Boleophthalmus pectinirostris: Antimicrobial activity and
immunomodulatory activity on monocytes/macrophages**

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NK-lysin (NKL) is a cationic host defense peptide that plays an important role in host immune responses against various pathogens. However, the immunomodulatory activity of NKL in fishes is rarely investigated. In this study, we characterized a cDNA sequence encoding an NK-lysin homolog (BpNKL) from the fish, mudskipper (*Boleophthalmus pectinirostris*). Sequence analysis revealed that BpNKL is most closely related to tiger puffer (*Takifugu rubripes*) NKL. BpNKL transcript was detected in all the tested tissues, with the highest level in the gill, followed by the spleen and kidney. Upon *Edwardsiella tarda* infection, the mRNA expression of BpNKL in the mudskipper was significantly upregulated in the spleen, kidney, and gill. A peptide, BpNKLP40, was then chemically synthesized and its biological functions were investigated. BpNKLP40 exhibited a direct antibacterial activity against some gram-negative bacteria, including *E. tarda*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, and *Vibrio harveyi*, and induced hydrolysis of *E. tarda* genomic DNA. Intraperitoneal injection of 1.0 µg/g BpNKLP40 significantly improved the survival of mudskipper following *E. tarda* infection and reduced the bacterial burden in tissues and blood. Moreover, 1.0 µg/ml BpNKLP40 treatment had an enhanced effect on the intracellular killing of *E. tarda* by monocytes/macrophages (MO/MΦ) as well as on the mRNA expression of pro-inflammatory cytokines in MO/MΦ. In conclusion, our study revealed that BpNKL plays a role

against *E. tarda* infection in the mudskipper by not only directly killing bacteria but also through an immunomodulatory activity on MO/MΦ.

Keywords: Antibacterial activity; *Edwardsiella tarda*; Monocyte/macrophage; mRNA expression; Mudskipper; NK-lysin; Sequence analysis

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Ubiquitylation & Cancer Signaling Pathways

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Protein ubiquitylation regulates many biological activities. Dysregulation of protein ubiquitylation has been linked to many human diseases. Our research focuses on the roles of ubiquitylation in cancer signaling pathways and inflammation. Our previous studies indicated the p97/Ufd11/NPL4 protein complex regulates post-ubiquitylational process of cytokine-induced degradation of I κ B α , a major inhibitor of NF- κ B transcription factors. This protein complex utilizes the induced-interaction between p97 and SCF ^{β TRCP}, the ubiquitin ligase of I κ B α to recognize polyubiquitin chains of I κ B α , and then delivers the ubiquitylated I κ B α to the 26S proteasome for degradation. More recently, together with Dr. Xin-Hua Feng's lab, we identified the CRL4^{DCAF3} ubiquitin ligase as a positive regulator of the TGF- β signaling pathway. We found that the CRL4^{DCAF3} ubiquitin ligase conjugates non-proteolytic polyubiquitin chains on Smad4, an essential transcription factor of the TGF- β signaling pathway, and enhances its DNA-binding activity. Furthermore, we observed that DCAF3 promotes TGF- β -induced EMT, migration, invasion and bone metastasis of breast cancer cells.

Human SERINC4 anti-HIV-1 mechanism

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Serine incorporator (SERINC) family has five homologous members that are type III integral membrane proteins with 9-11 transmembrane domains. Recently, SERINC5 was identified as a novel retroviral restriction factor. SERINC5 is incorporated into HIV-1 virions and strongly inhibits HIV-1 replication by directly targeting Env proteins. Nonetheless, SERINC5 is effectively antagonized by HIV-1 Nef and other retroviral accessory proteins. Here, we investigated SERINC4 expression and antiviral activity. Unlike the other four SERINC members, human SERINC4 is poorly expressed at protein levels due to proteasome-targeting. When expression was normalized at protein levels, SERINC4 reduced HIV-1 infectivity as strongly as SERINC5 in a Nef-dependent manner. Consistently, Nef decreased both SERINC4 and SERINC5 protein expression via the lysosomal pathway. Although SERINC4 proteins are conserved within primates or rodents, their N-terminal regions are highly variable between these two species. Notably, unlike human SERINC4, murine SERINC4 is stably expressed but does not have antiviral activity. Via making stable chimeras between human and murine SERINC4 proteins, we found that the human SERINC4 1-34 amino acid region determines levels of antiviral activity, whereas its 35-92 region determines levels of protein expression. Like SERINC5, SERINC4 was found in HIV-1 virions and restricted Tier 1 HIV-1 virus more effectively than Tier 3 viruses. Finally, we detected SERINC4 interaction with Env by immunoprecipitation and found that the interaction was independent of its two N-terminal regions. Collectively, SERINC4 shares a similar anti-HIV-1 mechanism as SERINC5 by directly targeting Env and its antiviral activity is antagonized by Nef.

Risks of MERS-cluster coronaviruses in China

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Middle East respiratory syndrome coronavirus (MERS-CoV) has represented a human health threat since 2012. The discovery of varieties of bat MERS-cluster CoV implied that MERS-CoV originated in bats. Although genetically distinct, some of the bat MERS-cluster CoV could use human MERS-CoV entry receptor, dipeptidyl peptidase 4 (DPP4), conferring a risk of spillover to human society. We screened 1,059 bat samples from at least 30 bat species collected in different regions in south China and identified 89 strains of lineage C betacoronaviruses, including *Tylonycteris pachypus* coronavirus HKU4, *Pipistrellus pipistrellus* coronavirus HKU5, and MERS-related CoVs. We also found spike proteins from two of the MERS-related CoVs bind to the human receptor DPP4 directly. In addition, a list of recombinant MERS-CoV that express HKU4 receptor-binding domains (RBD) were rescued. They showed infectivity to a list of cells from human and other species. Lastly, in vitro experiment also proved HKU4-MERS-RBD virus (HKU4-CoV backbone with MERS-CoV RBD) is pathogenic, same as MERS-CoV. These studies demonstrated those bats MERS-cluster CoVs in China are widespread, and potentially pathogenic to human if which acquired necessary changes. Long-term monitoring these viruses in bats is necessary.

The function and regulation of multicopy immune gene CXCR3 and CXCR4 in teleost

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The study of multiple copies of chemokine receptor genes in various teleosts has long appealed to investigators seeking to understand the evolution of the immune system. CXCR3 is preferentially expressed on immune cells to aid in cell migration to the sites of inflammation. Here, we illustrated the sub-functionalization of CXCR3 and CXCR4 genes in teleost immune system. We found that CXCR3.1 and CXCR3.2 differentially contributed to macrophage polarization in the teleosts: ayu (*Plecoglossus altivelis*), grass carp (*Ctenopharyngodon idella*), and spotted green pufferfish (*Tetraodon nigroviridis*). In ayu macrophages, the *P. altivelis* CXCR3.1 (PaCXCR3.1) gene was constitutively expressed, whereas the *P. altivelis* CXCR3.2 (PaCXCR3.2) gene was induced post-infection with *Escherichia coli*. Upon *E. coli* infection, PaCXCR3.1⁺ and PaCXCR3.2⁺ macrophages showed an M1 and an M2 phenotype, respectively. CXCL9–11-like proteins mediated M1 and M2 polarization by interacting with the PaCXCR3.1 and PaCXCR3.2 proteins on macrophages, respectively. The transcription factors *P. altivelis* STAT1 and *P. altivelis* STAT3 were activated in PaCXCR3.1⁺ and PaCXCR3.2⁺ macrophages, respectively. Furthermore, the prognosis of septic ayu adoptively transferred with PaCXCR3.2⁺ macrophages was improved. Moreover, CXCR4 represents the sole chemokine receptor in hematopoietic stem cells to mediate migration/chemotaxis. Here, we found that CXCR4b was highly expressed in ayu hematopoietic stem and progenitor cells, while CXCR4a was lowly expressed in health HSPC and upregulated after infection. Furthermore, CXCR4a bound LPS much more efficiently than CXCR4b, while CXCR4b bound SDF1 much more efficiently than CXCR4a. Our data reveal a previously unknown mechanism for CXCR3 and CXCR4 genes to regulate immune responses in teleost, suggesting that redundant genes may regulate crucial functions in the teleost immune system.

TAK-ing aim at tumorigenesis: the emerging role of MAP3K7 in pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer mortality in the USA with about 56,770 new cases in 2019 and is projected to surpass breast, colorectal, and prostate cancers as the second leading cause of cancer-related deaths by 2030 [1]. The 5-year survival rate has remained at 1%-3% for the past 30 years [2]. Current therapy is largely ineffective because PDAC cells are resistant to apoptosis and effective therapy is lacking in treating metastasis thus far. Hence, discovery of therapeutic targets for inhibiting signaling pathway driving PDAC metastasis is one of the key steps to improve the patient survival.

Our recent studies reveal the following finding on mechanisms of PDAC development: (1) knocking down TAK1 (TGFbactivated kinase-1, MAP3K7) in PDAC cells significantly reduces tumorigenesis in orthotopic mouse models (Melisi et al., 2011); (2) knocking out IKK2/bor TAK1 in pancreas of Pdx1-cre/ Kras^{LSL-G12D}/p53^{LSL-H172R} (KPC) mice resulted in PDAC-free survival for over a year without any sight of the disease, suggesting that IKK2/band TAK1 are required for PDAC initiation (Ling et al., 2012); (3) Kras^{G12D} activates NF-kB and its target genes IL-1 and p62, to initiate IL-1/p62 feedforward loops that induce and sustain high levels of NF-kB activity, which is frequently detected in the specimens from PDAC patients, revealing that TAK1/NF-kB pathway is required for mutant Kras to induce PDAC (Ling et al., 2012); and (4) Cell lineage tracing experiments with PDX1- or p48-cre indicate defects in acinar cell differentiation and epithelial cell proliferation in the absence of TAK1; (5) The activity of TAK1 is regulated by acetylation and de-acetylation steps. (6) About 5% of the TAK1-knockout KPC mice still developed PDAC, suggesting activation of alternative pathways in either ductal epithelial cells or acinar cells. Protein kinases phosphorylation array identified three tyrosine kinases: fibroblast growth factor

receptor-1 (FGFR1), epidermal growth factor (EGFR), and Bruton's tyrosine kinase (BTK) as potential kinase that compensates the functions of TAK1.

Therefore, these findings provide the groundwork for filling in the major gap in our understanding of how TAK1 and TAK1 independent pathways enable mutant Kras to induce PDAC.

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Twist-Regulated Gene Expression and Breast Cancer Metastasis

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Twist is a basic helix-loop-helix domain-containing transcription factor that either activates or suppresses its target genes. Many studies based on cultured cells have demonstrated that Twist-regulated gene expression promotes epithelial-mesenchymal transition (EMT), stem cell feature, migration and invasion of breast cancer cells. However, the expression pattern of Twist and its intrinsic role in EMT and metastasis during the entire breast tumor initiation, growth and metastasis process have not been investigated during spontaneous mammary tumorigenesis. The molecular mechanisms responsible for Twist to regulate gene expression and the direct target genes of Twist are still not fully understood.

We developed a mouse model with the oncogene-induced mammary tumors containing either wild type (WT) or tumor cell-specific knockout Twist alleles. In both types of early stage tumors, Twist, vimentin and fibroblast-specific protein (FSP), three of the mesenchymal markers, were undetectable in tumor cells, and lung metastasis was also not present in mice at this early stage. In the large tumors, Twist protein was detected in about 6% of tumor cells with WT Twist, while Twist protein was undetectable in Twist knockout tumor cells. In these advanced tumor cells, Twist expression negatively associated with epithelial markers but positively associated with mesenchymal markers. Circulating tumor cells and lung metastasis were developed in mice with the late stage WT tumors but it was largely diminished in mice bearing the same stage tumors with tumor cell-specific knockout of Twist. These results demonstrate that breast tumor progression-induced Twist expression plays a crucial role to promote tumor cell EMT and metastasis.

Twist expression is associated with basal-like breast cancer (BLBC) with poor prognosis due to its role in promoting epithelial-to-mesenchymal transition (EMT), invasiveness and metastasis, while Foxa1 expression is linked with luminal breast cancer (LBC) with good prognosis. However, the

regulatory and functional relationships between Twist and Foxa1 in breast cancer progression are unknown. We found that in the estrogen receptor (ER)-positive LBC cells Twist silences Foxa1 expression, which plays an essential role in relieving Foxa1-arrested migration, invasion and metastasis of breast cancer cells. Mechanistically, Twist binds to Foxa1 proximal promoter and recruits the NuRD transcriptional repressor complex to de-acetylate H3K9 and repress RNA Polymerase II recruitment. Twist also silences Foxa1 promoter by inhibiting AP-1 recruitment. Twist expression in MCF7 cells silenced Foxa1 expression, which was concurrent with the induction of EMT, migration, invasion and metastasis of these cells. Importantly, restored Foxa1 expression in these cells largely inhibited Twist-promoted migration, invasion and metastasis. Restored Foxa1 expression did not change the Twist-induced mesenchymal cellular morphology and the expression of Twist-regulated E-cadherin, beta-catenin, vimentin and Slug, but it partially rescued Twist-silenced ER and cytokeratin 8 expression and reduced Twist-induced integrin alpha5, integrin beta1 and MMP9 expression. In a xenografted mouse model, restored Foxa1 also increased Twist-repressed LBC markers and decreased Twist-induced BLBC markers. Furthermore, Twist expression is negatively correlated with Foxa1 in the human breast tumors. The tumors with high Twist and low Foxa1 expressions are associated with poor distant metastasis-free survival. These results demonstrate that Twist's silencing effect on Foxa1 expression is largely responsible for Twist-induced migration, invasion and metastasis but less responsible for Twist-induced mesenchymal morphogenesis and expression of certain EMT markers.

**Utilizing integrative taxonomy uncovers a new cryptic stygobiont
Macrobrachium species (Crustacea: Caridea: Palaemonidae) from a
karst cave of Guangxi Zhuang Autonomous Region, China**

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Stygobitic species of *Macrobrachium* is a land-locked macroinvertebrate species of freshwater prawn, which inhabits karstic areas and displays great ecological significance in cave ecosystems. To date, 15 stygobitic species of *Macrobrachium* have been discovered in taxonomy and 3 species in genus *Macrobrachium* of family Palaemonidae are known to be cave-dwelling from China. Evidence-based information is the foundation for addressing urgent global challenges in conservation and sustainable management of the biodiversity. In order to better understand the cave shrimps and related living environments in the karst terrain, we investigated the karst caves in Guangxi Zhuang Autonomous Region. Four species taxa were recorded during the dedicated surveys of this study. Based on morphological, molecular, and ecological evidences, a fourth species of stygobitic prawn *Macrobrachium tenuipus* Guo & Zheng sp. nov. is described from Guangxi Zhuang Autonomous Region, southwestern China. This new species with smooth carapace and the extremely slender pereopods, it can be separated from other congeners by the shape of rostrum; the segmental ratios, the arrangement of teeth on the cutting edge of the fingers of second pereopod; the longer and narrower scaphocerite; and the longer uropodal diaeresis spine. A molecular phylogeny based on mitochondrial cytochrome c oxidase subunit I (COI) and nuclear genomes 18S rRNA, supports the morphology-based description of the new species, which can also be clearly distinguished by pairwise genetic distance.

ASB13 Inhibits Breast Cancer Progression and Metastasis through SNAI2 Degradation and Transcriptional Regulation of YAP

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Metastasis is responsible for the majority of cancer-related deaths. The transcription factor SNAI2 promotes metastasis by facilitating tumor cell invasion and tumor initiating activity. However, its post-translational regulation is less studied. We performed a dual luciferase-based, genome-wide E3 ligase siRNA library screening and identified ASB13 as an E3 ubiquitin ligase that targets SNAI2 for ubiquitination and degradation. ASB13 knockout in breast cancer cells leads to increased cell migration and decreased F-actin polymerization; while overexpression of ASB13 suppresses lung metastasis formed by LM2 cells. Furthermore, we discover that ASB13 knockout decreases YAP expression, which is dependent on increased SNAI2 protein level. YAP functions as a potential tumor suppressor gene in breast cancer, as YAP knockout increases tumorsphere formation, anchorage-independent colony formation, and cell migration and increased lung

metastasis in vivo. Clinical data analysis reveals that ASB13 expression is positively correlated with overall survival in breast cancer patients. These findings establish the ASB13-SNAI2-YAP axis as a regulatory mechanism for breast cancer migration and metastasis.

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A vitamin-C-derived DNA modification catalysed by an algal TET homologue

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Methylation of cytosine to 5-methylcytosine (5mC) is a prevalent DNA modification found in many organisms. Sequential oxidation of 5mC by ten-eleven translocation (TET) dioxygenases results in a cascade of additional epigenetic marks and promotes demethylation of DNA in mammals. However, the enzymatic activity and function of TET homologues in other eukaryotes remains largely unexplored. Here we show that the green alga *Chlamydomonas reinhardtii* contains a 5mC-modifying enzyme (CMD1) that is a TET homologue and catalyses the conjugation of a glyceryl moiety to the methyl group of 5mC through a carbon-carbon bond, resulting in two stereoisomeric nucleobase products. The catalytic activity of CMD1 requires Fe(II) and the integrity of its binding motif His-X-Asp, which is conserved in Fe-dependent dioxygenases. However, unlike previously described TET enzymes, which use 2-oxoglutarate as a co-substrate, CMD1 uses L-ascorbic acid (vitamin C) as an essential co-substrate. Vitamin C donates the glyceryl moiety to 5mC with concurrent formation of glyoxylic acid and CO₂. The vitamin-C-derived DNA modification is present in the genome of wild-type *C. reinhardtii* but at a substantially lower level in a CMD1 mutant strain. The fitness of CMD1 mutant cells during exposure to high light levels is reduced. LHCSR3, a gene that is critical for the protection of *C. reinhardtii* from photo-oxidative damage under high light conditions, is hypermethylated and downregulated in CMD1 mutant cells compared to wild-type cells, causing a reduced capacity for photoprotective non-photochemical quenching. Our study thus identifies a eukaryotic DNA base modification that is catalysed by a divergent TET homologue and unexpectedly derived from vitamin C, and describes its role as a potential epigenetic mark that may counteract DNA methylation in the regulation of photosynthesis.

The molecular landscape of histone lysine methyltransferases and demethylases in non-small cell lung cancer

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Background: Lung cancer is one of the most common malignant tumors. Histone methylation was reported to regulate the expression of a variety of genes in cancer. However, comprehensive understanding of the expression profiles of histone methyltransferases and demethylases in lung cancer is still lacking.

Methods: We analyzed the expression profile of methyltransferases and demethylases in non-small cell lung cancer (NSCLC) using TCGA and cBioportal databases. The mutation, expression level, association with survival and clinical parameters of histone methyltransferases and demethylases were determined.

Results: We found overall upregulation of histone regulators in NSCLC. Mutation and copy number alteration of histone methylation related genes both exist in NSCLC. The expression of certain histone methylation related genes were significantly associated with overall survival and clinical attributes.

Conclusions: Our result suggests that alteration of histone methylation is strongly involved in NSCLC. Some histone methylation related genes might serve as potential prognosis predictor or therapeutic target for NSCLC. The significance of some histone methylation related genes was contrary to the literature and awaits further validation.

m⁶A RNA modification modulate PI3K/Akt/mTOR signal pathway in gastrointestinal cancer

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Methylation at the N6 position of adenosine (m⁶A) is the most prevalent RNA modification within protein-coding mRNAs in mammals, and it is a reversible modification with various important biological functions. The formation and function of m⁶A are regulated by methyltransferases (writers), demethylases (erasers), and special binding proteins (readers) as key factors. However, the underlying modification mechanisms of m⁶A in gastrointestinal (GI) cancer remain unclear. We performed comprehensive molecular profiling of the nine known m⁶A writer, eraser, and reader proteins in GI cancer with data from The Cancer Genome Atlas and Gene Expression Omnibus databases. We found that genomic alterations in m⁶A significantly influence the overall survival in patients with GI cancer. We further analyzed the association between m⁶A modification and alterations in GI cancer-related pathways, and charted the detailed landscape of these pathways in major human cancer types. The phosphatidylinositol-3-kinase (PI3K)/Akt and mammalian target of rapamycin (mTOR) signaling pathways were found to be potentially affected by m⁶A modification in most human cancers, including GI cancer, which was further validated by the human phospho-MAPK array. Our findings suggest that m⁶A RNA modification has a fundamental role in the regulation of PI3K/Akt and mTOR signaling pathway function in cancer.

DNA base editing induces substantial DNA&RNA off-target mutations and eliminated by mutagenesis

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DNA base editors holds promise for correcting pathogenic mutations and the evaluation of the off-target effects are necessary for their application. However, it is difficult to determine DNA and RNA off-target effects due to single-nucleotide polymorphisms among individuals. Here we developed a method named GOTI (genome-wide off-target analysis by two-cell embryo injection) to detect DNA off-target mutations by editing one blastomere of two-cell mouse embryos using either CRISPR-Cas9 or base editors. Comparison of the whole-genome sequences of progeny cells of edited and nonedited blastomeres at embryonic day 14.5 showed that off-target single-nucleotide variants (SNVs) were rare in embryos edited by CRISPR-Cas9 or adenine base editor (ABE), with a frequency close to the spontaneous mutation rate. By contrast, cytosine base editor (CBE) induced SNVs at more than 20-fold higher frequencies, requiring a solution to address its fidelity. As for RNA off-target mutations, we quantitatively evaluated the RNA SNVs induced by CBEs and ABEs. We found that both the cytosine base editor BE3 and the adenine base editor ABE7.10 generate tens of thousands of off-target RNA SNVs. Fortunately, by engineering deaminases, we found that three CBE variants and one ABE variant reduced off-target RNA SNVs to the base level while maintaining their DNA on-target efficiency. This study reveals a previously overlooked aspect of off-target effects in DNA editing and also demonstrates that such effects can be eliminated by engineering deaminases of the base editors.

Increased Akt-Driven glycolysis is the basis for the higher potency of CD137L-DCs

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CD137L-DCs are a novel type of DCs that have shown remarkable potency at activating anti-tumor T cell responses *in vitro*. This high potency is evidenced by the polarization of Th1 and Tc1 responses in allogenic MLR, and further supported by the superior killing of virus-associated tumor cells by autologous DC-activated T cells. Using an unbiased screening and live cell metabolic assays, I find a higher activity of the Akt-mTOR1 pathway and a higher glycolysis rate in CD137L-DCs than moDCs. Further, this higher activity of the Akt-mTORC1 pathway is responsible for the significantly higher glycolysis rate in CD137L-DCs than in moDCs. Inhibition of Akt during maturation or the inhibition of glycolysis during and after maturation result in suppression of inflammatory DCs, with mature CD137LDCs being the most affected ones. In contrast to supporting lipid synthesis as found in LPS-activated murine BMDCs, higher glycolytic rates in CD137L-DCs lead to an accumulation of succinate and serine. These findings emphasize the notion that metabolic reprogramming underlies immune activations and that higher Akt-driven glycolysis contributes to the higher potency of CD137L-DCs.

Identifying the pleiotropic risk function modules for cancers by a prior knowledge-based analysis of methylation beadchips

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Objective: As we all know, cancer is a common and complex disease, with the further research of cancer, the role of epigenetic modification has attracted more and more attention in the occurrence and development of cancer. This study, which is based on prior knowledge, gene interaction and network analysis, was applied to mine hub genes and functional risk modules for cancers and tries to find the molecular mechanism of cancer methylation and predicts the risk of cancer.

Methods: First, the methylation data of 33 cancers was extracted from TCGA and differential methylated genes was screened out by using the T-test and rank test. The pleiotropic methylated gene of cancer was taken as seed gene, pleiotropic methylated gene interaction network of cancer was built up, guided by PPI (protein-protein interaction) knowledge. Then, the Cytoscape mapping software was applied to construct a specific gene-gene interaction network map. Next, Newman spectrum algorithm was used to decompose network for its modules (Subnet-network) with high modularization. Finally, each sub module was annotated to the GO database and KEGG database in order to explore the biological process and molecular function of module.

Results: Follow the steps above, 3778 statistical significantly genes were obtained. Then, multiple independent different size gene networks were constructed in the guide of PPI knowledge, which contained 5980 nodes and 13868 edges (the primary network has 5778 nodes and 13757 edges). Next, based on Newman spectrum algorithm, we resolved the primary network into 23 modules, from which we identified 286 hub genes by using a Poisson test. By analyzing 23 modules, we found the biological process of methylation in the pathogenesis of cancer, such as DNA replication (GO:0006260), protein degradation (GO:0006508), signaling pathways (GO:0009968), inflammation and immune response (GO:0002224), KEGG pathway involving

MAPK (mitogen activated protein kinase, has04010) signal pathway, Wnt signaling pathway (hsa04310) etc. Other enriched functions may be the underlying pathogenesis of cancer, such as drug metabolism, hormone receptor signaling pathways and so on.

Conclusion: In brief, this study identified 23 pleiotropic gene methylation modules, which is based on functional analysis of cancer risk pleiotropic methylation functional module. And it suggesting that the molecular mechanisms of cancer involved multiple biological processes and pathways, which might provide important reference for the diagnosis and the treatment of early cancer.

Key words: Cancer; Methylation; Pleiotropic modules; Network

Characterization of cellular receptors for Epstein-Barr virus entry of nasopharyngeal epithelial cells

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Epstein-Barr virus (EBV) is implicated as an etiological factor in B lymphomas and undifferentiated nasopharyngeal carcinoma (NPC), a malignant epithelial cancer occurring frequently in South China and Southeast Asia. However, the mechanisms of cell-free EBV infection of nasopharyngeal epithelial cells (NEPCs) remain elusive, mainly due to the deficiency of highly susceptible NPECs model. We focus on establishing the highly susceptible NPECs model and identifying cellular factors responsible for EBV infection. We found EGF treatment and premalignant NPECs-Bmi1 cells grown as sphere-like cells (SLCs) significantly enhanced EBV infection. We then used cDNA array, siRNA library and pull-down assay followed by liquid chromatography-tandem MS to screen the key cellular factors involved in EBV infection of epithelial cells. We found that non-muscle myosin heavy chain IIA (NMHCIIA) interacts with EBV gH/gL and mediates EBV attachment with the epithelial cells. EphA2 interacts with both gH/gL and gB and mediates EBV fusion of cellular membrane, while neuropilin 1 (NRP1) interacts with EBV gB and triggers the activation of EGFR/RAS/ERK signaling induced by EBV. Taken together, NMHCIIA, EphA2 and NRP1 are identified as cellular factors in EBV infection of NPECs, indicative of their capacity to serve as targets for blocking EBV infection.

Macrophage Polarity in Metabolic Liver Diseases

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Alcoholic and non-alcoholic liver disease are significant clinical problems. Kupffer cells (liver resident macrophages) play crucial roles in the inflammatory responses of alcoholic and non-alcoholic liver diseases and contribute to the development of hepatic steatosis and injury in a paracrine manner. Macrophages have distinct functional states with pro-inflammatory M1 type and anti-inflammatory M2 type. The mechanisms that govern this classical polarization remain to be fully elucidated.

Nogo-B, also known as Reticulon 4B, is an endoplasmic reticulum (ER) resident protein that has been implicated in maintaining ER structure. In the liver, Nogo-B is restricted to non-parenchymal cells including Kupffer cells, liver sinusoidal endothelial cells and hepatic stellate cells, but not in hepatocytes. We showed that Nogo-B levels correlate with the severity of alcoholic liver disease in patients. Nogo-B levels in Kupffer cells were positively associated with M1 polarization and negatively with M2 polarization in human liver specimens. In mice, the absence of Nogo-B resulted in significantly lower levels of hepatic steatosis and injury than wildtype (WT) mice in response to an ethanol diet or high fat diet. Kupffer cells from Nogo-B knockout (KO) mice showed significantly decreased expression of M1 markers, including inducible nitric oxide synthase (iNOS), interleukin 1b (IL1b) and tumor necrosis factor α (TNF α), but exhibited significantly increased M2 markers, such as CD163 and arginase-1, compared to their WT counterparts. Importantly, iNOS, IL1b and TNF α have been reported to enhance hepatic steatosis in alcoholic or non-alcoholic settings and are induced by nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B). Nogo-B KO Kupffer cells exhibited significantly increased ER stress, a factor that induces M2 polarization.

In conclusion, Nogo-B is permissive of M1 polarization of Kupffer cells, thereby accentuating liver injury in alcoholic and non-alcoholic liver disease in humans and mice. Nogo-B in Kupffer cells may represent a new therapeutic target for alcoholic and non-alcoholic liver diseases.

Genetic Polymorphism of 20 Autosomal STR Loci in Yunnan Yi Population in Southwest China

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Objective: Yunnan, located in the southwest of China. It is one of the important birthplace of human civilization. Yunnan is the province with the most ethnic minorities in China. There are 56 ethnic groups in China, and Yunnan Province includes 26 of them. Among the many ethnic minorities, the Yi people have the largest population, reaching 4.71 million. Thus, the purpose of this research is to study the genetic polymorphisms of 20 autosomal STR loci in 650 healthy unrelated individuals from Yunnan Yi nationality. Calculate population genetic parameters, and establish genetic basis data of Yunnan Yi ethnic group. Aim to provide scientific evidence for forensic material certificate and personal identification.

Methods: The sample DNA was extracted by Chelex-100 method, amplified by PowerPlex®21System kit. The PCR composite amplification product was analyzed by ABI 3130XL automatic genetic analyzer. STR genotyping analysis was performed by ABI GeneMapper v3.2 software. The typing and nomenclature assignment of DNA were based on ISFG recommendations. Control DNA (2800 M) and ddH₂O were used as positive and negative controls. Statistical analysis of forensic genetic parameters and Hardy-Weinberg equilibrium test were performed using Power-States software.

Results: This study investigated 650 cases of Yunnan Yi nationality unrelated individuals. Genotyping of 20 autosomal STR loci in D3S1358, D1S1656, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, FGA and D6S1043. A total of 248 alleles and 933 genotypes were detected, and 8 (in D3S1358, D16S539, TH01, D5S818 and TPOX) to 20 (in Penta E, D21S11 and FGA)

alleles for each locus were observed. The observed heterozygosity (Hobs) ranged from 0.6108 (in TPOX) to 0.8815 (in Penta E), and the polymorphism information content (PIC) ranged from 0.5675 (TPOX) to 0.8851 (Penta E). The power of discrimination (PD) ranged from 0.8082 (TPOX) to 0.9779 (Penta E), with a combined power of discrimination (CPD) value of 0.99999999999999999996. The power of exclusion (PE) ranged from 0.3040 (TPOX) to 0.7579 (Penta E), with a combined power of exclusion (CPE) value of 0.999999907. Except D18S51, Penta D and FGA, the genotype distribution accorded with Hardy-Weinberg equilibrium ($P > 0.05$).

Conclusion: The 20 autosomal loci have high polymorphism and good personal recognition ability in the Yunnan Yi population, could provide scientific genetic basic data for forensic material certificate and personal identification.

Advances in Microbial-Brain-Intestinal Axis and Alcohol Dependence Syndrome

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The trillions of microbes colonized in the human gut are key factors in regulating body health and disease conversion. In patients with alcohol dependent syndrome, after long-term drinking, the type and quantity of microbes in the intestines were changed, and intestinal permeability increases, causing intestinal-derived bacteria to translocate to other organs of the body, affecting the functions of the enteric nervous system and central nervous system. Alcohol dependent patients are often accompanied by varying degrees of emotional disorders such as depression and anxiety. At the same time, the central nervous system also plays a top-down regulation role, by regulating the gastrointestinal physiology affects the composition of the intestinal microbiota in alcohol dependent patients. The interaction between gut microbes and the brain indicates that the "microbial-brain-intestinal axis" plays an important role in the formation and pathogenesis of alcohol dependence. Based on the latest research progress at home and abroad, this paper firstly explains the causes of intestinal microbial changes in alcohol dependent patients, and then introduces the mechanism of microbial-brain-intestinal axis interaction, and finally summarizes the role of microbial-brain-intestinal axis in alcohol dependent syndrome and related psychiatric diseases. In order to provide a new strategy for the treatment of related mental diseases.

Population data of 20 autosomal STR loci in Chinese Han population from Yunnan Province, Southwest China

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Objective: To study the genetic polymorphism of 20 autosomal STR loci in 2220 healthy unrelated individuals in Yunnan Han nationality, calculate population genetic parameters, and establish genetic basis data of Yunnan Han population, and provide scientific basis for forensic material identification and individual identification.

Methods: The sample DNA was extracted by Chelex-100 method, PCR amplification was performed with PowerPlex®21 System kit, PCR composite amplification products were analyzed by ABI 3130XL automatic genetic analyzer, and STR genotyping analysis was performed with ABI Gene Mapper v3.2 software. Power-States software for statistical analysis of forensic genetic parameters and Hardy-Weinberg equilibrium test.

Results: A total of 300 alleles and 1263 genotypes were detected in 2220 samples. The number of alleles of 20 autosomal loci was observed to be between 9 (D16S539) and 29 (FGA), and the heterozygosity (H) ranged from 0.6129 (TPOX) to 0.8962 (Penta E). The polymorphism information content (PIC) ranged from 0.5609 (TPOX) to 0.9016 (Penta E). The range of power of discrimination (DP) ranged from 0.7997 (TPOX) to 0.9844 (Penta E). Except D1S1656 and Penta E, the genotype distribution accorded with Hardy-Weinberg equilibrium ($P > 0.05$). The cumulative probability of exclusion (CPE) was 0.999999907, and the cumulative probability of discrimination (CDP) was 0.99999999999999999998.

Conclusion: The 20 autosomal loci have high polymorphism and good personal recognition ability in Yunnan Han population, and can provide scientific genetic basis data for forensic personal identification and paternity identification.

Stavudine exposure results in developmental defects by causing DNA damage, inhibiting cell proliferation and inducing apoptosis in mouse embryos

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Stavudine is an anti-AIDS drug widely used to prevent HIV transmission from pregnant mothers to the fetuses in underdeveloped countries for its low price. However, there is still a controversy on whether stavudine affects embryo development. In the current study, embryotoxicity of stavudine was evaluated using cultured mouse embryos. The data indicated that stavudine exposure at early neurulation affected embryonic growth and development in the brain, heart, neural tube, flexion, and caudal neural tube. Stavudine exposure reduced somite numbers, yolk sac diameter, crown-rump length, and increased absorption rate of deformity. At the molecular level, stavudine produced DNA damage, increased the levels of the phospho-CHK1 and cleaved-caspase-3, and decreased the expression level of proliferating cell nuclear antigen. These changes indicated that stavudine caused a coordinated DNA damage response, inhibited cell proliferation, and induced apoptosis in the embryos. Collectively these results suggest that stavudine exposure disturbs the embryonic development, and its use in pregnant mothers should be re-examined.

Mitochondrial PKC β -p66shc-ROS pathway in non-alcoholic steatohepatitis

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Nonalcoholic steatohepatitis (NASH) is a necro-inflammatory response that ensues when hepatocytes are injured by lipids. NASH is a potential outcome of nonalcoholic fatty liver (NAFL), a condition that occurs when lipids accumulate in hepatocytes. However, there are no approved pharmacotherapies for the treatment of NAFLD other than managing life style and controlling diets. Extensive studies have demonstrated that multiple mechanisms are involved in free fatty acid (FFA)- and high fat diet (HFD)-induced hepatic injury, including mitochondrial dysfunction, activation of oxidative stress and endoplasmic reticulum (ER) stress and lysosome dysfunction. Src-homology-2-domain-containing transforming protein 1 (p66Shc) is a redox enzyme that mediates H₂O₂ formation via the oxidation of cytochrome c and plays a critical role in mitochondrial ROS production. A previous study reported that Isosteviol (ISV), a derivative of stevioside, prevents HFD-induced hepatic injury. In the present study, we found that ISV inhibited protein kinase C- β (PKC- β) activity, which was activated by FFA or HFD. We examined the potential cellular/molecular mechanisms underlying ISV-mediated protective effect against FFA-/HFD-induced hepatic lipotoxicity using both in vitro primary rat hepatocytes and the in vivo rat liver damage model. The results indicated that ISV inhibits FFA-/HFD-induced hepatic injury via reducing oxidative. Specifically, ISV inhibited the expression, activation and mitochondrial translocation of p66Shc, an adapter protein, which mediates oxidative stress-induced injury and is a substrate of PKC- β . However, ISV had no effect on the expression and activity of peptidyl-prolyl

cis-trans isomerase and phosphatase A2, isomerase and phosphorylase of p66Shc. ISV also increased AMPK and PPAR- α mRNA levels and decreased SREBP1 mRNA level which indicated that ISV might regulate the lipid metabolism balance in hepatocytes. We found that ISV prevents FFA/HFD-induced hepatic injury through modulating PKC- β /p66Shc/oxidative pathways. Therefore, this mitochondrial pathway involved the oxidative stress and lipid metabolism balance in hepatocyte. The compound which could block this pathway will be a promising therapeutic agent for NASH or NAFLD in the future.

MiRNA-223 controls liver inflammation and injury by targeting multiple inflammatory genes

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Nonalcoholic fatty liver disease (NAFLD) represents a spectrum of diseases ranging from simple steatosis to more severe forms of liver injury including nonalcoholic steatohepatitis (NASH), fibrosis, and hepatocellular carcinoma (HCC). In humans, only 20%-40% of patients with fatty liver progress to NASH, and mice fed a high-fat diet (HFD) develop fatty liver but are resistant to NASH development. To understand how simple steatosis progresses to NASH, we examined hepatic expression of anti-inflammatory microRNA-223 (miR-223) and found that this miRNA was highly elevated in hepatocytes in HFD-fed mice and in human NASH samples. Genetic deletion of miR-223 induced a full spectrum of NAFLD in long-term HFD-fed mice including steatosis, inflammation, fibrosis, and HCC. Furthermore, microarray analyses revealed that, compared to wild-type mice, HFD-fed miR-223 knockout (miR-223KO) mice had greater hepatic expression of many inflammatory genes and cancer-related genes, including (C-X-C motif) chemokine 10 (Cxcl10) and transcriptional coactivator with PDZ-binding motif (Taz), two well-known factors that promote NASH development. In vitro experiments demonstrated that Cxcl10 and Taz are two downstream targets of miR-223 and that overexpression of miR-223 reduced their expression in cultured hepatocytes. Hepatic levels of miR-223, CXCL10, and TAZ mRNA were elevated in human NASH samples, which positively correlated with hepatic levels of several miR-223 targeted genes as well as several proinflammatory, cancer-related, and fibrogenic genes. Conclusion: HFD-fed miR-223KO mice develop a full spectrum of NAFLD, representing a clinically relevant mouse NAFLD model; miR-223 plays a key role in controlling steatosis-to-NASH progression by inhibiting hepatic Cxcl10 and Taz expression and may be a therapeutic target for the treatment of NASH.

STAT5 Confers Drug Resistance to Tamoxifen and Adriamycin in Breast Cancer Cell Lines

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Introduction: Breast cancer is the most common malignant tumor in women, and luminal A type represents about 1/3 of all patients. Endocrine therapy is the preferred strategy to treat luminal A breast cancer patients. However, more than 30% ER+ breast cancer patients are hormone resistant at diagnosis, and up to 30% ER+ patients in the adjuvant setting will acquire resistance to the inhibitory effects of their endocrine treatment. Chemotherapy is also an important approach to reduce metastasis and recurrence in breast cancer, but chemoresistance exists in a considerable number of patients, which presents an important factor inducing failure of the treatment. Thus it's important and urgent to figure out the mechanism of drug-resistance and overcome it to improve the treatment for patients with luminal A breast cancer.

Aberrant expression and activation of signal transducer and activator of transcription 5 (STAT5) is not only implicated in tumorigenesis and cancer progression, but also contributes to chemoresistance in various cancers. It is well proved by previous studies that STAT5 functions in association with EGFR, whose amplification and mutation is well known to be involved in the initiation and development of various cancers, such as lung cancer, glioblastoma, and head and neck squamous cell carcinoma. Importantly, EGFR contributes to drug resistance in multiple cancers as well. However, in breast cancer, the role of STAT5 and its relationship with EGFR in drug resistance to endocrine therapy and chemotherapy still remains to be explored. Here we aimed to investigate the importance of STAT5 in drug resistance in breast cancer, and the role of EGFR involved in the underline mechanism.

Methods :Breast cancer cell line MCF-7, adriamycin (ADR)-resistant MCF-7 (MCF-7/ADR) and tamoxifen (TAM)-resistant MCF-7 (MCF-7/TAM) were used in this study. MCF-7/ADR and

MCF-7/TAM cells were established by culturing MCF-7 cells in medium with 1 μ g/ml ADR or 1 μ M TAM respectively for over 6 months. Tandem Mass Tag (TMT) proteomic analysis was applied for analysis of protein expressing profiles of wild type MCF7 cell line, MCF-7/ADR and MCF-7/TAM cell lines. IC50 of adriamycin or tamoxifen was determined by MTT assays. Expression levels of related proteins were determined by Western blot. Knocking down of STAT5a/b was achieved by transfecting small interfere RNA oligos. TCGA database was analyzed with the online tool GEPIA2 (gepia2.cancer-pku.cn).

Results: A panel of 6645 proteins was identified by the TMT proteomic analysis in which 5683 proteins could be quantified. Compared with protein profile in MCF7, 248 and 255 proteins were upregulated more than 2 folds and 377 and 409 proteins were downregulated more than 50% in MCF7/ADR and MCF7/TAM, respectively. Interestingly, MCF7/ADR and MCF7/TAM cell lines shared 187 upregulated proteins and 310 downregulated proteins, which suggested some common mechanism conducting the resistance to these two distinct drugs. Expression of EGFR in MCF7/ADR and MCF7/TAM is 3.821 and 3.7 times as that in MCF7, respectively. What's more, protein expression levels of ABCB1 and GSTP1, which were vital drug resistance associated genes and proved to be regulated by EGFR, were upregulated for 15.728 and 4.01 times or 12.903 and 9.129 times compared that in MCF7, in MCF7/ADR and MCF7/TAM, respectively. These results were further confirmed by Western blotting assays. Though expression of STAT5 showed no elevation by proteomics analysis, results of Western blotting showed that activated phosphorylated-STAT5 (p-STAT5) is elevated in drug-resistant cell lines. In addition, analysis of TCGA database suggested that expression of STAT5b is associated with poor prognosis in patients with luminal A breast cancer. Furthermore, p-STAT5 (Tyr694/699) and EGFR (Tyr1068) could be activated by the stimulation of adriamycin and tamoxifen in MCF7, ABCB1 and GSTP1 protein levels were also altered after the stimulation. Finally, knocking-down of STAT5 sensitized MCF-7/ADR and MCF-7/TAM cell lines to adriamycin and tamoxifen, respectively.

Conclusion: Inhibition of STAT5 could reverse the drug resistance to adriamycin and tamoxifen, possibly through an EGFR dependent manner. This study renders STAT5 a potential target to overcome drug resistance in luminal A breast cancer.

Epigenetic regulation of energy metabolism

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Mitochondria are powerhouses regulating cellular and systemic energy metabolism. Although mitochondria have their own DNA encoding 13 oxidative phosphorylation proteins, mitochondrial function is mainly regulated by more than 1,000 proteins encoded by the “mitochondrial genes” in the nuclear genome. While transcription factors have been the focus of research on mitochondrial gene regulation, how epigenetic factors are integrated into the transcriptional networks remain elusive. Here we show that knockout of the histone demethylase LSD1 in liver of adult mice (LSD1-LKO) reduces the expression of approximately one-third of all nuclear-encoded mitochondrial genes and decreases mitochondrial biogenesis and function. ChIP-seq analysis reveals that LSD1 and LSD1-regulated histone methylation modulate the enhancer and promoter activity of the nuclear-encoded mitochondrial genes. Interestingly, despite the reduced hepatic mitochondrial function, LSD1-LKO mice are protected from diet-induced hepatic steatosis and glucose intolerance, partially due to the induction of hepatic FGF21 expression. Thus, LSD1 serves as a master epigenetic modulator of mitochondrial gene expression to regulate cellular and systemic energy metabolism.

Role of melatonin signaling in biliary senescence and liver fibrosis in cholangiopathies

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Background/experimental approach: Biliary damage/senescence and liver fibrosis are common features in cholangiopathies including primary sclerosing cholangitis (PSC) and primary biliary cholangitis (PBC). During the progression of cholangiopathies enhanced biliary senescence is characterized by the secretion of Senescence-Associated Secretory Phenotypes (SASPs) such as TGF-beta1, IL6 and TNFalpha that activate hepatic stellate cells by paracrine mechanisms. Melatonin is synthesized by the pineal gland and peripheral organs including the liver and intestine through the enzymes serotonin N-acetyltransferase (AANAT) and acetyl-serotonin O-methyltransferase (ASMT); melatonin synthesis increases with dark exposure but decreases with exposure to light. Melatonin therapy or prolonged exposure to complete darkness reduces biliary hyperplasia and liver fibrosis in bile-duct ligated (BDL) rats by interacting with the melatonin subtype receptor, MT1; however, no information exists in PSC and PBC. We aimed to determine the therapeutic effects of prolonged dark therapy or melatonin administration on biliary mass (ductular reaction) and senescence, macrophage infiltration, liver fibrosis, angiogenesis and miR-200b (miRNA increased in PSC and PBC) in mouse models of PSC (*Mdr2*^{-/-}) and late stage PBC (dominant-negative transforming growth factor-beta receptor II, *dnTGFbRII*) treated with vehicle or melatonin or exposed to complete darkness or 12:12 light/dark cycles for 1 wk. In human samples from PSC and PBC, we evaluated the expression of AANAT, MT1, angiogenesis and miR-200b in total liver RNA and sections as well as melatonin serum levels. *Mdr2*^{-/-} mice were treated in vivo with miR-200binhibitor or control before evaluating biliary mass, liver fibrosis, and angiogenesis. After overexpression of AANAT or inhibition of miR-200b in cholangiocyte cell lines, we evaluated: (i) biliary proliferation, senescence and fibrosis; and (ii) the paracrine effect of cholangiocyte supernatant on the fibrosis mRNA expression of hepatic stellate cell lines

(HSCs). Results: After exposure to darkness or administration of melatonin, *Mdr2*^{-/-} and *dnTGFbRII* mice show elevated serum melatonin levels, reduction of MT1 and miR-200b expression, and inhibition of ductular reaction along with decreased macrophage infiltration, biliary senescence liver fibrosis and angiogenesis. In samples from PSC and PBC patients there was reduced AANAT expression and melatonin levels but enhanced MT1 and miR-200b expression. In vitro, overexpression of AANAT or inhibition of miR-200b in cholangiocyte lines decreased the expression of miR-200b, angiogenesis and fibrosis genes. In HSCs treated with supernatant from cholangiocytes (overexpressing AANAT or treated with miR-200b inhibitor) there was reduced fibrosis mRNA expression compared to control cholangiocyte supernatant. Supporting the concept that in PSC and PBC (characterized by gut dysfunction) changes in melatonin secretion from the intestine may affect liver function, we demonstrated pathological phenotypes (by H&E staining), reduced AANAT expression but increased MT1 expression in the small intestine of *Mdr2*^{-/-} and *dnTGFbRII* mice, changes that were reversed by dark exposure or melatonin treatment. Dark or melatonin therapy or targeting melatonin/miR-200b axis may be important in the management of biliary damage and liver fibrosis in cholangiopathies including PSC and PBC.

Pooled Genome-wide CRISPR/Cas9 Knockout Screening Uncovers Target Genes Required for ADI-PEG20 Resistance

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In more than 70% tumors, downregulation of the rate-limiting enzyme in arginine synthesis, argininosuccinate synthetase (ASS1), results in an inability to synthesize arginine for growth. Thus, this intrinsic dependence on extracellular arginine, arginine auxotrophy, emerges as a common Achilles' heel for cancer and a series of arginine depleting agents are developed accordingly to deprive cancer cells of arginine. Currently, the arginine metabolizing enzyme, PEGylated bacterial arginine deiminase (ADI-PEG20), is applied in numerous clinical trials in different cancers with excellent safety profile. However, resistance on ADI-PEG20 has been occasionally arisen upon prolonged treatment. Exploring the mechanism underlying the ADI-resistance could offer novel insights into arginine auxotrophy and therapeutically exploited. In this study, a pooled genome-wide CRISPR/Cas9 screening was performed in ADI-resistant MDA-MB-231 breast cancer cells and identified 353 putative genes required for ADI-resistance. Analysis by Ingenuity Pathway Analysis returned a number of regulatory networks involved, including PI3K signaling, chemokine signaling and p70S6K signaling. This study present the power of a genome-wide CRISPR/Cas9 screen to systematically identify biological pathways involved in drug resistance and to provide potential target genes as clinically actionable candidates.

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Biomarkers for Cancer immunotherapy response prediction

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Immunotherapy, represented by immune checkpoint inhibitors, is transforming the treatment of cancer. However, only a fraction of patients show response to immune checkpoint inhibitors, and there is an unmet need for biomarkers that will identify patients more likely to respond to immunotherapy.

Non-small cell lung cancer (NSCLC) is known to carry heavy mutation load. Besides smoking, cytidine deaminase APOBEC3B plays a key role in the mutation process of NSCLC. APOBEC3B is also reported to be upregulated and predicts bad prognosis in NSCLC. However, targeting APOBEC3B high NSCLC is still a big challenge. Here we show that APOBEC3B upregulation is significantly associated with immune gene expression, and APOBEC3B expression positively correlates with known immunotherapy response biomarkers, including: PD-L1 expression and T-cell infiltration in NSCLC. Importantly, APOBEC mutational signature is specifically enriched in NSCLC patients with durable clinical benefit after immunotherapy and APOBEC mutation count can be better than total mutation in predicting immunotherapy response. In together, this work provides evidence that APOBEC3B upregulation and APOBEC mutation count can be used as novel predictive markers in guiding NSCLC checkpoint blockade immunotherapy (Wang S. et al. APOBEC3B and APOBEC mutational signature as potential predictive markers for immunotherapy response in non-small cell lung cancer. *Oncogene*. 2018 Jul;37(29):3924-3936).

Tumor mutational burden (TMB) is an emerging predictive biomarker for immune checkpoint inhibitors response. Here we reported for the first time that the immunotherapy response prediction biomarker TMB shows significant sex differences. TMB's predictive power is significantly better for female than for male lung cancer patients. Receiver operating characteristic curve analysis was performed and the area under the curve (AUC) was reported to evaluate the predictive power of TMB in lung cancer ICI response. Hazard ratios (HR) of TMB-high vs. TMB-low patients were

compared between male and female patients. Both AUC and HR differences between female and male are significant in all available independent lung cancer datasets. However, the AUC of programmed death ligand 1 (PD-L1) expression does not show a difference between female and male, suggesting TMB, but not PD-L1 expression has a better predictive power for female than for male lung cancer patients. Our study suggests significant sex differences in the performance of TMB in cancer immunotherapy response prediction. Future development of cancer immunotherapy biomarker should consider sex differences and special efforts should be paid to improve the performance of immunotherapy predictive biomarkers for male lung cancer patients (Wang S. et al. The predictive power of tumor mutational burden in lung cancer immunotherapy response is influenced by patients' sex. *Int J Cancer*. 2019 Apr 10. doi: 10.1002/ijc.32327.).

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Circular RNA TLK1 acts as the sponge of miR-136-5p to promote renal cancer progression

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Background: Circular RNAs (circRNAs), a novel type of ncRNAs, are covalently linked to make up circular configuration via a loop structure. Accumulating evidence has indicated that circRNAs are potential biomarkers and key regulators in the development and progression of tumor. However, the precise role of circRNAs in Renal cancer remains unknown. **Methods:** Though circRNA high-throughout sequencing from renal cancer cell lines, we determined circRNA TLK1 (circTLK1) as a novel candidate circRNA derived from TLK1 gene. Quantitative real-time PCR (qRT-PCR) was performed to detect the expression levels of mRNAs, circRNAs and miRNA in renal cancer tissues and cells. Loss-of function experiments were executed to detect the biological roles of circTLK1 on the phenotypes of renal cancer cells in vitro and in vivo. RNA-FISH, RNA-pull down, dual-luciferase reporter assay, western blot and immunohistochemistry were used to investigate the molecular mechanisms underlying the functions of circTLK1. **Results:** CircTLK1 is overexpressed in renal cancer and positively correlated with distant metastasis and unfavorable prognosis. Silencing of circTLK1 significantly inhibits the proliferation, migration and invasion of renal cancer cells in vitro and in vivo. Mechanistically, circTLK1 is mainly distributed in the cytoplasm and positively regulates CBX4 expression by sponging miR-136-5p. In addition, forced expression of CBX4 reverses the phenotypes inhibition of renal cancer cells induced by circTLK1 suppression. Moreover, CBX4 expression is positively correlated with VEGF expression in renal cancer tissues. Knockdown of CBX4 significantly inhibited VEGF expression in renal cancer cells. **Conclusion:** Collectively, our findings demonstrate that circTLK1 plays a critical role in renal progression by

sponging miR-136-5p to increase CBX4 expression. CircTLK1 may act as a diagnostic biomarker and therapeutic target for renal cancer. Yaoting Gui, a researcher at Peking University Shenzhen Hospital, working as the director of the Guangdong Provincial Key Laboratory of Male Reproduction and Genetics and the director of the Institute of Urology, Peking University Shenzhen Hospital. In addition, he is the doctoral tutor at Peking University.

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Sequence Maps — Sophisticated Accelerometer Data Analysis

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Up to know epidemiological studies have focused on the potential health effects of total volume of physical activity (PA) or sedentary behavior (SB). However, the total volume of PA or SB from different person might be similar, while but accumulated sequence might different. The pattern of PA and SB might play an more important role than the total volume to health and diseases. Therefore we aim was to develop a sophisticated algorithm translating accelerometer data into detailed sequence maps considering, and the machine learning methods, and other tools could be applied on this data analysis with this approach.

We developed a novel approach to transfer accelerometer counts into a sequence map based on behavior states defined by a combination of intensity (SB, light, moderate, and vigorous intensity) and duration (sporadic accumulation or in bouts of different duration). Additionally, hierarchical cluster analysis and statistical analysis was applied to identify performance of children with similar behavioral sequence maps.

Clustering resulted in seven clusters of children with similar PA and SB sequence maps: an average cluster (33% of children); a cluster with relatively more SB, light and moderate PA in bouts (SB and PA bouters, 31%); a cluster characterized by more sporadic SB and light PA (light activity breakers, 26%); and four smaller clusters with 7% of the children or less.

This novel algorithm is a next step in sophisticated analyses of accelerometer data with combination the intension and duration of original sequence. The sequencing was reduced with this approach, which could be used for machine learning method. The application of this approach on many cohorts are presented.

Effects of protein kinase A catalytic subunit on sperm motility regulation in Pacific abalone *Haliotis discus hannai*

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Sperm motility describes the ability of sperm to move towards the egg, which ultimately determines the fertilization rate. Protein kinase A plays a conservative and central role in sperm motility regulation from echinoderms to mammals, but its regulatory role in mollusks is poorly studied. In this study, a protein kinase A catalytic subunit (designated as HdPKA-C) was identified from Pacific abalone *Haliotis discus hannai* and investigated to clarify its possible role in sperm motility regulation. The open reading frame of HdPKA-C was of 1077 bp, encoding a peptide of 358 amino acids with a typical protein kinase domain. HdPKA-C shared 82-87% sequence similarities with other PKA-Cs, and was clustered first with gastropod homologues in the phylogenetic tree. The mRNA transcripts of HdPKA-C were constitutively expressed in all examined tissues, with the highest level detected in hepatopancreas. Subcellular localization analysis showed that the phosphorylated form of HdPKA-C (p-HdPKA-C) was localized at the acrosome, connecting piece and along the flagellum of abalone spermatozoa with variable intensity. The phosphorylated substrates of HdPKA-C showed similar distribution pattern with p-HdPKA-C, and much lower intensity was observed at the connecting piece in comparison to that of p-HdPKA-C. Inhibition of HdPKA-C activity by H-89 led to a significant reduction in the percentage of motile sperm and sperm velocities. Western blot analysis showed that p-HdPKA-C was detected at 42 kDa with a uniform content in strip spawned sperm, naturally spawned sperm and H-89 treated sperm, whereas different phosphorylation patterns of HdPKA-C substrates were detected in different states of sperm. These results collectively indicated that HdPKA-C played an important role in the regulation of abalone sperm motility by altering its substrates phosphorylation.

What is aging and when does aging start?

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Aging is a subject where the theorists have found no consensus, and where every existing theory is challenged by some experimental finding that contradicts it. Historically, this has been the hallmark of a field ripe for new insights, often with ramifications that ripple through science. Using *C. elegans* as a model system, we address a fundamental question “what is aging?” My lab has taken an approach that starts with characterizing the aging process in a systematic and quantitative manner. This includes quantifying abundance changes of mRNAs, proteins, and metabolites on a global scale. By documenting the age-associated molecular changes in *C. elegans*, we aim to find the earliest sign of aging and the biomarkers of physiological age. I would like to discuss with fellow researchers the preliminary results obtained at this point and potential insights that could be gained from them.

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Population genomics of fungal adaptation to insect hosts

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The early studies of individual fungal genomes will benefit our understanding of fungal gene contents, sex nature and particular protein-family expansions/contractions in association with fungal life styles. We are studying ascomycete insect pathogenic fungi. Genome sequencing of the model species of *Metarhizium* and *Beauveria* indicated that insect pathogens evolved with the expanded families of proteases and chitinases to target insect protein- and chitin-rich cuticles. Phylogenomic analyses of *Metarhizium* species with different host ranges revealed that the generalists species evolved from specialists via transitional species with intermediate host ranges and that this shift paralleled insect evolution. Comparative analysis with the plant and mammalian pathogens indicated that, unexpectedly, more common orthologous protein groups are found to exist between the insect and plant pathogens than between the insect and mammalian pathogens. The accumulated evidence of substantial genetic divergence between fungal strains promotes the sequencing/resequencing of dozens to hundreds of isolates collected from different environments for a single species. We therefore performed population genetics study of *B. bassiana* after releases of exotic strains to control insect pests for more than 20 years. Our results revealed the marginal population differentiation between periodical populations, frequent host jumping, and gene flow between populations. It is of particular importance to find that the released strains persisted in the environment for a long time but with low recovery rates to infect non-target hosts. We also found that substantial population replacement occurred once a decade. However, the population evolved preferentially towards a balancing selection, i.e., the evidence of a trench warfare instead of an arms-race model of evolution of the insect pathogen population. The genetic evidence of the non-strict control of isolate host preference implied that the infection of non-target host is evitable by the released strains but endemically occurs akin to the local strains. Thus, the concerns of environmental safety regarding the biocontrol application of mycoinsecticides can be alleviated.

Poly(dA:dT) suppresses HSV-2 infection of human cervical epithelial cells through RIG-I activation

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Epithelial cells of the female reproductive tract (FRT) participate in the initial innate immunity against viral infections. Both DNA and RNA sensors have a crucial role in detecting viral infections and activating IFN-based immune response. Here we examined whether poly(dA:dT), a ligand for DNA sensors, can inhibit herpes simplex virus type 2 (HSV-2) infection of human cervical epithelial cells (HCEs). We demonstrated that poly(dA:dT) treatment of the cells could significantly inhibit HSV-2 replication in human cervical epithelial cells. This poly(dA:dT)-mediated HSV-2 inhibition is associated with the induction of the intracellular IFNs and the multiple antiviral IFN-stimulated genes (ISGs), including ISG56, OAS1 and OAS2. Mechanistically, poly(dA:dT)-mediated HSV-2 inhibition is through the activation of RIG-I, as evidenced by the findings that poly(dA:dT) had little effect on HSV-2 infection and IFNs and ISGs induction in RIG-I knockout cells. These observations for the first time demonstrate the key role of RIG-I in the DNA sensor ligand-mediated anti-HSV-2 activity in HCEs, indicating the potential for developing DNA sensor ligand-based treatment of HSV-2 infection.

Keywords: Herpes simplex virus type 2 (HSV-2); Human cervical epithelial cells (HCEs); Poly(dA:dT); IFN; IFN-stimulated genes (ISGs); CRISPR-Cas9

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Deacetylation of Serine Hydroxymethyl-transferase 2 by SIRT3 promotes Colorectal Carcinogenesis

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The conversion of serine and glycine that is accomplished by serine hydroxymethyltransferase 2 (SHMT2) in mitochondria is significantly upregulated in various cancers to support cancer cell proliferation. In this study, we observed that SHMT2 is acetylated at K95 in colorectal cancer (CRC) cells. SIRT3, the major deacetylase in mitochondria, is responsible for SHMT2 deacetylation. SHMT2-K95-Ac disrupts its functional tetramer structure and inhibits its enzymatic activity. SHMT2-K95-Ac also promotes its degradation via the K63-ubiquitin-lysosome pathway in a glucose-dependent manner. TRIM21 acts as an E3 ubiquitin ligase for SHMT2. SHMT2-K95-Ac decreases CRC cell proliferation and tumor growth in vivo through attenuation of serine consumption and reduction in NADPH levels. Finally, SHMT2-K95-Ac is significantly decreased in human CRC samples and is inversely associated with increased SIRT3 expression, which is correlated with poorer postoperative overall survival. Our study reveals the unknown mechanism of SHMT2 regulation by acetylation which is involved in colorectal carcinogenesis.

Novel Roles of SIRT6 in DNA Repair

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Sirtuin 6 (SIRT6) is a mammalian homolog of the yeast Sir2. In addition to its major roles in lifespan extension, it has also been demonstrated to participate in regulating DNA double strand break repair (DSBR) by homologous recombination (HR), alternative nonhomologous end joining (alt-NHEJ) and canonical NHEJ (c-NHEJ). However, whether it has roles in other types of DNA repair remains to be determined. Here, Here we show that similar to nucleotide excision repair (NER) factors including DDB2 and RAD23A high expression level of SIRT6 is associated with low recurrence rates of melanoma, suggesting that SIRT6 might directly participate in NER to prevent tumorigenesis. We demonstrated that SIRT6 is required for the repair of UV induced DNA damage. In response to UV irradiation SIRT6 was recruited to DNA lesions and interacted with more DDB2, the major sensor initiating the global genome NER (GG-NER). SIRT6 deacetylated DDB2 and promoted its segregation from chromatin, therefore facilitating the downstream signalling. In addition, we characterized several patient-derived SIRT6 mutations. The mutant proteins are associated with high mutation rates across the genomes in melanoma. These SIRT6 mutants ablate its stimulatory effect on NER and destabilize genomes due to (1) partial loss of its enzymatic activity (P27S, H50Y), (2) a nonsense mutation (R150*), (3) high turnover rates (G134W). In summary, we demonstrated that SIRT6 participates in NER pathway through deacetylating DDB2, therefore preventing the onset of melanoma tumorigenesis.

Epigenetic characteristic of female germline stem cell development

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High-throughput stage-specific transcriptomics provides an unbiased approach for understanding the process of cell development. Here, we report transcriptome analysis of primordial germ cell, female germline stem cell (FGSC), germinal vesicle and mature oocyte by performing RNA sequencing of freshly isolated cells in mice. As expected, these stages and gene-expression profiles are consistent with developmental timing. Analysis of genome-wide DNA methylation during female germline development was used for confirmation. By pathway analysis and blocking experiments, we demonstrate PI3K-AKT pathway is critical for FGSC maintenance. We also identify functional modules with hub genes and lncRNAs, which represent candidates for regulating FGSC self-renewal and differentiation. Remarkably, we note alternative splicing patterns change dramatically during female germline development, with the highest occurring in FGSCs. In addition, we have successfully differentiated mouse FGSCs into oocytes in vitro. Furthermore, we reveal chromatin structure features of these germ cells using high-throughput chromosome conformation capture assays. These findings are invaluable resource for dissecting the molecular pathways and processes into oogenesis and will be wider applications for other types of stem cell research.

Music making from biomarkers to functional patient profile: a way leading to personalized medicine?

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If we want to deliver on personalized medicine, we need to develop new forms of diagnostics and improve our way to process data. But how do we move from single marker to pattern recognition profiling? It requires the new ways of processing, analysing and visualizing dynamic patient data. It also needs the proper statistical tools for the interpretation of such large-scale biological data. From the immunological point of view, the principle of novel diagnostic tools includes the phenotype measurements of immune cells, phosphorylation status detection of cell signal transduction pathways, and the functional profiling and evaluation. Interpretation of modern biology, which is a data-rich science, driven by our ability to measure the detailed molecular characteristics of cells, organs, and individuals at many different levels, requires the detection of statistical dependencies and patterns in order to establish useful models of complex biological systems. Techniques from machine learning are key in this endeavour. Typical examples are the visualization of high-throughput (flow cytometric) data using dimensionality reduction methods, as well as classification and neural-network based model establishing. Here several examples will be discussed to show that standardization of data structure makes processing of data more easy, use of Shiny-server (R) gives access and real-time viewing of data, patient cohorts can be easily compared and classified. By using the immunophenotyping and the functional phosphoproteome phenotype of patients in clinical context, we expect to evaluate the effect of medication on identified profiles. Further data integration and processing moving forward with pattern recognition profiling is our final goal to realize.

Preliminary Study on Biocompatibility of Fluorescent Carbon Dots

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Fluorescent carbon dots are a new type of zero-dimensional carbon material with the grain size of about 10nm and torispherical shape, which have attracted much attention due to its excellent optical properties, environmental friendliness, wide source of raw materials, easy surface functionalization and good biocompatibility. At present, the researches of fluorescent carbon dots are mostly concentrated in cell imaging, drug delivery, molecular or ions detection, etc., however, there are relatively few researches on biocompatibility evaluation of CDs, especially the reports on the interaction between CDs and biomacromolecules, such as proteins and nucleic acids in biological systems. Moreover, the reports on the toxicity of carbon at the molecular level of biotechnology are likewise few. With respect to biocompatibility, ISO conference shows an exact definition and the 20 relevant standards numbered 10993. Biocompatibility refers to the capability of living tissue to react to inactive substances. As for biological materials implanted into the body, they should not only be stable and effective under physiological conditions, but also have no adverse effects on human tissues, blood and immune systems. In this context, with sucrose and glutamic acid as raw materials, the fluorescence carbon dots were synthesized by hydrothermal method by optimizing the reaction conditions. Firstly, the physical and chemical properties of the synthesized carbon dots were analyzed, and the structure of the prepared CDs was characterized in detail by Transmission Electron Microscope (TEM), Fourier Transform Infrared Spectrum (FT-IR), Ultraviolet-Visible Absorption Spectrum and Fluorescence Spectrum. Secondly, the cytotoxicity of carbon on human bladder cancer cell line T24 was studied at both cellular and molecular levels. The experimental results showed that the obtained CDs are mono-dispersed spherical particles with the average diameter of 2.16 nm and are hydrophilic with a large number of hydroxyl, amino and carboxyl groups on the surface, and excitation dependence. In addition, The toxicity of carbon dots were detected by CCK8 method, which showed that at low concentration (1 $\mu\text{mol/L}$), the inhibition

rate of CDs on T24 cell proliferation was lower than 10%. Western blotting assay detected the effect of CDs on the expression of apoptotic related proteins in cells, indicating that the expression of Bcl-2 and Cleaved-Caspase3 protein at concentrations of 1 $\mu\text{mol/L}$ and 10 $\mu\text{mol/L}$ was not significantly different from that of the blank control group. This above indicates that the prepared fluorescent carbon dots has good biocompatibility.

Transcriptome sequencing reveals the involvement of epigenetic modifications in the trans-generational immune priming from oyster

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Trans-generational immune priming (TGIP), whereby mothers and fathers transfer immunological experience to its progeny and contribute a trans-generational immune protection on offspring when encountered with same pathogens, has been demonstrated in several invertebrates system. It is currently unclear what specific materials and how such protection are transferred, and the potential underlying mechanism of transmission. We therefore conducted a double-mating experiment, mothers and fathers were immune-challenged with live bacteria or a control solution and assigned to one of three treatments in which one parent, or neither parents were immune-challenged. The results showed that parental immune challenge has significant consequences for the immunocompetence of their offspring when encountered with same pathogens. The catalase activity in offspring of immune primed fathers and mothers are both enhanced upon the same bacterial challenge, while the lysozyme activity, an important immune trait, was only significantly increased in the offspring from immune primed mothers. Transcriptomic analyses revealed that a series of differentially expressed genes, including oxidase, lysosomal protease, and immune receptors, were significantly up-regulated both in the offspring from immune primed mothers and fathers, confirming the evidence of enhanced immunity in the offspring from immune primed parents. GO annotation revealed that the differentially expressed genes are enriched in immunity, signal transduction, energy metabolism and development in immune primed male offspring. Specially, the expression levels of DNA methyltransferases (DNMT1 and DNMT3B) were significantly up-regulated during the blastula and trochophore stage periods in the immune primed female offsprings, while the expression levels of DNA methyltransferases (DNMT1 and DNMT3B) were comparatively significantly up-regulated after entering into D-shaped stage in the immune primed male offsprings, indicating that the potential involvement of DNA methylation in TGIP.

Key words: Trans-generational immune priming (TGIP); Catalase activity; Transcriptomic analyses; DNA methyltransferases;

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Oncogenic role of TAp73 in glucose metabolic remodeling and tumorigenesis

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Many cancer cells reprogram key metabolic pathways to fuel their high demands of biogenesis. Recently, we have identified a role for tumor suppressor p53 in modulating NADPH metabolism and ammonia metabolism in cancer cells. Unlike p53, another p53 family member TAp73, whose role in tumour cells had remained an enigma for many years, is frequently and highly expressed and almost never mutated in human cancers. Our recent studies also identify an oncogenic role of TAp73 in promoting tumor growth. We found that, by enhancing PPP flux, TAp73 increases the proliferating rate of tumour cells⁶. To further investigate the mechanism(s) for the pro-proliferative effect of TAp73, we explored the function of TAp73 in glycolysis and found that TAp73 stimulates the expression of phosphofructokinase-1, liver type (PFKL). Through this regulation, TAp73 enhances glycolytic flux, increases ATP production, bolsters anti-oxidant defense and promotes the Warburg effect. TAp73 deficiency results in a pronounced reduction in tumorigenic potential, which can be rescued by forced PFKL expression. These findings establish TAp73 as a critical regulator of glycolysis and reveal a mechanism by which tumor cells achieve the Warburg effect to enable oncogenic growth. Taken together, potentially our findings could explain why TAp73 is frequently overexpressed but never mutated in human cancers.

Heterogeneity in Hepatic Cancer Stem Cells

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Various cancer stem cell (CSC) biomarkers have been identified for hepatocellular carcinoma (HCC), but little is known about the implications of heterogeneity and shared/ or distinct molecular networks among the CSC population. These CSC biomarkers were EpCAM, CD90, CD133, CD44, and CD24 etc.

Through miRNA profile analysis in a HCC cohort (n=241) for five groups of CSC+ HCC tissues, i.e., EpCAM+, CD90+, CD133+, CD44+, and CD24+ HCC, we identified a 14-miRNA signature commonly altered among these five groups of CSC+ HCC and miRNAs uniquely altered in certain CSC positive HCCs. MiR-192-5p, the top ranked CSC-miRNA from 14-miRNA signature, was liver -abundant and -specific and markedly downregulated in all five groups of CSC+ HCC from two independent cohorts (n=613). Suppressing miR-192-5p in HCC cells significantly increased multiple CSC populations and CSC-related features. Loss of miR-192-5p in HCC cells also presented a highly enhanced glycolytic features including increased extracellular acidification rate (ECAR), glycolytic-related genes and metabolites, Glucose uptake, and Lactate production. A group of CSC and metabolism-related genes were evaluated as targets of miR-192-5p. Furthermore, both TP53 mutation and hyper-methylation of the mir-192 promoter impeded transcriptional activation of miR-192-5p in HCC cell lines and primary CSC+ HCC. Together these results reveal the genetic regulation of miR-192-5p and its role in regulating CSC stemness features via targeting PABPC4 and fueling CSCs with enhanced glycolysis for their successful infiltration.

We have also found some miRNAs were unique to certain CSC+ HCCs. miR-125b was significantly down-regulated CD24+ HCCs and EpCAM+ HCCs, we have currently revealed its role in miR-125b/YB-1/IRES/HIF1/pAKT circus, which was important to transarterial chemoembolization-resistance.

Our lab is continually working on these projects such as conditional miR-192KO mice work, and roles of miR-125b with its pathway in guiding HCC patients to choose proper therapy etc. Our final goal is to discover the CSC heterogeneity and understanding their potential hierarchical relationship in HCC.

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Gut microbiota and acute liver injury

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Gut microbiota has been recognized as a “novel” organ in the body and modulates acute liver damage. We summarized the underlying mechanism in which how gut microbiota regulates liver injury progression based on our findings. First, microbiota in the intestine could influence gut barrier integrity and further control microbial associate molecular pattern (MAMP) translocation. Translocated bacteria or their products may directly exert harmful effects. Second, intestinal bacterial metabolites could penetrate into the circulatory system and modulate redox balance of the host organ. For example, 1-phenyl-1,2-propanedione (PPD) derived from gut microbiota could exhaust hepatic glutathione (GSH) and sensitize liver to acetaminophen induced acute liver failure. Third, bacterial metabolites could also modulate host inflammatory responses. For example, granisetron (GA), a metabolite from gut microbiota could reduce p38 MAPK and Nf-kB activation, as well as NLRP3 inflammasome activation during LPS stimulation and further attenuate sepsis induced liver damage. These effects of gut microbiota provide mechanistic insights into the pathogenesis of organ damage in the context of multiply diseases development.

Protein ubiquitination in immune homeostasis and dysregulation

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Effective immune responses in our body are critical in defending invading pathogens and in operating surveillance against cancer, whereas such responses are balanced by tolerance mechanisms to prevent the immune system from attacking our own tissues. Loss of such balance could result in excessive tissue damage or malignant tumor formation. Our research for the last two decades has focused on E3 ubiquitin ligases in lymphocyte activation and tolerance induction. We previously showed that the E3 ubiquitin ligase VHL-hypoxic inducing factor (HIF) pathway is critical in controlling the stability and function of regulatory T cells by modulating interferon-production. We recently extended to the study of other cell types including innate lymphoid type 2 cells and lung macrophages, and found that the VHL-HIF axis is important in regulating their development and function via balancing the cellular glucose metabolism during lung inflammation. The latest results of our on-going research will be presented in this meeting. These findings will provide us with a unique opportunity in targeting the E3 ligases in different cell types for potential therapeutic intervention of human inflammatory diseases and cancer.

ALDH1A1 contributes to PARP inhibitor resistance in ovarian cancer via enhancing DNA repair

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Poly (ADP-ribose) Polymerase (PARP) inhibitors (PARPi) are approved to treat recurrent ovarian cancer with BRCA1 or BRCA2 mutations, and as maintenance therapy for recurrent platinum sensitive ovarian cancer (BRCA wild-type or mutated) after treatment with platinum. However, the acquired resistance against PARPi remains a clinical hurdle. Here, we demonstrated that PARP inhibitor (olaparib)-resistant epithelial ovarian cancer (EOC) cells exhibited an elevated aldehyde dehydrogenase (ALDH) activity, which is mainly contributed by increased expression of ALDH1A1 due to olaparib-induced expression of BRD4, a member of bromodomain and extraterminal (BET) family protein. We also revealed that ALDH1A1 enhanced microhomology-mediated end joining (MMEJ) activity in EOC cells with inactivated BRCA2, a key protein that promotes homologous recombination (HR) by using an intra-chromosomal MMEJ reporter. Moreover, NCT-501, an ALDH1A1 selective inhibitor, can synergize with olaparib in killing EOC cells carrying BRCA2 mutation in both in vitro cell culture and the in vivo xenograft animal model. Given MMEJ activity has been reported to be responsible for PARPi resistance in HR deficient cells, we conclude that ALDH1A1 contributes to the resistance to PARP inhibitors via enhancing MMEJ in BRCA2^{-/-} ovarian cancer cells. Our findings provide a novel mechanism underlying PARPi resistance in BRCA2 mutated EOC cells, and suggest that inhibition of ALDH1A1 could be exploited for preventing and overcoming PARPi resistance in EOC patients carrying BRCA2 mutation.

Regulation of Hepatitis B Virus Replication by Oncogenic Signaling

Pathway

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Hepatitis B virus (HBV) is a human tumor virus that causes hepatocellular carcinoma (HCC) through multiple mechanisms. While the chronic HBV infection is able to activate oncogenic signaling pathways that lead to tumorigenesis, on the other hand, HBV replication is also regulated by these oncogenic pathways. We have previously reported that the PI3K-Akt pathway suppresses HBV replication in hepatoma cells primarily through inhibiting viral transcription. In order to further elucidate the Akt-mediated antiviral mechanism, we conducted RNA-seq analyses and identified the hypoxia-inducible gene domain 1A (HIGD1A) protein as an Akt-induced host factor. In addition, HIGD1A was expressed at relative higher level in malignant than peritumoral liver tissue. Furthermore, ectopic HIGD1A expression significantly inhibited HBV replication, transcription and antigen expression in hepatoma cell lines, as well as in pAAV-HBV1.2 hydrodynamic injection mouse model. In contrast, HIGD1A knockdown led to enhanced HBV replication and antigen expression in vitro and in vivo. HIGD1A is mainly localized to mitochondria and relies on its TMR1 region for anti-HBV function. Mechanistically, HIGD1A activates NF- κ B pathway and upregulates NR2F1 expression to inhibit HBV core promoter activity, while the dominant-negative form of I κ B α or knockdown of NR2F1 blocked HIGD1A-mediated anti-HBV effect. Besides its effect on HBV, overexpression of HIGD1A in HCC cell lines promoted cells growth, proliferation, and G2/M cell cycle transition. Interestingly, HIGD1A knockdown also promoted the autophagosome formation, which might be beneficial for HBV replication. In conclusions, our results demonstrated that HIGD1A inhibits HBV transcription through NF- κ B-induced NR2F1, and it might represent an

intrinsic host antiviral factor that restricted HBV prolongation in hepatocytes. Additionally, the effect of HIGD1A on hepatocytes phenotype implicated its potential role in the hepatocellular carcinogenesis.

CRISPR-based liver cancer modeling and gene therapy

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The cancer genome is highly complex, with hundreds of point mutations, translocations, and chromosome gains and losses per tumor. Likewise, the clinical presentation of hepatocellular carcinoma (HCC), a major type of primary liver cancer, is highly heterogeneous and its diagnostic, prognostic and treatment assessment is complicated by both tumor biology and compromised liver function due to underlying chronic inflammatory liver diseases such as fibrosis and cirrhosis. To understand the effects of various cancer genes, precise models that incorporate both genomic changes in tumor cells and appropriate hepatic microenvironmental milieu are needed. However, the presence of considerable genomic alterations constitutes a bottleneck and poses an enormous challenge to effectively rank, triage and evaluate these candidate driver genes as druggable targets. Numerous genetically-modified mouse models for HCC are currently available. A critical question remains as to which mouse models are most relevant to human HCC. The recent development of the CRISPR-Cas9 system, a powerful genome-editing tool for efficient and precise genome engineering, is transforming mouse-model generation. We will describe how CRISPR-Cas9 has been used to create mouse models of liver cancer and for proof-of-concept gene therapy in mice. We will highlight the progress and challenges of such approaches, and how these models can be used to understand progression of liver tumors and identify new strategies for cancer treatment. The generation of precision liver cancer mouse models will provide a rapid avenue for functional cancer genomics and pave the way for precision cancer medicine.

Lysosomal Metabolic Signals in Orchestrating Cellular and Organism

Homeostasis

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Lysosomes are highly specialized cellular organelles with crucial functions in metabolic digestion and recycling, and carry diverse hydrolases to execute these functions. Work in my laboratory shows that lysosomal hydrolases are not only inert digestion tools, but also actively participate into signal transduction and mediate cellular communication among organelles. We discovered a lysosome-specific lipase that triggers lysosome-to-nucleus retrograde lipid signaling and promotes longevity, and revealed its critical roles in the metabolic and signaling crosstalk between lysosomes and mitochondria. We also discovered a lysosome-specific nucleotide phosphatase and its key functions in the control of ER proteostasis through interacting with mTOR signaling. These studies highlight the signaling role of lysosomal metabolism in the active coordination of cellular homeostasis and in the improvement of health aging.

DEVELOPMENT OF BROAD SPECTRUM ANTI-FILOVIRUS SMALL MOLECULE ENTRY INHIBITORS

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Ebola virus (EBOV) and Marburg virus (MARV) are filoviruses which are aggressive pathogens that cause highly lethal viral hemorrhagic fever syndrome. Currently, there are no FDA approved vaccines or antiviral drugs that are effective against EBOV/MARV infections in humans. Although several Ebola-specific vaccines show promising efficacy results in rodent or nonhuman primate (NHP) models, there is an urgent medical need to develop small molecule-based therapeutics that are efficacious, with long shelf-life (for the Strategic National Stockpile), low cost and easy to use in the field and clinics. Therefore we are developing broad-spectrum small molecule inhibitors at the viral entry step targeting the viral glycoprotein (GP). Here we show a novel mechanism of action (MOA) by a diverse small molecule inhibitors on viral entry of both Ebola and Marburg viruses, which provides a strategy for developing broad spectrum inhibitors. Optimization of the leads has produced new inhibitors with improved potency against both Ebola and Marburg virus.

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Genomic and Epigenomic Regulation of Prostate Cancer: Implications in Precision Medicine

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Gene expression profiling have revealed important epigenetic modifiers and transcriptional regulators such as AR, EZH2, and FOXA1 that are de-regulated in prostate cancer. Recent whole-exome sequencing studies further identified recurrent mutations to many of these genes including SPOP and FOXA1. My laboratory utilizes biochemical, genomic, and bioinformatics approaches to understand genomic and epigenomic regulations of prostate cancer. We focus on the late-stage of the disease including castration-resistant prostate cancer and neuroendocrine prostate cancer. Our studies have informed the molecular mechanisms by which AR, ERG, EZH2, FOXA1, and TRIM28 contribute to prostate cancer progression. Our findings have provided foundations for new therapeutic development.

Ion channels in neuroprotection and drug development

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Stroke is one of the leading causes of mortality in the world. Stroke research and the related neuroprotection studies have been focused on excitotoxicity and the traditional glutamate mechanism in stroke for decades. However, the inhibition of glutamate mechanism has not yet showed beneficial effects in stroke clinical trials even it shows sufficient neuroprotective effects from the bench. Thus, the drug development of stroke treatment is important in stroke research. Recently, the non-traditional non-glutamate mechanisms have attracted some attention in stroke research. Non-glutamate mechanisms, which include Transient receptor potential (TRP) channels, K(ATP) channels, Acid-sensing ion channel (ASIC), hemichannels, chloride channels, ion exchangers and other nonselective cation channels, etc., also lead to intracellular ionic imbalance and neuronal cell death in cerebral ischemia and stroke. Few examples of the non-glutamate mechanisms will be discussed in potential drug development target for cerebral ischemia and stroke.

Clinical Application Evaluation and Influencing Factors Analysis of Tuberculosis γ -Interferon Enzyme-Linked Immunosorbent Assay

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Background: This study focuses specifically on the discussion of gamma interferon, enzyme-linked immuno-assay release test results and influencing factors.

Methods: Data were collected from outpatient and inpatients who visited the First People's Hospital in Yunnan Province from October 2018 to March 2019, The process of which explores the clinical of gamma interferon accuracy and specificity, and the influence of other factors in the experiment.

Results: γ -Interferon comparison between experimental results and the final pathology confirmed results reveals a p value < 0.001 , signifying a statistically significant difference. Another statistically significant relationship ($p < 0.05$) was also established between factors such as age, interferon release a quantity, lymphocyte, CRP and mono-nuclear cell.

Conclusions: Gamma interferon has high consistency with pathological results; it can be used as a subsidiary to traditional clinical imaging and pathological judgment method; age, interferon release a quantity, lymphocyte, CRP and monocyte are influence factors of the experimental results.

Immune Properties of Mesenchymal Stromal/Stem Cells

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Mesenchymal stromal/stem cells (MSCs) are of great interests for their ability to regulate regeneration and inflammation processes and are being investigated as potential therapeutic tools for tissue repair and various autoimmune and inflammatory diseases. MSCs are capable of orchestrating innate and adaptive immune responses locally and systematically. The mechanisms by which MSCs exert their effects are multifactorial, but in general they are thought to respond to inflammation and changes of the tissue microenvironment and release various immune regulatory factors, metabolites, growth factors, chemokines and exosomes to modulate inflammation and tissue regeneration processes. It is believed that better understanding of the immune properties of MSCs will lead to novel strategies for unmet needs in the treatment of degenerative and inflammatory diseases.

A Novel Long Non-coding RNA as a Key Regulator of Tumor Metabolism

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Long non-coding RNA (LncR) has recently emerged as a novel biological entity contributing to oncogenesis. LncR range in size from a few hundred to tens of thousands of bases. A conservative estimate is that there are more than 30,000 such species rivaling the number of coding genes. While their detailed functions vary, the majority of lncRs associate with proteins and modulate the functions of the proteins. Some modulate the stability and the binding partners of the associated proteins, while others sequester or translocate the proteins from their resident sites. In other instances, LncRs, referred to as ceRNA (competing endogenous RNA) associate with microRNAs and become competitors against the targeted proteins, thereby indirectly regulating the abundance of the proteins. Like the proteins they modulate, LncRs can be oncogenic or tumor suppressing. Since LncR usually has multiple associated partners, it is not surprising that they display multiple functions and can be either oncoRNA or tumor suppressor depending on the cell context.

PKM2 as a generator of pyruvate plays a key regulatory role of glycolysis, oxidative phosphorylation and biosynthesis of macromolecules. The level and activity of PKM2 have a profound effect on how cells utilize energy and are thought to be determinants which dictate tumor metabolism. There is considerable evidence that PKM2 is overexpressed in tumor cells and exhibit tumorigenic properties. By contrast, PKM1, an alternatively spliced gene with a higher pyruvate

kinase activity is viewed as the isoform favoring metabolism operated in normal cells. While there is strong evidence that PKM2 contributes to tumorigenic properties in human malignancies, in certain transgenic mouse models PKM1, but not PKM2, is shown to facilitate tumorigenesis. These results suggest that the oncogenic activities of PKM2 is context dependent and begs for a more detailed understanding of the regulations and functions of PKM2 in oncogenic transformation.

Here, we performed RNA immunoprecipitation and revealed a novel LncR, LncR-PKM2, which interacts with PKM2 and “traps” the PKM2 in the tetrameric form and in the cytosol. As a consequence, the nuclear PKM2 is depleted. Knockdown of LncR-PKM2 increases nuclear translocation of PKM2 with concomitant increase of nuclear translocation of HIF-1 and b-catenin. This is accompanied by an increase of ECAR and decrease of OCR. An upregulation the glycolytic genes and cyclin D1 was also observed. In prostate cancer cell lines and tumor tissues, the expression of LncR-PKM2 is generally low compared to nonmalignant controls. Knockdown of LncR-PKM2 increases proliferation, migration, sphere-formation, and proinflammatory responses of prostate cancer cells. All the data together suggest that LncR-PKM2 is a tumor suppressor in prostate cancer by retaining PKM2 in the cytosol, and that nuclear PKM2 play a key role in oncogenesis.

Targeting DNA damage checkpoint in cancer therapy

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DNA double strand breaks (DSBs) are repaired by nonhomologous end joining (NHEJ) and homologous recombination (HR) pathways in mammalian cells. It is speculated that which pathway to use for DSB repair is mainly controlled by end resection process. This repair pathway choice is important for tumor response to PARP inhibition, which is now accepted therapeutic strategy for cancer patients carrying BRCA mutations. While BRCA1 promotes end resection and therefore favors HR repair, 53BP1 inhibits end resection and engages NHEJ pathway for DSB repair. We and others showed previously that RIF1 is a major downstream effector of 53BP1 and participates in 53BP1-dependent inhibition of end resection. Interestingly, while RIF1 accumulation at DSBs is antagonized by BRCA1 in S and G2 phases, the translocation of BRCA1 to damage sites in G1 cells is inhibited by RIF1, indicating that 53BP1-dependent pathway and BRCA1 counteract each other in a cell cycle-dependent manner. We showed that this cell cycle-dependent regulation is in part regulated by BRCA1-dependent inhibition of 53BP1 phosphorylation in S/G2 phase cells, which requires the E3 ubiquitin ligase activity of BRCA1. Besides RIF1, another DNA damage repair protein PTIP could also act downstream of 53BP1 and counteract BRCA1 function in DNA repair. We discovered that a nuclease SNM1C/Artemis associates with PTIP and functions to prevent end resection and HR repair. In addition, we and others demonstrated that REV7/MAD2L2 acts downstream of RIF1 and inhibits HR repair. Therefore, it is believed that 53BP1 controls RIF1-REV7 and PTIP-Artemis to promote NHEJ and suppress HR repair. We and others recently uncovered another 53BP1-binding protein, NUDT16L1 (also called Tudor Interacting Repair Regulator, TIRR), which associates with 53BP1 and regulates 53BP1 localization to DNA damage sites. We are now further investigating the regulation of DSB repair pathways and damage-induced checkpoint control. In addition, we are performing genome wide CRISPR/Cas9 screens and have identified RNASEH2 deficiency as potential biomarker for ATR inhibitor (ATRi)-based therapy.

Moreover, we showed that ATRi could potentiate radiation-induced anti-tumor immune response. Therefore, these studies reveal the interplays between DNA damage repair and multiple cellular processes, which will help improve therapeutic outcome for cancer patients.

Hap40 is an essential regulator of HTT's physiological functions and a pathogenic modifier of Huntington's disease

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Huntington's disease (HD) is caused by an abnormal expansion of the glutamine tract (polyQ) in Huntingtin (HTT) protein. As a large scaffold protein with numerous reported binding partners (HAPs), HTT is implicated in a growing list of cellular processes from vesicular transport, transcription to autophagy. However, little is known how these diverse functions are integrated through HTT and how HTT itself is regulated.

Converging evidence support that HAP40 is a central regulator of HTT. HAP40 was originally isolated from rat and mouse brains as the "most significantly correlated" partner of endogenous HTT protein, and binds HTT in a stochastic 1:1 molar ratio. Recently, HAP40 was found to stabilize the structure of HTT, converting it from a conformational heterogeneity status to a well-defined

globular structure. Importantly, in both primary fibroblasts and striatal tissues, a ~10-fold increase of the levels of endogenous HAP40 were observed in samples from HD patients as compared to healthy controls, implicating a pathogenic role in HD. Despite these strong biochemical and structural evidence, there is no reported functional evaluation of HAP40 in any physiological setting, its role on HTT functions, mutant HTT toxicity and HD pathogenesis remains unclear.

In a proteomic study for the HTT homolog (**dHtt**) in *Drosophila*, we isolated a novel ~40Kda protein as the strongest binding partner for endogenous dHtt. This 40Kda protein has significant sequence homology with mammalian HAP40 and is renamed as **dHap40** (*Drosophila*Hap40). The co-evolution of Hap40 and HTT in evolutionarily distant species from flies to humans not only supports the functional importance of HAP40 in HTT regulation, but also establishes *Drosophila* as a relevant genetic model to evaluate the physiological and pathological roles of HAP40. To this end, we have created several dhap40knockout mutants and also transgenic flies for dHap40 and human HAP40 overexpression, and have characterized the resulting phenotypes. Our results demonstrate HAP40 is an essential regulator of HTT's physiological functions and a modifier of HD pathogenesis.

UFMylation in the DNA damage response

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A proper DNA damage response (DDR) is essential to maintain genome integrity and prevent tumorigenesis. DNA double-strand breaks (DSBs) are the most toxic DNA lesion and their repair is orchestrated by the ATM kinase. ATM is activated via the MRE11-RAD50-NBS1 (MRN) complex along with its autophosphorylation at S1981 and acetylation at K3106. Activated ATM rapidly phosphorylates a vast number of substrates in local chromatin, providing a scaffold for the assembly of higher-order complexes that can repair damaged DNA. While reversible ubiquitination has an important role in the DSB response, modification of the newly identified ubiquitin-like protein UFM1 and the function of UFMylation in the DDR is largely unknown. Here, we found that MRE11 is UFMylated on K282 and this UFMylation is required for the MRN complex formation under unperturbed conditions and DSB-induced optimal ATM activation, homologous recombination-mediated repair and genome integrity. A pathogenic mutation MRE11(G285C) identified in uterine endometrioid carcinoma exhibited a similar cellular phenotype as the UFMylation-defective mutant MRE11(K282R). Taken together, MRE11 UFMylation promotes ATM activation, DSB repair, and genome stability and potentially serves as a therapeutic target.

Inflammation Controls the Differentiation Potential of Mesenchymal Stem Cells in Adipose Tissue

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Stem cells play pivotal roles in the maintenance of tissue cellular homeostasis by replacing dead or damaged cells. Chronic diseases are often accompanied by inflammation; thus, stem cells located in the tissue microenvironment always interact with inflammatory cells and cytokines. With our specific focus on the bidirectional communication between mesenchymal stem cells (MSCs), the progenitors of adipocytes and osteoblasts, and the inflammatory microenvironment, we investigated the role of such communication in deciding the remodeling process of the adipose tissue during obesity development and the subsequent insulin resistance. We found that the macrophage accumulation in obese adipose tissue depends on local proliferation of resident macrophages as well as the immigration of monocytes. Furthermore, CD11b, a key molecule in mediating monocyte immigration, played a critical role in obesity-induced insulin resistance through limiting proliferation and alternative activation of macrophages in the adipose tissue. CD11b^{-/-} mice showed less insulin resistance than wild type mice upon subjected to a high fat diet. Interestingly, these mice exhibited more adipose tissue. Further studies found that ICAM-1, the ligand for a receptor composed by CD11b and integrin β 2, is important in regulation of adipose tissue remodeling. The tight interaction between ICAM-1⁺ SVF cells and immune cells can be observed in obesity progression. Using bone marrow chimeric mice, we found that the absence of ICAM-1 in stromal cells promoted high fat diet induced obesity. Interestingly, the enhanced adipose tissue development was not through adipocyte hypertrophy, rather hyperplasia. Our findings on the crosstalk between stem cells and inflammatory components are important for the understanding and treatment of obesity and the associated diseases.

Rapid depletion of the ESCRT protein Vps4 underlies injury-induced autophagic impediment and Wallerian degeneration

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Axonal degeneration is a prominent feature of acute neural injury and chronic neurodegenerative diseases. In particular, the distal segment of injured axons undergoes an active and highly regulated self-destruction process, termed Wallerian degeneration. Recent work of Wallerian degeneration has been centered on the genes and pathways regulating NAD⁺ metabolism such as the NAD⁺ synthase Nmnat and the NAD⁺ hydrolase SARM1. However, apart from the NAD⁺ mechanism, other crucial signal transduction pathways involved in axonal injury and degeneration remain poorly understood.

In our previous work, we developed an *in vivo* model of nerve injury using the *Drosophila* wing (Fang et al., 2012; Fang et al., 2013; Fang and Bonini, 2015). In this study, we have combined multiple *in vitro* and *in vivo* neural injury models, including the *Drosophila* wing nerve, primary mouse DRG neurons, the mouse optic nerve, and the spinal cord of cynomolgus monkeys to investigate the role and regulation of autophagy in axonal degeneration. We found that the basal levels of axonal autophagy (evident by the formation of mCherry-Atg8a puncta) are low in general, even in aged flies. However, upon axotomy, there is a rapid and massive autophagy induction in the distal segment of the injured axons. The response can be seen as early as 3 hr after injury and is much earlier than when fly axons start to degenerate (12~24 hr).

Next, by performing a transgenic RNAi screen in flies, we identified the ESCRT component Vps4 as a novel essential gene for axonal integrity (Wang et al., 2019). We found that upregulation of Vps4 significantly delays degeneration of injured fly wing axons. We further revealed that Vps4 is required and sufficient to promote autophagic flux in axons and mammalian cells. Moreover, using both *in vitro* and *in vivo* models, we showed that the function of Vps4 in maintaining axonal

autophagy and suppressing Wallerian degeneration is conserved in mammals. Finally, we uncover that the Vps4 protein is rapidly depleted in injured mouse DRG axons as well as the spinal cord of monkeys, which may underlie the injury-induced autophagic impediment and the subsequent axonal degeneration. Together, Vps4 and ESCRT may represent a novel signal transduction mechanism in axonal injury and Wallerian degeneration.

Key reference:

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Circuit-dependent ROS feedback loop mediates glutamate excitotoxicity to sculpt motor system in *Drosophila*

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Overproduction of reactive oxygen species (ROS) are known to mediate glutamate excitotoxicity in neurological diseases. However, how ROS burdens can influence the circuit integrity remains unclear. Here we investigate the impact of excitotoxicity induced by depletion of *Drosophila* *eaat1*, encoding an astrocytic glutamate transporter, on the locomotor central pattern generator (CPG) activity, neuromuscular junction architecture, and motor function. We show that glutamate excitotoxicity triggers a circuit-dependent ROS feedback loop to sculpt motor system. Excitotoxicity initially elevates ROS to inactivate cholinergic interneurons, consequently changing CPG output activity to overexcite motor neurons and muscles. Remarkably, tonic motor neuron stimulation boosts muscular ROS, gradually dampening muscle contractility to feedback enhance ROS accumulation in the CPG circuit and subsequently exacerbate circuit dysfunction. Ultimately, excess premotor excitation to motor neurons promotes ROS-activated stress signaling to alter neuromuscular junction architecture. Collectively, our results reveal that the excitotoxicity-induced ROS can perturb motor system integrity by a circuit-dependent mechanism.

Type I interferon response to viral infection

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In addition to common viruses such as seasonal influenza, hepatitis viruses and human immunodeficiency virus (HIV), we often encounter numerous emerging viruses including avian influenza, severe acute respiratory syndrome (SARS), Ebola and most recent Zika virus (ZIKV). However, traditional methods to develop antiviral agents such as HIV Reverse Transcriptase and influenza Neuraminidase Inhibitors take too long to meet with urgent needs against these unpredictable emerging viruses. On the other hand, we know for a long time that IFN-I is the major host defense weapon with broad antiviral activities against different types of viruses. We have been studying IFN-I responses and downstream signaling for about 20 years and have identified multiple signaling pathways for IFN-I induction in the host response to infection with different pathogens, as well as near 300 interferon-stimulated genes (ISGs). However, the mechanisms by which individual ISGs inhibit specific viruses are still poorly understood. We have recently performed an unbiased screen of antiviral ISGs by individually overexpressing over 300 ISGs and measuring their antiviral activities against various viruses. This study identified overlapping and distinct subsets of ISGs that can strongly suppress different types of viruses. Among these antiviral ISGs, we identified cholesterol 25-hydroxylase (CH25H), a metabolic gene encoding the enzyme that converts cholesterol to 25-hydroxy-cholesterol (25HC), as a novel ISG with a broad antiviral activity. We further demonstrated that 25HC can block viral entry by preventing the fusion between the viral envelope and cell membrane, and have shown that it strongly suppresses infection of every enveloped virus that have tested so far including Ebola and ZIKV. In addition, our recent studies on gene expression profiles regulated by IFN-I revealed that IFN suppresses viral infection not only by upregulating ISGs, but also by downregulating many host metabolic genes involved in the synthesis of fatty acids, nucleotides and amino acids. In particular, we found that IFN-I strongly downregulates fatty acid synthase (FASN), a key enzyme involved in

the synthesis of long-chain fatty acids. We have subsequently shown that overexpression of FASN promotes viral replication, while knockdown or knockout of FASN reduces viral replication, suggesting that downregulation of FASN by IFN-I might represent a novel IFN-mediated antiviral strategy. Furthermore, FASN inhibitors, which are used for treating cancer, are found to have strong antiviral effects both in vitro and in vivo against different types of viruses including ZIKV. Therefore, understanding host immune response to different viral infection may provide insights to the development of novel broad antiviral agents against unpredictable emerging viruses.

Autophagy in host defense and tissue homeostasis: lessons from fly

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Autophagy is a highly conserved, degradative process that is pivotal for maintaining cellular homeostasis and survival under various stress conditions. Dysregulation of autophagy often leads to many pathological conditions, including infections, cancer, aging and neurodegenerative diseases. In *Drosophila*, autophagy has been shown to be essential for larval midgut degeneration during metamorphosis. Our recent findings indicate that the transmembrane protein Atg9 can directly interact with *Drosophila* tumor necrosis factor receptor-associated factor 2 (dTRAF2) to regulate c-Jun N-terminal kinase (JNK) activation and intestinal homeostasis in response to pathogen infection. Loss of Atg9 also leads to aberrant adult midgut morphology and cell growth. Moreover, we found that the midgut defects of Atg9 mutants could be rescued by the modulation of TOR signaling. Our results reveal a novel function of Atg9 in the regulation of cell growth and tissue homeostasis.

**Defining a molecular roadmap of *C. elegans* embryos: every cell,
every minute**

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Elucidating dynamic gene expression at high spatiotemporal resolution is critical to understand the logic and mechanism of in vivo cell differentiation. However, it remains challenging to determine and trace protein expression levels of regulatory genes in definitive single cells during long-term development. In this presentation, I will first describe a live-imaging-based method we have developed to quantify protein expression of specific genes in identity-resolved single cells at minute temporal resolution during *C. elegans* embryogenesis. Second, I will present unpublished data on how we apply this method to construct a comprehensive protein expression atlas of hundreds of conserved transcription factors in nearly all embryonic cells. Finally, I will describe our ongoing efforts on using this high-resolution expression dataset to understand the molecular regulation of cell differentiation at the systems level.

ATR Protects the Genome Against R Loops

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The ATR kinase is a master regulator of the DNA damage and replication stress response in human cells. We recently found that ATR is activated by R loops in human cells. R loops are transcription intermediates that contain DNA:RNA hybrids and displaced single-stranded DNA. Although R loops are important for a number of cellular processes, they impose a threat to genomic stability when present at high levels. To investigate how ATR is activated by R loops, we have taken multiple approaches to induce R loops in cells. We found that ATR is a general sensor of R loops. Furthermore, during S phase of the cell cycle, ATR is activated by the collisions of R loops and replication forks. The activation of ATR by R loops requires a group of proteins that remodel and process stalled replication forks. When activated at R loops, ATR plays an important role in suppressing the DNA damage arising from R loop-replication fork collisions. In the absence of ATR, replication forks are much more prone to DSB formation up collision with R loops, and cells are unable to survive when R loop levels are aberrant high. Our studies suggest that ATR is a key sensor of R loops and a critical suppressor of R loop-associated DNA damage, revealing a new function of ATR in the protection of genomic integrity.

Engineering protein-protein interactions to probe and rewire cell signaling

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Effective therapeutic strategies rely on our ability to interfere with cellular processes that are deregulated in human diseases. Thanks to the advance of genomic technologies in recent years, components essential for major biological pathways have been identified at the genetic level. Together they constitute signal transduction cascades relying on protein-protein interactions (PPIs) to elicit various biological functions. However, it is still poorly understood about the exact roles of individual PPI in controlling enzyme activity and complex assembly, especially in the context of diverse signaling networks. Traditional mutation-to-function studies have limitations in this regard due to unpredictable changes in protein folding and conformation, and difficulties in the identification of bona fide “separation-of-function” alleles. Hence, there is an urgent need for novel approaches that can selectively probe and investigate individual PPIs to dissect their biological roles.

To tackle this problem, I have devised a structure-based combinatorial protein design and engineering strategy to develop novel protein-based PPI modulators. In the past three years, we generated inhibitors and/or activators for more than 50 E3 ligases and deubiquitinases, enzymes that determine specificity of ubiquitination and deubiquitination, respectively (1-8). With the help of these synthetic molecules, we discovered new biochemical mechanisms and new biological functions of diverse protein families in the ubiquitination system. Importantly, we have established effective delivery methods for these intracellular probes and successfully target therapeutic-relevant genes in cells and organoids. I will present at the conference about our recent work on utilizing the protein engineering and synthetic biology platform to develop potent and highly specific PPI modulators to probe and rewire DNA repair signaling pathways with unprecedented precision for underlying molecular mechanisms and potential therapeutics.

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A HMT-independent role for EZH2 in prostate cancer

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Prostate cancer is the leading cause of cancer-related deaths in American men. It is estimated that each year more than 180,000 new prostate cancer patients will be diagnosed, and approximately 26,000 patients in the United States will die, primarily due to metastasis. This occurs despite advances in early detection and treatment. The available treatment options are limited, not very effective, and associated with severe side effects. Furthermore, prostate cancer patients can develop resistance to the currently available therapeutics. Therefore, this proposal will address the overarching challenge of developing effective treatments and address mechanisms of therapeutic resistance for men with metastatic prostate cancer.

Similar to other genetic diseases, prostate cancer develops in a background of dysregulated gene expression and is affected by environmental factors. These environmental factors can lead to epigenetic modifications, which affect DNA, RNA and protein components of chromosomes, called histones, in which DNA winds around, thus, regulating gene expression. The protein enzyme enhancer of zest homolog 2 (EZH2) specifically modifies the histone H3 protein at its lysine 27; thereby, tightly winding DNA and silencing gene expression. Our previous work showed that EZH2 is upregulated in advanced prostate carcinomas and metastatic prostate cancer, and prostate cancer patients who have higher expression levels of EZH2 have shorter survival times than prostate cancer patients with low or no expression of EZH2. Surprisingly, we recently discovered that dysregulation of EZH2 alters rRNA modifications and then regulates ribosome functions, which is totally different from the well-known EZH2's canonical function as a protein modifier only.

Critical Role of Autophagy and TFEB-Mediated Lysosomal

Biogenesis in Pancreatitis

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Alcohol abuse is the major cause of experimental and human pancreatitis but the molecular mechanisms remain largely unknown. We investigated the role of transcription factor EB (TFEB), a master regulator of lysosomal biogenesis, in the pathogenesis of pancreatitis. Using a chronic plus acute alcohol binge (referred to as Gao-binge) and cerulein mouse model, we analyzed pancreas injury, autophagic flux, zymogen granule removal, TFEB nuclear translocation and lysosomal biogenesis in acinar cell-specific Atg5 knockout (KO) and TFEB KO mice as well as their matched wild type mice. Pancreatic changes were also determined in mice that we overexpressed TFEB in pancreas followed by Gao-binge alcohol. We found that Gao-binge alcohol and cerulein induced typical features of pancreatitis in mice with increased serum amylase and lipase activities, pancreatic edema, infiltration of inflammatory cells, accumulation of zymogen granules (ZGs) and expression of inflammatory cytokines. While Gao-binge alcohol and cerulein increased the number of autophagosomes, they also concurrently inhibited TFEB nuclear translocation and TFEB-mediated lysosomal biogenesis resulting in insufficient autophagy. Acinar cell-specific Atg5 KO and acinar cell-specific TFEB KO mice developed severe inflammatory and fibrotic pancreatitis in both Gao-binge alcohol and cerulein-treated mice. In contrast, TFEB overexpression inhibited alcohol-induced pancreatic edema, accumulation of zymogen granules and serum amylase and lipase activities. In line with our findings in mice, decreased LAMP1 and TFEB nuclear staining were also observed in human alcoholic and non-alcoholic pancreatitis tissues. In conclusion, our results indicate that TFEB plays a critical role in maintaining pancreatic acinar cell homeostasis. Impairment of TFEB-mediated lysosomal biogenesis by alcohol may lead to insufficient autophagy and promote pancreatitis.

Dramatic evolution of chromosomes and neo-sex chromosomes in the muntjac deer

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Muntjac deer (*Muntiacus* spp) are a group of special deer species which had experienced dramatic chromosome rearrangements during their speciation. Particularly the black muntjac (*Muntiacus crinifrons*) evolved neo-sex chromosomes. It was proposed the ancient karyotype of all muntjac deer was probably $2n=70$, which is still retained by a distant deer, the Chinese water deer. The extant basal lineage of muntjac deer represented by the Chinese muntjac has a karyotype of $2n=46$. However, it is $2n=13/14$ for the fee's muntjac, $2n=8/9$ for the black muntjac and gongshan muntjac, and the Indian muntjac has the lowest diploid number ($2n=6/7$) in mammals. The reduction of chromosome numbers was mostly resulted from constant tandem fusions. Up to now, why the chromosomes had been keeping fusing and the impact on the regulations of genes around the fusion regions remain largely unknown. In the black muntjac, a big inversion happened in the homolog compared to its counterpart fused with X chromosome, which thereby had created a pair of neo-sex chromosomes segregating between males and females. The black muntjac only diverged from the gongshan muntjac less than 1 million years, and thus it represents the youngest neo-sex chromosome system in mammals and provide a unique and valuable opportunity to investigate the evolution patterns and mechanism of sex chromosomes at their early stage. We have sequenced and assembled the chromosomes of the Chinese water deer, the Chinese muntjac, and both the female and male black muntjacs using the second and third sequencing technology together with large quantity of Hi-C data. These data allow us to reveal the molecular mechanisms underlying the recurrent chromosome fusion and early evolution processes of mammalian sex chromosomes.

Persistent Activation of MTOR Exacerbates Alcohol-induced Liver Injury

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Alcohol-induced liver disease (ALD) is a world-wide health issue that claims two million lives in U.S. each year. Using a chronic feeding plus acute binge mouse model, we previously demonstrated that alcohol activated MTOR and inhibited transcription factor EB (TFEB)-mediated lysosomal biogenesis resulting in insufficient autophagy and liver injury. However, the role and mechanisms by which genetic persistent activation of MTOR in alcohol-induced autophagy and liver injury are not known. In the present study, liver-specific TSC1 knockout (KO) mice were generated by crossing TSC1 flox/flox mice with Albumin Cre mice, and the KO mice and their matched Cre negative wild type mice were subjected to chronic feeding plus acute binge alcohol model (Gao-binge model). As expected, we found that liver-specific TSC1 KO mice had marked elevated levels of phosphorylated 4EBP1 and S6, two substrate proteins of MTOR, suggesting persistent MTOR activation in liver-specific TSC1 KO mice. Surprisingly, levels of hepatic TFEB and its target lysosomal proteins increased in liver-specific TSC1 KO mice likely due to the increased protein translation due to persistent MTOR activation. Liver-specific TSC1 KO mice also had increased ductular reaction and inflammation, which were further exacerbated by Gao-binge alcohol treatment. Further, liver-specific TSC1 KO mice also had increased serum ALT and AST levels compared with their matched wild type mice after Gao-binge alcohol. In conclusion, TSC1 depletion accelerates alcohol-induced ductular reaction, inflammation and liver injury in mice despite the compensatory activation of TFEB.

Epigenomic reprogramming in embryonic development during animal evolution

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Major evolutionary transitions are enigmas and the most notable enigma is between invertebrates and vertebrates with numerous spectacular innovations. To search for the molecular connections, we ask whether global epigenetic changes may offer a clue by surveying the inheritance and reprogramming of parental DNA methylation across metazoans. We focus on gametes and early embryos, where the methylomes are known to evolve divergently between fish and mammals. Here, we find that methylome reprogramming during embryogenesis occurs neither in pre-bilaterians such as cnidarians nor in protostomes such as insects, but clearly presents in deuterostomes such as echinoderms and invertebrate chordates, and then becomes more evident in vertebrates. Functional association analysis suggests that DNA methylation reprogramming is associated with development, reproduction and adaptive immunity for vertebrates, but not for invertebrates. Interestingly, the single HOX cluster of invertebrates maintains unmethylated status in all stages examined. In contrast, the multiple HOX clusters show dramatic dynamics of DNA methylation during vertebrate embryogenesis. Notably, the methylation dynamics of HOX clusters are associated with the spatiotemporal expression in mammals. Our study reveals that DNA methylation reprogramming has evolved dramatically during animal evolution, especially after the evolutionary transitions from invertebrates to vertebrates, and then to mammals.

Thyroid hormone stimulates brown adipose tissue thermogenesis via autophagy

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Although it has long been known that thyroid hormone (TH; T3) activates thermogenesis in brown adipose tissue (BAT), its direct action(s) in BAT is not well understood. TH activates thermogenesis by uncoupling electron transport from ATP synthesis in brown adipose tissue (BAT) mitochondria. Here, we examined effects of T3 on mitochondrial activity, autophagy, and metabolomics in primary brown adipocytes and BAT and found that T3 increased fatty acid oxidation and mitochondrial respiration as well as stimulated autophagic flux, mitophagy, and mitochondrial biogenesis. Interestingly, we found no significant induction of intracellular reactive oxygen species (ROS) despite high mitochondrial respiration and UCP1 induction by T3. We treated hepatic cells with Atg5 siRNA to block autophagy, and observed that induction of mitochondrial respiration by T3 decreased, and was accompanied by significant ROS accumulation, demonstrating a critical role for autophagic mitochondrial turnover. We examined the role of BAT-specific autophagy in thermogenesis in vivo, hyperthyroid Atg5Flox/Flox mice were injected with Ucp1 promoter-driven Cre-expressing adenovirus. These autophagy-deficient mice exhibited lower body temperature than hyperthyroid or euthyroid Atg5Flox/Flox mice injected with control adenovirus. T3 increased short and long chain acylcarnitine levels in BAT, consistent with increased β -oxidation. T3 also decreased amino acid levels, and in conjunction with SIRT1 activation, decreased mTOR activity to stimulate autophagy. Similar dependence on autophagy for thermogenesis also was observed during cold exposure in mice. Our results show that T3 has direct effects on mitophagy, mitochondrial activity, and its turnover in mature BAT that are critical for thermogenesis. Stimulation of BAT activity by TH or its analogs may represent a novel potential therapeutic approach to treat obesity and metabolic diseases.

Biological insights into lung diseases from GWAS

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Despite of recent success of genome-wide association studies (GWAS) in identification of thousands of disease-associated with loci harboring variants predicting the disease variation, determination on the functional implications of these loci remains challenging because GWAS are incapable of distinguishing causal from non-causal variants and pointing to the disease causal genes directly. For example, recent studies have identified more than 80 loci associated with chronic obstructive pulmonary diseases (COPD), the fourth leading cause of death worldwide, but only a small portion of these loci have been studied in depth. We have applied integrative approaches to link non-coding GWAS regions associated with asthma or COPD to disease causal genes, followed by dissections on function of these novel GWAS genes in both cellular and mouse models.

In this presentation, I will use one of the most significant and well-replicated COPD GWAS loci at chr4q as an example to demonstrate biological insights we gleaned through understanding COPD GWAS loci. Our earlier work identified intergenic functional variants at the COPD GWAS locus near HHIP (hedgehog interacting protein) by demonstrating a chromatin loop between the DNA region spanning the functional variant and the promoter of HHIP. Subsequently, age-dependent spontaneous emphysema was identified in Hhip heterozygous mice that may involve increased oxidative stress and enhanced lymphocyte activation in Hhip^{+/-} murine lungs. Increased lymphoid aggregates seen only in Hhip^{+/-} mice not wild type control mice, resemble pathological changes in human COPD lungs. To understand molecular drivers for increased lymphoid aggregates in Hhip^{+/-} murine lungs, we performed single cell RNA-Seq in murine lungs from wild type and Hhip^{+/-} mice at four different age points. Molecular communications between lung fibroblasts and CD8T cells were revealed by receptor-ligand analysis. Our data suggested that increased IL-18 in Hhip^{+/-} lung fibroblasts may activate interferon gamma signaling pathway in CD8T cells, thereby promoting emphysema development. Consistently, we detected significant correlation

between HHIP genotype with IL-18 levels in serum samples from COPD patients, suggesting that molecular mechanism we found in mouse models were further corroborated by human findings. More and more evidence suggested that drugs that have genetic evidence supporting its mechanism usually have a higher successful rate in the clinical trials. We expect our translational genetic studies will eventually greatly expedite discoveries of novel drug targets to benefit COPD patients.

What is critical for the critical period in the development of the olfactory circuit?

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The critical period is a developmental window during which sensory experience have lasting impact on the anatomical connection and functions of neural circuits. In the mammalian species, the olfactory system is the only nervous system that continuously generate new neurons throughout the life of the animals. This unique characteristic suggests that the olfactory neurons may hold a secret of regenerative capacity that is lost in other neurons. In this regenerative nervous system, we discover a critical period in setting up the highly specific connections between sensory neurons and their central targets. Despite massive neurogenesis during the postnatal development, we found only a population of perinatally born sensory neurons are endowed with the ability to set the olfactory map, and to correct erroneously projecting axons. Here I will discuss the behavioral implication of a critical period in the olfactory system development, the identification of a population of navigator neurons during the critical period, the cellular mechanisms by which the navigators establish the olfactory map, and the molecular mechanism that control the timing of the critical period.

Understanding how precancerous lesions progress to breast cancer using mouse models

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There are distinct cell subpopulations in normal epithelial tissue, including stem cells, progenitor cells, and more differentiated cells, all of which have been extensively studied for their susceptibility to tumorigenesis and intertumoral heterogeneity. However, normal cells usually have to progress through a precancerous lesion state before becoming a full-blown tumor. Precancerous early lesions are heterogeneous too. It remains unclear whether distinct subsets of precancerous cells contribute to the eventual cancer and intertumoral heterogeneity. By using mouse models of breast cancer that are tailored to address this question, we demonstrated that both precancerous stem cells (PcSCs) and more differentiated cells in the precancerous lesion can be induced to form cancer readily upon introduction of constitutively active versions of either HRAS or BRAF. However, the resulting tumors were dramatically different in protein profiles and histopathology: PcSCs gave rise to adenocarcinoma while more differentiated precancerous cells yielded metaplastic carcinoma with severe squamous differentiation and more robust activation of MEK/ERK signaling. In contrast, constitutively activated PIK3CA caused both PcSCs and more differentiated precancerous cells to progress into adenocarcinoma. Therefore, both cell types in precancerous lesions and the genetic mutations that these cells suffer contribute to the characteristics of the resulting cancer. This work identifies previously unknown players in cancer heterogeneity and suggests that cancer prevention should target precancerous cells broadly and not be limited to PcSC.

Systematic Analysis of Differential Transcription Factor Binding to Non-Coding Variants in the Human Genome

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A large number of sequence variants have been linked to complex human traits and diseases, but deciphering their biological function remains a daunting challenge especially for the non-protein-coding variants. To fill this gap, we have systematically assessed the differential binding of transcription factors (TF) to different alleles of non-coding variants in the human genome. Using an ultra-high throughput multiplex protein-DNA binding assay, we successfully examined the binding of 270 human TFs to 95,886 common sequence variants within the 110 type 2 diabetes (T2D) risk loci. We then employed a machine-learning approach to derive computational models to predict differential DNA binding of 160 TFs to other common non-coding variants in the human genome. We showed that the newly derived models outperformed current position-weight matrices (PWM) in describing TF binding to non-coding variants, and facilitated discovery of potential causal variants and dysregulated molecular pathways in human diseases.

A striosomal direct pathway for aversive learning

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The striatum has been implicated in evaluation of behavioral outcomes, but the underlying mechanisms remain elusive. Here we identified a molecularly defined striatal population for evaluating aversive outcomes. These neurons are localized within the striosome compartment and belong to the “direct pathway”. However, contrasting with the known and hypothesized functions of the direct pathway, these neurons are predominantly excited by punishment rather than reward. Furthermore, inhibiting these neurons impairs learning driven by punishment, without affecting learning guided by reward, while activating them is aversive, suppresses movement and drives negative reinforcement. Thus, this distinctive striosomal direct pathway constitutes a critical neural substrate for aversive or punishment-based learning. Our findings establish a major function of the striosome and revise the conventional model of the striatum.

Genetic variation in glia-neuron signaling modulates ageing rate

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The rate of behavioural decline in the ageing population is remarkably variable among individuals. Despite the great interest in studying natural variation in ageing rate to identify factors that control healthy ageing, no such factor has yet been found. Here we report a genetic basis for variation in ageing rates in *Caenorhabditis elegans*. We find that *C. elegans* isolates show diverse lifespan and age-related declines in virility, pharyngeal pumping, and locomotion. DNA polymorphisms in a novel peptide-coding gene, named regulatory-gene-for-behavioural-ageing-1 (*rgba-1*), and the neuropeptide receptor gene *npr-28* influence the rate of age-related decline of worm mating behaviour; these two genes might have been subjected to recent selective sweeps. Glia-derived RGBA-1 activates NPR-28 signalling, which acts in serotonergic and dopaminergic neurons to accelerate behavioural deterioration. This signalling involves the SIR-2.1-dependent activation of the mitochondrial unfolded protein response, a pathway that modulates ageing. Thus, natural variation in neuropeptide-mediated glia–neuron signalling modulates the rate of ageing in *C. elegans*.

Comprehensive analysis of sex-specific molecular differences in cancer

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Sex is a key biological factor affecting the incidence, prognosis, mortality and treatment response of many cancer types. While individual molecular differences between male and female subpopulations have been reported, we recently performed a comprehensive analysis of molecular differences between male and female patients in various cancer types using multi-omics data of The Cancer Genome Atlas. Our results suggest two sex-effect groups associated with distinct incidence and mortality profiles. One group show a relatively limited number of sex-affected genes, whereas the other shows much more extensive sex-biased molecular signatures. Importantly, most of clinically actionable genes show sex-biased signatures, suggesting significant clinical implications. These results will advance a full molecular-level understanding of sex effects in diverse cancers and suggests a pressing need to develop sex-specific therapeutic strategies in certain cancer types.

From bench to bed---Basic and translational studies of ADSCs

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Adipose-derived stem cells (ADSCs) are one of the adult stem cells with the multi-potency of differentiating into adipose cells, chondrocytes and osteoblasts. Since ADSCs are abundant and can be obtained and expanded to large quantity relatively easily with low immunogenicity and high immunomodulatory capacity, they are becoming an ideal seed cells for stem cell transplantation therapies for many diseases. In the present study, we developed a protocol to differentiate hADSCs and huMSCs into hepatocytes and compared their therapeutic effects in acute liver failure (ALF) rat model. ADSCs demonstrated better hepatocyte differentiation potential and therapeutic effect in ALF model. We further discovered that high concentration of extracellular vesicles (EVs) derived from hADSCs could exert much better therapeutic effect in ALF model than cell transplantation itself. Gene sequencing of rat liver revealed an increase in human long-chain non-coding RNA (lncRNA) H19 after hADSC-derived EVs transplantation. When the H19 was silenced in hADSCs and EVs were then collected for treatment of rats with liver failure, we saw a decrease in the survival rate, compared to treatment with EVs generated from non-silenced hADSCs. These data indicate that lncRNA H19 may be a potential therapeutic target for the treatment of liver failure.

Metabolism, activity, and targeting of 2-hydroxyglutarates (2-HG)

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Isocitrate dehydrogenases (IDH1/2) are frequently mutated in multiple types of human cancer, resulting in neomorphic enzymes that convert α -ketoglutarate (α -KG) to 2-hydroxyglutarate (2-HG). The current view on the mechanism of IDH mutation holds that 2-HG acts as an antagonist of α -KG and competitively inhibits the activity of α -KG-dependent dioxygenases, including those involved in histone and DNA demethylation. Other studies have implicated 2-HG in activities beyond epigenetic modification. Many enzymes have been discovered that lack mutations but which can nevertheless produce 2-HG promiscuously under hypoxia or acidic conditions. Therapies are being developed to treat IDH-mutant cancers by either directly targeting the mutant IDH enzymes or the pathways sensitized by 2-HG.

RNA 5-Methylcytosine Facilitates Maternal-to-zygotic Transition through Preventing Maternal RNA Decay

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The maternal-to-zygotic transition (MZT) is a conserved and fundamental process during which the embryo undergoes dramatic reprogramming to convert maternal environment to embryonic-driven programing. However, how the maternally supplied transcripts are dynamically regulated during MZT remains largely unknown. Herein, through genome-wide profiling of RNA 5-methylcytosine (m⁵C) in zebrafish early embryos, we show that m⁵C methylated maternal mRNAs display higher stability during MZT. We identify that the Y box-binding protein 1 (Ybx1) prefers to recognize m⁵C-modified mRNAs through p-p interaction with a key residue Trp45 in its cold shock domain (CSD), which plays essential roles in maternal mRNA stability and early embryogenesis of zebrafish. Cooperated with an mRNA stabilizer Pabpc1a, Ybx1 promotes the stability of its target mRNAs in a m⁵C-dependent manner. Our study demonstrates a novel mechanism of RNA m⁵C methylation-regulated maternal mRNA stability during zebrafish MZT, highlighting the critical role of m⁵C mRNA methylation in early development.

Monoacylglycerol lipase regulates cannabinoid receptor 2-dependent macrophage activation and cancer progression

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Metabolic reprogramming greatly contributes to the regulation of macrophage activation. However, the mechanism of lipid accumulation and the corresponding function in tumor associated macrophages (TAMs) remain unclear. With primary investigation in colon cancer and confirmation in other cancer models, here we determine that deficiency of monoacylglycerol lipase (MGLL) results in lipid overload in TAMs. Functionally, macrophage MGLL inhibits CB2 cannabinoid receptor-dependent tumor progression in inoculated and genetic cancer models. Mechanistically, MGLL deficiency promotes CB2/TLR4-dependent macrophage activation, which further suppresses the function of tumor-associated CD8⁺ T cells. Treatment with CB2 antagonists delays tumor progression in inoculated and genetic cancer models. Finally, we verify that expression of macrophage MGLL is decreased in cancer tissues and positively correlated with the survival of cancer patients. Taken together, our findings identify MGLL as a switch for CB2/TLR4-dependent macrophage activation and provide potential targets for cancer therapy.

**Reduced energy metabolism as a major strategy for cisplatin
resistance revealed by genome-wide siRNA screening**

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Application for JOSEPH K.-K. LI Travel Awards

Cisplatin is widely used for the treatment of various types of cancers, yet drug resistance frequently occurs through various mechanisms. We have previously shown that despite the drug resistance, cisplatin treatment elicits strong inhibition effect on tumor metastasis. Thus, we conducted synthetic lethal screenings against several siRNA libraries in order to find conditions that can overcome cisplatin resistance while keeping its ability for inhibiting cancer metastasis. We first conducted a mini siRNA screen targeting 55 custom-selected genes, and found that ATP7A, which sequesters cisplatin in the cytoplasm for exporting out the cell, was be a top synthetic lethal gene as pre-target mechanism for cisplatin resistance. Then we used a kinase library containing 704 kinases and found that cisplatin activates ATR, CHK1 and WEE1, which shut down DNA replication and attenuate cisplatin induced-lethality, as an on-target mechanism for the resistance. We now have conducted a whole genome screening with 6400 siRNAs targeting 21585 human genes to identify synthetic lethality for overcoming cisplatin resistance. Upon cisplatin admission, cells adapt an energy saving stage, which apparently helps cell survival to the treatment as acute knockdown or long-term reduced expression of genes involved in oxidative phosphorylation increased cisplatin resistance. We further demonstrated that reduced energy metabolism as a post-target mechanism for cisplatin resistance, which can be overridden by the combination specific therapeutic drugs to benefit clinic application of cisplatin.

Chaperone control of histone acetylation

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Lysine acetylation is among the first post-translational modifications of histones to be discovered, and it plays prominent roles in chromatin-based regulation of gene expression. Enzymatic acetylation and deacetylation of histones provide major means of modulation of degrees of histone acetylation. Apart from spatiotemporal changes of enzyme abundances, conformational dynamics and protein-protein interaction can significantly influence enzymatic activities of certain histone acetyltransferases, which include the Hat1-Hat2 complex, the Sas2-Sas4-Sas5 complex, and the Rtt109-Vps75 complex in budding yeast. In all, histone chaperone Asf1 plays an important role in acetylation of histone lysine residues. We have studied the structural basis of Asf1's role in regulation of H3K56 acetylation by Rtt109. The study revealed interesting mechanistic insights into allosteric regulation of histone acetylation, and the finding suggests that histone chaperones may have more extensive roles in regulating histone modifications prior to assembly onto nucleosomes.

New cell death pathway interactions reducing cortical injury in stroke

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Over the past 30 years a number of animal models of cerebral ischemic injury have been developed. Middle cerebral artery occlusion (MCAO) in particular reproduces both ischemic and reperfusion elements and is widely utilized as a model of ischemic stroke in rodents. However substantial variability exists in this model even in clonal inbred mice due to stochastic elements of the cerebral vasculature creating significant irreducible variabilities with respect to the zone of injury as well as inducing considerable injury to sub-cortical structures, conditions not typically seen for the majority of human clinical strokes. An alternative model utilizes endothelin-1 application focally, resulting in an ischemic reperfusion injury which more closely mimics that seen in human clinical strokes. We demonstrate that intra-cortical administration of ET-1 results in a highly reproducible pattern of tissue injury limited to the cerebral cortex and characterize the early cellular, morphologic and molecular events occurring during the first 24 hour post-injury period demonstrating that caspase-3 is both necessary and sufficient to regulate a majority of cortical cell death observed during this period. The enhanced survival effects seen upon genetic deletion of caspase-3 appear to arise as a result of direct modification of cell autonomous PCD signaling as opposed to secondary effectors such as granulocyte infiltration or microglia activation. These findings highlight the early mechanistic features of endothelin-1-mediated ischemic injury demonstrating the presence of several connected programs of cell death in mammals. Funding: This study has been funded by grants awarded to J.H. from the Natural Sciences and Engineering Research Council of Canada (NSERC) and Heart and Stroke Society of Canada.

PA200-proteasome maintains stability of histone marks during transcription and aging

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The stability of histone marks is critical to epigenetic inheritance. How this stability is maintained still remains unclear. We showed previously that the proteasome activator PA200 catalyzes the acetylation-dependent degradation of the core histones during DNA repair and spermiogenesis. Here we show that PA200 promotes proteasomal degradation of the core histones during transcription and cellular aging, and is critical to the maintenance of histone marks. Deletion of PA200 dramatically altered deposition of the active transcriptional hallmarks and transcription, especially during cellular aging. Furthermore, deletion of PA200 or its yeast ortholog Blm10 accelerated cellular aging. Notably, the PA200-deficient mice displayed a range of aging-related deteriorations, including immune malfunction, motor dysfunction, and anxiety-like behaviors. Thus, the PA200-mediated proteolysis is critical to the maintenance of the stability of histone marks during transcription and aging.

Slit2 signaling contributes to hepatic injury and fibrosis in bile duct-ligation mice by activation of hepatic stellate cells

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Liver fibrosis, a precursor to cirrhosis and liver cancer, is still lacking effective therapeutic strategies. Here, we identified the Slit2 signaling as an important positive regulator in liver fibrosis. Our findings revealed that the serum levels and hepatic expression of Slit2 were significantly increased in liver biliary atresia (PBC) patients with liver fibrosis. And Slit2-Tg mice were much more vulnerable to BDL-induced liver injury and fibrosis when compared to WT mice. Moreover, we also defined the involvement of activated hepatic stellate cells (HSCs) in mediating the Slit2-dependent profibrotic impacts by determining the hepatic co-expression of Slit2 and HSCs marker (α -smooth muscle actin, α -SMA) in BDL-induced mice. In cellular levels, Slit2 up-regulation via Slit2 recombinant protein promoted the activation and proliferation, and inhibited the apoptosis of human HSCs cell line LX-2 in a process partly mediated by the p38 and ERK signaling. In contrast, these transdifferentiated phenotypes of resistance to apoptosis and enhanced proliferation in activated HSCs were reversed by Slit2 down-regulation via siRNA silencing. In conclusion, Slit2 intervened in the activation, proliferation and apoptosis of HSCs during liver fibrogenesis, highlighting Slit2 as a potential therapeutic target for liver fibrosis.

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**LongHu RenDan, a Chinese traditional compound medicine,
ameliorates chronic liver injury and fibrosis in mice**

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Longhu Rendan (LHRD) is a Chinese traditional patent medicine that was used to cure heatstroke, motion sickness, diarrhea etc. But other effects of Longhu Rendan was not discovered. We aimed to investigate the anti-fibrotic effect of LHRD in experimental liver fibrosis and its potential mechanism. Liver fibrosis was induced by bile duct ligation 15 days or intraperitoneally injection (i.p.) of carbon tetrachloride (CCl₄) for 6 weeks. Mice were also treated with 100 mg/kg LHRD daily by gavage. Twenty-four hours after the last administration, blood serum and Liver tissue were collected. The activities of ALT and AST in the serum were analyzed in two models. The degree of liver injury and fibrosis were examined by HE staining and Sirius red staining, and the protein expression of α -SMA was examined and mRNA expression of α -SMA and other fibrosis index were detected. Meanwhile, the oxidative stress and inflammation indicator, such as ROS, CD68, NF κ B, were also examined. LHRD significantly attenuated liver injury and hepatocyte damage. Furthermore, LHRD suppressed collagen deposition and HSC activation, as well as profibrotic gene expression. Mechanistically, LHRD inhibited NF- κ B signaling and inflammatory gene expression. In addition, LHRD diminished production of ROS and 4-HNE, companied with downregulation of NOX4. LHRD prevents chronic liver injury and fibrosis in mice through suppression of inflammation and oxidative stress.

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The Role and Mechanism of Longhu Rendan in Preventing Liver Injury Induced by Acetaminophen Overdose

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Objective: To explore the effect of Longhu Rendan, a Chinese traditional compound medicine, on mouse liver injury induced by acetaminophen overdose and its possible mechanism. **Methods:** mice were randomly divided into five groups: control group, APAP model group, NAC positive drug group and Longhu Rendan administration group with low dosage of 80 mg/kg and high dosage of 160 mg/kg. After administering for 6 days, mice were fasting for 22 h and injected with APAP at a dose of 250 mg/kg. The mouse serum and liver were collected 6 hours later. The ALT and liver tissue GSH activity in the serum of each group were measured; the hematoxylin-eosin staining of liver was performed and liver Glutamate-cysteine ligase catalytic (GCLC) protein expression was examined by Western Blot. **Results:** Experiments have shown that Longhu Rendan can alleviate excessive APAP-induced liver injury. Compared with the model group, the degree of liver injury in Longhu Rendan-administered groups were significantly reduced, ALT levels were decreased, and GSH levels were significantly increased. Meanwhile, Longhu Rendan enhanced anti-oxidative effect by increasing protein expression of GCLC. **Conclusion:** Longhu Rendan has an improved effect on the prevention and treatment of acute liver injury induced by acetaminophen. The mechanism is mainly related to anti-oxidative stress.

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Hepatocyte-Specific Deficiency of SphK1 Aggravates

Acetaminophen-Induced Liver Injury

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Acetaminophen (APAP) overdose is one of the leading causes of hepatotoxicity and acute liver failure worldwide. Accumulating evidence suggests that NLRP3 inflammasome plays an important role in APAP-induced liver injury. However, the mechanisms of APAP-induced NLRP3 inflammasome-mediated hepatocyte pyroptosis are not fully understood. In this study, we found that sphingosine kinase 1 (SphK1), a lipid mediator enhances cell growth and inhibits apoptosis, was markedly induced by APAP in human and mouse livers as well as primary mouse hepatocytes. Hepatocyte-specific deletion of SphK1 promoted NLRP3 inflammasome activation in hepatocytes and accelerated hepatocyte pyroptosis, and aggravated APAP-induced hepatocyte pyroptosis and liver injury in mice. Moreover, SphK1 deficiency in hepatocytes upregulates thioredoxin interacting protein (TXNIP), which is known to promote hepatocyte pyroptosis by binding to NLRP3 inflammasome. Furthermore, adenovirus-mediated SphK1 overexpression mitigated APAP-induced hepatocyte pyroptosis and liver injury. Conclusion: Our data demonstrate that SphK1 ameliorates APAP-induced acute liver injury via the suppression of hepatocyte pyroptosis mediated by TXNIP/NLRP3 inflammasome and provide a new strategy for the targeted treatment of APAP-induced acute liver injury with hepatocellular SphK1.

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Epigenetic regulation of the pluripotent to totipotent state transition in embryonic stem cells

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Mouse embryonic stem cells (ESCs) are pluripotent but not totipotent, as they only give rise to cells in the embryonic lineage, but not the extra-embryonic lineages. While the gene regulatory network underlying pluripotency has been extensively studied, how the totipotent state is regulated remains to be elucidated. Recently, it has been shown that a rare transient cell population within mouse ESCs expresses high levels of transcripts found in the two-cell (2C) embryos where both the two blastomeres are totipotent. More importantly, these 2C-like cells can contribute to both embryonic and extra-embryonic tissues and are therefore important for both basic research and translational research. Using 2C-like cells, we performed CRISPR-Cas9 knockout screens for epigenetic regulators that regulate the entering and exiting of 2C-like state. We also profiled genome-wide histone modifications and analyzed 3D chromatin structures in 2C-like totipotent cells. This talk will discuss our latest findings regarding epigenetic mechanisms in the pluripotent to totipotent state transition in ESCs.

Differential Roles of Sex Hormone Receptors In Bladder Cancer

Development and Treatment

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Epidemiological studies showed that women have a lower bladder cancer (BCa) incidence, yet higher muscle-invasive rates than men, suggesting that androgen and estrogen and their receptors may play differential roles in different stages of BCa.

We applied multiple *in vitro* and *in vivo* strategies to investigate the differential roles of the sex hormone receptors, estrogen receptor (ER) and androgen receptor (AR), in BCa initiation and progression. Data from the *in vitro* cell malignant transformation and *in vivo* gene knockout BBN-induced mouse BCa models support that androgen/AR signals promote BCa initiation. Interestingly, we further found the 2 types of ER, ERalpha and ERbeta, play differential roles, resulting in a Yin-and-Yang regulatory model to control the BCa initiation.

To test the BCG therapeutic efficacy for non-muscle invasive BCa, data collected from *in vitro* and *in vivo* strategies showed that anti-androgen or anti-estrogen could regulate integrin- $\alpha 5\beta 1$ expression and IL-6 release to regulate the recruitment of monocytes/macrophages toward BCa cells, which consequently increased TNF- α release to potentiate the anti-BCa effects of BCG treatment. In the advanced stage of BCa, both AR and ER signals could impact the BCa tumor microenvironment by recruiting the tumor associated immune cells (including macrophages, mast cells, and T cells) to affect the invasion and metastasis of BCa.

Together, our data suggest sex hormone receptors play differential roles at different stages of BCa. The study presents a road map for targeting sex hormone signals and the associated downstream signal pathways, which expands potential therapeutic strategies for BCa treatment.

Inactivation of p38 MAPK Contributes to Stem Cell-Like Properties of Non-Small Cell Lung Cancer

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Cancer stem cells (CSCs) are recognized as the major source for cancer initiation and recurrence. Yet, the mechanism by which the cancer stem cell properties are acquired and maintained in a cancer cell population is not well understood. In the current study, we observed that the level of active p38 MAPK is downregulated, while the level of the stemness protein SOX2 is upregulated in lung cancer tissues as compared to normal tissues. We further demonstrated that inactivation of p38 is a potential mechanism contributing to acquisition and maintenance of cancer stem cell properties in non-small cell lung cancer (NSCLC) cells. p38, in particular the p38 α and p38 β isoforms, suppresses the cancer stem cell properties and tumor initiating ability of NSCLC cells by promoting the ubiquitylation and degradation of stemness proteins such as SOX2, Oct4, Nanog, Klf4 and c-Myc, through MK2-mediated phosphorylation of Hsp27 that is an essential component of the proteasomal degradation machinery. In contrast, inactivation of p38 in lung cancer cells leads to upregulation of the stemness proteins, thus promoting the cancer stem cell properties of these cells. These findings have demonstrated a novel mechanism by which cancer stem cell properties are acquired and maintained in a cancer cell population, and have revealed a new function of the p38 pathway in suppressing cancer development. These studies have also identified a new pathway that can potentially serve as a target for cancer therapies aimed at eliminating CSCs. We will discuss unpublished data that provide the basis for such cancer therapies that take advantage of the p38-mediated suppression of CSCs.

Spy on monoaminergic neuromodulation with new genetically encoded fluorescent sensors

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Dopamine (DA) is a central monoamine neurotransmitter involved in many physiological and pathological processes. A longstanding yet largely unmet goal is to measure DA changes reliably and specifically with high spatiotemporal precision, particularly in animals executing complex behaviors. Here, we report the development of genetically encoded GPCR-activation-based-DA (GRAB_{DA}) sensors that enable these measurements. In response to extracellular DA, GRAB_{DA} sensors exhibit large fluorescence increases ($F/F_0 \sim 90\%$) with subcellular resolution, subsecond kinetics, nanomolar to submicromolar affinities, and excellent molecular specificity. GRAB_{DA} sensors can resolve a single-electrical-stimulus-evoked DA release in mouse brain slices and detect endogenous DA release in living flies, fish, and mice. In freely behaving mice, GRAB_{DA} sensors readily report optogenetically elicited nigrostriatal DA release and depict dynamic mesoaccumbens DA signaling during Pavlovian conditioning or during sexual behaviors. Thus, GRAB_{DA} sensors enable spatiotemporally precise measurements of DA dynamics in a variety of model organisms while exhibiting complex behaviors. Similar strategies can be harnessed to develop a plethora of GRAB sensors for other important neurotransmitters/neuromodulators.

Dissecting the functional complexity of cellular proteins by Protein

Knockout

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The ubiquitin-proteasome pathway is a major proteolytic machinery that selectively targets cellular proteins for degradation. We have developed the “protein knockout” technology, which harnesses the SCF (Skp1, CUL-1, and F-box-containing substrate receptor) ubiquitination machinery to direct the degradation of otherwise stable cellular proteins. The engineered ubiquitin ligase consists of the F-box-containing β TrCP fused in-frame to a binding peptide (BP) for the intended target. We have previously shown that such engineered β TrCP-BP ubiquitin ligases efficiently target cellular proteins for destruction in cultured mammalian cells and in mouse models of embryonic development and tumorigenesis. Although protein knockout and RNAi are both aimed toward reducing or eliminating desired cellular targets, the two technologies differ mechanistically in that protein knockout operates at the posttranslational level to directly destroy the target protein, while RNAi functions to block the biosynthesis of the protein of interest. As such, protein knockout is capable of performing specialized functional analysis that are not attainable by RNAi, including selective destruction of a subpopulation of target proteins either posttranslationally modified or residing in a specific subcellular environment. Protein knockout system was also shown to mediate simultaneous eradication of an entire family of protein and across different eukaryotic species. We have further exploited the complimentary properties of RNAi and protein knockout to achieve rapid and effective eradication of stable cellular proteins. Data will be presented on protein knockout or in combination with RNAi to deplete cellular proteins of interest and generate loss-of-function mutations in somatic cells.

Alternative RNA Splicing Regulates Neuroendocrine Prostate Cancer

Progression

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Although the new generation androgen receptor (AR) inhibitors improve survival of patients with prostate adenocarcinoma (AdPC), emerging evidence indicates that AdPC can evade into a highly aggressive and AR “indifferent” form of prostate cancer, called neuroendocrine prostate cancer (NEPC). AdPC and NEPC share high similarity in genotypes but are distinct in transcriptomes and proteomics, suggesting that altered epigenetics, RNA splicing, and transcription may be the molecular mechanisms of NEPC progression. By comparing global RNA splicing events between AdPC and NEPC, we have identified an NEPC specific splicing signature that is controlled predominantly by the neural RNA splicing factor, SRRM4. SRRM4 catalyzes inclusion of neural-specific exons into mRNAs to be translated into neural protein isoforms of the genes required for neurogenesis. We demonstrate that several histone modification enzymes and transcription factors are SRRM4 target genes. Alternative RNA splicing of these genes by SRRM4 can not only stimulate neuroendocrine trans-differentiation of AdPC cells but also promote cell proliferation and metastasis. SRRM4 can transform AdPC cells into NEPC xenografts in mice and its expression in patient tumors is upregulated by anti-AR therapies. SRRM4 expression is highly correlated with neuroendocrine marker expression and NEPC cell morphology. These findings highlight alternative RNA splicing can confer cancer cells phenotypic plasticity to escape anti-cancer therapies, and that SRRM4 is a multi-functional driver gene for NEPC. SRRM4 may be a therapeutic target for NEPC.

Nonmetallic functions of metabolic enzymes in cancer development

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Cancer cells uniquely reprogram their cellular activities to support their rapid proliferation and migration and to counteract metabolic and genotoxic stress during cancer progression. In this reprogramming, cancer cells' metabolism and other cellular activities are integrated and mutually regulated, and cancer cells modulate metabolic enzymes spatially and temporally so that these enzymes not only have altered metabolic activities but also have modulated subcellular localization and gain non-canonical functions. We discovered metabolism enzymes' newly acquired functions and the non-canonical functions of some metabolites as features of cancer cell metabolism, which play critical roles in various cellular activities, including gene expression, anabolism, catabolism, redox homeostasis, and DNA repair.

Proto-sex chromosomes and their genes in sexual differentiation

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Vertebrates have evolved highly distinctive genomes that have been shaped by multiple whole-genome duplications (WGDs). A core question in biology is how sex chromosomes evolve in extant vertebrates. Here, we combined both comparative genomic and phylogenomic analyses with chromosome assemblies of diverse lineages of vertebrates, and reconstructed an ancestral vertebrate genome. Comparative mapping of the conserved syntenic blocks and chromosome evolution facilitates tracing the formation trajectory of sex-associated loci/chromosomes and provides insights into sex determination in vertebrates. We identified the chromosome Fs, particularly on which were the *Rspo1*/Wnt signaling components, for female sex determination in the teleost lineages. Evolution analysis showed that sex-determining genes *Dmrt1*, *Rspo1* and *Sox3/Sry* and their chromosomes originated from ancestor chromosomes E, F and G independently, indicating three independent origins of the sex-determining loci/chromosomes in vertebrates. Our study provides the post-WGD evolutionary landscape for sex chromosomes in vertebrates.

Keywords: Sex chromosome evolution; Sex; Vertebrates

Systems epigenomics to identify transcriptional dependencies in prostate cancer

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The constitutively active androgen receptor (AR) splice variant 7 (AR-V7) plays an important role in the progression of castration-resistant prostate cancer (CRPC). Although biomarker studies established the role of AR-V7 in resistance to AR-targeting therapies, how AR-V7 mediates genomic functions in CRPC remains largely unknown. We performed AR-V7 ChIP-exo in two CRPC cell lines (22RV1 and LNCaP95) and three AR-V7 positive CRPC patient tissues. We show AR-V7 binds to distinct genomic regions and recognizes a full-length androgen responsive element (ARE) in CRPC cells and patient tissues. Remarkably, we find dramatic differences in AR-V7 cistromes across diverse CRPC cells and patient tissues. Integrative AR-V7 ChIP-exo and

RNA-seq analysis found that AR-V7 regulates different target gene sets involved in CRPC progression. Since our ChIP-exo-based screening of collaborating transcription factor motifs within the AR-V7 binding locations discovered a Homeobox motif that significantly co-occurred with AR-V7-bound AREs and our RNA-seq analysis found that HoxB13 is the most abundant transcript among Homeobox genes expressed in CRPC cells, we performed HoxB13 ChIP-exo in CRPC cell lines and patient tissues. Surprisingly, we discover that HoxB13 is universally required for and colocalizes with AR-V7 binding across CRPC genomes. To determine chromatin accessibility of HoxB13/AR-V7 binding regions, we performed the assay for transposase-accessible chromatin by sequencing (ATAC-seq) using hormone-depleted 22RV1 and LNCaP95 cells. Integrative analysis of ATAC-seq data with AR-V7/AR-full length (AR-FL) ChIP-exo data found that chromatin accessibility was markedly higher on AREs in HoxB13 and AR-V7 binding regions than on AREs in the AR-FL preferred cistrome, suggesting that chromatin accessibility may facilitate preferred genomic colocalization between HoxB13 and AR-V7 versus AR-FL. Our results also showed that HoxB13 pioneers AR-V7 binding through direct physical interaction, and collaborates with AR-V7 to up-regulate target oncogenes. Importantly, transcriptional co-regulation by HoxB13 and AR-V7 was further supported by their co-expression in CRPC tissues (determined by immunohistochemistry) and CRPC circulating tumor cells (determined by RT-PCR). We finally examined whether silencing HoxB13 decreases AR-V7 driven CRPC growth in vivo. We found that silencing of HoxB13 significantly decreases CRPC growth through inhibition of AR-V7 binding and oncogenic function. These results identify HoxB13 as a pivotal upstream regulator of AR-V7-driven transcriptomes that are often cell context-dependent in CRPC, suggesting that HoxB13 may serve as a novel therapeutic target for AR-V7-driven prostate tumors.

A Reproducible Multi-Donor Class of Influenza Antibodies Targeting the Sialic Acid Binding Site on Hemagglutinin of H1N1

A/California/07/2009

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The hemagglutinin (HA) on influenza virus interacts with sialic acid on host cell to initiate entry. Most reproducible influenza-neutralizing antibodies reported have been shown to target the conserved stem region of HA. However, reproducible neutralizing antibodies generated in response to infection or vaccination in multiple individuals targeting the immunodominant globular head have not yet been reported. Here, we report the identification of a multi-donor class of antibodies that were capable of neutralizing A/California/07/2009 (H1N1) (CA09) virus from vaccinees. Genetic analysis indicated that these reproducible antibodies utilized HV2-70 and HD4-17 genes and contained a “YGD” motif in the CDR H3. Negative stain EM of CA09 HA in complex with antibody lineage members and crystal structure of CA09 HA in complex with a representative antibody, LPAF22 Fab, revealed that this antibody class binds to the sialic acid binding pocket of HA head, suggesting that this antibody class neutralized the virus by competing with receptor binding. Mutagenesis studies indicated that conserved “YGD” motif is critical for the function of this reproducible class of influenza antibody. The CDR H3 YGD motif alone provided ~23% of the total binding surface, in addition, the Asp₉₉ OD2 mimicked the sialic acid O8 to form hydrogen bond with HA Gln226. Bioinformatic search based on the defined sequence signature HV2-70,

⁹⁹GD, and 14-15 aa CDR H3 identified antibodies from previous vaccination studies, the frequency of eliciting these reproducible antibodies increased post pandemic H1N1 vaccination while unvaccinated healthy individuals have very low frequency of this reproducible class of antibody. Over 95% of the vaccine-elicited influenza antibodies identified from the defined sequence signature search showed potent neutralization of CA09 virus. Our study showed that the frequency of elicitation of reproducible class of antibodies targeting the receptor-binding pocket of HA head can be increased by vaccination.

Adenosine A2B receptor controls erythroid lineage commitment in stress erythropoiesis by promoting metabolic reprogramming

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Hypoxia is a dangerous condition existing in normal individuals at high altitude and patients with cardiovascular, respiratory, renal and hemolytic diseases. Stress-induced erythropoiesis is an important adaptive response for survival under hypoxic conditions. Substantial research has focused on transcriptional and translational regulation in stress erythropoiesis. However, the metabolic controls in stress erythropoiesis remain unclear.

Adenosine, a hypoxic sensitive metabolite, plays numerous biological functions under hypoxia and stress conditions. However, its function in stress erythropoiesis and underlying mechanisms are

enigmatic. Here we report that genetic ablation of murine erythroid adenosine A2B receptor (*Adora2b^{ff}EpoR-Cre⁺*) attenuates stress erythropoiesis in response to hypoxia and anemia. Unbiased metabolomic screening, flux tracing analysis coupled with gene expression profiling revealed that its activation is required for erythroid lineage commitment by promoting erythroid glucose and glutamine uptake and subsequently metabolic reprogramming channeling glucose metabolism toward the pentose phosphate pathway (PPP) over glycolysis to generate more ribose phosphate as well as serving as a nitrogen donor for de novo nucleotide biosynthesis and stimulation of glutaminolysis under stress erythropoiesis. Supplementation with exogenous nucleosides and dimethyl- α -ketoglutarate restored insufficient erythropoiesis in cultured Epo-stimulated *Adora2b^{-/-}* mouse hematopoietic stem progenitor cells (HSPCs). Mechanistically, we showed that reciprocal upregulation of ADORA2B and HIF-1 α underlying erythroid lineage commitment from murine HSPCs under stress erythropoiesis and cultured EPO-stimulated human CD34⁺ cells by inducing the expression genes encoding transporters for glucose and glutamine and key enzymes of PPP and glutaminolysis. As such, stabilizing HIF-1 α by DMOG rescued the insufficient erythroid lineage commitment in *Adora2b^{ff}EpoR-Cre⁺* mice under stress erythropoiesis. Overall, our studies reveal that ADORA2B is a missing cofactor controlling erythroid lineage commitment in stress erythropoiesis via HIF-1 α -dependent upregulation of key genes to promote metabolic reprogramming. These findings add significant new insight to erythroid commitment and immediately provide new strategies to counteract hypoxia in cardiovascular, respiratory and hemolytic diseases via regulation of stress erythropoiesis.

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Sonic hedgehog signaling is involved in perineural invasion of gastric cancer

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Introduction: Perineural invasion (PNI) is a process by which cancer cells invade the perineurium neural fascicles, and it also has been called neurotropic carcinomatous spread and perineural spread; it was described as the spread of cancer cells in the perineural spaces of the nerves. Clinical studies suggest that frequency of PNI was high in patients with gastric cancer and the proportion of PNI positivity increased with progression and clinical stage of disease, and denervation can inhibit the progression and metastasis of gastric cancer. However, the molecular mechanism of PNI is still unclear due to the lack of effective PNI research model of gastric cancer.

Sonic hedgehog (SHH) is a protein of 45 kDa encoded by the SHH gene. Considerably, SHH plays an impressive role in regulating vertebrate organogenesis such as growth of digits on limbs and organization of the brain. Recent studies have shown that SHH signaling is abnormally activated in neuroblastoma, colorectal cancer, basal cell cancer, medulloblastoma, prostate cancer, ovarian cancer, pancreatic cancer, and other forms of cancer. Some studies have suggested that SHH signaling activation could contribute to carcinogenesis. Several studies confirmed that SHH pathway activation is associated with poorly differentiated and aggressive types of gastric cancer. Therefore, an increased understanding of SHH signaling in carcinogenesis could provide novel insights into gastric cancer treatment. However, how neurogenic SHH is involved in gastric cancer metastasis has not been clarified.

This study attempted to explore the underline mechanism of SHH in the metastasis of gastric cancer via PNI.

Methods: We constructed a PNI-related gene set from a meta analysis of literature, and established the PNI animal model presenting gastric cancer metastasis by using PDOX. Six human gastric cancer (GC) cell lines HGC-27, MGC823, AGS, SGC7901, NCI-N87 and the human

gastric epithelial cell line GES-1 were obtained from the Cell Bank of Chinese Academy of Medical Science. Cell counting Kit-8 (CCK-8) assay was used to detect GC cell proliferation, and cell migration of GC was measured using a scratch assay, and cell invasion was assessed using a modified Boyden chamber model. The mRNA levels of SHH signaling pathway in the gastric cancer cells were determined using quantitative real-time polymerase chain reaction (qRT-PCR) analysis.

Results: We have developed an orthotopic mouse model whereby gastric cancer cell lines MKN-45, AGS and PBS as negative control were injected into the subserosal layer of the stomach. Mice were monitored and weighed three times weekly. In MKN-45 group, five in six mice were observed tumor in stomach, and 75% of mice at 6-weeks post injection showed the phenomenon of gastric cancer PNI. In AGS group, mice showed tumors growth at the primary injection site but only one in six mice showed gastric cancer PNI by 6-weeks. Undifferentiated GC cell lines HGC-27 showed highest cell proliferation and migration, however, high differentiated GC cell lines NCI-N87 showed lowest cell proliferation and migration, which is consistent with their differentiation. The mRNA level of SHH signaling pathway up-regulated in poor differentiated GC cell lines compared with high differentiated GC cell lines. After the addition of SHH conditioned medium to GC cell lines for 24h, the expression of uPA/uPAR increased in HGC-27 and MGC823.

Conclusion: Our findings indicate that SHH signaling pathway is involved in gastric cancer PNI, probably through regulation of ECM degradation.

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Eosinophils dampen hepatic ischemia reperfusion injury through interleukine-33 signaling

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Eosinophils are myeloid cells known for their involvement in allergy and host defense against parasitic infections. Unexpectedly, we found that eosinophils rapidly accumulated in human liver grafts following hepatic transplantation. In contrast, eosinophils are not detectable in healthy liver tissues. Studies with genetic models of eosinophil deficiency (PHIL or Δ dblGata1 mice) or antibody-mediated eosinophil-depletion reveal exacerbated injury following hepatic ischemia and reperfusion. Adoptive transfer of bone marrow-derived eosinophils normalized liver injury of eosinophil-deficient mice or reduced hepatic ischemia and reperfusion injury in wild-type mice. Mechanistic studies combining genetic and adoptive transfer approaches identified a critical role for eosinophil-specific interleukin

(IL)-33 signaling and IL-13 production in eosinophil-mediated hepato-protection. Together, these studies provide insight into a novel mechanism of eosinophil-dependent liver protection that can be targeted therapeutically to improve outcomes of patients undergoing liver transplantation.

Tubular Cell Specific Mst1/Mst2 Deficiency Leads to Chronic Kidney Disease in Mice

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The Hippo pathway is a regulator of organ size. The core components of the Hippo pathway consist of Mammalian Ste20-like kinases 1/2 (MST1/2) and their scaffold protein SAV1, large tumor suppressor 1/2 (LATS1/2) and their scaffold proteins MOB1A/1B, and two downstream effectors YAP/TAZ. Several members of the Hippo pathway including SAV1, LATS1/2, YAP and TAZ have been found to be involved in embryonic kidney development or kidney disease. However, the role of MST1/2 in kidney remains unknown. Here, we showed for the first time that MST1 was highly expressed in all nephron segments and collecting ducts in mouse kidneys. We therefore generated tubular cell specific *Mst1/Mst2* double knockout (dKO) mice by intercrossing floxed *Mst1/Mst2* mice with *Ksp-Cre* transgenic mice. dKO mice showed increased kidney weights starting at 4 weeks of age. Body weights were comparable between WT and dKO mice up to 8 weeks of age. However, dKO mice exhibited a significant body weight loss at 6 months and later stages. Kidney structural abnormality and tubular injuries were seen as early as 4 weeks and aggravated with age, as indicated by immune cell infiltration, tubular cell death, thickening of glomerular basement membrane and tubular basement membrane, cast formation, and increased levels of urinary NGAL, a marker for kidney injury. At 6 months of age and later, protein levels of the fibrotic markers α -SMA, fibronectin 1 and collagen I α 1 were significantly increased in dKO kidneys, and Masson's trichrome staining showed much more collagen deposition in dKO kidneys, indicating dKO mice developed renal fibrosis. Consequently, renal function was impaired at 6 months and older ages, as shown by increased serum creatinine and blood urea nitrogen (BUN) levels in dKO mice. Moreover, dKO kidneys exhibited increased expression of inflammatory factors and infiltrations of macrophages into the interstitium.

YAP activity was significantly enhanced in dKO kidneys at 4 weeks of age, coupled with increased Ki67-positive tubular cell numbers. By generating *Mst1/Mst2/Yap* triple knockout (tKO) mice, we found that deletion of *Yap* restored the kidney weights in dKO mice to WT levels, and also fully rescued the expression of all the inflammatory factors measured except TNF α at 4 weeks of age. Notably, an increase in TNF α was found in dKO kidneys at 2 weeks of age, when YAP was not activated yet.

These results suggest that the increased kidney weights and most inflammatory responses observed in tubular *Mst1/Mst2* deficient mice are dependent on YAP while TNF α expression is induced via both YAP-dependent and -independent mechanisms.

Collectively, we found tubular *Mst1/Mst2* deficiency leads to tubular cell hyperproliferation, inflammation, tubular injury, renal fibrosis, and renal dysfunction, indicating that tubular MST1/2 play important roles in restraining renal overgrowth and inflammation and maintaining normal tubular structure and function.

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Pharmacological inhibition of EZH2 enhances prostate cancer cell sensitivity to genotoxic insults through suppressing DNA damage repair

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Agents that block the activity of the methyltransferase EZH2 demonstrate promising anticancer effects. We found that EZH2 inhibitors (EZH2i) abrogated the growth of prostate cancer cells, especially those that have become castration resistant. Loss-of-function genetic screens using CRISPR-Cas9-mediated gene knockout identified a group of EZH2 target genes that are prominently downregulated upon EZH2 inhibition. These genes underlie the inhibitory effects of EZH2i in prostate cancer, and their expression is significantly correlated with EZH2 level and cellular sensitivity to EZH2i in prostate cancer as well as other cancer types. These genes are enriched for components of the DNA repair machinery, especially the base excision repair (BER) pathway. Consistent with this finding, treatment with EZH2i dramatically enhanced responses of prostate cancer cells to genotoxic stresses. Our work revealed a previously underappreciated mechanism of action of EZH2 inhibitors and provides a mechanistic basis for potential new combination cancer therapies.

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Targeting KDM4, an oncogenic histone demethylase

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Histone lysine demethylase 4 members (KDM4A-KDM4C) that remove repressive methyl marks from methylated lysines on histone 3 (H3K9me_{3/2} and H3K36me_{3/2}) regulate chromatin organization and gene expression. KDM4A, KDM4B, and KDM4C are often overexpressed in various malignancies and their overexpressions contribute to cancer progression due to their function as a co-activator of transcription factors such as androgen receptor. Here, we show that the depletion of KDM4A or KDM4B greatly downregulates the expression of androgen receptor-responsive genes and curbs tumor growth in prostate cancer. Additionally, KDM4A coactivates E2F1 to regulate cell cycle and tumor metabolism. Furthermore, KDM4B, but not KDM4A/KDM4C, upregulates IL-8 production in conjunction with c-Jun. This effect is even

more pronounced under *Helicobacter pylori* challenge. The high levels of KDM4B and p-c-Jun are associated with worse overall survival in gastric cancer patients. Genetic and pharmacological inhibition of KDM4A/KDM4B significantly blocks cancer progression. Together, our results highlight that KDM4 serves as a novel target for cancer therapy.

PRMT1-mediated metabolic reprogramming promotes leukemogenesis

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Copious expression of protein arginine methyltransferase 1 (PRMT1) is associated with poor survival in acute myeloid leukemia. To study the role of PRMT1 in leukemogenesis, we used a leukemia mouse model established by transplantation of a leukemia cell line (6133) derived from RBM15-MKL1 knock-in mice. We discovered that 6133 cells contain a subpopulation expressing high level of PRMT1 that causes leukemia in mice with short incubation time. A PRMT1 inhibitor, MS023, cures leukemia in mice transplanted with 6133 cells. Seahorse analysis indicates that PRMT1 increases ECAR (extracellular acidification rate) level and decrease the OCR (oxygen consumption rate) level. Metabolomic analysis and FACS analysis with Bodipy493/503 indicates that PRMT1 stimulates the accumulation of fatty acids intracellularly. Consistent with fatty acid accumulation, attenuation of fatty acid oxidation by PRMT1 is further validated by the downregulation of the CPT1A protein level, which catalyzes the rate-limiting step in fatty acid oxidation. Glucose consumption is accelerated by PRMT1 with the accumulation of lactic acid.

6133 cells expressing higher level of PRMT1 are addicted to glucose for cell proliferation than 6133 parental cells. Taken together, we concluded that PRMT1 enhances glycolysis in 6133 cells. Therefore, we treated the leukemia mice with glucose analogue 2-deoxy-glucose (2-DG). The leukemia progression is delayed by 2-DG treatment. Thus, PRMT1 promotes leukemia progression via downregulation of fatty acid oxidation and enhancing glycolysis.

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TGF- β -regulated alternative splicing in cancer progression

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Transforming growth factor- β (TGF- β) is major inducer of epithelial to mesenchymal transition (EMT), which associates with cancer cell metastasis and resistance to chemotherapy and targeted drugs, through both transcriptional and non-transcriptional mechanisms. We previously reported that in cancer cells, heightened mitogenic signaling allows TGF- β -activated Smad3 to interact with poly(RC) binding protein 1 (PCBP1) and together they regulate many alternative splicing events that favors expression of protein isoforms essential for EMT, cytoskeletal rearrangement, and adherens junction signaling. One of these genes is the TGF- β -activated kinase 1 (TAK1). We found that TGF- β -induced exclusion of TAK1 exon 12 requires another RNA-binding protein, Fox-1 homolog 2 (Rbfox2), which binds intronic sequences in front of exon 12 independently of the Smad3-PCBP1 complex. Functionally, exon 12-excluded TAK1 Δ E12 and full length TAK1FL are distinct. The short isoform TAK1 Δ E12 is constitutively active and supports TGF- β -induced EMT and nuclear factor kappa B (NF- κ B) signaling, whereas the full-length isoform TAK1FL promotes TGF- β -induced apoptosis. These observations offer a harmonious explanation for how a single TAK1 kinase can mediate the opposing responses of cell survival and apoptosis in response to TGF- β . They also reveal a propensity of the alternatively spliced TAK1 isoform TAK1 Δ E12 to cause drug resistance due to its activity in supporting EMT and NF- κ B survival signaling.

Medulloblastoma, Hedgehog, and an unexpected role of Suppressor of Fused

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Medulloblastoma (MB) is the most common type of pediatric brain tumor originated in the cerebellum and a well-studied model for the cancer genomic research. Histologically, MB can be distinguished in five subgroups; this classification is both compatible and sufficient for the conventional treatment by surgical resection. About a decade ago, advances in genetics and early generation of genomic expression profiling technique led to the identification of 4 molecular subgroups of MBs including those that showed characteristic patterns of Sonic Hedgehog (SHH) and WNT pathway activities. This new classification allowed the SHH subtype to be tested in clinical trials for the efficacy of a SHH pathway blocker. However, results from those trials, while encouraging, revealed limitation of the new system because of unacceptably high relapses developed after initial positive response. Secondary mutations in the drug target Smoothed Muscle (SMO) and mutations in downstream pathway components have been proposed as the causes of resistance. A recent cancer genomic sequencing study of the SHH MBs has uncovered a large number of unique mutations within and without the SHH pathway, and showed that tumors carrying mutations downstream of *SMO* are resistant to the pathway blocker.

SHH is a morphogen that plays critical roles in shaping tissue patterns during embryonic development and maintaining adult tissue homeostasis. This pathway is linked to MB because the gene that encodes SHH receptor, *PTCH*, is a key tumor suppressor underlying multiple categories of MB. *PTCH* keeps the pathway repressed by repressing the intrinsic activity of SMO, but upon ligation to SHH, the inhibition is alleviated, allowing SMO to activate a family of 3 GLI transcriptional factors for target gene expression. We have identified two HECT-domain ubiquitin E3 ligases, Smurf1 and Smurf2, that direct the ligand-occupied *PTCH* coming out of the primary cilium to move from lipid rafts into the endocytic vacuolar system for lysosome-mediated turnover. Another important tumor suppressor, *SUFU*, which is a nexus of the SHH pathway to many types of drug resistant MB and skin cancers, encodes a GLI-binding protein.

Orthodoxically, *SUFU* has been regarded as a negative regulator of *SHH* signaling, a description befitting its tumor-suppressing role. However, in a *PTCH* heterozygotically mutated mouse model of MB, we found that *SUFU* expression was grossly up-regulated. Moreover, although new born mouse pups deficient of *PTCH* specifically in the cerebellum all developed MB-like out growth, those that further lacked *SUFU* surprisingly did not. Our data showed that *SUFU* accompanies and is required for all *GLI* factors at each step of their functions such that without *SUFU*, those *GLI* transcriptional activators become unstable and cannot support MB formation. In this regard, *SUFU* is a novel type of molecular chaperon, which may be counter-intuitively usurped for targeted MB treatment.

In Vivo Study of Phosphorylated Sufu Model Mice

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Hedgehog (Hh) was initially discovered by Christiane Nüsslein-volhard and Eric Wieschaus in 1980. Malfunction of Shh signaling pathway contributes to many human diseases including birth defects, such as Gorlin syndrome and Greig Cephalopolysyndactyly syndrome, and cancers, involving Basal Cell Carcinoma, Medulloblastoma, and so on.

As the core member of Shh signaling pathway, Sufu binding to all Gli transcription factors, has a fundamental function in the embryonic development of mammalian animals. The modification of Sufu affects the transduction of Shh signaling pathway, especially phosphorylated Sufu.

Therefore, in order to study the physiological function of double phosphorylation of Sufu by PKA and GSK3 β , we utilized the homologous recombination method to construct the transgenic mouse which Sufu^{S342/346D} (SD) mutant inserted into Rosa26. Then, Rosa26^{SD} mice mated with Sufu^{flox/flox} mice, and their heterozygous offspring mated with Ddx4-Cre mice. Finally, under the drive of Ddx4-Cre recombinant enzymes, we got nine genotypes of mice, such as Sufu^{+/+} (Wildtype), Sufu^{+/-} (1 copy of Sufu), Sufu^{-/-}; Rosa26^{+/-SD} (1 copy of Sufu), Sufu^{-/-}; Rosa26^{SD/SufuSD} (2 copies of Sufu), Sufu^{+/-}; Rosa26^{+/-SD} (2 copies of Sufu), Sufu^{+/+}; Rosa26^{+/-SD} (3 copies of Sufu), Sufu^{+/-}; Rosa26^{SD/SD} (3 copies of Sufu), Sufu^{+/+}; Rosa26^{SD/SD} (4 copies of Sufu) and Sufu^{-/-} (embryonic lethal). They were absolutely in line with Mendel's second genetic law.

A preliminary phenotype analysis of eight genotypes of viable mice (a total of 503 mice) found that Sufu^{+/+}; Rosa26^{SD/SD} mice were more heavier, and 76.3% of them had anterior polydactyly of hindlimb, 9.52% of them had the symptom of Hydrocephalus, and the fertility of this genotype male mice were weak. Then, we extracted the Sufu^{+/+} (Wildtype), Sufu^{+/-}; Rosa26^{SD/SD} (3 copies of Sufu), and Sufu^{+/+}; Rosa26^{SD/SD} (4 copies of Sufu) primary MEF cells from E13.5 embryo, and examined some molecules associated with Shh signaling pathway, and used the infinite Sufu^{-/-} cell line, which

featured the constitutive activation of Shh signaling pathway. We found that Shh signaling pathway has activated gradually along with the increase of the number of Sufu copies, indicated that Sufu could modulate the transduction of Shh signaling pathway positively.

This study will be helpful to roundly understand the role of Sufu in the Shh signaling pathway, and prove that Sufu is an ideal target for disturbing the activity of Shh signaling pathway, in order to provide new ideas for the development substitutes of Smo blocker.

Key words: Shh, Sufu, Phosphorylation

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Lifespan extension induced by exercise is mediated by autophagy via

PAQR-1/AMPK/SIR-2.1 signaling in *C. elegans*

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Regular exercise has a multi-system anti-aging effect among different species, including worms, flies, mice, and primates. Although numerous hypotheses have been proposed to explain the benefits of exercise on longevity, the exact molecular mechanisms are still poorly characterized. Here we show that exercise extend lifespan of *C. elegans* and induces autophagy in the hypodermal seam cells, intestine and neurons of worms. Knockdown of the autophagy-related genes significantly shortens the lifespan of worms by exercise. Furthermore, we find that the induction of autophagy is required for the PAQR-1 (adiponectin receptor AdipoR1 homolog)/AMPK/SIR-2.1 signaling. The signaling pathway promotes autophagy by enhancing the transcription activity of DAF-16/FOXO. Knockdown of *daf-16* significantly suppresses exercise-mediated lifespan extension. Taken together, our findings reveal that the PAQR-1 signaling together with transcription factor DAF-16/FOXO act as a regulator of autophagy to extend lifespan in *C. elegans* after exercises.

The role of glypican-3 in regulating Wnt in hepatocellular carcinoma

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Wnt signaling is one of the key regulators of hepatocellular carcinoma (HCC) tumor progression. Glypican-3 (GPC3) is overexpressed in HCC and functions as a Wnt coreceptor. These features make GPC3 an attractive target for liver cancer therapy. However, the precise interaction of GPC3 and Wnt and how GPC3, Wnt, and FZD cooperate with each other are poorly understood.

Our study found that: 1) Both the core protein and the HS chains of GPC3 contributed to the GPC3 modulated Wnt signaling. Residue F41 area of the core protein of GPC3 formed a Wnt-binding groove. The HS chains of GPC3 contributed to the activation of Wnt signaling through a unique region which was at least four disaccharides in length with 6-O sulfation or even

shorter when 3-O sulfation presented. 2) We developed single domain antibody HN3 recognized the reside F41 area of the core protein of GPC3, and antibody HS20 targeting the HS chains of GPC3. These two antibodies both inhibited the activation of Wnt signaling in vitro and exhibited significant anti-tumor activity in xenografted mice model, which provides potential therapeutic strategy for HCC therapy.

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Structural variability of bacterial and fungal communities from the soil of *Malania oleifera*

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Malania oleifera is being planted actively in Guangnan and Funing Counties of Yunnan Province because of its potential utilization value as woody energy oil plant. However, low survival rate of seedlings is one of the main factors affecting its industrial development. The variability of soil colonization are closely relative with the host plants innate immune system, nutrition and energy transmission, the plant growth-promoting and also soil-borne diseases. In order to understand the structural variability of soil microbiome of *M. oleifera*, its rhizosphere soil from main planting area in Guangnan County were sampled and the bacterial and fungal communities were unraveled using high-throughput sequencing. We found that the majority of the soil samples saturated around 950-1200 OTUs and 420-500 OTUs for bacterial and fungal communities, approximately 609 and 154 of all OTUs were shared, respectively. Furthermore, by defining the core microbiome as the 10 most abundant OTUs of soil samples at genus level, the dominant bacteria were observed, including *Acidobacteriaceae*, *Rhodanobacter*, *Sphingomonas*, *Acidibacter*, *Acidothermus*, *Burkholderia*, *Bradyrhizobium* and *Acidimicrobiales*. In addition, *Archaeorhizomyces* as a dominant fungus accounted for 19% of all OTUs, followed by *Oidiodendron*, *Clavulinopsis*, *Mortierella*, *Clavaria*, *Trichoderma* and *Leohumicola*. Noticeably, traditional techniques should be considered to obtain and identify some key strains, and the net effect of bacterial and fungal communities from soil should also be assessed thoroughly to better understand the effect of soil microbiome on the growth and development of *M. oleifera*.

Keywords: *Malania oleifera*, Rhizosphere soil, Bacterial community, Fungal community

Study on VIT-2 in senescence of reproductive system of

Caenorhabditis elegans

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Many biological functions associate with quality of life decline with age, but a decline in female reproduction is one of the earliest hallmarks of aging, including invertebrates and mammals. Similarly, reproduction of *Caenorhabditis elegans* ceases in mid-adulthood . Although somatic aging has been studied in both worms and humans, mechanisms regulating reproductive aging have not yet understood. *vit-2* encodes the vitellogenin homolog YP170 which is a large lipid transfer modules capable of transporting phospholipids and cholesterol to oocytes. Here we demonstrated that *vit-2* can regulate reproductive span of *C.elegans* by affected the quality of oocytes. The expression of *vit-2* was bimodal and began to express when there were eggs in the body. On the first day of oviposition, the expression of *vit-2* appeared in first peak. Once the oviposition was over, the expression of *vit-2* would rise again. Knockout *fshr-1* and *ceh-60* could prolong reproductive senescence compared with wild type, silencing *fshr-1* or *ceh-60* by RNAi can inhibit the mRNA levels of *vit-2*. In addition, *fshr-1*, *ceh-60* mutation activated *lipl-4* to slow down lysosome senescence and thus prolong reproductive senescence. *Vit-2* was homologous to human apolipoproteins B (APOB). The data of 7135 patients with APOB showed that the curve showed bimodal change with age, which was consistent with the expression curve of *vit-2*. At the age of 46, 50, it is in climacteric, when ApoB levels in the blood rise again as a node. Therefore, the genetic study of reproductive senescence by using nematode model organisms laid a solid foundation for elucidating the mechanism of women's reproductive senescence.

Keywords: reproductive senescence; *vit-2*; apolipoprotein B

**PRELP, FZD6, DKK3, S100A9, novel biomarkers for the ruptured
atherosclerotic plaque identified by weighted gene correlation
network analysis**

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The rupture of atherosclerotic plaques is essential for cardiovascular or cerebrovascular events. The present work was to identify biomarkers for ruptured plaques using methods of weighted gene correlation network analysis (WGCNA). The expression profile for stable and ruptured atherosclerotic plaques, GSE41571, was downloaded from GEO. After preprocessing, WGCNA was conducted to identify the modules and hub genes which were correlated to the ruptured plaques. The Gene Ontology (GO) and KEGG enrichment was used to uncover the functions of these hub genes. Protein-protein interaction network of these hub genes was constructed using STRING. The “limma” package in R software was used to identify the differentially expressed genes (DEGs). The overlapped genes between DEGs and the hub genes were considered as key genes for the ruptured plaques. As a result, six modules with 236 hub genes were identified through WGCNA analysis. Among these six modules, blue and brown modules were with the highest correlations with plaque traits (with a correlation of 0.82 and -0.9 respectively). Further GO and KEGG enrichment of the 72 hub genes from blue and brown modules indicated these genes were related to cell adhesion, extracellular matrix organization, cell growth, cell migration, leukocyte migration, PI₃K-Akt signaling, focal adhesion, ECM-receptor interaction. Further analysis discovered 46 genes were overlapped in the DEGs and hub genes. Among them, DKK3, FZD6, and PRELP were significantly

decreased in ruptured plaques while S100A9 was significantly increased compared to the stable plaques. In conclusion, DKK3, FZD6, PRELP and S100A9 might be the novel biomarkers for the ruptured atherosclerotic plaques.

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Enhanced autophagy contributes to protective effects of IL-22 against acetaminophen-induced liver injury

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Acute or acute-on-chronic liver failure is a leading cause of death in liver diseases without effective treatment. Interleukin-22 (IL-22) is currently in clinical trials for the treatment of severe alcoholic hepatitis, but the underlying mechanisms remain to be explored. Autophagy plays a critical role in alleviating liver injury. The aim of the current study is to explore the role of autophagy in IL-22-mediated hepato-protective effect against acetaminophen (APAP)-induced liver injury.

Methods: A model of acute liver injury induced by APAP was used in vivo. IL-22 was administered to the APAP-treated mice. Hepatocytes were pre-incubated with IL-22, followed by exposure to APAP for in vitro analyses.

Results: IL-22 administration significantly reduced serum ALT and AST, hepatic reactive oxygen species, and liver necrosis in APAP-challenged mice. APAP treatment increased hepatic autophagosomes, which was further intensified by IL-22 co-treatment. Hepatic LC3-II was moderately upregulated after APAP administration without obvious alteration of phosphorylation of AMP-activated kinase (p-AMPK). IL-22 pretreatment significantly upregulated hepatic LC3-II and p-AMPK in APAP-treated mice. IL-22 also alleviated APAP-induced cytotoxicity and upregulated LC3-II and p-AMPK expression in cultured hepatocytes treated with APAP in vitro. When

p-AMPK was blocked with compound C (an AMPK inhibitor), IL-22-mediated LC3-II conversion and protection against APAP-induced cytotoxicity was weakened.

Conclusions: Enhanced AMPK-dependent autophagy contributes to protective effects of IL-22 against APAP-induced liver injury.

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Tumor suppressor p53 in metabolism

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Tumor suppressor p53 plays a central role in tumor suppression. The tumor suppressive function of p53 has long been attributed to its ability to induce apoptosis, cell cycle arrest and senescence in cells. However, recent studies suggest that p53 regulates various metabolic pathways to maintain the metabolic homeostasis of cells, contributing to its function in tumor suppression. Our recent studies show that p53 regulates metabolism and suppression of cancer metastasis through its transcription activation of its targets GLS2 and Parkin. We found that p53 regulates GLS2 expression to regulate glutamine metabolism. Furthermore, GLS2 binds to small GTPase Rac1 to inhibit the Rac1 activity in promoting cancer cell metastasis. Through up-regulating GLS2 expression, p53 inhibits Rac1 activity, which in turn inhibits cancer metastasis. We also found that p53 regulates Parkin expression to suppress glycolysis. As an E3 ubiquitin ligase for HIF-1 α , Parkin binds to HIF-1 α and leads to ubiquitination-mediated degradation of HIF-1 α , which in turn suppresses glycolysis and cancer metastasis. Together, these results strongly suggest that the role of p53 in metabolic regulation contributes to p53's function in tumor suppression. Further understanding of p53 in metabolism will provide new opportunities for cancer therapy.

CgCLec-HTM-mediated Signaling Pathway Regulates LPS-induced CgIL17 and CgTNF Production in Oyster

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The immune signaling pathway mediated by Dectin-1 is important in mammal to modulate the production of interleukin17 (IL17) and tumor necrosis factor α (TNF- α). Recently, IL17 and TNF have also been characterized in invertebrates to play crucial roles in antibacterial immune responses, while the immune recognition and regulation mechanisms to produce IL17 and TNF are still not well investigated. In the present study, a new type C-type lectin receptor (named as CgCLec-HTM) with a signal peptide, a carbohydrate-recognition domain (CRD), a transmembrane (TM) domain and a non-classical immunoreceptor tyrosine based activation motif (hemITAM) in the cytoplasmic tail was identified from oyster *Crassostrea gigas*. CgCLec-HTM could bind lipopolysaccharide (LPS) and various bacteria. After binding to its ligands, CgCLec-HTM was associated with the Src homology 2 (SH2) domain of spleen tyrosine kinase (CgSyk) by the hemITAM in its cytoplasmic tail to promote extracellular signal-regulated kinase (CgERK) phosphorylation. The activated CgERK could interact with CgRel to induce CgRel nuclear translocation. The CgRel in nucleus eventually induced the transcription of CgIL17s and CgTNF. The results demonstrated that CgCLec-HTM with a broad binding spectrum of bacteria could be associated with CgSyk to transfer immune signals into intracellular ERK-Rel pathway to induce CgIL17 and CgTNF production.

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Vessels that encapsulate tumor clusters (VETC) in HCC metastasis and therapeutic response

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We observed that in human HCC tissues, there were two distinct vascular patterns: vessels with discrete lumens (the reported classical capillary vessels), and sinusoid-like vessels that formed a cobweb-like pattern and encapsulated tumor clusters (we named it VETC). Further investigations disclosed that the presence of VETC pattern was significantly related to the increased metastasis and recurrence of HCC; the VETC pattern worked as a novel mechanism for EMT-independent metastasis and abrogation of VETC formation significantly attenuated HCC metastasis. Moreover, VETC+ and VETC- HCC patients achieved significantly different survival benefit from sorafenib treatment.

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Analgesic efficacy of Patient-controlled analgesia among hepatectomy patient

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Objective: This study was designed to explore analgesic efficacy of patient-controlled analgesia used for transcatheter arterial chemoembolization, and obtain a good analgesic regimen after TACE.

Methods: Information of 20 cases liver cancer patients who were treated with TACE in Army Medical University affiliated Southwest hospital and Chongqing Shapingba district People's Hospital was collected. Among them, 10 patients were treated with patient-controlled analgesia, other 10 patients were treated analgesia as control. The analgesic effect, average stay in hospital, and incidence of complications after analgesia of two groups patients were observed, analyzed and counted. **Results:** In the experimental group, the postoperative analgesia effect among patients with self-controlled analgesia was better, average hospitalization time was shorter, and incidence of complications was lower than control ($P < 0.05$). **Conclusion:** Compared with drug analgesia, patient-controlled analgesia pump has a definite effect on analgesia after TACE, which would be expected to be a routine clinical analgesia regimen for patients after TACE.

Key words: Patient-controlled analgesia; transcatheter arterial chemoembolization; analgesia

Emerging CRPC phenotypes and therapeutic opportunities from UW

Prostate Cancer Rapid Autopsy Program

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Department of Urology, University of Washington, Seattle, USA Most castration-resistant prostate cancer (CRPC) still rely on androgen receptor signaling, and therefore responsive to the next-generation androgen pathway inhibitors abiraterone and enzalutamide. Following the FDA-approval for abiraterone and enzalutamide in 2011 and 2012, respectively, emerging treatment-resistant phenotypes has developed. From the University of Washington rapid autopsy program, we observed increasing number of liver metastasis. Moreover, phenotypes of CRPC evolved which now include androgen receptor-positive PC (ARPC), neuroendocrine prostate cancer (NEPC), and double negative prostate cancer (DNPC). We have recently conducted a preclinical trial using patient-derived xenografts and revealed supraphysiological testosterone (SPT) as a potential therapy on CRPC failing enzalutamide. Our results highlighted SPT repressed transcriptional programs of ARv7, E2F, and DNA damage response, and provided evidence to support sustained suppression of these programs underlying durable SPT response.

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Downregulation of LncRNA H19 by Liver X receptor attenuates rheumatoid arthritis via targeting miR-124a

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OBJECTIVE: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease, is characterized by hyperplasia of synovial lining cells and destruction of cartilage. Although the exact cause is not fully understood, it has been demonstrated that impaired apoptosis of Fibroblast-like synoviocytes (FLSs) causes synovial hyperplasia, facilitating destruction of cartilage in RA. This study aimed to elucidate the role and possible mechanism of long non-coding RNA H19 (lncRNA H19) in the synovial hyperplasia and cartilage destruction of RA.

MATERIAL AND METHODS: The expressions of lncRNA H19, miR-124a and target genes (CDK2 and MCP-1) in TNF- α treated human synoviocyte MH7A cells were determined. Moreover, the effects of aberrant expression of lncRNA H19 on MH7A cells viability, miR-124a and target genes (CDK2 and MCP-1) were investigated. Furthermore, the effects of LXR agonists on the expression of lncRNA H19, miR-124a and target genes were assessed. The regulatory relationships between lncRNA H19 and miR-124a as well as the target of miR-124a were explored. Finally, the role of lncRNA H19 in collagen-induced arthritis (CIA) was detected.

RESULTS: Inverse expression of lncRNA H19 (upregulated) and miR-124a (downregulated) was revealed in CIA mice and TNF- α induced MH7A cells. Ectopic expression of lncRNA H19 inhibited miR-124a expression, resulting in increased the expression of miR-124a target genes (CDK2 and MCP-1) and promoted proliferation of MH7A. Furthermore, LXRs agonists downregulated the expression of lncRNA H19 and suppressed MH7A proliferation, which

mediated by mir-124a. In addition, the in vivo experiments exhibited that knockdown of lncRNA H19 inhibited inflammation in CIA mice, which were demonstrated by upregulation of mir-124a, and down-regulation of CDK2 and MCP-1.

CONCLUSIONS: Our results indicate that lncRNA H19 is upregulated in RA, and its upregulation may promote MH7A proliferation and exacerbate inflammation via regulating miR-124a/ CDK2 and MCP-1 axis.

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Perylene Probes as Photosensitizer and Live Cell Imaging Agent

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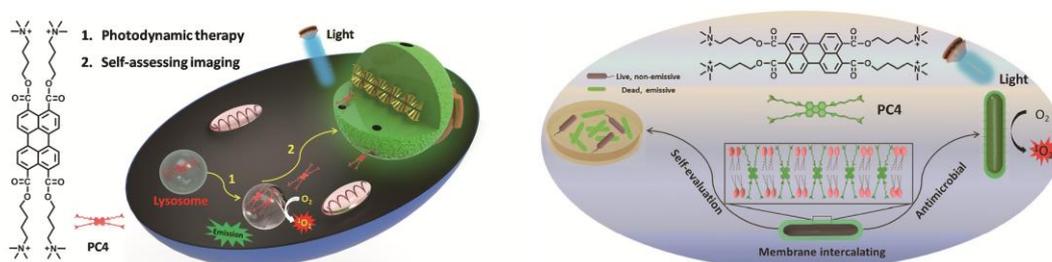
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Perylene derivatives containing a rigid and planar aromatic scaffold are known for their outstanding chemical and photostability, and they are promising building blocks for functional molecular and supramolecular systems. We have employed perylene probes and their controlled self-assembly properties for the development of a number of new biosensing techniques.

Here are some recent progresses:

(1) A cationic perylene probe PC4 was explored as a photosensitizer. The probe shows excellent water solubility, high chemical and photostability, and effective 1O_2 generation capability. It can specifically target lysosome with negligible dark toxicity ($IC_{50} > 500 \mu M$), and it shows excellent photo-toxicity ($IC_{50} = 2.8 \mu M$) upon low dose light irradiation ($4.2 J cm^{-2}$). More importantly, the movement of PC4 from lysosome in live cell to the nuclei in dead cell provides a facile and efficient way to monitor and assess the treatment effect. To the best of our knowledge, this is the first perylene probe reported as a smart lysosome-targeted photosensitizer and PDT imaging/assessing agent.

(2) PC4 was also explored as a MICOE (membrane intercalating conjugated oligoelectrolyte) and an antimicrobial agent. PC4 can spontaneously intercalate into the lipid membrane of the bacteria. And it can effectively kill Gram-positive bacteria, Gram-negative bacteria, and drug resistant bacteria via the generation of 1O_2 . Furthermore, PC4 can enter into the interior of dead bacterial cell and show bright fluorescence emission. Thus the therapeutic effect can be easily evaluated using a fluorescence microscope without the use of additional fluorescence dyes. PC4 can therefore serve both as a “killer” and a “marker”, namely a membrane-intercalating broad-spectrum antimicrobial agent and an excellent fluorescence imaging agent.



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4.

Synthetic biology platform for bioactive *Panax ginseng* and *Panax notoginseng* saponins production

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Ginsenosides are the main bioactive compounds of *Panax* plants; however, the low content in plants and unstable supply of ginsenosides has severely hindered their application. Synthetic biology offers an alternative way to manufacture natural products at large scale by reconstructing their biosynthesis pathway in microbial. We built a biosynthesis platform for bioactive Ginsenosides, including elements mining, synthesis pathways analysis, elements match and optimization, cell factory construction, fermentation conditions optimization, and so on. In the past 5 years, we characterized many key UDP-glycosyltransferases (UGTs) and NADPH-cytochrome P450 reductases (CPRs) involved in the biosynthesis of ginsenosides, and unveiled the biosynthesis pathway of many ginsenoside including Compound K, Rh2, Rg3, F1 and Rh1. We also developed yeast cell factories to produce these ginsenosides from glucose; however, the yield was too low for commercialization. Here, we optimize the cell factories systemically by boosting carbon flux to the precursor synthesis and tuning the expression and efficiency of key bioparts related to the pathway. We first develop a protopanaxadiol (PPD)-producing chassis via modular engineering of the mevalonic acid pathway and optimization of P450 expression. Based on this chassis, we established a series of cell factories to produce different rare ginsenosides. By optimizing the in vivo expression and performances of key UGT genes, the yield of all ginsenosides reached to >100 mg/L in shake flasks and > 2g/L in fed-batch fermentation. To the best of our knowledge, this is the highest ginsenosides production by engineered microbial. We believe that the gram scale per liter production of rare ginsenosides by yeast fermentation will make it possible to produce commercial feasible ginsenosides via environmental friendly and plant-free methods in the near future.

Histone demethylase JMJD1A in prostate cancer

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The androgen receptor (AR) pathway plays a central role in development of castration-resistant prostate cancer (CRPC). The H3K9 demethylase JMJD1A is overexpressed in CRPC and drives CRPC progression by functioning as an AR coactivator and promoting the generation of AR-V7 (a constitutively active AR variant). JMJD1A also promotes prostate cancer progression and radio-resistance by upregulating c-Myc activities. The function of JMJD1A can be modulated by several types of post-translational modifications including non-canonical ubiquitination, canonical ubiquitination and acetylation. These modifications provide means to target JMJD1A for the potential prostate cancer therapy.

The Multiple facets of the E3 ligase HUWE1 in diseases

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Ubiquitin (Ub) acts as an intracellular signal once tagged covalently to the target proteins and regulates a myriad of cellular processes. Ubiquitin is attached to the target protein by the concerted action of E1, E2, and E3 Ub ligase enzymes, in the presence of ATP. During this process, E3 Ub ligases are responsible for the final step of protein ubiquitination and play crucial roles in substrate selectivity and specificity. Therefore, understanding the structure and function of E3 Ub ligases provides valuable mechanistic information regarding their specific roles in Ub-mediated regulatory processes. In this talk, we aimed to investigate the structure and function of the human E3 Ub ligase, HUWE1. HUWE1 regulates multiple cellular pathways, including DNA damage response, apoptosis, and transcriptional regulation through controlling the stability and fate of various proteins involved in these pathways. Using multidisciplinary approaches, we studied the functional domains, the regulation, and the new cellular substrates of HUWE1. We report that HUWE1 harbors a previously uncharacterized tandem ubiquitin-binding motif (UBM). It contains three independently folded unique UBMs, and as a tandem, it binds three different Ub chains. Most significantly, this tandem UBM enhances HUWE1-mediated ubiquitination. We also uncovered the mechanism of a X-linked mental retardation mutation, R2981H, of HUWE1. Our results show this mutation disrupts the structure of the UBM that attenuates HUWE1 function. We also investigated the role of HUWE1 in the WNT signaling in colon cancer. We found that HUWE1 directly binds and ubiquitinates β -catenin, a master signal transducer in the canonical WNT signaling pathway. Overall, these findings identify a new HUWE1 substrate that provides new knowledge of HUWE1 function in cell regulation and development.

Regulation of hnRNP A1 by covalent and non-covalent PARylation

links to the pathogenesis of age-related neurological disorders

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Heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) is one of the known pathogenic proteins of amyotrophic lateral sclerosis (ALS) and an important RNA binding protein (RBP) component of stress granules (SGs). Previous studies have suggested that the transition of SGs into irreversible protein inclusions under disease conditions may contribute to the pathogenesis of ALS, but the underlying regulatory mechanism remains to be studied.

In our recent work, we have found that lowering the levels of poly(ADP-ribosylation) (PARylation) in cells can attenuate the hnRNP A1-mediated neurotoxicity, which is closely related to the function of PARylation in regulating the assembly and disassembly of SGs containing hnRNP A1. Further, we reveal that hnRNP A1 protein not only can be PARylated (covalent PARylation modification) but also contains specific functional domain to bind poly(ADP-ribose) (PAR) (non-covalent PARylation modification), which regulating the nucleocytoplasmic transport and response to stress of hnRNP A1 in the cell, respectively.

Furthermore, we utilize the proteomic approach to determine and compare the interactomes between the two different PARylation mutants and the wild-type hnRNP A1 protein. It reveals key proteins involved in the functions of hnRNP A1 in regulating important cellular events and processes such as stress response, nucleocytoplasmic transport, and SG localization by the covalent and non-covalent PARylation. Next, we will use human cell lines, mouse primary neurons and *in vivo* animal models to validate the findings and study the underlying mechanism. By conducting this study, we hope to cast novel insights into the pathogenesis of ALS and to provide new ideas for the development of therapeutic agents to treat ALS and related neurodegenerative diseases.

Identification of *SAD* and its essential role in glia for maintaining neural integrity during aging

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An integral nervous system is composed of both neurons and glia, which is important for the longevity and healthy aging. However, the mechanisms maintaining neural integrity during normal aging has yet to be explored. To reveal unknown genes and signaling pathways that are required for neural integrity during aging, we conducted a *Drosophila* genetic screen of brain-enriched genes and sought for the ones whose loss-of-function (LOF) led to shortened lifespan.

In the screen, we identified a functionally uncharacterized gene, whose adult-onset downregulation not only significantly shortened the lifespan but also caused age-dependent neurodegeneration in the fly brain. We have since named the gene *SAD*. Interestingly, in spite of high expression levels in the fly brain, downregulation of *SAD* in fly neurons did not show reduce the longevity or the integrity of the nervous system in flies. In contrast, knockdown of *SAD* in glia resulted in striking neurodegeneration and significantly shortened the lifespan. Further examinations indicated that only the cortex glia and the blood-brain barrier (BBB) glia are involved in the aging function of *SAD*. Moreover, immunohistochemistry analyses and the Dextran injection assay revealed that the fly glia matrix and the BBB were severely damaged in the RNAi-*SAD* flies.

To further investigate how *SAD* regulates glial functions and neural integrity, we performed the RNA-seq analyses. The results suggested that *SAD* might function as a chromatin repressor that keeps the innate immunity of the nervous system in check during aging. Without *SAD* repression, the immune response genes go unleashed, which, together with other detrimental effects, may lead to BBB disruption and neurodegeneration. Together, our study has revealed a glia-specific role of *SAD* in maintaining neural integrity during aging. By doing this study, we hope to cast new insights to the role and the mechanism of how glial regulation of innate immunity is involved in the process of aging of the nervous system.

Quantitative Analysis of site-specific N-glycan heterogeneity on biopharmaceuticals by mass spectrometry based on MS2 diagnostic ion filtering

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N-glycosylation is an important post-translational modification affects the stability and biological activity of glycoproteins^[1]. Most monoclonal antibodies (mAbs) and recombinant fusion proteins are glycoproteins emerge with characteristic N-glycosylation patterns and the N-glycanforms at each glycosylation site are heterogeneous. The recognition of glycans allows a heterogeneous population of glycoforms to participate in specific biological interactions^[2]. To analyze the site-specific N-glycosylation modification is a important processes in the quality control of biopharmaceutical production. The traditional methods, FLR-UPLC and HPAEC-PAD can not quantitatively analyze the N-glycan heterogeneity at different sites of glycoproteins. In our study, we established an analysis method of site-specific N-glycan heterogeneity based on data-dependent acquisition by mass spectrometry. We extracted the mass of intact N-glycopeptides containing modification sites, according the characteristic MS2 fragmentation (peptides+GlcNAc, Y1 ion). To determine N-glycoforms, we then matched the data of intact glycopeptides mass is to the theoretical mass. The relative content of N-glycan on each modification site is calculated next. Using this

method, we analyzed the site-specific N-glycosylation modification heterogeneity of Bevacizumab (mAbs) and Conbercept (a fusion protein). We also separated the 14 charged isomeric points of three batches of Conbercept protein using two-dimensional electrophoresis (2-DE) method and analyzed the N-glycosylation heterogeneity of each charge isome. In total, we identified 13 N-glycoforms modified in light chain of bevacizumab. G0F (84.1%) and G1F (8.1%) is the main N-glycoform of Bevacizumab. There are seven glycosylation sites in Conbercept protein and most abundant N-glycoform is the sialylated glycan. The main N-glycoform of Asn33, Asn65 and Asn270 sites is G2FS1, and the relative abundances are 37.0%, 38.2% and 32.8% respectively. The main N-glycoform of Asn120 and Asn193 sites is G2S1, and the relative abundances were 25.8% and 34.5% respectively. The main N-glycoform of Asn249 site is G2FS2, and the relative abundance is 37.7%. The main N-glycoform of Asn376 site is G1F and G0F respectively. Nine batches of Conbercept protein show the consistent distribution of N-glycoforms in each modified site. Moreover, the relative abundance of the highly sialylated glycoforms increases as the isomers are located to the acidic regions. The study provides an intuitive approach for site-specific N-glycan distribution and consistence analysis of biopharmaceutical based on mass spectrometry data.

Key words: Site-specific; N-glycosylation; Mass spectrometry; Quantitation.

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Target validation and drug discovery for prostate cancer

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Prostate cancer (PCa), a commonly diagnosed cancer, is a leading cause of cancer-related deaths. AR signaling is the major driver for progression to Castration-resistant prostate cancer (CRPC), after which most tumors continue to rely on AR signaling. Androgen-deprivation therapy (ADT) represents the gold standard treatment for PCa. However, the disease often progresses to CRPC and eventually develops resistance to second line drugs such as abiraterone and enzalutamide. Consequently, alternative strategies to elimination of AR signaling are needed for the treatment of CRPC. Here, we will present the identification and validation of new therapeutic targets for CRPC. By combining computational biology, computer-aided drug design with medicinal chemistry and structural biology, some nuclear receptors and epigenetic proteins were identified as targets for CRPC. As case studies, development of ROR γ , BRDs inhibitors against CRPC will be presented.

Key words: Target identification and validation, Nuclear receptor, Coregulator, Drug design

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Computer aided drug screening platform and its application

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Abstract: Lead compound discovery is the key step of innovative drug research and development. For large numbers of small molecules whose function are not clear, we have established a computer aided drug screening platform (www.vslead.com), which has been considered as a way for quick lead compound discovery and cost reduce. The platform adopted distributed architecture and integrated molecular docking softwares and a number of small molecule libraries. It is featured with data security, user friendly, and real time monitoring. Utilizing the facility provided by the platform, we found an active compound tested by wet lab experiment quickly. Therefore, user can have higher chances to find active compounds both time-saving and cost-efficiently.

LSD1 destabilizes FBXW7 and abrogates FBXW7 functions

independent of its demethylase activity

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FBXW7 acts as a typical tumor suppressor, with loss of function alterations in human cancers, by promoting ubiquitylation and degradation of many oncoproteins. Lysine-specific demethylase 1 (LSD1) is a well-characterized histone demethylase. Whether LSD1 has demethylase-independent activity remains elusive. Here we report that LSD1 directly binds to FBXW7 to destabilize FBXW7 independent of its demethylase activity. Specifically, LSD1 is a pseudo-substrate of FBXW7 and LSD1-FBXW7 binding does not trigger LSD1 ubiquitylation, instead promotes FBXW7 self-ubiquitylation by preventing FBXW7 dimerization. The self-ubiquitylated FBXW7 is subjected to degradation by proteasome as well as lysosome in a manner dependent of autophagy protein p62/SQSTM1. Biologically, LSD1 destabilizes FBXW7 to abrogate its functions in growth suppression, NHEJ repair and radioprotection. Collectively, our study revealed a previously unknown activity of LSD1, which likely contributes to its oncogenic function. Targeting LSD1 protein, not only its demethylase activity, might be a unique approach for LSD1-based drug discovery for anti-cancer application.

AMP-Activated Protein Kinase Plays an Essential Role in p-Syneprine Suppressing Hepatic Glucose Production

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In traditional Chinese herbs, Citrus aurantium is referred to as “Fructus aurantii immaturus,” “Zhi shi,” or “Zhi ke”, and has been used for hundreds of years for various digestive problems. And Citrus aurantium often uses as herbal or dietary supplement in various countries around the world. Its primary protoalkaloid, p-syneprine, exhibited lipolytic effects and energy expenditure. This work was designed to determine how p-syneprine regulates hepatic glucose production, and the underlying mechanisms in HepG2 cells. In this study, we showed that p-syneprine significantly inhibited hepatic glucose production in a dose-dependent manner. AMPK, ACC and FoxO1 phosphorylation were stimulated by different concentrations of p-syneprine. In addition, the enzyme activities of PEPCCK and G6Pase were all significantly suppressed. What is important is that these effects were partly reversed by (1) inhibition of AMPK activity by compound C, and by (2) suppression of AMPK α expression by siRNA. These results suggest that p-syneprine inhibits hepatic glucose production in HepG2 cells, and AMPK plays a critical role in p-syneprine ameliorating hyperglycemia through inhibition of hepatic gluconeogenesis. p-Syneprine could be utilized for the preventive and therapeutic uses against metabolic syndrome.

Keywords: p-syneprine; AMPK signaling pathway; glucose production

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**A novel combination of astilbin and low-dose methotrexate
respectively targeting A_{2A}AR and its ligand adenosine for the
treatment of collageninduced arthritis**

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Methotrexate (MTX) is widely used for rheumatoid arthritis (RA) treatment with frequently serious adverse effects. Therefore, combination of low-dose MTX with other drugs is often used in clinic. Unfortunately, some patients exhibit an incomplete response or flare of disease activity in receiving low-dose MTX. Therefore, we hope to find novel DMARDs to make patients complete response to low-dose MTX in combination therapy. Here, we investigated the potential of astilbin in low-dose MTX combination therapy and the underlying mechanism of synergistic effects. In this study, we investigated the improvement of astilbin and low-dose MTX combination on collagen-induced arthritis in DBA/1J mice. Results showed that the clinic score, incidence rate, paw swelling, pathological changes of joints and rheumatoid factors were more alleviated in combination therapy than MTX or astilbin alone group. Elevated antibodies (IgG, IgG1, IgG2a, IgM and anti-collagen IgG) and pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α , IFN- γ and IL-17A) in serum were significantly inhibited, while anti-inflammatory cytokine, IL-10, was enhanced by combination therapy. Further studies indicated that combination therapy significantly decreased Th1 and Th17 cell differentiation and increased Treg cell differentiation. Mechanisms analysis demonstrated combination therapy greatly inhibited ConA-activated MAPK and inflammatory transcriptional signals. Moreover, MTX activated adenosine release and astilbin specifically up-regulated A_{2A} adenosine receptor (A_{2A}AR) expression simultaneously, which most probably contributed to the synergistic efficacy of combination therapy. ZM241385, a specific antagonist of A_{2A}AR, greatly blocked the effects of combination therapy on T cell

functions and downstream pathways. All these findings suggest that astilbin is a valuable candidate for low-dose MTX combined therapy in RA via increasing A_{2A}AR/adenosine system and decreasing ERK/NFκB/STATs signals. Astilbin exerted its anti-inflammatory effect via enhancing expression of A_{2A}AR, and MTX simultaneously increased adenosine release. This ligand and receptor system greatly contribute to the synergistic effect of astilbin and MTX combination therapy, which may be more effective than other MTX combination medicine.

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Apelin-13 ameliorates cognitive deficit in a streptozotocin-induced rat model of Alzheimer's disease by suppressing neuroinflammation

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Background: Alzheimer' disease (AD), as a common age-related dementia, is an irreversible neurodegenerative disease characterized by impairments of cognitive function as a result of synaptic deficits and neuronal loss which is associated with inflammation. Several lines of evidence showed that apelin-13 is a multifunctional polypeptide with receptors present in various brain tissues such as hippocampus and has been associated with neuroprotection and memory processing. However, the underlying mechanism of apelin-13 protecting neurons to ameliorate cognitive deficits in AD is almost unknown. **Objective:** To investigate whether apelin-13 can protect neurons to ameliorate cognitive deficits in AD by inhibiting neuroinflammation. **Methods:** The AD model was induced by streptozocin (STZ) injection into the bilateral ventricle of rats on day 1 and day 3, respectively. 24h later, a single intracerebroventricular (icv) administration of apelin-13 for four weeks was performed before the detection of cognitive memory in novel object recognition (NOR) task and Morris water maze (MWM) task. Protein expression of apelin, APJ, microglial marker (IBA1), astroglia marker (GFAP), interleukin 1 beta (IL-1 β), tumor necrosis factor- α (TNF- α), synaptophysin (SYP) in the hippocampus were examined by western blotting or immunohistochemistry. And the gene expression of IBA1, GFAP IL-1 β , TNF- α and SYP were detected by real-time quantitative polymerase chain reaction (PCR). Inflammatory disorder in the hippocampus was tested by hematoxylin and eosin (H&E) staining. Furthermore, immunofluorescence was used to detect the proliferation of hippocampal cells labeled by BrdU.

Results: We observed that apelin/APJ signaling was downregulated in the hippocampus of rats administrated with STZ. Apelin-13 was found to significantly ameliorate STZ-induced AD-like phenotypes including cognitive deficit, the damage of neuron and synaptic plasticity, as well as proliferation deficit of neurons. Moreover, apelin-13 inhibited microglia and astrocyte activation and reduced IL-1 β and TNF- α expression in AD rats. **Conclusion:** These findings demonstrate that apelin-13 ameliorates cognitive deficit in a STZ-induced rat model of sporadic AD by attenuating inflammation and apelin-13 may serve as a novel agent for the treatment of AD.

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Galectin-3 promotes A oligomerization and A toxicity in a mouse model of Alzheimer's disease

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Amyloid- β (A β) oligomers largely initiate the cascade underlying the pathology of Alzheimer's disease (AD). Galectin-3 (Gal-3), which is a member of the galectin protein family, promotes inflammatory responses and enhances the homotypic aggregation of cancer cells. Here, we examined the role and action mechanism of Gal-3 in A β oligomerization and A β toxicities. Wild-type (WT) and Gal-3-knockout (KO) mice, APP/PS1;WT mice, APP/PS1;Gal-3^{+/-} mice and brain tissues from normal subjects and AD patients were used. We found that A β oligomerization is reduced in Gal-3 KO mice injected with A β whereas overexpression of Gal-3 enhances A β oligomerization in the hippocampi of A β -injected mice. Gal-3 expression shows an age-dependent increase that parallels endogenous A β oligomerization in APP/PS1 mice. Moreover, A β oligomerization, Iba1 expression, GFAP expression and amyloid plaque accumulation are reduced in APP/PS1;Gal-3^{+/-} mice compared with APP/PS1;WT mice. APP/PS1;Gal-3^{+/-} mice also show better acquisition and retention performance compared to APP/PS1;WT mice. In studying the mechanism underlying Gal-3-promoted A β oligomerization, we found that Gal-3 primarily co-localizes with Iba1, and that microglia-secreted Gal-3 directly interacts with A β . Gal-3 also interacts with triggering receptor expressed on myeloid cells-2 (TREM2), which then mediates the ability of Gal-3 to activate microglia for further Gal-3 expression. Immunohistochemical analyses show that the distribution of Gal-3 overlaps with that of endogenous A β in APP/PS1 mice and partially overlaps with that of amyloid plaque. Moreover, the expression of the A β -degrading enzyme, neprilysin, is increased in Gal-3 KO mice and this is associated with enhanced

integrin-mediated signaling. But the expression level of two other enzymes, transthyretin and insulin-degrading enzyme, was not altered in Gal-3 KO mice. Consistently, Gal-3 expression is also increased in the frontal lobe of AD patients, in parallel with elevated A oligomerization and amyloid plaque. Because Gal-3 expression is dramatically increased as early as 3 months of age in APP/PS1 mice and anti-A oligomerization is believed to protect against A toxicity, Gal-3 could be considered a novel therapeutic target in efforts to combat AD.

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Glutamic-oxaloacetic transaminase 2 inhibits HBV replication by modulating the production of low-level inflammation

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The low-level inflammation in the liver triggered by chronic infection with the hepatitis B virus (HBV) is associated with the dysfunction of antiviral innate immunity and adaptive immune. However, the molecular mechanisms involved in the production of low-level inflammation in the liver triggered by HBV remain to be fully elucidated. Here, we found that glutamic-oxaloacetic transaminase 2 (GOT2) inhibits HBV replication in HepG2.2.15 cells and in HBV-infected HLCZ01 cells. Mechanistically, GOT2 promoted the activation of MAPK, NF- κ B and AKT signaling pathways to enhance the production of pro-inflammatory cytokine TNF- α and type I IFN in HBV-infected HLCZ01 cells by interacting with lactate dehydrogenase B (LDHB) and preventing the degradation of the latter. The result of RNA sequencing shows that LDHB, a key metabolizing enzyme for catalyzing lactate to pyruvate in liver, was significantly down-regulated by HBV in HBV-infected HLCZ01 cells. Moreover, LDHB overexpression promotes the production of TNF- α but inhibits HBV replication in HepG2.2.15 cells and in HBV-infected HLCZ01 cells. Our data suggest that GOT2 is a critical regulator for HBV triggered low-level inflammation and may be a potential target for the prevention and treatment of chronic hepatitis B.

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Roles of the Y chromosome Genes in Human Cancers

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The human Y chromosome harbors approximately 80 male specific protein-coding genes within its male specific region Y (MSY). Many MSY genes are multiple-copy amplicons, and therefore only a total of 23 distinct proteins are coded by these MSY genes. The MSY genes are expressed either specifically/predominantly in testis or ubiquitously in all tissues. Recent studies have revealed that the expression patterns of the MSY genes could be altered in various cancer types, including hepatocellular carcinoma (HCC), prostate cancer, and gonadoblastoma/testicular germ cell tumors. Prostate cancer is a male specific cancer and HCC is a male-dominant cancer. Gonadoblastoma is a benign germ cell tumor, frequently developed in the dysgenetic gonads of patients with disorders of sex development (DSDs) harboring the Y chromosome materials. Accordingly, the altered expressions of MSY genes could affect the oncogenic processes of these cancer types in male-specific manners.

One of the key MSY genes is the testis specific protein Y-encoded (TSPY), which is a putative oncogene on the Y-chromosome. TSPY is tandemly repeated and located at the critical region of the gonadoblastoma locus on the Y chromosome (GBY). Under normal condition, TSPY is predominantly expressed in the gonocytes of embryonic testis and spermatogonia in mature testis, and it could play specific roles in testicular germ cell proliferation and development. However, it is aberrantly expressed in the dysgenetic gonads of XY females or DSDs patients harboring the TSPY sequences, and could promote cell proliferation and other oncogenic processes leading to gonadoblastoma development. Similarly, TSPY is expressed in maturation-disturbed testicular germ cells, and could promote the development of type-II and III testicular germ cell tumors, such as seminoma and intratubular germ cell neoplasia. The TSPY protein is a member of SET translocation/nucleosome assembly protein1 (SET/NAP1) family harboring a highly conserved SET/NAP- domain. SET/NAP proteins serve a wide range of biological functions, such as histone/chromatin modification, gene regulation and cell cycle regulation. Indeed, the ectopically

expressed TSPY could accelerate cell proliferation in various cancer cell lines. TSPY binds to the cyclin-B/CDK1 complex and stimulates its activity, thereby shortening the G₂/M transition in the cell cycle. As noted, TSPY is ectopically expressed in various somatic cancers, e.g. prostate cancer and HCC, suggesting that it could also exert similar oncogenic functions in these cancer types.

Prostate cancer is the second most common cancer in men, and androgen receptor (AR) plays key roles in the initiation and progression of prostate cancer by stimulating numerous genes involved in cell proliferation and oncogenesis. TSPY could directly interact with AR, and stimulates the transactivation function of AR, resulting in acceleration of proliferation in a prostate cancer cells. In addition, TSPY could interact with the constitutively active AR variants, such as AR-V7, lacking the ligand binding domain (LBD), and stimulate their transactivation activities in a ligand independent manner. Since these AR variants are postulated to play key roles in drug resistance and development of castration resistant prostate cancer (CRPC), the ectopically expressed TSPY may be potentially involved in such drug resistance and CRPC development. Liver cancer is a somatic cancer with significant male dominance. TSPY expression in selected male HCCs suggests its potential contribution to the male-bias in this deadly cancer. Indeed, TSPY expression level is significantly correlated with the survival ratio; the male patients with high-TSPY expression have a worse prognosis than the TSPY-negative patients. Importantly, ectopically expressed TSPY could upregulate various cell-cycle related genes whose expression levels are also closely correlated with cancer survival. These findings suggest that TSPY is a key MSY gene potentially contributing to various oncogenic functions in both male-specific and male dominant cancers.

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PRMT5 Is Critical for Intestinal Development and Function

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Background: Protein arginine methyltransferase 5 (PRMT5), which catalyzes the symmetric dimethylation of arginines on histone and non-histone proteins, plays essential roles in development and cancers. PRMT5 expression increased in many types of cancers, including colorectal cancer, breast cancer etc. Targeting prmt5 may represent efficient way to improve cancer therapy. But due to the embryonically lethal effect of Prmt5 knockout (KO), the role of Prmt5 in the intestine has not been analyzed yet. **Methods and results:** To investigate the important role of prmt5 on intestinal development, we generated conditional knockout mice of prmt5 with Villin-Cre recombinase (prmt5^{fl/fl}; VillinCre). We found prmt5 in the intestine was almost disappeared, but the expression of prmt5 in kidney, which also expressing villin, was not changed. We observed that prmt5 conditional KO mice showed a growth defect after their birth, with a higher death rate, a smaller body size and a lighter body weight compared with their wild type counterpart (prmt5^{wt/wt}VillinCre) as well as the heterozygous mice (prmt5^{fl/wt}VillinCre). Aimed at explaining these phenomena, we measured the intestinal length and found that the intestine of KO mice was shorter than control mice. And we found that prmt5 KO clearly damaged the intestinal morphology, with misaligned villus and shorter villus height. Whereas, we didn't see any change in the density or distribution of lymphocytes, Paneth cells, and goblet cells. Moreover, prmt5 KO inhibited intestinal epithelial cell proliferation as determined by Ki 67 immunohistochemical staining. **Conclusion:** Collectively, our data suggested that prmt5 KO disrupted the structure and function of the intestinal mucosa by modulating the proliferation of intestinal epithelial cell, thus leading to intestinal defects.

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Functional screens for host factors that regulate maintenance and lytic reactivation of Epstein-Barr virus

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Epstein-Barr virus (EBV) is one of the most common human herpesviruses, which infects over 90% of the entire world's population. In a few individuals, EBV infection can be oncogenic leading to malignancies such as Hodgkin's lymphoma, Burkitt's lymphoma, gastric carcinoma and nasopharyngeal carcinoma. EBV preferentially infects epithelial and B cells where it switches between latent and lytic states of replication cycle. Mucosal epithelial cells serve as the primary site of EBV infection followed by underlying circulating B cells. After primary infection, EBV is persistently present throughout life in dormant form until lytic reactivation. Host specificity of EBV suggests the importance of cellular host factors for its maintenance and replication within the cell but very little is known about these cellular factors involved in EBV replication cycle, especially in epithelial cells. This study aims to investigate the host cellular factors playing a potential role in EBV maintenance and lytic reactivation using CRISPR/Cas9-based whole genome screening approach. To study EBV maintenance, GFP-tagged EBV+ NPC cells were used and subjected to whole genome knockout using Toronto CRISPR knockout library. The cells with brightest GFP signal were sorted out and subjected to CRISPR knockout followed by sorting of GFP-negative cells. Such GFP⁻ cells were considered as EBV⁻ NPC cells and were predicted to have lost the EBV genome, suggesting that the knockout genes were playing a crucial role in EBV genome maintenance inside the cells. Bioinformatics analysis was performed to find target candidate genes, with a potential role in EBV maintenance in epithelial cells, which will be further validated by individual gene knockout experiments. Besides EBV genome maintenance, the regulation of EBV lytic reactivation will be studied using a fluorescent reporter plasmid for EBV lytic reactivation, where an EBV early lytic gene promoter will be used to drive the expression of a fluorescent protein.

The reporter assay along with CRISPR whole genome activation library screening will enable us to perform more functional screens to identify cellular factors facilitating EBV lytic reactivation. On the other hand, the use of CRISPR whole genome knockout library will be done to identify the cellular genes, which potentially inhibit lytic reactivation and therefore, maintain EBV latency within the host cell.

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Lysine specific demethylase LSD1 mediates epithelial to mesenchymal transition and chemoresistance in colon cancer

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Chemoresistance remains major burden for colorectal cancer (CRC) recurrence and patient death. In this study, we investigated the epigenetic mechanism of colon cancer chemoresistant. 5-Fluorouracil (5-FU) resistant models were established from two representative colon cancer cell lines. We found the resistant cells grow slower and have increased stem cell surface marker compared with parental cells. In addition, 5-FU resistant cells show morphological change toward mesenchymal phenotype. The epithelial to mesenchymal transition (EMT) is further supported by the decrease of E-cadherin and the increase of N-cadherin in resistant cells. Therefore, our data suggest that 5-FU resistance in colon cancers can promote EMT and reprogram cells to a more aggressive, stem-like state. We further investigated the epigenetic mechanism that promotes EMT in resistant cells. We found that the expression of lysine specific H3K4 demethylase LSD1 increased and histone H3K4 dimetylation decreased in resistant cells. LSD1 has been reported as a main driver for EMT. Overexpression of LSD1 in parental cells promotes chemoresistance and EMT. RNAseq analysis also shows that LSD1 overexpressed cells has gene expression profiles enriched in cell survival pathways which is similar to pathways enriched in resistant cells. However,

LSD1 did not promote cell stemness phenotype. We further show that inhibition or knocking-down of LSD1 in resistant cells can promote cell growth and resensitize cells to 5-FU in vitro and in xenograft mouse model. Thus, our results uncovered the epigenetic mechanism for chemoresistance and potential treatment for chemoresistant colon cancer.

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Local and systemic effects of cancer-secreted extracellular vesicles

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Across the many levels of intercellular crosstalk between cancer and niche, extracellular vesicles (EVs) and their cargo molecules such as miRNAs play important roles. We have previously shown that miR-105 is highly expressed by metastatic breast cancer cells and is secreted at high levels in the EVs from these cells. Higher levels of miR-105 in the circulation of early-stage breast cancer patients are associated with metastatic progression. We have also demonstrated that EV transfer of cancer-secreted miR-105 downregulates tight junctions in endothelial monolayers to induce vascular leakiness and metastasis. Given the highly context-dependent functioning pattern of miRNAs, we set out to further determine the function of miR-105 and other pro-metastatic miRNAs in a variety of non-cancer niche cells. Our data show that breast-cancer-secreted miRNAs reprogram niche cell metabolism to favor a cancer microenvironment through various mechanisms: (1) biasing the “competition” toward cancer cells by suppressing the consumption of nutrients by niche cells and favoring nutrient uptake by cancer cells (e.g., miR-122-mediated suppression of glucose uptake in lung fibroblasts and astrocytes); (2) establishing a “symbiosis” between cancer and niche cells through which niche-produced energy-rich metabolites feed anabolic cancer cells (observed in miR-105-reprogrammed cancer-associated fibroblasts); and (3) recruiting “scavengers” to support a rapidly increasing population of cancer cells by accelerating waste elimination by adjacent niche cells and by converting cancer-produced metabolic by-products into non-toxic metabolites to re-enter cancer bioenergetics (observed in miR-105-reprogrammed cancer-associated fibroblasts). These metabolic interactions may exist in different tumor types, tumor compartments, and/or tumor stages. The mechanism may operate differently at the primary vs. metastatic sites and may depend on the particular metabolic pattern of cancer cells and the dynamic metabolic conditions (nutrients and by-products) in the tumor microenvironment.

Regulation of evolved nuclear-localized tyrosine kinase MET in prostate cancer

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Nuclear receptor tyrosine kinases (nRTKs) are dysregulated in many types of cancer. We previously showed androgen deprivation therapy (ADT) can induce nuclear-localized MET (nMET) but less membrane MET in prostate cancer (PCa) patient specimens of castration resistant prostate cancer (CRPC). On the contrary, more membrane MET is elevated in primary tumors as we showed before. Recently we suggested that nMET degradation in dead cells most likely enhanced cell survival which fits the natural selection. Protein-protein interaction maps revealed the evolved nMET in prostate cancer through AR. Targeting nMET by carbon nanodots through proteasome degradation, dysregulation of phosphorylation would be promising in green therapy mediated cell death. For instance, carbon nanodrug combined with MET inhibitor induced damage on actin cytoskeleton. Moreover, carbon nanodots induced crosstalk pathways of MET by mass spectrometry (MS) analysis. Taken together, our reported and ongoing experimental data would suggest a novel target of nMET in prostate cancer progression by green endogenous carbon therapy.

Knockdown of Pokemon inhibits cell proliferation of breast cancer through regulating the ubiquitination of estrogen receptor alpha

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Pokemon, a transcriptional repressor, was recently revealed to be closely associated with the ER- α signaling in the progression of breast cancer. Here, we further confirm that Pokemon shows a significant positive correlation with ER- α in clinical breast cancer samples by tissue microarray-based analysis. Mechanically, we identify that the inhibition of Pokemon could upregulate E3 ligase TRIM25 leading to enhancement of ER- α ubiquitination and proteasomal degradation, which could partly explain the correlation between Pokemon and ER- α . Besides, we uncover that Pokemon could also transcriptionally up-regulate the expression of ER- α via indirectly binding to the region +146 to +461 bp downstream of the transcription start site of ESR1 (ERpro315) in breast cancer cells. Furthermore, Pokemon is found to stimulate the expression of ER- α 's downstream genes and promote the growth of estrogen receptor alpha (ER- α)-positive breast cancer cells. Together, our data reveal the novel mechanisms through which Pokemon manipulates ER- α level and might provide a new avenue for endocrine therapy in breast cancer.

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***Faecalibacterium prausnitzii* produces butyrate to decrease
c-Myc-related metabolism and Th17 differentiation by inhibiting
histone deacetylase 3**

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Decreased levels of *Faecalibacterium prausnitzii* (*F. prausnitzii*), whose supernatant plays an anti-inflammatory effect, are frequently found in inflammatory bowel disease (IBD) patients. However, the anti-inflammatory products in *F. prausnitzii* supernatant and the mechanism have not been fully investigated. Here we found that *F. prausnitzii* and *F. prausnitzii*-derived butyrate were decreased in the intestines of IBD patients. Supplementation with *F. prausnitzii* supernatant and butyrate could ameliorate colitis in an animal model. Butyrate, but not other substances produced by *F. prausnitzii*, exerted an anti-inflammatory effect by inhibiting the differentiation of Th17 cells. The mechanism underlying the anti-inflammatory effects of the butyrate produced by *F. prausnitzii* involved the enhancement of the acetylation-promoted degradation of c-Myc through histone deacetylase 3 (HDAC3) inhibition. In conclusion, *F. prausnitzii* produced butyrate to decrease Th17 differentiation and attenuate colitis through inhibiting HDAC3 and c-Myc-related metabolism in T cells. The use of *F. prausnitzii* may be an effective new approach to decrease the level of Th17 cells in the treatment of inflammatory diseases.

Key words: *Faecalibacterium prausnitzii*; inflammatory bowel disease; T helper 17 cells; metabolism; c-Myc; butyrate; histone deacetylase.

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Isolation and identification of lactic acid bacteria from Xinjiang small reed (*Phragmites australias*) silage

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Using 16S rDNA gene sequence analysis, nine LAB strains were identified from 90-day silage of Xinjiang small reed. These strains belonged to four genera and six species, including 1 strain of *Pediococcus pentosaceus*, 1 strain of *Enterococcus faecium*, 3 strains of *Enterococcus faecium*, 1 strain of *Lactococcus garvieae*, 1 strain of *Weissella thailandensis* and 2 strains of *Lactococcus lactis*. Biochemical analysis revealed that these strains were all Gram-positive and catalase-negative cocci. All strains were able to grow between 5°C and 40°C, and in the pH range of 4 to 8. All strains produced lactic acid, and *Weissella thailandensis* had the strongest ability to produce acid. To the best of our knowledge, this is the first report of isolation and identification of LAB strains from Xinjiang small reed. These strains may be used as additives in the production of silage of Xinjiang small reed.

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Histone Acetyltransferase P300 Facilitates the Stimulation of Key Prostaglandin Synthase COX2 in Human Amnion Fibroblasts

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Background: The initiation of human parturition is determined by complex endocrine circuits. In the late pregnancy, amnion fibroblasts secrete abundant prostaglandin and cortisol, which two form a forward feedback loop and promote the progression of parturition. Cyclooxygenase-2 (COX-2) is a key rate-limiting enzyme for the synthesis of prostaglandin. In human amnion fibroblast, cortisol induces COX2 expression, and the activation of PKC-CREB1 signaling pathway is required in this step. Histone acetyltransferase P300 functions as a coactivator through increasing histone acetylation on the site of *cis*-element, opening the chromatin structure and promoting the gene expression. But whether and how P300 is involved in the regulation of COX2 transcription are unknown. **Hypothesis:** Histone acetyltransferase P300 facilitates the stimulation of key prostaglandin synthase COX2 in human amnion fibroblasts. **Methods:** Immunohistochemistry (IHC) was used to determine the expression pattern of P300 in fetal membrane. Luciferase assay was used to measure the 1012bp COX2 promoter activity. Western blotting was used to measure the protein level of COX2. Antagonistic assay (P300 activity inhibitor, C646) was used to test the function of P300 in COX2 expression regulation. Chromatin immunoprecipitation (ChIP) assay was used to measure the enrichment of P300 and acetylated H3K9 (H3K9ac) on COX2 promoter. PMA was used to activate the PKC-CREB1 signaling pathway. **Results:** P300 is mainly localized in the amnion fibroblast and decidual stromal cell, and low level in epithelial cells. In amnion fibroblasts, P300 accumulates in the nucleus, consistent with the canonical function of P300 as a transcriptional coactivator. In addition, cortisol promotes the P300 expression in primary cultured amnion fibroblasts, which is dependent on the glucocorticoid receptor. Luciferase assay and Western Blotting showed P300 antagonist C646 abolished the induction of COX2 promoter activity and protein expression by cortisol, suggesting that P300 is required for stimulation. ChIP assay showed that the presence of P300 and H3K9ac on the proximal promoter region of COX2 significantly increased after cortisol treatment, and this enrichment was blocked by C646 cotreatment. Finally, after the activation of the PKC-CREB1

signaling pathway by PMAs, the enrichment of P300 and H3K9ac on the COX2 promoter was also observed. **Conclusion:** P300 is an important epigenetic factor involved in the transcription activation of COX2. This mechanism can be applied in other important factors for human parturition. Therefore, high-throughput analysis of P300-regulated genes will help to analyze the physiological mechanisms of labor initiation and screen potential targets for preterm intervention.

miRNA-mediated metabolic regulation by thyroid hormone in skeletal muscle

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Thyroid hormone (TH) signaling has profound effects on a variety of physiologic processes. Myopathic symptoms are very common among hyperthyroid or hypothyroid patients, suggesting that skeletal muscle is an important target organ of TH. It has been widely accepted that TH signaling can be controlled at multiple levels. MicroRNAs (miRNAs) are a group of highly conserved noncoding RNAs, which function primarily as negative regulators of gene expression at the post-transcriptional level. miRNAs not only confer signaling robustness as amplifiers, balancers, or buffers but also play important roles in signaling crosstalk and coordination as nodes of signaling networks. Years ago, Olson's group published a research article in *Science*, linking the role of miRNA to TH signaling for the first time. Recently, more and more studies have shed light on the contributions of miRNAs to TH signaling. MiR-378 is located in the first intron of its host gene peroxisome proliferator-activated receptor gamma coactivator-1 β (PGC1 β). MiR-378 has been functionally implicated in mitochondrial metabolism, systemic energy homeostasis, classic brown adipose tissue-specific expansion, and hepatic insulin signaling. Here, we reported that the levels of miR-378 in skeletal muscle of mice were altered under different thyroid hormone status, suggesting that miR-378 might also play a role in the metabolic regulation of skeletal muscle by TH. By using both in vitro and in vivo models, we found that miR-378 could maintain normal muscle homeostasis by coordinating autophagy and apoptosis. Mechanistic studies revealed that miR-378 could induce autophagy through its direct target phosphoinositide-dependent protein kinase 1 (PDK1) and inhibit apoptosis via its direct target Caspase 9. Thus, we speculated that TH-regulated miR-378 might play a role in the metabolic regulation of myocyte death, while muscle weakness observed under different thyroid hormone status might be partly due to an imbalance between autophagy and apoptosis.

Acute inflammation and stress as risk factors for lung cancer recurrence

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Patients with early-stage non-small cell lung cancer (NSCLC) have a high rate of recurrence after curative resection, supporting early tumor cell dissemination (ETCD) in NSCLC. However, its underlying mechanism and biological significance remain largely unknown. Here we report that the combination of acute stress and inflammation induced ETCD in NSCLC mouse models. A small number of the early disseminated tumor cells (<100) at the adenoma stage were capable of inducing metastatic tumor growth. We identified a molecular pathway that inflammation and stress signals activated peripheral monocytes to stimulate both the invasiveness of premalignant tumor cells and increase vascular permeability via induction of TGF- β 1 and MMP-9. We further showed that use of aspirin and β -adrenergic antagonist together blocked ETCD in mice and was associated with reduced risk of cancer recurrence in NSCLC patients. Our study provides a molecular pathway for ETCD in NSCLC and supports the utility of history of aspirin and beta-blocker usage as an indicator for assessing recurrence risk in NSCLC patients. Our results further suggest that targeting stress and inflammation pathways may represent a valid approach to prevent ETCD and recurrence in people with high risk of developing lung cancer.

**Shared functional modular mechanisms for acute myocardial
infarction and acute cerebral infarction, identified by network
analysis of transcriptomes**

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Objective: Despite it is well known that acute myocardial infarction (AMI) and acute cerebral infarction (ACI) are two clinically highly correlated diseases, the underlying molecular mechanisms to link the two diseases remain largely unknown. Hence, the aim of this present study was to identify the common modular mechanisms for AMI and ACI by using large-scale transcriptomic data.

Methods: First, three microarray datasets for AMI and two datasets for ACI were obtained from GEO database, which consisted of the transcriptomic data for 97 AMI patients, 59 ACI patients and 141 healthy controls. Then, the lists of differentially expressed genes (DEGs) were identified by meta analysis of the multiple datasets for two diseases, separately. The overlapped genes between two lists were defined as the pleiotropic genes. Next, guided by PPI knowledge, these pleiotropic genes were expanded into a gene network(s). In order to explore the more detailed modular mechanisms shared by the two diseases, Newman's spectrum algorithm was utilized to decompose the primary network into the most compacted modules (subnets) with high modularity. Finally, enrichment analysis of GO database and KEGG database were performed to assess their functional meanings of the newly identified modules.

Results: A total 899 DEGs of AMI and 1024 DEGs of ACI were identified, respectively, of which 290 DEGs were common to the two diseases. Then, these overlapped genes were translated into a primary network consisting of 1244 nodes (genes) and 1667 edges, and 41 small subnets of few

gene nodes. The primary network was then decomposed into 31 compacted modules. Topological analysis of these modules identified a total of 61 hub genes, most of which (e.g. PECAM1, ALDH2, TLR4, ABCA1) had been proved to be associated with AMI and/or ACI, while the rest might be novel risk genes for the two diseases. Further bioinformatic functional analysis of GO and KEGG databases revealed that these modules were not only involved in some well known pathogenic biological processes/pathways for either AMI or ACI (e.g. inflammatory response, toll-like receptor signaling pathway, ECM-receptor interaction, etc.), but also a few unreported ones (e.g. Fc gamma R-mediated phagocytosis, NOD-like receptor signaling pathway).

Conclusion: In short, this study demonstrates that AMI and ACI have some similar molecular bases, and the identified pleiotropic modules may shape the complicated molecular interplays underlying the two clinically correlated diseases.

Key words: Acute myocardial infarction; Acute cerebral infarction; Microarrays; Network analysis; Pleiotropism.

Endothelial cell-CXCR4 regulates tumor angiogenesis and angiocrine effect to modulate immunosuppressive tumor microenvironment in hepatocellular carcinoma

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C-X-C chemokine receptor 4 (CXCR4) is known to be involved in both developmental and hematopoiesis; however, its role in tumor angiogenesis and immunosuppression remains largely unknown. Here, we showed that the chemokine receptor CXCR4 was specifically expressed in tumor vascular endothelial cells (TEC) and promoted the formation of vessel encapsulating tumor cell cluster (VETC) structure, which was shown to be a distinct vascular pattern that was positively associated with metastasis by promoting angiogenesis in hepatocellular carcinoma (HCC). Clinically, high expression of endothelial cell-CXCR4 stratified HCC patients into those with poor prognosis, and more importantly, high density of CXCR4⁺ blood vessels in resected tumor tissues before sorafenib treatment was strongly correlated with prolonged survival in patients with advanced HCC after treated with sorafenib, providing that endothelial cell-CXCR4 could be a prominent regulator and predictor of HCC progression and drug response. At the molecular level, we revealed that the expression of endothelial cell-CXCR4 was controlled by the inflammatory cytokines (TNF- α) secreted from tumor associated monocytes/macrophages. Remarkably, we showed that these CXCR4⁺ TEC regulated the infiltration of regulatory T cells (Treg cells) in HCC tumors via angiocrine effect (did CXCR4 induce the expression of cytokines related to Treg cells?). Further animal studies indicated that HCC tumor bearing mice treated with combination of sorafenib and ZA (a depletion drug of Mo/Mf) significantly enhanced the anti-tumor efficacy of sorafenib via the inhibition of endothelial cell-CXCR4 expression and its mediated Treg cell infiltration. Taken together, these data revealed that the CXCR4⁺ TEC acts as a bridge between tumor angiogenesis and immunosuppressive microenvironment, establishing the theoretical basis and concepts of combining anti-angiogenesis and immunotherapy in HCC treatment.

Sphingolipid metabolic reprogramming in NSCLC patients determines cancer growth and progression

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Genome-wide association studies have ranked sphingolipid (SPL) metabolism as the top dysregulated pathways in non-small cell lung cancer (NSCLC), suggesting that circulating sphingolipidomics can be prominent determinants and markers of cancer growth and progression. However, the correlation between circulating levels of SPLs and cancer progression is still poorly understood. Here we provide clinical data from multiple databases and our cohort, establishing that the alteration in expressions of SPL metabolic enzymes, including CERS6, ASAH2, CERK, SPHK, UGCG, UGT8, B3GNT5 and GAL3ST1, stratifies NSCLC patients into those with poor prognosis. By combining our integrative multi-enrichment analysis with LC-MS based sphingolipidomic approach (IMESP), we have successfully detected and confirmed the change in circulating concentrations of their metabolites, including ceramide, ceramide-1P, sphingosine-1P and glycosphingolipids in NSCLC patients. Further sphingolipidomic analysis of this alteration reveals the existence of a circular network of co-regulated ceramide and glycosphingolipids in NSCLC patients, and its alteration significantly correlates to cancer growth and progression. Importantly, deep mining study illustrates that B3GNT5, and their metabolites, glycosphingolipids, are the key regulators of this circular network, which directly affects cancer cell adhesion, proliferation, signal transduction pathway and glycoprotein production. Overall, these findings provide new insights whereby sphingolipid metabolic reprogramming determines tumor growth and progression, providing prominent markers and therapeutics targets for NSCLC patients.

PD-L1 (B7-H1) regulate the DNA damage response and can be targeted to sensitize to radiation or chemotherapy

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Programmed death ligand 1 (PD-L1, also called B7-H1) is an immune checkpoint protein that inhibits immune function through its binding of the programmed cell death protein 1 (PD-1) receptor. Clinically approved antibodies block extracellular PD-1/PD-L1 binding, yet the role of intracellular PD-L1 in cancer remains poorly understood. Here, we discovered that intracellular PD-L1 acts as an RNA binding protein that regulates the mRNA stability of NBS1, BRCA1, and other DNA damage related genes. Through competition with the RNA exosome, intracellular PD-L1 protects targeted RNAs from degradation, thereby increasing cellular resistance to DNA damage. RNA immunoprecipitation and RNA-seq experiments demonstrated that PD-L1 regulates RNA stability genome-wide. Furthermore, we developed a PD-L1 antibody, H1A that promotes PD-L1 degradation. Intracellular PD-L1 may be a potential therapeutic target to enhance the efficacy of radiotherapy and chemotherapy in cancer through inhibition of DNA damage response and repair.

The regulation of homologous recombination and chemotherapy

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Homologous recombination (HR) is important for maintaining genomic stability. However, elevated HR is highly related to chemoresistance in cancer. Therefore, inducing HR deficiency (HRD) in HR proficient cancers causes synthetic lethality with DNA damaging agents and overcomes chemoresistance in cancer. A bromodomain containing protein, BRD9, is previously reported to regulate chromatin remodeling and transcription. Here we clarify a new role of BRD9 in HR through regulating RAD51 in response to DNA damage. Overall, our results demonstrated a transcriptional regulation independent molecular mechanism of BRD9 in HR regulation and identify BRD9 as a potential therapeutic target for overcoming chemoresistance in HR proficient ovarian cancers.

Paneth cell dysfunction contributes to alcoholic hepatitis through promoting bacterial translocation: Role of zinc deficiency

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Background & Aims: Enteric dysbiosis is associated with alcoholic hepatitis with mechanisms yet to be elucidated. Intestinal Paneth cells are antimicrobial peptide-secreting innate immune cells and play crucial role in control of gut microbiota homeostasis. Up to date, the involvement of Paneth cells in alcohol-induced dysbiosis and hepatitis remains obscure. The present study aimed to determine the effects of alcohol and zinc deficiency on Paneth cell antimicrobial function and to define the link between Paneth cell dysfunction and alcoholic hepatitis.

Methods: Translocation of pathogen-associated molecular patterns (PAMPs) was determined in patients with severe alcoholic hepatitis and in mouse model of alcoholic liver disease. Microbial composition and Paneth cell function were examined in mice. The link between alpha-defensin dysfunction and alcoholic hepatitis was investigated in alpha-defensin deficient mice. Recombinant human alpha-defensin 5 (HD5) was orally given to the alcohol-fed mice to test the therapeutic potential. The role of zinc deficiency in alpha-defensin was evaluated in acute and chronic mouse models of zinc deficiency.

Results: Hepatic inflammation was associated with PAMP translocation, and upregulation of lipocalin-2 and CXCL1 in patients with alcoholic hepatitis. Antibiotic administration, lipopolysaccharide injection, and in vitro studies showed that PAMPs, but not alcohol, directly induced lipocalin-2 and CXCL1 expression. Chronic alcohol feeding caused systemic dysbiosis, downregulated Paneth cell alpha-defensin expression, and decreased intestinal antimicrobial activity. Knockout functional alpha-defensins synergistically affected alcohol-perturbed bacterial composition and gut barrier, and exacerbated PAMP translocation and liver damage. Administration of HD5 effectively altered cecal microbial composition, especially increased *Akkermansia muciniphila*, and reversed alcohol-induced deleterious effect. Zinc regulated Paneth cell

homeostasis at multiple levels, including transcriptional regulation and posttranscriptional modification, and dietary zinc deficiency exaggerated the deleterious effect of alcohol on Paneth cell antibactericidal activity.

Conclusion: Taken together, the present study demonstrated that alcohol-induced Paneth cell dysfunction is a pathophysiological factor in alcohol-induced dysbiosis and hepatitis, whereas cellular zinc deficiency contributes to alcohol-induced Paneth cell dysfunction. The results suggest that HD5 administration may represent a novel and promising therapeutic approach for treating alcoholic hepatitis.

YAP circular RNA, circYAP, attenuates cardiac fibrosis via binding with tropomyosin-4 and gamma-actin

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Cardiac fibrosis, characteristics of pathological myocardial remodeling, is a worldwide health problem associated with nearly all etiologies of heart diseases. Upon cardiac fibrosis, the compliance of heart tissue was decreased and the progression of heart failure was accelerated. However, the molecular mechanisms underlying cardiac fibrosis remain unclear. Yes-associated protein (YAP) is the key component of Hippo pathway which plays crucial roles in cardiac regeneration. The YAP circular RNA, circYAP, is generated from exon 5 and exon 6 of YAP pre-mRNA. In our previous study, we found that circYAP played an essential role in cell proliferation and survival. In the present study, we examined whether circYAP modulated heart remodeling. We analyzed the heart tissue specimens from 90 patients with tetralogy of fallot (TOF) and 25 patients with heart failure and found circYAP levels in these patients' heart were significantly decreased compared to 25 hearts without detectable disease. For further investigation, pressure overload animal model was established by transverse aortic constriction (TAC) in mice hearts. Cardiac fibrosis was induced by pressure overload after 8 week in TAC mice. We found that the circYAP levels were significantly decreased in TAC mouse hearts. Upon circYAP plasmid injection, the circYAP levels were brought back to the similar levels as sham group. Meanwhile, the heart function was improved and cardiac fibrosis associated factors, such as collagen I and III and TGF- β , were attenuated by the circYAP plasmid injection. To further investigate the underlying mechanisms by which circYAP functions in cardiac fibrosis, the potential proteins that could bind to circYAP in the cardiac fibroblasts and cardiomyocytes were identified with mass spectrometry. Our results indicated that circYAP probe could pull down more tropomyosin 4 (TMP4) and gamma-actin (ACTG) in cells with circYAP overexpression than vector control. We further confirmed the binding of circYAP with TPM4 and ACTG in cardiac fibroblasts and cardiomyocytes

with RNA immunoprecipitation and RNA pull-down assay. Such bindings led to an increased TPM4 interaction with ACTG, which might stabilize actin filaments and inhibit actin-myosin complex formation. We also detected decreased levels of TPM4 and ACTG pulled down by circYAP probes in the TAC mouse hearts due to the decreased circYAP levels. The binding sites of circYAP with TPM4 and ACTG were identified by a computational approach and confirmed experimentally. Collectively, our study uncovered a novel molecule that could regulate cardiac remodeling during cardiac fibrosis and implicated a new function of circular RNA. Our findings also support the pursuit of circYAP as a potential tool for future intervention of cardiac disease.

Mechanism of Hepatitis C Virus-induced Hepatocarcinogenesis

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Hepatitis C virus (HCV) is a major cause of hepatocellular carcinoma (HCC). It can induce HCC via multiple pathways. These pathways include the dysregulation of lipid metabolism to cause fatty liver, which can progress to liver cirrhosis and HCC, the induction of oxidative stress to cause DNA damage, the suppression of DNA repair, the induction of expression of proinflammatory cytokines such as TNF- α via toll-like receptors to activate immune cells and cause chronic hepatitis, and the activation of AKT, a protein kinase that promotes cell growth. In this report, the molecular mechanisms of some of these research findings that are related to HCV-induced HCC will be discussed.

Bile acids and mast cell regulation of NAFLD/NASH

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Non-alcoholic fatty liver disease (NAFLD), a growing epidemic, can progress to non-alcoholic steatohepatitis (NASH), which is poorly understood. We have shown that (i) mast cells (MCs) migrate to portal areas during cholestasis induced by bile duct ligation (BDL) and (ii) MC-deficient ($\text{Kit}^{\text{W-sh}}$) and l-histidine decarboxylase knockout ($\text{HDC}^{-/-}$) mice subjected to BDL have reduced biliary damage and MC infiltration. In addition, $\text{HDC}^{-/-}$ mice fed a high fat diet (HFD) have decreased biliary damage and hepatic fibrosis. Patients with cholestatic liver injury have increased levels of circulating total bile acids (TBAs) and altered FXR/FGF signaling which contribute to disease progression. The FXR agonist, obeticholic acid is currently being used as a potential therapy in patients with NAFLD. The current.

Aim of the study was to evaluate signaling mechanisms and BA pathways regulating hepatic MC migration during NAFLD.

Methods: WT and $\text{Kit}^{\text{W-sh}}$ mice were fed control diet (CD) or HFD for 16 weeks. Hepatic steatosis, biliary damage and fibrosis were determined by Oil Red O staining, CK-19 immunohistochemistry and Fast Green/Red Sirius staining, respectively. Biliary senescence was measured by SA-b-Gal staining, and immunohistochemistry (IHC) in liver sections and qPCR in isolated cholangiocytes for p16, p18, p21 and p53. Biliary senescence-associated secretory phenotype (SASP) was measured in liver sections and in isolated cholangiocyte supernatants by EIA for stem cell factor, IL-6 and IL-10. MCs were detected by IHC for mouse mast cell protease-1. TBAs were measured in all groups of mice in both serum and snap liver. The expression of FXR, SHP and FGF15/19 was determined in total liver by qPCR. FGF15/19 secretion was measured in serum by EIA. In vitro, cholangiocytes were treated with free fatty acids (FFAs), palmitic and stearic acid and senescence/SASP markers measured by qPCR and EIA. Cholangiocytes, hepatocytes and hepatic stellate cells (HSCs) were plated in Boyden chambers and treated with FFAs prior to measuring MC

migration. Isolated cholangiocyte supernatants from mice were added to MC cultures; activation was evaluated by histamine EIA and qPCR for chymase, tryptase and c-Kit. By IHC and qPCR, human control, NAFLD and NASH samples were evaluated for (i) biliary damage (CK-19); senescence markers and (ii) MC migration by staining for chymase and tryptase; (iii) TBAs and FXR/FGF signaling.

Results: WT HFD mice have increased hepatic steatosis, biliary damage and fibrosis coupled with enhanced biliary senescence/SASP and MC infiltration localized to portal areas compared to CD mice. These parameters were reduced in HFD Kit^{W-sh} mice. TBAs were increased in WT mice fed HFD compared to WT CD, which was decreased in HFD Kit^{W-sh} mice. FXR/SHP signaling was enhanced in WT HFD mice, but was reduced in HFD Kit^{W-sh} mice. Cholangiocytes treated with FFAs had increased senescence/SASP, and FFA-treated cholangiocytes (but not hepatocytes or HSCs) induced MC migration. Cholangiocyte supernatants from WT HFD mice increased MC activation, which was reduced with cholangiocyte supernatant from HFD Kit^{W-sh} and HDC^{-/-} mice. Human NAFLD and NASH had increased (i) biliary damage/senescence; (ii) MC infiltration near portal areas; (iii) TBAs and FXR/FGF signaling.

Conclusion: HFD-associated damage causes increased biliary damage/senescence/SASP inducing MC migration near portal areas which drives hepatic steatosis and fibrosis. Our data suggest that damaged, senescent cholangiocytes may attract MCs, which contribute to increased TBAs and FXR/FGF signaling. Therapeutics targeting MC migration and activation could benefit NAFLD and NASH patients.

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IL-17A signaling promotes CD8 T cell effector function against West Nile virus infection via mTOR metabolic pathway

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West Nile virus (WNV) is a mosquito-transmitted, neuroinvasive flavivirus that continues to cause significant morbidity and mortality in the United States. Although CD8⁺T lymphocytes are essential for clearance of virally infected cells, the knowledge how the effector function of CD8⁺T cells is regulated remains elusive. We found that IL-17A deficient mice (Il17a^{-/-}) were more susceptible to WNV infection compared to wild-type (WT) controls, and CD8⁺T cells purified from WNV-infected Il17a^{-/-} mice were also less cytotoxic to WNV-antigen-expressing target cells and expressed lower levels of cytotoxic mediator genes (perforin, granzyme and fas-l). Conversely, exogenous treatment with mouse recombinant IL-17A (rIL-17A) increased expression of these cytotoxic mediator genes in purified CD8⁺T cells of both WNV-infected Il17a^{-/-} and WT mice. In addition, injection of rIL-17A into WNV-infected WT mice on day 6 post-infection increased the expression of these cytotoxic mediator genes in CD8⁺T cells, profoundly reduced viral burden in the brain and enhanced the survival, suggesting a therapeutic potential of IL-17A in treatment of WNV infection. Moreover, RNA sequencing (RNA-seq) analysis on the splenic CD8⁺T cells purified from WNV-infected WT and IL-17A receptor gene knockout (Il17ra^{-/-}) mice indicated that IL-17A might facilitate CD8⁺T cell effector function through activating mammalian target of rapamycin (mTOR) kinase signaling pathway.

Intestinal NDRG2 deletion aggravates colonic inflammation via paracellular barrier disruption

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Paracellular barriers play an important role in the pathogenesis of IBDs, such as ulcerative colitis (UC) and Crohn's disease (CD), and maintain gut homeostasis. N-myc downstream-regulated gene 2 (NDRG2) has been reported to be a tumor suppressor gene and suppresses epithelial-mesenchymal transition (EMT) and colorectal cancer metastasis. However, whether NDRG2 affects colitis initiation is unclear. Our study aimed to elucidate the role of NDRG2 in epithelial barrier construction and colitis initiation using an *NdrG2* knockout mouse model. We generated intestine-specific *NdrG2* knockout mice and assessed the function of *NdrG2* in established experimental colitis models. Intestine-specific *NdrG2* deficiency caused more severe inflammation in dextran sodium sulfate (DSS)-treated mice and increased pro-inflammatory cytokine levels and neutrophil and macrophage recruitment. NDRG2 loss led to adherens junction (AJ) destruction via E-cadherin expression attenuation, resulting in diminished epithelial barrier function and enhanced intestinal epithelial permeability. In human UC patients, NDRG2 expression was negatively correlated with CD68⁺ macrophage recruitment and positively correlated with E-cadherin expression. These findings suggest that NDRG2 is an essential colonic epithelial barrier regulator and plays a role in gut homeostasis maintenance.

Exploring regulatory networks of hypoxia-induced

LncHIFCAR-intronic miR-31 in pancreatic cancer progression

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Tumor hypoxia triggers a complex HIF-1 signaling network in cancer cells, contributing to cell mobility, metastasis and therapy resistance. Previously, we have identified an oncogenic, hypoxia-inducible long noncoding RNA, LncHIFCAR/MIR31HG as HIF-1 α co-activator crucial for cancer progression. Although the oncogenic property of the lncRNA LncHIFCAR/MIR31HG and its intronic microRNA miR-31 have been reported in pancreatic ductal adenocarcinoma (PDAC), the functional involvement of miR-31 in pancreatic cancer still remain elusive. In this study, to explore the regulatory network of miR-31 in PDAC progression, three algorithmic platforms, miRDB, miRWalk and miRTarBase, were included to confine our predictions of the potential miR-31 downstream targets. Meanwhile, we analyzed the gene expression profiles in online PDAC datasets, with the aim of obtaining a set of the differentially expressed putative target genes negatively correlated with miR-31/MIR31HG levels in PDAC samples for the subsequent

experimental validation. In addition, to further reflect the transcriptional regulation of LncHIFCAR/MIR31HG and its intronic miR-31 in vivo, the dCas9-KRAB transcriptional repression system targeting MIR31HG promoter was established to suppress the expression of these two non-coding RNAs simultaneously. Based on the comprehensive PDAC RNA-seq dataset from The Cancer Genome Atlas at cBioPortal, combined with bioinformatics computational approaches, several miR-31 potential target genes were identified. These results highlighted a clinically relevant regulatory network of miR-31, which will facilitate further experimental characterization and could be used to refine biomarker predictions for developing novel therapeutic strategies in pancreatic cancer.

Identification of Schizophrenia Functional Modules Based on Biomolecular Network Analysis

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Schizophrenia (SCZ) is a complex mental illness that affects human health and quality of life. Nevertheless, until recently, the pathogenesis of SCZ is still unclear. Therefore, it is important to study the pathogenesis at the molecular level and to identify functional modules related to SCZ. Protein-protein interaction knowledge was used as a guide to expand the SCZ risk genes into a SCZ-specific gene network. Next the network was decomposed by using Newman spectral algorithm to obtain functional modules. Then the topological properties and hub genes of each module were identified according to the topological analysis and Poisson distribution test. Enrichment analysis of each functional module was performed, evaluating the interactions between the functional modules based on the enriched pathway categories. A total of 14 functional modules have been obtained, all of which have scale-free properties. Topological analysis of these modules identified a total of 102 hub genes, most of which (e.g. EGFR, HAX1, IL1R1, RALGDS, etc.) had been proved to be associated with SCZ, while the rest (e.g. SVIL, DNAJA1, RABAC1, STX6, etc.) would be novel risk genes. Further bioinformatic functional analysis of KEGG databases revealed that these modules were involved multiple pathogenic biological pathways (e.g. Apoptosis, ErbB signaling pathway, Cell cycle, Phospholipase D signaling pathway, PI3K-Akt signaling pathway, etc). Functional modules analysis showed that most of these specific modules did not appear to function in an isolated way, interacting with each other to influence the occurrence and development of SCZ instead, with a shared pathogenesis.

Key words: schizophrenia; functional module; protein-protein interaction knowledge; hub gene; biological pathway

Role of intestinal barrier dysfunction in diet induced diabetes

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Metabolically-induced diseases such as Type 2 diabetes (T2DM) and atherosclerosis that underlie the development of cardiovascular diseases are a major cause of global mortality and morbidity. Regular consumption of high fat, high cholesterol containing Western diets (WD) is thought to underlie the induction of these diseases not only by promoting dyslipidemia but by also causing local intestinal inflammation and intestinal barrier dysfunction. An intact intestinal barrier is required to prevent translocation of bacterial endotoxin, lipopolysaccharide (LPS), from the lumen to systemic circulation. Intestinal alkaline phosphatase (IAP) is present on the apical side of epithelial cells and considered the first layer of the intestinal barrier. IAP catalyzes the removal of one of the two phosphate groups from the toxic lipid A moiety of LPS producing monophosphoryl-LPS that results in detoxification of the downstream Toll-like receptor (TLR)-4 dependent inflammatory cascade; dephospho-LPS still binds to TLR4 but predominantly as an antagonist. We have demonstrated that WD significantly reduces IAP activity leading to disruption of the intestinal barrier and increase in translocation of LPS to the systemic circulation. Oral supplement of curcumin, the active ingredient in the Asian spice turmeric, significantly increase IAP and improves WD-induced barrier dysfunction. Increase in systemic LPS levels as a result of intestinal barrier dysfunction lead to a low-grade chronic inflammatory state that underlie the development of metabolic diseases such as T2DM and atherosclerosis. Consistently, improvement of barrier function by curcumin supplementation attenuated WD-induced glucose intolerance and atherosclerosis. To further establish the role of IAP as the first layer of the intestinal barrier, we developed intestine-specific IAP transgenic mice expressing chimeric human IAP. In contrast to wild type C57BL/6 mice where IAP expression declines along the length of the intestine from duodenum to colon, these mice showed consistent expression of IAP along the entire length. This increased IAP expression led to improved barrier function, reduced intestinal inflammation and

attenuation of WD-induced glucose intolerance. These studies establish the role of IAP in modulating intestinal barrier function and identify IAP as a potential therapeutic target. While loss of activity and instability of IAP at pH<5.0 (or the gastric pH) precludes oral supplementation, enteric coated tablets or use of agents such as curcumin represent a novel and viable approach to increase IAP and for treatment of diet-induced T2DM.

Knowledge-based analysis of the online OMIM database to search for the pleiotropic modules shared by coronary artery disease and diabetes mellitus

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Objective Coronary artery disease and diabetes mellitus are two clinically highly correlated diseases, while the underlying molecular mechanisms to link the two diseases remain largely unknown. The objective of the present study is thus to develop a prior knowledge-based approach to search for the pleiotropic modules shared by coronary artery disease and diabetes mellitus.

Methods First, we extracted the lists of the susceptible genes for the two diseases from the OMIM database, respectively, and defined the 121 overlapped genes as the initial seed genes. Second, we used PPI knowledge as a guide to expand these genes into a network(s). Third, to identify the pleiotropic modules for the two diseases, we used the Newman's spectrum algorithm to decompose the primary network into the most compacted modules (sub-networks) with high modularity. Finally, we assessed the functional involvements of the newly identified modules by enrichment analysis of the GO database and KEGG database.

Result Totally, we identified 19 pleiotropic modules for the two diseases, most of which (15 modules) followed the power-law distribution, typically for a scale-free network. By further network analysis of these modules, we identified 42 hub genes, most of which (e.g. APOA1, LDLR, LPL and MBL2) are linked to the known molecular mechanisms while the rest of the hub genes (e.g.) might be the unknown pleiotropic genes for two diseases. Finally, bioinformatic functional analysis revealed that these modules are involved a list of biological processes (for examples, lipid metabolism, glucose metabolism, and inflammatory responses) and undertook molecular functions of nuclear hormone receptor binding, protein kinase activity, serine-type endopeptidase activity and so on. Their activities most occurred in intracellular organelle lumen,

platelet alpha granule and cytosol. Interestingly, we also found that these modules interacted with several pathways for cancers (e.g. renal cell carcinoma, prostate cancer, and pancreatic cancer), and for rheumatoid diseases (Fc epsilon RI signaling pathway), indicating that these modules may have broader effects on the molecular etiologies for multiple chronic diseases.

Conclusion This study suggests that coronary artery disease and diabetes mellitus share several molecular mechanisms. Also, this study reveals that the two diseases may link to several malignant tumors and rheumatoid diseases from the underlying molecular networks.

Key words:Coronary artery disease; Diabetes mellitus; Functional modules; Pleiotropism; OMIM

Sexual Dimorphism Factor Screen for Genitalia Development

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Background: External genitalia are derived from peri-cloaca mesenchymal progenitor. In human, the most common congenital defect in external genitalia is hypospadias, about 1 in 200 kids. Its etiological factors are complex, associated with developmental and hormonal factors. e.g. Loss function of *Wnt*-signaling inhibitor *Dkk1* leads to hypospadias genitalia in mouse. In addition, inhibition of androgen signaling predisposed the hypospadias at critical window of e16.5 but not earlier than e15.5. Yet the molecule profiling of the critical sex developmental stage of genitalia or pathological condition are largely unknown. Here, we report our data from a translating mRNA affinity purification (TRAP) based high throughout screen of transcriptome in dorsal peri-cloaca mesenchymal cell (dPCM), a critical cell lineage for sexual dimorphism of genitalia.

Hypothesis: The transcriptome screen of specific genitalia lineage in critical sex developmental stage or pathological condition will identify pivotal genes for sexual dimorphism and pathogenesis of Hypospadias.

Methods: *Lbx2*-Cre/AP mice were generated. *Lbx2*-Cre mice were bred with *Dkk1*^{-/+} and *Rosa26*^{Trap/+} or *Rosa26*^{LacZ/+} mice to generate WT and *Dkk1* mutant. Genitalia from both sexes was collected at e15.5. TRAP was performed with about 100 samples for each immunoprecipitation, the pulldown mRNA was verified by qPCR and sent to RNAseq. DEG, Venn Diagram and IPA were used for the bioinformatics analyses.

Results: AP staining shown that *Lbx2* gene *in situ* expression starts as early as e9.0. *Rosa26*^{LacZ/+} reporter lineage tracing shown *Lbx2*-Cre labelled all progeny of dorsal peri-cloaca mesenchymal progenitor and this lineage cells exhibited a sexual dimorphic fate in adult stage. In adult male, *Lbx2*-Cre; *Rosa26*^{Trap/+} revealed that *Lbx2*- lineage contributes both smooth muscle and mesenchymal cells in dorsal region of penis. The qPCR confirmed that *Lbx2*-Cre enriched but *Shh* gene decreased in TRAP-IPed samples, suggesting that the pull-down through TRAP protocol is successful. At e15.5, whole genitalia comparison between male and female shown 27 DEGs, *Lbx2*- lineage comparison between male and female revealed 34 DEGs. Venn diagram analysis

shown 13 DEGs are common between genitalia and *Lbx2*- lineage, 5 male specific and 8 female specific. Twenty-one DEGs are *Lbx2*- lineage specific, 8 male specific and 13 female specific. Fourteen DEGs are genitalia specific and all are female specific. In *Dkk1* mutant, we identified 99 common DEGs in *Lbx2*- lineage, 24 DEGs are commonly upregulated in both male and female.

Conclusion: Taking the advantage of genetic engineering mouse, we identified 42 novel sexual dimorphic genes in wild type mouse and identified 24 novel putative pathological candidates for hypospadias. Future studying the function of those genes may help us to elucidate the molecular mechanism of sexual dimorphism and hypospadias.

Regulate Cullin-RING E3 Ubiquitin Ligases by Small Molecule

Modulators

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Cullin-RING E3 Ubiquitin (Ub) Ligases (CRLs) are the largest RING-type of E3 family consisting of ≈ 300 members, nearly half of E3s identified in humans. CRL targets many regulators critical for cell division and signaling. Canonical CRLs are modular complexes, in which the N-terminal domain of a cullin (CUL) subunit assembles interchangeably with different CUL-specific substrate receptors capable of binding a substrate. On the other hand, the C-terminal half of a CUL (CUL CTD) binds a RING finger protein, ROC1/RBX1 for CUL1-CUL4 or ROC2/RBX2 for CUL5, respectively, to form a core ligase complex. CRL's core ligase can collaborate with specific E2 conjugating enzymes for transferring ubiquitin to the bound substrate and polyubiquitination. I shall discuss our recent progress in targeting E3 CRLs using small molecule modulators.

Role of Wnt signaling in regulating lipid metabolism in *Drosophila*

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Although critical roles of the Wnt signaling cascade in regulating normal development and tumorigenesis have been well appreciated, its function in regulating fat metabolism is less understood. Given that hyperactivated Wnt signaling can lead to drastic defects in adipocyte function, medicinal intervention might be beneficial to patients with deregulated Wnt activities. To address these issues, we have established a *Drosophila* model with hyperactivated-Wnt (Wingless or Wg in *Drosophila*) signaling caused by partial loss of Axin (Axn). We find that Axn mutant larvae are transparent, accompanied by severe reduction of fat accumulation. These defects are caused by ectopic accumulation of Armadillo (β -catenin in mammals) and upregulation of Wg signaling target genes. Through screening a compound library and subsequent analyses, we identified proteasome inhibitor Bortezomib (BTZ) as well as additional peptide boronic proteasome inhibitors that can potently rescue the adipocyte defects of Axn mutants. Mechanistically, BTZ suppresses Wg activities by stabilizing α -catenin: ectopic expression of α -catenin is sufficient to rescue the adipocyte defects in Axn mutants; conversely, depletion of α -catenin in adipose tissue abolished the rescuing effects of BTZ on Axn mutants. These results suggest that peptide boronic acids such as BTZ can preferentially stabilize α -catenin, which inhibits the extra β -catenin caused by Axn mutation, thereby attenuating β -catenin-stimulated transcription and rescuing defective fat metabolism in *Drosophila*. These findings indicate that pharmacological modulation of β -catenin activity through α -catenin is an attractive approach to attenuate Wnt signaling in vivo.

内生细菌对金铁锁种子萌发及促生特性的研究

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摘要:植物内生细菌是存在于植物体内能产生药理活性、增强植物抵抗能力、促进植物生长的微生物。金铁锁是中国西南特有的传统药用植物,需求量大,但其野生资源分布区域小,自然更新能力差,根部生长缓慢。为了金铁锁资源可持续利用与保护,本文以金铁锁根部作为研究材料,对其进行预处理后,采用组织块分离法、稀释涂抹法对金铁锁根部内生细菌进行分离及纯化,以幼苗生长、萌芽率为指标挑选出促进金铁锁生长的内生细菌。结果表明,从金铁锁植株体内共分离出 17 株细菌,其中有 5 株内生细菌能促进金铁锁种子的萌发,5 株内生细菌在促进金铁锁幼苗生长上显著高于对照组,种子萌发促生效果最佳的是第 11 号菌株组,种子萌芽率为 73.33%,促进根部生长效果最佳的是 13 号菌株组。具有溶磷能力的菌株有 6 株,能产生生长素的菌株有 7 株,具有固氮效果菌株有 7 株。该结果为进一步发展人工栽培技术提高金铁锁产量及根部的药用成分含量的研究提供新的思路。

关键词: 金铁锁; 内生细菌; 促生作用

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表观遗传因子 P300 辅助糖皮质激素诱导环氧酶-2

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研究背景: 人类分娩启动是由复杂的内分泌因子的此消彼长来决定。妊娠晚期, 人羊膜成纤维细胞所分泌的前列腺素和糖皮质激素所形成的正反馈通路尤为重要。环氧酶-2 (COX-2) 是合成前列腺素的关键限速酶, 在人羊膜成纤维细胞中, COX2 能够被糖皮质激素诱导表达, 该调控与 PKC-CREB1 信号通路激活有关。P300 是转录激活辅因子, 通过增加组蛋白乙酰化从而促进顺式元件开放和基因的表达。但 P300 是否参与人羊膜 COX2 转录调控以及分子机制尚不清楚。**假说:** 表观遗传 P300 和组蛋白的乙酰化参与糖皮质激素所诱导的 COX2 转录表达。**实验方法:** 免疫组化测定 P300 表达的细胞类型; P300 活性抑制剂 (C646) 拮抗实验测定 P300 是否影响 COX2 的转录调控; 染色质免疫共沉淀测定 P300 和乙酰化 H3K9 (H3K9ac) 在 COX2 启动子富集程度; PMA 用于激活 PKC-CREB1 信号通路; 过表达失活突变体 CREB1 (dn-CREB1) 用于测定 CREB1 是否影响 COX2 的转录调控。**实验结果:** 在 P300 特异性抗体杂交的人羊膜组织中, 发现 P300 主要分布于羊膜组织的成纤维细胞和蜕膜成纤维细胞中, 上皮细胞有一定表达。值得关注的是, 在人羊膜成纤维细胞中, P300 主要积聚在细胞核中, 与 P300 传统的细胞核内乙酰化组蛋白的功能一致。有趣的是, 糖皮质激素可以促进 P300 的表达, 该诱导作用依赖于糖皮质激素受体。P300 的拮抗剂实验中, C646 阻断了糖皮质激素对 COX2 启动子活性和蛋白表达的诱导作用, 提示 P300 在此调控中扮演重要角色。染色质免疫共沉淀 (CHIP) 检测发现糖皮质激素处理后, P300 和 H3K9ac 在 COX2 启动子近端的富集显著性增加, 该富集可以被 C646 所阻断, 提示内源性的 COX2 启动子也受到 P300 的影响和调控。最后, 单独激活糖皮质激素下游 PKC-CREB1 信号通路, P300 和 H3K9ac 在 COX2 启动子的富集也出现显著性的增加, 进一步支持 P300 是一个调控 COX2 转录激活的重要因子。**结论:** 本研究证明 P300 是参与 COX2 转录活化的重要表观遗传因子, 该机制可能还参与其他重要的因子的表达调控, 因此高通量分析 P300 调控的基因将有助于解析分娩启动生理机制和早产干预的潜在靶点筛选。

损伤修复共性表观遗传因子调节膀胱上皮细胞再生

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背景:膀胱上皮组织是隔离有害物质的第一层屏障,经常暴露于物理生化的损伤状态中,该组织的损伤会导致膀胱疼痛发炎。反复炎症则有可能进一步发展成为恶性的病理情况,诸如慢性膀胱炎和膀胱癌等疾病。膀胱上皮组织中含有干细胞,能够在损伤后快速的修复和再生,但是其分子机制仍未完全阐明。表观遗传调控干细胞的发育和干性,然而表观遗传如何调控膀胱上皮细胞的损伤再生尚未阐明。**假说:**损伤修复共性表观遗传因子影响膀胱上皮细胞再生。**研究方法:**为了探索控制膀胱上皮细胞干性的表观遗传调控机制,建立了三种膀胱上皮损伤模型,分别是:细菌感染(UTI)、化学损伤(CPP)和复合损伤(CPP+UTI)。为了筛选损伤修复共性表观遗传因子,采用RNAseq分析三种损伤情况下分别与PBS对照组比较产生的差异表达基因(DEG)。为了研究候选基因的功能,建立膀胱上皮特异性敲除的小鼠,在化学损伤条件下观察是否影响干细胞的表型。为进一步解释表型,采用翻译mRNA富集法(TRAP)和RNAseq来测定分析最有可能影响表型的下游因子及信号通路。**结果:**通过免疫组化分析UTI、CPP及UTI+CPP损伤的膀胱,发现损伤后一天,膀胱上皮的表层特化细胞-伞细胞脱落。有意思的是,UTI和CPP的损伤在第七天伞细胞恢复并完成修复;而CPP+UTI损伤,第七天伞细胞仍未完全修复,提示生化复合损伤有叠加效应。通过RNAseq分析,发现在三种损伤模型中,共计320个损伤修复共性DEGs,其中有两个是表观遗传调节因子:*Ezh2*和*Xist*。*Ezh2*是多梳复合体2(PRC2)的核心蛋白,具有甲基转移酶活性,能够沉默基因的表达。膀胱上皮特异性敲除PRC2活性并不影响上皮细胞的发育和分化;但是在CPP损伤模型中,条件敲除的上皮细胞中Ki67显著性下降,说明PRC2促进上皮细胞的增殖。在没有损伤条件下,对比条件性敲除和野生型,TRAP-seq鉴定出27个DEGs,其中包含细胞周期抑制因子*Cdkn2a*。生物信息学分析(GO-terms, IPA信号通路和上游因子分析)显示上述差异表达基因与细胞代谢事件和信号通路异常相关。**结论:**通过对三种损伤模型的对比分析,我们发现两个损伤修复共性表观遗传因子*Ezh2*和*Xist*。经证实,*Ezh2*参与干细胞增殖的调控。该表型与*Ezh2*调控的下游因子,诸如*Cdkn2a*等的异常表达有关。进一步分析筛选的因子和*Ezh2*下游因子将对解析膀胱上皮细胞的干性调控和维持具有重要意义。

去泛素化酶 USP14 在肝脏甘油三酯沉积中的作用及机制研究

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目的:肝脏甘油三酯的过量沉积是非酒精性脂肪肝病(NAFLD)的特征性表现, 然而其分子机制尚未完全阐明。本研究拟探讨去泛素化酶 14(Ubiquitin Specific Peptidase 14, USP14)在肥胖引发肝脏脂质沉积中的作用和调控机制。**方法:**1.通过基因表达谱芯片、实时定量 PCR、蛋白免疫印迹等方法, 比较肥胖小鼠和正常小鼠肝脏组织中 USP14 的表达差异。2.借助腺病毒系统, 分别在正常小鼠和肥胖小鼠的肝脏中过表达和干扰表达 USP14 基因, 进而检测小鼠的肝脏重量、肝脏甘油三酯含量等脂代谢表型。3.利用免疫沉淀-质谱和蛋白表达差异芯片等方法, 筛选和鉴定 USP14 调控的靶蛋白, 从而明确其调控甘油三酯代谢的机制。

结果:1.我们首次发现: 同对照组小鼠相比, 肥胖小鼠(高脂饮食诱导、瘦素受体缺乏)肝脏组织中 USP14 的表达显著上调。2.在正常 C57BL/6 小鼠肝脏组织中过表达 USP14 基因后, 肝重/体重比增加, 肝脏甘油三酯含量增加, 肝细胞中脂滴聚集增加。3.沉默瘦素受体缺乏的肥胖小鼠肝脏组织中的 USP14 基因后, 肝重/体重比降低, 肝脏三酯含量降低, 并伴有血糖的降低和胰岛素抵抗的改善。4.USP14 通过和脂肪酸合成酶(FASN)的蛋白-蛋白相互作用, 抑制 FASN 蛋白的泛素化修饰, 从而稳定 FASN 蛋白, 促进脂肪酸和甘油三酯的合成。**结论:**本研究首次发现去泛素化酶 USP14 在肝脏甘油三酯代谢中的作用, 即: 肥胖时, USP14 通过稳定 FASN 蛋白, 促进甘油三酯的合成和过量堆积。因此, 我们的结果提示, 抑制 USP14 的表达或活性, 可能是干预 NAFLD 等代谢性疾病的有效手段和策略。

关键字:非酒精性脂肪肝病、肥胖、甘油三酯、去泛素化酶 14、脂肪酸合成酶

一个遗传性痉挛性截瘫家系致病基因的研究

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背景与目的: 遗传性痉挛性截瘫(Hereditary Spastic Paraplegia, HSP)是一类以下肢缓慢进行性肌肉紧张、肌无力和痉挛性截瘫为典型症状的神经系统变性疾病。HSP 具有显著的遗传异质性和临床表现异质性, 目前已有超过 80 个致病基因位点, 呈现多种遗传方式, 其发病机制尚不完全清楚, 从而导致临床和基因诊断困难。本研究通过临床及遗传学和分子生物学分析, 筛选和鉴定一个中国哈尼族 HSP 家系中的致病基因突变, 为 HSP 的基因诊断和分子病理机制研究提供实验依据。

材料与方法: 收集到一个来自中国云南省偏远山区的哈尼族 HSP 家系, 包含 3 代共 16 人, 其中患者 5 人。我们对先证者和患者进行病史采集、体格检查和颅脑核磁共振 (MRI) 等临床检查, 采集外周血进行 EB 病毒永生淋巴母细胞转化和培养以及基因组 DNA 分离, 用高通量全基因组外显子 (WES) 测序技术结合生物信息学分析筛选先证者中可疑致病基因突变位点, 用 Sanger 测序的方法验证家系中所有成员以及 96 名同地区同民族健康对照者中候选基因位点的基因型, 用基因克隆、细胞转染和免疫印迹分析候选基因突变对基因功能的影响。

结果: 临床检测发现家系患者均存在幼年发病、病情进展缓慢、双下肢无力、痉挛步态等症状。病情严重程度总体上随年龄增高而加重, 但同一代患者中的临床表现具有明显差异, 重症患者双下肢瘫痪、上肢肌张力明显增高、伴有轻度认知障碍, 而轻症患者仅表现为痉挛步态。但临床表型的显著差异不能完全用年龄和性别因素进行解释。遗传分析发现 GBA2 基因第 1 外显中的一个无义突变在该家系中与临床表型呈现共分离, 家系中所有 5 名患者均为该突变的纯合子, 突变在家系中呈现出常染色体隐性遗传模式, 此前尚未见关于该突变的报道, 在正常对照样本中未检测到该突变。生物信息分析表明此无义突变导致第 1 外显子中一个新的终止密码子的形成, 从而产生一个仅有 20 多个氨基酸的截短蛋白, 是致病性基因突变。EB 病毒转化的永生 B 淋巴细胞 (EBV-LCL) mRNA 分析表明该突变不引起 mRNA

稳定性的改变。在体外细胞中高表达 GBA2 基因 mRNA 后,野生型检测到显著增高的 GBA2 蛋白表达,而突变纯合子不能检测到蛋白表达。

结论: 结合临床表现和遗传分析结果,新发现的一个 GBA2 基因无义突变是该 HSP 家系中患者的致病基因突变,患者所患疾病为复杂型遗传性痉挛性截瘫 (SPG46),可能存在其他因素导致该突变在同一家系不同成员中的外显度差异。本研究进一步丰富了 HSP 的基因突变谱和临床表型多样性数据,为 HSP 的发病机制研究提供了新的实验证据。

一种新型 β -木糖苷酶的功能及机理研究

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1. 本研究获得了一种新型 β -木糖苷酶, 其具有耐盐、耐乙醇、耐胰蛋白酶、低 pH、低温、转糖基及 β -1, 2-糖苷键水解活性; 特别适合应用于酵母发酵酒精的糖化过程、含木糖的醇类制备、及将三七皂苷 R_1 和 R_2 转化为稀少的人参皂苷 R_{g1} 和 R_{h1} 。

我们从具有广泛适应性的新型菌株 *Sphingomonas* sp. JB13 中克隆得到 GH39 β -木糖苷酶基因 *jb13GH39*, 将其与 pEasy-E2 连接并转化大肠杆菌, 该基因成功表达得到 β -木糖苷酶 (JB13GH39)。纯化重组酶功能如下: 最适 pH 4.5, 最适温度 50 °C; 在 0-20 °C 内仍能保持 10.0%-50.0% 的活性; 大多数盐和化学试剂对其无影响, 例如在反应体系中加入 3.0%-20.0% (w/v) 的 NaCl, 该酶的活性不受影响; 在 10.0% 和 15.0% (v/v) 的乙醇中, 该酶分别具有 71.9% 和 55.2% 的活性; 该酶经 3.0%-30.0% (w/v) 的 NaCl、3.0%-20.0% (v/v) 的乙醇或 2.2-87.0 mg/mL 的胰蛋白酶在低于 60 °C 条件下处理一段时间, 其活性均能保持稳定; 该酶能催化木糖基转移到特定的糖或醇上, 形成新的功能产物, 例如木糖乙醇、木糖甘油; 该酶具有 β -1, 2-木糖苷酶活性, 能将三七皂苷 R_1 和 R_2 转化为稀少的人参皂苷 R_{g1} 和 R_{h1} 。

2. 探讨该酶耐盐、耐乙醇、具低温活性的机理及 β -1, 2-木糖苷酶活性催化机理。

分析 JB13GH39 及同家族 β -木糖苷酶的二级结构和三级结构, 该酶具有高比例的小氨基酸 (ACDGNSTV) 和无规则卷曲, 使其高级结构具有高度柔性, 以平衡高盐环境所导致的增强疏水作用、平衡高乙醇环境所导致的增强静电相互作用、降低催化所需要的能障, 这是其具有耐盐、耐乙醇和低温活性的机理。

此外, 对 β -木糖苷酶进行系统发育分析、序列比对、酶-配体结构分子对接分析, 发现 β -1, 2-木糖苷酶催化活性所需要的 7 个保守氨基酸位点, 经突变分析, 验证了这 5 个位点与 β -1, 2-木糖苷酶催化活性相关, 同时提出, GH39 β -木糖苷酶应该都具有 β -1, 2-木糖苷酶催化活性, 该活性应该列为一个新的水解酶子类并以 GH39 β -木糖苷酶作为本类代表。

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