HIV-1感染T细胞外泌体介导宫颈癌浸润转移的分子机制研究

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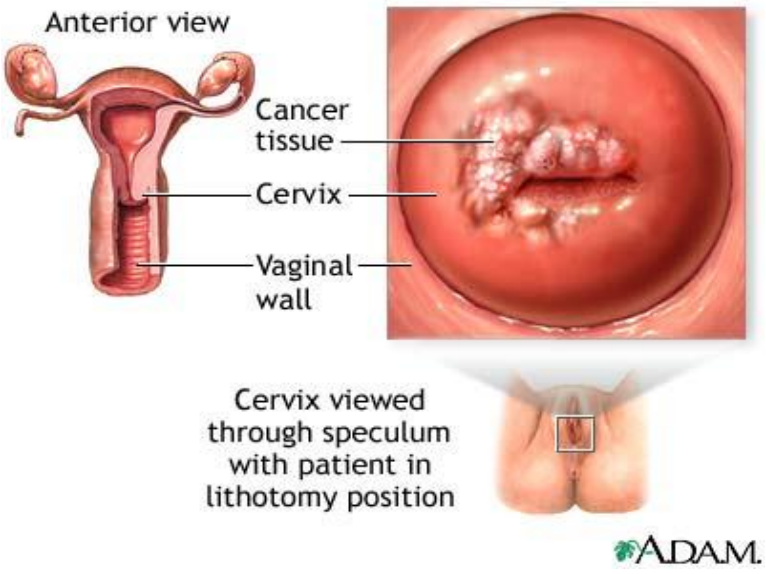
宫颈癌特点

高发性：全球新发病例约50万/年，我国宫颈癌每年新发病例13.2万，约占全球的28%；

高危性：女性的第二大恶性肿瘤，死亡约25万/年；

年轻化：50%在35 ~ 55岁。



Bray F, et al. Cancer J Clin. 2018;68:394-424.

HIV感染者多种癌症风险明显升高

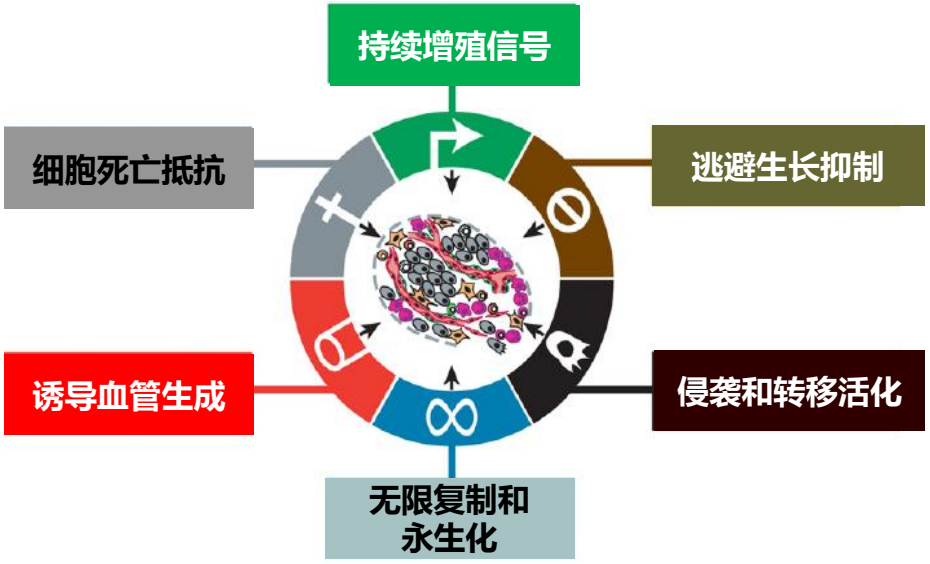
	分类	癌种举例	HIV携带者是非携带人群风险倍数
HIV携带者患癌症风险更高	AIDS定义性癌症	• 卡波西肉瘤	440-500倍
		• 非霍奇金淋巴瘤	12倍
		• 宫颈癌	7倍
	非AIDS定义性癌症	• 肛门癌	19-30倍
		• 霍奇金淋巴病	8倍
		• 肝癌	3倍
		• 肺癌	2倍
		• 口腔癌	2倍

1.HIV Infection and Cancer Risk. https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hiv-fact-sheet

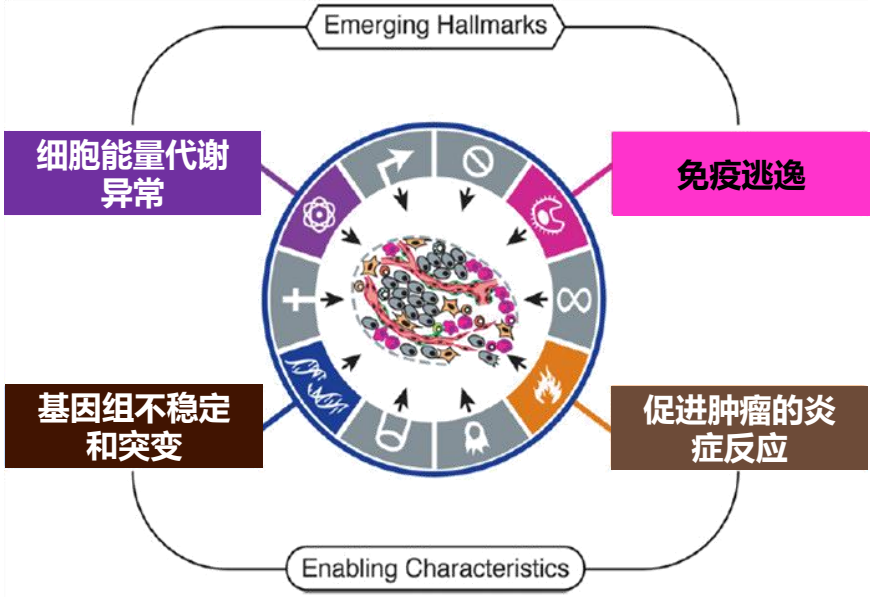
2.Silverberg MJ, et al. Cumulative incidence of cancer among persons with HIV in North America: a cohort study. Ann Intern Med. 2015;163(7):507–518

浸润转移在肿瘤发生发展中重要特征之一

肿瘤的六大特征（2000）



肿瘤的十大特征（2011）

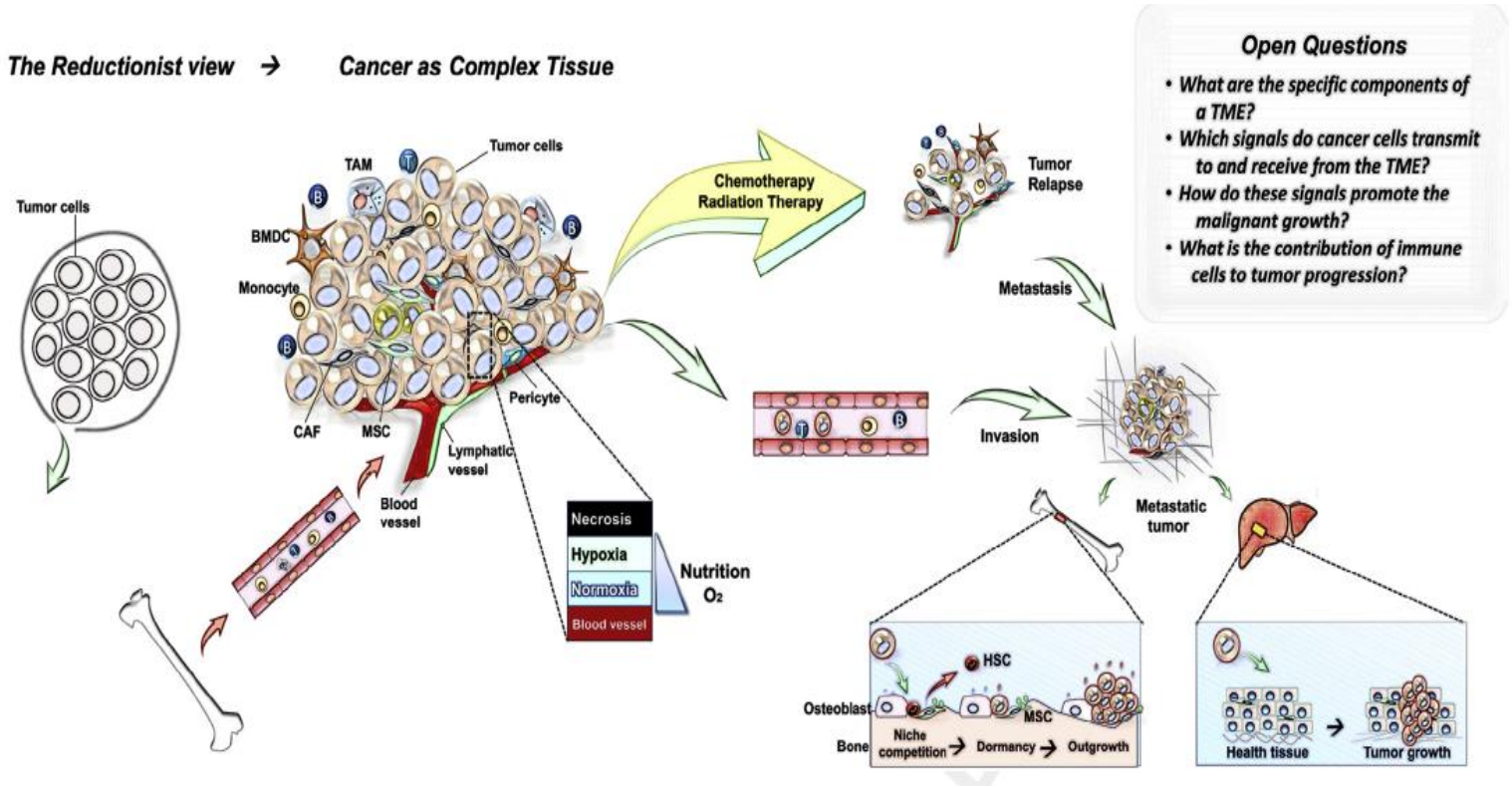


浸润转移在肿瘤的发生和发展中发挥着关键作用。

Hanahan D, et al. Cell 2000;100:57-70.

Hanahan D, et al. Cell 2011;144:646-74.

肿瘤转移与肿瘤微环境



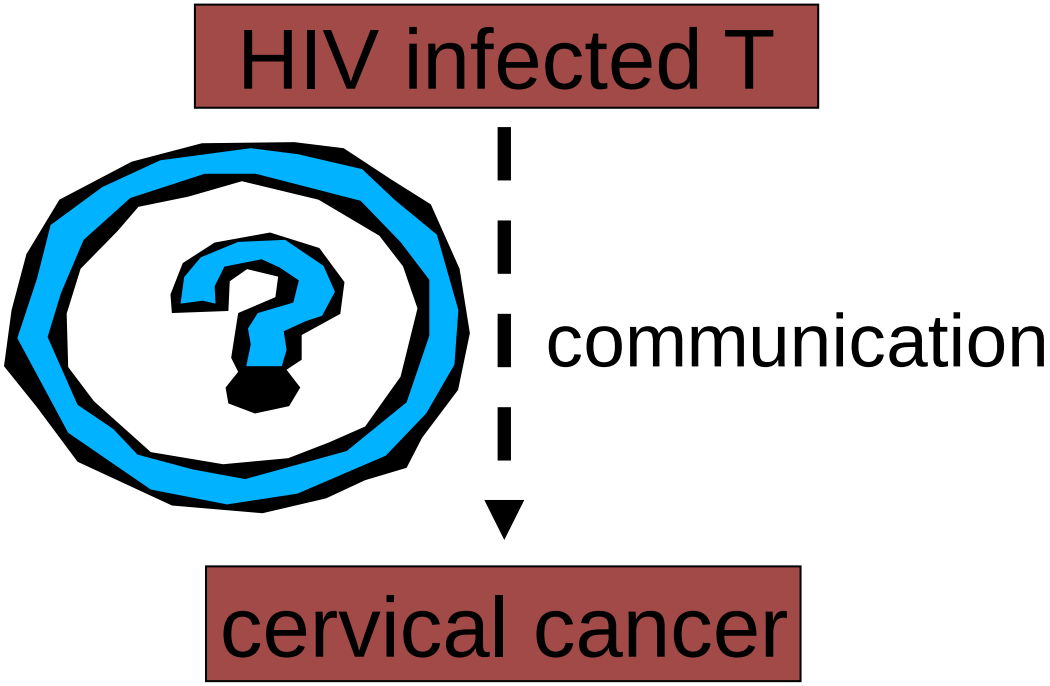
HIV感染促进肺癌的浸润转移，且免疫缺陷不是其主要原因。

ART治疗并未能降低宫颈癌发生率。

Hui L., et al, Cancer Lett. 2015; 1;368(1):7-13.

宫颈癌浸润转移机制仍不清楚

进一步研究HIV感染与宫颈癌发生发展的分子机理，开发新的干预与治疗方法，具有理论和现实意义。



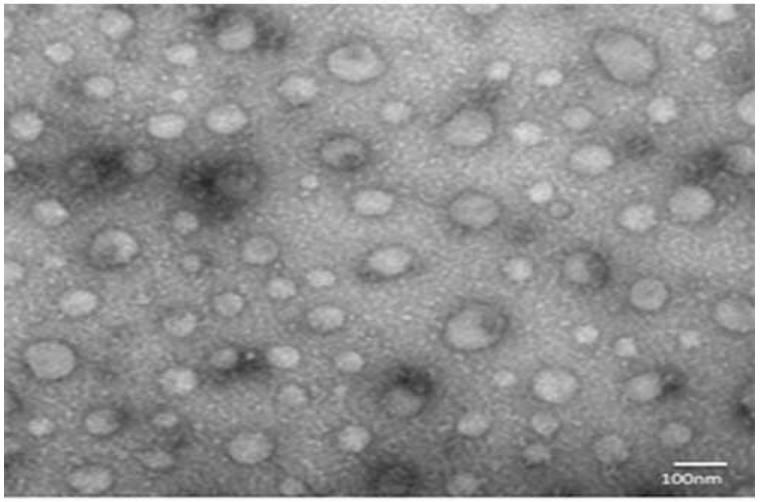
外泌体（Exosomes）

- 1986年，绵羊红细胞上清液中发现了一种有膜结构为40~100纳米的小囊泡,命名为外泌体；
- 1996年，B细胞分泌外泌体，可通过MHC激活T细胞；
- 现已证实哺乳动物的所有细胞均可分泌外泌体；
- 2013年诺贝尔生物/医学奖。

外泌体成分复杂

- 蛋白质：GPC-1…
- 细胞因子：IL-6, IL-8…
- RNA：microRNA…
- 生长因子：EGF…
- CD9，CD63, CD81

Exosomes(外秘体）形态完整



细胞上清提取的Exosomes(外秘体)。扫描电镜下，Exosomes形态完整，大小在20-200nM。

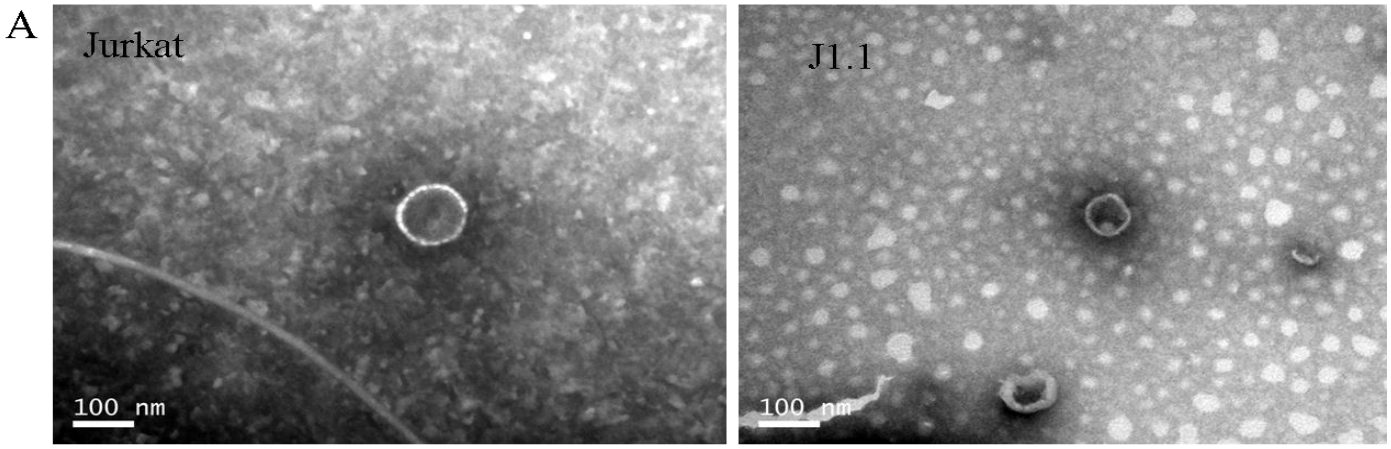
外泌体功能

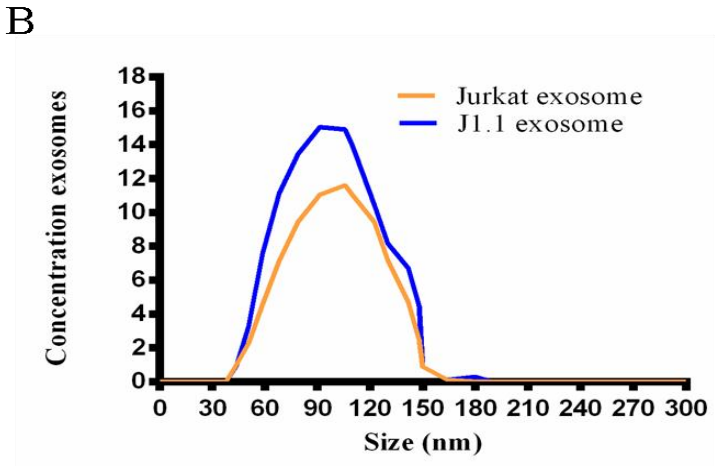
- 可以携带蛋白，运送RNA，在细胞间物质和信息转导中起重要作用；
- 可能通过调控免疫功能，促进肿瘤血管新生和肿瘤转移；
- 通过直接作用于肿瘤细胞等途径，影响肿瘤的进展。

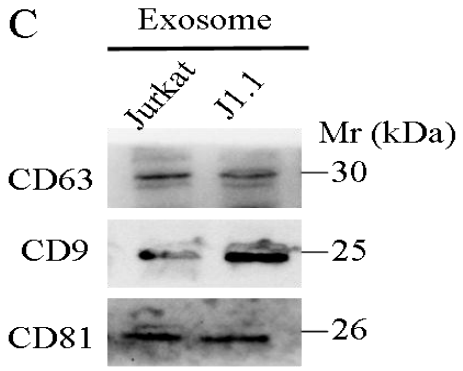
本研究拟解决的科学问题

1. HIV感染T细胞外泌体是否介导了宫颈癌的恶性进展？
2. HIV感染T细胞外泌体microRNA的变化如何？
3. HIV感染T细胞来源的外泌体microRNA参与宫颈癌细胞增殖和转移分子机制是什么？

HIV感染Jurkat细胞外泌体的提取及鉴定

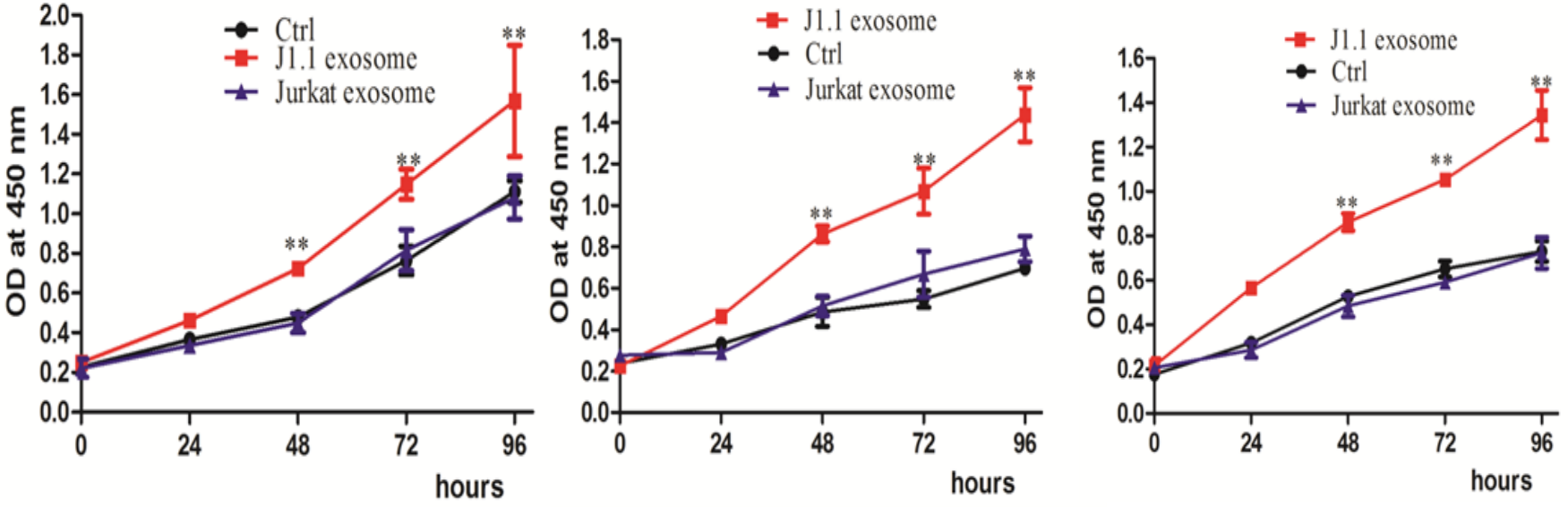






J1.1为HIV-1的假病毒颗粒感染Jurkat的潜伏模型，采用低温超高速离心法提取了Jurkat和J1.1细胞上清液的外泌体

HIV感染T细胞外泌体促进宫颈癌细胞增殖

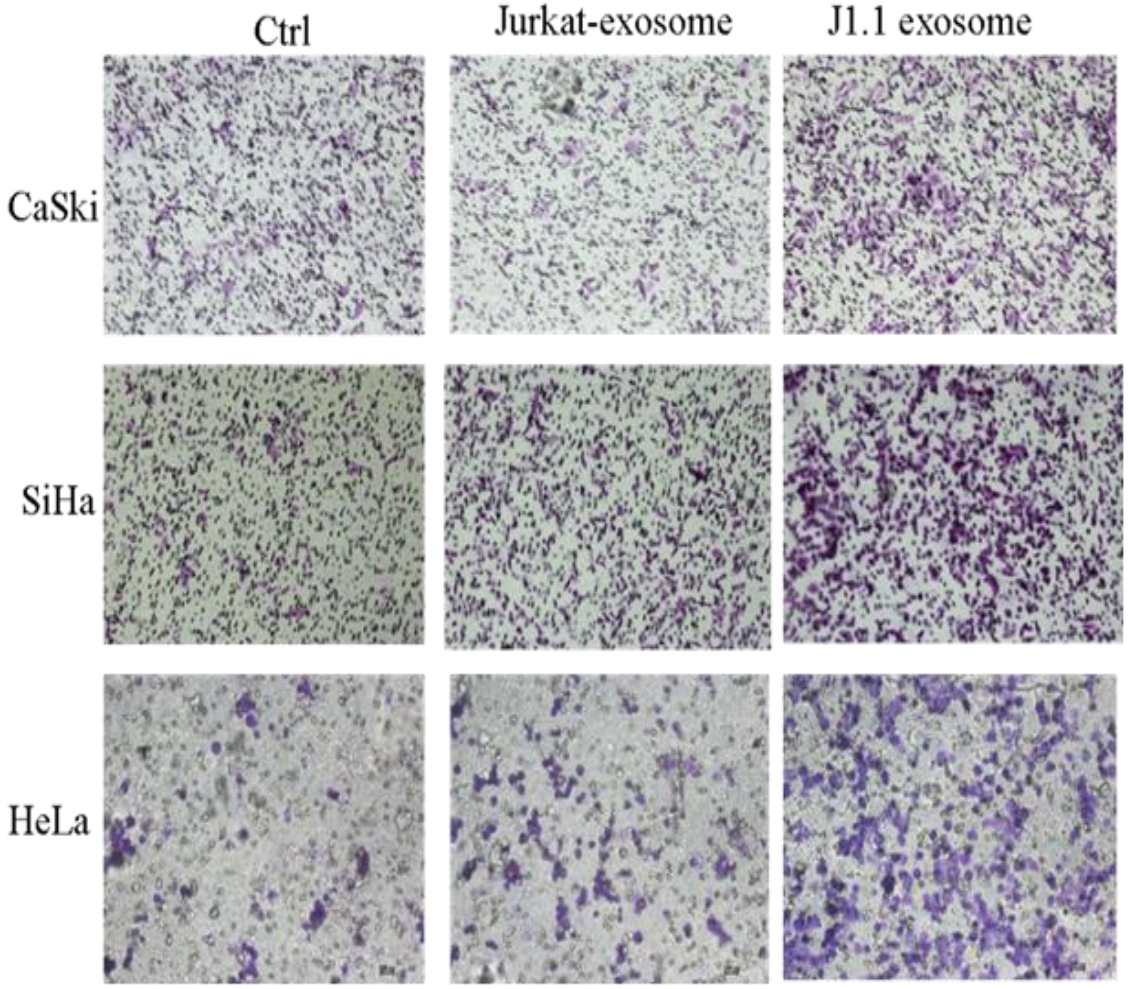


CaSki　　SiHa　　HeLa

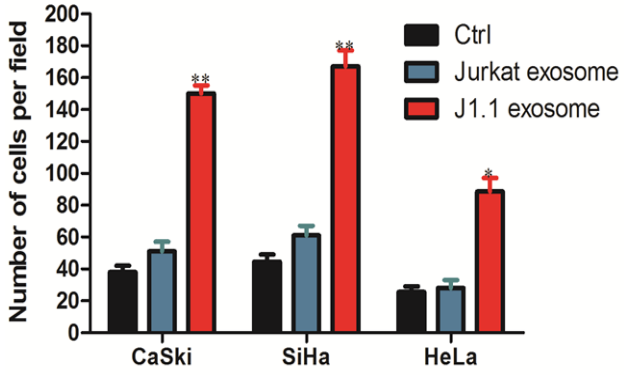
注：Hela（HPV18+）Siha(HPV16+) CaSki(HPV16/18+)

将Jurkat和J1.1提取的外泌体和宫颈癌细胞CaSki、SiHa和HeLa共培养，CCK8（cell counting kit）实验检测发现来源J1.1外泌体促进了宫颈癌细胞的增殖能力

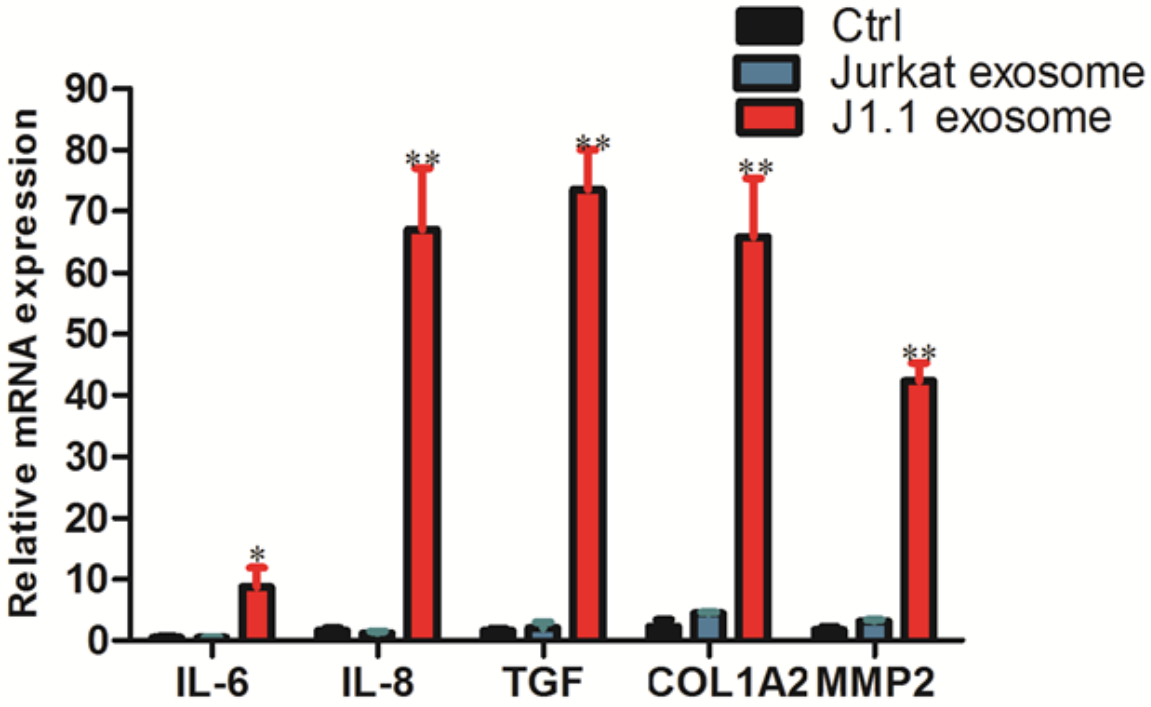
HIV感染T细胞外泌体促进宫颈癌细胞侵袭



将Jurkat和J1.1提取的外泌体和宫颈癌细胞共培养，Transwell侵袭实验检测发现，来源J1.1外泌体和宫颈癌细胞共培养后侵袭能力明显增强

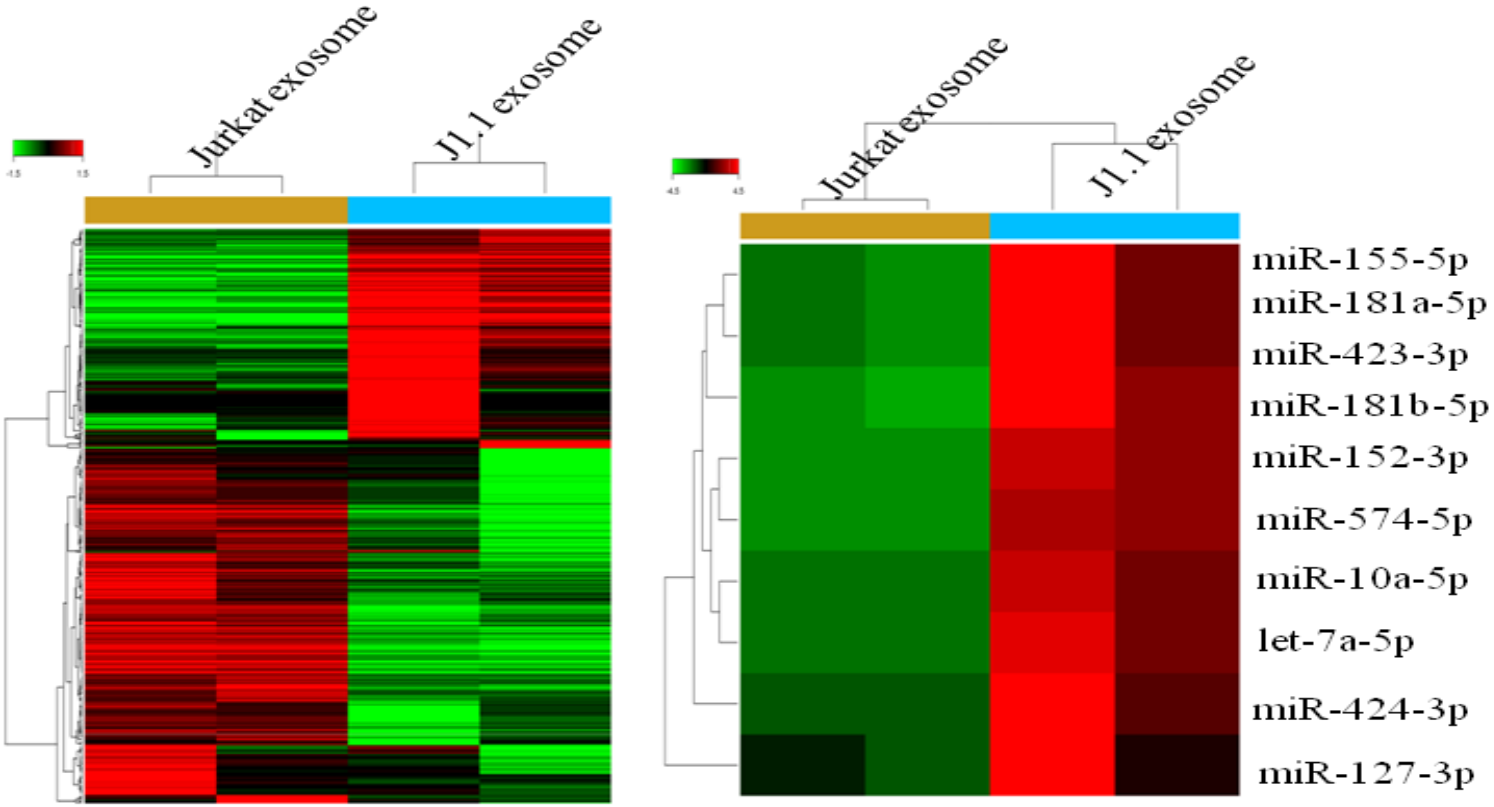


HIV感染T细胞外泌体促进宫颈癌细胞炎性因子分泌



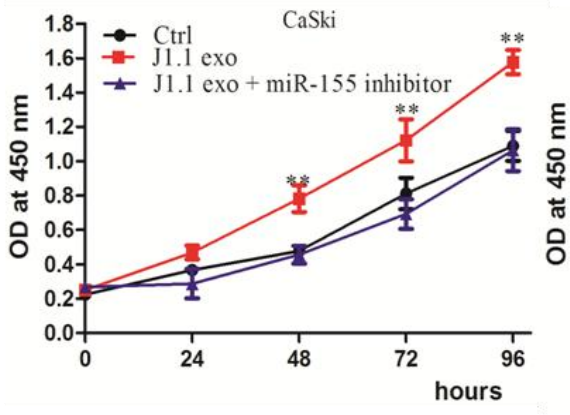
Jurkat和J1.1提取的外泌体和宫颈癌细胞共培养，qRT-PCR检测发现J1.1外泌体促进宫颈癌细胞CaSki炎性因子的分泌，说明来源HIV-1感染的J1.1的外泌体影响了宫颈癌细胞微环境。

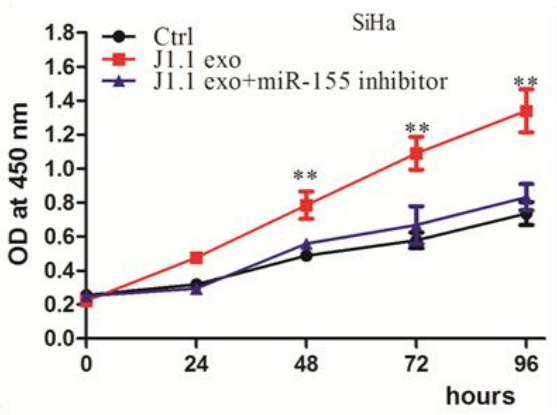
HIV感染T细胞外泌体携带关键microRNA

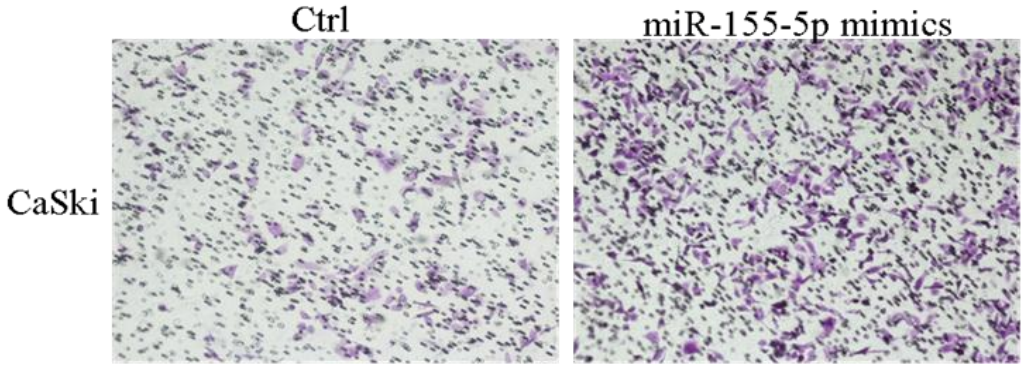


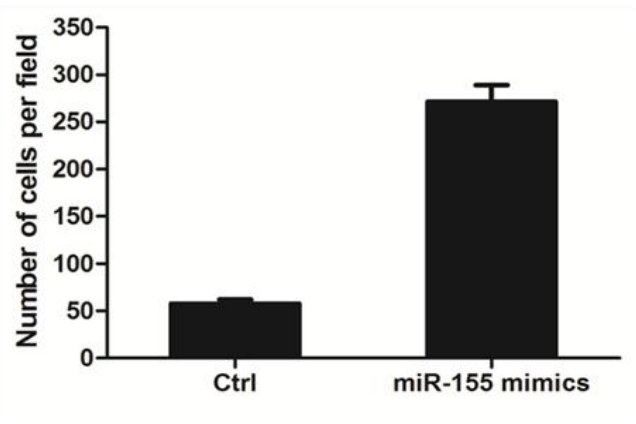
对Jurkat和J1.1提取的外泌体进行测序，以差异倍数$|\log_2| \geq 2$ $q < 0.05$共发现在J1.1外泌体中有274个差异miRNA，高表达有113，低表达161，其中以miR-155差异最为明显，提示miR-155可能在外泌体发挥生物学功能中起关键作用。

miR-155-5p促进宫颈癌细胞增殖和侵袭



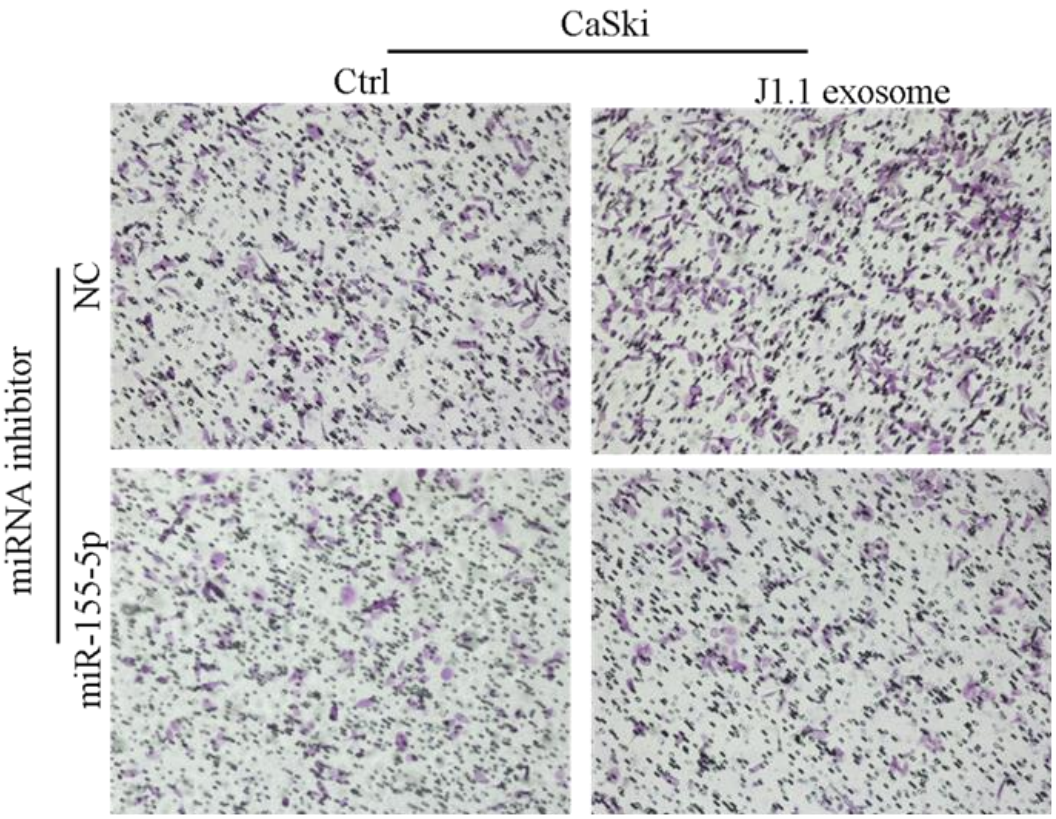


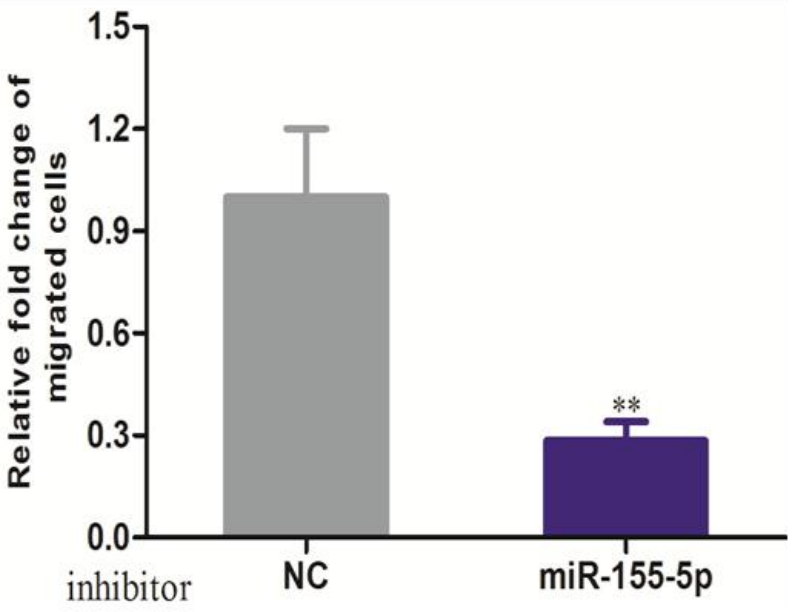




为了进一步探讨miR-155在宫颈癌中的生物学功能，在CaSki和SiHa细胞中过表达miR-155后，发现其促进宫颈癌细胞增殖和侵袭能力

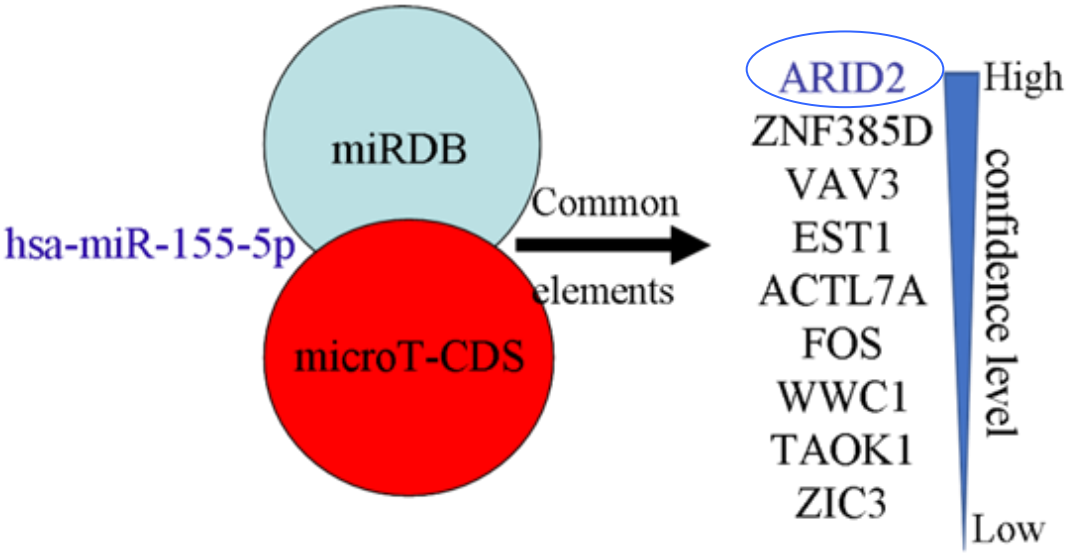
miR-155抑制剂可抑制HIV感染T细胞外泌体促宫颈癌细胞侵袭效应

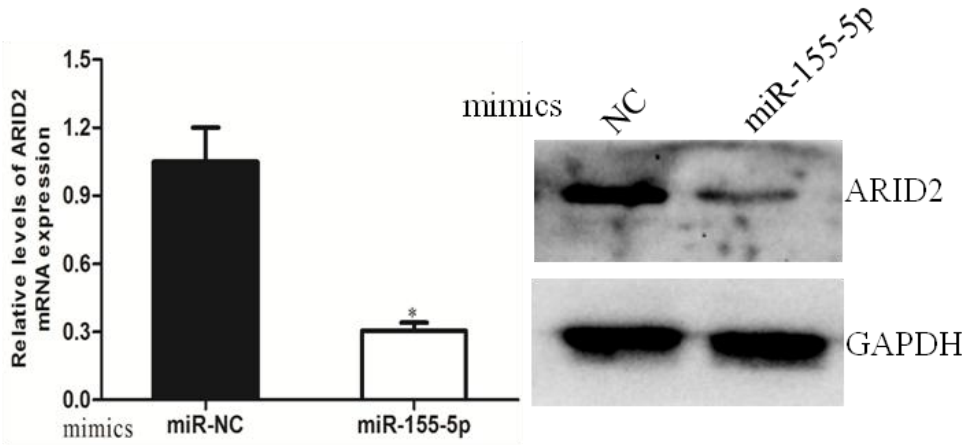




将J1.1提取的外泌体和宫颈癌CaSki细胞共培养的同时，再加入miR-155的抑制剂，发现miR-155的抑制剂能够消除J1.1外泌体促宫颈侵袭能力，说明miR-155是J1.1外泌体促宫颈癌侵袭的关键分子

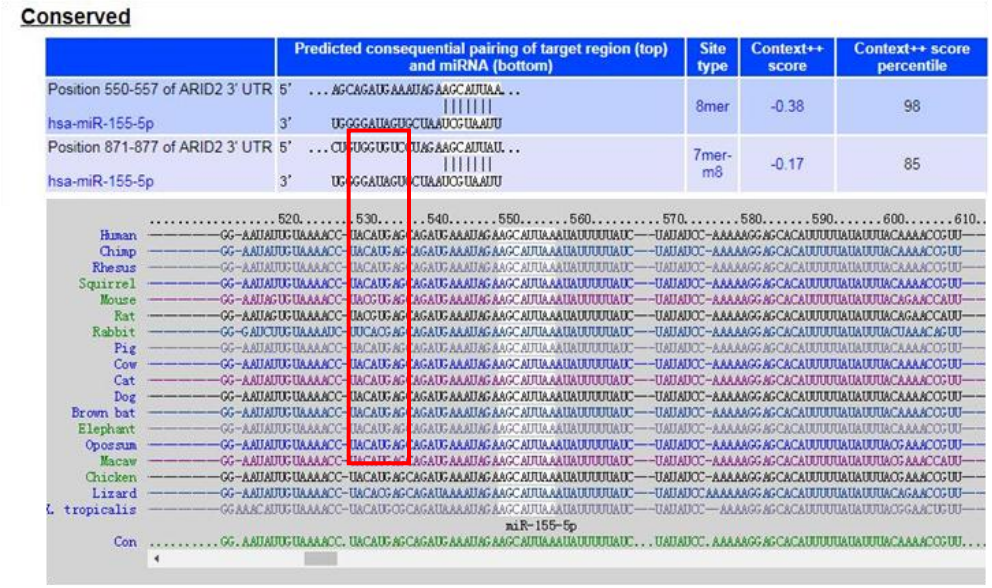
生物学信息学分析miR-155下游靶基因及鉴定

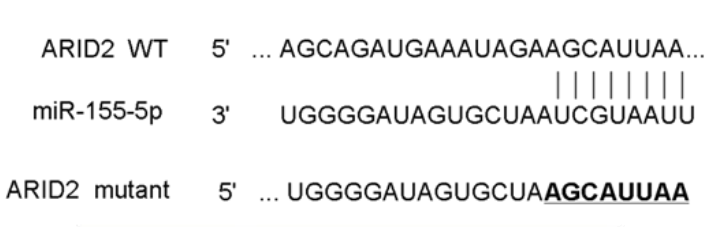


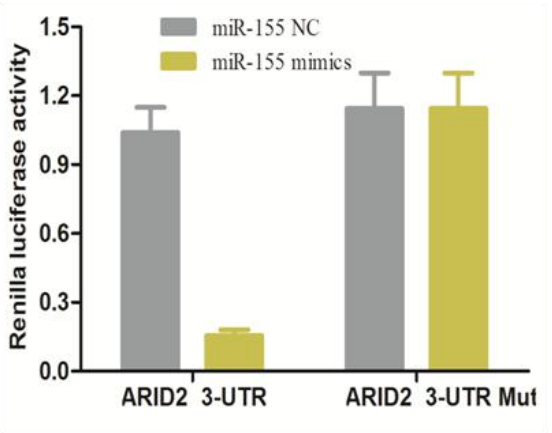


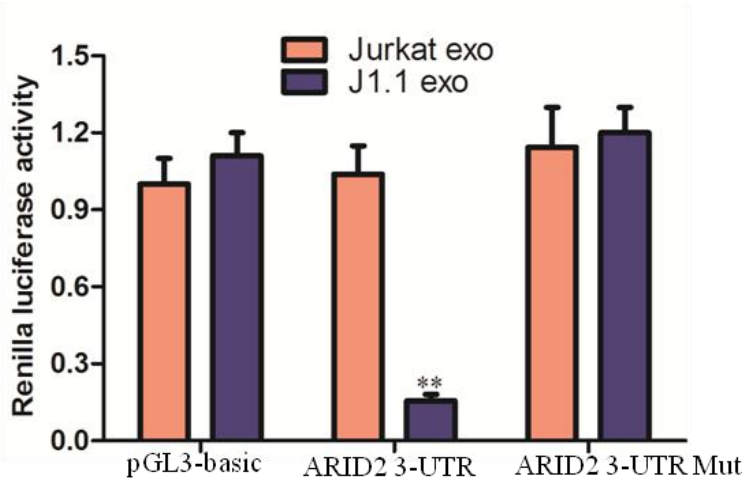
在宫颈癌细胞CaSki中过表达miR-155能够抑制ARID2 mRNA和蛋白水平的表达，说明ARID2可能是其下游作用的靶基因。

双荧光素酶实验验证miR-155直接靶向ARID2 3-UTR



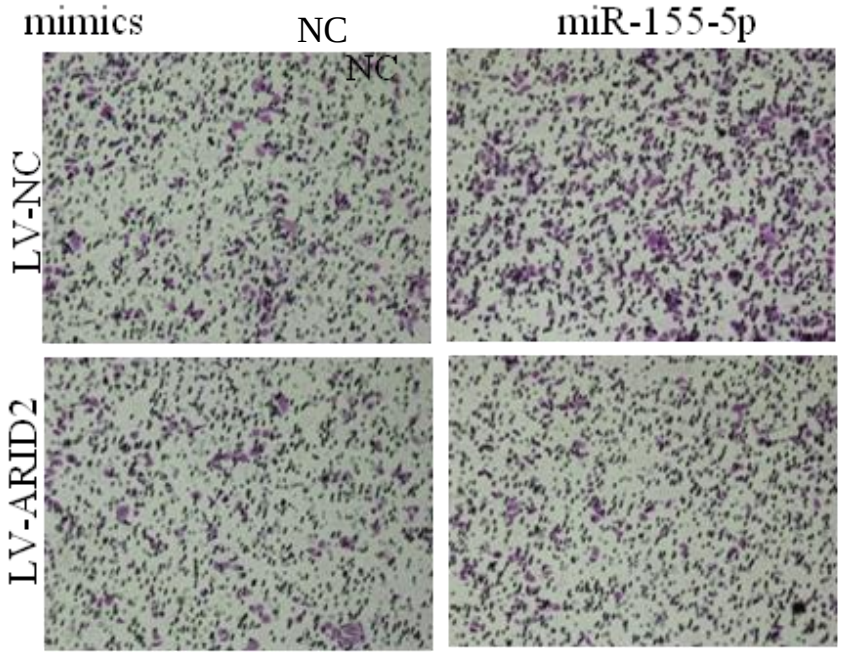


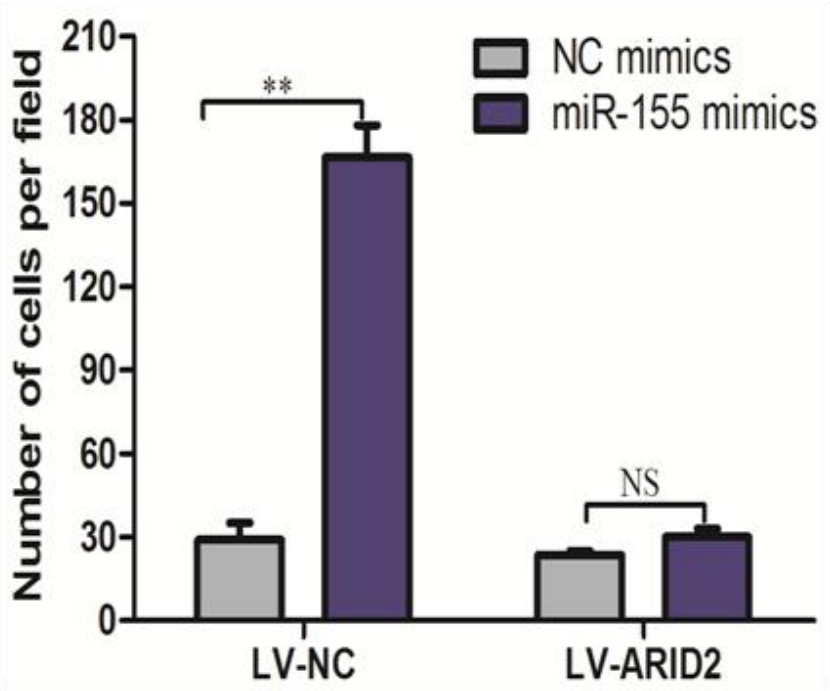




miR-155和J1.1外泌体对野生型ARID2 3-UTR活性明显抑制（$p<0.05$），而对突变型ARID2 3-UTR活性无抑制作用，体外证实了ARID2是其下游作用的靶蛋白。

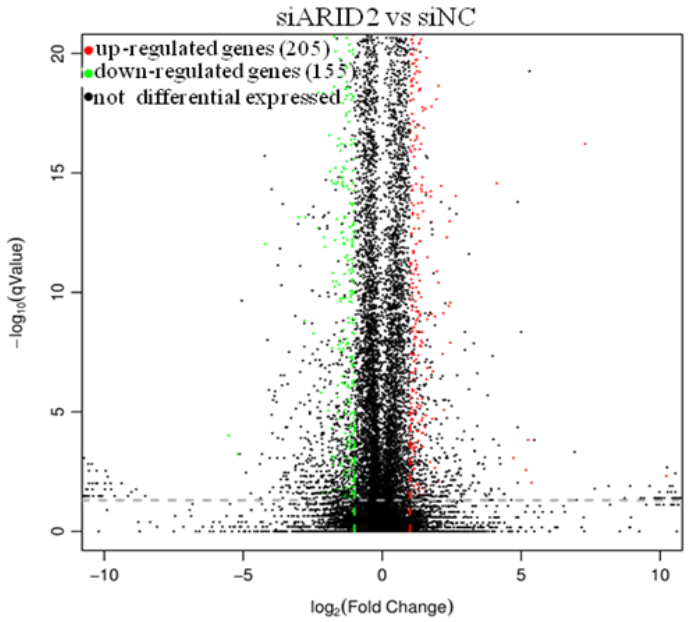
ARID2是miR-155促进宫颈癌细胞侵袭的关键下游靶基因

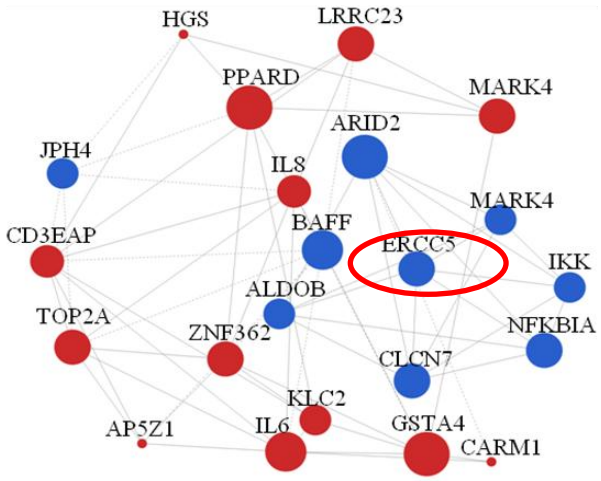




过表达ARID2能够消除miR-155促宫颈癌细胞侵袭效应,说明miR-155是通过ARID2发挥生物学功能。

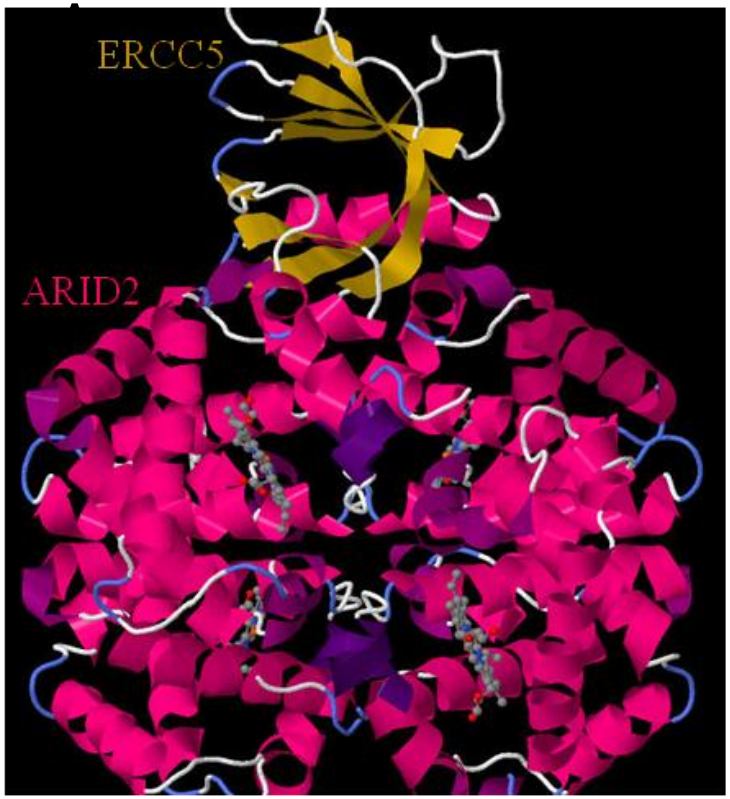
生物信息学分析ARID2下游靶蛋白及信号通路



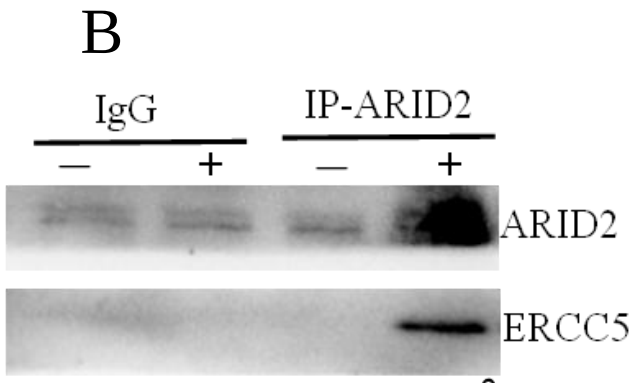


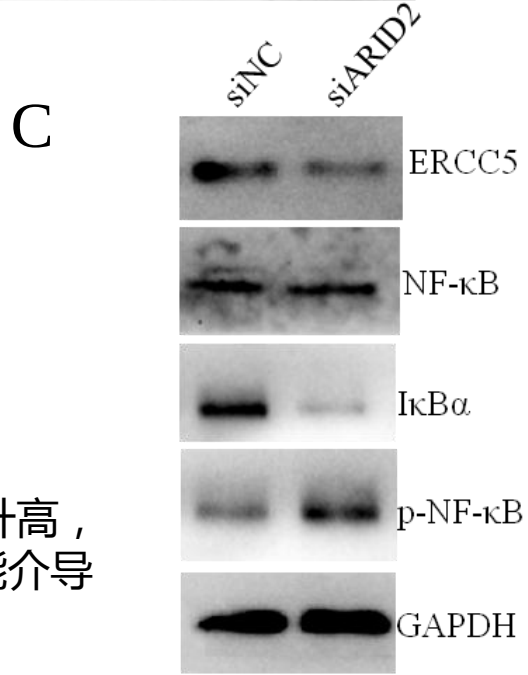
ARID2和ERCC5相互结合促进下游靶基因激活

A:分子对接（ molecular docking ）
预测ARID2和ERCC5存在结合



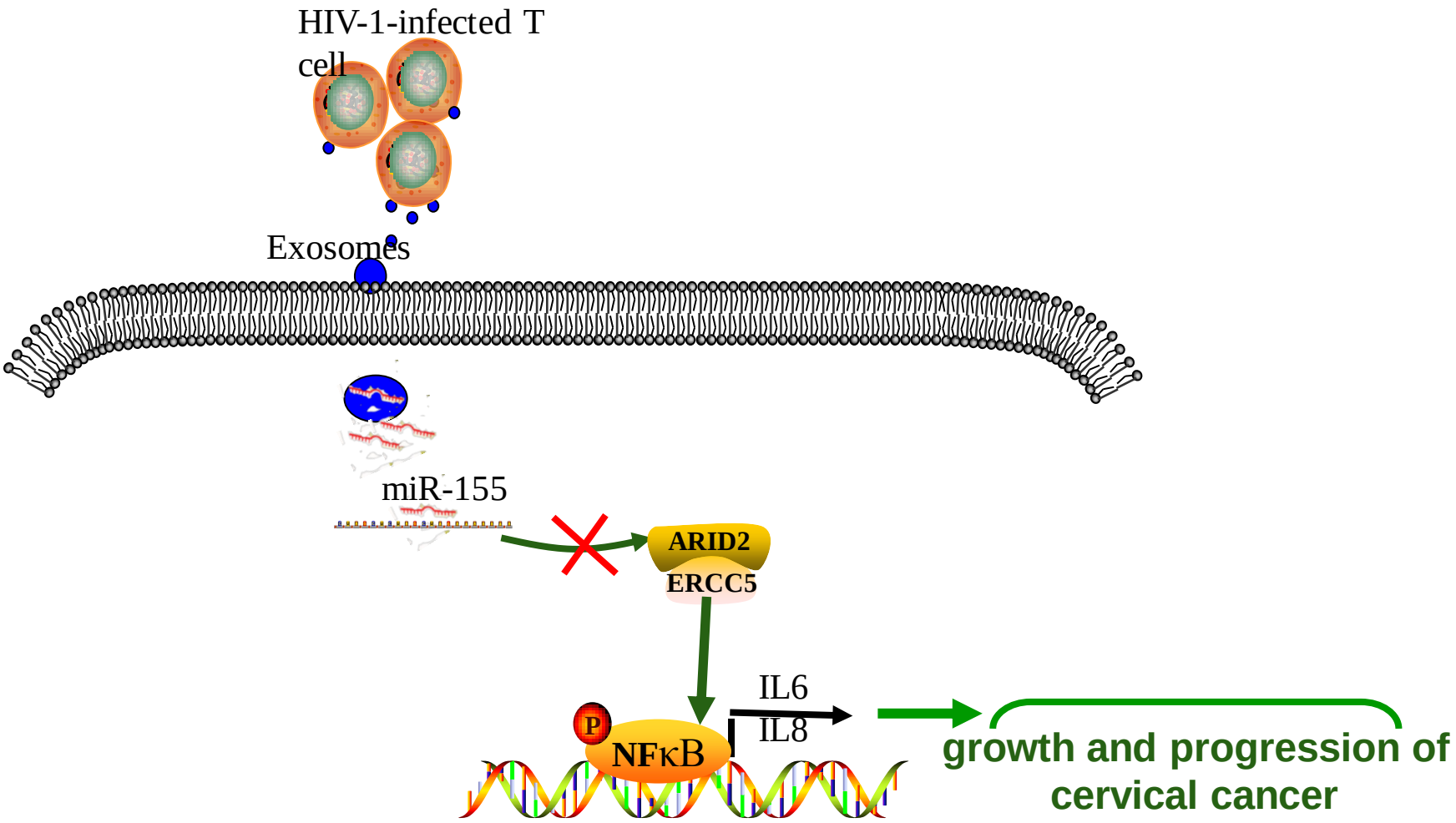
B:CO-IP实验
证实ARID2和ERCC5存在结合





C：干扰ARID2表达后，NF-KB的磷酸化水平升高，IkBa表达下降，说明ARID2/ERCC5复合物可能介导NF-KB通路

研究结论


HIV-1-infected T cell
Exosomes
miR-155
ARID2
ERCC5
IL6
IL8
P
NFκB
growth and progression of cervical cancer

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Research Paper

HIV-1-infected cell-derived exosomes promote the growth and progression of cervical cancer

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Abstract

Background: Women infected with HIV are more likely to have aggressive cervical cancer, and patients with HIV infection are often more severely ill than those without HIV infection. However, the underlying mechanism for the progression of cervical cancer is not yet fully understood and requires further research.

Methods: Exosomes were isolated from cell culture supernatants using differential ultracentrifugation. Confirmation of exosome isolation was based upon identification by electron microscopy and NanoSight particle tracking analysis of the purified fraction. The function of exosomes derived from HIV-infected T-cells in cervical cancer was determined by CCK8 and Transwell invasion assays.

Results: Exosomal miR-155-5p derived from HIV-infected T-cells promotes the proliferation, migration and invasion of cervical cancer cells. Furthermore, we found that HIV-infected T-cells secrete exosomal miR-155-5p that directly targets ARID2 degradation, leading to activation of the NF-κB signaling pathway. MiR-155-5p promotes cervical cancer progression by secreting proinflammatory cytokines, including IL-6 and IL-8.

Conclusions: In conclusion, we demonstrate that intercellular crosstalk between HIV-infected T-cells and cervical cancer is mediated by exosomes from HIV-infected T-cells that contribute to the malignant progression of cervical cancer, providing potential targets for the prevention and treatment of HIV-associated cervical cancer.

Key words: cervical cancer; HIV-infected T-cell; exosome; miR-155-5p

下一步研究计划

- 从HIV感染者血液标本中提取外泌体，并与宫颈癌细胞共培养，进一步确证上述研究结论。


Thanks