



读书报告

张文雅

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Hepatocellular Carcinoma Cell-Secreted Exosomal MicroRNA-210 Promotes Angiogenesis *In Vitro* and *In Vivo*

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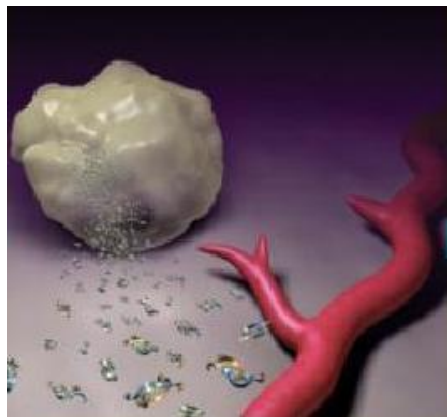
01

研究背景

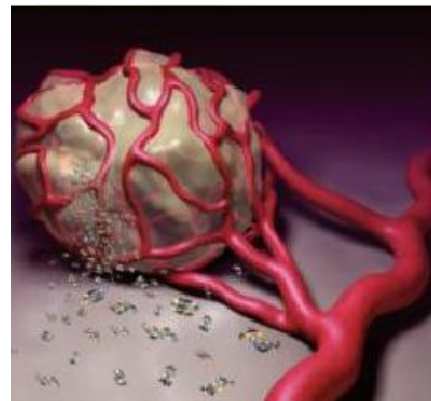




无血供，仅靠弥散获取营养，体积不超过 2mm^3 ，静息期。



肿瘤细胞分泌血管内皮生长因子（VEGF），促使供应肿瘤的血管生成。



有血供的肿瘤迅速生长并发生侵袭和转移。





肝细胞癌(Hepatocellularcarcinoma, HCC)是一种常见的恶性肿瘤。先前的研究中发现肝癌患者血清中有19种miRNAs水平显著升高。有研究表明,外泌体miRNAs的细胞间转移对肿瘤的生长、迁移、侵袭有调节作用。然而,这些研究仅提供了体外实验证据,主要集中在不同肝癌细胞间的通讯。外泌体miRNA在肝癌发展中的意义尚不清楚,尤其是对HCC的血管生成,目前尚未报道。

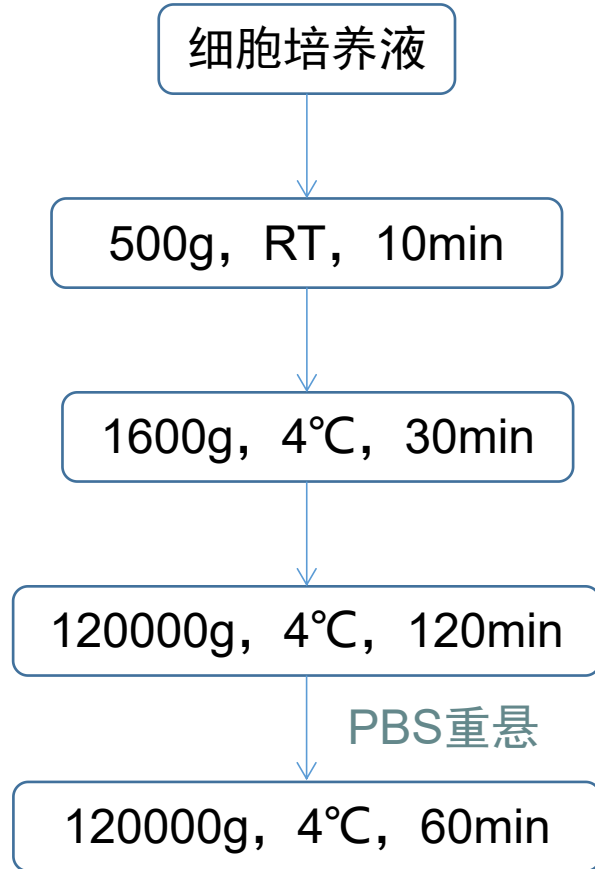


02

材料方法



1.外泌体分离



2.DiIC₁₆ 染色

QGY-7703细胞接种后24小时，用1×pBS清洗，然后在含有DiIC₁₆的无血清DMEM中孵育48小时。



3. 毛细管形成试验

用RNA双螺旋转染HUVECs或用外泌体处理HUVECs 48小时后，将HUVECs细胞重新悬浮在含1%FBS的SFM中，加入到涂有Matrigel的包被板中。孵化6小时。在光学显微镜下捕捉到毛细血管样结构的形成。

4. 荧光素酶报告实验

50 nM 的miR-210或对照RNA，2 ng 的 pRL-CMV及50 ng含目的基因（野生型或突变型）3'UTR的荧光素酶报告质粒共转染293T细胞。



03

研究结果



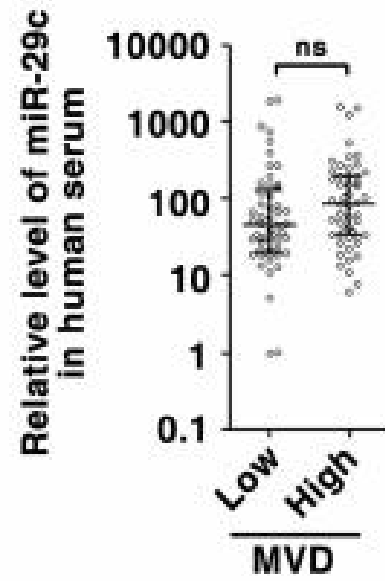
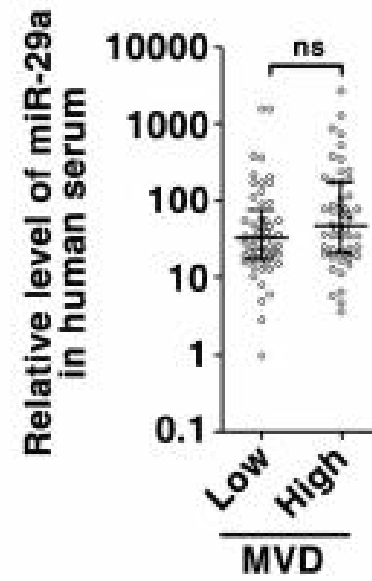
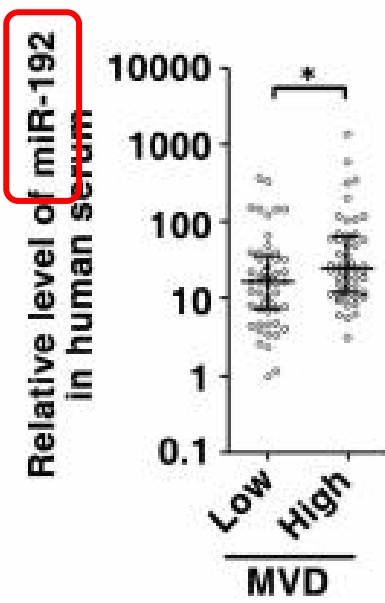
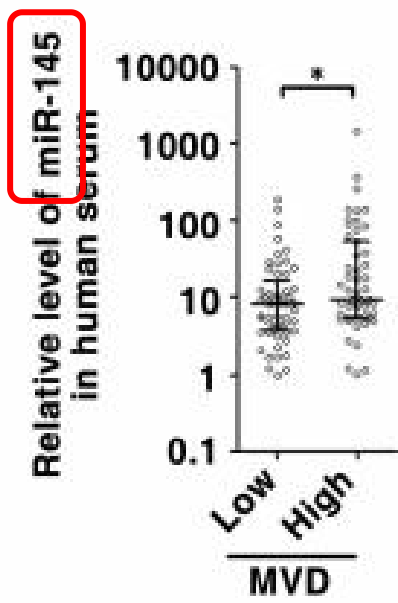
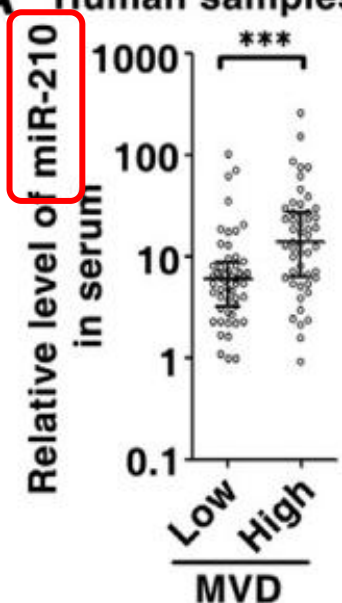
肝癌细胞miRNA

Table S1. Detection of miRNAs in the conditioned media of hepatoma cells.*

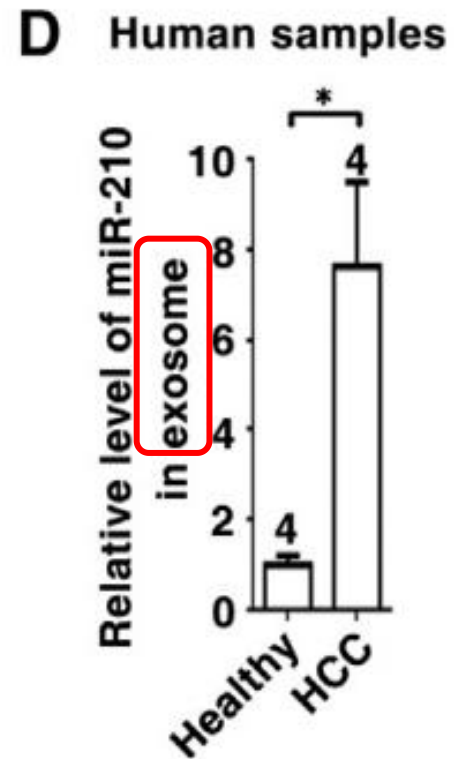
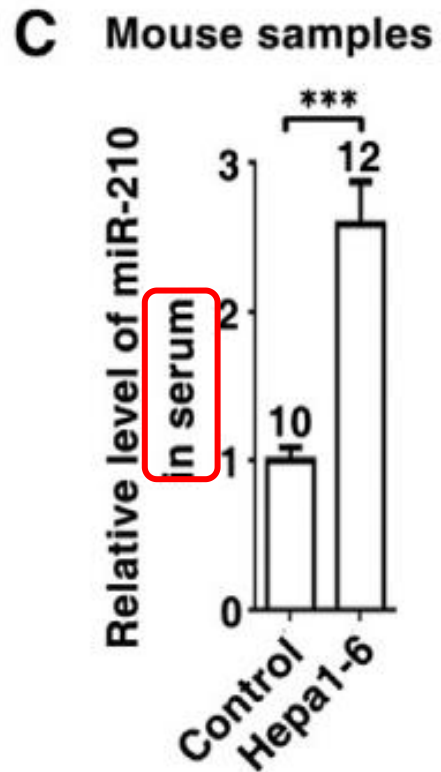
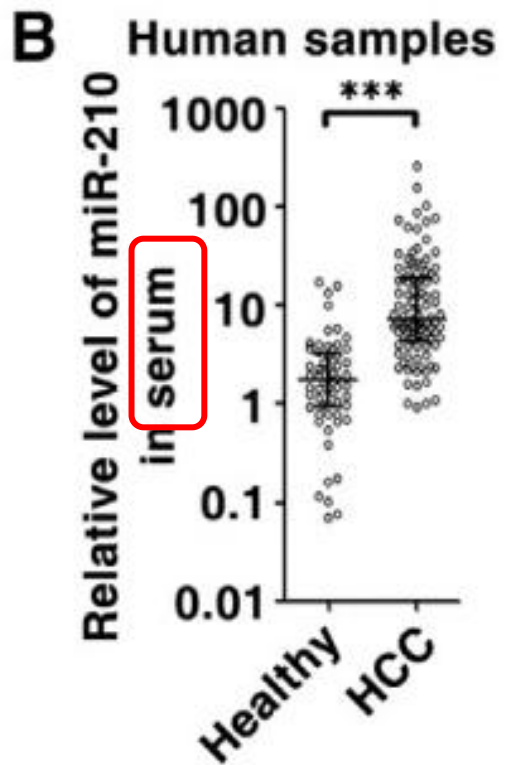
miRNAs	QGY-7703	HepG2	SK-Hep-1	Huh-7
hsa-miR-29a	+	+	+	+
hsa-miR-29c	+	+	+	+
hsa-miR-145	+	+	+	+
hsa-miR-192	+	+	+	+
hsa-miR-210	+	+	+	+
hsa-miR-505	+	+	+	-
hsa-miR-218	-	-	+	+
hsa-miR-452	+	+	-	-
hsa-miR-769-5p	+	-	+	-
hsa-miR-122	-	-	-	+
hsa-miR-143	-	+	-	-
hsa-miR-193a-5p	-	-	+	-
hsa-miR-497	-	-	+	-
hsa-miR-1	-	-	-	-
hsa-miR-133a	-	-	-	-
hsa-miR-136-3p	-	-	-	-
hsa-miR-145-3p	-	-	-	-
hsa-miR-214	-	-	-	-
hsa-miR-214-5p	-	-	-	-

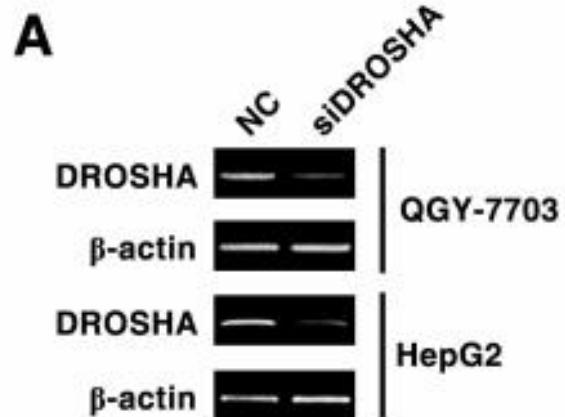
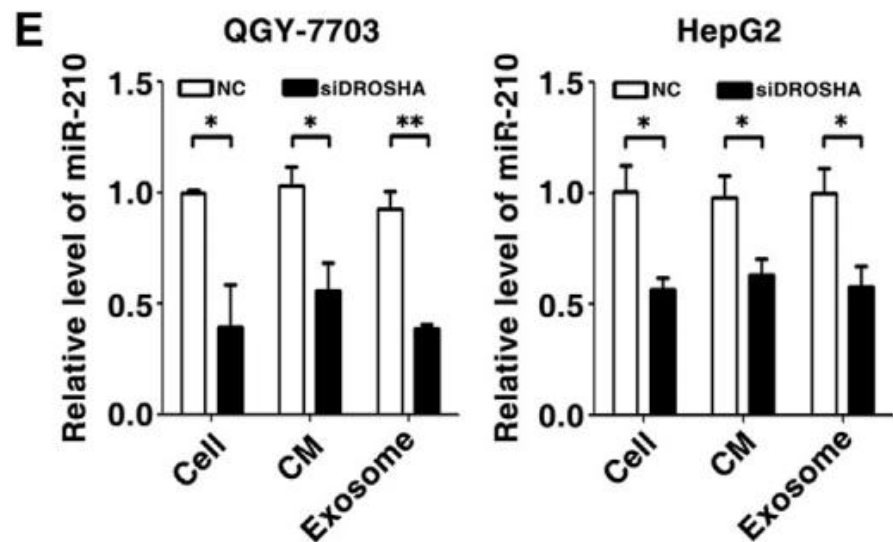
微血管密度与miRNA的关系

A Human samples



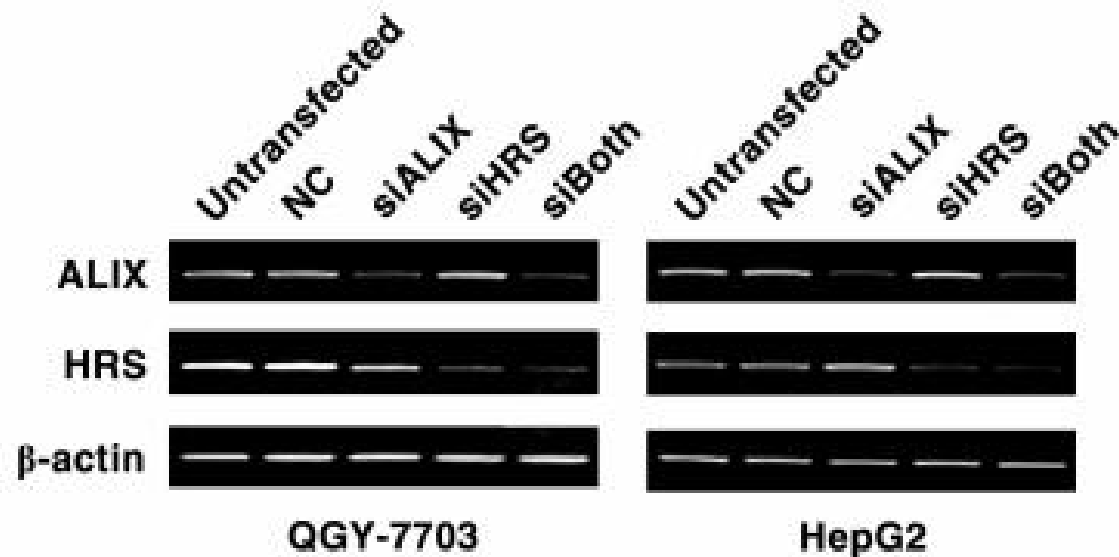
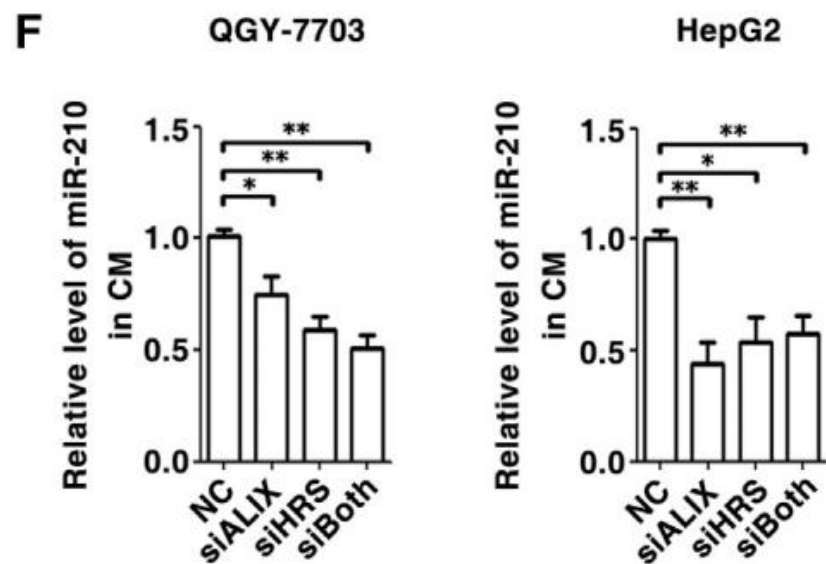
miR-210在肝癌细胞中的表达量





DROSHA是miRNA生物生成所必需的调节因子。

ALIX和HRS是外泌体分泌的两个关键成分。

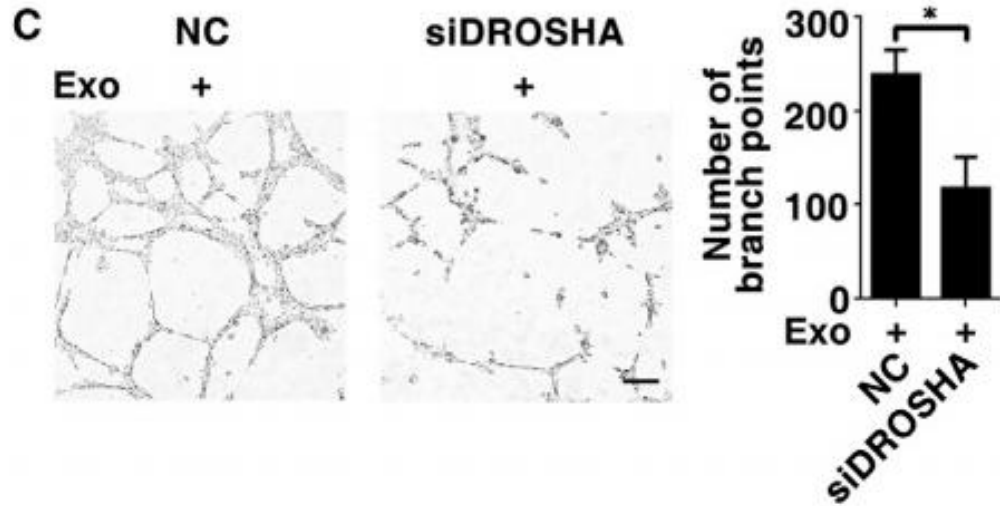
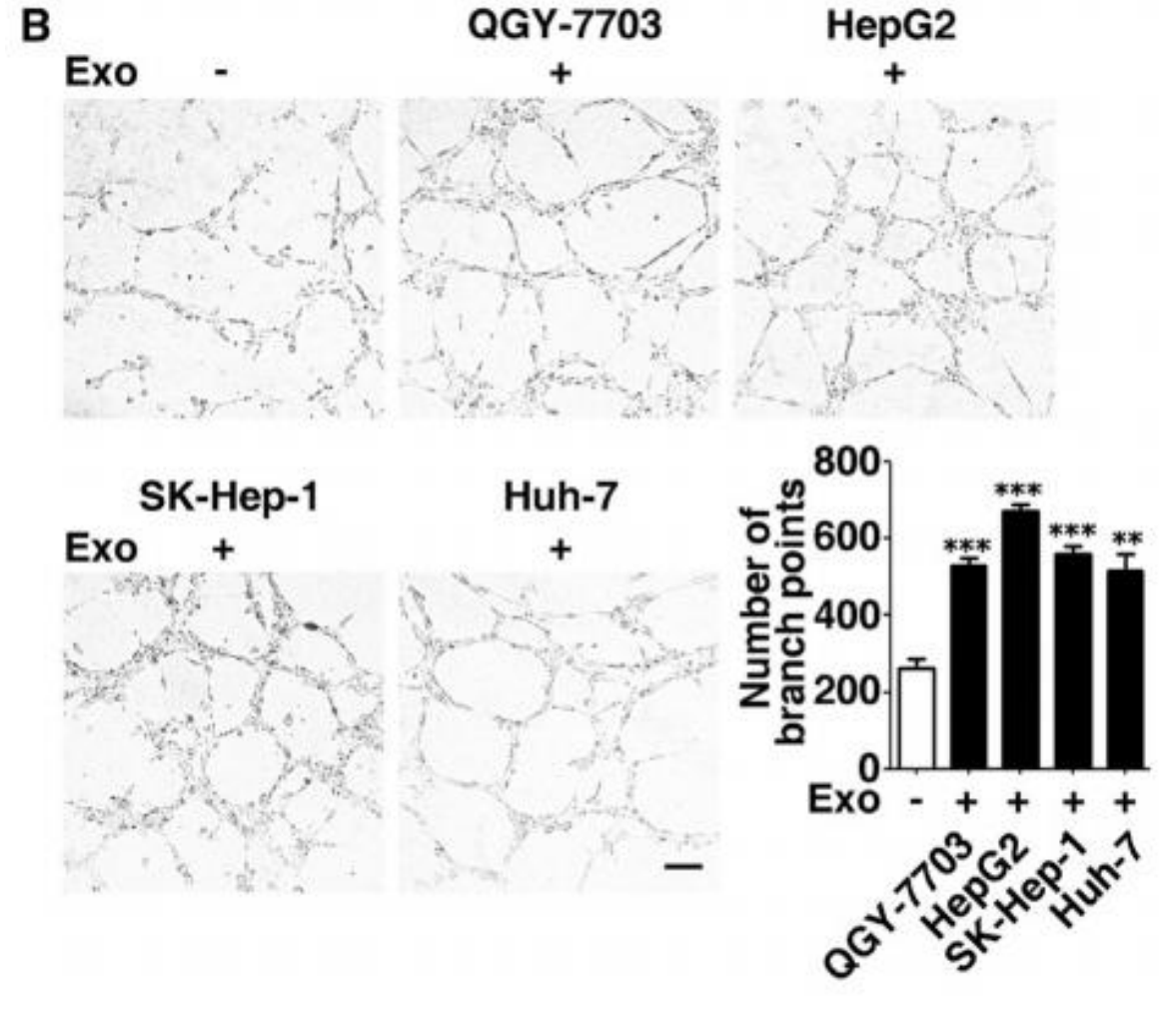
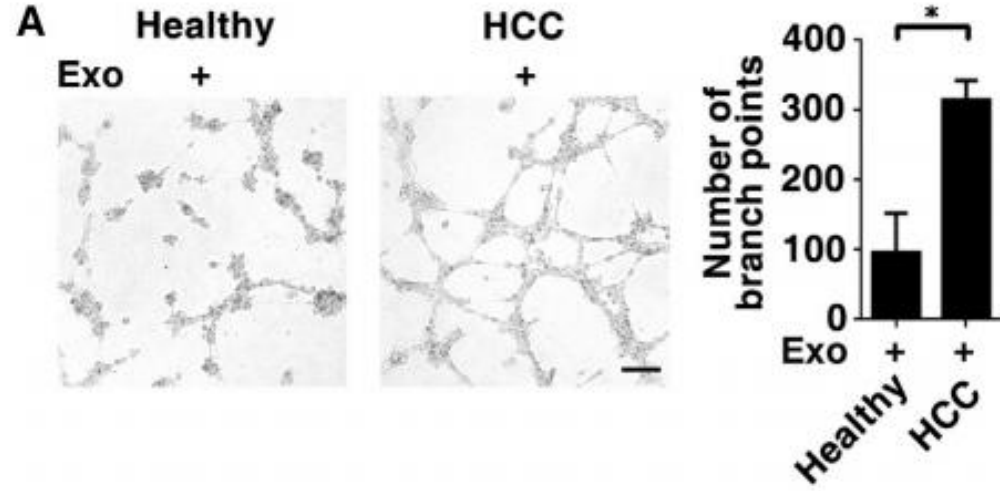




小结

肝癌细胞可能通过外泌体分泌miR-210，而分泌的miR-210可能参与了肝癌的血管生成。

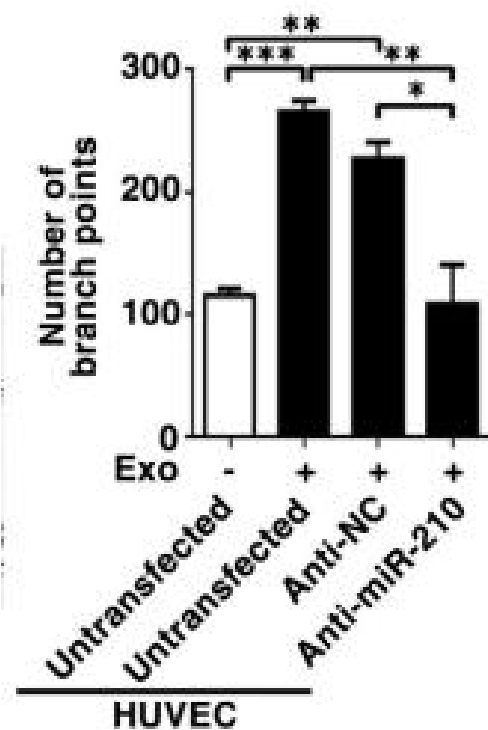
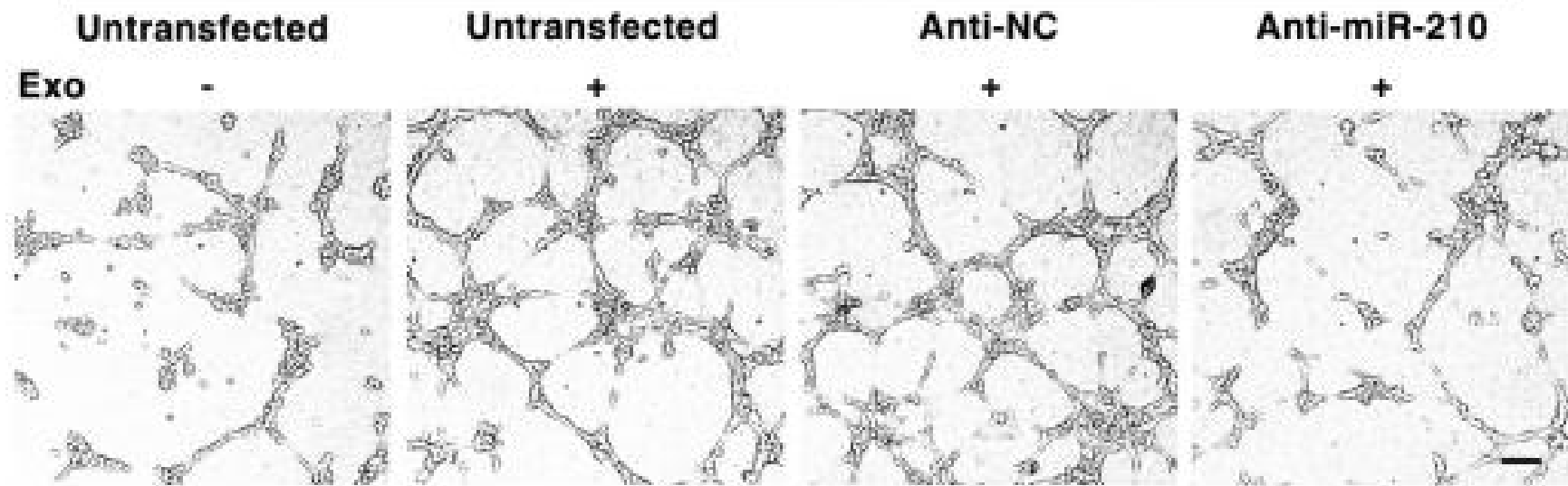
肝癌细胞外泌体促进内皮细胞的血管形成



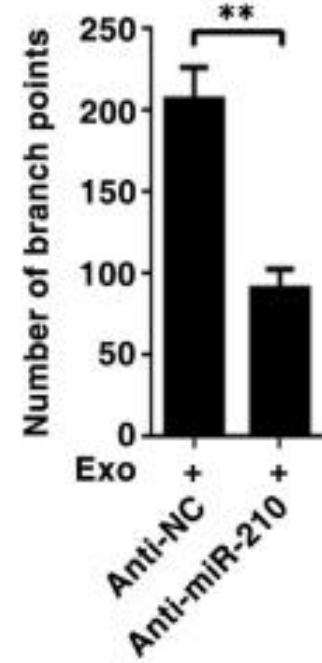
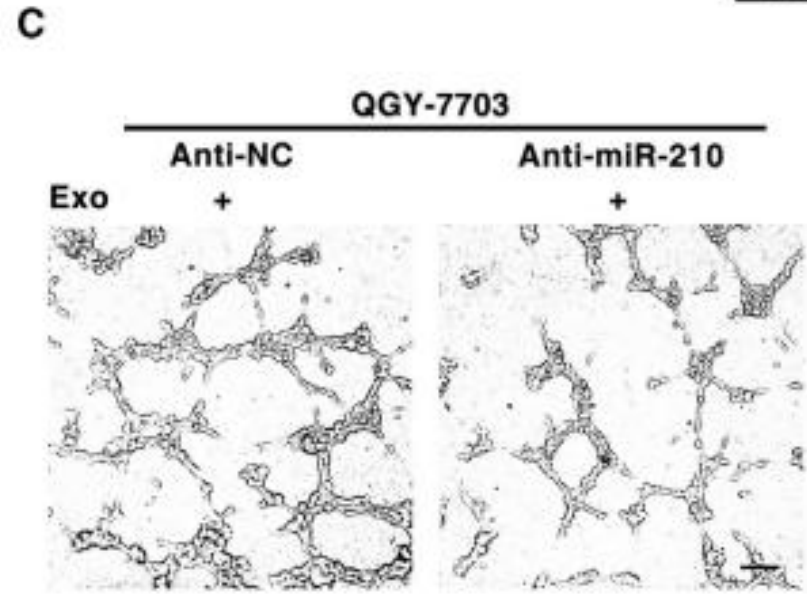
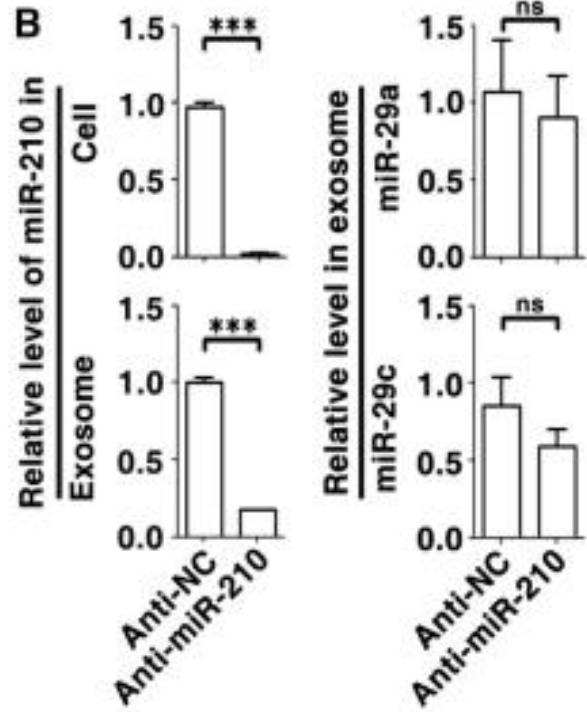
肝癌细胞外泌体miR-210促进内皮细胞的血管形成

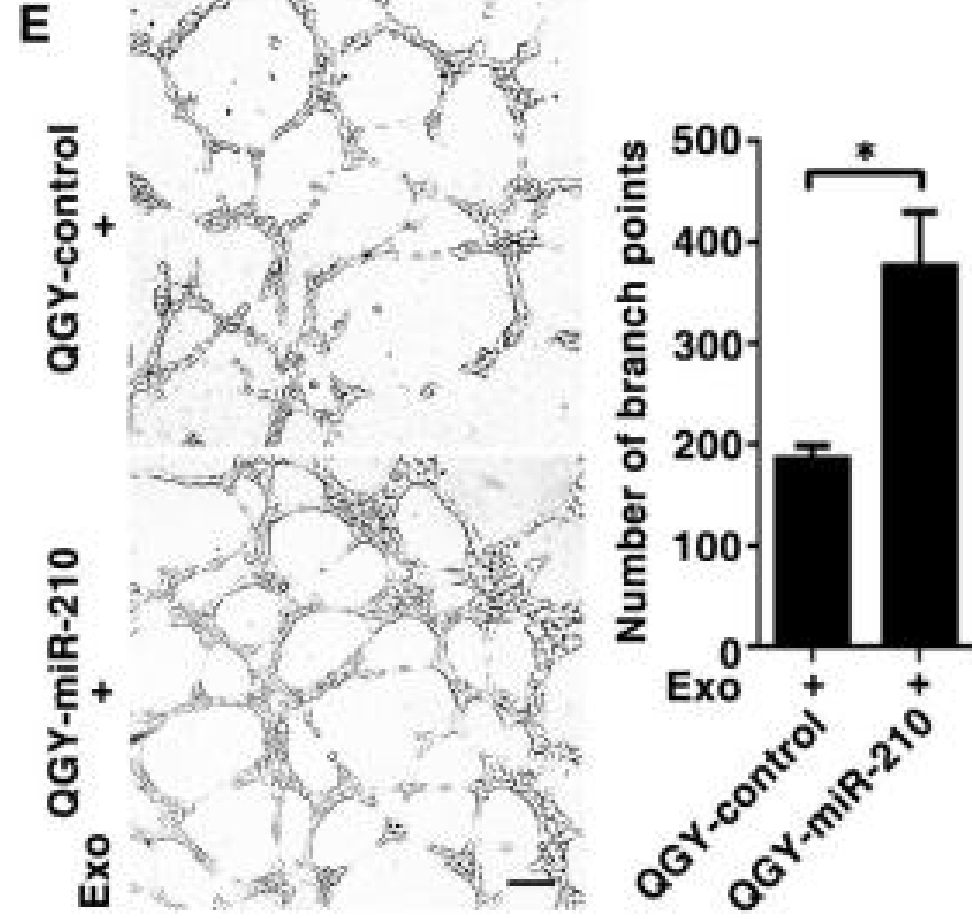
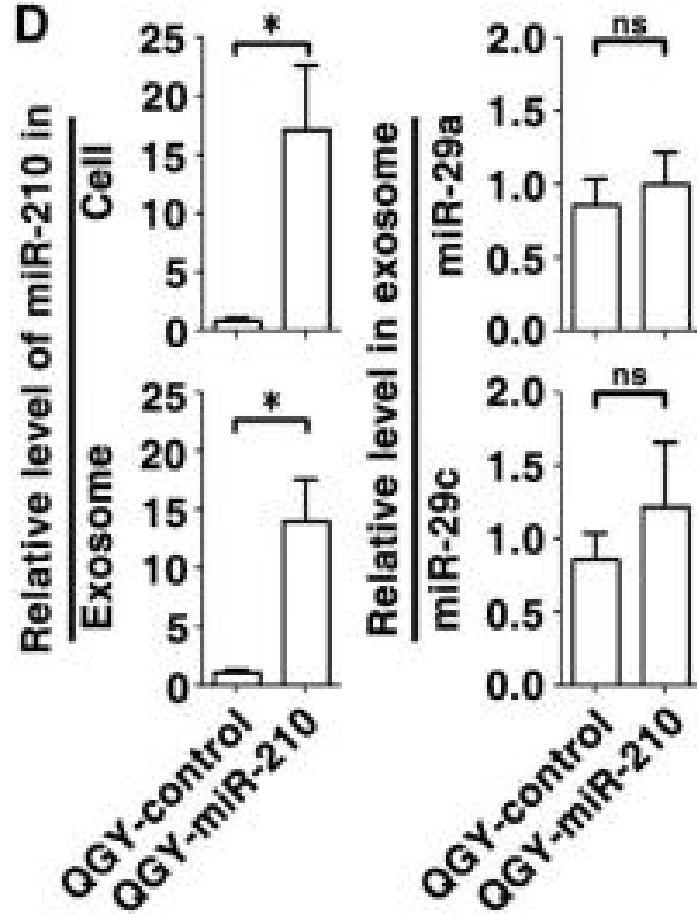
A

HUVEC

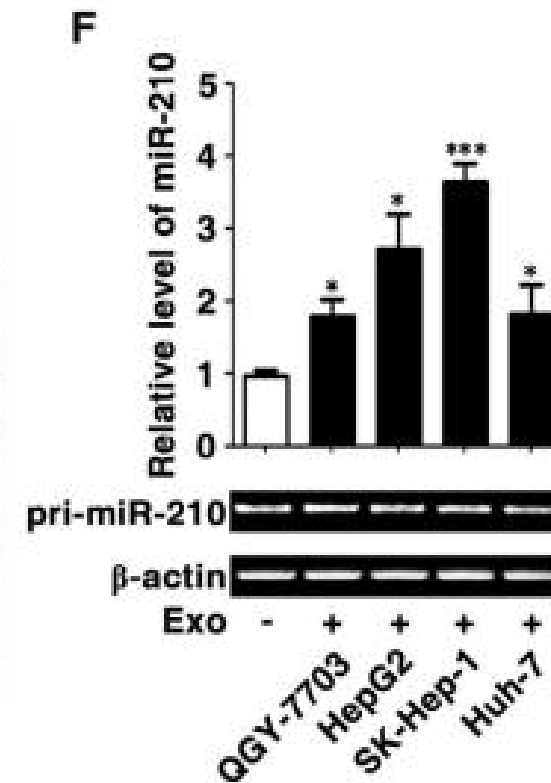
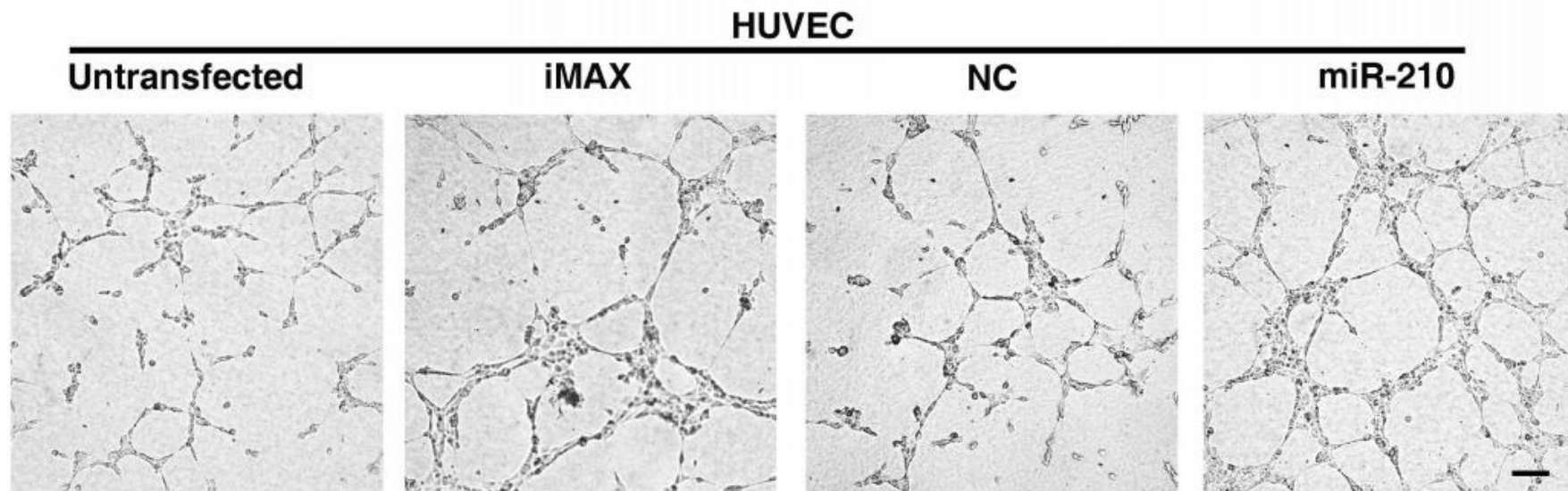


肝癌细胞外泌体miR-210促进肝癌细胞的血管形成





外源性miR-210促进内皮细胞的血管形成，而非内源性miR-210

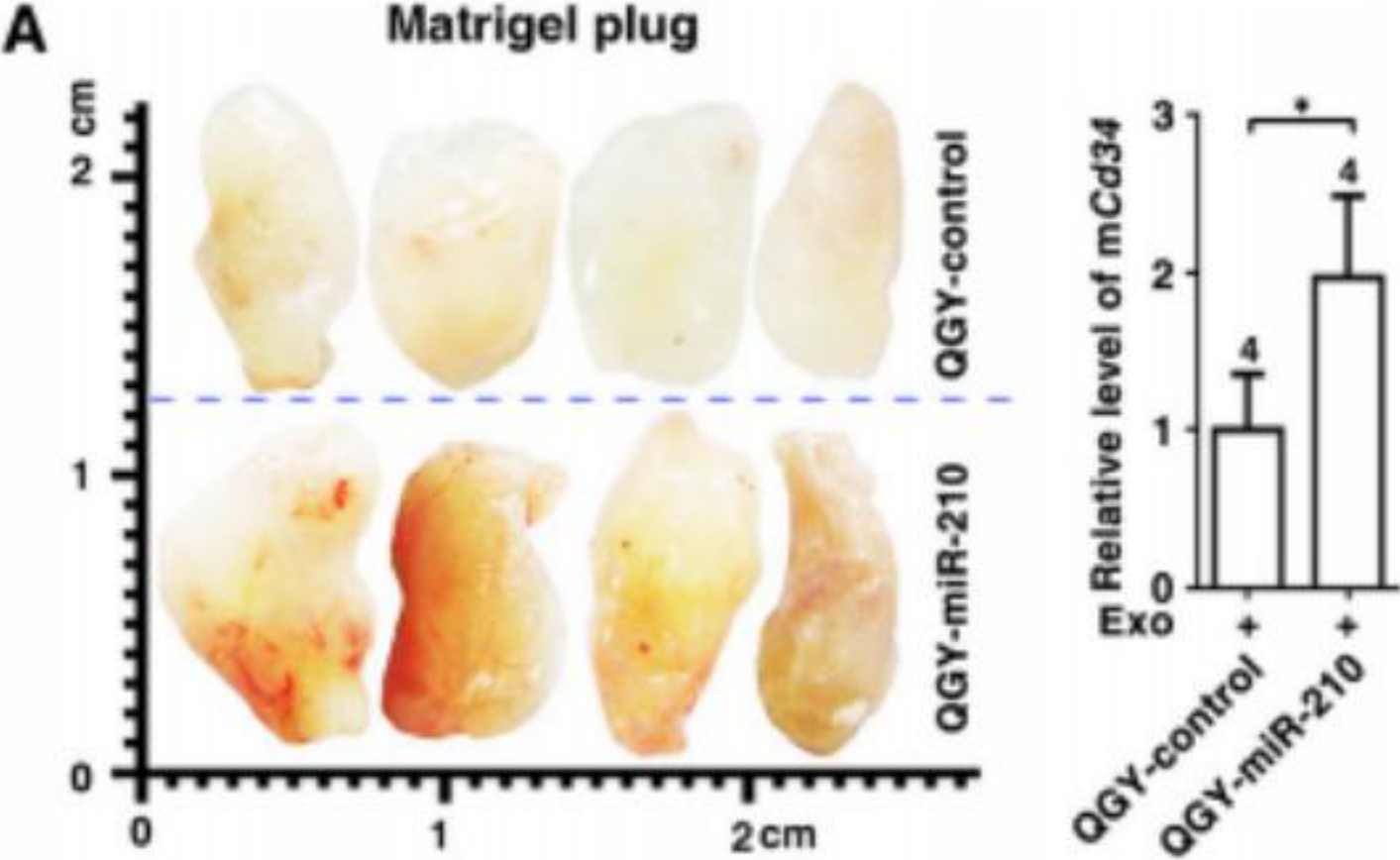




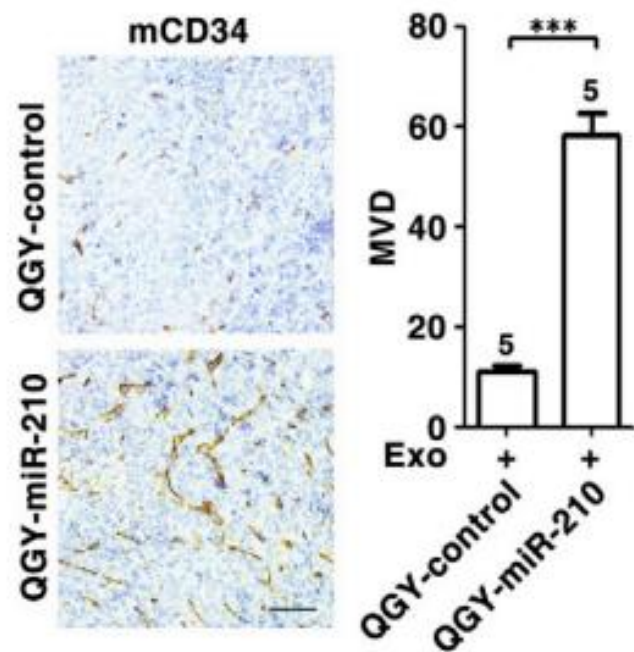
小结

肝癌细胞来源的外泌体miR-210在体外促进内皮细胞的血管形成。

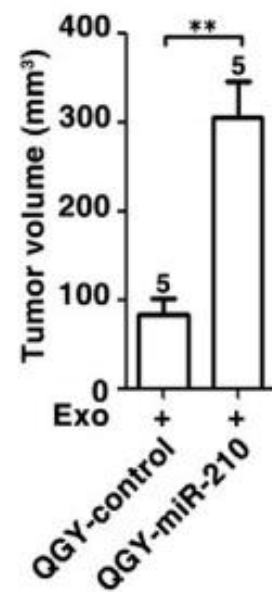
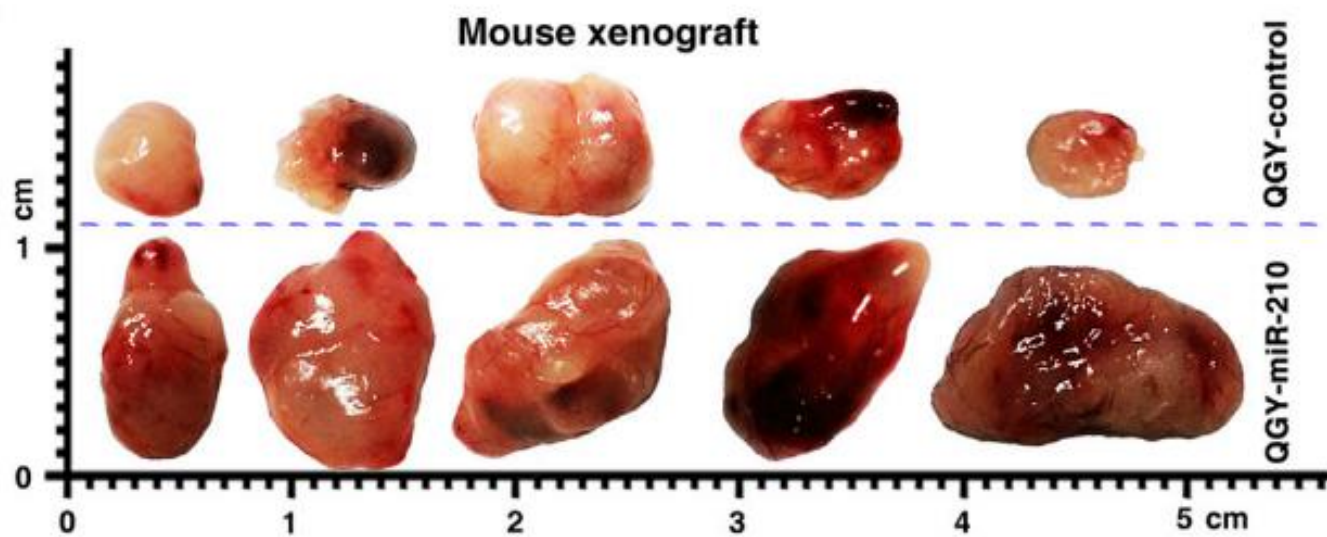
体内基质胶塞实验验证肝癌细胞外泌体miR-210促进内皮细胞血管生成

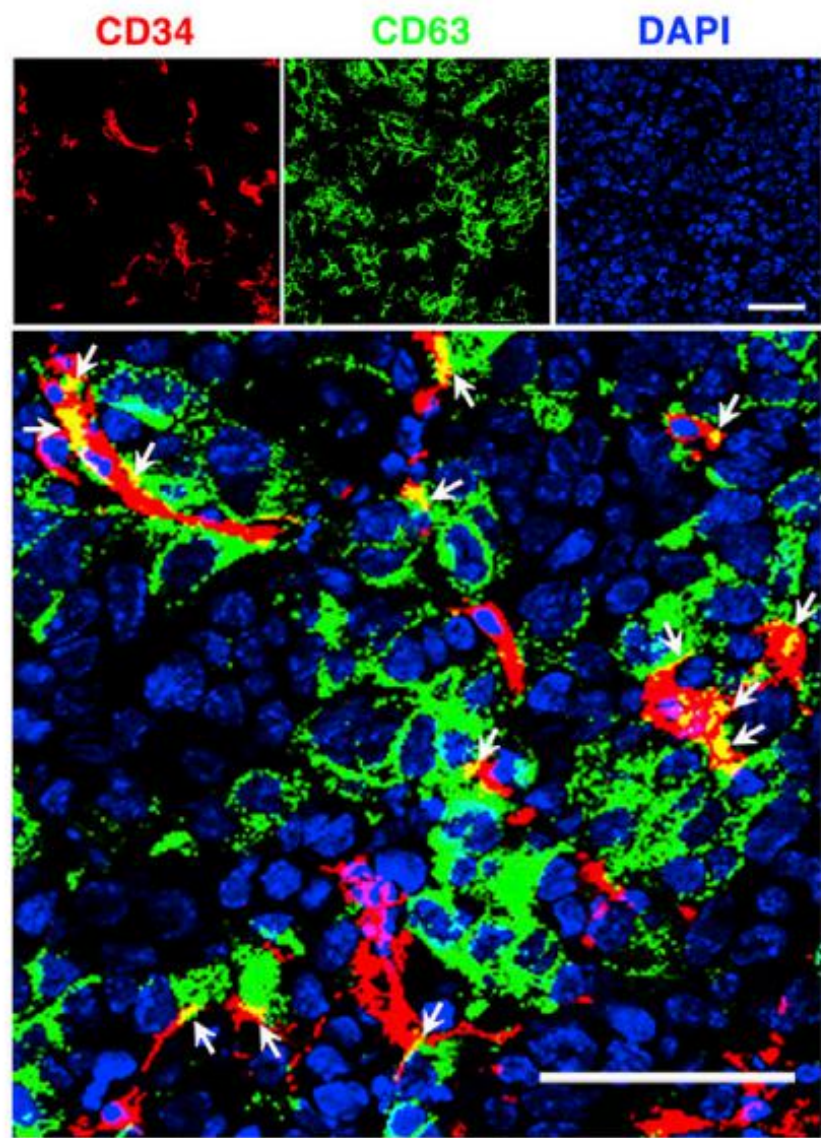


B Mouse xenograft

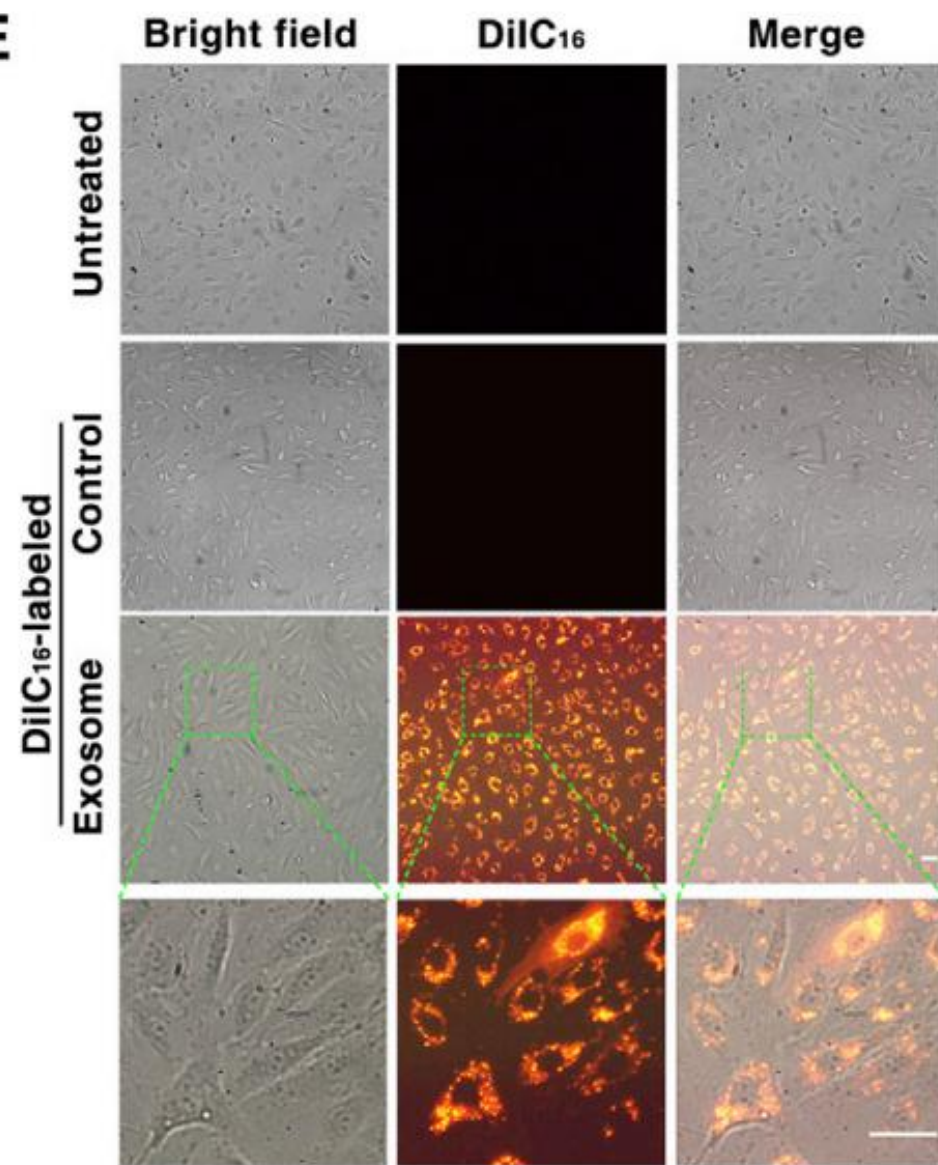


C





E

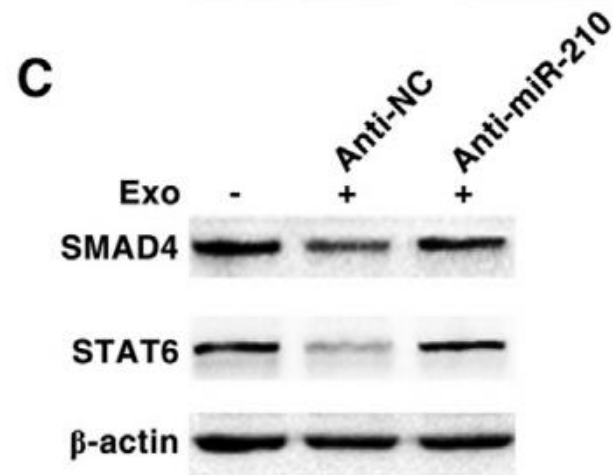
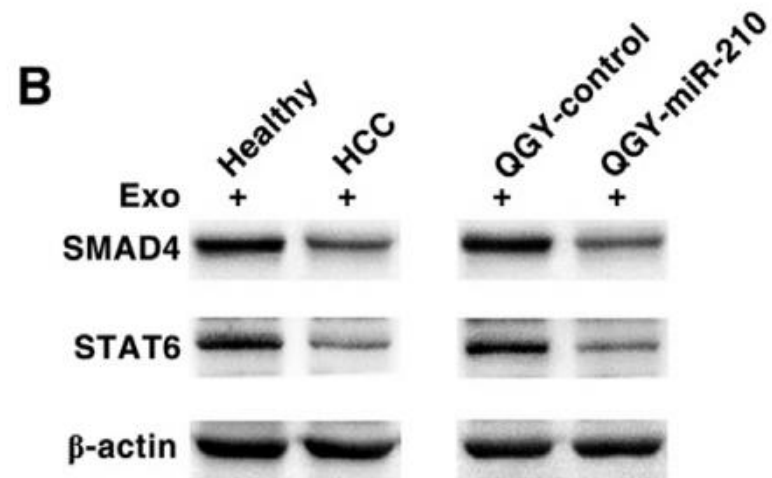
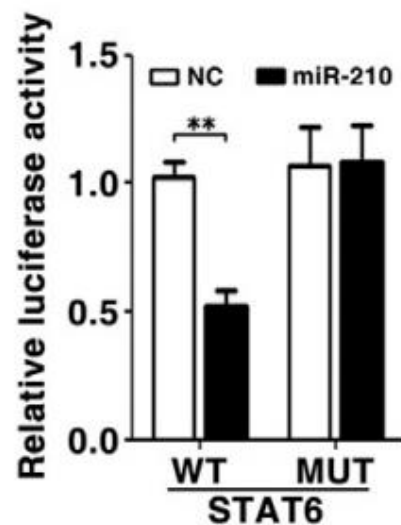
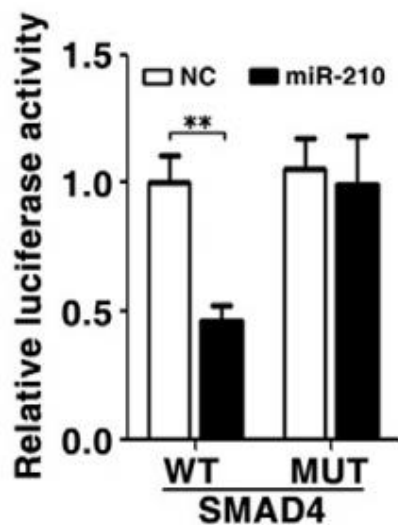
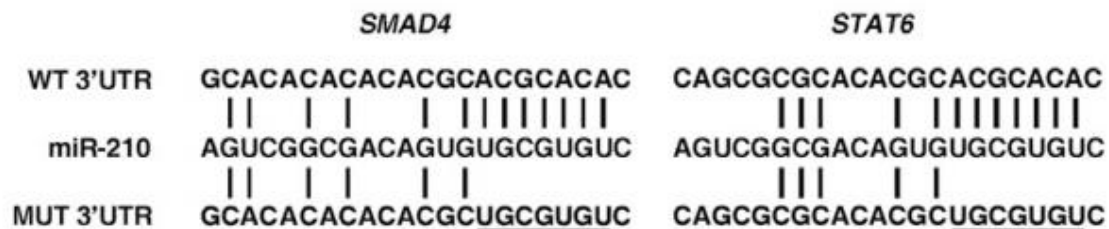




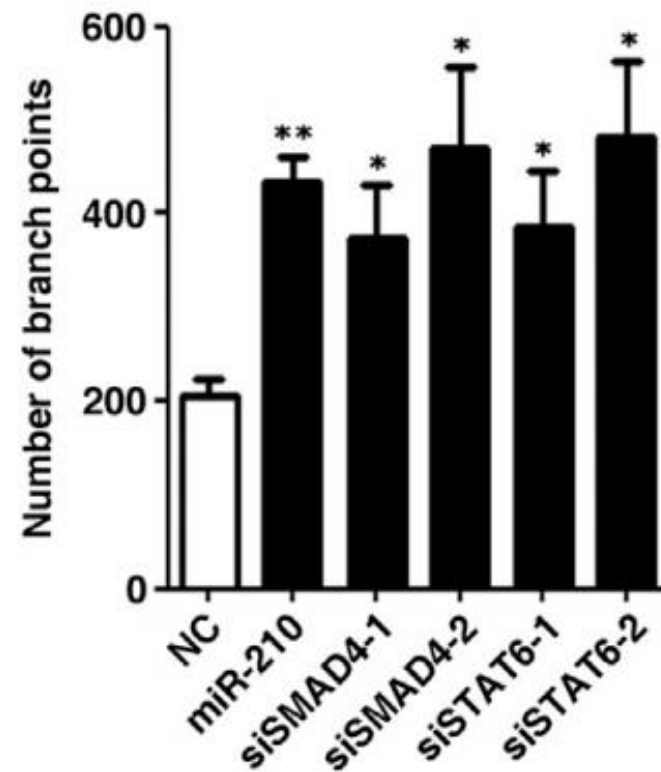
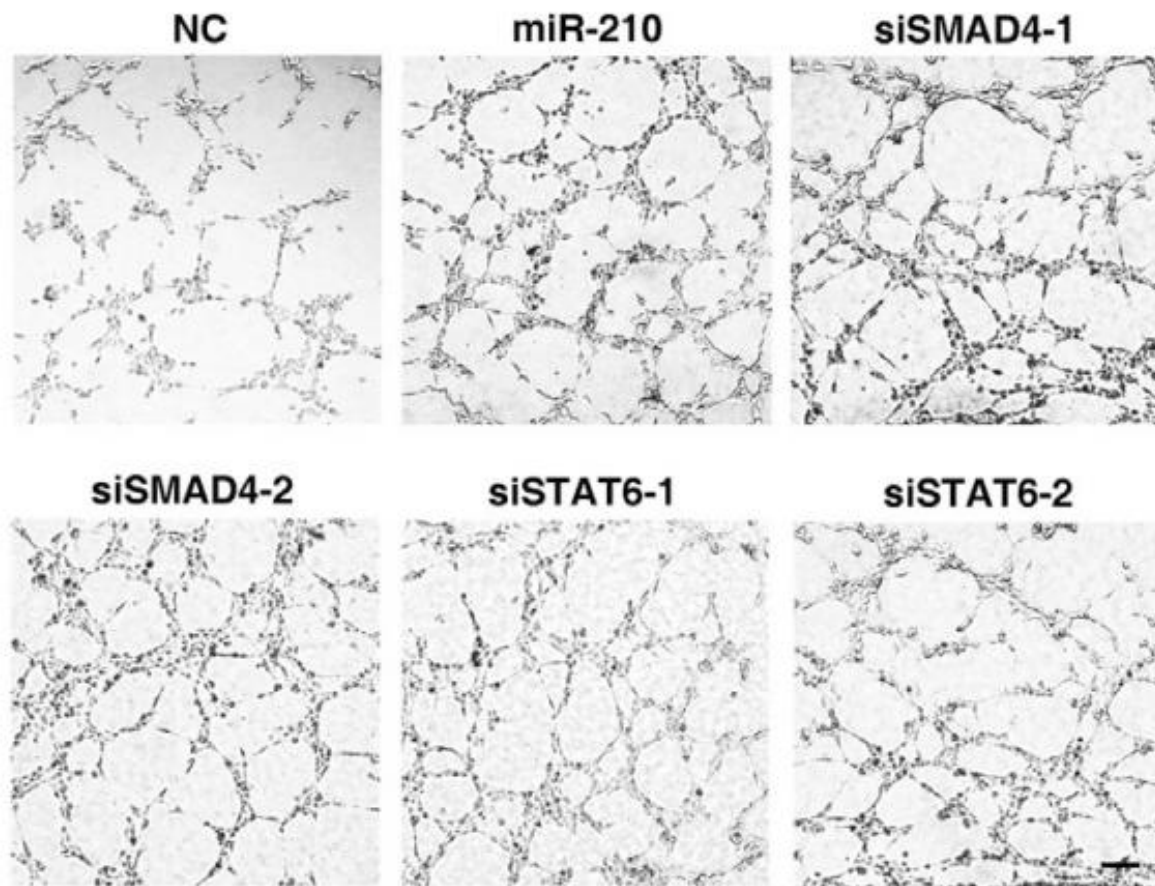
小结

肝癌细胞分泌带有miR-210的外泌体可能被内皮细胞摄取，并在体内促进血管生成。

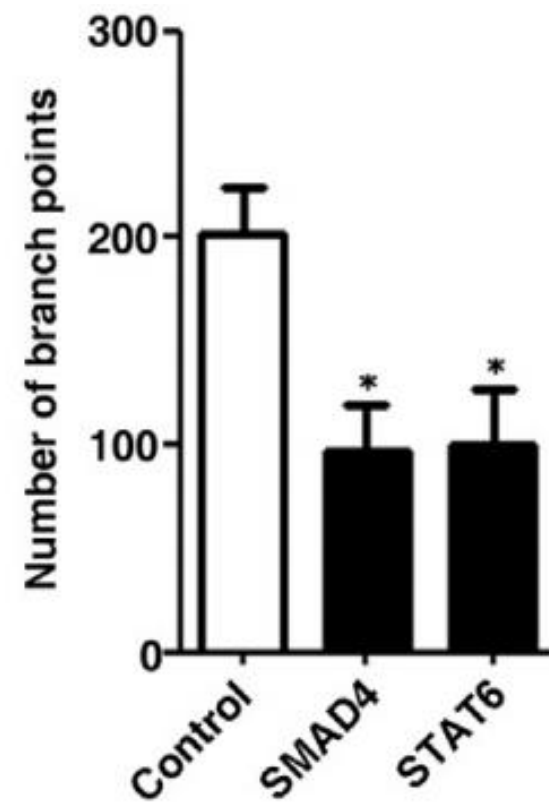
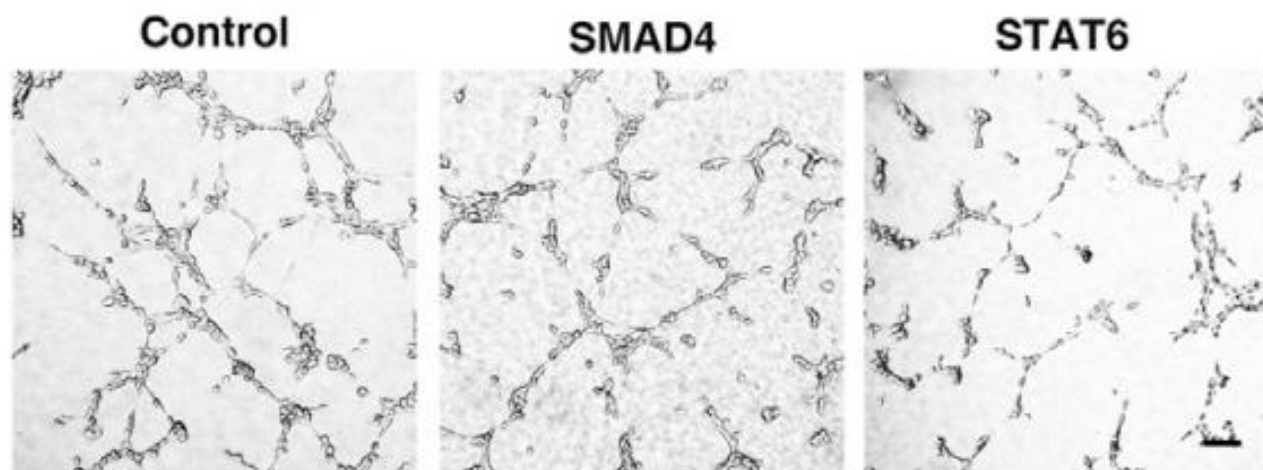
验证SMAD4和STAT6是miR-210的靶基因



敲除SMAD4和STAT6促进血管生成



过表达SMAD4和STAT6促进血管生成





小结

肝癌细胞的外泌体miR-210可能通过抑制内皮细胞的smad 4和stat 6来促进肿瘤血管生成。



04

总结分析





总结：

- 肝癌细胞分泌的miR-210参与血管生成，可以被内皮细胞摄取（体内和体外），从而通过靶向SMAD4和STAT6发挥促进血管生成的作用。
- 肝癌细胞衍生的外泌体可以将miRNAs传递到受体细胞中，从而促进肝癌细胞的生长、迁移和侵袭。



讨论

- 19种miRNAs在肝癌患者的血清中显著增加。然而，只有mir-29a、mir-29c、mir-145、miR-192和miR-210在所有四种肝癌细胞系的条件培养基和外泌体中都能检测到，而其余的miRNAs是未知的。



THANKS YOU!!!

