



读书报告

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Exosomes from Adipose-Derived Stem Cells Promotes VEGF-C-Dependent Lymphangiogenesis by Regulating miRNA-132/TGF- β Pathway

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01

研究背景





淋巴管生成在成人组织炎症、伤口愈合和肿瘤转移等病理过程中发生，它也是肠道、皮肤、心脏和气道炎症过程的一个公认特征。在急性和慢性炎症环境中，淋巴管生成可促进组织水肿的解决和炎症细胞的动员。相反，淋巴管生成也会加重免疫疾病，如移植后的排斥反应。因此，淋巴管生成反应在病理条件下可能是有害的，也可能是有益的，这取决于基础疾病的病理生理学。



血管内皮生长因子-C(VEGF-C)是最强大的淋巴管生成因子。淋巴管生成是通过VEGF-C与VEGFR-3的结合而介导的。有研究表明VEGF-C过表达诱导淋巴管新生，减轻原发性淋巴水肿小鼠模型的水肿程度。此外，IBD动物模型中的抗淋巴管治疗也被证明会加重炎症。



02

材料方法





1. 细胞增殖评估

LECs在96孔板中，每孔细胞数约为 $2\sim 5 \times 10^4$ 个， $100\mu\text{L}$ EGM-2-MV培养。去除培养基后，用PBS洗涤3次，用 $100\mu\text{L}$ EBM-2作阴性对照或指示试剂(外泌子，miR-132或LY 2109761)。450 nm处用细胞计数kit-8检测吸光值。

2. 细胞迁移试验

经指示试剂处理后，将悬浮于无血清培养基中的 2×10^5 个LECs细胞转入上腔内进行迁移试验，将添加10%FBS的培养基放入下腔内。通过膜向下表面迁移的细胞在48h后固定、染色和计数。



3.管形成试验

基质凝胶(Matrigel)放置在24孔板中37°C, 30min。将 2×10^5 个LECs接种到24孔包模板中, 在5%CO₂浓度下, 加入500 μL的EBM-2, 含指示试剂的EBM-2在37°C下培养24h。在光学显微镜下观察并拍照。

4.荧光素酶报告实验

构建了pGL3-Smad7-3'-UTR(野生型)或pGL3-Smad7-3'-UTR(突变型)重组质粒。40nM的miR-132或对照miRNA共转染, 2 ng 的 pRL-TK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HepG 2细胞。



03

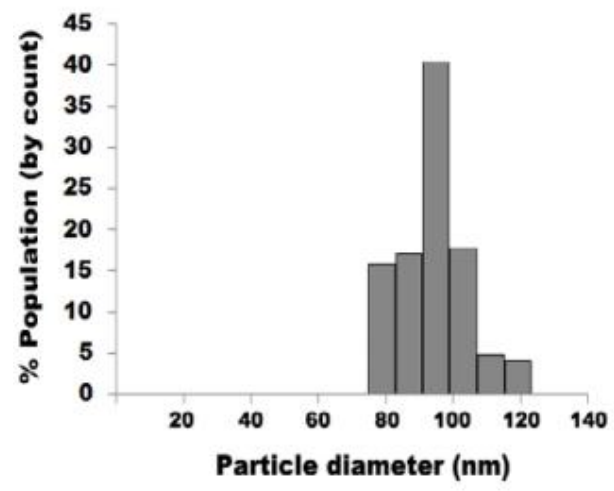
研究结果



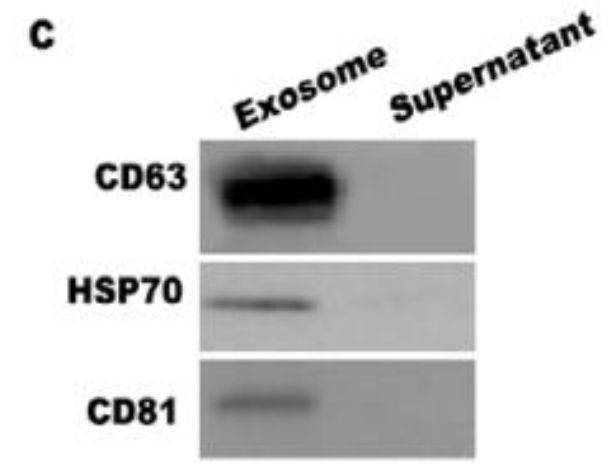
ADSCs/VEGF-C外泌体



透射电镜



NTA

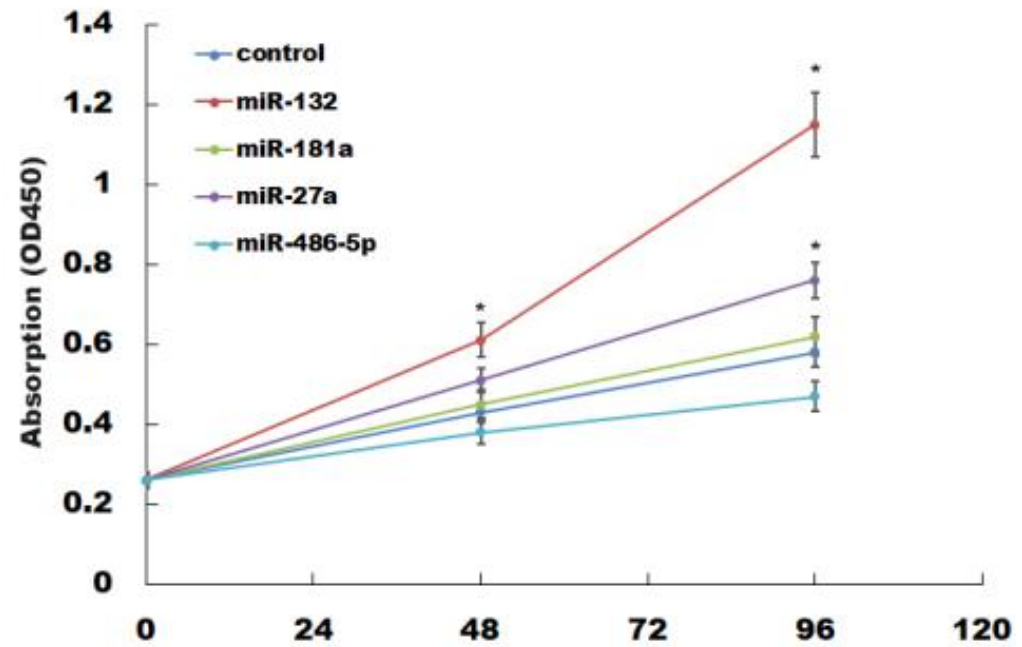


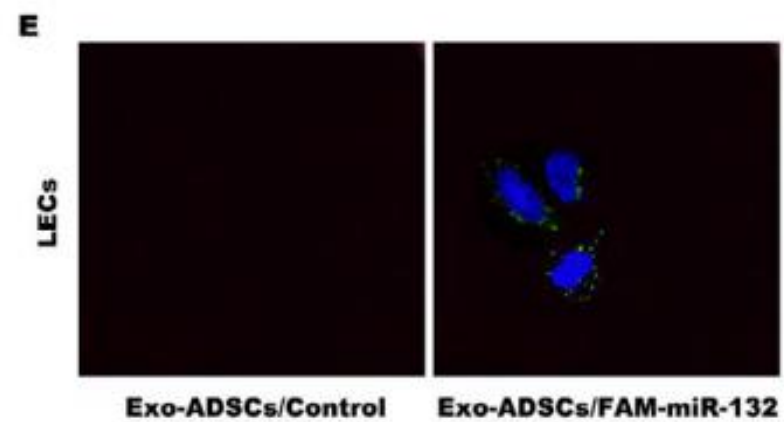
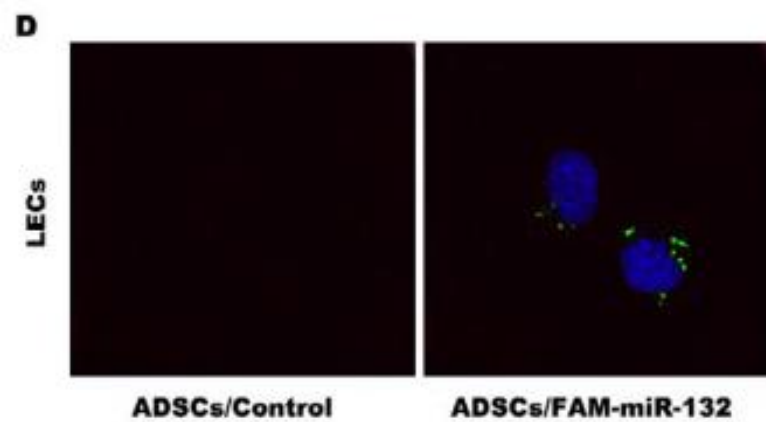
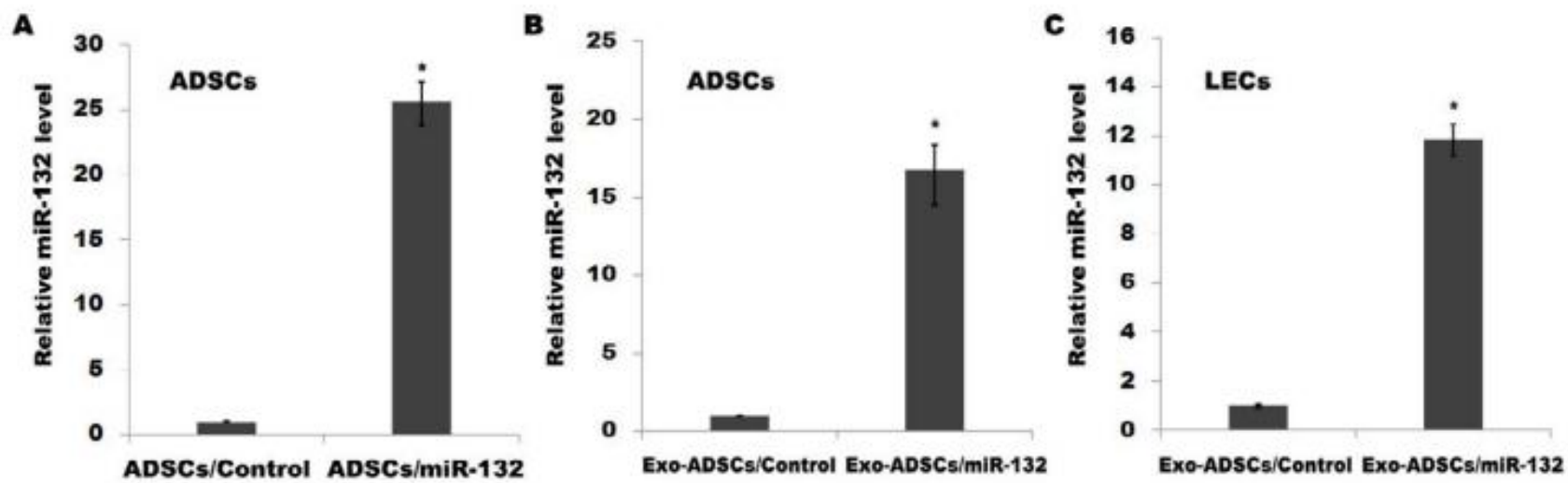
WB

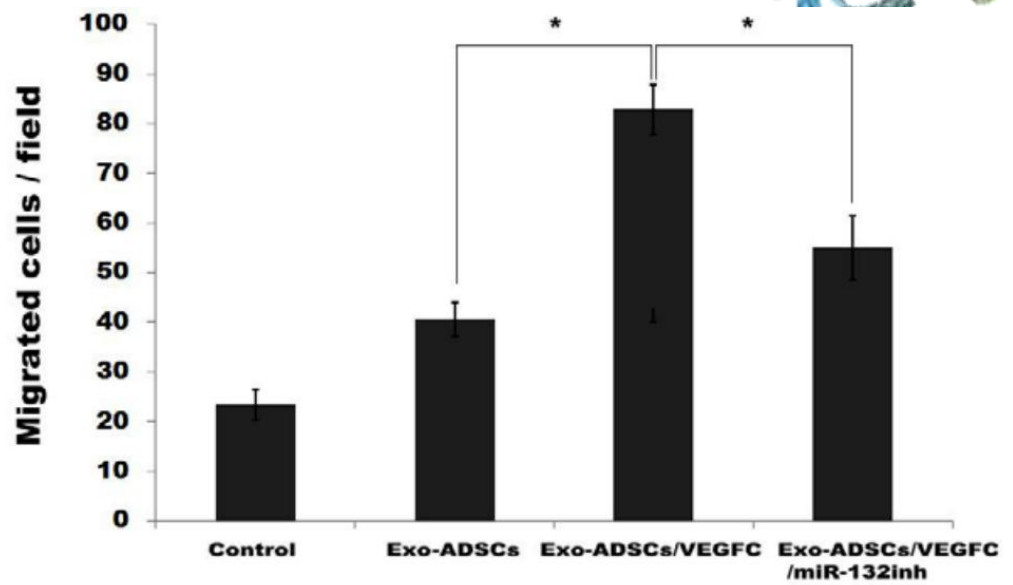
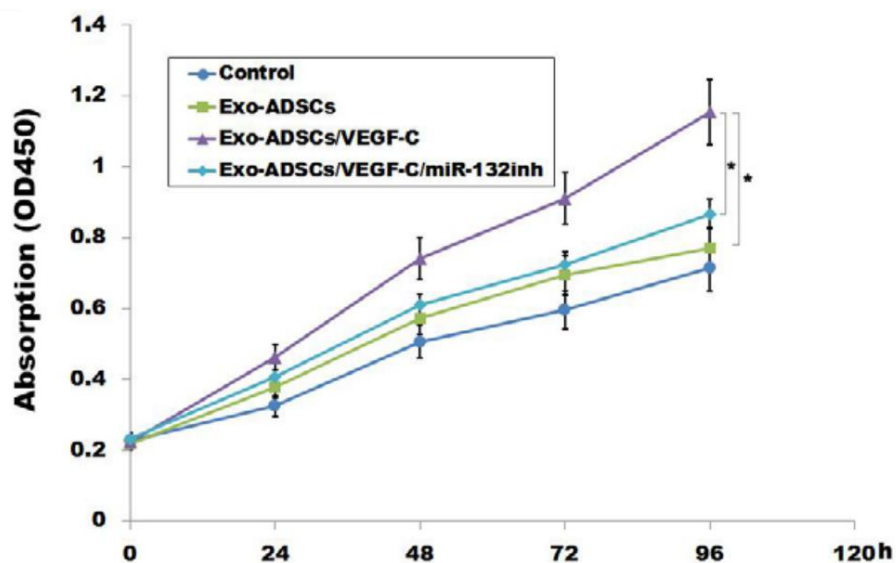


	Exo/ADSC control	Exo/ADSC+VEGFC	Up or Down
miR-126	153.9508	101.71	DOWN
	124.2514	111.3253	
miR-132	101.4858	118.9297	UP
	6.020987	17.15981	
	5.038304	16.6152	
miR-9	5.528374	15.32034	
	5.528374	5.320338	
	8.987999	10.61512	
miR-1236	9.872763	12.17262	DOWN
	60.5865	38.34589	
	57.14203	35.47851	
miR-181a	56.6141	34.5948	UP
	37.66102	41.51627	
	39.47288	54.10103	
miR-31	40.27287	45.8273	
	112.674	131.2645	
	114.281	134.393	
miR-466	115.8297	125.5152	
	1.641713	2.091984	
	2.748301	2.802094	
miR-146a	1.857362	3.110209	
	1.902637	1.128296	
	1.702726	1.030202	
miR-146b	1.826356	1.100204	
	4.902637	3.128296	
	5.702726	4.030202	
miR-155	4.826356	5.100204	DOWN
	91.51627	37.66102	
	94.10103	39.47288	
miR-221	95.8273	40.27287	
	0.632529	1.016558	
	0.741817	1.229374	
miR-222	0.600063	0.637263	
	0.832529	1.023514	
	0.784652	1.115246	
miR-27a	0.686587	0.965874	UP
	112.674	171.2645	
	114.281	174.393	
miR-486-5p	115.8297	175.5152	UP
	3.021657	13.05356	
	3.602155	15.33022	
miR-93	3.852031	17.03127	
	28.60254	18.81746	
	28.03936	27.81728	
	26.83836	17.01827	

15种与淋巴管生成有关的miRNA





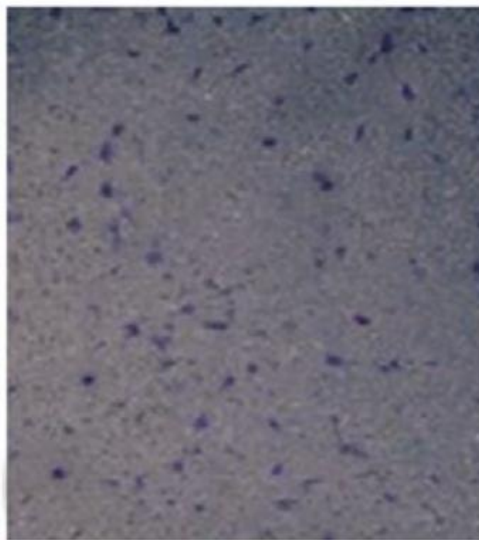
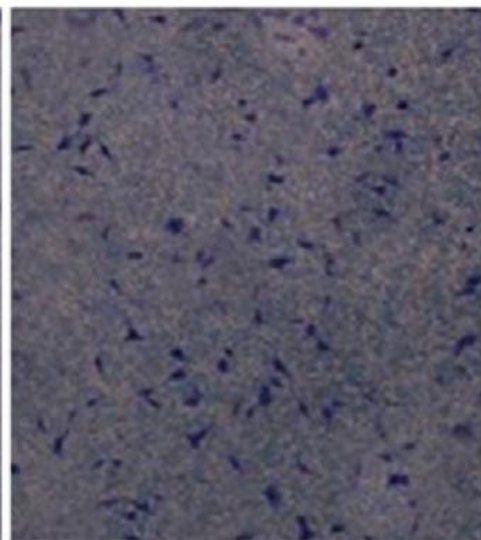
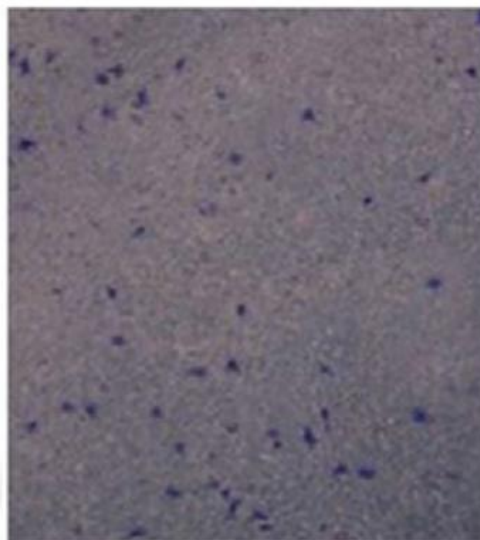


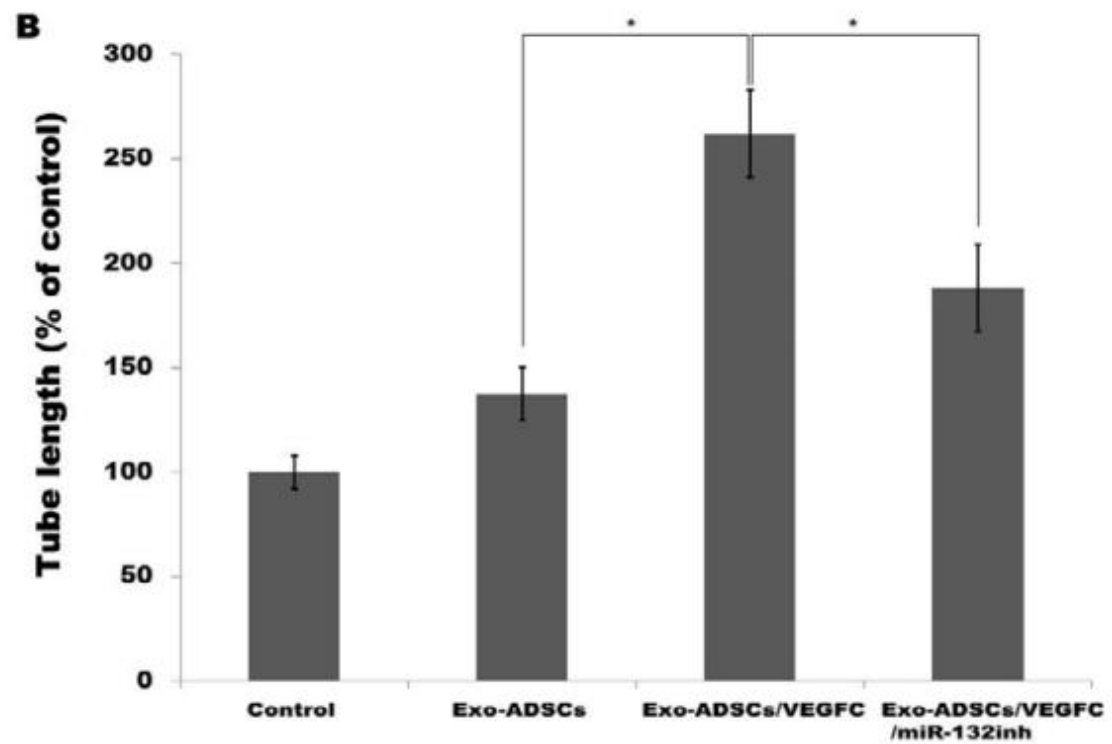
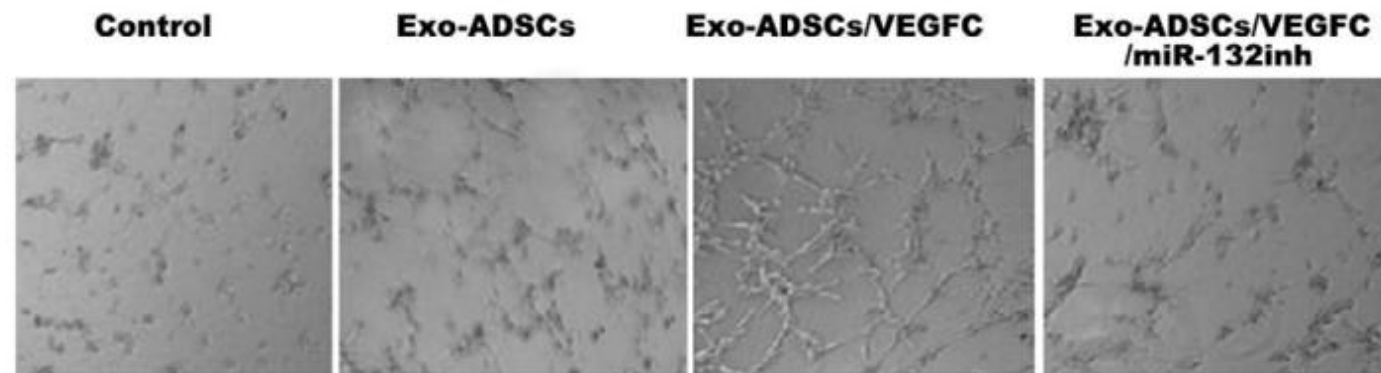
Control

Exo-ADSCs

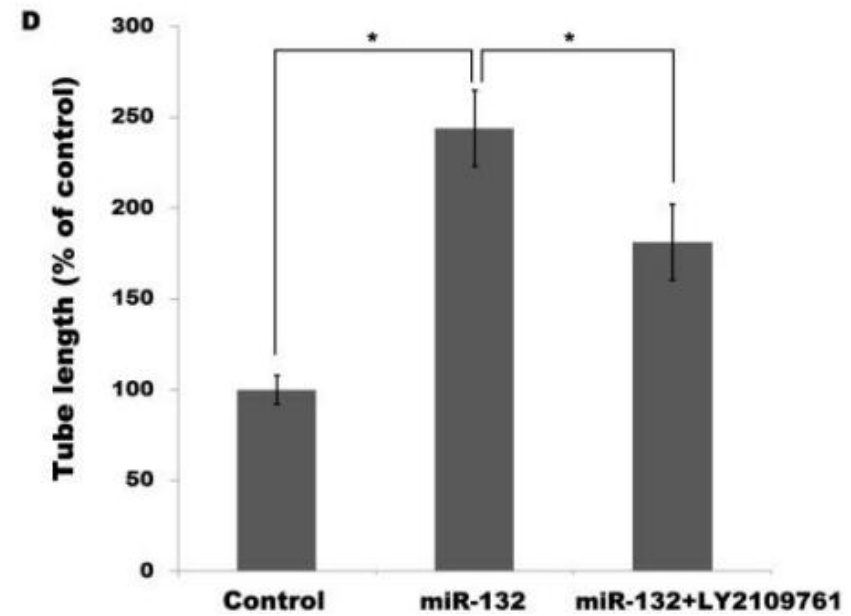
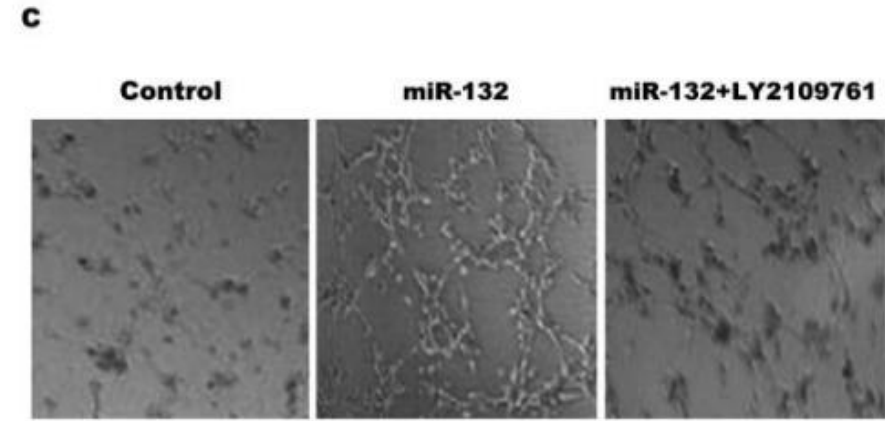
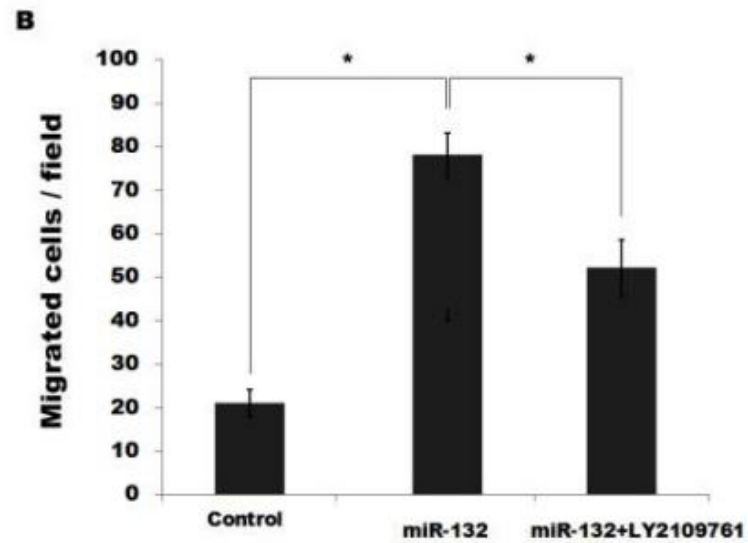
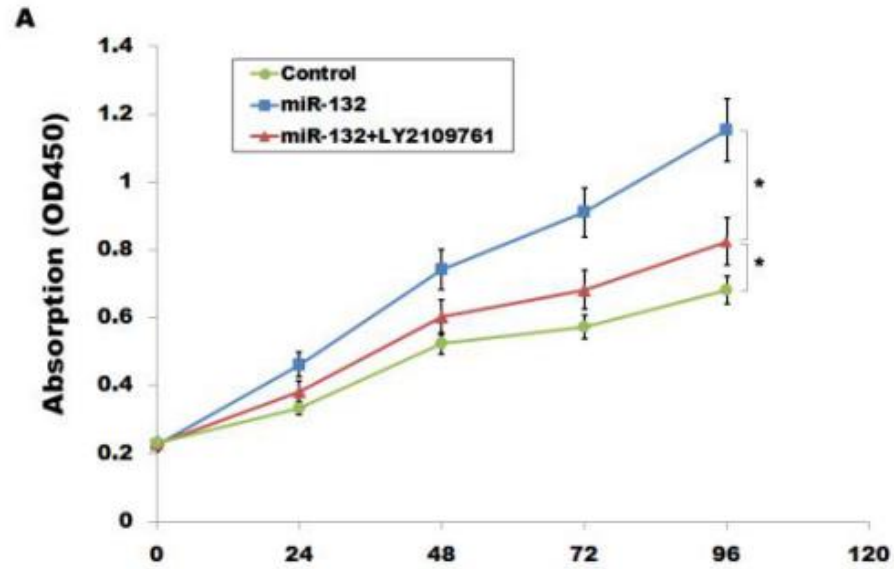
Exo-ADSCs/VEGFC

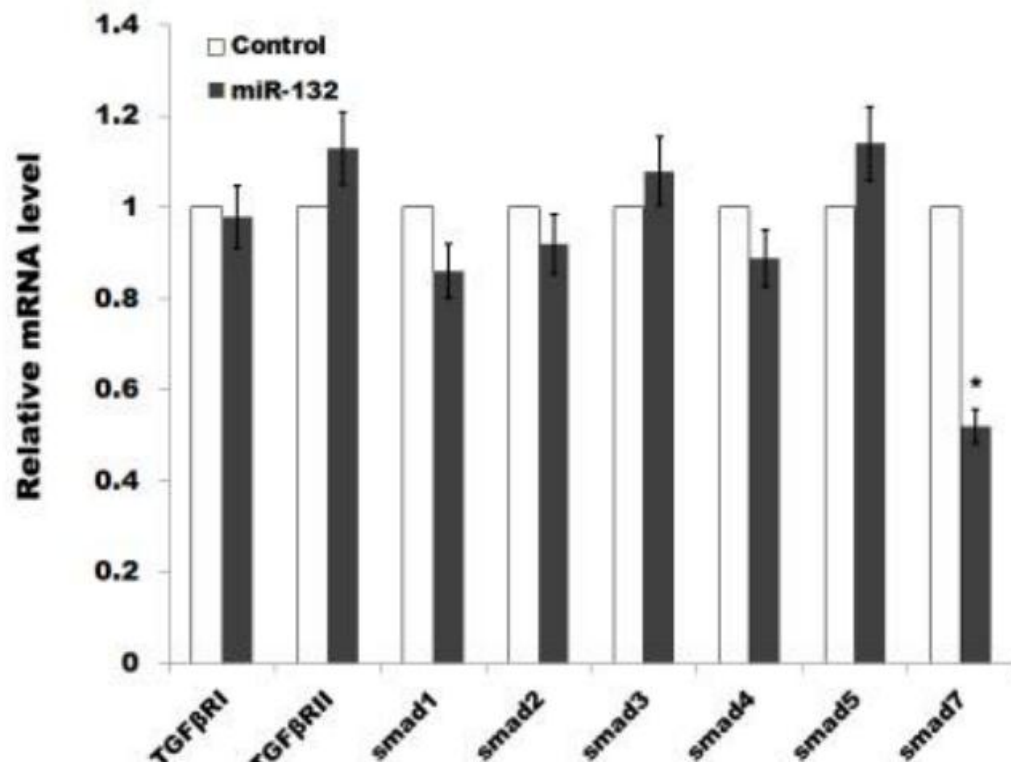
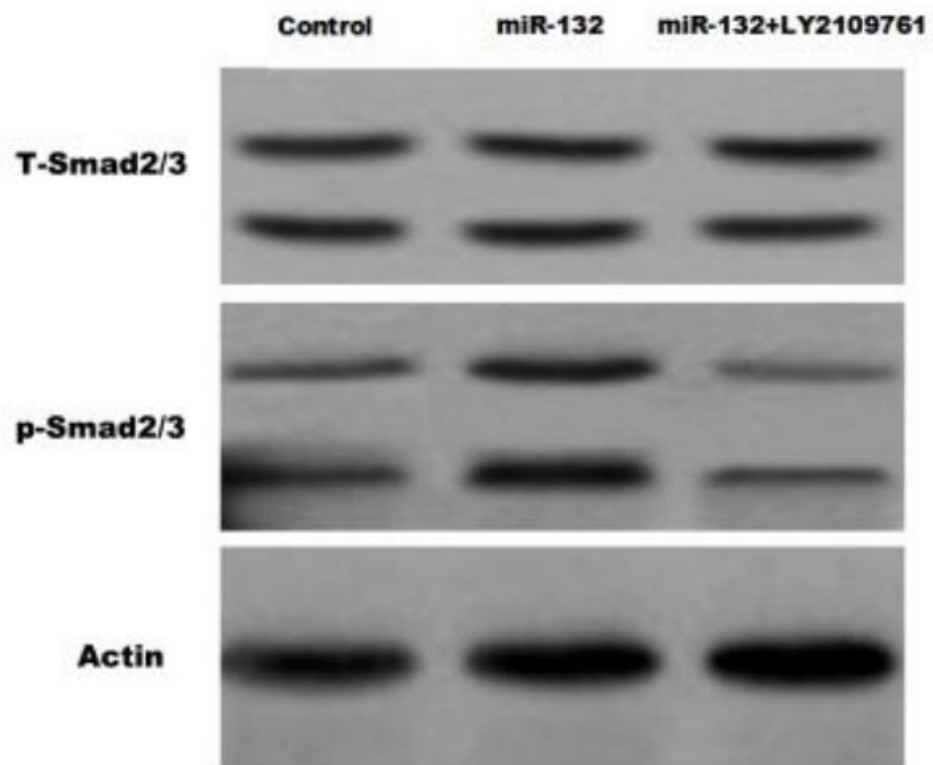
Exo-ADSCs/VEGFC/miR-132inh

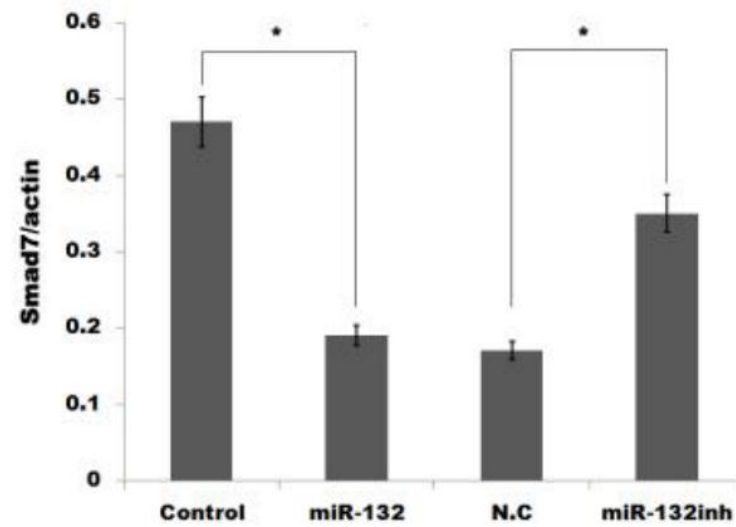
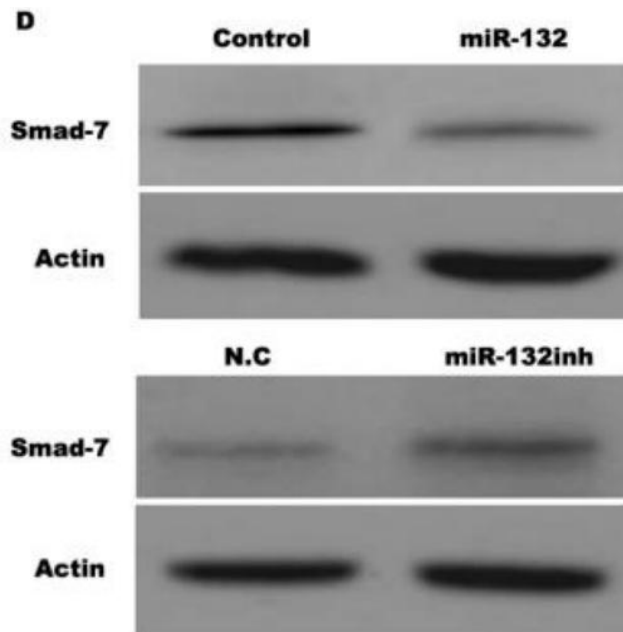
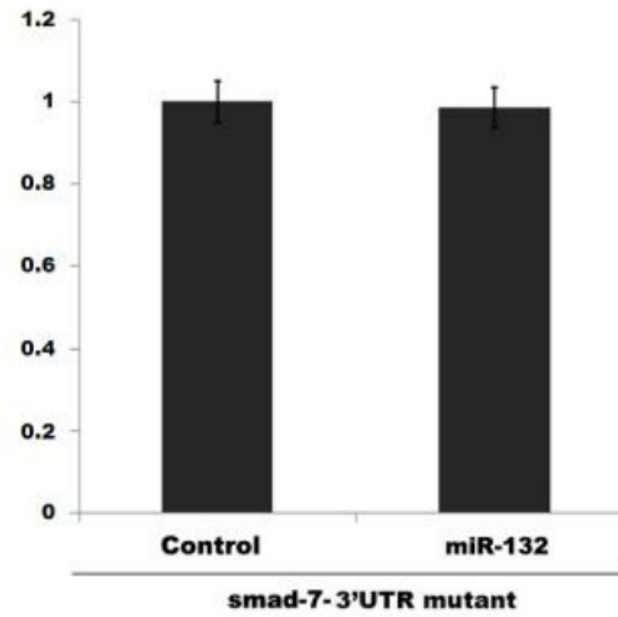
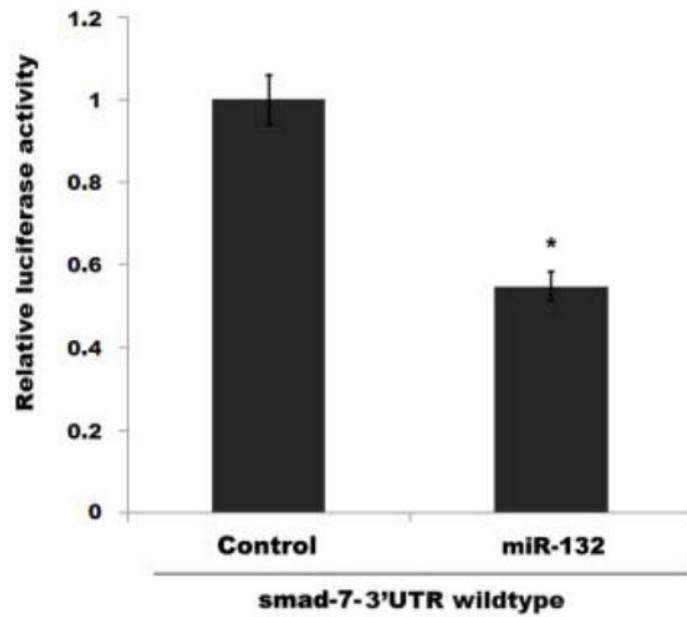




LY2109761是TGF- β /Smad的特异性抑制剂





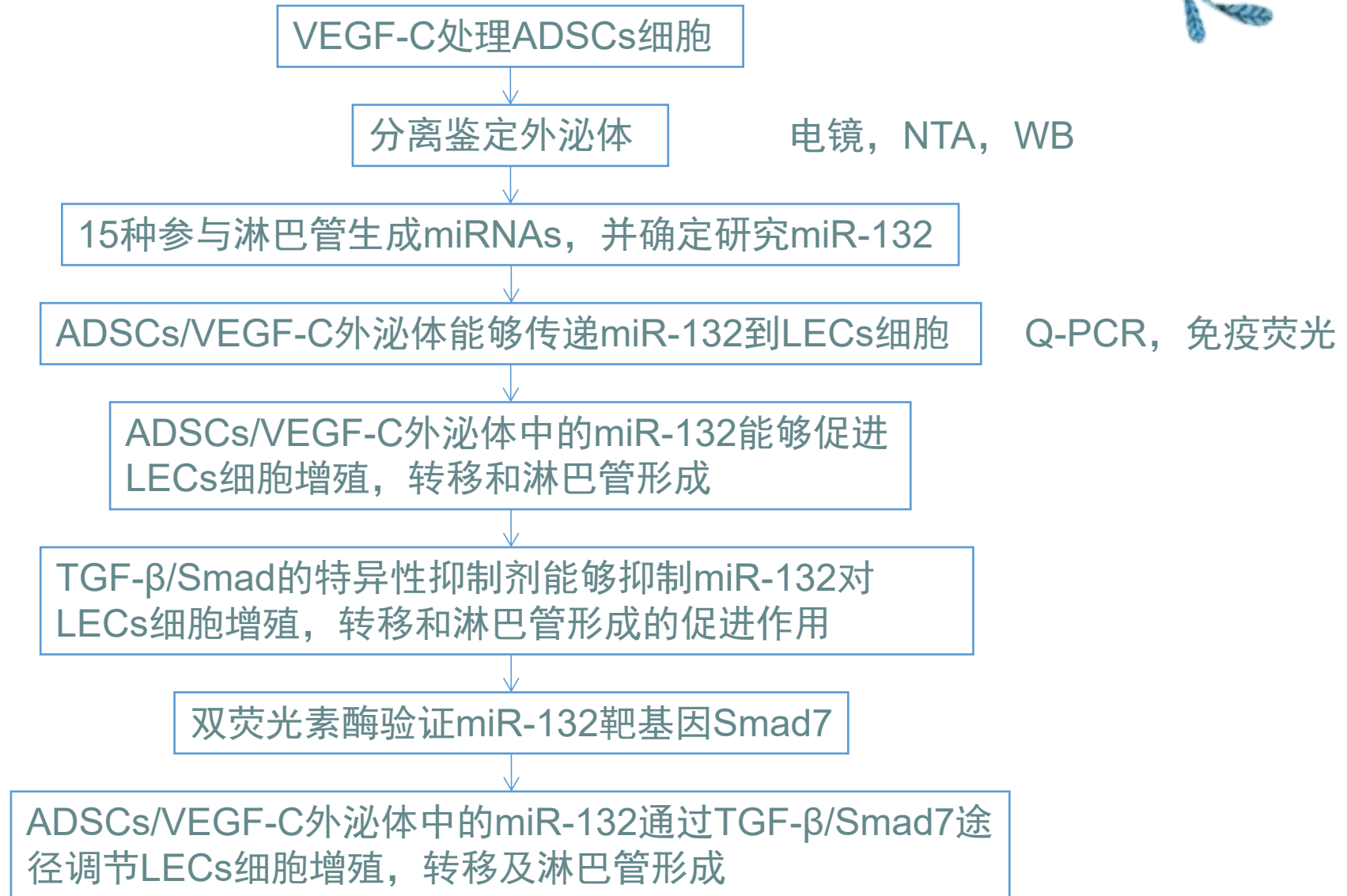




04

总结







THANKS YOU!!!

