lncRNA DBH-AS1通过靶向miR-1291影响婴幼儿血管瘤内皮细胞增殖和凋亡

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摘要 该文旨在探讨长链非编码RNA(lncRNA) DBH-AS1对婴幼儿血管瘤内皮细胞(HemECs)增殖和凋亡的影响及可能机制。收集49个婴幼儿的血管瘤组织及瘤旁正常皮下组织, 利用qRT-PCR检测血管瘤组织及瘤旁正常皮下组织中DBH-AS1和miR-1291的表达情况, 通过Pearson相关性分析评价婴幼儿血管瘤患者血管瘤组织中DBH-AS1和miR-1291表达水平的相关性。CCK-8法和克隆形成实验检测细胞增殖情况, 流式细胞术检测细胞凋亡情况, Western blot检测增殖、凋亡相关蛋白的表达情况。血管瘤组织中DBH-AS1的表达水平高于瘤旁正常皮下组织(P<0.05), 而miR-1291表达水平低于瘤旁正常皮下组织(P<0.05), Pearson相关性分析结果显示, 血管瘤组织中DBH-AS1表达水平与miR-1291表达水平呈负相关(r=–0.887, P<0.01)。与si-NC组或miR-NC组比较, si-DBH-AS1组和miR-1291组细胞增殖活性和Ki-67、PCNA蛋白表达水平降低(P<0.05), 细胞凋亡率和cleaved-caspase9、cleaved-caspase3蛋白表达水平升高(P<0.05)。DBH-AS1可靶向结合miR-1291, 且si-DBH-AS1组HemECs中miR-1291表达水平高于si-NC组(P<0.05)。下调miR-1291逆转干扰DBH-AS1对HemECs增殖和凋亡的作用(P<0.05)。干扰DBH-AS1表达可阻碍HemECs增殖, 并促进细胞凋亡, 其可能通过负调控miR-1291发挥作用。

关键词 血管瘤; DBH-AS1; miR-1291; 细胞增殖; 凋亡

lncRNA DBH-AS1 Affects the Proliferation and Apoptosis of Infantile Hemangioma Endothelial Cells by Targeting miR-1291

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Abstract This study aims to investigate the effect of lncRNA (long non-coding RNA) DBH-AS1 on the proliferation and apoptosis of infantile HemECs (hemangioma endothelial cells) and its possible mechanism. Hemangioma tissue and normal subcutaneous tissue adjacent to tumor in 49 infants were collected. qRT-PCR was used to detect the expression of DBH-AS1 and miR-1291 in hemangioma tissue and adjacent normal subcutaneous tissue, and Pearson correlation analysis was used to evaluate the correlation between the expression levels of DBH-AS1 and miR-1291 in hemangioma tissues of infant hemangioma patients. Cell proliferation was detected by CCK-8 assay and clonal formation assay. Cell apoptosis was detected by flow cytometry. The expressions of proliferation and apoptosis related proteins were detected by Western blot. Dual-luciferase reporter assay was used to verify the regulatory relationship between DBH-AS1 and miR-1291. The expres-

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sion of DBH-AS1 in hemangioma tissue was higher than that in adjacent normal subcutaneous tissue (P<0.05), while the expression of miR-1291 was lower than that in adjacent normal subcutaneous tissue (P<0.05). Pearson correlation analysis showed that the expression level of DBH-AS1 in hemangioma tissue was negatively correlated with that of miR-1291 (r=–0.887, P<0.01). Compared with the si-NC group or miR-NC group, the proliferation activity of HemECs and the protein expression levels of Ki-67 and PCNA in the si-DBH-AS1 group and miR-1291 group were reduced (P<0.05), but the apoptosis rate and the protein expression levels of cleaved-caspase9 and cleaved-caspase3 were increased (P<0.05). DBH-AS1 could target miR-1291, and the expression level of miR-1291 in HemECs in the si-DBH-AS1 group was higher than that in the si-NC group (P<0.05). Downregulation of miR-1291 reversed the effect of interfering DBH-AS1 on the proliferation and apoptosis of HemECs (P<0.05). Interference with DBH-AS1 expression inhibits HemECs proliferation and promotes apoptosis, possibly through the negative regulation of miR-1291.

Keywords hemangioma; DBH-AS1; miR-1291; cell proliferation; apoptosis

血管瘤是婴幼儿最常见的良性血管肿瘤, 其发生发展与血管瘤内皮细胞(hemangioma endothelial cells, HemECs)的异常增生有关, 但其分子机制尚不完全明确[1-2]。长链非编码RNA(long non-coding RNA, lncRNA)参与调控多种肿瘤细胞的恶性行为, 能够作为肿瘤治疗的分子靶点[3]。多巴胺β羟化酶反义RNA1(dopamine beta hydroxylase antisense RNA 1, DBH-AS1)是一种lncRNA, 在多种肿瘤中表达不同, 如在非小细胞肺癌中表达下调, 上调其表达可抑制体外非小细胞肺癌细胞增殖, 其在非小细胞肺癌中发挥抑癌基因作用[4]。DBH-AS1在弥漫性大B细胞淋巴瘤、骨肉瘤、和肝癌等肿瘤中过表达, 发挥促癌基因作用[5-7]。而DBH-AS1对婴幼儿血管瘤的影响未知。靶基因预测显示, DBH-AS1可能与miR-1291存在靶向关系, miR-1291在前列腺癌[8]、肾癌[9]等肿瘤中表达量降低, 过表达miR-1291可抑制肿瘤细胞的恶性表型, miR-1291起抑癌基因作用。目前miR-1291对婴幼儿血管瘤的影响还未知。本研究首先检测了婴幼儿血管瘤组织中DBH-AS1和miR-1291的表达水平, 观察了DBH-AS1和miR-1291对HemECs增殖和凋亡的影响及DBH-AS1能否通过靶向miR-1291而发挥作用。

1 材料与方法

1.1 临床资料

取2018年5月至2020年5月于本院行手术切除术的49例婴幼儿的血管瘤组织及瘤旁正常皮下组织。其中男18例, 女31例, 年龄3个月~5岁, 平均年龄(3.06±0.85)岁。本研究经我院伦理委员会批准通过(批准号: 20171226), 所有样品采集均取得受试者监护人知情同意。

1.2 细胞和试剂

人HemECs(批号: 1067-74-9)购自上海弘顺生物科技有限公司; EGM-2MV培养液购自瑞士Lonza公司; 逆转录试剂盒、RNA抽提试剂盒、PCR试剂盒均购自宝生物工程(大连)有限公司; 凋亡试剂盒、BCA试剂盒、Lipofectamine™ 2000试剂盒、双荧光素酶试剂盒均购自北京索莱宝科技有限公司; 胎牛血清(fetal bovine serum, FBS)购自浙江天杭生物科技有限公司; 兔抗人Ki-67、PCNA、cleaved-caspase3、cleaved-caspase9、GAPDH抗体, 山羊抗兔二抗均购自英国Abcam公司。

1.3 方法

1.3.1 qRT-PCR检测DBH-AS1、miR-1291的表达情况 将样本组织在液氮中充分研磨, 用RNA抽提试剂盒提取组织中的总RNA, 将其逆转录为cDNA, 行PCR扩增。反应体系(20 μL): 9.0 μL SYBR mix、0.5 μL正向引物、0.5 μL反向引物、2.0 μL cDNA模板、8.0 μL RNase free dH_2O。反应条件: 95 °C预变性10 min; 95 °C变性15 s, 60 °C退火1 min, 共40个循环; 72 °C延伸1 min。DBH-AS1上游引物5′-CTT GTT GCA GTG CAT GGA G-3′, 下游引物5′-CTT CGG GAT CCT CTG TTC C-3′; miR-1291上游引物5′-ACA CTC CAG CTG GGT GGC CCT GAC TGA AGA CC-3′, 下游引物5′-TGG TGT CGT GGA GTC G-3′。甘油醛-3-磷酸脱氢酶(glyceraldehyde-3-phosphate dehydrogenase, *GAPDH*)上游引物5′-GGA AAG CTG TGG CGT GAT-3′, 下游引物5′-AAG GTG GAA GAA TGG GAG TT-3′; *U6*上游引物

5′-CTC GCT TCG GCA GCA CAT ATA CT-3′, 下游引物5′-ACG CTT CAC GAA TTT GCG TGT C-3′。*GAPDH*(用于DBH-AS1)和*U6*(用于miR-1291)为内参。用$2^{-\Delta\Delta Ct}$法计算DBH-AS1、miR-1291的表达量。

1.3.2 细胞培养和转染 将血管瘤内皮细胞在含10% FBS的EGM-2MV培养液中培养。将细胞接种于6孔板并培养24 h, 用Lipofectamine™ 2000脂质体法, 转染si-DBH-AS1(si-DBH-AS1组)、si-NC(si-NC组)、miR-1291 mimics(miR-1291组)、miR-NC(miR-NC组), 共转染si-DBH-AS1与anti-miR-1291(si-DBH-AS1+anti-miR-1291组)、si-DBH-AS1与anti-miR-NC(si-DBH-AS1+anti-miR-NC组); 另取正常培养的血管瘤内皮细胞为对照组(Con组)。

1.3.3 CCK-8检测细胞增殖情况 将各组细胞接种于96孔板, 在37 ℃下培养24 h, 向每孔中加入10 μL CCK-8溶液, 继续在37 ℃下孵育2 h, 酶标仪测量各孔在450 nm波长处的光密度(*D*)值。

1.3.4 克隆形成实验 将各组细胞以每孔800个细胞的密度接种到6孔板中, 在37 ℃、5% CO_2细胞培养箱中培养14天。当克隆体肉眼可见时, 终止培养。弃去培养基, 加入4%多聚甲醛室温固定细胞15 min, 弃去固定液后, 加入结晶紫室温染色15 min。在显微镜下观察并计数细胞克隆数(每个克隆大于50个细胞)。

1.3.5 流式细胞术检测细胞凋亡情况 将各组细胞培养24 h, PBS洗涤后重悬于1× 结合缓冲液中。然后, 将细胞(5×10^5个细胞)在黑暗中用5 μL Annexin-V-FITC和5 μL PI染色15 min, 上流式细胞仪检测细胞凋亡情况。早期和晚期细胞凋亡百分比之和(右下象限+右上象限)为细胞凋亡率。

1.3.6 Western blot检测细胞中Ki-67、cleaved-caspase9和cleaved-caspase3的表达情况 用RIPA试剂提取各组细胞的总蛋白, BCA法检测蛋白浓度, SDS-PAGE分离等量蛋白样品, 然后转移到聚偏二氟乙烯膜上, 将膜用5%脱脂牛奶室温封闭1 h, 随后, 于4 ℃下将膜与相应的Ki-67(1:1 000)、cleaved-caspase9(1:500)、cleaved-caspase3(1:500)和GAPDH(1:1 000)一抗孵育过夜, 用山羊抗兔二抗(1:1 000)室温孵育2 h。加显影液, 避光显影、拍照, ImageJ软件分析目的蛋白的表达量。

1.3.7 双荧光素酶报告基因实验 用starBase数据库(http://starbase.sysu.edu.cn/)预测DBH-AS1与miR-1291的互补位点。用PCR技术扩增含miR-1291结合位点的DBH-AS1核苷酸序列, 再将其插入pGL3空载体, 构建DBH-AS1野生型荧光素酶报告基因载体(WT-DBH-AS1); 同时将结合位点突变后的DBH-AS1核苷酸序列插入pGL3空载体, 构建DBH-AS1突变型荧光素酶报告基因载体(MUT-DBH-AS1), 该过程由上海生工生物工程有限公司完成。取6孔板, 每孔加2.5 mL血管瘤内皮细胞悬液(2.5×10^4个/mL), 培养24 h后, 用Lipofectamine™ 2000分别将WT-DBH-AS1与miR-1291 mimics或miR-NC、MUT-DBH-AS1与miR-1291 mimics或miR-NC共转染至血管瘤内皮细胞。转染48 h后, 收集并裂解细胞, 使用双荧光素酶报告基因分析系统检测荧光素酶活性, 结果以萤火虫与海肾的荧光强度比值表示。

1.4 统计学分析

用SPSS 22.0进行统计学分析。计量资料以均值±标准差($\bar{x}\pm s$)表示。两组间比较用*t*检验, 三组及以上的组间比较用单因素方差分析和LSD-*t*检验, 通过Pearson相关性分析评价婴幼儿血管瘤患者血管瘤组织中DBH-AS1和miR-1291表达水平的相关性。$P<0.05$表示差异有统计学意义。

2 结果

2.1 DBH-AS1、miR-1291在血管瘤组织中的表达情况

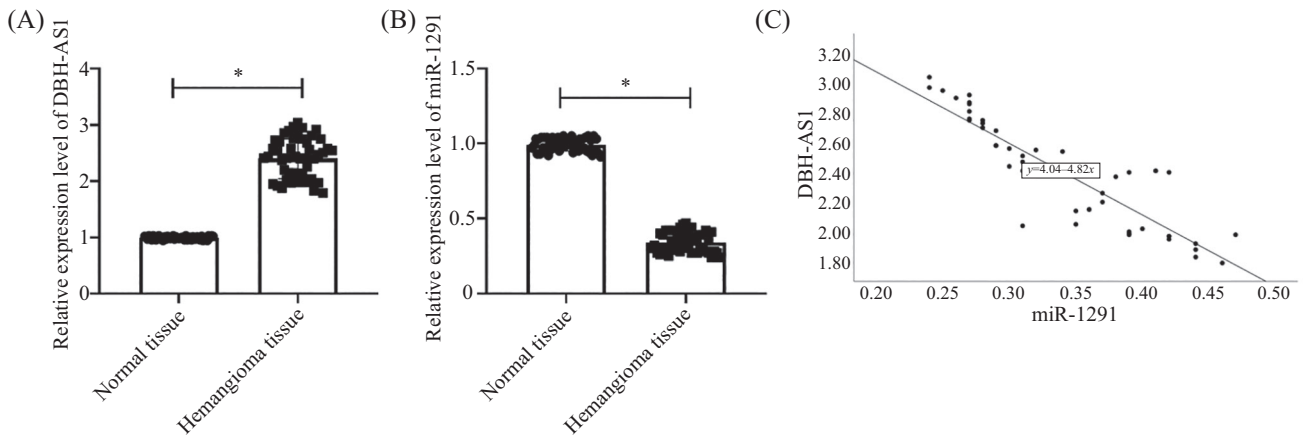
血管瘤组织中DBH-AS1表达水平高于血管瘤周围正常皮下组织, miR-1291表达水平低于正常皮下组织, 差异均具有统计学意义($P<0.05$, 图1A和图1B)。Pearson相关性分析结果显示, 血管瘤组织中DBH-AS1表达水平与miR-1291表达水平呈负相关($r=-0.887$, $P<0.01$, 图1C)。

2.2 干扰DBH-AS1表达对HemECs增殖的影响

si-DBH-AS1组HemECs中DBH-AS1表达水平低于Con组和si-NC组, 差异均具有统计学意义($P<0.05$), 说明si-DBH-AS1转染成功, si-DBH-AS1组HemECs中DBH-AS1表达受到干扰。si-DBH-AS1组HemECs增殖活性、克隆形成数和Ki-67、PCNA蛋白表达水平均低于Con组和si-NC组($P<0.05$, 图2)。该结果提示干扰DBH-AS1表达可抑制HemECs增殖。

2.3 干扰DBH-AS1表达对HemECs凋亡的影响

si-DBH-AS1组HemECs凋亡率和cleaved-caspase9、cleaved-caspase3蛋白表达水平均高于Con组和si-NC组($P<0.05$, 图3)。该结果提示干扰DBH-AS1表



A: DBH-AS1在血管瘤组织和血管瘤周围正常皮下组织中的表达水平; B: miR-1291在血管瘤组织和血管瘤周围正常皮下组织中的表达水平; C: 血管瘤组织中DBH-AS1表达水平与miR-1291表达水平的相关性。*P<0.05; n=49。

A: the expression level of DBH-AS1 in hemangioma tissue and normal subcutaneous tissue surrounding hemangioma; B: the expression levels of miR-1291 in hemangioma tissue and normal subcutaneous tissue surrounding hemangioma; C: correlation between the expression level of DBH-AS1 and miR-1291 in hemangioma tissue. *P<0.05; n=49.

图1 DBH-AS1、miR-1291在血管瘤组织中的表达水平及相关性分析

Fig.1 Expression and correlation analysis of DBH-AS1 and miR-1291 in hemangioma tissue

达可诱导HemECs凋亡。

2.4 DBH-AS1靶向调控miR-1291的表达

starBase数据库预测的DBH-AS1与miR-1291的结合位点见图4A。双荧光素酶结果显示, 与miR-NC和WT-DBH-AS1共转染组相比, WT-DBH-AS1与miR-1291 mimics共转染组的荧光素酶活性显著降低(P<0.05), 与miR-NC和MUT-DBH-AS1共转染组相比, MUT-DBH-AS1与miR-1291 mimics共转染组的荧光素酶活性没有显著差异(P>0.05, 图4B)。与si-NC组相比, si-DBH-AS1组HemECs中miR-1291的表达水平显著升高(t=25.821, P<0.05, 图4C)。该结果提示DBH-AS1可靶向负调节miR-1291的表达。

2.5 过表达miR-1291对HemECs增殖和凋亡的影响

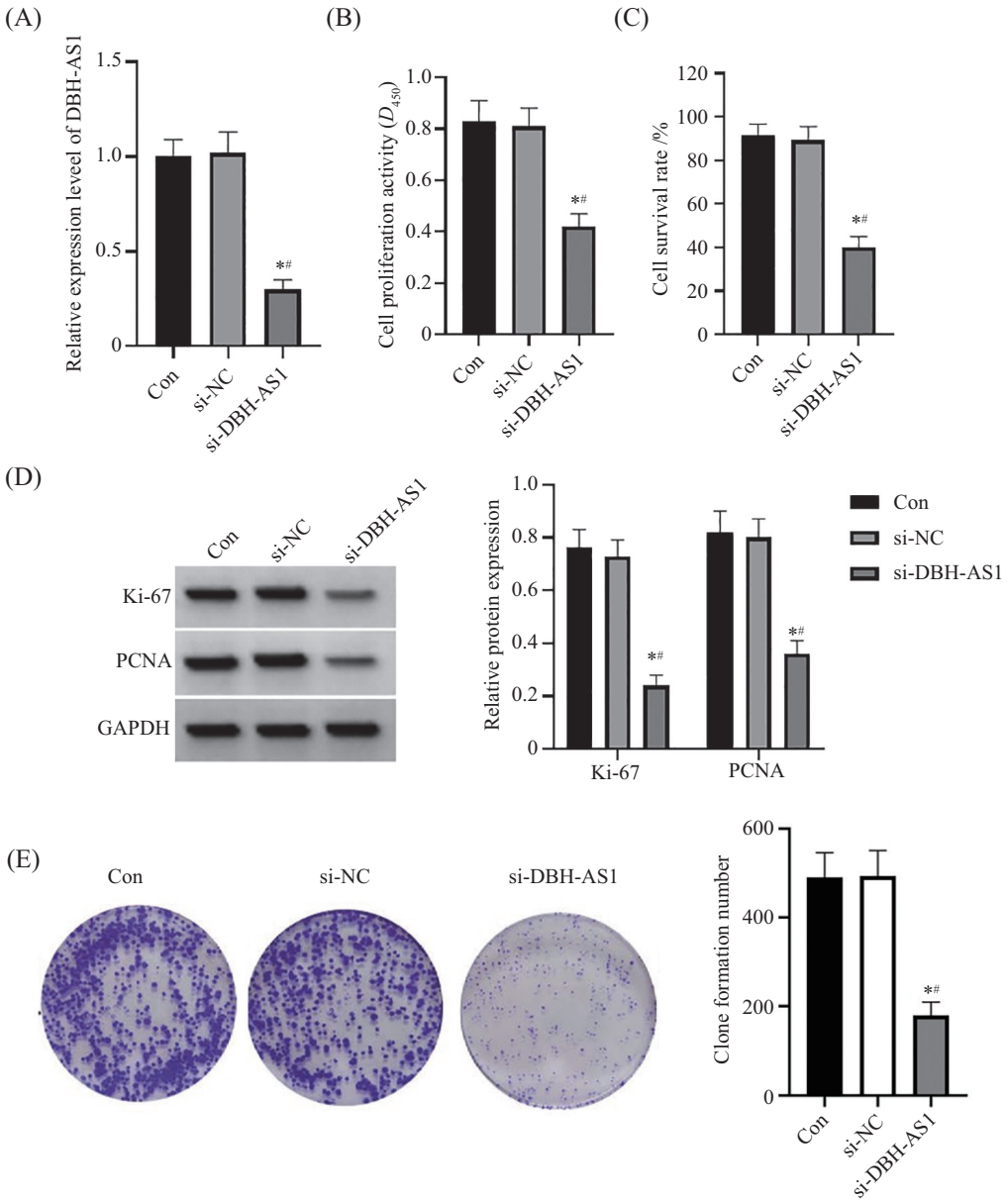
miR-1291组HemECs中miR-1291表达水平高于Con组和miR-NC组, 差异具有统计学意义(P<0.05), 说明miR-1291 mimics转染成功, miR-1291组HemECs中miR-1291过表达。miR-1291组增殖活性、克隆形成数和Ki-67、PCNA蛋白表达水平低于Con组和miR-NC组, 凋亡率和cleaved-caspase9、cleaved-caspase3蛋白表达水平高于Con组和miR-NC组(P<0.05, 图5和图6)。该结果提示过表达miR-1291可抑制HemECs增殖, 并诱导其凋亡。

2.6 下调miR-1291逆转干扰DBH-AS1对HemECs增殖和凋亡的作用

si-DBH-AS1+anti-miR-1291组HemECs中miR-1291表达水平低于si-DBH-AS1+anti-miR-NC组, 差异具有统计学意义(P<0.05), 说明anti-miR-1291转染成功, si-DBH-AS1+anti-miR-1291组HemECs中miR-1291表达受到抑制。si-DBH-AS1+anti-miR-1291组HemECs增殖活性、克隆形成数和Ki-67、PCNA蛋白表达水平均高于si-DBH-AS1+anti-miR-NC组, 细胞凋亡率和cleaved-caspase9、cleaved-caspase3蛋白表达水平均低于si-DBH-AS1+anti-miR-NC组, 差异均具有统计学意义(P<0.05, 图7和图8)。该结果提示干扰DBH-AS1表达对HemECs增殖、凋亡的影响可能是通过上调miR-1291表达实现的。

3 讨论

血管瘤的主要病理特征为血管过度生成。内皮细胞异常增生是血管瘤发生发展的主要原因, 抑制HemECs增殖并诱导其凋亡对血管瘤的治疗尤为重要[10-11]。临床诊断、治疗血管瘤的方法有多种, 但治疗效果差, 导致患者预后不良[12]。近年发现, 血管瘤中存在的异常表达lncRNA, 参与调控HemECs增殖和凋亡, 为血管瘤的治疗提供了分子靶点[13]。例如, 血管瘤组织中lncRNA NEAT1表达量升高, 敲减其表达可削弱HemECs的增殖、迁移和侵袭能力, 其作用机制与竞争性结合miR-33a-5p并正向调控缺氧诱导因子1α(hypoxia inducible factor 1α, HIF1α)的表达有关[14]; LINC00152可通过上调VEGFR2表达促进Hem-



A: HemECs中DBH-AS1的相对表达水平; B、C: 干扰DBH-AS1表达对HemECs增殖活性、存活率的影响; D: 干扰DBH-AS1表达对HemECs中Ki-67、PCNA蛋白表达水平的影响; E: 干扰DBH-AS1表达对HemECs克隆形成的影响。*P<0.05, 与si-NC组比较; #P<0.05, 与Con组比较; n=6。

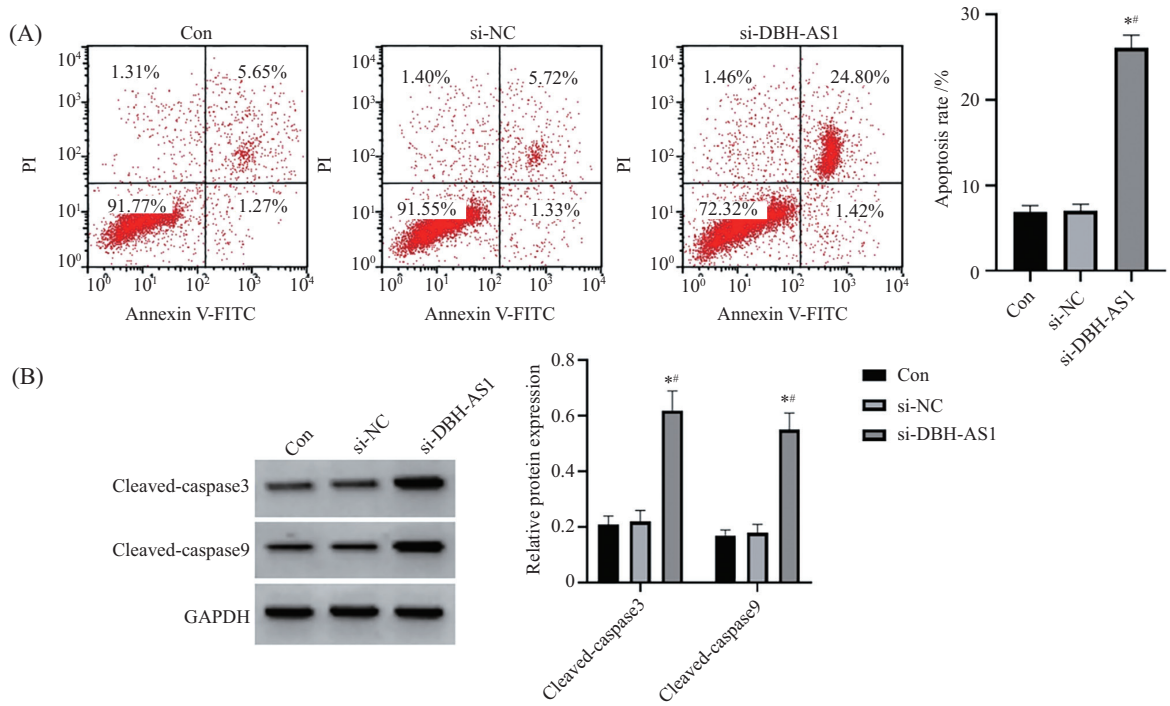
A: the relative expression level of DBH-AS1 in HemECs; B,C: the effect of interference with DBH-AS1 expression on the proliferation activity and survival rate of HemECs; D: influence of interference with DBH-AS1 expression on Ki-67 and PCNA protein expression levels in HemECs; E: the effect of interference with DBH-AS1 expression on HemECs cloning. *P<0.05 compared with si-NC group; #P<0.05 compared with Con group; n=6.

图2　干扰DBH-AS1表达对HemECs增殖的影响

Fig.2　Effect of interference with DBH-AS1 expression on the proliferation of HemECs

ECs增殖、迁移和侵袭[15]。DBH-AS1是新发现的一种lncRNA, 其在血管瘤中的作用机制未知。

本研究结果显示, 血管瘤组织中DBH-AS1表达水平高于正常皮下组织, 通过干扰HemECs中DBH-AS1的表达发现, 干扰DBH-AS1可降低HemECs的增殖能力, 并诱导细胞凋亡, 这提示DBH-AS1可能成为血管瘤治疗的潜在靶点。细胞增殖核抗原Ki-67和PCNA, 是细胞增殖标志性蛋白[16]。caspase级联反应参与调控细胞凋亡, caspase9是级联反应启动因子, 被活化后生成cleaved-caspase9, 传递凋亡信号。caspase3是级联反应核心分子, 其被上游凋亡信号刺激后活化生成cleaved-caspase3, 进而促进细胞凋亡[17]。本研究显示, 干扰DBH-AS1可减少Ki-67和PCNA蛋白表达量, 增加cleaved-caspase9和cleaved-caspase3蛋白表达量, 这进一步说明干扰DBH-AS1可抑制HemECs增殖及促进细胞凋亡。

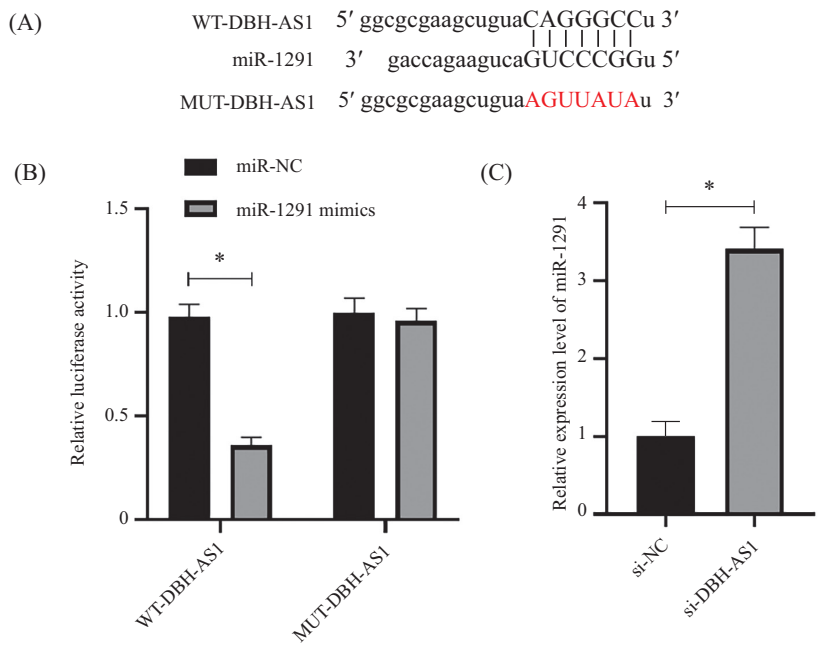


A: 干扰DBH-AS1后, HemECs的凋亡流式图及细胞凋亡率; B: 干扰DBH-AS1后, HemECs中cleaved-caspase9和cleaved-caspase3的蛋白表达条带图及相对蛋白表达水平。*P<0.05, 与si-NC组比较; #P<0.05, 与Con组比较; n=6。

A: flow cytometry and apoptosis rate of HemECs after DBH-AS1 interference; B: protein expression band maps and relative protein expression levels of cleaved-caspase9 and cleaved-caspase3 in HemECs were observed after interference with DBH-AS1. *P<0.05 compared with si-NC group; #P<0.05 compared with Con group; n=6.

图3 干扰DBH-AS1对HemECs凋亡的影响

Fig.3 Effect of interference with DBH-AS1 on apoptosis of HemECs

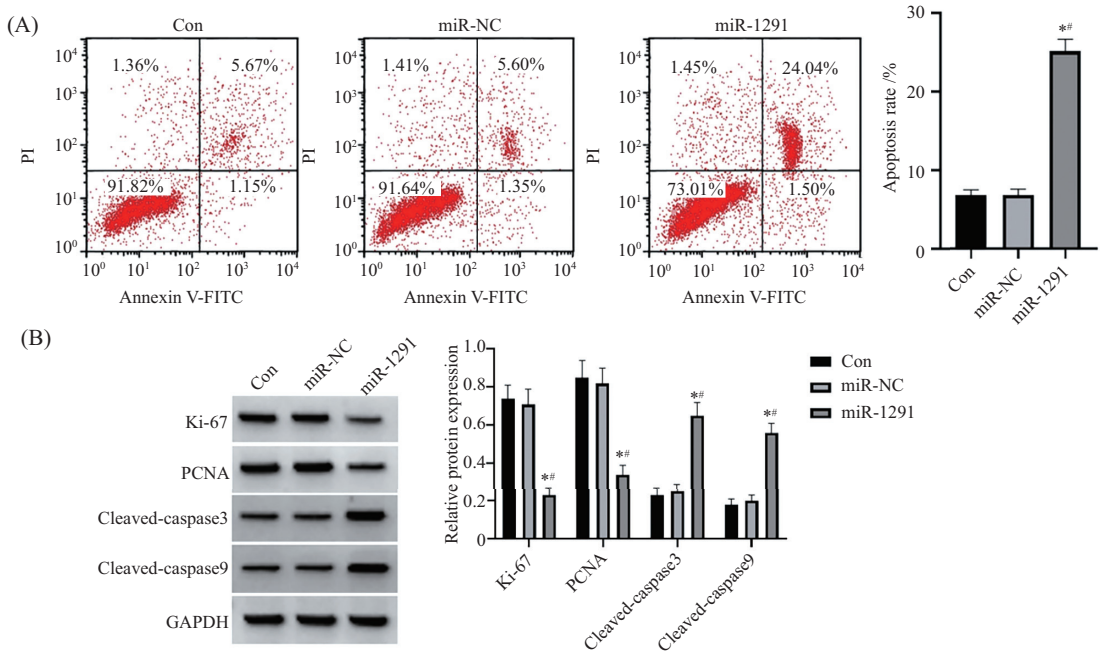


A: starBase数据库中预测的DBH-AS1和miR-1291的靶向结合序列, 红色为DBH-AS1突变序列的突变位点; B: 用于验证DBH-AS1和miR-1291之间关系的双荧光素酶检测的结果; C: 干扰DBH-AS1后, HemECs中miR-1291的表达水平。*P<0.05; n=6。

A: the target binding sequence of DBH-AS1 and miR-1291 predicted in starBase database, red is the mutation site of DBH-AS1 mutation sequence; B: double luciferase test results used to verify the relationship between DBH-AS1 and miR-1291; C: the expression level of miR-1291 in HemECs after interfering with DBH-AS1. *P<0.05; n=6.

图4 DBH-AS1靶向调控miR-1291的表达

Fig.4 DBH-AS1 targets the expression of miR-1291

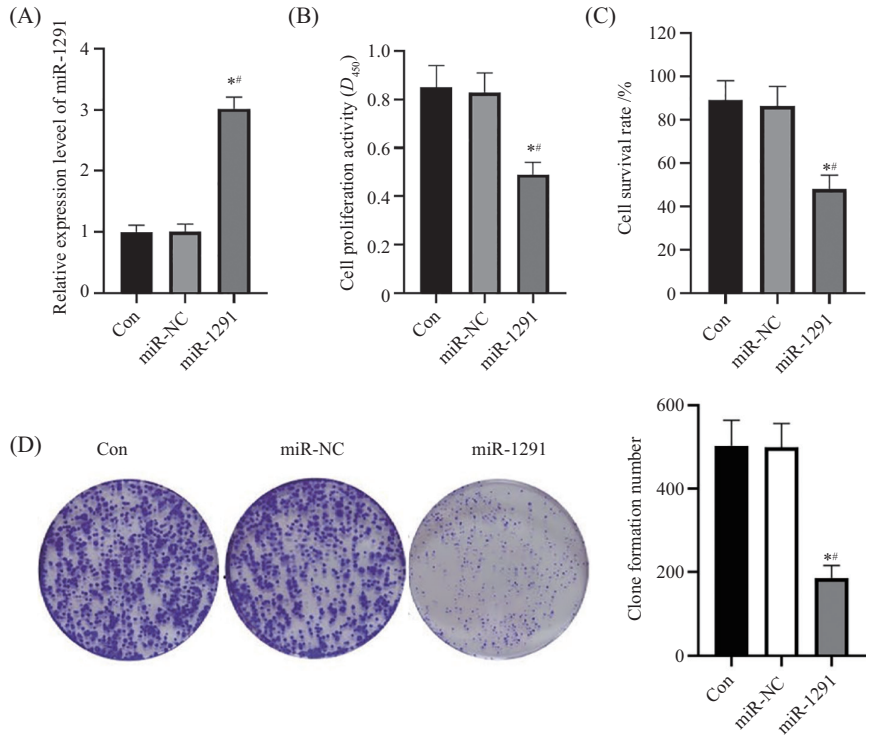


A: 过表达miR-1291后, HemECs的凋亡流式图及细胞凋亡率; B: 过表达miR-1291后, HemECs中Ki-67、PCNA、cleaved-caspase9、cleaved-caspase3的蛋白表达条带图及蛋白相对表达量。*P<0.05, 与miR-NC组比较; #P<0.05, 与Con组比较; n=6。

A: flow cytometry and apoptosis rate of HemECs after overexpression of miR-1291; B: after overexpression of miR-1291, the protein expression band map of Ki-67, PCNA, cleaved-caspase9, cleaved-caspase3 and their relative expression levels in HemECs. *P<0.05 compared with miR-NC group; #P<0.05 compared with Con group; n=6.

图5 过表达miR-1291对HemECs凋亡以及增殖、凋亡相关蛋白表达的影响

Fig.5 Effects of overexpression of miR-1291 on apoptosis and expression of proliferation and apoptosis related protein of HemECs

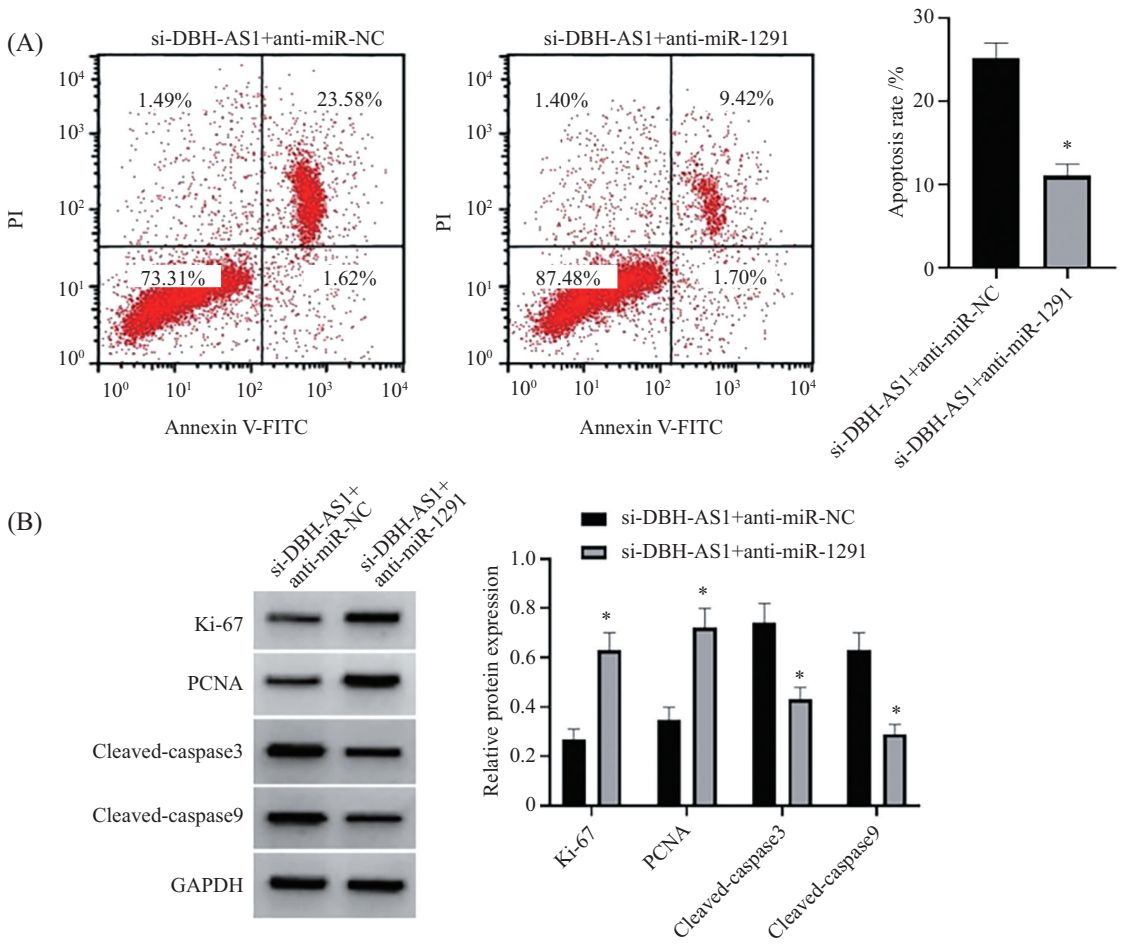


A: HemECs中miR-1291的相对表达水平; B、C: 过表达miR-1291对HemECs细胞增殖活性及存活率的影响; D: 过表达miR-1291对HemECs克隆形成的影响。*P<0.05, 与miR-NC组比较; #P<0.05, 与Con组比较; n=6。

A: relative expression level of miR-1291 in HemECs; B,C: effects of overexpression of miR-1291 on proliferative activity and survival rate of HemECs cells; D: effect of overexpression of miR-1291 on HemECs clone formation. *P<0.05 compared with miR-NC group; #P<0.05 compared with Con group; n=6.

图6 过表达miR-1291对HemECs增殖的影响

Fig.6 Effect of overexpression of miR-1291 on proliferation of HemECs



A: 下调miR-1291逆转干扰DBH-AS1对HemECs细胞凋亡的影响; B: 下调miR-1291逆转干扰DBH-AS1对HemECs细胞中Ki-67、PCNA、cleaved-caspase9和cleaved-caspase3蛋白表达的影响; *P<0.05, 与si-DBH-AS1+anti-miR-NC组比较; n=6。

A: down-regulating miR-1291 reverses the effect of interfering DBH-AS1 on apoptosis of HemECs cells; B: down-regulatimg of miR-1291 reverses the effect of interference with DBH-AS1 on the expression of Ki-67, PCNA, cleaved-caspase9, and cleaved-caspase3 proteins in HemECs cells; *P<0.05 compared with si-DBH-AS1+anti-miR-NC group; n=6.

图7 下调miR-1291逆转干扰DBH-AS1对HemECs凋亡的作用

Fig.7 Downregulation of miR-1291 reverses the effect of interference with DBH-AS1 on apoptosis of HemECs

lncRNA可调控miRNA/miRNA表达发挥生物学调控作用, 在肿瘤发生中起重要作用[18]。本研究进一步探究了干扰DBH-AS1阻碍HemECs增殖并诱导其凋亡的分子机制, 证实了DBH-AS1可靶向结合并负调控miR-1291, 这与本文血管瘤组织中DBH-AS1表达量升高而miR-1291表达量降低的结果一致。有报道称, 食管癌组织样本中miR-1291表达量降低, 且miR-1291低表达与食管癌患者淋巴结转移和临床分期密切相关, 上调miR-1291可通过靶向抑制黏蛋白1(mucin 1, MUC1)的表达阻碍食管癌细胞生长和侵袭, 并促进食管癌细胞凋亡, miR-1291有可能成为食管癌早期诊断的生物标志物及治疗靶点[19]。本研究结果显示, 过表达miR-1291对HemECs增殖起抑制作用, 对其凋亡起促进作用, 这提示上调miR-1291有可能抑制血管瘤的发展进程。此外, 本研究结果表明, 下调miR-1291减弱了干扰DBH-AS1对HemECs增殖的阻碍作用及凋亡促进作用, 这进一步提示DBH-AS1通过靶向负调控miR-1291来影响HemECs增殖和凋亡。

综上, 在婴幼儿血管瘤组织中干扰DBH-AS1可阻碍HemECs增殖, 并诱导其凋亡, 作用机制可能与DBH-AS1靶向负调控miR-1291有关。

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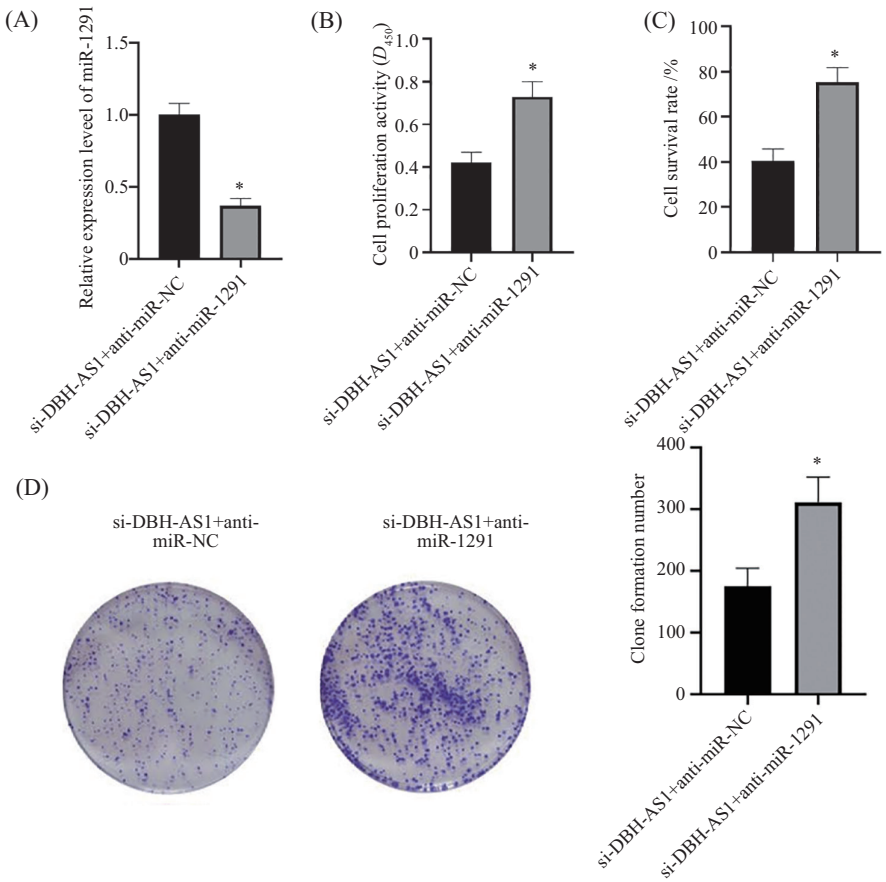

A: HemECs中miR-1291的相对表达水平; B、C: 下调miR-1291逆转干扰DBH-AS1对HemECs细胞增殖活性及存活率的影响; D: 下调miR-1291逆转干扰DBH-AS1对HemECs克隆形成的影响。*P<0.05, 与si-DBH-AS1+anti-miR-NC组比较; n=6。

A: relative expression level of miR-1291 in HemECs; B,C: down-regulating miR-1291 reverses the effects of interfering DBH-AS1 on the proliferative activity and survival rate of HemECs cells; D: downregulation of miR-1291 reverses the effect of interference with DBH-AS1 on HemECs cloning. *P<0.05 compared with si-DBH-AS1+anti-miR-NC group; n=6.

图8 下调miR-1291逆转干扰DBH-AS1对HemECs增殖和凋亡的作用

Fig.8 Downregulation of miR-1291 reverses the effect of interference with DBH-AS1 on the proliferation and apoptosis of HemECs

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