

长链非编码 RNA GAS5 抑制内皮细胞功能

孙玉聪¹⁾, 楚海荣²⁾, 李宏¹⁾, 官秀梅¹⁾, 张晓芸³⁾, 崔晓栋³⁾, 成敏^{3)*}

(潍坊医学院临床医学院,¹⁾生物化学教研室;²⁾医学检验实验室;³⁾生理学教研室,山东 潍坊 261053)

摘要 最新研究表明,长链非编码 RNA GAS5(lncRNA GAS5)可调节血管内皮细胞的凋亡,但对内皮细胞其他功能的调控并不明确。本研究旨在了解 lncRNA GAS5 对内皮细胞的增殖、成血管、NO 分泌及内皮标志分子 CD31 和 vWF 表达的影响及可能机制。将 LncRNA GAS5 干扰慢病毒(LV-GAS5-RNAi)转染人脐静脉内皮细胞株(EA.hy926)后,采用 CCK8 及 Matrigel 胶分别检测 EA.hy926 的增殖和成血管能力;硝酸还原酶法检测 NO 的分泌情况;real-time RT-PCR 检测 CD31、vWF 及 miR-21 的表达;Western 印迹检测 PTEN 在蛋白质水平的表达。结果显示:与对照组比较,LV-GAS5-RNAi 组 EA.hy926 增殖能力无明显变化(0.34 ± 0.01 vs. 0.34 ± 0.04 , $P > 0.05$),而其成血管能力升高(133.70 ± 12.64 vs. 100.00 ± 4.65 , $P < 0.05$),NO 的分泌量亦增加(28.54 ± 2.75 $\mu\text{mol/L}$ vs. 15.11 ± 1.19 $\mu\text{mol/L}$, $P < 0.01$);内皮标志分子 CD31(是对照组的 1.46 倍)及 vWF(是对照组的 2.94 倍)的基因表达量均显著升高。同时,miR-21 表达亦明显升高(是对照组的 1.42 倍),而 miR-21 下游靶基因 PTEN 蛋白质的表达量则显著降低(0.13 ± 0.05 vs. 0.38 ± 0.03 , $P < 0.01$)。以上结果提示,LncRNA GAS5 抑制了内皮细胞的功能,miR-21、PTEN 信号分子可能参与其中的调节。

关键词 LncRNA GAS5; miR-21; 血小板内皮细胞黏附分子; 血管性血友病因子
中图分类号 R543.1

LncRNA GAS5 Inhibits the Functions of Endothelial Cells

SUN Yu-Cong¹⁾, CHU Hai-Rong²⁾, LI Hong¹⁾, GUAN Xiu-Mei¹⁾,
ZHANG Xiao-Yun³⁾, CUI Xiao-Dong³⁾, CHENG Min^{3)*}

(Clinical Medical College of Weifang Medical University, ¹⁾Department of Biochemistry;

²⁾Specialized Lab for Medical Examination, ³⁾Department of Pathophysiology, Weifang 261053, Shandong, China)

Abstract Recent research showed that LncRNA GAS5 regulated the apoptosis of vascular endothelial cells in atherosclerosis. However, it is still unclear whether LncRNA GAS5 could influence other functions of endothelial cells. In this study, we investigated the effects of LncRNA GAS5 on endothelial cell proliferation, vascular genesis, NO secretion and the expression of CD31 and vWF. After transfecting LV-GAS5-RNAi into endothelial cells (EA.hy926), the proliferation and tube formation ability of EA.hy926 were detected by CCK8 and Matrigel assays, respectively. Nitric oxide levels in the supernatant were measured by the NO assay kit. The mRNA expression of CD31, vWF and miR-21 were determined by real-time RT-PCR. Western blotting was used to analyze the protein expression of PTEN. The results

收稿日期: 2018-01-21; 修回日期: 2018-02-23; 接受日期: 2018-06-14

国家自然科学基金(No. 31570941, No. 31270993, No. 81700406); 山东省自然科学基金项目(No. ZR2013CQ032, No. ZR2014JL018, No. ZR2016CM20); 山东省中医药管理局项目(No. 2015-239)和山东省卫生厅项目(No. 2016WS0667)资助

* 通讯作者 Tel: 0536-8462286; E-mail: mincheng@wfmc.edu.cn

Received: January 21, 2018; Revised: February 23, 2018; Accepted: June 14, 2018

Supported by National Natural Science Foundation of China (No. 31570941, No. 31270993, No. 81700406); Natural Science Foundation of Shandong Province (No. ZR2013CQ032, No. ZR2014JL018, No. ZR2016CM20); Administration of Traditional Chinese Medicine of Shandong Province (No. 2015-239); and Health Department of Shandong Province (No. 2016WS0667)

* Corresponding author Tel: 0536-8462286; E-mail: mincheng@wfmc.edu.cn

showed that compared with the control group, there was no significant difference in the proliferation of EA.hy926 in the LV-GAS5-RNAi group (0.34 ± 0.01 vs. 0.34 ± 0.04 , $P > 0.05$), while the tube formation ability (133.70 ± 12.59 vs. 100.00 ± 4.65 , $P < 0.05$) and the secretion of NO (28.54 ± 2.75 $\mu\text{mol/L}$ vs. 15.11 ± 1.19 $\mu\text{mol/L}$, $P < 0.01$) significantly increased. In the meantime, the mRNA expression of CD31 (1.46-fold higher than that in control group) and vWF (2.94-fold higher than that in control group) were markedly upregulated. Furthermore, the expression of miR-21 was also significantly increased (1.42-fold higher than that in control group), while the protein expression of PTEN (one of the targets of miR-21) decreased (0.13 ± 0.05 vs. 0.38 ± 0.03 , $P < 0.01$). These results suggested that LncRNA GAS5 inhibited the functions of endothelial cells, which might be mediated by signaling molecules such as miR-21 and PTEN.

Key words LncRNA GAS5; miR-21; platelet endothelial cell adhesion molecule-1 (CD31); von-Willebrand factor(vWF)

动脉粥样硬化(atherosclerosis, AS)导致的血管事件是心脑血管疾病致死致残的主要原因^[1]。研究显示,动脉粥样硬化的形成在一定程度上是由内皮细胞功能紊乱引起,血管内皮损伤将导致动脉壁上脂质堆积、内皮通透性增大等一系列病理改变^[2]。因此,探讨内皮细胞功能的调控因素,对防治动脉粥样硬化引起的心血管疾病具有重要的意义。

长链非编码RNA(long non-coding RNAs, lncRNAs)是指不编码蛋白质,且长度大于200 nt的RNA分子,主要通过染色体修饰、转录调控、转录后调控等方式调节细胞内多种病理生理过程^[3,4]。越来越多的研究发现,lncRNAs能调节内皮细胞功能、血管炎症和新陈代谢等^[5]。LncRNA GAS5是一种与细胞增生相关的lncRNA,它在调控哺乳动物细胞周期和凋亡过程中发挥着重要作用,在多种恶性肿瘤中异常表达^[6]。近年来,lncRNA GAS5在心血管疾病中的生物学功能逐渐被研究者认识。最新研究表明,lncRNA GAS5可调节血管内皮细胞的凋亡^[7],但是否调控内皮细胞其他功能仍不明确。本研究旨在探讨LncRNA GAS5对人脐静脉内皮细胞株(EA.hy926)的增殖、成血管、NO分泌及内皮标志分子CD31和血管性血友病因子(von-Willebrand factor, vWF)表达的影响及可能的机制,为临床防治动脉粥样硬化引起的心血管疾病提供新的作用靶点和实验依据。

1 材料与方法

1.1 材料

1640培养基、胎牛血清和胰蛋白酶均购自美国HyClone公司;LncRNA GAS5干扰慢病毒(LV-GAS5-RNAi)及其对应的阴性对照病毒、polybrene由上海吉凯基因化学技术有限公司合成;TRIzol购

自Invitrogen公司;CD31、vWF、miR-21引物及real-time RT-PCR试剂购自宝生物工程(大连)有限公司;CCK8购自广州锐博生物科技有限公司;NO检测试剂盒购自南京建成生物工程研究所;PTEN一抗购自CST公司; β -肌动蛋白一抗购自Proteintech公司;HRP-山羊抗兔二抗、HRP-山羊抗小鼠二抗均购自碧云天生物技术有限公司。

1.2 细胞培养

EA.hy926细胞用含10%胎牛血清的1640培养液,置于37℃、5% CO₂的细胞培养箱中恒温培养,根据细胞生长状态及时更换新鲜培养基,当细胞融合达到80%~90%时进行传代,取生长状态良好的对数生长期细胞用于后续转染及功能检测。

1.3 LncRNA GAS5干扰慢病毒转染与分组

胰酶消化对数生长期的EA.hy926细胞,以 $3 \sim 5 \times 10^4/\text{mL}$ 密度接种到6孔细胞培养板中。待细胞生长至30%~50%融合时进行病毒转染,分为干扰组和对照组。干扰组和对照组细胞分别转染LncRNA GAS5干扰慢病毒及空载体慢病毒。病毒转染,加入5 $\mu\text{g/mL}$ polybrene以增加感染效率。24 h后换液,继续培养48 h后检测各项指标。

1.4 CCK8法检测细胞增殖

将消化的细胞,5 000个/100 μL /孔接种于96孔板中。置37℃、5% CO₂培养箱培养,24 h后取出,弃原培养液,按1:9的比例加入CCK8试剂和培养基的混合液100 μL ,在培养箱内孵育1 h,酶标仪测定450 nm波长的吸光度A值。

1.5 细胞成血管能力的测定

取70 μL Matrigel胶于96孔板中,置于37℃、5% CO₂培养箱中孵育1 h,每孔加入100 μL 细胞数为 1.5×10^4 的细胞悬液。6 h后倒置显微镜下观察并拍照,计数形成的管腔样结构。

1.6 细胞培养液中 NO 含量的测定

收集细胞培养液,以 4 000 r/min 离心 10 min,取上清液。按硝酸还原酶法一氧化氮试剂盒说明书操作,在酶标仪测定细胞培养液中 NO 含量。

1.7 Real-time RT-PCR 检测 mRNA 水平

TRIzol 提取细胞总 RNA,用 TaKaRa 公司逆转录试剂盒逆转录成 cDNA,real-time RT-PCR 进行扩增。 $2^{-\Delta\Delta CT}$ 法计算基因相对表达量。

(1) 检测 LncRNA GAS5、CD31、vWF 时,以 GAPDH 为内参,反应条件:LncRNA GAS5:95℃ 30 s;95℃ 5 s,60℃ 30 s,共 40 个循环;CD31 和 vWF:95℃ 3 min;95℃ 30 s,60℃ 30 s,72℃ 30 s,共 40 个循环;

(2)检测 miR-21 时,以 U6 作为内参。miR-21 逆转录机制,在 Mir-X cDNA 合成反应中,用试剂盒中的 mRQ 酶混合物,以 polyA 聚合酶进行 RNAs 加尾,通过修饰过的 Oligo dT 引物和 MMLV 逆转录酶进行逆转录。逆转录条件:37℃ 60 min;85℃ 5 s;real-time RT-PCR 反应:95℃ 30 s;95℃ 5 s,60℃ 30 s,共 40 个循环。

1.8 Western 印迹法检测 PTEN 在蛋白质水平的表达

加 RIPA 细胞裂解液提取总蛋白质,BCA 法测定蛋白质浓度,上样缓冲液高温变性后进行 SDS-PAGE。电泳后转至 PVDF 膜,5% BSA 封闭 1 h,PTEN 一抗(1:1 000),β-肌动蛋白(1:1 500)分别 4℃ 孵育过夜。一抗孵育完成后,TBST 漂洗 10 min × 5 次,加入 TBST 稀释的二抗(1:2 000),37℃ 恒温摇床孵育 1 h。二抗孵育完成后 TBST 漂洗 10 min × 3 次,采用凝胶成像系统发光显色。以 β-肌动蛋白为内参,分析 PTEN 蛋白质的相对表达量。

1.9 统计学方法

实验数据以均值 ± 标准差(mean ± SD)表示,采用 SPSS 16.0 统计软件处理。两两比较采用 LSD-*t* 检验, $P < 0.05$ 表示有统计学差异。

2 结果

2.1 LV-GAS5-RNAi 慢病毒有效抑制 LncRNA GAS5 在 EA.hy926 中的表达

LV-GAS5-RNAi 转染 48 h 后收集细胞,real-time RT-PCR 检测 LncRNA GAS5 在 EA.hy926 中的表达。结果显示,在 EA.hy926 中表达有 LncRNA GAS5。与对照组相比, LV-GAS5-RNAi 组中 LncRNA GAS5 的表达量明显降低(是对照组的

56%),提示 LV-GAS5-RNAi 有效抑制了 LncRNA GAS5 在 EA.hy926 中的表达(Fig. 1)。

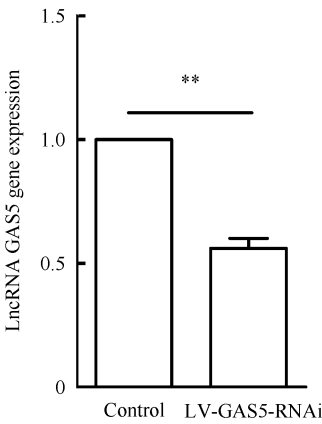


Fig. 1 LV-GAS5-RNAi effectively inhibited the expression of LncRNA GAS5 in EA.hy926 EA.hy926 was transfected with LV-GAS5-RNAi or LV-RNAi. Total RNAs were isolated after transfection for 48 hours. The mRNA expression of LncRNA GAS5 was analyzed by real-time RT-PCR. GAPDH was used as an internal control. Data represent mean ± SD ($n = 3$). ** $P < 0.01$

2.2 干扰 LncRNA GAS5 对 EA.hy926 增殖无明显影响

收集转染后的 EA.hy926 细胞,接种于 96 孔板中,CCK8 法检测细胞增殖能力。结果显示,抑制 LncRNA GAS5 的表达,对 EA.hy926 增殖无明显影响(0.34 ± 0.01 vs. 0.34 ± 0.04 , $P > 0.05$)(Fig. 2)。

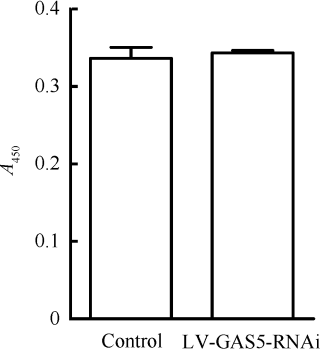


Fig. 2 LV-GAS5-RNAi has no effect on the proliferation of EA.hy926 EA.hy926 was plated onto 96-well plates and transfected with LV-GAS5-RNAi or LV-RNAi. The proliferation was measured by the CCK8 method. The absorbance value was tested by the microplate reader. Data represent mean ± SD ($n = 3$)

2.3 干扰 LncRNA GAS5 促进 EA.hy926 成血管能力

转染 LV-GAS5-RNAi 后 48 h,收集 EA.hy926 细胞,接种于 Matrigel 基质胶上。结果显示,LV-GAS5-

RNAi 组 EA. hy926 细胞体外成血管能力明显强于对照组 (133.70 ± 12.59 vs. 100.00 ± 4.65 , $P < 0.05$),提示 LncRNA GAS5 抑制 EA. hy926 细胞的体外生成血管能力(Fig. 3)。

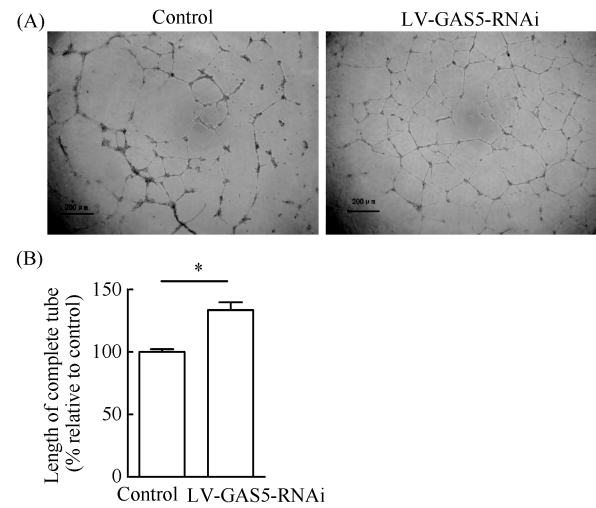


Fig. 3 LV-GAS5-RNAi increased the tube formation ability of EA. hy926 EA. hy926 transfected with LV-GAS5-RNAi or LV-RNAi were plated onto 96-well plates precoated with matrigel for 6 hours. (A) Images of the tube formation under optical microscopes; (B) The analysis of the tube formation length. Data represent mean \pm SD ($n = 3$). * $P < 0.05$

2.4 干扰 LncRNA GAS5 促进 EA. hy926 细胞分泌 NO

细胞转染 LV-GAS5-RNAi 48 h,收集培养液上清,测定 NO 含量。结果显示, LV-GAS5-RNAi 组 EA. hy926 细胞培养液中 NO 含量明显高于对照组 (28.54 ± 2.75 μmol/L vs. 15.11 ± 1.19 μmol/L, $P < 0.01$),提示 LncRNA GAS5 抑制 EA. hy926 分泌 NO(Fig. 4)。

2.5 干扰 LncRNA GAS5 促进 EA. hy926 中 CD31 和 vWF mRNA 表达

转染 LV-GAS5-RNAi 后 48 h,收集 EA. hy926 细胞,提取总 RNA。Real-time RT-PCR 结果显示,与对照组相比, LV-GAS5-RNAi 组内皮标志分子 CD31 (是对照组的 1.46 倍)和 vWF (是对照组的 2.94 倍)的 mRNA 表达量明显升高,提示 LncRNA GAS5 是 EA. hy926 细胞中 CD31 和 vWF 表达的抑制性因素(Fig. 5)。

2.6 干扰 LncRNA GAS5 上调 EA. hy926 中 miR-21 的表达

LncRNA GAS5 干扰慢病毒转染之后,收集细胞提取 RNA,应用 microRNA 逆转录试剂盒转录成

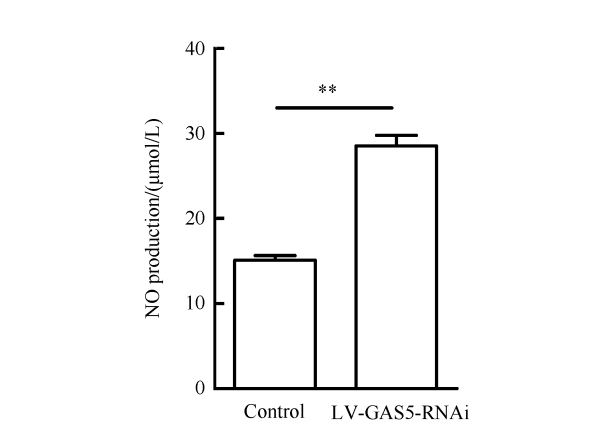


Fig. 4 LV-GAS5-RNAi promoted NO secretion of EA. hy926 EA. hy926 was plated onto 6-well plates and transfected with LV-GAS5-RNAi or LV-RNAi. NO was assessed indirectly by measuring NO_3^- and NO_2^- in the culture media. Data represent mean \pm SD ($n = 3$). ** $P < 0.01$

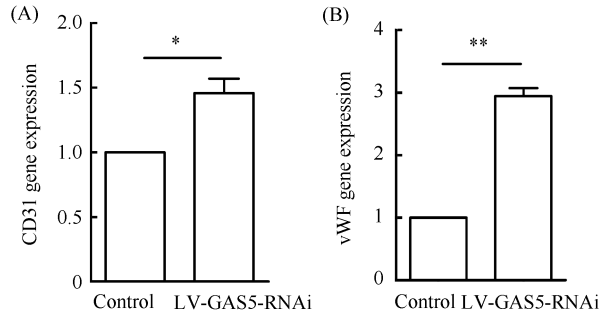


Fig. 5 LV-GAS5-RNAi increased the mRNA expression of CD31 and vWF in EA. hy926 EA. hy926 was plated onto 6-well plates and transfected with LV-GAS5-RNAi or LV-RNAi. Total RNAs were isolated after transfection for 48 hour and the mRNA expression were determined by real-time RT-PCR. (A) The relative expression of CD31; (B) The relative expression of vWF. GAPDH was used as an internal control. Data represent mean \pm SD ($n = 3$). * $P < 0.05$, ** $P < 0.01$

cDNA,miR-21 特异性引物进行 real-time PCR。结果显示,和对照组相比, LV-GAS5-RNAi 组 miR-21 的表达量显著升高 (是对照组的 1.42 倍),提示 LncRNA GAS5 可负向调控 miR-21 的表达(Fig. 6)。

2.7 干扰 LncRNA GAS5 抑制 EA. hy926 中 PTEN 蛋白质的表达水平

Western 印迹结果显示,与对照组相比,转染 LV-GAS5-RNAi 之后,PTEN 蛋白质的表达量较对照组明显降低 (0.13 ± 0.05 vs. 0.38 ± 0.03 , $P < 0.01$),提示 LncRNA GAS5 可促进 EA. hy926 中 PTEN 蛋白质的表达(Fig. 7)。

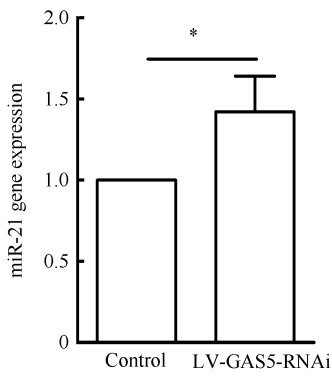


Fig. 6 LV-GAS5-RNAi up-regulates the expression of *miR-21* in EA. hy926 EA. hy926 was plated onto 6-well plates and transfected with LV-GAS5-RNAi or LV-RNAi. Total RNAs were isolated after transfection for 48 hour. The expression of *miR-21* was analyzed by real-time RT-PCR. *U6* was used as an internal control. Data represent mean \pm SD ($n = 3$). * $P < 0.05$

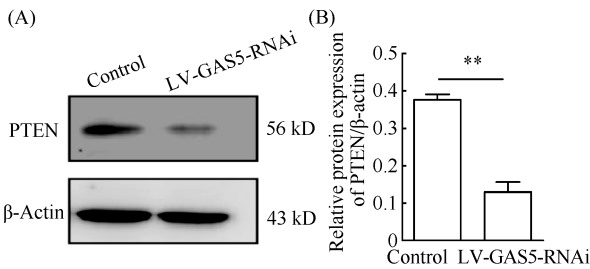


Fig. 7 LV-GAS5-RNAi decreases the expression of PTEN proteins in EA. hy926 EA. hy926 was plated onto 6-well plates and transfected with LV-GAS5-RNAi or LV-RNAi. (A) The levels of PTEN proteins was detected by Western blotting. (B) Quantified data of PTEN protein levels normalized to β -actin. Data represent mean \pm SD ($n = 3$). ** $P < 0.01$

3 讨论

近年的研究表明,心血管疾病在我国的发生率和死亡率呈逐年上升趋势,已成为威胁我国公众健康的重要疾病之一^[8]。内皮细胞在多种病理因素(如高血脂、氧自由基、低血流切应力等)刺激下,血管内皮依赖性舒张反应减弱甚至消失、粘附分子表达异常等导致的血管痉挛、异常收缩、血栓形成及血管增生^[9]是形成动脉粥样硬化的早期表现。因此,分析其细胞和分子生物学机制,寻找有效的治疗靶点,是预防和治疗心血管疾病发生发展的重要途径。

长期以来,LncRNAs被人们视为基因组转录的“暗物质”。有研究表明,LncRNA GAS5在细胞生长停滞、细胞增殖以及细胞凋亡等生物进程中发挥重

要的作用^[10,11]。Wang等^[12]发现,LncRNA GAS5在内皮细胞高度表达,而高血压患者体内的LncRNA GAS5表达显著下调^[13]。Chen等^[7]研究发现,LncRNA GAS5在动脉粥样斑块中的表达明显升高,且包含LncRNA GAS5的外泌体可导致内皮细胞的凋亡。本文的研究结果显示,应用慢病毒抑制LncRNA GAS5的表达之后,EA. hy926细胞的增殖能力无明显变化,但其成血管能力、NO分泌量及内皮标志分子CD31和vWF的表达均明显增加,以上结果提示,LncRNA GAS5对内皮细胞而言,可能是一种抑制性调控因素。

Song等^[14]研究发现,LncRNA GAS5可通过分子“海绵”效应负性调控*miR-21*的表达,进而在骨关节炎的发生发展中发挥重要作用。进一步研究显示,*miR-21*和LncRNA GAS5能够通过一种类似于microRNA介导靶mRNA沉默的方式进行彼此调节^[15,16]。我们应用生物信息学分析显示,LncRNA GAS5存在靶向结合*miR-21*的种子序列,而*miR-21*可与其靶基因*PTEN*的3'非翻译区(3'UTR)不完全互补配对。本文研究表明,抑制LncRNA GAS5表达后,*miR-21*的表达量明显升高。

大量研究表明,*miR-21*的异常表达与心血管生理和病理过程密切相关。*miR-21*可通过抑制PTEN,减少内皮细胞缺氧后的凋亡^[17,18]。另有研究证实,抑制TGF- β 1诱导的早期反应基因的表达,能够导致PTEN表达量降低,使内皮细胞的增殖分化能力增强^[19,20]。本文的结果表明,抑制LncRNA GAS5的表达后,*miR-21*的表达明显上调,而PTEN蛋白质的表达量则明显减少,这可能是抑制LncRNA GAS5后内皮细胞功能增强的原因。

综合以上结果,抑制LncRNA GAS5表达后,内皮细胞成血管和NO分泌能力增加,而且内皮标志分子CD31和vWF表达量亦明显上调,提示LncRNA GAS5可抑制内皮细胞功能。此外,抑制LncRNA GAS5表达,*miR-21*表达上调,而其靶基因PTEN蛋白质表达量则明显降低,提示LncRNA GAS5对内皮细胞功能的调控可能是通过*miR-21*、PTEN信号分子来发挥,而*miR-21*、PTEN信号分子如何发挥其调控作用还有待进一步深入研究。

参考文献 (References)

[1] Rafieian-Kopaei M, Setorki M, Dousti M, et al. Atherosclerosis: process, indicators, risk factors and new hopes[J]. Int J Prev Med, 2014, 5(8): 927-946

[2] Novák J, Bienertová-Vásků J, Kúra T, et al. MicroRNAs

- involved in the lipid metabolism and their possible implications for atherosclerosis development and treatment [J]. *Mediators Inflamm*, 2014, **2014**: 275867
- [3] 张旭慧,孔鹏,韩梅. MicroRNA 和 lncRNA:衰老相关心血管疾病的重要调控因子[J]. 中国生物化学与分子生物学报 (Zhang XH, Kong P, Han M. MicroRNA and LncRNA: Important regulatory factors of aging related cardiovascular diseases[J], *Chin J Biochem Mol Biol*), 2017, **33**(7): 645-649
- [4] 杨倩,黄宏斌,龚朝建,等. lncRNAs 参与基因表达调控机制的研究进展[J]. 中南大学学报(医学版) (Yang Q, Huang HB, Gong CJ, *et al.* Advances in regulation of gene expression mediated by lncRNAs[J], *J Cent South Univ (Med Sci)*), 2014, **39**(1): 91-95
- [5] Zhou T, Ding J, Wang X, *et al.* Long noncoding RNAs and atherosclerosis[J]. *Atherosclerosis*, 2016, **248**: 51-61
- [6] Li Y, Li Y, Huang S, *et al.* Long non-coding RNA growth arrest specific transcript 5 acts as a tumour suppressor in colorectal cancer by inhibiting interleukin-10 and vascular endothelial growth factor expression [J]. *Oncotarget*, 2017, **8**(8): 13690-13702
- [7] Chen L, Yang W, Guo Y, *et al.* Exosomal lncRNA GAS5 regulates the apoptosis of macrophages and vascular endothelial cells in atherosclerosis[J]. *PLoS One*, 2017, **12**(9): e0185406
- [8] 成捷,刘新光,熊兴东. miRNA,lncRNA 与心血管疾病[J]. 中国生物化学与分子生物学报 (Cheng J, Liu XG, Xiong XD. MiRNA and lncRNA in cardiovascular diseases [J]. *Chin J Biochem Mol Biol*), 2014, **30**(4): 328-335
- [9] 李丹,李玉洁,杨庆,等. 血管内皮功能障碍与动脉粥样硬化研究进展[J]. 中国实验方剂学杂志 (Li D, Li YJ, Yang Q, *et al.* Research progress of endothelial dysfunction and atherosclerosis[J]. *Chin J Exp Tradit Med Formulae*), 2012, **18**(8): 272-276
- [10] Li S, Zhou J, Wang Z, *et al.* Long noncoding RNA GAS5 suppresses triple negative breast cancer progression through inhibition of proliferation and invasion by competitively binding miR-196a-5p[J]. *Biomed Pharmacother*, 2018, **104**: 451-457
- [11] Yang Y, Shen Z, Yan Y, *et al.* Long non-coding RNA GAS5 inhibits cell proliferation, induces G0/G1 arrest and apoptosis, and functions as a prognostic marker in colorectal cancer[J]. *Oncol Lett*, 2017, **13**(5): 3151-3158
- [12] Wang YN, Shan K, Yao MD, *et al.* Long noncoding RNA-GAS5: A novel regulator of hypertension-induced vascular remodeling [J]. *Hypertension*, 2016, **68**(3): 736-748
- [13] Qi M, Zhou Q, Zeng W, *et al.* Analysis of long non-coding RNA expression of lymphatic endothelial cells in response to type 2 diabetes[J]. *Cell Physiol Biochem*, 2017, **41**(2): 466-474
- [14] Song J, Ahn C, Chun CH, *et al.* A long non-coding RNA, GAS5, plays a critical role in the regulation of miR-21 during osteoarthritis[J]. *J Orthop Res*, 2014, **32**(12): 1628-1635
- [15] Zhang Z, Zhu Z, Watabe K, *et al.* Negative regulation of lncRNA GAS5 by miR-21 [J]. *Cell Death Differ*, 2013, **20**(11): 1558-1568
- [16] Cao L, Chen J, Ou B, *et al.* GAS5 knockdown reduces the chemo-sensitivity of non-small cell lung cancer (NSCLC) cell to cisplatin (DDP) through regulating miR-21/PTEN axis [J]. *Biomed Pharmacother*, 2017, **93**: 570-579
- [17] Zhang JY, Ma J, Yu P, *et al.* Reduced beta 2 glycoprotein I prevents high glucose-induced cell death in HUVECs through miR-21/PTEN[J]. *Am J Transl Res*, 2017, **9**(9): 3935-3949
- [18] 单娟,李鸿生,孙桂香,等. MicroRNA-21 通过抑制 PTEN 减少人脐静脉内皮细胞缺氧后的凋亡[J]. 中国病理生理杂志 (Shan J, Li HS, Sun GX, *et al.* MicroRNA-21 reduces hypoxia induced apoptosis in human umbilical vein endothelial cells by inhibiting PTEN [J]. *Chin J pathophysiol*), 2012, **28**(11): 2032
- [19] Cen M, Hu P, Cai Z, *et al.* TIEG1 deficiency confers enhanced myocardial protection in the infarcted heart by mediating the Pten/Akt signalling pathway[J]. *Int J Mol Med*, 2017, **39**(3): 569-578
- [20] Koide M, Ikeda K, Akakabe Y, *et al.* Apoptosis regulator through modulating IAP expression (ARIA) controls the PI3K/Akt pathway in endothelial and endothelial progenitor cells[J]. *Proc Natl Acad of Sci U S A*, 2011, **108**(23): 9472-9477