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·基础研究·

lncRNA SNHG16靶向调控miR-182-5p对缺氧/复氧诱导的心肌细胞凋亡的影响

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[摘 要] **目的** 探讨lncRNA SNHG16对缺氧/复氧(H/R)诱导的心肌细胞凋亡的影响及对miR-182-5p的调控作用。**方法** 将细胞分为对照组(正常培养)、模型组(H/R诱导)、si-NC组、si-SNHG16组、si-SNHG16+anti-NC组、si-SNHG16+anti-miR-182-5p组,除对照组与模型组以外,其余各组细胞均在H/R诱导后转染相应质粒。Edu实验检测细胞增殖;流式细胞术及AO/EB染色检测细胞凋亡;ELISA检测心肌细胞损伤、炎症反应及氧化应激相关因子水平;DCFDA荧光探针法检测细胞活性氧(ROS)生成量;Western blot检测凋亡相关蛋白表达;qRT-PCR检测各组细胞lncRNA SNHG16、miR-182-5p水平;双荧光素酶报告基因实验及RNA pull-down实验检测lncRNA SNHG16与miR-182-5p的靶向关系。**结果** 与对照组比较,模型组细胞Edu阳性率、超氧化物歧化酶(SOD)活性、miR-182-5p水平降低($P<0.05$),细胞凋亡率、凋亡细胞占比、乳酸脱氢酶(LDH)、肌酸激酶(CK)、白细胞介素(IL)-6、IL-1β、肿瘤坏死因子-α(TNF-α)、ROS、丙二醛(MDA)、lncRNA SNHG16及裂解型胱天蛋白酶-3(C-Caspase-3)、B细胞淋巴瘤-2相关X蛋白(Bax)/B细胞淋巴瘤-2(Bcl-2)表达水平升高($P<0.05$);与si-NC组比较,si-SNHG16组细胞Edu阳性率、SOD活性、miR-182-5p水平升高($P<0.05$),细胞凋亡率、凋亡细胞占比、LDH、CK、IL-6、IL-1β、TNF-α、ROS、MDA、lncRNA SNHG16及C-Caspase-3、Bax/Bcl-2表达水平降低($P<0.05$);与si-SNHG16+anti-NC组比较,si-SNHG16+anti-miR-182-5p组细胞Edu阳性率、SOD活性、miR-182-5p水平降低($P<0.05$),细胞凋亡率、凋亡细胞占比、LDH、CK、IL-6、IL-1β、TNF-α、ROS、MDA及C-Caspase-3、Bax/Bcl-2表达水平升高($P<0.05$)。RNA pull-down实验发现lncRNA SNHG16与miR-182-5p之间存在相互作用;与WT-SNHG16和miR-NC共转染的心肌细胞相比,WT-SNHG16和miR-182-5p mimics共转染的心肌细胞相对荧光素酶活性降低($P<0.05$)。**结论** 沉默lncRNA SNHG16可通过上调miR-182-5p减少H/R诱导的心肌细胞凋亡。

[关键词] lncRNA SNHG16;miR-182-5p;缺氧/复氧;心肌细胞;凋亡

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Effect of lncRNA SNHG16 targeting miR-182-5p on hypoxia/reoxygenation-induced cardiomyocyte apoptosis

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Abstract: Objective To explore the effect of lncRNA SNHG16 on cardiomyocyte apoptosis induced by hypoxia/reoxygenation (H/R) and its regulatory role on miR-182-5p. **Methods** The cells were divided into the control group (normal culture), the model group (induced by H/R), the si-NC group, the si-SNHG16 group, the si-SNHG16+anti-NC group, and the si-SNHG16+anti-miR-182-5p group. Except for the control group and the model group, the cells in the other groups were transfected with the corresponding plasmids after H/R induction. Edu assay was used to detect the cell proliferation. Flow cytometry and AO/EB staining were used to detect the cell apoptosis. ELISA was used to detect the levels of injury, inflammatory response and oxidative stress-related factors in myocardial cells. DCFDA fluorescence probe method was used to detect the production of reactive oxygen species (ROS) in cells. Western blot was used to detect the expression of apoptosis-related proteins. qRT-PCR was used to detect the levels of lncRNA SNHG16 and miR-182-5p in each group of cells. Dual-luciferase reporter gene assay and RNA pull-down assay was used to detect the targeting relationship between lncRNA SNHG16 and miR-182-5p. **Results** Compared with the control group, the Edu positive rate, superoxide dismutase (SOD) activity and miR-182-5p level in the model group decreased ($P<0.05$), the apoptosis rate, the proportion of apoptotic cells, the levels of lactate dehydrogenase (LDH), creatine kinase (CK), interleukin (IL)-6, IL-1β, tumor necrosis factor-alpha (TNF-α), ROS, malondialdehyde (MDA), lncRNA SNHG16, and the expressions of cleaved caspase-3 (C-Caspase-3) and B-cell lymphoma-2-associated X protein (Bax)/B-cell lymphoma-2 (Bcl-2) ratio increased ($P<0.05$). Compared with the si-NC group, the Edu positive rate, SOD activity and miR-182-5p level in the si-SNHG16 group increased ($P<0.05$), the apoptosis rate, the proportion of apoptotic cells, the levels of LDH, CK, IL-6, IL-1β, TNF-α, ROS, MDA, lncRNA SNHG16, and the expressions of C-Caspase-3 and Bax/Bcl-2 ratio decreased ($P<0.05$). Compared with the si-SNHG16+anti-NC group, the Edu positive rate, SOD activity and miR-182-5p level in the si-SNHG16+anti-miR-182-5p group decreased ($P<0.05$), the apoptosis rate, the proportion of apoptotic cells, the levels of LDH, CK, IL-6, IL-1β, TNF-α, ROS, MDA, and the expressions of C-Caspase-3 and Bax/Bcl-2 ratio increased ($P<0.05$). The RNA pull-down experiment revealed that there was an interaction between lncRNA SNHG16 and miR-182-5p. Compared with the cardiomyocytes co-transfected with WT-SNHG16 and miR-NC, the cardiomyocytes co-transfected with WT-SNHG16 and miR-182-5p mimics showed a decrease in relative luciferase activity ($P<0.05$). **Conclusion** Silencing lncRNA SNHG16 can reduce H/R-induced cardiomyocyte apoptosis by upregulating miR-182-5p.

Keywords: lncRNA SNHG16; miR-182-5p; hypoxia/reoxygenation; cardiomyocytes; apoptosis

缺血性心脏病是全球最常见的死亡原因，也是全球最大的健康负担。急性心肌梗死作为其最严重、最危急的类型，主要是在冠状动脉粥样硬化病变的基础上，斑块突然破裂形成血栓，堵塞血管，导致供血不足，引发心肌区域坏死，若不及时治疗，将发展为心力衰竭，甚至导致死亡[1]。通过经皮冠状动脉介入和外科冠状动脉旁路移植术进行早期再灌注，可显著改善急性心肌梗死患者的预后，但再灌注过程中可加重心肌损伤，形成缺血/再灌注(ischemia/reperfusion, I/R)损伤[2]。目前，临床上仍缺乏针对I/R损伤的有效治疗策略。近年来研究发现，表观遗传调控在I/R损伤的研究和治疗进程中发挥重要的作用[3]。其中抑制lncRNA SNHG16可减轻缺氧/复氧(hypoxia/reoxygenation, H/R)诱导的心肌细胞凋亡及心肌I/R损伤[4]。miR-182-5p可减轻H/R诱导的炎症反应，减少心肌梗死面积，改善心脏功能，对心肌I/R损伤发挥保护作用[5]。Starbase网站预测lncRNA SNHG16与miR-182-5p之间存在结合位点；另有研究发现，沉默lncRNA SNHG16可通过靶向上调miR-182-5p抑制血管紧张素Ⅱ引起的心脏肥大[6]。推测lncRNA SNHG16可通过靶向调控miR-182-5p影响H/R诱导的心肌细胞凋亡，从细胞层面丰富了I/R损伤的靶向治疗机制。

1 材料与方法

1.1 主要材料

H9C2心肌细胞(批号：HT-X1594，深圳市豪地华拓生物科技有限公司)；乳酸脱氢酶(lactate dehydrogenase, LDH) ELISA试剂盒(批号：KTE4059，亚科因生物技术有限公司)；肌酸激酶(creatine kinase, CK)、肿瘤坏死因子-α(tumor necrosis factor-alpha, TNF-α) ELISA试剂盒(批号：EK-R38852、EK-R31197，上海酶研生物科技有限公司)；白细胞介素(interleukin, IL)-6、

IL-1β ELISA试剂盒(批号:B20431、KB10760,上海江莱生物科技有限公司);丙二醛(malondialdehyde,MDA)、超氧化物歧化酶(superoxide dismutase,SOD)ELISA试剂盒(批号:YM-mj1921、YM-S1922,上海远慕生物科技有限公司);裂解型胱天蛋白酶-3(cleaved caspase-3,C-Caspase-3)、B细胞淋巴瘤-2(B-cell lymphoma-2,Bcl-2)、Bcl-2相关X蛋白(Bcl-2-associated X protein,Bax)抗体(批号:40500、24245、34260,南京赛戈巍生物科技有限公司)。

1.2 细胞培养与处理

将H9C2细胞接种于DMEM培养液(含10%胎牛血清),并置于含5%CO_2的37 ℃恒温培养箱孵育,待细胞生长密度达70%,加入胰蛋白酶消化传代,选择第3代对数期细胞进行后续研究。

将H9C2细胞分为对照组(正常培养)、模型组(H/R诱导)、si-NC组、si-SNHG16组、si-SNHG16+anti-NC组、si-SNHG16+anti-miR-182-5p组。H/R诱导[7]:将对数期细胞接种于96孔板,待细胞生长密度达70%时,更换为不含血清的培养液,并置于含5%CO_2、95%N_2的37 ℃恒温培养箱缺氧处理21 h,再更换为DMEM培养液(含15%胎牛血清),置于含5%CO_2的37 ℃恒温培养箱复氧处理6 h。除对照组与模型组以外,其余各组细胞均在H/R诱导后采用Lipo-fectamineTM 3000转染相应质粒48 h。

1.3 Edu实验

将各组细胞接种于24孔板培养24 h,加入Edu溶液于37 ℃反应2 h,洗涤后4%多聚甲醛固定,再用0.5% Triton X-100室温通透10~20 min,随后进行DAPI室温避光染核,清洗后加入封片剂,置于显微镜下观察分析Edu阳性率。

1.4 流式细胞术

将各组细胞接种于24孔板培养24 h,收集细胞并进行胰蛋白酶消化,离心弃上清后,用PBS洗涤并加入Binding Buffer重悬细胞,依次进行Annexin V-FITC和PI室温避光反应,最后流式细胞仪检测细胞凋亡。

1.5 AO/EB染色

将各组细胞接种于24孔板培养24 h,加入AO/EB染液避光孵育,荧光显微镜下观察分析凋亡细胞(早期凋亡细胞核呈亮绿色荧光,晚期凋亡或坏死细胞呈橙红色荧光)所占比例。

1.6 ELISA实验

将各组细胞接种于24孔板培养24 h,收集细胞,离心取上清,ELISA试剂盒检测心肌损伤(LDH、CK)、炎症反应(IL-6、IL-1β、TNF-α)及氧化应激(MDA、SOD)相关因子水平。

1.7 DCFDA实验

将各组细胞接种于96孔板培养24 h,加入100 μL DCFDA避光孵育45 min,更换为不含酚红的完全培养基,酶标仪检测485 nm激发光波长与535 nm发射光波长处的荧光强度,根据485 nm激发光谱/535 nm发射光谱计算活性氧(reactive oxygen species,ROS)相对荧光强度。

1.8 Western blot实验

使用预冷的蛋白裂解液提取各组细胞总蛋白并进行定量,将蛋白经沸水浴变性后,进行SDS-PAGE凝胶电泳分离并转至PVDF膜后封闭,将封闭膜与Bax、Bcl-2、β-actin一抗于4 ℃孵育过夜,再加入HRP标记二抗室温孵育1 h,ECL显影后,收集图像并进行蛋白分析。

1.9 qRT-PCR实验

提取各组细胞RNA,反转录并扩增,采用$2^{-\Delta\Delta Ct}$法计算lncRNA SNHG16(GAPDH)、miR-182-5p(U6)水平。lncRNA SNHG16正向引物GCAGAATGCCATGGTTTCCC,反向引物GGACAGCTGGCAAGAGACTT;GAPDH正向引物CATGAGAAGTATGACAACAGCCT,反向引物AGTCCTTCCACGATACCAAAGT;miR-182-5p正向引物GTCGTATCCAGTGCGTGTCGTGGAGTC,反向引物GGCAATTGCACTGGATACGACAGTGTG;U6正向引物CTCGCTTCGGCAGCACA,反向引物AACGCTTCACGAATTTGCGT。

1.10 双荧光素酶报告基因实验

将WT-SNHG16与MUT-SNHG16目的片段扩增到pGL3 vector载体,然后分别与miR-NC、miR-182-5p mimics质粒共转染至H9C2细胞48 h,根据双荧光素酶报告基因实验操作分析细胞相对荧光素酶活性。

1.11 RNA pull-down

使用RIPA裂解液处理H/R诱导的H9C2细胞,加入RNase抑制剂防止RNA降解,加入蛋白酶抑制剂确保蛋白质活性。生物素标记(bio)WT-SNHG16与MUT-SNHG16及阴性对照(NC),并加入链霉亲和素磁珠室温反应1 h,加入细胞裂解液于4 ℃下反应2~4 h,提取pull-down复合物中RNA,反转录扩增,qRT-PCR检测miR-182-5p的富集情况。

1.12 统计学方法

采用SPSS 25.0进行统计分析,计量资料符合正态分布,以均数±标准差($\bar{x}\pm s$)描述,多组间比较采用单因素方差分析,组间两两比较采用*SNK-q*检验。$P<0.05$表示差异有统计学意义。

2 结果

2.1 沉默lncRNA SNHG16对细胞增殖的影响

与对照组比较，模型组细胞Edu阳性率降低（$P<0.05$）；与si-NC组比较，si-SNHG16组细胞Edu阳性率升高（$P<0.05$）；与si-SNHG16+anti-NC组比较，si-SNHG16+anti-miR-182-5p组细胞Edu阳性率降低（$P<0.05$），见图1、2。

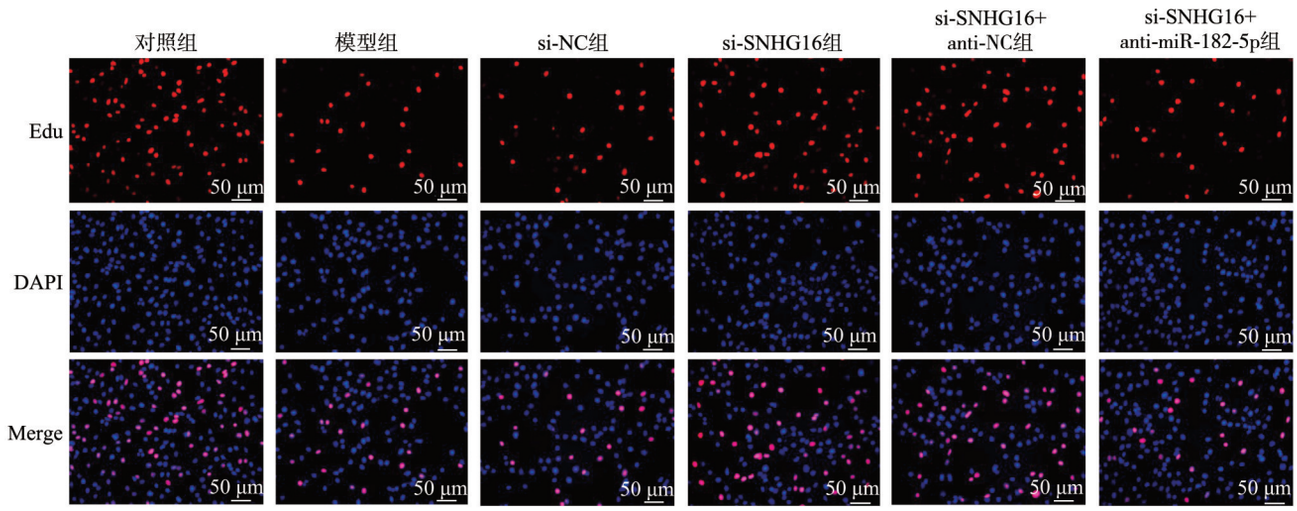
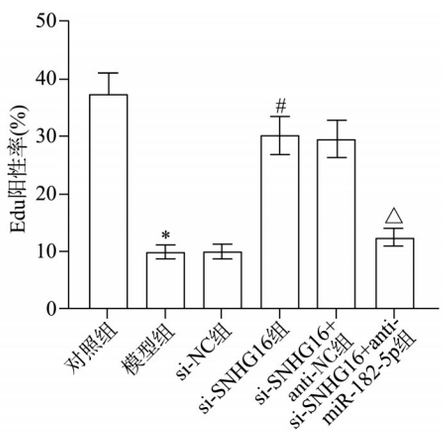


图1 Edu实验染色图片



*：与对照组比较，$P<0.05$；#：与si-NC组比较，$P<0.05$；△：与si-SNHG16+anti-NC组比较，$P<0.05$

图2 Edu检测细胞增殖

2.2 沉默lncRNA SNHG16对细胞凋亡的影响

与对照组比较，模型组细胞凋亡率、凋亡细胞占比升高（$P<0.05$）；与si-NC组比较，si-SNHG16组细胞凋亡率、凋亡细胞占比降低（$P<0.05$）；与si-SNHG16+anti-NC组比较，si-SNHG16+anti-miR-182-5p组细胞凋亡率、凋亡细胞占比升高（$P<0.05$），见图3、4。

2.3 沉默lncRNA SNHG16对心肌细胞损伤的影响

与对照组比较，模型组细胞LDH、CK水平升高（$P<0.05$）；与si-NC组比较，si-SNHG16组细胞LDH、CK水平降低（$P<0.05$）；与si-SNHG16+anti-NC组比较，si-SNHG16+anti-miR-182-5p组细胞LDH、CK水平升高（$P<0.05$），见表1。

2.4 沉默lncRNA SNHG16对细胞炎症因子的影响

与对照组比较，模型组细胞IL-6、IL-1β、TNF-α水平升高（$P<0.05$）；与si-NC组比较，si-SNHG16组细胞IL-6、IL-1β、TNF-α水平降低（$P<0.05$）；与si-SNHG16+anti-NC组比较，si-SNHG16+anti-miR-182-5p组细胞IL-6、IL-1β、TNF-α水平升高（$P<0.05$），见表2。

2.5 沉默lncRNA SNHG16对细胞氧化应激的影响

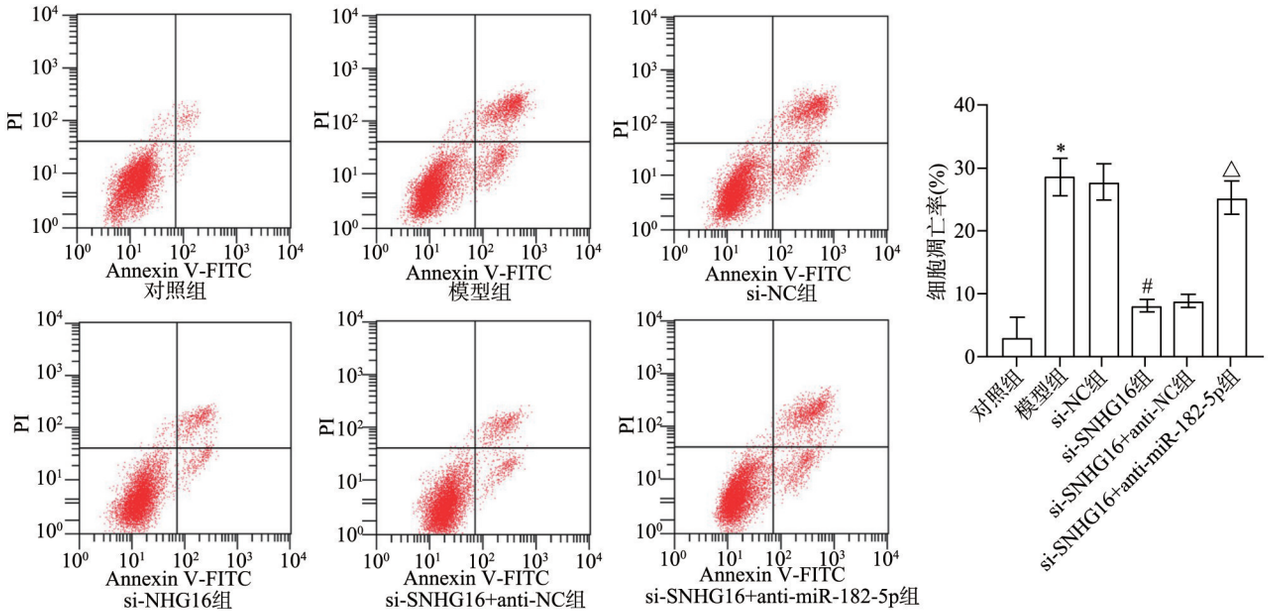
与对照组比较，模型组细胞ROS、MDA水平升高（$P<0.05$），SOD活性降低（$P<0.05$）；与si-NC组比较，si-SNHG16组细胞ROS、MDA水平降低（$P<0.05$），SOD活性升高（$P<0.05$）；与si-SNHG16+anti-NC组比较，si-SNHG16+anti-miR-182-5p组细胞ROS、MDA水平升高（$P<0.05$），SOD活性降低（$P<0.05$），见图5、表3。

2.6 沉默lncRNA SNHG16对细胞凋亡相关蛋白表达的影响

与对照组比较，模型组细胞C-Caspase-3、Bax/Bcl-2表达升高（$P<0.05$）；与si-NC组比较，si-SNHG16组细胞C-Caspase-3、Bax/Bcl-2表达降低（$P<0.05$）；与si-SNHG16+anti-NC组比较，si-SNHG16+anti-miR-182-5p组细胞C-Caspase-3、Bax/Bcl-2表达升高（$P<0.05$），见表4、图6。

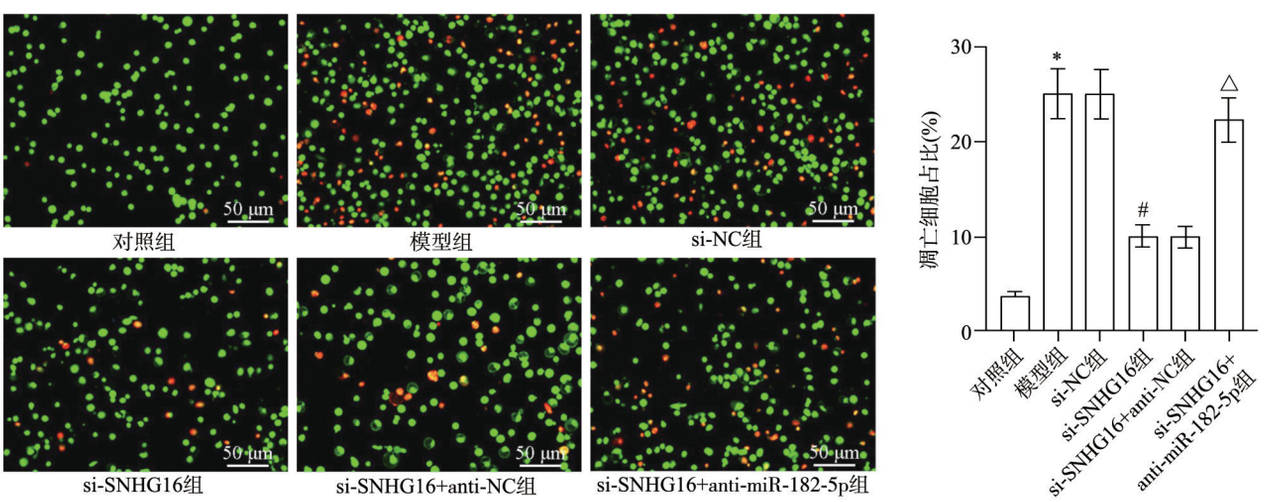
2.7 沉默lncRNA SNHG16对细胞lncRNA SNHG16、miR-182-5p表达的影响

与对照组比较，模型组细胞lncRNA SNHG16水平升高（$P<0.05$），miR-182-5p水平降低（$P<0.05$）；与



*:与对照组比较,$P<0.05$;#:与si-NC组比较,$P<0.05$;△:与si-SNHG16+anti-NC组比较,$P<0.05$

图3 流式细胞术检测细胞凋亡



*:与对照组比较,$P<0.05$;#:与si-NC组比较,$P<0.05$;△:与si-SNHG16+anti-NC组比较,$P<0.05$

图4 AO/EB染色检测细胞凋亡

表1 各组细胞LDH、CK水平比较($\bar{x}\pm s$,U/L)

组别	LDH	CK
对照组	18.42±1.97	10.31±1.26
模型组	95.27±9.83*	75.48±7.72*
si-NC组	93.12±9.52	74.36±7.68
si-SNHG16组	26.79±2.76#	16.90±1.85#
si-SNHG16+anti-NC组	27.15±2.84	17.08±1.94
si-SNHG16+anti-miR-182-5p组	80.32±8.39△	64.25±6.63△

*:与对照组比较,$P<0.05$;#:与si-NC组比较,$P<0.05$;△:与si-SNHG16+anti-NC组比较,$P<0.05$

si-NC组比较,si-SNHG16组细胞lncRNA SNHG16水平降低($P<0.05$),miR-182-5p水平升高($P<0.05$);与si-SNHG16+anti-NC组比较,si-SNHG16+anti-miR-182-5p组细胞lncRNA SNHG16无显著变化($P>0.05$),miR-182-5p水平降低($P<0.05$),见表5。

2.8 lncRNA SNHG16与miR-182-5p靶向关系验证

Starbase网站预测lncRNA SNHG16与miR-182-5p之间存在结合位点;RNA pull-down实验发现lncRNA SNHG16与miR-182-5p之间存在相互作用;与WT-SNHG16和miR-NC共转染的心肌细胞相比,WT-SNHG16和miR-182-5p mimics共转染的心肌细胞相对荧光素酶活性降低($P<0.05$),见图7、8及表6。

3 讨论

本研究通过H/R处理心肌细胞,从细胞层面模拟体外I/R损伤模型,结果显示,模型组细胞Edu阳性率

表2 各组细胞IL-6、IL-1β、TNF-α水平比较($\bar{x}\pm s$,pg/mL)

组别	IL-6	IL-1β	TNF-α
对照组	53.49±5.59	87.93±9.25	229.36±23.14
模型组	185.31±19.24*	305.24±31.22*	514.28±52.17*
si-NC组	180.22±19.07	296.58±30.14	502.17±51.83
si-SNHG16组	70.14±7.35#	123.47±13.29#	283.45±30.24#
si-SNHG16+anti-NC组	72.63±7.56	130.56±13.56	291.02±31.39
si-SNHG16+anti-miR-182-5p组	167.28±17.28△	271.15±28.17△	480.39±49.26△

*:与对照组比较,$P<0.05$;#:与si-NC组比较,$P<0.05$;△:与si-SNHG16+anti-NC组比较,$P<0.05$

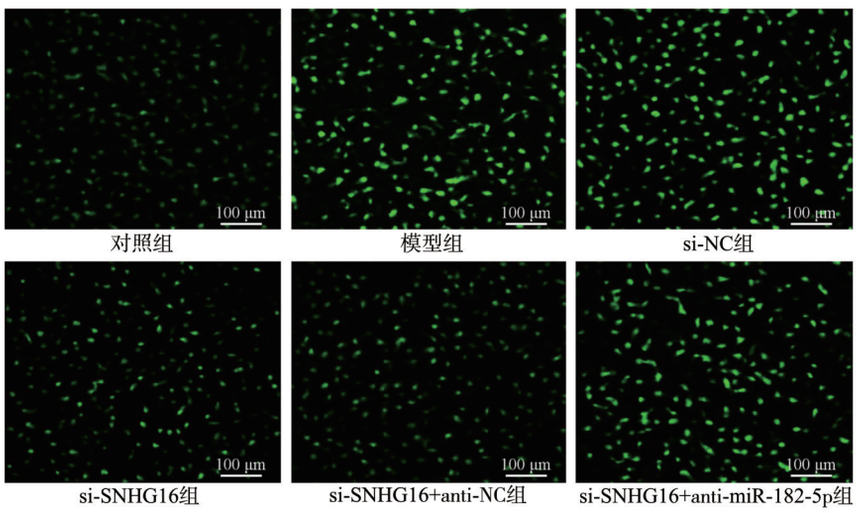


图5 DCFDA荧光探针检测细胞ROS水平

表3 各组细胞ROS、MDA、SOD水平比较($\bar{x}\pm s$)

组别	ROS(RFU×10³)	MDA(μmol/L)	SOD(U/L)
对照组	7.46±1.02	30.15±3.52	55.37±5.70
模型组	27.11±2.81*	87.26±8.96*	18.29±2.08*
si-NC组	26.84±2.75	86.74±8.78	19.03±2.17
si-SNHG16组	9.35±1.19#	35.18±3.72#	50.24±5.25#
si-SNHG16+anti-NC组	9.48±1.23	35.94±3.85	50.09±5.19
si-SNHG16+anti-miR-182-5p组	23.59±2.61△	73.29±7.62△	22.51±2.46△

*:与对照组比较,$P<0.05$;#:与si-NC组比较,$P<0.05$;△:与si-SNHG16+anti-NC组比较,$P<0.05$

表4 各组细胞凋亡相关蛋白表达比较($\bar{x}\pm s$)

组别	C-Caspase-3	Bax	Bcl-2	Bax/Bcl-2
对照组	0.65±0.08	0.57±0.07	1.05±0.12	0.54±0.06
模型组	1.17±0.13*	1.08±0.12*	0.52±0.06*	2.08±0.22*
si-NC组	1.15±0.13	1.07±0.12	0.53±0.06	2.02±0.21
si-SNHG16组	0.72±0.08#	0.64±0.07#	0.90±0.10#	0.71±0.08#
si-SNHG16+anti-NC组	0.76±0.09	0.66±0.08	0.85±0.10	0.78±0.09
si-SNHG16+anti-miR-182-5p组	1.02±0.11△	0.95±0.11△	0.60±0.07△	1.58±0.17△

*:与对照组比较,$P<0.05$;#:与si-NC组比较,$P<0.05$;△:与si-SNHG16+anti-NC组比较,$P<0.05$

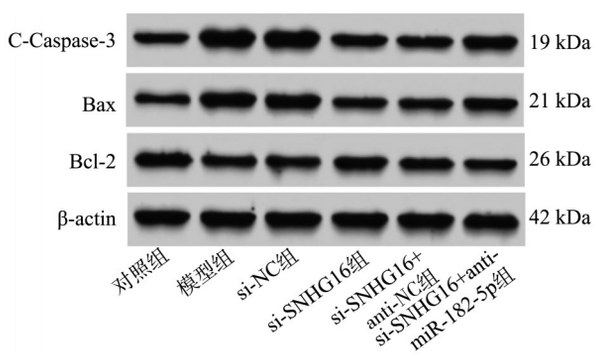


图6 Western blot检测C-Caspase-3、Bax、Bcl-2表达

表5 各组细胞lncRNA SNHG16、miR-182-5p水平比较($\bar{x}\pm s$)

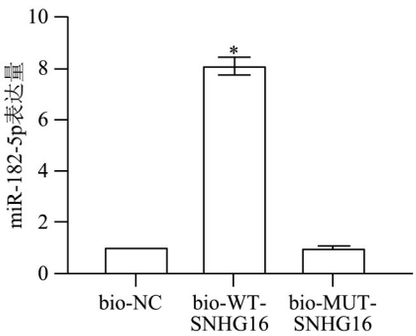
组别	lncRNA SNHG16	miR-182-5p
对照组	1.00±0.00	1.00±0.00
模型组	1.31±0.14*	0.54±0.06*
si-NC组	1.30±0.14	0.55±0.07
si-SNHG16组	1.05±0.12#	0.93±0.10#
si-SNHG16+anti-NC组	1.06±0.12	0.91±0.10
si-SNHG16+anti-miR-182-5p组	1.07±0.12	0.60±0.07△

*:与对照组比较,$P<0.05$;#:与si-NC组比较,$P<0.05$;△:与si-SNHG16+anti-NC组比较,$P<0.05$

has-miR-182-5p↑	ENSG00000163597	SNHG16↑	lncRNA↑	chr17:76564818-76564842[+]	↑

Target: 5′ GGAGGGACUGUUUAAGGUUGCCAAG 3′↑
miRNA: 3′ UCACACUCA-AGAUGGUAACGGUUU 5′

图7 Starbase网站预测lncRNA SNHG16与miR-182-5p的靶向关系



*:与bio-NC比较,$P<0.05$

图8 各组miR-182-5p富集倍数比较

表6 相对荧光素酶活性比较($\bar{x}\pm s$)

质粒	WT-SNHG16	MUT-SNHG16
miR-NC	1.00±0.11	1.01±0.11
miR-182-5p mimics	0.55±0.06*	0.98±0.11

*:与WT-SNHG16+miR-NC比较,$P<0.05$

降低,细胞凋亡率、凋亡细胞占比及LDH、CK水平显著升高,提示H/R细胞模型构建成功。在I/R损伤发展进程中,炎症反应贯穿全程,其中促炎因子IL-6、IL-1β、TNF-α发挥着重要作用。本研究模型组细胞中TNF-α、IL-1β、IL-6水平显著升高。研究显示,在心肌缺血再灌注损伤中,下调IL-6、IL-1β、TNF-α表达可抑制炎症反应,减少梗死面积和细胞凋亡,改善I/R后大鼠的心功能[8-9]。提示抑制炎症对于改善I/R损伤具有重要作用。氧化应激是I/R损伤的另一核心机制,主要是缺血导致线粒体功能受损,再灌注时大量氧气涌入及NADPH氧化酶等被激活,导致ROS爆发,氧化与抗氧化反应严重失衡,进而引发细胞氧化损伤,甚至死亡。本研究模型组细胞中ROS、MDA、LDH水平显著升高,SOD活性降低。研究显示,降低ROS、MDA、LDH水平,提高SOD活性,可减轻氧化应激反应与H/R诱导的心肌细胞损伤[10-11]。提示氧化应激在I/R损伤进程中发挥重要作用。此外,细胞凋亡是I/R损伤的重要病理机制,也是炎症与氧化应激反应的最终表现,其中细胞线粒体凋亡途径尤为关键[12]。本研究模型组细胞中C-Caspase-3表达、Bax/Bcl-2比值升高。Bax、Bcl-2作为线粒体凋亡途径关键蛋白,两者相互拮抗,Bax/Bcl-2比值是反映细胞凋亡的重要指标。C-Caspase-3是细胞凋亡执行蛋白的活化形式。研究显示,下调C-Caspase-3、Bax表达,上调Bcl-2表达,可抑制H/R诱导的H9C2细胞凋亡[13]。此外,体内外实验研究显示,降低LDH、CK、TNF-α、IL-1β、IL-6、ROS、MDA水平及C-Caspase-3表达、Bax/Bcl-2比值,提高SOD活性,可减轻炎症及氧化应激反应,改善线粒体功能障碍,减少细胞凋亡,从而保护心肌免受I/R损伤[14-15]。提示抑制细胞凋亡对于改善I/R损伤至关重要。

近年来,表观遗传调控在心血管疾病研究中备受关注。lncRNA SNHG16作为表观遗传调控分子之一,被发现与包括I/R损伤在内的多种心血管疾病的发生发展与治疗密切相关[16]。本研究发现lncRNA SNHG16在H/R诱导的心肌细胞中高表达,沉默lncRNA SNHG16可下调LDH、CK、TNF-α、IL-1β、IL-6、ROS、MDA水平及C-Caspase-3表达、Bax/Bcl-2比值,同时提高SOD活性。提示沉默lncRNA SNHG16可能成为I/R损伤的潜在治疗机制。

miRNA同样是表观遗传调控的重要组成部分。研究显示,miR-182-5p可作为识别心脏损伤和药物诱导心脏毒性的早期生物标志物,其过表达可抑制炎症反应,改善心功能,减轻小鼠心肌梗死诱导的心肌损伤[17-18]。miRNA通常受lncRNA靶向调控,进而影响下游基因表达,参与I/R损伤的发展及治疗[19]。其中lncRNA CHRF可负向调控miR-182-5p表达,使LDH水平升高,进而促进心肌细胞凋亡,加重心肌I/R损伤[20]。另外,Starbase网站预测lncRNA SNHG16与miR-182-5p之间存在结合位点,推测lncRNA SNHG16可能通过调控miR-182-5p影响心肌I/R损伤发展进程。本研究通过RNA pull-down实验及双荧光素酶报告基因实验发现,lncRNA SNHG16与miR-182-5p之间存在靶向关系,而且miR-182-5p在H/R诱导的心肌细胞中低表达,沉默lncRNA SNHG16可上调miR-182-5p水平,在此基础上沉默miR-182-5p可部分逆转沉默lncRNA SNHG16对H/R诱导的心肌细胞凋亡的改善作用。这进一步证实,lncRNA SNHG16可能通过负向调控miR-182-5p,减轻H/R诱导的心肌细胞凋亡。

综上,沉默lncRNA SNHG16可通过上调miR-182-5p减少H/R诱导的心肌细胞凋亡。本研究从细胞层面丰富了I/R损伤的治疗机制,但其发生机制较复杂,后续仍需完善实验设计,并从动物水平深入探究论证,以期推动向临床转化。

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