过表达SNHG5对高糖诱导HK-2细胞凋亡及氧化应激的影响

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摘要 该文探讨小核仁RNA宿主基因5(small nucleolar RNA host gene 5, *SNHG5*)对高糖(high glucose, HG)诱导的HK-2凋亡、氧化应激的影响及分子机制。采用30 mmol/L葡萄糖处理HK-2细胞48 h, 将pcDNA、pcDNA-SNHG5、anti-miR-阴性对照(negative control, NC)、微小RNA-196a(microRNA-196a, miRNA/miR-196a) inhibitor、pcDNA-SNHG5分别与miR-NC、miR-196a mimic共转染至HK-2细胞中, 用30 mmol/L葡萄糖处理48 h。实时荧光定量PCR(quantitative real-time PCR, RT-qPCR)检测*SNHG5*和miR-196a的表达水平; 细胞计数试剂盒8(cell counting kit-8, CCK-8)检测细胞增殖; 流式细胞术检测细胞凋亡情况; Western blot法检测蛋白表达水平; 丙二醛(malondialdehyde, MDA)、超氧化物歧化酶(superoxide dismutase, SOD)、谷胱甘肽过氧化物酶(glutathione peroxidase, GSH-Px)试剂盒分别检测MDA含量及SOD、GSH-Px活性; 双荧光素酶报告实验检测*SNHG5*和miR-196a的靶向关系。该研究得出, 高糖诱导的HK-2细胞活性以及细胞中的*SNHG5*表达水平、B细胞淋巴瘤/白血病-2(B cell lymphoma/lewkmia-2, Bcl-2)表达水平均降低, miR-196a表达水平、凋亡率、活化的含半胱氨酸的天冬氨酸蛋白水解酶3(Cleaved cysteinyl aspartate specific proteinase 3, Cleaved-caspase3)表达水平、Bcl-2相关X蛋白(Bcl-2 associated X protein, Bax)表达水平、MDA含量均升高, SOD、GSH-Px活性降低($P<0.05$)。过表达*SNHG5*或抑制miR-196a表达后, 高糖诱导的HK-2的细胞活性显著上升且细胞死亡率降低, Bcl-2表达水平显著上升, Bax、Cleaved-caspase3与MDA含量明显降低, SOD、GSH-Px活性升高($P<0.05$)。SNHG5靶向调控miR-196a, miR-196a过表达可逆转*SNHG5*对高糖诱导的HK-2细胞活性、凋亡和氧化应激的影响。总之, 过表达*SNHG5*可能通过下调miR-196a抑制高糖诱导的HK-2凋亡及氧化应激。

关键词 SNHG5; miR-196a; 糖尿病肾病; 肾小管上皮细胞; 凋亡; 氧化应激

Effects of Overexpression of SNHG5 on High Glucose-Induced Apoptosis and Oxidative Stress in HK-2 Cells

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Abstract This study investigated the effect and molecular mechanism of *SNHG5* (small nucleolar RNA host gene 5) on HG (high glucose)-induced HK-2 apoptosis and oxidative stress. HK-2 cells were treated with 30 mmol/L glucose for 48 h. pcDNA, pcDNA-SNHG5, anti-miR-NC (negative control), microRNA-196a (miRNA/miR-196a)

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inhibitor, pcDNA-SNHG5 were co-transfected with miR-NC or miR-196a mimic into HK-2 cells, respectively. The expression levels of *SNHG5* and miR-196a were detected by RT-qPCR (quantitative real-time PCR); cell proliferation was detected by CCK-8 (cell counting kit-8); flow cytometry was performed to determine cell apoptosis; protein expression detected by Western blot; MDA (malondialdehyde), SOD(superoxide dismutase), glutathione peroxidase GSH-Px (glutathione peroxidase) kits were implemented to monitor the content of MDA and the activities of SOD and GSH-Px; the targeting relationship between *SNHG5* and miR-196a was detected by dual-luciferase reporter assay. The study concluded that the expression level of *SNHG5*, cell viability, and expression level of Bcl-2 (B cell lymphoma/lewkmia-2) in HK-2 cells induced by high glucose decreased, miR-196a expression level, apoptosis rate, expression of activated Cleaved-caspase3 (Cleaved cysteinyl aspartate specific proteinase 3) and Bax (Bcl-2 associated X protein) level and MDA content increased, while SOD and GSH-Px activities decreased (P<0.05). After overexpression of SNHG5 or inhibition of miR-196a expression, high glucose-induced HK-2 cell activity significantly increased and cell death rate decreased, Bcl-2 expression level increased significantly, Bax, Cleaved-caspase3 and MDA contents significantly decreased, SOD, GSH-Px activity increased (P<0.05). SNHG5 can target and regulate miR-196a expression, and miR-196a overexpression can reverse the effects of SNHG5 on high glucose-induced HK-2 cell viability, apoptosis and oxidative stress. In conclusion, overexpression of SNHG5 may inhibit high glucose-induced HK-2 apoptosis and oxidative stress by downregulating miR-196a.

Keywords SNHG5; miR-196a; diabetic nephropathy; renal tubular epithelial cells; apoptosis; oxidative stress

糖尿病肾病是终末期肾病的主要原因, 在该病早期, 可观察到肾小管细胞的氧化损伤和凋亡, 肾小管上皮细胞出现氧化应激反应, 这导致其分泌多种细胞因子, 从而引发间质炎症和纤维化, 介导糖尿病肾病发生与发展[1-2]。因此, 通过研究阐明肾小管损伤的分子机制, 对于确定药物干预靶点, 延缓糖尿病肾病的进展具有重要意义。研究表明长链非编码RNA(long noncoding RNA, lncRNA)异常表达与包括糖尿病肾病在内的多种疾病相关, 可能在肾小管上皮细胞损伤的病理生理过程中起作用, 或可作为糖尿病肾病的分子标志物[3]。小核仁RNA宿主基因5(small nucleolar RNA host gene 5, *SNHG5*)作为细胞质lncRNA, 全长为524 bp, 研究报道lncRNA *SNHG5*可促进人脐静脉内皮细胞增殖, 抑制细胞黏附[4]。过表达*SNHG5*对高糖(hiuantitativegh glucose, HG)缺氧引起的视网膜血管内皮细胞凋亡百分比增高和迁移面积增大具有抑制作用[5]。*SNHG5*通过增加星形胶质细胞和小胶质细胞的活力来促进脊髓损伤[6]。但目前尚未完全阐明*SNHG5*在糖尿病肾病中的作用及其机制。研究表明, 微小RNA(microRNA, miRNA/miR)在各种类型肾脏细胞中异常表达, 通过对其含量的测定, 有助于糖尿病肾病的早期诊断及对疾病发展的预测[7]。研究报道miR-196a水平与糖尿病肾病进展呈正相关, 分析原因可能其是糖尿病肾病患者肾纤维化的非侵入性预后标志物[8]。糖尿病肾病小鼠肾脏中miR-196a表达水平明显升高, miR-196a可能通过调节膜联蛋白1(Annexin 1, ANX1)、叉头转录因子1(forkhead box O1, FOXO1)的表达水平来参与糖尿病肾病的发生发展[9]。但目前miR-196a对肾小管上皮细胞凋亡和氧化应激的作用机制尚未有研究阐明。生物学软件预测发现, *SNHG5*与miR-196a有结合位点, 因此, 本实验用高糖处理肾小管上皮细胞HK-2并构建损伤模型, 研究*SNHG5*是否通过调控miR-196a影响高糖诱导的小管上皮细胞HK-2凋亡和氧化应激。

1 材料与方法

1.1 材料

肾小管上皮细胞HK-2(货号: CL1093)购自无锡欣润生物科技有限公司; DMEM培养基(货号: 31600034)购自上海联硕生物科技有限公司; 葡萄糖(货号: CS0798)购自北京凯瑞基生物科技有限公司; Lipofectamine™ 2000(货号: 11668-019)购自美国Invitrogen; 实时荧光定量PCR(quantitative real-time PCR, RT-qPCR)试剂盒(货号: 218073)购自北京杰辉博高生物技术有限公司; 细胞计数试剂盒8(cell counting kit-8, CCK-8, 货号: Lvn10031)购自北京利维宁生物科技有限公司; 磷脂酰结合蛋白V-FITC(Annexin V-

FITC)/碘化丙锭(propidium iodide, PI)凋亡检测试剂盒(货号: P-CA-201)购自武汉益普生物科技有限公司; 放射免疫沉淀法(Radio-Immunoprecipitation Assay, RIPA)蛋白裂解液(货号: QCB-3201-1)购自上海钦诚生物科技有限公司; 增强化学发光(enhanced chemiluminescence, ECL)液(货号: PE0010)购自上海恒斐生物科技有限公司; 丙二醛(malondialdehyde, MDA)含量检测试剂盒(货号: BC0020)购自北京索莱宝科技有限公司; 超氧化物歧化酶(superoxide dismutase, SOD)活性检测试剂盒(货号: SBJ-1616)、谷胱甘肽过氧化物酶(glutathione peroxidase, GSH-Px)活性检测试剂盒(批号: SBJ-1581)购自南京森贝伽生物科技有限公司; 双荧光素酶报告基因检测试剂盒(货号: AD0010-100T)购自上海吉至生化科技有限公司; B细胞淋巴瘤/白血病-2(B cell lymphoma/lewkmia-2, Bcl-2)、Bcl-2相关X蛋白(Bcl-2 associated X protein, Bax)抗体(货号: 251711、251834)购自美国Abbiotec; 活化的含半胱氨酸的天冬氨酸蛋白水解酶3(Cleaved cysteinyl aspartate specific proteinase 3, Cleaved-caspase3)抗体(货号: 1050S)购自美国CST; 山羊抗兔IgG-HRP(货号: L153A)购自美国GeneCopoeia。

1.2 细胞处理与分组

HK-2细胞用含10%胎牛血清的DMEM培养基培养, 用30 mmol/L葡萄糖处理HK-2 24 h构建损伤模型, 记为HG组, 不作处理的细胞作为Con组; 将pcDNA、pcDNA-SNHG5、anti-miR-阴性对照(negative control, NC)、miR-196a inhibitor转染至HK-2细胞后用30 mmol/L葡萄糖处理, 记为HG+pcDNA组、HG+pcDNA-SNHG5组、HG+anti-miR-NC组、HG+miR-196a inhibitor组; 将pcDNA-SNHG5分别与miR-NC、miR-196a mimic共转染至HK-2细胞中, 用30 mmol/L葡萄糖处理, 记为HG+pcDNA-SNHG5+miR-NC组、HG+pcDNA-SNHG5+miR-196a mimic组。其中, pcDNA-SNHG5载体通过pcDNA构建。具体转染步骤: 培养细胞使其密度为80%左右, 并将质粒与无血清DMEM培养皿进行稀释; 5 min内将其混合, 室温静置20 min; 将复合物加入到含细胞的培养板中, 37 ℃孵育6 h; 然后用30 mmol/L葡萄糖处理。

1.3 对SNHG5和miR-196a的表达水平采用RT-qPCR法进行检测

定量提取细胞RNA, 反转录成cDNA后进行实时荧光定量PCR检测, 设置循环条件参数并重复40次并计算。*SNHG5*以甘油醛-3-磷酸脱氢酶(glyceraldehyde-3-phosphate dehydrogenase, *GAPDH*)作为内部参照, miR-196a以*U6*作为内部参照, *SNHG5*上下游引物序列分别为: 5′-CGC TTG GTT AAA ACC TGA CAC T-3′, 5′-CCA AGA CAA TCT GGC CTC TAT C-3′; *GAPDH*上游引物序列: 5′-CAA GGT CAT CCA TGA CAA CTT TG-3′, 下游引物序列: 5′-GTC CAC CAC CCT GTT GCT GTA G-3′; miR-196a上下游引物序列分别为: 5′-ACC TGC GTA GGT AGT TTC ATG T-3′, 5′-CGT CAG AAG GAA TGA TGC ACA G-3′; *U6*上游引物序列: 5′-CTC GCT TCG GCA GCA CA-3′, 下游引物序列: 5′-TGG TGT CGT GGA GTC G-3′; 引物由上海生工生物工程有限公司合成。

1.4 CCK-8检测细胞增殖

各组转染后培养48 h的细胞(2×10^4个/mL), 取100 μL接种于96孔板中, 在37 ℃恒温培养箱培养48 h, 每孔加入10 μL CCK-8试剂, 37 ℃恒温培养箱继续培养, 2 h后使用酶标仪基于450 nm处检测到的吸光度(*D*)值来测量细胞活性。*D*值与细胞活性成正比。

1.5 流式细胞术检测细胞凋亡

将细胞培养48 h后进行收集, 并使用冷却好的磷酸盐缓冲液(phosphate buffered saline, PBS)洗涤液反复冲洗, 加入500 μL结合缓冲液进行重悬, 根据Annexin V-FITC/PI凋亡检测试剂盒操作规程, 加入10 μL的Annexin V-FITC和5 μL PI, 37 ℃黑暗中反应15 min后对细胞凋亡率采用流式细胞仪检测。

1.6 使用蛋白免疫印迹法检测蛋白表达水平

通过RIPA蛋白裂解液对血液中细胞总蛋白进行提取, 使用对应试剂盒定量蛋白, 聚丙烯酰胺凝胶电泳后转膜, 并使用5%脱脂牛奶于室温进行2 h封闭处理, 再加入一抗(Bcl-2、Bax、Cleaved-caspase3)(1:800) 4 ℃孵育过夜, 洗膜后加入二抗(山羊抗兔IgG-HRP) (1:1 500)室温孵育2 h, PBS洗涤3次, 每次10 min, 加入ECL电化学发光液显影, ImageJ软件检测灰度值, 以GAPDH作为参照计算蛋白水平。

1.7 MDA、SOD、GSH-Px试剂盒检测MDA含量及SOD、GSH-Px活性

采用比色法测定MDA含量及SOD与GSH-Px活性。对细胞培养48 h后进行收集, 随后对照相关试剂盒说明书操作, 分别加入MDA、SOD、GSH-Px的提取试剂, 在4 ℃下以8 000 ×*g*离心10 min。分别

加入MDA、SOD和GSH-Px检测试剂, 通过各自*D*值计算MDA含量、SOD活性与GSH-Px活性。

1.8 双荧光素酶报告实验检测*SNHG5*和miR-196a的靶向关系

使用PCR扩增包含miR-196a结合位点的*SNHG5*序列片段UUGUUGCCG, 并将其构建至荧光素酶表达载体中, 获得*SNHG5*野生型(wild type, wt)载体(wt-SNHG5), 将*SNHG5*序列UUGUUGCCG突变为AAAGCCAGA, 获得*SNHG5*突变型(mutant type, mut)载体(mut-SNHG5), 将wt-SNHG5和mut-SNHG5分别与miR-NC、miR-196a共转染至HK-2细胞, 连续放置48 h后按照相关说明书操作对其荧光素酶活性进行检测。

1.9 统计学分析

将纳入研究的所有数据进行收集并整理, 放入SPSS 20.0进行统计学数据分析, 计量资料符合正态分布后使用均数±标准差($\bar{x}\pm s$)表示, 组间数据差异行*t*检验, 单因素方差比较多组间数据差异, LSD-*t*检验两组间差异。$P<0.05$时具有统计学意义。

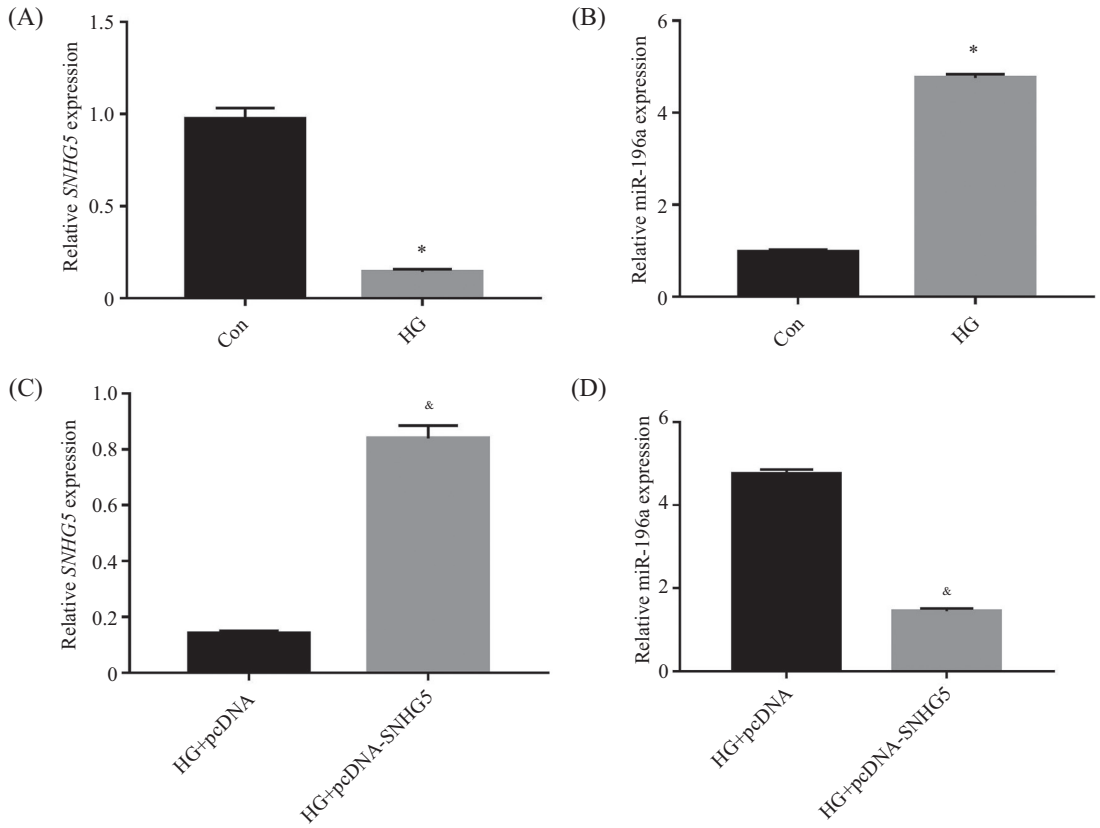
2 结果

2.1 *SNHG5*和miR-196a在高糖诱导的HK-2细胞中的表达

RT-qPCR对*SNHG5*和miR-196a表达水平的检测结果显示, 与Con组相比, HG组HK-2中*SNHG5*表达水平降低, miR-196a表达水平升高($P<0.05$); 与HG+pcDNA组相比, HG+pcDNA-SNHG5组HK-2中*SNHG5*表达水平升高, miR-196a表达水平降低($P<0.05$)(图1)。

2.2 SNHG5对高糖诱导的HK-2细胞活性和凋亡的影响

对细胞活性和凋亡的检测结果显示, 与Con组



A: RT-qPCR法检测*SNHG5*的表达量; B: RT-qPCR法检测miR-196a的表达量; C: RT-qPCR法检测*SNHG5*过表达转染效果; D: RT-qPCR法检测*SNHG5*过表达对miR-196a表达量的影响。*$P<0.05$, 与Con组相比; &$P<0.05$, 与HG+pcDNA组相比。

A: RT-qPCR was used to detect the expression of *SNHG5*; B: RT-qPCR was used to detect the expression of miR-196a; C: RT-qPCR was used to detect the transfection effect of *SNHG5* overexpression; D: RT-qPCR was used to detect the effect of *SNHG5* overexpression on the expression of miR-196a. *$P<0.05$ compared with Con group; &$P<0.05$ compared with HG+pcDNA group.

图1 *SNHG5*和miR-196a的表达

Fig.1 Expression of *SNHG5* and miR-196a

相比, HG组HK-2细胞活性降低, 凋亡率升高, Bcl-2表达水平呈下降趋势, Cleaved-caspase3、Bax表达水平呈显著上升趋势(P<0.05); 转染pcDNA-SNHG5过表达后, 与HG组、HG+pcDNA组相比, HG+pcDNA-SNHG5组HK-2细胞活性升高, 凋亡率降低, Bcl-2表达水平明显上升, Cleaved-caspase3、Bax表达水平显著下降(P<0.05)(图2~图4)。

2.3 SNHG5对高糖诱导的HK-2细胞氧化应激的影响

转染pcDNA-SNHG5过表达后, HG组HK-2中MDA含量较Con组显著上升且SOD、GSH-Px活性明显下降(P<0.05); 与HG组、HG+pcDNA组相比, HG+pcDNA-SNHG5组HK-2中MDA含量降低, SOD、GSH-Px活性升高(P<0.05)(图5)。

2.4 *SNHG5*和miR-196a靶向关系的验证

miR-196a与*SNHG5*含有互补的核苷酸序列(图6)。荧光素酶报告实验对二者关系进一步验证, wt-SNHG5与miR-196a共转染后的细胞荧光素酶活性降低(P<0.05); 而mut-SNHG5与miR-196a共转染后的细胞荧光素酶活性无显著变化(P>0.05)(图6)。

2.5 抑制miR-196a对高糖诱导的HK-2细胞活性和凋亡的影响

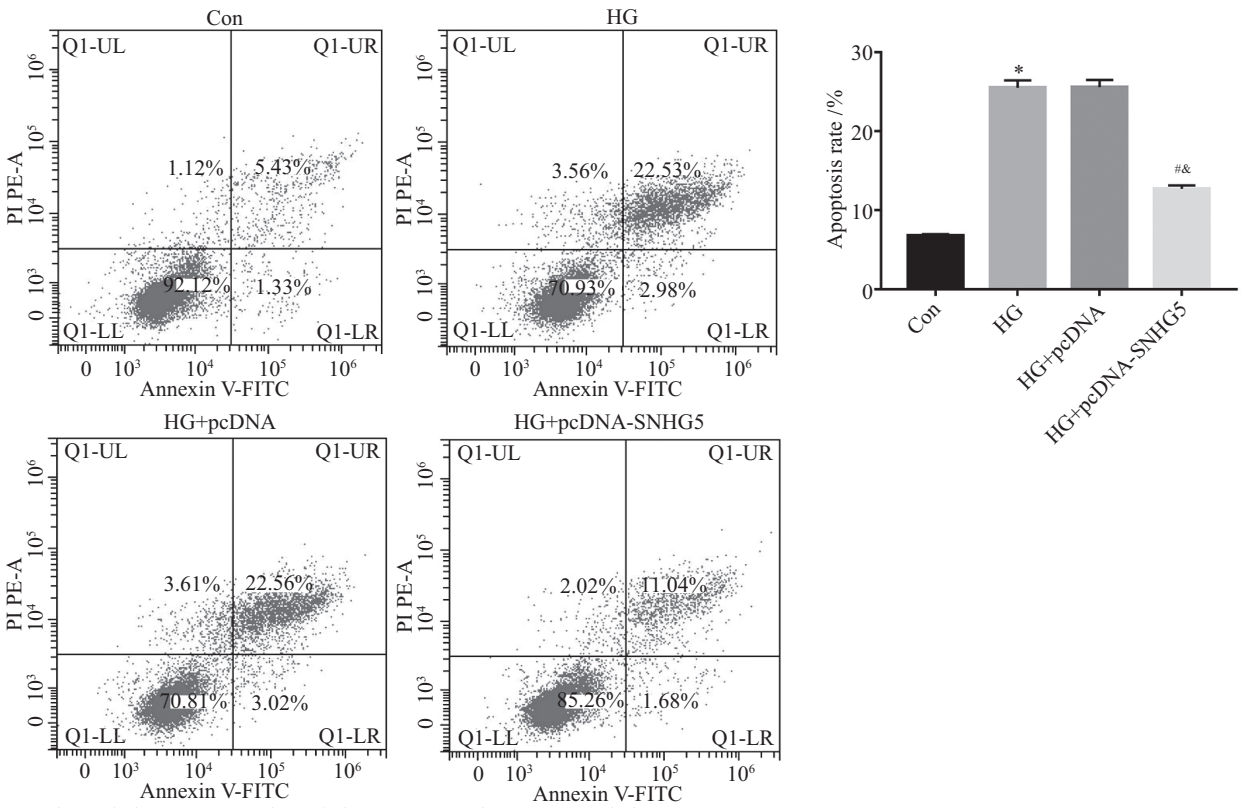
抑制miR-196a后, 与HG组和HG+anti-miR-NC组相比, HG+miR-196a inhibitor组HK-2细胞活性升高, 凋亡率降低, Bcl-2表达水平升高, Cleaved-caspase3、Bax表达水平降低(P<0.05)(图7~图9)。

2.6 抑制miR-196a对高糖诱导的HK-2细胞氧化应激的影响

抑制miR-196a后, 与HG组和HG+anti-miR-NC组相比, HG+miR-196a inhibitor组HK-2细胞中MDA含量降低, SOD、GSH-Px活性升高(P<0.05)(图10)。

2.7 miR-196a可逆转SNHG5对高糖诱导的HK-2细胞活性和凋亡的影响

与HG+pcDNA-SNHG5组和HG+pcDNA-SNHG5+miR-NC组相比, HG+pcDNA-SNHG5+miR-196a mimic组HK-2细胞活性降低, 凋亡率升高, Bcl-2表达水平降低, Cleaved-caspase3、Bax表达水平升高(P<0.05)(图11~图13)。

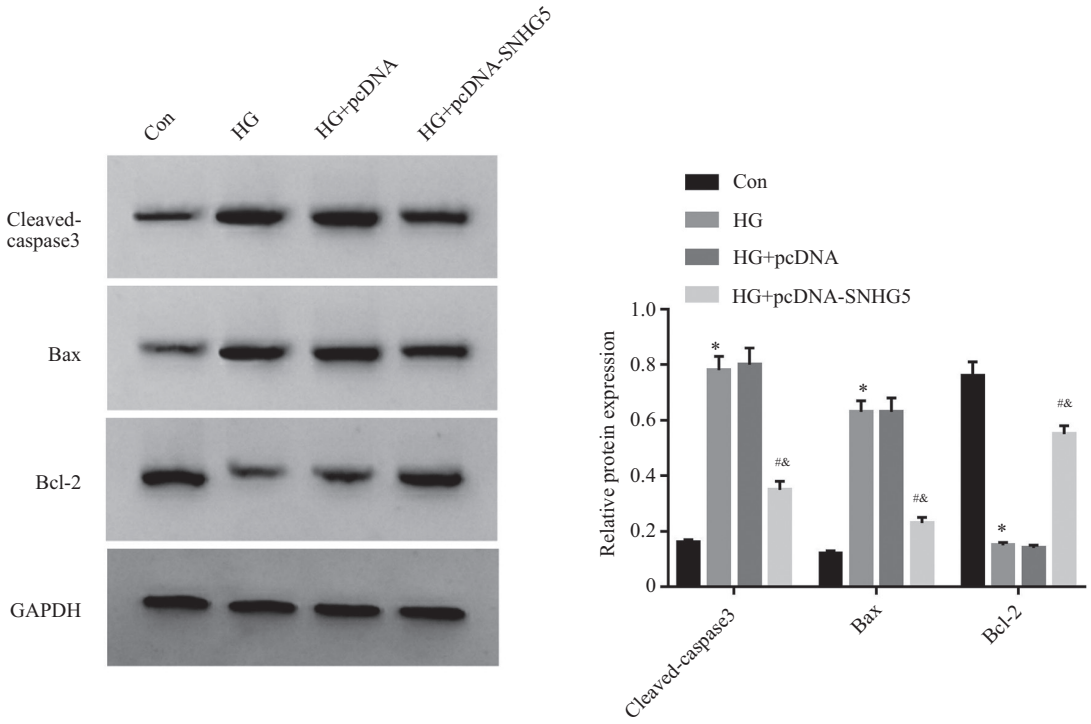


*P<0.05, 与Con组相比; #P<0.05, 与HG组相比; &P<0.05, 与HG+pcDNA组相比。

*P<0.05 compared with Con group; #P<0.05 compared with HG group; &P<0.05 compared with HG+pcDNA group.

图2 SNHG5对高糖诱导的HK-2细胞凋亡的影响

Fig.2 The effect of SNHG5 on HK-2 apoptosis induced by high glucose

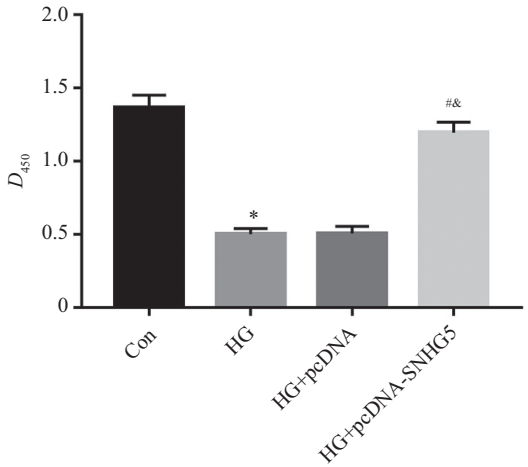


*P<0.05, 与Con组相比; #P<0.05, 与HG组相比; &P<0.05, 与HG+pcDNA组相比。

*P<0.05 compared with Con group; #P<0.05 compared with HG group; &P<0.05 compared with HG+pcDNA group.

图3 SNHG5对高糖诱导的HK-2凋亡蛋白表达的影响

Fig.3 The effect of SNHG5 on the expression of HK-2 apoptotic protein induced by high glucose



*P<0.05, 与Con组相比; #P<0.05, 与HG组相比; &P<0.05, 与HG+pcDNA组相比。

*P<0.05 compared with Con group; #P<0.05 compared with HG group; &P<0.05 compared with HG+pcDNA group.

图4 SNHG5对高糖诱导的HK-2细胞活性的影响

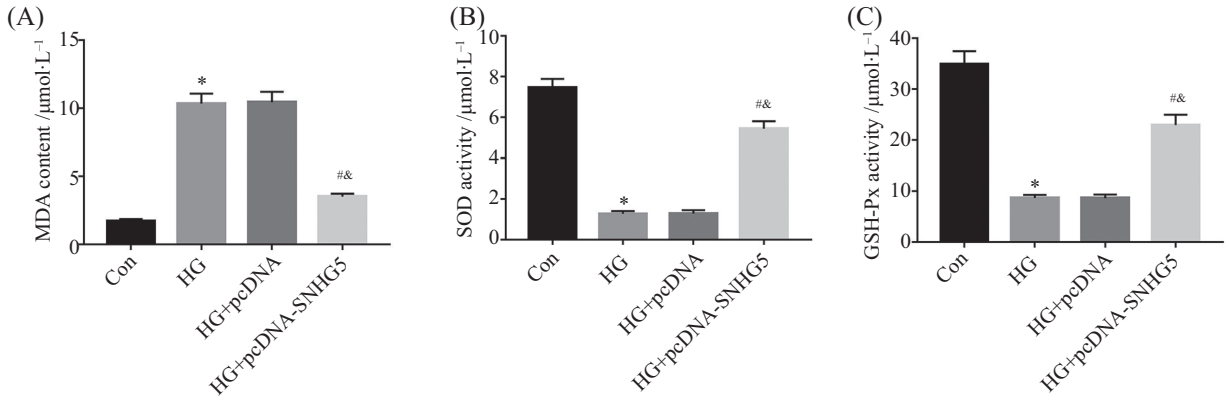
Fig.4 The effect of SNHG5 on the activity of HK-2 cells induced by high glucose

2.8 miR-196a可逆转SNHG5对高糖诱导的HK-2细胞氧化应激的影响

与HG+pcDNA-SNHG5组和HG+pcDNA-SNHG5+miR-NC组相比, HG+pcDNA-SNHG5+miR-196a mimic组HK-2细胞中MDA含量升高, SOD、GSH-Px活性降低(P<0.05)。

3 讨论

糖尿病肾病是糖尿病引起的慢性微血管并发症之一, 具有死亡率较高的特点, 肾小管损伤作为糖尿病肾病发展过程中的一个重要环节, 深入研究其分子机制可更好地预测糖尿病肾病的进展和预后[10]。据研究表明, 高糖可以促进HK-2细胞发生上皮–间

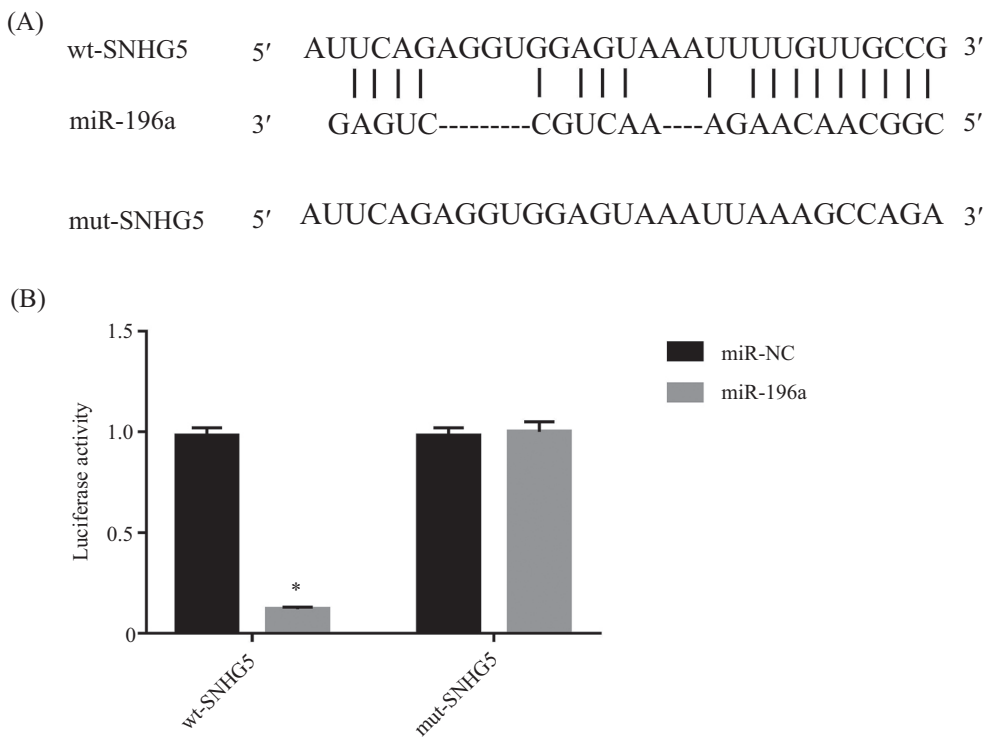


A: MDA试剂盒检测MDA含量; B: SOD试剂盒检测SOD活性; C: GSH-Px试剂盒检测GSH-Px活性; **P*<0.05, 与Con组相比; #*P*<0.05, 与HG组相比; &*P*<0.05, 与HG+pcDNA组相比。

A: MDA kit was used to detect MDA content; B: SOD kit was used to detect SOD activity; C: GSH-Px kit was used to detect GSH-Px activity; **P*<0.05 compared with Con group; #*P*<0.05 compared with HG group; &*P*<0.05 compared with HG+pcDNA group.

图5 SNHG5对高糖诱导的HK-2细胞氧化应激的影响

Fig.5 Effects of SNHG5 on HK-2 cells oxidative stress induced by high glucose



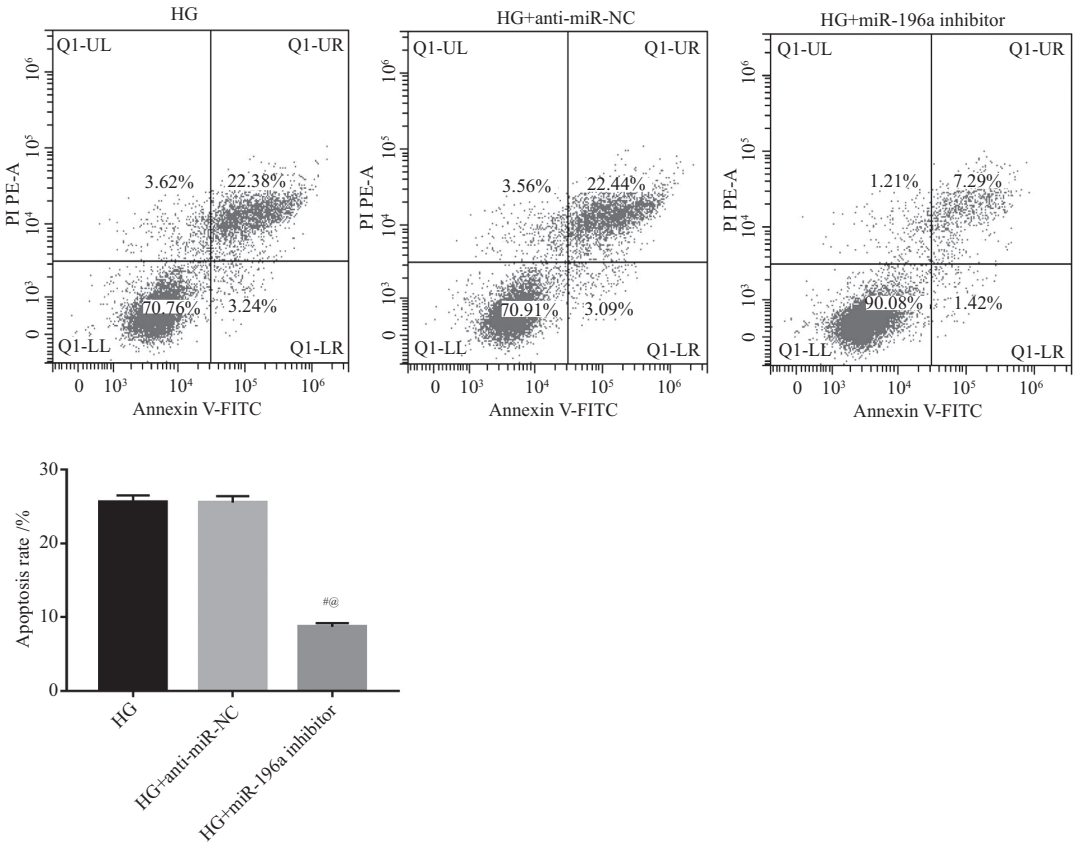
A: *SNHG5*与miR-196a存在结合位点; B: 双荧光素酶报告检测结果。**P*<0.05, 与miR-NC组相比。

A: there was a binding site between *SNHG5* and miR-196a; B: the detection result of dual luciferase reporter; **P*<0.05 compared with miR-NC group.

图6 *SNHG5*靶向miR-196a及荧光素酶报告实验

Fig.6 *SNHG5* targets miR-196a and double luciferase report experiment

质转化, 造成其纤维化, 引起肾小管损伤[11]。细胞凋亡作为糖尿病肾病高血糖损伤的重要机制之一[12]。本实验用高糖处理HK-2细胞构建损伤模型, 结果显示, 高糖诱导的HK-2细胞活性下降且细胞死亡率上升, Bcl-2表达水平呈下降趋势, Bax表达水平呈上升趋势; 与其他学者研究报道的高糖会降低HK-2细胞活力, 从而加速HK-2细胞凋亡[12]一致, 表明高糖可诱导HK-2细胞加速死亡。同时, 氧化应激作为肾小管间质病理损伤的过程之一, 不仅影响肾小管重吸收功能, 还会影响其分泌和间质纤维化相关作用[13]。研

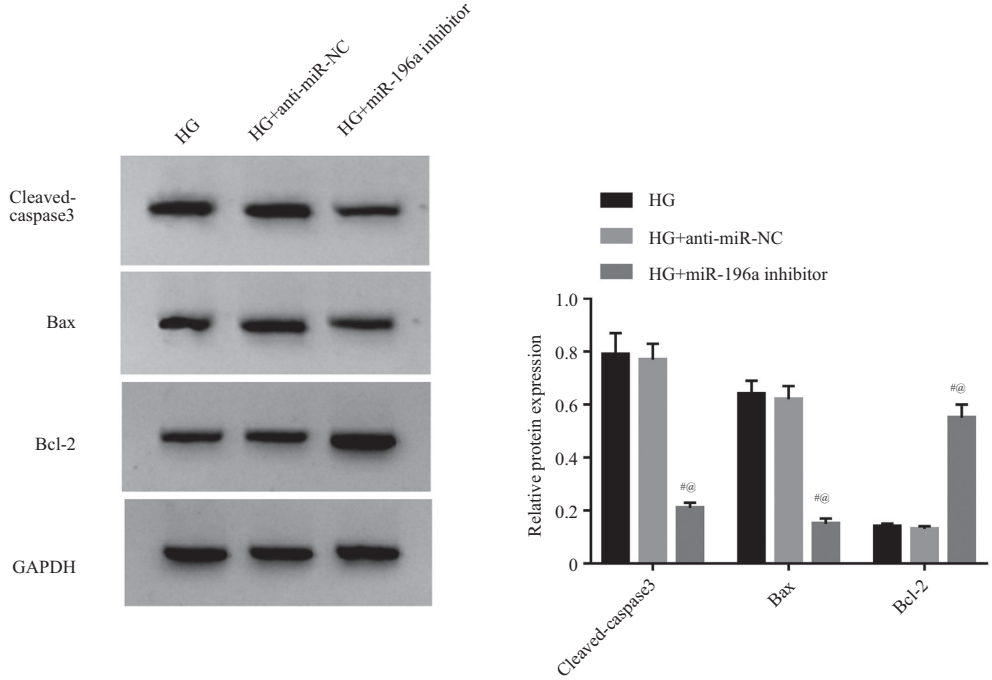


#P<0.05, 与HG组相比; @P<0.05, HG+anti-miR-NC组相比。

#P<0.05 compared with HG group; @P<0.05 compared with HG+anti-miR-NC group.

图7 抑制miR-196a对高糖诱导的HK-2细胞凋亡的影响

Fig.7 The effect of inhibiting miR-196a on HK-2 cells apoptosis induced by high glucose

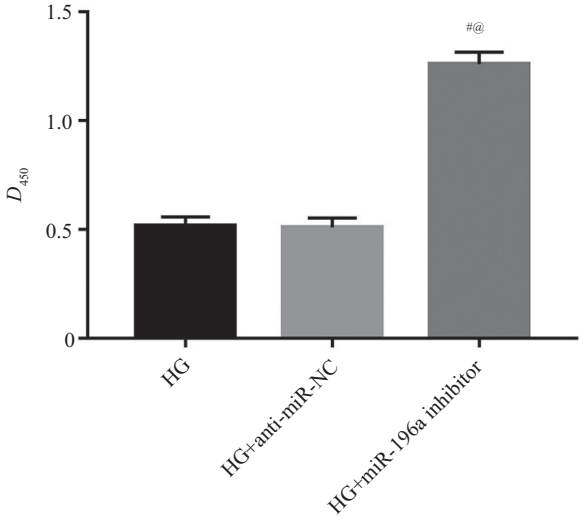


#P<0.05, 与HG组相比; @P<0.05, HG+anti-miR-NC组相比。

#P<0.05 compared with HG group; @P<0.05 compared with HG+anti-miR-NC group.

图8 抑制miR-196a对高糖诱导的HK-2细胞凋亡蛋白表达的影响

Fig.8 The effect of inhibiting miR-196a on the expression of HK-2 cells apoptotic protein induced by high glucose

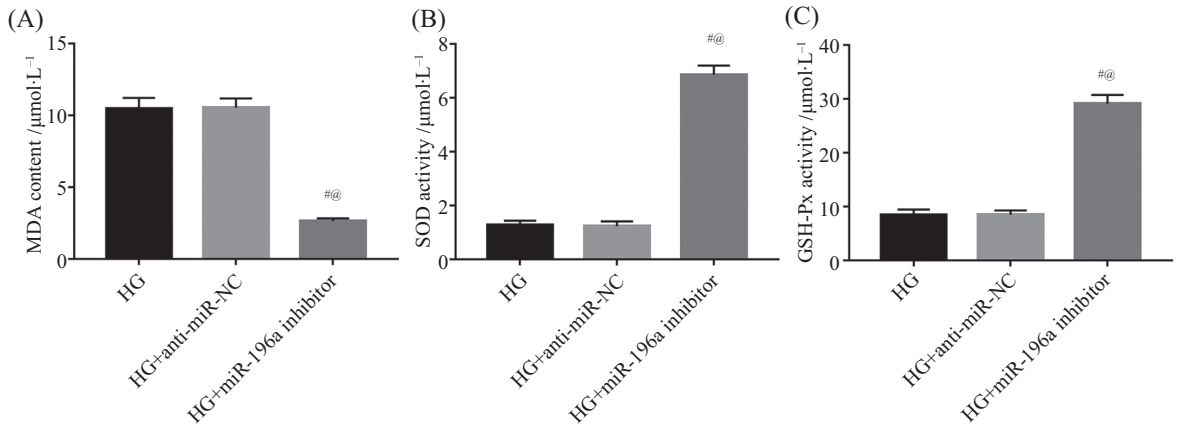


#P<0.05, 与HG组相比; @P<0.05, HG+anti-miR-NC组相比。

#P<0.05 compared with HG group; @P<0.05 compared with HG+anti-miR-NC group.

图9 抑制miR-196a对高糖诱导的HK-2细胞活性的影响

Fig. 9 The effect of inhibiting miR-196a on the activity of HK-2 cells induced by high glucose



A: MDA试剂盒检测MDA含量; B: SOD试剂盒检测SOD活性; C: GSH-Px试剂盒检测GSH-Px活性; *P<0.05, 与Con组相比; #P<0.05, 与HG组相比; &P<0.05, 与HG+anti-miR-NC组相比。

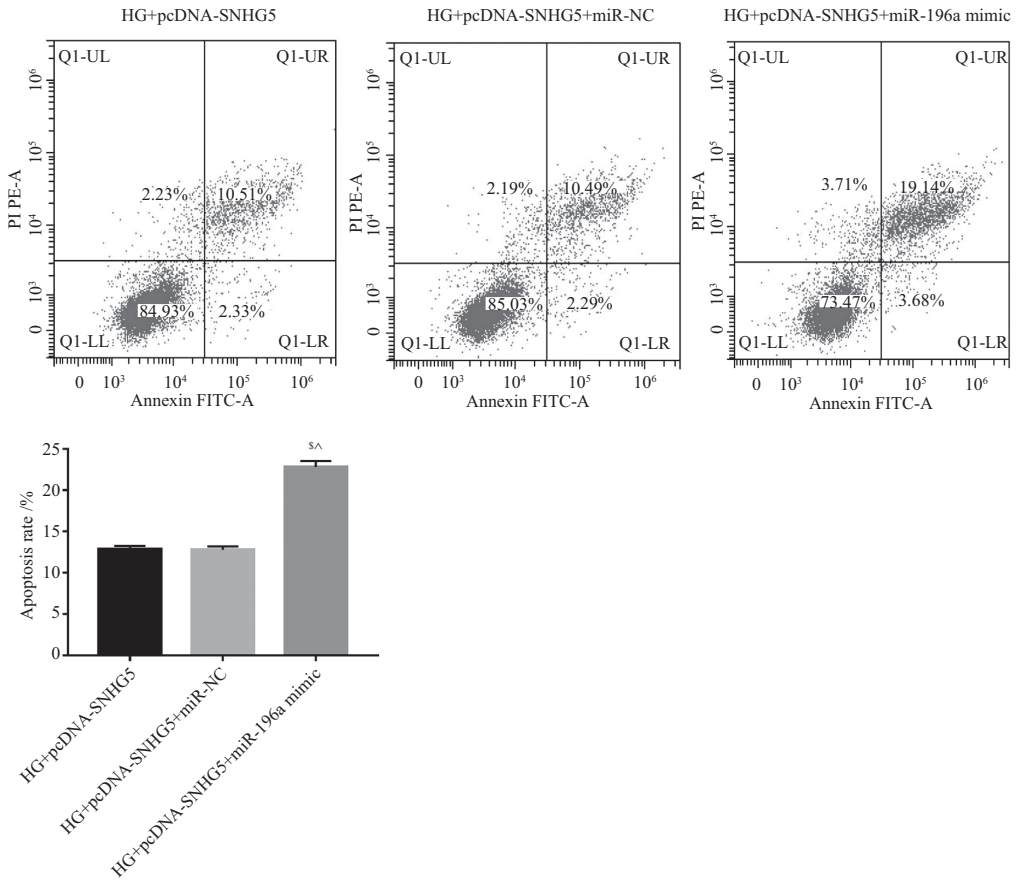
A: MDA kit was used to detect MDA content; B: SOD kit was used to detect SOD activity; C: GSH-Px kit was used to detect GSH-Px activity; *P<0.05 compared with Con group; #P<0.05 compared with HG group; @P<0.05 compared with HG+anti-miR-NC group.

图10 抑制miR-196a对高糖诱导的HK-2细胞氧化应激的影响

Fig.10 The effect of inhibiting miR-196a on HK-2 cells oxidative stress induced by high glucose

究表明, 持续高血糖会增加活性氧的产生, 该作用超过内源性抗氧化剂, 导致MDA产生和随后的细胞损伤。SOD和GSH-Px作为重要的抗氧化物质, 它们的水平在高糖损伤后HK-2细胞中降低[14-15]。因此, MDA、SOD和GSH-Px是检测糖尿病肾病氧化应激的3种生物标志物。本实验结果也显示, 高糖诱导的HK-2细胞中MDA含量升高, SOD、GSH-Px活性降低, 表明高糖也可诱导HK-2细胞氧化应激的发生, 与前人研究[14-15]吻合。

此前的研究表明lncRNA参与调控肾小管上皮细胞损伤过程[16], 如lncRNA ZFAS1通过靶向miR-588促进糖尿病肾病中的肾小球系膜细胞增殖、氧化应激[17]。KCNQ1OT1的下调通过上调miR-506-3p的表达来抑制高糖诱导的HK-2细胞的炎症、氧化应激和细胞凋亡[18]。沉默lncRNA GAS5可通过调节miR-27a减轻高糖诱导的人肾小管上皮HK-2细胞损伤[19]。本实验结果显示, 高糖诱导的HK-2细胞中lncRNA SNHG5表达水平降低, 提示lncRNA SNHG5或与高糖诱导的HK-2细胞损伤有关。为了明确lncRNA SNHG5对高糖诱导的HK-2细胞损伤的影响,

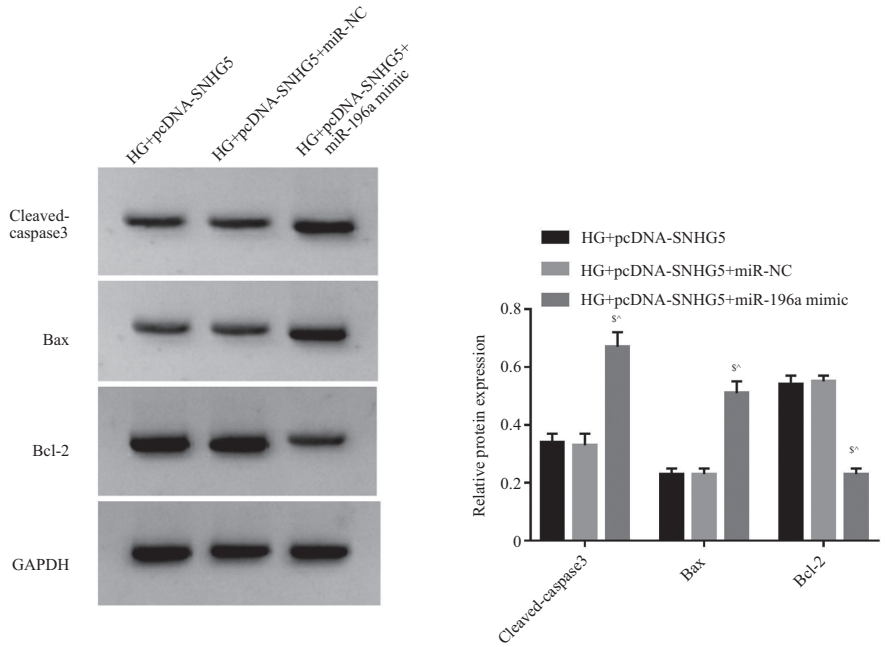


$P<0.05, 与HG+pcDNA-SNHG5组相比; ^P<0.05, HG+pcDNA-SNHG5+miR-NC组相比。

$P<0.05 compared with HG+pcDNA-SNHG5 group; ^P<0.05 compared with HG+pcDNA-SNHG5+miR-NC group.

图11 miR-196a可逆转SNHG5对高糖诱导的HK-2凋亡的影响

Fig.11 miR-196a can reverse the effect of SNHG5 on HK-2 apoptosis induced by high glucose

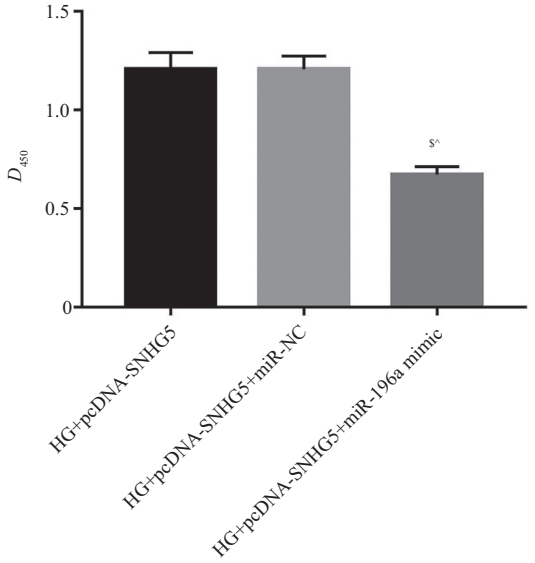


$P<0.05, 与HG+pcDNA-SNHG5组相比; ^P<0.05, HG+pcDNA-SNHG5+miR-NC组相比。

$P<0.05 compared with HG+pcDNA-SNHG5 group; ^P<0.05 compared with HG+pcDNA-SNHG5+miR-NC group.

图12 miR-196a可逆转SNHG5对高糖诱导的HK-2细胞凋亡蛋白表达的影响

Fig.12 miR-196a can reverse the effect of SNHG5 on the expression of HK-2 cells apoptotic proteins induced by high glucose

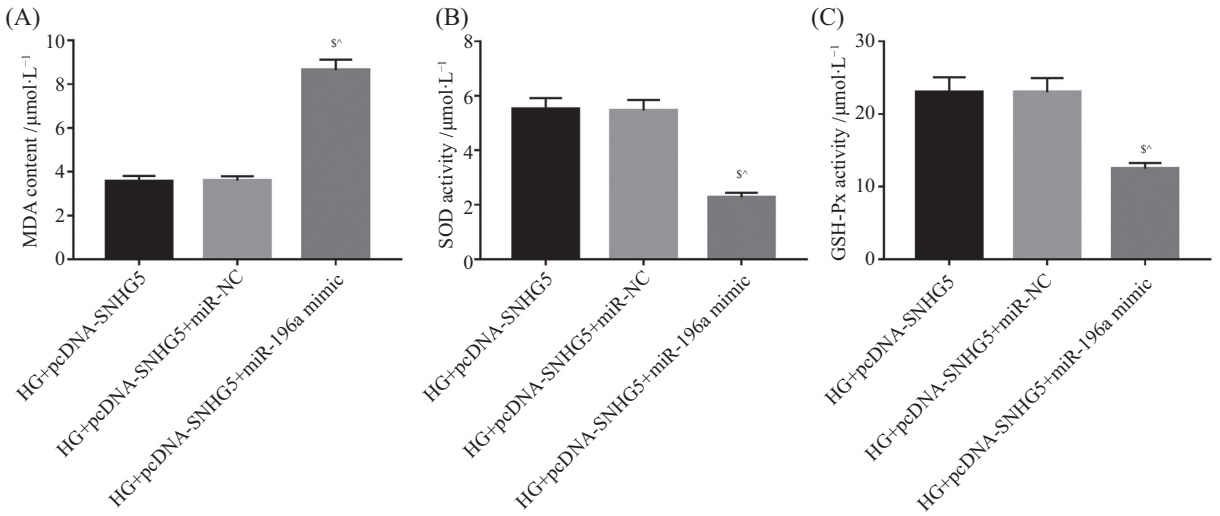


$^{\$}P$<0.05, 与HG+pcDNA-SNHG5组相比; $^{\wedge}P$<0.05, HG+pcDNA-SNHG5+miR-NC组相比。

$^{\$}P$<0.05 compared with HG+pcDNA-SNHG5 group; $^{\wedge}P$<0.05 compared with HG+pcDNA-SNHG5+miR-NC group.

图13 miR-196a可逆转SNHG5对高糖诱导的HK-2细胞活性的影响

Fig.13 miR-196a can reverse the effect of SNHG5 on the activity of HK-2 cells induced by high glucose



A: MDA试剂盒检测MDA含量; B: SOD试剂盒检测SOD活性; C: GSH-Px试剂盒检测GSH-Px活性; $^{\$}P$<0.05, 与HG+pcDNA-SNHG5组相比; $^{\wedge}P$<0.05, HG+pcDNA-SNHG5+miR-NC组相比。

A: MDA kit was used to detect MDA content; B: SOD kit was used to detect SOD activity; C: GSH-Px kit was used to detect GSH-Px activity; $^{\$}P$<0.05 compared with HG+pcDNA-SNHG5 group; $^{\wedge}P$<0.05 compared with HG+pcDNA-SNHG5+miR-NC group.

图14 miR-196a可逆转SNHG5对高糖诱导的HK-2细胞氧化应激的影响

Fig.14 miR-196a can reverse the effect of SNHG5 on HK-2 cells oxidative stress induced by high glucose

本研究利用pcDNA-SNHG5增加SNHG5表达量, 结果显示, 过表达SNHG5后, 高糖诱导的HK-2细胞活性、凋亡均与上述研究结果中的变化具有一致性且MDA含量下降, SOD、GSH-Px活性升高; 表明过表达SNHG5可抑制高糖诱导的HK-2凋亡和氧化应激。

本实验还发现, 高糖诱导的HK-2细胞中miR-196a表达水平升高, 并且SNHG5与miR-196a存在结合位点, 暗示miR-196a可能参与SNHG5调节高糖损伤HK-2细胞的机制。既往研究报道, miR-196a水平与糖尿病肾病进展呈正相关[8], 尿液miR-196a是局灶节段性肾小球硬化患者发生终末期肾病的独立危险因素[20]。可通过上调肾脏miR-196a/b表达抑制转

化生长因子-β-Smad信号转导, 从而进一步减轻肾纤维化[21]。此外, lncRNA LOC105374325过表达会降低miR-196a/b水平, 诱导小鼠蛋白尿和局部节段性病变[22]。但miR-196a在高糖损伤HK-2细胞中的具体功能尚未明确。本研究中, 抑制miR-196a表达后, 高糖诱导的HK-2细胞活性显著提升且细胞死亡率下降, MDA含量减少, SOD、GSH-Px活性升高; 表明抑制miR-196a表达可抑制高糖诱导的HK-2凋亡和氧化应激。研究表明, lncRNA和mRNA可能起到miRNA海绵的作用。它们通过miRNA反应元件相互竞争, 并调节许多疾病的进展[23]。如lncRNA SNHG1通过直接与miR-196a相互作用调控血管内皮细胞增殖和血管生成[24]。此外, SNHG5靶向上调miR-196a表达可逆转SNHG5对高糖诱导的HK-2细胞活性、凋亡和氧化应激的影响。

综上所述, 过表达SNHG5可能通过下调miR-196a抑制高糖诱导的HK-2细胞凋亡及氧化应激。

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