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Myod1通过调节lncRNA SNHG15和miR-24-3p对氧糖剥夺SH-SY5Y细胞增殖及凋亡的影响

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[摘 要] **目的：**探讨肌源性分化蛋白1（Myod1）对氧糖剥夺（OGD）诱导的SH-SY5Y细胞增殖抑制和凋亡的影响，并阐明其作用机制。**方法：**采用实时荧光定量PCR（RT-qPCR）法检测正常对照组研究对象和缺血性脑梗死组患者外周血及正常培养的SH-SY5Y细胞（对照组）和OGD细胞模型（OGD组）细胞中Myod1和长链非编码RNA（lncRNA）小核仁RNA宿主基因15（SNHG15）mRNA表达水平。分别采用si-Myod1、pcDNA3.0-Myod1、si-SNHG15、pcDNA3.0-SNHG15、si-NC、空载质粒（Vector）、miR-NC和miR-24-3p模拟物（miR-mimics）质粒转染SH-SY5Y细胞后，进行OGD处理，将SH-SY5Y细胞分为对照组、OGD组、OGD＋Vector组、OGD＋Myod1组、OGD＋si-NC组、OGD＋si-Myod1组、OGD＋si-SNHG15组、OGD＋si-SNHG15＋Vector组、OGD＋si-SNHG15＋Myod1组、OGD＋miR-NC组、OGD＋miR-mimics组、OGD＋miR-mimics＋Vector组和OGD＋miR-mimics＋SNHG15组。采用CCK-8法检测各组细胞活性，采用5-乙炔基-2′-脱氧尿苷（EdU）染色法检测各组EdU阳性细胞率，采用原位末端转移酶标记（TUNEL）法检测各组TUNEL阳性细胞率，采用Western blotting法检测各组细胞中裂解的含半胱氨酸的天冬氨酸蛋白水解酶3（cleaved caspase-3）、裂解的含半胱氨酸的天冬氨酸蛋白水解酶9（cleaved caspase-9）、B细胞淋巴瘤2（Bcl-2）和Bcl-2相关X蛋白（Bax）蛋白表达水平。染色质免疫共沉淀（CHIP）法评估Myod1和SNHG15之间的关联。双荧光素酶报告基因实验评估Myod1与SNHG15及SNHG15与miR-24-3p的靶向关系。**结果：**与正常对照组比较，缺血性脑梗死组患者外周血中Myod1和SNHG15 mRNA表达水平均升高（$P<0.05$）。与对照组比较，OGD组细胞中Myod1和SNHG15 mRNA表达水平均明显升高（$P<0.05$）。与OGD组比较，48和72 h时OGD＋Myod1组细胞活性和EdU阳性细胞率均降低（$P<0.01$），TUNEL阳性细胞率升高（$P<0.01$）；OGD＋si-Myod1组细胞活性和EdU阳性细胞率均升高（$P<0.01$），TUNEL阳性细胞率降低（$P<0.01$）。Myod1可与SNHG15的启动子序列结合。SNHG15可吸附miR-24-3p，Myod1与SNHG15及SNHG15与miR-24-3p存在靶关系。敲低SNHG15后，与OGD组比较，48和72 h时OGD＋si-SNHG15组细胞活性和EdU阳性细胞率均升高（$P<0.01$），TUNEL阳性细胞率降低（$P<0.01$），细胞中Bax、cleaved caspase-3和cleaved caspase-9蛋白表达水平降低（$P<0.01$），Bcl-2蛋白表达水平升高（$P<0.01$）；与OGD＋si-SNHG15组比较，48和72 h时OGD＋si-SNHG15＋Myod1组细胞活性和EdU阳性细胞率降低（$P<0.05$），TUNEL阳性细胞率升高（$P<0.05$），细胞中Bax、cleaved caspase-3和cleaved caspase-9蛋白表达水平升高（$P<0.05$），Bcl-2的蛋白表达水平降低（$P<0.05$）。过表达miR-24-3p和SNHG15后，与OGD组比较，48和72 h时OGD＋miR-mimics组细胞活性和EdU阳性细胞率升高（$P<0.01$），TUNEL阳性细胞率降低（$P<0.01$），细胞中Bax、cleaved caspase-3和cleaved caspase-9蛋白表达水平降低（$P<0.01$），Bcl-2蛋白表达水平升高（$P<0.01$）；与OGD＋miR-mimics组比较，48和72 h时OGD＋miR-mimics＋SNHG15组细胞活性和EdU阳性细胞率降低（$P<0.05$），TUNEL阳性细胞率升高（$P<0.05$），细

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胞中Bax、cleaved caspase-3和cleaved caspase-9蛋白表达水平升高（$P<0.05$），Bcl-2蛋白表达水平降低（$P<0.05$）。**结论：**Myod1可通过与SNHG15启动子区结合进而吸附miRNA-24，促进OGD诱导的SH-SY5Y细胞的增殖抑制和细胞凋亡。

[关键词] 肌源性分化蛋白1；小核仁RNA宿主基因15；微小RNA-24-3p；SH-SY5Y细胞；脑梗死
[中图分类号] R743; R363.2 **[文献标志码]** A

Effect of Myod1 on proliferation and apoptosis of oxygen-glucose-deprived SHSY5Y cells by regulating lncRNA SNHG15 and miR-24-3p

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ABSTRACT **Objective:** To investigate the effect of myogenic differentiation protein 1 (Myod1) on the proliferation inhibition and apoptosis of the SH-SY5Y cells induced by oxygen-glucose deprivation (OGD), and to elucidate its mechanism. **Methods:** Real-time quantitative fluorescence PCR (RT-qPCR) method was used to detect the mRNA levels of Myod1 and long non-coding RNA (lncRNA) small nucleolar RNA host gene 15 (SNHG15) in peripheral blood of the subjects in normal group and the patients in ischemic cerebral infarction group as well as the normal cultured SH-SY5Y cells (control group) and the cells in OGD model (OGD group). After transfecting SH-SY5Y cells with si-Myod1, pcDNA3.0-Myod1, si-SNHG15, pcDNA3.0-SNHG15、si-NC, Vector, miR-NC, and miR-24-3p mimics, the cells were treated with OGD, and then the SH-SY5Y cells were divided into control group, OGD group, OGD+Vector group, OGD+Myod1 group, OGD+si-NC group, OGD+si-Myod1 group, OGD+si-SNHG15 group, OGD+si-SNHG15+Vector group, OGD+si-SNHG15+Myod1 group, OGD+miR-NC group, OGD+miR-mimics group, OGD+miR-mimics+Vector group, and OGD+miR-mimics+SNHG15 group. CCK-8 method was used to detect the cell activities in various groups; 5-ethynyl-2′-deoxyuridine (EdU) staining was used to detect the rates of EDU positive cells in various groups; the rates of TdT-mediated dUTP nick end labeling (TUNEL) positive cells in various groups were detected by TUNEL staining; Western blotting method was used to detect the expression levels of cleaved caspase-3, cleaved caspase-9, B-cell lymphoma 2 (Bcl-2) and Bcl-2 associated X protein (Bax) proteins in the cells in various groups; the association between Myod1 and SNHG15 was evaluated by chromatin immunoprecipitate (CHIP); dual luciferase reporter gene experiment was used to evaluate the targeting relationships between Myod1 and SNHG15 as well as SNHG15 and miR-24-3p. **Results:** Compared with normal control group, the expression levels of Myod1 and SNHG15 mRNA in peripheral blood of the patients in ischemic cerebral infarction group were significantly increased ($P<0.05$). Compared with control group, the expression levels of Myod1 and SNHG15 mRNA in the SH-SY5Y cells in OGD group were significantly increased ($P<0.05$). Compared with OGD group, the cell activities and rates of EdU positive cells in OGD+Myod1 group at 48 and 72 h were decreased ($P<0.01$), and the rates of TUNEL positive cells were increased ($P<0.05$); the cell activities and rates of EdU positive cells in OGD+si-Myod1 group were increased ($P<0.05$), while the rates of TUNEL positive cells were decreased ($P<0.01$). Myod1 binded to the promoter sequence of SNHG15. SNHG15 could absorb miR-24-3p, and there were target relatronships between Myod1 and SNHG15 as well as SNHG15 and miR-24-3p. After SNHG15 knockdown, compared with OGD group, the cell activities and rates of EdU positive cells in OGD+si-SNHG15 group at 48 and 72 h

were increased ($P<0.01$), and the rates of TUNEL positive cells were decreased ($P<0.01$), the expression levels of Bax, cleaved caspase-3 and cleaved caspase-9 proteins were decreased ($P<0.01$), and the expression levels of Bcl-2 protein were increased ($P<0.01$). Compared with OGD+si-SNHG15 group, the cell activities and rates of EdU positive cells in OGD+si-SNHG15+Myod1 group at 48 and 72 h were decreased ($P<0.05$), the rates of TUNEL positive cells were ($P<0.05$), the expression levels of Bax, cleaved caspase-3, and cleaved caspase-9 proteins were increased ($P<0.05$), and the expression levels of Bcl-2 were decreased ($P<0.05$). After over-expression of miR-24-3p and SNHG15, compared with OGD group, the cell activities and rates of EdU positive cells in OGD+miR-mimics group at 48 and 72 h were increased ($P<0.01$), the rates of TUNEL positive cells were significantly decreased ($P<0.01$), the protein expression levels of Bax, cleaved caspase-3 and cleaved caspase-9 were decreased ($P<0.05$), and the expression levels of Bcl-2 were increased ($P<0.01$). Compared with OGD+miR-mimics group, the cell activities and rates of EdU positive cells in OGD+miR-mimics+SNHG15 group at 48 and 72 h were decreased ($P<0.05$), and the rates of TUNEL positive cells were increased ($P<0.05$), the expression levels of Bax, cleaved caspase-3 and cleaved caspase-9 proteins were increased ($P<0.05$), and the expression levels of Bcl-2 protein were decreased ($P<0.05$). **Conclusion**: Myod1 can promote the proliferation inhibition and apoptosis of OGD-induced SH-SY5Y cells by binding to the SNHG15 promoter region and then absorbing miRNA-24.

KEYWORDS Myogenic differentiation 1; Small nuclear RNA host gene 15; MicroRNA-24-3p; SH-SY5Y cell; Cerebral infarction

脑血管病是与心肌梗死和恶性肿瘤并列的三大疾病，目前其发病率呈逐年上升趋势[1]。在所有脑血管病患者中，脑梗死最为常见，约占陈旧性血管病的60%～70%[2-3]。缺氧缺血不仅是脑梗死的主要原因，也是导致脑损伤的病理生理过程[4]。肺腺癌转移相关转录因子1（metastasis-associated lung adenocarcinoma transcript 1，MALAT1）、HOX转录反义RNA（HOX transcript antisense RNA，HOTAIR）、H19和小核仁RNA宿主基因14（small nuclear RNA host gene 14，SNHG14）等多种长链非编码RNAs（long non-coding RNAs，lncRNAs）在缺血性脑损伤过程中异常表达[5-10]。近期研究[11-13]表明：小核仁RNA宿主基因15（small nuclear RNA host gene 15，SNHG15）可作为微小RNA（microRNA，miRNAs）的“分子海绵”在脊柱结核、缺血性中风和心力衰竭中发挥作用。此外，急性脑缺血患者外周血中SNHG15表达上调[14]。尽管上述研究提示SNHG15可能在脑梗死中发挥作用，但其确切的生物学效应和机制尚未明确，同时Myod1在脑梗死患者脑组织中的表达水平和功能尚不清楚。本研究通过对比脑梗死患者和正常对照者外周血中Myod1和SNHG15 mRNA表达水平的差异，并分析其在氧糖剥夺（oxygen-glucose deprivation，OGD）细胞模型中的作用，旨在揭示Myod1和SNHG15在脑梗死中的作用及其分子机制，为脑梗死患者的治疗提供新的靶点。

1 材料与方法

1.1 外周血标本来源 收集来自齐齐哈尔医学院附属第三医院19例缺血性脑梗死患者（缺血性脑梗死组）的外周血样本，同时收集19例同期参加体检的健康人外周血样本作为正常对照组。本研究已获得齐齐哈尔医学院附属第三医院伦理委员会批准，所有患者签署知情同意书。

1.2 细胞、主要试剂和仪器 人神经母细胞瘤SH-SY5Y细胞系购自美国模式培养物保藏中心（American Type Culture Collection，ATCC）。胎牛血清、MEM培养基和F-12培养基购自美国Gibco公司，RNAiso试剂和实时荧光定量PCR（real-time fluorescence quantitative PCR，RT-qPCR）检测试剂盒购自日本TaKaRa公司，靶向Myod1和SNHG15的小干扰RNA（small interfering RNA，siRNA）质粒（si-Myod1和si-SNHG15）及阴性对照质粒（si-NC）、miR-24-3p模拟物（miR-mimics）和miR-NC质粒由上海吉玛制药技术有限公司构建，Lipofectamine 2000试剂和引物购自美国Invitrogen公司，pGL3-Basic和双荧光素酶报告基因检测系统购自美国Promega公司，CCK-8细胞计数试剂盒购自日本Dojindo公司，5-乙炔基-2′-脱氧尿苷

（5-ethynyl-2′-deoxyuridine，EdU）增殖检测试剂盒、原位末端转移酶标记法（TdT-mediated dUTP nick end labeling，TUNEL）染色试剂盒和染色质免疫共沉淀（chromatin immunoprecipitation，CHIP）分析试剂盒购自上海碧云天生物技术有限公司，裂解的含半胱氨酸的天冬氨酸蛋白水解酶3（cleaved cysteinyl aspartate specific proteinase-3，cleaved caspase-3）、裂解的含半胱氨酸的天冬氨酸蛋白水解酶9（cleaved cysteinyl aspartate specific proteinase-9，cleaved caspase-9）、B细胞淋巴瘤2（B-cell lymphoma-2，Bcl-2）和Bcl-2相关X蛋白（Bcl-2 associated X protein，Bax）抗体购自美国CST公司，GAPDH抗体和HRP-羊抗兔IgG购自武汉博士德生物工程有限公司。Mx3000P定量PCR仪（美国Agilent公司），Infinite F50酶标仪（瑞士Tecan公司），BX51荧光显微镜（日本Olympus公司），Microtek中晶BIO-6000平板式凝胶成像扫描仪（上海中晶科技有限公司）。

1.3 OGD细胞模型制备 将SH-SY5Y细胞接种于含10%胎牛血清和1%链霉素-青霉素的45%MEM培养基和45%F-12培养基中，于常规条件（95%大气，5%CO_2，37 ℃）下培养。按参考文献［15］中的方法构建OGD细胞模型：生长状态良好的SH-SY5Y细胞置于缺糖培养基中并转移至无氧环境（5%CO_2，95%N_2，37 ℃）中孵育6 h，作为OGD组。同时设正常培养的SH-SY5Y细胞为对照组。

1.4 RT-qPCR法检测外周血和SH-SY5Y细胞中Myod1和SNHG15 mRNA表达水平 采用RNAiso试剂从外周血中和SH-SY5Y细胞中提取总RNA。将RNA反转录为cDNA。加模板和引物，采用RT-qPCR试剂盒进行检测。引物序列：Myod1，正向引物5′-CGG-CATGATGGACTACAGCG-3′，反向引物5′-CAGGC-AGTCTAGGCTCGAC-3′；SNHG15，正向引物5′- GGTCTTCGGCAGTCT-AGTCA-3′，反向引物5′-CAGGAACTGGCAGC-TAACAC-3′；GAPDH，正向引物5′-TGTTCGT-CATGGGTGTGAAC-3′，反向引物5′-ATGGCAT-GGACTGTGGTCAT-3′。反应条件：95 ℃、2 min；94 ℃、20 s，58 ℃、20 s，72 ℃ 20 s，40个循环。以GAPDH为内参，采用$2^{-\triangle\triangle Ct}$法计算目的基因mRNA表达水平。

1.5 细胞转染和分组 将Myod1编码序列克隆至pcDNA3.0质粒中（pcDNA3.0-Myod1），以pcDNA3.0空载体质粒（Vector）作为阴性对照。按照转染试剂说明书操作步骤，用Lipofectamine 2000试剂将si-Myod1、pcDNA3.0-Myod1、si-SNHG15、pcDNA3.0-SNHG15、si-NC、Vector、miR-mimics和miR-NC分别转染入SH-SY5Y细胞，48 h后收集各组细胞经Western blotting法验证SH-SY5Y细胞中Myod1过表达和敲低效率。将上述转染的细胞进行OGD处理，分为对照组、OGD组、OGD＋Vector组、OGD＋Myod1组、OGD＋si-NC组、OGD＋si-Myod1组、OGD＋si-SNHG15组、OGD＋si-SNHG15＋Vector组、OGD＋si-SNHG15＋Myod1组、OGD＋miR-NC组、OGD＋miR-mimics组、OGD＋miR-mimics＋Vector组和OGD＋miR-mimics＋SNHG15组。

1.6 双荧光素酶报告基因实验验证Myod1与SNHG15及SNHG15与miR-24-3p的靶向关系 由Starbase数据库（http：//starbase. sysu. edu. cn/）预测miR-24-3p与SNHG15的结合区域。将SNHG15的野生型启动子区域片段及预测的SNHG15-3′非翻译区（untranslated regions，UTR）区域片段分别克隆至pGL3-Basic中构建各自的pGL3-SNHG15野生型（pGL3-SNHG15-WT）载体质粒。将SNHG15的突变启动子区域片段和突变SNHG15-3′UTR片段克隆至pGL3-Basic中，分别构建pGL3-SNHG15突变型（pGL3-SNHG15-MUT）载体质粒。将pGL3-SNHG15-WT和pGL3-SNHG15-MUT（100 ng）分别与pcDNA3.0-Myod1（1 mg·L^{-1}）、pcDNA3.0（Vector，1 mg·L^{-1}）质粒及miR-NC、miR-mimics共同转染至SH-SY5Y细胞（6孔细胞培养板，密度为1.0×10^6 cm^{-2}）中。48 h后，采用双荧光素酶报告基因检测系统检测并分析各组SH-SY5Y细胞荧光素酶活性。

1.7 CHIP法评估Myod1与SNHG15之间的关联 由美国Invitrogen公司根据JASPAR数据库（http：//jaspar. genereg. net/）预测的Myod1与SNHG15结合区域设计引物。按照CHIP分析试剂盒说明书进行操作，将SH-SY5Y细胞交联后进行胞核制备和核染色质消化，留取部分染色质制备物稀释后作为input组，再分别设置IgG组和Myod1组；染色质洗脱解交联后进行DNA回收纯化，分别将上述3组DNA纯化产物用结合位点的引物进行琼

脂糖凝胶电泳，电泳条件为电压110 V和电流40 mA，待电泳条带到达凝胶合适位置时停止电泳，在凝胶成像扫描仪中曝光拍照并对灰度值进行分析。

1.8 CCK-8法和EdU染色法检测各组细胞活性和EdU阳性细胞率 采用CCK-8细胞计数试剂盒检测各组细胞活性：按说明书中方法，各组细胞培养24、48和72 h后，加入CCK-8试剂，采用酶标仪检测450 nm波长处吸光度（A）值，以A值代表各组细胞活性。采用EdU增殖检测试剂盒检测各组细胞中EdU阳性细胞率：按说明书中方法，各组细胞加入EdU试剂孵育2 h，用DAPI染色标记细胞核，荧光显微镜下计数EdU阳性细胞，并计算每个视野中EdU阳性细胞率。EdU阳性细胞率＝EdU阳性细胞数/总细胞数×100%。

1.9 TUNEL染色法检测各组细胞TUNEL阳性细胞率 各组细胞培养48 h后加入TUNEL检测试剂，在室温避光条件下染色30 min，用DAPI染色标记细胞核，荧光显微镜下计数TUNEL阳性细胞，计算每个视野中TUNEL阳性细胞率，代表细胞凋亡情况。TUNEL阳性细胞率＝TUNEL阳性细胞数/总细胞数×100%。

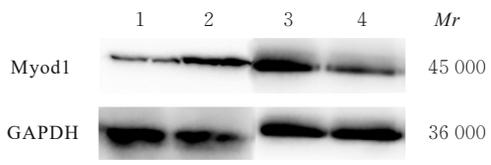
1.10 Western blotting法检测各组细胞中Myod1、cleaved caspase-3、cleaved caspase-9、Bax和Bcl-2蛋白表达水平 提取各组细胞总蛋白，在10% SDS-PAGE中进行分离并将蛋白转移到PVDF膜上，用5%脱脂奶的封闭处理。加一抗（Myod1、cleaved caspase-3、cleaved caspase-9、Bax和Bcl-2，1∶1 000）和兔抗-GAPDH单克隆抗体（1∶10 000）孵育过夜，TBST洗涤（共3次，每次5 min）。加HRP羊抗兔IgG（1∶2 000）孵育2 h，洗涤。用ECL发光底物使条带曝光，用凝胶成像扫描仪成像。采用Image J软件计算目的蛋白表达水平。目的蛋白表达水平＝目的蛋白条带灰度值/GAPDH蛋白条带灰度值；验证SH-SY5Y细胞中Myod1过表达和敲低效率时，Myod1蛋白表达水平＝Myod1蛋白条带灰度值/GAPDH蛋白条带灰度值×100%。

1.11 统计学分析 采用SPSS 22.0统计软件进行统计学分析。缺血性正常对照组研究对象和脑梗死组患者外周血中Myod1和SNHG15 mRNA表达水平，各组细胞活性、EdU阳性细胞率、TUNEL阳性细胞率、荧光素酶活性和Myod1及凋亡相关蛋白表达水平均符合正态分布，以$\bar{x}\pm s$表示，2组间样本均数比较采用t检验，多组间样本均数比较采用单因素方差分析，组间样本均数两两比较采用Bonferroni法。以$P<0.05$为差异有统计学意义。

2 结 果

2.1 正常对照组研究对象和缺血性脑梗死组患者外周血中及2组SH-SY5Y细胞中Myod1和SNHG15 mRNA表达水平 与正常对照组（1.18±0.80和1.21±0.89）比较，脑梗死组患者外周血中Myod1和SNHG15 mRNA表达水平（2.19±0.93和3.43±1.40）均升高（P<0.05）。采用OGD细胞模型模拟体外脑缺血模型，与对照组（1.01±0.13和1.00±0.11）比较，OGD组SH-SY5Y细胞中Myod1和SNHG15 mRNA表达水平（9.57±0.69和5.88±0.37）均升高（$P<0.05$）。

2.2 各组SH-SY5Y细胞中Myod1蛋白表达水平 与Vector组（102.65% ± 14.05%）比较，pcDNA3.0-Myod1组SH-SY5Y细胞中Myod1蛋白表达水平（406.53%±36.28%）升高（$t=9.732$，$P<0.05$）。与si-NC组（99.79%±10.12%）比较，si-Myod1组SH-SY5Y细胞中Myod1蛋白表达水平（21.36%±3.41%）降低（$t=13.124$，$P<0.05$）。见图1。



Lane 1: Vector group; Lane 2: PcDNA3.0-Myod1 group; Lane 3: Si-NC group; Lane 4: Si-Myod1 group.

图1 过表达和敲低Myod1后各组SH-SY5Y细胞中Myod1蛋白表达电泳图

Fig. 1 Electrophoregram of expressions of Myod1 protein in SH-SY5Y cells in various groups after over-expression and knockdown of Myod1

2.3 过表达和敲低Myod1后各组细胞活性、EdU阳性细胞率和TUNEL阳性细胞率 与对照组比较，48和72 h时OGD组细胞活性及EdU阳性细胞率降低（$P<0.05$），TUNEL阳性细胞率升高（$P<0.05$）。与OGD组比较，48和72 h时OGD＋Myod1组细胞活性和EdU阳性细胞率降低（$P<0.05$），TUNEL阳性细胞率升高（$P<0.05$）；OGD＋si-Myod1组细胞活性和EdU阳性细胞率升高（$P<0.05$），TUNEL阳性细胞率降低（$P<0.05$）。见图2、3和表1～3。

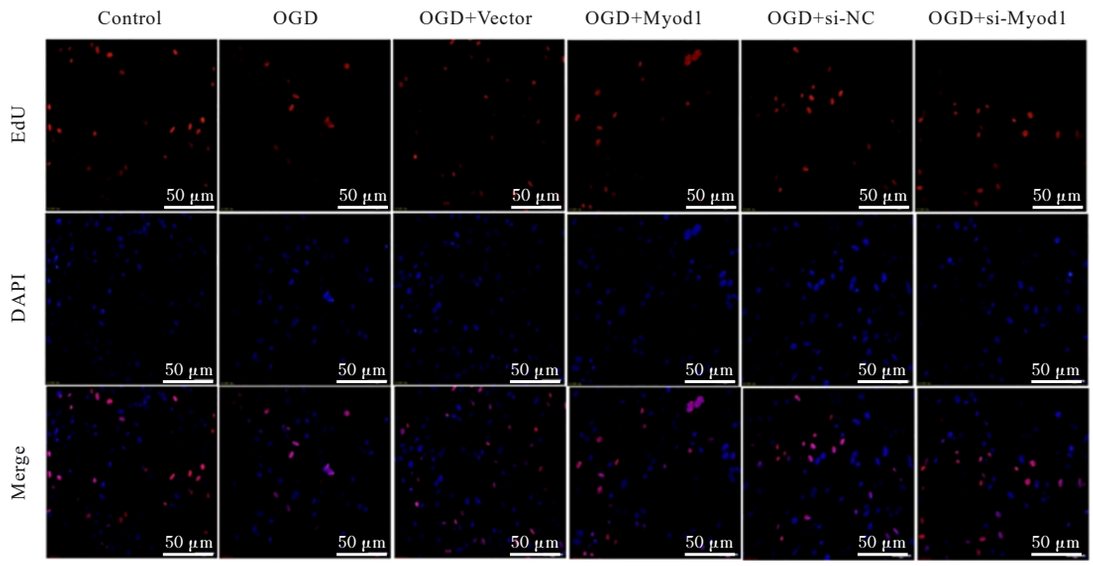
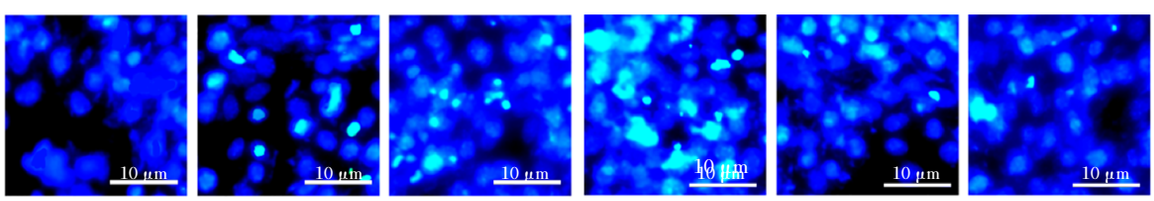


图 2 过表达和敲低 Myod1 后各组细胞 EdU 染色情况

Fig. 2 EdU staining of cells in various groups after over-expression and knockdown of Myod1



A：Control group；B：OGD group；C：OGD＋Vector group；D：OGD＋Myod1 group；E：OGD＋si-NC group；F：OGD＋si-Myod1 group.

图 3 过表达和敲低 Myod1 后各组细胞 TUNEL 染色情况

Fig. 3 TUNEL staining of cells in various groups after over-expression and knockdown of Myod1

表 1 过表达 Myod1 后各组细胞活性

Tab. 1 Cell activities in various groups after over-expression of Myod1 （$n=3, \bar{x}\pm s$）

Group	Cell activity		
	（t/h）24	48	72
Control	0.52±0.03	0.91±0.06	1.15±0.03
OGD	0.44±0.02	0.59±0.05*	0.82±0.04*
OGD＋Vector	0.43±0.02	0.56±0.02	0.64±0.02
OGD＋Myod1	0.43±0.03	0.48±0.03△	0.62±0.04△

*$P<0.05$ *vs* control group；△$P<0.01$ *vs* OGD group.

表 2 敲低 Myod1 后各组细胞活性

Tab. 2 Cell activities in various groups after knockdown of Myod1 （$n=3, \bar{x}\pm s$）

Group	Cell activity		
	（t/h）24	48	72
Control	0.51±0.03	0.81±0.05	1.16±0.05
OGD	0.42±0.02	0.51±0.05*	0.58±0.03*
OGD＋si-NC	0.40±0.02	0.50±0.05	0.58±0.04
OGD＋si-Myod1	0.43±0.02	0.71±0.04△	1.05±0.06△

*$P<0.05$ *vs* control group；△$P<0.01$ *vs* OGD group.

2.4 双荧光素酶报告基因实验和 CHIP 实验验证 Myod1 与 SNHG15 之间的关联 与 pcDNA3.0-vector 组（1.03±0.09）比较，pcDNA3.0-Myod1 组细胞中 SNHG15 mRNA 表达水平（16.98±0.96）升高（$P<0.05$）。与 si-NC 组（1.00±0.06）比较，si-Myod1 组细胞中 SNHG15 mRNA 表达水平（0.43±0.03）降低（$P<0.05$）。生物信息学预测：SNHG15 的启动子区域有 Myod1 的结合位点（图 4A）。双荧光素酶报告基因实验结果显示：与 pcDNA3.0-Vector＋pGL3-SNHG15-WT 组（1.02±0.10）比较，pcDNA3.0-Myod1＋pGL3-SNHG1515-WT 组细胞荧光素酶活性（1.98±0.05）升高（$P<0.05$）；与 pcDNA3.0-Vector＋pGL3-SNHG15-MUT 组（0.97±0.16）比较，

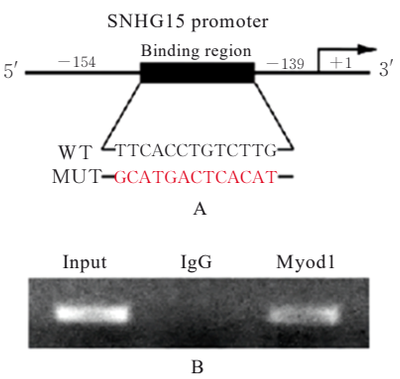
表3 过表达和敲低Myod1后各组EdU阳性细胞率和TUNEL阳性细胞率

Tab. 3 Rates of EdU positive cells and rates of TUNEL positive cells in various groups after over-expression and knockdown of Myod1 ($n=3, \bar{x}\pm s, \eta/\%$)

Group	Rate of EdU positive cells	Rate of TUNEL positive cells
Control	42.14±2.01	3.17±0.29
OGD	12.34±1.11*	12.05±1.05*
OGD+Vector	11.12±0.44	13.12±0.82
OGD+Myod1	3.56±0.96△	17.92±1.01△
OGD+si-NC	13.89±1.31	11.37±0.91
OGD+si-Myod1	32.98±1.46△	9.13±0.67△

*$P<0.05$ *vs* control group; △$P<0.01$ *vs* OGD group.

pcDNA3.0-Myod1+pGL3-SNHG15-MUT组细胞荧光素酶活性（0.98±0.05）差异无统计学意义（$P>0.05$）。CHIP实验结果显示：Myod1在SNHG15启动子区域上调（图4B）。



A: Wild type and mutant sequences of Myod1 binding region in SNHG15 promoter; B: Interaction between Myod1 and SNHG15 promoter regions determined by CHIP analysis.

图4 Myod1与SNHG15启动子区域的相互作用

Fig. 4 Interaction between Myod1 and promoter region of SNHG15

2.5 敲低SNHG15和过表达Myod1后各组细胞活性、EdU阳性细胞率、TUNEL阳性细胞率和细胞中凋亡相关蛋白表达水平 与si-NC组（1.03±0.06）比较，si-SNHG15组细胞中SNHG15 mRNA表达水平（0.25±0.04）降低（$t=10.496$，$P<0.05$）。与对照组比较，48和72 h时OGD组细胞活性和EdU阳性细胞率降低（$P<0.05$），TUNEL阳性细胞率升高（$P<0.05$），细胞中Bax、cleaved caspase-3和cleaved caspase-9蛋白表达水平升高（$P<0.05$），Bcl-2蛋白表达水平降低（$P<0.05$）；与OGD组比较，48和72 h时OGD+si-SNHG15组SH-SY5Y细胞活性和EdU阳性细胞率升高（$P<0.01$），TUNEL阳性细胞率降低（$P<0.01$），细胞中Bax、cleaved caspase-3和cleaved caspase-9蛋白表达水平降低（$P<0.01$），Bcl-2蛋白表达水平升高（$P<0.01$）；与OGD+si-SNHG15组比较，48和72 h时OGD+si-SNHG15+Myod1组细胞活性和EdU阳性细胞率降低（$P<0.05$），TUNEL阳性细胞率升高（$P<0.05$），细胞中Bax、cleaved caspase-3和cleaved caspase-9蛋白表达水平升高（$P<0.05$），Bcl-2蛋白表达水平降低（$P<0.05$）。见表4～6和图5。

表4 敲低SNHG15和过表达Myod1后各组细胞活性

Tab. 4 Cell activities in various groups after knockdown of SNHG15 and over-expression of Myod1 ($n=3, \bar{x}\pm s$)

Group	Cell activity		
	(t/h) 24	48	72
Control	0.38±0.01	0.87±0.03	1.17±0.03
OGD	0.28±0.01	0.49±0.02*	0.59±0.02*
OGD+si-NC	0.30±0.01	0.52±0.02	0.64±0.02
OGD+si-SNHG15	0.34±0.01	0.73±0.03△	0.96±0.04△
OGD+si-SNHG15+Vector	0.33±0.02	0.76±0.03	0.93±0.04
OGD+si-SNHG15+Myod1	0.33±0.02	0.55±0.02#	0.72±0.01#

*$P<0.05$ *vs* control group; △$P<0.01$ *vs* OGD group; #$P<0.05$ *vs* OGD+si-SNHG15 group.

表5 敲低SNHG15和过表达Myod1后各组EdU阳性细胞率及TUNEL阳性细胞率

Fig. 5 Rates of EdU positive cells and rates of TUNEL positive cells after knockdown of SNHG15 and over-expression of Myod1 ($n=3, \bar{x}\pm s, \eta/\%$)

Group	Rate of EdU positive cells	Rate of TUNEL positive cells
Control	28.34±2.53	2.64±0.26
OGD	8.02±1.78*	27.73±2.08*
OGD+si-NC	7.54±1.22	29.18±2.95
OGD+si-SNHG15	19.43±2.88△	14.84±1.15△
OGD+si-SNHG15+Vector	21.25±3.43	16.05±1.22
OGD+si-SNHG15+Myod1	9.32±2.05#	22.53±2.07#

*$P<0.05$ *vs* control group; △$P<0.01$ *vs* OGD group; #$P<0.05$ *vs* OGD+si-SNHG15 group.

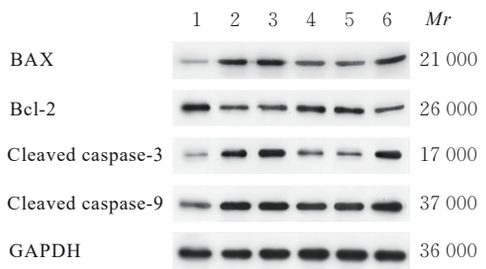
2.6 SNHG15与miR-24-3p的靶向关系 Starbase软件预测结果显示：SNHG15与miR-24-3p具有靶

表 6 敲低 SNHG15 和过表达 Myod1 后各组细胞中凋亡相关蛋白表达水平

Tab. 6 Expression levels of apoptosis-related proteins in cells in various groups after knockdown of SNHG15 and over-expression of Myod1 ($n=3, \bar{x}\pm s$)

Group	Bax	Bcl-2	Cleaved caspase-3	Cleaved caspase-9
Control	1.06±0.13	0.98±0.09	3.13±0.39	0.98±0.09
OGD	4.21±0.29*	0.33±0.05*	17.21±1.80*	2.57±0.37*
OGD+si-NC	4.68±0.34	0.29±0.03	17.98±1.70	2.38±0.41
OGD+si-SNHG15	2.23±0.18△	0.72±0.08△	8.83±1.01△	1.38±0.12△
OGD+si-SNHG15+Vector	2.15±0.14	0.82±0.05	9.91±1.16	1.69±0.20
OGD+si-SNHG15+Myod1	3.77±0.29#	0.43±0.04#	22.18±2.8#	2.11±0.16#

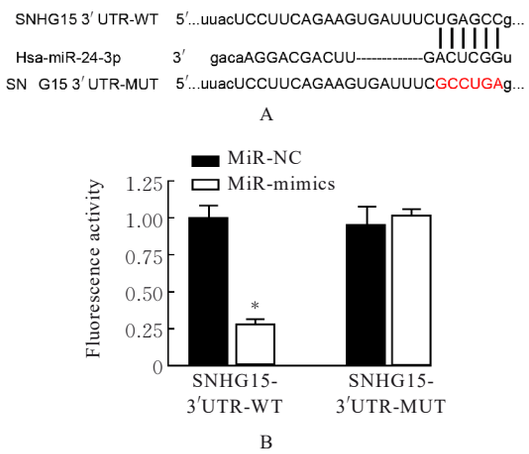
*$P<0.05$ *vs* control group; △$P<0.01$ *vs* OGD group; #$P<0.05$ *vs* OGD+si-SNHG15 group.



Lane 1: Control group; Lane 2: OGD group; Lane 3: OGD+si-NC group; Lane 4: OGD+si-SNHG15 group; Lane 5: OGD+si-SNHG15+vector group; Lane 6: OGD+si-SNHG15+Myod1 group.

图 5 敲低 SNHG15 和过表达 Myod1 后各组细胞中凋亡相关蛋白表达电泳图

Fig. 5 Electrophoregram of expressions of apoptosis-related proteins in cells in various groups after knockdown of SNHG15 and over-expression of Myod1



A: Starbase software predicted the complementary sequences of miR-24-3p and SNHG15 3′-UTR binding sites; B: Gene interaction was detected by double luciferase reporter gene assay. *$P<0.05$ *vs* miR-NC+SNHG15-3′UTR-WT group.

图 6 SNHG15 与 miRNA-24-3p 的靶向关系

Fig. 6 Target relationship between SNHG15 and miRNA-24-3p

向关系，SNHG15 可吸附 miR-24-3p（图 6A）。双荧光素酶报告基因实验结果显示：与 miR-NC+SNHG15-3′UTR-WT 组比较，miR-mimics+SNHG15-3′UTR-WT 组细胞荧光素酶活性降低（$P<0.05$）；与 miR-NC+SNHG15-3′UTR-MUT 组比较，miR-mimics+SNHG15-3′UTR-MUT 组细胞荧光素酶活性差异无统计学意义（$P>0.05$）（图 6B）。

2.7 过表达 miR-24-3p 和 SNHG15 后各组细胞活性、EdU 阳性细胞率、TUNEL 阳性细胞率和细胞中凋亡相关蛋白表达水平 与对照组比较，48 和 72 h 时 OGD 组细胞活性和 EdU 阳性细胞率降低（$P<0.05$），TUNEL 阳性细胞率升高（$P<0.05$），细胞中 Bax、cleaved caspase-3 和 cleaved caspase-9 蛋白表达水平升高（$P<0.05$），Bcl-2 蛋白表达水平降低（$P<0.05$）；与 OGD 组比较，48 和 72 h 时 OGD+miR-mimics 组细胞活性和 EdU 阳性细胞率升高（$P<0.01$），TUNEL 阳性细胞率降低（$P<0.01$），细胞中 Bax、cleaved caspase-3 和 cleaved caspase-9 蛋白表达水平降低（$P<0.01$），Bcl-2 蛋白表达水平升高（$P<0.01$）；与 OGD+miR-mimics 组比较，48 和 72 h 时 OGD+miR-mimics+SNHG15 组细胞活性和 EdU 阳性细胞率降低（$P<0.05$），TUNEL 阳性细胞率升高（$P<0.05$），细胞中 Bax、cleaved caspase-3 和 cleaved caspase-9 蛋白表达水平升高（$P<0.05$），Bcl-2 蛋白表达水平降低（$P<0.05$）。见表 7～9 和图 7。

3 讨 论

脑缺血已被证实是造成脑梗死相关疾病脑损伤的主要原因[4]，但其作用机制尚不明确。本研究结

表7 过表达miR-24-3p和SNHG15后各组细胞活性

Tab. 7 Cell activities in various groups after over-expression of miR-24-3p and SNHG15 ($n=3, \bar{x}\pm s$)

Group	Cell activity		
	(t/h)24	48	72
Control	0.80±0.03	1.32±0.03	1.64±0.05
OGD	0.68±0.04	0.93±0.05*	1.10±0.04*
OGD+miR-NC	0.77±0.01	1.03±0.01	1.24±0.05
OGD+miR-mimics	0.75±0.01	1.18±0.01△	1.43±0.04△
OGD+miR-mimics+Vector	0.79±0.01	1.22±0.02	1.39±0.05
OGD+miR-mimics+SNHG15	0.81±0.01	0.99±0.02#	1.16±0.02#

*$P<0.05$ *vs* control group; △$P<0.01$ *vs* OGD group; #$P<0.05$ *vs* OGD+miR-mimics group.

表8 过表达miR-24-3p和SNHG15后各组EdU阳性细胞率和TUNEL阳性细胞率

Tab. 8 Rates of EdU positive cells and rates of TUNEL positive cells in various groups after over-expression of miR-24-3p and SNHG15 ($n=3, \bar{x}\pm s, \eta/\%$)

Group	Rate of EdU-positive cells	Rate of TUNEL-positive cells
Control	24.57±3.16	3.18±0.46
OGD	6.42±1.11*	27.49±2.74*
OGD+miR-NC	5.78±0.34	25.32±2.16
OGD+miR-mimics	14.85±1.83△	13.18±1.21△
OGD+miR-mimics+Vector	15.72±1.37	14.67±2.02
OGD+miR-mimics+SNHG15	8.21±1.42#	20.30±2.57#

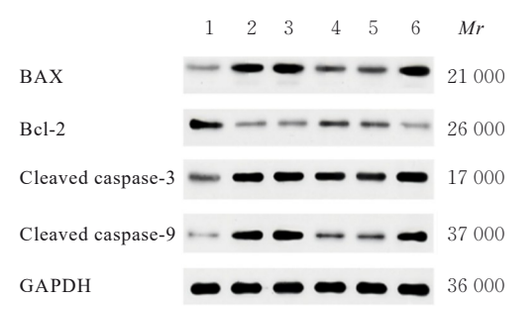
*$P<0.05$ *vs* control group; △$P<0.01$ *vs* OGD group; #$P<0.05$ *vs* OGD+miR-mimics group.

表9 过表达miR-24-3p和SNHG15后各组细胞中凋亡相关蛋白表达水平

Tab. 9 Expression levels of apoptosis-related proteins in cells in various groups after over-expression of miR-24-3p and SNHG15 ($n=3, \bar{x}\pm s$)

Group	Bax	Bcl-2	Cleaved caspase-3	Cleaved caspase-9
Control	1.04±0.05	0.99±0.09	1.01±0.09	0.98±0.09
OGD	4.82±0.51*	0.34±0.05*	2.88±0.28*	3.52±0.28*
OGD+miR-NC	4.17±0.38	0.37±0.07	2.77±0.29	3.40±0.37
OGD+miR-mimics	1.89±0.19△	0.47±0.09△	1.98±0.15△	1.89±0.16△
OGD+miR-mimics+Vector	2.14±0.25	0.43±0.06	1.83±0.20	2.03±0.24
OGD+miR-mimics+SNHG15	3.88±0.42#	0.39±0.05#	3.00±0.42#	2.95±0.27#

*$P<0.05$ *vs* control group; △$P<0.01$ *vs* OGD group; #$P<0.05$ *vs* OGD+miR-mimics group.



Lane 1: Control group; Lane 2: OGD group; Lane 3: OGD+miR-NC group; Lane 4: OGD+miR-mimics group; Lane 5: OGD+miR-mimics+Vector group; Lane 6: OGD+miR-mimics+SNHG15 group.

图7 过表达miR-24-3p和SNHG15后各组细胞中凋亡相关蛋白表达电泳图

Fig. 7 Electrophoregram of expressions of apoptosis-related proteins in cells in various groups after over-expression of miR-24-3p and SNHG15

果显示：在脑梗死患者外周血和OGD模型SH-SY5Y细胞中Myod1表达上调，表明Myod1可能参与调节缺血性脑损伤。已有研究[11, 16-17]显示：在急性脑卒中患者外周血、脑缺血相关的动物模型和细胞模型中SNHG15表达水平均上调，敲低SNHG15可减轻脑缺血相关的脑损伤。本研究结果显示：脑梗死患者外周血和OGD模型SH-SY5Y细胞中Myod1表达上调，同时伴随着SNHG15表达上调，且Myod1与SNHG15启动子区域存在结合位点，可形成DNA结合异二聚体，进而促进SNHG15表达；此外，敲低SNHG15可部分减轻OGD诱导的SH-SY5Y细胞增殖抑制和凋亡，而过表达Myod1可部分逆转敲低SNHG15的作用。本研究结果提示：Myod1可能通过与SNHG15启动子结合上调SNHG15表达，进而参与调节脑缺血造成的脑损伤。

lncRNAs可作为竞争性内源RNA（competing

endogenous RNAs, ceRNA）通过竞争性结合miRNAs来调节基因表达[18-19]。本研究通过Starbase预测软件和双荧光素酶报告基因实验证实：SNHG15为miR-24-3p的ceRNA。miR-24-3p与脑血管相关疾病的发生发展有密切关联[20-21]。在缺血性卒中中，miR-24-3p抑制剂通过调节Bax、caspase-3和热休克蛋白70（heat shock protein 70, HSP70）表达抑制细胞凋亡[20]。本研究结果显示：miR-24-3p过表达可促进OGD模型SH-SY5Y细胞增殖，并抑制其凋亡。miR-24-3p可通过调节Bax、Bcl-2、caspase-3和caspase-9相关的凋亡信号通路抑制细胞凋亡[22-24]。本研究结果显示：miR-24-3p可降低OGD模型SH-SY5Y细胞中Bax、cleaved caspase-3和cleaved caspase-9蛋白表达水平，并上调Bcl-2蛋白表达水平，即miR-24-3p对OGD诱导的SH-SY5Y细胞增殖抑制和细胞凋亡具有改善作用，而过表达SNHG15能削弱miRNA-24-3p的作用，表明SNHG15和miR-24-3p参与调节OGD诱导的SH-SY5Y细胞增殖抑制和细胞凋亡。

综上所述，脑梗死患者外周血和OGD细胞中Myod1和SNHG15表达上调。Myod1可与SNHG15启动子区域交联进而促进SNHG15表达，而SNHG15作为miRNA-24-3p的ceRNA，可促进OGD诱导的SH-SY5Y细胞增殖抑制和细胞凋亡。

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