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【基础研究】

miR-21 抑制剂对海马神经元氧糖剥夺/复氧损伤的影响

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摘要：　目的　探讨 miR-21 抑制剂对海马神经元离体氧糖剥夺/复氧(OGD/R)损伤的影响及作用机制。**方法**　将小鼠海马神经元 HT22 细胞接种于达尔伯克改良伊格尔培养基,置于含体积分数 95% 空气和 5% CO_2 培养箱中培养 2 d,然后将细胞分为正常组、OGD/R 6 h 组、OGD/R 12 h 组和 OGD/R 24 h组。正常组细胞置于常氧培养箱培养,OGD/R 6 h 组、OGD/R 12 h 组和 OGD/R 24 h 组细胞建立 OGD/R 模型,然后分别培养 6、12、24 h。取培养 24 h 的 HT22 细胞分为正常组、OGD/R + miRNA-NC 组、OGD/R + miR-21 mimics 组和 OGD/R + miR-21 inhibitor 组;正常组细胞置于常氧培养箱培养;OGD/R + miRNA-NC 组细胞转染 miRNA 阴性对照质粒,OGD/R + miR-21 mimics 组细胞转染 miR-21 mimics 质粒,OGD/R + miR-21 inhibitor 组细胞转染 miR-21 inhibitor 质粒,转染后置于培养箱孵育 2 d。实时荧光定量反转录聚合酶链反应(qRT-PCR)检测正常组、OGD/R 6 h 组、OGD/R 12 h 组和 OGD/R 24 h 组细胞中 miR-21 表达。qRT-PCR 检测正常组、OGD/R + miRNA-NC 组、OGD/R + miR-21 mimics 组和 OGD/R + miR-21 inhibitor 组细胞中 miR-21 和脑源性神经营养因子(BDNF) mRNA 表达,细胞计数试剂盒-8 检测各组细胞活性,Hoechst33342/碘化丙啶双染法检测各组细胞凋亡情况,酶联免疫吸附试验检测各组细胞中肿瘤坏死因子-α(TNF-α)和白细胞介素-1β(IL-1β)的水平。**结果**　OGD/R 6 h 组、OGD/R 12 h 组和 OGD/R 24 h 组细胞中 miR-21 相对表达量均显著低于正常组($P<0.05$)。OGD/R + miRNA-NC 组和 OGD/R + miR-21 inhibitor 组细胞中 miR-21 相对表达量显著低于正常组($P<0.05$),OGD/R + miR-21 mimics 组细胞中 miR-21 相对表达量显著高于正常组($P<0.05$)。OGD/R + miR-21 mimics 组细胞中 miR-21 相对表达量显著高于 OGD/R + miRNA-NC 组($P<0.05$);OGD/R + miR-21 inhibitor 组细胞中 miR-21 相对表达量显著低于 OGD/R + miRNA-NC 组和 OGD/R + miR-21 mimics 组($P<0.05$)。OGD/R + miRNA-NC 组和 OGD/R + miR-21 inhibitor 组细胞中 BDNF mRNA 相对表达量显著高于正常组($P<0.05$);OGD/R + miR-21 mimics 组细胞中 BDNF mRNA 相对表达量与正常组比较差异无统计学意义($P>0.05$)。OGD/R + miR-21 mimics 组细胞中 BDNF mRNA 相对表达量显著低于 OGD/R + miRNA-NC 组($P<0.05$);OGD/R + miR-21 inhibitor 组细胞中 BDNF mRNA 相对表达量显著高于 OGD/R + miRNA-NC 组和 OGD/R + miR-21 mimics 组($P<0.05$)。OGD/R + miRNA-NC 组和 OGD/R + miR-21 mimics 组细胞活性显著低于正常组($P<0.05$);OGD/R + miR-21 inhibitor 组细胞活性与正常组比较差异无统计学意义($P>0.05$)。OGD/R + miR-21 mimics 组细胞活性显著低于 OGD/R + miRNA-NC 组($P<0.05$),OGD/R + miR-21 inhibitor 组细胞活性显著高于 OGD/R + miRNA-NC 组和 OGD/R + miR-21 mimics 组($P<0.05$)。OGD/R + miRNA-NC 组、OGD/R + miR-21 mimics 组和 OGD/R + miR-21 inhibitor 组细胞凋亡率均显著高于正常组($P<0.05$)。OGD/R + miR-21 mimics 组细胞凋亡率显著高于 OGD/R + miRNA-NC 组($P<0.05$);OGD/R + miR-21 inhibitor 组细胞凋亡率显著低于 OGD/R + miRNA-NC 组和 OGD/R + miR-21 mimics 组($P<0.05$)。OGD/R + miRNA-NC 组、OGD/R + miR-21 mimics 组和 OGD/R + miR-21 inhibitor 组细胞中炎症因子 TNF-α 和 IL-1β 水平均显著高于正常组($P<0.05$)。OGD/R + miR-21 mimics 组细胞中 TNF-α、IL-1β 水平显著高于 OGD/R + miRNA-NC 组($P<0.05$);OGD/R + miR-21 inhibitor 组细胞中 TNF-α、IL-1β 水平显著低于 OGD/R + miRNA-NC 组和 OGD/R + miR-21 mimics 组($P<0.05$)。**结论**　miR-21 抑制剂可改善 OGD/R 诱导的海马神经元缺血/再灌注损伤,其机制可能与上调 BDNF 信号通路、抑制炎症因子信号途径有关。

关键词：　miR-21 抑制剂;氧糖剥夺/复氧;海马神经元;脑源性神经营养因子;肿瘤坏死因子-α;白细胞介素-1β

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Effects of microRNA-21 inhibitors on oxygen-glucose deprivation/reoxygenation injury of hippocampal neurons

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Abstract: **Objective** To investigate the effect and mechanism of miR-21 inhibitors on oxygen-glucose deprivation/reoxygenation (OGD/R) injury in hippocampal neurons in vitro. **Methods** HT22 cells of mouse hippocampal neurons were inoculated into Dulbecco's modified Eagle's medium and cultured in incubator which contained volume fraction 95% air and 5% CO_2 for 2 days. Then the cells were divided into normal group, OGD/R 6 h group, OGD/R 12 h group and OGD/R 24 h group. The cells in normal group were cultured in normoxia incubator; the cells in OGD/R 6 h group, OGD/R 12 h group and OGD/R 24 h group were established the OGD/R model and then cultured for 6, 12, 24 h respectively. The HT22 cells which cultured for 24 hours were taken and divided into normal group, OGD/R + miRNA-NC group, OGD/R + miR-21 mimics group and OGD/R + miR-21 inhibitor group. The cells in the normal group were cultured in the normoxia incubator, the cells in the OGD/R + miRNA-NC group were transfected with miRNA negative control plasmids, the cells in the OGD/R + miR-21 mimics group were transfected with miR-21 mimics plasmids, the cells in the OGD/R + miR-21 inhibitor group were transfected with miR-21 inhibitor plasmids; then the above cells were cultured in incubator for 2 days. The expression of miR-21 mRNA in HT22 cells in normal group, OGD/R 6 h group, OGD/R 12 h group and OGD/R 24 h group was detected by real-time fluorescence quantitative reverse transcription polymerase chain reaction (qRT-PCR). The expression of miR-21, brain derived neurotrophic factor (BMNF) mRNA in the normal group, OGD/R + miRNA-NC group, OGD/R + miR-21 mimics group and OGD/R + miR-21 inhibitor group were detected by qRT-PCR; the cell activity in above each group was detected by cell count kit-8; the apoptosis in above each group was detected by Hoechst33342 / propidium iodide double staining; the levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in the cells in above each group were detected by enzyme linked immunosorbent assay. **Results** The relative expression of miR-21 in the OGD/R 6 h group, OGD/R 12 h group and OGD/R 24 h group was significantly lower than that in the normal group ($P<0.05$). The expression of miR-21 in the OGD/R + miRNA-NC group and OGD/R + miR-21 inhibitor group was significantly lower than that in the normal group ($P<0.05$), and the expression of miR-21 in the OGD/R + miR-21 mimics group was significantly higher than that in the normal group ($P<0.05$). The expression of miR-21 in the OGD/R + miR-21 mimics group was significantly higher than that in the OGD/R + miRNA-NC group ($P<0.05$); the expression of miR-21 in the OGD/R + miR-21 inhibitor group was significantly lower than that in the OGD/R + miRNA-NC group and OGD/R + miR-21 mimics group ($P<0.05$). The expression of BDNF mRNA in the OGD/R + miRNA-NC group and OGD/R + miR-21 inhibitor group was significantly higher than that in the normal group ($P<0.05$); there was no significant difference in BDNF mRNA expression between the OGD/R + miR-21 mimics group and normal group ($P>0.05$). The expression of BDNF mRNA in the OGD/R + miR-21 mimics group was significantly lower than that in the OGD/R + miRNA-NC group ($P<0.05$); the expression of BDNF mRNA in the OGD/R + miR-21 inhibitor group was significantly higher than that in the OGD/R + miRNA-NC group and OGD/R + miR-21 mimics group ($P<0.05$). The cell activity in the OGD/R + miRNA-NC group and OGD/R + miR-21 mimics group was significantly lower than that in the normal group ($P<0.05$); there was no significant difference in the cell activity between the OGD/R + miR-21 inhibitor group and the normal group ($P>0.05$). The cell activity in the OGD/R + miR-21 mimics group was significantly lowrer than that in the OGD/R + miRNA-NC group ($P<0.05$); the cell activity of the OGD/R + miR-21 inhibitor group was significantly higher than that in the OGD/R + miRNA-NC group and OGD/R + miR-21 mimics group ($P<0.05$). The apoptosis rate in the OGD/R + miRNA-NC group, OGD/R + miR-21 mimics group and OGD/R + miR-21 inhibitor group was significantly higher than that in the normal group ($P<0.05$). The apoptosis rate in the OGD/R + miR-21 mimics group was significantly higher than that in the OGD/R + miRNA-NC group ($P<0.05$); the apoptosis rate in the OGD/R + miR-21 inhibitor group was significantly lower than that in the OGD/R + miRNA-NC group and OGD/R + miR-21 mimics group ($P<0.05$). The levels of TNF-α and IL-1β in cells in the OGD/R + miRNA-NC group, OGD/R + miR-21 mimics group and OGD/R + miR-21 inhibitor group were significantly higher than those in the normal group ($P<0.05$). The levels of TNF-α and IL-1β in cells in the OGD/R + miR-21 mimics group were significantly higher than those in the OGD/R + miRNA-NC group ($P<0.05$); the levels of TNF-α and IL-1β in cells in the OGD/R + miR-21 inhibitor group were significantly lower than those in the OGD/R + miRNA-NC group and OGD/R + miR-21 mimics group ($P<0.05$). **Conclusion** MiR-21 inhibitor can improve OGD/R-induced ischemia/reperfusion injury in hippocampal neurons, which may be related to up-regulation of BDNF signaling pathway and inhibition inflammatory cytokine signaling pathway.

Key words: miR-21 inhibitor; oxygen glucose deprivation/reoxygenation; hippocampal neurons; brain derived neurotrophic factor; tumor necrosis factor α; interleukin-1β

脑缺血再灌注损伤是一种常见临床疾病,在世界范围内具有较高的致残率和病死率。脑缺血/再灌注损伤可造成多种神经系统后遗症,包括失语症、偏瘫和痴呆等[1-2]。但目前尚缺乏有效预防脑缺血再灌注损伤的治疗策略。脑缺血再灌注损伤病理过程复杂,目前研究证实,脑源性神经营养因子(brain derived neurotrophic factor,BDNF)干预可能是治疗脑缺血再灌注损伤的一种有前景的方法[3-4]。但 BDNF 的毒副作用和应用复杂性使其临床应用受到一些限制[5]。

MiRNAs 是一组小的非编码 RNA,以转录后方式调节基因表达。其通过与目的 mRNA 的 3′非翻译区结合,导致 mRNA 降解或翻译抑制。MiRNAs 通过靶向不同的基因及相关的信号通路,参与了多种生理和病理过程[6-7]。研究表明,miRNAs 参与了脑缺血再灌注损伤的病理过程,并调节神经元存活和凋亡过程中各种关键基因的表达,成为脑缺血再灌注损伤新的生物标志物和治疗靶点[7-8]。因此,探讨 miRNA 分子表达和功能可以有效揭示脑缺血再灌注损伤的分子机制。

XIE 等[9]研究证实,miR-21 基因敲除能够促进脊髓损伤后神经元再生和功能恢复,该研究还发现,miR-21 能够通过靶向调控 BDNF 信号通路促进损伤后神经元增殖和迁移,但其在脑缺血再灌注损伤中对海马神经元的作用及调控机制至今未见报道。基于此,本研究拟构建氧糖剥夺与复氧(oxygen-glucose deprivation and reoxygenation,OGD/R)海马神经元体外缺血再灌注损伤模型,观察转染 miR-21 模拟物和抑制剂对 OGD/R 细胞活性和细胞凋亡的作用,探讨 miR-21 抑制剂对缺血再灌注损伤海马神经元的作用机制,为临床治疗脑缺血再灌注损伤提供新的靶点。

1 材料与方法

1.1 细胞、试剂与仪器 小鼠海马神经元 HT22 细胞购自中国广州吉妮欧生物科技有限公司;miRNA-NC、miR-21 mimics、miR-21 inhibitor 购自美国 ABM 公司,细胞计数试剂盒-8(cell counting kit-8,CCK-8)、达尔伯克改良伊格尔培养基(Dulbecco′s modified Eagle′s medium,DMEM)、胎牛血清、青霉素、链霉素、碘化丙啶(propidium iodine,PI)、赫斯特荧光染料 33342(Hoechst33342)、胰蛋白酶购自美国 Sigma公司,肿瘤坏死因子-α(tumor necrosis factor-α,TNF-α)和白细胞介素-1β(interleukin-1β,IL-1β)测定酶联免疫吸附试验(enzyme linked immunosorbent assay,ELISA)试剂盒购自美国 R&D Systems 公司,所用引物由宝日医生物技术(北京)有限公司合成,SYBR Green PCR Master Mix 购自美国 Applied Biosystems 公司,cDNA 合成试剂盒购自德国 Qiagen 公司,反转录定量聚合酶链反应(quantitative reverse transcription polymerase chain reaction,qRT-PCR)检测试剂盒、TRIzol 试剂盒、Lipofectamine 2000 购自美国 Invitrogen 公司;C21 三气缺氧细胞培养箱购自美国 Biospherix 公司,3550UV 型酶标仪购自美国 BIO-RAD公司,Olympus AX70 荧光显微镜购自日本 Olympus 公司。

1.2 实验方法

1.2.1 细胞培养 将小鼠海马神经元 HT22 细胞接种于含体积分数 10% 胎牛血清、4.5 g·L^{-1}葡萄糖和体积分数 1% 青霉素/链霉素的 DMEM 中,置于 37 ℃、含体积分数 95% 空气和 5% CO_2 培养箱中培养 2 d,备用。

1.2.2 海马神经元 OGD/R 模型的建立 将 HT22 细胞分为正常组、OGD/R 6 h组、OGD/R 12 h 组和 OGD/R 24 h 组。正常组细胞置于常氧培养箱(37 ℃、含体积分数 95% 空气和 5% CO_2)培养;OGD/R 6 h 组、OGD/R 12 h组和 OGD/R 24 h 组细胞建立 OGD/R 模型。OGD/R 模型制备步骤为:将 HT22 细胞接种于无糖平衡盐溶液中,置于 37 ℃三气缺氧细胞培养箱(含体积分数 1% O_2、94% N_2、5% CO_2)培养 6 h,用含4.5 g·L^{-1}葡萄糖的培养基代替无糖平衡盐溶液,置于 37 ℃含体积分数 95% 空气和 5% CO_2 培养箱中培养;OGD/R 6 h 组、OGD/R 12 h 组和 OGD/R 24 h 组细胞分别培养 6、12、24 h。将培养 24 h 细胞作为 OGD/R 模型细胞进行后续实验[8]。

1.2.3 qRT-PCR 检测正常组、OGD/R 6 h 组、OGD/R 12 h 组和 OGD/R 24 h 组细胞中 miR-21 表达 应用 TRIzol 裂解溶液提取正常组、OGD/R 6 h组、OGD/R 12 h 组和 OGD/R 24 h 组细胞总 RNA,检测 OGD/R 后 miR-21 mRNA 表达;具体操作严格按照试剂盒说明书进行。采用反转录试剂盒和反转录酶反转录 cDNA,U6 作为 miR-21 内参照。反应体系包括 10 mL SYBR Green PCR Master Mix、2 mL引物和 8 mL cDNA。MiR-21 上游引物序列为 5′- CCGCTCGAGATCCCAGTAATGGAATGAAG-3′,下游引物序列为 5′- ATAAGAATGCGGCCGCCATCACTTATTATTGCCTATGT-3′;U6 上游引物序列为 5′-

CTCGCTTCGGCAGCACA-3′,下游引物序列为 5′-AACGCTTCACGAATTTGCGT-3′。采用 $2^{-\triangle\triangle Ct}$ 法分析 miR-21 相对表达量。实验重复 4 次,取均值。

1.2.4　细胞转染　将 OGD/R 模型细胞分为正常组、OGD/R + miRNA-NC 组、OGD/R + miR-21 mimics组和 OGD/R + miR-21 inhibitor 组。OGD/R + miRNA-NC 组细胞转染 miRNA 阴性对照质粒,OGD/R + miR-21 mimics 组细胞转染 miR-21 mimics 质粒,OGD/R + miR-21 inhibitor 组细胞转染 miR-21 inhibitor 质粒,转染后置于 37 ℃含体积分数 5% CO_2 培养箱孵育 2 d 用于后续实验;正常组细胞置于 37 ℃含体积分数 95% 空气和 5% CO_2 常氧培养箱中培养。

1.2.5　qRT-PCR 检测正常组、OGD/R + miRNA-NC 组、OGD/R + miR-21 mimics 组和 OGD/R + miR-21 inhibitor 组细胞中 miR-21 和 BDNF mRNA 表达　应用 TRIzol 试剂盒提取各组细胞总 RNA,检测 miR-21 和 BDNF mRNA 相对表达量;具体操作严格按照试剂盒说明书进行。U6 作为 miR-21 内参照,甘油醛-3-磷酸脱氢酶(glyceraldehyde-3-phosphate dehydrogenase,GAPDH)作为 BDNF 内参照。PCR 反应体系:10 mL SYBR Green PCR Master Mix、2 mL 引物和 8 mL cDNA。miR-21 上游引物序列为 5′-CCGCTCGAGATCCCAGTAATGGAATGAAG-3′,下游引物序列为 5′- ATAAGAATGCGGCCGCCATCACTTATTATTGCCTATGT-3′;U6 上游引物序列为 5′- CTCGCTTCGGCAGCACA-3′,下游引物序列为 5′- AACGCTTCACGAATTTGCGT-3′;BDNF 上游引物序列为 5′-CTGTATCAAAAGGCCAACTGAA-3′,下游引物序列为 5′-GTGTCTATCCTTATGAATCGCCA-3′; GAPDH 上游引物序列为 5′-TGGTGGGTATGGGTCAGAAGGACTC-3′,下游引物序列为 5′- CATGGCTGGGGTGTTGAAGGTCTCA-3′。每个样本重复 4 次,取均值。采用 $2^{-\triangle\triangle Ct}$ 法计算 miR-21 和 BDNF mRNA 相对表达量。实验重复 6 次,取均值。

1.2.6　CCK-8 检测各组细胞活性　应用 CCK-8 比色法测定各组细胞活力。将 4 组细胞接种于 96 孔板中并培养过夜,每孔加入 10 mL CCK-8 溶液于 37 ℃下培养 2 h,应用酶标仪检测各组细胞 450 nm 处吸光度,并计算各组细胞活性。细胞活性 = 干预组吸光度值/正常组吸光度值 ×100%,数值越大,细胞活性越高。实验重复 3 次,取均值。

1.2.7　Hoechst33342/PI 双染法检测各组细胞凋亡情况　各组细胞接种于 6 孔板,每孔 1×10^6 个细胞。加入 1 mL Hoechst33342 孵育 0.5 h,应用 50 mg · L^{-1} PI 孵育 15 min,在 Olympus AX70 荧光显微镜下用紫外光激发,于高倍显微镜下(×400)每组随机选取 3 个视野,计数凋亡细胞数和细胞总数(每组不少于 1 000 个),计算细胞凋亡率,细胞凋亡率 = 凋亡细胞数/细胞总数 ×100%。实验重复 3 次,取均值。

1.2.8　ELISA 法检测各组细胞中 TNF-α 和 IL-1β 的表达　使用 2.5 g · L^{-1} 胰蛋白酶消化液消化细胞,显微镜下观察见细胞收缩后去除消化液,收集各组细胞,1 500 r · min^{-1} 离心 5 min,取各组细胞离心培养液,应用 TNF-α 测定 ELISA 试剂盒和 IL-1β 测定 ELISA 试剂盒检测各组细胞中 TNF-α 和 IL-1β 水平,具体操作严格按照说明书进行。加入样品 10 mL,加入 40 μL 稀释液进行稀释,应用酶标仪在波长 450 nm 处检测各样本孔吸光度值,用各干预组吸光度值与正常组吸光度值的比值来计算 TNF-α 和 IL-1β 相对表达水平。实验重复 6 次,取均值。

1.3　统计学处理　应用 SPSS 13.0 软件进行统计学分析,计量资料以均数 ± 标准差($\bar{x}\pm s$)表示,组间比较采用单因素方差分析,多组间两两比较采用 SNK 法,$P<0.05$ 为差异有统计学意义。

2　结果

2.1　正常组、OGD/R 6 h 组、OGD/R 12 h 组和 OGD/R 24 h 组细胞中 miR-21 相对表达量比较　正常组、OGD/R 6 h 组、OGD/R 12 h 组和 OGD/R 24 h 组细胞中 miR-21 相对表达量分别为 1.083 ± 0.099、0.790 ± 0.079、0.498 ± 0.055、0.389 ± 0.079;OGD/R 6 h 组、OGD/R 12 h 组和 OGD/R 24 h 组细胞中 miR-21 相对表达量均显著低于正常组,差异有统计学意义($P<0.05$)。

2.2　正常组、OGD/R + miRNA-NC 组、OGD/R + miR-21 mimics 组和 OGD/R + miR-21 inhibitor 组细胞中 miR-21 和 BDNF mRNA 相对表达量比较　结果见表 1。OGD/R + miRNA-NC 组和 OGD/R + miR-21 inhibitor 组细胞中 miR-21 相对表达量显著低于正常组,OGD/R + miR-21 mimics 组细胞中 miR-21 相对表达量显著高于正常组,差异均有统计学意义($P<0.05$)。与 OGD/R + miRNA-NC 组比较,OGD/R + miR-21 mimics 组细胞中 miR-21 相对表达量显著升高,OGD/R + miR-21 inhibitor 组细胞中 miR-21 相对表达量显著降低,差异均有统计学意义($P<0.05$)。OGD/R + miR-21 inhibitor 组细胞中 miR-21 相对表达量显著低于 OGD/R + miR-21 mimics 组,差异有统计学意义($P<0.05$)。

OGD/R + miRNA-NC 组和 OGD/R + miR-21 inhibitor组细胞中 BDNF mRNA 相对表达量显著高于正常组,差异有统计学意义($P<0.05$);OGD/R + miR-21 mimics 组细胞中 BDNF mRNA 相对表达量

与正常组比较差异无统计学意义($P>0.05$)。与 OGD/R + miRNA-NC 组比较,OGD/R + miR-21 mimics 组细胞中 BDNF mRNA 相对表达量显著降低,OGD/R + miR-21 inhibitor 组细胞中 BDNF mRNA 相对表达量显著升高,差异均有统计学意义($P<0.05$);OGD/R + miR-21 inhibitor 组细胞中 BDNF mRNA 相对表达量显著高于 OGD/R + miR-21 mimics 组,差异有统计学意义($P<0.05$)。

表 1 4 组细胞中 miR-21 和 BDNF mRNA 相对表达量比较

Tab. 1 Comparison of the relative expression of miR-21 and BDNF mRNA among the four groups ($\bar{x}\pm s$)

组别	n	miR-21	BDNF mRNA
正常组	6	1.003 ± 0.074	1.201 ± 0.232
OGD/R + miRNA-NC 组	6	0.408 ± 0.079[a]	7.212 ± 0.860[a]
OGD/R + miR-21 mimics 组	6	6.705 ± 0.580[ab]	0.945 ± 0.347[b]
OGD/R + miR-21 inhibitor 组	6	0.043 ± 0.026[abc]	12.720 ± 2.746[abc]
F		450.500	59.480
P		0.000	0.000

注:与正常组比较[a]$P<0.05$;与 OGD/R + miRNA-NC 组比较[b]$P<0.05$;与 OGD/R + miR-21mimics 组比较[c]$P<0.05$。

2.3 4 组细胞活性和细胞凋亡率比较 结果见表 2 和图 1。OGD/R + miRNA-NC 组和 OGD/R + miR-21 mimics 组细胞活性显著低于正常组,差异有统计学意义($P<0.05$)。OGD/R + miR-21 inhibitor 组细胞活性与正常组比较差异无统计学意义($P>0.05$)。与 OGD/R + miRNA-NC 组比较,OGD/R + miR-21 mimics 组细胞活性显著降低,OGD/R + miR-21 inhibitor 组细胞活性显著增高,差异均有统计学意义($P<0.05$);OGD/R + miR-21 inhibitor 组细胞活性显著高于 OGD/R + miR-21 mimics 组,差异有统计学意义($P<0.05$)。

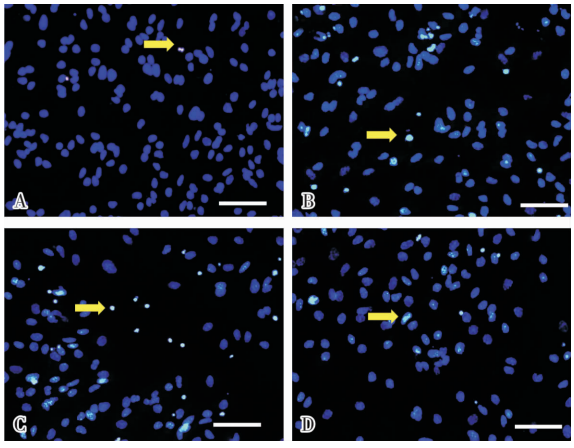
OGD/R + miRNA-NC 组、OGD/R + miR-21 mimics组和 OGD/R + miR-21 inhibitor 组细胞凋亡率均显著高于正常组,差异有统计学意义($P<0.05$)。与 OGD/R + miRNA-NC 组比较,OGD/R + miR-21 mimics 组细胞凋亡率显著增高,OGD/R + miR-21 inhibitor 组细胞凋亡率显著降低,差异均有统计学意义($P<0.05$);OGD/R + miR-21 inhibitor 组细胞凋亡率显著低于 OGD/R + miR-21 mimics 组,差异有统计学意义($P<0.05$)。

表 2 4 组细胞活性和细胞凋亡率比较

Tab. 2 Comparison of the cell viability and cell apoptosis rate among the four groups ($\bar{x}\pm s$)

组别	n	细胞活性/%	细胞凋亡率/%
正常组	3	99.832 ± 4.241	10.497 ± 3.194
OGD/R + miRNA-NC 组	3	59.051 ± 5.245[a]	69.052 ± 5.186[a]
OGD/R + miR-21 mimics 组	3	13.523 ± 2.961[ab]	90.190 ± 2.875[ab]
OGD/R + miR-21 inhibitor 组	3	95.955 ± 10.440[bc]	22.623 ± 5.890[abc]
F		118.600	213.600
P		0.000	0.000

注:与正常组比较[a]$P<0.05$;与 OGD/R + miRNA-NC 组比较[b]$P<0.05$;与 OGD/R + miR-21 mimics 组比较[c]$P<0.05$。



A:正常组;B: OGD/R + miRNA-NC 组;C: OGD/R + miR-21 mimics 组;D: OGD/R + miR-21 inhibitor 组;黄色箭头示凋亡细胞。

图 1 各组细胞凋亡情况(Hoechst33342/PI 双染法,×100)

Fig. 1 Cell apoptosis in each group (Hoechst33342/PI double staining, ×100)

2.4 4 组细胞中炎症因子水平比较 结果见表 3。OGD/R + miRNA-NC 组、OGD/R + miR-21 mimics 组和 OGD/R + miR-21 inhibitor 组细胞中炎症因子 TNF-α 和 IL-1β 水平均显著高于正常组,差异有统计学意义($P<0.05$)。与 OGD/R + miRNA-NC 组比较,OGD/R + miR-21 mimics 组细胞中 TNF-α、IL-1β 水平显著增高,OGD/R + miR-21 inhibitor 组细胞中 TNF-α、IL-1β 水平显著降低,差异均有统计学意义($P<0.05$);OGD/R + miR-21 inhibitor 组细胞中 TNF-α、IL-1β 水平显著低于 OGD/R + miR-21 mimics组,差异有统计学意义($P<0.05$)。

表 3 4 组细胞中 TNF-α 和 IL-1β 水平比较

Tab. 3 Comparison of the levels of TNF-α and IL-1β in cells among the four groups ($\bar{x}\pm s$)

组别	n	TNF-α	IL-1β
正常组	6	1.035 ± 0.028	1.118 ± 0.044
OGD/R + miRNA-NC 组	6	1.588 ± 0.039[a]	2.017 ± 0.128[a]
OGD/R + miR-21 mimics 组	6	1.930 ± 0.101[ab]	2.548 ± 0.108[ab]
OGD/R + miR-21 inhibitor 组	6	1.245 ± 0.059[abc]	1.526 ± 0.189[abc]
F		114.200	69.310
P		0.000	0.000

注:与正常组比较[a]$P<0.05$;与 OGD/R + miRNA-NC 组比较[b]$P<0.05$;与 OGD/R + miR-21 mimics 组比较[c]$P<0.05$。

3 讨论

脑缺血再灌注损伤是一个复杂的病理过程,涉及炎症、蛋白合成抑制以及线粒体功能与能量代谢受损,其可导致神经元凋亡和脑损伤[2]。因此,单一药物干预效果有限。MiRNAs 是一种小的内源性非编码 RNA 分子,在转录后调节基因表达。这些小分子通过调节增殖、分化和凋亡等多种细胞功能,在各种生理和病理过程中发挥作用。大量研究证明,多种 miRNAs 在脑缺血再灌注损伤中发挥神经元保

护作用[4,7-9]。

有研究表明,miR-21 在心、肺、肾和肝脏纤维化瘢痕形成中发挥作用[10-11],miR-21 与转化生长因子-β/SMAD 蛋白信号通路组成的调控网络可以调节脊髓损伤后纤维化疤痕的形成[9]。研究发现,通过抑制 miR-21 表达可激活 TrkA 受体信号通路,进而促进脊髓背根神经节神经元轴突生长[12];敲除 miR-21 能够靶向调控 BDNF,显著降低脊髓损伤部位的炎症反应,促进运动功能恢复[9]。但 miR-21 在脑缺血再灌注损伤中的作用机制尚不明确。

本研究首先评估了 miR-21 在海马神经元 OGD/R 损伤后表达情况,结果显示,OGD/R 损伤后,海马神经元 miR-21 的表达显著降低,表明 miR-21 可能是参与调节 OGD/R 诱导的海马神经元损伤的新型 miRNA。进一步检测 miR-21 模拟物和抑制剂对细胞活力和凋亡的影响,结果显示,miR-21 抑制剂能显著降低 OGD/R 诱导的细胞凋亡,同时增加细胞活性;而 miR-21 模拟物具有相反的效果。这些结果表明,抑制 miR-21 对 OGD/R 处理的神经元具有神经保护作用。

有研究发现,miR-21 能够通过调控靶基因 BDNF信号通路[9-10,13],降低神经损伤后炎症反应。BDNF 在神经营养素家族中具有重要地位,BDNF 在周围神经系统和中枢神经系统神经元的发育和存活中发挥关键作用。但 BDNF 用于神经再生的临床应用受到限制,因为目前难以克服其对人体的不良反应,包括肌肉疼痛、注射部位痛觉过敏以及高剂量时可能的致瘤性。此外,BDNF 是一个大分子,不能跨越血脑屏障,且半衰期相对较短[5,14]。因此,通过 miRNA 调控增加内源性 BDNF 分泌可作为一种替代疗法,代替外源性基因载体诱导 BDNF 高表达。基于此,本研究观察了 miR-21 对海马神经元产生 BDNF的影响,结果发现,与 OGD/R + miRNA-NC 组比较,OGD/R + miR-21 mimics 组 miR-21、TNF-α 和 IL-1β 表达显著增高,BDNF 表达显著降低;而 OGD/R + miR-21 inhibitor 组与之相反,再次证实 miR-21 在 OGD/R 细胞模型中可靶向调控 BDNF 表达,推测在 OGD/R 损伤过程中 miR-21 抑制剂调节机制可能是通过靶向上调 BDNF 信号通路,进而下调炎症细胞因子 TNF-α 和 IL-1β 表达实现的。

综上所述,本研究证实了体外 miR-21 抑制剂对海马神经元缺血再灌注损伤具有保护作用,其机制可能与上调 BDNF 信号通路、抑制 OGD/R 诱导的炎症因子信号途径及细胞凋亡有关。miR-21 有望成为治疗脑缺血再灌注损伤的新靶点。

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