CircOGDH通过miR-24-3p介导的HOXA1上调
对缺氧复氧诱导的神经元损伤的影响

陈玉兰[1]*　　陈萌[2]　　朱正萍[2]

([1]南京市浦口区中医院, 发热门诊, 南京 211800; [2]南京市浦口区中医院, 神经内科, 南京 211800)

摘要　　该文探讨了CircOGDH通过miR-24-3p靶向调控HOXA1对缺氧复氧(H/R)诱导的神经元损伤的作用机制。以Ht22细胞为研究对象, 利用缺氧复氧诱导神经元细胞损伤, 将细胞分为阴性对照组(si-NC组)、CircOGDH沉默组(si-CircOGDH组)、过表达阴性对照组(miR-NC组)、miR-24-3p过表达组(miR-24-3p minic组)、CircOGDH沉默+抑制剂阴性对照组(si-CircOGDH+anti-miR-NC组)、CircOGDH沉默+miR-24-3p抑制剂组(si-CircOGDH+anti-miR-24-3p组), 另设置未转染的对照组(control组)、H/R组。qRT-PCR法检测CircOGDH、miR-24-3p、*HOXA1* mRNA表达水平; CCK-8法检测细胞活性; 氧化应激水平检测采用微量法; 流式细胞仪检测细胞凋亡; Western blot检测HOXA1、Bax、Bcl-2蛋白表达。结果显示, H/R组Ht22细胞CircOGDH、HOXA1表达上调, miR-24-3p表达下调, 细胞凋亡率以及Bax、LDH、MDA含量升高, 细胞存活率以及Bcl-2水平、SOD活性、GSH-Px活性下降($P<0.05$); 沉默CircOGDH可以上调H/R诱导的Ht22细胞中miR-24-3p表达, 下调HOXA1表达, 提高细胞存活率和Bcl-2蛋白、SOD、GSH-Px水平, 降低细胞凋亡率以及Bax、LDH、MDA含量($P<0.05$); 过表达miR-24-3p能够下调HOXA1表达, 提高细胞存活率及以Bcl-2、SOD、GSH-Px水平, 降低细胞凋亡率及以Bax、LDH、MDA含量($P<0.05$); 抑制miR-24-3p表达能够一定程度上逆转沉默CircOGDH对HT22细胞损伤的保护作用。由此提示, 沉默CircOGDH可能通过上调miR-24-3p表达, 下调HOXA1表达, 改善H/R诱导的神经元氧化损伤, 抑制其凋亡。

关键词　　CircOGDH; miR-24-3p; HOXA1; 神经元损伤

Effect of CircOGDH on Hypoxia Reoxygenation Induced Neuronal Damage through miR-24-3p Mediated HOXA1 Upregulation

CHEN Yulan[1]*, CHEN Meng[2], ZHU Zhengping[2]

([1]*Fever Clinic, Nanjing Pukou District TCM Hospital, Nanjing 211800, China;*
[2]*Department of Neurology, Nanjing Pukou District TCM Hospital, Nanjing 211800, China*)

Abstract　　This study aimed to investigate the mechanism of CircOGDH on hypoxic reoxygenation (H/R) induced neuronal damage by targeting and regulating HOXA1 through miR-24-3p. Ht22 cells were used as the research object, hypoxia reoxygenation was used to induce neuronal damage, and the cells were divided into negative control group (si-NC group), CircOGDH silencing group (si-CircOGDH group), overexpression negative control group (miR-NC group), miR-24-3p overexpression group (miR-24-3p minic group), CircOGDH

silencing+inhibitor negative control group (si-CircOGDH+anti-miR-NC group), and CircOGDH silencing +miR-24-3p inhibitor group (si-CircOGDH+anti-miR-24-3p group). Control group and H/R group without transfection were also set up. The expression of CircOGDH, miR-24-3p and *HOXA1* mRNA was detected by qRT-PCR; the cell activity was detected by CCK-8 method; the oxidative stress level was detected by micro method; apoptosis was detected by flow cytometry; Western blot was used to detect the expression of HOXA1, Bax and Bcl-2 proteins. The results showed that the expression of CircOGDH and HOXA1 was up-regulated and the expression of miR-24-3p was down-regulated in Ht22 cells in H/R group, the apoptosis rate, the expression of Bax protein, and the contents of LDH and MDA increased, the cell survival rate, the expression of Bcl-2 protein, the activities of SOD and GSH-Px decreased ($P<0.05$); silencing CircOGDH could up-regulate the expression of miR-24-3p and down-regulate the expression of HOXA1 in H/R-induced Ht22 cells, improve cell survival rate and the levels of Bcl-2 protein, SOD, and GSH-Px, reduce the apoptosis rate, Bax protein, the contents of LDH, MDA ($P<0.05$); overexpression of miR-24-3p could reduce the expression of HOXA1, increase the cell survival rate, the levels of Bcl-2 protein, SOD, GSH-Px, and reduce the apoptosis rate, Bax protein, contents of LDH and MDA ($P<0.05$); inhibiting the expression of miR-24-3p could partially reverse the protective effect of silencing CircOGDH on HT22 cells damage. In conclusion, silencing CircOGDH may up-regulate the expression of miR-24-3p, down-regulate the expression of HOXA1, improve the oxidative damage of neurons induced by H/R, and inhibit their apoptosis.

Keywords CircOGDH; miR-24-3p; HOXA1; neuron damage

缺血性中风是由脑血流量减少引起脑组织缺血、缺氧，而发生坏死的脑血管疾病，涉及氧化应激、炎症、细胞凋亡等多种病理机制[1]。从分子层面寻找治疗靶点来抑制氧化应激和细胞凋亡对于改善缺血性脑损伤意义重大[2]。研究发现，环状RNA OGDH(CircOGDH)是调节缺血神经元活力的潜在治疗靶点，在急性缺血性中风患者中可作为预测缺血半暗带神经元损伤的生物标志物，对于缺血性中风的临床诊断和治疗具有重要价值[3]。miRNA参与大脑发育和脑损伤疾病(包括缺血性中风)的发生和发展[4-5]。据报道，miR-24-3p在氧糖剥夺/复氧诱导的受损神经元中低表达，上调miR-24-3p表达会增加细胞活性，抑制氧化应激和神经元凋亡[6]。同源异形盒基因A1(homologous heteromorphic box gene A1, *HOXA1*)是一种DNA结合转录因子，在胚胎大脑的发育中发挥作用，可通过基因表达调节多种生物过程，包括神经发生。研究显示，在缺氧缺血诱导的神经元凋亡中，HOXA1 mRNA和蛋白水平升高[7]。由生物信息学网站预测得到，*HOXA1*是miR-24-3p的靶基因，并且CircOGDH与miR-24-3p也存在结合位点，因此本研究利用H/R诱导神经元损伤，观察CircOGDH对HT22细胞活性、氧化应激、凋亡的影响，以及miR-24-3p/HOXA1的调控机制。

1 材料和方法

1.1 材料

Ht22细胞、DEME培养基均购自武汉普诺赛生命科技有限公司；胎牛血清(FBS)购自北京缔一生物科技有限公司；青/链霉素双抗购自上海麦克林生化科技股份有限公司；TRIzol试剂购自上海创赛科技有限公司；Annexin V-FITC/PI凋亡试剂盒购自ThermoFisher Scientific公司；CCK-8细胞活性检测试剂盒购自上海尚宝生物科技有限公司；SOD、GSH-Px、LDH、MDA试剂盒购自北京索莱宝科技有限公司；双荧光素酶报告基因实验检测试剂盒购自北京百奥莱博科技有限公司；抗体Bax、Bcl-2、HOXA1、GAPDH购自英国Abcam公司。

全自动酶标仪、流式细胞仪、凝胶成像系统均购自ThermoFisher Scientific公司。

1.2 方法

1.2.1 细胞分组与处理

将Ht22细胞培养于DEME培养基(含有10% FBS和1%青/链霉素双抗)中，置于恒温细胞培养箱(37 ℃、5% CO₂)中培养至对数期，利用脂质体转染法转染对应核酸序列至Ht22细胞中，分别记为阴性对照组(si-NC组)、Cir-

cOGDH沉默组 (si-CircOGDH组)、过表达阴性对照组 (miR-NC组)、miR-24-3p过表达组 (miR-24-3p minic组)、CircOGDH沉默+抑制剂阴性对照组 (si-CircOGDH+anti-miR-NC组)、CircOGDH沉默+miR-24-3p抑制剂组 (si-CircOGDH+anti-miR-24-3p组),另设置未转染的对照组 (control组)、缺氧复氧 (H/R组)。除control组外,其余各组Ht22细胞均建立H/R模型:将细胞用无FBS、无糖的DEME培养基培养2 h后,转移至缺氧培养基中(含有94% N_2、5% CO_2、1% O_2)培养6 h,再次更换培养基为正常的DEME培养基继续培养24 h、48 h、72 h。实验设计见表1。

1.2.2 qRT-PCR法检测 CircOGDH、miR-24-3p、*HOXA1* mRNA表达 收集1.2.1培养24 h的细胞,加

入TRIzol试剂提取总RNA,用反转录试剂盒将其逆转录为cDNA,然后进行PCR扩增,反应条件:95 ℃预变性5 min;94 ℃变性10 s,65 ℃退火50 s,70 ℃延伸2 min,共38个循环。引物设计见表2。CircOGDH、miR-24-3p以*U6*为内参,*HOXA1*以*GAPDH*为内参。mRNA相对表达量用$2^{-\Delta\Delta Ct}$法计算。引物序列见表2。

1.2.3 CCK-8法检测细胞活性 在1.2.1培养的细胞中,分别在24 h、48 h、72 h时,根据CCK-8试剂盒说明书操作,加入CCK-8溶液,继续培养2 h后,在波长为450 nm下读取D_{450}值,根据D_{450}值计算细胞存活率。

1.2.4 细胞氧化应激水平检测 收集1.2.1培养48 h的细胞,LDH水平检测:取细胞培养液,3 000 r/min离心

表1 实验设计
Table 1 Experimental design

实验 Experiment	分组 Groups	检测指标 Detection index
Experiment 1	Control group, H/R group	Detection of CircOGDH, miR-24-3p and *HOXA1* mRNA expression by qRT-PCR (experiment was repeated for 3 times)
Experiment 2	Control group, H/R group, si-NC group, si-CircOGDH group	mRNA expression of CircOGDH, miR-24-3p and *HOXA1* was detected by qRT-PCR; cell activity, oxidative stress and apoptosis were detected by CCK-8; protein expression was detected by Western blot (experiment was repeated for 3 times)
Experiment 3	Control group, H/R group, miR-NC group, miR-24-3p minic group	mRNA expression of CircOGDH, miR-24-3p and *HOXA1* was detected by qRT-PCR; cell activity, oxidative stress and apoptosis were detected by CCK-8; protein expression was detected by Western blot (experiment was repeated for 3 times)
Experiment 4	si-CircOGDH+anti-miR-NC group, si-CircOGDH+anti-miR-24-3p group	mRNA expression of CircOGDH, miR-24-3p and *HOXA1* was detected by qRT-PCR; cell activity, oxidative stress and apoptosis were detected by CCK-8; protein expression was detected by Western blot (experiment was repeated for 3 times)

表2 引物设计
Table 2 Primer design

引物名称 Primer name	引物序列 Primer sequence
CircOGDH	Forward primer: 5′-ATC AGA TAC GAG GGC ACC ATG T-3′
	Reverse primer: 5′-GGC TCC GGC ATT CGT GTT G-3′
miR-24-3p	Forward primer: 5′-TGG CTC AGT TCA GCA GGA ACA-3′
	Reverse primer: 5′-GAT CCA GTC TCA GGG TCC GAG-3′
U6	Forward primer: 5′-CTC GCT TCG GCA GCA CA-3′
	Reverse primer: 5′-AAC GCT TCA CGA ATT TGC GT-3′
HOXA1	Forward primer: 5′-CGG CTT CCT GTG CTA AGT CT-3′
	Reverse primer: 5′-TTC ATT GTG CCA TCC ATC AC-3′
GAPDH	Forward primer: 5′-CTG GGC TAC ACT GAG CAC C-3′
	Reverse primer: 5′-AGT GGT CGT TGA GGG CAA TG-3′

10 min保留上清, 根据LDH试剂盒检测其水平; SOD、MDA、GSH-Px水平检测: 去除细胞培养基, 加入细胞裂解液, 12 000 r/min离心5 min取上清, 根据SOD、MDA、GSH-Px各自对应的试剂盒检测其水平。

1.2.5 流式细胞仪检测细胞凋亡 收集1.2.1培养24 h的细胞, 使用不含EDTA的胰酶在37 °C下消化细胞3 min, 1 000 r/min离心5 min后弃去上清, PBS清洗后, 再次离心(1 000 r/min、5 min), 加入100 μL结合缓冲液, 混匀后分别加入Annexin V-FITC和PI, 各5 μL, 混匀, 室温下避光孵育10 min, 收集细胞至流式管中, 待流式细胞仪检测。

1.2.6 Western blot检测蛋白表达 收集1.2.1培养24 h的细胞, 加入PIPA提取总蛋白, BC试剂盒定量蛋白后, 取30 μg蛋白上样然后进行电泳, 随后转膜至PVDF, 5%脱脂牛奶室温封闭2 h, 洗膜后加入一抗HOXA1(1:1 000)、Bax(1:1 000)、Bcl-2(1:1 000), 以GAPDH(1:1 000)为内参, 4 °C孵育过夜后, 洗膜加入二抗(1:2 000), 室温下继续孵育2 h后, 加入ECL显色, 利用蛋白条带灰度值计算目的蛋白表达量。实验重复3次。

1.2.7 双荧光素酶报告基因实验 根据miR-24-3p分别与CircOGDH和HOXA1的结合位点, 分别构建CircOGDH和HOXA1的野生型(WT)及突变型(MUT)质粒, 将其分别与miR-NC、miR-24-3p minic共转染至Ht22细胞。转染海肾质粒为内参, 转染48 h后用双荧光素酶报告基因试剂盒检测荧光素酶活性。

1.3 统计分析

实验重复3次。采用SPSS 26.0进行数据分析, 所有计算数据用平均值±标准差($\bar{x}\pm s$)表示, 多组间比较采用单因素方差分析, 两两间比较采用SNK-q检验, $P<0.05$为差异有统计学意义。

2 结果

2.1 各组Ht22细胞中CircOGDH、miR-24-3p、HOXA1 mRNA

如图1所示, 与control组比较, H/R组Ht22细胞中CircOGDH、HOXA1 mRNA表达量显著增加, miR-24-3p表达量显著下降($P<0.05$)。

### 2.2 沉默CircOGDH对各组Ht22细胞miR-24-3p、HOXA1表达的影响

如图2所示, 与H/R组、si-NC组比较, si-CircOGDH组CircOGDH、HOXA1 mRNA和HOXA1蛋白表达水平显著降低, miR-24-3p表达水平显著升高($P<0.05$)。

2.3 沉默CircOGDH对各组Ht22细胞损伤的影响

如图3、图4、表3、表4所示, 与control组比较, H/R组HT22细胞凋亡率、促凋亡蛋白Bax水平、LDH含量、MDA含量显著升高, 细胞存活率、抗凋亡蛋白Bcl-2水平、SOD活性和GSH-Px活性显著下降($P<0.05$); 与H/R组、si-NC组比较, si-CircOGDH组HT22细胞凋亡率、促凋亡蛋白Bax水平、LDH含量、MDA含量显著下降, 细胞存活率、抗凋亡蛋白Bcl-2水平、SOD活性和GSH-Px活性显著升高($P<0.05$)。

2.4 过表达miR-24-3p对各组Ht22细胞损伤及HOXA1表达的影响

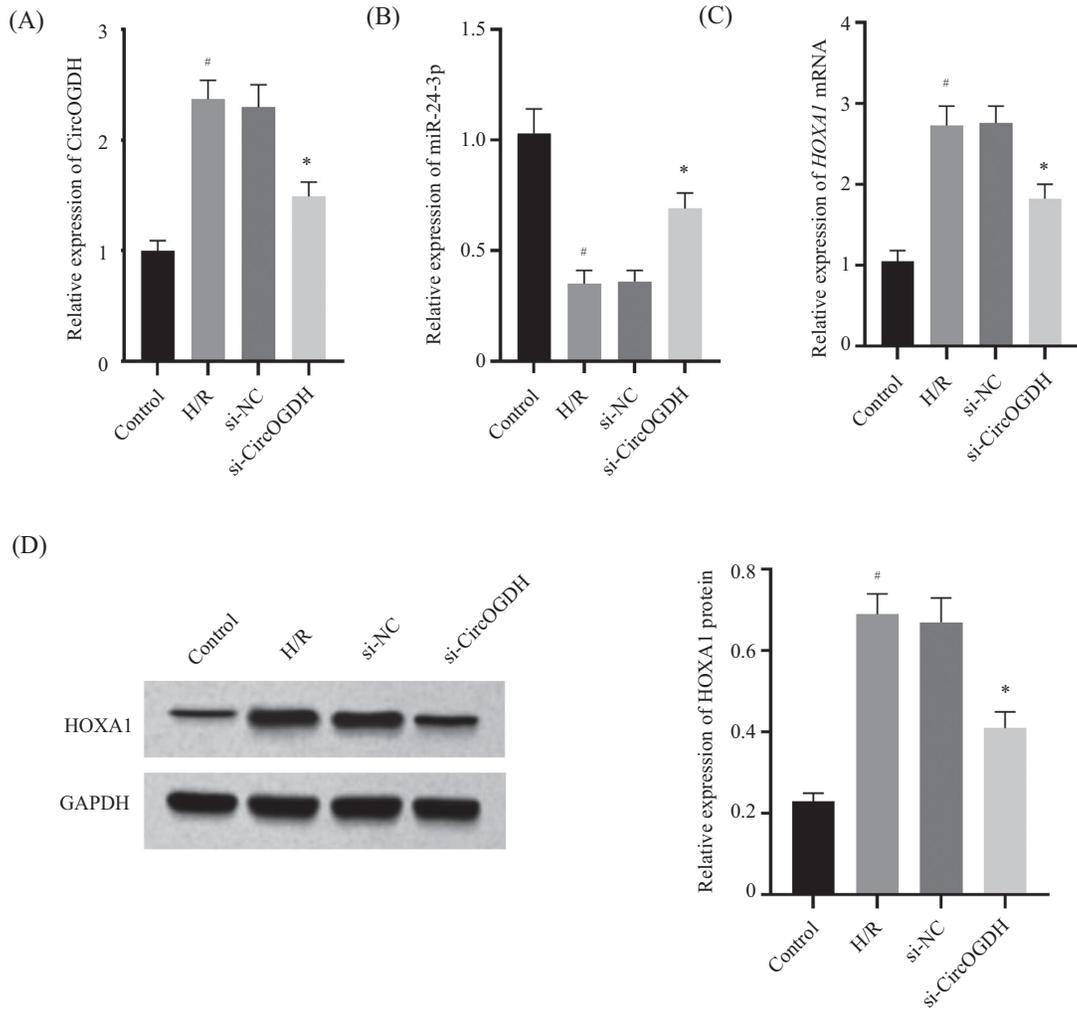如图5、图6、表5和表6所示, 与control组比较, H/R组HT22细胞HOXA1 mRNA及HOXA1蛋白表
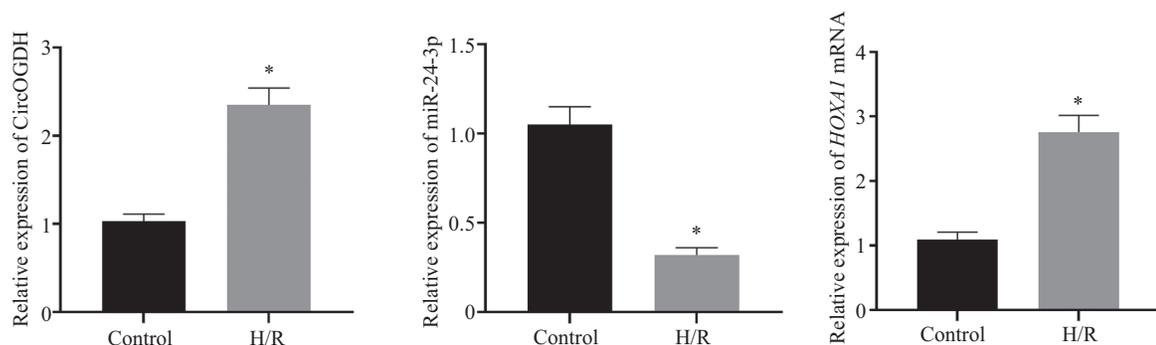
*$P<0.05$, 与control组比较。$n=6$。
*$P<0.05$ compared with control group. $n=6$.

图1 各组Ht22细胞CircOGDH、miR-24-3p、HOXA1 mRNA比较
Fig.1 Comparison of CircOGDH, miR-24-3p, and HOXA1 mRNA in Ht22 cells of each group

A: CircOGDH相对表达量; B: miR-24-3p相对表达量; C: *HOXA1* mRNA相对表达量; D: HOXA1蛋白表达条带图和HOXA1蛋白相对表达量;
#*P*<0.05, 与control组比较; **P*<0.05, 与si-NC组比较。*n*=6。

A: relative expression of CircOGDH; B: relative expression level of miR-24-3p; C: relative expression level of *HOXA1* mRNA; D: HOXA1 protein expression band map and HOXA1 protein relative expression level; #*P*<0.05 compared with control group; **P*<0.05 compared with si-NC group. *n*=6.

图2 各组Ht22细胞CircOGDH、miR-24-3p、HOXA1表达比较

Fig.2 Comparison of CircOGDH, miR-24-3p and HOXA1 expression in Ht22 cells of each group
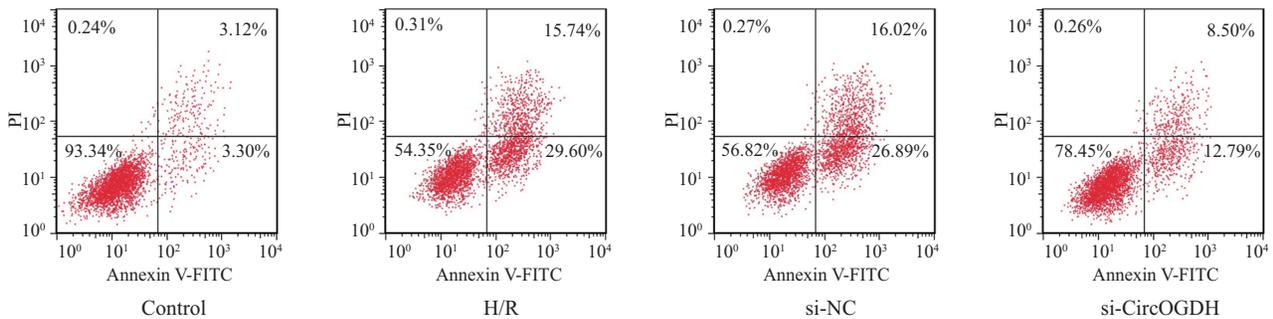

图3 各组Ht22细胞凋亡情况

Fig.3 Apoptosis of Ht22 cells in each group

达水平显著升高(*P*<0.05); 与H/R组、miR-NC组比较, miR-24-3p minic组HT22细胞凋亡率、Bax蛋白表达水平、LDH含量、MDA含量、*HOXA1* mRNA 和HOXA1蛋白表达水平显著下降, miR-24-3p表达水平、细胞存活率、Bcl-2蛋白表达水平、SOD活性和GSH-Px活性显著升高(*P*<0.05)。
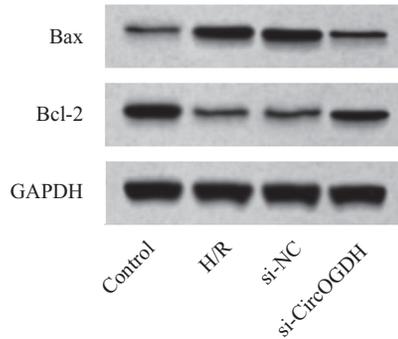
图4 各组Ht22细胞中Bax、Bcl-2蛋白条带

Fig.4 Bax and Bcl-2 protein bands in Ht22 cells of each group

表3 各组Ht22细胞存活与凋亡情况比较

Table 3 Comparison of survival and apoptosis of Ht22 cells in each group

组别 Groups	存活率/% Survival rate /%	凋亡率/% Apoptosis /%	Bax/GAPDH	Bcl-2/GAPDH
Control	98.34±6.31	6.34±0.71	0.15±0.03	0.87±0.07
H/R	54.23±5.17[#]	45.30±3.86[#]	0.62±0.06[#]	0.25±0.03[#]
si-NC	56.18±5.69	43.01±4.05	0.64±0.05	0.27±0.03
si-CircOGDH	76.36±6.60*	21.32±2.57*	0.26±0.02*	0.55±0.04*

[#]$P<0.05$, 与control组比较; *$P<0.05$, 与si-NC组比较。

[#]$P<0.05$ compared with control group; *$P<0.05$ compared with si-NC group.

表4 各组Ht22细胞氧化损伤指标比较

Table 4 Comparison of oxidative damage indicators of Ht22 cells in each group

组别 Groups	LDH /U·L^{-1}	SOD /U·mg^{-1}	MDA /nmol·mg^{-1}	GSH-Px /U·mg^{-1}
Control	96.32±8.34	55.23±4.71	0.89±0.05	75.46±6.87
H/R	323.25±21.02[#]	18.32±1.99[#]	6.60±0.33[#]	20.33±2.65[#]
si-NC	330.55±23.41	17.76±1.80	6.23±0.25	21.34±2.03
si-CircOGDH	202.35±20.38*	38.16±3.02*	2.14±0.12*	59.82±4.66*

[#]$P<0.05$, 与control组比较, [#]$P<0.05$; *$P<0.05$, 与si-NC组比较。

[#]$P<0.05$ compared with control group; *$P<0.05$ compared with si-NC group.
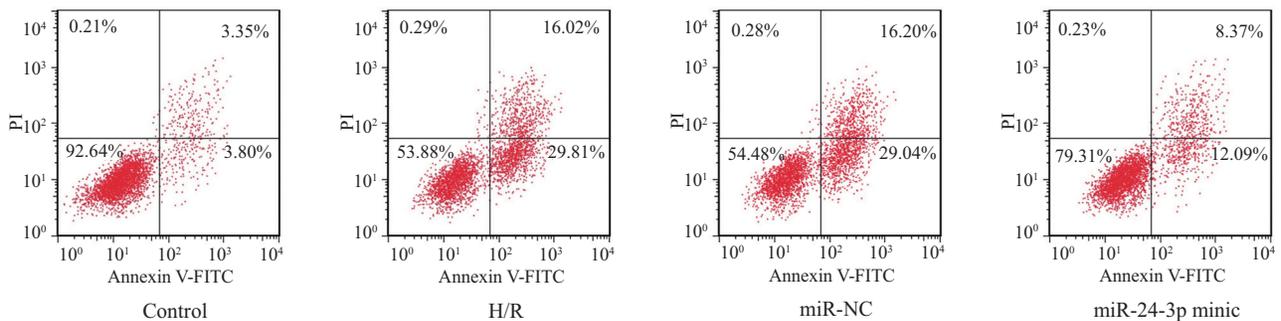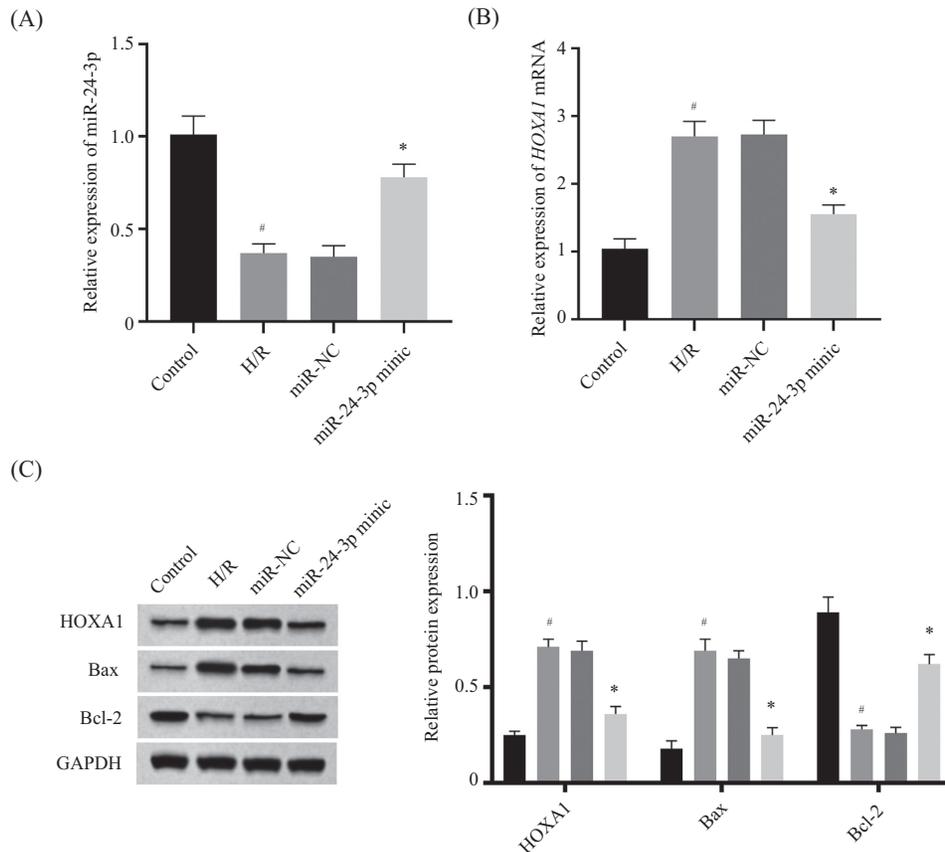

图5 各组Ht22细胞凋亡情况

Fig.5 Apoptosis of Ht22 cells in each group

2.5 抑制miR-24-3p会逆转si-CircOGDH对HT22细胞损伤的保护

如图7、图8、表7和表8所示, 与si-CircOGDH+anti-miR-NC组比较, si-CircOGDH+anti-miR-24-3p组中细胞存活率和Bcl-2蛋白表达量、miR-24-3p表达量、SOD活性和GSH-Px活性降低, 细胞凋亡率和

A: miR-24-3p相对表达量; B: *HOXA1* mRNA相对表达量; C: HOXA1、Bax、Bcl-2蛋白表达条带图和蛋白相对表达量; #*P*<0.05, 与control组比较; *P<0.05, 与miR-NC组比较。

A: relative expression level of miR-24-3p; B: *HOXA1* mRNA relative expression level; C: HOXA1, Bax, Bcl-2 protein expression band map and protein relative expression levels; #*P*<0.05 compared with control group; *P<0.05 compared with miR-NC group.

图6 各组Ht22细胞miR-24-3p、HOXA1、Bax、Bcl-2表达比较

Fig.6 Comparison of miR-24-3p, HOXA1, Bax, and Bcl-2 expression in Ht22 cells of each group

表5 各组Ht22细胞存活与凋亡情况比较

Table 5 Comparison of survival and apoptosis of Ht22 cells in each group

组别 Groups	存活率/% Survival rate /%	凋亡率/% Apoptosis /%	Bax/GAPDH	Bcl-2/GAPDH
Control	97.60±7.31	7.34±0.68	0.18±0.04	0.89±0.08
H/R	53.23±6.17#	45.78±3.76#	0.69±0.06#	0.28±0.02#
miR-NC	54.18±6.00	45.01±4.25	0.65±0.04	0.26±0.03
miR-24-3p minic	75.36±6.78*	20.38±3.01*	0.25±0.03*	0.62±0.05*

#*P*<0.05, 与control组比较; *P<0.05, 与miR-NC组比较。

#*P*<0.05 compared with control group; *P<0.05 compared with miR-NC group.

表6 各组Ht22细胞氧化损伤指标比较

Table 6 Comparison of oxidative damage indicators of Ht22 cells in each group

组别 Groups	LDH /U·L⁻¹	SOD /U·mg⁻¹	MDA /nmol·mg⁻¹	GSH-Px /U·mg⁻¹
Control	97.32±8.67	56.23±4.71	0.89±0.06	78.46±6.27
H/R	330.25±22.02#	19.32±1.79#	6.70±0.35#	21.33±2.15#
miR-NC	335.55±25.62	18.76±1.91	6.45±0.22	21.78±2.10
miR-24-3p minic	193.35±20.38*	40.13±3.05*	2.35±0.13*	61.12±4.76*

#*P*<0.05, 与control组比较; *P<0.05, 与miR-NC组比较。

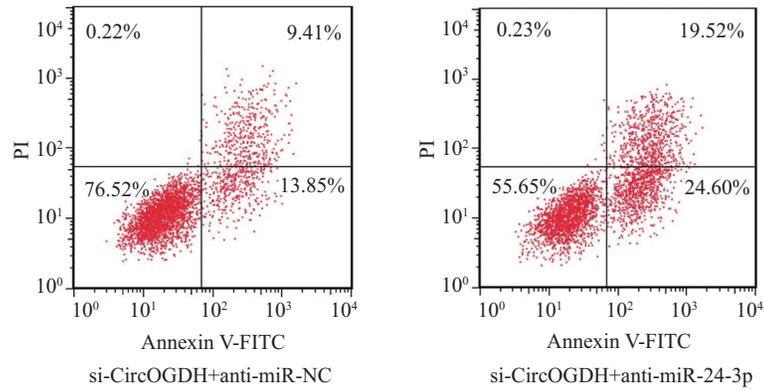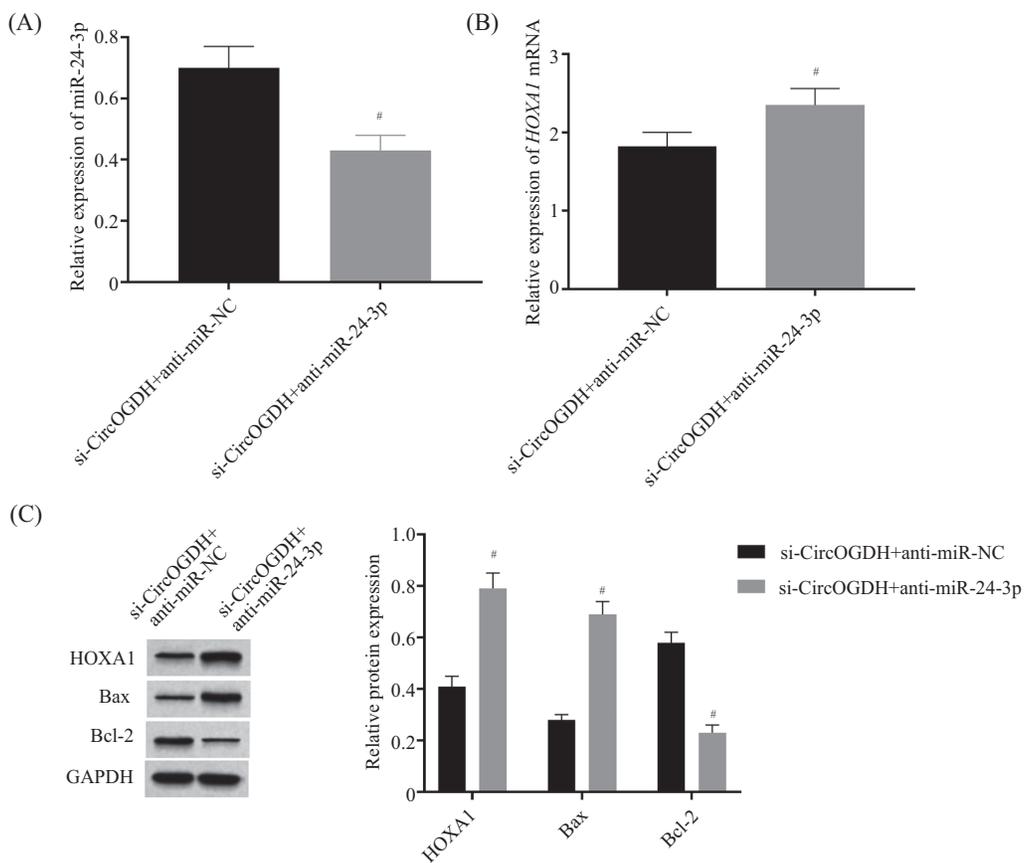#*P*<0.05 compared with control group; *P<0.05 compared with miR-NC group.

图7 各组Ht22细胞凋亡情况

Fig.7 Apoptosis of Ht22 cells in each group

A: miR-24-3p相对表达量; B: *HOXA1* mRNA相对表达量; C: HOXA1、Bax、Bcl-2蛋白表达条带图和蛋白相对表达量; [#]P<0.05, 与si-CircOGDH+anti-miR-NC组比较。n=6。

A: relative expression level of miR-24-3p; B: *HOXA1* mRNA relative expression level; C: HOXA1, Bax, Bcl-2 protein expression band map and protein relative expression levels; [#]P<0.05 compared with si-CircOGDH+anti-miR-NC group. n=6.

图8 各组Ht22细胞miR-24-3p、HOXA1、Bax、Bcl-2比较

Fig.8 Comparison of miR-24-3p, HOXA1, Bax and Bcl-2 in Ht22 cells of each group

Bax蛋白水平、*HOXA1* mRNA水平、HOXA1蛋白表达水平、LDH含量、MDA含量升高(P<0.05)。

2.6 miR-24-3p与CircOGDH、miR-24-3p与*HOXA1*的靶向关系验证

如图9、图10所示, 通过TargetScan数据库推测

miR-24-3p与CircOGDH、miR-24-3p与*HOXA1*存在结合位点; 如表9所示, miR-24-3p minic与CircOGDH-WT及*HOXA1*-WT共转染组的细胞荧光素酶活性显著低于miR-NC与CircOGDH-WT及*HOXA1*-WT共转染组 (P<0.05); miR-24-3p minic、miR-NC与

表7 各组Ht22细胞存活与凋亡情况比较
Table 7 Comparison of survival and apoptosis of Ht22 cells in each group

组别 Groups	存活率/% Survival rate /%	凋亡率/% Apoptosis /%	Bax/GAPDH	Bcl-2/GAPDH
si-CircOGDH+ anti-miR-NC	75.36±6.50	23.32±2.67	0.28±0.02	0.58±0.04
si-CircOGDH+ anti-miR-24-3p	56.45±5.26[#]	44.03±3.98[#]	0.69±0.05[#]	0.23±0.03[#]

[#]$P<0.05$, 与si-CircOGDH+anti-miR-NC组比较。

[#]$P<0.05$ compared with si-CircOGDH+anti-miR-NC group.

表8 各组Ht22细胞氧化损伤比较
Table 8 Comparison of oxidative damage of Ht22 cells in each group

组别 Groups	LDH /U·L^{-1}	SOD /U·mg^{-1}	MDA /nmol·mg^{-1}	GSH-Px /U·mg^{-1}
si-CircOGDH+anti-miR-NC	205.35±21.38	39.26±3.08	2.48±0.19	57.42±4.03
si-CircOGDH+anti-miR-24-3p	251.32±20.35[#]	25.48±2.12[#]	4.89±0.23[#]	32.25±3.01[#]

[#]$P<0.05$, 与si-CircOGDH+anti-miR-NC组比较。

[#]$P<0.05$ compared with si-CircOGDH+anti-miR-NC group.

```
CircOGDH   5′ CUGCACCUGUUGCUGCUGAGCCC 3′
              | | | | : | |    : | | | | | | |
miR-24-3p  3′ GACAAGGACGAC- UUGACUCGGU 5′
```

图9 miR-24-3p与CircOGDH 3′UTR结合位点
Fig.9 miR-24-3p and CircOGDH 3′UTR binding sites

```
HOXA1      5′ AUCAGUUAUCCUUUGUGAGCCU 3′
              | | | | | |
miR-24-3p  3′ GACAAGGACGACUUGACUCGGU 5′
```
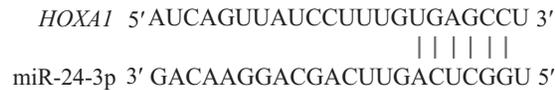
图10 miR-24-3p与HOXA1 3′UTR结合位点
Fig.10 miR-24-3p and HOXA1 3′UTR binding sites

表9 双荧光素酶报告实验
Table 9 Report experiment of double Luciferase

组别 Groups	荧光素酶活性 Luciferase activity			
	CircOGDH-WT	CircOGDH-MUT	HOXA1-WT	HOXA1-MUT
miR-NC	1.05±0.12	1.02±0.10	1.03±0.09	1.00±0.10
miR-24-3p minic	0.58±0.06*	0.97±0.08	0.44±0.06*	0.98±0.08

*$P<0.05$, 与miR-NC组比较。

*$P<0.05$ compared with miR-NC group.

CircOGDH-MUT及HOXA1-MUT共转染组的细胞荧光素酶活性无显著差异($P>0.05$)。

3 讨论

缺血性中风是最具破坏性的神经系统疾病,治疗过程中缺血再灌注会产生大量活性氧,消耗抗氧化物质,造成氧化应激,引起细胞凋亡,加重脑组织损伤[8-9]。CircRNA属于一类新型的非编码RNA,由基因外显子通过替代mRNA剪接产生,可通过调节神经炎症、氧化应激和细胞凋亡参与缺血性中风的发生与发展,可作为缺血性中风诊断和预后生物标志物[10-12]。本研究发现,CircOGDH在H/R诱导的Ht22损伤细胞中高表达,并且沉默CircOGDH表达可以改善H/R诱导的Ht22细胞损伤。CircRNA和miRNA是

密切相关的, CircRNA通常靶向miRNA参与疾病的病理生理过程[13]。许多药物在对疾病发挥作用时, miRNA表达也发生了变化, 药物靶向治疗越来越成为热点, 如山楂叶总黄酮通过调控miR-133b改善缺氧复氧诱导的神经细胞损伤[14], 而黄芩苷通过上调miR-190表达缓解缺氧缺糖对神经细胞的损伤[15]。研究发现miR-24-3p可以从减轻氧化应激、抑制心肌细胞凋亡角度, 来减弱阿霉素诱导的小鼠心脏毒性[16]。miR-24-3p还可以抑制脂质积累和活性氧产生, 从而改善非酒精性脂肪肝疾病[17]。在氧糖剥夺/复氧诱导的受损神经元中miR-24-3p呈低表达, 上调miR-24-3p表达可抑制氧化应激和神经元凋亡[6]。基于CircOGDH和HOXA1在细胞中的作用, 以及二者均与miR-24-3p存在靶向关系, 我们经过研究发现CircOGDH和HOXA1在H/R诱导的Ht22细胞中均上调, miR-24-3p下调, 沉默CircOGDH可以促进miR-24-3p表达上调, 负向调控HOXA1表达, 改善Ht22细胞损伤。

有研究证明miRNA参与了缺血性脑卒中的氧化应激、细胞凋亡、再灌注损伤等病理生理过程, 如miR-142a-5p能够通过靶向MFN1诱导线粒体功能障碍、线粒体自噬和细胞凋亡[18], 而miR-24-3p可以靶向STAT3, 抑制氧化应激和细胞凋亡, 减轻神经元损伤, 改善缺血性中风[19]。先前已有研究表明miR-24-3p在脑缺血再灌注损伤细胞中低表达[20]。在本研究中, miR-24-3p在H/R诱导的Ht22细胞中低表达, 而沉默CircOGDH可以促进miR-24-3p表达, 减轻Ht22细胞损伤, 抑制miR-24-3p表达则会一定程度逆转沉默CircOGDH对H/R诱导的Ht22细胞损伤的保护作用。这说明CircOGDH可以调控miR-24-3p表达, 逆转H/R诱导的Ht22细胞损伤。

在一项关于氧化应激对于干细胞维持和神经元分化的实验中发现, 氧化剂可提高活性氧的水平, 增强HOXA1等神经元分化标志物的自发表达能力, 加入抗氧化剂GSH, 均可以显著抑制HOXA1的表达和活性氧的产生[21]。在本研究中, HOXA1在H/R诱导的Ht22损伤细胞中高表达, 同时过氧化产物MDA、LDH水平上升, 抗氧化物质SOD和GSH-Px活性下降, 沉默CircOGDH或者过表达miR-24-3p均可以抑制HOXA1表达, 促进抗氧化物质生成, 抑制氧化应激。双荧光素酶报告实验显示miR-24-3p可以与 *HOXA1* 3′UTR结合, 负调控HOXA1表达; miR-24-3p

与CircOGDH 3′UTR也有结合位点, 沉默CircOGDH可以促进miR-24-3p表达, 下调HOXA1, 与本研究中对于miR-24-3p、HOXA1表达水平的检测结果相符合。

miR-24-3p也与细胞凋亡的生理过程相关, 在急性淋巴细胞白血病中, 小檗碱通过上调miR-24-3p表达, 促进癌细胞凋亡[22]。miR-24-3p还可以促进BPNT1产生, 从而减弱紫外线照射诱导的晶状体上皮细胞凋亡, 缓解白内障疾病[23]。研究发现HOXA1在细胞凋亡中也扮演重要角色, 它在骨关节炎软骨细胞中高表达, miR-18a-3p靶向 *HOXA1*, 沉默HOXA1可以抑制软骨细胞凋亡[24]。此外, HOXA1可通过调控PI3K/AKT/mTOR信号通路影响口腔鳞状细胞癌细胞凋亡[25]。在本研究中, miR-24-3p可以靶向 *HOXA1*, 负调控HOXA1表达, 抑制Ht22细胞凋亡。沉默CircOGDH或者过表达miR-24-3p, 都可以抑制HOXA1表达, 从而抑制细胞凋亡。这提示沉默CircOGDH可能通过上调miR-24-3p表达, 下调HOXA1表达, 逆转H/R诱导的神经元氧化损伤, 抑制其凋亡。

综上所述, 沉默CircOGDH或者过表达miR-24-3p, 可以抑制HOXA1表达, 从而抑制H/R诱导的Ht22细胞氧化应激损伤和细胞凋亡。缺乏人体数据和动物实验是本研究的不足, 下一步需要进一步研究动物实验中CircOGDH、miR-24-3p、HOXA1三者的表达和调节作用, 为临床脑缺血损伤疾病提供更多可能的生物靶标。此外, PI3K/AKT/mTOR信号通路是调节缺血缺氧诱导的神经元氧化应激损伤和凋亡的重要机制[26], HOXA1是否能通过PI3K/AKT/mTOR信号通路参与神经元损伤仍有待进一步分析。

参考文献 (References)

[1] GRECO P, NENCINI G, PIVA I, et al. Pathophysiology of hypoxic-ischemic encephalopathy: a review of the past and a view on the future [J]. Acta Neurol Belg, 2020, 120(2): 277-88.

[2] LI C, SUN T, JIANG C. Recent advances in nanomedicines for the treatment of ischemic stroke [J]. Acta Pharm Sin B, 2021, 11(7): 1767-88.

[3] LIU Y, LI Y, ZANG J, et al. CircOGDH is a penumbra biomarker and therapeutic target in acute ischemic stroke [J]. Circ Res, 2022, 130(6): 907-24.

[4] CAN U, MARZIOGLU E, AKDU S. Some miRNA expressions and their targets in ischemic stroke [J]. Nucleosides Nucleotides Nucleic Acids, 2022, 41(11): 1224-62.

[5] GUGLIANDOLO A, SILVESTRO S, SINDONA C, et al. miRNA: involvement of the MAPK pathway in ischemic stroke. A

promising therapeutic target [J]. Medicina, 2021, 57(10): 1053.

[6] DI G, YANG X, CHENG F, et al. CEBPA-AS1 knockdown alleviates oxygen-glucose deprivation/reperfusion-induced neuron cell damage by the microRNA 24-3p/BOK axis [J]. Mol Cell Biol, 2021, 41(8): e0006521.

[7] ZHAO J, HE L, YIN L. lncRNA NEAT1 binds to miR-339-5p to increase HOXA1 and alleviate ischemic brain damage in neonatal mice [J]. Mol Ther Nucleic Acids, 2020, 20: 117-27.

[8] HUANG L, LI X, LIU Y, et al. Curcumin alleviates cerebral ischemia-reperfusion injury by inhibiting NLRP1-dependent neuronal pyroptosis [J]. Curr Neurovasc Res, 2021, 18(2): 189-96.

[9] ORELLANA-URZÚA S, ROJAS I, LÍBANO L, et al. Pathophysiology of ischemic stroke: role of oxidative stress [J]. Curr Pharm Des, 2020, 26(34): 4246-60.

[10] LIU X, WANG Q, ZHAO J, et al. Inflammation-related circRNA polymorphism and ischemic stroke prognosis [J]. J Mol Neurosci, 2021,71(10): 2126-33.

[11] HU X, MA R, CAO J, et al. CircSAMD4A aggravates H/R-induced cardiomyocyte apoptosis and inflammatory response by sponging miR-138-5p [J]. J Cell Mol Med, 2022, 26(6): 1776-84.

[12] ZHOU D, HUANG Z, ZHU X, et al. Circular RNA 0025984 ameliorates ischemic stroke injury and protects astrocytes through miR-143-3p/TET1/ORP150 pathway [J]. Mol Neurobiol, 2021, 58(11): 5937-53.

[13] KIM K W, KIM S W, LIM S, et al. Neutralization of hexokinase 2-targeting miRNA attenuates the oxidative stress-induced cardiomyocyte apoptosis [J]. Clin Hemorheol Microcirc, 2021, 78(1): 57-68.

[14] 李丽静, 王领弟, 吴晓光. 山楂叶总黄酮通过调控miR-133b改善缺氧复氧诱导的神经细胞损伤[J]. 中成药(LI L J, WANG L D, WU X G. Total flavonoids of Hawthorn leaf can improve nerve cell injury induced by hypoxia and reoxygenation by regulating miR-133b [J]. Chin Tradit Pat Med), 2020, 42(6): 1443-9.

[15] 吴艳荣, 刘光伟, 刘全忠, 等. 黄芩苷通过上调miR-190表达缓解缺氧缺糖对神经细胞损伤的研究[J]. 中草药(WU Y R, LIU G W, LIU Q Z, et al. Study on the effects of baicalin on hypoxic and glucose-deficient nerve cells by up-regulating the expression of miR-190 [J]. Chin Tradit Herb Drugs), 2021, 52(10): 3009-17.

[16] FAN D, CHEN H B, LENG Y, et al. MiR-24-3p attenuates doxorubicin-induced cardiotoxicity via the Nrf2 pathway in mice [J]. Curr Med Sci, 2022, 42(1): 48-55.

[17] DU X, LI H, HAN X, et al. Mesenchymal stem cells-derived exosomal miR-24-3p ameliorates non-alcohol fatty liver disease by targeting Keap-1 [J]. Biochem Biophys Res Commun, 2022, 637: 331-40.

[18] YANG X, XUE P, CHEN H, et al. Denervation drives skeletal muscle atrophy and induces mitochondrial dysfunction, mitophagy and apoptosis via miR-142a-5p/MFN1 axis [J]. Theranostics, 2020, 10(3): 1415-32.

[19] CHEN Z, FAN T, ZHAO X, et al. Depleting SOX2 improves ischemic stroke via lncRNA PVT1/microRNA-24-3p/STAT3 axis [J]. Mol Med, 2021, 27(1): 107.

[20] KUAI F, ZHOU L, ZHOU J, et al. Long non-coding RNA THRIL inhibits miRNA-24-3p to upregulate neuropilin-1 to aggravate cerebral ischemia-reperfusion injury through regulating the nuclear factor κB p65 signaling [J]. Aging, 2021, 13(6): 9071-84.

[21] HU Q, KHANNA P, EE WONG B S, et al. Oxidative stress promotes exit from the stem cell state and spontaneous neuronal differentiation [J]. Oncotarget, 2017, 9(3): 4223-38.

[22] LIU J, CHEN Z, CUI Y, et al. Berberine promotes XIAP-mediated cells apoptosis by upregulation of miR-24-3p in acute lymphoblastic leukemia [J]. Aging, 2020, 12(4): 3298-311.

[23] ZHOU C, HUANG X, LI X, et al. Circular RNA erythrocyte membrane protein band 4.1 assuages ultraviolet irradiation-induced apoptosis of lens epithelial cells by stimulating 5'-bisphosphate nucleotidase 1 in a miR-24-3p-dependent manner [J]. Bioengineered, 2021, 12(1): 8953-64.

[24] DING B, XU S, SUN X, et al. miR-18a-3p encourages apoptosis of chondrocyte in osteoarthritis via HOXA1 pathway [J]. Curr Mol Pharmacol, 2020, 13(4): 328-41.

[25] 卜一, 张硕, 钱旭东, 等. 基于PI3K/AKT/mTOR信号通路探讨半枝莲总黄酮对脑缺血再灌注损伤大鼠神经功能和氧化应激损伤的影响[J]. 现代生物医学进展(BU Y, ZHANG S, QIAN X D, et al. Effects of total flavonoids of Machidium chinensis on neural function and oxidative stress injury in rats with cerebral ischemia reperfusion injury based on PI3K/AKT/mTOR signaling pathway [J]. Advances in Modern Biomedicine), 2022, 22(18): 3440-6.

[26] 郭佳, 冯素亚, 路学文. HOXA1通过调控PI3K/AKT/mTOR信号通路影响口腔鳞状细胞癌细胞增殖、凋亡及侵袭能力[J]. 东南大学学报(医学版)(GUO J, FENG S Y, LU X W. HOXA1 affects the proliferation, apoptosis and invasion ability of oral squamous cell carcinoma cells by regulating PI3K/AKT/mTOR signaling pathway [J]. J Southeast Univ, Med Sci Ed), 2020, 39(2): 203-7.