

doi: 10.13241/j.cnki.pmb.2024.23.002

利拉鲁肽改善 SH-SY5Y 细胞氧化应激、线粒体功能障碍、自噬损伤的机制研究*

路瑶 杨晶晶 万尖尖 马雪莲 赵子暄

(新疆医科大学第二附属医院内分泌科 新疆 乌鲁木齐 830000)

摘要 目的: 探讨利拉鲁肽(Lira)干预人神经母细胞瘤(SH-SY5Y)细胞对氧化应激、线粒体功能及自噬损伤的机制。**方法:** 将 SH-SY5Y 细胞分为对照组、高糖(HG)组、HG+LI-L 组、HG+LI-H 组、HG+LI-H+3-甲基腺嘌呤(3MA)组。检测细胞增殖能力、Glu、丙二醛(MDA)、三磷酸腺苷(ATP)和超氧化物歧化酶(SOD)水平、细胞凋亡、活性氧(ROS)水平和线粒体膜电位、微管相关蛋白 1 轻链 3(LC3B)和 p62 蛋白表达及定位、磷脂酰肌醇 3 激酶(PI3K)、p-PI3K、哺乳动物雷帕霉素靶蛋白(mTOR)、p-mTOR、蛋白激酶 B(Akt)、p-Akt 蛋白表达。**结果:** 与 HG 组比较, HG+LI(500 nM)组细胞增殖活力最为显著, HG+LI(500 nM)组 Glu 和 MDA 降低, SOD 升高, HG+LI-L 组和 HG+LI-H 组细胞存活率、ATP 和 SOD 升高, 细胞凋亡率、ROS 荧光强度、Glu、MDA 和 JC-1 下降, 且 HG+LI-H 组细胞存活率、ATP 和 SOD 高于 HG+LI-L 组, 细胞凋亡率、ROS 荧光强度、Glu、MDA、JC-1 低于 HG+LI-L 组, HG+LI-H 组 p-mTOR 和 p62 蛋白表达降低, p-PI3K、p-Akt 和 LC3B 蛋白表达升高($P<0.05$)。与对照组比较, HG 组 Glu、MDA、细胞凋亡率、细胞 ROS 荧光强度、Glu、MDA、JC-1、p-mTOR、p62 蛋白表达升高, SOD、ATP、细胞存活率、p-PI3K、p-Akt 和 LC3B 蛋白表达降低($P<0.05$)。与 HG+LI-H 组比较, HG+LI-H+3MA 组细胞存活率、ATP、SOD、p-PI3K、p-Akt 和 LC3B 蛋白表达下降, 细胞凋亡率、ROS 荧光强度、Glu、MDA、JC-1、p-mTOR、p62 蛋白表达升高($P<0.05$)。**结论:** Lira 可能是以剂量依赖的方式降低 SH-SY5Y 细胞内氧化应激, 改善线粒体功能和 PI3K/AKT/mTOR 自噬通路, 发挥抗凋亡和保护神经细胞作用。

关键词: 利拉鲁肽; 神经母细胞瘤; 氧化应激; 线粒体功能; 自噬

中图分类号: R-33; R587.2 文献标识码: A 文章编号: 1673-6273(2024)23-4408-06

Mechanism of Liraglutide Improving Oxidative Stress, Mitochondrial Dysfunction and Autophagy Damage in SH-SY5Y Cells*

LU Yao, YANG Jing-jing, WAN Jian-jian, MA Xue-lian, ZHAO Zi-xuan

(Department of Endocrinology, Second Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang, 830000, China)

ABSTRACT Objective: To investigate the mechanism of liraglutide (Lira) intervention on oxidative stress, mitochondrial function and autophagy damage in human neuroblastoma (SH-SY5Y) cells. **Methods:** SH-SY5Y cells were divided into control group, high glucose (HG) group, HG+LI-L group, HG+LI-H group, HG+LI-H+3-methyladenine (3MA) group. The cell proliferation ability, the levels of Glu, malondialdehyde (MDA), adenosine triphosphate (ATP), superoxide dismutase (SOD), Cell apoptosis, reactive oxygen species (ROS) level, mitochondrial membrane potential. The expression and localization of microtubule-associated protein 1 light chain 3 (LC3B), p62, The expression of phosphatidylinositol 3-kinase (PI3K), p-PI3K, mammalian rapamycin target protein (mTOR) p-mTOR, protein kinase B (Akt) and p-Akt protein were detected. **Results:** Compared with HG group, the cell proliferation activity in HG+LI (500 nM) group was the most significant, Glu and MDA decreased and SOD increased in HG+LI (500 nM) group, the cell viability, ATP and SOD were increased, and the apoptosis rate, ROS fluorescence intensity, Glu, MDA and JC-1 were decreased in HG+LI-L group and HG+LI-H group, and the cell viability, ATP and SOD in HG+LI-H group were higher than those in HG+LI-L group, and the apoptosis rate, ROS fluorescence intensity, Glu, MDA and JC-1 were lower than those in HG+LI-L group, p-mTOR and p62 proteins expression were decreased, p-PI3K, p-Akt and LC3B proteins expression were increased in the HG+LI-H group ($P<0.05$). Compared with control group, Glu, MDA, apoptosis rate, ROS fluorescence intensity, Glu, MDA, JC-1, P-mTOR and p62 protein expression in HG group increased, while SOD, ATP, cell survival rate, p-PI3K, p-Akt and LC3B protein expression decreased ($P<0.05$). Compared with HG+LI-H group, the cell viability, ATP, SOD, p-PI3K, p-Akt and LC3B protein expression were decreased, and the apoptosis rate, ROS fluorescence intensity, Glu, MDA JC-1, p-mTOR and p62 protein expression were increased in HG+LI-H+3MA group ($P<0.05$). **Conclusion:** Lira may reduce oxidative stress in SH-SY5Y cells in a dose-dependent manner, improve mitochondrial function and PI3K/AKT/mTOR autophagy pathway, Play a role in resist apoptosis and protect nerve cells.

* 基金项目: 省部共建中亚高发成因与防治国家重点实验室代谢病专项基金项目(SKL-HIDCA-2022-DX6);

新疆维吾尔自治区自然科学基金项目(2020D01C183)

作者简介: 路瑶(1980-), 女, 硕士, 副主任医师, 研究方向: 内分泌代谢性疾病, E-mail: Luyao_12345678@126.com

(收稿日期: 2024-06-18 接受日期: 2024-07-10)

Key words: Liraglutide; Human neuroblastoma; Oxidative stress; Mitochondrial dysfunction; Autophagy

Chinese Library Classification(CLC): R-33; R587.2 **Document code:** A

Article ID: 1673-6273(2024)23-4408-06

前言

糖尿病是常见的代谢性疾病之一,可并发认知功能障碍,与机体氧化应激、线粒体功能及自噬损伤有关^[1]。利拉鲁肽(Lira)是一种胰高血糖素样肽-1(GLP-1)类似物,是可用于治疗糖尿病患者的降糖药物,同时其对神经退行性疾病具有增强记忆和神经保护作用。动物实验发现,Lira可降低阿尔茨海默病小鼠模型 β -淀粉样蛋白低聚物水平和tau蛋白过度磷酸化,减轻学习记忆和突触可塑性受损,促进神经生长,Lira可降低2型糖尿病(T2DM)小鼠Morris水迷宫试验的逃避潜伏期,改善海马海马区受损的神经元形态,减少神经元和突触损伤^[2]。GLP-1类似物可以防止海马等神经元和人神经母细胞瘤(SH-SY5Y)细胞在缺氧、兴奋性毒性损伤、神经毒素和毒胡萝卜素诱导的内质网应激下的异常凋亡。一项临床试验表明,GLP-1类似物可持续改善帕金森病患者的认知和运动功能^[3]。上述研究表明Lira可有效介导多种组织或细胞在不同条件下恢复受损的神经元细胞和改善认知功能方面具有潜力。然而关于Lira治疗糖尿病性认知功能障碍的机制尚有待阐明。因此,本研究探讨Lira干预SH-SY5Y细胞对氧化应激、线粒体功能及自噬损伤的机制,旨在为预防和治疗糖尿病认知功能障碍提供临床研究证据。

1 材料与方法

1.1 细胞、试剂和仪器

SH-SY5Y细胞;Lira、3-甲基腺嘌呤(3MA);丙二醛(MDA)、超氧化物歧化酶(SOD)、活性氧(ROS)和葡萄糖(Glu)检测试剂盒、线粒体膜电位JC-1单体(JC-1);三磷酸腺苷(ATP)、膜联蛋白VPE/7AAD试剂盒;Anti-PI3 Kinase p85 alpha抗体、Anti-PI3 Kinase p85 alpha(phospho Y607)抗体、重组Anti-LC3B抗体、重组Anti-SQSTM1/p62抗体、Phospho-Akt(Ser473)抗体、重组Anti-mTOR抗体、重组Anti-mTOR(phospho S2448)抗体、山羊抗兔IgG H&L(Alexa Fluor® 488)、山羊抗鼠IgG H&L(Alexa Fluor® 594);Akt(pan)(C67E7)Rabbit mAb #4691;DMEM/F12培养基;Eclipse TS100-F荧光倒置显微镜;BF-700 B4流式细胞仪;LSM 5 PASCAL共聚焦显微镜;Bio-rad酶标仪。

1.2 细胞模型建立和分组方法

SH-SY5Y细胞予DMEM/F12培养基+15%胎牛血清+1%PS,37℃,5%CO₂,饱和湿度,传代培养。给予不同浓度Lira(1 nM、10 nM、100 nM、500 nM、1000 nM)预处理细胞2 h,暴露于50 mM高糖(HG)24 h确定低、高浓度。随机分为对照组:SH-SY5Y细胞正常培养24 h;HG组:50 mM浓度Glu培养基中培养24 h;HG+LI-L组:100 nM Lira干预2 h,50 mM浓度Glu共培养24 h;HG+LI-H组:500 nM Lira干预2 h,50 mM浓度Glu共培养24 h;HG+LI-H+3MA组:500 nM Lira干预2 h,1 h后予5 mM 3-MA干预1 h,再予50 mM浓度Glu共培养24 h。

1.3 实验方法

1.3.1 光镜下细胞形态观察 在96孔板下,用荧光倒置显微镜于100倍镜下拍照。

1.3.2 细胞计数试剂盒(CCK)-8法检测细胞增殖能力 将各组细胞置入96孔板中,将100 μ L的CCK-8溶液分别加入每个孔中后置入培养箱中孵育,孵育时间为2 h,再使用酶标仪测定并记录450 nm处的光密度(OD)值。

1.3.3 酶联免疫吸附法检测细胞Glu、MDA、SOD、ATP水平 细胞处理后,收集各组细胞及上清液,参考Glu、MDA、SOD、ATP试剂盒说明书进行操作,使用酶标仪进行检测。

1.3.4 流式细胞术检测细胞凋亡情况、细胞ROS水平和线粒体膜电位 细胞干预完成后,离心(1000 rpm,5 min),细胞凋亡情况:加入500 μ L 1 \times Binding Buffer重悬细胞,过200目筛网,制成单细胞悬液,每管加入5 μ L Annexin V-PE和10 μ L 7-AAD,于4℃避光放置10 min。细胞ROS水平:每孔加入2 mL磷酸盐缓冲液(PBS)与2 μ L DCFH-DA,DCFH-DA终浓度为10 μ M,37℃孵育30 min,离心(1000 rpm,5 min),加入500 μ L PBS重悬细胞,过200目筛网,制成单细胞悬液。线粒体膜电位:加入500 μ L 1 \times JC-1染色液重悬细胞,37℃ 30 min,加入500 μ L PBS重悬细胞,过200目筛网,制成单细胞悬液。均在30 min内使用流式细胞仪进行细胞凋亡、细胞ROS水平和线粒体膜电位检测。

1.3.5 免疫荧光检测微管相关蛋白1轻链3(LC3B)和p62蛋白表达及定位 细胞滴培养24 h至细胞贴壁,细胞干预完成后固定10 min,0.5%Triton X-100处理20 min,1%BSA室温封闭40 min,甩干封闭液;滴加80 μ L浓度为2 μ g/mL的Anti-LC3B、Anti-P62抗体,4℃孵育过夜,滴加80 μ L浓度为2 μ g/mL的二抗山羊抗兔IgG H&L(Alexa Fluor® 488)、山羊抗小鼠IgG H&L(Alexa Fluor® 594),37℃孵育1 h,滴加80 μ L浓度为1 μ g/mL的4',6-二脒基-2-苯基吲哚(DAPI),孵育5 min(室温环境下),封片选用浓度为50%的甘油,再于显微镜观察LC3B和p62蛋白表达及定位,并用ImageJ计算其荧光强度。

1.3.6 Western Blot检测磷脂酰肌醇3激酶(PI3K)、p-PI3K、蛋白激酶B(Akt)、p-Akt、哺乳动物雷帕霉素靶蛋白(mTOR)、p-mTOR、LC3-II、p62蛋白表达 采用RIPA裂解法提取细胞总蛋白并行蛋白定量。调整蛋白浓度,根据目的蛋白分子量,配胶,上样,电泳条件(70 v,30 min \rightarrow 110V,150 min),采用湿转法恒流定时(200 mA,90 min)转膜,封闭,洗膜,加入一抗、二抗孵育,ECL加膜上,曝光成像,应用Image J软件对条带的灰度值进行定量分析。

1.4 数据统计

SPSS26.0行统计分析。制图采用GraphPad Prism 5.0软件,图片处理采用Image J软件。计量资料以($\bar{x}\pm s$),多组间比较行单因素方差分析,两组间比较行t检验。检验标准=0.05。

2 结果

2.1 各组细胞存活率比较

HG 组细胞的存活率较对照组更低 ($P<0.05$), 随 Lira 浓度的增加细胞存活率先增加后降低 ($P<0.05$)。与 HG 组比较, HG+LI(500 nM) 组细胞增殖活力最为显著 ($P<0.05$)。见图 1。

2.2 各组上清液 Glu 及细胞内 MDA、SOD 水平比较

HG 组 Glu 和 MDA 较对照组更高, SOD 较对照组更低 ($P<0.05$); 与 HG 组比较, HG+LI(500 nM) 组 Glu 和 MDA 较 HG 组更低, SOD 较 HG 组更高 ($P<0.05$)。见图 2。

2.3 细胞状态和存活率比较

HG 组细胞存活率较对照组更低 ($P<0.05$); HG+LI-L 组和 HG+LI-H 组细胞存活率较 HG 组更高, 且 HG+LI-H 组细胞存活率较 HG+LI-L 组更高 ($P<0.05$); 与 HG+LI-H 组比较, HG+LI-H+3MA 组细胞存活率较 HG+LI-H 组更低 ($P<0.05$)。见图 3~4。

2.4 各组细胞凋亡比较

HG 组细胞凋亡率较对照组更高 ($P<0.05$); 与 HG 组比较, HG+LI-L 组和 HG+LI-H 组细胞凋亡率较 HG 组更低, 且 HG+LI-H 组细胞凋亡率较 HG+LI-L 组更低 ($P<0.05$); HG+LI-H+3MA 组细胞凋亡率较 HG+LI-H 组更高 ($P<0.05$)。见图 5。

2.5 各组细胞 ROS 水平比较

HG 组细胞 ROS 荧光强度较对照组更高 ($P<0.05$); HG+LI-L 组和 HG+LI-H 组细胞 ROS 荧光强度较 HG 组更低, 且 HG+LI-H 组细胞 ROS 荧光强度较 HG+LI-L 组更低 ($P<0.05$); HG+LI-H+3MA 组细胞 ROS 荧光强度较 HG+LI-H 组更高 ($P<0.05$)。见图 6。

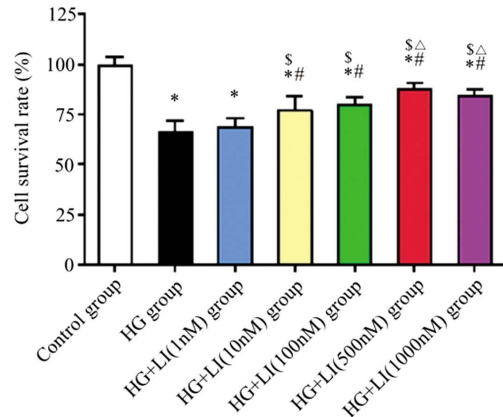


图 1 各组细胞存活率

Fig.1 Cell survival rate in each group

Note: Compared with control group, * $P<0.05$; Compared with HG group, # $P<0.05$; Compared with HG+LI (1 nM) group, § $P<0.05$; Compared with HG+LI (10 nM) group, △ $P<0.05$.

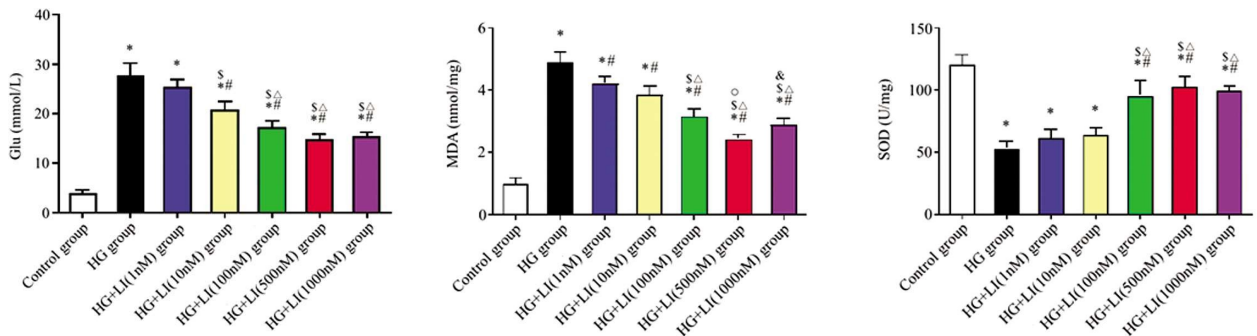


图 2 各组上清液 Glu 及细胞内 MDA、SOD 水平

Fig. 2 Supernatant Glu and intracellular MDA and SOD levels in each group

Note: Compared with control group, * $P<0.05$; Compared with HG group, # $P<0.05$; Compared with HG+LI (1 nM) group, § $P<0.05$; Compared with HG+LI (10 nM) group, △ $P<0.05$; Compared with HG+LI (100 nM) group, ° $P<0.05$; Compared with HG+LI (500 nM) group, & $P<0.05$.

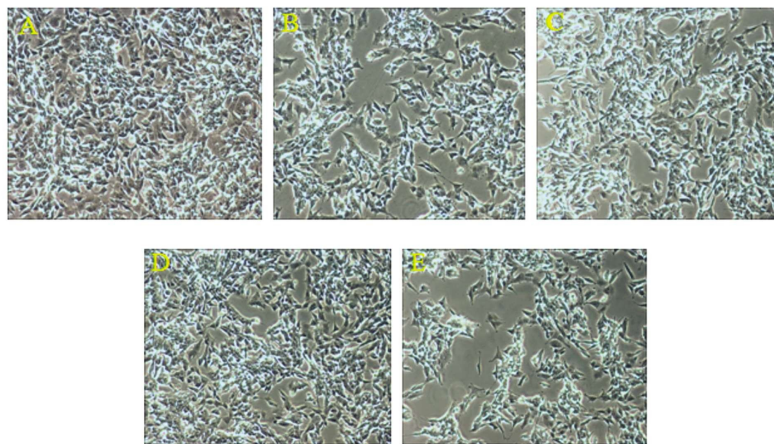


图 3 观察各组细胞存活状态(×100)

Fig.3 Cell survival status in each group (×100)

Note: A: Control group; B: HG group; C: HG+LI-L group; D: HG+LI-H group; E: HG+LI-H+3MA group.

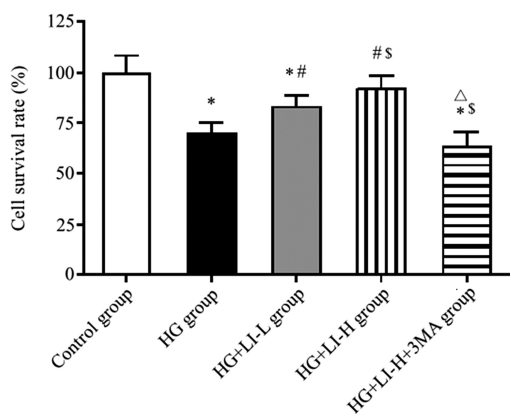


图 4 各组细胞存活率

Fig. 4 Cell survival rate in each group

Note: Compared with control group, * $P < 0.05$; Compared with HG group, # $P < 0.05$; Compared with HG+LI-L group, s $P < 0.05$; Compared with HG+LI-H group, [△] $P < 0.05$.

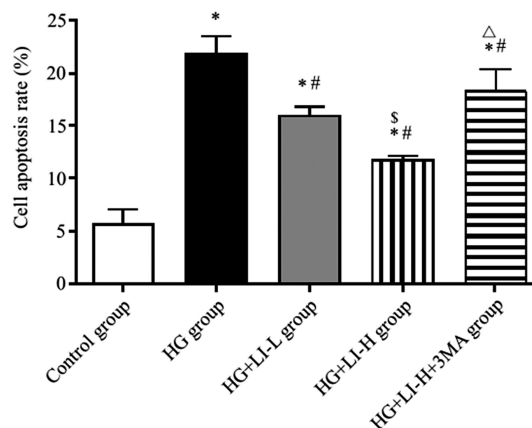


图 5 各组细胞凋亡率柱状图

Fig. 5 Bar graph of cell apoptosis rate in each group

Note: Same as Fig. 4.

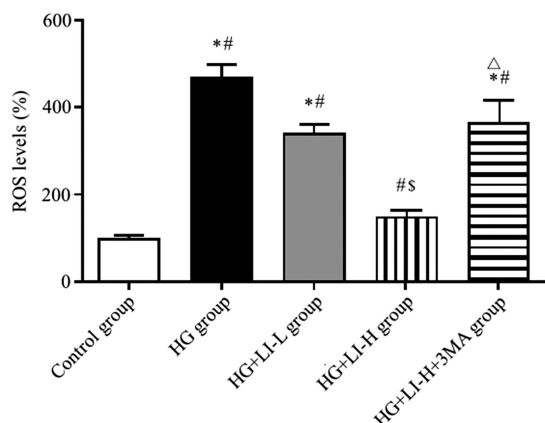


图 6 各组细胞 ROS 水平柱状图

Fig.6 Bar graph of the ROS levels of the cells in each group

Note: Same as Fig. 4

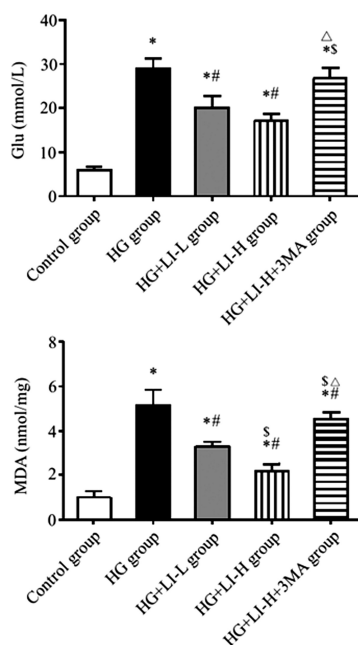


图 7 各组 Glu、MDA、SOD、ATP 水平

Fig.7 Levels of Glu, MDA, SOD and ATP in each group

Note: Same as Fig.4.

2.6 各组上清液 Glu 及细胞内 MDA、SOD、ATP 水平比较

与对照组比较, HG 组 Glu 和 MDA 水平较对照组更高, ATP 和 SOD 较对照组更低 ($P < 0.05$), 与 HG 组比较, HG+LI-L 组和 HG+LI-H 组 Glu 和 MDA 较 HG 组更低, ATP 和 SOD 较 HG 组更高, 且 HG+LI-H 组 Glu 和 MDA 较 HG+LI-L 组更低, ATP 和 SOD 较 HG+LI-L 组更高 ($P < 0.05$); 与 HG+LI-H 组比较, HG+LI-H+3MA 组 Glu 和 MDA 较 HG+LI-H 组更高, ATP 和 SOD 较 HG+LI-H 组更低 ($P < 0.05$)。见图 7。

2.7 各组细胞膜电位水平比较

HG 组 JC-1 较对照组更高, 提示线粒体膜电位较对照组更低 ($P < 0.05$); HG+LI-L 组及 HG+LI-H 组 JC-1 均较 HG 组更低, 且 HG+LI-H 组 JC-1 较 HG+LI-L 组更低, 提示线粒体膜电位升高 ($P < 0.05$); HG+LI-H+3MA 组 JC-1 增加, 提示线粒体膜电位较 HG+LI-H 组更低 ($P < 0.05$)。见图 8。

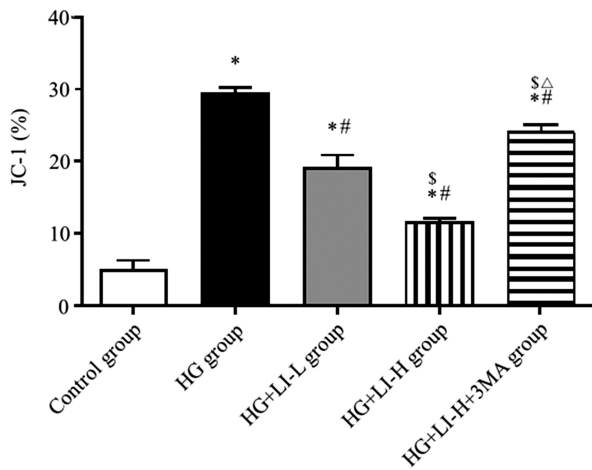


图 8 各组细胞膜电位水平柱状图

Fig. 8 Bar graph of the membrane potential level of the cells in each group

Note: Same as Fig. 4.

2.8 各组 LC3B 和 p62 共定位及表达比较

HG 组细胞内 LC3B 蛋白荧光强度较对照组更低, p62 蛋白荧光强度较对照组更高 ($P<0.05$); HG+LI-L 组及 HG+LI-H 组 LC3B 蛋白荧光强度较 HG 组更高, 且 HG+LI-H 组 LC3B

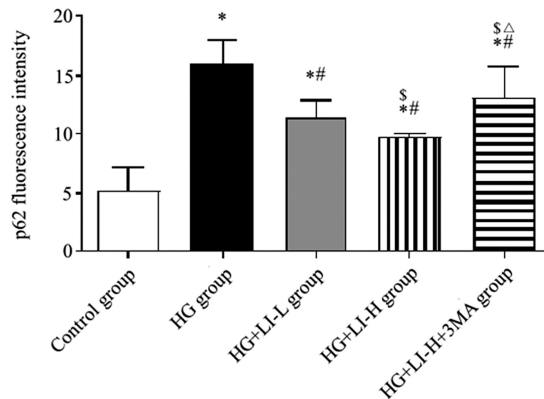
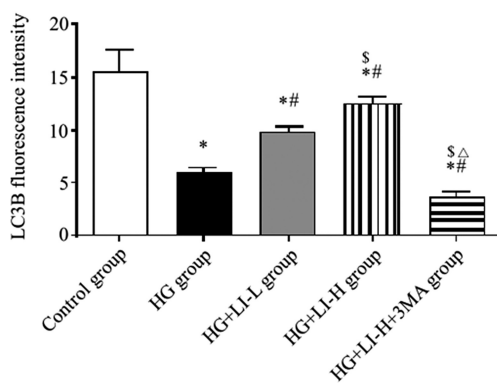


图 9 各组 LC3B、p62 荧光强度表达

Fig.9 The fluorescence intensity expression of LC3B and p62 in each group

Note: Same as Fig. 4.

高血糖、游离脂肪酸等可诱导 DM 患者机体 ROS 的过量生成, 损害脂质、蛋白质和 DNA, 参与癌症、心血管疾病和神经退行性疾病发生发展^[9], 研究显示, T2DM 患者血清 MDA 水平较健康受试者升高, 血清抗氧化剂水平降低^[9]。本研究显示 Lira 能改善高糖诱导的 SH-SY5Y 细胞 Glu、MDA、ROS 水平升高, SOD 活性降低, 而 3MA 可削弱 Lira 的作用。

多项研究提示线粒体功能障碍与 DM 及帕金森病有关, 线粒体氧化损伤、功能变化是细胞坏死与凋亡中的一个关键的环节, 线粒体复合体以电化学偶联的方式氧化磷酸化, 而解耦连蛋白(UCP)通过降低线粒体膜电位、调节钙超载、降低 ROS 而保护神经, 过表达 UCP-2 的皮层神经元凋亡率较正常组织降低^[7]。本研究表明 Lira 可改善高糖诱导的 SH-SY5Y 细胞线粒体膜电位 JC-1 增加和膜电位降低, 而 3MA 则削弱 Lira 调节线粒体功能障碍作用。

自噬主要负责有害细胞成分的系统降解和再循环, 是神经

蛋白荧光强度较 HG+LI-L 组更高, p62 蛋白荧光强度较 HG+LI-L 组更低, 且 HG+LI-H 组 LC3B 蛋白荧光强度较 HG+LI-H 组更低 ($P<0.05$); 与 HG+LI-H 组比较, HG+LI-H+3MA 组细胞内 LC3B 蛋白荧光强度较 HG+LI-H 组更低, p62 蛋白荧光强度较 HG+LI-H 组更高 ($P<0.05$)。见图 9~10。

2.9 各组 PI3K、p-PI3K、Akt、p-Akt、mTOR、p-mTOR、LC3-II、p62 蛋白表达比较

HG 组 p-PI3K、p-Akt 和 LC3B 蛋白表达较对照组更低, p-mTOR 和 p62 蛋白表达较对照组更高 ($P<0.05$); HG+LI-H 组 p-PI3K、p-Akt 和 LC3B 蛋白表达较 HG 组更高, p-mTOR 和 p62 蛋白表达较 HG 组更低 ($P<0.05$); HG+LI-H+3MA 组 p-PI3K、p-Akt 和 LC3B 蛋白表达较 HG+LI-H 组更低, p-mTOR 和 p62 蛋白表达较 HG+LI-H 组更高 ($P<0.05$)。见图 11。

3 讨论

认知功能障碍是 DM 早期临床表现, 发病机制与糖代谢紊乱导致氧化应激、线粒体损伤和自噬等有关^[4]。本研究发现 Lira 能显著降低高糖诱导 SH-SY5Y 细胞糖毒性和细胞的凋亡, 提高细胞存活率。

元稳态所必需的生理过程^[8]。受损蛋白和细胞器的积累可导致自噬通量的缺陷, 表现为自噬结构标志物 LC3B 和抑制标志物 p62 的改变, 以及 LC3B-II/I 比值的变化^[9]。Lira 可提高 LC3B-II/I 比值和 Beclin1 蛋白表达, 降低 p62 蛋白表达, 调节高糖诱导的皮层原代神经元的自噬通量^[10]。同时 PI3K/Akt/mTOR 信号通路可调控许多细胞活动, 与糖尿病及神经退行性疾病密切相关^[11]。PI3K 可激活 AKT、糖原合酶激酶、Forkhead 转录因子、mTOR 等分子引起信号通路级联反应^[12]。PI3K 的特异性抑制剂 wortmanin 和转染 PI3K 显性负性突变体均可以抑制 Akt 的活性^[13]。活化的 PI3K/Akt 信号通路能在体外保护细胞抵抗 Aβ 的毒性, 而 PI3K 特异性抑制剂 LY294002 预处理 30 分钟则显著阻断硫辛酸诱导的 Akt 活性, 减弱神经保护作用^[14]。本研究也发现 Lira 可使高糖诱导的 SH-SY5Y 细胞 LC3B 荧光强度降低, p62 荧光强度增强, LC3B 和 p-PI3K/Akt 蛋白表达降低, p-mTOR 和 p62 蛋白表达增强, PI3K 抑制剂

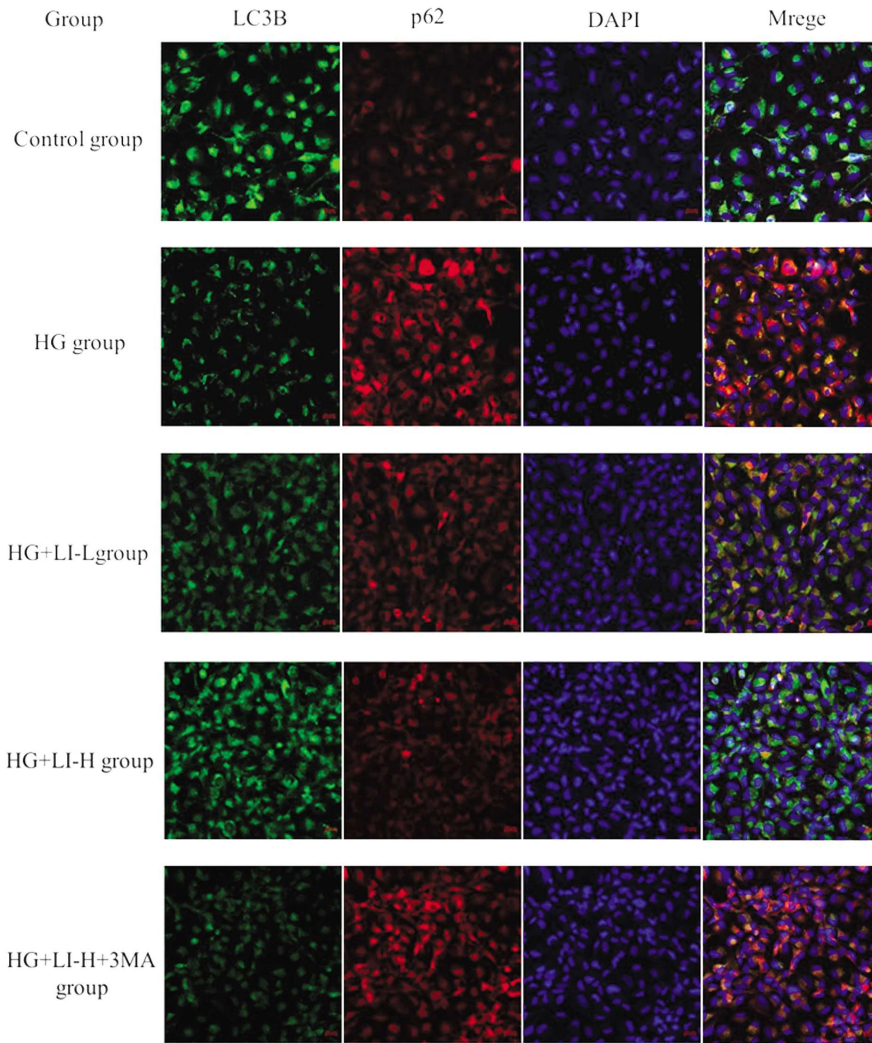


图 10 各组 LC3B 和 P62 及其双重免疫荧光标记与定位

Fig. 10 Analysis of LC3B and P62 and their dual immunofluorescent labeling and localization in each group

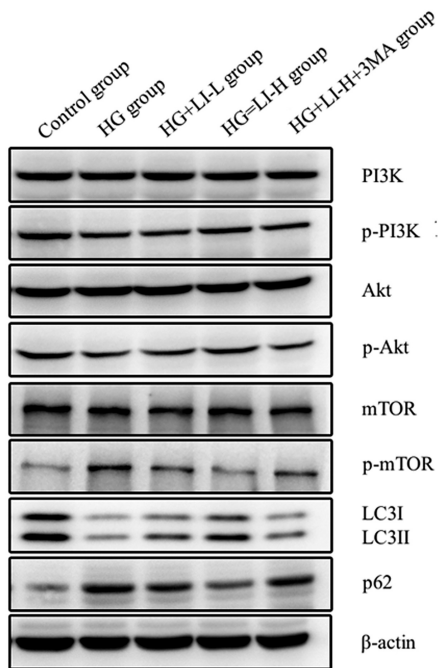


图 11 各组蛋白条带

Fig. 11 Each histone band

3MA 明显削弱了 Lira 对自噬通量与 PI3K/Akt/mTOR 自噬通路的调控和神经保护作用。

综上所述, Lira 在保护神经、改善认知方面的作用越来越受到关注。Lira 降低高糖诱导的 SH-SY5Y 细胞糖毒性, 改善氧化应激、线粒体功能障碍、激活 PI3K/Akt/mTOR 通路, 改善自噬缺陷。

参考文献(References)

[1] Srikanth V, Sinclair AJ, Hill-Briggs F, et al. Type 2 diabetes and cognitive dysfunction-towards effective management of both comorbidities[J]. *Lancet Diabetes Endocrinol*, 2020, 8(6): 535-545.

[2] Zhang M, Yan W, Yu Y, et al. Liraglutide ameliorates diabetes-associated cognitive dysfunction via rescuing autophagic flux [J]. *J Pharmacol Sci*, 2021, 147(3): 234-244.

[3] Glotfelty EJ, Olson L, Karlsson TE, et al. Glucagon-like peptide-1 (GLP-1)-based receptor agonists as a treatment for Parkinson's disease [J]. *Expert Opin Investig Drugs*, 2020, 29(6): 595-602.

[4] 蔡宏桂, 姚南珍, 李思韵, 等. 茵陈五苓散联合利拉鲁肽对肥胖型 2 型糖尿病患者糖脂代谢、胰岛素敏感性和氧化应激的影响[J]. *现代生物医学进展*, 2024, 24(1): 162-166.

- [13] Nakanishi M, Watanabe Y, Honda N, et al. Dynamics of host and graft after cell sheet transplantation: Basic study for the application of amyotrophic lateral sclerosis[J]. *Brain Res*, 2019, 1724: 146444.
- [14] Yu-Taeger L, Stricker-Shaver J, Arnold K, et al. Intranasal Administration of Mesenchymal Stem Cells Ameliorates the Abnormal Dopamine Transmission System and Inflammatory Reaction in the R6/2 Mouse Model of Huntington Disease [J]. *Cells*, 2019, 8(6).
- [15] Mei J, Niu C. Effects of engineered conserved dopamine neurotrophic factor-expressing bone marrow stromal cells on dopaminergic neurons following 6-OHDA administrations [J]. *Mol Med Rep*, 2015, 11(2): 1207-1213.
- [16] Yan L, Jiang B, Niu Y, et al. Intrathecal delivery of human ESC-derived mesenchymal stem cell spheres promotes recovery of a primate multiple sclerosis model[J]. *Cell Death Discov*, 2018, 4: 28.
- [17] Seo JH, Jang IK, Kim H, et al. Early Immunomodulation by Intravenously Transplanted Mesenchymal Stem Cells Promotes Functional Recovery in Spinal Cord Injured Rats [J]. *Cell Med*, 2011, 2(2): 55-67.
- [18] Bae JS, Jin HK, Lee JK, et al. Bone marrow-derived mesenchymal stem cells contribute to the reduction of amyloid- β deposits and the improvement of synaptic transmission in a mouse model of pre-dementia Alzheimer's disease [J]. *Curr Alzheimer Res*, 2013, 10(5): 524-531.
- [19] Harach T, Jammes F, Muller C, et al. Administrations of human adult ischemia-tolerant mesenchymal stem cells and factors reduce amyloid beta pathology in a mouse model of Alzheimer's disease[J]. *Neurobiol Aging*, 2017, 51: 83-96.
- [20] Zappa Villar MF, López Hanotte J, Pardo J, et al. Mesenchymal Stem Cells Therapy Improved the Streptozotocin-Induced Behavioral and Hippocampal Impairment in Rats [J]. *Mol Neurobiol*, 2020, 57(2): 600-615.
- [21] Sarmah D, Datta A, Kaur H, et al. Sirtuin-1 - Mediated NF- κ B Pathway Modulation to Mitigate Inflammasome Signaling and Cellular Apoptosis is One of the Neuroprotective Effects of Intra-arterial Mesenchymal Stem Cell Therapy Following Ischemic Stroke[J]. *Stem Cell Rev Rep*, 2022, 18(2): 821-838.
- [22] Zhang Z, Sheng H, Liao L, et al. Mesenchymal Stem Cell-Conditioned Medium Improves Mitochondrial Dysfunction and Suppresses Apoptosis in Okadaic Acid-Treated SH-SY5Y Cells by Extracellular Vesicle Mitochondrial Transfer [J]. *J Alzheimers Dis*, 2020, 78(3): 1161-1176.
- [23] Wu L, Chen L, Li L. Apelin/APJ system: A novel promising therapy target for pathological angiogenesis [J]. *Clin Chim Acta*, 2017, 466: 78-84.
- [24] Hosoya M, Kawamata Y, Fukusumi S, et al. Molecular and functional characteristics of APJ. Tissue distribution of mRNA and interaction with the endogenous ligand apelin [J]. *J Biol Chem*, 2000, 275(28): 21061-21067.
- [25] Masri B, Morin N, Pedebernade L, et al. The apelin receptor is coupled to Gi1 or Gi2 protein and is differentially desensitized by apelin fragments[J]. *J Biol Chem*, 2006, 281(27): 18317-18326.
- [26] Bortolato B, Miskowiak KW, Köhler CA, et al. Cognitive remission: a novel objective for the treatment of major depression? [J]. *BMC Med*, 2016, 14: 9.

(上接第 4413 页)

- [5] Rendra E, Riabov V, Mossel DM, et al. Reactive oxygen species (ROS) in macrophage activation and function in diabetes [J]. *Immunobiology*, 2019, 224(2): 242-253.
- [6] Altoum AEA, Abbas MY, Osman AL, et al. The Influence of Oral Multivitamins Supplementation on Selected Oxidative Stress Parameters and Lipid Profiles among Sudanese Patients with Type-2 Diabetes[J]. *Open Access Maced J Med Sci*, 2019, 7(5): 775-778.
- [7] Barnstable CJ, Zhang M, Tombran-Tink J. Uncoupling Proteins as Therapeutic Targets for Neurodegenerative Diseases[J]. *Int J Mol Sci*, 2022, 23(10): 5672.
- [8] Kanamori H, Naruse G, Yoshida A, et al. Morphological characteristics in diabetic cardiomyopathy associated with autophagy [J]. *J Cardiol*, 2021, 77(1): 30-40.
- [9] Li R, Du JH, Yao GM, et al. Autophagy: a new mechanism for regulating VEGF and PEDF expression in retinal pigment epithelium cells[J]. *Int J Ophthalmol*, 2019, 12(4): 557-562.
- [10] Kong FJ, Wu JH, Sun SY, et al. Liraglutide ameliorates cognitive decline by promoting autophagy via the AMP-activated protein kinase/mammalian target of rapamycin pathway in a streptozotocin-induced mouse model of diabetes[J]. *Neuropharmacology*, 2018, 131(3): 316-325.
- [11] Ramasubbu K, Devi Rajeswari V. Impairment of insulin signaling pathway PI3K/Akt/mTOR and insulin resistance induced AGEs on diabetes mellitus and neurodegenerative diseases: a perspective review[J]. *Mol Cell Biochem*, 2023, 478(6): 1307-1324.
- [12] Glaviano A, Foo ASC, Lam HY, et al. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer[J]. *Mol Cancer*, 2023, 22(1): 138.
- [13] Kma L, Baruah TJ. The interplay of ROS and the PI3K/Akt pathway in autophagy regulation [J]. *Biotechnol Appl Biochem*, 2022, 69(1): 248-264.
- [14] Chen S, Peng J, Sherchan P, et al. TREM2 activation attenuates neuroinflammation and neuronal apoptosis via PI3K/Akt pathway after intracerebral hemorrhage in mice [J]. *J Neuroinflammation*, 2020, 17(1): 168.