论著·基础研究

缺氧诱导因子-1α抑制剂YC-1改善糖尿病肾病小鼠肾脏损伤的机制研究

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［摘要］目的·研究缺氧诱导因子-1α（hypoxia inducible factor-1α，HIF-1α）抑制剂YC-1对小鼠糖尿病肾病（diabetic nephropathy，DN）进展的影响及潜在机制。方法·将10周龄的雄性db/db小鼠（DN模型）和同窝野生型（WT）小鼠按是否给予YC-1分为4组，即WT组、WT+YC-1组、DB组、DB+YC-1组，每组6只。YC-1干预组予以YC-1（20 mg/kg，1次/d）腹腔注射8周，非干预组同时予以等体积二甲基亚砜腹腔注射。干预8周后，检测小鼠血糖、体质量和肾脏质量，并收集血清、尿液、肾组织标本。检测小鼠血肌酐、尿白蛋白/肌酐比（urinary albumin-to-creatinine ratio，UACR）、尿中性粒细胞明胶酶相关脂质运载蛋白（neutropil gelatinase-associated lipocalin，NGAL）水平。肾脏行苏木精-伊红（H-E）染色、过碘酸-雪夫（PAS）染色观察组织病理损伤；马松（Masson）染色检测纤维化情况，免疫组织化学（免疫组化）法检测Ⅰ型胶原蛋白，Western blotting检测α-平滑肌肌动蛋白（α-smooth muscle actin，α-SMA）水平；免疫组化法和Western blotting检测HIF-1α表达；TUNEL染色和Western blotting检测细胞凋亡水平；试剂盒检测肾脏超氧化物歧化酶（superoxide dismutase，SOD）活性和丙二醛（malondialdehyde，MDA）含量；Western blotting检测内质网应激（endoplasmic reticulum stress，ERS）标志物免疫球蛋白重链结合蛋白（immunoglobulin heavy chain binding protein，BiP；又称GRP78）、磷酸化蛋白激酶样内质网激酶（phospho-protein kinase R-like endoplasmic reticulum kinase，p-PERK）、总PERK、磷酸化真核起始因子2α（phospho-eukaryotic initiation factor 2α，p-eIF2α）、总eIF2α、激活转录因子4（activating transcription factor 4，ATF4）和C/EBP同源蛋白（C/EBP homologous protein，CHOP）的表达。结果·与WT组小鼠相比，DB组小鼠血糖升高，肾功能下降，肾脏病理损伤和纤维化加重，肾脏HIF-1α表达、氧化应激和ERS激活程度增加。与DB组小鼠相比，DB+YC-1组小鼠血糖无明显变化，但肾/体质量比、血肌酐、UACR、尿NGAL水平显著下降，肾脏病理损伤和纤维化程度显著减轻，Ⅰ型胶原蛋白和α-SMA表达显著降低，肾脏HIF-1α表达显著降低，肾脏TUNEL阳性细胞数减少，促凋亡蛋白BAX和活化的胱天蛋白酶（cleaved caspase-3）表达显著下降，抑制凋亡蛋白BCL-2表达显著升高，肾脏SOD活性显著升高，MDA含量显著降低，肾脏ERS标志物GRP78、p-PERK、p-eIF2α、ATF4和CHOP表达显著下降（均P<0.05）。结论·HIF-1α抑制剂YC-1能够改善DN小鼠肾脏氧化应激和ERS的异常激活，抑制细胞凋亡和肾脏纤维化，减轻肾脏病理损伤，保护肾功能。

［关键词］糖尿病肾病；缺氧诱导因子-1α；氧化应激；内质网应激
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Renal protective effect and mechanism research of hypoxia inducible factor-1α inhibitor YC-1 in diabetic nephropathy mice

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[Abstract] Objective·To investigate the effect of hypoxia inducible factor-1α (HIF-1α) inhibitor YC-1 on the progression of diabetic nephropathy (DN) in mice and the potential mechanism. Methods·Ten-week-old male db/db mice (DN model) and their nondiabetic wild-type (WT) littermates were divided into 4 groups (n=6) according to whether treated with YC-1 or not: WT group,

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WT+YC-1 group, DB group, and DB+YC-1 group. The treatment groups were intraperitoneally injected with YC-1 (20 mg·kg^{-1}) once a day, while the non-treatment groups received the same volumes of DMSO injection. After a total of 8 weeks of intervention, blood glucose, body weight, and kidney weight of all mice were measured. Serum, urine and kidney tissue samples were harvested. Serum creatinine, urinary albumin-to-creatinine ratio (UACR), and urine neutropil gelatinase-associated lipocalin (NGAL) levels were detected. The kidneys were stained with hematoxylin-eosin (H-E) and periodic acid-Schiff (PAS) to observe the pathological changes. Masson staining was used to detect fibrosis, collagen-Ⅰ was detected by immunohistochemistry, and α-smooth muscle actin (α-SMA) was detected by Western blotting. The expression of HIF-1α was detected by both Western blotting and immunohistochemistry. TUNEL staining and Western blotting for apoptosis-related proteins were used to observe the cell apoptosis level. Superoxide dismutase (SOD) activity and malondialdehyde (MDA) level were detected by the kits. Endoplasmic reticulum stress (ERS) markers, including immunoglobulin heavy chain binding protein (BiP, also known as GRP78), phospho-protein kinase R-like endoplasmic reticulum kinase (p-PERK), total PERK, phospho-eukaryotic initiation factor 2α (p-eIF2α), total eIF2α, activating transcription factor 4 (ATF4), and C/EBP homologous protein (CHOP), were determined by Western blotting. **Results·** Compared with the WT group, the DB group showed significant rise of blood glucose, loss of renal function, severe kidney histopathology injuries and kidney fibrosis, increase of renal HIF-1α expression, and aggravated oxidative stress and ERS. Whilst there were no significant changes in blood glucose, YC-1 treatment notably reduced kidney weight/body weight ratio, serum creatinine, UACR, and urine NGAL levels in db/db mice. YC-1 treatment ameliorated kidney histopathology injuries and kidney fibrosis, and decreased the expressions of collagen-Ⅰ and α-SMA. YC-1 treatment also reduced the number of TUNEL positive cells, the expression of HIF-1α and pro-apoptotic proteins including BAX and cleaved caspase-3, and MDA level in the kidneys of db/db mice, while promoting anti-apoptotic protein BCL-2 expression and SOD activity. The expressions of ERS markers GRP78, p-PERK, p-eIF2α, ATF4, and CHOP were likewise significantly decreased in DB+YC-1 group. **Conclusion·** HIF-1α inhibitor YC-1 inhibits oxidative stress and abnormal activation of ERS, improving cell apoptosis and fibrosis in the kidneys of DN mice, which would attenuate the aggravation of pathological damage and loss of kidney function.

[Key words] diabetic nephropathy (DN); hypoxia inducible factor-1α (HIF-1α); oxidative stress; endoplasmic reticulum stress (ERS)

糖尿病肾病（diabetic nephropathy，DN）是糖尿病最严重的并发症之一，也是全世界导致慢性肾脏疾病（chronic kidney disease，CKD）进展至终末期肾脏病（end stage renal disease，ESRD）的最主要病因[1]。DN的发病机制复杂，且缺乏有效的治疗手段。在高血糖、炎症、氧化应激等多种病理因素的作用下，肾实质发生广泛缺氧，这将引起肾脏结构病变并最终导致纤维化[2-3]。缺氧诱导因子-1α（hypoxia inducible factor-1α，HIF-1α）是驱动细胞缺氧适应性反应的重要分子，在肾脏中高表达，并与肾脏疾病的发生、发展密切相关[4-5]。YC-1（Lificiguat）是一种HIF-1α特异性抑制剂，既往用于多种癌症的治疗，并对炎症性疾病具有潜在疗效[6]。本研究采用db/db小鼠作为2型DN动物模型，探讨应用YC-1抑制HIF-1α的表达后对DN小鼠肾功能和病理损伤的影响，并揭示潜在的分子机制，以期为探索DN的发病机制和治疗提供新的靶点和方向。

1 材料与方法

1.1 实验动物

6周龄、SPF级雄性db/db小鼠（C57BLKS/J-*Lepr*$^{db/db}$）及同窝野生型小鼠（C57BLKS/J-*Lepr*$^{WT/WT}$）购自江苏集萃药康生物科技股份有限公司［实验动物生产许可证号为SCXK（苏）2019-0009］，饲养于上海交通大学医学院附属第六人民医院动物实验室［实验动物使用许可证号为SYXK（沪）2011-0128］；饲养温度为22~26 ℃，相对湿度40%~70%，12 h昼夜更替，自由摄食饮水。

1.2 主要试剂与仪器

YC-1（S7958，美国Selleck），DMSO（A610163，上海生工），小鼠白蛋白ELISA试剂盒（E99-134，美国Bethyl），肌酐试剂盒（DICT-500，美国BioAssay Systems），小鼠中性粒细胞明胶酶相关脂质运载蛋白（neutropil gelatinase-associated lipocalin，NGAL）ELISA试剂盒（JL11556，上海江莱生物），荧光素原位凋亡检测试剂盒（S7110，美国Sigma），脂质过氧化产物丙二醛（malondialdehyde，MDA）检测试剂盒（S0131，上海碧云天），总超氧化物歧化酶（superoxide dismutase，SOD）活性检测试剂盒（S0101，上海碧云天），β-肌动蛋白（β-actin）兔单克隆抗体（#8457，美国Cell Signaling Technology），Ⅰ型胶原蛋白（collagen-Ⅰ）兔多克隆抗体（A1352，

武汉爱博泰克），α-平滑肌肌动蛋白（α-smooth muscle actin，α-SMA）兔单克隆抗体（A17910，武汉爱博泰克），HIF-1α兔多克隆抗体（20960-1-AP，武汉三鹰），活化的胱天蛋白酶（cleaved caspase-3）兔多克隆抗体（#9661，美国Cell Signaling Technology），促凋亡蛋白BAX兔单克隆抗体（A19684，武汉爱博泰克），抑制凋亡蛋白BCL-2兔单克隆抗体（#3498，美国Cell Signaling Technology），免疫球蛋白重链结合蛋白（immunoglobulin heavy chain binding protein，BiP；又称GRP78）兔单克隆抗体（#3177，美国Cell Signaling Technology），磷酸化蛋白激酶样内质网激酶（phospho-protein kinase R-like endoplasmic reticulum kinase，p-PERK）兔多克隆抗体（PA5-37773，美国Invitrogen），PERK兔单克隆抗体（#3192，美国Cell Signaling Technology），磷酸化真核起始因子2α（phospho-eukaryotic initiation factor 2α，p-eIF2α）兔单克隆抗体（#3597，美国Cell Signaling Technology），eIF2α兔多克隆抗体（A0764，武汉爱博泰克），激活转录因子4（activating transcription factor 4，ATF4）兔单克隆抗体（#11815，美国Cell Signaling Technology），C/EBP同源蛋白（C/EBP homologous protein，CHOP）兔多克隆抗体（15204-1-AP，武汉三鹰）。

血糖仪及血糖试纸（Performa，瑞士Roche），电子天平（Quintix224-1CN，德国Sartorius），正置荧光显微镜（BX43，日本Olympus），酶标仪（Synergy H1，美国BioTek），冷冻研磨仪（JXFSTPRP-CL，上海净信），全自动化学发光图像分析系统（4600，上海天能）。

1.3 实验方法

1.3.1 动物分组及给药 将野生型小鼠（WT）和db/db小鼠（DB）随机分为4组，即WT组、WT+YC-1组、DB组、DB+YC-1组，每组6只。适应性喂养至10周龄后，YC-1处理组小鼠予以YC-1（20 mg/kg，1次/d）腹腔注射，WT组和DB组小鼠予以等体积二甲基亚砜（DMSO）腹腔注射。每周检测小鼠体质量以调整用药量，总计干预8周。

1.3.2 血糖、体质量检测和标本采集 累计干预满8周，于小鼠18周龄时检测体质量、血糖，随后用心脏灌注法处死小鼠，并留取血清和尿液标本，置于−80 ℃冻存。将两侧肾脏剥离肾包膜后称重，随后将左肾分离出肾皮质并置于液氮冻存，右肾放入4%多聚甲醛固定。

1.3.3 尿蛋白、尿NGAL和血肌酐检测 将血清和尿液上清液解冻后，采用ELISA法检测尿液白蛋白和NGAL含量。NGAL是一种检测急、慢性肾脏疾病肾小管间质损伤的新型生物学标志物，尿液中NGAL的含量能够反映DN肾小管的早期损伤[7]。采用比色法检测血肌酐（serum creatinine，Scr）、尿肌酐水平。尿蛋白表示为尿白蛋白/肌酐比（urinary albumin-to-creatinine ratio，UACR）。所有检测步骤严格按照试剂盒说明书进行。

1.3.4 肾组织病理标本制作和病理学评分 对多聚甲醛固定的肾脏组织依次进行梯度脱水、石蜡包埋和切片，随后采用不同染色液对肾脏切片分别进行苏木精-伊红（H-E）染色、过碘酸-雪夫（PAS）染色和马松（Masson）染色。每只小鼠高倍镜（×400）下随机选取10个不同视野进行拍摄。采用Image-Pro Plus 6.0软件测量肾小球面积和PAS阳性区域，并计算肾小球系膜基质评分（PAS阳性区域/肾小球面积×100%）[8]。肾小管损伤评分参考既往研究[9]，由2名观察者分别观察肾小管间质区域并进行半定量评分：0分，肾小管间质正常；1分，肾小管间质损伤区域占视野比例<10%；2分，损伤区域占视野10%~<25%；3分，损伤区域占视野25%~<50%；4分，损伤区域占视野50%~<75%；5分，损伤区域占视野≥75%。

1.3.5 免疫组织化学检测 将石蜡切片进行梯度脱蜡后采用EDTA抗原热修复法进行抗原修复，并采用3%过氧化氢封闭过氧化物酶。用10%山羊血清PBS溶液进行封闭后，4 ℃下一抗孵育过夜。复温后PBST洗涤，孵育二抗后再次PBST洗涤。使用DAB显色液进行显色后苏木精染液复染，最后进行梯度脱水，并用中性树胶封片。镜下观察并拍摄。

1.3.6 肾脏TUNEL染色 石蜡切片经过梯度脱蜡后用蛋白酶K进行预处理，PBS漂洗后滴加平衡液平衡。用末端转移酶孵育1 h后加入反应终止液终止反应。PBS漂洗后以地高辛抗体孵育，最后DAPI染核并封片。采用荧光显微镜观察并拍摄。蓝色荧光为细胞核，核内有绿色荧光颗粒的细胞为凋亡阳性细胞。每组小鼠高倍镜（×400）下随机选取20个不同视野进行拍摄，对视野内凋亡阳性细胞进行定量

计数并作统计分析。

1.3.7 肾组织SOD活性、MDA水平检测 肾皮质组织研磨匀浆后，采用WST-8（一种水溶性四唑盐）法检测SOD活性，采用硫代巴比妥酸（TBA）法检测肾组织MDA含量。所有检测步骤严格按照试剂盒说明书进行。

1.3.8 Western blotting检测蛋白表达量 称取适量肾皮质组织研磨匀浆，二喹啉甲酸（BCA）法测定蛋白浓度。取30 μg蛋白样品通过10%聚丙烯酰胺凝胶电泳分离，半干转法转膜至PVDF膜。采用5%牛血清白蛋白（BSA）的TBST溶液封闭后4 ℃一抗孵育过夜，洗涤，二抗孵育后再次洗涤。采用ECL发光液上机显影并拍摄。使用ImageJ软件对蛋白条带进行灰度值定量，以内参蛋白β-actin或其他合适蛋白进行标准化，结果表示为条带相对灰度。

1.4 统计学分析

使用GraphPad Prism 9.0软件和SPSS 22.0软件对数据进行统计分析和绘图。定量资料表示为$\bar{x}\pm s_{\bar{x}}$，并采用Shapiro-Wilk检验和Levene检验进行正态性和方差齐性检验，正态分布且符合方差齐性的数据采用双因素方差分析（Two-way ANOVA）和Tukey多重比较法进行组间差异性分析，不满足正态分布或方差齐性的数据采用Kruskal-Wallis秩和检验和Dunn多重比较法进行组间非参数统计。P<0.05表示差异具有统计学意义。

2 结果

2.1 YC-1对db/db小鼠肾功能指标的影响

研究结果显示，在小鼠18周龄时，DB组小鼠的体质量和血糖显著高于WT组小鼠。处死小鼠并进行体液检测后，我们发现DB组小鼠肾/体质量比、Scr、UACR和尿NGAL水平显著升高。在连续使用8周YC-1腹腔注射干预后，DB+YC-1组小鼠肾/体质量比、Scr、UACR和尿NGAL水平显著下降。同时，YC-1的干预未对db/db小鼠的体质量和血糖产生影响。对于WT小鼠，YC-1没有造成小鼠血糖、体质量和各项肾功能指标的明显变化。以上结果表明，YC-1能够显著改善db/db小鼠肾功能的恶化（表1）。

表1 YC-1对WT和db/db小鼠一般情况和肾功能指标的影响（n=6）

Tab 1 Effect of YC-1 on general physical signs and kidney function indexes in the WT and db/db mice（n=6）

Index	WT group	WT+YC-1 group	DB group	DB+YC-1 group
RBG/(mmol·L^{-1})	8.10±0.39	8.25±0.43	28.57±1.18①	29.15±1.29①
BW/g	27.47±0.58	25.98±0.65	56.72±1.05①	54.53±1.02①
KW/BW/(mg·g^{-1})	9.97±0.23	10.19±0.23	12.25±0.22①	11.31±0.14①②
Scr/(mg·dL^{-1})	0.239±0.010	0.263±0.012	0.473±0.017①	0.414±0.016①③
UACR/(μg·mg^{-1})	61.68±12.18	67.46±10.71	1 445.61±63.10①	663.94±60.19①②
uNGAL/(μg·mL^{-1})	23.70±1.09	23.04±0.95	118.66±2.98①	86.02±3.44①②

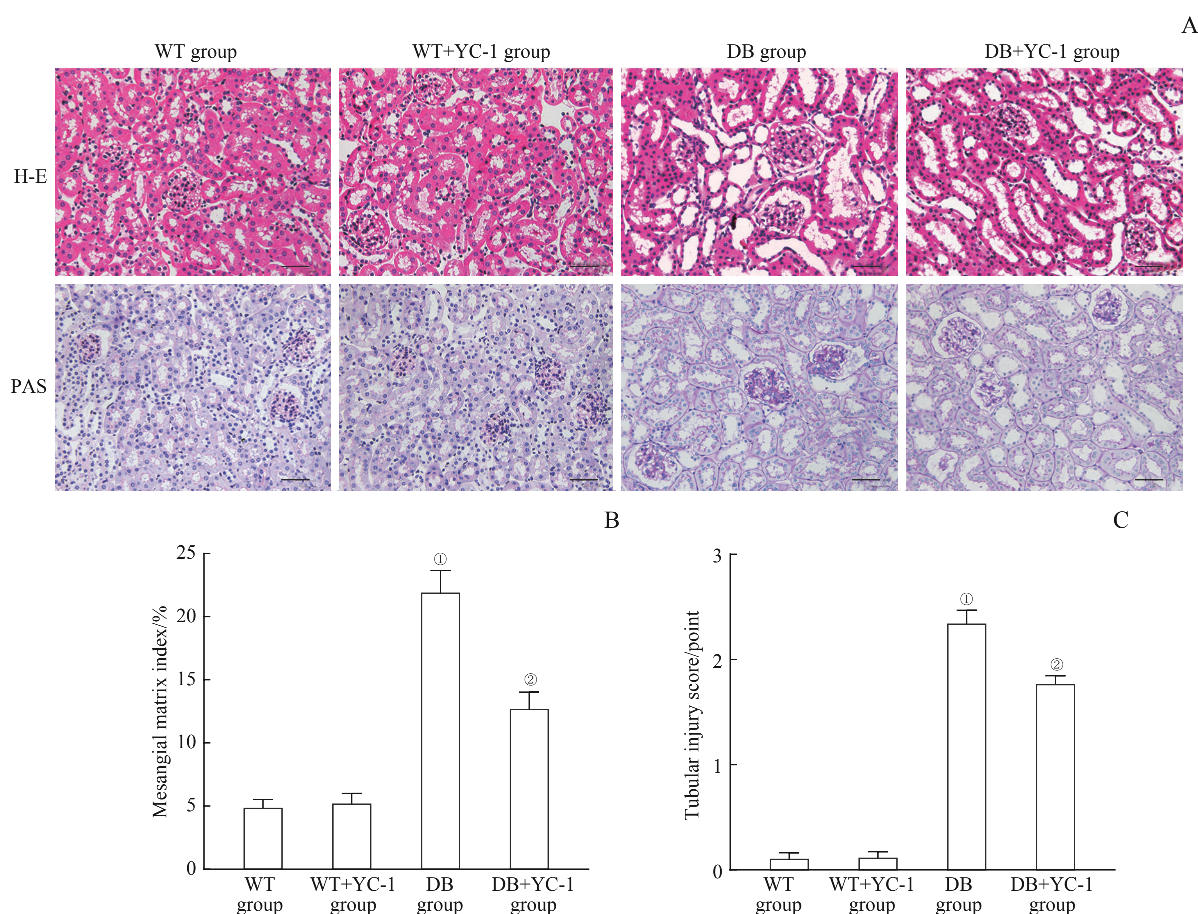
Note: RBG—random blood glucose; BW—body weight; KW/BW—kidney weight-to-body weight ratio; uNGAL—urine NGAL. ①P=0.000, compared with the WT group; ②P=0.023, ③P=0.039, compared with the DB group.

2.2 YC-1对db/db小鼠肾脏病理损伤的影响

组织形态方面，H-E和PAS染色显示db/db小鼠出现了明显的肾小球基底膜增厚、系膜基质增生硬化、肾小管损伤和间质炎症细胞浸润，肾小球系膜基质评分和肾小管损伤评分相较WT小鼠明显升高；YC-1的干预显著减轻了db/db小鼠的肾脏病理损伤，降低了肾小球系膜基质评分和肾小管损伤评分（图1）。

2.3 YC-1对db/db小鼠肾脏纤维化的影响

肾脏纤维化是DN重要的病理特征，也是DN向ESRD进展的关键事件，因此我们检测了YC-1对db/db小鼠肾脏纤维化的影响（图2）。Masson染色显示，db/db小鼠肾小管间质胶原纤维明显增多，YC-1的干预改善了胶原纤维沉积。Ⅰ型胶原蛋白是肾脏胶原纤维的重要成分，免疫组织化学（免疫组化）结果显示db/db小鼠肾脏Ⅰ型胶原蛋白阳性面积显著增加，而YC-1的干预减少了Ⅰ型胶原蛋白的表达。



Note: A. Renal histology evaluations of different groups were performed with H-E staining and PAS staining (×200, scale bar=50 μm). B. Mesangial matrix index expressed as the ratio of mesangial matrix area to glomerular tuft area. C. Analysis of tubular injury score. ①P=0.000, compared with WT group; ②P=0.000, compared with DB group.

图1　YC-1对db/db小鼠肾脏组织病理损伤的影响

Fig 1　Effect of YC-1 on kidney histopathology injuries in the db/db mice

α-SMA是间质特异性蛋白，也是肾脏纤维化的重要标志物。Western blotting结果表明，db/db小鼠肾脏α-SMA含量相较WT小鼠显著增多，而YC-1能够下调α-SMA的蛋白表达。以上结果提示，YC-1能够延缓db/db小鼠肾脏纤维化。

2.4　YC-1对db/db小鼠肾脏HIF-1α表达的影响

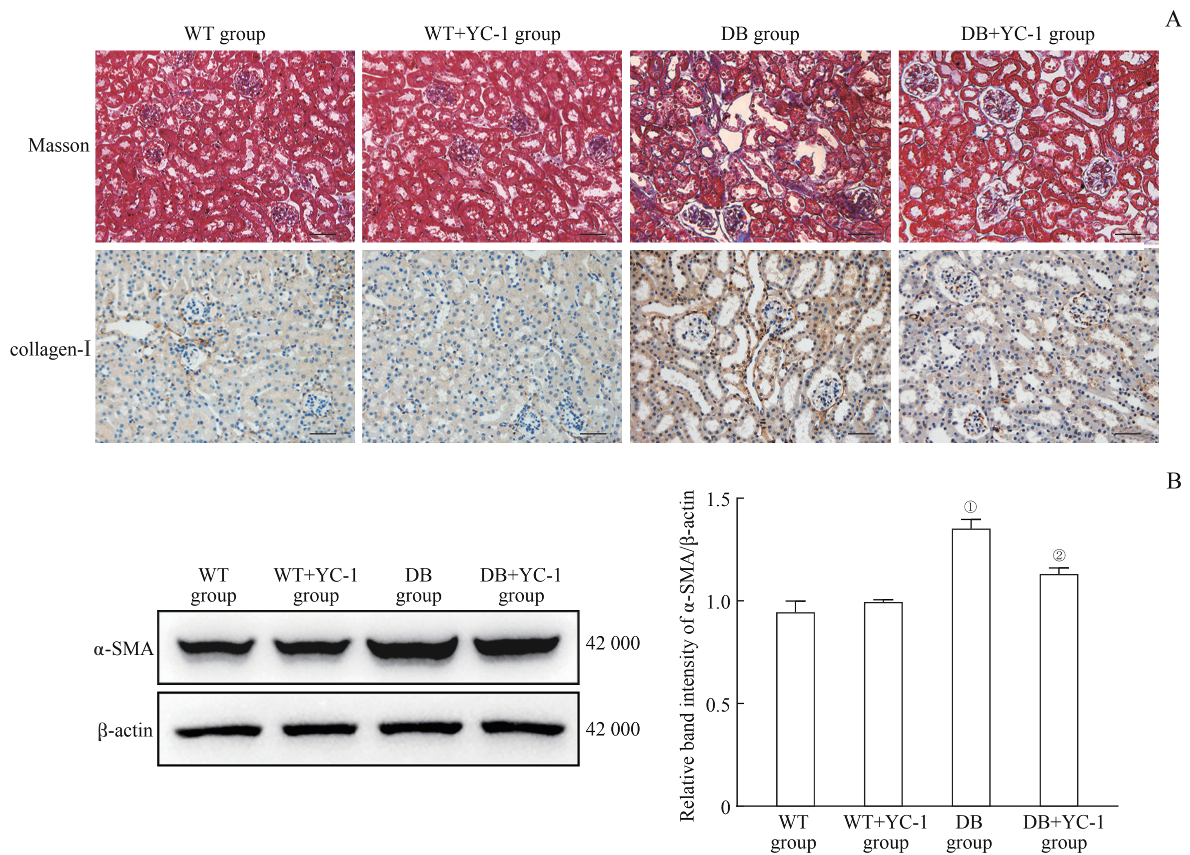
YC-1是HIF-1α特异性抑制剂。为了明确YC-1的干预对肾脏HIF-1α的抑制作用，我们通过免疫组化和Western blotting检测了HIF-1α的表达。结果证实，YC-1的干预使WT小鼠和db/db小鼠肾脏HIF-1α的表达均显著下降（图3）。

2.5　YC-1对db/db小鼠肾脏细胞凋亡的影响

细胞凋亡是DN组织损伤和肾功能恶化的重要表现和诱因。TUNEL染色显示，db/db小鼠肾脏TUNEL染色阳性细胞数量明显增多，而YC-1的干预显著减少了凋亡细胞数量（图4A）。Western blotting显示，db/db小鼠促凋亡蛋白cleaved caspase-3和BAX表达显著升高、抗凋亡蛋白BCL-2表达显著降低，YC-1的干预逆转了凋亡相关蛋白的异常表达（图4B）。这些结果提示，YC-1能够降低db/db小鼠肾脏细胞凋亡水平。

2.6　YC-1对db/db小鼠肾脏氧化应激的影响

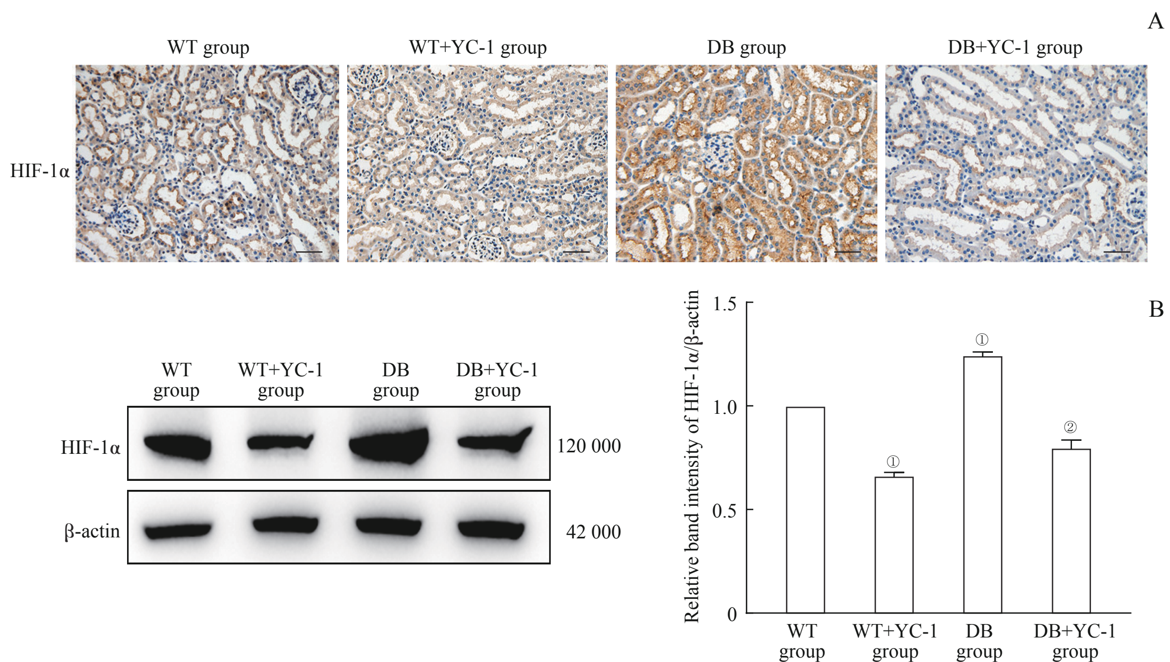
MDA是氧化应激诱导脂质过氧化产生的最终产物，而SOD是体内重要的氧自由基清除剂，MDA和SOD间的失衡能够反映细胞氧自由基代谢水平。我们发现，相较于WT小鼠，db/db小鼠肾脏MDA含量显著上升，SOD活性显著下降；YC-1的干预能够恢复MDA的异常生成和SOD的酶活性（表2）。这提示YC-1能够显著改善db/db小鼠肾脏氧化应激反应。



Note: A. Extracellular matrix accumulation and collagen fiber deposition were determined with Masson trichrome staining and immunohistochemical staining of collagen-Ⅰ (×200, scale bar=50 μm). B. Western blotting analysis and quantification of the expression of the profibrotic molecule α-SMA. ①P=0.000, compared with the WT group; ②P=0.009, compared with the DB group.

图2　YC-1对db/db小鼠肾脏纤维化的影响

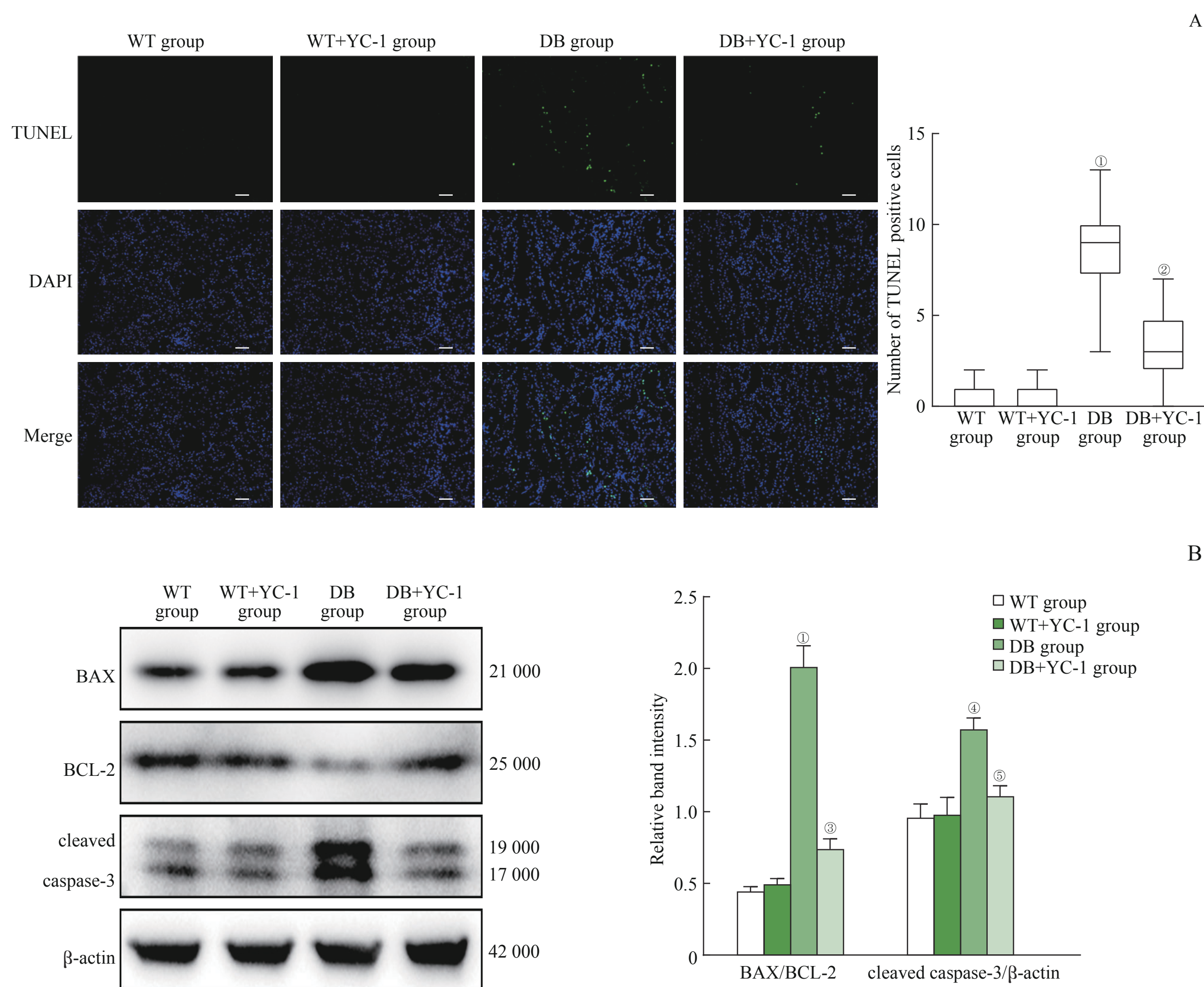
Fig 2　Effect of YC-1 on kidney fibrosis in the db/db mice



Note: A. Immunohistochemical staining of HIF-1α in kidney tissues (×200, scale bar=50 μm). B. Western blotting analysis and quantification of HIF-1α expression. ①P=0.000, compared with the WT group; ②P=0.000, compared with the DB group.

图3　YC-1对db/db小鼠肾脏HIF-1α表达的影响

Fig 3　Effect of YC-1 on HIF-1α expression in the kidneys of db/db mice



Note: A. Representative TUNEL staining photographs (×200, scale bar=50 μm) and quantification analysis of TUNEL positive cells under each high power field. The data are presented as boxplot. B. Western blotting analysis and quantification of the expression of BAX, BCL-2, and cleaved caspase-3. ①*P*=0.000, ④*P*=0.004, compared with the WT group; ②*P*=0.011, ③*P*=0.000, ⑤*P*=0.021, compared with the DB group.

图4　YC-1对db/db小鼠肾脏细胞凋亡的影响

Fig 4　Effect of YC-1 on cell apoptosis in the kidneys of the db/db mice

表2　YC-1对db/db小鼠肾脏氧化应激的影响（*n*=6）

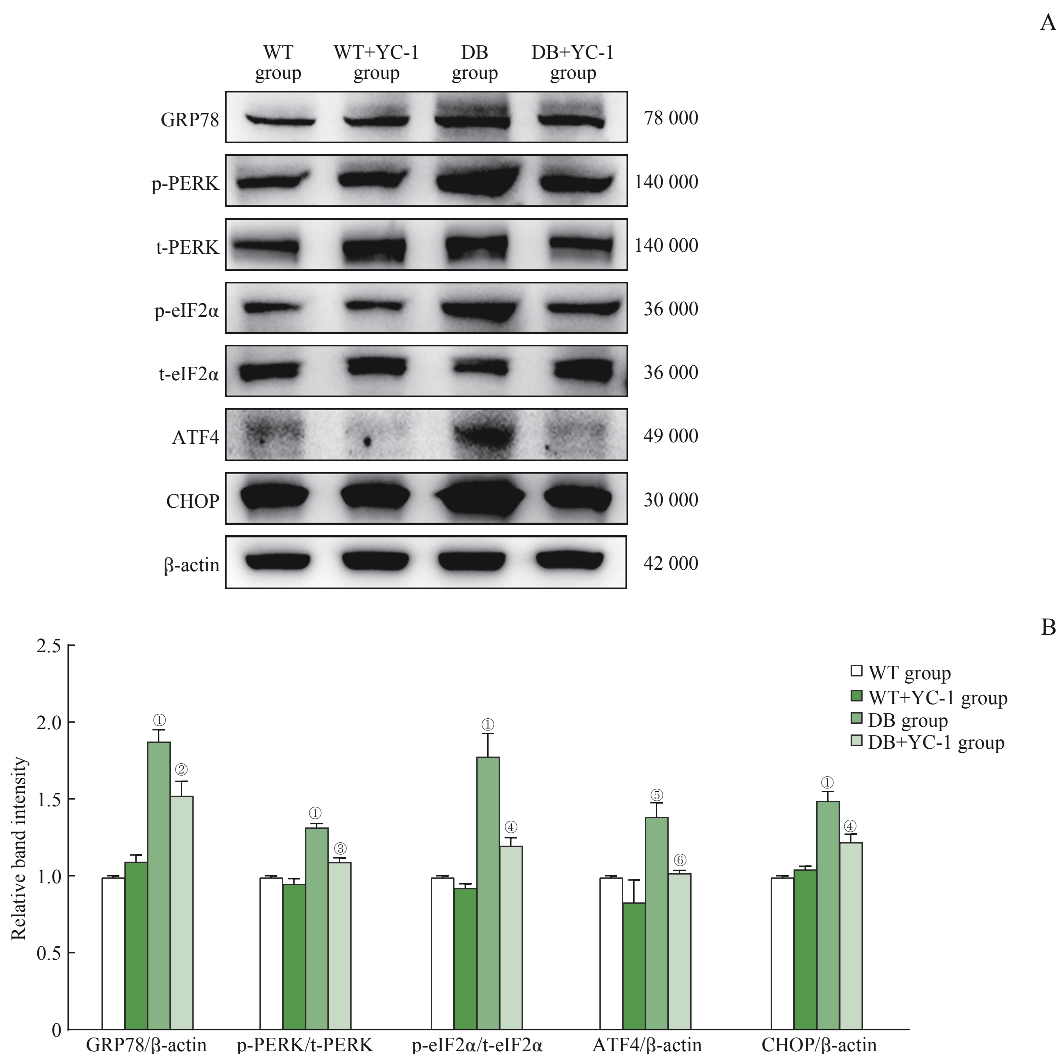
Tab 2　Effect of YC-1 on oxidative stress in the kidneys of db/db mice（*n*=6）

Index	WT group	WT+YC-1 group	DB group	DB+YC-1 group
MDA level/(nmol·mg^{-1})	6.69±0.53	5.78±0.82	14.05±1.56①	9.65±0.71②
SOD activity/(U·mg^{-1})	117.28±11.90	115.29±6.72	41.71±3.19①	79.40±2.52③

Note: ①*P*=0.000, compared with the WT group; ②*P*=0.024, ③*P*=0.007, compared with the DB group.

2.7　YC-1对db/db小鼠肾脏内质网应激的影响

内质网应激（endoplasmic reticulum stress，ERS）是细胞凋亡的重要诱因，与DN的进展密切相关[10]。因此，我们进一步检测了YC-1对db/db小鼠肾脏ERS的影响。Western blotting结果显示，db/db小鼠ERS标志物GRP78、p-PERK、p-eIF2α、ATF4和CHOP表达显著升高，YC-1的干预显著下调了小鼠肾脏各ERS标志物的异常表达（图5）。



Note：A. Representative Western blotting bands. t-PERK—total-PERK; t-eIF2α—total-eIF2α. B. Quantification of Western blotting bands. ①*P*=0.000, ⑤*P*=0.035, compared with the WT group; ②*P*=0.011, ③*P*=0.000, ④*P*=0.002, ⑥*P*=0.048, compared with the DB group.

图5　YC-1对db/db小鼠肾脏ERS的影响

Fig 5　Effect of YC-1 on ERS in the kidneys of db/db mice

3　讨论

DN的发病机制复杂，涉及高糖、晚期糖基化终末产物（advanced glycation end products，AGE）累积、肾素血管紧张素系统（renin-angiotensin system，RAS）激活，以及炎症、氧化应激等多因素的共同作用[2]。这些病理因素使肾小球毛细血管受损，从而影响输氧通路，最终导致肾脏的广泛缺氧状态[3]。缺氧不仅是造成肾脏结构受损和肾功能恶化的关键因素，也是推动肾脏各部分损伤加重，进展为ESRD的最终途径[11]。此时，肾脏细胞将激活一系列适应性反应以应对摄氧量的减少。

HIF是细胞适应缺氧以及调节氧稳态的重要转录因子[4]。它由HIF-α和HIF-β 2个亚基组成，其中HIF-α是其活性亚基。常氧状态下，HIF-α受到脯氨酰羟化酶（prolyl hydroxylase，PHD）和泛素-蛋白酶体途径的调控；缺氧状态下，HIF-α的降解过程受阻，稳定的HIF-α能够入核与HIF-β装配，形成具有转录因子活性的HIF[4]。HIF-α有3种不同的亚型：HIF-1α、HIF-2α、HIF-3α。其中，HIF-1α是细胞缺氧适应性反应的驱动因子，在缺氧时最先激活并可激活下游一系列的代谢反应[4,12]。肾脏是HIF-1α高度富集的器官，在肾小管的表达尤其广泛[12]。HIF-1α在肾脏中的功能具有两面性：一方面，HIF-1α能够改善肾脏代谢，增强细胞对缺氧的适应力和修复能力；另一方面，HIF-1α通过促炎、促纤维化机制加重CKD的进展[5,12]。

既往研究[5,13]表明，HIF-1α与DN的进展密切

相关，能够通过多种途径参与肾小球和肾小管的损伤过程。DN患者和DN动物模型肾脏中HIF-1α表达均明显升高[5]。异常激活的HIF-1α将诱导足细胞发生上皮-间充质转化（epithelial-mesenchymal transition，EMT）、细胞骨架紊乱、足突消失和裂隙隔膜功能障碍，促使足细胞功能受损[13]。在小管间质中，HIF-1α能够激活炎症因子和氧化应激，促进肾小管EMT和间质广泛纤维化，并增加DN小鼠单侧输尿管梗阻（unilateral ureteral obstruction，UUO）模型的病理损伤[14-16]。此外，HIF-1α能够促进巨噬细胞糖酵解，进一步加重DN肾脏炎症和纤维化[17]。加权基因共表达网络分析（weighted correlation network analysis，WGCNA）显示，DN患者肾小管间质HIF-1α表达与其估计肾小球滤过率（estimated glomerular filtration rate，eGFR）呈负相关，与纤维化信号呈正相关[18]。本研究采用HIF-1α抑制剂YC-1对db/db小鼠进行干预，观察阻断HIF-1α对DN病程发展的影响。我们发现，经YC-1干预的db/db小鼠肾功能和病理损伤明显改善，同时肾脏纤维化程度减轻，细胞凋亡水平下降，这提示HIF-1α参与了DN进展过程，能够诱导肾小管损伤和间质纤维化。YC-1能够直接阻断HIF-1α通路发挥肾脏保护作用，延缓DN进展，这与既往研究结果[19]相一致。实际上，HIF-1α作为DN的潜在治疗靶点已逐渐受到人们的关注。如近期研究[20]发现，新型降糖药钠-葡萄糖协同转运蛋白2（sodium-dependent glucose transporters 2，SGLT2）抑制剂能够通过逆转DN诱导的HIF-1α和HIF-2α失衡，缓解肾脏缺氧和氧化应激，抑制炎症和纤维化。

HIF-1α的激活与氧化应激密切相关。活性氧（reactive oxygen species，ROS）是常氧状态下维持HIF-1α稳定的重要原因，能够抑制PHD活性并激活磷脂酰肌醇3-激酶（phosphatidylinositide 3-kinase，PI3K）/蛋白激酶B（又称AKT）和细胞外调节蛋白激酶（extracellular regulated protein kinase，ERK）通路阻断HIF-1α的降解[21]；HIF-1α的累积将促进烟酰胺腺嘌呤二核苷酸磷酸氧化酶4（nicotinamide adenine dinucleotide phosphate oxidase 4，NOX4）的转录，进一步驱动ROS的生成[22]。我们的研究进一步证实，YC-1能够调节db/db小鼠肾脏MDA和SOD的失衡，抑制氧化应激，发挥对肾脏的保护作用。

ERS是导致DN发生和进展的重要致病机制[10]。在高血糖、AGE、RAS激活等各种病理因素作用下，内质网未折叠或错误折叠蛋白质堆积并触发未折叠蛋白反应（unfolded protein response，UPR），导致内质网分子伴侣GRP78与下游传感器分离，从而激活下游3条ERS信号通路：PERK/eIF2α/ATF4通路、肌醇需求酶-1α（inositol requiring enzyme-1 α，IRE-1α）/X-盒结合蛋白1（X-box binding protein 1，XBP1）通路和ATF6通路[10,23]。适度的ERS有助于恢复细胞稳态，但ERS的过度激活是细胞凋亡的关键诱因。PERK通路是UPR重要的促凋亡通路，能够激活下游重要促凋亡因子CHOP，启动内源性凋亡途径[23]。既往研究发现，HIF-1α与ERS可能具有直接联系。HIF-1α能够激活ERS促进间歇性缺氧诱导的心肌细胞凋亡[24]；此外，HIF-1α诱导的ERS被发现是导致主动脉血管重塑以及特发性肺纤维化等疾病的重要机制[25-26]。目前在肾脏病机制研究中尚无有关HIF-1α与ERS互作调控的报道。本研究揭示了DN中HIF-1α对ERS的调控作用，我们发现YC-1的干预可显著抑制PERK/eIF2α/ATF4/CHOP通路的激活，改善db/db小鼠肾脏ERS反应。HIF-1α诱导ERS的机制可能是通过发挥转录活性，促进凋亡相关基因的表达从而激活ERS[27]；也有研究[28]表明HIF-1α能够直接参与PERK通路，与ATF4发生交互协同作用。因此，HIF-1α可能是DN环境下诱导ERS的关键分子，能够直接参与DN细胞凋亡过程。

综上，HIF-1α抑制剂YC-1能够改善db/db小鼠肾脏氧化应激和ERS的异常激活，抑制细胞凋亡和肾脏纤维化，延缓肾脏病理损伤和肾功能下降。本研究揭示了HIF-1α作为肾脏中高度表达的缺氧适应性分子，对DN进展可能产生的不良影响，这将为后续的临床和药理学研究提供更多的启发和警示。

利益冲突声明/Conflict of Interests

所有作者声明不存在利益冲突。

All authors disclose no relevant conflict of interests.

伦理批准和动物权利声明/Ethics Approval and Animal Right

本实验涉及的所有动物实验均通过上海交通大学医学院附属第六人民医院动物伦理委员会审批（文件号：2021-0183）。所有实验操作均符合实验动物学3R原则。

All experimental animal protocols in this study were reviewed and approved by the Laboratory Animals Ethical Committee of Shanghai Sixth People's Hospital, Shanghai Jiao Tong University School of Medicine (Approval No. 2021-0183), and all the experimental operations were compliant with the laboratory animal rules of 3R.

作者贡献/Authors' Contributions

范瑛、汪年松参与实验设计，贾君杰、邢海帆、张群子参与实验操作，贾君杰、刘奇烨参与数据分析，贾君杰、范瑛参与论文写作和修改。所有作者均阅读并同意了最终稿件的提交。

The study was design by FAN Ying and WANG Niansong. The experimental operations were completed by JIA Junjie, XING Haifan, and ZHANG Qunzi. The data were analysed by JIA Junjie and LIU Qiye. The manuscript was drafted and revised by JIA Junjie and FAN Ying. All the authors have read the last version of paper and consented for submission.

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