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·基础研究·

caveolin-1 表达在糖尿病大鼠下肢缺血中的作用及其与 eNOS/NO 通路的关系

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摘 要

背景与目的：研究表明 caveolin-1 可促进血管新生，并与糖尿病病理过程密切相关。本研究推测 caveolin-1 可能在糖尿病下肢缺血中发挥作用，并在糖尿病大鼠急性下肢缺血模型实验中探讨其作用及机制。

方法：选用 80 只健康雄性 SD 大鼠，应用 STZ 法构建 1 型糖尿病大鼠模型，取建模成功的大鼠随机分为 4 组，分别行单纯股动脉游离（假手术组）、股动脉及分支离断（模型组）、股动脉及分支离断 + 尾静脉注射含 caveolin-1 的质粒（转染组）、股动脉及分支离断 + 尾静脉注射不含 caveolin-1 质粒脂质体溶液（空转组）。于术后 14 d 取各组大鼠腓肠肌组织标本，HE 染色观察组织形态及炎性细胞浸润情况；ELISA 法检测组织中 NO 水平；免疫组织化学染色检测组织中 caveolin-1、eNOS 和 CD34 标记的微血管密度（MVD）。

结果：与假手术组比较，模型组及空转组的腓肠肌组织萎缩明显，组织中有较多的炎症细胞浸润，转染组肌肉萎缩不明显，炎症细胞浸润较少；模型组和空转组的腓肠肌组织中 NO 表达明显降低，转染组 NO 表达明显增加（均 $P<0.01$）；模型组、空转组、转染组 caveolin-1 表达均明显升高，转染组升高更为明显（均 $P<0.01$）；模型组和空转组 eNOS 表达无明显变化（均 $P>0.05$），转染组 eNOS 表达明显升高（$P<0.01$）；模型组、空转组、转染组 MVD 均有增加，转染组升高更为明显（均 $P<0.01$）；以上指标在模型组与空转组间的差异均无统计学意义（均 $P>0.05$）。

结论：caveolin-1 高表达对糖尿病下肢缺血有明显改善作用，其机制可能是通过活化 eNOS/NO 通路，从而促进 NO 生成有关。

关键词 缺血；下肢；糖尿病血管病变；窖蛋白 1；大鼠

中图分类号：R654.3

Effects of caveolin-1 expression on lower limb ischemia in diabetic rat and its association with eNOS/NO pathway

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Abstract

Background and Aims: Studies have demonstrated that caveolin-1 is associated increased angiogenesis, and also closely related to the pathological process of diabetes. Based on speculation that caveolin-1 may play a role in diabetic lower limb ischemia, this study conducted an experiment in acute lower limb ischemia in diabetic rats to investigate its effect and mechanism.

Methods: Eighty healthy male SD rats were used to construct diabetes models by STZ induction. Then, rats with successful modeling were randomly divided into 4 groups, and underwent femoral artery isolation only (sham operation group), division of the femoral artery and its branches (model group), division of the femoral artery and its branches plus tail vein injection of plasmid containing caveolin-1 (transfection group), and division of the femoral artery and its branches plus tail vein injection of liposome solution without caveolin-1-bearing plasmid (empty transfection group), respectively. After 14 days, gastrocnemius muscle tissues in rats of each group were harvested, the morphological alterations and infiltration of inflammatory cells were observed by HE staining, the NO level was detected by ELISA assay, and the expressions of caveolin-1 and eNOS as well as the CD34 marked microvascular density (MVD) were determined by immunohistochemical staining, respectively.

Results: Compared with sham operation group, obvious atrophy and marked inflammatory cell infiltration of the gastrocnemius muscle tissue were observed in model group and empty transfection group, while the muscle atrophy was not obvious and inflammatory cell infiltration was attenuated in transfection group; the NO levels in the gastrocnemius muscle tissue of model group and empty transfection group were significantly decreased, while the NO level in transfection group was significantly increased (all $P<0.01$); the expression levels of caveolin-1 in model group, empty transfection group and transfection group were all increased, but the increasing amplitude in transfection group was significantly greater (all $P<0.01$); the expression levels of eNOS showed no significant differences in model group and empty transfection group (both $P>0.05$), but was significantly increased in transfection group ($P<0.01$); the MVD values in model group, empty transfection group and transfection group were all increased, but the increasing amplitude in transfection group was significantly greater (all $P<0.01$). All differences in above parameters showed no statistical significance between model group and empty transfection group (all $P>0.05$).

Conclusion: The high expression of caveolin-1 can effectively improve the diabetic lower limb ischemia, and its mechanism may be related to its activating eNOS/NO pathway and thereby increasing the NO production.

Key words　Ischemia; Lower Extremity; Diabetic Angiopathies; Caveolin 1; Rats

CLC number: R654.3

糖尿病下肢血管病变是糖尿病患者常见的并发症之一[1]，约占糖尿病并发症的20%，是导致患者截肢的主要原因，严重影响糖尿病患者的生命健康[2]。而目前无论药物、外科转流术以及介入治疗远期疗效都不能令人满意[3-4]。近年来，通过促血管新生改善缺血部位血液循环，已成为治疗糖尿病下肢缺血重要方法[5-7]。小凹蛋白1（caveolin-1）是一种信号转导蛋白，通过分子表面的“脚手架”区能与多种信号分子相互作用，在多种疾病中起着重要调控作用[8-9]。有研究报道，caveolin-1可促进血管新生，并参与糖尿病病理过程[10-11]，笔者推测caveolin-1可能在糖尿病下肢缺血中发挥着重要作用，因此，本研究主要探讨caveolin-1对糖尿病下肢缺血部位血管新生的作用及机制。

1　材料与方法

1.1　实验材料

1.1.1　实验动物　80只健康SD大鼠，雄性，（200±20）g，8~10周，从南华大学动物实验部购买。

1.1.2　实验仪器　SZ-93自动双重纯水蒸馏器（亚荣，上海）；三用恒温水浴箱（中国）；Eppendorf型高速离心机（5804型、5804R 德国）；ELX800型酶标仪（BioTEk，美国）；微量移液

器（Select Bioproducts，美国）；OLYMPUS显微镜与显微摄像系统（BX51型，日本）；Sartorius电子天平（BSA223S，美国）；Sanyo –80 ℃冰箱（日本）。

1.1.3 实验试剂 pCDNA3.1（+）caveolin-1（南华大学药物药理研究所赠送）；链脲菌素（STZ，Sigma，美国）；兔抗大鼠caveolin-1抗体（博奥森生物，中国）；兔抗大鼠eNOS抗体（博奥森生物，中国）；NO检测试剂盒（建成生物公司，中国）；DAB显色试剂盒（艾杰生物，中国）；柠檬酸三钠（大茂化学，中国）；柠檬酸（大茂化学，中国）；青霉素（鲁抗医药，中国）。

1.2 实验方法

1.2.1 1型糖尿病大鼠模型建立 根据文献[12]方法，大鼠禁食12 h后，将配好的STZ溶液（0.1 mol/L）经腹腔快速注射入大鼠体内，剂量为50 mg/kg。分别术后第3、7、14、21天采尾静脉血测空腹血糖，当血糖浓度均高于16.8 mmol/L为建模成功，最终有44只大鼠造模成功。

1.2.2 实验分组 根据随机化原则，将糖尿病模型大鼠分为4组：假手术组、手术缺血组（模型组）、手术缺血空转染组（空转组）、缺血+caveilin-1转染组（转染组），每组11只。假手术组则仅将右侧腹股沟皮肤切开，暴露股动脉后缝合，不离断股动脉；模型组单纯结扎离断股动脉及分支；空转染组在模型组的基础上，通过尾静脉注射不含caveolin-1质粒的脂质体溶液；转染组为股动脉离断术后，从大鼠尾静脉注射含caveolin-1的质粒进行转染。

1.2.3 建立糖尿病大鼠急性下肢缺血模型 建模方法：各组大鼠应用3%戊巴比妥钠以（1.5 mL/Kg）腹腔注射麻醉，麻醉成功后，将大鼠固定于手术台，在右侧腹股沟区做一2 cm纵行切口，逐层分离并游离股动脉的上下端，除假手术组外，各组于股动脉起始部至股动脉末端结扎离断，避免损伤并行的静脉及神经，依次缝合切口（图1）。于术后14 d处死大鼠，取术侧腓肠肌组织进行下一步检测。

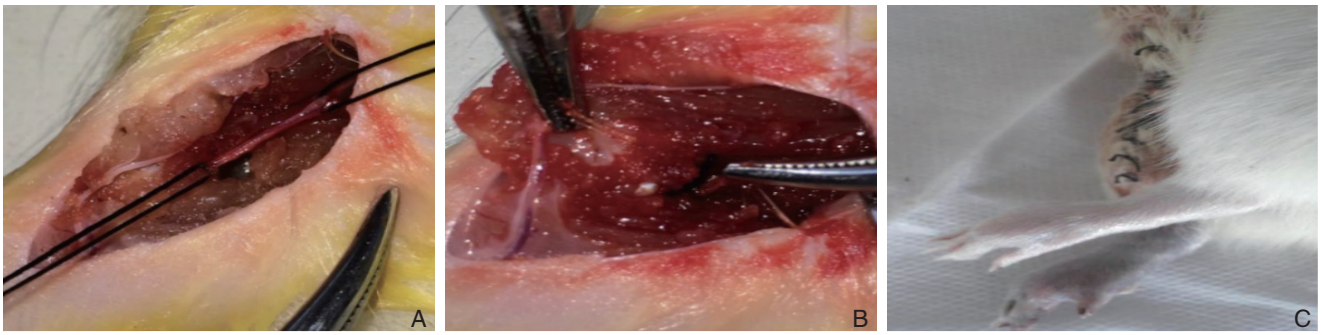

图1 建立大鼠下肢缺血模型 A：分离股动脉；B：结扎股动脉及分支；C：术后大鼠出现肢体发绀

Figure 1 Establishment of rat model of limb ischemia A: Separation of the femoral artery; B: Ligation of the femoral artery and the branches; C: Limb cyanosis after operation

1.2.4 Caveolin-1质粒转染方法 备好的质粒和lipofectamine™2000（Invitrogen，美国）按1:3的比例稀释于2个无血清培养基中，室温静置4 min后两者混合，再放置20 min备用。空转染组：尾静脉注射不含质粒的脂质体溶液；转染组：通过尾静脉注射含有caveolin-1的质粒的脂质体溶液进行转染；每只浓度为caveolin-1 5×10^{9} pfu。

1.2.5 ELISA法检测肌肉组织中NO的表达 术后14 d处死各组大鼠后，取出右下肢腓肠肌组织捣碎，高速离心后取上清液100 μL，根据试剂盒说明书，采用硝酸还原酶法检测一氧化氮（nitric oxide，NO）的含量，试验重复3次取平均值。

1.2.6 缺血组织标本检测 术后第14 d处死各组大鼠取右下肢腓肠肌标本，剥离周围的肌腱和脂肪组织进行HE染色；免疫组化检测caveolin-1和eNOS蛋白的表达。用不同倍数光学显微镜（40、100、200倍）观察切片，选取5个视野观察肌纤维形态学变化，并拍照留下图像。使用Image J 1.52V软件对免疫组化样本图像进行分析，测其平均光密度值（average optical density，AOD）进行半定量分析。

1.2.7 微血管计数（MVD） 腓肠肌组织进行CD34免疫组化检测，以染成棕黄色的单个内皮细胞或内皮细胞簇作为1个血管计数，在低倍镜

下（40 倍）选择微血管密度最高的 3 个区域，在高倍镜（200 倍）下计数微血管数，取其平均数为该标本的微血管数。

1.3 统计学处理

采用SPSS 22.0软件对数据进行统计学分析，计量资料以均数 ± 标准差（$\bar{x} \pm s$）表示，两组间的比较采用独立样本的t检验，P<0.05为差异有统计学意义。

2 结　果

2.1 一般情况

大鼠在注射STZ溶液后第3~4天开始出现饮水量、尿量、摄食量明显增加，体质量逐渐减轻、活动量降低、皮毛干燥无光泽。急性下肢缺血模型建立后，大鼠出现下肢皮肤温度降低、紫绀等症状，大鼠后肢屈曲，走路不稳，手术侧肌肉肌肉萎缩，肌张力明显弱于健侧，在观察期间转染组和模型组各死亡1只大鼠，死亡原因考虑为糖尿病酮症酸中毒和（或）切口感染所致。

2.2 HE 染色观察腓肠肌组织形态及炎细胞浸润情况

取各术侧组腓肠肌组织进行HE染色，将玻片放入不同倍数的显微镜下观察，腓肠肌细胞形无明显充血水肿，模型组及空转染组有多量炎症细胞浸润，且肌肉组织萎缩明显，转染组炎症细胞浸润较少，肌肉萎缩不明显（图2）。

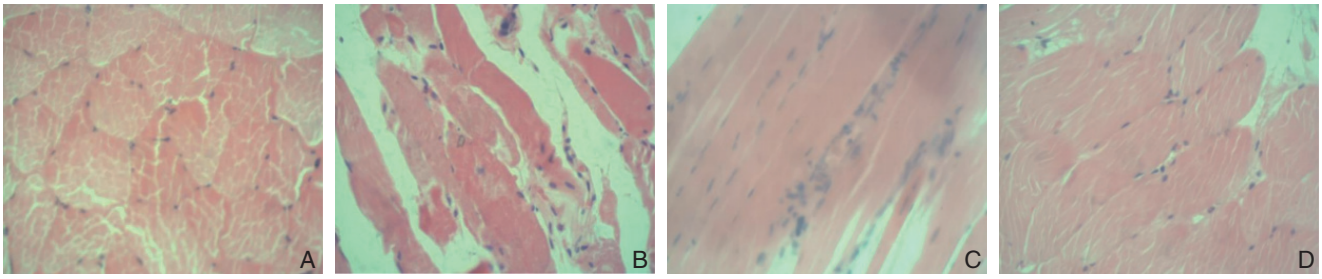

图 2　各组腓肠肌组织 HE 染色（×200）　A：假手术组；B：模型组；C：空转组；D：转染组

Figure 2　HE staining of gastrocnemius muscle tissue of each group (×200)　A: Sham operation group; B: Model group; C: Empty transfection group; D: Transfection group

2.3 ELISA 检测各组 NO 表达变化

NO在各组均有一定程度的表达，其中模型组、空转染组中的NO的表达低于假手术组（均P<0.01）；而转染组NO水平明显高于其他各组（均P<0.01），模型组与空转组比较，差异无统计学意义（P>0.05）（表1）。

表 1　各组腓肠肌肌肉组织中 NO 的浓度（$\bar{x} \pm s$）

Table 1　Concentrations of NO in gastrocnemius muscle tissues of each group ($\bar{x} \pm s$)

组别	n	NO（μmol/L）
假手术组	11	19.2476 ± 1.89
模型组	10	15.7433 ± 1.34[1)]
空转组	11	15.5737 ± 1.37[1)]
转染组	10	42.2661 ± 3.56[1), 2)]

注：1）与假手术组比较，P<0.01；2）与模型组比较，P<0.01

Note: 1) P<0.01 *vs.* sham operation group; 2) P<0.01 *vs.* model group

2.4 免疫组化染色检测腓肠肌组织中 caveolin-1 和 eNOS 的表达

P染色法检测各组腓肠肌组织中caveolin-1和eNOS的表达情况，并用Image J 1.52V图像分析软件进行半定量分析。结果显示，caveolin-1在各组均有表达，但转染组标本中caveolin-1蛋白的表达水平明显高于其他各组（P<0.01）；而与假手术组比较，模型组、空转染组caveolin-1蛋白的表达升高（P<0.01）；模型组与空转组比较，差异无统计学意义（P>0.05）（图3）（表2）。同样方法检测eNOS蛋白，结果显示，假手术组、空转染组和模型组的eNOS表达无统计学意义（P>0.05），而转染组的eNOS表达明显高于各组（P<0.01）（图4）（表3）。

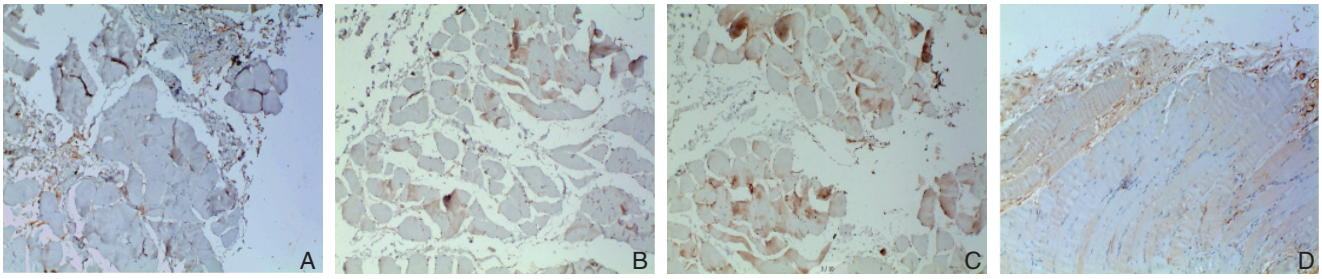

图 3 各组腓肠肌组织 caveolin-1 免疫组化染色（×100） A：假手术组；B：模型组；C：空转组；D：转染组

Figure 3 Immunohistochemical staining for caveolin-1 in the gastrocnemius muscle tissues in each group (×100) A: Sham operation group; B: Model group; C: Empty transfection group; D: Transfection group

表 2 免疫组化检测各组腓肠肌组织中 caveolin-1 AOD 值（$\bar{x} \pm s$）

Table 2 AOD values of immunohistochemical staining for caveolin-1 in the gastrocnemius muscle tissues of each group ($\bar{x} \pm s$)

组别	*n*	caveolin-1 AOD
假手术组	11	140.9326 ± 2.8504
模型组	10	153.3185 ± 2.3596[1)]
空转组	11	159.1245 ± 3.2122[1)]
转染组	10	171.0315 ± 3.3738[1), 2)]

注：1）与假手术组比较，*P*<0.01；2）与模型组比较，*P*<0.01

Note: 1) *P*<0.01 *vs.* sham operation group; 2) *P*<0.01 *vs.* model group

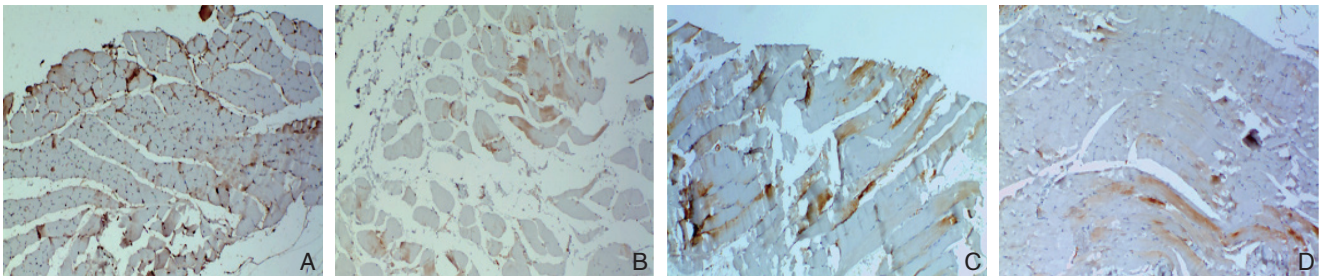

图 4 免疫组化检测各组腓肠肌组织中 eNOS 表达（×100） A：假手术组；B：模型组；C：空转组；D：转染组

Figure 4 Immunohistochemical staining for eNOS in the gastrocnemius muscle tissues in each group (×100) A: Sham operation group; B: Model group; C: Empty transfection group; D: Transfection group

表 3 免疫组化检测各组腓肠肌组织中 eNOS AOD 值（$\bar{x} \pm s$）

Table 3 AOD values of immunohistochemical staining for eNOS in the gastrocnemius muscle tissues of each group ($\bar{x} \pm s$)

组别	*n*	eNOS AOD 值
假手术组	11	140.2806 ± 2.0644
模型组	10	138.3015 ± 2.3596
空转组	11	139.1245 ± 3.2122
转染组	10	171.0315 ± 3.3738[1), 2)]

注：1）与假手术组比较，*P*<0.01；2）与模型组比较，*P*<0.01

Note: 1) *P*<0.01 *vs.* sham operation group; 2) *P*<0.01 *vs.* model group

2.5 CD34 免疫组化检测腓肠肌组织中 MVD

免疫组化检测各组腓肠肌组织中CD34的表达并计数MVD，与假手术组比较，模型组及空转组棕褐色颗粒增加，CD34的表达量明显增加（均*P*<0.01）；与模型组比较，转染组，CD34的表达量明显增加（*P*<0.01），模型组与空转组比较，差异无统计学意义（*P*>0.05）（图5-6）。

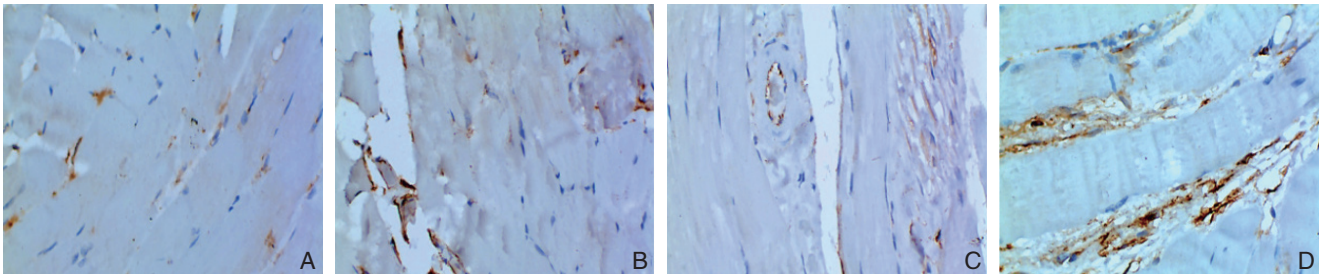

图 5　CD34 免疫组化检测各组腓肠肌组织中 MVD（×200）　A：假手术组；B：模型组；C：空转组；D：转染组

Figure 5　Immunohistochemical staining for CD34 marked MVD in the gastrocnemius muscle tissues of each group (×200)　A: Sham operation group; B: Model group; C: Empty transfection group; D: Transfection group

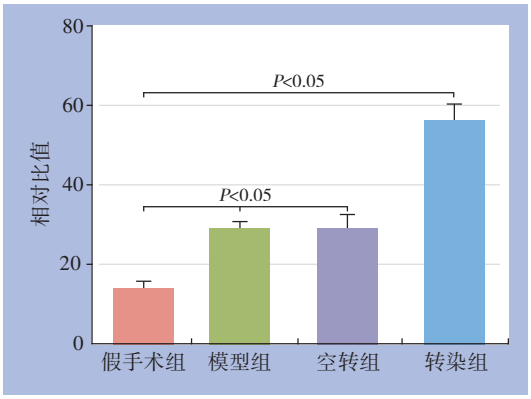

图 6　各组腓肠肌组织中 CD34 标记的 MVD 比较

Figure 6　Comparison of the values of CD34 marked MVD in the gastrocnemius muscle tissues among groups

3　讨　论

糖尿病下肢血管病变是由于机体内长期处于高血糖环境，引起血管内皮细胞的的脂质过氧化、蛋白质变性及炎性损伤，诱导动脉粥样硬化、中小动脉血栓形成最终导致血管狭窄或闭塞[13-15]。研究显示，糖尿病患者合并有下肢血管病变者高达50%以上[16]，其截肢率是非糖尿病患者的15~30倍[17-18]。而目前手术及药物治疗糖尿病下肢血管病变的效果并不理想[19]。因此，如何促进糖尿病肢体血液循环，改善患者生活质量，降低致死致残率发生，是临床急需解决的问题。

caveolin-1是小凹（caveolae）表面的信号传递蛋白能，诱导多种信号蛋白的活化或沉默，广泛表达于血管内皮细胞、平滑肌细胞等细胞中，在心血管疾病中发挥重要的生物学效应[20-21]。研究[22]表明，机体出现组织缺血时caveolin-1的高表达可促进血管新生，并对组织缺血、血流再灌注发挥有益的效应。Jasmin等[23]发现，将被敲除caveolin-1基因的大鼠结扎股动脉后缺血明显加重，肢体出现不可逆的坏死，而提高caveolin-1基因表达后血管再生能力则恢复，下肢缺血得以改善。另外，caveolin-1还参与了糖尿病病变过程，敲除了大鼠caveolin-1基因后，大鼠的血糖明显增高，组织中胰岛素受体水下降可高达90%的[24]。而caveolin-1在糖尿病下肢缺血中的作用如何，目前较少报道，实验通过结扎股动脉建立糖尿病大鼠下肢缺血模型，转染caveolin-1使其高表达，结果显示caveolin-1转染后微血管形成增多，肢体缺血缓解，提示caveolin-1在糖尿病下肢缺血中可诱导血管新生，改善局部血流灌注。

NO是机体内一种作用广泛的气信号分子，可调节血管舒缩[25]，抑制血小板聚集[26]，促进血管新生[27]，减轻氧化应激损伤[28]，是重要血管保护因子。NO是由内皮型一氧化氮合酶（endothelial nitric oxide synthase，eNOS）催化左旋精氨酸和氧反应生成，eNOS/NO信号通路在血管新生过程中起着多重促进效应[29-30]。有研究[31]表明，NO的生成及利用率降低引起内皮依赖性血管舒张因子功能障碍是糖尿病周围血管病变重要原因。Caveolin-1是eNOS的“分子伴娘”，eNOS位于caveolae内并与caveolin-1结合形成复合体[32]，caveolin-1可以促进AKT的磷酸化，使其eNOS 发生丝氨酸磷酸化，从而增加 NO的生成[33]。那么caveolin-1促进糖尿病大鼠下肢缺血部位血管新生是否与eNOS/NO通路相关？从实验图5可以发现，糖尿病下肢缺血大鼠转染caveolin-1后，eNOS和NO表达明显升高，说明caveolin-1在糖尿病下肢缺血模型中促进血管新生作用是通过活化eNOS促进NO表达其作用。但是，本实验存在很多不足，如仅仅在动物水平阐述caveolin-1对糖尿病下肢缺血大鼠的作用，缺乏细胞水平及其更加详细的分子

机制研究，我们将在后续工作中进一步完成。

综上所述，caveolin-1的高表达在糖尿病大鼠下肢缺血部位具有促进血管新生的作用，其机制可能与活化eNOS/NO通路有关，但其具体分子机制仍需进一步研究。

参考文献

[1] Choke E, Tang TY, Cheng SC, et al. Treatment of lower limb ischaemia in patients with diabetes[J]. Diabetes Metab Res Rev, 2020, 36(Suppl 1):e3262. doi: 10.1002/dmrr.3262.

[2] van Reijen NS, Ponchant K, Ubbink DT, et al. Editor's Choice - The Prognostic Value of the WIfI Classification in Patients with Chronic Limb Threatening Ischaemia: A Systematic Review and Meta-Analysis[J]. Eur J Vasc Endovasc Surg, 2019, 58(3):362–371. doi: 10.1016/j.ejvs.2019.03.040.

[3] Cheun TJ, Jayakumar L, Sideman MJ, et al. Outcomes of isolated inframalleolar interventions for chronic limb-threatening ischemia in diabetic patients[J]. J Vasc Surg, 2020, 71(5):1644–1652. doi: 10.1016/j.jvs.2019.07.094.

[4] Conte MS, Bradbury AW, Kolh P, et al. Global vascular guidelines on the management of chronic limb-threatening ischemia[J]. J Vasc Surg, 2019, 69(6S):3S-125S. doi: 10.1016/j.jvs.2019.02.016.

[5] 谢保城, 王清辉, 许周媚, 等. 自体干细胞移 植联合血管成形术治疗糖尿病足或肢体缺血性疾病的系统评价[J]. 中国普通外科杂志, 2017, 26(12):1589–1598. doi:10.3978/j.issn.1005–6947.2017.12.013.
Xie BC, Wang QH, Xu ZM, et al. Systematical evaluation of autologous stem cell transplantation combined with angioplasty therapy in treatment of diabetic foot or limb ischemia[J]. Chinese Journal of General Surgery, 2017, 26(12):1589–1598. doi:10.3978/j.issn.1005–6947.2017.12.013.

[6] Park IS, Mahapatra C, Park JS, et al. Revascularization and limb salvage following critical limb ischemia by nanoceria-induced Ref-1/APE1-dependent angiogenesis[J]. Biomaterials, 2020, 242:119919. doi: 10.1016/j.biomaterials.2020.119919.

[7] Mathew SA, Naik C, Cahill PA, et al. Placental mesenchymal stromal cells as an alternative tool for therapeutic angiogenesis[J]. Cell Mol Life Sci, 2020, 77(2):253–265. doi: 10.1007/s00018–019–03268–1.

[8] Volonte D, Galbiati F. Caveolin-1, a master regulator of cellular senescence[J]. Cancer Metastasis Rev, 2020, 39(2):397–414. doi: 10.1007/s10555–020–09875-w.

[9] Hou K, Li S, Zhang M, et al. Caveolin-1 in autophagy: A potential therapeutic target in atherosclerosis[J]. Clin Chim Acta, 2021, 513:25–33. doi: 10.1016/j.cca.2020.11.020.

[10] Haddad D, Al Madhoun A, Nizam R, et al. Role of Caveolin-1 in Diabetes and Its Complications[J]. Oxid Med Cell Longev, 2020, 2020:9761539. doi: 10.1155/2020/9761539.

[11] Saad MAE, Fahmy MIM, Al-Shorbagy M, et al. Nateglinide Exerts Neuroprotective Effects via Downregulation of HIF-1α/TIM-3 Inflammatory Pathway and Promotion of Caveolin-1 Expression in the Rat's Hippocampus Subjected to Focal Cerebral Ischemia/Reperfusion Injury[J]. Inflammation, 2020, 43(2):401–416. doi: 10.1007/s10753–019–01154–3.

[12] Dmitriev AV, Henderson D, Linsenmeier RA. Diabetes Alters pH Control in Rat Retina[J]. Invest Ophthalmol Vis Sci, 2019, 60(2):723–730. doi: 10.1167/iovs.18–26073.

[13] 李文东, 倪海真, 周敏, 等. 血管平滑肌细胞在糖尿病血管病变中的作用[J]. 中国普通外科杂志, 2018, 27(6):776–782. doi:10.3978/j.issn.1005–6947.2018.06.018.
Li WD, Ni HZ, Zhou M, et al. Role of vascular smooth muscle cells in diabetic vascular disease[J]. Chinese Journal of General Surgery, 2018, 27(6):776–782. doi:10.3978/j.issn.1005–6947.2018.06.018.

[14] Thor M, Yu A, Swedenborg J. Markers of inflammation and hypercoagulability in diabetic and nondiabetic patients with lower extremity ischemia[J]. Thromb Res, 2002, 105(5):379–383. doi: 10.1016/s0049–3848(02)00037–3.

[15] Kuo YR, Chien CM, Kuo MJ, et al. Endothelin-1 Expression Associated with Lipid Peroxidation and Nuclear Factor-κB Activation in Type 2 Diabetes Mellitus Patients with Angiopathy and Limb Amputation[J]. Plast Reconstr Surg, 2016, 137(1):187e-195e. doi: 10.1097/PRS.0000000000001886.

[16] Darling JD, O'Donnell TFX, Deery SE, et al. Outcomes after first-time lower extremity revascularization for chronic limb-threatening ischemia in insulin-dependent diabetic patients[J]. J Vasc Surg, 2018, 68(5):1455–1464. doi: 10.1016/j.jvs.2018.01.055.

[17] Liang P, Soden PA, Zettervall SL, et al. Treatment outcomes in diabetic patients with chronic limb-threatening ischemia[J]. J Vasc Surg, 2018, 68(2):487–494. doi: 10.1016/j.jvs.2017.11.081.

[18] Farber A, Eberhardt RT. The Current State of Critical Limb Ischemia: A Systematic Review[J]. JAMA Surg, 2016, 151(11):1070–1077. doi: 10.1001/jamasurg.2016.2018.

[19] 苏少飞, 田玉峰, 陈林宝, 等. 抗凝联合抗血小板 聚集治疗预防糖尿病下肢动脉硬化闭塞症支架植入后再狭窄的疗效分析[J]. 中国普通外科杂志, 2015, 24(6):823–827. doi:10.3978/j.issn.1005–6947.2015.06.011.
Su SF, Tian YF, Chen LB, et al. Combined anticoagulation and antiplatelet therapy for prophylaxis of restenosis after stent placement for leg atherosclerosis obliterans in diabetic patients[J].

Chinese Journal of General Surgery, 2015, 24(6):823–827. doi:10.3978/j.issn.1005–6947.2015.06.011.

[20] Zhang X, Ramírez CM, Aryal B, et al. Cav-1 (Caveolin-1) Deficiency Increases Autophagy in the Endothelium and Attenuates Vascular Inflammation and Atherosclerosis[J]. Arterioscler Thromb Vasc Biol, 2020, 40(6):1510–1522. doi: 10.1161/ATVBAHA.120.314291.

[21] Marudamuthu AS, Bhandary YP, Fan L, et al. Caveolin-1-derived peptide limits development of pulmonary fibrosis[J]. Sci Transl Med, 2019, 11(522):eaat2848. doi: 10.1126/scitranslmed.aat2848.

[22] Ito A, Shiroto T, Godo S, et al. Important roles of endothelial caveolin-1 in endothelium-dependent hyperpolarization and ischemic angiogenesis in mice[J]. Am J Physiol Heart Circ Physiol, 2019, 316(4):H900–910. doi: 10.1152/ajpheart.00589.2018.

[23] Jasmin JF, Malhotra S, Singh Dhallu M, et al. Caveolin-1 deficiency increases cerebral ischemic injury[J]. Circ Res, 2007, 100(5):721–729. doi: 10.1161/01.RES.0000260180.42709.29.

[24] Boothe T, Lim GE, Cen H, et al. Inter-domain tagging implicates caveolin-1 in insulin receptor trafficking and Erk signaling bias in pancreatic beta-cells[J]. Mol Metab, 2016, 5(5):366–378. doi: 10.1016/j.molmet.2016.01.009.

[25] Hu L, Feng Y, Liu W, et al. Botulinum toxin type A suppresses arterial vasoconstriction by regulating calcium sensitization and the endothelium-dependent endothelial nitric oxide synthase/soluble guanylyl cyclase/cyclic guanosine monophosphate pathway: An in vitro study[J]. Exp Biol Med (Maywood), 2019, 244(16):1475–1484. doi: 10.1177/1535370219878143.

[26] Tejero J, Shiva S, Gladwin MT. Sources of Vascular Nitric Oxide and Reactive Oxygen Species and Their Regulation[J]. Physiol Rev, 2019, 99(1):311–379. doi: 10.1152/physrev.00036.2017.

[27] Thom CS, Devine M, Kleinman S, et al. Neonatal platelet count trends during inhaled nitric oxide therapy[J]. Br J Haematol, 2020, 188(3):e28–30. doi: 10.1111/bjh.16301.

[28] Yamamoto N, Oyaizu T, Enomoto M, et al. VEGF and bFGF induction by nitric oxide is associated with hyperbaric oxygen-induced angiogenesis and muscle regeneration[J]. Sci Rep, 2020, 10(1):2744. doi: 10.1038/s41598–020–59615-x.

[29] Gazyakan E, Hirche C, Reichenberger MA, et al. Modulation of Nitric Oxide Bioavailability Attenuates Ischemia-Reperfusion Injury in Type II Diabetes[J]. J Plast Reconstr Aesthet Surg, 2021, 74(1):183–191. doi: 10.1016/j.bjps.2020.08.021.

[30] Bai J, Wang Q, Qi J, et al. Promoting effect of baicalin on nitric oxide production in CMECs via activating the PI3K-AKT-eNOS pathway attenuates myocardial ischemia-reperfusion injury[J]. Phytomedicine, 2019, 63:153035. doi: 10.1016/j.phymed.2019.153035.

[31] Icli B, Wu W, Ozdemir D, et al. MicroRNA-615–5p Regulates Angiogenesis and Tissue Repair by Targeting AKT/eNOS (Protein Kinase B/Endothelial Nitric Oxide Synthase) Signaling in Endothelial Cells[J]. Arterioscler Thromb Vasc Biol, 2019, 39(7):1458–1474. doi: 10.1161/ATVBAHA.119.312726.

[32] Shamsaldeen YA, Ugur R, Benham CD, et al. Diabetic dyslipidaemia is associated with alterations in eNOS, caveolin-1, and endothelial dysfunction in streptozotocin treated rats[J]. Diabetes Metab Res Rev, 2018, 34(5):e2995. doi: 10.1002/dmrr.2995.

[33] Li X, Xing W, Wang Y, et al. Upregulation of caveolin-1 contributes to aggravated high-salt diet-induced endothelial dysfunction and hypertension in type 1 diabetic rats[J]. Life Sci, 2014, 113(1/2):31–39. doi: 10.1016/j.lfs.2014.07.027.

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