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磷酸化PKM2调控糖尿病内皮依赖性血管舒张功能

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[摘 要] 目的：内皮依赖性血管舒张功能障碍是糖尿病性大血管病变的病理基础，内皮细胞对于高糖的利用及适应性改变决定了内皮细胞的功能状态。糖酵解途径是内皮细胞的主要能量来源，糖酵解异常在高糖诱导的内皮依赖性舒张功能障碍中发挥重要作用。M2型丙酮酸激酶(pyruvate kinase isozyme type M2，PKM2)是糖酵解途径关键酶之一，磷酸化可使其活性下降从而影响葡萄糖的糖酵解过程。TEPP-46可使PKM2稳定在四聚体形态，从而抑制其二聚体的形成和磷酸化。本研究采用TEPP-46作为抑制PKM2磷酸化的工具药探讨高糖条件下磷酸化PKM2(phosphorylated PKM2，p-PKM2)对内皮依赖性血管舒张功能的影响和潜在机制，以期为寻找糖尿病性大血管病变的新干预靶点提供理论依据。**方法：**将小鼠分为3组，其中野生型组(对照组，C57BL/6小鼠)和糖尿病组(db/db组，db/db小鼠)用羧甲基纤维素钠溶液每日灌胃1次；TEPP-46组(治疗组，db/db小鼠)用TEPP-46(30 mg/kg)和羧甲基纤维素钠溶液每日灌胃1次。各组小鼠分别处理12周后，检测胸主动脉p-PKM2和PKM2的蛋白质表达和血浆一氧化氮(nitric oxide，NO)水平以及胸主动脉内皮依赖性血管舒张功能。高糖(30 mmol/L)伴或不伴TEPP-46(10 μmol/L)、甘露醇孵育人脐静脉内皮细胞(human umbilical vein endothelial cells，HUVECs)72 h后，检测上清液中NO水平、细胞内NO含量及p-PKM2和PKM2的蛋白质表达水平。最后，在细胞水平和动物水平检测TEPP-46对内皮型一氧化氮合酶(endothelial nitric oxide synthase，eNOS)磷酸化的影响。**结果：**与野生型组相比，糖尿病组小鼠胸主动脉p-PKM2表达增加(P<0.05)。糖尿病组小鼠胸主动脉对乙酰胆碱(acetylcholine，ACh)的反应性较对照组降低了47%(P<0.05)，TEPP-46组小鼠胸主动脉对ACh的反应性较糖尿病组提高了28%(P<0.05)；3组小鼠胸主动脉对硝普钠(sodium nitroprusside，SNP)的反应性差异无统计学意义(P>0.05)。与对照组相比，糖尿病组小鼠血清NO水平降低；与糖尿病组相比，TEPP-46组胸主动脉p-PKM2表达降低的同时血清NO水平升高(均P<0.05)。高糖而非甘露醇可诱导HUVECs中p-PKM2表达增加，同时降低上清液中NO水平(均P<0.05)。TEPP-46和高糖同时孵育HUVECs，在抑制PKM2磷酸化的同时可逆转高糖所诱导的NO生成和分泌减少(均P<0.05)。在细胞水平和动物水平，TEPP-46均可逆转高糖所诱导的eNOS(Ser1177)磷酸化下降(均P<0.05)。**结论：**p-PKM2可能通过抑制p-eNOS(Ser1177)/NO途径参与2型糖尿病内皮依赖性血管舒张功能障碍的过程。

[关键词] 糖尿病；M2型丙酮酸激酶；内皮依赖性血管舒张功能；内皮型一氧化氮合酶；一氧化氮

Phosphorylated PKM2 regulates endothelium-dependent vasodilation in diabetes

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ABSTRACT

Objective: Endothelium-dependent vasodilation dysfunction is the pathological basis of diabetic macroangiopathy. The utilization and adaptation of endothelial cells to high glucose determine the functional status of endothelial cells. Glycolysis pathway is the major energy source for endothelial cells. Abnormal glycolysis plays an important role in endothelium-dependent vasodilation dysfunction induced by high glucose. Pyruvate kinase isozyme type M2 (PKM2) is one of key enzymes in glycolysis pathway, phosphorylation of PKM2 can reduce the activity of pyruvate kinase and affect the glycolysis process of glucose. TEPP-46 can stabilize PKM2 in its tetramer form, reducing its dimer formation and phosphorylation. Using TEPP-46 as a tool drug to inhibit PKM2 phosphorylation, this study aims to explore the impact and potential mechanism of phosphorylated PKM2 (p-PKM2) on endothelial dependent vasodilation function in high glucose, and to provide a theoretical basis for finding new intervention targets for diabetic macroangiopathy.

Methods: The mice were divided into 3 groups: a wild-type (WT) group (a control group, C57BL/6 mice) and a db/db group (a diabetic group, db/db mice), which were treated with the sodium carboxymethyl cellulose solution (solvent) by gavage once a day, and a TEPP-46 group (a treatment group, db/db mice+TEPP-46), which was gavaged with TEPP-46 (30 mg/kg) and sodium carboxymethyl cellulose solution once a day. After 12 weeks of treatment, the levels of p-PKM2 and PKM2 protein in thoracic aortas, plasma nitric oxide (NO) level and endothelium-dependent vasodilation function of thoracic aortas were detected. High glucose (30 mmol/L) with or without TEPP-46 (10 μmol/L), mannitol incubating human umbilical vein endothelial cells (HUVECs) for 72 hours, respectively. The level of NO in supernatant, the content of NO in cells, and the levels of p-PKM2 and PKM2 protein were detected. Finally, the effect of TEPP-46 on endothelial nitric oxide synthase (eNOS) phosphorylation was detected at the cellular and animal levels.

Results: Compared with the control group, the levels of p-PKM2 in thoracic aortas of the diabetic group increased (P<0.05). The responsiveness of thoracic aortas in the diabetic group to acetylcholine (ACh) was 47% lower than that in the control group (P<0.05), and that in TEPP-46 treatment group was 28% higher than that in the diabetic group (P<0.05), while there was no statistically significant difference in the responsiveness of thoracic aortas to sodium nitroprusside (SNP). Compared with the control group, the plasma NO level of mice decreased in the diabetic group, while compared with the diabetic group, the phosphorylation of PKM2 in thoracic aortas decreased and the plasma NO level increased in the TEPP-46 group (both P<0.05). High glucose instead of mannitol induced the increase of PKM2 phosphorylation in HUVECs and reduced the level of NO in supernatant (both P<0.05). HUVECs incubated with TEPP-46 and high glucose reversed the reduction of NO production and secretion induced by high glucose while inhibiting PKM2 phosphorylation (both P<0.05). At the cellular and animal levels, TEPP-46 reversed the

decrease of eNOS (ser1177) phosphorylation induced by high glucose (both $P<0.05$).
Conclusion: p-PKM2 may be involved in the process of endothelium-dependent vasodilation dysfunction in Type 2 diabetes by inhibiting p-eNOS (ser1177)/NO pathway.

KEY WORDS diabetes; pyruvate kinase isozyme type M2; endothelium-dependent vasodilation; endothelial nitric oxide synthase; nitric oxide

糖尿病性大血管病变可表现为冠心病、中风、外周动脉病变，超过50%的糖尿病患者死于糖尿病大血管病变[1-2]。血管内皮是血液和血管壁的物质与信息交换界面，最早感受血糖浓度变化；内皮依赖性舒张功能障碍是糖尿病大血管病变的病理基础，是导致糖尿病患者出现各种血管并发症的关键步骤[3]。高糖通过诱导氧化应激降低一氧化氮(nitric oxide，NO)生物利用度从而引起内皮依赖性舒张功能障碍[4-5]，然而循证医学证据[6]显示单纯的抗氧化药物治疗并不能改善肱动脉血流介导的血管舒张功能。寻找氧化应激之外高糖诱导内皮依赖性舒张功能障碍的机制将为防治糖尿病性大血管病变提供新思路。

内皮细胞对高糖的利用及适应性改变决定了内皮细胞的功能状态[7]，而内皮细胞85%以上的能量来源于糖酵解，糖酵解途径异常可能在高糖诱导的内皮依赖性舒张功能障碍中发挥重要作用[8]。丙酮酸激酶是调控糖酵解过程中的关键酶，可以催化磷酸烯醇式丙酮酸生成丙酮酸。丙酮酸激酶活性降低可导致其上游的中间产物磷酸烯醇式丙酮酸增加；磷酸烯醇式丙酮酸可转化为二酰基甘油(diacylglycerol，DAG)，DAG的增加会激活蛋白激酶C(protein kinase C，PKC)信号通路[9]。内皮细胞中PKC可以下调内皮型一氧化氮合酶(endothelial nitric oxide synthase，eNOS)(Ser1177)的磷酸化水平，而PKC抑制剂可以提高内皮细胞eNOS(Ser1177)的磷酸化水平[10]，从而影响NO的生物利用度。但高糖状态是否能影响内皮细胞丙酮酸激酶活性并借此调控eNOS(Ser1177)磷酸化和NO水平还有待进一步研究。

丙酮酸激酶有4种同工酶(M1、M2、L和R)，其中M2型丙酮酸激酶(pyruvate kinase isozyme type M2，PKM2)主要在内皮细胞表达[11]。PKM2有3种构型，分别为四聚体、二聚体和单体，其中四聚体PKM2具有较高的丙酮酸激酶活性，二聚体PKM2活性明显降低，PKM2的磷酸化能够促进PKM2二聚体形成[12]。TEPP-46(一种有效的选择性PKM2激活剂)、丝氨酸和果糖-1,6-二磷酸都能促进PKM2四聚体形成，使磷酸化PKM2(phosphorylated PKM2，p-PKM2)水平降低。本研究拟采用db/db小鼠观察高糖对PKM2及p-PKM2的影响，通过TEPP-46干预后观察其对p-PKM2和内皮依赖性血管舒张功能的影响，并在内皮细胞中验证抑制PKM2磷酸化可否改善高糖诱导的eNOS(Ser1177)磷酸化和NO水平，以期为寻找糖尿病性大血管病变的早期干预策略提供理论依据。

1 材料与方法

1.1 材料

1.1.1 动物

8周龄雄性C57BL/6(C57)小鼠(购自湖南斯莱克景达实验动物有限公司)及8周龄雄性db/db小鼠(购自南京大学模式动物研究所)饲养于中南大学实验动物中心。小鼠饲养环境：SPF级，12 h昼夜交替，温度为20~25 ℃。小鼠自由饮水、进食，适应性饲养7 d后开始正式实验。本研究已通过中南大学动物伦理委员会批准(审批号：2018 sydwo44)。

1.1.2 细胞与试剂

人脐静脉内皮细胞(human umbilical vein endothelial cells，HUVECs)购自美国ATCC公司，兔单克隆抗体PKM2、p-PKM2、eNOS和p-eNOS(货号分别为4053、3827、32027、9570)购自美国CST公司，NO荧光探针(货号为S0019)购自上海碧云天生物技术有限公司，NO检测试剂盒(货号为A012)购自南京建成生物工程研究所有限公司。

1.2 方法

1.2.1 动物分组

选用野生型(wild-type，WT)C57BL/6小鼠和db/db小鼠进行研究。小鼠分为3组，其中WT组和糖尿病组(db/db组)用羧甲基纤维素钠溶液每日灌胃1次；TEPP-46组(治疗组，db/db小鼠)用TEPP-46(30 mg/kg)和羧甲基纤维素钠溶液每日灌胃1次[13]，各组均连续干预12周。

1.2.2 收集样本

收集小鼠血液，离心10 min(4 ℃，3 000 g)后取上层血清置于−80 ℃冰箱储存。取血后解剖小鼠，小

心分离胸主动脉，一部分胸主动脉用于血管张力实验，另一部分存于−80 ℃冰箱用于蛋白质印迹检测。

1.2.3 血管张力实验

麻醉后固定小鼠，充分暴露胸腔，用剪刀和镊子取下心脏和胸主动脉，迅速将其放置在预冷的Kreb's缓冲液中。将每条胸主动脉分割成2~3 mm的小段，装载到微血管张力测定仪器(DMT 620M)的单个小室中，稳定后调零血管张力，之后将张力调为9.8 mN，稳定至少30 min。用氯化钾(终浓度50 mmol/L)刺激血管30 min，洗脱，静置约30 min待张力恢复到9.8 mN，此时血管达到稳定状态[14]。接下来加入去氧肾上腺素(1 μmol/L)，待张力数值稳定30 min后，加入累积剂量(10^{-9}~10^{-5} mol/L)的乙酰胆碱(acetylcholine, ACh)。待张力恢复到基线(9.8 mN)并稳定后，加入同样累积剂量(10^{-9}~10^{-5} mol/L)的硝普钠。用PowerLab (LabChart 7)采集变化的张力数值。

1.2.4 细胞培养与分组

HUVECs在含有5%胎牛血清和1%生长因子的内皮细胞培养基中培养。培养箱条件为5% CO_2、37 ℃。将细胞随机分为对照组(5.5 mmol/L的*D*-葡萄糖)，高糖组(30 mmol/L的*D*-葡萄糖)和甘露醇组(渗透压为30 mOsm/L)，以检测高糖对内皮细胞p-PKM2表达和NO释放的影响；另外再随机设置3组，分别为Control组，HG组和TEPP-46组(30 mmol/L的*D*-葡萄糖和10 μmol/L[11]TEPP-46共同孵育)，以检测TEPP-46对高糖诱导后内皮细胞p-PKM2表达和NO释放的影响。各组相应干预72 h后进行后续实验。

1.2.5 蛋白质印迹法

用含蛋白酶和磷酸酶抑制剂的放射免疫沉淀分析(radio-immunoprecipitation assay，RIPA)裂解液提取胸主动脉和细胞中的总蛋白质，BCA法测定蛋白质的含量，即行SDS-PAGE凝胶电泳，转膜，随后用5%的牛奶或BSA封闭1 h，分别加入PKM2(1∶1 000)、p-PKM2(1∶1000)、eNOS(1∶500)和p-eNOS(1∶500)一抗，在4 ℃下孵育过夜，PBST漂洗3次，HRP抗兔IgG抗体在室温下孵育1.5 h。洗膜后用ECL显影，使用Bio-Rad凝胶成像系统成像，Image lab软件分析灰度值。

1.2.6 NO荧光探针实验

选择干预72 h后细胞密度接近2×10^6个/孔的6孔板进行下一步操作。配置DAF-FM DA溶液(稀释液∶DAF-FM DA=1 000∶1)，浓度为5 μmol/L。DAF-FM本身仅有很弱的荧光，与NO结合后会产生强烈的绿色荧光。去除细胞培养液，用PBS清洗后，加入适当体积荧光液，在培养箱中孵育20 min左右，PBS洗掉多余荧光液，整个过程避光操作，每组设置相同的拍照条件，荧光显微镜摄片。

1.2.7 NO含量检测

通过检测亚硝酸盐水平来间接反映NO含量。具体步骤为：抽取0.1 mL血清和细胞上清液，离心5 min(4 ℃，12 000 r/min)，取上清液进行后续操作。整个操作严格按照试剂盒说明书在暗室中进行，最后酶标仪测定样本吸光度值(波长550 nm)。根据标准曲线计算样本NO含量(μmol/L)。

1.3 统计学处理

采用GraphPad Prism 5软件进行数据分析，符合正态分布的计量资料以均数±标准差($\bar{x}\pm s$)表示，2组比较采用独立样本*t*检验，多组比较采用单因素方差检验，$P<0.05$为差异具有统计学意义。

2 结 果

2.1 TEPP-46改善db/db小鼠内皮依赖性血管舒张功能，同时抑制PKM2的磷酸化

蛋白质印迹法结果显示：糖尿病组小鼠胸主动脉中p-PKM2/PKM2表达较WT组明显增加($P<0.05$)，而2组小鼠总的PKM2表达水平差异无统计学意义($P>0.05$)，TEPP-46组小鼠胸主动脉p-PKM2/PKM2水平明显降低($P<0.05$，图1A)。

糖尿病组小鼠胸主动脉血管对ACh依赖性血管舒张功能反应显著降低，相较WT组小鼠最大舒张率降低了47%($P<0.05$)；使用TEPP-46干预后，糖尿病组小鼠胸主动脉血管对ACh的反应明显增加，TEPP-46组小鼠最大舒张率较糖尿病组小鼠提高了28%($P<0.05$，图1B)。3组小鼠血管对硝普钠的反应性舒张曲线几乎重叠，说明3组血管对硝普钠的反应无差异，使用TEPP-46干预后也无明显变化($P<0.05$，图1C)。相较WT组，糖尿病组小鼠血清中NO的含量明显减少($P<0.05$，图1D)；相较糖尿病组，TEPP-46组小鼠血清中NO含量增加，差异有统计学意义($P<0.05$，图1D)。

2.2 高糖诱导内皮细胞p-PKM2表达增加和NO释放减少

对照组、高糖组、甘露醇组细胞总PKM2表达水平接近；与对照组比较，高糖组p-PKM2水平明显上调($P<0.05$)，甘露醇组p-PKM2水平没有明显变化($P>0.05$，图2A)。甘露醇组与高糖组之间p-PKM2的表达含量差异具有统计学意义($P<0.05$，图2A)。与对照组相比，高糖孵育72 h后，HUVECs上清液中NO含量明显减少($P<0.05$，图2B)。

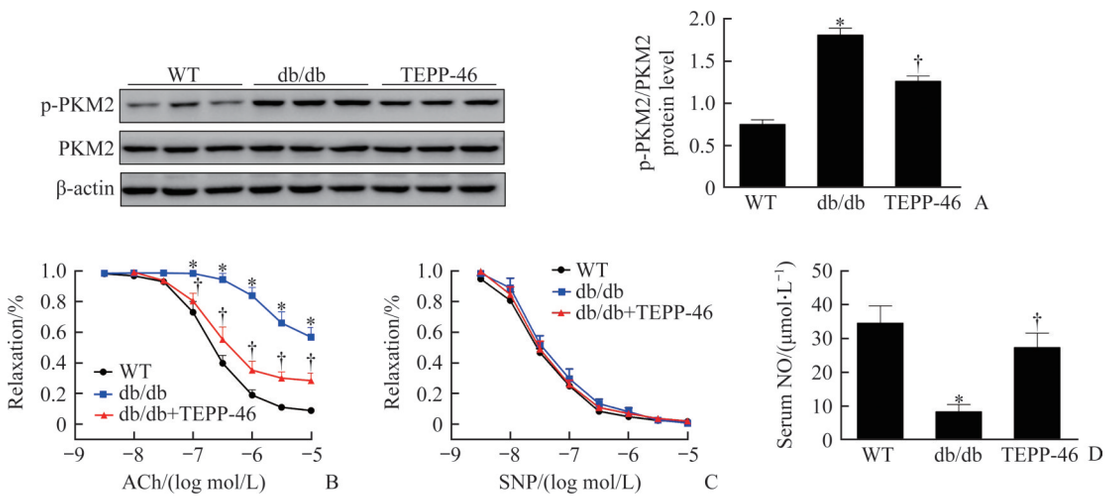

图1　TEPP-46改善db/db小鼠内皮依赖性血管舒张功能，同时抑制PKM2的磷酸化

Figure 1　TEPP-46 alleviates endothelium-derived relaxing impairment in db/db mice and inhibits the PKM2 phosphorylation of the thoracic aortas

A: Western blotting showing the expression of p-PKM2 and PKM2 in thoracic aortas of mice in the 3 groups. B: Acetylcholine (ACh)-induced endothelium-dependent vasodilation of aortic rings of mice in the 3 groups. C: Sodium nitroprusside (SNP)-induced endothelium-independent vasodilation of aortic rings of mice in the 3 groups. D: The level of NO in the serum of mice in the 3 groups. *P<0.05 vs the wild-type (WT) group, †P<0.05 vs the db/db group (n=5). PKM2: Pyruvate kinase isozyme type M2; p-PKM2: Phosphorylated PKM2.

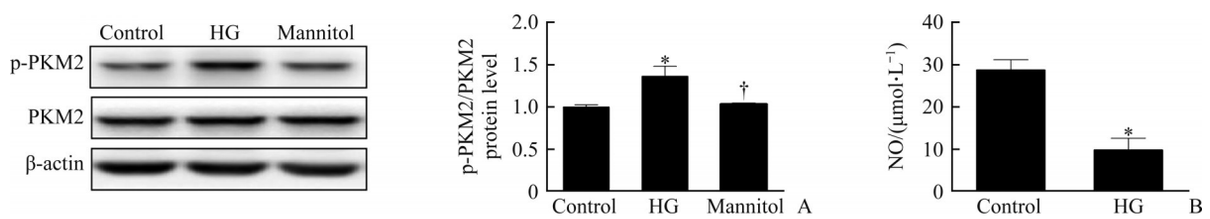

图2　高糖诱导内皮细胞p-PKM2表达增加和NO释放减少

Figure 2　High glucose (HG) induces the increase of p-PKM2 in HUVECs and decreases NO release

A: Western blotting showing the expression of p-PKM2 and PKM2 in HUVECs treated with HG or mannitol for 72 h. B: Total NO level in supernatant of HUVECs in the 2 groups. *P<0.05 vs the control group, †P<0.05 vs the HG group (n=3). PKM2: Pyruvate kinase isozyme type M2; p-PKM2: Phosphorylated PKM2; HUVECs: Human umbilical vein endothelial cells.

2.3　TEPP-46抑制PKM2磷酸化，同时促进内皮细胞NO释放

蛋白质印迹法结果表明：TEPP-46组较高糖组p-PKM2/PKM2下降了30%(P<0.05，图3A)，细胞上清液中NO含量增加130%(P<0.05，图3B)。与高糖组相比，TEPP-46组绿色荧光强度明显增强(P<0.05，图3C)。

2.4　TEPP-46逆转高糖诱导的eNOS(Ser1177)磷酸化下降

在细胞水平，与对照组相比，高糖组eNOS(Ser1177)磷酸化水平下降了64%；而TEPP-46干预后，eNOS(Ser1177)磷酸化水平增高了93%，差异均有统计学意义(均P<0.05，图4A)。在动物水平，与对照组比较，糖尿病组小鼠eNOS(Ser1177)磷酸化水平降低了51%；而与糖尿病组相比，TEPP-46组小鼠胸主动脉eNOS(Ser1177)磷酸化水平增加了62%(均P<0.05，图4B)。

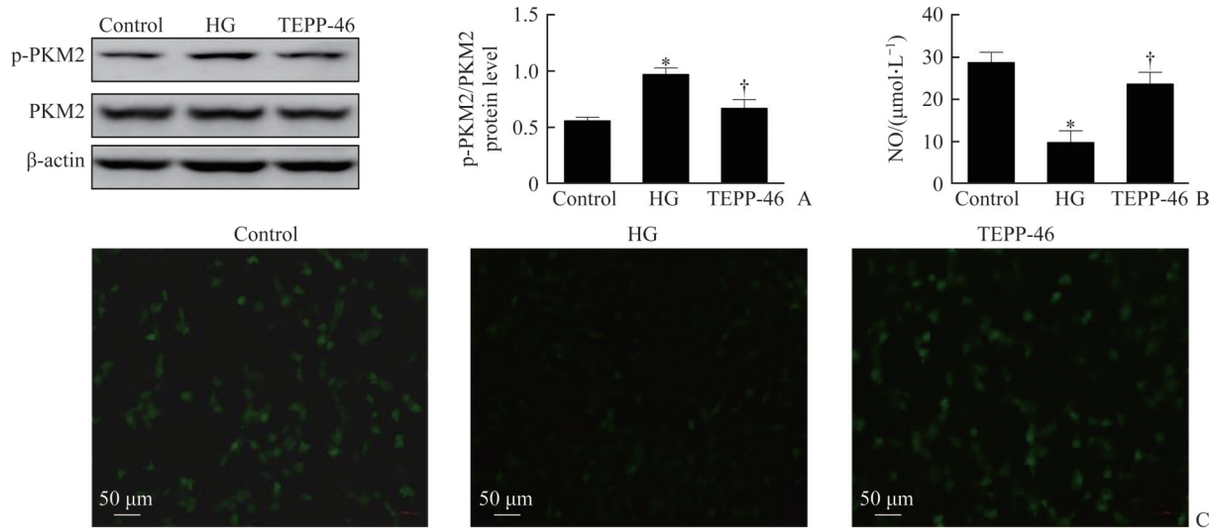

图3 TEPP-46抑制PKM2磷酸化的同时促进内皮细胞NO释放

Figure 3 TEPP-46 inhibits PKM2 phosphorylation and increases NO release

A: Western blotting showing the expression of p-PKM2 and PKM2 of HUVECs in the 3 groups. B: Total NO level in supernatant of HUVECs in the 3 groups. C: NO generation was measured by DAF-FM DA fluorescence of HUVECs in the 3 groups. *P<0.05 vs the control group, †P<0.05 vs the HG group (n=3). PKM2: Pyruvate kinase isozyme type M2; p-PKM2: Phosphorylated PKM2; HUVECs: Human umbilical vein endothelial cells.

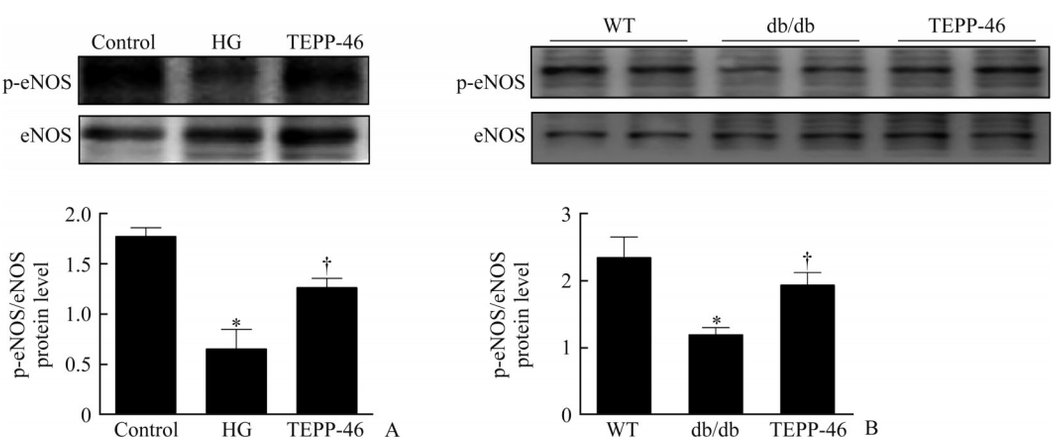

图4 TEPP-46可逆转高糖诱导的eNOS(Ser1177)磷酸化下降

Figure 4 TEPP-46 reverses the decrease of eNOS (ser1177) phosphorylation induced by high glucose (HG)

A: Western blotting showing the expression of p-eNOS and eNOS of HUVECs in the 3 groups. *P<0.05 vs the control group, †P<0.05 vs the HG group (n=3). B: Western blotting showing the expression of p-eNOS and eNOS in thoracic aortas of mice in the 3 groups. *P<0.05 vs the wild-type (WT) group, †P<0.05 vs the db/db group (n=5). PKM2: Pyruvate kinase isozyme type M2; p-PKM2: Phosphorylated PKM2; HUVECs: Human umbilical vein endothelial cells.

3 讨 论

本研究发现糖尿病小鼠胸主动脉内皮依赖性舒张功能下降并伴有p-PKM2表达增加，TEPP-46抑制p-PKM2表达并改善内皮依赖性舒张功能障碍；在体水平和离体水平均证明TEPP-46可增加高糖状态下内皮细胞eNOS(Ser1177)磷酸化和NO释放。

PKM2从四聚体构型向二聚体构型转变过程中表现为PKM2磷酸化增加，其丙酮酸激酶活性降低，PKM2这种构型转变参与多种心血管疾病的发生、发展。肺高压患者和动物模型肺组织中p-PKM2增加，抑制PKM2磷酸化后可抑制肺动脉平滑肌细胞迁移和增殖，从而改善肺高压小鼠肺血管重构和右心功能[15]。在动脉粥样硬化性冠心病患者单核-巨噬细胞

中，增加的葡萄糖摄取和糖酵解通量可促进PKM2磷酸化并入核，核PKM2作为蛋白激酶，磷酸化信号转导和转录激活因子3(signal transducer and activator of transcription3，STAT3)，从而促进白细胞介素-6(interleukin-6，IL-6)和白细胞介素-1β(interleukin-1β，IL-1β)的产生[16]。本课题组[17-18]前期发现p-PKM2可介导高糖诱导的内皮细胞基质金属蛋白酶-1和炎症因子(IL-6、IL-1β)的表达，抑制PKM2磷酸化可以改善高糖诱导的内皮细胞功能障碍。本研究进一步发现p-PKM2能介导高糖诱导的内皮依赖性血管舒张功能，并能抑制内皮细胞eNOS(Ser1177)磷酸化和NO释放，这一发现有助于加深对糖尿病大血管病变发病机制的理解。p-PKM2的增加伴随着丙酮酸激酶活性降低，导致糖酵解中间产物DAG累积，DAG的增加会激活PKC信号通路[9]；p-PKM2还可以从细胞质进入细胞核参与基因调控[16]。动物实验研究[19]发现PKC抑制剂可显著增高ob/ob小鼠主动脉中eNOS(Ser1177)的磷酸化水平，并改善ob/ob小鼠主动脉内皮依赖性血管舒张功能。p-PKM2是否通过DAG/PKC/eNOS(Ser1177)途径调节NO和血管内皮依赖性血管舒张功能，以及p-PKM2是否存在其他调控机制有待进一步研究。

TEPP-46是一种小分子药物，可与PKM2亚基界面处的袋状结构结合促进PKM2亚基形成稳定的四聚体而维持其丙酮酸激酶活性，同时可减少二聚体形成及PKM2磷酸化[20]。TEPP-46对PKM2具有高选择性，这一特性决定了它是一个研究PKM2功能的主要工具药[21]。研究[18]发现：维持PKM2活性对多种炎症性疾病具有保护作用，TEPP-46通过抑制内皮细胞炎症因子表达及巨噬细胞极化减轻糖尿病肾病症状。TEPP-46可通过下调p-STAT3抑制NOD样受体热蛋白结构域相关蛋白3(NOD-like receptor thermal protein domain associated protein 3，NLRP3)的活化，从而显著减弱小鼠模型中胸主动脉瘤和夹层的进展[22]；TEPP-46预处理巨噬细胞可使其对脂多糖刺激耐受，保护小鼠免受内毒素血症和败血症的影响[23]。研究[24]还发现TEPP-46可以通过抑制PKM2二聚体形成减少血小板活化、聚集，发挥抗血栓的作用。本课题组前期研究[18]发现使用TEPP-46干预后糖尿病小鼠口服葡萄糖耐量实验2 h血糖值与未干预的糖尿病组小鼠之间并无明显差异，提示TEPP-46可能通过非血糖调节途径参与糖尿病小鼠内皮依赖性血管舒张功能的调控。在本研究中，无论是在动物水平还是在细胞水平，TEPP-46都能促进高糖条件下内皮细胞eNOS(Ser1177)磷酸化和NO释放。但本研究也存在一些不足，如因小鼠动脉样本数量有限，未能使用原代主动脉内皮细胞进行研究，也未能阐明p-PKM2调控eNOS磷酸化的具体机制。我们将在后续研究中使用主动脉内皮细胞，阐明p-PKM2影响p-eNOS的潜在机制。

总之，本研究发现糖尿病小鼠胸主动脉中p-PKM2的表达增加，PKM2活性的降低可能通过抑制p-eNOS(Ser1177)/NO途径参与2型糖尿病内皮依赖性血管舒张功能障碍，这可能为寻找糖尿病大血管病变的潜在靶点提供了依据。

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参考文献

[1] Einarson TR, Acs A, Ludwig C, et al. Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence from across the world in 2007—2017[J]. Cardiovasc Diabetol, 2018, 17(1): 83. https://doi.org/10.1186/s12933-018-0728-6.

[2] Wei JH, Tian JX, Tang C, et al. The influence of different types of diabetes on vascular complications[J]. J Diabetes Res, 2022, 2022: 3448618. https://doi.org/10.1155/2022/3448618.

[3] Zhu ZW, Peng XF, Li XP, et al. HMGB1 impairs endothelium-dependent relaxation in diabetes through TLR4/eNOS pathway [J]. FASEB J, 2020, 34(6): 8641-8652. https://doi.org/10.1096/fj.202000242R.

[4] Liu F, Fang SH, Liu XX, et al. Omentin-1 protects against high glucose-induced endothelial dysfunction via the AMPK/PPARδ signaling pathway[J]. Biochem Pharmacol, 2020, 174: 113830. https://doi.org/10.1016/j.bcp.2020.113830.

[5] Takeda Y, Matoba K, Sekiguchi K, et al. Endothelial dysfunction in diabetes[J]. Biomedicines, 2020, 8(7): 182. https://doi.org/10.3390/biomedicines8070182.

[6] Tromba L, Perla FM, Carbotta G, et al. Effect of alpha-lipoic acid supplementation on endothelial function and cardiovascular risk factors in overweight/obese youths: a double-blind, placebo-controlled randomized trial[J]. Nutrients, 2019, 11(2): 375. https://doi.org/10.3390/nu11020375.

[7] Li XR, Sun XD, Carmeliet P. Hallmarks of endothelial cell metabolism in health and disease[J]. Cell Metab, 2019, 30(3): 414-433. https://doi.org/10.1016/j.cmet.2019.08.011.

[8] Lennicke C, Cochemé HM. Redox metabolism: ROS as specific molecular regulators of cell signaling and function[J]. Mol Cell, 2021, 81(18): 3691-3707. https://doi.org/10.1016/j.molcel.2021.08.018.

[9] Kolczynska K, Loza-Valdes A, Hawro I, et al. Diacylglycerol-

evoked activation of PKC and PKD isoforms in regulation of glucose and lipid metabolism: a review[J]. Lipids Health Dis, 2020, 19(1): 113. https://doi.org/10.1186/s12944-020-01286-8.

[10] Signorello MG, Segantin A, Passalacqua M, et al. Homocysteine decreases platelet NO level via protein kinase C activation[J]. Nitric Oxide, 2009, 20(2): 104-113. https://doi.org/10.1016/j.niox.2008.11.005.

[11] Kim B, Jang C, Dharaneeswaran H, et al. Endothelial pyruvate kinase M2 maintains vascular integrity[J]. J Clin Invest, 2018, 128(10): 4543-4556. https://doi.org/10.1172/JCI120912.

[12] Zhang Z, Deng XY, Liu YD, et al. PKM2, function and expression and regulation[J]. Cell Biosci, 2019, 9(1): 1-25. https://doi.org/10.1186/s13578-019-0317-8.

[13] Qi WE, Keenan HA, Li Q, et al. Pyruvate kinase M2 activation may protect against the progression of diabetic glomerular pathology and mitochondrial dysfunction[J]. Nat Med, 2017, 23 (6): 753-762. https://doi.org/10.1038/nm.4328.

[14] Zhu ZW, Peng XF, Li XP, et al. Association between radiation exposure and endothelium-dependent vasodilation: results from clinical and experimental studies[J]. J Vasc Interv Radiol, 2020, 31(1): 42-48. https://doi.org/10.1016/j.jvir.2019.09.017.

[15] Zhang AK, Yu FF, Yu WD, et al. Pyruvate kinase M2 activation protects against the proliferation and migration of pulmonary artery smooth muscle cells[J]. Cell Tissue Res, 2020, 382(3): 585-598. https://doi.org/10.1007/s00441-020-03245-2.

[16] Shirai T, Nazarewicz RR, Wallis BB, et al. The glycolytic enzyme PKM2 bridges metabolic and inflammatory dysfunction in coronary artery disease[J]. J Exp Med, 2016, 213 (3): 337-354. https://doi.org/10.1084/jem.20150900.

[17] 唐磊, 徐逸洲, 刘晓靖, 等. 高糖通过mTORC2-PKM2信号通路促进内皮细胞MMP-1的表达[J]. 中南大学学报(医学版), 2020, 45(3): 305-313. https://doi. org/10.11817/j. issn. 1672-7347.2020.190108.
TANG Lei, XU Yizhou, LIU Xiaojing, et al. High glucose up-regulates MMP-1 expression in endothelial cells via mTORC2-PKM2 pathway[J]. Journal of Central South University. Medical Science, 2020, 45(3): 305-313. https://doi. org/10.11817/j.issn.1672-7347.2020.190108.

[18] Li L, Tang L, Yang XP, et al. Gene regulatory effect of pyruvate kinase M2 is involved in renal inflammation in type 2 diabetic nephropathy[J]. Exp Clin Endocrinol Diabetes, 2020, 128(9): 599-606. https://doi.org/10.1055/a-1069-7290.

[19] Taguchi K, Kobayashi T, Matsumoto T, et al. Dysfunction of endothelium-dependent relaxation to insulin via PKC-mediated GRK2/Akt activation in aortas of ob/ob mice[J]. Am J Physiol Heart Circ Physiol, 2011, 301(2): H571-H583. https://doi. org/10.1152/ajpheart.01189.2010.

[20] Lee SA, Ho C, Troxler M, et al. Non-metabolic functions of PKM2 contribute to cervical cancer cell proliferation induced by the HPV16 E7 oncoprotein[J]. Viruses, 2021, 13(3): 433. https://doi.org/10.3390/v13030433.

[21] Anastasiou D, Yu YM, Israelsen WJ, et al. Pyruvate kinase M2 activators promote tetramer formation and suppress tumorigenesis[J]. Nat Chem Biol, 2012, 8(10): 839-847. https://doi.org/10.1038/nchembio.1060.

[22] Le S, Zhang H, Huang XF, et al. PKM2 activator TEPP-46 attenuates thoracic aortic aneurysm and dissection by inhibiting NLRP3 inflammasome-mediated IL-1β secretion[J]. J Cardiovasc Pharmacol Ther, 2020, 25(4): 364-376. https://doi.org/10.1177/1074248420919966.

[23] Yi ZJ, Wu YL, Zhang WF, et al. Activator-mediated pyruvate kinase M2 activation contributes to endotoxin tolerance by promoting mitochondrial biogenesis[J]. Front Immunol, 2020, 11: 595316. https://doi.org/10.3389/fimmu.2020.595316.

[24] Nayak MK, Ghatge M, Flora GD, et al. The metabolic enzyme pyruvate kinase M2 regulates platelet function and arterial thrombosis[J]. Blood, 2021, 137(12): 1658-1668. https://doi.org/10.1182/blood.2020007140.

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