论著·基础研究

甲基转移酶3调控pri-miR-21甲基化修饰在糖尿病肾病肾脏纤维化中的作用

吴佳晋，钟　晨，李大伟，陈若洋，瞿俊文，张　明
上海交通大学医学院附属仁济医院泌尿外科，上海 200127

［摘要］**目的**·探讨甲基转移酶3（methyltransferase like 3，METTL3）调控pri-miR-21的N^6-甲基腺苷（N^6-methyladenosine，m^6A）甲基化修饰在糖尿病肾病（diabetic nephropathy，DN）小鼠肾脏纤维化发病机制中的作用。**方法**·采用8周龄雄性db/db小鼠作为DN模型小鼠，db/m小鼠作为对照，同时按照是否经尾静脉注射S-腺苷高半胱氨酸水解酶抑制剂3-脱氮腺苷（3-deazaadenosine，DAA），共随机分为4组（5只/组），分别为db/m组、db/db组、db/m+DAA组和db/db+DAA组；8周龄开始注射DAA，注射1次/5 d，共注射8次。DAA干预结束后继续饲养小鼠至19周龄，收取各组小鼠血、尿、肾脏组织标本。检测血糖、血肌酐、尿白蛋白肌酐比（albumin-to-creatinine ratio，ACR），肾脏行苏木精-伊红（H-E）染色、Masson染色及天狼星红染色观察病理变化；试剂盒检测肾脏总RNA中m^6A的甲基化水平；Western blotting检测肾脏METTL3及纤维化相关蛋白表达；实时定量PCR检测肾脏总pri-miR-21和成熟miR-21；使用免疫磁珠富集肾脏组织中m^6A甲基化RNA，并通过PCR检测其中m^6A甲基化的pri-miR-21。**结果**·相较于db/m组，db/db组小鼠血糖，血肌酐，ACR，肾脏METTL3、m^6A甲基化修饰水平、纤维化相关蛋白、总pri-miR-21、m^6A甲基化pri-miR-21和成熟miR-21表达水平均显著增加（均$P<0.05$），小鼠肾脏系膜基质增多、肾小球基底膜增厚、胶原纤维累积显著增加。相较于db/db组，db/db+DAA组血糖，血肌酐，ACR，肾脏m^6A甲基化修饰水平、纤维化相关蛋白、m^6A甲基化pri-miR-21和成熟miR-21表达水平均显著下降（均$P<0.05$），总pri-miR-21表达水平显著升高（$P=0.000$），METTL3蛋白表达水平未见显著变化，小鼠肾脏损伤及纤维化程度显著减轻。**结论**·pri-miR-21的m^6A甲基化修饰促进miR-21成熟，进而促进DN小鼠肾脏纤维化的发生发展；抑制METTL3可通过调控pri-miR-21的m^6A甲基化修饰抑制DN小鼠肾脏纤维化。

［关键词］糖尿病肾病；肾脏纤维化；N^6-甲基腺苷；甲基转移酶3；pri-miR-21；miR-21
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Role of methyltransferase like 3 regulating pri-miR-21 methylation in renal fibrosis of diabetes nephropathy

WU Jiajin, ZHONG Chen, LI Dawei, CHEN Ruoyang, QU Junwen, ZHANG Ming
Department of Urology, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200127, China

[Abstract] Objective·To investigate the role of methyltransferase like 3 (METTL3) acting on N^6-methyladenosine (m^6A) and regulating pri-miR-21 methylation in the renal fibrosis of diabetic nephropathy (DN). **Methods**·Eight-week-old male db/db mice were used as DN models, and db/m mice were used as controls. The mice were randomly divided into 4 groups according to whether they received the treatment of 3-deazaadenosine (DAA) by tail vein injection or not (5 mice/group): db/m group, db/db group, db/m+DAA group and db/db+DAA group. From the age of 8 weeks, DAA was injected once per 5 d for a total of 8 times. After the DAA intervention, the mice were kept until they were 19 weeks old. The blood, the urine and the kidney tissue samples of the mice were collected, and blood glucose (BG), serum creatinine (Scr), and urinary albumin-to-creatinine ratio (ACR) were detected. The kidneys were stained with hematoxylin-eosin (H-E), Masson and sirius red to observe the pathological changes. The methylation level of m^6A in total RNAs of the kidney was detected with the kit. The expression levels of METTL3 and fibrosis-related proteins in the kidney were detected by Western blotting. The overall pri-miR-21 and the mature miR-21 were detected by real-time quantitative PCR. After enrichment of the m^6A-methylated RNAs in the kidney by immunomagnetic beads, the methylated pri-miR-21 at m^6A was detected by PCR. **Results**·Compared with the db/m group, the levels of BG, Scr, and ACR, and METTL3, m^6A methylation level, fibrosis-related proteins, overall pri-miR-21, m^6A-

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[作者简介] 吴佳晋（1992—），男，硕士；电子信箱：625760805@qq.com。
[通信作者] 张　明，电子信箱：drmingzhang@126.com。
[Funding Information] National Natural Science Foundation of China (81900680).
[Corresponding Author] ZHANG Ming, E-mail: drmingzhang@126.com.

methylated pri-miR-21 and mature miR-21 in the kidney in the db/db group significantly increased (P<0.05). Furthermore, the mesangial matrix in the kidney increased, glomerular basement membrane thickened, and the accumulation of collagen fibers increased significantly in the db/db group. Compared with the db/db group, the levels of BG, Scr, and ACR, and m^6A methylation level, fibrosis-related proteins, m^6A-methylated pri-miR-21 and mature miR-21 in the kidney in the db/db+DAA group decreased significantly (P< 0.05) and the degree of renal injury and fibrosis was significantly reduced, but the expression level of overall pri-miR-21 significantly increased (P=0.000). The expression level of METTL3 protein did not change significantly. **Conclusion·**The m^6A methylation modification of pri-miR-21 promotes the maturation of miR-21, thereby promoting the occurrence and development of renal fibrosis in DN mice; inhibition of METTL3 can inhibit renal fibrosis in DN mice by regulating m^6A methylation of pri-miR-21.

[Key words] diabetic nephropathy (DN); renal fibrosis; N^6-methyladenosine (m^6A); methyltransferase like 3 (METTL3); pri-miR-21; miR-21

糖尿病肾病（diabetic nephropathy，DN）是糖尿病常见的慢性微血管并发症之一[1-3]，其病理特征为细胞外基质（extracellular matrix，ECM）蛋白的沉积[4]。多种因素参与了DN发病，其中微RNA（microRNA，miRNA）在DN中的调控作用近年来备受关注。研究[5]证实miR-21与DN肾脏纤维化密切相关，它能促进肾脏的纤维生成和上皮损伤，是抗肾脏纤维化治疗的候选靶点。已有研究[6-7]证实miR-21可通过上调Sma和Mad相关蛋白3（Sma- and Mad-related protein，*SMAD3*）、下调*SMAD7*，以及与靶基因磷酸酯酶与张力蛋白同源物（phosphatase and tensin homolog，*PTEN*）基因结合抑制其表达等多种途径促进肾脏纤维化，但miR-21的上游调节机制对DN的影响仍不清楚。

N^6-甲基腺苷（N^6-methyladenosine，m^6A）是一种丰富的mRNA修饰。研究[8-9]表明，哺乳动物的m^6A是动态调节的，参与各种生物学进展，并与代谢性疾病密切相关，包括糖尿病和肥胖。已有研究[10]表明，miR-21基因转录后生成的pri-miR-21向成熟miR-21转化受到甲基转移酶3（methyltransferase like 3，METTL3）催化的m^6A甲基化修饰调控，但miR-21转录后的m^6A甲基化修饰在DN中的病理作用和调控机制尚未阐明，揭示其在DN肾脏纤维化中的作用将有助于为DN患者开发新的治疗策略。本研究采用db/db糖尿病小鼠作为DN模型小鼠，探究pri-miR-21的m^6A甲基化修饰在DN肾脏纤维化中的生物学功能和潜在的分子调控机制，旨在为DN肾脏纤维化的治疗提供新的靶点。

1 材料与方法

1.1 实验动物

db/db小鼠及同窝db/m小鼠（对照小鼠）各10只，雄性，8周龄，体质量20~25 g，购自南京大学-南京生物医药研究院，实验动物生产许可证号为SCXK（苏）2015-0001。动物饲养于上海交通大学医学院附属仁济医院SPF级标准动物房，实验动物使用许可证号为SYXK（沪）2018-0013。饲养条件：环境温度为18~29 ℃，日温差≤3 ℃，相对湿度为40%~70%，单笼常规饲料喂养，照明模拟自然光照，12 h昼夜更替。

1.2 主要试剂及仪器

3-脱氮腺苷（3-deazaadenosine，DAA；上海Perfemiker，货号6736-58-9）、肌酐（creatinine，Cr）测定试剂盒（南京建成，货号C011-2-1）、TRIzol试剂（美国Invitrogen，货号15596026）、EpiQuik m^6A RNA甲基化定量试剂盒（美国Epigentek，货号P-9013-48）、RIPA裂解缓冲液（上海碧云天，货号P0013B）、BCA蛋白质测定试剂盒（上海碧云天，货号P0012S）、兔抗鼠METTL3抗体（英国Proteintech，货号15073-1-AP）、兔抗鼠α-平滑肌肌动蛋白（α-smooth muscle actin，α-SMA）抗体（英国Abcam，货号ab32575）、兔抗鼠纤连蛋白（fibronectin，FN）抗体（英国Abcam，货号ab124964）、兔抗鼠Ⅰ型胶原蛋白（collagen Ⅰ，COL-Ⅰ）抗体（英国Abcam，货号ab34710）、兔抗鼠Ⅳ型胶原蛋白（collagen Ⅳ，COL-Ⅳ）抗体（英国Abcam，货号ab6586）、兔抗鼠甘油醛-3-磷酸脱氢酶（glyceraldehyde-3-phosphate dehydrogenase，GAPDH）抗体（英国Abcam，ab8245）、辣根过氧化物酶标记的抗兔IgG抗体（英国Abcam，货号ab288151）、ECL Western blotting底物试剂盒（美国Biovision，货号K820-500）、One Step PrimeScript® miRNA cDNA合成试剂盒（日本TaKaRa，货号D350A）、SYBR Green Realtime PCR Master Mix（上海联迈生物，货号LM-0049）、蛋白A/G磁珠（美国MCE，货号HY-K0202）、m^6A抗体

（德国Synaptic Systems，货号202-003）。

酶标仪（MK3，美国Thermo Fisher Scientific）、凝胶图像分析仪（FR-980A，上海复日科技有限公司）、血糖仪及血糖试纸（美国强生）、全自动生化分析系统（型号3500，日本Hitachi）。尿白蛋白及Cr水平检测由上海交通大学医学院附属仁济医院检验科完成。

1.3　实验方法

1.3.1　动物分组和给药　将小鼠随机分为4组，分别为db/m组、db/db组、db/m+DAA组、db/db+DAA组，每组5只。将小鼠适应性饲养至8周龄。DAA在DN模型中的应用暂无文献报道，参考DAA在其他疾病模型中的使用剂量及方法[11-12]，本研究以每次20 mg/kg的剂量通过尾静脉注射小鼠，1次/5 d，共注射8次，为期5周。db/m组和db/db组同时注射同体积的二甲基亚砜（DMSO）溶剂。

1.3.2　标本收集及生化指标分析　DAA干预结束后继续观察6周，即小鼠19周龄时收取血、尿、肾脏组织标本。使用尿液留置装置，收集各组小鼠24 h尿液，检测评估小鼠尿白蛋白肌酐比（albumin-to-creatinine ratio，ACR）。各组小鼠行尾静脉取血，使用快速血糖仪检测小鼠血糖浓度；通过摘除一侧眼球取血0.5~1 mL，静置并离心后取血清，使用Cr测定试剂盒（肌氨酸氧化酶法）检测小鼠血清Cr水平（Scr）。各组小鼠以颈椎脱臼法处死后开腹摘取双侧肾脏，将一侧肾脏放入1 mL EP管后于液氮中保存，另一侧肾脏去除肾包膜并纵向切开后放入4%多聚甲醛溶液备用。

1.3.3　肾脏病理组织学观察　肾组织包埋切片后，分别行苏木精-伊红（H-E）染色、Masson染色和天狼星红染色，光学显微镜下观察肾小球和肾小管的病理改变，以及肾脏纤维化情况。

1.3.4　甲基化定量　使用EpiQuik m^6A RNA甲基化定量试剂盒对肾脏总RNA中m^6A的甲基化水平进行定量检测，按说明书操作。

1.3.5　Western blotting　用RIPA裂解缓冲液提取肾脏蛋白质，并用BCA蛋白质测定试剂盒进行蛋白定量。采用10%十二烷基硫酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE）分离总蛋白，并转移至聚偏二氟乙烯（PVDF）膜；室温下以5%脱脂奶粉封闭1 h；添加一抗［METTL3抗体（1∶1 000）、α-SMA抗体（1∶1 000）、FN抗体（1∶500）、COL-Ⅰ抗体（1∶1 000）、COL-Ⅳ抗体（1∶1 000）或GAPDH抗体（1∶1 000）］于4 ℃下孵育过夜，清洗后与辣根过氧化物酶偶联的二抗（1∶2 000）孵育2 h；清洗后使用ECL Western blotting底物试剂盒显色。

1.3.6　实时定量PCR　使用TRIzol试剂从肾脏组织中提取总RNA，使用One Step PrimeScript® miRNA cDNA合成试剂盒反转录为cDNA，然后使用SYBR Green Realtime PCR Master Mix进行实时定量PCR（qPCR）检测。引物序列见表1。PCR反应条件为：95 ℃，15 min；95 ℃ 10 s，60 ℃ 30 s，72 ℃ 60 s，共40个循环；72 ℃ 7 min。每个样品重复3次。

表1　实时qPCR引物序列

Tab 1　Primer sequences for real-time qPCR

Gene	Forward primer	Reverse primer
miR-21	5′-ACGTTGTGTAGCTTATCAGACTG-3′	5′-AATGGTTGTTCTCCACACTCTC-3′
pri-miR-21	5′-ACAGGCCAGAAATGCCTGGG-3′	5′-GATGGTCAGATGAAAGATAC-3′
U6	5′-CTCGCTTCGGCAGCACA-3′	5′-AACGCTTCACGAATTTGCGT-3′
Gapdh	5′-AATCCCATCACCATCTTCCAG-3′	5′-AAATGAGCCCCAGCCTTC-3′

Note: U6—small nuclear RNA U6.

1.3.7　免疫共沉淀　提取肾脏总RNA后将A/G免疫磁珠和m^6A抗体按照试剂盒的标准步骤进行预混，配制免疫磁珠抗体预混液，将预混好的免疫磁珠加入总RNA中，m^6A抗体会与发生m^6A甲基化修饰的RNA结合。采用磁力架对m^6A免疫磁珠进行富集。用蛋白酶对富集到的RNA-抗体复合物进行消化，m^6A抗体被消化完后只剩下RNA。采用pri-miR-21的特异性引物，对剩下的RNA进行qPCR。

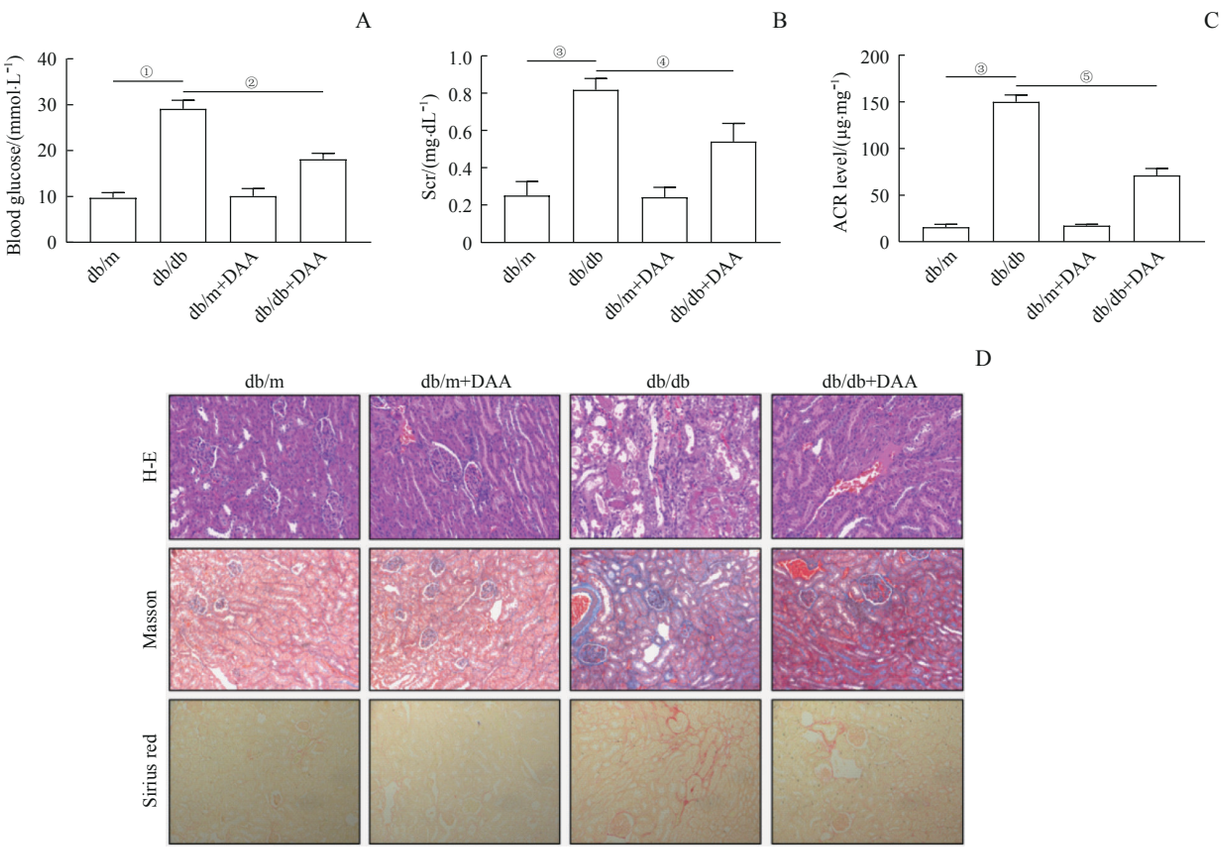
1.4　统计学分析

利用GraphPad Prism 7软件进行统计分析，实验数据以$\bar{x}\pm s$表示，组间比较采用t检验以及单因素方差分析。P<0.05表示差异具有统计学意义。

2 结果

2.1 各组血尿生化指标及肾脏病理变化

相较于db/m组，db/db组血糖、Scr及ACR水平均显著升高，差异均有统计学意义（均P<0.05）；相较于db/db组，db/db+DAA组血糖、Scr及ACR水平均显著降低，差异均有统计学意义（均P<0.05）。各组小鼠肾脏H-E染色、Masson染色及天狼星红染色结果显示：db/m组和db/m+DAA组小鼠肾脏均未见明显病理性改变；相较于db/m组，db/db组小鼠肾脏系膜基质增多，肾小球基底膜增厚，肾小球和肾小管中胶原蛋白明显聚集，提示肾小球和肾小管发生间质纤维化；db/db+DAA组小鼠肾脏损伤及胶原纤维累积较db/db组明显减轻。具体详见图1。



Note: A. Analysis of blood glucose levels in the 4 groups. B. Analysis of Scr levels in the 4 groups. C. Analysis of ACR levels in the 4 groups. D. H-E staining, Masson staining and sirius red staining of kidneys in the 4 groups (×200). ①P=0.006, ②P=0.033, ③P=0.000, ④P=0.016, ⑤P=0.023.

图1 各组小鼠血糖、Scr、ACR及肾脏病理变化

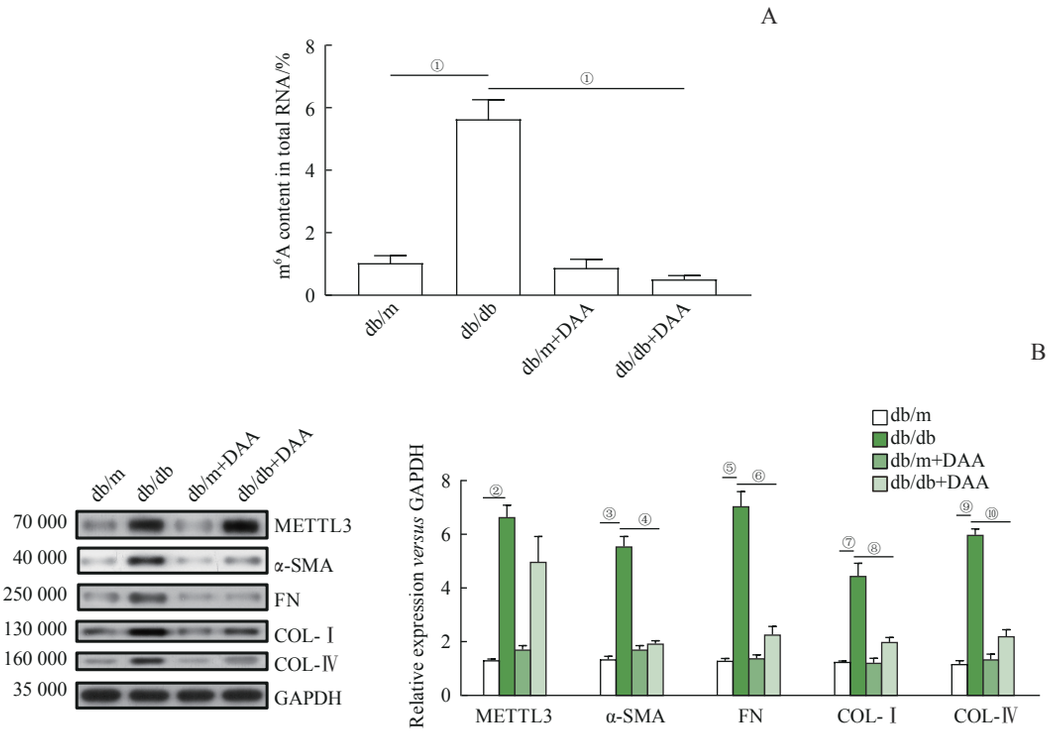
Fig 1 Changes of blood glucose, Scr, ACR and kidney pathology in the mice of the 4 groups

2.2 各组小鼠肾脏m^{6}A甲基化修饰水平、METTL3水平及纤维化相关蛋白表达情况

相较于db/m组，db/db组小鼠肾脏中m^{6}A甲基化修饰水平，METTL3、α-SMA、FN、COL-Ⅰ、COL-Ⅳ蛋白表达水平均显著上调，差异均具有统计学意义（均P<0.05）；而db/db+DAA组的m^{6}A甲基化，α-SMA、FN、COL-Ⅰ、COL-Ⅳ表达水平比db/db组均显著下降（均P<0.05），METTL3蛋白表达水平未见显著变化（图2）。

2.3 各组小鼠肾脏甲基化pri-miR-21、成熟miR-21及总体pri-miR-21表达情况

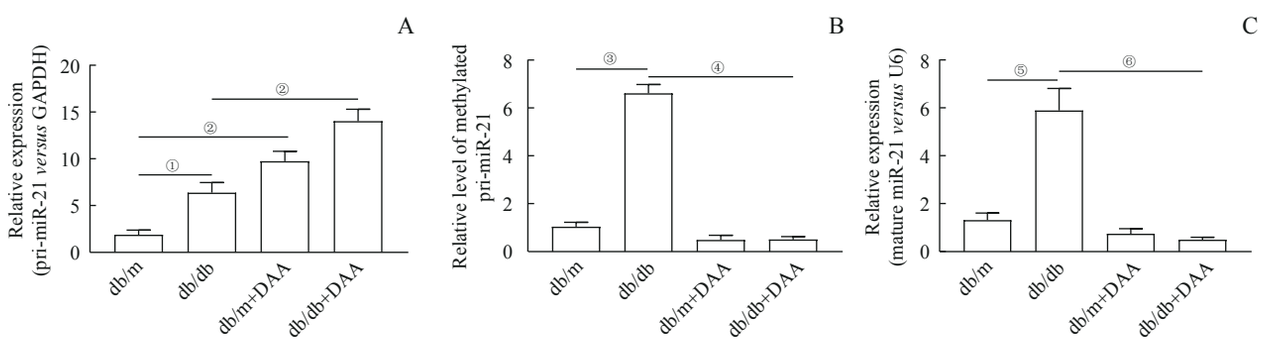
相较于db/m组，db/db组小鼠肾脏中总pri-miR-21、m^{6}A甲基化pri-miR-21和成熟miR-21表达水平均显著升高，差异具有统计学意义（均P<0.05）；而db/m+DAA组中甲基化pri-miR-21和成熟miR-21表达水平与db/m组差异无统计学意义，总pri-miR-21表达水平则显著升高（P=0.000）。相较于db/db组，db/db+DAA组中甲基化pri-miR-21和成熟miR-21表达水平显著下调（均P<0.05），总pri-miR-21表达水平则显著升高（P=0.000，图3）。



Note: A. The m^{6}A modification levels in the 4 groups. B. The protein expression levels of METTL3, α-SMA, FN, COL-Ⅰ, and COL-Ⅳ in the 4 groups. ①P=0.000, ②P=0.019, ③P=0.022, ④P=0.038, ⑤P=0.007, ⑥P=0.009, ⑦P=0.021, ⑧P=0.039, ⑨P=0.017, ⑩P=0.045.

图2　各组小鼠肾脏m^{6}A甲基化修饰水平、METTL3水平及纤维化相关蛋白表达变化

Fig 2　Changes of levels of m^{6}A methylation modification, METTL3 and fibrosis-related proteins in the 4 groups of mice kidneys



Note: A. The levels of pri-miR-21 in the renal tissues in the 4 groups detected by qPCR. B. The levels of m^{6}A methylated pri-miR-21 in the renal tissues in the 4 groups detected by immunocoprecipitation. C. The levels of mature miR-21 in the renal tissues in 4 groups detected by qPCR. ①P=0.008, ②P=0.000, ③P=0.004, ④P=0.001, ⑤P=0.006, ⑥P=0.005.

图3　各组小鼠肾脏总pri-miR-21、甲基化pri-miR-21及成熟miR-21的表达变化

Fig 3　Changes of total pri-miR-21, methylated pri-miR-21, and mature miR-21 in the 4 groups of mice kidneys

3　讨论

m^{6}A甲基化修饰是真核生物中常见的mRNA以及非编码RNA的转录后修饰[13-14]，METTL3/METTL14异二聚体是典型的m^{6}A甲基转移酶复合物，负责大部分哺乳动物细胞内mRNA的m^{6}A甲基化修饰[15]。研究[16]表明与无尿蛋白的糖尿病患者相比，尿蛋白阳性的糖尿病患者肾脏组织中总体mRNA甲基化水平显著升高；研究[17]发现db/m小鼠与db/db小鼠之间多个基因甲基化状态存在明显差异，通过反转录qPCR检测表明其mRNA表达也有明显不同。相关研究[18]表明METTL3作为调控m^{6}A修饰最主要的甲基化转移酶参与DN肾脏损伤，是引起DN足细胞损伤的重要分子。本研究中DN模型小鼠（db/db小鼠）肾脏组织中m^{6}A甲基化水平及METTL3表达水平均显著升高，一定程度上证实了METTL3介导的

m^{6}A甲基化修饰与DN发生发展的相关性。

S-腺苷高半胱氨酸是METTL3/METTL14异二聚体复合物抑制剂，DAA可通过抑制S-腺苷高半胱氨酸水解从而抑制m^{6}A基团插入mRNA底物中，进而抑制mRNA发生甲基化[19]。相关研究[20]证实METTL14仅是METTL3/METTL14复合物中无活性的分子伴侣，因此推测DAA抑制m^{6}A甲基化主要通过抑制METTL3的活性发挥作用。本研究通过DAA抑制m^{6}A甲基化修饰显著降低了DN模型小鼠的血糖、Scr及ACR水平，减轻了肾脏病理损伤，同时抑制了肾脏组织中ECM蛋白表达，提示抑制METTL3/METTL14异二聚体复合物介导的m^{6}A甲基化修饰可改善DN模型小鼠肾功能并减轻肾脏纤维化程度。此外，相关研究[21-22]表明甲基化修饰与肥胖及2型糖尿病的胰岛素抵抗相关，DAA可以通过抑制甲基化进而抑制糖异生及产糖基因的表达，改善糖耐量及胰岛素抵抗[23]，这可能是本研究中DAA处理可降低db/db小鼠血糖水平的原因。

相关研究[24]已证实miR-21与DN肾脏纤维化相关，miR-21在肾小管上皮细胞的过度表达可以促进高糖介导的肾脏间质纤维化或炎症标志物的产生；相关研究[25]显示miR-21在DN小鼠模型中的过表达可通过增强转化生长因子-β1（transforming growth factor-β1，TGF-β1）诱导的上皮-间充质转化（epithelial-mesenchymal transition，EMT），加剧肾脏损伤；研究[26]发现miR-21高表达会抑制*PTEN*的表达从而加剧肾脏间质纤维化。多项研究[5,25-26]表明，miR-21通过作用于靶基因来促进DN的肾纤维化，但miR-21的上游调节机制仍不清楚。本研究未进一步探究miR-21促进DN模型小鼠发生肾脏纤维化的下游通路，而将重点放在探究m^{6}A甲基化修饰在pri-miR-21向成熟miR-21转化过程中作用。本研究发现DN模型小鼠肾脏中成熟miR-21水平及甲基化pri-miR-21表达水平显著增加，给予DAA干预后显著抑制其表达，证实了miR-21的高表达与DN肾脏纤维化的相关性，同时表明pri-miR-21的甲基化水平与成熟miR-21的表达水平相关。

相关研究[27-28]已证实m^{6}A修饰可促进pri-miRNA向成熟miRNA转化，且miRNA的成熟对其发挥生物学效应至关重要。本研究通过进一步量化各组小鼠肾脏组织中总pri-miR-21表达水平，发现DN模型小鼠肾脏组织中pri-miR-21的总体水平增加；抑制m^{6}A甲基化修饰后，小鼠肾脏中pri-miR-21总表达水平进一步上调，而成熟miR-21的表达水平下降。这提示抑制pri-miR-21的甲基化修饰后，其无法转化为成熟的miR-21，从而出现pri-miR-21累聚而总体水平升高。本研究尚存在一定不足，如未能采用*METTL3*敲除小鼠及体外细胞实验进一步进行验证，未能进一步探究miR-21促进DN模型小鼠发生肾脏纤维化的下游通路等。

总之，本项研究发现pri-miR-21的m^{6}A甲基化修饰促进miR-21成熟，进而促进DN肾脏纤维化的发生发展，METTL3参与了pri-miR-21的m^{6}A甲基化修饰，这将为DN的治疗提供新的靶点。

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

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All experimental animal protocols in this study were reviewed and approved by Laboratory Animal Ethics Committee in Renji Hospital, Shanghai Jiao Tong University School of Medicine (Approval Letter No. RJ2021-0219), and all experimental animal protocols were carried out by following *Laboratory Animal—Guideline for Ethical Review of Animal Welfare.*

作者贡献/Authors' Contributions

吴佳晋、张明、李大伟参与了实验设计以及论文的写作和修改；吴佳晋、钟晨、陈若洋、瞿俊文负责实验操作与数据分析。所有作者均阅读并同意了最终稿件的提交。
The study was designed by WU Jiajin, ZHANG Ming and LI Dawei. The manuscript was drafted and revised by WU Jiajin, ZHANG Ming and LI Dawei. The experiments were performed by WU Jiajin, ZHONG Chen, CHEN Ruoyang and QU Junwen. The data were analyzed by WU Jiajin, ZHONG Chen, CHEN Ruoyang and QU Junwen. All the authors have read the last version of paper and consented for submission.

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参·考·文·献

[1] CHANG Y E, MORADI H, KALANTAR-ZADEH K. Emerging paradigms of treating diabetic nephropathy[J]. Lancet Diabetes Endocrinol, 2018, 6(12): 912-913.

[2] CHERNEY D Z I, ODUTAYO A, VERMA S. A big win for diabetic kidney disease: CREDENCE[J]. Cell Metab, 2019, 29(5): 1024-1027.

[3] DE BOER I H. A new chapter for diabetic kidney disease[J]. N Engl J Med, 2017, 377(9): 885-887.

[4] JARDINE M J, MAHAFFEY K W, PERKOVIC V. Canagliflozin and renal outcomes in diabetic nephropathy. Reply[J]. N Engl J Med, 2019, 381(11): 1089-1090.

[5] CHAU B N, XIN C Y, HARTNER J, et al. MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways[J]. Sci Transl Med, 2012, 4(121): 121ra18.

[6] WANG J Y, GAO Y B, ZHANG N, et al. Tongxinluo ameliorates renal structure and function by regulating miR-21-induced epithelial-to-mesenchymal transition in diabetic nephropathy[J]. Am J Physiol Renal Physiol, 2014, 306(5): F486-F495.

[7] DEY N, DAS F, MARIAPPAN M M, et al. MicroRNA-21 orchestrates high glucose-induced signals to TOR complex 1, resulting in renal cell pathology in diabetes[J]. J Biol Chem, 2011, 286(29): 25586-25603.

[8] YANG C, HU Y Y, ZHOU B, et al. The role of m^6A modification in physiology and disease[J]. Cell Death Dis, 2020, 11(11): 960.

[9] MATHIYALAGAN P, ADAMIAK M, MAYOURIAN J, et al. FTO-dependent N^6-methyladenosine regulates cardiac function during remodeling and repair[J]. Circulation, 2019, 139(4): 518-532.

[10] LIU E P, LV L, ZHAN Y H, et al. METTL3/N^6-methyladenosine/miR-21-5p promotes obstructive renal fibrosis by regulating inflammation through SPRY1/ERK/NF-κB pathway activation[J]. J Cell Mol Med, 2021, 25(16): 7660-7674.

[11] CHEN J, ZHANG M J, ZHANG X, et al. EZH2 inhibitor DZNep modulates microglial activation and protects against ischaemic brain injury after experimental stroke[J]. Eur J Pharmacol, 2019, 857: 172452.

[12] OVECHKIN A V, TYAGI N, SEN U, et al. 3-Deazaadenosine mitigates arterial remodeling and hypertension in hyperhomocysteinemic mice[J]. Am J Physiol Lung Cell Mol Physiol, 2006, 291(5): L905-L911.

[13] MEYER K D, SALETORE Y, ZUMBO P, et al. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons[J]. Cell, 2012, 149(7): 1635-1646.

[14] NIU Y M, ZHAO X, WU Y S, et al. N^6-methyl-adenosine (m^6A) in RNA: an old modification with a novel epigenetic function[J]. Genom Proteom Bioinform, 2013, 11(1): 8-17.

[15] LIU J Z, YUE Y N, HAN D L, et al. A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation[J]. Nat Chem Biol, 2014, 10(2): 93-95.

[16] MAGHBOOLI Z, LARIJANI B, EMAMGHOLIPOUR S, et al. Aberrant DNA methylation patterns in diabetic nephropathy[J]. J Diabetes Metab Disord, 2014, 13(1): 69.

[17] MARUMO T, YAGI S, KAWARAZAKI W, et al. Diabetes induces aberrant DNA methylation in the proximal tubules of the kidney[J]. J Am Soc Nephrol, 2015, 26(10): 2388-2397.

[18] JIANG L, LIU X Q, HU X R, et al. METTL3-mediated m^6A modification of TIMP2 mRNA promotes podocyte injury in diabetic nephropathy[J]. Mol Ther, 2022, 30(4): 1721-1740.

[19] LI Q H, ZHU L Q, YAN Y M, et al. S-adenosyl homocysteine hydrolase (SAHH) accelerates flagellar regeneration in *Dunaliella salina*[J]. Curr Microbiol, 2013, 67(2): 249-254.

[20] ZACCARA S, RIES R J, JAFFREY S R. Reading, writing and erasing mRNA methylation[J]. Nat Rev Mol Cell Biol, 2019, 20(10): 608-624.

[21] DE JESUS D F, ZHANG Z J, KAHRAMAN S, et al. m^6A mRNA methylation regulates human β-cell biology in physiological states and in type 2 diabetes[J]. Nat Metab, 2019, 1(8): 765-774.

[22] XIE W, MA L L, XU Y Q, et al. METTL3 inhibits hepatic insulin sensitivity *via* N6-methyladenosine modification of *Fasn* mRNA and promoting fatty acid metabolism[J]. Biochem Biophys Res Commun, 2019, 518(1): 120-126.

[23] 张丹亭. S-腺苷高半胱氨酸水解酶抑制剂在肝脏葡萄糖糖代谢中的作用[D]. 长春: 东北师范大学, 2019.
ZHANG D T. 3-Deazaadenosine, mechanism of action in liver glucose metabolism[D]. Changchun: Northeast Normal University, 2019.

[24] ZHONG X, CHUNG A C K, CHEN H Y, et al. miR-21 is a key therapeutic target for renal injury in a mouse model of type 2 diabetes[J]. Diabetologia, 2013, 56(3): 663-674.

[25] WANG J Y, GAO Y B, ZHANG N, et al. miR-21 overexpression enhances TGF-β1-induced epithelial-to-mesenchymal transition by target smad7 and aggravates renal damage in diabetic nephropathy[J]. Mol Cell Endocrinol, 2014, 392(1-2): 163-172.

[26] SEKAR D, VENUGOPAL B, SEKAR P, et al. Role of microRNA 21 in diabetes and associated/related diseases[J]. Gene, 2016, 582(1): 14-18.

[27] DIAO L T, XIE S J, LEI H, et al. METTL3 regulates skeletal muscle specific miRNAs at both transcriptional and post-transcriptional levels[J]. Biochem Biophys Res Commun, 2021, 552: 52-58.

[28] MICHLEWSKI G, CÁCERES J F. Post-transcriptional control of miRNA biogenesis[J]. RNA, 2019, 25(1): 1-16.

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