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EGR3通过抑制PRMT1/p-STAT3通路减轻肥胖相关性肾病足细胞炎症损伤

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[摘 要] 目的： 肥胖会导致肥胖相关性肾病(obesity related glomerulopathy，ORG)，但其发病机制并不明确。本研究拟检测早期生长反应蛋白3(early growth response protein 3，EGR3)在ORG患者和高脂饮食诱导的肥胖小鼠肾皮质组织中的表达，并探讨EGR3抑制棕榈酸(palmitic acid，PA)诱导的人足细胞炎症损伤的分子机制。**方法：** 收集排除其他疾病导致的肾损害并经组织病理学证实的ORG患者(*n*=6)和高脂饮食诱导的肥胖小鼠的肾皮质组织(*n*=10)。使用150 μmol/L PA干预人和小鼠足细胞48 h；人足细胞中分别过表达或沉默EGR3。采用酶联免疫吸附试验(enzyme linked immunosorbent assay，ELISA)检测白细胞介素(interleukin，IL)-6和IL-1β的含量；real-time RT-PCR检测*EGR3*、足细胞分子标志*NPHS1*(nephrosis 1)、*NPHS2*(nephrosis 2)、足糖萼蛋白(podocalyxin，*PODXL*)、平足蛋白(podoplanin，*PDPN*)mRNA的表达；RNA-seq检测人足细胞过表达EGR3并150 μmol/L PA干预后与对照组的差异表达基因(differentially expressed genes，DEGs)；免疫共沉淀(co-immunoprecipitation，Co-IP)+液相色谱串联质谱(liquid chromatography tandem mass spectrometry，LC-MS)检测EGR3可能的相互作用蛋白质，并与RNA-seq的结果取交集；Co-IP验证EGR3与蛋白精氨酸甲基转移酶1(protein arginine methyltransferases 1，PRMT1)的相互作用；沉默EGR3和PRMT1抑制剂干预后检测PA诱导的足细胞培养液中IL-6和IL-1β的含量；蛋白质印迹法检测分别过表达或沉默EGR3后磷酸化信号转导及转录激活蛋白3(phosphorylated signal transducer and activator of transcription 3，p-STAT3)的蛋白质表达。**结果：** EGR3在ORG患者和高脂饮食诱导的肥胖小鼠肾皮质组织中的表达均显著上调(均*P*<0.01)，150 μmol/L PA干预人和小鼠足细胞48 h后显著上调2种细胞EGR3的表达(均*P*<0.05)。人足细胞过表达或沉默EGR3分别抑制或促进PA干预后细胞培养液中IL-6和IL-1β的分泌，并分别上调或下调*NPHS1*、*PODXL*、*NPHS2*及*PDPN*的表达(均*P*<0.05)。RNA-seq结果显示共有988个DEGs，Co-IP+LC-MS共发现238个可能与EGR3相互作用的蛋白质，且Co-IP证实PRMT1为EGR3的相互作用蛋白质。PRMT1抑制剂能部分减少人足细胞沉默EGR3后PA诱导的IL-6及IL-1β的分泌(均*P*<0.05)；此外，过表达或沉默EGR3负调控PRMT1及p-STAT3的表达。**结论：** EGR3可能通过抑制PRMT1/p-STAT3通路减轻ORG足细胞炎症损伤。

[关键词] 早期生长反应蛋白3；棕榈酸；肥胖相关性肾病；足细胞；炎症因子

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EGR3 reduces podocyte inflammatory damage in obesity related glomerulopathy by inhibiting the PRMT1/p-STAT3 pathway

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ABSTRACT

Objective: Obesity related glomerulopathy (ORG) is induced by obesity, but the pathogenesis remains unclear. This study aims to investigate the expression of early growth response protein 3 (EGR3) in the renal cortex tissues of ORG patients and high-fat diet-induced obese mice, and to further explore the molecular mechanism of EGR3 in inhibiting palmitic acid (PA) induced human podocyte inflammatory damage.

Methods: Renal cortex tissues were collected from ORG patients (n=6) who have been excluded from kidney damage caused by other diseases and confirmed by histopathology, and from obese mice induced by high-fat diet (n=10). Human and mouse podocytes were intervened with 150 μmol/L PA for 48 hours. EGR3 was overexpressed or silenced in human podocytes. Enzyme linked immunosorbent assay (ELISA) was used to detcet the levels of interleukin-6 (IL-6) and interleukin-1β (IL-1β). Real-time RT-PCR was used to detect the mRNA expressions of *EGR3*, podocytes molecular markers nephrosis 1 (*NPHS1*), nephrosis 2 (*NPHS2*), podocalyxin (*PODXL*), and podoplanin (*PDPN*). RNA-seq was performed to detect differentially expressed genes (DEGs) after human podocytes overexpressing EGR3 and treated with 150 μmol/L PA compared with the control group. Co-immunoprecipitation (Co-IP) combined with liquid chromatography tandem mass spectrometry (LC-MS) was used to detect potential interacting proteins of EGR3 and the intersected with the RNA-seq results. Co-IP confirmed the interaction between EGR3 and protein arginine methyltransferases 1 (PRMT1), after silencing EGR3 and PRMT1 inhibitor intervention, the secretion of IL-6 and IL-1β in PA-induced podocytes was detected. Western blotting was used to detect the expression of phosphorylated signal transducer and activator of transcription 3 (p-STAT3) after overexpression or silencing of EGR3.

Results: EGR3 was significantly upregulated in renal cortex tissues of ORG patients and high-fat diet-induced obese mice (both P<0.01). In addition, after treating with 150 μmol/L PA for 48 hours, the expression of EGR3 in human and mouse podocytes was significantly upregulated (both P<0.05). Overexpression or silencing of EGR3 in human podocytes inhibited or promoted the secretion of IL-6 and IL-1β in the cell culture supernatant after PA intervention, respectively, and upregulated or downregulated the expression of *NPHS1*, *PODXL*, *NPHS2*, and *PDPN* (all P<0.05). RNA-seq showed a total of 988 DEGs, and Co-IP+LC-MS identified a total of 238 proteins that may interact with EGR3. Co-IP confirmed that PRMT1 was an interacting protein with EGR3. Furthermore, PRMT1 inhibitors could partially reduce PA-induced IL-6 and IL-1β secretion after EGR3 silencing in human podocytes (both P<0.05). Overexpression or silencing of EGR3 negatively regulated the expression of PRMT1 and p-STAT3.

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Conclusion: EGR3 may reduce ORG podocyte inflammatory damage by inhibiting the PRMT1/p-STAT3 pathway.

KEY WORDS　early growth response protein 3; palmitic acid; obesity related nephropathy; podocyte; inflammatory factors

肥胖的发病率逐年上升，肥胖常合并冠心病、高血压、糖尿病等慢性疾病，严重危害人类健康[1]。肥胖导致的肾脏疾病被称为肥胖相关性肾病(obesity related glomerulopathy，ORG)，早期的ORG起病隐匿，初期无明显临床表现，然而随着病情的进展，逐渐发展为以肾小球肥大和局灶节段性肾小球硬化为病理特点的肾损伤，临床表现出现蛋白尿和肾功能受损，最终导致终末期肾病[2]。ORG的发病机制并不明确，其中血流动力学改变、肾素-血管紧张素-醛固酮系统激活、高脂诱导的脂毒性、氧化应激及炎症因子增加等机制的研究较为深入[3]。在ORG患者的肾脏组织中，炎症因子白细胞介素(interleukin，IL)-6、肿瘤坏死因子-α(tumor necrosis factor-α，TNF-α)和IL-1β显著增加[4]。在体外实验中，棕榈酸(palmitic acid，PA)激活足细胞NLRP3炎症小体及核因子-κB(nuclear factor κB，NF-κB)通路，促进炎症因子如IL-6、TNF-α、单核细胞趋化蛋白-1(monocyte chemotactic protein-1，MCP1)、IL-18等表达，导致足细胞凋亡增加、足细胞表面标志分子表达下调[5-6]。

早期生长反应蛋白(early growth response protein，EGR)3属EGRs家族成员，EGRs包括EGR1~4，是典型的C2H2型锌指蛋白转录因子，EGR3参与调控肌肉、神经、淋巴细胞的发育及细胞的生长、分化和迁移等[7-8]。EGR3与炎症因子信号通路密切相关，在T细胞中，EGR3通过与p50及p65相互作用调控NF-κB通路介导炎症因子产生[9]。此外，在*EGR3*敲减鼠的B细胞和T细胞中，磷酸化信号转导及转录激活蛋白3(phosphorylated signal transducer and activator of transcription 3，p-STAT3)的活性显著增加[10]，p-STAT3是经典炎症JAK-STAT通路的关键转录因子[11]。然而，在16HBE细胞中沉默EGR3可减少香烟提取物诱导的IL-6、TNF-α和IL-1β的分泌[12]。

目前，关于EGR3参与调控足细胞功能的研究较少。本研究拟检测EGR3在ORG患者和高脂饮食诱导的肥胖小鼠肾皮质组织中的表达，并探讨EGR3减轻PA诱导的足细胞炎症损伤的分子机制，旨在为ORG的治疗提供新的靶点。

1 材料与方法

1.1 材料

1.1.1 动物

4周龄的雄性C57BL/6J小鼠购自湖南斯莱克景达实验动物有限公司，动物饲养在中南大学湘雅医学院实验动物学部。

1.1.2 细胞、试剂

人足细胞系由英国Bristol大学Moin A. Saleem教授惠赠；小鼠足细胞系MPC5由中南大学湘雅三医院内分泌科实验室提供。

胎牛血清购自以色列Bioind公司；TRIzol RNA抽提试剂购自美国Invitrogen公司；反转录试剂盒购自美国Fermentas公司；引物和SYBR Green qPCR Mix购自北京擎科生物科技股份有限公司；RPMI 1640培养基和BCA蛋白质定量试剂盒购自美国Thermo Fisher公司；干扰素-γ(interferon-γ，INF-γ)、PA和胰蛋白酶购自美国Sigma公司；蛋白精氨酸甲基转移酶1(protein arginine methyltransferases 1，PRMT1)抑制剂(MS023)购自美国Selleck Chemicals公司；蛋白质提取试剂盒购自南京凯基生物科技发展有限公司；免疫共沉淀(co-immunoprecipitation，Co-IP)试剂盒购自广州辉骏生物科技股份有限公司；人慢病毒EGR3过表达质粒(lentiviral-EGR3，LV-EGR3)、慢病毒阴性对照质粒(lentiviral-negative control，LV-NC)、人慢病毒EGR3沉默质粒(lentiviral shRNA-EGR3，LV-shRNA-EGR3)、慢病毒沉默阴性对照质粒(lentiviral shRNA-negative control，LV-shRNA-NC)、PRMT1过表达和阴性对照质粒均由上海吉凯基因医学科技股份有限公司构建及合成；小鼠IL-6及IL-1β的酶联免疫吸附试验(enzyme linked immunosorbent assay，ELISA)试剂盒购自武汉爱博泰克生物科技有限公司；EGR3抗体(sc-390967)购自美国Santa Cruz Biotechnology公司；p-STAT3抗体(#9145)购自美国Cell Signaling Technology公司；PRMT1抗体(11279-1-AP)、STAT3抗体(10253-2-AP)、β-actin抗体(81115-1-RR)和相关二抗均购自武汉三鹰生物技术有限公司；普通饲料(1010001)及高

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脂饲料(XTHF60；含60%脂肪、20%蛋白质及20%碳水化合物)购自江苏省协同医药生物工程有限公司。

1.2 方法

1.2.1 人ORG肾组织获取

收集长沙市第一医院肾脏内科经组织病理学证实、排除其他疾病所致肾损害的ORG患者(*n*=6)的肾皮质活检组织，并收集同年龄、同性别外伤后肾损伤正常体重对照组的肾皮质组织标本(*n*=6)，取2组标本提取RNA。所有参与者均签署知情同意书，本研究经长沙市第一医院伦理委员会批准(审批号：2021-79)。

1.2.2 建立肥胖小鼠模型

4周龄的雄性C57BL/6J小鼠(*n*=20)适应性喂养1周后，分别给予普通饮食(chow diet，*n*=10)及高脂饮食(high-fat diet，*n*=10)喂养12周，检测体重、血脂及血糖，证实造模成功后，分为普通饮食组(*n*=10)及高脂饮食组(*n*=10)，取2组小鼠肾皮质组织提取RNA。

1.2.3 人和小鼠足细胞培养及PA干预

人足细胞培养在含有10%的胎牛血清、100 U/mL青霉素、0.1 mg/mL链霉素的RPMI 1640培养基中，培养条件为33 ℃、5% CO_2，待细胞达到70%~80%融合后，将细胞在37 ℃、5% CO_2条件下培养15 d，使其分化为成熟的人足细胞。

小鼠足细胞系MPC5培养在含有10%的胎牛血清、100 U/mL青霉素、0.1 mg/mL链霉素和10 U/mL IFN-γ的RPMI 1640培养基中，培养条件为33 ℃、5% CO_2，待细胞达到70%~80%的融合后，用不含IFN-γ的RPMI 1640培养基，以37 ℃、5% CO_2的条件培养细胞15 d使其分化为成熟的小鼠足细胞。

用150 μmol/L PA干预人和小鼠成熟足细胞48 h后提取RNA。

1.2.4 人足细胞转染

人足细胞分别转染LV-NC、LV-EGR3、LV-shRNA-NC及LV-shRNA-EGR3，操作步骤按操作说明书执行。

1.2.5 RNA提取和real-time RT-PCR

研磨人或小鼠肾脏皮质组织，将细胞接种于6孔培养板，每孔中加入3 mL预冷的PBS，轻柔冲洗细胞表面3遍；每孔加入1 mL预冷的TRIzol，反复吹打混匀，收集液体于1.5 mL EP管中；加入200 μL氯仿，吹打混匀后静置10 min，离心10 min后取上清液，加入600 μL的异丙醇，吹打混匀后静置10 min，离心10 min后弃上清液，风干；加入30 μL水溶解RNA。cDNA合成根据反转录试剂盒操作说明书执行。

采用real-time RT-PCR检测各目的基因mRNA的表达，以*GAPDH*为内参，$2^{-\Delta\Delta Ct}$法计算各目的基因的相对表达量。引物序列见表1。

表1 Real-time PCR引物序列

Table 1 Sequences of primers for real-time PCR

Gene names	Primer sequence
Human *EGR3*	Forward: 5'-GACATCGGTCTGACCAACGAG-3'
	Reverse: 5'-GGCGAACTTTCCCAAGTAGGT-3'
Mouse *Egr3*	Forward: 5'-TTGCCTGACAATCTGTACCCC-3'
	Reverse: 5'-TAATGGGCTACCGAGTCGCT-3'
Human *NPHS1*	Forward: 5'-CTGCCTGAAAACCTGACGGT-3'
	Reverse: 5'-GACCTGGCACTCATACTCCG-3'
Human *PODXL*	Forward: 5'-TCCCAGAATGCAACCCAGAC-3'
	Reverse: 5'-GGTGAGTCACTGGATACACCAA-3'
Human *NPHS2*	Forward: 5'-ACCAAATCCTCCGGCTTAGG-3'
	Reverse: 5'-CAACCTTTACGCAGAACCAGA-3'
Human *PDPN*	Forward: 5'-AACCAGCGAAGACCGCTATAA-3'
	Reverse: 5'-CGAATGCCTGTTACACTGTTGA-3'
Human *GAPDH*	Forward: 5'-GGAGCGAGATCCCTCCAAAAT-3'
	Reverse: 5'-GGCTGTTGTCATACTTCTCATGG-3'
Mouse *GAPDH*	Forward: 5'-AGGTCGGTGTGAACGGATTTG-3'
	Reverse: 5'-GGGGTCGTTGATGGCAACA-3'

EGR3: Early growth response protein 3; *PODXL*: Podocalyxin; *PDPN*: Podoplanin; *GAPDH*: Glyceraldehyde-3-phosphate dehydrogenase.

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1.2.6 蛋白质印迹法

用预冷PBS轻柔冲洗细胞3次，蛋白质提取试剂盒抽提细胞总蛋白质，BCA法定量蛋白质浓度，煮沸使蛋白质变性。将30 μg总蛋白质上样进行凝胶电泳1.5 h，以300 mA恒流转膜1 h，5%脱脂奶粉封闭2 h，一抗孵育过夜，二抗孵育1 h，ECL发光液发光，Gel Doc XR凝胶成像分析系统进行条带分析。

1.2.7 RNA-seq测序

分别用LV-shRNA-NC及LV-shRNA-EGR3转染人足细胞72 h后加入150 μmol/L PA干预48 h，抽提RNA后送北京擎科生物科技股份有限公司测序和进行生物信息学分析。使用R包中的Limma包对基因进行分析，得到差异表达基因(differentially expressed genes，DEGs)，使用京都基因与基因组数据库(Kyoto Encyclopedia of Genes and Genomes，KEGG)进行DEGs的信号通路富集分析。

1.2.8 Co-IP

用PRMT1过表达或阴性对照转染人足细胞，提取细胞总蛋白质，加入PRMT1一抗，于4 ℃孵育过夜，加入protein G琼脂糖珠，于4 ℃孵育3 h，4 ℃离心1 min后弃上清液，洗涤液洗涤5次，此后按蛋白质印迹法步骤进行。

1.2.9 Co-IP+液相色谱串联质谱鉴定EGR3的相互作用蛋白

采用Co-IP+液相色谱串联质谱(liquid chromatography tandem mass spectrometry，LC-MS)鉴定EGR3的相互作用蛋白质(由北京百泰派克生物科技有限公司完成)。分别用LV-EGR3和LV-NC转染人足细胞72 h，收集2组细胞，抽提细胞总蛋白质，按照Co-IP方法收集富集的蛋白，用胰蛋白酶酶解处理后，采用LC-MS检测可能与EGR3相互作用的蛋白质，使用MaxQuant(1.6.2.10)软件进行结果分析。

1.2.10 细胞培养液中IL-6及IL-1β的测定

收集各组细胞培养液，按ELISA试剂盒说明书进行IL-6及IL-1β含量的测定。

1.3 统计学处理

采用SPSS 24.0软件进行数据分析。计量资料均以均数±标准差($\bar{x}\pm s$)表示，2组间比较采用*t*检验。*P*<0.05为差异有统计学意义。

2 结　果

2.1 *EGR3*在ORG患者肾脏皮质组织和PA干预的足细胞中表达显著下调

与正常体重者相比，*EGR3*在ORG患者肾皮质组织中的表达显著下调；与正常饮食小鼠相比，*EGR3*在高脂饮食诱导的肥胖小鼠肾皮质组织中的表达显著下调(均*P*<0.01，图1A和1B)。此外，150 μmol/L PA干预人和小鼠足细胞48 h，*EGR3*的表达较其对照组均下调(均*P*<0.05，图1C和1D)。

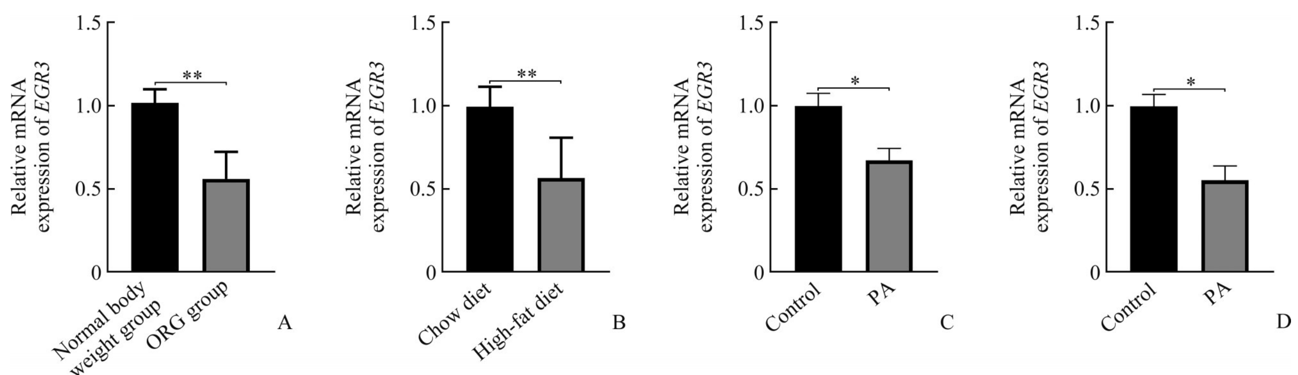

图1　PA诱导足细胞*EGR3*表达下调

Figure 1　*EGR3* is down-regulated in podocytes induced by PA

A: *EGR3* is down-regulated in human renal cortex tissues of the ORG group (*n*=6) compared with the normal body weight group (*n*=6). B: *EGR3* is down-regulated in mice renal cortex tissues of the high-fat diet group (*n*=10) compared with the chow diet group (*n*=10). C and D: *EGR3* is down-regulated in human (C) and mice (D) podocytes after treating with 150 μmol/L PA for 48 hours. *P<0.05, **P<0.01. EGR3: Early growth response protein 3; PA: Palmitic acid; ORG: Obesity related glomerulopathy.

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2.2 EGR3负调控PA诱导的足细胞炎症因子分泌及足细胞损伤

人足细胞中分别过表达或沉默EGR3，72 h后用150 μmol/L PA干预48 h，结果发现：过表达或沉默EGR3分别减少或增加PA诱导的人足细胞IL-6及IL-1β的分泌(均*P*<0.05，图2A和2B)；此外，过表达或沉默EGR3分别上调或下调PA诱导的*NPHS1*、*PODXL*、*NPHS2*及*PDPN* mRNA的表达(均*P*<0.05，图2C~2F)。

2.3 PRMT1为EGR3的相互作用蛋白质

为探讨EGR3保护PA诱导的炎症损伤的分子机制，在人足细胞中过表达EGR3，RNA-seq筛选下游调控分子，结果发现共有988个DEGs，其中630个基因上调，358个基因下调(图3A和3B)。DEGs通路分析显示：DEGs在JAK-STAT、PI3K-Akt及类风湿关节炎等信号通路显著富集(图3C)。此外，通过Co-IP+LC-MS检测共发现238个可能与EGR3相互作用的蛋白质，列出评分最高的10个可能的相互作用蛋白质(表2)，将RNA-seq及Co-IP+LC-MS结果取交集，共获得16个基因(图3D)，通过Co-IP进一步证实PRMT1为EGR3的相互作用蛋白质(图3E)。

2.4 EGR3通过PRMT1/p-STAT3通路调控PA诱导的足细胞炎症因子分泌

ELISA结果显示：PRMT1抑制剂能部分减少人足细胞沉默EGR3后PA诱导的IL-6及IL-1β的分泌(均*P*<0.05，图4A和4B)。此外，尽管过表达或沉默EGR3不影响SATA3的表达，但EGR3负调控PRMT1及p-STAT3的表达(图4C)。

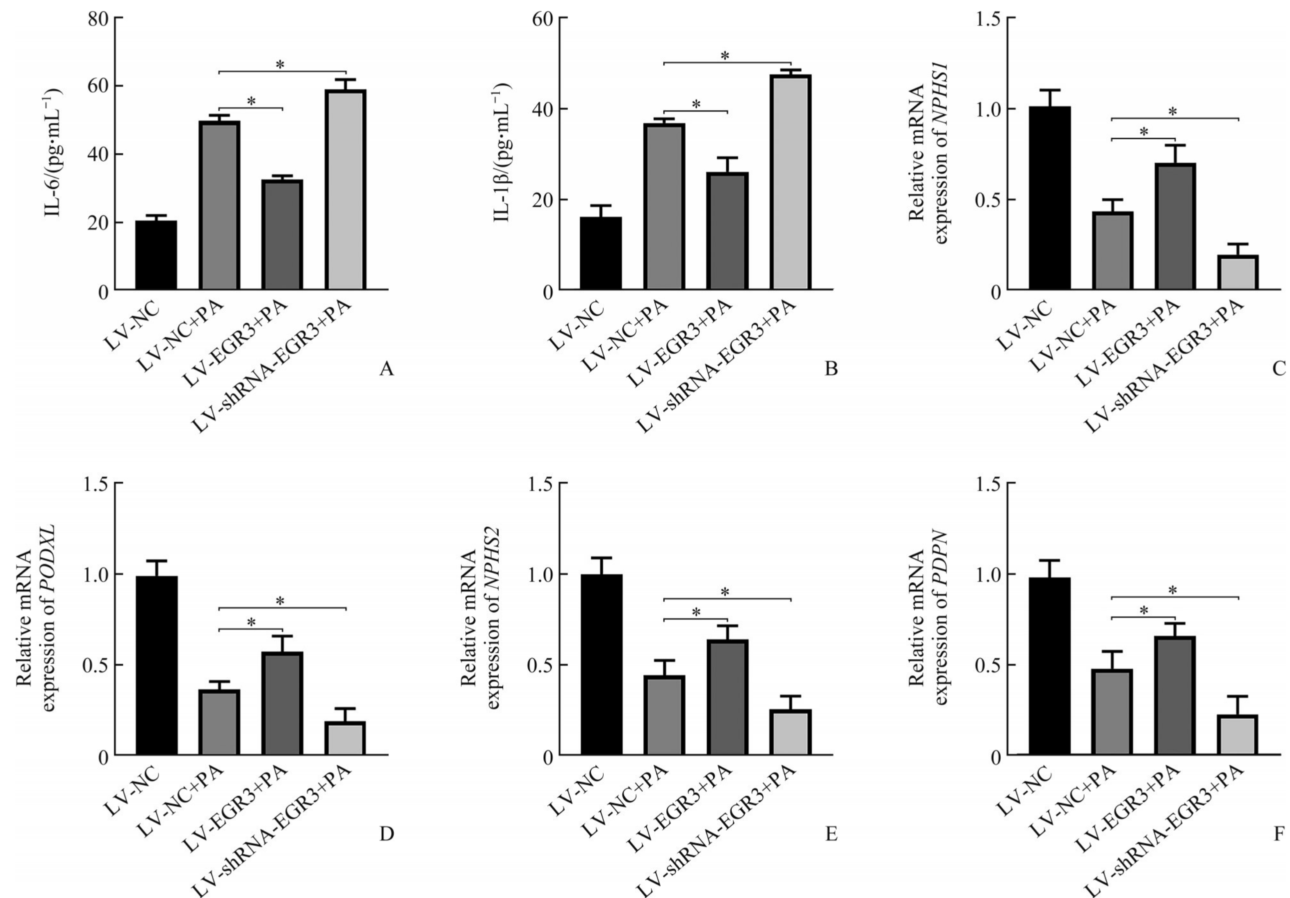

图2　EGR3负调控PA诱导的炎症因子分泌及足细胞损伤

Figure 2　EGR3 negatively regulates PA induced inflammation factors secretion and podocytes damage

A and B: EGR3 decreases PA induced IL-6 (A) and IL-1β (B) in human podocytes. C−F: EGR3 upregulates the mRNA expressions of *NPHS1* (C), *PODXL* (D), *NPHS2* (E), and *PDPN* (F) in human podocytes inhibited by PA. **P*<0.05. EGR3: Early growth response protein 3; PA: Palmitic acid; IL-6: Interleukin-6; IL-1β: Interleukin-1β.

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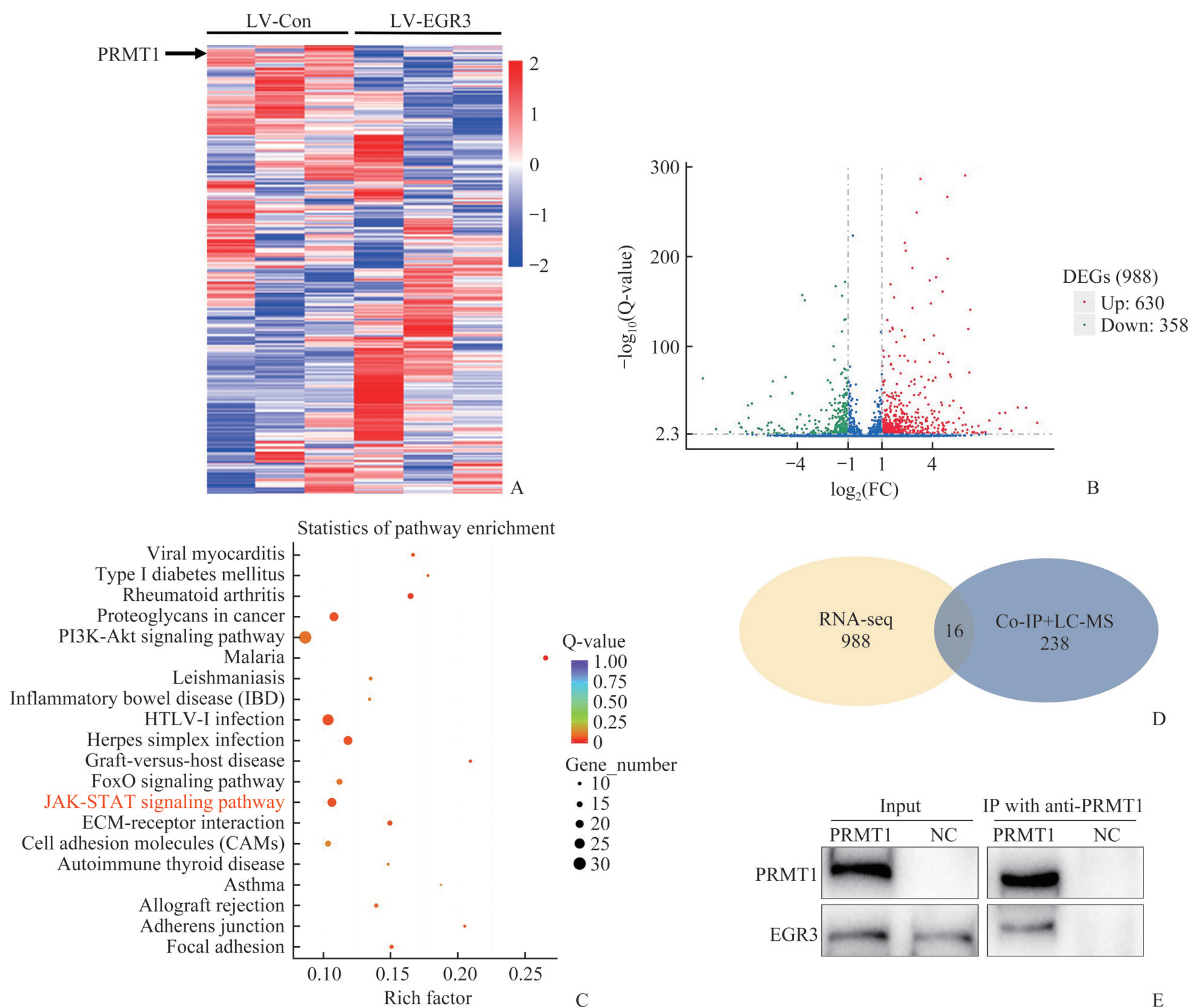

图3　Co-IP+LC-MS联合RNA-seq测序筛选EGR3下游靶基因

Figure 3　Co-IP+LC-MS combined with RNA-seq sequencing for screening downstream target genes of EGR3

A: Heatmap analysis of DEGs; B: Scatter plot of DEGs; C: Pathway analysis of DEGs; D: Co-IP+LC-MS analysis; E: EGR3 interact with PRMT1 using Co-IP. EGR3: Early growth response protein 3; DEGs: Differentially expressed genes; Co-IP: Co-immunoprecipitation; LC-MS: Liquid chromatography tandem mass spectrometry; PRMT1: Protein arginine methyltransferases 1.

表2　Co-IP+LC-MS鉴定EGR3的相互作用蛋白质(部分)

Table 2　Identification of EGR3 interacting proteins (partial) with Co-IP+LC-MS

Protein names	Gene names	Q-value	Score
Keratin, type II cytoskeletal 1	*KRT1*	0	323.31
Keratin, type I cytoskeletal 10	*KRT10*	0	261.91
Keratin, type I cytoskeletal 9	*KRT9*	0	237.23
F-box only protein 22	*FBXO22*	0	187.14
Actin, cytoplasmic 1	*ACTG1*	0	173.37
Protein arginine N-methyltransferase 1	*PRMT1*	0	167.69
Phosphoglycerate kinase 1	*PGK1*	0	158.55
Creatine kinase B-type	*CKB*	0	144.46
Aspartate aminotransferase	*GOT2*	0	139.41
40S ribosomal protein SA	*RPSA*	0	138.31

Co-IP: Co-immunoprecipitation; LC-MS: Liquid chromatography tandem mass spectrometry; EGR3: Early growth response protein 3.

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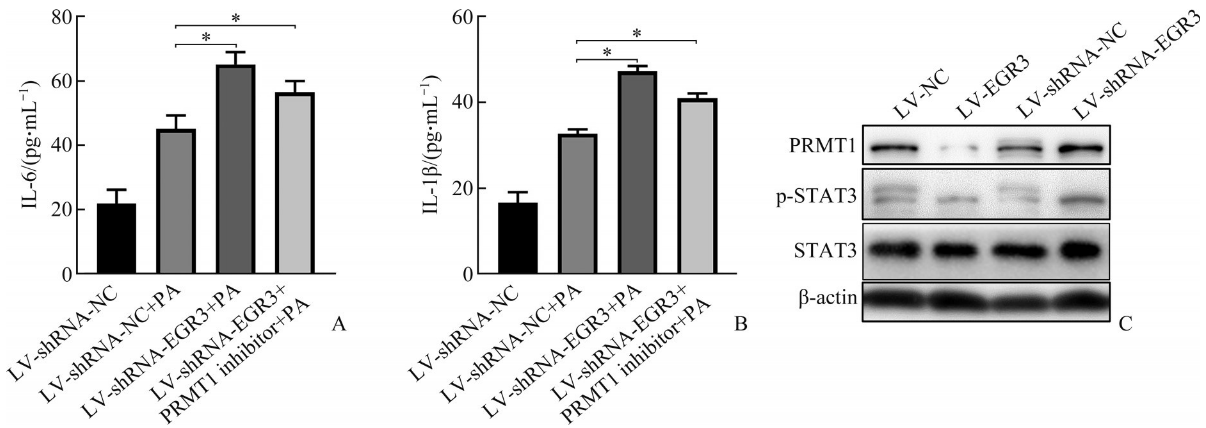

图4 EGR3抑制PRMT1/p-STAT3通路减少PA诱导的足细胞炎症因子分泌

Figure 4 EGR3 inhibits PRMT1/p-STAT3 pathway and reduces PA induced inflammatory factor secretion in podocytes

A and B: PRMT1 inhibitor partially rescues PA induced IL-6 (A) and IL-1β (B) secretion due to silence of EGR3. C: Overexpression of EGR3 decreases PRMT1 and p-STAT3 and silencing of EGR3 increases PRMT1 and p-STAT3, but not STAT3. *$P<0.05$. EGR3: Early growth response protein 3; PA: Palmitic acid; IL-6: Interleukin-6; IL-1β: Interleukin-1β; PRMT1: Protein arginine methyltransferases 1; p-STAT3: Phosphorylated signal transducer and activator of transcription 3.

3 讨 论

研究[13]表明，脂毒性所致的肾足细胞损伤是OGR发病机制的重要原因之一，脂滴在足细胞胞质内沉积，导致细胞内氧化应激增加，激活炎症通路，促进炎症因子的表达及分泌，最终引起足细胞功能的受损。

EGR3是调控细胞生长、分化与凋亡、昼夜节律等一系列重要生理活动的分子，在组织中广泛表达，作为关键的转录因子，能被各种刺激，如细胞因子、炎症因子、电离辐射和某些化合物如佛波酯迅速而短暂地激活[14]。EGR3在各种病理过程中扮演不同的角色：EGR3在前列腺肿瘤中低表达，过表达EGR3显著抑制前列腺肿瘤细胞的上皮向间质转化、迁移及侵袭[7]；EGR3在维甲酸诱导的SH-SY5Y细胞神经分化过程中表达下调，过表达EGR3抑制神经突生长[15]；EGR3在肝肿瘤细胞中下调，而过表达EGR3抑制肝肿瘤细胞的增殖[16]。为探讨EGR3在ORG中的作用，本研究检测了EGR3在ORG患者和肥胖小鼠肾皮质组织中的mRNA表达，发现EGR3均显著下调。此外，PA诱导人和小鼠足细胞48 h显著下调EGR3的表达，因此，笔者推测EGR3可能在ORG足细胞损伤过程中扮演关键角色。

为探讨EGR3在PA诱导的足细胞损伤中的作用，本研究在人足细胞中分别过表达或沉默EGR3后发现，过表达EGR3抑制PA诱导的足细胞IL-6及IL-1β的分泌，而沉默EGR3的结果则相反。此外，本研究还发现，过表达EGR3增加足细胞分子标志*NPHS1*、*NPHS2*、*PODXL*和*PDPN*的mRNA表达，证明EGR3可能在PA诱导的足细胞炎症因子损伤中具有保护作用。

已有研究[17]报道EGR3与炎症通路呈负相关，如EGR3在大鼠脑缺血/再灌注损伤及氧糖剥夺/复糖复氧诱导的神经元中表达显著下调，且过表达EGR3减轻神经元炎症。但在某些组织，EGR3正调控炎症因子分泌：在前列腺肿瘤细胞中，人EGR3直接结合IL-6及IL-8的启动子并促进IL-6及IL-8的表达[18]；在肺纤维化模型，过表达EGR3促进γδ T细胞产生IL-17[19]。因此，尽管EGR3参与炎症通路的调控，但在不同组织，可能存在不同的病理生理作用。

本研究进一步探讨EGR3在抑制PA诱导的足细胞炎症损伤中的分子机制，采用Co-IP+LC-MS联合RNA-seq筛选EGR3可能的相互作用蛋白质，发现共有16个基因既能与EGR3相互作用，又能受到EGR3表达的调控，而在RNA-seq的通路分析中，JAK-STAT通路显著富集。因此，笔者推测EGR3可能通过JAK-STAT通路调控下游的信号通路。JAK-STAT通路是经典的炎症调控通路，而IL-6等炎症因子是JAK-STAT通路的上游分子，一旦STAT分子被激活，p-STAT入核后启动凋亡、程序性细胞死亡和自噬等程序，导致细胞功能受损[20-21]。

本研究采用Co-IP+LC-MS联合RNA-seq筛选出16个基因，并采用Co-IP证实PRMT1是EGR3的相互作用蛋白质。PRMT1被证明与炎症通路密切相关，在髓系特异性*PRMT1*基因敲低小鼠中，肝脏中IL-10和IL-6的表达显著下调[22]。PRMT1通过抑制NF-κB

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通路可减轻脂多糖诱导的小鼠中IL-1β、IL-6和TNF-α的产生[23-24]。敲低*PRMT1*通过IL-6/STAT3通路可减轻脓毒症诱导的肾小管上皮细胞损伤[25]。此外，PRMT1抑制剂能部分减少沉默EGR3后PA诱导的足细胞IL-6及IL-1β的分泌。本研究还检测了IL-6下游分子STAT3分子的表达，发现EGR3可抑制p-STAT3的表达。尽管本研究验证了EGR3与PRMT1这2种蛋白质存在相互作用，但EGR3影响PRMT1/p-STAT通路的分子机制和在信号转导中的作用仍需进一步研究。

EGR3是否通过其他分子机制影响PRMT1/p-STAT通路并不清楚，例如，EGR3作为转录因子，能否结合PRMT1的启动子影响PRMT1的转录需要进一步验证，但因目前国内外的抗体公司暂无法提供Chip级别的EGR3抗体，这一分子机制暂无法证实；另外，EGR3是否直接影响PRMT1的活性或是否通过其他分子介导PRMT1/p-STAT通路仍不清楚，本研究团队将在未来的实验中进一步证实。

综上，本研究发现EGR3通过与PRMT1直接相互作用并下调PRMT1/p-STAT3通路抑制PA诱导的足细胞炎症因子分泌。本研究可能为EGR3如何调控ORG足细胞炎症损伤提供新的思路，并为探讨ORG的分子机制提供实验依据及治疗靶点。

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参考文献

[1] Endalifer ML, Diress G. Epidemiology, predisposing factors, biomarkers, and prevention mechanism of obesity: a systematic review[J]. J Obes, 2020, 2020: 6134362. https://doi. org/10. 1155/2020/6134362.

[2] Wei LF, Li Y, Yu Y, et al. Obesity-related glomerulopathy: from mechanism to therapeutic target[J]. Diabetes Metab Syndr Obes, 2021, 14: 4371-4380. https://doi. org/10.2147/DMSO. S334199.

[3] Yang ST, Cao CQ, Deng T, et al. Obesity-related glomerulopathy: a latent change in obesity requiring more attention[J]. Kidney Blood Press Res, 2020, 45(4): 510-522. https://doi.org/10.1159/000507784.

[4] Manabe I. Chronic inflammation links cardiovascular, metabolic and renal diseases[J]. Circ J, 2011, 75(12): 2739-2748. https://doi.org/10.1253/circj.cj-11-1184.

[5] Yu Y, Mo HY, Zhuo H, et al. High fat diet induces kidney injury via stimulating Wnt/β-catenin signaling[J]. Front Med, 2022, 9: 851618. https://doi.org/10.3389/fmed.2022.851618.

[6] Martínez-García C, Izquierdo-Lahuerta A, Vivas Y, et al. Renal lipotoxicity-associated inflammation and insulin resistance affects actin cytoskeleton organization in podocytes[J/OL]. PLoS One, 2015, 10(11): e0142291[2024-09-01]. https://doi. org/10.1371/journal.pone.0142291.

[7] Shin SH, Kim I, Lee JE, et al. Loss of EGR3 is an independent risk factor for metastatic progression in prostate cancer[J]. Oncogene, 2020, 39(36): 5839-5854. https://doi. org/10.1038/ s41388-020-01418-5.

[8] Kim KH, Son ED, Kim HJ, et al. EGR3 is a late epidermal differentiation regulator that establishes the skin-specific gene network[J]. J Invest Dermatol, 2019, 139(3): 615-625. https:// doi.org/10.1016/j.jid.2018.09.019.

[9] Wieland GD, Nehmann N, Müller D, et al. Early growth response proteins EGR-4 and EGR-3 interact with immune inflammatory mediators NF-kappaB p50 and p65[J]. J Cell Sci, 2005, 118(Pt 14): 3203-3212. https://doi.org/10.1242/jcs.02445.

[10] Li SL, Miao TZ, Sebastian M, et al. The transcription factors Egr2 and Egr3 are essential for the control of inflammation and antigen-induced proliferation of B and T cells[J]. Immunity, 2012, 37(4): 685-696. https://doi. org/10.1016/j. immuni. 2012. 08.001.

[11] Hu YS, Han X, Liu XH. STAT3: a potential drug target for tumor and inflammation[J]. Curr Top Med Chem, 2019, 19(15): 1305-1317. https://doi.org/10.2174/1568026619666190620145052.

[12] Chen PF, Jiang P, Chen JN, et al. XIST promotes apoptosis and the inflammatory response in CSE-stimulated cells via the miR-200c-3p/EGR3 axis[J]. BMC Pulm Med, 2021, 21(1): 215. https://doi.org/10.1186/s12890-021-01582-8.

[13] Schelling JR. The contribution of lipotoxicity to diabetic kidney disease[J]. Cells, 2022, 11(20): 3236. https://doi. org/ 10.3390/cells11203236.

[14] Kwon Y, Kim M, Kim Y, et al. EGR3-HDAC6-IL-27 axis mediates allergic inflammation and is necessary for tumorigenic potential of cancer cells enhanced by allergic inflammation-promoted cellular interactions[J]. Front Immunol, 2021, 12: 680441. https://doi. org/10.3389/fimmu. 2021.680441.

[15] Nie FY, Zhang QX, Ma J, et al. Schizophrenia risk candidate EGR3 is a novel transcriptional regulator of RELN and regulates neurite outgrowth via the Reelin signal pathway in vitro[J]. J Neurochem, 2021, 157(6): 1745-1758. https://doi.org/ 10.1111/jnc.15225.

[16] Zhang SJ, Xia C, Xu C, et al. Early growth response 3 inhibits growth of hepatocellular carcinoma cells via upregulation of Fas ligand[J]. Int J Oncol, 2017, 50(3): 805-814. https://doi.org/ 10.3892/ijo.2017.3855.

[17] Han B, Wang J, Hu FY, et al. Functional mechanism of EGR3 in cerebral ischemia/reperfusion injury in rats by modulating transcription of pri-miR-146a/146b to miR-146 and suppressing SORT1 expression[J]. Brain Res, 2022, 1797:

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148096. https://doi.org/10.1016/j.brainres.2022.148096.

[18] Baron VT, Pio R, Jia Z, et al. Early growth response 3 regulates genes of inflammation and directly activates IL6 and IL8 expression in prostate cancer[J]. Br J Cancer, 2015, 112(4): 755-764. https://doi.org/10.1038/bjc.2014.622.

[19] Parkinson RM, Collins SL, Horton MR, et al. Egr3 induces a Th17 response by promoting the development of γδ T cells[J/OL]. PLoS One, 2014, 9(1): e87265[2024-09-01]. https://doi.org/10.1371/journal.pone.0087265.

[20] Ivanov VN, Bhoumik A, Krasilnikov M, et al. Cooperation between STAT3 and c-Jun suppresses Fas transcription[J]. Mol Cell, 2001, 7(3): 517-528. https://doi. org/10.1016/s1097-2765(01)00199-x.

[21] Shen SS, Niso-Santano M, Adjemian S, et al. Cytoplasmic STAT3 represses autophagy by inhibiting PKR activity[J]. Mol Cell, 2012, 48(5): 667-680. https://doi. org/10.1016/j. molcel.2012.09.013.

[22] Zhao J, O'Neil M, Vittal A, et al. PRMT1-dependent macrophage IL-6 production is required for alcohol-induced HCC progression[J]. Gene Expr, 2019, 19(2): 137-150. https://doi.org/10.3727/105221618X15372014086197.

[23] Liu HB, Jiang JH, Zhao LL. Protein arginine methyltransferase-1 deficiency restrains depression-like behavior of mice by inhibiting inflammation and oxidative stress via Nrf-2[J]. Biochem Biophys Res Commun, 2019, 518(3): 430-437. https://doi.org/10.1016/j.bbrc.2019.08.032.

[24] Kim E, Jang J, Park JG, et al. Protein arginine methyltransferase 1 (PRMT1) selective inhibitor, TC-E 5003, has anti-inflammatory properties in TLR4 signaling[J]. Int J Mol Sci, 2020, 21(9): 3058. https://doi.org/10.3390/ijms21093058.

[25] Zhu Y, Wang LM, Liu R, et al. Inhibition of PRMT1 alleviates sepsis-induced acute kidney injury in mice by blocking the TGF-β1 and IL-6 trans-signaling pathways[J]. FEBS Open Bio, 2023, 13(10): 1859-1873. https://doi. org/10.1002/2211-5463.13684.

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