

• 基础研究 •

## Sublytic C5b-9 上调 KLF5 促进 Thy-1 肾炎大鼠肾小球系膜细胞生成 IL-23 的作用

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**[摘要]** 目的: 研究亚溶解型 C5b-9(sublytic C5b-9)上调转录因子 Krüppel 样因子 5(Krüppel-like factor 5, KLF5)促进 Thy-1 肾炎(Thy-1 nephritis, Thy-1N)大鼠肾小球系膜细胞(glomerular mesangial cell, GMC)产生炎症因子白细胞介素(interleukin, IL)-23 的作用。方法: ①建立大鼠 Thy-1N 模型和体外培养大鼠 GMC, 用 Western blot(WB)检查 Thy-1N 大鼠肾组织和受 sublytic C5b-9 刺激的 GMC 中 KLF5 和 IL-23 的表达。②分别将 KLF5 过表达质粒(pIRES2-KLF5)或 KLF5 小干扰质粒(shKLF5)转染 GMC, 通过实时荧光定量 PCR 和 WB 检测 KLF5 和 IL-23 的 mRNA 和蛋白水平。③将 IL-23 全长启动子荧光素酶报告基因质粒(pGL3-IL-23-FL)转染 GMC, 再给予 sublytic C5b-9 刺激, 或将 pGL3-IL-23-FL 与 pIRES2-KLF5 或 shKLF5 共转染 GMC, 用荧光素酶报告基因实验检测 IL-23 启动子活性的变化。④将慢病毒(lentivirus, LV)包装的 LV-shKLF5 和 LV-shCTR 行肾动脉灌注术导入大鼠肾组织, 经小动物脏器可见光三维成像和冰冻切片观察 GFP 表达, 证实 LV-shCTR 在肾组织中富集效率。之后再复制大鼠 Thy-1N, 用 WB 检查肾组织中 KLF5 和 IL-23 的蛋白表达。结果: ①Thy-1N 大鼠的肾组织和 sublytic C5b-9 刺激的 GMC 中, KLF5 和 IL-23 的表达均显著升高, 且 KLF5 的表达高峰稍早于 IL-23。②在 GMC 中过表达或敲低 KLF5 能分别引起 IL-23 表达的升高或降低。③Sublytic C5b-9 刺激或 KLF5 过表达均可增加 GMC 中 IL-23 启动子的活性, 但敲低 KLF5 后可明显下调由 sublytic C5b-9 刺激 GMC 诱导的 IL-23 启动子活性。④敲低 Thy-1N 大鼠肾组织中 KLF5 的表达后, 其肾组织中 IL-23 的表达水平明显降低。结论: 大鼠 Thy-1N 发病早期, sublytic C5b-9 刺激 GMC 后可通过上调 KLF5 促进 IL-23 基因的转录与表达。

**[关键词]** Thy-1 肾炎; 亚溶解型 C5b-9; 肾小球系膜细胞; krüppel 样因子 5; 白细胞介素-23**[中图分类号]** R692.3**[文献标志码]** A**[文章编号]** 1007-4368(2024)09-1198-09**doi:** 10.7655/NYDXBNSN240571

## Sublytic C5b-9 induces IL-23 production in glomerular mesangial cells of rats with Thy-1 nephritis via up-regulated KLF5 expression

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**[Abstract]** **Objective:** To explore the role of sublytic C5b-9 upregulating the transcription factor Krüppel-like factor 5 (KLF5) in promoting the production of the inflammatory cytokine interleukin-23 (IL-23) in glomerular mesangial cells (GMCs) of rats with Thy-1 nephritis (Thy-1N). **Methods:** ① A rat model of Thy-1N was established and rat GMCs were cultured *in vitro*. The expression levels of KLF5 and IL-23 in the renal tissues of Thy-1N rats and in GMCs stimulated by sublytic C5b-9 were detected by using Western blot (WB). ② The levels of mRNA and protein of KLF5 and IL-23 in the GMCs transfected with either a KLF5 overexpressing plasmid (pIRES2-KLF5) or a KLF5 small interfering plasmid (shKLF5) were examined by RT-qPCR and WB. ③ The full-length IL-23 promoter luciferase reporter gene plasmid (pGL3-IL-23-FL) was transfected into GMCs, followed by stimulation with sublytic C5b-9.

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Alternatively, pGL3-IL-23-FL was co-transfected with either pIRES2-KLF5 or shKLF5 into GMCs, and changes in IL-23 promoter activity were measured using a luciferase reporter gene assay. ④The LV-shKLF5 and LV-shCTR lentivirus vectors were respectively perfused into rat renal tissues *via* the artery perfusion. After confirming that LV-shCTR could enrich in rat kidney through animal imaging system and frozen section, the Thy-1N was reproduced, and the KLF5 and IL-23 expression in the renal tissues were measured by WB. **Results:** ①The expressions of KLF5 and IL-23 were significantly increased in the renal tissues of Thy-1N rats and in GMCs stimulated by sublytic C5b-9, with KLF5 expression peaking slightly earlier than IL-23. ②Overexpression or knockdown of KLF5 in GMCs led to an increase or decrease in IL-23 expression, respectively. ③Sublytic C5b-9 stimulation or KLF5 overexpression upregulated the activity of IL-23 promoter, while KLF5 knockdown markedly reduced the IL-23 promoter activity induced by sublytic C5b-9. ④IL-23 expression in the renal tissues of the rats treated by knocking down of renal KLF5 gene was significantly downregulated. **Conclusion:** In the early stage of Thy-1N in rats, sublytic C5b-9 stimulates GMCs to promote the transcription and expression of the IL-23 gene by upregulating KLF5.

[Key words] Thy-1 nephritis; sublytic C5b-9; glomerular mesangial cell; krüppel-like factor 5; interleukin-23

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系膜增生性肾小球肾炎(mesangial proliferative glomerulonephritis, MsPGN)是一种免疫相关的肾脏疾病(如IgA型肾病),其主要病理特征是:肾组织早期炎症以及病程中后期的肾小球系膜细胞(glomerular mesangial cell, GMC)增生和细胞外基质(extracellular matrix, ECM)积聚,最终可引起肾脏的纤维化,导致患者的肾功能衰竭<sup>[1-3]</sup>。大鼠Thy-1肾炎(Thy-1 nephritis, Thy-1N)的病理改变与人MsPGN的病变十分相似,是目前公认的研究MsPGN的动物模型<sup>[4]</sup>。有文献报道,无论是MsPGN患者还是Thy-1N大鼠,其肾组织中不仅可见补体C5b-9复合物沉积于GMC表面,而且还可见促炎细胞因子的增多<sup>[5-8]</sup>。此外,研究证实,Thy-1N大鼠的GMC病变具有C5b-9依赖性<sup>[9-10]</sup>。

已知补体系统激活后产生的C5b-9攻击靶细胞的方式可分为全溶解型(lytic)和亚溶解型(sublytic)两种,前者可直接引起细胞膜穿孔破坏,而后者虽不直接溶解靶细胞,但能激活胞内多种信号通路,促使细胞产生不同的生物学效应<sup>[7-8, 11]</sup>。本课题组以往的研究发现,Thy-1N大鼠发病早期,其GMC表面沉积的C5b-9多为sublytic C5b-9。体外用sublytic C5b-9刺激大鼠GMC后也能活化或上调细胞内某些信号分子或转录因子,促进多种靶基因的转录和表达,最终引起GMC增生或某些促炎细胞因子的合成与释放<sup>[8-10]</sup>。

Krüppel样因子5(Krüppel-like factor 5, KLF5)属于KLF家族成员,是一种含有锌指结构的转录因子<sup>[12]</sup>,在机体多种细胞中表达,参与调控组织和细胞的炎症反应。有研究发现,当人子宫肌层细胞受到白介素(interleukin, IL)-1 $\beta$ 刺激时,其KLF5的表

达显著升高,而当沉默KLF5基因后,由IL-1 $\beta$ 诱导的促炎因子IL-6和IL-8的生成水平则明显降低<sup>[13]</sup>。另有研究证实,在糖尿病小鼠的血管平滑肌细胞中,KLF5是诱导TNF- $\alpha$ 和IL-1 $\beta$ 表达所必需的因素<sup>[14]</sup>。此外,在大鼠GMC中,敲低KLF5表达可下调由sublytic C5b-9诱导的IL-36 $\alpha$ 的表达水平<sup>[15]</sup>。由此可见,KLF5在某些促炎因子的生成过程中发挥了重要的促进效应,加剧了组织的炎症。

IL-23属于IL-12家族的一个成员,其主要功能是参与多种组织的炎症反应,诱导炎症肠病、强直性脊柱炎和狼疮性肾炎<sup>[16-18]</sup>。研究报道,伴有间质性肾炎的小鼠和人肾小管上皮细胞中IL-23表达显著升高,而IL-23高表达既可重塑肾脏固有细胞代谢,又能促进肾组织的炎症<sup>[18]</sup>。系统性红斑狼疮(systemic lupus erythematosus, SLE)患者血清中IL-23的水平亦显著升高,且其升高程度与狼疮性肾炎的活动程度呈正相关<sup>[19]</sup>。此外,在一些自身免疫性疾病(如类风湿关节炎、多发性硬化症和银屑病)中,IL-23能明显加重慢性免疫性炎症,且IL-23/IL-17轴还可加速其炎症病变的进展<sup>[20]</sup>。以上研究提示,IL-23表达升高起到了重要的促炎作用。

由于大鼠Thy-1N是研究MsPGN的动物模型,其病变具有sublytic C5b-9依赖性<sup>[4, 8-9]</sup>,且本课题组前期实验也证实,Thy-1N大鼠的肾组织和受sublytic C5b-9刺激的GMC中,KLF5和IL-23的表达显著上调,且KLF5的升高峰值还稍早于IL-23的表达高峰。此外,国内外学者发现,KLF5能调控多种促炎因子的表达<sup>[13-15]</sup>,但其能否促进IL-23的表达尚不清楚。因此,本研究利用Thy-1N大鼠模型和体外培养的大鼠GMC,探讨KLF5上调对sublytic C5b-9刺

激GMC诱导IL-23表达的影响,拟揭示Thy-1N大鼠肾组织早期炎症的参与因素,为MsPGN的深入研究提供必要的实验依据。

## 1 材料和方法

### 1.1 材料

雄性SD大鼠(180~200 g)来源于南京医科大学实验动物中心,动物实验操作经南京医科大学实验动物伦理委员会批准(IACUC-2011007)。大鼠GMC细胞株HBZY-1由武汉大学中国典型培养物保藏中心提供。多克隆兔抗大鼠胸腺细胞血清(anti-thymocyte serum, ATS)由本实验室制备(用56℃水浴30 min灭活补体)。正常人血清(normal human serum, NHS)来源于30位健康志愿者。单克隆鼠源性KLF5和IL-23抗体(Santa Cruz公司,美国),RIPA裂解液(上海碧云天生物科技有限公司),Lipofectamine 2000(Thermo Fisher Scientific公司,美国),Hieff qPCR SYBR Green Master Mix(上海翊圣生物科技有限公司),双荧光素酶报告基因检测试剂盒、pGL3-basic、pRL-SV40质粒(Promega公司,美国),pIRES2-EGFP质粒(Clontech公司,美国)。IL-23全长启动子荧光素酶报告基因质粒(pGL3-IL-23-FL)、KLF5过表达质粒(pIRES2-KLF5)、KLF5小干扰质粒(shKLF5)、shRNA对照质粒(shCTR)、慢病毒包装的shKLF5(LV-shKLF5)和shCTR(LV-shCTR)由本课题组前期分别委托通用生物(安徽)股份有限公司和上海吉玛制药技术有限公司构建。

### 1.2 方法

#### 1.2.1 Thy-1N大鼠模型的复制

取雄性SD大鼠(180~200 g),经尾静脉注射含Thy-1抗体(Thy-1 antibody, Thy-1 Ab)的ATS(剂量为0.75 mL/100 g),在注射后1、2、3、6、12 h取大鼠的肾组织( $n=5$ /时间点),实验以0 h无处理的肾组织作对照。之后,提取肾组织中的蛋白质以检测目的蛋白的表达。

#### 1.2.2 GMC的培养

将大鼠GMC细胞株接种于含10%胎牛血清的MEM完全培养液中,37℃、5% CO<sub>2</sub> 孵箱内培养48 h。当细胞密度达80%时,PBS洗涤后予0.25%胰蛋白酶消化细胞。1 000 r/min离心5 min,弃去上清,再按1:3比例稀释进行传代培养。

#### 1.2.3 Sublytic C5b-9刺激GMC的剂量

采用棋盘滴定法<sup>[8-10]</sup>,即先以不同浓度的ATS致敏GMC 30 min,再添加不同浓度的NHS孵育

30 min。行乳酸脱氢酶(lactate dehydrogenase, LDH)法检查GMC溶解的百分率(GMC溶解率<5%时被视为形成了sublytic C5b-9复合物)。本实验确定以5% ATS与4% NHS作为形成sublytic C5b-9的最佳剂量。

#### 1.2.4 质粒转染与表达验证

用Lipofectamine 2000将shCTR质粒转染GMC,48 h后观察绿色荧光蛋白(green fluorescent protein, GFP)的表达,以评估其转染效率。之后,同法给GMC转染pIRES2-EGFP或pIRES2-KLF5质粒48 h,或转染shCTR或shKLF5质粒48 h,再给予sublytic C5b-9刺激6 h。提取细胞蛋白,Western blot(WB)检测KLF5和IL-23的表达水平。

#### 1.2.5 WB实验

大鼠肾组织或GMC中加RIPA裂解液,离心收集上清测定其蛋白浓度。然后,取30 μg蛋白上样于10%的SDS-PAGE中,先用50 V电泳30 min(浓缩蛋白),再用120 V电泳2 h。接着用0.3 A恒流湿转2 h。PVDF膜用脱脂牛奶封闭2 h,加KLF5或IL-23抗体,4℃孵育过夜。次日,用TBST洗涤3次,再用HRP标记的二抗室温孵育1 h,最后加入ECL发光液并予曝光。

#### 1.2.6 实时荧光定量PCR(RT-qPCR)

用TRIzol提取GMC的总mRNA,逆转录为cDNA。另用Primer Premier 5软件设计大鼠IL-23的PCR引物,并委托南京金斯瑞生物技术有限公司合成。IL-23引物序列是:上游,5'-TGTGCCTAGGAG-TAGCAGTC-3',下游,5'-TCCATTTGTCCCCTGGT-GT-3'。RT-qPCR反应体系为cDNA模板1 μL, Hieff qPCR SYBR Green Master Mix 10 μL,上下游引物各0.4 μL, DEPC水补足至20 μL。在ABI StepOne-Plus™ PCR仪上行扩增反应,程序为:保温阶段(95℃ 5 min)、循环阶段(95℃ 10 s、60℃ 30 s,共40个循环)和溶解曲线阶段(95℃ 15 s、60℃ 60 s、95℃ 15 s)。每样本设3复孔,β-actin做内参基因,采用2<sup>-ΔΔCt</sup>计算相对倍数。

#### 1.2.7 荧光素酶报告基因实验

将GMC用sublytic C5b-9刺激6 h,或转染pIRES2-EGFP或pIRES2-KLF5质粒及pGL3-IL-23-FL联同pRL-SV40内参质粒48 h。另将shCTR或shKLF5质粒与pGL3-IL-23-FL和pRL-SV40质粒共转染GMC 48 h,再加sublytic C5b-9刺激6 h(针对shCTR或shKLF5转染组)。之后,用1×被动裂解缓冲液(passive lysis buffer, PLB)处理上述GMC,行荧

光素酶报告基因实验测定IL-23的启动子活性。即先在检测管中加入25  $\mu$ L 荧光素酶检测试剂II, 放入荧光检测仪中, 接着转移5  $\mu$ L 1 $\times$ PLB裂解的细胞液到检测管中, 检测萤火虫荧光素酶的活性, 最后向检测管中加25  $\mu$ L 1 $\times$ Stop&Glo<sup>®</sup>试剂, 混匀后再测定海肾荧光素酶的活性。萤火虫荧光值与海肾荧光值的比值即为最终结果。

### 1.2.8 LV-shCTR感染GMC

收集大鼠GMC悬液, 接种于96孔板(5 $\times$ 10<sup>3</sup>个孔), 培养24 h。用MEM培养液将LV-shCTR稀释成3个浓度, 即1 $\times$ 10<sup>7</sup>、1 $\times$ 10<sup>6</sup>、1 $\times$ 10<sup>5</sup> TU/mL。每个培养孔内加100  $\mu$ L的LV-shCTR稀释液, 12 h后换液, 继续培养60 h, 置荧光显微镜下观察GFP表达情况, 以选取最佳滴度的LV-shRNA。

### 1.2.9 肾动脉灌注术

经腹腔注射3 mg/mL戊巴比妥钠(1 mg/100 g), 麻醉大鼠后, 在其剑突下约0.5 cm处切开腹部。在腹腔中部找到腹主动脉旁的肾动脉。先后在两侧肾动脉开口的上端和下端用动脉夹夹闭。行头皮针穿刺并迅速将生理盐水稀释的1 mL病毒悬液(含1 $\times$ 10<sup>7</sup> TU/mL的LV-shCTR或LV-shKLF5)推注到肾动脉内。用纱布按压创口5 min使其感染肾脏细胞。5 min后松开腹主动脉上的动脉夹, 用明胶海绵按压止血。最后分层缝合, 用碘伏消毒创口<sup>[9,22]</sup>。

### 1.2.10 可见光三维成像及冰冻切片检查

为了确认上述肾动脉灌注术的成功率, 在肾动脉灌注的第72 h时取大鼠的心、肝、脾、肺和肾5种脏器, 快速置于小动物活体成像系统(PerkinElmer公司, 美国), 进行可见光三维成像检查, 即通过5种脏器的荧光强度判定肾动脉灌注LV-shCTR后的器官分布。此外, 取大鼠肾组织行冰冻切片, 再用荧光显微镜观察肾组织中GFP的表达与分布。

### 1.2.11 基因沉默的体内实验

取20只雄性SD大鼠(180~200 g), 随机分为4组(每组n=5), 即正常血清组(normal serum, NS)组、Thy-1N组、LV-shCTR+Thy-1N组和LV-shKLF5+Thy-1N组。前两组的处理是: 分别给大鼠尾静脉注射正常兔血清或ATS(剂量均为0.75 mL/100 g), 后两组的处理是: 大鼠行肾动脉灌注术分别导入LV-shCTR或LV-shKLF5 72 h后, 再注射ATS诱导Thy-1N。当Thy-1N大鼠发病6 h时, 取其肾组织, 用WB检测KLF5和IL-23蛋白的表达。

### 1.3 统计学方法

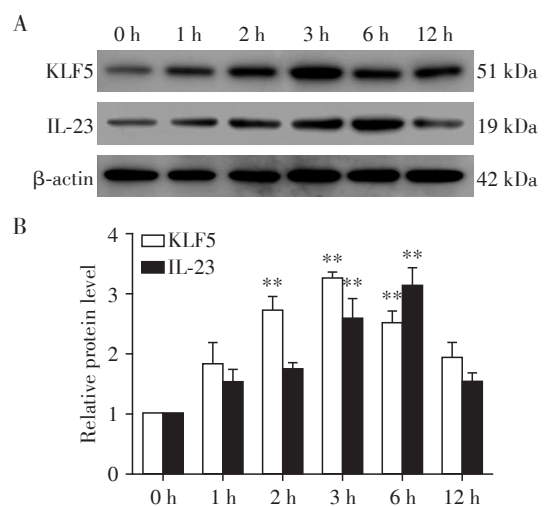
所有实验均独立重复3次, 所得定量数据以均

数 $\pm$ 标准误( $\bar{x} \pm s_x$ )表示, 两两比较采用配对t检验, 组间比较则采用单因素方差分析。应用GraphPad Prism软件(版本5.01)进行统计学分析,  $P < 0.05$ 为差异有统计学意义。

## 2 结果

### 2.1 Thy-1N大鼠发病不同时间的肾组织中KLF5和IL-23蛋白的表达

SD大鼠尾静脉注射含Thy-1Ab的ATS, 复制Thy-1N大鼠模型, 并分别于注射ATS后1、2、3、6、12 h收取大鼠的肾组织, 以0 h无处理的肾组织作对照, WB实验检测KLF5和IL-23蛋白的表达。结果发现, 两种蛋白的表达在1 h开始上升, 2 h时上升显著, 3 h时KLF5的表达呈现高峰, 而IL-23的表达则在6 h时升到峰值, 但在12 h时两种蛋白的表达均已回落(图1)。



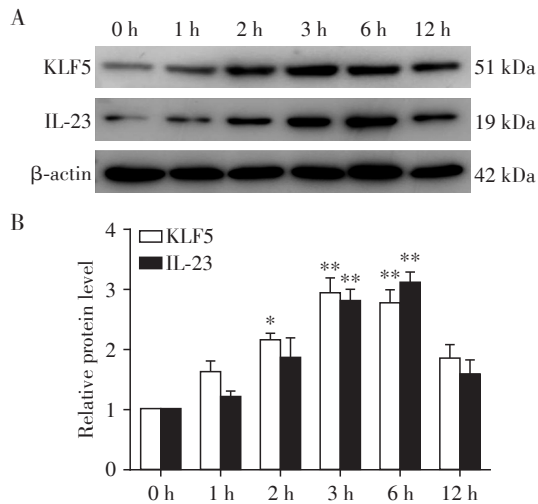
A: The protein levels of KLF5 and IL-23 in the renal tissues of Thy-1N rats at 0, 1, 2, 3, 6 and 12 h were examined by WB. B: Semi-quantitative analysis of WB. Compared with the 0 h group, \*\* $P < 0.01$  ( $n=3$ ).

图1 Thy-1N大鼠发病不同时间的肾组织中KLF5和IL-23蛋白的表达

Figure 1 The expression levels of KLF5 and IL-23 protein in the renal tissues of Thy-1N rats at different time

### 2.2 Sublytic C5b-9刺激GMC不同时间KLF5和IL-23蛋白的表达

予sublytic C5b-9刺激大鼠GMC 0、1、2、3、6、12 h, 提取GMC蛋白质, WB检测KLF5和IL-23两种蛋白的表达。结果显示, sublytic C5b-9刺激GMC后1 h, KLF5和IL-23蛋白的表达开始上调, 2 h时上升明显, 并分别在3 h和6 h达到峰值, 12 h时两种蛋白的表达已见回落(图2)。



A: The protein levels of KLF5 and IL-23 in rat GMC stimulated with sublytic C5b-9 for 0, 1, 2, 3, 6 and 12 h were examined by WB. B: Semi-quantitative analysis of WB. Compared with the 0 h group, \* $P < 0.05$  and \*\* $P < 0.01$  ( $n=3$ ).

图2 Sublytic C5b-9刺激GMC不同时间KLF5和IL-23蛋白的表达

Figure 2 The expression levels of KLF5 and IL-23 protein in rat GMC stimulated by sublytic C5b-9 at different time

### 2.3 KLF5过表达或小干扰质粒转染GMC后KLF5的表达

将KLF5过表达质粒(pIRES2-KLF5)和对照质粒(pIRES2-EGFP)转染大鼠GMC 48 h,或将KLF5小干扰质粒(shKLF5)及对照质粒(shCTR)转染GMC 48 h时再行sublytic C5b-9刺激6 h。以shCTR

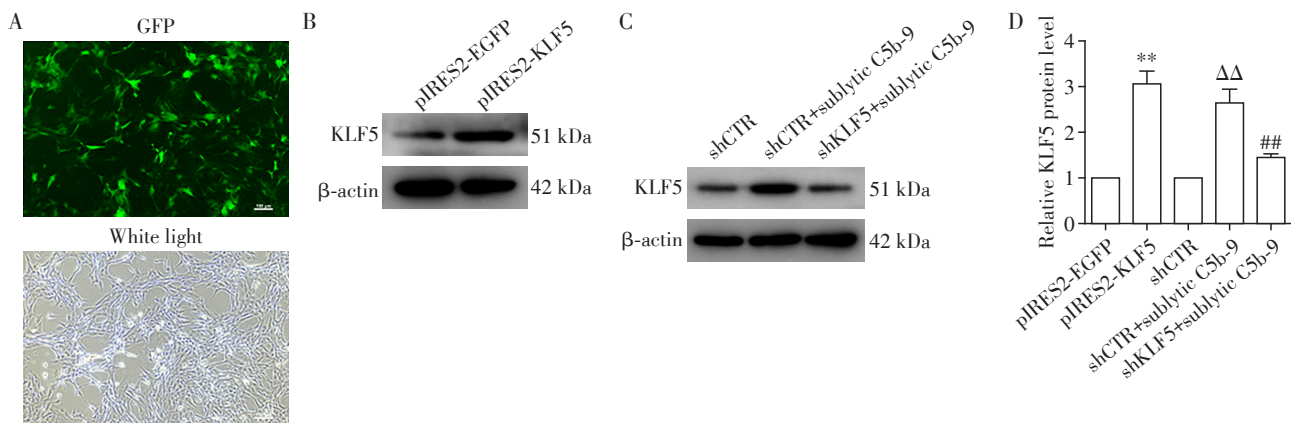
质粒为代表检查GFP表达情况,发现其转染效率可达80%左右(图3A)。之后,分别提取前述GMC中的蛋白质,用WB检查KLF5的过表达和沉默水平。结果证实,与pIRES2-EGFP转染组相比,pIRES2-KLF5转染细胞后能显著表达KLF5。shCTR+sublytic C5b-9组的KLF5表达量显著高于shCTR组,而与shCTR+sublytic C5b-9组相比,shKLF5+sublytic C5b-9组的KLF5表达则未见显著升高(图3B~D),提示,sublytic C5b-9刺激GMC能显著升高KLF5蛋白的表达,而用shKLF5则可沉默KLF5基因的表达。

### 2.4 过表达或敲低KLF5对sublytic C5b-9刺激GMC诱导的IL-23表达的影响

将pIRES2-EGFP和pIRES2-KLF5质粒分别转染GMC 48 h,或将shCTR和shKLF5质粒分别转染GMC后48 h再给予sublytic C5b-9刺激6 h。提取细胞的mRNA和蛋白,行RT-qPCR和WB实验。结果表明,转染pIRES2-KLF5过表达质粒的GMC,IL-23 mRNA和KLF5及IL-23的蛋白水平均明显升高,而转染shKLF5质粒的GMC,其由sublytic C5b-9诱导的IL-23 mRNA以及蛋白水平均显著低于shCTR+sublytic C5b-9组(图4)。

### 2.5 Sublytic C5b-9刺激和高低表达KLF5对IL-23启动子活性的影响

将IL-23全长启动子荧光素酶报告基因质粒(pGL3-IL-23-FL)转染GMC 48 h,再用sublytic C5b-9刺激GMC 6 h,或将pGL3-IL-23-FL质粒分别与pIRES2-KLF5或shKLF5质粒(包括各自的对照质



A: Rat GMCs were transfected with shCTR plasmid for 48 h, and GFP expression showed that the transfection efficiency was up to 80% ( $\times 100$ , scale bar=100  $\mu\text{m}$ ). B-C: GMCs were transfected with pIRES2-KLF5 plasmid for 48 h (B) or shKLF5 plasmid for 48 h followed by sublytic C5b-9 stimulation for 6 h (C), and the protein level of KLF5 was detected by WB. D: Semi-quantitative analysis of WB (figure 3 B and C). Compared with the pIRES2-EGFP group, \*\* $P < 0.01$ ; compared with the shCTR group,  $\Delta\Delta P < 0.01$ ; compared with the shCTR+sublytic C5b-9 group, ## $P < 0.01$  ( $n=3$ ).

图3 GMC质粒转染效率以及KLF5过表达或小干扰质粒转染GMC后KLF5的表达

Figure 3 The efficiency of plasmid transfection into GMC and the expression of KLF5 in the GMC transfected with pIRES2-KLF5 or shKLF5 plasmids

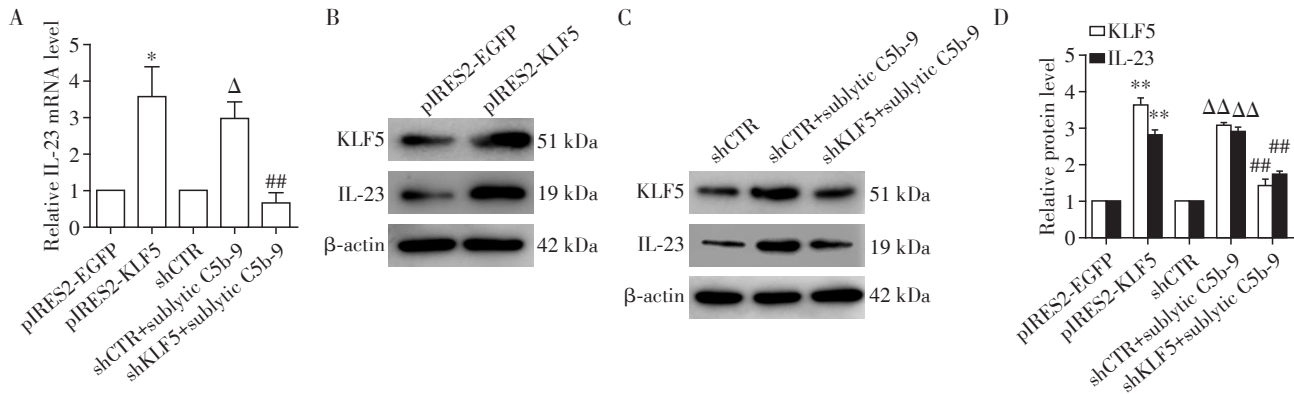


Figure 4 GMC中过表达或敲低KLF5后对IL-23表达的影响  
Figure 4 Effect of KLF5 overexpression or knockdown on IL-23 expression in the GMC

粒)共同转染GMC 48 h,其中转染shKLF5的GMC再加sublytic C5b-9刺激6 h。测定IL-23启动子的活性发现,sublytic C5b-9刺激的GMC(图5A)或在GMC中过表达KLF5(图5B)均可明显升高IL-23启动子的活性,但沉默KLF5基因后由sublytic C5b-9刺激诱导的IL-23启动子活性则未见显著提高(图5B)。

### 2.6 LV-shRNA 感染 GMC 的剂量及大鼠肾脏导入 LV-shRNA 的确定

先将LV-shCTR制备成3种滴度(即 $1 \times 10^7$ 、 $1 \times 10^6$ 、 $1 \times 10^5$  TU/mL),分别感染GMC 72 h,观察GFP表达发现,3种滴度的LV-shCTR均能感染大鼠GMC,但以 $1 \times 10^7$  TU/mL滴度感染后GFP表达最强,效率可达90%以上(图6A)。选用最佳滴度的LV-shCTR行大鼠动脉灌注术<sup>[22]</sup>,将其导入肾组织,同时设生理盐水灌注对照组。实验72 h时,取出大鼠心、肝、脾、肺和肾,进行可见光三维成像。结果显示,LV-shCTR灌注大鼠的肾脏荧光强度显著高于生理盐水灌注大鼠的肾脏,而其他脏器的荧光强度未见明显增强(图6B)。另取大鼠肾脏进行冰冻切片,行荧光显微镜观察证实,大鼠肾小球和肾小管部位均见GFP荧光,其中肾小球更为显著(图6C)。以上结果表明,LV-shCTR不仅能有效地感染GMC,而且经肾动脉灌注后能将其有效地导入大鼠肾组织。

### 2.7 敲低KLF5表达对Thy-1N大鼠肾组织中IL-23蛋白表达的影响

将大鼠分为NS、Thy-1N、LV-shCTR+Thy-1N和LV-shKLF5+Thy-1N 4组,后两组先经肾动脉灌注相应的LV-shCTR或LV-shKLF5,于灌注后72 h注射

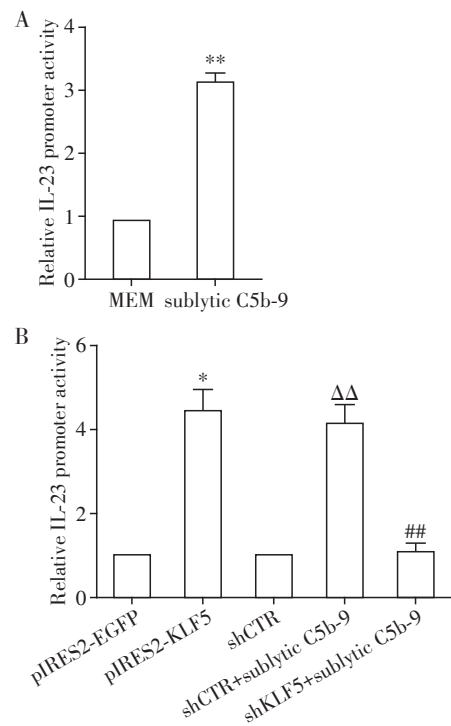
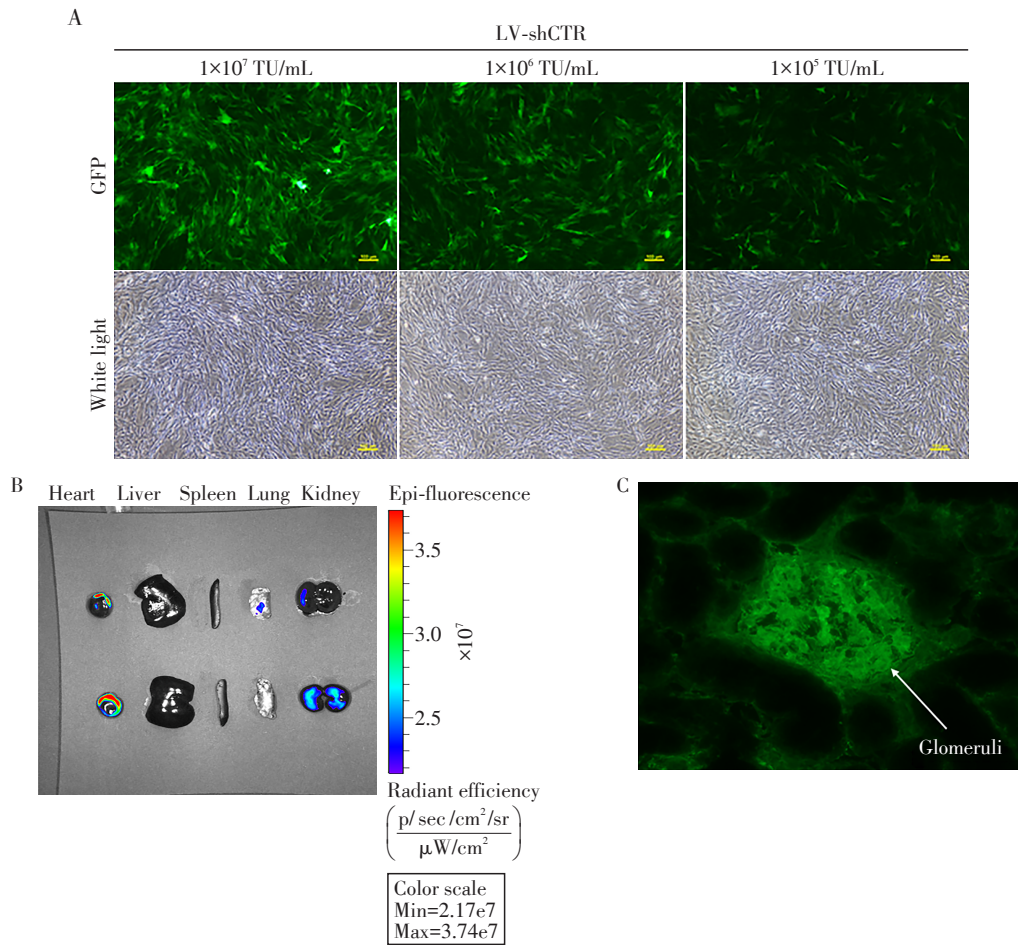


Figure 5 Sublytic C5b-9刺激GMC和高低表达KLF5后IL-23启动子活性的变化  
A: GMCs were transfected with pGL3-IL-23-FL plasmid for 48 h followed by sublytic C5b-9 stimulation for 6 h, and IL-23 promoter activity was determined by luciferase reporter assay. B: GMCs were co-transfected with pGL3-IL-23-FL and pIRES2-KLF5 or shKLF5 plasmids, then IL-23 promoter activity was determined by luciferase reporter assay. Compared with the MEM or the pIRES2-EGFP group, \* $P < 0.05$ , \*\* $P < 0.01$ ; compared with the shCTR group, <sup>Δ</sup> $P < 0.01$ ; compared with the shCTR+sublytic C5b-9 group, <sup>##</sup> $P < 0.01$  ( $n=3$ ).

Figure 5 The change of IL-23 promoter activity in the GMC after sublytic C5b-9 stimulation and KLF5 overexpression or knockdown



**图6 LV-shCTR感染GMC和大鼠组织的效率**  
**Figure 6 Efficiency of LV-shCTR infection in GMC and rat tissues**

A: Rat GMCs were infected with LV-shCTR at three different titers for 72 h, and the examination of GMCs under fluorescent microscopy showed that 1×10<sup>7</sup> TU/mL had the best infection into GMCs, with an infection efficiency of over 90% (×100, scale bar=100 μm). B: The normal saline (upper) and LV-shCTR (lower) were respectively perfused into rat renal tissues *via* the renal artery perfusion for 72 h, then imaging of five rat organs confirmed that the kidney fluorescence intensity of the rats perfused with LV-shCTR was significantly higher than that of rats perfused with normal saline. C: Renal frozen section examination showed GFP fluorescence location in both glomeruli and renal tubules (×400).

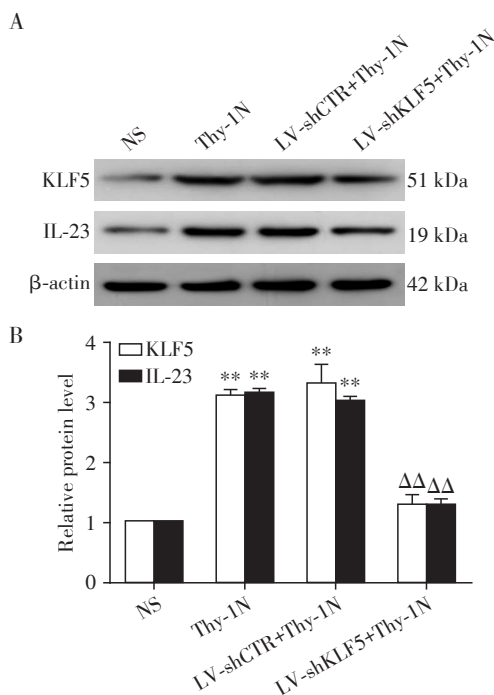
ATS,复制大鼠Thy-1N。当Thy-1N诱导后6 h时,取各组大鼠的肾组织,提取蛋白质行WB检测。结果发现,Thy-1N组和LV-shCTR+Thy-1N组,其大鼠肾组织中的KLF5和IL-23的表达量显著高于NS组,但敲低KLF5表达的LV-shKLF5+Thy-1N组,其KLF5和IL-23的表达水平则明显低于LV-shCTR+Thy-1N组(图7)。这一实验表明,敲低大鼠肾组织中KLF5表达后,其肾内促炎因子IL-23的表达受到了明显抑制,提示Thy-1N大鼠肾组织中过表达KLF5上调了IL-23的表达。

### 3 讨论

Thy-1N大鼠是研究人MsPGN的动物模型,其本质上属于一种免疫性肾小球炎症<sup>[3-4]</sup>。该模型的制

作原理是,给大鼠注射含Thy-1抗体的ATS,该抗体能随血循环进入肾小球内,与GMC表面的Thy-1抗原结合,形成免疫复合物,继而激活补体系统,产生C5b-9复合物并引起病变。本课题组前期研究已证实,在Thy-1N大鼠发病的早期,不仅可测到GMC表面有sublytic C5b-9的形成以及某些促炎因子(如IL-6、IL-17)的表达增多,而且体外用sublytic C5b-9刺激大鼠GMC后还能上调促炎因子IL-6和IL-17的表达,其机制涉及转录因子(如KLF4或C/EBPβ)的正向调控<sup>[8,21]</sup>。提示,Thy-1N大鼠早期肾组织的炎症病变与sublytic C5b-9形成后诱导GMC上调某些转录因子和促炎因子的表达明显相关。

基于课题组前期研究及国内外相关文献的报道<sup>[22-23]</sup>,本研究先检测了Thy-1N大鼠发病早期的肾



A: The protein levels of KLF5 and IL-23 in rat renal tissues of NS, Thy-1N, LV-shCTR+Thy-1N, and LV-shKLF5+Thy-1N groups were detected by WB. B: Semi-quantitative analysis of WB. Compared with the NS group, \*\* $P < 0.01$ ; compared with the LV-shCTR+Thy-1N group,  $\Delta\Delta\Delta\Delta P < 0.01$  ( $n=3$ ).

图7 敲低KLF5表达后Thy-1N大鼠肾组织中IL-23蛋白表达的变化

Figure 7 The change of IL-23 protein expression in the renal tissues of Thy-1N rats after KLF5 knock-down

组织和受sublytic C5b-9刺激的GMC中,转录因子KLF5和促炎因子IL-23的蛋白表达。结果表明,无论是体内还是体外,其KLF5和IL-23的表达水平均明显上升,且KLF5的表达时相还略早于IL-23。已知,KLF5是一种C端含有3个连续锌指结构DNA结合结构域的转录因子,其表达增加能调控下游靶基因的转录<sup>[12]</sup>。为进一步探讨Thy-1N大鼠发病早期由sublytic C5b-9刺激GMC后上调KLF5表达对IL-23生成的影响,本研究利用KLF5过表达质粒(pIRES2-KLF5)和发夹状小干扰RNA质粒(shKLF5)分别转染GMC,检查KLF5和IL-23的表达。结果证实,过表达KLF5蛋白后,GMC中IL-23的mRNA和蛋白水平也明显升高。而shKLF5沉默KLF5基因后,由sublytic C5b-9刺激GMC诱导的IL-23合成显著减少。这一实验表明,KLF5的表达水平影响GMC中IL-23的合成。

一般情况下,转录因子调控靶基因表达的关键在于其能与靶基因启动子结合,并提高靶基因的启

动子活性<sup>[21-22]</sup>。为明确KLF5作为转录因子,其表达升高或降低能否影响IL-23的启动子活性,本研究将大鼠IL-23全长启动子荧光素酶报告基因质粒(pGL3-IL-23-FL)转染GMC,一方面,行sublytic C5b-9刺激,另一方面,将其与KLF5过表达质粒共转染GMC。结果显示,无论是sublytic C5b-9刺激GMC,还是在GMC中过表达KLF5,其IL-23的启动子活性均见显著增加。此外,将shKLF5质粒与IL-23启动子质粒共转染GMC,再行sublytic C5b-9刺激,其IL-23的启动子活性则未见明显升高。以上实验表明,作为Thy-1N致病始动因素,sublytic C5b-9刺激GMC后确能上调IL-23基因的启动子活性,而KLF5过表达与sublytic C5b-9刺激的效果基本一致。但当KLF5基因被沉默后,由sublytic C5b-9诱导IL-23启动子的活性则明显受到了抑制,提示sublytic C5b-9刺激GMC后上调的KLF5能促进IL-23基因的转录与表达。

尽管体外细胞学实验的结果影响因素相对简单,且实验条件易于控制,但是体外实验的结果往往需要体内实验(如动物模型)加以验证<sup>[4,8-9]</sup>。本课题组应用慢病毒包装shKLF5和shCTR,在体外选定LV-shCTR转染GMC的最佳浓度后,行大鼠肾动脉灌注术,将上述LV-shRNA分别导入大鼠的肾组织<sup>[9,21]</sup>。结果证实,不仅相应的LV-shRNA能有效地富集于大鼠的肾脏中,而且LV-shKLF5还能沉默肾组织中的KLF5基因表达。与此同时,Thy-1N大鼠肾组织中促炎因子IL-23的表达水平也相应降低。这一体内实验再次确证,敲低大鼠肾组织中KLF5的表达能明显抑制Thy-1N大鼠肾组织中IL-23的生成。

综上所述,本研究发现,在Thy-1N大鼠发病早期,由sublytic C5b-9刺激GMC后诱导上调的KLF5能促进IL-23基因的转录与表达。然而,Thy-1N大鼠肾组织早期发生炎症的机制较为复杂<sup>[4,8-9]</sup>,本研究仅初探了KLF5表达升高对sublytic C5b-9刺激GMC诱导促炎因子IL-23生成的影响,至于KLF5调控IL-23表达的具体机制以及其他影响因素等,仍需进一步探究。

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