·论著·

芹菜素通过miR-181a-5p/SOCS3信号通路调节Treg/Th17细胞平衡减轻类风湿关节炎进展

焦宁[1],陈迎[1],常利华[2]
(中国医科大学 1.附属第一医院药学部,沈阳 110002;2.附属盛京医院风湿免疫科,沈阳 110004)

摘要 目的 探讨芹菜素调节miR-181a-5p/SOCS3信号通路对类风湿关节炎大鼠关节炎的影响及其可能的作用机制。**方法** 将Wistar大鼠随机分为对照组、模型组[胶原诱导性关节炎(CIA)组]、芹菜素组(API组)、芹菜素+敲减对照组(API+sh-NC组)和芹菜素+敲减SOCS3组(API+sh-SOCS3组)。CIA组、API组、API+sh-NC组和API+sh-SOCS3组大鼠注射乳化剂制备CIA模型,对照组同时间注射等量的生理盐水。采用关节炎指数评分评价各组大鼠关节炎症情况;采用HE染色比较各组大鼠关节滑膜病理损伤情况;采用ELISA检测各组大鼠血清中Th17相关细胞因子白细胞介素(IL)-17A和IL-6以及Treg相关细胞因子肿瘤坏死因子(TGF)-β和IL-10水平;流式细胞术检测大鼠脾细胞中Th17细胞($CD4^+$ $IL\text{-}17A^+$)和Treg细胞($CD25^+$ $Foxp^{3+}$)的细胞比例。采用RT-qPCR和Western blotting检测大鼠滑膜组织中miR-181a-5p和SOCS3表达情况。双萤光素酶报告基因实验验证miR-181a-5p与SOCS3之间的结合关系。**结果** 与对照组比较,CIA组大鼠足部肿胀程度和踝关节滑膜病理损伤加重,关节炎指数评分、血清中IL-17A和IL-6水平以及脾细胞中$CD4^+$ $IL\text{-}17A^+$细胞数明显升高,血清中TGF-β和IL-10水平以及脾细胞中$CD25^+$ $Foxp^{3+}$细胞数明显降低(均$P<0.05$);与CIA组比较,API组和API+sh-NC组大鼠足部肿胀程度和踝关节滑膜病理损伤改善,关节炎指数评分、血清中IL-17A和IL-6水平以及脾细胞中$CD4^+$ $IL\text{-}17A^+$细胞数明显降低,血清中TGF-β和IL-10水平以及脾细胞中$CD25^+$ $Foxp^{3+}$细胞数明显升高(均$P<0.05$);与API组和API+sh-NC组比较,API+sh-SOCS3组大鼠足部肿胀程度和踝关节滑膜病理损伤加重,关节炎指数评分、血清中IL-17A和IL-6水平以及脾细胞中$CD4^+$ $IL\text{-}17A^+$细胞数明显升高,血清中TGF-β和IL-10水平以及脾细胞中$CD25^+$ $Foxp^{3+}$细胞数明显降低($P<0.05$)。与对照组比较,CIA组大鼠滑膜组织中miR-181a-5p表达明显升高,*SOCS3* mRNA和蛋白表达明显降低(均$P<0.05$)。与CIA组比较,API组大鼠滑膜组织中miR-181a-5p表达明显降低,*SOCS3* mRNA和蛋白表达明显升高(均$P<0.05$)。与mimics NC组比较,miR-181a-5p mimics组转染SOCS3-WT的HEK-293T细胞相对萤光素酶活性明显降低($P<0.05$),而转染SOCS3-MUT的HEK-293T细胞相对萤光素酶活性无明显差异($P>0.05$)。**结论** 芹菜素通过下调miR-181a-5p表达上调其下游靶基因*SOCS3*表达来改善类风湿关节炎中Treg/Th17细胞失衡状态,从而改善类风湿关节炎诱导的关节损伤。

关键词 芹菜素;类风湿关节炎;Treg/Th17细胞平衡;miR-181a-5p/SOCS3信号通路
中图分类号 R684.3 **文献标志码** A **文章编号** 0258-4646(2024)12-1080-08
网络出版地址 https://link.cnki.net/urlid/21.1227.R.20241206.1532.030
DOI: 10.12007/j.issn.0258-4646.2024.12.004

Apigenin regulates Treg/Th17 cells balance reduces the progress of rheumatoid arthritis via miR-181a-5p/SOCS3 signaling pathway

JIAO Ning[1], CHEN Ying[1], CHANG Lihua[2]
(1. Department of Pharmacy, The First Hospital of China Medical University, Shenyang 110001, China; 2. Department of Rheumatology and Immunology, Shengjing Hospital of China Medical University, Shenyang 110004, China)

Abstract Objective To investigate the protective effects of apigenin (API) on the balance of Treg/Th17 cells mediated by the miR-181a-5p/SOCS3 signal pathway in a rat model of rheumatoid arthritis (RA). **Methods** Wistar rats were randomly divided into control, collagen induced arthritis (CIA), API, API + knockdown control (API+sh-NC), and API + SOCS3 knockdown (API+sh-SOCS3) groups. Inflammatory symptoms of arthritis were evaluated using the arthritis index score and gross observations of paw swelling. Pathological injury to the rat synovium was observed using HE staining. The levels of Th17-related cytokines interleukin (IL)-17A and IL-6 and Treg-related cytokines tumor growth factor (TGF)-β and IL-10 in rat serum were detected using ELISA. The proportions of $CD4^+$ $IL17A^+$ Th17 cells and $CD25^+$ $Foxp^{3+}$ Treg cells in the rat spleen were determined using flow cytometry. The expression of miR-181a-5p and SOCS3 in the synovial tissue of rats was detected using real-time fluorescence quantitative PCR (RT-qPCR) and Western blotting. Dou-

基金项目: 辽宁省自然科学基金(2023-MS-01)
作者简介: 焦宁(1988-),女,护师,大专.
通信作者: 常利华,E-mail:304043895@qq.com
收稿日期: 2024-01-19
网络出版时间: 2024-12-09 12:12:17

ble-luciferase reporter gene experiment was used to verify the combination of miR-181a-5p and SOCS3. **Results** Compared with the control group, in CIA group, paw swelling and pathological injury of ankle synovium were aggravated, arthritis index score, serum IL-17A and IL-6 levels and $CD4^+$ $IL\text{-}17A^+$ cells in spleen cells were significantly increased, while serum TGF-β and IL-10 levels and $CD25^+$ $Foxp^{3+}$ cells in spleen cells were significantly decreased (all $P < 0.05$). Compared with CIA group, paw swelling and pathological injury of ankle synovium in API group and API+sh-NC group were improved, arthritis index score, IL-17A and IL-6 levels in serum and $CD4^+$ $IL\text{-}17A^+$ cells in spleen cells were significantly decreased, while TGF-β and IL-10 levels in serum and $CD25^+$ $Foxp^{3+}$ cells in spleen cells were significantly increased (all $P < 0.05$). Compared with the control group, the expression of miR-181a-5p in the synovial tissue of the CIA group was significantly increased, and the expression of *SOCS3* mRNA and protein was significantly decreased (all $P < 0.05$). Compared with the CIA group, the expression of miR-181a-5p in the synovial tissue of the API group was significantly decreased, and the expression of *SOCS3* mRNA and protein was significantly increased (all $P < 0.05$). Compared with API group and API+sh-NC group, API+sh-SOCS group paw swelling and pathological injury of ankle synovium were aggravated, arthritis index score, serum IL-17A and IL-6 levels and $CD4^+$ $IL\text{-}17A^+$ cells in spleen cells were significantly increased, while serum TGF-β and IL-10 levels and $CD25^+$ $Foxp^{3+}$ cells in spleen cells were significantly decreased (all $P < 0.05$). Compared with the mimics NC group, the relative luciferase activity of HEK-293T cells transfected with SOCS3-WT in the miR-181a-5p mimics group decreased significantly ($P < 0.05$); however, there was no significant difference in the relative luciferase activity of HEK-293T cells transfected with SOCS3-MUT ($P > 0.05$). **Conclusion** Apigenin can improve the imbalance of Treg/Th17 cells in RA by downregulating the expression of miR-181a-5p and upregulating the expression of its downstream target gene *SOCS3*, thus improving joint injury induced by RA.

Keywords apigenin; rheumatoid arthritis; Treg/Th17 cell balance; miR-181a-5p/SOCS3 signal pathway

类风湿关节炎（rheumatoid arthritis，RA）是常见的慢性自身免疫性炎症疾病，其特征表现为持续性滑膜炎和关节破坏[1]。RA基本病变是大量炎性细胞因子产生和滑膜增生，Treg/Th17细胞失衡影响炎性细胞因子水平，进而导致RA进展[2-3]。研究[4-5]显示，Th17细胞通过分泌白细胞介素（interleukin，IL）-17促进炎症反应加速RA进展，而Treg细胞通过分泌IL-10发挥抗炎作用抑制RA进展。因此，调节Treg和Th17细胞平衡可能抑制RA发展。芹菜素（apigenin，API）是植物中最丰富的黄酮类化合物之一，具有抗氧化、抗癌和抗炎等作用[6]。已有研究[7]表明，API可通过抑制滑膜增生、血管生成、骨细胞生成在RA中发挥保护作用。此外，还有研究[8]发现API处理的树突状细胞共培养的T细胞中Th17细胞比例降低，Treg细胞比例增加。目前，API能否通过调节Treg/Th17细胞平衡来改善RA症状及其作用机制尚不清楚。本研究探讨API对胶原诱导性关节炎（collagen induced arthritis，CIA）大鼠关节炎的影响及其对Treg/Th17细胞平衡的调节作用，并进一步分析可能的作用机制。

1　材料与方法

1.1　实验动物、主要试剂和仪器

Wistar大鼠（6周龄，体重140~180 g，雄性，共50只）购自北京维通利华实验动物技术有限公司。HEK-293T细胞购自武汉普诺赛生命科技有限公司。胎牛血清和DMEM高糖培养基购自美国Gibco公司，慢病毒购自上海吉凯基因医学科技股份有限公司，Lipofectamine 3000转染试剂、细胞培养箱和流式细胞仪购自美国Thermo公司，双萤光素酶报告基因检测试剂盒和化学发光仪购自美国Promega公司，Western blotting相关试剂购自上海碧云天生物技术股份有限公司，实时荧光定量PCR（real-time fluorescence quantitative PCR，RT-qPCR）相关试剂购自南京诺唯赞生物科技股份有限公司，SOCS3和GAPDH抗体购自美国abcam公司，CD4、CD25、IL-17和Foxp3抗体购自武汉伊莱瑞特生物科技股份有限公司，HE染色试剂盒购自上海碧云天生物技术股份有限公司，酶标仪购自美国BioTek公司，凝胶成像系统购自美国Bio-Rad公司。

1.2　方法

1.2.1　CIA大鼠模型建立：取乳化剂（0.2 mL牛Ⅱ型胶原和弗氏完全佐剂乳剂等比例混合乳化）经大鼠尾部注射进行首次免疫。首次免疫后7 d取乳化剂（0.4 mL牛Ⅱ型胶原和弗氏不完全佐剂乳剂等比例混合乳化）经大鼠尾部注射进行加强免疫[3]。通过观察大鼠足部肿胀程度、检测关节炎指数评分以及HE染色观察踝关节组织病理损伤来评价模型是否

建立成功。

1.2.2　动物分组及处理：将50只Wistar大鼠随机分为对照组（Control组）、模型组（CIA组）、API组、API+敲减对照组（API+sh-NC组）和API+敲减SOCS3组（API+sh-SOCS3组），每组10只。CIA组、API组、API+sh-NC组和API+sh-SOCS3组大鼠按1.2.1方法制备CIA模型。对照组大鼠于相同时间注射乳化剂等量的生理盐水。API组、API+sh-NC组和API+sh-SOCS3组大鼠于第1次注射乳化剂前1 d开始腹腔注射API（20 mg/kg），持续50 d。API+sh-NC组和API+sh-SOCS3组于第10天和第30天经尾静脉注射敲减对照和敲减SOCS3慢病毒（50 μL，滴度为1×10^8 TU/mL）。各组大鼠于最后1次给予API后第2天脱颈椎法处死，取血清、脾脏、踝关节和滑膜组织用于后续实验。

1.2.3　关节炎指数评分：各组大鼠于造模后第7天至第50天进行关节炎指数评分[9]。评分标准：0分，正常；1分，跗关节或脚踝出现红肿；2分，红肿和轻度肿胀从脚踝蔓延到跗关节；3分，发红和中度肿胀从脚踝蔓延到跖骨关节；4分，遍及整个足部的严重肿胀和四肢僵硬。

1.2.4　HE染色：按HE染色试剂盒说明书对大鼠踝关节组织进行染色。多聚甲醛固定的踝关节组织用EDTA脱钙后，经脱水、透明、浸蜡和包埋后切成厚4 μm的石蜡切片。石蜡切片烘干后二甲苯脱蜡、梯度乙醇水化，然后依次进行苏木素染色、盐酸酒精分化和伊红染色，染色后切片经梯度乙醇脱水和二甲苯透明后，中性树胶封片，光学显微镜下观察并拍照。

1.2.5　酶联免疫吸附试验（enzyme-linked immunosorbent assay，ELISA）：严格按照ELISA试剂盒说明书检测大鼠血清中IL-17A、IL-6、TGF-β和IL-10水平。

1.2.6　流式细胞术：取大鼠脾脏，无菌PBS匀浆，用200目细胞过滤器过滤细胞悬液，300 *g*离心并弃上清，红细胞溶解液重悬细胞。取1×10^6个脾细胞，用PE偶联的抗CD4抗体和ER780偶联的抗CD25抗体4 ℃染色45 min。PBS洗涤后，细胞内固定和渗透缓冲液用于固定和渗透脾细胞。PBS洗涤脾细胞，并与APC偶联的抗IL-17抗体和FITC偶联的抗Foxp3抗体4 ℃染色45 min。PBS洗涤后流式细胞仪中检测Treg/Th17细胞。

1.2.7　RT-qPCR：大鼠滑膜组织液氮冷冻后经研钵研碎，用TRIzol试剂收集研磨后的滑膜组织并提取组织中总RNA。将提取的RNA反转录为cDNA，以cDNA为模板进行RT-qPCR反应，以*U6*和*GAPDH*为内参，采用$2^{-\Delta\Delta Ct}$法计算目的基因的相对表达量。

1.2.8　Western blotting：大鼠滑膜组织液氮冷冻后经研钵研碎，用RIPA裂解液收集研磨后的滑膜组织并提取组织总蛋白，BCA法进行蛋白定量。取20 μg蛋白样本煮样使其充分变性后进行SDS-聚丙烯酰胺凝胶电泳及转膜，5%脱脂牛奶室温封闭1 h，SOCS3（1 ∶ 1 000稀释）和GAPDH（1 ∶ 5 000稀释）抗体4 ℃孵育过夜。PBST洗膜3次，二抗室温孵育1 h，PBST洗膜3次，ECL化学发光，使用ImageJ软件进行灰度分析。

1.2.9　双萤光素酶报告基因实验：取对数生长期的HEK-293T细胞均匀铺板于24孔板中，培养过夜后，将细胞分为SOCS3-WT和mimics NC共转染组、SOCS3-WT和miR-181a-5p mimics共转染组、SOCS3-MUT和mimics NC共转染组和SOCS3-MUT和miR-181a-5p mimics共转染组，各组细胞严格按照Lipofectamine 3000转染试剂说明书进行转染，转染后细胞继续培养24 h后弃培养基，PBS洗去残余培养基，加入裂解液后室温摇晃15 min，并通过相应试剂盒检测细胞的萤光素酶活性。

1.3　统计学分析

采用SPSS 22.0软件进行统计学分析。符合正态分布的计量资料采用$\bar{x}\pm s$表示，多组间比较采用单因素方差分析，并采用Tukey事后检验进行组间两两比较。$P<0.05$为差异有统计学意义。

2　结果

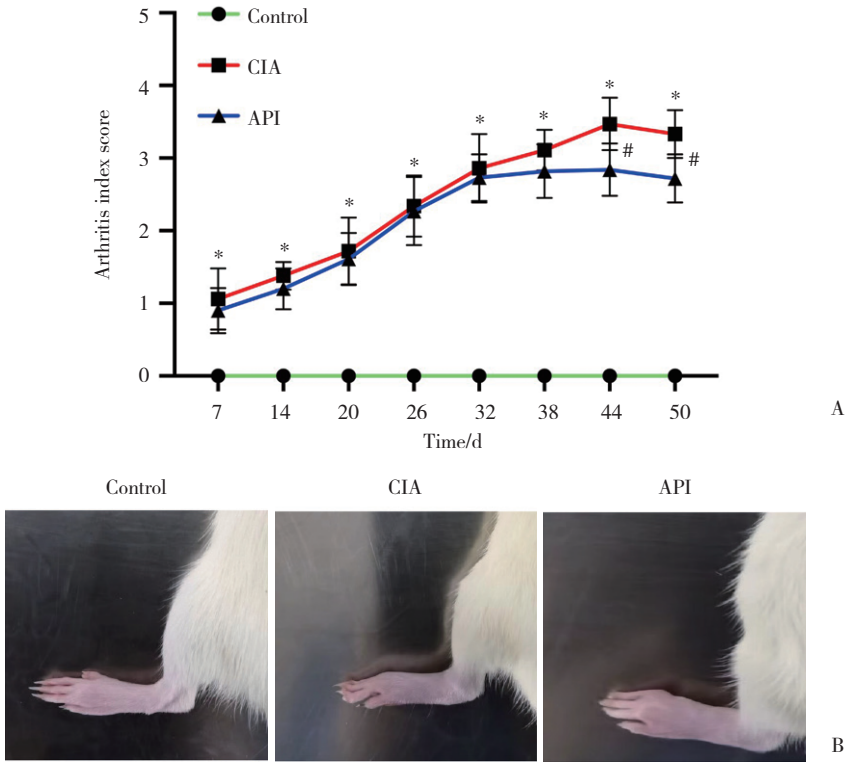
2.1　API对CIA大鼠关节炎症状的影响

Control组大鼠关节炎指数为0，且随着时间的推移无改变，足部未发现水肿；CIA组大鼠关节炎指数随时间的推移迅速增加，并于第44天达到峰值，足部水肿严重；API组大鼠关节炎指数于第44天开始明显低于CIA组（$P<0.05$），足部的水肿程度与CIA组比较明显降低。见图1。

2.2　API对CIA大鼠踝关节滑膜损伤的影响

对照组大鼠未见关节滑膜损伤。CIA组大鼠关节腔可见大量炎症细胞浸润及增生的滑膜组织。与CIA组比较，API组大鼠关节滑膜损伤改善。见图2。

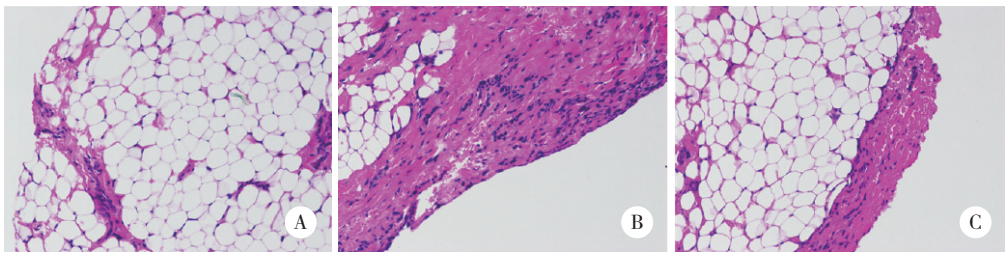
2.3　API对CIA大鼠Treg/Th17细胞平衡的影响



A, arthritis index score of rat in each group; B: gross observation results of paws of rat in each group. * $P < 0.05$ vs. control group; # $P < 0.05$ vs. CIA group.

图1　各组大鼠关节炎炎症症状

Fig.1　Arthritis inflammatory symptoms of rat in each group



A, control group; B, CIA group; C, API group.

图2　HE染色检测各组大鼠踝关节滑膜损伤×200

Fig.2　The synovial injury of ankle joint of rat in each group by HE staining ×200

与Control组比较，CIA组大鼠血清中IL-17A和IL-6水平以及脾细胞中$CD4^+$ $IL17A^+$细胞数均明显升高，血清中TGF-β和IL-10水平以及脾细胞中$CD25^+$ $Foxp^{3+}$细胞数均明显降低（$P < 0.05$）。与CIA组比较，API组大鼠血清中IL-17A和IL-6水平以及脾细胞中$CD4^+$ $IL17A^+$细胞数明显降低，血清中TGF-β和IL-10水平以及脾细胞中$CD25^+$ $Foxp^{3+}$细胞数明显升高（均$P < 0.05$）。见图3。

2.4　API对CIA大鼠滑膜中miR-181a-5p和SOCS3表达的影响

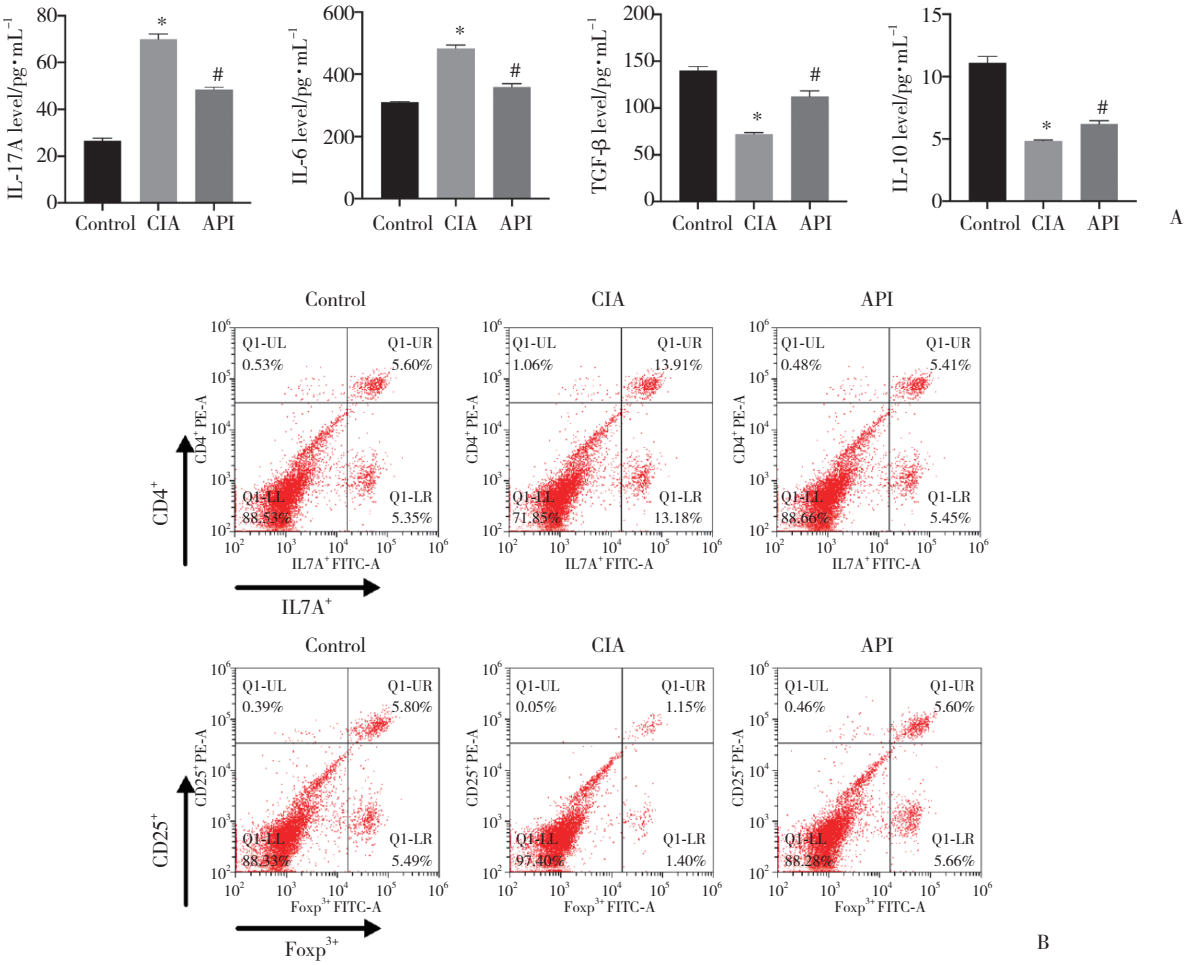
与Control组比较，CIA组大鼠滑膜组织中miR-181a-5p表达水平明显升高，*SOCS3* mRNA和蛋白表达水平明显降低（$P < 0.05$）。与CIA组比较，API组大鼠滑膜组织中miR-181a-5p表达水平明显降低，*SOCS3* mRNA和蛋白表达水平明显升高（$P < 0.05$）。见图4。

2.5　miR-181a-5p与SOCS3间的靶向关系

与mimics NC组比较，miR-181a-5p mimics组转染SOCS3-WT的HEK-293T细胞的萤光素酶活性明显降低（$P < 0.05$），而转染SOCS3-MUT的HEK-293T细胞的萤光素酶活性无明显差异（$P > 0.05$）。见图5。

2.6　API介导SOCS3影响CIA大鼠关节炎症状和踝关节滑膜损伤

与API+sh-NC组比较，API+sh-SOCS3组大鼠滑膜组织中miR-181a-5p表达水平无统计学差异（$P>0.05$），*SOCS3* mRNA和蛋白表达水平明显降低（均$P<0.05$）。API+sh-NC组大鼠关节炎指数随时间推移增加，并于第38天达到峰值，足部水肿程度和关节滑膜损伤较轻。API+sh-SOCS3组大鼠关节炎指数于第26天开始明显高于API+sh-NC组（$P<0.05$），足部水肿和关节滑膜损伤程度重于API+sh-NC组。见图6。



A，Th17 and Treg-related cytokines levels in serum of rat in each group；B，the proportion of Th17 and Treg cells in spleen cells of rat in each group. * $P<0.05$ vs. control group；# $P<0.05$ vs. CIA group.

图3　ELISA和流式细胞术检测各组大鼠Treg/Th17细胞平衡

Fig.3　Treg/Th17 cells balance of rat in each group by ELISA and flow cytometry

2.7　API介导SOCS3影响CIA大鼠Treg/Th17细胞平衡

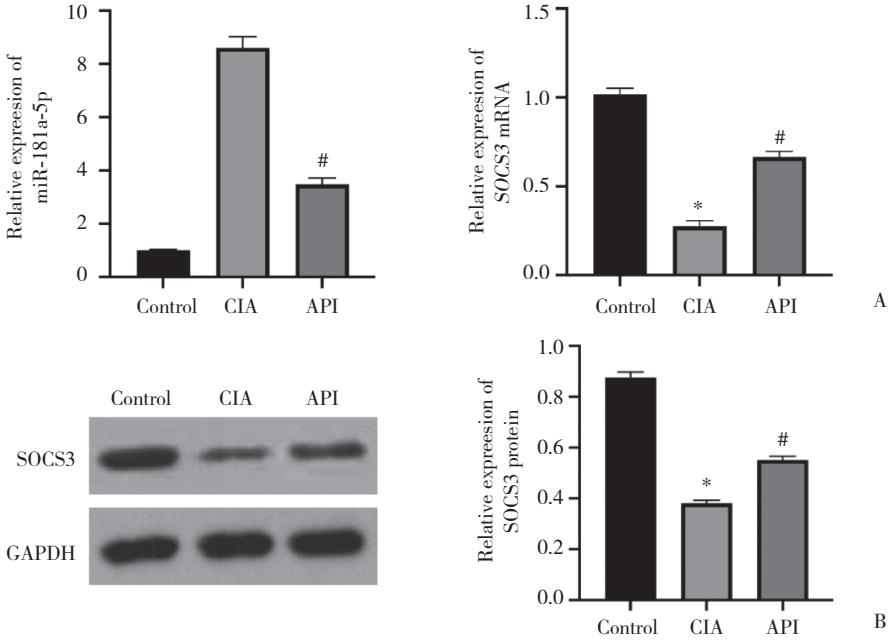
与API+sh-NC组比较，API+sh-SOCS3组大鼠血清中IL-17A和IL-6水平以及脾细胞中$CD4^+$ $IL17A^+$细胞数明显升高，血清中TGF-β和IL-10水平以及脾细胞中$CD25^+$ $Foxp^{3+}$细胞数明显降低（$P<0.05$）。见图7。

3　讨论

API对RA的改善作用已有报道。LI等[7]研究发现API抑制CIA小鼠滑膜增生，通过下调血管内皮细胞生长因子及其受体抑制CIA小鼠滑膜组织中的血管生成，并通过抑制RANKL/RANK/OPG信号通路抑制CIA小鼠滑膜组织中的破骨细胞生成。SUN等[10]研究发现，API抑制LPS诱导的巨噬细胞释放IL-6和TNF-α，并抑制ConA诱导的脾T淋巴细胞释放IFN-γ和IL-2，表明API抑制RA中免疫细胞的炎症反应。本研究结果显示，API降低CIA大鼠的关节炎指数评分，改善CIA诱导的大鼠足部肿胀和滑膜病理损伤。此外，本研究结果显示，API降低CIA大鼠血

清IL-17A和IL-6水平并增加TGF-β和IL-10水平，降低大鼠脾细胞中$CD4^+$ $IL17A^+$ Th17细胞比例并增加$CD25^+$ $Foxp^{3+}$ Treg细胞比例，表明API改善了CIA诱导的Treg/Th17细胞失衡状态。

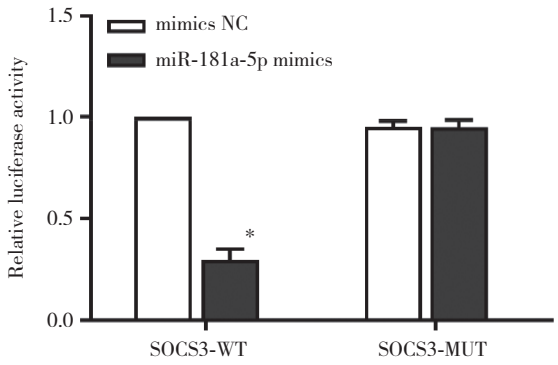
最近，多项研究表明miRNA调节RA进展中Treg/Th17细胞平衡。JIN等[11]研究发现，Maresin 1通过上调miR-21增加RA关节中Treg细胞比例并降低Th17细胞比例，改善Treg/Th17细胞失衡状态，减少关节炎症和损伤。XIE等[12]研究发现NF-κB诱导miR-34a表达抑制其靶基因*Foxp3*表达，破坏Treg/Th17细胞平衡，从而促进CIA小鼠关节损伤。本研究结果显示，API下调CIA大鼠滑膜组织中miR-181a-5p表达。此外，本研究还鉴定了miR-181a-5p的下游靶基因*SOCS3*；并发现*SOCS3* mRNA的3'-UTR存在miR-181a-5p的潜在结合位点，而且API上调CIA大鼠滑膜组织中SOCS3表达。说明API改善RA中的Treg/Th17细胞失衡状态可能是通过下调miR-181a-5p表达上调其下游靶基因*SOCS3*表达来实现的。



A，the expression of miR-181a-5p and *SOCS3* mRNA in synovial tissue of rat in each group；B，the expression of SOCS3 protein in synovial tissue of rat in each group.* $P < 0.05$ vs. control group；# $P < 0.05$ vs. CIA group.

图4　各组大鼠滑膜组织中miR-181a-5p和SOCS3表达情况

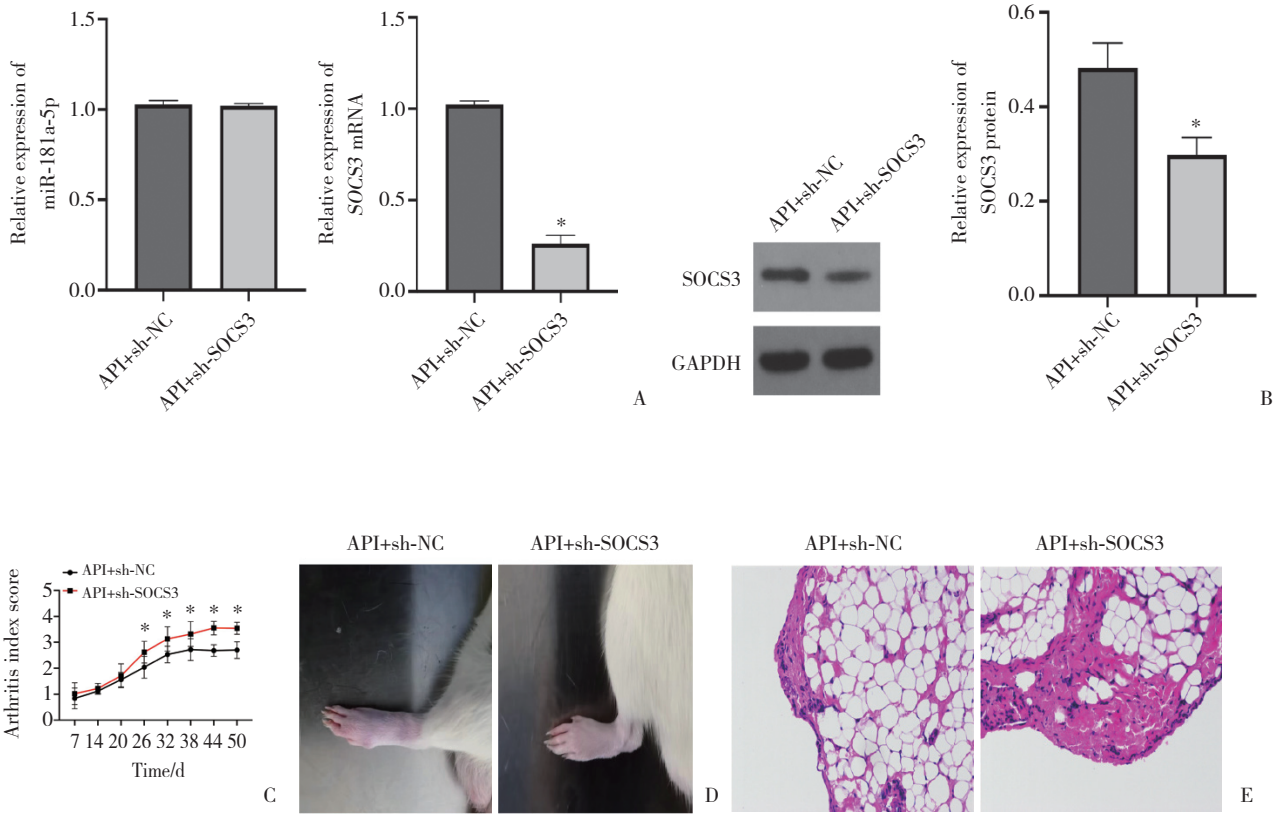
Fig.4　Expression of miR-181a-5p and SOCS3 in synovial tissue of rat in each group



* $P < 0.05$ vs. mimics NC+SOCS3-WT group.

图5　miR-181a-5p与SOCS3间的靶向关系

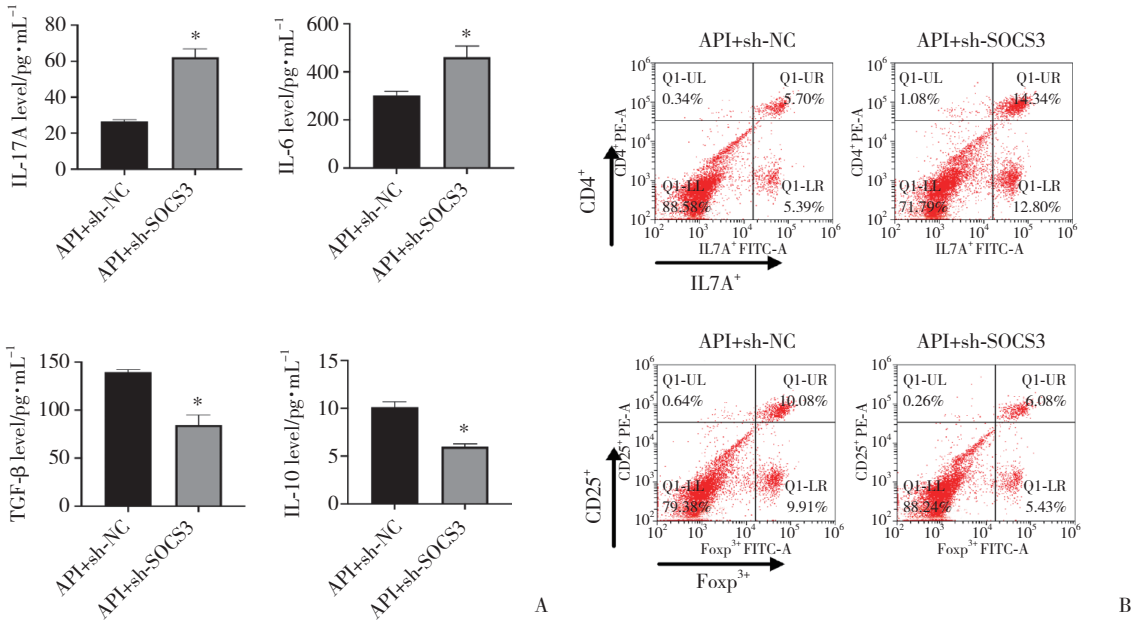
Fig.5　Targeting relationship between miR-181a-5p and SOCS3



A, the expression of miR-181a-5p and *SOCS3* mRNA in synovial tissue of rat in each group; B, the expression of SOCS3 protein in synovial tissue of rat in each group; C, arthritis index score of rat in each group; D, gross observation results of paws of rat in each group; E, the synovial injury of ankle joint of rat in each group by HE staining (× 200). * $P < 0.05$ vs. API+sh-NC group.

图6　API治疗基础上敲减*SOCS3*后CIA大鼠关节炎炎症症状以及踝关节滑膜损伤情况

Fig.6　Inflammatory symptoms of arthritis and synovial injury of ankle joint in CIA rats after knocking down *SOCS3* on the treatment of API



A, Th17 and Treg-related cytokines levels in serum of rat in each group; B, the proportion of Th17 and Treg cells in spleen cells of rat in each group. * $P < 0.05$ vs. API+sh-NC group.

图7　ELISA和流式细胞术检测各组大鼠Treg/Th17细胞平衡

Fig.7　Treg/Th17 cells balance of rat in each group by ELISA and flow cytometry

SOCS3在RA中的保护作用已广泛报道。研究[13]表明SOCS3通过抑制信号转导和转录激活因子3/类视黄醇相关的孤儿核受体γt信号通路抑制Th17细胞分化。ZHAO等[14]研究发现，艾灸可以通过调节SOCS3的表达水平来调节Treg/Th17细胞平衡，并减轻RA进展；且敲低SOCS3逆转了艾灸在缓解RA和调节Treg/Th17细胞平衡中的保护作用。AARTS等[15]研究发现，过表达IL-22可增强SOCS3表达降低CIA发病率和关节严重程度。还有研究[16]发现西洛他唑联合塞来昔布治疗通过激活IL-10和SOCS3协同作用来抑制RA患者滑膜成纤维细胞中促炎细胞因子的分泌。本研究结果显示，敲减*SOCS3*逆转了API对CIA大鼠滑膜损伤的改善作用，也逆转了API对CIA大鼠Treg/Th17细胞失衡的改善作用。

综上所述，API对RA诱导的关节损伤具有改善作用；其机制可能是通过下调miR-181a-5p表达上调其下游靶基因*SOCS3*表达，从而改善Treg/Th17细胞失衡状态来实现的。然而，本研究仅验证了敲减*SOCS3*部分逆转API对RA诱导的关节损伤的改善作用，API是否介导miR-181a-5p调节SOCS3表达发挥作用有待在后续实验中进一步验证。

参考文献：

[1] JANG S，KWON EJ，LEE JJ. Rheumatoid arthritis：pathogenic roles of diverse immune cells [J]. Int J Mol Sci，2022，23 (2)：905. DOI：10.3390/ijms23020905.

[2] MIN HK，KIM S，LEE JY，et al. IL-18 binding protein suppresses IL-17-induced osteoclastogenesis and rectifies type 17 helper T cell/regulatory T cell imbalance in rheumatoid arthritis [J]. J Transl Med，2021，19 (1)：392. DOI：10.1186/s12967-021-03071-2.

[3] FAN ZX，ROSS RP，STANTON C，et al. Lactobacillus casei CCFM1074 alleviates collagen-induced arthritis in rats via balancing treg/Th17 and modulating the metabolites and gut microbiota [J]. Front Immunol，2021，12：680073. DOI：10.3389/fimmu.2021.680073.

[4] PARADOWSKA-GORYCKA A，WAJDA A，ROMANOWSKA-PRÓCHNICKA K，et al. Th17/treg-related transcriptional factor expression and cytokine profile in patients with rheumatoid arthritis [J]. Front Immunol，2020，11：572858. DOI：10.3389/fimmu.2020.572858.

[5] XU K，MA D，ZHANG GL，et al. Human umbilical cord mesenchymal stem cell-derived small extracellular vesicles ameliorate collagen-induced arthritis via immunomodulatory T lymphocytes [J]. Mol Immunol，2021，135：36-44. DOI：10.1016/j.molimm.2021.04.001.

[6] SALEHI B，VENDITTI A，SHARIFI-RAD M，et al. The therapeutic potential of apigenin [J]. Int J Mol Sci，2019，20 (6)：1305. DOI：10.3390/ijms20061305.

[7] LI YX，YANG B，BAI JY，et al. The roles of synovial hyperplasia，angiogenesis and osteoclastogenesis in the protective effect of apigenin on collagen-induced arthritis [J]. Int Immunopharmacol，2019，73：362-369. DOI：10.1016/j.intimp.2019.05.024.

[8] GINWALA R，BHAVSAR R，MOORE P，et al. Apigenin modulates dendritic cell activities and curbs inflammation via RelB inhibition in the context of neuroinflammatory diseases [J]. J Neuroimmune Pharmacol，2021，16 (2)：403-424. DOI：10.1007/s11481-020-09933-8.

[9] ZHAO C，LI XY，YANG Y，et al. An analysis of Treg/Th17 cells imbalance associated microRNA networks regulated by moxibustion therapy on Zusanli (ST36) and Shenshu (BL23) in mice with collagen induced arthritis [J]. Am J Transl Res，2019，11 (7)：4029-4045.

[10] SUN YW，BAO Y，YU H，et al. Anti-rheumatoid arthritis effects of flavonoids from Daphne genkwa [J]. Int Immunopharmacol，2020，83：106384. DOI：10.1016/j.intimp.2020.106384.

[11] JIN SW，CHEN HJ，LI YS，et al. Maresin 1 improves the Treg/Th17 imbalance in rheumatoid arthritis through miR-21 [J]. Ann Rheum Dis，2018，77 (11)：1644-1652. DOI：10.1136/annrheumdis-2018-213511.

[12] XIE MX，WANG JZ，GONG W，et al. NF-κB-driven miR-34a impairs Treg/Th17 balance via targeting Foxp3 [J]. J Autoimmun，2019，102：96-113. DOI：10.1016/j.jaut.2019.04.018.

[13] SHI DM，YANG J，WANG Q，et al. SOCS3 ablation enhances DC-derived Th17 immune response against Candida albicans by activating IL-6/STAT3 in vitro [J]. Life Sci，2019，222：183-194. DOI：10.1016/j.lfs.2019.03.009.

[14] ZHAO C，LI XY，LI ZY，et al. Moxibustion regulates T-regulatory/T-helper 17 cell balance by modulating the microRNA-221/suppressor of cytokine signaling 3 axis in a mouse model of rheumatoid arthritis [J]. J Integr Med，2022，20 (5)：453-462. DOI：10.1016/j.joim.2022.06.002.

[15] AARTS J，ROELEVELD DM，HELSEN MM，et al. Systemic overexpression of interleukin-22 induces the negative immune-regulator SOCS3 and potently reduces experimental arthritis in mice [J]. Rheumatology，2021，60 (4)：1974-1983. DOI：10.1093/rheumatology/keaa589.

[16] LEE YS，LEE SY，PARK SY，et al. Cilostazol add-on therapy for celecoxib synergistically inhibits proinflammatory cytokines by activating IL-10 and SOCS3 in the synovial fibroblasts of patients with rheumatoid arthritis [J]. Inflammopharmacology，2019，27 (6)：1205-1216. DOI：10.1007/s10787-019-00605-5.

（编辑　武玉欣）