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·论　著·

类风湿关节炎湿热痹阻证患者外周血单核细胞中circRNA 0003353的变化及其对炎症反应的影响*

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【摘要】 **目的**　观察类风湿关节炎（rheumatoid arthritis, RA）湿热痹阻证患者外周血单核细胞（peripheral blood mononuclear cells, PBMCs）中circRNA 0003353的表达，探究其对成纤维样滑膜细胞（fibroblast-like synoviocytes, FLS）炎症反应的影响。**方法**　收集本院RA湿热痹阻证患者55例、正常人30例PBMCs及血清，观察circRNA 0003353表达及其与临床指标的相关性。构建circRNA 0003353过表达质粒和小干扰RNA，转染至RA-FLS中；RT-qPCR检测circRNA 0003353表达；酶联免疫吸附法（ELISA）检测白细胞介素（interleukin, IL）-10、IL-17的表达；Western blot检测Janus激酶2（Janus kinase 2, JAK2）、磷酸化（p）-JAK2、信号转导和转录激活因子3（signal transducers and activators of transcription 3, STAT3）、p-STAT3蛋白的表达；CCK-8法检测细胞活力；Transwell实验检测细胞迁移能力。**结果**　①与正常对照组相比，circRNA 0003353在RA湿热痹阻证患者PBMCs中表达升高（$P<0.05$）。②Pearson相关性分析表明，RA湿热痹阻证患者circRNA 0003353与红细胞沉降率（erythrocyte sedimentation rate, ESR）、类风湿因子（rheumatoid factor, RF）、核因子κ B受体活化因子配体（receptor activator of nuclear factor-κ B ligand, RANKL）、DAS28呈正相关，circRNA 0003353与IL-10呈负相关（$P<0.05$）。③关联规则结果表明，circRNA 0003353的升高与ESR、IL-17、CRP、免疫球蛋白（Ig）G升高存在显著相关。④Logistic回归分析结果表明，circRNA 0003353是RANKL、CRP、ESR的危险因素。⑤RT-qPCR结果显示，与pcDNA3.1-NC组相比，pcDNA3.1-circRNA 0003353组circRNA 0003353表达升高（$P<0.05$），与si-NC组相比，si-circRNA 0003353组circRNA 0003353表达降低（$P<0.05$）。⑥ELISA和Western blot结果显示，与pcDNA3.1-NC组相比，pcDNA3.1-circRNA 0003353组IL-10表达降低、IL-17表达升高，p-JAK2/JAK2、p-STAT3/STAT3比值升高（$P<0.05$）；与si-NC组相比，si-circRNA 0003353组IL-10表达升高、IL-17、JAK2表达降低，p-JAK2/JAK2、p-STAT3/STAT3比值降低（$P<0.05$）。⑦CCK-8和Transwell实验结果表明，与pcDNA3.1-NC组相比，pcDNA3.1-circRNA 0003353组RA-FLS细胞活力和迁移能力升高（$P<0.05$）；与si-NC组相比，si-circRNA 0003353组RA-FLS细胞活力和迁移能力降低（$P<0.05$）。**结论**　circRNA 0003353在RA湿热痹阻证患者中表达上调，通过激活JAK2/STAT3信号通路，促进炎症反应，从而参与RA的发病机制。

【关键词】 类风湿关节炎　湿热痹阻证　circRNA　成纤维样滑膜细胞　炎症

Correlation between circRNA0003353 in Peripheral Blood Mononuclear Cells and Immune Inflammation in Rheumatoid Arthritis Patients with Damp Heat Obstruction Syndrome

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【**Abstract**】 **Objective**　To investigate the expression of circRNA 0003353 in the peripheral blood mononuclear cells (PBMCs) of rheumatoid arthritis (RA) patients with dampness heat obstruction syndrome and to examine its effect on inflammatory response of fibroblast-like synoviocytes (FLS). **Methods**　The PBMCs and serum samples of 55 RA patients with dampness heat obstruction syndrome and 30 healthy volunteers were collected. The expression of circRNA 0003353 and its correlation with clinical indexes were examined. The circRNA 0003353 overexpression plasmid and siRNA were constructed and transfected into RA-FLS cell line. RT-qPCR was used to determine the expression of circRNA 0003353 mRNA. The expressions of interleukin (IL)-4, IL-10 and IL-17 were examined by ELISA. The expressions of Janus kinase 2 (JAK2), p-JAK2, signal transducers and activators of transcription 3 (STAT3) and p-STAT3 were exmained by Western blot. CCK-8 assay was used to assess cell viability. Cell migration was assessed with Transwell migration assay. **Results**　1) Compared with that of the normal group, the expression of circRNA 003353 in the PBMCs of RA patients with damp heat obstruction syndrome was significantly increased ($P<0.05$). 2) Pearson correlation analysis showed that circRNA 0003353 was positively correlated with erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), receptor activator of nuclear factor-κ B ligand (RANKL) and DAS28, and circRNA 0003353 was negatively

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correlated with IL-10 (P<0.05). 3) The findings on the association patterns showed that the increase in circRNA 0003353 was significantly correlated with the increase of ESR, IL-17, CRP and immunoglobulin (Ig) G. 4) Logistic regression analysis showed that circRNA 0003353 was a risk factor for RANKL, CRP and ESR. 5) RT-qPCR results showed that the expression of circRNA 003353 mRNA in pcDNA3.1-circRNA 0003353 group was significantly higher than that in pcDNA3.1-NC group (P<0.05), and that the expression of circRNA 003353 mRNA in si-circRNA 0003353 group was significantly lower than that in si-NC group (P<0.05). 6) ELISA and Western blot results showed that, compared with those of pcDNA3.1-NC group, the expression of IL-10 in pcDNA3.1-circRNA 0003353 group significantly decreased, the expression of IL-17 increased, and p-JAK2/JAK2 and p-STAT3/STAT3 ratios significantly increased (P<0.05). Compared with those of si-NC group, the expression of IL-10 in si-circRNA 0003353 group significantly increased, the expression of IL-17 and JAK2 decreased, and p-JAK2/JAK2 and p-STAT3/STAT3 ratios significantly decreased (P<0.05). 7) The results of CCK-8 and Transwell assays showed that the viability and migration of RA-FLS in pcDNA3.1-circRNA 0003353 group were higher than those in pcDNA3.1-NC group (P<0.05). Compared with those of si-NC group, the viability and migration ability of RA-FLS in si-circRNA 0003353 group decreased (P<0.05). **Conclusion** The expression of circRNA 0003353 is up-regulated in RA patients with damp heat obstruction syndrome, and it is involved in the pathogenesis of RA by activating the JAK2/STAT3 signaling pathway and promoting the inflammatory response.

【Key words】 Rheumatoid arthritis Dampness heat obstruction syndrome circRNA Fibroblast-like synoviocytes Inflammation

类风湿关节炎(rheumatoid arthritis, RA)是一种以对称性多关节炎为主要临床表现的自身免疫性疾病,以关节滑膜炎症、关节进行性破坏为特征[1-2],严重影响患者的生活质量和精神状况[3-4]。RA患者多属湿热痹阻证,主要临床表现为关节肿痛而热,屈伸不利;发热、口渴、小便黄等外在热象[5-6]。成纤维样滑膜细胞(fibroblast-like synoviocytes, FLS)是RA病理变化过程中的关键效应细胞,伴随FLS增殖过度、凋亡不足,炎性细胞浸润,最终导致关节畸形、功能障碍[7-8]。circRNAs是一类内源性环状RNA分子,其在FLS的增殖与凋亡、成骨细胞与破骨细胞分化、分子信号通路、免疫炎症反应等方面具有调控作用[9-12]。Janus激酶2/信号转导和转录激活因子3(Janus kinase 2/signal transducers and activators of transcription 3, JAK2/STAT3)是参与多种细胞生长、分化、凋亡的重要信号转导通路[13],研究表明其异常活化可导致RA患者体内免疫炎症反应升高[14]。不同形式的炎症因子在RA的发生发展中扮演着重要角色,根据不同功能将其分为促炎因子和抑炎因子,常见的促炎因子有肿瘤坏死因子(tumor necrosis factor, TNF)-α、白细胞介素(interleukin, IL)-1β、IL-6、IL-17等,抑炎因子主要有IL-4、IL-10、IL-13等[15-16]。本团队前期通过高通量测序及GO分析研究,认为circRNA 0003353是参与RA炎症反应的关键circRNA[17]。但是RA湿热痹阻证患者体内circRNA 0003353表达如何,以及circRNA 0003353表达与患者炎症指标、骨代谢指标等是否存在关联尚不明确。

本研究通过观察RA湿热痹阻证患者体内circRNA 0003353表达变化,分析其与炎症、骨代谢指标等的相关性,再通过体外细胞培养探究circRNA 0003353干扰或过表达状态下对RA-FLS炎症反应的影响,为RA的发病机制的阐释提供实验基础。

1 材料与方法

1.1 病例来源

55例RA湿热痹阻证患者来自安徽中医药大学第一附属医院风湿免疫科住院患者(2020年5–12月),其中男性8例,女性47例,平均年龄(59.63±12.27)岁;30例正常人来自我院健康体检中心,其中男性4例,女性26例,平均年龄(55.84±16.39)岁,两组基本情况一致,差异无统计学意义。本研究经安徽中医药大学第一附属医院伦理委员会批准(伦理编号:2019AH-12)。

1.2 观察指标

①一般性指标:年龄、性别、病程;②骨代谢、免疫炎症指标:骨钙素(bone gla-protein, BGP),护骨素(osteoprotegerin, OPG),核因子κ B受体活化因子配体(receptor activator of nuclear factor-κ B ligand, RANKL);红细胞沉降率(erythrocyte sedimentation rate, ESR),C-反应蛋白(C-reactive protein, CRP),类风湿因子(rheumatoid factor, RF),免疫球蛋白(immunoglobulin, Ig)A, IgG, IgM; IL-10, IL-17, DAS28积分。

1.3 细胞系和主要试剂

RA-FLS购自北纳生物公司;pcDNA3.1-circRNA 0003353、pcDNA3.1-NC、si-circRNA 0003353(共3条,siRNA1、siRNA2、siRNA3)、si-NC购自上海GenePharma公司;实时定量PCR(RT-qPCR)引物合成由Sangon Biotech公司合成;JAK2、STAT3、磷酸化(p)-JAK2、p-STAT3山羊抗兔抗体(ab39636、ab68153、ab32101、

ab32143, abcam)；β-actin山羊抗小鼠抗体(TA-09, ZsBIO)；TNF-α(80693, MedChemExpress)；双抗(青链霉素)(SV30010, 碧云天公司)；DMEM培养基(Hyclone)；二甲基亚砜(DMSO, Sigma公司)；胎牛血清(10099141, Gibco公司)；逆转录试剂盒(RR047A, TaKaRa)；DAPI(Solarbio)；Fluoromount-G荧光封片剂(SourthernBiotech)；ELISA试剂盒IL-10、IL-17(JYM0142Hu、JYM0155Hu, 武汉基因美科技有限公司)；Transwell小室(724301, NEST)；CCK-8试剂盒(BB-4202-01, Bestbio-贝博)。

1.4　实验方法

1.4.1　细胞培养及分组　留取RA湿热痹阻证患者及健康对照者静脉血4 mL, 利用Ficoll提取外周血单个核细胞(peripheral blood mononuclear cell, PBMCs), 保存至−80 ℃冰箱备用；RA-FLS是由SV40过表达慢病毒转染RA原代FLS, 产生的永生化细胞系, 于含100 U/mL青霉素、0.1 mg/mL链霉素的RPMI-1640培养基中培养并传代。分别将过表达载体pcDNA3.1-circRNA 0003353与其阴性对照pcDNA3.1-NC, 3条敲降载体siRNA1、siRNA2、siRNA3与其阴性对照si-NC用Lipofectamine2000转染至RA-FLS中。实验分组：RA-FLS组：细胞培养48 h；RA-FLS+TNF-α组：使用10 ng/mL TNF-α刺激细胞48 h；RA-FLS+TNF-α+pcDNA3.1-NC组：在10 ng/mL TNF-α刺激的同时, RA-FLS中加入pcDNA3.1-NC质粒共同孵育48 h；RA-FLS+TNF-α+pcDNA3.1-circRNA 0003353组：在10 ng/mL TNF-α刺激的同时, RA-FLS中加入pcDNA3.1-circRNA 0003353质粒共同孵育48 h；RA-FLS+TNF-α+si-NC组：在10 ng/mL TNF-α刺激的同时, RA-FLS中加入si-NC质粒共同孵育48 h；RA-FLS+TNF-α+si-circRNA 0003353组：在10 ng/mL TNF-α刺激的同时, RA-FLS中加入si-circRNA 0003353质粒共同孵育48 h。

1.4.2　RT-qPCR检测circRNA 0003353的表达　Trizol提取RA湿热痹阻证患者及健康对照者PBMCs及各组RA-FLS总RNA, 使用PrimeScript™RT reagent Kit with gDNA Eraser进行RT反应, 采用Novostart SYBR qPCR SuperMix Plus进行PCR反应(反应条件：95 ℃预变性1 min, 95 ℃变性20 s, 60 ℃退火1 min, 40个循环, 每组设6个重复)。引物：内参β-actin F：5′-CCCTGGAGAAGAGCTACGAG-3′, R：5′-GGAAGGAAGGCTGGAAGAGT-3′；circRNA 0003353 F：5′-CCTGACTCCGCAATCAAC-3′, R：5′-GGCATACAGAAGCCCAAA′。采用$2^{-\Delta\Delta Ct}$进行相对定量分析。

1.4.3　ELISA检测IL-10和IL-17的表达　收集各组细胞的上清液, 设置离心时间为10 min, 用差速离心机以1 000 r/min离心。在37 ℃的温度下, 于酶标板中滴入离心后上层清液, 孵育90 min。具体步骤严格按照试剂盒说明进行。

1.4.4　Western blot检测JAK2、STAT3和p-JAK2、p-STAT3蛋白的表达　收集各组细胞样本, 经过离心、煮沸、上样和电泳后, 再与Western洗涤液漂洗封闭。参考一抗说明书, 按照1∶500(JAK2)、1∶1 000(p-JAK2、STAT3)、1∶2 000(p-STAT3)用一抗稀释液进行稀释、洗涤。参考二抗说明书, 按照1∶10000(β-actin)、1∶3000(JAK2、p-JAK2、STAT3、p-STAT3)用二抗稀释液稀释辣根过氧化物酶标记的二抗。孵育、漂洗后用ECL发光试剂盒检测蛋白。Image J读取目的蛋白和内参的灰度值, 目的蛋白的灰度值/内参的灰度值即为目的蛋白的相对表达量。

1.4.5　CCK-8法检测RA-FLS的细胞活性　将各组细胞以2×10^3/孔接种到96孔板中, 置于培养箱中过夜培养(体积分数为5% CO_2, 37 ℃)。分组(同1.4.1)培养至指定时间点(0、24和48 h)将CCK-8(10 μL)装载到每个孔上, 37 ℃下再次培养4 h。使用微孔板读取器在450 nm处测量光密度(optical density, OD)值。本实验独立重复5次。

1.4.6　Transwell实验检测RA-FLS的细胞迁移能力　在Transwell小室中添加200 μL RA-FLS细胞悬液(细胞密度为2×10^5 mL^{-1}), 再加入500 μL含趋化因子的培养基, 置于培养箱中过夜培养(体积分数为5% CO_2, 37 ℃), 再进行分组(同1.4.1)处理48 h。将上室液体除去, PBS洗涤, 甲醛固定后再次清洗, 使用含有0.5%的结晶紫试剂染色20 min, 擦去未迁移细胞, 拍照计数。使用微孔板读取器在450 nm处测量OD值。本实验独立重复3次。

1.5　统计学方法

计量资料以$\bar{x}\pm s$表示。两组间比较采用两独立样本t检验或秩和检验, 多组间比较采用one-way ANOVA检验或Kruskal-Wallis H检验；circRNA 0003353与炎症、骨代谢指标、DAS28积分的相关性分析采用Pearson分析；circRNA 0003353指标上升与炎症、骨代谢指标、DAS28积分变化关联规则分析采用SPSS Modeler 18.0中Aprior挖掘分析；采用logistic回归分析circRNA 0003353指标对炎症、骨代谢指标和DAS28积分的影响。P<0.05为差异有统计学意义。

2　结果

2.1　circRNA 0003353在RA湿热痹阻证患者中的表达

RA湿热痹阻证患者PBMCs中circRNA 0003353相对表达量为2.71±0.06, 在健康对照者PBMCs中相对表达量为1.09±0.03(P<0.05), 故circRNA 0003353在RA湿热痹阻

证患者PBMCs中表达上调。

2.2 circRNA 0003353与RA湿热痹阻证患者各指标的相关性

Pearson相关性分析结果显示(图1), RA湿热痹阻证患者circRNA 0003353与ESR(r=0.482)、RF(r=0.524)、RANKL(r=0.379)、DAS28(r=0.328)均呈正相关(P<0.05), circRNA 0003353与IL-10(r=−0.347)呈负相关(P<0.05)。

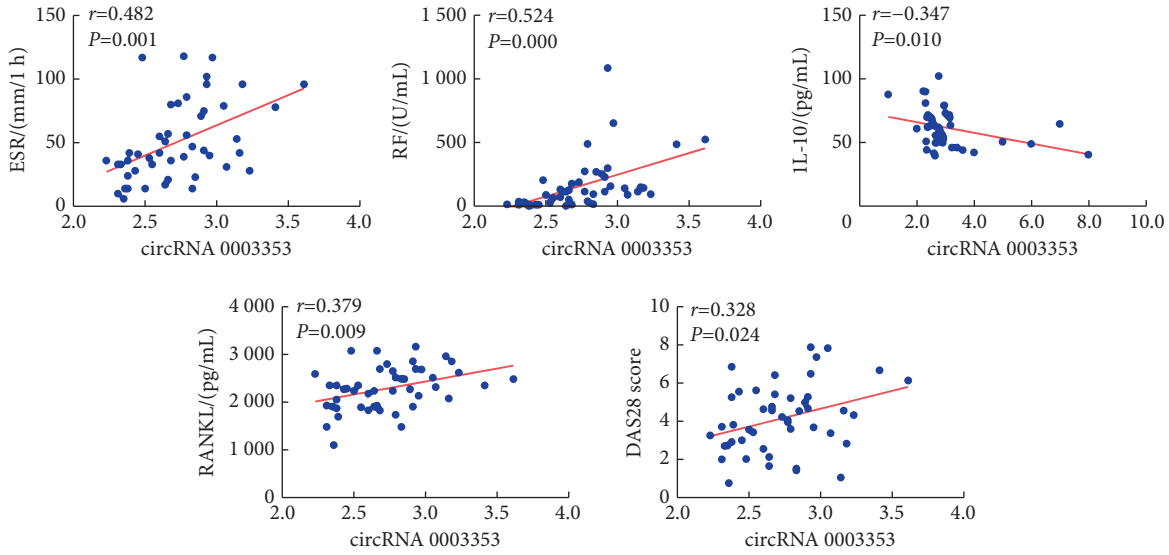

图 1 circRNA 0003353与RA湿热痹阻证患者各指标的相关性分析（ n=55 ）

Fig 1 Correlation analysis of circRNA 0003353 and various indexes of RA patients with damp heat obstruction syndrome (n=55)

2.3 circRNA 0003353与RA湿热痹阻证患者临床指标间的关联规则分析

关联规则结果显示, circRNA 0003353的升高与RA湿热痹阻证患者ESR、IL-17、CRP、IgG升高的支持度、置信度和提升度较高(P< 0.01)。见表1。

表 1 circRNA 0003353与RA湿热痹阻证患者各指标的关联规则分析

Table 1 Analysis of patterns of association between circRNA 0003353 and various indexes of RA patients with damp heat obstruction syndrome

LHS	RHS	Support/%	Confidence/%	Lift
circRNA 0003353	ESR	81.82	90.00	1.03
circRNA 0003353	IL-17	78.18	87.76	1.01
circRNA 0003353	CRP	72.73	90.91	1.04
circRNA 0003353	IgG	72.73	88.89	1.02

LHS: Left-hand-side; RHS: Right-hand-side.

2.4 circRNA 0003353与RA湿热痹阻证患者各指标间的logistic回归分析

将circRNA 0003353设为因变量(以健康对照circRNA 0003353相对表达量的均值作为分界点, 设circRNA 0003353相对表达量> 1.09为1, 相对表达量≤1.09为0), 炎症、骨代谢指标等设为自变量。结果显示, 偏回归系数(B)> 0、P< 0.05、OR> 1的自变量为RANKL、CRP、ESR(均为连续型变量)。故circRNA 0003353是RA湿热痹阻证患者RANKL、CRP、ESR的危险因素, circRNA 0003353升高与RANKL、CRP、ESR异常表达密切相关。见表2。

表 2 circRNA 0003353与RA湿热痹阻证患者各指标间的logistic回归分析

Table 2 Logistic regression analysis between circRNA 0003353 and various indexes of RA patients with damp heat obstruction syndrome

Independent variable	B	SE	Wald	P	OR	95% CI
RANKL	2.648	0.635	17.390	0.000	14.739	12.089-17.388
CRP	1.936	0.528	13.444	0.000	9.242	7.307-11.181
ESR	1.592	0.423	14.165	0.004	7.084	5.940-8.677

B: Partial regression coefficient; SE: Standard error; OR: Odds ratio; RANKL: Receptor activator of nuclear factor-κB ligand; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate.

2.5 circRNA 0003353 mRNA在RA-FLS中的表达

RT-qPCR结果显示(图2), 与RA-FLS组相比, 经TNF-α刺激后, RA-FLS+TNF-α组circRNA 0003353 mRNA表达升高(P< 0.01); 与RA-FLS+TNF-α+pcDNA3.1-NC组相比, RA-FLS+TNF-α+pcDNA3.1-circRNA 0003353组circRNA 0003353 mRNA表达升高(P< 0.01); 与RA-FLS+TNF-α+si-NC组相比, RA-FLS+TNF-α+si-circRNA 0003353组circRNA 0003353 mRNA表达均降低(P< 0.05), 其中siRNA1组circRNA 0003353降低程度最高, 故选择siRNA1进行后续实验。

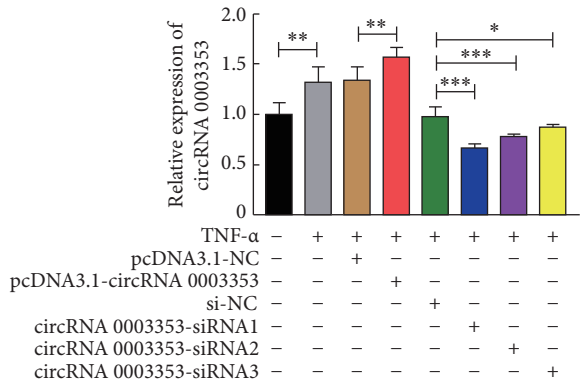

图 2　circRNA 0003353 mRNA在RA-FLS中的表达（*n*=6）

Fig 2　Expression of circRNA 0003353 mRNA in RA-FLS (*n*=6)

*P<0.05, **P<0.01, ***P<0.001.

2.6　circRNA 0003353对RA-FLS中IL-10和IL-17表达的影响

ELISA结果显示(图3), 与RA-FLS组相比, 经TNF-α刺激后, RA-FLS+TNF-α组IL-10表达下降、IL-17表达升高(P< 0.001); 与RA-FLS+TNF-α+pcDNA3.1-NC组相比, RA-FLS+TNF-α+pcDNA3.1-circRNA 0003353组IL-10表达下降、IL-17表达升高(P< 0.001); 与RA-FLS+TNF-α+si-NC组相比, RA-FLS+TNF-α+si-circRNA 0003353组IL-10表达升高、IL-17表达下降(P< 0.01)。

2.7　circRNA 0003353对RA-FLS中JAK2、STAT3及其磷酸化蛋白表达的影响

Western blot结果显示(图4), 与RA-FLS组相比, 经TNF-α刺激后, RA-FLS+TNF-α组p-JAK2和p-STAT3表达升高(P< 0.001); 与RA-FLS+TNF-α+pcDNA3.1-NC组相比, RA-FLS+TNF-α+pcDNA3.1-circRNA 0003353组p-JAK2和p-STAT3表达升高, p-JAK2/JAK2、p-STAT3/STAT3比值升高(P< 0.001); 与RA-FLS+TNF-α+si-NC组相比, RA-FLS+TNF-α+si-circRNA 0003353组p-JAK2和p-STAT3表达降

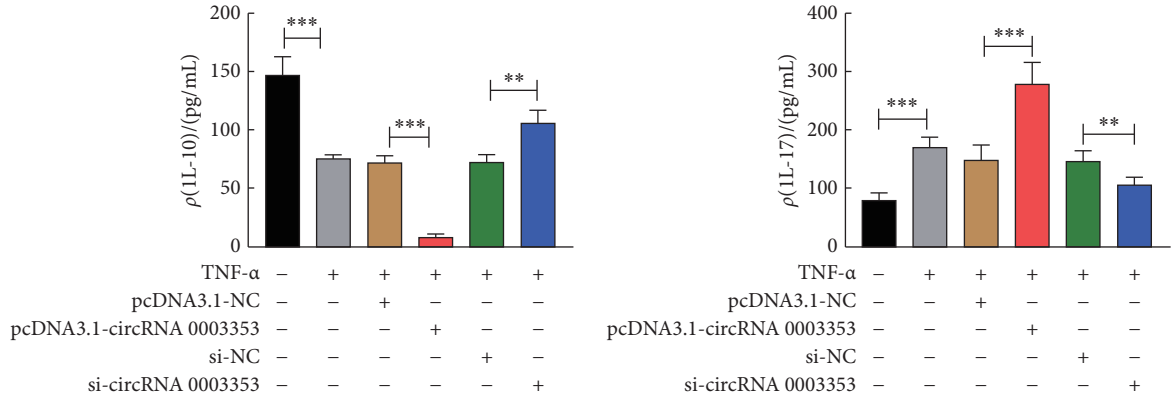

图 3　circRNA 0003353对RA-FLS中IL-10、IL-17表达的影响（*n*=6）

Fig 3　Effect of circRNA 0003353 on the expression of IL-10 and IL-17 in RA-FLS (*n*=6)

**P<0.01, ***P<0.001.

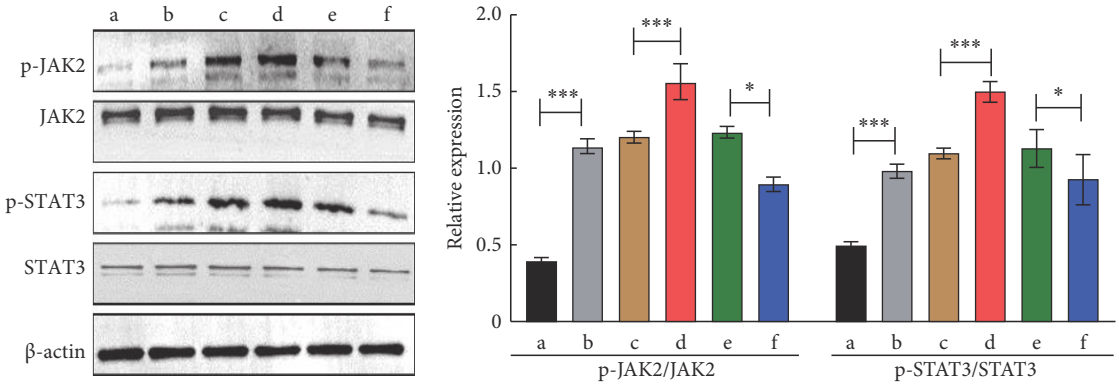

图 4　circRNA 0003353对RA-FLS中p-JAK2、JAK2、p-STAT3和STAT3蛋白表达的影响（*n*=3）

Fig 4　Effect of circRNA 0003353 on the expression of p-JAK2, JAK2, p-STAT3 and STAT3 proteins in RA-FLS (*n*=3)

a: RA-FLS; b: RA-FLS+TNF-α; c: RA-FLS+TNF-α+pcDNA3.1-NC; d: RA-FLS+TNF-α+pcDNA3.1-circRNA 0003353; e: RA-FLS+TNF-α+si-NC; f: RA-FLS+TNF-α+si-circRNA 0003353. *P<0.05, ***P<0.001.

低, p-JAK2/JAK2、p-STAT3/STAT3比值降低($P<0.05$)。

2.8 circRNA 0003353对RA-FLS细胞活力、迁移的影响

CCK-8和Transwell实验结果显示(图5), 与RA-FLS组相比, 经TNF-α刺激后, 24 h和48 h时, RA-FLS+TNF-α组RA-FLS细胞活力升高($P<0.05$), 48 h时细胞迁移能力增强($P<0.001$); 与RA-FLS+TNF-α+pcDNA3.1-NC组相比, 24 h和48 h时RA-FLS+TNF-α+pcDNA3.1-circRNA 0003353组RA-FLS细胞活力升高($P<0.05$), 48 h时细胞迁移能力增强($P<0.001$); 与RA-FLS+TNF-α+si-NC组相比, 24 h和48 h时RA-FLS+TNF-α+si-circRNA 0003353组RA-FLS细胞活力降低($P<0.05$), 48 h时细胞迁移能力减弱($P<0.01$)。

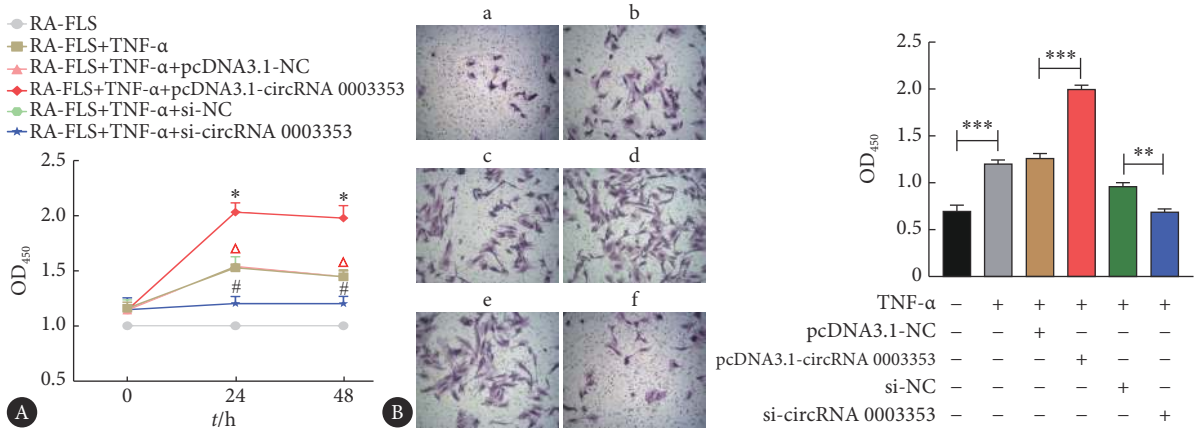

图 5 circRNA 0003353对RA-FLS细胞活力(A, n=5)、迁移(B, n=3, ×100)的影响

Fig 5 Effects of circRNA 0003353 on the viability (A, n=5) and migration (B, n=3, ×100) of RA-FLS

a-f: The denotations are the same as those in Fig 4. *P<0.05, vs. RA-FLS+TNF-α+pcDNA3.1-NC; #P<0.05, vs. RA-FLS+TNF-α+si-NC; △P<0.05, vs. RA-FLS. **P<0.01, ***P<0.001.

3 讨论

RA主要表现为对称性多关节炎、关节肿胀、免疫炎症指标明显升高等, 严重影响患者生活质量[18]。本研究以55例RA湿热痹阻证患者为研究对象, 结果表明circRNA 0003353在RA湿热痹阻证患者PBMCs中表达显著升高; 且circRNA 0003353表达与ESR、RF、RANKL、DAS28呈正相关, 与IL-10呈负相关; 关联规则结果显示, circRNA 0003353的升高与RA湿热痹阻证患者ESR、IL-17、CRP、IgG升高的支持度、置信度和提升度较高; logistic回归分析显示, circRNA 0003353升高与RANKL、CRP、ESR异常表达密切相关。WEN等[17]和OUYANG等[19]通过高通量测序表明多种circRNAs在RA-PBMCs中表达异常, 并与RA炎症存在密切联系。ZHONG等[20]通过RT-qPCR验证表明, circRNA 0088036在RA中表达上调, 促进RA-FLS增殖和迁移。上述结论与本研究结果类似, 且本研究表明circRNA 0003353与免疫炎症指标ESR、IL-17、CRP、IgG及骨代谢指标RANKL等有关。故circRNA 0003353在RA中可能通过调控炎症反应, 进而影响RA的发生发展, 因此接下来通过构建circRNA 0003353的过表达质粒和小干扰RNA转染至RA-FLS中探究circRNA 0003353在RA中的具体作用机制。

细胞实验结果表明与RA-FLS+TNF-α+pcDNA3.1-NC组相比, RA-FLS+TNF-α+pcDNA3.1-circRNA 0003353组RA-FLS中抑炎因子IL-10表达下降、促炎因子IL-17升高, p-JAK2/JAK2、p-STAT3/STAT3比值均升高, 且RA-FLS细胞活力和迁移能力亦升高; 与RA-FLS+TNF-α+si-NC组相比, RA-FLS+TNF-α+si-circRNA 0003353组IL-10表达升高、IL-17表达降低, p-JAK2/JAK2、p-STAT3/STAT3比值均降低, 且RA-FLS细胞活力和迁移能力亦降低。CAI等[21]研究表明circRNA 0088194在RA-FLS中上调, 过表达circRNA 0088194促进RA-FLS的迁移和侵袭, 而敲除circRNA 0088194则作用相反。YANG等[22]研究表明过表达circRNA 09505促进TNF-α、IL-6和IL-12的表达, 并促进巨噬细胞的增殖。LIU等[23]研究表明敲除长链非编码(lnc)RNA XIST可通过炎症信号通路抑制RA-FLS增殖, 增加细胞凋亡率。上述研究与本研究结果类似, 非编码RNA(circRNAs、lncRNA)在参与RA发生发展中有着重要作用, 可以通过调控炎症信号通路和炎症指标, 调节细胞迁移、增殖、凋亡等。

综上所述, circRNA 0003353在RA湿热痹阻证患者中表达升高, 与炎症指标显著相关。circRNA 0003353可以

激活JAK2/STAT3信号通路, 促进炎症反应, 提高细胞活力、迁移能力。接下来我们将从分子生物学角度进一步研究circRNA 0003353是否靶向调控下游基因、通过RNA修饰等途径参与RA发病。

*　　*　　*

利益冲突　所有作者均声明不存在利益冲突

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