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类风湿关节炎湿热痹阻证患者LINC00638的表达及其调控炎症和氧化应激的机制

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[摘 要] 目的：类风湿关节炎(rheumatoid arthritis，RA)是以慢性炎症和关节破坏为主要特征的自身免疫性疾病。炎症反应、氧化应激是RA发病的重要环节，氧化损伤能够诱发和加重免疫炎症失衡，促进细胞和组织损伤。本研究观察长链非编码RNA (long non-coding RNA，lncRNA) LINC00638在RA湿热痹阻证患者外周血中的表达，旨在研究LINC00638与疾病活动度、免疫炎症、氧化应激指标的相关性，探讨LINC00638在过表达和干扰情况下调控RA滑膜样成纤维细胞(fibroblast-like synoviocyte，FLS)的炎症反应与氧化应激的机制。**方法：**纳入安徽中医药大学第一附属医院风湿科48例RA湿热痹阻证患者和27例正常健康者，将其分为RA组和对照组。采用real-time PCR检测受试者外周血单个核细胞(peripheral blood mononuclear cell，PBMC)中LINC00638的表达。采用酶联免疫吸附法(enzyme linked immunosorbent assay，ELISA)检测血清中白介素(interleukin，IL)-10、IL-17、肿瘤坏死因子-α(tumor necrosis factor -α，TNF-α)、丙二醛(malondialdehyde，MDA)、血红素氧合酶1(heme oxygenase-1，HO-1)、超氧化物歧化酶2(superoxide dismutase 2，SOD2)的表达。采用Spearman方法研究RA患者LINC00638与红细胞沉降率(erythrocyte sedimentation rate，ESR)、C反应蛋白(C-reactive protein，CRP)、类风湿因子(rheumatoid factor，RF)、抗环瓜氨酸肽抗体(anti-cyclic citrullinated peptide antibody，anti-CCP)的相关性，并观察其与28处关节疾病活动评分(Disease Activity Scores for 28 Joints，DAS28)、RA湿热证候量化积分、视觉模拟评分(Visual Analogue Scale，VAS)、焦虑自评量表(Self-rating Anxiety Scale，SAS)、抑郁自评量表(Self-rating Depression Scale，SDS)评分的关系。以RA-PBMC诱导RA-FLS，建立RA体外细胞实验模型，构建LINC00638过表达质粒和小干扰RNA(small interfering RNA，siRNA)，并将其转染至RA-FLS中。细胞实验分为4组：pcDNA3.1-对照组、pcDNA3.1-LINC00638组、siRNA-对照组、siRNA-LINC00638组。采用real-time PCR检测过表达质粒和siRNA转染效率，ELISA法检测各组TNF-α、IL-10表达，免疫荧光法检测各组抗氧化蛋白HO-1、SOD2的表达。**结果：**与对照组相比，LINC00638在RA组外周血中呈低表达(P<0.01)，LINC00638的受试者工作特征(receiver operating characteristic，ROC)曲线下面积(area under curve，AUC)为0.9271。RA组DAS28评分为5.70(5.40~6.50)，RA湿热证候量化积分为20.00(17.00~23.00)，VAS评分为7.00(6.30~8.00)。与对照组相比，RA组ESR、CRP、RF、anti-CCP、SAS评分、SDS评分均显著升高(均P<0.01)。Spearman相关性分析结果显示：RA湿热痹阻证患者LINC00638与ESR(r=−0.532，P<0.01)、CRP(r=−0.367，P<0.05)、TNF-α(r= −0.375，P<0.01)、MDA(r=−0.295，P<0.05)、DAS28 (r=−0.450，P<0.01)均呈负相关，与SOD2呈正

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相关(r=0.370， P<0.05)。RA-FLS经RA-PBMC诱导后，LINC00638表达水平显著降低(P<0.01)，表明PBMC的刺激能够有效降低RA-FLS的LINC00638表达，故可采用PBMC诱导的RA-FLS实验模型。与pcDNA3.1-对照组相比，pcDNA3.1-LINC00638组LINC00638、IL-10、SOD2、HO-1表达均显著升高(均P<0.01)，TNF-α的表达降低(P<0.01)；与siRNA对照组相比，siRNA-LINC00638组LINC00638、IL-10、SOD2、HO-1均显著降低(均P<0.01)，TNF-α显著升高(P<0.01)。**结论：**LINC00638在RA湿热痹阻证患者外周血中呈低表达，与疾病活动度、免疫炎症、氧化应激指标存在相关性。过表达LINC00638能够降下调促炎因子，上调抑炎因子，提高抗氧化酶活性，从而可改善RA炎症与氧化应激。LINC00638是本课题组前期对RA患者外周血PBMC进行全转录组高通量测序，以及临床样本验证所得到的差异lncRNA。为深化该基因在分子生物学中研究，可从竞争性内源RNA角度进一步研究LINC00638靶向的微小RNA与mRNA。

[关键词] 类风湿关节炎；湿热痹阻证；炎症；氧化应激；LINC00638

Expression of LINC00638 in rheumatoid arthritis patients with damp-heat obstruction syndrome and the regulatory mechanisms for inflammation and oxidative stress

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ABSTRACT

Objective: Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation and joint destruction. Both inflammatory response and oxidative stress contribute to the pathogenesis of RA. Oxidative damage can induce and aggravate the imbalance of immune inflammation and promote cell and tissue damage. In this study, the expression of long non-coding RNA (lncRNA) LINC00638 in peripheral blood of patients with RA damp-heat arthralgia syndrome was observed, and the correlation between LINC00638 and disease activity, immune inflammation and oxidative stress indicator was investigated. Subsequently, the mechanisms for LINC00638 in regulating the inflammatory response and oxidative stress in RA fibroblast-like synoviocyte (FLS) under the condition of overexpression and interference were further explored.

Methods: In this study, 48 RA patients with damp-heat arthralgia syndrome and 27 normal healthy subjects, who came from Department of Rheumatology, First Affiliated Hospital of Anhui University of Chinese Medicine, were included; and they were divided into a RA group and a control group. The expression of LINC00638 in peripheral blood mononuclear cells (PBMC) from the subjects was detected by real-time PCR. Enzyme linked immunosorbent assay (ELISA) was used to detect serum interleukin (IL)-10, IL-17, tumor necrosis factor-α (TNF-α), malondialdehyde (MDA), heme oxygenase 1 (HO-1) and superoxide dismutase 2 (SOD2) expression. Spearman method was used to study the relationship between LINC00638 and erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), anti-cyclic citrullinated peptide antibody (anti-CCP), and to observe the relation between LINC00638 and the Disease Activity Score of 28 Joint (DAS28), Quantitative Score of Damp Heat Syndrome, Visual Analogue Scale

(VAS), Self-rating Anxiety Scale (SAS) and Self-rating Depression Scale (SDS). RA-FLS was induced by RA-PBMC, and the RA in vitro cell experimental model was established. LINC00638 overexpression plasmid and small interfering RNA (siRNA) were constructed and transfected into RA-FLS. The cell experiments were divided into 4 groups: a pcDNA3. 1-control group, a pcDNA3.1-LINC00638 group, a siRNA-control group, and a siRNA-LINC00638 group. The transfection efficiency of overexpression plasmid and siRNA was detected by real-time PCR, the expression of TNF-α and IL-10 was detected by ELISA, and the expression of antioxidant proteins HO-1 and SOD2 was detected by immunofluorescence.

Results: Compared with the control group, the expression of LINC00638 in the RA group was lower (P<0.01). The area under the curve (AUC) of the receiver operating characteristic (ROC) curve of LINC00638 was 0.9271. The DAS28 in RA group was 5.70 (5.40−6.50), the Quantitative Score of Damp-heat Syndrome was 20.0 (17.0−23.0), and the VAS score was 7.0 (6.3−8.0). Compared with the control group, the ESR, CRP, RF, anti-CCP, SAS and SDS scores in the RA group were significantly increased (all P<0.01). Spearman correlation analysis showed that: LINC00638 was negatively correlated with ESR (r=−0.532, P<0.01), CRP (r=−0.367, P<0.05), TNF-α (r=−0.375, P<0.01), MDA (r=−0.295, P<0.05), DAS28 (r=−0.450, P<0.01), and which was positively correlated with SOD2 (r=0.370, P<0.05). After the induction of RA-FLS, the expression level of LINC00638 was significantly decreased (P<0.01), indicating that the stimulation of PBMC could effectively reduce the expression of LINC00638 in RA-FLS, so the experimental model of RA-FLS-induced by PBMC was utilized. Compared with the pcDNA3.1-control group, the expressions of LINC00638, IL-10, SOD2, and HO-1 in the pcDNA3.1-LINC00638 group were significantly increased (all P<0.01), and the expression of TNF-α was decreased (P<0.01). Compared with siRNA-control group, LINC00638, IL-10, SOD2 and HO-1 in the siRNA-LINC00638 group were significantly decreased (all P<0.01), and TNF-α was significantly increased (P<0.01).

Conclusion: LINC00638 is down-regulated in the peripheral blood of RA patients with damp-heat arthralgia syndrome, which is correlated with disease activity, immune inflammation and oxidative stress. Overexpression of LINC00638 can down-regulate pro-inflammatory factors, up-regulate anti-inflammatory factors, and increase antioxidant enzyme activity, thereby improving inflammation and oxidative stress in RA. LINC00638 is the differential lncRNA obtained by the research group's previous high-throughput sequencing of the whole transcriptome of peripheral blood PBMCs in RA patients and validation of clinical samples. In order to deepen the molecular biology research of this gene, the microRNA and mRNA targeted by LINC00638 can be further studied from the perspective of competing endogenous RNAs.

KEY WORDS rheumatoid arthritis; damp-heat arthralgia syndrome; inflammation; oxidative stress; LINC00638

类风湿关节炎(rheumatoid arthritis，RA)是一种以慢性炎症和关节破坏为主要特征的自身免疫性疾病[1-3]，以滑膜过度增生、滑膜炎、血管翳生成为主要病理基础，具有较高的致畸率和致残率[4-5]。RA全身性免疫失衡伴随滑膜和关节局部炎症浸润贯穿整个病程[6]。氧化应激的发生、发展与抗氧化剂和促氧化剂的失衡有关，RA氧化应激可诱发或加重炎症反应，可进一步促进组织、细胞损伤。

RA属于中医“痹证”范畴，湿热痹阻证是临床最常见的证候分型[7]，临床多表现为关节肿胀、疼

痛，常伴发热，关节局部发红、活动受限，舌质红、苔黄腻，脉滑数等，实验室指标常表现为高水平的疾病活动和免疫炎症反应。长链非编码RNA(long non-coding RNA，lncRNA)是长度为200~300个核苷酸的非编码RNA分子，不具备或具备极低的编码蛋白质能力，可通过与微RNA(microRNA)、蛋白质等下游分子结合，对靶基因转录及转录后水平进行调控，参与细胞的生理及病理过程。课题组前期研究[8-9]发现LINC00638是参与RA湿热痹阻证免疫炎症、氧化应激的重要的lncRNA。

本研究通过检测LINC00638在RA湿热痹阻证患者外周血单个核细胞(peripheral blood mononuclear cell，PBMC)中的表达，分析基因与临床免疫炎症指标、细胞因子、氧化应激指标、患者感受(Self-Perception of Patients，SPP)量表评分的相关性，通过RA湿热痹阻证患者PBMC诱导RA患者滑膜样成纤维细胞(fibroblast-like synoviocyte，FLS)，建立RA体外细胞实验模型，通过转染LINC00638过表达质粒与小干扰RNA(small interfering RNA，siRNA)，探讨LINC00638对RA-FLS细胞因子及抗氧化蛋白的影响。

1 资料与方法

1.1 临床资料

收集2021年1月至2021年4月就诊于安徽中医药大学第一附属医院风湿科48例RA湿热痹阻证患者作为RA组，其中男13例，女35例，年龄为(56.48±6.42)岁，病程为5.00 (1.00~10.00)年。另选择27例正常健康者作为对照组，其中男6例，女21例，年龄为(55.85±10.49)岁，两组年龄、性别差异均无统计学意义(均P>0.05)。本研究在美国临床试验数据库完成注册(网址：https://www.clinicaltrials.gov/，注册号：NCT04136262)，所有患者均获得知情同意，本研究经安徽中医药大学第一附属医院伦理委员会批准后实施(2019AH-12)。

临床指标：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)、抗环瓜氨酸肽抗体(anti-cyclic citrullinated peptide antibody，anti-CCP)、28处关节疾病活动评分(Disease Activity Scores for 28 Joints，DAS28)。

细胞因子及氧化应激指标：白介素-10(interleukin-10，IL-10)、白介素17(interleukin-17，IL-17)、肿瘤坏死因子-α(tumor necrosis factor -α，TNF-α)、丙二醛(malondialdehyde，MDA)、血红素氧合酶1(heme oxygenase-1，HO-1)、超氧化物歧化酶2(superoxide dismutase 2，SOD2)。

SPP评分：采用RA湿热证候量化积分、视觉模拟评分(Visual Analogue Scale，VAS)、焦虑自评量表(Self-rating Anxiety Scale，SAS)、抑郁自评量表(Self-rating Depression Scale，SDS)进行评分，评分越高，说明患者存在湿热、疼痛、焦虑、抑郁的程度越重。

1.2 纳入、排除标准

纳入标准：符合RA西医诊断标准[10]和RA病证结合指南湿热痹阻证诊断标准[7]。排除标准：1)实验室指标不完善者；2)未成年及妊娠期患者；3)合并肿瘤、感染、其他自身免疫性疾病、肝肾功能损伤、严重心血管疾病者；4)病变严重、无生活自理能力者；5)确诊患有精神疾病者。

1.3 实验试剂及仪器

DMEM培养基购自美国Hyclone公司；人外周血淋巴细胞分离液购自美国GE Healthcare公司；RA-FLS细胞株购自上海赛百慷生物技术股份有限公司；ELISA试剂盒购自武汉基因美生物科技公司；反转录试剂盒购自日本TaKaRa公司；SOD2抗体(兔抗人1∶200)、HO-1抗体(小鼠抗人1∶400)均购自美国Santa Cruz公司；pcDNA3.1-LINC00638、siRNA-LINC00638与各自阴性对照均购自上海吉玛制药技术有限公司；DAPI染色液购自上海碧云天生物技术有限公司；荧光定量PCR仪购自美国Thermo Scientific公司；酶标仪购自深圳雷杜生命科学股份有限公司；高速台式冷冻离心机购自安徽嘉文仪器装备有限公司。

1.4 RA-PBMC提取、诱导及RA-FLS转染方法

采集48例RA组患者、27例对照组受试者静脉血各5 mL，每组均加入等体积的生理盐水进行稀释。取等量淋巴细胞分离液，缓慢加入配好的样本中，以2 000 r/min离心20 min；吸取离心管中白色絮状物；加入等量的PBS混匀，以800 r/min离心8 min，清洗，重复2次，得到RA-PBMC，孵育备用。

消化对数生长期的RA-FLS细胞，终止消化后离心(1 000 r/min，5 min)，弃去培养液，用PBS洗2遍，用DMEM培养基重悬，按照3×10^5个细胞/孔接种到6孔板中。采用Transwell小室将RA-PBMC与RA-FLS以3∶1比例接种培养，PBMC放置于上室，FLS放置于下室，放入培养箱中培养24 h，观察细胞汇合达到70%~90%时，去除各孔中的Transwell小室。根据操作说明，采用Lipofectamine 2000将过表达质粒、siRNA以及相应的阴性对照转染至RA-PBMC诱导的RA-FLS。细胞实验分为4组：pcDNA3.1-对照组、

pcDNA3.1-LINC00638组、siRNA-对照组、siRNA-LINC00638组。转染后继续培养48 h，收集细胞及上清用于后续检测。

1.5 Real-time PCR检测

提取细胞总RNA，反转录合成cDNA。Real-time PCR反应体系为10 μL，包括SYBR Green mixture染料5 μL，正向、反向引物各1 μL，cDNA 1 μL，DEPC处理水2 μL。样品设置3个复孔，以β-actin作为内参基因。本实验的分析方法为相对定量研究，ΔCt值=目的基因的平均Ct值−内参基因的平均Ct值，采用$2^{-\Delta\Delta Ct}$计算RNA相对表达量。具体引物序列见表1。

表1　Real-time PCR引物序列

Table 1　Real-time PCR primer sequence

基因名	产物长度/bp	正向引物(5'−3')	反向引物(5'−3')
β-actin	96	CCCTGGAGAAGAGCTACGAG	GGAAGGAAGGCTGGAAGAGT
LINC00638	120	CCATAGCCGATTAGCTGTCA	AATGCCGAACTGGAGGTG

1.6 ELISA检测

收集血清及FLS培养上清液，严格按照各试剂盒说明书进行操作，检测IL-10、IL-17、TNF-α、MDA、HO-1、SOD2的表达。

1.7 免疫荧光法检测

将细胞悬液滴加在6孔板中，用冷丙酮固定10 min；山羊血清封闭30 min；甩掉山羊血清，滴加一抗，于37 ℃孵育1 h；将片子甩干，加入荧光二抗(稀释比例1∶400)，于37 ℃避光孵育20 min；DAPI复染5 min；行抗荧光淬灭、封片剂封片，在荧光显微镜下观察切片、采集图像。荧光显微镜下观察FLS细胞核为蓝色，阳性表达为绿光。

1.8 统计学处理

采用SPSS 24. 0统计学软件进行数据分析，采用GraphPad Prism 8.0.1软件绘图，计数资料采用χ^2检验，正态分布的计量资料采用均数±标准差($\bar{x}\pm s$)表示，两组间比较采用两独立样本t检验；不符合正态分布的数据采用中位数(第1四分位数，第3四分位数)[$M(P_{25}, P_{75})$]表示，两组间比较采用秩和检验，以P<0.05为差异有统计学意义。

2 结　果

2.1 LINC00638在RA湿热痹阻证患者PBMC中的表达及诊断效用的评估

与对照组相比，RA组PBMC中LINC00638的表达水平显著降低(P<0.01)。采用受试者工作特征(receiver operating characteristic，ROC)曲线评估LINC00638的诊断价值，ROC曲线下面积(area under curve，AUC)为0.9271(95% CI：0.87~0.98)，表明LINC00638具有较好的辅助诊断效用。区分RA组和对照组的最佳截断值为0.91，对应的敏感性为85.42%，特异性为88.89%(图1)。

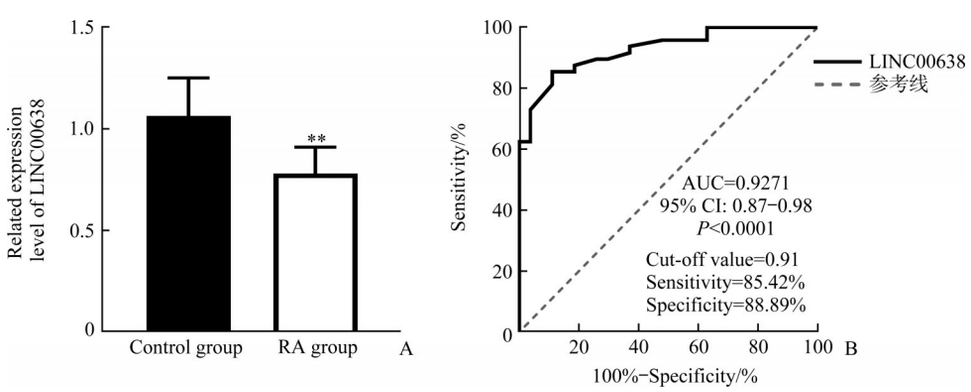

图1　LINC00638在RA组PBMC中的表达及ROC曲线分析

Figure 1　Expression of LINC00638 in PBMC and ROC curve analysis in the RA group

A: Low expression of LINC00638 in PBMC in the RA group by real-time PCR; B: Diagnostic utility of LINC00638 by ROC curve. **P<0.01 vs the control group.

2.2 RA组外周血中细胞因子及氧化应激指标的表达

与对照组相比，RA组外周血中IL-10、SOD2表达水平显著降低(均P<0.01)，IL-17、TNF-α、MDA、HO-1表达水平显著升高(均P<0.01，图2)。

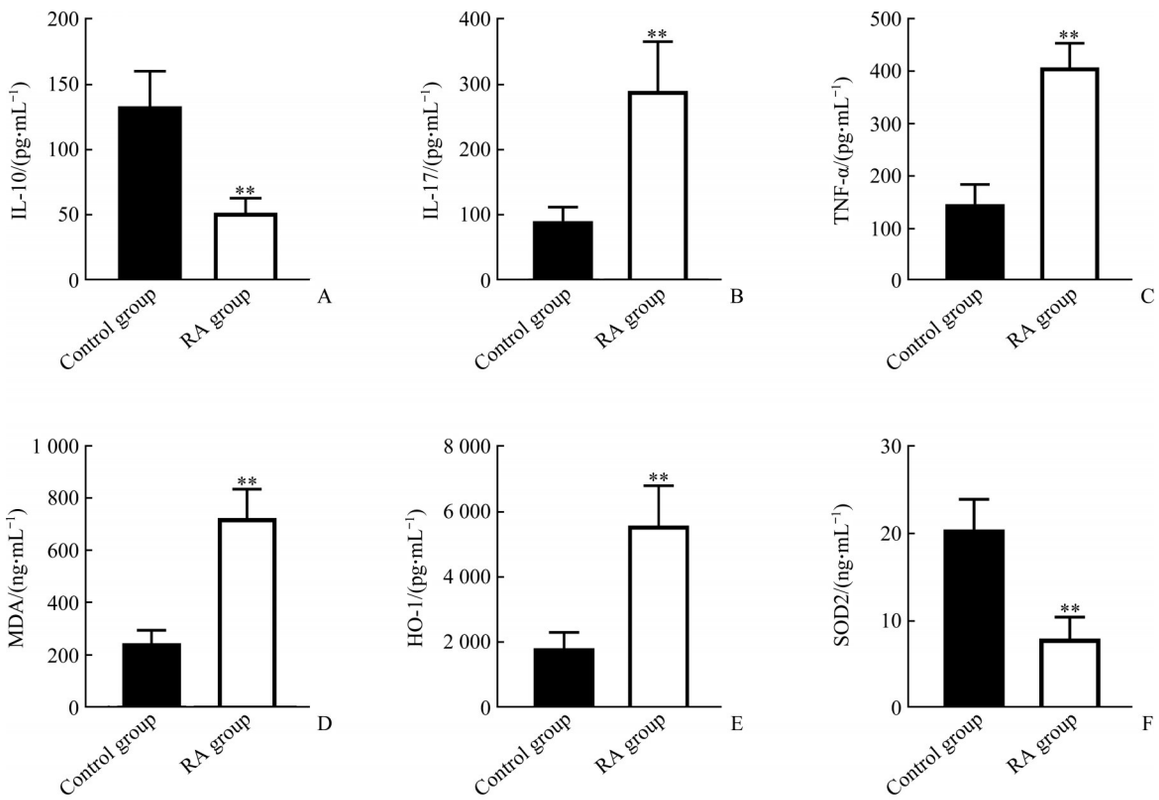

图2 RA和对照组血清胞因子及氧化应激指标表达的比较

Figure 2 Comparison of expression of serum cytokines and oxidative stress indicators between the RA group and the control group

A: IL-10; B: IL-17; C: TNF-α; D: MDA; E: HO-1; F: SOD2. **P<0.01 vs the control group.

2.3 RA组临床免疫炎症指标、患者SPP评分的变化

RA组DAS28评分为5.70(5.40~6.50)，RA湿热证候量化积分为20.00(17.00~23.00)，VAS评分为7.00(6.30~8.00)。与对照组相比，RA组ESR、CRP、RF、anti-CCP、SAS评分、SDS评分均显著升高(均P<0.01，表2)。

表2 RA组临床免疫炎症指标、SPP评分的变化

Table2 Changes of clinical immune inflammatory indexes and SPP scores in the RA group

组别	n	ESR/(mm·h^{-1})	CRP/(mg·L^{-1})	RF/(U·mL^{-1})	anti-CCP/(U·mL^{-1})
对照组	27	8.41±4.35	3.56±1.28	9.39±3.98	2.51±0.94
RA组	48	36.00(26.00~64.00)	13.36(4.38~37.53)	106.40 (48.80~222.10)	111.5(38.40~295.50)
t/Z		−6.775	−4.416	−6.766	−5.392
P		<0.01	<0.01	<0.01	<0.01

表2(续)

组别	SAS评分	SDS评分	DAS28评分	RA湿热证候量化积分	VAS评分
对照组	40.83±6.66	37.22±13.09	–	–	–
RA组	52.50(46.25~61.88)	61.82±13.57	5.70(5.40~6.50)	20.00(17.00~23.00)	7.00(6.30~8.00)
t/*Z*	−0.484	−7.627	–	–	–
P	<0.01	<0.01	–	–	–

ESR：红细胞沉降率；CRP：C反应蛋白；RF：类风湿因子；anti-CCP：抗环瓜氨酸肽抗体；SAS评分：焦虑自评量表评分；SDS评分：抑郁自评量表评分；DAS28：28处关节疾病活动评分；VAS评分：视觉模拟评分。

2.4 LINC00638与临床免疫炎症指标、细胞因子、氧化应激指标的相关性分析

Spearman相关性分析结果显示：RA组LINC00638的表达水平与ESR(r=−0.532，P<0.01)、CRP(r=−0.367，P<0.05)、TNF-α(r=−0.375，P<0.01)、MDA(r=−0.295，P<0.05)、DAS28(r=−0.450，P<0.01)呈负相关，与SOD2呈正相关(r=0.370，P<0.05；图3)。

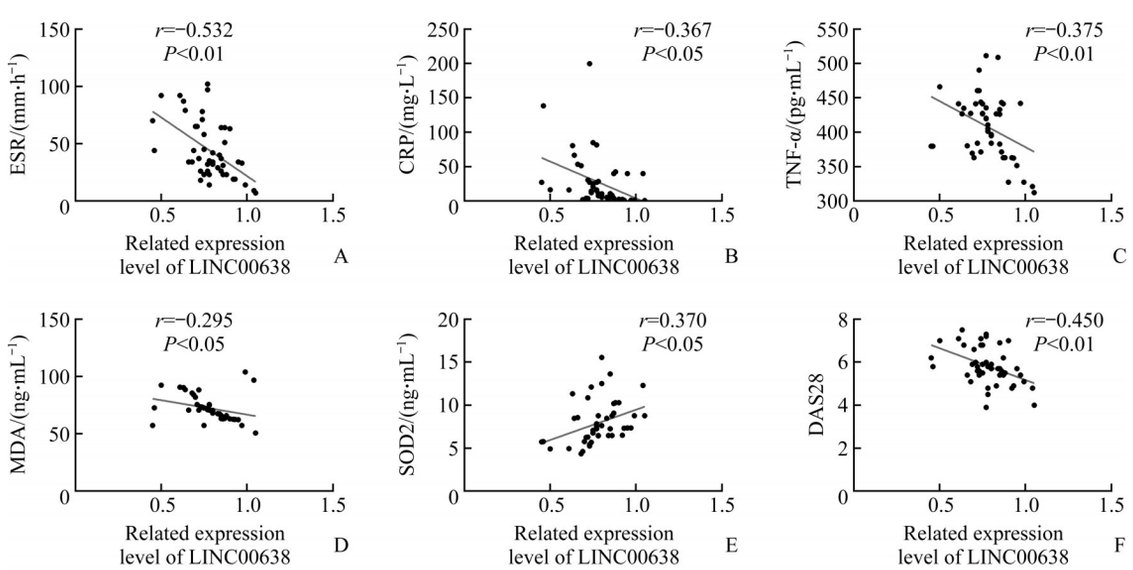

图3　LINC00638与RA组临床指标的相关性分析

Figure 3　Correlation analysis between LINC00638 and clinical indexes in the RA group

A: Correlation analysis between LINC00638 and ESR; B: Correlation analysis between LINC00638 and CRP; C: Correlation analysis between LINC00638 and TNF-α; D: Correlation analysis between LINC00638 and MDA; E: Correlation analysis between LINC00638 and SOD2; F: Correlation analysis between LINC00638 and DAS28.

2.5 LINC00638过表达质粒与siRNA的转染效率

与RA-FLS相比，经RA-PBMC诱导RA-FLS后，LINC00638的表达水平显著降低(P<0.01)，表明PBMC的刺激能够有效降低FLS的LINC00638表达，故以下各实验均采用PBMC诱导的RA-FLS模型。与pcDNA3.1-对照组相比，pcDNA3.1-LINC00638组LINC00638表达显著升高(P<0.01)；与siRNA-对照组相比，si-LINC00638组LINC00638表达显著降低(P<0.01，图4)。

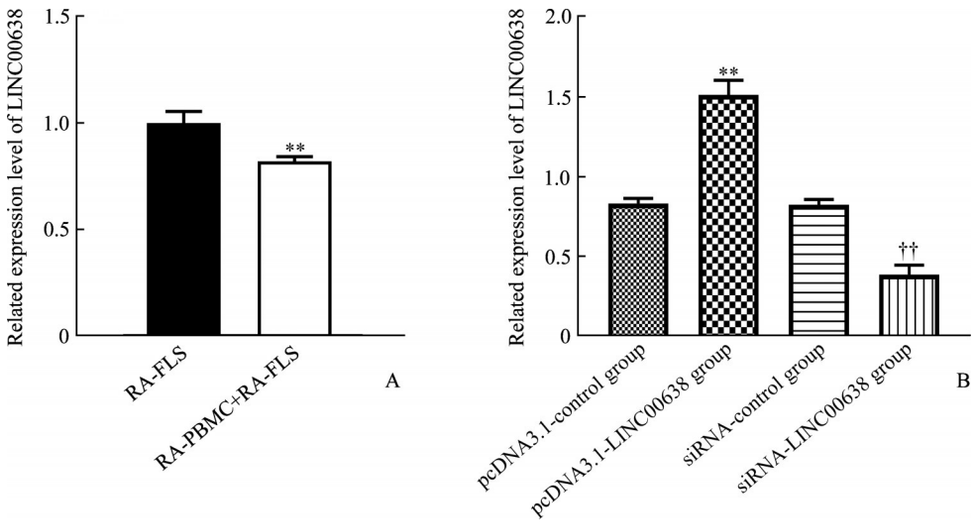

图4 LINC00638过表达质粒与小干扰RNA的转染效率

Figure 4 Transfection efficiency of LINC00638 overexpressed plasmid and siRNA

A: LINC00638 expression in RA-FLS stimulated by RA-PBMC. ***P*<0.01 vs the RA-FLS. B: Detection efficiency of LINC00638 overexpressed plasmid and siRNA by real-time PCR. ***P*<0.01 vs the pcDNA3.1-control group; ††*P*<0.01 vs the siRNA-control group.

2.6 LINC00638对RA-FLS中TNF-α、IL-10表达的影响

与pcDNA3.1-对照组相比，pcDNA3.1-LINC00638组TNF-α表达显著降低(*P*<0.01)，IL-10表达显著升高(*P*<0.01)；与siRNA-对照组相比，siRNA-LINC00638组TNF-α表达显著升高(*P*<0.01)，IL-10表达显著降低(*P*<0.01，图5)。

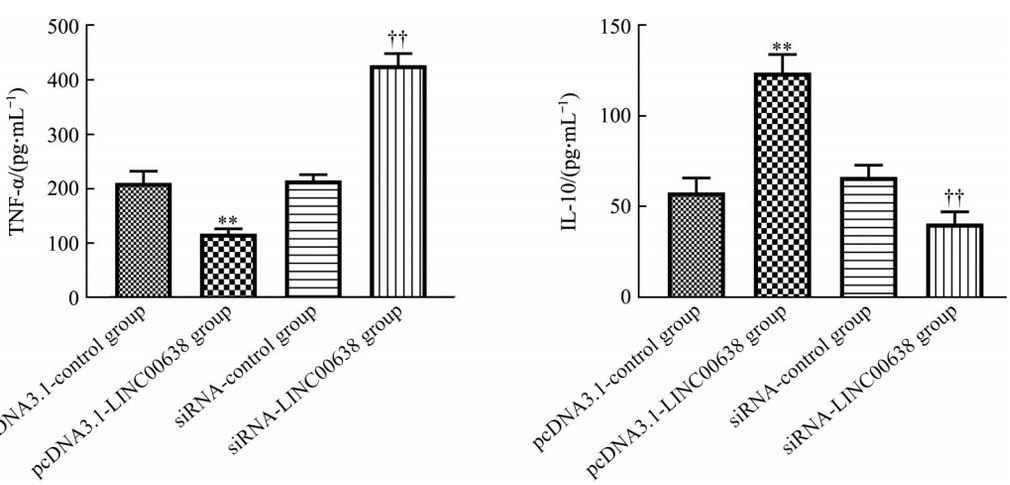

图5 LINC00638对RA-FLS中TNF-α、IL-10表达的影响

Figure 5 Effect of LINC00638 on the expression of TNF-α and IL-10 in RA-FLS

A: Expression of TNF-α by ELISA; B: Expression of IL-10 by ELISA. ***P*<0.01 vs the pcDNA3.1-control group; ††*P*<0.01 vs the siRNA-control group.

2.7 LINC00638对RA-FLS中HO-1、SOD2蛋白质表达的影响

与pcDNA3.1-对照组相比，pcDNA3.1-LINC00638组抗氧化蛋白HO-1、SOD2表达显著升高(*P*<0.01)；与siRNA-对照组相比，siRNA-LINC00638组抗氧化蛋白HO-1、SOD2表达均显著降低(*P*<0.01，图6)。

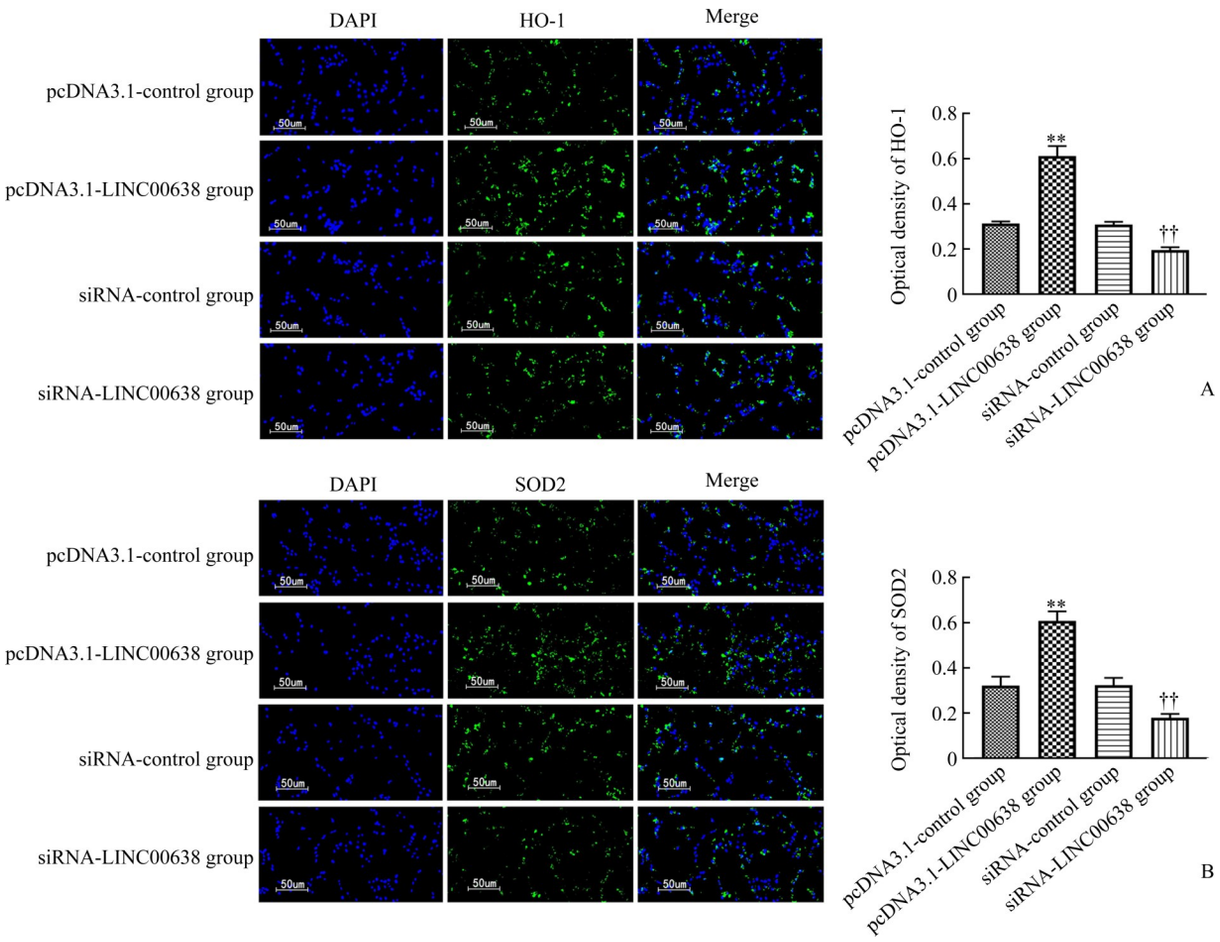

图6　LINC00638对RA-FLS的HO-1和SOD2蛋白表达的影响

Figure 6　Effect of LINC00638 on HO-1 and SOD2 protein expression in RA-FLS

A: Expression of HO-1 protein by immunofluorescence assay; B: Expression of SOD2 protein by immunofluorescence assay. Merge: Dyeing superposition. ***P*<0.01 vs the pcDNA3.1-control group; ††*P*<0.01 vs the siRNA-control group.

3 讨　论

RA是可累及全身多系统的慢性、炎症性的自身免疫性疾病，发病与遗传、环境、应激等多种因素有关。LncRNA在自身免疫性疾病、肿瘤等多种疾病中受到越来越多的关注，多项研究[11-13]表明lncRNA在RA患者外周血、滑膜组织或细胞中存在异常表达，参与调控RA疾病过程。本课题组前期研究[14]表明LINC00638与RA炎症、氧化应激有关，本实验进一步通过临床体内实验、细胞实验来研究其调控机制。本研究显示LINC00638在RA湿热痹阻证患者PBMC中呈低表达，ROC曲线下面积0.9271，根据Youden指数，LINC00638在RA组与对照组的最佳截断值为0.91，对应的敏感性为85.42%，特异性为88.89%，表明LINC00638可能参与RA进展，并且具有较高的辅助诊断价值。此外，研究[15-18]表明其在新生儿脓毒症、腰椎间盘退变、食管癌、牙周膜干细胞成骨分化等疾病过程中也发挥重要作用。

氧化应激引起大量氧化中间产物ROS、活性氮自由基蓄积，抗氧化能力减弱或相对不足，引起一系列毒性反应，导致组织、细胞损伤。氧化损伤能够诱发、加重体内炎症反应，引起机体抗氧化能力降低，参与RA疾病的发展过程[19-20]。MDA是脂质过氧化的代谢产物，其水平高低反映了机体的脂质代谢水平，SOD是体内抗氧化系统的重要成员，能够特异性地清除氧化自由基，SOD活性反映机体抗氧化能力的强弱。HO-1是体内保护性分子，具有抗炎、抗氧化作用。在本研究中，RA湿热痹阻证患者外周血IL-10、SOD2表达水平降低，IL-17、TNF-α、MDA，HO-1表达水平升高，表明RA患者体内促炎因子水平升高，抑炎因子水平降低，氧化应激水平升高，而抗氧化能力减弱。HO-1水平的升高是机体对氧化刺激作出的保护性反应。研究[21-23]表明：HO-1在RA患者外周血、滑膜组织中有不同程度的升高，与RA疾病活动、免疫炎症存在相关性。在本研究中，RA湿热痹阻证患者疾病活动性高，免疫炎症反

应明显增强，且具有不同程度上的焦虑、抑郁情况，相关性分析表明LINC00638与ESR、CRP、TNF-α、MDA、DAS28呈负相关，与SOD2呈正相关，进一步表明LINC00638与RA炎症、氧化应激相关。多项研究[24-25]表明RA患者存在一定程度的焦虑、抑郁情况，与疾病活动度、年龄、经济情况等密切相关。

FLS是RA炎症形成的主要效应细胞，具有肿瘤样生物学行为，如细胞活性增强、过度增生、具有较高的侵袭和迁移能力，在细胞增殖、迁移、分泌炎症因子的方面发挥重要作用[26-27]。为进一步研究LINC00638对RA-FLS炎症、氧化应激的影响，本研究采用RA湿热痹阻证患者PBMC与RA-FLS共培养，模拟RA患者体内炎症与氧化应激状态。通过构建并转染LINC00638的过表达和siRNA至RA-FLS，检测结果表明过表达LINC00638能够显著促进RA-FLS中IL-10、SOD2、HO-1表达，降低TNF-α表达，而沉默LINC00638表现出相反的表达趋势。细胞实验则表明LINC00638过表达有利于减轻RA-FLS诱导的炎症反应、氧化应激损伤。

综上所述，LINC00638在RA湿热痹阻证患者外周血中呈低表达，并且与疾病活动度、免疫炎症、氧化应激指标存在相关性，以RA-PBMC诱导RA-FLS构建细胞实验模型，通过对LINC00638进行过表达和干扰，表明LINC00638能够下调TNF-α，上调IL-10、HO-1、SOD2表达，降低促炎因子，升高抑炎因子，提高抗氧化酶活性，从而改善RA炎症与氧化应激。

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