European Review for Medical and Pharmacological Sciences 2019; 23: 8186-8193

MicroRNA-421 promotes inflammatory response of fibroblast-like synoviocytes in rheumatoid arthritis by targeting SPRY1

F. JIANG[1], H.-Y. ZHOU[2], L.-F. ZHOU[1], Y.-H. WEN[1], H.-H. GAI[1], G.-M. WU[1]

[1]Department of General Medicine, Beijing Luhe Hospital, Capital Medical University, Beijing, China
[2]Department of Anesthesiology, Tongzhou Maternal and Child Health Hospital of Beijing, Beijing, China

Abstract. – OBJECTIVE: The aim of this study was to investigate whether microRNA-421 could participate in the proliferative, migratory and inflammatory changes of fibroblast-like synoviocytes (FLS) in rheumatoid arthritis by targeting SPRY1.

PATIENTS AND METHODS: The expressions of microRNA-421 and SPRY1 in synovial tissues and FLS were detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) and Western blot, respectively. The binding condition between microRNA-421 and SPRY1 was verified by the Dual-Luciferase reporter gene assay. MicroRNA-421 mimics and inhibitor were constructed and transfected. The levels of extracellular interleukin-1 (IL-1), IL-6, and COX2 in FLS after microRNA-421 mimics or inhibitor transfection were detected by enzyme-linked immunosorbent assay (ELISA). The regulatory effect of microRNA-421 on the proliferation and migration of FLS was detected using cell counting kit-8 (CCK-8) and transwell assay, respectively. Furthermore, collagen-induced RA mouse model was constructed to confirm the specific effect of microRNA-421 on regulating RA development.

RESULTS: MicroRNA-421 was highly expressed in the synovial tissues of RA patients. SPRY1 expression in FLS was negatively regulated by microRNA-421. Moreover, the overexpression of microRNA-421 significantly promoted proliferative, invasive potentials and inflammatory response of FLS. *In vivo,* RA mouse model indicated that downregulated microRNA-421 and upregulated SPRY1 were observed in mice injected with cortisone and microRNA-421 inhibitor when compared with those of controls.

CONCLUSIONS: MicroRNA-421 promotes the inflammatory response of fibroblast-like synoviocytes in rheumatoid arthritis by downregulating the SPRY1 expression.

Key Words:
Rheumatoid arthritis (RA), MicroRNA-421, SPRY1, Fibroblast-like synoviocytes.

Introduction

Rheumatoid arthritis (RA) is a long-term systemic inflammatory disease. However, its pathogenesis has not been well elucidated. Currently, RA is considered as an autoimmune disease[1,2] which is characterized by hyperplasia of synovial tissues, vasospasm formation and bone erosion. The main symptoms of RA patients include symmetrically swollen joint, joint pain, morning stiffness and deformity[3]. Fibroblast-like synoviocytes (FLS), also known as synovial fibroblasts (SFs), are B-type cells distributed in the lining layer of normal synovial tissues. FLS have similar characteristics to cancer cells, reflecting the abnormal proliferation and apoptosis under pathological conditions of RA[4]. Meanwhile, FLS in RA patients release various cytokines, resulting in abnormal proliferation of synovial tissues, infiltration of inflammatory cells and erosion of cartilage and bone. This may eventually lead to joint destruction and deformity[5]. Therefore, abnormal proliferation and apoptosis of FLS are considered as major causes for RA[6].

MicroRNA is an important molecule that is capable of regulating target gene expressions. Accumulating evidence has indicated that microRNAs participate in signal transduction processes of innate immunity, adaptive immunity and inflammatory factors[7]. MicroRNAs are endogenous, non-coding, small RNA with 22 nucleotides in length. Previous studies have indicated that microRNA is highly conserved in evolution. Meanwhile, it is encoded by genomic DNA and transcribed by RNA polymerase[8]. Some certain microRNAs have already been found to be involved in the occurrence and progression of RA. For example, Stanczyk et al[9] have found that mi-

Corresponding Author: Guang-Ming Wu, MD; e-mail: 1034556389@qq.com

croRNA-155 is highly expressed in synovial fluid and tissue of RA patients when compared with those of osteoarthritis patients. Alsaleh et al[10] have pointed out that microRNA-346 expression is significantly increased in FLS of RA, which can indirectly mediate IL-18 release. However, the potential effect of microRNA-421 on regulating FLS functions in RA has rarely been reported.

SPRY is a regulatory feedback factor of receptor tyrosine kinases (RTKs), which inhibits RTK-mediated ERK pathway[11,12]. SPRY exerts an essential role in cell proliferation, differentiation and survival. SPRY family in mammals contains four members (SPRY 1-4), which vary greatly in distributions and activities[13]. Studies[14-16] have reported that SPRY expression is downregulated in breast cancer, prostate cancer and liver tissue, suggesting that SPRY may inhibit tumor development. SPRY1 is considered to inhibit tumorigenesis of multiple malignancies, including prostate cancer and hepatocellular carcinoma[17]. Meanwhile, SPRY1 downregulation inhibits the ERK activity[18]. Furthermore, SPRY1 particularly suppresses receptor tyrosine kinase (RTK)-mediated Ras-ERK/MAPKS signaling[19]. However, the potential role of SPRY1 in RA has not been fully elucidated.

The aim of this study was to investigate the role of microRNA-421/SPRY1 in the pathogenesis of RA. Moreover, the regulatory effects of microRNA-421 and SPRY1 on cell proliferation, migration and inflammatory response of FLS were explored. Our work might provide evidence for the prediction and treatment of RA.

Patients and Methods

Sample Collection

This study was approved by the Ethics Committee of Beijing Luhe Hospital. Informed content was obtained from each subject before the study. A total of 30 RA patients and 30 normal subjects were enrolled for synovial histology examination. FLS were first isolated from synovial tissues by fluorescence-activated cell sorting (FACS). Subsequently, FLS were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, NY, USA) in a 5% CO_2, 37°C incubator. Cultured FLS were assigned into 3 groups, including the control group, microRNA-421 mimics group and microRNA-421 inhibitor group.

Quantitative Real Time-Polymerase Chain Reaction (QRT-PCR)

Total RNA in treated FLS was extracted using the TRIzol method (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed in strict accordance with PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). The RNA concentration was detected using a spectrometer (Hitachi, Tokyo, Japan). QRT-PCR was performed based on the instructions of SYBR Premix Ex Taq TM (TaKaRa, Otsu, Shiga, Japan), with 3 replicates in each group. The relative expression of miRNAs was calculated using the $2^{-\Delta\Delta CT}$ method. Primer sequences used in this study were as follows: SPRY1, F: 5'-CCAGGAACCCCTCCTTACTC-3', R: 5'-GATGTGTCCGCTAGGAAGGA-3'; microRNA-421, F: 5'-CAAGTGTAAACATCCTCGACTG-3', R: 5'-GCCTGCGTGTCGTGGAGTCG-3'; IL-1: F: CGCCATAGCTGGAGAGTGTGG, R: AGTGCTCTGCTTGAGAGGTGCT; IL-6, F: 5'-GCGGCATTAGCATGGACAA-3', R: 5'-CGAATAGCGACGTGCAGTTCT-3'; COX2: F: CCTATCCTTGTAGAAGCACAAC, R: ACAGCGTCATTTCCCCTGTGA; U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Western Blot

Total protein was extracted from cells by radio-immunoprecipitation assay (RIPA) solution (Beyotime, Shanghai, China). After separation, proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Then, the membranes were blocked with skimmed milk and incubated with primary antibodies (Cell Signaling Technology, Danvers, MA, USA) overnight at 4°C. On the next day, the membranes were incubated with secondary antibody at room temperature for 1 h. The exposure of protein blots on the membrane was obtained by enhanced chemiluminescence.

Dual-Luciferase Reporter Gene Assay

Wild-type (SPRY1 WT) and mutant-type SPRY1 (SPRY1 MUT) were first constructed. FLS were co-transfected with microRNA-421 mimics or microRNA-421 inhibitor and SPRY1 WT or SPRY1 MUT, respectively. The Luciferase activity was detected according to the instructions of relative commercial kits (Promega, Madison, WI, USA).

Cell Counting Kit-8 (CCK-8) Assay

FLS were seeded into 96-well plates at a density of 2×10^3/μL. Briefly, 10 μL of CCK-8 solution (Dojindo, Kumamoto, Japan) was added to each well at 6, 24, 48, 72 and 96 h, respectively. The absorbance at 490 nm was measured by a microplate reader (Bio-Rad, Hercules, CA, USA). 3 replicates were set in each group.

Transwell Assay

FLS were pretreated with 10 μg/mL Rhodamine 123 in a water bath at 37°C for 10 min. 100 μL of pre-melt Matrigel was added in transwell chamber on ice. Subsequently, 1 mL of cell suspension (1×10^6/mL) and 400 μL of DMEM containing 10 ng/mL VEGF were added in the upper and lower chamber, respectively. After culturing for 5.5 h, the number of penetrating cells was calculated.

Enzyme-Linked Immunosorbent Assay (ELISA)

Transfected cells were centrifuged at 6000 rpm/min for 10 min. The expression levels of IL-1, IL-6, and COX2 in the culture medium were detected using ELISA detection kit (Abcam, Cambridge, MA, USA). Finally, the absorbance at 490 nm was recorded.

RA Mouse Model

Female DBA/1 mice with SPF level (13 to 15-week-old) were used for RA mouse model construction. Briefly, 0.1 mL of collagen emulsion was intracutaneously injected into multiple sites of mouse tail. After fundamental immunization for 21 days, 0.1 mL of collagen emulsion was intraperitoneally injected to enhance immunization. To accelerate RA development, 20 μg LPS was intraperitoneally injected on the 28th and 32nd day, respectively. Mice in the adjuvant group received an intracutaneous injection of isodose CFA (complete Freund's adjuvant diluted in 0.1 mol/L glacial acetic acid solution) on the 1st and 21st day, respectively. Corticosterone (10 mg S.C., Sigma-Aldrich, St. Louis, MO, USA), microRNA-421 inhibitor (2107 transduced units, GenePharma, Shanghai, China) or normal saline was injected into 3 mice, respectively. The expression levels of microRNA-421 and SPRY1 were detected in synovial tissues of the above 9 experimental and 3 normal mice. This study was approved by the Animal Ethics Committee of the Capital Medical University Animal Center.

Statistical Analysis

GraphPad Prism 6 (La Jolla, CA, USA) was used for all statistical analyses. Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). The *t*-test was used to compare the difference between the two groups. $p<0.05$ was considered statistically significant (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

Results

Expressions of MicroRNA-421 and SPRY1 in Synovial Tissues

QRT-PCR results showed that the expression of microRNA-421 in synovial tissues of RA patients was significantly higher than that of normal subjects ($p<0.001$, Figure 1A). Western blot results indicated that SPRY1 was markedly downregulated in synovial tissues of RA patients when compared with that of normal subjects ($p<0.001$, Figure 1 B). Subsequently, the possible interaction between microRNA-421 and SPRY1 in RA was detected. Transfection efficacies of microRNA-421 mimics and inhibitor were first verified by qRT-PCR and Western blot ($p<0.01$, Figure 1C). Both protein and mRNA levels of SPRY1 were significantly decreased after microRNA-421 overexpression in FLS (Figure 1D and 1E), suggesting the regulatory effect of microRNA-421 on SPRY1.

SPRY1 Was the Target Gene of MicroRNA-421

TargetScan predicted that SPRY1 might be the target gene of microRNA-421 (Figure 2A). Subsequently, the target condition was further verified by Dual-Luciferase reporter gene assay. The results demonstrated that FLS co-transfected with SPRY1 WT and microRNA-421 mimics showed remarkably lower Luciferase activity than those co-transfected with SPRY1 MUT and microRNA-421 mimics ($p<0.001$, Figure 2B). This indicated that SPRY1 was the target gene of microRNA-421.

MicroRNA-421 Promoted Proliferation, Migration and Inflammation of SFs

CCK-8 results showed that microRNA-421 overexpression significantly promoted FLS viability (Figure 3A). The transwell assay demonstrated that migratory ability of FLS was remark-

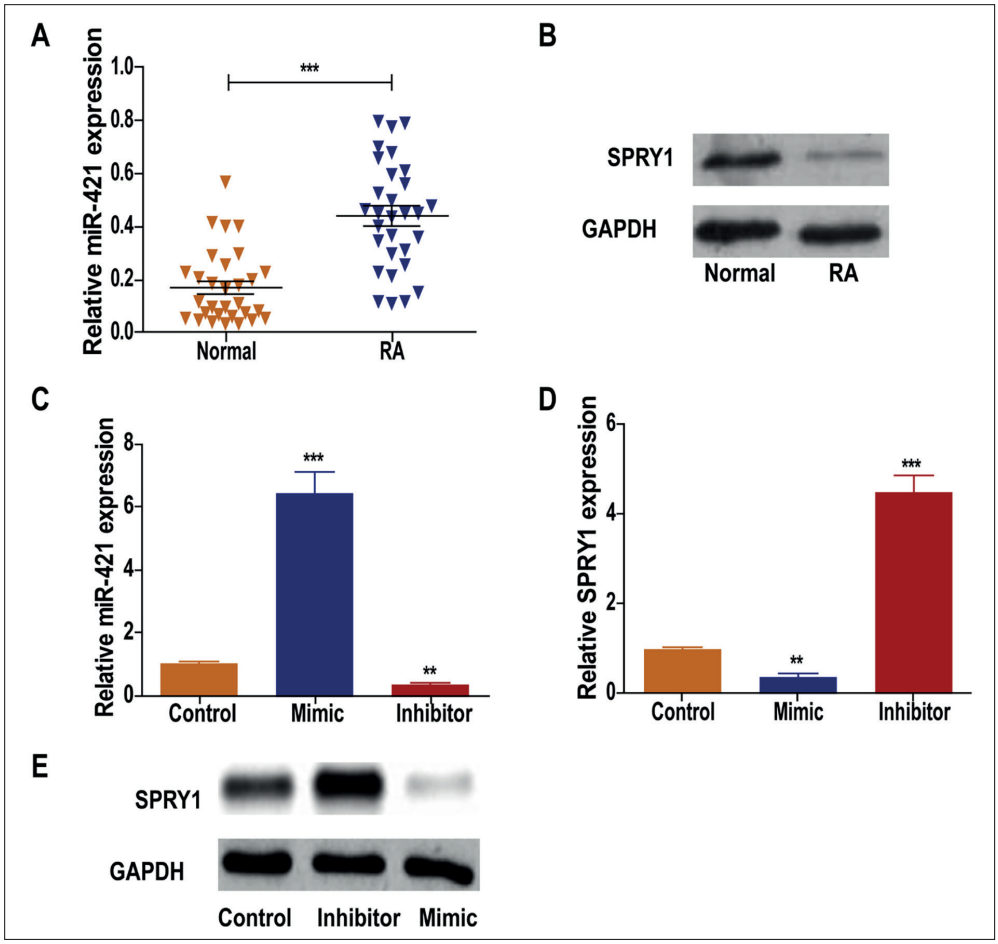

Figure 1. Expressions of microRNA-421 and SPRY1 in synovial tissues. **A,** QRT-PCR indicated that microRNA-421 was overexpressed in synovial tissues of RA patients than that of the normal group. **B,** Western blot results indicated that SPRY1 was downregulated in synovial tissues of RA patients compared with that of the normal group. **C,** Transfection efficacies of microRNA-421 mimics and inhibitor were verified. **D, E,** Both protein **D,** and mRNA **E,** levels of SPRY1 were significantly decreased after microRNA-421 overexpression in FLS.

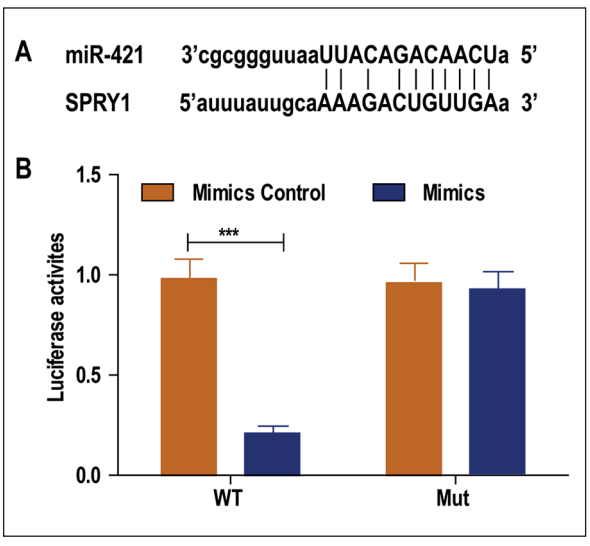

Figure 2. SPRY1 was the target gene of microRNA-421. **A,** TargetScan predicted that SPRY1 might be the target gene of microRNA-421. **B,** Luciferase activity of FLS co-transfected with SPRY1 WT and microRNA-421 mimics was markedly lower than those co-transfected with SPRY1 MUT and microRNA-421 mimics.

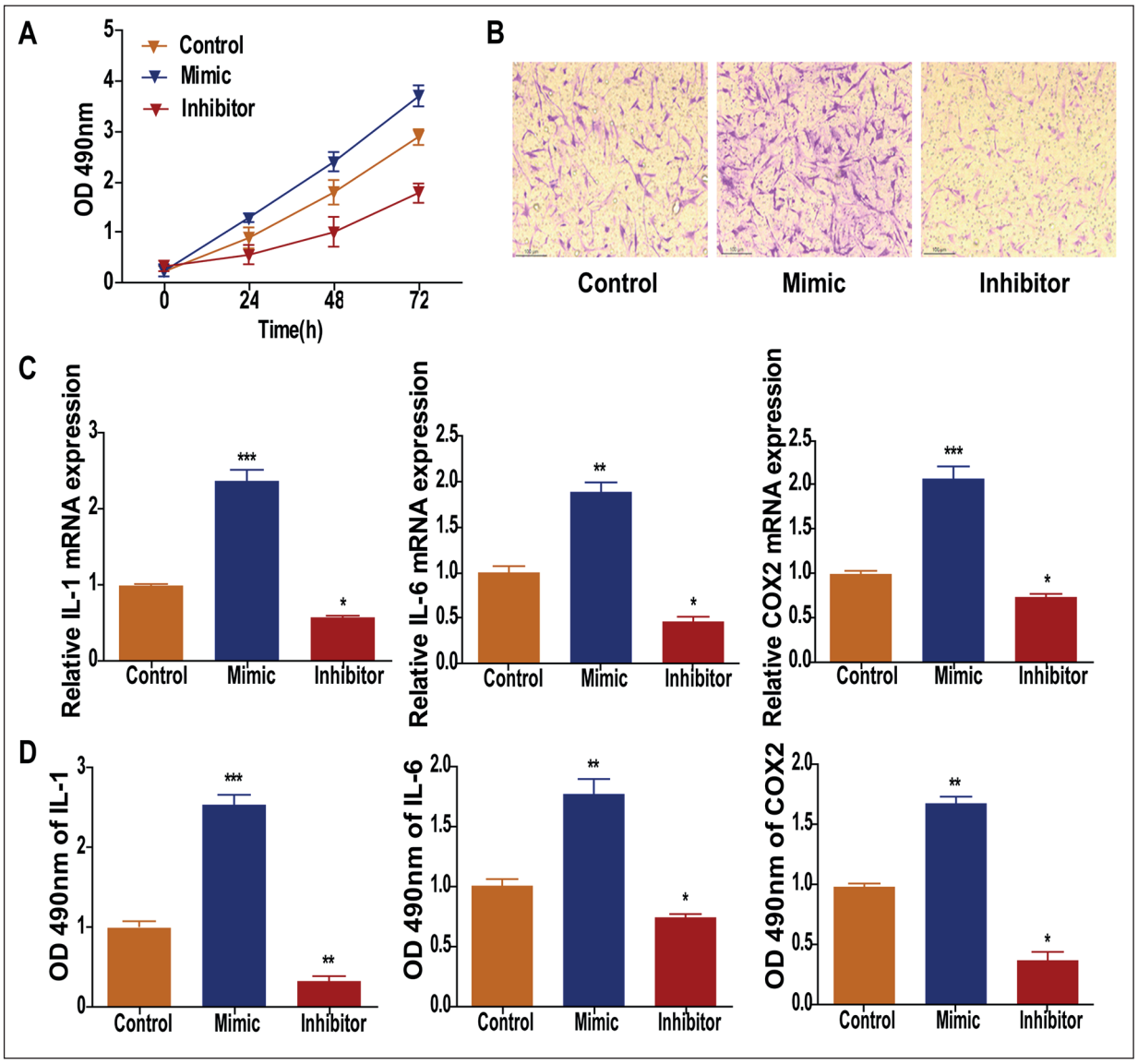

Figure 3. MicroRNA-421 promoted proliferation, migration and inflammation of FLS. **A,** CCK-8 results showed that microRNA-421 overexpression promoted FLS viability. **B,** The transwell assay demonstrated that migratory ability of FLS was remarkably elevated after transfection of microRNA-421 mimics. **C,** The mRNA levels of IL-1, IL-6, and COX2 were significantly increased in FLS transfected with microRNA-421 mimics. **D,** The levels of IL-1, IL-6, and COX2 were remarkably increased in culture medium of FLS transfected with microRNA-421 mimics.

ably elevated after transfection of microRNA-421 mimics (Figure 3B). The expressions of inflammatory factors in FLS were detected by qRT-PCR and ELISA, respectively. We found that mRNA levels of IL-1, IL-6 and COX2 were markedly increased in FLS transfected with microRNA-421 mimics when compared with those of controls (Figure 3C). Similar results were obtained in ELISA detection (Figure 3D).

MicroRNA-421 Inhibitor and Corticosterone Regulated Expressions of MicroRNA-421 and SPRY1 in RA Mouse Model

In vivo, RA mouse model was constructed to explore whether microRNA-421 inhibitor could regulate RA development. No significant differences in the expression levels of microRNA-421 and SPRY1 were observed

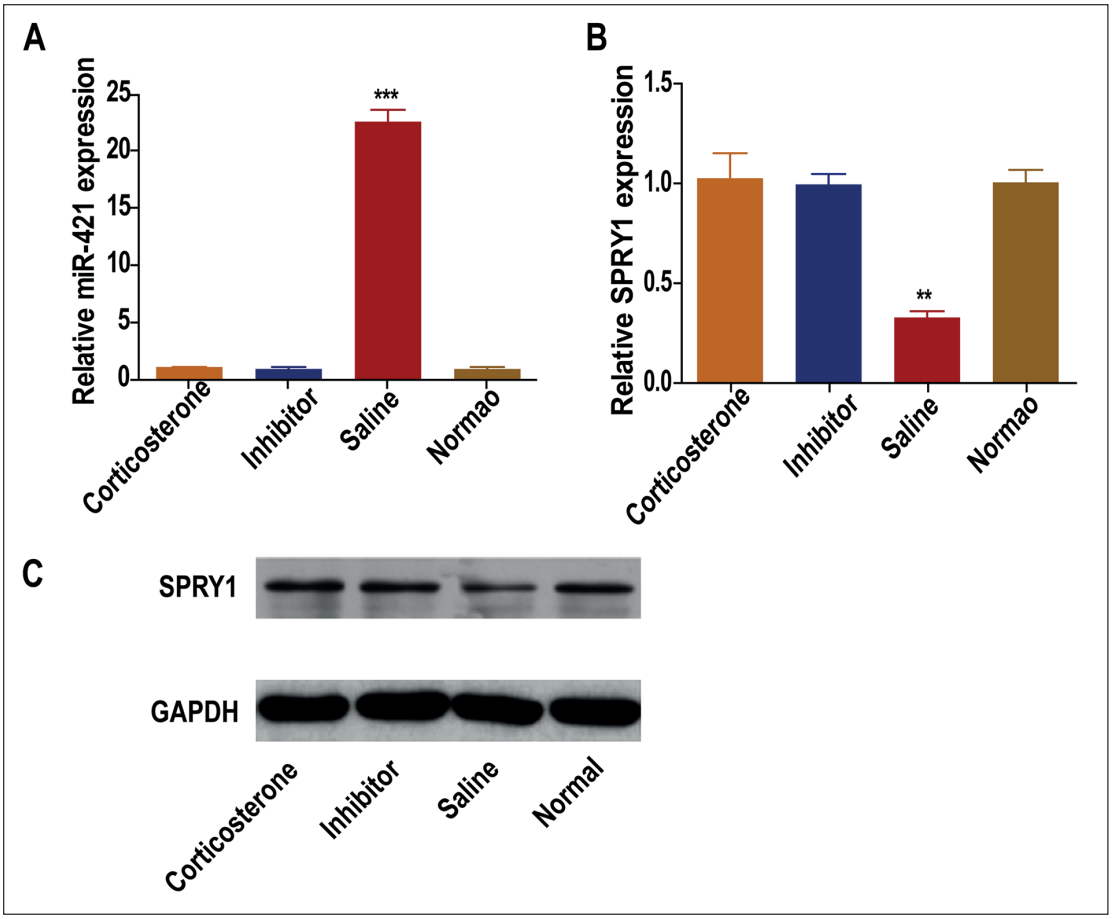

Figure 4. MicroRNA-421 inhibitor and corticosterone affected expressions of microRNA-421 and SPRY1 in RA mouse model. **A, B,** Compared with mice injected with saline, downregulated microRNA-421 **A,** and upregulated SPRY1 **B,** were observed in mice injected with cortisone and microRNA-421 inhibitor. **C,** Higher protein expression of SPRY1 was observed in mice injected with cortisone and microRNA-421 inhibitor than those injected with saline.

in mice injected with cortisone and microRNA-421 inhibitor. Compared with mice injected with saline, significantly downregulated microRNA-421 and upregulated SPRY1 were observed in mice injected with corticosterone and microRNA-421 inhibitor (Figure 4A and 4B). However, both corticosterone and microRNA-421 inhibitor administration could only partially reverse the original expressions of microRNA-421 and SPRY1. The protein expression of SPRY1 in synovial tissues was also detected by Western blot. The results showed that higher protein expression of SPRY1 was observed in mice injected with corticosterone and microRNA-421 inhibitor than those injected with saline (Figure 4C). The above data suggested that microRNA-421 inhibition exerted similar therapeutic effects of the anti-inflammatory drug as corticosterone.

Discussion

MicroRNA-421 significantly promoted proliferative and migratory potentials of FLS in RA *via* downregulating SPRY1. Our study revealed that microRNA-421 exerted its biological function by directly binding to SPRY1, which might provide new references for RA treatment. The pathogenesis of RA has not been thoroughly studied. However, evidence has shown that some certain microRNAs are closely related to the pathogenesis of RA. Li et al[20] have found that the levels of microRNA-155, microRNA-146a, microRNA-132 and microRNA-16 are higher in peripheral blood of RA patients compared with those of osteoarthritis patients. Besides, the expression levels of microRNA-146a and microRNA-16 are correlated with the severity of RA. Dong et al[21] have found that microRNA-21 concentration in peripheral

blood of RA patients is significantly downregulated. Meanwhile, microRNA-21 concentration is negatively correlated with the ratio of Th17/Treg cells in peripheral blood. *In vitro* studies have also demonstrated that lipopolysaccharide treatment downregulates microRNA-21 concentration in peripheral blood of RA patients.

Currently, microRNA-421 has been well studied in gastric cancer. Meanwhile, relative studies[22-24] have also confirmed its role in neuroblastoma, pancreatic and prostate cancers and other malignant tumors. Jiang et al[25] have analyzed the relationship between microRNA-421 expression and clinical data in gastric cancer. Moreover, Zhou et al[26] have found that microRNA-421 knockdown remarkably inhibits the development of gastric cancer. However, the specific effect of microRNA-421 on RA is rarely reported. Our work demonstrated that microRNA-421 was overexpressed in synovial tissues of RA patients. MicroRNA-421 up-regulation could significantly accelerate proliferative, migratory potentials and inflammatory response of FLS. Hence, we speculated that microRNA-421 might be involved in RA development.

Recent studies have shown that SPRY1, as a direct target of microRNA-21, can regulate various diseases, including cancer[27], atrial fibrillation[28], vascular and metabolic diseases[29]. Furthermore, SPRY is also capable of inducing cell senescence[30]. In this work, microRNA-421 was confirmed to regulate cellular behaviors of FLS *via* directly binding to SPRY1. Similar results were obtained *in vivo*. Therefore, we considered that microRNA-421 could serve as an anti-inflammatory drug for the treatment of RA.

Conclusions

Our results showed that microRNA-421 promotes the inflammatory response of FLS by downregulating SPRY1 expression in RA.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding

This study was supported from the Foundation of Science and Technology of Tongzhou, Beijing (No. 20181346).

References

1) Wong JB, Ramey DR, Singh G. Long-term morbidity, mortality, and economics of rheumatoid arthritis. Arthritis Rheum 2001; 44: 2746-2749.
2) Lajas C, Abasolo L, Bellajdel B, Hernandez-Garcia C, Carmona L, Vargas E, Lazaro P, Jover JA. Costs and predictors of costs in rheumatoid arthritis: a prevalence-based study. Arthritis Rheum 2003; 49: 64-70.
3) Martinez A, Orozco G, Varade J, Sanchez Lopez M, Pascual D, Balsa A, Garcia A, de la Concha EG, Fernandez-Gutierrez B, Martin J, Urcelay E. Macrophage migration inhibitory factor gene: influence on rheumatoid arthritis susceptibility. Hum Immunol 2007; 68: 744-747.
4) Firestein GS. Invasive fibroblast-like synoviocytes in rheumatoid arthritis. Passive responders or transformed aggressors? Arthritis Rheum 1996; 39: 1781-1790.
5) Kinne RW, Brauer R, Stuhlmuller B, Palombo-Kinne E, Burmester GR. Macrophages in rheumatoid arthritis. Arthritis Res 2000; 2: 189-202.
6) Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. Immunol Rev 2010; 233: 233-255.
7) Liu Y, Zhang XL, Li XF, Tang YC, Zhao X. MiR-212-3p reduced proliferation, and promoted apoptosis of fibroblast-like synoviocytes via down-regulating SOX5 in rheumatoid arthritis. Eur Rev Med Pharmacol Sci 2018; 22: 461-471.
8) Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 2008; 9: 102-114.
9) Stanczyk J, Pedrioli DM, Brentano F, Sanchez-Pernaute O, Kolling C, Gay RE, Detmar M, Gay S, Kyburz D. Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. Arthritis Rheum 2008; 58: 1001-1009.
10) Alsaleh G, Suffert G, Semaan N, Juncker T, Frenzel L, Gottenberg JE, Sibilia J, Pfeffer S, Wachsmann D. Bruton's tyrosine kinase is involved in miR-346-related regulation of IL-18 release by lipopolysaccharide-activated rheumatoid fibroblast-like synoviocytes. J Immunol 2009; 182: 5088-5097.
11) Edwin F, Anderson K, Ying C, Patel TB. Intermolecular interactions of Sprouty proteins and their implications in development and disease. Mol Pharmacol 2009; 76: 679-691.
12) Mason JM, Morrison DJ, Basson MA, Licht JD. Sprouty proteins: multifaceted negative-feedback regulators of receptor tyrosine kinase signaling. Trends Cell Biol 2006; 16: 45-54.
13) Christofori G. Split personalities: the agonistic antagonist Sprouty. Nat Cell Biol 2003; 5: 377-379.
14) Fong CW, Chua MS, McKie AB, Ling SH, Mason V, Li R, Yusoff P, Lo TL, Leung HY, So SK, Guy GR. Sprouty 2, an inhibitor of mitogen-activated pro-

tein kinase signaling, is down-regulated in hepatocellular carcinoma. Cancer Res 2006; 66: 2048-2058.

15) Chen Y, Takita J, Hiwatari M, Igarashi T, Hanada R, Kikuchi A, Hongo T, Taki T, Ogasawara M, Shimada A, Hayashi Y. Mutations of the PTPN11 and RAS genes in rhabdomyosarcoma and pediatric hematological malignancies. Genes Chromosomes Cancer 2006; 45: 583-591.

16) Yoo J, Robinson RA. H-ras and K-ras mutations in soft tissue sarcoma: comparative studies of sarcomas from Korean and American patients. Cancer 1999; 86: 58-63.

17) Macia A, Gallel P, Vaquero M, Gou-Fabregas M, Santacana M, Maliszewska A, Robledo M, Gardiner JR, Basson MA, Matias-Guiu X, Encinas M. Sprouty1 is a candidate tumor-suppressor gene in medullary thyroid carcinoma. Oncogene 2012; 31: 3961-3972.

18) Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, Galuppo P, Just S, Rottbauer W, Frantz S, Castoldi M, Soutschek J, Koteliansky V, Rosenwald A, Basson MA, Licht JD, Pena JT, Rouhanifard SH, Muckenthaler MU, Tuschl T, Martin GR, Bauersachs J, Engelhardt S. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature 2008; 456: 980-984.

19) Sabatel C, Cornet AM, Tabruyn SP, Malvaux L, Castermans K, Martial JA, Struman I. Sprouty1, a new target of the angiostatic agent 16K prolactin, negatively regulates angiogenesis. Mol Cancer 2010; 9: 231.

20) Li X, Tian F, Wang F. Rheumatoid arthritis-associated microRNA-155 targets SOCS1 and upregulates TNF-alpha and IL-1beta in PBMCs. Int J Mol Sci 2013; 14: 23910-23921.

21) Dong L, Wang X, Tan J, Li H, Qian W, Chen J, Chen Q, Wang J, Xu W, Tao C, Wang S. Decreased expression of microRNA-21 correlates with the imbalance of Th17 and Treg cells in patients with rheumatoid arthritis. J Cell Mol Med 2014; 18: 2213-2224.

22) Hu H, Du L, Nagabayashi G, Seeger RC, Gatti RA. ATM is down-regulated by N-Myc-regulated microRNA-421. Proc Natl Acad Sci U S A 2010; 107: 1506-1511.

23) Hao J, Zhang S, Zhou Y, Liu C, Hu X, Shao C. MicroRNA 421 suppresses DPC4/Smad4 in pancreatic cancer. Biochem Biophys Res Commun 2011; 406: 552-557.

24) Ostling P, Leivonen SK, Aakula A, Kohonen P, Makela R, Hagman Z, Edsjo A, Kangaspeska S, Edgren H, Nicorici D, Bjartell A, Ceder Y, Perala M, Kallioniemi O. Systematic analysis of microRNAs targeting the androgen receptor in prostate cancer cells. Cancer Res 2011; 71: 1956-1967.

25) Jiang Z, Guo J, Xiao B, Miao Y, Huang R, Li D, Zhang Y. Increased expression of miR-421 in human gastric carcinoma and its clinical association. J Gastroenterol 2010; 45: 17-23.

26) Zhou H, Xiao B, Zhou F, Deng H, Zhang X, Lou Y, Gong Z, Du C, Guo J. MiR-421 is a functional marker of circulating tumor cells in gastric cancer patients. Biomarkers 2012; 17: 104-110.

27) Jin XL, Sun QS, Liu F, Yang HW, Liu M, Liu HX, Xu W, Jiang YY. microRNA 21-mediated suppression of Sprouty1 by Pokemon affects liver cancer cell growth and proliferation. J Cell Biochem 2013; 114: 1625-1633.

28) Cardin S, Guasch E, Luo X, Naud P, Le Quang K, Shi Y, Tardif JC, Comtois P, Nattel S. Role for microRNA-21 in atrial profibrillatory fibrotic remodeling associated with experimental postinfarction heart failure. Circ Arrhythm Electrophysiol 2012; 5: 1027-1035.

29) Cheng Y, Zhang C. MicroRNA-21 in cardiovascular disease. J Cardiovasc Transl Res 2010; 3: 251-255.

30) Macia A, Vaquero M, Gou-Fabregas M, Castelblanco E, Valdivielso JM, Anerillas C, Mauricio D, Matias-Guiu X, Ribera J, Encinas M. Sprouty1 induces a senescence-associated secretory phenotype by regulating NFkappaB activity: implications for tumorigenesis. Cell Death Differ 2014; 21: 333-343.