European Review for Medical and Pharmacological Sciences 2018; 22: 914-920

UCA1 impacts progress of rheumatoid arthritis by inducing the apoptosis of fibroblast-like synoviocyte

Z.-F. YAN[1], X.-Y. ZHAO[2], W. LIU[1], X.-P. LIU[3]

[1]CHENLU Traditional Chinese Medicine Internal Clinic, Shenzhen, China
[2]Geriatric Department, Beijing University of Chinese Medicine Third Affiliated Hospital, Beijing, China
[3]Rheumatology, Dongfang Hospital Beijing University of Chinese Medicine, Beijing, China

Zhifan Yan and Xuying Zhao equally contributed to this work

Abstract. – OBJECTIVE: Rheumatoid arthritis is a chronic autoimmune joint disease, which is characterized by the proliferation of fibroblast-like synoviocyte. Long non-coding RNA (lncRNA) has been reported to play an important role in the progression of many different diseases. The main objective of this research was to find out whether the lncRNAs influence the activity of fibroblast-like synoviocyte and the progression of this disease.

PATIENTS AND METHODS: qRT-PCR was used to detect the expression of UCA1 in fibroblast-like synoviocyte from normal people and rheumatoid arthritis patients. MTT assay was used to detect the viability of cells. Apoptosis was detected by Caspase-3 Colorimetric Activity Assay Kit (Millipore, Billerica, MA, USA). Western blot was used to analyze the relationship of UCA1 and apoptosis.

RESULTS: We found that the UCA1 was highly expressed in the normal fibroblast-like synoviocyte (NFLS), compared with the fibroblast-like synoviocyte of rheumatoid arthritis (RAFLS). We also found that the decrease in UCA1 expression increased the viability in NFLS and overexpressed UCA1 level in RAFLS decreased the viability. Caspase-3 was highly expressed in cells with higher viability. What's more, UCA1 could affect the viability of FLS by changing the expression of Wnt6.

CONCLUSIONS: According to the results, we found that UCA1 was closely related to rheumatoid arthritis, which could be a potential target for treating it.

Key Words:
UCA1, Fibroblast-like synoviocytes, Rheumatoid arthritis, Apoptosis, NFLS, RAFLS

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune joint disease, which is characterized by the proliferation of synoviocytes that produce inflammatory cytokines and chemokines. It typically results in warm, swollen, and painful joints[1-3]. Pain and stiffness often worsen following rest. Most commonly, the wrist and hands are involved, with the same joints typically involved on both sides of the body. This disease impairs patients' bodies and decreases the quality of life. The pathogenesis of RA is leukocytic infiltration of the synovium and the expansion of fibroblast-like synoviocytes (FLS). FLS are involved in the important pathological progression in RA, thus solving the problem has become a hot-spot[4,5]. Long non-coding RNAs (lncRNAs) are a novel class of molecules, with a length longer than 200 nucleotides (nt)[6-8]. In recent years, accumulating evidence has demonstrated that lncRNAs participated in the regulation of various biological processes, such as cell proliferation, differentiation, apoptosis, motility and so on. Furthermore, Pan et al[3] reported that MALAT1 expression was significantly upregulated in the quercetin-treated RAFLS and the knockdown of MALAT1 inhibited the apoptosis of RAFLS, leading to the activation of the PI3K/AKT pathway. These results pointed out that quercetin promotes RAFLS apoptosis by up-regulating lncRNA MALAT1. Whether it could be used in clinical trails still remains to be elucidated. Urothelial carcinoma associated 1 (UCA1) was a novel lncRNA gene with three exons and two introns, which is located in the chromosome 19p13.12. The expression of lncRNA UCA1 was firstly discovered to be increased in the carcinogenesis of bladder cancer in 2006 (9,10). In recent years, accumulating evidence has shown that UCA1 played a part in the development of many diseases. Therefore, we wanted to know whether

Corresponding Author: Xiaoping Liu, MD; e-mail: wslxp-001@163.com

the lncRNA was related to the development of RA. In this investigation, we detected the expression of UCA1 in the normal fibroblast-like synoviocyte and the fibroblast-like synoviocyte from RA patients. The effect of UCA1 on the viability of two cell lines was also measured. Finally, the possible mechanism of UCA1 in influencing activity of FLS was discussed.

Materials and Methods

Cell Culture and Treatment

Rheumatoid arthritis fibroblast-like synoviocytes (RAFLS) and normal fibroblast-like synoviocytes (NFLS) used in this study were purchased from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). The cells were cultured in Dulbecco's modified eagle's Medium (DMEM) Gibco (Grand Island, NY, USA), which contained 10% fetal bovine serum (FBS) HyClone (South Logan, UT, USA) in a humidified cell incubator with 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified environment of 5% CO_2 and 95% air. The medium was changed every 24 hours, and cells at passages 3 to 6 were used in the following experiments. Each experiment was repeated three times. We stored the specimens at -80°C for further use.

RNA Extraction and Real-time Quantitative PCR Assays

Total RNA from cell lines was extracted by using RNAiso Plus (TaKaRa, Otsu, Shiga, Japan), following the manufacturer's protocol. The concentration of RNA was detected and cDNA was synthesized through RT reaction by using PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Otsu, Shiga, Japan). The expression of UCA1 in RAFLS and NFLS cell lines was detected by standard qRT-PCR protocol, using SYBR Premix Ex Taq (TaKaRa, Otsu, Shiga, Japan).

Plasmid Transfection

We seeded the target cells into 6-well plate at 60-80% confluent before transfection. Then, we put 1 μg of plasmid and 1 μl of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) into 200 μl of medium without fetal bovine serum (FBS), respectively. Diluted plasmid and lipofectamine 2000 were mixed and incubated for 15 min. Finally, the plasmid-lipid complex was added to the cells.

Cell Viability Assay

NFLS and RAFLS were plated at 10^4 cells per well on a 96-well plate and incubated overnight in Dulbecco's modified eagle's Medium supplemented with 10% v/v FBS. Then, the medium was removed, and the fresh medium was added. After 48 h, the cell viability was assayed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Dojindo Laboratories (Shanghai, China) assay as previously described.

Caspase 3 Activity Assays

We used the Caspase-3 Colorimetric Activity Assay Kit (Millipore, Billerica, MA, USA) to detect the apoptosis situation of cell lines following the Standard Assay Instructions.

Western Blot

We used 10% resolving gel and a 5% stacking gel for immune-blotting experiments. After loading prepared samples in the gel, electrophoresis was performed through the stacking gel at 60 V for 40 min and through the resolving gel at 110 V for 75 min. The gel was transferred to a membrane using a low-temperature constant current of 350 mA for 2 hours. The membrane was sealed with bovine serum albumin (BSA) for 1 h and incubated with a primary antibody overnight at 4°C. After that, the membrane was developed and imaged using a gel documentation system (Bio-Rad, Hercules, CA, USA). The membrane was incubated with a secondary antibody for 2 h at 25°C, and the results were analyzed.

Statistical Analysis

We used the software Graphpad 6 (GraphPad Software, La Jolla, CA, USA) to perform statistical analysis. Data were reported as mean ± standard deviation. Two-sample means were compared using a Student's *t*-test, multiple-sample means were compared using complete randomized block one-way ANOVA, and pairwise multiple-sample means were compared using LSD and Bonferroni tests. $p<0.05$ was considered to be statistically significant.

Results

UCA1 was Highly Expressed in the NFLS and Decreased the Viability of Cells

In order to estimate the effect of UCA1 in progress of rheumatoid arthritis, we used qRT-PCR to detect the expression of UCA in NFLS and RAFLS. We found the expression of UCA1

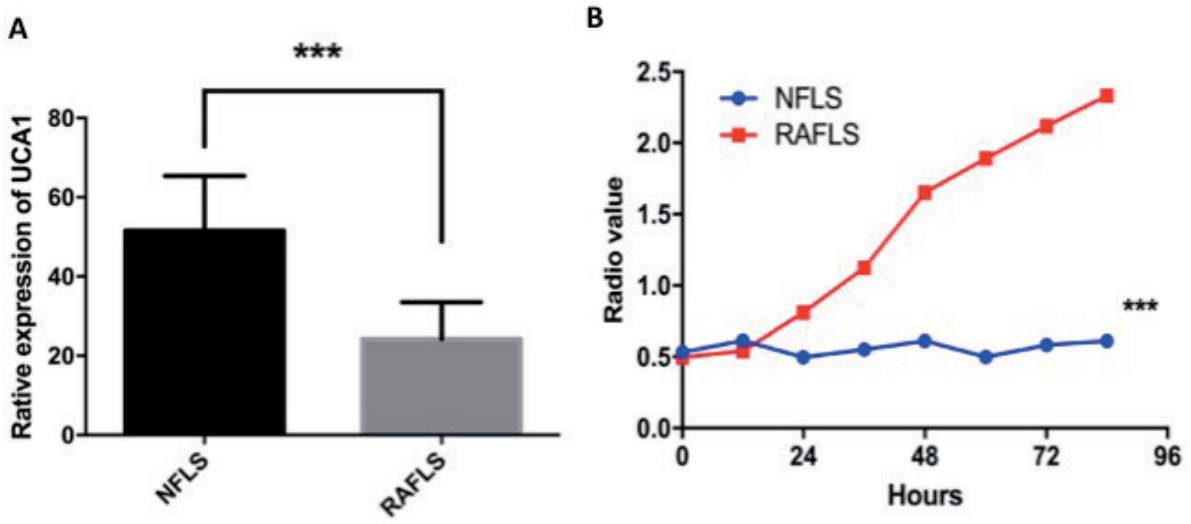

Figure 1. UCA1 was highly expressed in the NFLS and decreased the viability of cells. ***(A)*** The expression of UCA1 in the NFLS and RAFLS was detected by qRT-PCR assay. $^{***}p<0.001$; ***(B)*** The viability of NFLS and RAFLS was detected by MTT assays. $^{***}p<0.001$.

was highly expressed in NFLS compared with that in the RAFLS. Then, we examined the viability of cell lines and we found that the viability of RAFLS was increased (Figure 1). These results showed that UCA1 was involved in the development of RA, but the mechanism remained unclear.

Alter the Expression of UCA1 Influences the Viability of FLS

Our observations of altered expression level of UCA1 in FLS suggested that more investigation of those factors was needed. We selected the RAFLS and NFLS to conduct additional experiments. We up-regulated UCA1 expression in RAFLS and knocked down UCA1 expression in NFLS. We observed that the overexpression of UCA1 in RAFLS inhibited the viability of cells. However, down-regulation of UCA1 promoted the cells viability in NFLS (Figure 2). These results indicated that expression level of UCA1 was implicated in cells viability.

Cells Apoptosis Affects the Cells Viability

Cell apoptosis is a key factor influencing cell viability so that we wanted to know whether the expression of caspase-3 would show any difference in two cell lines. We detected the apoptosis by Caspase-3 Colorimetric Activity Assay Kit and we found that caspase-3 was decreased in RAFLS, compared with NFLS. Furthermore, the caspase-3 was decreased in NFLS when we knocked down the UCA1 while the caspase-3 was increased in RAFLS when we overexpressed the UCA1 (Figure 3). The results showed that UCA1 might induce cell apoptosis leading to the changes in cell viability.

UCA1 Regulated Cell Viability of FLS by Wnt Pathway

In previous experiments, we found that UCA1 was closely related to viability of FLS, but the mechanism remained unclear. Fan et al[11] reported that UCA1 promoted cisplatin resistance by up-regulating Wnt6 expression in bladder cancer. The activation of Wnt signaling contributed to development of proliferation, migration, invasion and chemo-resistance in human cancers. We investigated whether UCA1 could affect the viability of FLS via transforming Wnt6. We found that UCA1 suppression in NFLS could improve the expression of Wnt6 while UCA1 overexpression in RAFLS could reduce the expression of Wnt6. Furthermore, we transfected a small interfering plasmid of Wnt6 into the NFLS, in which UCA1 was knocked down and a over-expression plasmid of Wnt6 was transfected into RAFLS, in which UCA1 was overexpressed. We found that the viability of NFLS was decreased and the viability of RAFLS was increased, via MTT assay (Figure 4). These results indicated that

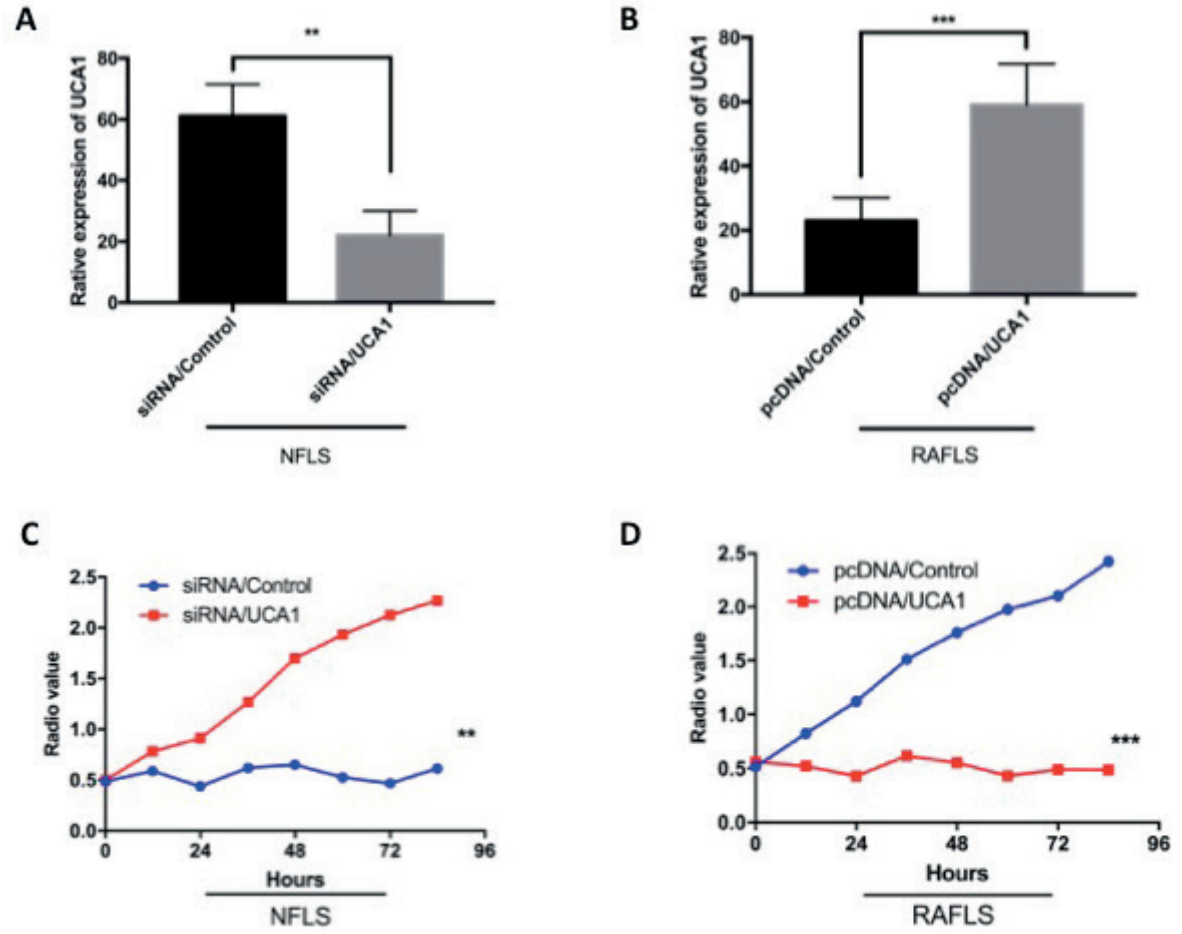

Figure 2. Altering the expression of UCA1 influenced the viability of FLS. ***(A-B)*** Relative expression of UCA1 was detected by PCR, ***p <0.001. ***(C-D)*** The viability of NFLS and RAFLS was detected by MTT assays. ***p <0.001.

UCA1 affected the cell viability via altering the expression of Wnt6.

Discussion

RA is a chronic type of inflammatory arthritis and is characterized by the presence of activated T-lymphocytes, synoviocytes and macrophages. It mainly leads to disability. RA is also related to cardiovascular disease by improving the risk rate[12,13]. FLS are major mesenchymal cells of the synovial joints that are regarded to play a key role in the development of RA[14]. It can rapidly proliferate and invade cartilage and bone in the period of RA progression. It's a hot-spot to find how to control the viability of FLS and improve the patients' quality of life. Recent studies have shown that lncRNAs played important roles in diverse biological processes including development, cell growth and tumorigenesis. For examples, lncRNA LINC00152 was reported to promote the proliferation of hepatocellular carcinoma (HCC) cells by inhibition of EpCAM expression via regulating the mTOR signaling pathway, which suggested that LINC00152 acted as an oncogene in HCC[15]. Furthermore, evidence indicated that lncRNAs have influence on different kinds of biological activity, which revealed a possible target of treating diseases.

In this study, we investigated whether UCA1 could affect the progress of RAFLS, as well as its underlying mechanism. We found that UCA1 was expressed in NFLS, and the expression level in RAFLS was higher in pathologic condition than that in normal condition. Our results showed that the cell viability was increased in RAFLS. For further verification,

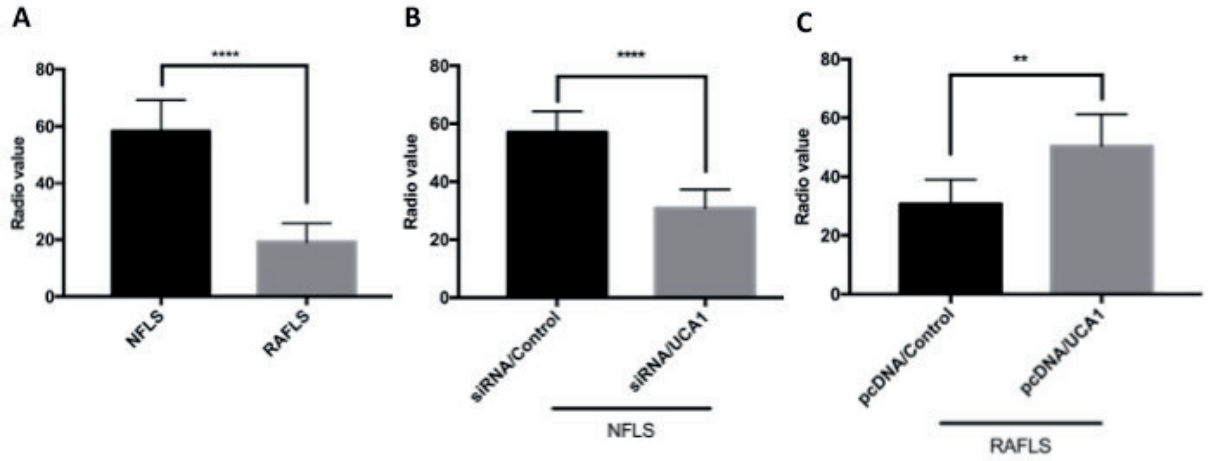

Figure 3. Cells apoptosis affected the cells viability. ***(A-C)*** The expression of caspase-3 was detected Caspase 3 Activity assays in FLS. $^{***}p$ <0.001;

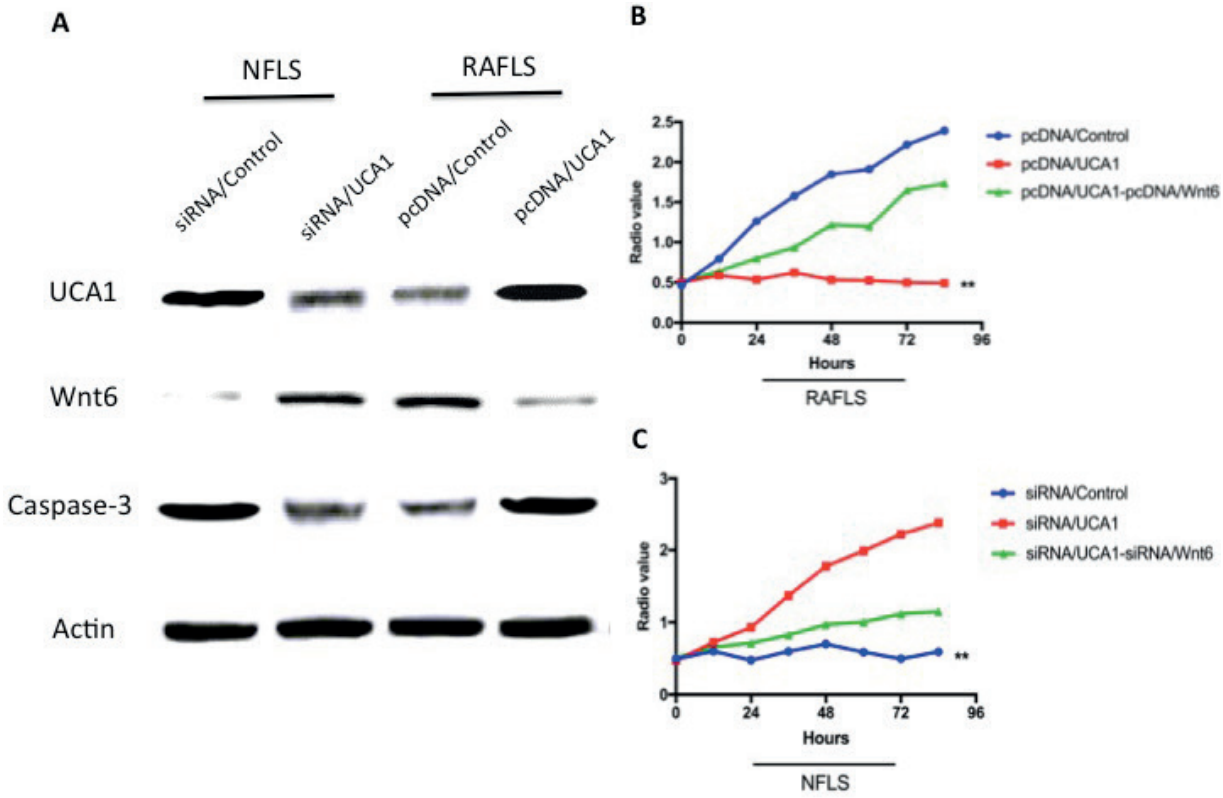

Figure 4. UCA1 regulated cell viability of FLS by influencing the expression of Wnt6. ***(A)*** Relative expression of Wnt6, UCA1, and caspase-3 was detected by Western blot. ***p <0.001; ***(B-C)*** The viability of FLS was detected by MTT assays. $^{***}p$ <0.001.

we knocked down UCA1 in NFLS, which resulted in increasing the cell viability, while overexpressed UCA1 in RAFLS reduced the cell viability. These results showed that UCA1 was a closely related with the viability of FLS. Cell viability is an equilibrium of cell proliferation and apoptosis[16,17]. Since FLS does not have the ability to grow like tumors, we mainly

paid attention to the cell apoptosis that was the major problem causing RA. We detected the expression of caspase-3 in FLS and found that UCA1 and caspase-3 had a positive correlation. UCA1 overexpression in RAFLS promoted the expression of caspase-3, and the knocking down of UCA1 in NFLS decreased the expression of caspase-3. These results showed that UCA1 was closely related with the apoptosis of FLS. We also found that UCA1 could cause apoptosis in FLS, promoting the progression of RA. However, the possible mechanism remained unclear. Recently, Fan et al[11] found up-regulated UCA1 increased Wnt6 expression and activated Wnt signaling, which resulted in cisplatin resistance. According to those works, we knew that UCA1 could promote the resistance in bladder cancer by increasing the expression of Wnt6 via activation of Wnt signaling. We then found that the expression of Wnt6 was increased after suppressing the expression of UCA1 in NFLS and its expression was reduced after overexpressing UCA1 in RAFLS. Furthermore, we found that the viability of RAFLS, in which the expression of UCA1 was increased, recovered after we transfected the over-expression plasmid of Wnt6 into it. These results indicated that UCA1 could influence the progression of RA via inducing the apoptosis of fibroblast-like synoviocyte. In summary, UCA1 could be a potential and independent factor influencing the progress of RA whose expression changed viability of FLS via regulating Wnt signaling pathway. This research provided us with a novel treatment strategy for rheumatoid arthritis.

Conclusions

We found that UCA1 was closely related to the rheumatoid arthritis and could be used as a promising prognostic marker. UCA1 could decrease the expression of Wnt6 to down-regulate the viability of RAFLS.

Acknowledgement

This work was supported by Beijing Natural Science Foundation (7164275).

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Nanki T, Nagasaka K, Hayashida K, Saita Y, Miyasaka N. Chemokines regulate IL-6 and IL-8 production by fibroblast-like synoviocytes from patients with rheumatoid arthritis. J Immunol 2001; 167: 5381-5385.
2) Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. Immunol Rev 2010; 233: 233-255.
3) Pan F, Zhu L, Lv H, Pei C. Quercetin promotes the apoptosis of fibroblast-like synoviocytes in rheumatoid arthritis by upregulating lncRNA MALAT1. Int J Mol Med 2016; 38: 1507-1514.
4) Kurowska M, Rudnicka W, Kontny E, Janicka I, Chorazy M, Kowalczewski J, Ziółkowska M, Ferrari-Lacraz S, Strom TB, Maðliðski W. Fibroblast-like synoviocytes from rheumatoid arthritis patients express functional IL-15 receptor complex: endogenous IL-15 in autocrine fashion enhances cell proliferation and expression of Bcl-x(L) and Bcl-2. J Immunol 2002; 169: 1760-1767.
5) Chen S, Yang Y, Feng H, Wang H, Zhao R, Liu H. Baicalein inhibits interleukin-1β-induced proliferation of human rheumatoid arthritis fibroblast-like synoviocytes. Inflammation 2014; 37: 163-169.
6) Lalevee S, Feil R. Long noncoding RNAs in human disease: emerging mechanisms and therapeutic strategies. Epigenomics 2015; 7: 877-879.
7) Zhou Y, Wang DL, Pang Q. Long noncoding RNA SPRY4-IT1 is a prognostic factor for poor overall survival and has an oncogenic role in glioma. Eur Rev Med Pharmacol Sci 2016; 20: 3035-3039.
8) Luo J, Tang L, Zhang J, Ni J, Zhang HP, Zhang L, Xu JF, Zheng D. Long non-coding RNA CARLo-5 is a negative prognostic factor and exhibits tumor pro-oncogenic activity in non-small cell lung cancer. Tumour Biol 2014; 35: 11541-11549.
9) Liu FT, Dong Q, Gao H, Zhu ZM. The prognostic significance of uca1 for predicting clinical outcome in patients with digestive system malignancies. Oncotarget 2017; 20: 40620-40632.
10) Wang XS, Zhang Z, Wang HC, Cai JL, Xu QW, Li MQ, Chen YC, Qian XP, Lu TJ, Yu LZ, Zhang Y, Xin DQ, Na YQ, Chen WF. Rapid identification of UCA1 as a very sensitive and specific unique marker for human bladder carcinoma. Clin Cancer Res 2006; 12: 4851-4858.
11) Fan Y, Shen B, Tan M, Mu X, Qin Y, Zhang F, Liu Y. Long non-coding RNA UCA1 increases chemoresistance of bladder cancer cells by regulating Wnt signaling. FEBS J 2014; 281: 1750-1758.
12) Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003; 423: 356-361.
13) McInnes IB, al-Mughales J, Field M, Leung BP, Huang FP, Dixon R, Sturrock RD, Wilkinson PC, Liew FY. The role of interleukin-15 in T-cell migration and activation in rheumatoid arthritis. Nat Med 1996; 2: 175-182.
14) Noss EH, Brenner MB. The role and therapeutic implications of fibroblast-like synoviocytes in in-

flammation and cartilage erosion in rheumatoid arthritis. Immunol Rev 2008; 223: 252-270.

15) Ji J, Tang J, Deng L, Xie Y, Jiang R, Li G, Sun B. LINC00152 promotes proliferation in hepatocellular carcinoma by targeting EpCAM via the mTOR signaling pathway. Oncotarget 2015; 6: 42813-42824.

16) Tolboom TC, van der Helm-Van Mil AH, Nelissen RG, Breedveld FC, Toes RE, Huizinga TW. Invasiveness of fibroblast-like synoviocytes is an individual patient characteristic associated with the rate of joint destruction in patients with rheumatoid arthritis. Arthritis Rheum 2005; 52: 1999-2002.

17) 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.