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

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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. 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. Long non-coding RNAs (lncRNAs) are a novel class of molecules, with a length longer than 200 nucleotides (nt). 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. reported that MALAT1 expression was significantly up-regulated 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 trials 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. In recent years, accumulating evidence has shown that UCA1 played a part in the development of many diseases. Therefore, we wanted to know whether
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% CO₂ 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 Prime-Script™ 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 10⁴ 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
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. 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
UCA1 affects 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. FLS are major mesenchymal cells of the synovial joints that are regarded to play a key role in the development of RA. 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 IncRNAs played important roles in diverse biological processes including development, cell growth and tumorigenesis. For examples, IncRNA 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. Furthermore, evidence indicated that IncRNAs 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, we can see that the viability of FLS was detected by MTT assays. **p < 0.001.**
Cell viability is an equilibrium of cell proliferation and apoptosis. Since FLS does not have the ability to grow like tumors, we mainly 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 closely related with the viability of FLS. Cell viability was an equilibrium of cell proliferation and apoptosis. Since FLS does not have the ability to grow like tumors, we mainly

**Figure 3.** Cells apoptosis affected the cells viability. (A-C) The expression of caspase-3 was detected by 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.
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We found that UCA1 was closely related to the apoptotic mechanisms of RA and could be used as a promising prognostic marker. UCA1 could decrease the expression of Wnt6 to down-regulate the viability of RAFLS.

Conclusions

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

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Conflict of Interest

The Authors declare that they have no conflict of interest.

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