European Review for Medical and Pharmacological Sciences 2019; 23: 9729-9737

LncRNA ANRIL impacts the progress of osteoarthritis *via* regulating proliferation and apoptosis of osteoarthritis synoviocytes

X. LI, T.-L. HUANG, G.-D. ZHANG, J.-T. JIANG, P.-Y. GUO

Orthopedics, First Affiliated Hospital of Kunming Medical University, Kunming, China

Xi Li and *Tianlong Huang* co-first authors

Abstract. – OBJECTIVE: Long noncoding RNAs (lncRNAs) have been indicated to play an important role in many different diseases. Osteoarthritis (OA) is a disease which causes a change of morphology and function in articular cartilage and synovium, leading to cartilage degradation. Synovitis is a common pathological feature of OA, owing to the proliferation of synoviocytes. In this research, we want to verify the role of lncRNA ANRIL in osteoarthritis.

PATIENTS AND METHODS: qRT-PCR was used to detect the expression of lncRNA ANRIL in normal synoviocytes and osteoarthritis synoviocytes. The cell proliferation in normal synoviocytes and osteoarthritis synoviocytes after transfection with lncRNA-NC or lncRNA-ANRIL were tested. The apoptosis rate and cell cycle in normal synoviocytes and osteoarthritis synoviocytes were detected by the Flow Cytometry analysis. Western blot was used to analyze the possible mechanism that ANRIL regulated the cells' proliferation in osteoarthritis.

RESULTS: We indicated that the expression of ANRIL was significantly improved in OAS compared to NS. The expression of ANRIL was decreased and the cell proliferation was reduced in OAS after transfected with siRNA. And the cell cycle was suspended in G0/G1 phase and the cell apoptosis was improved in OAS after transfected with siRNA. Moreover, ANRIL could regulate the proliferation and apoptosis of OAS *via* miR-122-5p/DUSP4 axis.

CONCLUSIONS: We suggest that lncRNA ANRIL was closely related to osteoarthritis. ANRIL may be involved in the development and progression of osteoarthritis and become a potential target for diagnosis and treatment in OA.

Key Words:
LncRNA ANRIL, Osteoarthritis, Synovitis.

Introduction

Osteoarthritis (OA) is a common prevalent chronic joint disease, leading to degradation of articular cartilage and disability of structure and function[1,2]. Accumulating evidence has paid attention to chondrocyte survival, which is closely associated with OA patients, but the effect of treatment was not ideal. Emerging pieces of reports have indicated metalloprotease activity and synovial inflammation play an important role during OA pathogenesis[3]. Synovitis is a common pathological feature of OA, accompanied by the proliferation of osteoarthritis synoviocytes (OAS), which secret interleukin-1β, interleukin-6 and tumor necrosis factor α, resulting in the destruction of bone and cartilage[4]. Controlling and recovering the function of the synovial membrane has become a novel hotspot of treatment for OA.

Long noncoding RNAs (lncRNAs) are a class of molecules without protein-coding potential, which are more than 200 nucleotides (nt) in length. LncRNAs play important roles in various biological processes, such as cell differentiation and apoptosis, tumorigenesis and metastasis[5-8]. Alterations in lncRNA can result in abnormal expression of genes involved in biological function and disease[9]. A large variety of studies have showed that lncRNAs were associate with OA[10,11]. Zhang et al[12] demonstrated that MALAT1 contributed to OA progression by promoting cell proliferation and cartilage formation, inhibiting cell apoptosis and ECM degradation. Zhang et al[13] suggested that UFC1 facilitates proliferation and inhibits apoptosis in a miR-34a-dependent manner in OA chondrocytes. Kang et al[14] indicated that overexpression of PCGEM1 in human synoviocytes suppressed miR-770 by direct binding and suggested that PCGEM1 may act as a sponge for miR-770. LncRNA antisense noncoding RNA in the INK4 locus (ANRIL) is identified as a shared genetic susceptibility region related to several human diseases including cancers, which had been con-

Corresponding Author: Peiyu Guo, MD; e-mail: ails1344@163.com

firmed to predict a poor prognosis in patients with hepatocellular carcinoma (HCC)[15]. Salmena et al[16] firstly presented a unifying hypothesis that the long noncoding RNAs "talk" to each other using miRNA response elements (MREs) as letters of a new language, which was called "competing endogenous RNA " (ceRNA). This process plays important roles in pathological conditions and Tay et al[17] also suggested that these RNA transcripts act as competing endogenous RNAs (ceRNAs) or natural microRNA sponges - they communicate with and co-regulate each other by competing for binding to shared microRNAs that are important post-transcriptional regulators of gene expression. However, the underlying mechanisms of lncRNA ANRIL involved in OA remain to be elucidated at present. Accumulated reports demonstrated that lncRNA plays an important role in regulating gene expression by acting as miRNA sponges. Ma et al[15] indicated that ANRIL promotes carcinogenesis by acting as a miR-122-5p sponge in HCC.

In the present study, we aimed to explore the function of ANRIL and its underlying mechanisms in OA. At first, we detected the expression of ANRIL in the normal synoviocyte and osteoarthritis synoviocytes. Then, the effect of ANRIL on the proliferation and apoptosis of two cell lines was also tested. Finally, we explored the probable mechanism of ANRIL for impacting OA patients.

Patients and Methods

Patients and Samples

The OA cartilage tissues were obtained from the knee joints of 28 patients undergoing total knee replacement surgery from June 2015 to June 2017. OA was diagnosed by the American College of Rheumatology criteria. The normal articular cartilage was obtained from the knee joints of 23 patients with traumatic emergency amputation without OA or rheumatic arthritis at the same time. All 51 tissue samples taken into this research were provided by patients who signed informed consent. The work was approved by the Human Ethics Committee of Hospital of Kunming Medical University, China.

Cell Culture and Treatment

Normal synoviocytes (NS) and osteoarthritis synoviocytes (OAS) were purchased from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). After centrifugation, the primary chondrocytes were isolated from articular cartilage samples, which were digested at 37°C with 0.2% collagenase II (Invitrogen, Carlsbad, CA, USA) in Dulbecco's modified Eagle's medium (DMEM) for 4-6 h with stirring every 25 minutes after 1 h. All the cells were maintained under the recommended conditions that cultured with the complete culture medium and incubated at 37°C in a humidified atmosphere of 5% CO_2 for 6-7 days before used.

RNA Extraction and Real-Time Quantitative PCR Assays

Total RNAs were severally isolated from cells using a TRIzol kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The concentration of RNA was detected, and the RNA solution was stored at -80°C for further use. Then cDNA was obtained by reverse transcription using the TaKaRa Reverse Transcriptase kit (TaKaRa, Otsu, Shiga, Japan). The expression level of ANRIL in synoviocytes and chondrocytes was evaluated and quantified using Real-time qPCR with SYBR Premix Ex Taq (TaKaRa, Otsu, Shiga, Japan). β-actin was used as an endogenous control. The following primer pairs were used for the qPCR: for ANRIL, 5′-TTGTGAAGCCCAAGT ACTGC-3′(forward), 5′-TTCACTGTGGAGAC-GTTGGT-3′(reverse); for miR-122-5p,5′-GT-GACAATGG-TGGAATGTGG-3′ (forward), 5′-AAAGCAAACGATGCCAAGAC-3′ (reverse); for DUSP4, 5'-TGGTTCATGGAAGCCATA-GAG-3'(forward), 5'-CCCGTTTCTT-CAT-CATCAGG-3' (reverse); for U6, 5'- GCTTC-GGCAGCACATATACTAAAAT-3'(forward), 5'-CGCTTCACGAATTTGCGTGTCAT-3' (reverse); for β-actin, 5'-GACCTCTATGCCAACA-CAGT-3' (sense) and 5'-AGTACTTGCGCTCAG-GAGG-A-3' (antisense). All experiments were repeated three times at least.

Cell Proliferation Assay

Cell proliferation was detected by the CCK-8 assay. The cells were added in 96-well plates at a density of 10^3 per well with 200 ul cell suspension. Then we inoculated 10 μL CCK-8 solution (Dojindo Laboratories, Kumamoto, Japan) to each well and the plate was kept for 2 hours at 37°C in a 5% CO_2 incubator. They were tested in absorbance at 450 nm. All experiments were repeated 3 times.

Cell Cycle Analysis and Apoptosis Analysis

Cells were seeded into six-well plates with a concentration of 3×10^5 cells/well. Then, we collected cells with low-speed centrifugation (1200 rpm, 5 min) at 4°C and cell pellets were re-suspended in 1 ml of PBS solution. Cells were lysed, centrifuged and re-suspended in propidium iodide (PI, Sigma-Aldrich, St. Louis, MO, USA) staining buffer with 50 μl/ml of PI and 250 μl/ml of RNase A before the analysis of flow cytometry (FCM). At last, the cell mixture was evaluated for cell cycle and stained with 5 μL of annexin V-FITC and apoptosis was detected by fluorescence activated cell sorting (FACS) technique (Beckman Coulter, Brea, CA, USA) incubating for 30 minutes at 4°C and avoiding light. All experiments were repeated 3 times.

Luciferase Assays

To construct ANRIL-WT, ANRIL-MUT, DUSP4-WT and DUSP4-MUT luciferase reporters, 3′UTR of ANRIL-WT, ANRIL-MUT, DUSP4-WT and DUSP4-MUT were amplified and inserted into the pmirGLO vector (Invitrogen, Carlsbad, CA, USA). Wt-ANRIL/mut-ANRIL and wt-DUSP4/mut-DUSP4 sequences were amplified and cloned into the downstream of the stop codon of the firefly luciferase in basic vector (Promega, Madison, WI, USA). Total RNA was reverse transcribed into cDNA, extracting from OAS. The potential binding sites of pmiR-ANRIL-WT, pmiR-DUSP4-WT and mutant sequence pmiR-ANRIL-MT, pmiR-DUSP4-MT were synthesized into pmiR-GLO (Promega, Madison, WI, USA). Next, miR-122-5p mimics and miR-122-5p negative control (NC) were co-transfected into OAS with pmiR-GLO for 24 h. Then, we transfected Renilla expression vector into each group, serving as a normalized control. After 48 hours, firefly and Renilla luciferase activities were detected by Dual Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Plasmid Transfection

Cells were placed into 6-well plates at 60-80% confluency and seeded in a fresh culture medium without fetal bovine serum (FBS) 2 hours before transfection. Respectively, 1 μg of plasmid and 1 μl of lipofectamine 2000 were added into 250 μl of medium without fetal bovine serum (FBS) and incubated for 10-15 minutes. Diluted plasmid and lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) were mixed and incubated for 15-25 minutes. Plasmid was transfected into cells by Lipofectamine 2000 for 8 hours according to the manufacturer's protocol. At last, the plasmid-lipid complex was mixed to the cells.

Western Blot

All cells were seeded in the lysis buffer in the presence of Aprotinin, Leupeptin, Phenylmethanesulfonyl fluoride (PMSF, Sigma-Aldrich, St. Louis, MO, USA) and phosphatase inhibitor mix II and III (Sigma-Aldrich). Next, we used 10% resolving gel and a 5% stacking gel to immune-blot experiments. After that, prepared samples were loaded in the gel and electrophoresis was performed through the stacking gel at 60 V for 45 min and through the resolving gel at 110 V for 90 min. The membrane was dealt with bovine serum albumin (BSA, HyClone, South-Logan, UT, USA) for 90 minutes, incubated at 4°C in a primary antibody overnight and developed and imaged by a gel documentation system (Bio-Rad, Hercules, CA, USA). Finally, the secondary antibody was added to the samples for 1 hour and incubated at room temperature until the results were observed.

Statistical Analysis

All the data were expressed as mean±SD (standard deviation, SD) and all the statistical analysis was performed using the software Graphpad 6 (GraphPad, San Diego, CA, USA). Each assay was applied at least three independent experiments or replicates. Student's *t*-test, one-way analysis of variance (ANOVA) and multiple comparison between the groups were performed by SNK method. p-value<0.05 was considered statistically significant.

Results

ANRIL is Up-Regulated in OA Patients and Highly Expressed in the OAS

To determine the role of lncRNA ANRIL in OA, we firstly performed qRT-PCR to examine the expression of ANRIL in OA and normal cartilage tissues. The results showed that the expression of ANRIL was significantly upregulated in OA cartilage tissues compared with that of normal tissues (Figure 1A). To further identify which cell plays a key role in OA, we detected the expression of ANRIL in chondrocytes and synoviocytes. The data indicated that ANRIL was significantly increased in OAS compared with NS, while no differences

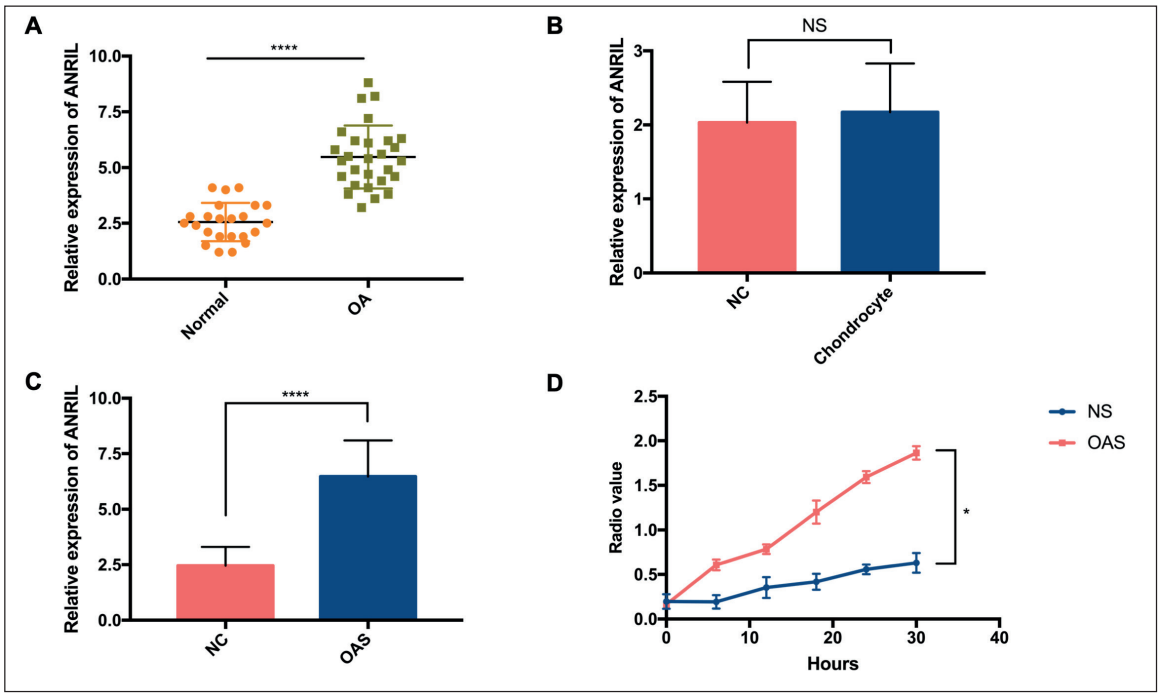

Figure 1. ANRIL is up-regulated in OA patients and highly expressed in the OAS. **A,-C,** The expression of ANRIL in the cartilage tissues, chondrocytes and synoviocytes was detected by qRT-PCR assay. ****$p<0.0001$; **D,** The proliferation of NS and OAS was detected by CCK-8 assays. *$p<0.05$.

had been discovered in chondrocytes (Figure 1B-C). Next, we tested the proliferation of cell and we found that OAS had a much greater proliferation rate compared with NS (Figure 1D). From these results, we suggested that ANRIL might play an important role in osteoarthritis and OAS could lead the process of disease.

Regulating the Expression of ANRIL Affects the Proliferation of Synoviocytes

To explore the functions of ANRIL in osteoarthritis synoviocytes, ANRIL was up-regulated in NS and down-regulated the expression of ANRIL in OAS (Figure 2A-B). Then we found that overexpressed ANRIL in NS improved the proliferation of cells and silencing ANRIL expression with siRNA reduced the proliferation in OAS (Figure 2C-D). The reports demonstrated that altered expression of ANRIL could influence the proliferation in synoviocytes.

The Cell Cycle Was Suspended and the Apoptosis Was Enhanced in OAS After the Expression of ANRIL Was Down-Regulated

To further determine that ANRIL enhanced the process of proliferation in OAS, we detected the cell cycle distribution and apoptosis rate in synoviocytes after the expression of ANRIL was regulated by the Flow cytometry analysis. These reports demonstrated that when ANRIL was up-regulated, the percentage of cell was significantly reduced in G0/G1 phase. Thus, cell percentage was significantly improved in S phase and inhibited cell apoptosis in NS compared to control group (Figure 3A). Meanwhile, we examined the level of mRNA and protein-related with apoptosis to estimate the effect of ANRIL in synoviocytes. The data clarified that cleaved caspase-3 and Bax, which were the apoptosis biomarkers, were significantly suspended and the Bcl-2, the anti-apoptotic biomarker, was significantly increased in NS compared to control group (Figure 3C-D). Next, when ANRIL was down-regulated, the percentage of cell was significantly enhanced in G0/G1 phase and the percentage of cell was significantly reduced in S phase and improved cell apoptosis in OAS compared with control group (Figure 3B). Respectively, the cleaved caspase-3 and Bax were markedly increased and the Bcl-2 was significantly increased in OAS compared with control group (Figure 3E-F). These results indicated that ANRIL regulated the cell proliferation and apoptosis by altering cell cycle in OA.

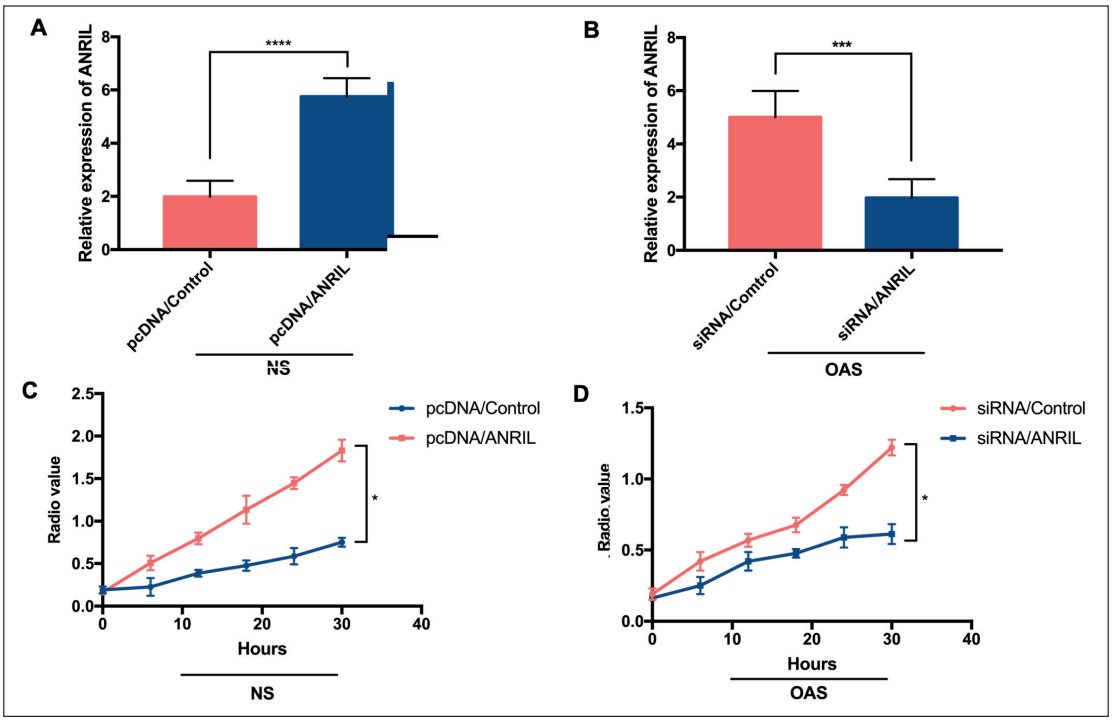

Figure 2. Regulating the expression of ANRIL affects the proliferation of synoviocytes. **A,-B,** Relative expression of ANRIL was detected by PCR. ****p<0.0001; ***p<0.001. **C,-D,** The proliferation of NS and OAS was detected by CCK-8 assays. *p<0.05.

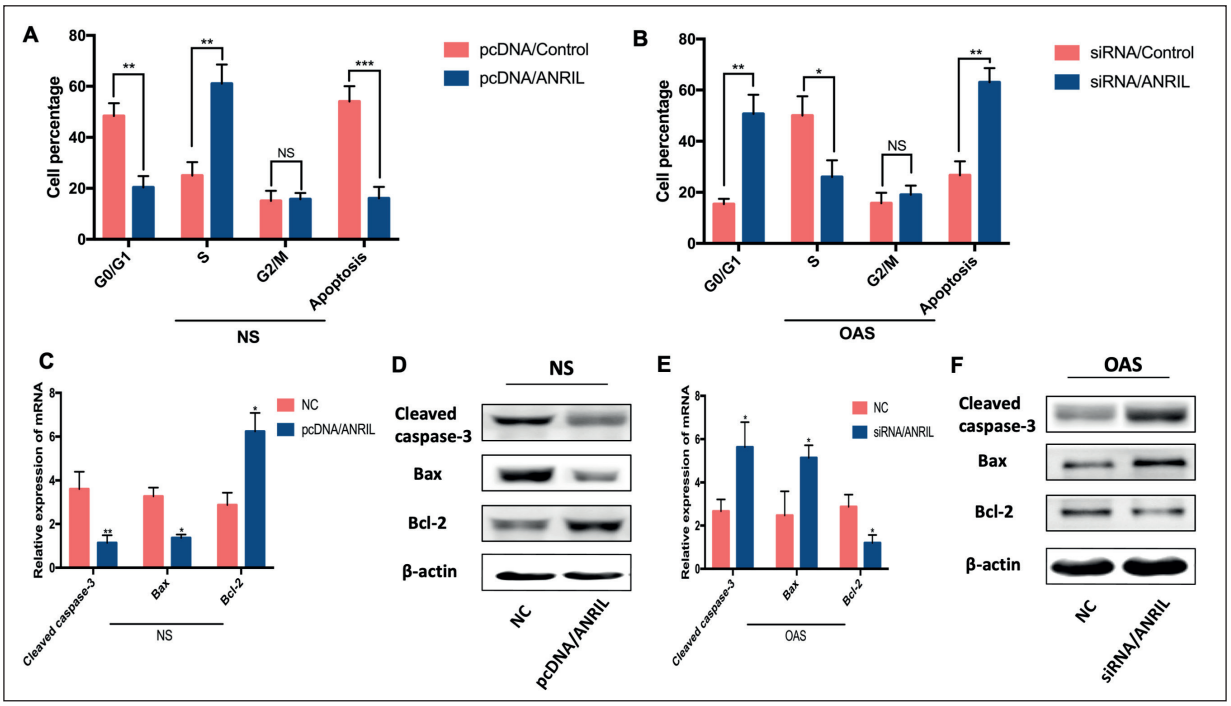

Figure 3. The cell cycle was suspended, and the apoptosis was enhanced in OAS after the expression of ANRIL was down-regulated. **A,-B,** The cell cycle was detected by flow cytometry assays in NS and OAS. ***p <0.001; **p<0.01. **C,-D,** After overexpressed ANRIL, the mRNA and protein levels of apoptotic and anti-apoptotic genes were detected by RT-PCR and WB. **p<0.01; *p<0.05. **E,-F,** After down-regulated ANRIL, the mRNA and protein levels of apoptotic and anti-apoptotic genes were detected by RT-PCR and WB. *p<0.05.

LncRNA ANRIL Could Directly Binds with miRNA-122-5p in OAS

The luciferase reporter assay was used to investigate whether ANRIL could bind with miR-122-5p. The potential binding sequence was synthesized into pmiR-GLO vector, which was pmiR-ANRIL-WT. We also synthesized the mutant binding sequence into pmiR-GLO vector, which was pmiR-ANRIL-MT (Figure 4A). After transfected miR-122-5p mimics and miR-122-5p NC into OAS for 24 h, we examined the relative luciferase activity. The results indicated that the luciferase activity of cells transfected with pmiR-ANRIL-WT was significantly reduced compared to pmiR-GLO vector. Meanwhile, the luciferase activity in pmiR-ANRIL-MT was improved, compared with transfected pmiR-ANRIL-WT (Figure 4B). To further explore the potential association of the biological functions between ANRIL and miR-122-5p, the serum samples of OA patients were exerted to research the relationship between the ANRIL and miR-122-5p. The reports suggested that the expression of miR-122-5p was negatively correlated with the lncRNA-ANRIL (Figure 4C). Further, we overexpressed ANRIL and found that significantly inhibited the expression of miR-122-5p. We suspended the expression of ANRIL and the expression of miR-122-5p was enhanced in OAS (Figure 4D). Moreover, we transfected miRNA-122-5p mimics into OAS, in which ANRIL was overexpressed. The result indicated that the viability of OAS was reduced by CCK-8 assay (Figure 4E).

LncRNA ANRIL Regulated the Expression of DUSP4 by Targeting miRNA-122-5p

In the previous reports, miR-122-5p inhibited cell migration and invasion in gastric cancer by down-regulating DUSP4[18]. However, the underlying mechanisms of miRNA-122-5p/DUSP4 axis in OA require further investigation. Subsequent dual-luciferase reporter assay indicated that transfection with miR-122-5p mimics significantly attenuated the luciferase activity of DUSP4-WT but not that of DUSP4-MUT reporter (Figure 5A-B). Moreover, we exerted the serum samples of OA patients to test the relationship between the miR-122-5p and DUSP4, ANRIL and

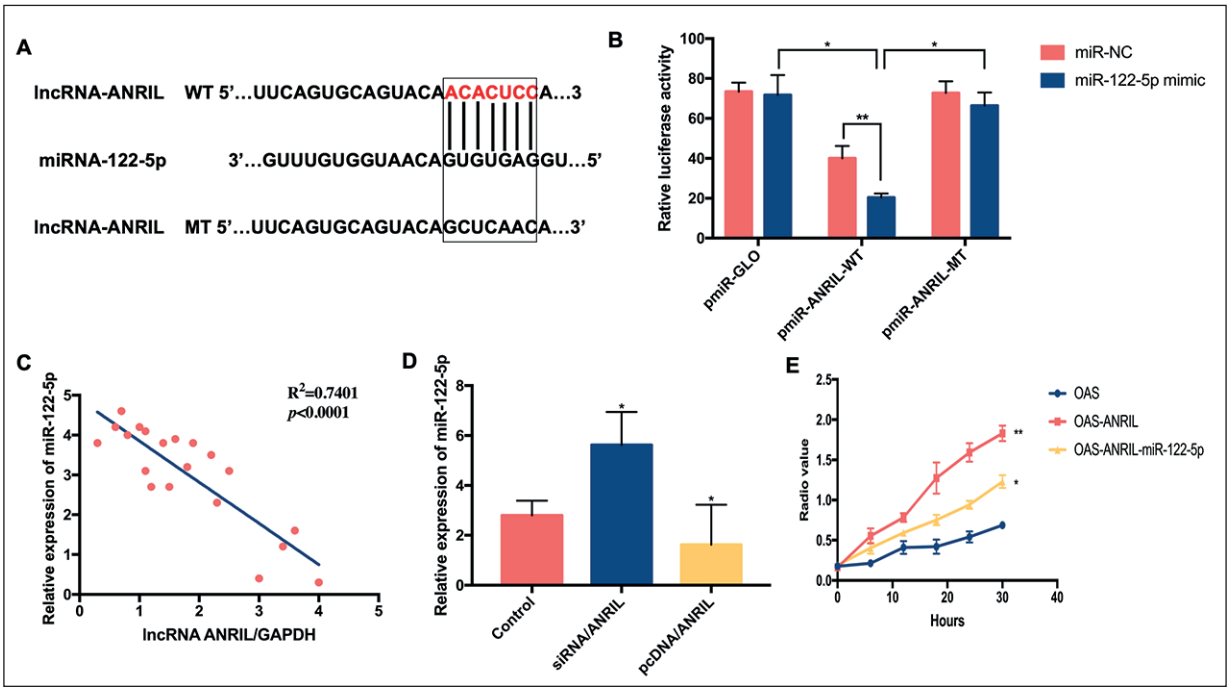

Figure 4. LncRNA ANRIL could directly binds with miRNA-122-5p in OAS. **A,** Bioinformatics analysis predicted binding sites between ANRIL and miRNA-122-5p. **B,** The luciferase reporter assay. Co-transfection with miR-122-5p and ANRIL WT significantly increased the luciferase activity of OAS cells compared with others. $^{**}p<0.01$, $^{*}p<0.05$. **C,** The LncRNA ANRIL expression level was negatively correlated with miRNA-122-5p expression in OAS. $R^2=0.7401$, $^{****}p<0.0001$. **D,** Up-regulation of LncRNA ANRIL significantly reduced the expression of miRNA-122-5p, while knocked-down of LncRNA ANRIL significantly increased the expression of miRNA-122-5p in OAS. $^{*}p<0.05$. **E,** The proliferation of NS and OAS was detected by CCK-8 assays. $^{**}p<0.01$, $^{*}p<0.05$.

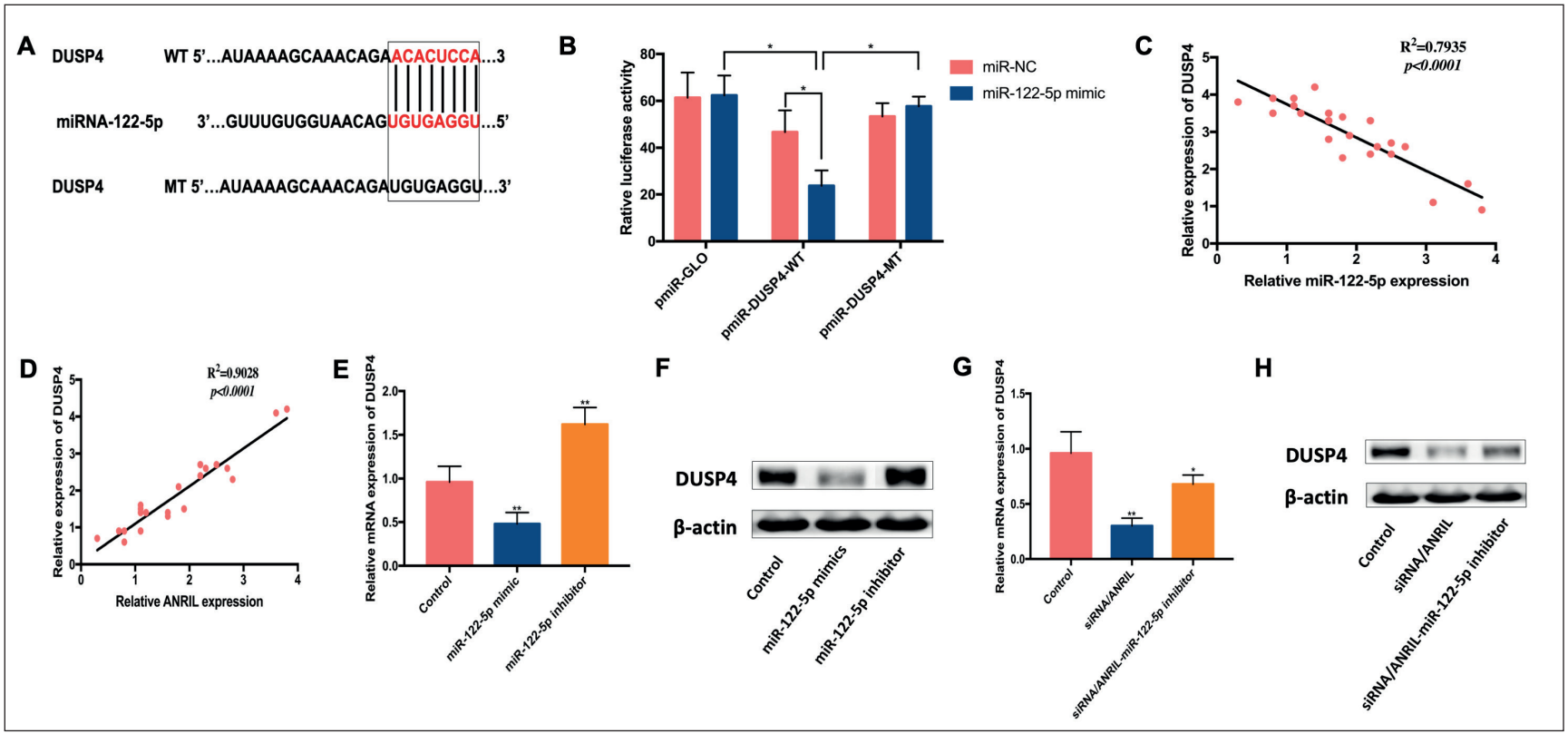

Figure 5. LncRNA ANRIL regulated the expression of DUSP4 by targeting miRNA-122-5p. **A,** Bioinformatics analysis predicted binding sites between miRNA-122-5p and DUSP4. **B,** The luciferase reporter assay. Co-transfection with miR-122-5p and DUSP4 WT significantly increased the luciferase activity of OAS cells compared with others. *p<0.05. **C,** The DUSP4 expression level was negatively correlated with miRNA-122-5p expression in OAS. R^2=0.7935, *** p<0.0001. **D,** The expression of ANRIL was negatively correlated with DUSP4 expression in OAS. R^2=0.9028, ****p<0.0001. **E,-F,** Adjusted miRNA-122-5p level significantly regulated the mRNA and protein expression of DUSP4 in OAS. **p <0.01. **G,-H**, Down-regulation of ANRIL significantly reduced the expression of DUSP4, while transfection of si-ANRIL reduced DUSP4 level, which was reversed by co-transfection of miRNA-122-5p inhibitor in OAS. **p<0.01, *p<0.05.

DUSP4, respectively. The Spearman's correlation analysis demonstrated a significantly negative correlation between miR-122-5p and DUSP4, and a significantly positive correlation between ANRIL and DUSP4 in OA, revealing that DUSP4 could be a direct target gene of miR-122-5p in OA (Figure 5C-D). In addition, RT-qPCR and Western blotting suggested that transfection of miR-122-5p mimics in OAS down-regulated DUSP4 level, which was reversed by co-transfection of miRNA-122-5p inhibitor. Further, the transfection of si-ANRIL in OAS reduced DUSP4 level, which was reversed by co-transfection of miRNA-122-5p inhibitor (Figure 5E-H). Above that, we demonstrated that lncRNA ANRIL could regulate the proliferation and apoptosis of synoviocytes *via* miR-122-5p/DUSP4 axis.

Discussion

The formation of osteoarthritis (OA) is affected by a series of risk factors, including aging, overweight and obese[19]. OA mainly influences patients older than 50 years, and its incidence is even higher in 65 years old patients[20]. Nowadays, OA has become a major public health problem[21]. Most works on OA have focused on a larger amount of treatments including drug treatment, stem cell treatment and so on[22-25]. However, OA is difficult to cure without a detail molecular mechanism. It is necessary to expound the deep mechanisms of OA to obtain more efficiency of treatment.

Recently, lncRNAs have been considered as a novel class of regulatory RNAs, involved in a wide range of biological and pathological processes. Previous researches also offered evidence that lncRNAs also implicated in pathological physiological activities of OA. Pearson et al[26] found that lncRNA-PACER has been characterized as a chondrocyte inflammation-associated lncRNA and chondrocyte inflammation contributes to OA. Shen et al[27] showed that lncRNA small nucleolar RNA host gene 5 (SNHG5) promotes the proliferation of chondrocytes *via* miR-26a/SOX2 signal axis in OA. However, the underlying mechanisms of OA are still lacking, making the treatment of OA very difficult. Although some researchers have explored the function of ANRIL in many diseases[28,29], its role in OA remains largely unclear. In our study, we wanted to know whether lncRNA ANRIL was related to osteoarthritis and the underlying mechanism. We investigated the expression of ANRIL in articular cartilage, chondrocytes and synoviocytes. The results determined that ANRIL was only overexpressed in synoviocytes except for chondrocytes. From changing the expression of ANRIL, we found that down-expressed or overexpressed, the lncRNA could regulate the proliferation and apoptosis of synoviocytes. To further discover the detail molecular mechanism, we verified the interaction between ANRIL and miR-122-5p by miRNA profiling from previous reports. In this research, we found that ANRIL and miR-122-5p were negatively correlated. Finally, we transfected with interfering plasmid of ANRIL into OAS and significantly up-regulated the expression of miR-122-5p, while overexpressed ANRIL significantly reduced the expression of miR-122-5p. Following these data, we further explored a possible interaction of ANRIL and miR-122-5p for regulating the proliferation of synoviocytes. When we co-transfected with miR-122-5p mimics, it significantly suppressed the proliferation of OAS, which was overexpressed ANRIL. Finally, we verified that ANRIL regulated the expression of DUSP4 by targeting miRNA-122-5p. Above that, the results indicated that ANRIL impacts the progression of OA *via* miR-122-5p/DUSP4 axis.

Conclusions

We firstly demonstrated that lncRNA ANRIL was highly expressed in OAS and had a close relationship with the proliferation of OAS. We also found that lncRNA ANRIL might adjust cell apoptosis to regulate the proliferation of OAS *via* miR-122-5p/DUSP4 axis. These results determined that lncRNA ANRIL might be a potential target of treatment for OA in the future.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Berenbaum F. Osteoarthritis is an inflammatory disease (osteoarthritis is not osteoarthrosis!). Osteoarthritis Cartilage 2013; 21: 16-21.

2) Sacitharan PK, Vincent TL. Cellular ageing mechanisms in osteoarthritis. Mamm Genome 2016; 27: 421-429.

3) Sun S, Bayjensen AC, Karsdal MA, Siebuhr AS, Zheng Q, Maksymowych WP, Christiansen TG, Henriksen K.

The active form of MMP-3 is a marker of synovial inflammation and cartilage turnover in inflammatory joint diseases. BMC Musculoskelet Disord 2014; 15: 93.

4) Lambert C, Dubuc J E, Montell E, Vergés J, Munaut C, Noël A, Henrotin Y. Gene expression pattern of cells from inflamed and normal areas of osteoarthritis synovial membrane. Arthritis Rheumatol 2014; 66: 960-968.
5) Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet 2016; 17: 47-62.
6) Chen X, Yan CC, Zhang X, You ZH. Long non-coding RNAs and complex diseases: from experimental results to computational models. Brief Bioinform 2017; 18: 558-576.
7) Li J, Meng H, Bai Y, Wang K. Regulation of lncRNA and its role in cancer metastasis. Oncol Res 2016; 23: 205-217.
8) Zhu JJ, Fu HJ, Wu YG, Zheng XF. Function of lncRNAs and approaches to lncRNA-protein interactions. Sci China Life Sci 2013; 56: 876-885.
9) Orly W, Howard YC. Long noncoding RNAs and human disease. Trends Cell Biol 2011; 21: 354-361.
10) Wang X, Hunter D, Xu J, Ding C. Metabolic triggered inflammation in osteoarthritis. Osteoarthritis Cartilage 2015; 23: 22-30.
11) Zhang C, Wang P, Jiang P, Lv Y, Dong C, Dai X, Tan L, Wang Z. Upregulation of lncRNA HOTAIR contributes to IL-1β-induced MMP overexpression and chondrocytes apoptosis in temporomandibular joint osteoarthritis. Gene 2016; 586: 248-253.
12) Zhang Y, Wang FY, Chen G, He R, Yang L. LncRNA MALAT1 promotes osteoarthritis by modulating miR-150-5p/AKT3 axis. Cell Biosci 2019; 9: 54.
13) Zhang G, Wu Y, Xu D, Yan XF. Long noncoding RNA UFC1 promotes proliferation of chondrocyte in osteoarthritis by acting as a sponge for miR-34a. DNA Cell Biol 2016; 35: 691-695.
14) Kang Y, Song J, Kim D, Ahn C, Park S, Chun CH, Jin EJ. PCGEM1 stimulates proliferation of osteoarthritic synoviocytes by acting as a sponge for miR-770. J Orthop Res 2016; 34: 412-418.
15) Ma J, Li T, Han X, Yuan HF. Knockdown of LncRNA ANRIL suppresses cell proliferation, metastasis, and invasion via regulating miR-122-5p expression in hepatocellular carcinoma. J Cancer Res Clin Oncol 2018; 144: 205-214.
16) Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta stone of a hidden RNA language? Cell 2011; 146: 353-358.
17) Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. Nature 2014; 505: 344-352.
18) Xu X, Gao F, Wang J, Tao L, Ye J, Ding L, Ji W, Chen X. MiR-122-5p inhibits cell migration and invasion in gastric cancer by down-regulating DUSP4. Cancer Biol Ther 2018; 19: 427-435.
19) Silverwood V, Blagojevic-Bucknall M, Jinks C, Jordan JL, Protheroe J, Jordan KP. Current evidence on risk factors for knee osteoarthritis in older adults: a systematic review and meta-analysis. Osteoarthritis Cartilage 2015; 23: 507-515.
20) Blaney Davidson EN, van Caam AP, van der Karaam PM. Osteoarthritis year in review 2016: biology. Osteoarthritis Cartilage 2017; 25: 175-180.
21) Jinks C, Jordan K, Croft P. Osteoarthritis as a public health problem: the impact of developing knee pain on physical function in adults living in the community: (KNEST 3). Rheumatology (Oxford) 2007; 46: 877-881.
22) Majeed MH, Sherazi SAA, Bacon D, Bajwa ZH. Pharmacological treatment of pain in osteoarthritis: a descriptive review. Curr Rheumatol Rep 2018; 20: 88.
23) Zhang S, Guan X. Latest development of external treatment with traditional Chinese medicine for knee osteoarthritis. Chin J Osteoporosis 2016; 22: 907-911.
24) Richmond J, Hunter D, Irrgang J, Jones MH, Snyder-Mackler L, Van Durme D, Rubin C, Matzkin EG, Marx RG, Levy BA, Watters WC 3rd, Goldberg MJ, Keith M, Haralson RH 3rd, Turkelson CM, Wies JL, Anderson S, Boyer K, Sluka P, St Andre J, McGowan R; American Academy of Orthopaedic Surgeons. American Academy of Orthopaedic Surgeons clinical practice guideline on the treatment of osteoarthritis (OA) of the knee. J Bone Joint Surg 2010; 92: 990-993.
25) Brosseau L, Wells G, Marchand S, Gaboury I, Stokes B, Morin M, Casimiro L, Yonge K, Tugwell P. Randomized controlled trial on low level laser therapy (LLLT) in the treatment of osteoarthritis (OA) of the hand. Lasers Surg Med 2005; 36: 210-219.
26) Pearson MJ, Philp AM, Heward JA, Roux BT, Walsh DA, Davis ET, Lindsay MA, Jones SW. Long intergenic noncoding RNAs mediate the human chondrocyte inflammatory response and are differentially expressed in osteoarthritis cartilage. Arthritis Rheumatol 2016; 68: 845-856.
27) Shen H, Wang Y, Shi W, Sun G, Hong L, Zhang Y. LncRNA SNHG5/miR-26a/ SOX2 signal axis enhances proliferation of chondrocyte in osteoarthritis. Acta Biochim Biophys Sin (China) 2018; 50: 191-198.
28) Zhang Z, Wang J, Li J, Wang X, Song W. MicroRNA-150 promotes cell proliferation, migration, and invasion of cervical cancer through targeting PDCD4. Biomed Pharmacother 2018; 97: 511-517.
29) Feng J, Yang Y, Zhang P, Wang F, Ma Y, Qin H, Wang Y. miR-150 functions as a tumour suppressor in human colorectal cancer by targeting c-Myb. J Cell Mol Med 2014; 18: 2125-2134.