

Expression of long non-coding RNA NONHSAT227927.1 and its effect on the JAK2/STAT3 signaling pathway and inflammation in patients with ankylosing spondylitis

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Abstract. Long non-coding RNAs (lncRNAs) assume pivotal roles in various autoimmune diseases including ankylosing spondylitis (AS). Inflammation affects the progression of multiple diseases. The current research aimed at dissecting out the possible role and mechanism of lncRNAs NONHSAT227927.1 in the pathogenesis of AS. In clinical trials, 50 patients with AS and 30 healthy persons were enrolled, followed by the extraction of peripheral blood mononuclear cells (PBMCs). NONHSAT227927.1 expression was detected using reverse transcription quantitative PCR. Enzyme-linked immunosorbent assay was used to determine the levels of inflammatory cytokines. Human fibroblast-like synoviocytes (FLSs) were induced by PBMC supernatant, after which the activity of FLSs was measured by Cell Counting Kit-8 and the signaling pathway were detected by western blotting. Cell migratory capacity was assessed by Transwell migration assay. NONHSAT227927.1 expression was obviously enhanced in the PBMCs of AS patients. NONHSAT227927.1 expression was positively correlated with immunoglobulin A (IgA), erythrocyte sedimentation rate (ESR), complement C3 (C3), visual analog scale, IL-17, and IL-23 Levels but was negatively correlated with IL-10 level. The results of association rules showed that the increase of NONHSAT227927.1 expression was strongly associated with the elevation of IgA, C3, ESR, self-rating anxiety scale and IL-17 levels. Overexpression of NONHSAT227927.1 remarkably augmented the proliferation and migration of AS-PBMCs stimulated by AS-FLSs,

facilitated the levels of IL-17 and IL-23, and reduced the IL-10 level. By contrast, NONHSAT227927.1 knockdown decreased cell proliferation and migration and cell viability as well as the levels of IL-17 and IL-23, but increased the level of IL-10. overexpression of NONHSAT227927.1 promoted the phosphorylation of JAK2 and STAT3 proteins, while knockout of NONHSAT227927.1 decreased their phosphorylation. Conclusively, lncRNA NONHSAT227927.1 was overexpressed in AS, which activated the JAK2/STAT3 signaling pathway to upregulate inflammatory factors.

Introduction

As an inflammatory immune disease, ankylosing spondylitis (AS) is slow in taking effect that progresses over time, with the global prevalence rate ranging from 0.1-1.4% (1). AS mainly afflicts the sacroiliac joints and lumbar vertebrae, leading to fibrosis, spinal fusion, calcification and hence loss of flexibility (2). Spinal fusion due to chronic inflammation gradually restricts spinal activity to result in the partial loss of function, which causes the loss of normal working ability and substantially debases the life quality of sufferers with AS (3,4). Previous studies have reported the alteration of cytokines in AS. Proinflammatory cytokines and tumor necrosis factor- α (TNF- α) are involved in osteoclast stimulation. However, the exact mechanism underlying AS remains enigmatic. It is generally accepted that AS is related to heredity, immunity and infection. It has been documented that the abnormal change of cell factors is a significant characteristic of the development of AS (5,6). Among all interleukins (ILs) secreted by T helper type 17 (Th17) cells, IL-23 and IL-17 have the strongest response to Th17 cells (7,8) and IL-10 are an important anti-inflammatory factor. The Janus kinase 2/signal transducers and activators of transcription 3 (JAK2/STAT3) signaling pathway is one of the critical pathways in inflammation and is implicated in the etiology and pathogenesis of AS. The JAK2/STAT3 signaling pathway induces the expression of TNF- α and other proinflammatory cytokines (9,10). JAK1/2/3, tyrosine kinase 2 (TYK2), and STAT are the main factors for the signal transduction of proinflammatory and anti-inflammatory cytokines. The JAK2/TYK2-STAT3 signaling pathway has been identified

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to participate in IL-23-mediated Th17 cell regulation (11,12). Therefore, the JAK2/STAT3 signaling pathway can be regarded as the major promising target of AS treatment (13).

The synovium is an important structure connecting bone and joint capsule, which is prone to inflammatory erosion and hyperplasia in inflammatory diseases (14). Fibroblast-like synoviocytes (FLSs) decisively participate in the pathophysiology of synovitis in numerous joint diseases including AS. FLSs can secrete a variety of inflammatory cytokines, including tumor necrosis factor- α (TNF- α) (15) and interleukin-6 (IL-6) (16), to trigger inflammatory signaling cascades and exacerbate inflammatory responses (16,17), thus resulting in persistent local inflammation and joint destruction (18). Peripheral blood mononuclear cells (PBMCs) mainly include lymphocytes, monocytes, phagocytes, dendritic cells and a small number of other cell types. PBMCs are commonly used to simulate the blood immune environment *in vitro*. Numerous studies have indicated the critical role of monocytes and their secreted cytokines in the pathogenesis of AS (19). In the present study, AS-PBMCs were co-cultured with AS-FLSs. As a result, inflammatory factors in AS-PBMCs can promote the inflammation and proliferation of FLSs and respond in an improved way to the internal environment of AS patients (20).

Long non-coding RNAs (lncRNAs) act as novel regulators of gene expression to orchestrate biological processes including chondrocyte proliferation and death, as well as inflammation in PBMCs (12). LncRNAs have attracted increasing attention in the field of AS. Previously (21), lncRNA NONHSAT227927.1 was identified by the authors as a key lncRNA involved in the immune-inflammation of AS through high-throughput sequencing and bioinformatics analysis. Despite intensive studies on lncRNAs, their functional and pathological significance remains largely unknown. In the present study, NONHSAT227927.1 was selected as the object of the study to detect its expression in PBMCs of AS patients and its relationship with immune-inflammation and self-rating depression and anxiety levels of patients. In order to explore the possible mechanism, AS-FLSs were further utilized to assess the impact of altered NONHSAT227927.1 on the inflammatory response.

Materials and methods

Subjects. AS patients and sex- and age-matched healthy controls (HCs) were recruited from the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine between March 2021 and May 2021. Patients who did not meet the 1984 American College of Rheumatology diagnostic criteria (22) with severe mental illness, significantly impaired liver or renal function, pregnancy, or immunosuppressants, were excluded. All participants provided written informed consents. The present study was approved (approval no. 2015-AH20) by the Medical Ethics Committee of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine (Hefei, China).

Culture of FLSs. Human primary FLSs isolated from sacroiliac joints (cat. no. RAB-iCell-s004) and AS-FLSs (cat. no. JDBG200752) were purchased from Subikang iCell Bioscience Inc. (Shanghai, China) and cultured in RPMI-1640

medium [Saibaikang (Shanghai) Biotechnology Co., Ltd.] containing 1% penicillin-streptomycin and 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) in a 37°C incubator with 5% CO₂ and 100% humidity (11). The medium was renewed once every 2-3 days. When the cells were cultured to 80-90% confluence, they were washed twice with phosphate buffer saline, digested with 0.25% trypsin, followed by the observation of the digestion under a x200 light microscope. The trypsin was discarded when the cells adhered to the wall and became loose. Following the addition of the complete medium, the cell layer was blown.

Culture of PBMCs. The venous blood (4 ml) of AS patients was collected using an anticoagulant tube and mixed well with 4 ml of PBS. Then, 4 ml Ficoll solution (cat. no. 17-1440-02; GE Healthcare) was added into a 15-ml centrifuge tube. The diluted blood was added gently and slowly to the upper layer of Ficoll solution to avoid mixing the two solutions. The tube was placed in a horizontal centrifuge for centrifugation at 1,150 x g at 37°C for 20 min. After successful centrifugation, the PBMCs were located in the second white layer from top to bottom. All the cells were moved to a new centrifuge tube, added with 10-15 ml of PBS, and placed in a centrifuge for centrifugation at 640 x g at 37°C for 10 min. After removing the supernatant, 5-10 ml of PBS was added to repeat the same procedures. The cells were resuspended by adding 1 ml of PBS, transferred to a 1.5-ml EP tube, and set aside.

AS-PBMC induction and AS-FLS transfection. AS-PBMCs and AS-FLSs were seeded and cultured in a Transwell chamber at a ratio of 3:1. PBMCs were added into the apical chamber and FLSs were placed in the basolateral chamber. The chamber was placed in the incubator for 24 h. Upon the cell confluence reaching 70-90%, cells in each Transwell well were taken out. Following the operating instructions, According to the manufacturer's instructions, pcDNA3.1-negative control (NC)(C11022), pcDNA3.1-NONHSAT227927.1 (C05008), small interfering RNA (siRNA)-NC (A06001), and siRNA-NONHSAT227927.1 (A01001) (both Shanghai Jima Pharmaceutical Technology Co., Ltd.) were transfected into AS-FLSs using Lipofectamine® 2000 (cat. no. 11668-019; Thermo Fisher Scientific, Inc.) and cultured at 37°C for 24 h. The transfection concentration of siRNA was 50 pmol/ml. According to the manufacturer's instructions, >5 μ g nucleic acid was used, and then the cells were collected after incubation at 37°C for 48 h. RT-qPCR was then used to observe cell transfection efficiency. The oligonucleotide sequences were as follows: siRNA-NC forward, 5'-UUCUCCGAACGUGUC ACGUTT-3' and reverse, 5'-ACGUGACACGUUCGGAGA ATT-3'; and siRNA-NONHSAT227927.1 forward, 5'-CGA CUGACUCGAUCUUUGAAG-3' and reverse, 5'-UCAAG AUCGAGUCAGUCGGG-3'.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from AS-FLSs with TRIzol® (cat. no. 15596026; Thermo Fisher Scientific, Inc.), Reverse transcription of lncRNA was performed using Novostart SYBR qPCR SuperMix Plus (cat. no. E096-01B; Novoprotein Scientific, Inc.) according to the manufacturer's instructions. PrimeScript™ RT Reagent Kit with gDNA

Eraser kit (cat. no. RR047A; Takara Biotechnology Co., Ltd.) was used according to the manufacturer's instructions for qPCR (reaction conditions: 95°C pre-denaturation for 1 min, 95°C denaturation for 20 sec and 60°C annealing for 1 min, for 40 cycles). Relative quantitative analysis was performed using the $2^{-\Delta\Delta C_q}$ method (23) with β -actin as an internal reference. The sequences of primers used were as follows: NONHSAT227927.1 forward, 5'-TGGGAAGCTCCTGAGCATACC-3' and reverse, 5'-ATGCTCCAGCAAGTCAGGAT-3'; and β -actin forward, 5'-CCCTGGAGAAGAGCTACGAG-3' and reverse, 5'-GGAAGGAAGGCTGGAAGAGT-3'.

Enzyme-linked immunosorbent assay (ELISA). The levels of IL-17 (JYM0082Hu), IL-23 (JYM0077Hu) and IL-10 (JYM0155Hu) in the serum of patients or the supernatant of FLSs were evaluated through ELISA according to the manufacturer's instructions (Wuhan Genomics Technology Co., Ltd.). Each sample was examined three times.

Cell counting Kit-8 (CCK-8) assay. Cell viability was measured using a CCK-8 assay kit (BIOSS) following the manufacturer's protocols. At first, 3×10^4 AS-FLSs were seeded into 96-well plates and cultured until 70 and 90% confluence. Logarithmically growing cells were transfected with the aforementioned method with three wells in each group. Cells were cultured for 0, 12, 24, 48 and 72 h, respectively. Then, 10 μ l CCK-8 solution was supplemented into each well at each experimental site, followed by 1-4 h of cell culture at 37°C. Cell viability was assessed by measuring the optical density at 450 nm.

Western blot analysis. A total of 600 μ l RIPA lysate (cat. no. P0013B; Beyotime Institute of Biotechnology) was used to extract the total protein in the cell. SDS-PAGE gel preparation kit (cat. no. S8010; Beijing Solarbio Science & Technology Co., Ltd.) was used to prepare the gel (5% stacking gel, 10% separating gel concentration), and then 30 μ g protein per lane was added for electrophoresis on PVDF membranes. The membranes were blocked with 5% skimmed dry milk for 2 h at room temperature, and then incubated in anti-phosphorylated (p)-STAT3 (1:500; cat. no. ab76315; Abcam), p-JAK2 (1:1,000; cat. no. ab32101; Abcam), JAK2 (1:500; cat. no. ab39636; Abcam) and STAT3 (1:1,000; cat. no. ab68153; Abcam) overnight with slow shaking at 4°C. After washing, goat anti-mouse (cat. no. ZB-2305; IgG; OriGene Technologies, Inc.) and goat anti-rabbit (cat. no. ZB-2301; IgG; OriGene Technologies, Inc.) secondary antibodies (labeled with horseradish peroxidase) were added at a dilution of 1:10⁴, and the membrane was re-probed at room temperature for 2 h. After washing, proteins were visualized using an ECL luminescence kit (cat. no. 34094; Thermo Fisher Scientific, Inc.). Image capture was then performed. The relative expression of target proteins was calculated as the ratio of absorbance of GAPDH. ImageJ software (ImageJ 180; National Institutes of Health) was used for band density analysis.

Transwell migration assays. All cells were incubated overnight in serum-free DMEM, the cell concentration was adjusted to 2×10^5 /ml, 100 μ l of cell suspension was added to

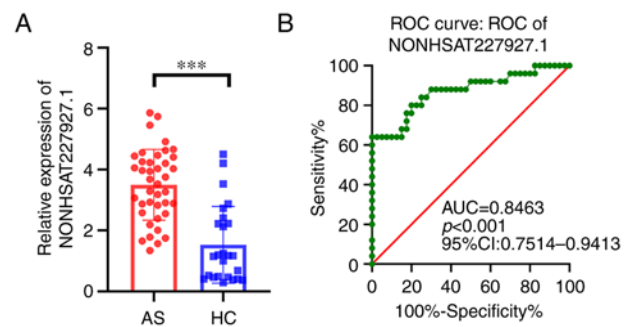


Figure 1. NONHSAT227927.1 expression is high in the PBMCs of AS patients. (A) NONHSAT227927.1 expression in AS patients was significantly higher than that in HCs. (B) ROC curve analysis of NONHSAT227927.1 in PBMCs from AS patients and HCs. ***P<0.001. PBMCs, peripheral blood mononuclear cells; AS, ankylosing spondylitis; HCs, healthy controls; ROC, receiver operating characteristic; CI, confidence interval.

the Transwell chamber, The lower cavity of the Transwell contained complete fibroblast medium (containing 10% FBS and 1% double antibody). The upper Transwell chamber was 6.5 mm in diameter, with an 8.0- μ m pore size PC membrane, while the lower Transwell chamber was 6.5 mm in diameter, with a 0.4- μ m pore size PC membrane. The culture was continued for 24 h. Cells were fixed with 4% paraformaldehyde for 30 min at room temperature followed by staining with 0.5% crystal violet for 15-30 min. Cells were rinsed several times with clean water, the chamber was dried at room temperature, images were captured from randomly selected fields of view and counted under a x200 light microscope.

Statistical analysis. GraphPad Prism (version 8.2; Dotmatics) was utilized for statistical analysis and image rendering. The differences between groups were analyzed using the paired Student's t-test or the Kruskal-Wallis non-parametric test, followed by a Steel-Dwass post-hoc test. Explanatory data were compared using the χ^2 test. Correlation analysis was conducted using the Pearson's correlation coefficient method. Data are presented as the mean \pm standard deviation. P<0.05 was considered to indicate a statistically significant difference.

Results

NONHSAT227927.1 expression is upregulated in the PBMCs of AS patients. To investigate NONHSAT227927.1 expression in AS, RT-qPCR was performed in the PBMCs from 50 AS patients and 30 HCs. The results demonstrated that under different environments, NONHSAT227927.1 was upregulated in AS (Fig. 1A). Subsequently, the receiver operating characteristic (ROC) curve analysis was implemented to evaluate NONHSAT227927.1 diagnostic utility, and the area under the ROC curve (AUC) was 0.8463 [95% confidence interval (CI): 0.7514-0.9413; P<0.001] (Fig. 1B), suggesting that NONHSAT227927.1 had significant diagnostic utility.

Changes of clinical immune-inflammatory index and perception scale of patients with AS. Compared with normal subjects, there were substantial increases of C-reactive

Table I. Clinical immune-inflammatory markers and perception score of patients with AS and NC. Data were expressed as the n or mean \pm standard deviation.

Characteristics	NC (n=30)	AS (n=50)	P-value
Sex			0.952
Male	19	32	
Female	11	18	
Age, years		56.50 (47.00-65.50)	<0.01
Course of disease, years		9.30 (4.54)	-
CRP, mg/dl	7.13 (5.90)	24.28 (12.34-55.56)	<0.01
ESR, mm/h	6.5 (2.00-30.00)	39.90 (23.37)	<0.01
IgA, mmol/l	1.33 (0.63-4.41)	1.65 (1.32-4.54)	<0.01
IgG, mmol/l	8.99 (7.97-10.78)	10.63 (9.00-12.08)	<0.01
IgM, mmol/l	1.05 (0.70-1.58)	1.31 (0.93-1.84)	0.02
C3, g/l	1.01 (1.00-1.37)	1.27 (1.13-1.40)	0.245
C4, g/l	0.33 (0.25-0.37)	0.35 (0.25-0.40)	0.636
SAS	23.54 (15.32-51.56)	48.75 (47.50-52.50)	<0.01
SDS	23.54 (15.04-46.44)	56.55 (23.63)	<0.01
VAS	5.81 (4.49-6.84)	6.80 (5.82-7.28)	<0.01

AS, ankylosing spondylitis; NC, normal controls; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; C3, complement C3; C4, complement C4; SAS, self-rating anxiety scale; SDS, self-rating depression scale; VAS, visual analog scale.

Table II. Association rules analysis of laboratory indexes of NONHSAT227927.1 in patients with ankylosing spondylitis.

Items (LHS \Rightarrow RHS)	Confidence (%)	Support (%)
\uparrow (NONHSAT227927.1 \uparrow) \Rightarrow (CRP \uparrow)	81.08	60
\uparrow (NONHSAT227927.1 \uparrow) \Rightarrow (VAS \uparrow)	78.38	58
\uparrow (NONHSAT227927.1 \uparrow) \Rightarrow (ESR \uparrow)	70.27	52
\uparrow (NONHSAT227927.1 \uparrow) \Rightarrow (C3 \uparrow)	62.16	46
\uparrow (NONHSAT227927.1 \uparrow) \Rightarrow (IL-17 \uparrow)	56.76	46

CRP, C-reactive protein; VAS, visual analog scale; ESR, erythrocyte sedimentation rate; C3, complement C3.

protein (CRP), erythrocyte sedimentation rate (ESR), immunoglobulin A (IgA), immunoglobulin G, immunoglobulin M, self-rating anxiety scale (SAS) scores, self-rating depression scale scores in AS patients, and visual analog scale (VAS). ($P < 0.05$; Table I).

Correlations between NONHSAT227927.1 and clinical indicators in AS patients. The results of correlation analysis manifested the positive correlation of NONHSAT227927.1 expression with the levels of IgA, C3, ESR, SAS and IL-23 ($P < 0.05$) and its negative correlation with IL-10 expression ($P < 0.05$) in AS patients (Fig. 2A-F).

Association rules analysis between laboratory indexes and NONHSAT227927.1 in AS patients. Association rules analysis displayed that the upregulation of NONHSAT227927.1 in patients with AS was strongly correlated with the elevation in the levels of CRP, VAS, ESR, C3 and IL-17, and

the support was $>55\%$ and the confidence level was $>23\%$ (Table II).

Expression of inflammatory cytokines in patients with AS. ELISA analysis was performed to detect the level of inflammation in patients with AS. The levels of IL-23 and IL-17 in AS patients were significantly increased ($P < 0.01$) compared with those in the HC, while IL-10 level was significantly reduced ($P < 0.01$) (Fig. 3A-C).

Expression of NONHSAT227927.1 in AS-FLSs and its effect on cell viability. In order to ascertain whether NONHSAT227927.1 was successfully transfected and expressed in AS-FLSs, NONHSAT227927.1 mRNA expression was examined by RT-qPCR (Fig. 4A). CCK-8 (Fig. 4B) and migration experiments (Fig. 4C) were performed to evaluate the effect of NONHSAT227927.1 on the viability and migratory abilities of AS-FLS cells, respectively. NONHSAT227927.1 silencing

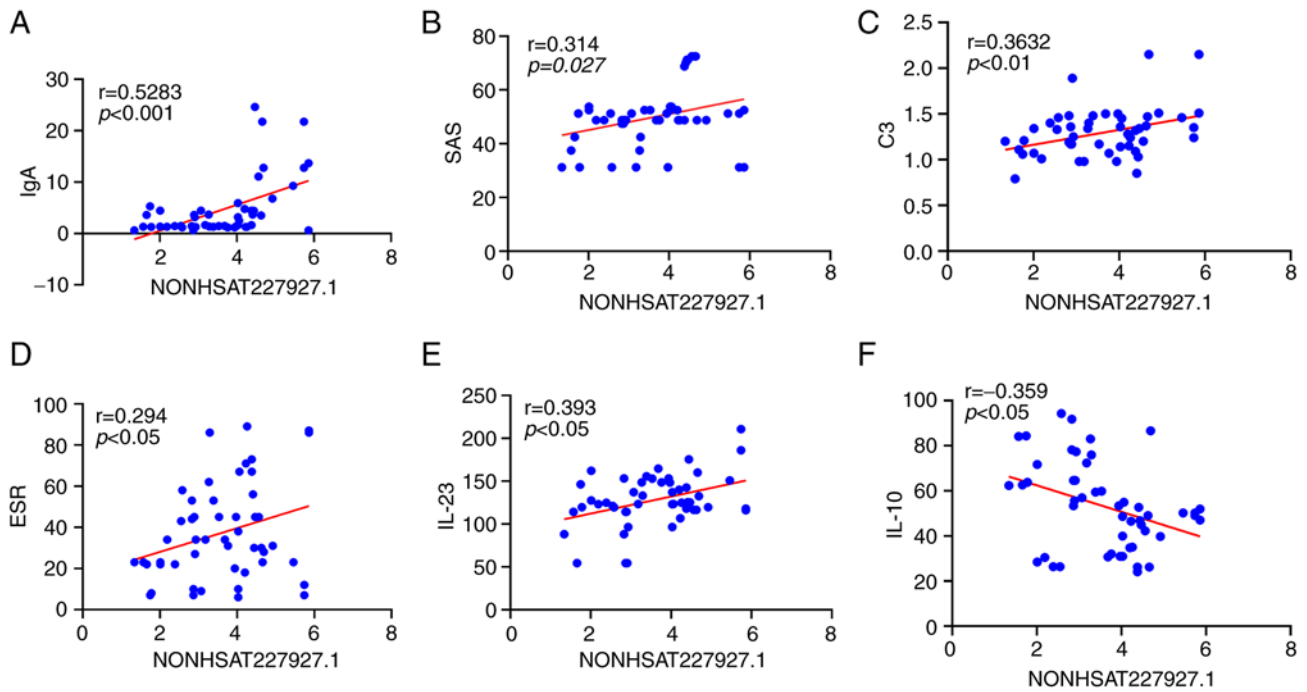


Figure 2. Correlation analysis of NONHSAT227927.1 with the clinical indicators in AS patients. (A-F) Correlation analysis of NONHSAT227927.1 expression in PBMCs of AS patients with (A) IgA, (B) SAS, (C) C3, (D) ESR, (E) IL-23 and (F) IL-10 levels was conducted. AS, ankylosing spondylitis; IgA, immunoglobulin A; SAS, self-rating anxiety scale; C3, complement C3; ESR, erythrocyte sedimentation rate.

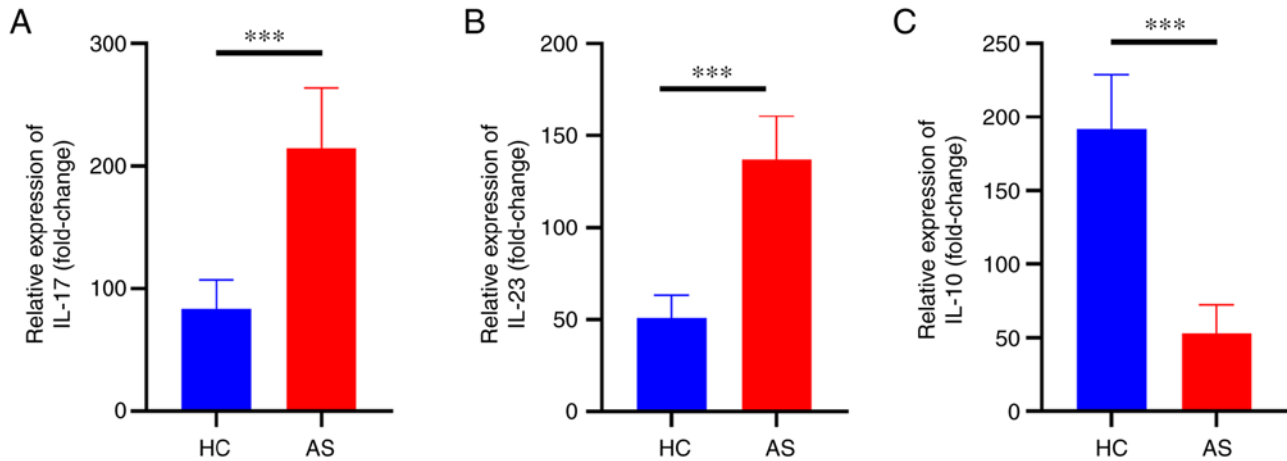


Figure 3. Levels of inflammatory cytokines in AS patients and normal controls. (A) IL-17 (B), IL-23, and (C) IL-10 in serum of AS patients and normal controls were determined. *** $P < 0.01$. AS, ankylosing spondylitis; HC, healthy control; si-, small interfering.

significantly reduced AS-FLS migration and cell viability (Fig. 4).

The effects of NONHSAT227927.1 on inflammatory cytokines in AS-FLS. The expression of inflammatory cytokines was measured by ELISA, which exhibited that NONHSAT227927.1 overexpression augmented the expression of IL-17 and IL-23 but diminished IL-10 expression, which was contrary after silencing NONHSAT227927.1 (Fig. 5).

The impacts of NONHSAT227927.1 on the expression of JAK2, STAT3, P-JAK2, and P-STAT3 in AS-PBMC-induced AS-FLSs. In order to identify whether the JAK2/STAT3 signaling pathway is involved in NONHSAT227927.1-manipulated cell

inflammation in AS, western blot analysis was performed. The results demonstrated that the overexpression of NONHSAT227927.1 elevated the phosphorylation of JAK2 and STAT proteins in AS-FLSs (Fig. 6).

Discussion

AS facilitates bone deterioration and aberrant bone density due to inflammation and the pathogenesis of the disease (24). Mechanical, inflammatory and metabolic factors are non-negligible (25). Inflammatory cells including macrophages and lymphocytes penetrate the joints, which contributes to fibrosis and synovial thickening, thus the joints and bones stiffening (26,27). RANKL signaling leads to

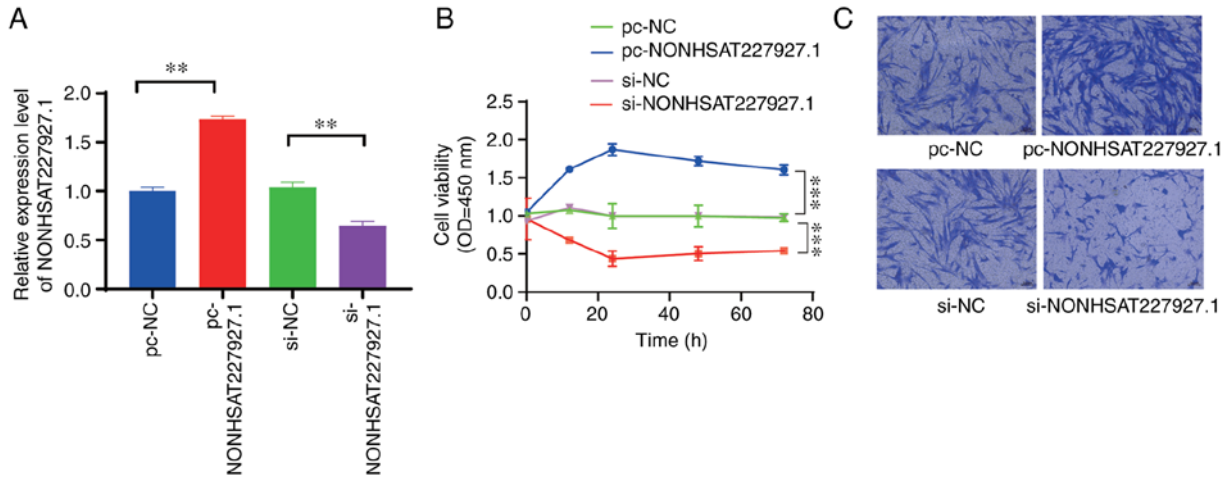


Figure 4. Effects of NONHSAT227927.1 on the proliferation of AS-FLSs. (A) Reverse transcription-quantitative PCR was performed to detect the transfection efficiency of NONHSAT227927. (B) Cell Counting Kit-8 was used to detect the effect of NONHSAT227927 on AS-FLS viability. (C) Transwell migration assay was conducted to examine the effect of NONHSAT227927 on the migratory capacity of AS-FLS. All experiments were repeated three times and data were expressed as the mean \pm standard deviation. ** $P < 0.01$ and *** $P < 0.001$. AS, ankylosing spondylitis; FLSs, fibroblast-like synoviocytes; NC, negative control; si-, small interfering.

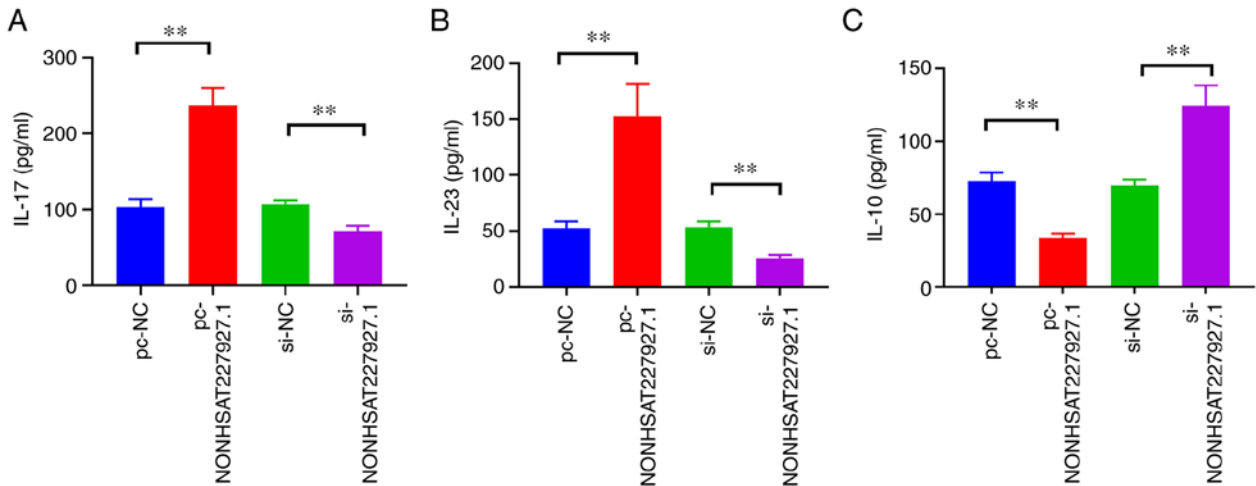


Figure 5. Effect of NONHSAT227927.1 on the expression of IL-17 and IL-23 induced by AS-PBMCs in AS-FLSs. (A-C) The contents of (A) IL-17, (B) IL-23 and (C) IL-10 were assessed in AS-FLSs by ELISA. All experiments were repeated three times and data were expressed as the mean \pm standard deviation. ** $P < 0.01$. AS, ankylosing spondylitis; PBMCs, peripheral blood mononuclear cells; FLSs, fibroblast-like synoviocytes.

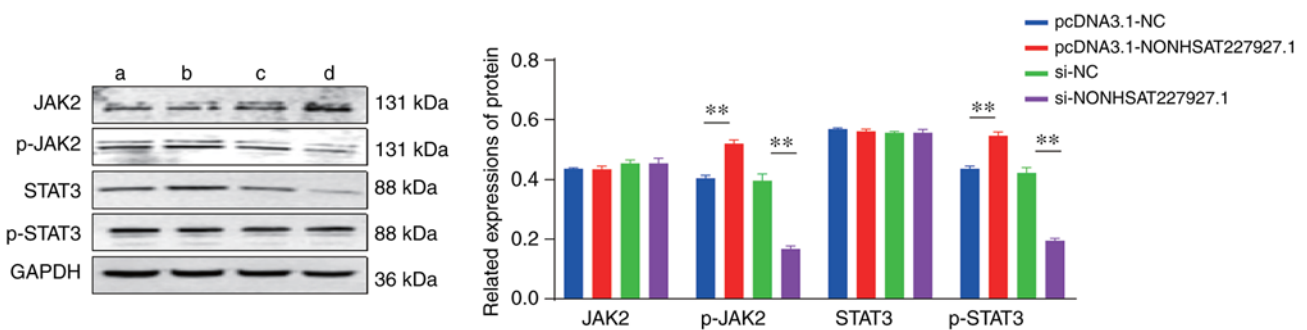


Figure 6. The influences of NONHSAT227927 on the JAK2/STAT3 signaling pathway in AS-FLSs. The effects of NONHSAT227927 on the expression of JAK2, STAT3, p-JAK2, and p-STAT3 were evaluated by western blot analysis. All experiments were repeated three times, and the data were expressed as mean \pm standard deviation. ** $P < 0.01$. AS, ankylosing spondylitis; FLSs, fibroblast-like synoviocytes; p-, phosphorylated.

disease progression by promoting osteoclast formation (28,29). Genetic factors have an obvious causal relationship, and it is

crucial to dissect out the underlying mechanism and the way leading to disease from a genetic perspective (30). To expand

the number of the research records, lncRNAs participate in the orchestration of inflammatory reactions and cell processes and are aberrantly expressed in a range of immune-mediated diseases (31). However, the meaningful understanding of the function of lncRNAs can only be realized through relevant in-depth researches (32).

Initially, the current research provided evidence of obvious high expression of NONHSAT227927.1 in PBMCs, PBMC-induced AS-FLSs, and AS patients, and ROC curve analysis elucidated that the AUC value was 0.8463, illustrating that NONHSAT227927.1 had great diagnostic utility. There is an imbalance between inflammatory cytokines in the serum of patients with AS. IL-17 and IL-23 increased prominently, and IL-10 decreased noticeably. In addition, clinical trials unraveled that NONHSAT227927.1 expression shared a positive correlation with IL-23 expression and had an inverse correlation with IL-10 expression. In order to further ascertain the influences of NONHSAT227927.1 on AS-FLS viability and the JAK2/STAT3 signaling pathway, AS-PBMCs were utilized to stimulate AS-FLSs as a model to implement *in vitro* cell experiments. CCK-8 was applied to assess the impacts of NONHSAT227927.1 on the function of AS-FLSs. The results showed that after transfection of NONHSAT227927.1 overexpression plasmid, the activity and migration ability of AS-FLS were enhanced. By contrast, AS-FLS viability and migration ability were reduced after NONHSAT227927.1 silencing. Under the condition of the overexpression or silencing of NONHSAT227927.1, the inflammatory cytokines were further measured using ELISA. The results manifested that the overexpression of NONHSAT227927.1 conspicuously increased the levels of IL-17 and IL-23 and decreased IL-10 level. On the contrary, the silencing of NONHSAT227927.1 exhibited the opposite trends. These results suggested that NONHSAT227927.1 may be the key lncRNA in the progress of AS.

However, there are also a large number of studies elaborating the potential relevance and mechanism of lncRNAs at the level of AS-FLSs. lncRNAs are mainly involved in the inflammatory reaction and osteogenic differentiation of AS through the sponge microRNA (33). They primarily participate in the manipulation of gene expression after gene transcription and post-transcription, and also are implicated in the progression of AS. Li *et al* (34) found that MEG3 was downregulated in patients with AS, and Wang *et al* (21) observed that lncRNA H19 was also related to the pathogenesis of inflammatory diseases. The data obtained by Gong *et al* (35) documented that lncRNAs (326C3.7 and 122K13.12) elevated considerably in AS patients with osseous bridges. Inflammatory factors afflict the severity and potential course of AS (36). A previous study has unveiled that in patients with AS at the active stage, the high level of inflammation (ESR and CRP) is positively correlated with the percentage of regulatory T cells (Tregs) in PMBCs (37). Another research demonstrated that young AS patients had a reduced Vd Tregs ratio in their PBMCs, which suppressed naive CD4 T cell proliferation and the secretion of produced interferon-g through lowering IL-10 level (38). The pathophysiology of AS has been proven to involve an aberrant inflammatory cytokine pathway (39). Stem cell-based bone tissue regeneration has shown promising therapeutic potential

in AS (40). Besides, it has been reported that JAK is the major sensor in Th17 cells (41,42). Th17 cells assume critical roles in AS, which secrete a variety of inflammatory mediators, including IL-17 to 22, IL-6, IL-26, interferon, Gamma and TNF- α (43). Additionally, the shaft of activating IL-23 and IL-17 cytokines has been pointed out (44). Melis *et al* (45) demonstrated that AS patients possessed increased serum IL-23 level. The combination of the JAK2/STAT3 signaling pathway and IL-23 has been documented to stimulate IL-23R (13). The inhibition of the JAK2/STAT3 signaling pathway is hypothesized to be facilitated by IL-23. The JAK/STAT signaling pathway plays a crucial role in growth factor and cytokine-activated signal transduction (46).

In summary, the present data provided the notion that NONHSAT227927.1 expression was high in AS, and that its abnormal expression could modulate the viability of AS-FLSs and mediate inflammatory response through the JAK2/STAT3 signaling pathway, thus influencing the occurrence and development of AS. Therefore, NONHSAT227927.1 may function as a biomarker for AS and provide new insights into the pathogenesis of AS.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XD and JL were both involved in the research design. XD participated in the data analysis, authored the first draft and made revisions to the paper. YQS participated in the collection and analysis of experimental data. JL and YQS supervised the project and contributed to the manuscript revision. JL and YQS confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved (approval no. 2015-AH20) by the Medical Ethics Committee of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine (Hefei, China). All procedures in the present study were conducted in accordance to the approved protocols of the Medical Ethics Committee of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine.

Patient consent for publication

Written informed consent was obtained from the patients for their anonymized information to be published in this article.

Competing interests

The authors declare that they have no competing interests.

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