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Original Article

Clinical significance and mechanism of LncRNA GAS-5 in osteoarthritis

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Abstract: Objective: To investigate the clinical significance and mechanism of LncRNA GAS-5 in osteoarthritis. Methods: 67 patients with knee osteoarthritis (the case group) and 60 patients who underwent physical examination (the healthy group) were selected to evaluate the expression level of lncRNA GAS-5 in peripheral blood mononuclear cells. Cell experiments were conducted that took THP-1 cells carrying NC-shRNA (negative lentivirus) as the control group, and THP1 cells carrying GAS5-shRNA (lentivirus infection) as the study group; we evaluated the expression of lncRNA GAS-5 gene, and the expression of immune-related cytokines. Results: (1) The expression of lncRNA GAS-5 in the case group was lower than in the healthy group ($P<0.05$). (2) The expression level of lncRNA GAS-5 in the case group versus control group, had an inhibition rate of 65.49% ($P<0.05$). The expression levels of 18 cytokines such as IL-1, IL-2, IL-6, IL-7, IL-17, G-CSF, M-CSF, and TGF-β1, in the study group were higher than in the control group ($P<0.05$), but the expression of IL-10 and IL-13 were significantly lower than in the control group ($P<0.05$). Conclusion: The expression of lncRNA GAS-5 is low in osteoarthritis patients. While the expression of lncRNA GAS-5 is inhibited, related immune and inflammatory factors are also affected, so lncRNA GAS-5 may affect the occurrence and progression of osteoarthritis through immune regulation. A low level of lncRNA GAS-5 may be a marker for the occurrence and progression of osteoarthritis.

Keywords: Osteoarthritis, LncRNA GAS-5, clinical significance, mechanism

Introduction

Osteoarthritis (OA) is a joint condition in which the initiation may be attributed to the fibrosis of joint cartilage, leading to rhagadia, ulcer, or even loss in the knees, spine and the medulla, ankle joints, hands and other weight-bearing joints [1-3]. The OA pathogenesis has not been completely clarified, but the involvement of cartilage cytokines in bone metabolism is an important cause of OA. Under the action of a variety of cytokines in the joint, the immune response system is further activated, which aggravates lesions of articular cartilage and injury. More articular cartilage tissues and cells are presented in the immune response system, forming a vicious circle. OA is formed and continuously exacerbated [4-6]. Growth arrest-specific transcript-5 (GAS-5) is a common lncRNA encoded by the GAS5 with the same name. It is involved in and regulates both the biologic behaviors of tumor cells, including proliferation, invasion, metastasis and apoptosis, and various pathogenic processes such as myocardial fibrosis and cerebral ischemia. Moreover, it can participate in the immune response and related occurrence and progression by regulating immune cells [7-10]. However, there are few reports on whether lncRNA GAS-5 plays a regulatory role in the occurrence of osteoarthritis. In the current study, we investigated the clinical significance of lncRNA GAS-5 in osteoarthritis and the regulation of related immune factors, and preliminarily explored its clinical significance and mechanism in osteoarthritis.

Materials and methods

Subjects of study

A total of 67 patients with knee osteoarthritis admitted to our hospital from January 2019 to January 2020 were selected as the case group, including 30 males and 37 females aged 41-76

Table 1. Comparison of general data from patients in the two groups

Data	Case group (n = 67)	Healthy group (n = 60)	χ^2/t	*P*
Gender				
Male	30	26	0.027	0.870
Female	37	34		
Age (years)	58.39±9.74	59.05±8.93	0.396	0.693
BMI (kg/m^2)	24.39±2.87	24.83±2.06	0.982	0.328

years, with an average of (58.39±9.74) year. Inclusion criteria: Being diagnosed with osteoarthritis, signing an informed consent form; Exclusion criteria: Complicated by heart, brain, liver, kidney and other important organ serious disorders, or rheumatoid arthritis and other autoimmune disorders, inflammatory disorders, and hematologic disorders. 60 patients who underwent physical examination in our hospital at the same time were selected as the healthy group, including 26 males and 34 females aged 40-77 years, with an average of (59.05±8.93) years. Inclusion criteria: Having healthy results by physical examination, signing an informed consent form. Exclusion criteria: Complicated by heart, brain, liver, kidney and other important organ serious disorders, or rheumatoid arthritis and other autoimmune disorders, inflammatory disorders, and hematologic disorders. This study has been approved by the Ethics Committee of our hospital. There were no significant differences in gender, age, or body mass index (BMI) of patients between the two groups (P>0.05) (**Table 1**).

Materials

Experimental cells: The investigational THP-1 human monocytic leukemia cell lines were purchased from the Cell Bank of Chinese Academy of Sciences.

Main reagents and instruments: RPMI-1640 culture medium, fetal bovine serum, and β-mercaptoethanol were purchased from Gibco; human cytokine antibody array was purchased from Wayen Biotechnologies (Shanghai), Inc.; human peripheral blood lymphocyte isolation solution was purchased from Solarbio; the design and synthesis of lncRNAGAS5shRNA and the packaging of lentivirus (EGFR) were entrusted to Cyagen; Trizol reagent, primers, fluorescent quantitative polymerase chain reaction (PCR) kit and PrimeScript reverse transcription kit were purchased from Thermo Fisher. The fluorescent quantitative PCR instrument was purchased from Thermo Fisher; dual-energy X-ray absorptiometry was purchased from Perkin Elmer; microarray scanner was purchased from NatureGene Corp; and nucleic acid detector was purchased from Eppendorf.

Methods

Isolation of peripheral blood mononuclear cells and extraction of RNAs: The isolation and extraction of peripheral blood mononuclear cells from human peripheral blood lymphocyte isolation solution were conducted. Total RNA was extracted using the Trizol method, and the purity of the RNA solution was determined through reading the absorbance values using a spectrophotometer (at 260 nm and 280 nm). The RNA integrity was detected by 1% formaldehyde denaturing agarose gel electrophoresis.

Detection of lncRNA GAS-5: 1 μg of each sample was collected for reverse transcription of total RNAs, and the primer sequences are detailed in **Table 2**. The reaction system of fluorogenic quantitative PCR was as follows: 12.5 μL of SYBR PremixExTaq™ GC, 0.5 μL of each upstream and downstream primer, 9.5 μL of dH_2O, 2 μL of cDNA, and 25 μL of system. The reaction conditions were as follows: pre-denaturation at 95°C for 10 s, denaturation at 95°C for 5 s, annealing and extension at 60°C for 31 s, 40 cycles in total.

The expression of lncRNA GAS-5 was detected and analyzed by PCR instrument. Formula: Relative expression level of lncRNA GAS-5 = $2^{-\Delta\Delta Ct}$, where ΔΔCt value = target gene Ct value - GAPDH Ct value. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used as an internal reference, and each group was performed in triplicate and averaged.

Culture of THP-1 cells and infection with lentivirus: THP-1 cells (logarithmic growth phase) were collected and inoculated into 96-well plates. THP-1 cells carrying NC-shRNA (negative lentivirus) were used as the control group, and THP1 cells carrying GAS5-shRNA (lentivirus infection) were used as the study group.

Table 2. Primer sequences

Item	Gene	
	GAS5	GAPDH
Primer sequences	5'-CTTGCCTGGACCAGCTTAAT-3'	5'-GAGAGTGGCGCCTCCGCCAT-3'
	5'-CAGCCTCAAGATCATCAGCA-3'	5'-GAGAGTGGCGCCTCCGCTCAT-3'
Primer length (bp)	122	106

Table 3. Expression of lncRNA GAS-5 in the two groups

Group	Expression level of lncRNA GAS-5	t	*P*
Case group (n = 67)	0.22±0.03	7.459	<0.001
Healthy group (n = 60)	0.29±0.07		

The GAS5-shRNA interference sequence was ACTTGCCTGGACCAGCTTAAT. The multiplicity of infection in the two groups was 100 moi. After 24 h, the cells were transfected in normal culture medium for another 72 h. If the infection rate was estimated to be more than 80% by fluorescence microscopy, the cells were collected for real-time fluorescent quantitative PCR assay to assess the effect of gene silencing.

Detection of cytokine antibody array: Total cellular protein was extracted using Raybiotech lysate, and protein quantification was performed by the bicinchoninic acid (BCA) assay. A total of 50 μg of cellular protein from each sample was collected for microarray-based detection. Blocking was done with 100 μL of blocking solution at room temperature for 30 min. Later, sample was added and then placed on a horizontal shaker overnight at 70 r/min at 4°C. We added 150 μL of washing solution I, gently shaked and washed 3 times at room temperature (2 min/time). We put the microarray into the washing box, added sufficient washing solution I followed by placing on a horizontal shaker at 70 r/min, then washed at room temperature (2 times). Subsequently, we added biomarker antibodies and incubated for 2 h at room temperature. This was washed and added to the blocking solution. Later, we added the diluted Streptavin-Fluor, and incubated for 2 h at room temperature, protected from light. The microarray was washed again andscanned by the scanner (532 nm).

Statistical analysis

Data were processed using SPSS 23.0 statistical software and figures were drawn by GraphPad prism 8.0. Qualitative data were expressed as cases (rates) and analyzed by the x^2 test; quantitative data were expressed as mean ± standard deviation, and t-test was used for comparison between the two groups. *P*<0.05 suggested a significant difference.

Results

Expression levels of lncRNA GAS-5 in the case group and healthy group

The expression of lncRNA GAS-5 in the case group and the healthy group were (0.22±0.03) and (0.29±0.07), significantly lower in the case group compared with the healthy group (t = 7.459, P<0.05) (**Table 3**).

Effect of lncRNA GAS5 on bone metabolism-related cytokine levels

It was shown by fluorescence microscopy that the positive rate of fluorescent protein was >80% in the two groups. In the present study, the human cytokine antibody array contained 40 bone metabolism-related cytokines, and the cytokines with different expression were screened by fold change (FC) based on following conditions: ① FC>1.5; ② *P*<0.05. The expression levels of 18 cytokines including IL-1, IL-2, IL-6, IL-7, IL-17, G-CSF, M-CSF, and TGF-β1, in the study group were higher than that in the control group (P<0.05), but the expression levels of IL-10 and IL-13 were lower than that in the control group (P<0.05) (**Table 4**).

Discussion

Long non-coding RNA (lncRNA) is an RNA molecule that is longer than 200 nucleotides and is not translated into a protein [11]. With the intensive study of lncRNA, lncRNA has been shown to be involved in diverse biologic processes, including histone modification, chro-

Table 4. Effect of lncRNA GAS5 on bone metabolism-related cytokine levels

Cytokine	Control group (OD532 nm)	Study group (OD532 nm)	Difference, fold	Direction
IL-1	79.62±1.96	52.69±1.25	1.51	↑
IL-2	139.22±1.35	83.95±1.29	1.66	↑
IL-6	153.91±1.12	75.33±2.62	2.04	↑
IL-7	155.61±1.09	47.36±1.28	3.29	↑
IL-11	56.38±1.25	23.69±1.08	1.29	↑
IL-15	149.32±1.58	83.69±1.27	1.59	↑
IL-17	69.78±0.88	28.65±1.12	2.44	↑
G-CSF	131.65±1.98	65.39±3.33	2.01	↑
GM-CSF	126.55±3.12	79.27±2.65	1.42	↑
M-CSF	124.19±2.37	29.81±1.88	4.17	↑
MCP-1	169.85±1.34	98.41±1.06	1.57	↑
MIP-1a	41.58±1.09	23.69±0.98	1.23	↑
MIG	131.43±1.58	51.66±1.75	1.43	↑
sTNF RI	261.64±2.37	158.21±1.96	1.56	↑
sTNF RII	357.28±2.17	208.15±2.98	1.58	↑
TGF-β1	93.18±1.39	51.62±1.01	1.81	↑
TIMP-2	187.62±1.59	90.17±1.06	1.56	↑
TNF-α	101.39±2.89	51.64±1.84	1.96	↑
IL-10	57.39±1.62	119.88±2.12	2.09	↓
IL-13	50.35±1.17	81.57±1.32	1.62	↓

matin remodeling, and DNA methylation, and to function as a regulator of the expression of target genes in terms of transcription, post-transcription, and epigenetics [12-14]. Growth arrest-specific transcript 5 (GAS5) is a common lncRNA, and is often found in cancer, with a wide range of inhibitory effects on tumor cells [15, 16]. In recent years, it has been found that there is a correlation between lncRNA-Gas-5 and immune and inflammatory disorders. The 1q25d locus of lncRNA GAS-5 chromosome is involved in the pathogenesis of systemic lupus erythematosus, and lncRNA GAS-5 is significantly down-regulated in both CD4+ T cells and B cells [17, 18]. LncRNA GAS-5 can be involved in the progression of multiple sclerosis by regulating the downstream NR3C1 gene [19]. Additionally, LncRNA GAS-5 has the capability of participating in relevant immune responses through binding to glucocorticoid responsive elements [20]. Osteoarthritis is mainly characterized by articular cartilage injury, subchondral bone and other tissue changes, and osteophyte formation, with clinical manifestations of pain, joint deformity, and limited activity. Its occurrence and progression are closely related to cell synthesis and metabolic factors, inflammatory factors and hormones. Yue et al found [21] that lncRNA is abnormally expressed in the pathogenesis of psoriatic arthritis, and lnc-RP11-701H24.7 and lnc-RNU12 might be novel biomarkers for psoriatic arthritis risk and activity. The above studies showed that lncRNA GAS-5 is involved in the immune regulation of arthrosis, providing a reference for exploring its mechanism in osteoarthritis in terms of immunity and inflammation. Therefore, the expression levels of lncRNA GAS-5 were detected from osteoarthritis patients and healthy subjects in this study, and the results showed that the expression level of lncRNA GAS-5 in osteoarthritis patients was lower than that of healthy subjects ($P<0.05$). This suggests that expression levels of lncRNA GAS-5 were inhibited in osteoarthritis patients. To explore the mechanism of lncRNA GAS-5 in osteoarthritis, the effect of lentivirus-mediated RNA interference on lncRNA GAS-5 in THP-1 cells was investigated in the current study. The results showed that the expression level of lncRNA GAS-5 in the study group was lower than that in the control group under lentivirus-mediated RNA interference, and the expression levels of 18 cytokines such as IL-1, IL-2, IL-6, IL-7, IL-17, G-CSF, M-CSF, and TGF-β1, etc. in the study group were significantly up-regulated compared

with those of the control group, but the expression levels of IL-10 and IL-13 were down-regulated, which suggested that the levels of bone metabolism-related cytokines were also affected under the inhibition of lncRNA GAS-5. The cytokines IL-1, IL-6, IL-17, and TGF-β caused by the down-regulation of lncRNA GAS-5 are key mediators of bone resorption and bone formation-related regulation in arthrosis [22]. As a consequence, lncRNA GAS-5 may play a crucial role in the occurrence and progression of osteoarthritis by regulating bone immunity, and inflammation-related pathways. The study by Jinsoo et al concluded that lncRNA GAS-5 plays a key role in osteoarthritis through the regulation of miR-21 through mouse model experiments [23]. This also illustrated that lncRNA GAS-5 do have an important clinical significance in osteoarthritis, and a lower level of lncRNA GAS-5 may act as a marker for the occurrence and progression of osteoarthritis.

In conclusion, the expression of lncRNA GAS-5 is low in osteoarthritis patients. While the expression level of lncRNA GAS-5 is inhibited, related immune and inflammatory factors are also affected, so lncRNA GAS-5 may affect the occurrence and progression of osteoarthritis through immune dysfunction. A low level of lncRNA GAS-5 may be a marker for the occurrence and progression of osteoarthritis, but specific regulatory mechanisms demand further study.

Disclosure of conflict of interest

None.

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