Expression of Long Noncoding RNA HOTAIR and Its Influence on the PTEN/PI3K/AKT Pathway and Inflammation in Patients With Osteoarthritis

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Abstract

Objective: Our research was designed to investigate the correlations among expression of PTEN/PI3K/AKT pathway, clinical-related indicators, and long noncoding RNA HOX transcript antisense RNA (lncRNA HOTAIR) in osteoarthritis (OA).

Methods: The expression of immune-inflammatory indicators was detected in OA patients and normal people, and peripheral blood mononuclear cells (PBMCs) were extracted to induce human chondrocytes (CHs). Reverse transcription-quantitative polymerase chain reaction was performed to measure lncRNA HOTAIR expression. The levels of inflammatory cytokines and adiponectin were detected using enzyme-linked immunosorbent assay. Cell Counting Kit-8 was used to assess the viability of CHs. Western blot analysis was utilized to evaluate related protein expression.

Results: LncRNA HOTAIR showed high expression in PBMCs of OA patients with the sensitivity and specificity of receiver-operating characteristics (ROC) curve according to the area under the ROC curve, indicating that lncRNA HOTAIR might act as a biomarker of OA. Moreover, lncRNA HOTAIR was positively correlated with total cholesterol, high sensitivity C-reactive protein, immunoglobulin G, tumor necrosis factor-α (TNF-α), and visual analog scale score, which were found to be independent risk factors for lncRNA HOTAIR expression. Besides, overexpression of lncRNA HOTAIR diminished cell viability and IL-10 expression but augmented TNF-α expression in OA-CHs stimulated by OA-PBMCs. Meanwhile, lncRNA HOTAIR overexpression elevated the levels of PI3K, AKT proteins and reduced PTEN protein expression in OA-CHs.

Conclusions: Conclusively, lncRNA HOTAIR was upregulated in PBMCs of OA patients, which facilitated the inflammatory response of OA by orchestrating inflammatory cytokines and the PTEN/PI3K/AKT pathway.

1 Introduction

Osteoarthritis (OA) is a kind of degenerative disease featured by "low level" cartilage and synovial inflammation [1, 2], which is based on joint degeneration or aging and leads to the disappearance of joint structure and gradual aggravation of cartilage. It is a chronic arthritic disease with systemic pain in large joints and swelling or dysfunction of elbow and knee joints as the main clinical manifestations. It has been reported that there exist several risk factors for OA, including age, joint trauma, obesity, and genetic susceptibility [3]. To a certain extent, obesity is related to inflammation, namely, hyperlipidemia that is a common metabolic change and is characterized by an abnormal increase in serum total cholesterol (TC), triglycerides, and low-density lipoprotein cholesterol, as well as an abnormal decrease in high-density lipoprotein cholesterol and apolipoprotein A1, all of which contribute to the inflammatory response and OA deterioration. In addition, body fat, insulin resistance index, and body mass index are negatively related to adiponectin (APN) [4, 5]. APN, a recently discovered cytokine released by adipose tissues, is a regulator for the repression of the inflammatory response in OA [6]. Through an animal experiment [7], long
noncoding RNAs (IncRNAs) are involved in the inflammation, lipid metabolism, and other processes of OA, which has become a research hotspot [8].

A prior research [9] has observed that IncRNA HOTAIR expression in OA-chondrocytes (CHs) was apparently higher than that in normal CHs and that the proliferation capacity was significantly higher in OA-CHs transfected with small interfering RNA (si)-IncRNA HOX transcript antisense RNA (HOTAIR) than in those transfected with si-negative control (NC). The tumor suppressor gene phosphatase and tensin homolog deleted on chromosome ten (PTEN) can dephosphorylate and inactivate phosphatidylinositol-3,4,5-trisphosphate (PIP3), an important molecule in the phosphoinositide 3-kinase (PI3K)/AKT pathway, to downregulate the PI3K/AKT pathway. Therefore, PTEN and the PI3K/AKT pathway together are called the PTEN/PI3K/AKT pathway, a classic inflammatory pathway. A recent study reported that PTEN can downregulate the expression of fatty acid synthase by inhibiting the PTEN/PI3K/AKT pathway [10]. Interleukin (IL)-10 and tumor necrosis factor-α (TNF-α) are critical pro-inflammatory factors and anti-inflammatory factors of OA [11], respectively. Of note, the PTEN/PI3K/AKT pathway can increase TNF-α expression and decrease IL-10 expression [12]. Furthermore, it has been noted in a previous research that the content of TNF-α is obviously reduced and the content of IL-10 is remarkably enhanced after silencing IncRNA HOTAIR [13]. However, although IncRNA HOTAIR and the PTEN/PI3K/AKT pathway participate in the inflammation of OA, there has hitherto been little report on whether the former can assume a role in immune regulation by affecting the latter in the inflammatory response of OA.

Therefore, this study was conducted to focus on observing IncRNA HOTAIR expression in OA patients and analyzing its correlation with clinical indicators and visual analog scale (VAS) scores. Finally, IncRNA HOTAIR was altered in cell experiments to assess the changes in the expression of the PTEN/PI3K/AKT pathway, inflammatory factors, and lipid metabolism indexes.

2 Materials And Methods

2.1 Subjects and samples

OA patients and healthy control (HC) subjects matched on sex and age were enrolled from the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine between November 2020 and May 2021. Patients were excluded from the present research if they did not meet the following criteria: not conforming to the 2019 Guidelines for the diagnosis of OA [14], severe mental illness, significantly impaired liver or renal function, the administration of immunosuppressive drugs, or pregnancy. The research was conducted following the relevant provisions of the Declaration of Helsinki [15] and was approved by the Ethical Committee of Scientific Research of Anhui University of Traditional Chinese Medicine's First Affiliated Hospital [2014AH-06(J)].

2.2 Co-culture of peripheral blood mononuclear cells (PBMCs) with CHs from OA patients and their transfection
Totally, 5 mL venous blood was collected from normal healthy subjects and OA patients, and diluted with an equal volume of normal saline. The same amount of lymphocyte separation solution was slowly added into the prepared samples and centrifuged at 2000 r/min for 20 min. The white floc in the centrifuge tube was transferred to the clean centrifuge tube. The same amount of normal saline was added into the tube and mixed, followed by 8-min centrifugation 800 r/min and two washes. After blowing, the samples were transferred into a culture flask and incubated for later use.

The CHs were purchased from Saibaikang Biotechnology Co., Ltd. (Shanghai, China). PBMCs were extracted and seeded into the Transwell chamber, followed by the addition of 75 µL Dulbecco's Modified Eagle Medium (DMEM). CHs were digested and centrifuged. Then, the medium was discarded, and CHs were washed twice with phosphate buffer saline, resuspended with medium, and seeded into the apical chamber, followed by the supplementation of 100 µL DMEM. Following cell adherence, the cells were activated with TNF-α (10 ng/mL), and OA-PBMCs and OA-CHs were treated at the ratio of 0.5:1. Co-culture was conducted in Roswell Park Memorial Institute-1640 media containing penicillin (the final concentration of 100 U/mL) and streptomycin (the final concentration of 0.1 mg/mL) with 5% CO₂ at 37°C until the CH confluence reached 70%-90%. OA-CHs were transfected with PCDNA3.1-IncRNA HOTAIR plasmids, si-IncRNA HOTAIR plasmids, and their corresponding NC plasmids as per the manuals of Lipofectamine 2000, followed by 48-h incubation. All plasmids were purchased from Shanghai GenePharma Company (Shanghai, China).

2.3 Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total OA-CH RNA was obtained using Trizol, followed by reverse transcription reaction and amplification reaction. Agarose-gel electrophoresis was implemented for semi-quantitative analysis of PCR products using the Gelpro32 gel image analysis software. Relative quantitative analysis was performed using 2^−ΔΔCt with β-actin as an internal reference. All used primers were as follows: lncRNA HOTAIR: the forward primer: 5'-CCATAGCCGATTAGCTGTCA' and the reverse primer: 5'-AATGCCGAACTGGAGGTG-3'; β-actin: the forward primer: 5'-GGCAAATGTCAGAGGGTTCT-3' and the reverse primer: 5'-TTCTTAAATTGGGCTGGGT-3'.

2.4 Enzyme-linked immunosorbent assay (ELISA)

Following collection, the supernatant of OA-CHs was centrifuged at 2500 r/min for 20 min, and the precipitation was discarded. The supernatant was added to the ELISA plate (50 µL per well) and incubated at 37°C for 30 minutes after the plate was sealed with a sealing plate membrane. The levels of TNF-α, IL-10, APN, and adiponectin receptor 2 (ADIPOR2) were detected using the ELISA method strictly in the light of the kit instructions.

2.5 Cell Counting Kit-8 (CCK-8) assay

Cell viability was measured with a CCK-8 assay kit (BIOSS, Beijing, China). At first, 3 × 10⁴ OA-CHs were seeded into each well of 96-well plates and cultured until 70% and 90% confluence. Logarithmically
growing cells were transfected by the above method. Three wells in each group were cultured for 0, 12, 24, 48, and 72 h, respectively. Next, 10 mL CCK-8 solution was supplemented into each well at each experimental site, followed by 1-4 h of cell incubation at 37°C. The viability of OA-CHs was determined by measuring the absorbance value of each well at 450 nm.

2.6 Western blot analysis

Cells were lysed with Radio-Immunoprecipitation assay lysis solution and centrifuged at 12000 r/min for 10 min to harvest total proteins. A sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel preparation kit (Beyotime, Shanghai, China) was utilized. The 5 × SDS-PAGE protein loading buffer was added into cells at the ratio of 1:4 to obtain protein samples. The samples were heated for 10 min in a boiling water bath and supplemented into SDS-PAGE gel wells (5-10 µL per well). Filter papers and polyvinylidene fluoride membrane of the same size as the rubber strip were cut in advance (soaked in methanol for 2-3 min in advance) and immersed in the rotating film buffer for 5 min. Bubbles were removed at every step and the flow membrane was constant. After the protein was transferred into the membranes, the membranes were cooled to room temperature before the insertion of the membranes into the prepared Western washing solution and the removal of the membrane transfer solution by 5-min washing. The Western sealing solution (5% skim milk powder) was added, slowly shaken in the shaker, closed at room temperature for 2 h, and then washed with phosphate-buffered saline containing Tween 20 three times with 10 min for each time. The membrane underwent overnight probing with primary pig antibodies to phosphorylation (p)-AKT, PI3K, PTEN, AKT, P-PI3K, APN, ADIPOR1, and ADIPOR2 at 4°C. The horseradish peroxidase-tagged secondary antibody was diluted with secondary antibody diluent at the ratio of 1:20,000 and incubated with the membranes at room temperature for 1 h. The ratio of absorbance of target proteins to that of glyceraldehyde-3-phosphate dehydrogenase was calculated after electrogenerated chemiluminescence coloring, exposure in the darkroom, fixation, and photography.

2.7 Statistical analysis

SPSS statistical software version 23.0 (IBM Corp. Armonk, NY, USA) was applied for statistical analysis, and GraphPad Prism software version 8.2 (GraphPad Software, La Jolla, CA, USA) was utilized to capture images. The significant difference between groups was compared with matching double tail Student t-test or Kruskal Wallis nonparametric tests. Classification variables were compared by the chi-square test. The Spearman correlation analysis was employed to evaluate the correlation of IncRNA HOTAIR with erythrocyte sedimentation rate (ESR), high sensitivity C-reactive protein (hs-CRP), immunoglobulin A (IgA), and other indicators. Data were expressed as mean ± standard deviation or median (quartile range). p < 0.05 was considered to be significantly different.

3 Results

3.1 Clinical characteristics of OA patients and HC subjects
A total of 80 subjects were enrolled, including 30 HC subjects (10 males and 20 females, the mean age of 59.24 ± 11.94 years) in the control group and 50 OA patients (17 males and 33 females, the mean age of 65.54 ± 14.10 years) in the OA group. There were no statistically significant differences in age and gender distribution between the two groups but significant differences in ESR, TC, triglyceride, immunoglobulin G (IgG), complement C3 (Table 1).

### Table 1

Changes in clinical lipid metabolism indexes and immune-inflammatory indexes in OA patients

| Indexes     | OA (n = 50)          | HC (n = 30)            | p     |
|-------------|----------------------|------------------------|-------|
| Age (years) | 55.85 ± 10.49^a      | 56.48 ± 6.42^a         | 0.655 |
| ESR (mm/h)  | 19.4 (11.2, 31)^b    | 9.50 (5.66, 11.62)^b   | < 0.01|
| TC (mmol/L) | 5.25 ± 1.29^c        | 2.20 (1.53, 3.10)^b    | < 0.01|
| TG (mmol/L) | 1.00 (1.35, 1.73)^b  | 0.50 (0.34, 1.03)^b    | < 0.01|
| IgA (g/L)   | 2.48 ± 1.12^c        | 1.61 (1.20, 2.20)^b    | < 0.01|
| C3 (g/L)    | 1.15 ± 0.22^c        | 1.05 (0.94, 1.09)^b    | < 0.01|
| SDS score   | 53.09 ± 7.11^c       | NA                     | -     |
| VAS score   | 5.72 ± 0.60^c        | -                      | -     |
| SAS score   | 43.38 (40.62, 47.67)^b| -                      | -     |

Notes: a, Student’s t-test. b, Wilcoxon signed-rank test, median (25–75th percentile). c, mean ± standard error. NA, not applicable; NC, negative control; OA, osteoarthritis; ESR, erythrocyte sedimentation rate; TC, total cholesterol; TG, triglyceride; IgA, immunoglobulin A; C3, complement C3; SDS, Self-rating Depression Scale; SAS, Self-rating Anxiety Scale; VAS, visual analog scale; HC, healthy control.

### 3.2 LncRNA HOTAIR expression is upregulated in the PBMCs of OA patients

This study intended to assess lncRNA HOTAIR expression in OA patients. LncRNA HOTAIR expression in the PBMCs of 50 OA patients and 30 HC subjects was detected using RT-qPCR. The findings revealed that lncRNA HOTAIR expression was increased in the PBMCs of OA patients (Figure 1A). The diagnostic efficacy of lncRNA HOTAIR was evaluated using a receiver-operating characteristic (ROC) curve analysis, and the area under the ROC curve (AUC) was 0.8310 [95% confidence interval (CI): 0.74-0.92]. According to the Youden index, the ideal cut-off value for the separation of OA from HC subjects was 1.185 with a sensitivity of 60.00% and a specificity of 92.67% (Figure 1B).
3.3 Differential expression of inflammatory cytokines and APN in OA with a positive correlation with IncRNA HOTAIR expression

The levels of IL-10 and TNF-α in the OA group were dramatically lowered ($p < 0.01$) and substantially increased ($p < 0.01$), respectively, as compared to the normal group, showing that there were alterations in the levels of pro-inflammatory and anti-inflammatory factors in OA (Figure 2A-B). Furthermore, the expression of APN and ADIPOR2 was considerably reduced in the OA group compared with the normal group ($p < 0.01$; Figure 2C-D). Then, to further explore the possible relationship between IncRNA HOTAIR and related research indicators, we conducted correlation analyses of IncRNA HOTAIR with TC, hs-CRP, IgG, TNF-α, and VAS respectively. The results displayed that IncRNA HOTAIR was a risk factor for TC, hs-CRP, IgG, TNF-α, and VAS [16] (Figure 2E-I). These results further suggested the close relationship between IncRNA HOTAIR and the occurrence and development of OA.

3.4 Association rules analysis of IncRNA HOTAIR with laboratory indexes in OA patient

Association rule analysis showed that the elevation of IncRNA HOTAIR was strongly correlated with the elevation of GSH, ESR, and VAS in OA patients. There was a strong correlation between the increase of IncRNA HOTAIR and the decrease of LDL-C, with a support degree greater than 20% and a confidence degree greater than 30%. The results are shown in Table 2.

| Items (LHS $\Rightarrow$ RHS) | Support (%) | Confidence (%) | Lift | $p$ value |
|------------------------------|-------------|----------------|------|-----------|
| \{IncRNA HOTAIR↑\}$\Rightarrow$\{Hospitalization days↓\} | 63.636 | 76.087 | 1.02 | $< 0.01$ |
| \{IncRNA HOTAIR↑\}$\Rightarrow$\{sex (female)\} | 56.364 | 67.391 | 1.05 | $< 0.01$ |
| \{IncRNA HOTAIR↑\}$\Rightarrow$\{SDS↑\} | 63.636 | 76.087 | 1.03 | $< 0.01$ |
| \{IncRNA HOTAIR↑\}$\Rightarrow$\{ESR↑\} | 61.818 | 73.913 | 1.03 | $< 0.01$ |
| \{IncRNA HOTAIR↑\}$\Rightarrow$\{VAS↑\} | 67.273 | 80.435 | 1.01 | $< 0.01$ |
| \{IncRNA HOTAIR↑\}$\Rightarrow$\{LDL-C↑\} | 27.273 | 32.609 | 1.02 | $< 0.01$ |

Notes: For correlations, Aprior module analysis was used. The minimal level of support was set at 20%, while the minimum level of confidence was set at 30%. When the degree of the lift was set to greater than one, it was deemed important.

3.5 LncRNA HOTAIR overexpression enhanced the viability of OA-CHs
LncRNA HOTAIR expression in the PCDNA3.1-IncRNA HOTAIR group was substantially higher \((p < 0.01)\) than that in the PCDNA3.1-NC group, whereas IncRNA HOTAIR expression in the si-IncRNA HOTAIR group was significantly lower than that in the si-NC group \((p < 0.01)\). The effect of IncRNA HOTAIR on the viability of OA-CHs was assessed by CCK-8. The results exhibited that the PCDNA3.1-IncRNA HOTAIR group had dramatically boosted cell viability when compared to the PCDNA3.1-NC group. In contrast to the si-NC group, cell viability of the si-IncRNA HOTAIR group was evidently diminished. Among the four groups, cell viability of the si-IncRNA HOTAIR group was the highest and that of the IncRNA HOTAIR group was the lowest. As shown in figure 3.

### 3.6 LncRNA HOTAIR overexpression facilitated TNF-\(\alpha\) expression but repressed IL-10 expression in OA-CHs

The level of cytokines was evaluated by ELISA. As a result, versus the PCDNA3.1-NC group, TNF-\(\alpha\) expression in the PCDNA3.1-IncRNA HOTAIR group was prominently augmented and IL-10 expression was remarkably decreased \((p < 0.01)\). Compared with the si-NC group, TNF-\(\alpha\) expression was markedly lowered and IL-10 expression was noticeably elevated in the si-IncRNA HOTAIR group \((p < 0.01; \text{Figure 4A-B})\).

### 3.7 LncRNA HOTAIR manipulated the PTEN/PI3K/AKT pathway in OA-CHs

In order to ascertain the impacts of IncRNA HOTAIR on the PTEN/PI3K/AKT pathway, Western blot analysis was adopted to identify the expression of PI3K, p-AKT, p-PI3K, AKT, APN, ADIPOR1, ADIPOR2, and PTEN. As discovered in Figure 5, overexpression of IncRNA HOTAIR enhanced the expression of p-PI3K and p-AKT and reduced the expression of APN, ADIPOR1, and PTEN. On the contrary, IncRNA HOTAIR silencing contributed to the opposite trends. However, the overexpression or silence of IncRNA HOTAIR did not significantly change the expression of PI3K, AKT, and ADIPOR2.

### 4 Discussion

As one of the most frequent types of arthritis, OA is a chronic condition that causes cartilage loss, pain, and stiffness \([17, 18]\). OA is particularly common among the elderly and obese, which is associated with a significant risk of joint injuries \([19]\). Joint space narrowing is a symptom of OA, which indicates cartilage loss. The function of inflammation has just recently been introduced in the pathophysiology of OA. Several studies \([20, 21, 22]\) completed in the last ten years have documented a correlation between synovial inflammation and pain on one hand and radiographic progression on the other hand, demonstrating the critical role of inflammation in OA by resulting in the changes in the levels of inflammatory factors such as IL-10 and TNF-\(\alpha\). TNF-\(\alpha\) is an essential pro-inflammatory factor involved in OA, which can activate osteoclasts of endothelial cells, leading to continuous inflammatory responses occurring in the gradual destruction of cartilage. IL-10 level was inhibited in the progression of OA. Existing studies \([23, 24]\) have shown that lipid metabolism imbalance plays an important role in the occurrence and development of
OA, which is a risk factor for the occurrence of OA and hyperlipidemia and is closely related to the immune-inflammatory response of OA patients. APN, an indicator related to lipid metabolism, induces inflammatory responses \cite{25}. Kabalyk et al. \cite{26} confirmed that hyperlipidemia directly acts on articular tissues and causes cellular stress, manifested as changes in morphological and functional characteristics of CHs. Processes, including cell death, pathological mineralization of articular cartilages, and enhanced pathological angiogenesis can be observed in hyperlipidemia. Chen et al. constructed a lipopolysaccharide-induced human articular CH C28/I2 cell model and found that the expression and quantity of inflammatory cytokines (including IL-1β, IL-6, IL-8, and TNF-α) augmented in this cell model compared with the corresponding control group \cite{27,28}. LncRNAs mediate critical mechanisms in the pathogenesis of OA, and these regulatory effects of lncRNA HOTAIR have also begun to attract extensive attention \cite{29,30}, which is conducive to the development and progression of OA \cite{31}. LncRNA HOTAIR, for example, is upregulated in OA, thus participating in IL-1β-induced matrix metalloproteinase overexpression and promoting inflammatory responses in CHs \cite{32}. A prior research has elucidated the PI3K/AKT pathway assumes a key role in the development of inflammation in OA \cite{33}. Moreover, another research has unraveled that the PI3K/AKT pathway exerts a certain effect on lipid metabolism \cite{34}.

This research provided evidence of substantially high expression of IncRNA HOTAIR in PBMCs from OA patients and OA-CHs induced by PBMCs of OA patients with the AUC of 0.8310, the optimal truncation value of 1.185, the sensitivity of 60.00%, and the specificity of 92.67%, illustrating that IncRNA HOTAIR had the high diagnostic value. Our results also exhibited that IL-10, APN, and ADIPOR2 levels were diminished and TNF-α level was augmented in OA patients. It has been documented that APN can control inflammation, thus functioning as an additional potential therapeutic target \cite{35}. All in all, changes in levels of both cytokines and APN and its receptors can be indicated as the deregulation of pro-inflammatory and anti-inflammatory factors. In addition, clinical trials depicted that the level of IncRNA HOTAIR shared a positive correlation with TNF-α, clinical immune-inflammatory indicators, lipid metabolism index, and patient perception score scales, like TC, hs-CRP, IgG, and VAS. It has been reported that the imbalance of inflammatory factors is associated with IncRNA HOTAIR. In order to further dissect out the effects of IncRNA HOTAIR on the proliferation, inflammation, and lipid metabolism of OA-CHs, OA-PBMCs were utilized to stimulate OA-CHs as a cell model to conduct in vitro cell experiments.

Subsequently, the impacts of IncRNA HOTAIR on the function of OA-CHs were assessed using CCK-8 assay. The results showed that the viability of OA-CHs was conspicuously decreased after overexpressing IncRNA HOTAIR. On the contrary, cell viability was enhanced following the transfection of IncRNA HOTAIR overexpression plasmids. Under the condition of IncRNA HOTAIR overexpression and silencing, inflammatory cytokines were further measured by the ELISA method. The results displayed that overexpression of IncRNA HOTAIR significantly enhanced TNF-α level and reduced IL-10 level. In contrast, the deletion of IncRNA HOTAIR triggered an opposite tendency to inflammation. These results suggested that si-IncRNA HOTAIR might be implicated in the occurrence and development of OA through orchestrating the levels of inflammatory factors in patients.
Studies\textsuperscript{[36, 37, 38, 39]} have unveiled that IncRNA HOTAIR knockout can restrain cell viability through the PI3K/AKT pathway, reduce the cellular inflammatory response, inhibit drug resistance, and improve the quality of life of patients.

Therefore, we next explored how IncRNA HOTAIR was involved in the progression of OA by manipulating the PTEN/PI3K/AKT pathway. The current study noted that the potential correlation of IncRNA HOTAIR with the level of the PTEN/PI3K/AKT pathway in OA-CHs through cell experiments. Western blot analysis was implemented to determine the influence of IncRNA HOTAIR on the pathway-related proteins and APN and its receptors proteins, manifesting that overexpression of IncRNA HOTAIR elevated the expression of p-PI3K and p-AKT but lowered the expression of PTEN, APN, and ADIPOR1 proteins. The cause of the insignificant effect on ADIPOR2 expression may be that ADIPOR1 is expressed abundantly in cartilages, bones, and synovial tissues, whereas ADIPOR R2 is rarely detected\textsuperscript{[40]}.

In summary, our obtained findings uncovered that IncRNA HOTAIR expression was high in the PBMCs of OA patients. Furthermore, its abnormal expression could modulate the viability of OA-CHs, which participated in the inflammatory response of OA by influencing the levels of inflammatory cytokines, APN, and the PTEN/PI3K/AKT pathway in OA-CHs, thus afflicting the occurrence and development of OA. Therefore, IncRNA HOTAIR may be utilized as a biomarker for OA and our research provides new insights into the pathogenesis of OA. The specific mechanism of action needs to be further explored.

**Declarations**

**Acknowledges**

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Contributions

CXL and LJ contributed to the study design. CXL contributed to data analysis, wrote the first draft, and revised the manuscript. SYQ contributed to the specimen and data collection. ZXH, ZQ, and DX supervised the project and contributed to the manuscript revision. All authors reviewed and accepted the content of the final manuscript.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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**Figures**

**Figure 1**

LncRNA HOTAIR expression is high in the PBMCs of OA patients. (A) LncRNA HOTAIR expression was considerably higher in OA patients than in HC subjects. (B) Comparison of ROC curves of LncRNA HOTAIR expression between PBMCs in OA patients and HC patients. **p < 0.01.
Figure 2

**Differential expression of inflammatory cytokines in OA.** (A-D) The levels of TNF-α (A), IL-10 (B), APN (C), and ADIPOR2 (D) in serum of OA patients and normal subjects. ***p < 0.001. (F-I) Correlation analyses of IncRNA HOTAIR with TC (E), hs-CRP (F), IgG (G), TNF-α (H), and VAS (I) in the PBMCs of OA patients.
LncRNA HOTAIR upregulation facilitates the viability of OA-CHs. (A) The expression of IncRNA HOTAIR in OA-CHs measured by RT-qPCR. (B) The effect of IncRNA HOTAIR on the viability of OA-CHs tested by CCK-8. All tests were repeated three times and results were expressed as mean ± standard deviation. **p < 0.01, ***p < 0.001.
Figure 4

LncRNA HOTAIR upregulation causes TNF-\(\alpha\) upregulation and IL-10 downregulation in OA-CHs. (A-B) The levels of IL-10 (A) and TNF-\(\alpha\) (B) in OA-CHs determined by ELISA. ***p < 0.001.
Figure 5

Effects of lncRNA HOTAIR on the PTEN/PI3K/AKT pathway in OA-CHs. (A) The representative Western blots of PI3K, p-AKT, p-PI3K, AKT, APN, ADIPOR1, ADIPOR2, and PTEN in OA-CHs. (B) The quantitative analysis of the expression of PI3K, p-AKT, p-PI3K, AKT, and PTEN in OA-CHs. (C) The quantitative analysis of the expression of APN, ADIPOR1, and ADIPOR2 in OA-CHs. All experiments were repeated three times and data were expressed as mean ± standard deviation. **p < 0.01, ***p < 0.001.