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LncRNA HOTAIR alleviates rheumatoid arthritis by targeting miR-138 and inactivating NF-κB pathway

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic and autoimmune-mediated inflammatory disease. We aimed to investigate the regulation of lncRNA HOTAIR in LPS-treated chondrocytes and RA mouse. Our results showed that HOTAIR expression was significantly reduced in LPS-treated chondrocytes. The HOTAIR was then over-expressed in chondrocytes by transfecting recombinant lentivirus carrying sequences encoding HOTAIR. The LPS-induced reduction of cell proliferation rate and production of two inflammatory factors interleukin (IL)-17, IL-23 were markedly inhibited. Enforced expression of HOTAIR also led to the upregulation of proliferation-related protein Ki67 and proliferating cell nuclear antigen (PCNA). Moreover, a negative correlation was detected between the expression of HOTAIR and microRNA (miR)-138, and the expression of miR-138 was significantly increased in LPS-induced chondrocytes. The effects of HOTAIR over-expression on the proliferation and inflammation were partly reversed by miR-138 overexpression. Furthermore, the overexpression of HOTAIR significantly inhibited the activation of nuclear transcription factor-κB (NF-κB) in LPS-treated chondrocytes by suppressing p65 to cell nucleus, resulting in the down-regulation of IL-1β and tumor necrosis factor (TNF)-α. In addition, the *in vivo* experiments exhibited that overexpression of HOTAIR increased cell proliferation and inhibited inflammation in RA rats, which were demonstrated by upregulation of Ki67 and PCNA, reduced $CD4^+$ $IL\text{-}17^+$,$CD4^+$ $IL\text{-}23^+$ cells, and down-regulation of p-p65, IL-1β and TNF-α. In summary, our study suggests HOTAIR plays a protective role in RA by increasing proliferation rate and inhibiting inflammation, which may be related with the regulation of miR-138 expression and NF-κB signaling pathway. These results suggest that the regulation of HOTAIR may be a promising therapeutic strategy for RA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic and autoimmune-mediated inflammatory disease, which is characterized by systemic inflammation, articular cartilage destruction, joint damage and bone erosion [1]. A variety of investigations have shown that RA could be caused by the combination of genetic and environmental risk factors [2,3]. In the present, RA affects approximately 1% of the population worldwide, and seriously decreased the quality of life of RA patients and their families [4]. Increased studies demonstrated that cytokine networks and associated cells were closely associated with the development of RA. Currently, many treatment strategies for RA have gained a great progress, such as antagonists of interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF) α signaling pathway [4–6], suggesting that inhibition of inflammation is an effective strategy to suppress the progression of RA. However, the underlying regulatory mechanism of inflammation in RA needs further exploring.

In recent years, non-protein coding RNAs have been attracted much attention for their involvement in a wide range of biological and pathological processes, such as proliferation, apoptosis, inflammation and immunity [7]. Long noncoding RNAs (lncRNAs) are a class of transcripts with > 200 nucleotides and emerging studies have shown that lncRNAs play a crucial role in many inflammatory disease including RA [8,9]. For example, in the CAIA mouse model of RA, shikonin could inhibit inflammatory response via regulating lncRNA-NR024118 [10]. And quercetin promotes the apoptosis of fibroblast-like synoviocytes in RA by upregulating lncRNA MALAT1 via inhibiting the activation of the

Abbreviations: RA, rheumatoid arthritis; miR, microRNA; lncRNA, long non-coding RNA; PCNA, proliferating cell nuclear antigen; IL, interleukin; NF-κB, nuclear transcription factor-κB; TNF-α, tumor necrosis factor (TNF)-α

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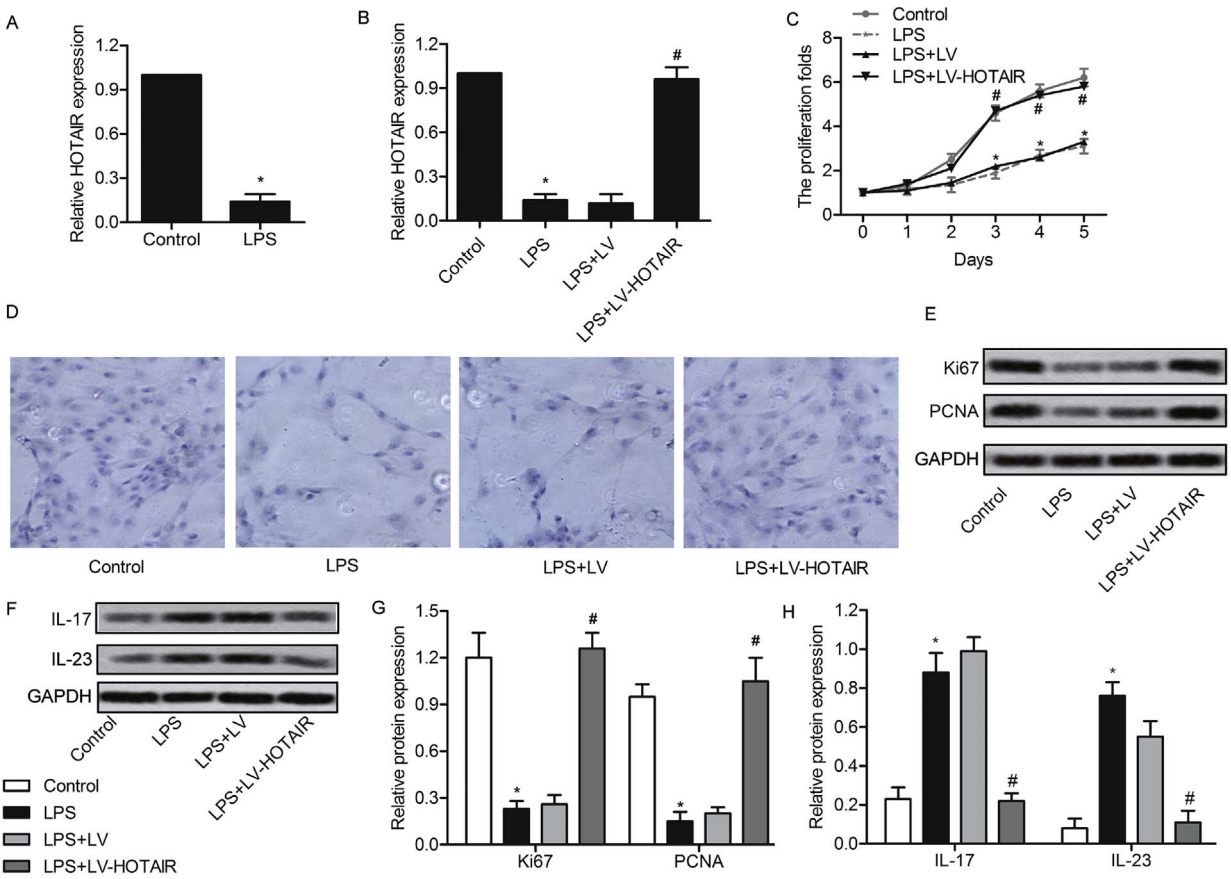

Fig. 1. Effects of HOTAIR on the proliferation and inflammation of chondrocytes induced by LPS. (A) Expression of HOTAIR was measured by RT-PCR assay. (B) Chondrocytes were infected with LV-HOTAIR or negative control, and then treated with LPS for 48 h. Expression of HOTAIR was measured by RT-PCR assay. (C) Cell proliferation was assessed by CCK-8 assay. (D) Chondrocytes were stained with Crystal violet. (E) The protein expression of Ki67 and PCNA were determined by Western blot. GAPDH was used as an endogenous reference. (F) The protein expression of IL-17 and IL-23 were determined by Western blot. (G) The relative protein expression of Ki67 and PCNA are shown in the bar graphs. (H) The relative protein expression of IL-17 and IL-23 are shown in the bar graphs. $^{*}P < 0.05$ vs control group, $^{\#}P < 0.05$ vs LPS group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

PI3K/AKT pathway [11]. MicroRNAs (miRNAs) are small non-coding RNAs with 20–22 nt in length, and the underlying regulation mechanisms that are able to target the mRNAs of target genes by sequence complementarity have been extensively investigated [12–14]. Due to the important role of miRNAs in various cellular processes, miRNA expression is strictly controlled. It has been reported that there may be a regulation relationship between lncRNAs and miRNAs [15]. One report suggests that lncRNA H19 acts as a metabolic correlate by influencing the miR-675-mediated COL2A1 levels in cartilage and cultured chondrocytes [16]. Therefore, to investigate the effects of lncRNAs and miRNAs may contribute to clarify the pathogenesis of RA.

HOTAIR is an lncRNA that has been identified to be involved in the development of several diseases such as various cancers [17,18] and cardiovascular disease [19]. Recently, one exploratory study provides novel empirical evidence that HOTAIR could be one of potential biomarkers for diagnosing RA [20]. However, the special functions and mechanisms of HOTAIR in RA still need further investigations. MiR-138 is has been demonstrated to be involved in maintaining the chondrocyte phenotype, suggesting miR-138 may associated with the development of RA. In the present study, we aim to observe the effect of HOTAIR on the proliferation and inflammation in LPS-induced chondrocytes and RA and further explore the potential mechanism.

2. Materials and methods

2.1. Chondrocyte isolation and cell culture

The cartilage tissues obtained from SD rats (SPF, male, 200–280 g, provided from ZiBo central hospital), and the chondrocytes were isolated from cartilage as following described. Cartilage was washed 3 times using DMEM, cut into 1 mm^3 small pieces and digested with filter-sterilized collagenase A overnight at 37 °C. The following day, cells were filtered through a 70 mM nylon cell strainer (BD Falcon), counted, and resuspended in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Upon confluence, cells were split once and cultivated to confluence again, for experimental use. Cells were cultured in an atmosphere of 5% CO_2 at 37 °C. Then, we performed preliminary experiments (CCK-8 assay) to select the treatment concentration of LPS by setting up serial concentration gradients including 1 ng/mL, 10 ng/mL, 100 ng/mL, 1 μg/mL, 10 μg/mL. The results showed that LPS induced cell viability injury and HOTAIR expression reduction in a concentration-dependent manner in chondrocytes, and 10 μg/mL LPS treatment induced the most significant changes. Therefore, we chose 10 μg/mL concentration of LPS (Sigma-Aldrich, St. Louis, MO, USA) for the following studies.

2.2. Infection of lentiviral particles

Recombinant lentiviruses carrying sequences encoding HOTAIR (LV-HOTAIR) and a negative control sequence (LV) were constructed according to previous manuscripts [20]. Chondrocytes were infected with either LV-HOTAIR or LV by 5 μg/mL polybrene (GENECHEM, Shanghai, China). After 48 h, the infection efficiency was detected by qRT-PCR.