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Long noncoding RNA maternally expressed gene 3 knockdown alleviates lipopolysaccharide-induced inflammatory injury by up-regulation of miR-203 in ATDC5 cells

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ABSTRACT

Background: Osteoarthritis (OA) is a common degenerative joint disease, which seriously impacts the health of elderly. However, there is no effective treatment for curing this disease until now. Numerous studies reported that long noncoding RNAs (lncRNAs) are closely related to the pathogenesis of OA. Therefore, the study aims to investigate the effect of maternally expressed gene 3 (MEG3) on lipopolysaccharide (LPS)-induced inflammatory injury of ATDC5 cells.

Methods: Different concentrations (0, 1, 5, and 10 μg/ml) of LPS were used to induce ATDC5 cells injury. The specific expressing vectors were then transfected into ATDC5 cells to alter MEG3, Sirt1 and miR-203 expressions. Flow cytometry, luciferase reporter, qRT-PCR and western blot assays were used to detect cell viability, apoptosis, and the expressions of apoptosis-related proteins and pro-inflammatory factors (IL-1β, IL-6, IL-8 and TNF-α). Meanwhile, ELISA was used for analyzing the concentrations of inflammatory cytokines in culture supernatant. Besides, the key pathways of PI3K/AKT and NF-κB were examined by western blot.

Results: LPS decreased cell viability, increased cell apoptosis, promoted the release of pro-inflammatory factors, and down-regulated MEG3 expression, Moreover, MEG3 knockdown alleviated LPS-induced inflammatory injury. MEG3 acted as a competing endogenous RNAs (ceRNA) for miR-203, and MEG3 knockdown reduced inflammatory injury by regulating miR-203. Furthermore, miR-203 positively regulated Sirt1 expression, and Sirt1 alleviated LPS-induced inflammatory injury via mediating PI3K/AKT and NF-κB pathways.

Conclusion: This study showed that MEG3 knockdown alleviated LPS-induced inflammatory injury in ATDC5 cells by regulating miR-203 expression. Hence, the findings may offer a potential treatment perspective of OA.

1. Introduction

Osteoarthritis (OA) is a degenerative joint disease, which commonly occurs in elder [1]. The direct cause of OA is the degradation of articular cartilage, reactive hyperplasia of subchondral bone, synovitis and other tissue lesions [2,3]. It is affected by various factors, including aging, obesity, strain, trauma, joint congenital abnormality and joint deformity, which can lead to joint pain, stiffness, joint swelling, and limited activity [2,4,5]. To date, there is a variety of therapeutic methods for OA, such as platelet-rich plasma kits [6], Ai Chi [7] and traditional Chinese medicine (TCM) [8], but joint replacement is still the most common treatment [3]. Although the treatment is constantly improving, OA still cannot completely cure. Hence, the molecular mechanisms regarding chondrocyte inflammatory injury are urgently needed to be revealed for understanding the pathogenesis and clinical treatment of OA.

Long noncoding RNAs (lncRNAs) is a kind of non-coding RNAs (ncRNAs) with a length of more than 200 nt and present in eukaryotic cells [9]. Japanese scientists Okazaki et al. first proposed that lncRNA was originated from mice in 2002 [10], then until 2007, Rinn et al. found that lncRNA could be modified chromatin and inhibited HOX gene transcription, thereby regulating the physiological function of the organism [11]. With the continuous attention to lncRNA, the study of various diseases is also carried out gradually, such as cancers,

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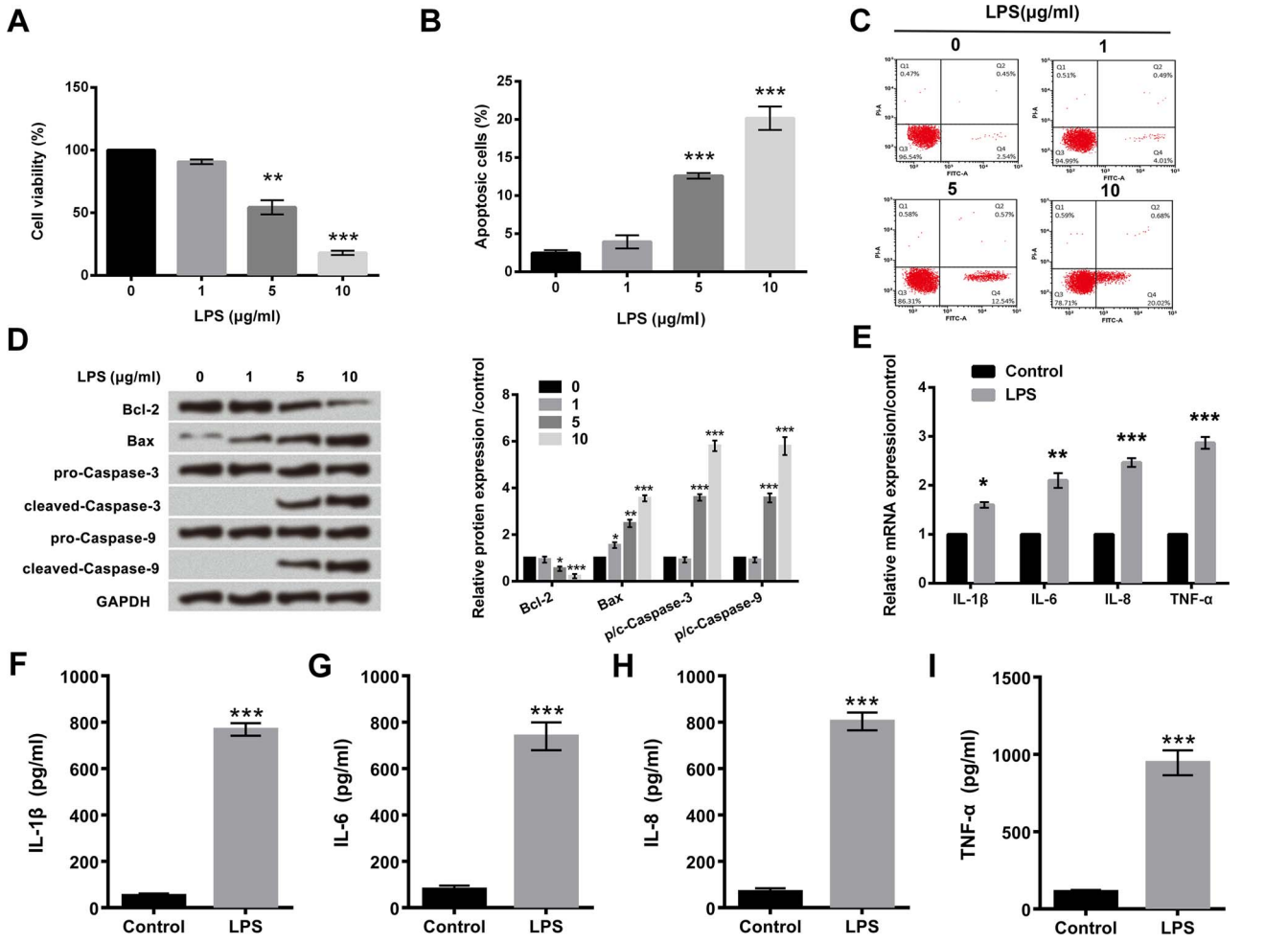

Fig. 1. LPS induced ATDC5 cells inflammatory injury. Different concentrations of LPS (0, 1, 5, and 10 μg/ml) were used to treat cells for 12 h. (A,B) Cell viability and apoptosis were measured by using CCK-8 and flow cytometry, respectively. (C) The representative dot-plot images of flow cytometric results. (D) The protein levels of apoptosis-related factors were tested by using western blot analysis. (E) The mRNA levels of inflammatory cytokines of IL-1β, IL-6, IL-8 and TNF-α were examined using qRT-PCR. (F-I) The concentrations of IL-1β, IL-6, IL-8 and TNF-α in the culture supernatant were detected using ELISA after 5 μg/ml LPS stimulated cells. * $p < .05$, ** $p < .01$, *** $p < .001$.

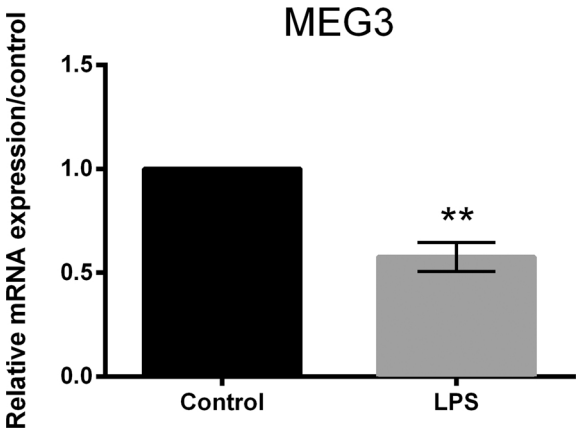

Fig. 2. MEG3 expression was down-regulated in LPS-treated ATDC5 cells. The mRNA expression level of MEG3 was tested by using qRT-PCR. ** $p < .01$.

inflammatory damage, and immune disease [12]. A great deal of evidence has shown that lncRNA is closely related to the pathogenesis of OA [13]. For instance, lncRNA plasmacytoma variant translocation 1 (PVT1) could promote the apoptosis of chondrocytes in OA [14], and lncRNA UFC1 was also reported to increase the proliferation of OA chondrocytes [15]. Maternally expressed gene 3 (MEG3) is a novel lncRNA, which is associated with tumor progression [16]. The regulatory mechanisms of MEG3 have been investigated in liver fibrogenesis [16], hepatocellular carcinoma [17], esophageal cancer [18]. Only one study referred to the role of MEG3 in OA, and described MEG3 as a pivotal modulator in regulating angiogenesis [19].

MicroRNAs (miRNAs) are another ncRNA with about 22 nt in length, and involved in post transcriptional regulation of biological gene expression in different creatures [2]. Various diseases will be caused when the functions of miRNAs is disturbed [20]. In orthopedic diseases, several miRNAs might form a network to regulate the steady state, catabolism and repair of cartilage [21]. Mounting studies revealed that a variety of miRNAs might play critical roles in OA chondrocyte, such as miR-29b-3p [2], miR-138 [22] and miR-140 [23]. These miRNAs positively regulate chondrocyte apoptosis to facilitate inflammatory damage, while miR-146 overexpression inhibits inflammation of chondrocytes by regulating cytokine signaling [23]. A previous study has demonstrated that the apoptosis of chondrocytes was regulated by lncRNA through acting as a sponge for miRNA in OA [14]. In this study, we aimed to uncover the role of MEG3 in the regulation of inflammatory injury in chondrocytes, and to reveal whether MEG3 affects chondrocytes via sponging other miRNAs.