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Lipopolysaccharide-induced expression of astrocyte elevated gene-1 promotes degeneration and inflammation of chondrocytes *via* activation of nuclear factor-κB signaling

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ABSTRACT

Osteoarthritis is an inflammatory disease characterized by joint degeneration and inflammation. Astrocyte elevated gene-1 (AEG-1) has been suggested as a novel inflammation-related factor in the pathological processes of various inflammatory diseases. To date, little is known about the role of AEG-1 in osteoarthritis. The aim of the present study was to explore the potential role of AEG-1 in the regulation of lipopolysaccharide-induced apoptosis and inflammation of chondrocytes. The results showed that AEG-1 expression was significantly up-regulated in chondrocytes following exposure to lipopolysaccharide. Knockdown of AEG-1 increased the survival and decreased the expression of matrix metalloproteinases in chondrocytes treated with lipopolysaccharide. Moreover, silencing of AEG-1 restricted the lipopolysaccharide-induced production of proinflammatory cytokines. In contrast, AEG-1 overexpression caused opposite effects. Notably, we found that AEG-1 inhibition blocked the lipopolysaccharide-induced activation of nuclear factor-κB signaling through impeding the nuclear translocation of nuclear factor-κB p65 subunit. Additionally, inhibition of nuclear factor-κB partially reversed the AEG-1-mediated promotion of lipopolysaccharide-induced inflammatory injury in chondrocytes. In conclusion, our results demonstrate that inhibition of AEG-1 expression attenuates lipopolysaccharide-induced degeneration and inflammation of chondrocytes through suppressing the activation of nuclear factor-κB signaling. This work therefore highlights a potential role of AEG-1 in the pathogenesis of osteoarthritis, and indicates its potential as a therapeutic target.

1. Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease characterized by cartilage degradation and inflammation, which affects a large proportion of the aged population [1,2]. The pathology of OA is associated with multiple factors, including environmental and genetic factors [3,4]. However, the precise molecular mechanism underlying the pathology of OA remains elusive. Chondrocytes play an important role in regulating joint integrity, and the degeneration and inflammation of chondrocytes contribute to the pathogenesis of OA [5,6]. Therefore, research into underlying chondrocyte degeneration and inflammation is of great significance to the clinical management and treatment of OA.

OA is an inflammatory disease which involves the NF-κB signaling pathway [7,8]. The NF-κB proteins are a family of ubiquitously expressed transcription factors involved in inflammation, immune response, and stress response [9,10]. During unstimulated conditions, NF-κB proteins are sequestered in the cytosol by the binding of inhibitor of NF-κB (IκB) proteins [11]. In response to stimulating signals, IκB proteins are degraded by IκB kinases (IKKs), triggering the translocation of NF-κB into the nucleus and subsequent transcription of target genes. Activation of NF-κB signaling in chondrocytes induces excessive expression of tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and matrix metalloproteinases (MMPs), which promote cartilage inflammation, degradation and apoptosis of OA chondrocytes [12]. Therefore, NF-κB signaling has been highlighted as a promising target

Abbreviations: OA, osteoarthritis; AEG-1, astrocyte elevated gene-1; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; IκB, inhibitors of NF-κB; IKKs, activation of IκB kinases; TNF-α, tumor necrosis factor-α; IL, interleukin; MMP, matrix metalloproteinases

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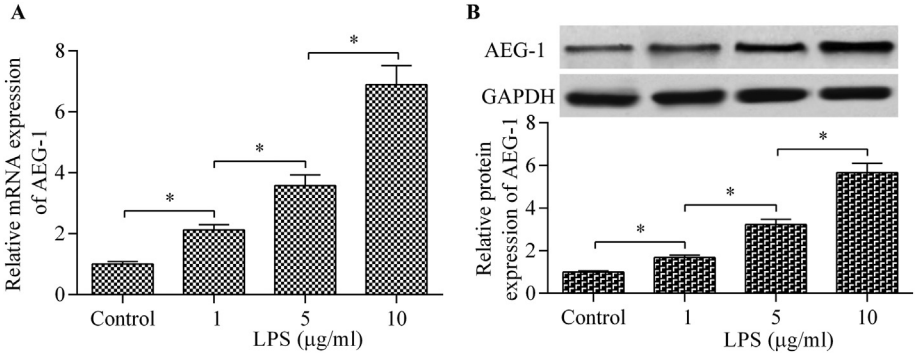

Fig. 1. Induction of AEG-1 by LPS in chondrocytes. The C28/I2 chondrocytes were stimulated with LPS (1, 5, and 10 μg/ml) for 6 h. (A) The effect of LPS on AEG-1 mRNA expression was examined by RT-qPCR analysis. (B) The effect of LPS on AEG-1 protein expression was determined by western blot analysis. *$p < 0.05$.

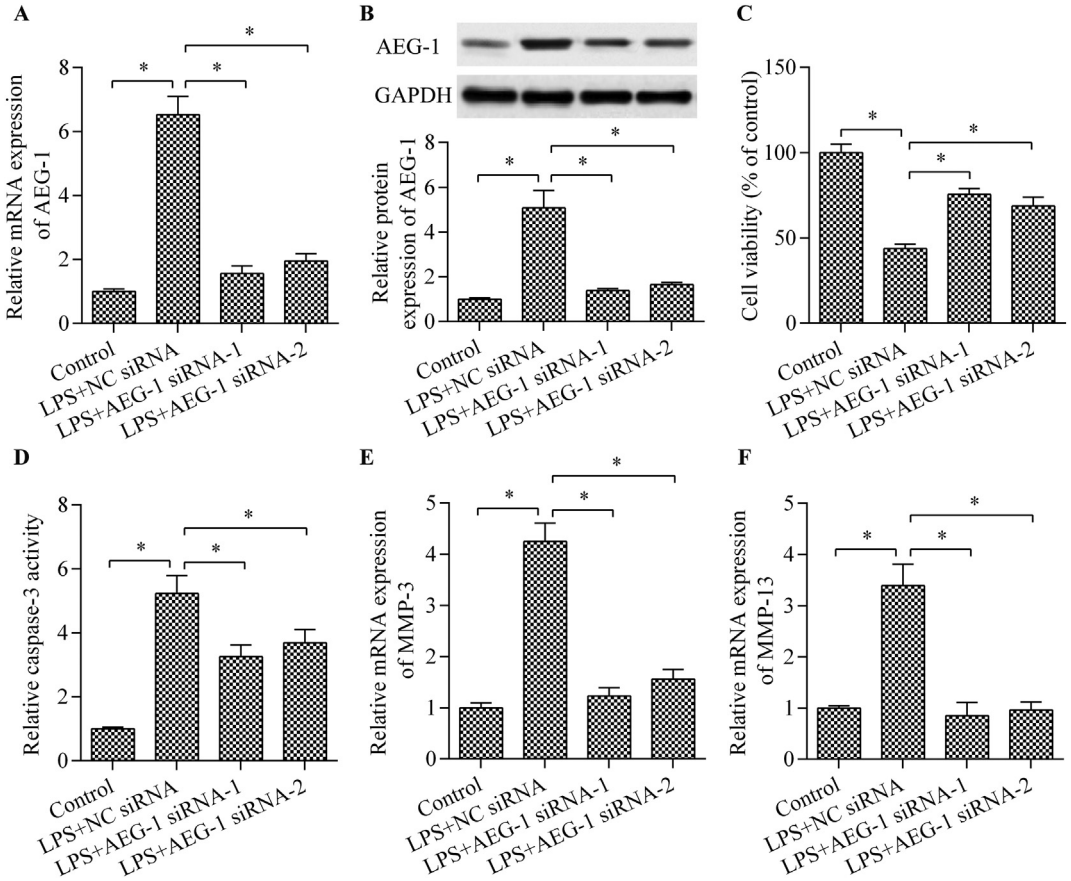

Fig. 2. Knockdown of AEG-1 reduces LPS-induced apoptosis and degeneration of chondrocytes. Cells were transfected with AEG-1 siRNA or NC siRNA for 48 h and then stimulated with 10 μg/ml of LPS for 6 h. (A) Relative mRNA and (B) protein expression levels of AEG-1 were examined by RT-qPCR and western blot analysis, respectively. (C) The effect of AEG-1 knockdown on cell survival was evaluated by MTT assay. (D) The effect of AEG-1 knockdown on cell apoptosis was assessed by caspase-3 activity assay. Relative mRNA expression levels of MMP-3(E) and MMP-13 (F) in AEG-1 siRNA transfected cells were evaluated by RT-qPCR analysis. *$p < 0.05$.

for the development of OA therapeutics [13].

Astrocyte elevated gene-1 (AEG-1) is originally reported to be a TNF-α or human immunodeficiency virus (HIV)-inducible gene in human fetal astrocytes [14]. AEG-1 gene is located at 8q22 encoding a putative 582 amino acid protein with a calculated molecular mass of 64 kDa [15]. The AEG-1 gene is ubiquitously expressed in a variety of tissues and cells, and plays an important role in diverse physiological and pathological processes. AEG-1 has emerged as an oncogene that is overexpressed in multiple cancers and plays an important role in tumorigenesis [16]. Furthermore, elevated AEG-1 expression contributes to neurodegeneration by promoting glutamate accumulation and excitotoxicity [17,18]. AEG-1 is a multifunctional protein that is involved in the regulation of immune-senescence, oxidative stress, and lipid homeostasis [19,20]. Notably, AEG-1 is fundamentally required for the activation of NF-κB signaling, and AEG-1 modulates NF-κB at multiple levels [21–23]. Moreover, AEG-1 inhibition abrogates lipopolysaccharide (LPS)-induced NF-κB activation [24]. Therefore, AEG-1-mediated NF-κB signaling may play a crucial role in inflammation-related diseases.

Multiple studies have demonstrated that AEG-1 is dysregulated in various inflammatory diseases [25,26]. However, whether AEG-1 is involved in the pathogenesis of OA remains unclear. LPS-mediated

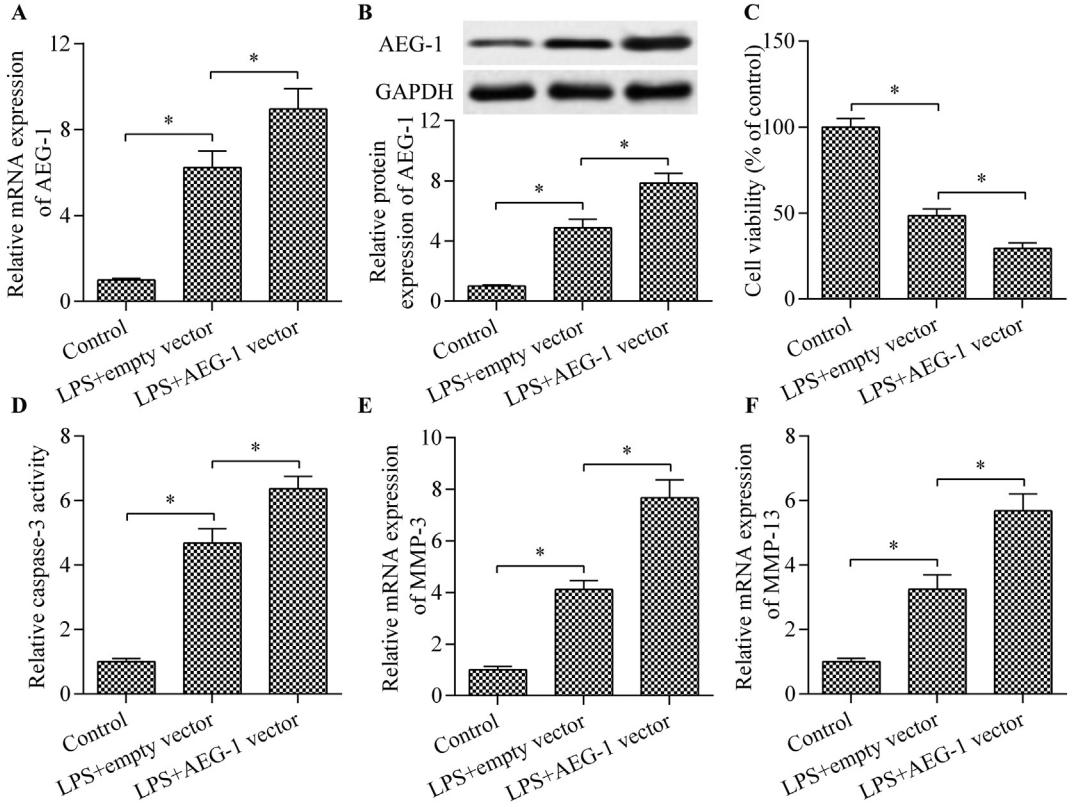

Fig. 3. Overexpression of AEG-1 exacerbated LPS-induced apoptosis and degeneration of chondrocytes. Cells were transfected with pcDNA3.1/AEG-1 vector or pcDNA3.1 empty vector for 48 h and then stimulated with 10 μg/ml of LPS for 6 h. (A) Relative mRNA and (B) protein expression levels of AEG-1 were determined by RT-qPCR and western blot analyses. (C) Cell survival was evaluated using the MTT assay. (D) Cell apoptosis was assessed by a caspase-3 activity assay. Relative mRNA expression levels of MMP-3 (E) and MMP-13 (F) were assessed by RT-qPCR analysis. *$p < 0.05$.

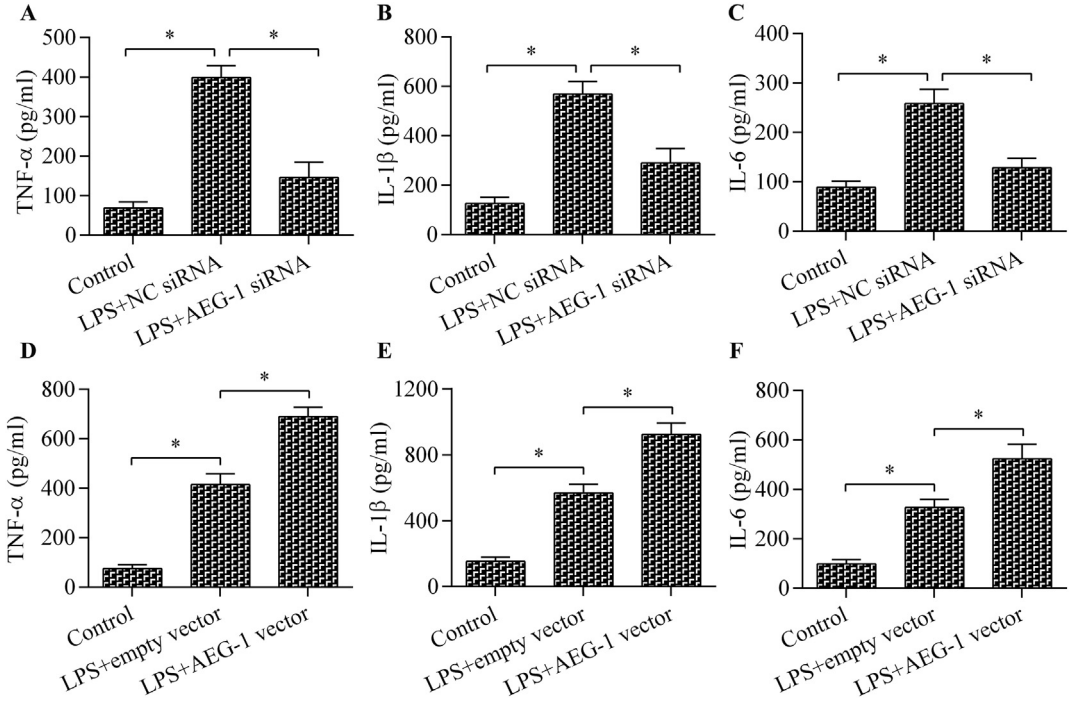

Fig. 4. The expression of AEG-1 regulates LPS-induced release of inflammatory cytokines. The effect of AEG-1 knockdown on the release of TNF-α (A), IL-1β (B), and IL-6 (C) was determined by ELISA. Cells were transfected with AEG-1 siRNA or NC siRNA for 48 h and then stimulated with 10 μg/ml of LPS for 6 h. The effect of AEG-1 overexpression on the release of TNF-α (D), IL-1β (E), and IL-6 (F) was determined by ELISA. Cells were transfected with pcDNA3.1/AEG-1 vector or pcDNA3.1 empty vector for 48 h and then stimulated with 10 μg/ml of LPS for 6 h. *$p < 0.05$.

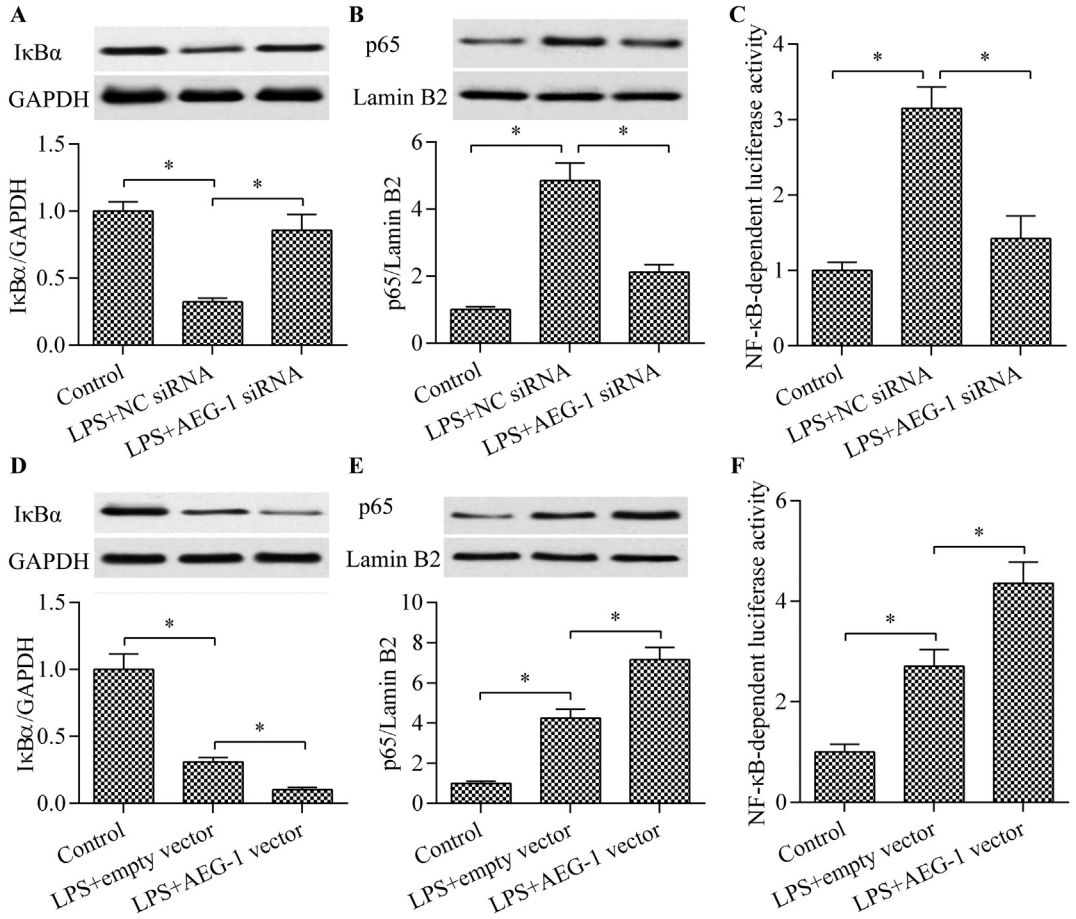

Fig. 5. AEG-1 contributes to regulation of NF-κB signaling in LPS-stimulated chondrocytes. The effects of AEG-1 knockdown on IκBα expression (A) and nuclear expression of NF-κB p65 (B) in LPS-stimulated chondrocytes were determined by western blot. (C) The effect of AEG-1 knockdown on LPS-induced NF-κB signaling activation was monitored by luciferase reporter assay. Cells were transfected with AEG-1 siRNA or NC siRNA for 48 h and then stimulated with 10 μg/ml of LPS for 6 h. The effects of AEG-1 overexpression on IκBα expression (D) and nuclear expression of NF-κB-p65 (E) in LPS-stimulated chondrocytes were detected by western blot. (F) The effect of AEG-1 overexpression on LPS-induced NF-κB signaling activation was assessed by luciferase reporter assay. Cells were transfected with pcDNA3.1/AEG-1 vector or pcDNA3.1 empty vector for 48 h and then stimulated with 10 μg/ml of LPS for 6 h. *$p < 0.05$.

inflammation plays an important role in OA pathogenesis [27]. LPS-induced degeneration and inflammation of chondrocytes has been widely used as an *in vitro* model of OA [28–31]. The present study aimed to explore the potential function of AEG-1 in regulating LPS-induced degeneration and inflammation of chondrocytes *in vitro*.

2. Materials and methods

2.1. Cell culture

The human chondrocyte cell line C28/I2 was purchased from Sigma-Aldrich (St. Louis, MO, USA) and cultured in Dulbecco's Modified Eagle's Medium (DMEM)/F12 medium (Gibco, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS). Cells were maintained in a humidified incubator at 5% CO_2 and 37 °C.

2.2. Cell transfection

AEG-1 siRNA and negative control (NC) siRNA were synthesized by Thermo Fisher Scientific, Inc. (Waltham, MA, USA). The open reading frame of AEG-1 was inserted into a pcDNA3.1 vector to generate AEG-1 expression vector. Cell transfection was carried out using the Lipofectamine 3000 Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Briefly, cells were seeded into a 24-well plate at a density of 1×10^5 cells/well. Transfection was performed when cells reached to 70–90% confluence. After transfection of 48 h, transfection efficacy was determined by real-time quantitative polymerase chain reaction (RT-qPCR) and Western blot analysis. For detection of LPS treatment on AEG-1 expression, cells were treated with LPS (1, 5, and 10 μg/ml; Sigma-Aldrich) for 6 h as previously described [30]. Moreover, 10 μg/ml of LPS treatment was chosen for the following functional experiments of AEG-1. The concentration of endotoxin in original culture medium, FBS and culture dishes was measured before the experiments to ensure the endotoxin is not exceed.

2.3. RNA extraction and RT-qPCR analysis

Total RNA from chondrocytes was isolated using TRIzol (Invitrogen), and reverse transcribed into cDNA using a cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The synthesized cDNA was used as template for RT-qPCR using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) with appropriate primers. The primer sequences were as follows: AEG-1, forwards: 5′-AAATGGGCGGACTGTTGAAGT-3′ and reverse: 5′-CTGTTTTGCACTGCTTTAGCAT-3′; MMP-3, forwards: 5′-CTGGACTCCGACACTCTGGA-3′ and reverse: 5′-CAGGAAAGGTTCTGAAGTGACC-3′; MMP-13, forwards: 5′-TCCTGATGTGGGTGAATACAATG-3′ and reverse: 5′-GCCATCGTGAAGTCTGGTAAAAT-3′; glyceraldehyde 3-phosphate dehydrogenase (GAPDH), forward: 5′-ACAACTTTGGTATCGTGGAAGG-3′ and reverse: 5′-GCCATCACGCC

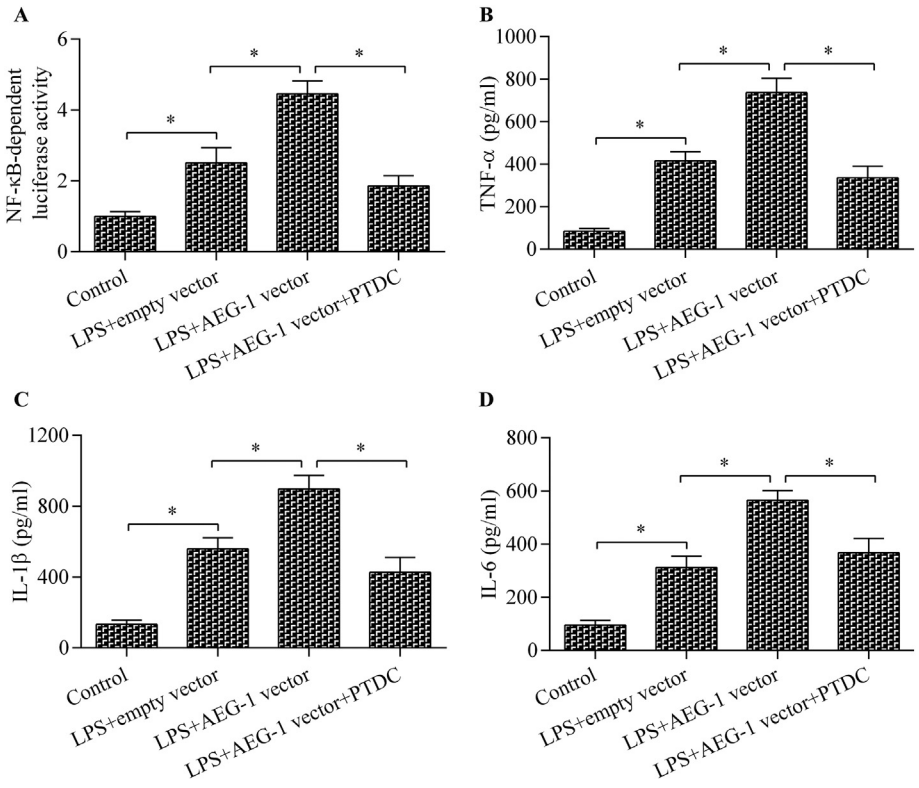

Fig. 6. Inhibition of NF-κB reverses the effects of AEG-1 on LPS-induced inflammation. Cells were transfected with AEG-1 expression vector in the presence of NF-κB inhibitor PTDC for 48 h and treated with 10 μg/ml of LPS for 6 h. (A) The activity of NF-κB was measured by luciferase reporter assay. The release of TNF-α (B), IL-1β (C), and IL-6 (D) was examined by ELISA. *$p < 0.05$.

ACAGTTTC-3′. The PCR reaction was carried out using an Applied Biosystems AB7500 Real-Time PCR system with the following thermocycler program: 10 min at 95 °C then 40 cycles of 15 s at 95 °C, 60 s at 60 °C. GAPDH was used as an internal reference for normalizing the expression of target gene. Data were calculated by the $2^{-\Delta\Delta Ct}$ method.

2.4. Western blot analysis

Total proteins were isolated using radioimmunoprecipitation assay lysis buffer. Nuclear fractions were obtained using a Nuclear and Cytoplasmic Extraction Kit (Beyotime Institute of Biotechnology, Shanghai, China) according to the manufacturer's protocols. Protein expression was measured by Pierce BCA Protein Assay Kit (Pierce, Rockford, IL, USA). Equivalent amounts of proteins from each group were loaded on sodium dodecyl sulfate polyacrylamide gel for separation by electrophoresis. The separated proteins were transferred onto a polyvinylidene difluoride membrane which was then incubated with 5% skim milk in Tris-buffered saline-Tween (TBST) at 37 °C for 1 h to block non-specific binding sites. The membrane was incubated with primary antibodies to AEG-1 (1:1000), NF-κB p65 (1:2000), GAPDH (1:10000), and Lamin B2 (1:1000) (Abcam, Cambridge, MA, USA) and incubated overnight at 4 °C. Then, the membrane was washed with TBST for three times and then incubated with horseradish peroxidase-conjugated secondary antibodies (Abcam) for 1 h at room temperature. Afterwards, the membrane was washed with TBST for three times and protein band was develop using ECL Western Blotting Substrate (Thermo Fisher Scientific, Inc.). Protein band intensity was determined using Image-Pro Plus 6.0 software.

2.5. Cell viability assay

Cell viability was detected using an MTT Cell Viability Assay Kit (RiboBio Co., Ltd., Guangzhou, China) as per manufacturer's instructions. Briefly, cells were plated into 96-well plates at a density of 1×10^4 cells/well and cultured overnight. After the indicated transfection and treatment, the old medium was removed by aspiration and discarded, and fresh medium was added. Then, 10 μl of MTT solution (5 mg/ml) was added to each well and incubated for 4 h at 37 °C. Afterwards, the resulting formazan crystals were dissolved by adding 100 μl of dissolution reagent of the kit. The optical density (OD) value was measured at 570 nm using a microplate spectrophotometer (Molecular Devices, Sunnyvale, CA, USA).

2.6. Cell apoptosis assay

Cell apoptosis was monitored by measuring caspase-3 activity using a commercial colorimetric kit (Beyotime Institute of Biotechnology). A total of 2×10^6 cells were collected after treatment and lysed in 100 μl of lysis buffer of the kit. The supernatants were collected by centrifugation at 4 °C, 16000*g* for 15 min. About 50 μl of supernatants were transferred to a new 96-well plate and treated with 10 μl of 2 mM Ac-DEVD-pNA and 40 μl of detection buffer. After incubation of 2 h at 37 °C, the OD value at 405 nm was determined using a microplate spectrophotometer (Molecular Devices).

2.7. Enzyme-linked immunosorbent assay

The concentrations of TNF-α, IL-1β, and IL-6 in the supernatants obtained from different groups were analyzed using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions.

2.8. NF-κB-dependent luciferase reporter assay

Cells were seeded into 48-well plates at a density of 5×10^4 cells/well and cotransfected with pGL4.32/NF-κB reporter vector (Promega, Madison, WI, USA), pRL-TK plasmid and either AEG-1 siRNA or AEG-1 expression vector for 48 h. After stimulation with LPS for 6 h, luminescent signals were determined using the Dual-Luciferase Reporter Assay System (Promega) as per the manufacturer's instructions. Relative luciferase activity was calculated by normalizing Firefly luciferase activity against Renilla luciferase activity.

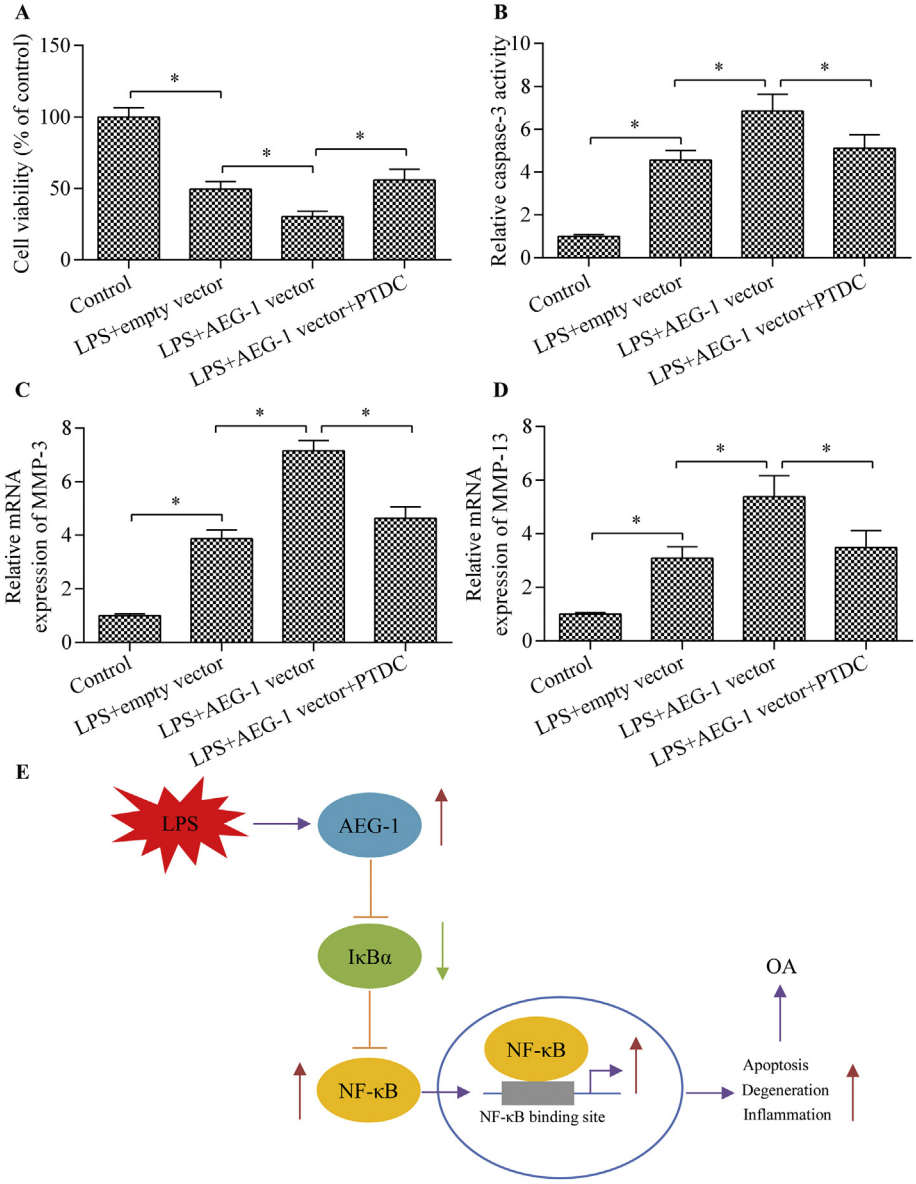

Fig. 7. Inhibition of NF-κB reverses the effects of AEG-1 on LPS-induced apoptosis and degeneration of chondrocytes. Cells were transfected with AEG-1 expression vector in the presence of NF-κB inhibitor PTDC for 48 h and treated with 10 μg/ml of LPS for 6 h. (A) Cell survival was monitored by MTT assay. (B) Cell apoptosis was determined by measuring caspase-3 activity. Relative mRNA expression levels of MMP-3(C) and MMP-13 (D) were detected by RT-qPCR analysis. *$p < 0.05$. (E) A model of AEG-1-mediated NF-κB signaling in the regulation of LPS-induced damage of chondrocytes.

2.9. Statistical analysis

Transfection experiments were performed in triplicate. Data are expressed as means ± standard deviation (SD). Statistical analysis was carried out using SPSS Statistics Version 19.0 software (SPSS Inc., Chicago, IL, USA). Multi-group comparisons were analyzed by one-way analysis of variance (ANOVA) followed by a *post hoc* Bonferroni test. A p value of < 0.05 was considered to indicate a statistically significant difference.

3. Results

3.1. Lipopolysaccharide causes induction of AEG-1 expression in chondrocytes

To investigate the potential role of AEG-1 in LPS-induced inflammatory injury in chondrocytes, we first detected the expression of AEG-1 in LPS-stimulated cells. Analysis by RT-qPCR revealed that expression of AEG-1 mRNA was markedly increased following LPS exposure compared with the control (Fig. 1A). Moreover, the level of AEG-1 protein also increased following LPS stimulation (Fig. 1B). These data indicate that AEG-1 expression is induced by LPS in chondrocytes.

3.2. Suppression of AEG-1 alleviates LPS-induced apoptosis and degeneration of chondrocytes

To explore the function of AEG-1 in LPS-stimulated chondrocytes, we depleted AEG-1 by transfecting specific siRNA. Results of AEG-1 silencing revealed that transfection of AEG-1 siRNA caused significant downregulation of expression in chondrocytes treated with or without LPS (Fig. 2A–B and Supplemental Fig. 1). Stimulation by LPS significantly lowered the survival of chondrocytes and induced apoptosis of the cells. Notably, downregulation of AEG-1 improved the survival and attenuated apoptosis of chondrocytes that was observed following LPS stimulation (Fig. 2C and D). In addition, AEG-1 knockdown also led to a reduction in the LPS-induced expression of MMP-3 and MMP-13

(Fig. 2E and F). Collectively, these data suggest that inhibition of AEG-1 expression alleviates LPS-induced apoptosis and degeneration of chondrocytes.

3.3. Overexpression of AEG-1 exacerbates LPS-induced apoptosis and degeneration of chondrocytes

To validate AEG-1 regulates LPS-induced apoptosis and degeneration of chondrocytes, we performed gain-of-function experiments involving transfection of the pcDNA3.1/AEG-1 expression vector. Overexpression of AEG-1 in chondrocytes treated with or without LPS was confirmed by RT-PCR and western blot analyses (Fig. 3A–B and Supplemental Fig. 2). Results showed that AEG-1 overexpression caused the decrease in survival and increase in apoptosis of chondrocytes treated with LPS (Fig. 3C and D). Moreover, the LPS-induced expressions of MMP-3 and MMP-13 were significantly upregulated by AEG-1 overexpression (Fig. 3E and F). These data confirm that AEG-1 overexpression exacerbated LPS-induced apoptosis and degeneration of chondrocytes.

3.4. LPS-induced inflammatory cytokine release in chondrocytes is regulated by AEG-1 expression

To further investigate the function of AEG-1 in regulating LPS-induced damage of chondrocytes, we measured the regulatory effect of AEG-1 on the release of proinflammatory cytokines, including TNF-α, IL-1β and IL-6. Analysis of proinflammatory cytokines revealed that the LPS-induced release of TNF-α, IL-1β, and IL-6 was significantly attenuated by AEG-1 knockdown (Fig. 4A–C). In contrast, AEG-1 overexpression enhanced the LPS-induced release of TNF-α, IL-1β, and IL-6 in chondrocytes (Fig. 4D–F). These data suggest that AEG-1 expression contributes to the regulation of LPS-induced inflammation in chondrocytes.

3.5. Regulation of the activation of NF-κB signaling in LPS-stimulated chondrocytes by AEG-1 expression

To elucidate the molecular mechanism underlying AEG-1-mediated LPS-induced inflammatory injury in chondrocytes, we investigated the regulatory effect of AEG-1 on the activation of NF-κB signaling, a proinflammatory signaling for LPS-induced damage. Knockdown of AEG-1 expression led to significantly increased IκBα expression and decreased nuclear translocation of NF-κB p65 in LPS-stimulated cells (Fig. 5A and B). Moreover, the LPS-induced activation of NF-κB was significantly reduced following AEG-1 knockdown (Fig. 5C). In contrast, overexpression of AEG-1 had the opposite effect, resulting in an increase in LPS-induced NF-κB signaling (Fig. 5D–F). Collectively, these results suggest that AEG-1 is involved in the regulation of NF-κB signaling in LPS-stimulated chondrocytes.

3.6. Regulation of LPS-induced damage of chondrocytes by AEG-1 via NF-κB signaling

To investigate whether AEG-1 regulates LPS-induced damage of chondrocytes through NF-κB signaling, we detected the effect of NF-κB inhibition on the AEG-1-mediated effect in LPS-induced injury. Results demonstrated that treatment with an NF-κB inhibitor significantly reduced the effect of AEG-1 overexpression on LPS-induced activation of NF-κB signaling (Fig. 6A). Inhibition of NF-κB also partially reversed the AEG-1 overexpression-mediated effect on LPS-induced inflammatory cytokine release (Fig. 6B–D). Moreover, the effects of AEG-1 overexpression on LPS-induced apoptosis and degeneration of chondrocytes were also significantly reversed by NF-κB inhibition (Fig. 7A–D). Overall, these results suggest that AEG-1 contributes LPS-induced damage of chondrocytes through activation of NF-κB signaling.

4. Discussion

Chondrocyte degeneration and inflammation both play an important role in OA pathogenesis. Our study reports that AEG-1 is a novel regulator of chondrocyte degeneration and inflammation. The results presented here reveal that expression of the gene is induced by LPS in chondrocytes, and that high expression contributes to the LPS-induced inflammatory response through activation of NF-κB signaling (Fig. 7E). Our study suggests that AEG-1 may participate in the pathogenesis of OA by modulating NF-κB signaling.

The multifunctional protein AEG-1 is involved in various pathological processes, especially in inflammation related diseases. Expression of AEG-1 in astrocytes is induced by HIV-1, TNF-α, and IL-1β and involved in neuroinflammation, which can lead to excitotoxic neuronal damage [26,32]. Downregulation of AEG-1 is involved in the ginsenoside Rg1-mediated anti-inflammatory capacity in the hippocampus [33]. Upregulation of AEG-1 results in uncontrolled inflammation, which promotes the progression of various cancers, including gastric cancer and hepatocellular carcinoma [34,35]. Notably, AEG-1 ablation renders macrophages functionally anergic, and restricts inflammation during tumorigenesis [36]. Hepatocyte-specific overexpression of AEG-1 has been demonstrated to promote the development of nonalcoholic steatohepatitis induced by a high-fat diet in transgenic mice [25]. AEG-1 expression has been found to be upregulated in a rat model of pulpitis [37]. Moreover, silencing of AEG-1 reduces LPS-induced intestinal inflammation through inhibition of inflammasome activation [38]. These findings suggest that AEG-1 is a critical regulator of inflammatory diseases. However, whether expression of *the gene* is involved in OA remains unknown. In this study, we demonstrated that knockdown of AEG-1 caused a reduction in the LPS-induced inflammatory response in chondrocytes, thus indicating a potential role in OA. A recent study has suggested that AEG-1 is involved in in the inflammatory process of rheumatoid arthritis [39], which is consistent with the hypothesis that AEG-1 dysregulation is involved in joint inflammation. It is reported that AEG-1 expression is highly expressed in synovial tissues of rheumatoid arthritis patients [39]. Notably, AEG-1 inhibition attenuates TNF-α-induced inflammation and MMP-3 expression in fibroblast-like synoviocytes [39].

In-depth molecular analysis revealed the important role of AEG-1 expression in the regulation of NF-κB signaling. Expression of AEG-1 promotes the activation of NF-κB signaling by increasing the degradation of IκBα and the nuclear translocation of p65 [22,23], an effect which is induced by the AEG-1-mediated increase in p65 phosphorylation [40]. Interestingly, Sarkar et al. reported that AEG-1 functioned as a coactivator of NF-κB by interacting with the cyclic AMP-responsive element binding protein [21]. Deletion of AEG-1 impedes tumor progression by a mechanism involving the suppression of NF-κB signaling [24,41,42]. Moreover, AEG-1 knockdown decreases the release of proinflammatory cytokines and the expression of MMPs through inactivation of NF-κB signaling [37,39,43,44]. Consistent with these findings, our results support that AEG-1 is an important regulator of NF-κB signaling. Our study confirmed that expression of AEG-1 led to decreased expression of IκBα and increased nuclear translocation of p65, which then activated the expression of TNF-α, IL-1β, IL-6, and MMP-3/13. However, blockade of NF-κB signaling significantly attenuated AEG-1-mediated chondrocyte degeneration and inflammation. Considering that NF-κB signaling plays an important role in OA [45,46], targeting AEG-1 with the aim of inhibiting NF-κB signaling may represent a novel therapeutic approach for the treatment of OA.

Accumulating evidence suggests that AEG-1 is an LPS-responsive gene and is involved in the LPS-induced inflammatory response. In LPS-stimulated promonocytic cells, AEG-1 expression was induced with consequent promotion of prostaglandin E and TNF-α production *via* the regulation of NF-κB signaling [47]. In human gastric cancer cells, LPS-induced Toll-like receptor 4/AEG-1 signaling decreased the expression of suppressor of cytokine signaling 1 protein and promoted the

activation of NF-κB signaling [34]. Moreover, knockdown of AEG-1 has been shown to impede LPS-induced hepatic fibrosis by regulating the profibrogenic phenotype of hepatic stellate cells [48]. Notably, AEG-1 knockdown downregulates the LPS-induced synthesis of pro-inflammatory cytokines in dental pulp cells by impeding the nuclear translocation of p65 [37]. Consistent with these previous studies, our results demonstrated that AEG-1 expression was induced by LPS in chondrocytes and contributed to the inflammatory response. LPS-induced proinflammation is considered a major hidden risk factor for OA [27]. In addition to LPS, other pro-inflammatory factors, such as IL-1β, TNF-α, and virus infection, also contribute to induction of AEG-1 [26,39], indicating that AEG-1 may be used as a classic pro-inflammation related gene.

In conclusion, our results demonstrate that inhibition of AEG-1 expression alleviates LPS-induced inflammatory injury in chondrocytes *via* inactivation of NF-κB signaling. Our study suggests that AEG-1/NF-κB signaling may play an important role in the pathogenesis of OA. Targeting AEG-1 with the aim of inhibiting NF-κB signaling may have potential application for the development of therapeutics for OA. However, the precise role of AEG-1 in regulating OA requires further study using animal models *in vivo*.

Acknowledgments

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Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.03.006.

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