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Gastrodia elata attenuates inflammatory response by inhibiting the NF-κB pathway in rheumatoid arthritis fibroblast-like synoviocytes

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ABSTRACT

Gastrodia elata (GE), which belongs to the Orchidaceae family, was found to possess anti-inflammatory activity. However, the effect of GE on inflammatory response in rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS) remains largely unknown. Thus, the aim of this study was to investigate the effects of GE on tumor necrosis factor-α (TNF-α)-induced inflammatory response in RA-FLS and the underlying molecular mechanism was also explored. Our results demonstrated that GE significantly attenuated TNF-α-induced IL-6 and IL-8 production in RA-FLS. GE also inhibited TNF-α-induced MMP-3 and MMP-13 expression in RA-FLS. Furthermore, pretreatment with GE significantly attenuated TNF-α-induced the expression of p-p65 and IκBα degradation in RA-FLS. In conclusion, this study demonstrated for the first time that GE attenuated inflammatory response by inhibiting the NF-κB pathway signaling in RA-FLS. Thus, GE might have a therapeutic potential towards the treatment of RA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease and afflicts 0.5–1.0% of population all over the world [1]. It is characterized by inflammation in the joint lining and destruction of cartilage and bone [2]. Despite significant therapeutic advances, there are no effective pharmacological treatments to treat RA [3–5].

Fibroblast-like synoviocytes (FLS) is a specialized cell type located in synovial joints, play crucial roles in the damage, destruction and deformation of cartilage and joints [6]. A growing body of evidence indicates that activated RA-FLS play a central role in both initiating and driving RA [7–9]. In addition, tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine produced by monocytes/macrophage that plays a pathological role in RA. It was reported that TNF-α is abundant in the RA synovium and is necessary for the initiation of chronic and persistent inflammatory responses in RA [10]. Therefore, targeting inflammation may be a crucial therapeutic strategy for preventing and treating RA.

Gastrodia elata (GE), which belongs to the Orchidaceae family, is a traditional Chinese herbal medicine and mainly distributed in the mountainous areas of eastern Asia, such as China, Korea, Japan and India [11]. Many previous studies have demonstrated that GE possesses anti-asthmatic, anti-oxidative, immune-regulation, anti-inflammatory and anti-tumor activities [12–15]. For example, Kim et al. reported that GE extract inhibited lipopolysaccharide (LPS)-stimulated production of inflammatory cytokines in BV-2 cells [16]. However, the effect of GE on inflammatory response in RA-FLS remains largely unknown. Thus, the aim of this study was to investigate the effects of GE on TNF-α-induced inflammatory response in RA-FLS and the underlying molecular mechanism was also explored.

2. Materials and methods

2.1. Cell culture

FLS were isolated from synovial tissues from RA patients as previously described [17]. In brief, tissues were minced and digested with a solution containing 0.15 mg/ml DNase, 0.15 mg/ml hyaluronidase (type I-S) and 1 mg/ml collagenase I (Sigma-Aldrich, St. Louis, MO, USA) in Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL) at 37 °C for 90 min. Then, FLS were cultured in DMEM supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), 100 U/mL penicillin and 0.1 g/mL streptomycin (Sigma, St. Louis, MO, USA), and were used between passages 3 and 8 for all experiments.

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2.2. Preparation of the GE

GE was purchased in the traditional herb market and the ethanol extract of GE was obtained as previously described [18]. The dried GE (400 g) was extracted with 4 l of 95% ethanol at room temperature for 1 week. The extract was filtered through Whatman no. 3 filter paper (Whatman International Ltd., England) and concentrated using rotary evaporator. The resulting extract was lyophilized by using a freeze drier.

2.3. Cell viability assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to detect cell viability. In brief, RA-FLS at a density of 1×10^4 cells/well were treated with different concentrations (1, 5 10 and 20 μg/ml) of GE for 24 h. Then 20 μl of MTT solution (5 mg/ml in PBS; Sigma, St. Louis, MO, USA) was added to each well and incubated for 4 h. The supernatant was removed, and the crystals were dissolved by the addition of dimethyl sulfoxide (100 μl/well; Sigma, St. Louis, MO, USA). The absorbance at 490 nm was determined using an automatic enzyme-linked immunosorbent assay reader (Bio-Rad, Hercules, CA, USA).

2.4. Enzyme-linked immunosorbent assay (ELISA)

RA-FLS were pretreated with different concentrations (1, 5 and 10 μg/ml) of GE for 1 h and exposed to TNF-α (10 ng/ml) for 24 h. The supernatants were collected, centrifuged, and analyzed for the production of IL-6 and IL-8 using commercial ELISA kits (Sigma, St. Louis, MO, USA) according to the manufacturer's instructions.

2.5. Quantitative real-time RT-PCR (qRT-PCR)

Total RNA was extracted from RA-FLS using RNeasy Mini Kits (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed to cDNA using the PrimeScript® cDNA synthesis kit (TaKaRa, Japan) according to the manufacturer's instructions. qRT-PCR was performed using an ABI Prism 7500 System (Applied Biosystems) with the SYBR Green Supermix (Invitrogen, Carlsbad, CA, USA). The sense and antisense primers used as follows: for MMP-3, S: 5′-GCATTGGCTGAGTGAAAGAGAC TGTATC-3′, A: 5′-ATGATGAAC-GATGGACAGATGA-3′; for MMP-13, S: 5′-AGTAGTTCCAAAGGCTA-CAACTTGTTT-3′, A: 5′-GGAGTGGTCAAGCCCTAAGGA-3′; for β-actin, S: 5′-GAT CAT TGC TCC TCC TGA GC-3′, A: 5′-ACT CCT GCT TGC TGA TCC AC-3′. β-actin served as the internal control. Cycle threshold (Ct) values were used to analyze expression data with the $2^{-D\Delta Ct}$ method [19].

2.6. Western blot

RA-FLS were washed with ice-cold PBS and lysed with RIPA lysis buffer (Beyotime, Nantong, China). The protein concentration in the lysates was determined using the Bradford method. Protein aliquots (30 μg) were separated on 12% SDS-PAGE gels and transferred to a PVDF membrane (Millipore, Boston, MA, USA). The membranes were then each blocked with 5% non-fat milk in Tris-buffered saline containing 0.2% Tween 20 (TBST; Invitrogen, Carlsbad, CA, USA), and then incubated with specific primary antibodies [anti-MMP-3, anti-MMP-13, anti-p-NF-κB p65, anti-NF-κB p65, anti-IκBα, and anti-GAPDH] (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4 °C overnight. Subsequently, the blots were incubated with horseradish peroxidase- (HRP-) labelled goat anti-mouse secondary antibody (1:3000 dilution, Santa Cruz Biotechnology) for 1 h at room temperature. The specific protein bands

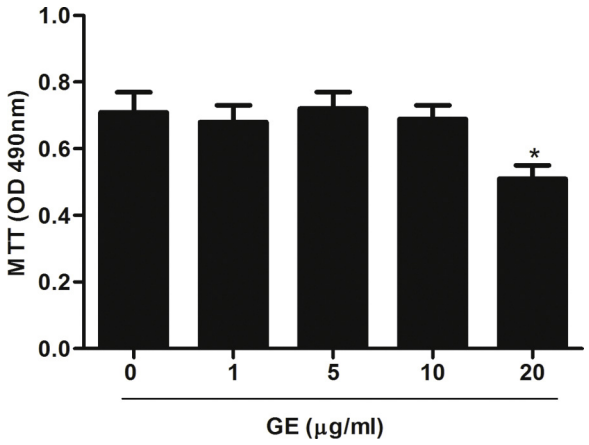

Fig. 1. Effect of GE on RA-FLS viability. RA-FLS were treated with different concentrations (1, 5, 10 and 20 μg/ml) of GE for 24 h. Cell viability was evaluated by the MTT assay. The data are representative of three experiments. *P < 0.05 vs. control group.

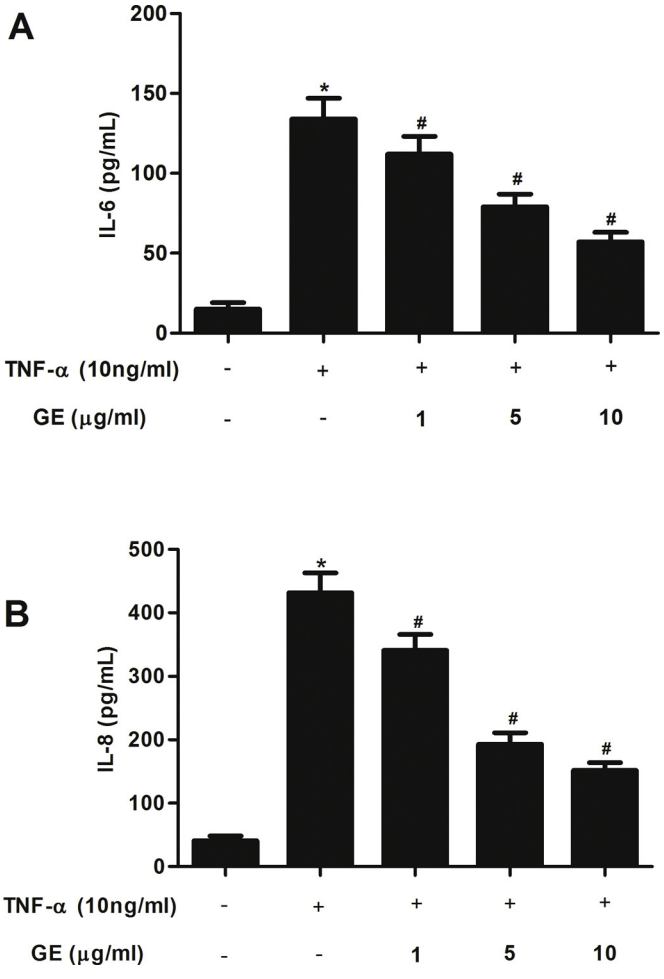

Fig. 2. GE inhibited TNF-α-induced IL-6 and IL-8 production in RA-FLS. RA-FLS were pretreated with different concentrations (1, 5 and 10 μg/ml) of GE for 1 h and exposed to TNF-α (10 ng/ml) for 24 h. (A, B) The production of IL-6 and IL-8 in the cell-free culture supernatants were then evaluated via enzyme-linked immunosorbent assay. The data are representative of three experiments. *P < 0.05 vs. control group, #P < 0.05 vs. TNF-α group.