Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Up-regulation of TRAF2 inhibits chondrocytes apoptosis in lumbar facet joint osteoarthritis

Jiawei Jiang [1], Jinlong Zhang [1], Chunshuai Wu, Xiaofeng Guo, Chu Chen, Guofeng Bao, Yuyu Sun, Jiajia Chen, Pengfei Xue, Guanhua Xu*, Zhiming Cui**

Department of Spine Surgery, The Second Affiliated Hospital of Nantong University, Nantong, Jiangsu, 226001, China

ARTICLE INFO

Article history:
Received 15 July 2018
Accepted 19 July 2018
Available online xxx

Keywords:
TRAF2
Lumbar facet joint
Osteoarthritis
Apoptosis
Tumor necrosis factor-alpha

ABSTRACT

Tumor necrosis factor receptor-associated factor 2 (TRAF2) has been demonstrated that it plays a significant role in cell death receptor signal transduction. The purpose of this study was to investigate the expression of TRAF2 and its possible role in FJOA. We observed an up-regulation of TRAF2 in FJOA by immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR) compared to normal tissues. In vitro, we used TNF-α to stimulate Human SW1353 chondrosarcoma cells to establish the chondrocytes injury model. Western blot analysis revealed significant expression of TRAF2 and cleaved caspase-3/8 in SW1353 cells. Co-localization of TRAF2/cleaved caspase-3/8 was detected in the cells injury model by double-labeling immunofluorescent staining. We demonstrated a possible anti-apoptotic effect of TRAF2 in chondrocyte apoptosis in FJOA by knockdown of its expression with siRNA. Moreover, TRAF2 knockdown was demonstrated to enhance TNF-α-induced apoptosis by flow cytometry assay. In conclusion, our results show that the up-regulation of TRAF2 may play an important role in the inhibition of chondrocyte apoptosis of FJOA.

1. Introduction

Lumbar facet joint osteoarthritis (FJOA) is a spinal joint disease that is characterized by articular cartilages degenerative changes in the lumbar spine [1]. According to reports, 15%–40% of patients with low back pain are caused by FJOA [2,3]. Just like the knee joints, lumbar facet joint is a synovial joint composed of cartilage, synovial membrane, and a joint capsule [1,4]. The occurrence of osteoarthritis (OA) is affected by a number of inflammation-related factors such as tumor necrosis factor-alpha (TNF-α)、interleukin-1β (IL-1β) [5]. In the progression of FJOA, these Pro-inflammatory cytokines have been thought of as a key factor promoting the apoptosis of chondrocytes. Moreover, it has been demonstrated that the activation of caspase-8 and caspase-3 involves in the development of OA [6]. However, lack of basic research for evaluating FJOA and its specific molecular mechanisms have existed for many years. Therefore, it will be necessary and helpful to better understand the molecular mechanisms and function of proteins related to pathological processes of chondrocytes apoptosis for treatment of lumbar facet joint osteoarthritis.

TRAF2, a classical and the most widely expressed member of the TRAF family, is transcribed in almost all tissues. It is also one of the most compelling and well-studied key factors [7,8]. TRAF2 is a common target of pro-inflammatory and tumor-derived factors in the 7 members of the known TRAF superfamily [9], and as an adaptor protein, it can transduce signals after binding to certain cytokine receptors, including those that bind TNF [10]. The family of TNF and its ligands function primarily by binding to their respective receptors. Together, these receptor molecules constitute the TNFR superfamily (TNFRs) such as TNF receptor-2(TNFR2) and TNF receptor-1 (TNFR1). The activation of multiple intracellular signaling cascades can be triggered by both receptors, just like the canonical NF-κB pathway [11,12]. To form the TNFR1 signaling complex I, TNFR1 recruits the TNFR1-associated death domain (TRADD) protein, and TRADD recruits receptor-interacting protein 1 (RIP1) and TRAF2 by its death domain in turn [13,14]. The RING domains of TRAF2 are nearly always related to ubiquitin E3 ligase activity [15] and can advance ubiquitylation of RIPK1 in TNFR1 signaling complexes by recruiting E3 ligases such as Cellular Inhibitor of apoptosis 1 and 2 (cIAP1/2) proteins to TNFR1 signaling

* Corresponding author.
** Corresponding author.
E-mail addresses: xghspine@163.com (G. Xu), zhimingcui@126.com (Z. Cui).
[1] These authors contribute equally to the work.

https://doi.org/10.1016/j.bbrc.2018.07.096
0006-291X/

complex I [16,17]. In addition, the TNFR1 signaling complex II formed by the association of the Fas-associated death domain protein (FADD) and caspase-8 with the TRADD/RIP1/TRAF2 complex dissociating from TNFR1 plays a role in promoting apoptosis in the cytoplasm [14]. The complex, in turn, recruits and leads to the activation of the upstream initiator caspases, such as caspase-2,-8,-9, which activates effector caspases (caspase-3,-6,-7) [18,19]. Meanwhile, TRAF2 can suppress apoptosis by interfering with activating effector caspases downstream of the initial protease caspases [20]. These findings indicate that TRAF2 has the potential to regulate apoptosis, while it is still unknown that its expression profile and possible roles in lumbar facet joint osteoarthritis.

In the current study, we investigated whether TRAF2 could regulate chondrocyte apoptosis and the pathophysiology of FJOA. Considering similar phenotypes between SW1353 cells and human chondrocytes and successful use in previous scientific experiments, We resolved to use the SW1353 cell line for a series of experiments [21]. As far as we know, this is the first research to evaluate the differential expression, localization, and explore the role of TRAF2 in FJOA.

2. Materials and methods

2.1. Clinical samples

Lumbar facet joints were obtained from 38 patients (median age: 51.38 years) at the time of lumbar fusion surgery. The collection of human facet joints was performed with the patients' informed consent and was approved by the Human Ethics Committee of No.2 People Hospital Affiliated to Nantong University. All lumbar facet joints were grouped according to the Weishaupt grade [22], as determined by computed tomography (CT) and magnetic resonance imaging (MRI). Lumbar facet joints were divided into four groups: Grade 0(normal group, n = 8); Grade 1 (mild degeneration group, n = 10); Grade 2(moderate degeneration group, n = 10); Grade 3 (severe degeneration group, n = 10). Part of the Samples were stored at −80 °C after surgery until use. The remaining samples were fixed in 10% neutral formalin-buffered solution and decalcified in EDTA solution, and embedded in paraffin. The tissue sections were obtained by sagittally cutting the articular cartilage of the lumbar facet joints. Cartilage degeneration of lumbar facet joint was evaluated by OARSI Score [23].

2.2. Histological

Hematoxylin and eosin (H&E) staining was used to detect the articular cartilage of the lumbar facet joints in the tissue sections. Lumbar facet joint samples were fixed in 10% neutral formalin-buffered solution. After fixation, the samples were decalcified in EDTA solution which was replaced with the new fluid every day until the pin could be easily pierced, and dehydrated in graded alcohol, and embedded longitudinally in paraffin, then cut into 4-μm tissue sections. The sections were prepared and stained with H&E and examined with a ZEISS optical microscope (Germany).

2.3. Immunohistochemistry

All groups of the sections were baked in a 60 °C incubator for 1 h, deparaffinized in xylene and rehydrated in graded ethanol and washed twice in 0. 01 M PBS for 5min. Pepsase are used as antigen retrieval and sections were incubated with endogenous peroxidase blocking solution for 10 min at room temperature (RT) and incubated with a primary monoclonal antibody against TRAF2 (1:100; Abcam) for 1 h at 37 °C incubator, followed by the biotinylated secondary antibody (Vector Laboratories, USA). Negative control slides were processed in parallel with the antibody-peroxidase system but without primary antibody. All sections were visualized with DAB (Vector Laboratories) and examined by ZEISS optical microscope (Germany).

2.4. RNA isolation and RT-PCR analysis

Total RNA was isolated from collected facet joint tissues by using TRIzol reagent (Invitrogen, Carlsbed, CA) The ThermoScript RT-PCR system (Invitrogen) was used to reverse-transcribe the total RNA. PCR analysis was performed using SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) (TaKaRa) according to the manufacturer's instructions. The primer sequences for qRT-PCR were as follows: the forward primer for TRAF2 was 5′-CACACCTGTCCCTCTTCTTTG-3′ and the reverse primer was 5′-TTCTGGTCGAGCAGCATTAAG-3′, with GAPDH as the internal control. The forward primer for GAPDH was 5′-TGCACCACCAACTGCTTAGC-3′ and the reverse primer was 5′-GGCATGGACTGTGGTCATGAG-3'. Reaction products were run on 1.0% agarose gels and visualized under UV light.

2.5. Cell culture and stimulation

Human SW1353 chondrosarcoma cells (Institute of Biochemistry and Cell Biology, Shanghai, China) were cultured in leibovitz's L-15 medium, supplemented with 10% fetal bovine serum at 37 °C in humidified air with 5% CO2. The cells were passaged every 3–4 days. For stimulation experiments, 20 ng/ml human TNF-α (PeproTech) was added in the cells for 0, 6, 12, 24, 36, or 48 h before harvest [24,25].

2.6. SiRNAs transfection

The TRAF2-siRNA was obtained from Guangzhou RiboBio Co., Ltd. The siRNA targeting TRAF2 sequences were: 5′-GGACCAAGACAAGAUUGAATT-3′, and the control sequences were: 5′-TCGTTGGAGGCCATGTGGGTCAT-3'. The SW1353 cells were transfected with TRAF2-siRNA as per manufacturer's instructions. The transfected cells were collected for the subsequent experiments 48 h after transfection.

2.7. Western blot analysis

After necessary treatment, the protein samples were collected from the SW1353 cells with the cell lysis buffer. 50 μg of total protein was loaded onto 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto a polyvinylidene difluoride (PVDF) membrane. The membranes were blocked with 5% nonfat milk at room temperature for 1 h, and incubated with primary antibodies against TRAF2(anti-rabbit, 1:500; Abcam), cleaved caspase-3 (anti-rabbit, 1:1000; Cell Signaling), cleaved caspase-8 (anti-rabbit, 1:1000; Cell Signaling) or β-actin (anti-rabbit, 1:1000; Santa Cruz) at 4 °C overnight. After incubating with horseradish peroxidase (HRP)-conjugated secondary antibodies, the protein was visualized using an enhanced chemiluminescence kit (ECL, Pierce Company).

2.8. Double immunofluorescent staining

Cells were plated on coverslips (2×10^4 cells/coverslip) in 24-well plates and treated with TNF-α (20 ng/ml) for 36 h. Then co-localization of TRAF2 and cleaved caspase-3/8 in SW1353 cells was assessed with TRAF2 and cleaved caspase-3/8 primary antibody and fluorescence-labeled secondary antibody. Finally the cells were examined with a ZEISS fluorescence microscope (Germany) or Leica Confocal microscope (Germany).

Please cite this article in press as: J. Jiang, et al., Up-regulation of TRAF2 inhibits chondrocytes apoptosis in lumbar facet joint osteoarthritis, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.07.096