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Mechanism of TNF- α inducing apoptosis and autophagy of chondrocytes by activating NF- κ B signal pathway

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ABSTRACT

This study aimed to explore the mechanism of apoptosis and autophagy of chondrocytes induced by tumor necrosis factor α (TNA-α) by activating the NF-κB signal pathway. For this purpose, 24 SD rats were selected for feeding. The knee cartilage was cut by ophthalmology and the chondrocytes were extracted. The chondrocytes were randomly divided into a control group (CG) and an observation group (OG). TNF-α of 50ng/mL was added before the beginning of the study, while the control group did not receive any treatment. The levels of IL-1, IL-6, IL-12, autophagy markers (Atg5, Atg7, LC3II/I), apoptosis-related indexes (Bax, Bcl-2), NF-κB signal pathway-related indexes (p-p65, p65, IκBα) protein expression, mRNA expression and apoptosis rate in chondrocytes were compared in each group. Results showed that the levels of IL-1, IL-6 and IL-12 in the OG were raised than those in the CG. The expression levels of autophagy markers Atg5, Atg7, LC3II/I and mRNA in the OG were reduced than those in the CG. The apoptosis rate and the expression of BaxmRNA and protein in the OG were higher than those in the CG, while the expression of Bcl-2mRNA and protein were lower than those in the CG. The p-p65, p65, IκBα protein and mRNA related to NF-κB signal pathway in the OG were raised than those in the CG. In conclusion, TNF-α can induce apoptosis and autophagy of chondrocytes by activating the NF-κB signal pathway.

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Introduction

Osteoarthritis (OA) is one of the common joint diseases in the elderly. It has been reported that about 10% of elderly men and 18% of elderly women suffer from pain caused by OA for a long time. At present, the main purpose of the treatment of OA is to relieve pain and improve joint function, but it brings huge economic burden to patients and threatens the patients life (1,2). In the past, people thought that OA was only joint damage caused by simple cartilage degeneration. However, with further research, it was found that its onset, progress and outcome were the result of many mechanical mechanisms and inflammatory factors. Its pathophysiological process is extremely complex (3). Chondrocytes play a critical role in regulating the mechanism of mechanical and biochemical responses, releasing a series of inflammatory mediators such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNA-α). Among them, TNA-α is one of the key factors leading to cartilage damage in OA, and it can be differentially expressed in different sites along with the receptor during the onset and progression of OA. Nucleartranscriptionfactor, NF-κB family plays a critical role in biological regulation, including cell differentiation, apoptosis, proliferation and so on. Some scholars believe that TNA-α can further promote the transformation of chondrocytes into mast cells by mediating NF-κB signal pathway, and finally achieve the purpose of destroying articular cartilage structure (4-7). Therefore, this study aims to explore the mechanism of chondrocyte apoptosis and autophagy induced by TNF-α by activating NF-κB signal pathway.

Materials and Methods

Experimental subjects

24 SD rats were purchased from Zhaoyan New Drug Research Center Co., Ltd., weighing (240 ±20) g for about 7 days. The rats were fed in cages, drank and fed freely, and fed day and night. Adaptive feeding was carried out one week before the experiment began.

Mainly reagents and instruments

Reagent

PBS powder purchased from Xiamen Yuhe Chemical Co., Ltd.; Type II collagen purchased from Jinxing Langfang Venus Chemical Co., Ltd.; Fetal Bovine Serum purchased from Shanghai Yiji Industrial Co., Ltd.; Anti-fluorescence quenching tablet purchased from Shanghai Bidingta Biotechnology Co., Ltd.; TNF-α purchased from Shenzhen Zike Biotechnology Co., Ltd.; protein Marker purchased from Shenzhen Kanglixin Biotechnology Co., Ltd. The rapid cell lysis solution was purchased from Shenzhen Sanhe Boda Mechanical and Electrical Technology Co., Ltd., and the BCA protein concentration determination kit was purchased from Weifang Qixiang Biotechnology Co., Ltd.

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Instrument

CO2 constant temperature incubator is purchased from Jinan Xinbexi Biotechnology Co., Ltd.; Clean desk is purchased from Kunshan Kelaisheng Environmental Protection Technology Co., Ltd.; Scroll Mixer is purchased from Xihua instrument Technology Co., Ltd.; ordinary optical microscope is purchased from Zhejiang Nader Scientific instrument Co., Ltd.; inverted microscope is purchased from Shanghai Zhongyong Inspection equipment Co., Ltd.; automatic chemiluminescence analyzer is purchased from Deyang Lida instrument Co., Ltd.

Method

The rats were anesthetized in the abdominal cavity and then disinfected in 75% ethanol solution. 10 minutes later, the cartilage tissue of the knee joint was cut off by ophthalmology and cut into small fragments with a width of about 2 mm. Put the small fragments of cartilage into the centrifuge tube, rinse with PBS solution, then centrifuge, and remove the supernatant. The bone and joint fragments were digested again with 0.25% trypsin, centrifuged 1 hour later, and the supernatant was removed. Add 0.2% type II collagenase and put it in a constant temperature incubator and blow it every 30 minutes until the fragments are completely dissolved. When most of the cells were free, the cells were filtered and collected by stainless steel screen and centrifuged to remove the supernatant in a cryogenic centrifuge; added to the medium containing 10% fetal bovine serum and blown the cell suspension to make it evenly distributed; it was planted in the culture bottle (containing polylysine) with the concentration of 1x106, the liquid was replaced, and the non-adherent cells were removed at the same time, and the cells were taken for experiment.

Chondrocytes were randomly divided into control group and experimental group. TNF- α of 50ng/mL was added in the experimental group before the beginning of the study, while the control group did not receive any treatment.

The levels of IL-1, IL-6 and IL-12 in chondrocytes of the two groups were determined by ELISA.

The expression of autophagic markers [Atg5, Atg7, LC3II/I], NF-κB signal pathway related indexes (p-p65, p65, IκBα), NF-κB signal pathway related indexes (p-p65, p65, IκBα) and mRNA in chondrocytes of the two groups were determined by western blot and RT-PCR methods, respectively.

The ability of chondrocyte apoptosis in the two groups was measured by Calcein-AM/PI double staining, and the expression of apoptosis-related indexes (Bax, Bcl-2) protein and mRNA were measured by western blot and RT-PCR methods.

Statistical methods

The levels of inflammatory factors and apoptosis rate in the two groups were expressed by ($\bar{x}\pm s$), and the t-test was used for comparison between the two groups. All data in this study were analyzed by SPSS23.0.

Results

The levels of inflammatory factors in each group

The IL-1, IL-6 and IL-12 in the OG were raised than those in the control group (P<0.05) (Table 1).

The protein and mRNA levels of autophagy markers between the two groups

The expression levels of autophagy markers Atg5, Atg7, LC3II/I and mRNA in the OG were reduced than those in the CG (P<0.05) (Figure 1, Table 2).

Comparison of apoptosis rate, expression of apoptosis-related proteins and mRNA between the two groups

The apoptosis rate and the Bax mRNA and protein in the OG were raised than those in the CG (P<0.05), while the expression of Bcl-2mRNA and protein were reduced than those in the CG (P<0.05) (Figures 2 and 3, Table 3).

Protein expression and mRNA comparison of NF-κB signal transduction pathway

The p-p65, p65, IκBα protein and mRNA related to NF-κB signal pathway in the OG were raised than those in the CG (P<0.05) (Figure 4, Table 4).

Table 1. The levels of inflammatory factors in each group ($\bar{x}\pm s$).

Group	IL-1 (pg/mL)	IL-6 (pg/mL)	IL-12 (pg/mL)
CG	176.17±11.87	62.35±21.12	49.52±9.07
OG	489.07±30.89	229.84±24.09	168.02±22.46
t	13.372	7.394	6.919
P	0.006	0.018	0.020

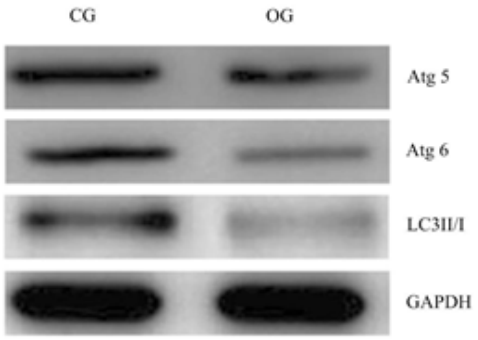

Figure 1. Comparison of autophagy marker protein expression levels in two groups of cells.

Table 3. Comparison of cell apoptosis rate, apoptosis-related protein expression and mRNA between the two groups ($\bar{x}\pm s$).

Group	Apoptosis rate (%)	Bax mRNA	Bcl-2 mRNA
CG	18.56±1.90	0.36±0.05	1.00
OG	46.34±5.71	0.98±0.12	0.13±0.06
t	6.528	6.745	20.227
P	0.023	0.021	0.002

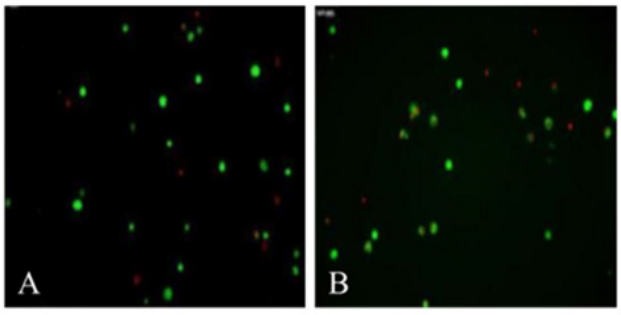

Figure 2. Comparison of cell apoptosis between two groups.

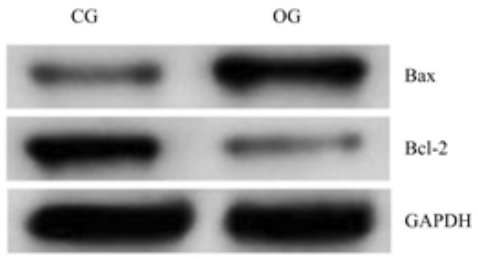

Figure 3. Comparison of apoptosis-related protein expression between the two groups.

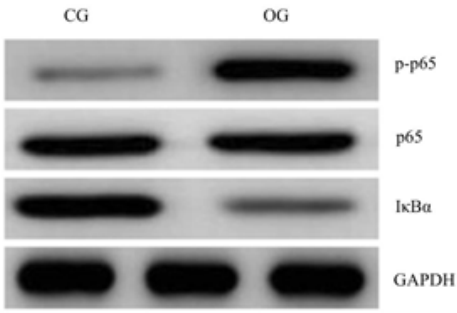

Figure 4. Comparison of protein expression of the related index of NF-κB signaling pathway.

Table 4. Comparison of mRNA expression and related index protein of NF-κB signaling pathway ($\bar{x}\pm s$).

Group	p-p65 mRNA	p65 mRNA	IκBα mRNA
CG	1.31±0.25	0.40±0.14	0.65±0.21
OG	0.38±0.13	0.39±0.13	0.33±0.10
t	4.667	0.050	4.378
P	0.043	0.948	0.048

Discussion

In OA, autophagy and apoptosis of chondrocytes play a critical role. As part of the pathological process of OA, many inflammatory mediators can induce the release of different types of inflammatory mediators, and further cause a cascade of inflammatory reactions around chondrocytes. TNF-α is the key factor inducing the disease (8,9). Clinical studies have shown that blocking the expression of TNF-α is beneficial in alleviating the degeneration of extracellular matrix in human chondrocytes. In addition, overexpression of TNF- α can lead to the failure of autophagy, the release of reactive oxygen species and nitric oxide, and finally cell death. In this study, inflammatory factors were detected by ELISA. The results showed that the IL-1, IL-6 and IL-12 in the OG were raised than those in the CG. It is suggested that TNF-α in chondrocytes can promote the release of IL-1, IL-6 and IL-12 inflammatory mediators. It is important to maintain the normal physiological function and metabolism of chondrocytes through autophagy. In addition, autophagy can regulate the final stage of the cell cycle and promote chondrocyte differentiation. Atg5 and Atg7 are important proteins that can affect the process of autophagy. Clinical studies have shown that the risk of age-related OA is increased after knockout of mouse chondrocytes Atg5 (10-13). LC3 plays a critical role in the process of autophagy. Cytoplasmic LC3 can be transformed into membrane LC3 by enzymatic hydrolysis, and finally achieve the purpose of initiating autophagy. In this study, western blot and RT-PCR methods were used to detect the expression of autophagy-related proteins and mRNA. The expression levels of autophagy markers Atg5, Atg7, LC3II/I and mRNA in the OG were reduced than those in the CG. It is suggested that TNF-α can inhibit the expression of autophagy markers in chondrocytes.

Apoptosis is a normal physiological phenomenon of cells, which is strictly regulated by multiple genes. If the apoptosis process is disordered, it can lead to a variety of diseases (14,15). It has been reported that excessive apoptosis of chondrocytes is involved in the destruction process of OA. If the disease develops to a late stage, it can lead to a decrease in chondrocytes on the articular surface and lead to the formation of cavities. Bcl-2 and Bax are anti-apoptotic cells and pro-apoptotic cells respectively. Both of them are formed in mitochondria and play an important role in the control of apoptosis. In this study, apoptosis, apoptosis-related protein and mRNA in each group were determined by Calcein-AM/PI double staining, western blot and RT-PCR methods. The apoptosis rate, BaxmRNA and protein expression in the OG were raised than those in the CG, while the expression of Bcl-2mRNA and protein were reduced than those in the CG. It is suggested that TNF- α can promote apoptosis in chondrocytes. NF-κB can participate in the pathophysiological process of OA. Clinical studies have shown that it can be activated by inflammatory factors to participate in chondrocyte destruction and apoptosis (16-18). P65 and its phosphorylated form are members of the NF-κB family. As an inhibitor, IκBα binds to NF-κB, resulting in the inhibition of NF- κ B function. If stimulated by inflammatory factors, I κ B α is phosphorylated and finally activates the NF-κB signal pathway (19-22). The p-p65, p65, IκBα protein and mRNA related to NF-κB signal pathway in the OG were raised than those in the CG. It is suggested that TNF-α can activate the NF-κB signal pathway.

In a word, TNF-α can induce apoptosis and autophagy of chondrocytes by activating the NF-κB signal pathway.

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