Effect of TGF-β/Smads signaling in patients with osteoarthritis and osteoarthritis rat model

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Abstract: Osteoarthritis (OA) is serious to elderly health with complex pathogenesis, mainly including genetic, inflammation, environment, and other factors. TGF-β is an important regulatory factor that plays a critical role in cell proliferation, differentiation, and apoptosis. It was showed that the TGF-β has the vital significance in maintaining the normal articular cartilage function and joint repair. Smads is a key pathway, regulated by TGF-β, whereas its role in OA development is still unclear. A total of 52 cases of OA patients and 18 amputees because of traffic accident were enrolled. Joint cartilages obtained from surgery were stored in liquid nitrogen. Real time PCR was applied to test Smads and TGF-β1 mRNA expressions in patients and rat model. No statistical difference was observed on clinical information including gender, age, blood pressure, and BMI between OA and healthy control. Smad2, Smad3, and TGF-β1 mRNA expressions were significantly up-regulated in OA patients compared with control. Smad2, Smad3, and TGF-β1 mRNA levels were significantly increased in OA rat model compared with normal control following course prolongation. TGF-β1 mRNA expression exhibited positive correlation with Smad2 and Smad3 mRNA levels. TGF-β/Smads signaling pathway may be involved in OA progress. TGF-β1 could be treated as potential therapeutic target for OA.

Keywords: TGF-β/Smads, osteoarthritis, rat model

Introduction

Osteoarthritis (OA) is a chronic disease characterized as articular cartilage degeneration associated with or without synovitis and osteophyte. It shows higher incidence in the elderly and correlated with age and work in most cases. In 2010, knee arthritis affects 3.64% of population in the world and accounts for the fastest speed leading to disability [1]. Though the quality of life of patients with joint system disease has been greatly improved following the elevation of diagnosis and treatment level, there are still some patients can’t obtain effective cure, leading to disability.

Transforming growth factor-β (TGF-β) is an important growth factor repairing articular cartilage in arthritis [2, 3]. It promotes cartilage cells and osteoblasts proliferation and differentiation in both of healthy people and patients [4]. Current study also confirmed that Smad transduction has a critical role in TGF-β induced cartilage cells proliferation and differentiation [4-6]. However, the specific mechanism of its role in regulating the formation of cartilage is still unclear. TGF-β has a regulatory role in articular proliferation. Some scholars considered that different isomer exhibited different functions on cartilage tissue. TGF-β1 has a key role in regulating cartilage cell proliferation [7], while TGF-β2 and TGF-β3 increase in serum are positively correlated with pain, function, and radiological staging in OA patients [8]. The previous study showed that TGF-β accelerated the proliferation of cells in S phase, whereas inhibited proliferation of cells in G1 phase [9].

TGF-β family could be induced to cells through receptor I and II or Smads. It was found that Smad2 highly expressed in proliferated cartilage while Smad3 mainly expressed in mature cartilage [10]. It suggested that TGF-β/Smads played an important role in the development or proliferation process of bone joint. Therefore, this study analyzed the TGF-β/Smads expression of the OA patients.
Materials and methods

Main reagents and instruments

Trizol reagent was purchased from Invitrogen. Real time PCR kit was purchased from Takara. SYBR Green qPCR mix was purchased from ToYoBo. ABI 7500 real time PCR was purchased from Life Tech. High speed freezing centrifuge was purchased from Eppendorf. Protein electrophoresis system and transfer system were purchased from Invitrogen. BCA protein quantification kit was purchased from Sigma-Aldrich. Ultraviolet spectrometry photometer was purchased from Lengguang Tech.

Sample collection

A total of 52 cases of OA patients and 18 amputees because of traffic accident were enrolled in Jiulongpo district people’s hospital between Jun 2014 and Mar 2016. Joint cartilage obtained from surgery were stored in liquid nitrogen. The research was approved by the Ethic Committee of Jiulongpo district people’s hospital. All participants were required to sign the informed consent form.

Rat knee arthritis model establishment

SD rats in SPF grade and weighted 213.4±25.6 g were provided by Sun Yat-sen university laboratory animal center. The rats were used for experiment at 2 weeks after quarantine. A total of 30 rats were randomly divided into normal control and OA model. OA model was established according to Hulth method. The left knee accessory ligaments, anterior and the posterior cruciate ligament, and meniscus were cut off. Penicillin was injected for three days. The rats were killed after 6 weeks. HE staining was applied to observe knee arthritis preparation.

Morphology observation

The knee joint was exposed after killing the rat. The joint was fixed in 4% paraformaldehyde and decalcified in 10% EDTA for three weeks. After paraffin embedding, the tissue slice was stained by HE.

Methods

Joint soft tissue RNA extraction: The rats were killed at different time points (0, 4, 12 weeks) after modeling. The tissue was grinded in liquid nitrogen and cracked by Trizol. Total RNA was extracted upon phenol-chloroform method. The RNA was dissolved in DEPC water and quantified by ultraviolet spectrometry photometer. Next, RNA integrity was identified in 1% agarose gel electrophoresis. RNA with A260/280 nm between 1.8-2.0 was stored at -80°C and used for the following experiments.

Real-time PCR: Total RNA was reverse transcribed to cDNA using the kit from Takara. PCR reaction system contained 9 μl 2 × SYBR Green Mixture, 2 μl Primer 1 (5 μM), 2 μl Primer 2 (5 μM), 2 μl DNA, and 5 μl ddH2O. PCR reaction contained 95°C for 30 s, followed by 40 cycles of 95°C for 30 s, 55°C for 30 s, 55°C for 30 s, and 72°C for 60 s. The primers used were as Table 1.

Statistical analysis

All data analysis was performed on SPSS 13.0 software. T test was adopted for independent data comparison. Chi-square test was applied for enumeration data comparison. Correlations between the variables were tested by using the Spearman’s rank correlation rho (r) and Wilcoxon Mann-Whitney tests. P < 0.05 was depicted as statistical significance.

Results

Knee joint pathological observation

HE staining showed that cartilage cells presented as flat or circular in the normal articular cartilage. The boundary between calcification and cartilage layer was clear. Articular cartilage matrix hyperplasia, narrow matrix gap, fewer hyperplasia cells, and disturbance were observed in knee arthritis model group. Cartilage

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
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<tbody>
<tr>
<td>Smad2 Forward: 5’-CCACTACGGGTGGAGA-3’ Reverse: 5’-CTGCTGGAATTGTGT-3’</td>
<td></td>
</tr>
<tr>
<td>Smad3 Forward: 5’-GAGACATCTCTCCCTCCTC-3’ Reverse: 5’-GCCACAGAGATTCCATGCC-3’</td>
<td></td>
</tr>
<tr>
<td>TGF-β1 Forward: 5’-TGCGGTGAGATGGGCAA-3’ Reverse: 5’-AGTAACGCGGAGATTGTGCTA-3’</td>
<td></td>
</tr>
<tr>
<td>GAPDH Forward: 5’-GGCACAGTCAAGGCTGAAATG-3’ Reverse: 5’-ATGGTGAGTGGAAGCCAGTA-3’</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Primer sequences of Smad2, Smad3, and TGF-beta

TGF-β/Smads in OA
cells metaplasia was found, and no clear dividing line was observed between calcification and cartilage layers (Figure 1).

**TGF-β1, Smad2, and Smad3 mRNA expression in cartilage tissue from patients**

No statistical difference was observed on clinical information including gender, age, blood pressure, and BMI between OA patients and healthy control ($P > 0.05$, Figure 2; Table 2).

**TGF-β1, Smad2, and Smad3 mRNA expression in OA rat model**

Smad2, Smad3, and TGF-β1 mRNA levels obviously increased in OA rat model after 4 weeks compared with normal control. Their expressions reduced after 12 weeks (Figure 3).

**TGF-β1 mRNA expression was positively correlated with Smad2 and Smad3 mRNA expression**

The results showed that TGF-β1 mRNA expression was positively correlated with the Smad2 and Smad3 mRNA expressions in different time course (Table 3).
OA is complicated disease that seriously affects people’s health and quality of life. The leading role among its pathogenesis is still unknown at present. TGF-β is a known inflammatory factor that plays a critical role in a variety of inflammation and related diseases [11, 12]. TGF-β trimer can form tetramer with intracellular Smad2 or Smad3, thus completes the signal transduction in the nuclei [13]. Multiple growth factors including TGF-β1, IGF-1, and BMP-2 have been investigated in the process of cartilage occurrence and development [14]. However, the specific mechanism is still unclear. Our results showed TGF-β1 exhibited elevation in OA patients. Mouse experiment revealed that inhibition of TGF-β1 signaling pathway can protect the knee joint from arthritis [15]. Another study demonstrated that injection of engineering cartilage cells containing TGF-β1 to knee arthritis patients can improve the clinical effect, slow the disease progress, promote pain relief, and improve function [16]. Thus, it is speculated that the aim of TGF-β1 up-regulation in OA patients is to promote tissue self-healing, which is similar with Aref-Eshghi E results [17]. Moreover, it reported that TGF-β1 expression was positively correlated with Smad3 mRNA. It was showed that TGF-β1 and IL-1 can regulate miR-29 family, which can negative-ly regulate Smad and Wnt signaling pathway in cartilage from OA [18]. Our results suggested that TGF-β1, Smad2, and Smad3 mRNA obviously increased in OA, while the possible signaling pathway was still unclear. It is speculated that TGF-β1 plays a key role in OA occurrence through regulating Smad expression.

Due to the complexity and uncontrollability of clinical patients, this study applied rat OA model for investigation. TGF-β1, Smad2, and Smad3 mRNA elevated in the process of OA modeling and lasted for a period of time. However, their levels exhibited reducing trend following time extension. The benefits and inhibition of TGF-β in OA have been widely reported. TGF-β/Smad signaling pathway is extremely necessary in maintaining articular cartilage [19], and its polymorphism is associated with the occurrence of knee arthritis. According to mRNA expression trend in our results, it is speculated that TGF-β/Smad played a repair role in the early phase of OA. TGF-β/Smad expression was gradually down-regulated because of the reduction of normal cells. However, its specific regulatory role in OA still needs further exploration. TGF-β can activate ALK-5 or ALK/type I receptor, thus to affect Smad2/3 or Smad1/5/8 expression. It was revealed that ALK5/Smad2/3 signaling pathway can impact cartilage cell differentiation [20, 21].

A variety of signaling pathways are involved in the process of cartilage formation, thus to form a complex network of biological molecules. For instance, BMP plays an important role in OA formation participated by TGF-β [22-24]. TGF-β has been proved to be treated as a potential therapeutic target of OA [25]. Our results indicated that TGF-β/Smad signaling pathway plays
a critical role in cartilage cell proliferation, which may be one of the important pathogenesis of OA.

Conclusion

TGF-β/Smads showed statistical different expression in OA progress. TGF-β, Smad2, and Smad3 exhibited first increased then decreased expression trend in rat OA model, indicating that TGF-β/Smads participated in the occurrence of OA with divergent function in different stages.

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Disclosure of conflict of interest

None.

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