CORRELATION BETWEEN B - CATENIN SIGNALING PATHWAY AND CHONDROCYTE APOPTOSIS AND OSTEOARTHRITIS IN SOST TG MICE

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ABSTRACT

Objective: *To investigate the relationship between the β-catenin signalling pathway, chondrocyte apoptosis and osteoarthritis in osteosclerotin (SOST) transgene (TG) mice.*

Methods: *Sixty specific pathogen–free healthy SOST TG mice were selected and divided into normal, moderate osteoarthritis (moderate group) and severe osteoarthritis groups (severe group) according to the modified Mankin articular cartilage pathological score. The changes in articular cartilage, chondrocyte pathological changes, chondrocyte apoptosis and β-catenin expression in chondrocytes were compared.*

Results: *Compared with the normal group, the Mankin score in the moderate and severe groups was increased significantly, and with the aggravation of osteoarthritis, the Mankin score increased gradually (P<0.01). In the normal group, the articular cartilage surface was smooth, with normal thickness, uniform safranine O staining and there was no loss of proteoglycan in the femoral condyle and tibial plateau. In the moderate group, the articular surface was abraded and proteoglycan was lost, but it was lighter than that in the normal group. In the severe group, the non-calcified cartilage in the tibial plateau and femoral condyle essentially disappeared, and there was severe loss of proteoglycan. In the normal group, the surface of chondrocytes was complete, the structure was clear, and the arrangement was orderly. In the moderate group, the chondrocytes were hypertrophic, and the arrangement was disordered. In the severe group, the chondrocyte surfaces were damaged to varying degrees, and the chondrocytes were significantly reduced. Compared with the normal group, the positive rate of chondrocyte apoptosis in the moderate and severe groups was significantly higher (P<0.01). The normal group had fewer β-catenin staining–positive cells. In the moderate group, the β-catenin staining–positive cells were more concentrated on the cartilage surface. In the severe group, there were numerous β-catenin staining–positive cells on the surface and deep cartilage. Compared with the normal group, the moderate and severe groups had significantly increased β-catenin–positive cells (P<0.01). Pearson correlation analysis showed that the β-catenin expression level and the positive rate of chondrocyte apoptosis correlated positively with Mankin score. A higher Mankin score indicated a higher β-catenin expression level and positive rate of chondrocyte apoptosis (P<0.05).*

Conclusion: *The expression level of β-catenin and the positive rate of chondrocyte apoptosis in patients with osteoarthritis are significantly higher than those in normal people, and participate in the development of osteoarthritis.*

Keywords: *β-Catenin signalling pathway, chondrocyte apoptosis, SOST, TG mice, osteoarthritis, correlation.*

DOI: 10.19193/0393-6384_2020_6_507

Received *November 30, 2019;* ***Accepted*** *January 20, 2020*

Introduction

Osteoarthritis is a bone and joint degenerative disease that is common in the middle-aged and the elderly. It mainly occurs in the knee joint, hip joint, spine and other parts, with pain and joint dysfunction as the main manifestations. Long-term pain can induce local inflammatory reaction of the joint soft tissue, aggravate cartilage damage and greatly reduce quality of life[1]. Abnormal apoptosis of the articular chondrocytes and progressive destruction of the articular cartilage are the main pathological features of osteoarthritis, which may occur in the whole soft tissue of the joint. It has been suggested that the β-catenin signalling pathway and chondrocyte apoptosis are closely related to the pathogenesis of osteoarthritis. The β-chain protein (i.e. β-catenin) signalling pathway is widely recognized in the initial formation and development of embryos, and plays an important role in maintaining the homeostasis of bone and cartilage. β-Catenin is a multifunctional protein that plays an important role in the differenti-

ation, proliferation and apoptosis of bone cells[2]. In osteoarthritis, β-catenin is overactivated, which disrupts the metabolic balance of the bone system and destroys cartilage tissue, further aggravating the development of osteoarthritis. The abnormal apoptosis of chondrocytes, and extracellular matrix synthesis and degradation are important pathological characteristics of osteoarthritis, but the matrix regulating apoptosis of chondrocytes is unclear[3-4]. Here, the purpose of the present study is to explore the relationship between the β-catenin signalling pathway, chondrocyte apoptosis and osteoarthritis and to provide a theoretical basis for early diagnosis and treatment of osteoarthritis.

Materials and methods

Experimental animals

Sixty specific pathogen–free (SPF) osteosclerotin transgenic (SOST TG) mice (provided by Guangzhou Weibaxin Biotechnology Co., Ltd.) weighing 20.35±2.12 g.

Main instruments and reagents

Low-temperature high-speed centrifuge (Sigma, Germany, model: 3-18k); low-speed centrifuge (Changsha Xiangrui Centrifuge Co., Ltd., model: dt5-1b); 0.9% sodium chloride injection (Chengdu Qingshan Likang Pharmaceutical Co., Ltd., specification: 100 ml: 0.9 g, production batch number: 2011120917); low-temperature refrigerator (Qingdao Haier Group, model: bcd-470wdpg); incubator (Shanghai Hetian Science Instrument Co., Ltd., specification: hh-us); electron microscope (Shanghai Optical Instrument Factory, specification: xsp-63xdv); optical microscope (Shanghai Puhe Biotechnology Co., Ltd., model: bx53); slicer (Zhejiang Jinhua HuaSu Technology Co., Ltd.); fetal bovine serum (Shanghai Laichuang Biotechnology Co., Ltd., specification: z7185fbs-100); bovine serum albumin (Shanghai Haoran Biotechnology Co., Ltd.); safranin O powder (Shanghai Hengyuan Biotechnology Co., Ltd.); solid green powder (Best Reagent); β-catenin antibody (Shanghai Xinyu Biotechnology Co., Ltd.); tumour necrosis factor (TNF)-α detection kit (Moshak Biotechnology Co., Ltd.); papain (Shanghai Ruji Biotechnology Development Co., Ltd.); L-cysteine (Shanghai Jining Biotechnology Co., Ltd.).

Grouping and experimental methods

All mice were fed in an environment of 20±3°C, 58±12% humidity, 12-h light–dark alternation and free feeding and drinking water, and adaptive feeding for 4 weeks. A mixture containing 5% papain and 0.04 mol/L-cysteine (25 μL) was injected into the left knee joint cavity of the observation group and the control group at the same time. After 3 and 6 weeks of modelling, 75% ethanol was used to disinfect the skin of the knee joint, the hair around the knee joint was shaved and the knee joint was removed. The muscle tissue around the knee joint was removed, fixed, decalcified, dehydrated with alcohol, paraffin-embedded and sectioned for preservation.

According to the modified Mankin articular cartilage pathological score[5], the mice were divided into normal, moderate osteoarthritis (moderate group) and severe osteoarthritis groups (severe group), with 20 mice in each group.

The sections were dewaxed, stained, dehydrated and sealed with he and safranin O/solid green powder, and observed under a microscope.

β-Catenin was stained using immunohistochemistry. After the section was removed, it was rinsed with phosphate-buffered solution (PBS) three times at 3 min/time, with diaminobenzidine (DAB) for 3-5 min, restained with haematoxylin, dehydrated, cleared and sealed.

Observation indicators

The changes in articular cartilage were observed via the safranin O/solid green staining. He staining was used to observe the pathological changes of chondrocytes. Chondrocyte apoptosis was detected by terminal deoxynucleotidyl transferase (TDT)-mediated dUTP nick end labelling (TUNEL).

Statistical methods

SPSS 21.0 was used for statistical data analysis, and single-factor and multiple-sample means were used for measurement data comparison; the χ^2 test was used for counting data comparison. Pearson correlation test was used for correlation analysis. The statistical results showed that $P<0.05$ was statistically significant.

Results

Comparison of Mankin scores of articular cartilage improvement in each group

Compared with the normal group, the Mankin score in the moderate and severe groups was decreased significantly, and increased gradually with the aggravation of osteoarthritis; the difference was statistically significant ($P<0.01$) (Table 1).

Group	n	Mankin score	*P*-value, normal group vs. moderate group	*P*-value, normal group vs. severe group	*P*-value, moderate group vs. severe group
Normal	20	0.56±0.52			
Moderate	20	7.76±1.08	<0.001	<0.001	<0.001
Severe	20	11.94±1.43			

Table 1: Comparison of modified Mankin scores of articular cartilage of mice in each group ($\bar{x}\pm s$).

Changes of articular cartilage in each group

The articular cartilage surface of the normal group was smooth, with normal thickness, uniform safranine O staining, and there was no loss of proteoglycan in the femoral condyle and tibial plateau. In the moderate group, there was wear on the articular surface and proteoglycan loss, but it was lighter than that in the normal group. In the severe group, the non-calcified cartilage of the femoral condyle and tibial plateau had essentially disappeared, and proteoglycan loss was severe (Figure 1).

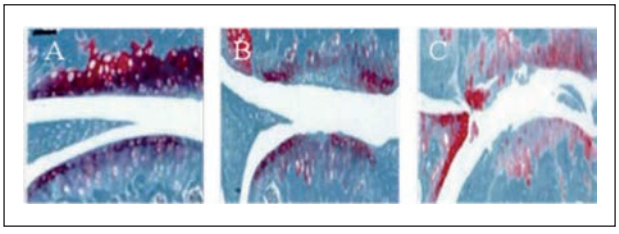

Figure 1: Changes of articular cartilage in each group. *A: Normal group; B: moderate group; C: severe group.*

Pathological changes of chondrocytes in each group

In the normal group, the surface of chondrocytes was complete, the structure was clear, and the arrangement was orderly. In the moderate group, chondrocytes were hypertrophic, and the arrangement was disordered. In the severe group, the surface of chondrocytes was damaged to different degrees, and the chondrocytes were significantly reduced (Figure 2).

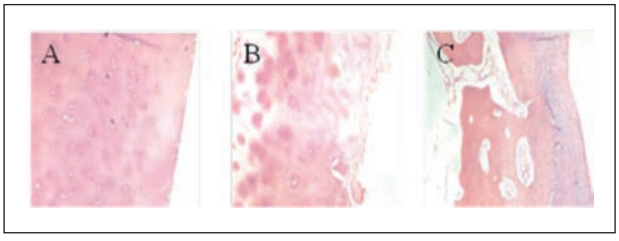

Figure 2: Changes of chondrocytes in each group. *A: Normal group; B: moderate group; C: severe group.*

Apoptosis of chondrocytes in each group

The positive substance of TUNEL is located in the nucleus, and is a purple-blue particle. The moderate and severe groups had significantly more positive cells than the normal group (Figure 3). Compared with the normal group, the positive rate of chondrocyte apoptosis was significantly higher in the moderate and severe groups (P<0.01) (Table 2).

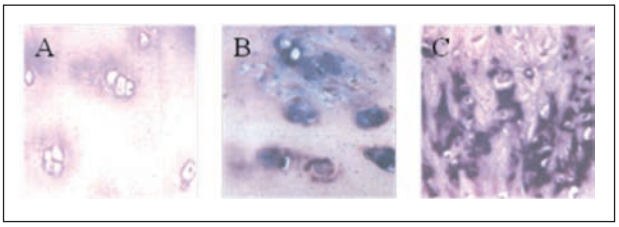

Figure 3: Apoptosis of chondrocytes in each group. *A: Normal group; B: moderate group; C: severe group.*

Group	n	Apoptosis-positive rate of chondrocytes	*P*-value, normal group vs. moderate group	*P*-value, normal group vs. severe group	*P*-value, moderate group vs. severe group
Normal	20	0.03±0.02			
Moderate	20	0.31±0.13	<0.001	<0.001	<0.001
Severe	20	0.52±0.06			

Table 2: Comparison of apoptosis-positive rate of chondrocytes in each group ($\bar{x}\pm s$).

β-Catenin expression in each group

The normal group had fewer β-catenin staining–positive cells. In the moderate group, the β-catenin staining–positive cells were mostly concentrated on the surface of the cartilage. In the severe group, a large number of β-catenin staining–positive cells could be seen on the surface and deep cartilage (Figure 4). Compared with the normal group, the moderate and severe groups had significantly increased β-catenin–positive cells (P<0.01) (Table 3).

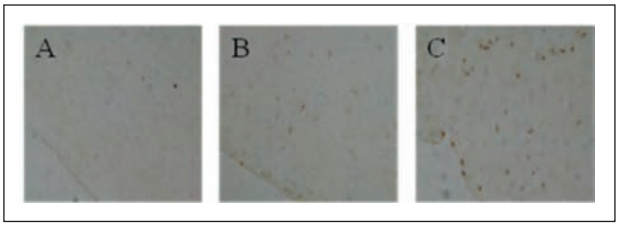

Figure 4: Apoptosis of chondrocytes in each group. *A: Normal group; B: moderate group; C: severe group.*

Group	n	β-catenin	*P*-value, normal group vs. moderate group	*P*-value, normal group vs. severe group	*P*-value, moderate group vs. severe group
Normal	20	0.21±0.01			
Moderate	20	0.42±0.16	<0.001	<0.001	<0.001
Severe	20	0.65±0.09			

Table 3: β-Catenin expression in each group.

Correlation analysis

Pearson correlation analysis showed that the β-catenin expression level and the positive rate of chondrocyte apoptosis correlated positively with Mankin score. A higher Mankin score indicated a higher β-catenin expression level and positive rate of chondrocyte apoptosis (P<0.05) (Table 4).

group	Mankin score	
	r	*P*
β-Catenin	0.324	<0.05
Positive rate of chondrocyte apoptosis	0.267	<0.05

Table 4: Correlation analysis.

Discussion

Osteoarthritis is a common clinical disease, of which the highest incidence is osteoarthritis of the knee joint caused by degenerative disease of the knee joint. In the early stage, it is mostly manifested as pain, stiffness, hypertrophy and limited activity of the diseased joint; in the late stage, it is caused by cartilage peeling, necrosis and hyperosteogeny, resulting in the loss of joint function[6]. Sex, age, heredity, inflammation, trauma and other factors are closely related to the pathogenesis of osteoarthritis. The pathophysiological changes are the imbalance of articular cartilage tissue synthesis and catabolism, which leads to chondrocyte overmaturation, osteocyte formation, articular cartilage destruction and joint space narrowing[7]. β-Catenin can induce chondrocyte terminal differentiation, accelerate cartilage decomposition and promote the expression of matrix metalloproteinase 13 (MMP-13)[8]. Apoptosis is a specific gene program in cells. With the development of molecular biology, apoptosis plays an important role in the pathogenesis of the bone and joints[9]. In the present study, the changes in the β-catenin signalling pathway and chondrocyte apoptosis in osteoarthritis were observed, and the relationship between the β-catenin signalling pathway and osteoarthritis in SOST TG mice was analysed.

The β-catenin signalling pathway, also known as a classical signalling pathway, is one of the three intracellular signalling pathways of Wnt, which regulates chondrocyte function and metabolism mainly through the Wnt protein family, β-catenin and related inhibitors[10]. When β-catenin signalling is activated, cell proliferative ability and chondrogenic differentiation ability are significantly reduced, showing aging phenomenon[11]. Zhou et al.[12] believed that after β-catenin is activated, chondrocytes would differentiate in advance, β-catenin would be highly expressed, chondrocytes would be hypertrophic, MMP-13 and bone morphogenetic protein 2 (BMP-2) expression levels would be significantly increased, subsequently aggravating osteoarthritis. When the β-catenin signalling pathway is blocked, chondrocyte apoptosis will also be accelerated. Some scholars[13] think that the high expression of β-catenin in articular chondrocytes may be an important reason for the articular cartilage destruction and the development of osteoarthritis. Here, the expression of β-Catenin in the normal group was significantly lower than that in the moderate and severe groups, and was significantly increased with the development of the disease. It is suggested that the β-catenin signalling pathway correlates positively with the severity of osteoarthritis in SOST TG mice. The β-catenin signalling pathway plays an important role in the pathological process of osteoarthritis. This is consistent with the results of Gallego-Delgado et al.[14]. Normal articular cartilage is mainly composed of chondrocytes, cartilage matrix and water. Cartilage matrix is mainly composed of proteoglycan and collagen. In the normal physiological state, apoptosis and cell proliferation work together to maintain the normal formation of tissues and organs and cell number stability. Hui et al.[15] found that when osteoarthritis occurs, the patient's chondrocytes express collagen X, and there is apoptosis of chondrocytes. TUNEL is a common molecular biological method for detecting apoptosis. In that study, the positive rate of chondrocyte apoptosis in osteoarthritis patients was significantly higher than that in the normal group, and increased significantly with the development of the disease. Chondrocyte apoptosis is an important pathological mechanism of osteoarthritis.

In conclusion, chondrocyte apoptosis plays a very important role in osteoarthritis. The β-catenin signalling pathway is closely related to the early development of osteoarthritis. The increase in β-catenin expression level is an important sign of early osteoarthritis. The overactivation of the β-catenin signalling pathway can accelerate chondrocyte apoptosis, subsequently aggravating the degeneration of articular cartilage, leading to osteoarthritis. The β-catenin signalling pathway and chondrocyte apoptosis correlate positively with the development of osteoarthritis.

References

1) Shimamura M, Iwata K, Mashiba T, Miki T, Yamamoto T. Accumulation of microdamage in subchondral bone at the femoral head in patients with end-stage osteoarthritis of the hip. J Bone Miner Metab, 2019; 37: 880-885.
2) Shi JY, Yi Z, Liu ZZ, JI L, Liu HT. Correlation Analysis of Wnt/β-catenin Signaling Pathway, OPN and MMP-13 in Varying Degrees of Osteoarthritis. Progress Mod Biomed 2017; 17: 49-54.
3) Choi MC, Maruyama T, Chun CH, Park Y. Alleviation of Murine Osteoarthritis by Cartilage-Specific Deletion of IkappaBzeta. Arthritis Rheumatol 2018; 70: 1440-1449.
4) Cui ZY. Effect of Aerobic Exercise on Apoptosis of Articular Cartilage Cells and Expression of Related Gene Proteins. Progress Vet Med 2017; 38: 82-87.
5) Mankin HJ. Biochemical and metabolic aspects of osteoarthritis. Orthop Clin N Am 2017; 2: 19-31.
6) Davatchi F, Abdollahi BS, Mohyeddin M, Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients. Int J Rheum Dis 2016; 19: 219-225.
7) Lin KY, Yang CC, Hsu CJ, Yeh ML, Renn JH. Intra-articular Injection of Platelet-Rich Plasma Is Superior to Hyaluronic Acid or Saline Solution in the Treatment of Mild to Moderate Knee Osteoarthritis: A Randomized, Double-Blind, Triple-Parallel, Placebo-Controlled Clinical Trial. Arthroscopy 2019; 35: 106-117.
8) Zhao YP, Liu B, Tian QY, Wei JL, Richbourgh B, et al. Progranulin protects against osteoarthritis through interacting with TNF-α and β-Catenin signalling. Ann Rheum Dis 2015; 74: 2244-2253.
9) Xie D. The mechanism of apoptosis of articular chondrocytes and osteoarthritis. J Clini Rehabil Tissue Eng Res 2018; 5: 111.
10) Wang YZ, Wang WY, Zhang L. Pathogenesis of osteoarthritis and its relationship with the Wnt/β - catenin pathway. Orthop J China 2017; 17: 1800-1803.
11) Liu SS, Zhou PU, Zhang Y. Abnormal expression of key genes and proteins in the canonical Wnt/β-catenin pathway of articular cartilage in a rat model of exercise-induced osteoarthritis. Mol Med Rep 2016; 13: 1999-2006.
12) Zhou Y, Chen M, Ricupero CL, He L, Wu J, et al. Profiling of Stem/Progenitor Cell Regulatory Genes of the Synovial Joint by Genome-Wide RNA-Seq Analysis. Biomed Res Int 2018; 2018: 9327487.
13) Diederichs S, Tonnier V, März M, Dreher SI, Geisbüsch A, et al. Regulation of WNT5A and WNT11 during MSC in vitro chondrogenesis: WNT inhibition lowers BMP and hedgehog activity, and reduces hypertrophy. Cell Mol Life Sci 2019; 76: 3875-3889.
14) Gallego-Delgado J, Basu-Roy U, Ty M, Fernandez-Arias C, Movila A, et al. Angiotensin receptors and β-catenin regulate brain endothelial integrity in malaria. J Clin Invest 2016; 126: 4016-4029.
15) Hui T, Zhou Y, Wang T, Li J, Zhang S, et al. Activation of β-catenin signaling in aggrecan-expressing cells in temporomandibular joint causes osteoarthritis-like defects. Int J Oral Sci 2018; 10: 13.

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