Osteoarthritis and Cartilage 25 (2017) 1372–1375

Osteoarthritis and Cartilage

Brief Report

Altered expression of chondroitin sulfate structure modifying sulfotransferases in the articular cartilage from adult osteoarthritis and Kashin-Beck disease

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ARTICLE INFO

Article history:
Received 17 May 2016
Accepted 21 February 2017

Keywords:
Kashin-Beck disease
Osteoarthritis
Adult cartilage
Chondroitin sulfate
Sulfation
Sulfotransferases

SUMMARY

Objective: To investigate the expression of enzymes involved in chondroitin sulfate (CS) sulfation in the articular cartilage isolated from adult patients with osteoarthritis (OA) and Kashin-Beck disease (KBD), using normal adults as controls.

Methods: Articular cartilage samples were collected from normal, OA and KBD adults aged 38–60 years old, and divided into three groups with six individual subjects in each group. The morphology and pathology grading of knee joint cartilage was examined by Safranin O staining. The localization and expression of enzymes involved in CS sulfation (CHST-3, CHST-11, CHST-12, CHST-13, carbohydrate (N-acetylgalactosamine 4-sulfate 6-O) sulfotransferase 15 – CHST-15, and uronyl 2-O-sulfotransferase – UST) were examined by immunohistochemical (IHC) staining and semi-quantitative analysis.

Results: Positive staining rates for anabolic enzymes CHST-3, CHST-12, CHST-15, and UST were lower in the KBD and OA groups than those in the control group. Meanwhile, reduced levels of CHST-11, and CHST-13 in KBD group were observed, in contrast to those in OA and control groups. The expressions of all six CS sulfation enzymes were less detected in the superficial and deep zones of KBD cartilage compared with control and OA cartilage.

Conclusion: The reduced expression of the CS structure modifying sulfotransferases in the chondrocytes of both KBD and OA adult patients may provide explanations for their cartilage damages, and therapeutic targets for their treatment.

Introduction

Chondroitin sulfate (CS), one of the two glycosaminoglycans (GAGs) involved in aggrecan composition, is a linear polysaccharide consisting of repeating disaccharide units of glucuronic acid (GlcA) and N-acetylgalactosamine. It has been reported that CS sulfation plays an important role in morphogenesis and tissue homeostasis[1], and undersulphation of CS proteoglycans (PGs) caused bone shortening and reduced chondrocyte proliferation[2]. CS also had a stimulatory effect on PG production[3], and CS-based scaffold-mediated microenvironment significantly up-regulated the expression of cartilage specific markers, such as Sox9, type II collagen and aggrecan[4].

Osteoarthritis (OA) is a progressive, degenerative joint disease causing joint pain and stiffness. In China, an endemic OA, Kashin-Beck disease (KBD) is manifested with osteochondrodysplasias, osteosclerosis and shortage of diaphysis[5]. Recently, similar pathological changes have been found in these two diseases, including degradation of extracellular matrix (ECM), degeneration of cartilage, and reduction and disruption of PGs[6,7]. The disruption of the

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http://dx.doi.org/10.1016/j.joca.2017.02.803
1063-4584/

cartilage has been associated with a reduction in CS content and an alteration in its sulfation in OA[6] and in some of the enzymes involved in CS metabolism of KBD patients[7]. Hence, our hypothesis was that dysfunction of CS sulfation enzymes may play a key role in the development of KBD and OA.

In this study, the presence of sulfotransferases including chondroitin 6-O-sulfotransferase 1 carbohydrate sulfotransferase 3 (CHST-3), chondroitin 4-O-sulfotransferase 1 carbohydrate sulfotransferase 11 (CHST-11), chondroitin 4-O-sulfotransferase 2 carbohydrate sulfotransferase 12 (CHST-12), chondroitin 4-O-sulfotransferase 3 carbohydrate sulfotransferase 13 (CHST-13), carbohydrate (N-acetylgalactosamine 4-sulfate 6-O) sulfotransferase 15 carbohydrate sulfotransferase 15 (CHST-15), and uronyl 2-O-sulfotransferase (UST) in human articular cartilage was examined by immunohistochemical (IHC) staining among adult OA, KBD and normal groups. The explicit explanations for the mechanism of CS sulfation process may provide new therapeutical targets for both OA and KBD patients.

Materials and methods

Sample collection and groups

Detailed information of the subjects included in this study is shown in Table I. The KBD cartilage samples were collected from six KBD patients' knees (three males and three females, age 44–58 years) diagnosed as Grade II or III based on the diagnosis criteria WS/T 207-2010 (http://www.moh.gov.cn/zwgkzt/s9500/201006/47920.shtml). The OA cartilage samples were obtained from OA patients who were graded and diagnosed based on histopathology[8] (three males and three females, age 45–60 years). Thus, the patient samples were obtained from the end stages of the disease. The normal cartilage samples were collected from the knees of the donors from non-KBD endemic regions (four males and two females, age 38–55 years), who had suffered from traffic accidents and had undergone total knee replacement surgery. This study was permitted by the Human Ethics Committee of Xi'an Jiaotong University. All the KBD, OA and normal donors had provided a written informed consent for the study participation and publication of their individual clinical details and images. The osteochondral tissues were collected within 8 h after surgery. All the tissues were obtained from similar topologic regions of the joint. After decalcification, the samples were embedded in paraffin. Safranin O staining was conducted for classification of OA and KBD cartilage tissues with comparison to normal cartilage tissues[8] (Table I).

IHC staining and quantification

Paraffin sections are deparaffinized and then rehydrated with a series of gradually decreasing concentrations of ethanol. The activity of endogenous peroxidase was destroyed using 0.3% (w/v) hydrogen peroxide (H_2O_2) for 10 min at room temperature, followed by incubation with 10 M urea for 20 min and then with 2 mg/ml hyaluronidase (Sigma–Aldrich, St. Louis, MO, USA) for 20 min. The non-specific binding was blocked with 10% (v/v) normal goat serum for 20 min. Tissue sections were incubated with primary antibodies [CHST-3 (18242-1-AP, Proteintech Group Inc., Rosemount, IL, USA), CHST-11 (ab188001, Abcam, Cambridge, England), CHST-12 (15341-1-AP, Proteintech Group Inc.), CHST-13 (ab110762, Abcam), CHST-15 (GTX120568, GeneTex Inc., Irvine, CA, USA) and UST (ab137624, Abcam), 1:50 dilution in 1% bovine serum albumin in PBS] at 4°C overnight. The negative controls (primary antibody replaced by Phosphate Buffered Saline (PBS), or negative control rabbit IgG) showed no non-specific staining. After washing, the sections were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:500 dilution in 1% bovine serum albumin in PBS) at 37°C for 20 min. After extensive washing, the sections were visualized using a diaminobenzidine kit according to the manufacturer's protocols and counterstained with hematoxylin.

Representative regions were selected and photographed using an Olympus BX51 fluorescence microscope (Olympus, Tokyo, Japan) equipped with digital image acquisition. Positively stained cells in the cytoplasm were quantified using Image J (NIH, USA). In each image, all cells and the positively stained cells in the whole cartilage depth were counted. The variation in topology was controlled by taking photos of parallel regions of the superficial, middle and deep zones as well. Six randomly selected fields in each zone, from one section of the patient, were chosen for each six subjects of the groups, and the percentage of positively stained cells were counted at 100× magnification. The positive staining rate was calculated as: The positive staining rate = (positively stained cells)/(all cells) × 100%.

Statistical analysis

SAS 9.3 software was used for the data entry and analysis of the positive staining rate. Since the data from KBD, OA and control groups were not in accordance with normal distribution by the test for normality and equal variance, the data from all the three groups were calculated as median (P25, P75). Rank-based Analysis of Variance (ANOVA) test followed by Bonferroni's post-test was performed for the multiple comparisons of discrepancy in the positive staining rate among the KBD, OA and control groups, and the results are represented by T value. Statistical significance was accepted when $P < 0.05$.

Results

For CHST-3 enzyme, the strongest positive staining in all three zones was observed in the control group, followed by the OA and

Table I
Characteristics of experiment subjects ($\chi^2_{gender} = 0.560$, $F_{age} = 2.485$, both p value > 0.05)

Control			OA				KBD			
Sample set	Age	Gender	Sample set	Age	Gender	Grade	Sample set	Age	Gender	Grade
1	38	F	1	48	F	3.0	1	44	F	II, 3.0
2	45	F	2	54	F	3.5	2	50	F	III, 3.5
3	45	M	3	60	F	4.0	3	58	F	III, 4.5
4	48	M	4	45	M	2.5	4	50	M	II, 3.0
5	54	M	5	58	M	3.0	5	55	M	III, 3.5
6	55	M	6	60	M	3.5	6	57	M	III, 4.0
Mean	47.5	–	Mean	54.2	–	–	Mean	52.3	–	–

Control: Normal adult; F: Female; M: Male.
The grading of OA cartilage damage was analyzed from Safranin O stained sections[8].
The grading of KBD cartilage damage was analyzed according to WS/T 207-2010 criteria and Safranin O staining.