Alterations in the Chondroitin Sulfate Chain in Human Osteoarthritic Cartilage of the Knee

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Disclosures:

Introduction: Osteoarthritis (OA) is a common joint disease characterized by progressive breakdown of articular cartilage. Chondroitin sulfate (CS) is one of the main components of aggrecan abundantly involved in the cartilage, and a glycosaminoglycan (GAG) that consists of repeating disaccharide units of N-acetylgalactosamine(GalNAc) and glucuronic acid (GlcUA) residues with sulfate residues at the 4 or 6-O-position of GalNAc residues. To date, 6 glycosyltransferases have been identified to be involved in CS biosynthesis in mammals: chondroitin sulfate synthase 1 (CSS1/CHSY1), chondroitin polymerizing factor (CHPF), chondroitin sulfate synthase 3 (CSS3), chondroitin polymerizing factor 2 (CHPF2), chondroitin sulfate N-acetylgalactosaminyltransferase 1 (CSGALNACT1), and chondroitin sulfate N-acetylgalactosaminytransferase 2 (CSGALNACT2). These glycosyltransferases collaboratively catalyze CS chain initiation and polymerization. Their biochemical characteristics have been validated both in vitro and in vivo studies. Recent studies involving knockout mouse models have shown the in vivo roles of these proteins in skeletogenesis and chondrocyte development. CSGalNAcT1 null mice exhibit slight dwarfism with shorter and disorganized chondrocyte column in the growth plate, and Css1/Chsy1 null mice display chondrodysplasia. These results indicate that CS glycosyltransferases and CS play important roles in skeletogenesis and chondrogenesis. Although abnormality of chondrocyte maturation such as chondrocyte hypertrophy-like changes is reported to play a crucial role in early and late stage OA by releasing of proteolytic enzymes, there has not been enough information about a structural change or regulation of biosynthesis of CS in the osteoarthritic cartilage. Therefore, in this study, we focused on the structure and biosynthesis of CS in the osteoarthritic cartilage. Our aim is to examine whether structure of CS relates to degree of cartilage degeneration in human osteoarthritic cartilage, and regulation of CS biosynthesis in the cartilage.

Methods: Human articular cartilage samples were obtained from primary end-stage OA patients including 28 knees of 24 patients (male: 1, female: 23) in performing total knee arthroplasty (age: 72.64±8.18 years old [mean ± standard deviation]). Two osteoarthritic cartilage samples were harvested from each femoral condyle and divided into 2 groups: “the lesion area” which was adjacent from a severe osteoarthritic cartilage and “the remote area” which was apart from the osteoarthritic cartilage as shown in Figure1A. Histologically, degree of cartilage degradation was determined by the modified Mankin score. With using CS solution extracted from the cartilage samples, each CS concentration and mean CS chain length (molecular weight) was measured by high performance liquid chromatography and gel filtration chromatography, respectively. In addition, gene expressions of CS synthases (CSS1, CHPF, CSS3, CHPF2, CSGALNACT1, CSGALNACT2) were measured by real-time polymerase chain reaction. CS composition

Results: According to the modified Mankin score, the cartilage was more degraded in the lesion area than that in the remote area (P < 0.05, 7.04±3.19 and 4.43±2.22, respectively) (Figure 1B). Mean CS amount was significantly smaller in the lesion area than that in the remote area (P < 0.01, 12.27±5.42 and 15.67±5.64µg/mg wet weight, respectively) (Figure 1C). The total amounts of chondroitin-0-sulfate (C0S) and chondroitin-4-sulfate (C4S) were significantly lower in the lesion area than in the remote area. With regard to the mean CS chain length, the CS chain length was significantly short in the lesion area compared with the remote area (P < 0.05, 5.3±1.4 vs 6.1±1.7 kDa, respectively), although the CS chain length was variable in each individual (Figure 2B, C). Of all 6 CS synthases related genes, 4 genes (CSS1, CHPF, CSGALNACT1, CSGALNACT2) were significantly down-regulated in the lesion area in the remote area (Figure 3). Especially, the expression level of CSGALNACT1 in the lesion area was most strongly down-regulated of 4 CS synthases related genes and 0.47-fold that in the remote area (P < 0.0001, 0.069±0.047 and 0.15±0.10, respectively).

Discussion: In this study, we could reveal that CS in the osteoarthritic cartilage showed a varied structural change depending on the cartilage degeneration, namely the mean CS concentration and CS chain length (molecular weight) was significantly lesser and shorter, respectively, in the lesion area than in the remote area. The expressions of CS synthase-related genes (CSS1, CHPF, CSGALNACT1, CSGALNACT2) significantly decreased in the lesion area. Notably, the total concentration of C0S and chondroitin-4-sulfate (C4S) was lower in the lesion area. Previous in vitro studies, CSS1 exhibits the highest glycosyltransferase-II activity, followed by CHPF, CSS3, and CHPF2, and hetero-oligomer formation of two of them is required to progress the CS polymerization, which indicates these enzymes can contribute to regulate CS chain length. Of all combination for these CS glycosyltransferases, the hetero-oligomer of CSS1 and CHPF exhibited the highest glycosyltransferase-II activity. Especially, CSS1
has a more powerful impact on CS polymerization compared with CHPF. It is suggested that the reduction of the expression for CS synthase-related genes (CSS1, CHPF) in the lesion area may influence on CS chain length in the lesion cartilage. Actually, the CS chain length was shorter in the lesion area than that in the remote area. In addition, various skeletal abnormalities with decreased CS have been reported. CSGalNAcT1-null mice with 50% CS levels in cartilage exhibit slight dwarfism. They show some osteoarthritic changes in the cartilage such as a decreased level of aggrecan, a rapid catabolism of aggrecan and abnormally aggregated type-II collagen fibers. In the human OA cartilage, CS glycosyltransferases’ gene expression decreased, and CS chain length and concentration was short and less, respectively. These observations support the notion that the reduction of CS synthases’ gene expression in the osteoarthritic cartilage may contribute to the progression of OA. Taken together, attenuation of CS synthases’ gene expression in the osteoarthritic cartilage may reduce CS chain length and it results in the progression of osteoarthritis.

**Significance:** In the osteoarthritic cartilage, amount of chondroitin sulfate (CS) and CS chain length in aggrecan decrease as cartilage degeneration proceeds, and CS synthases’ gene expression also decrease. Attenuation of CS glycosyltransferases’ gene expression in the osteoarthritic cartilage may reduce CS chain length and amount, which results in the progression of osteoarthritis.

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**References:**