Effects of the Number, Not the Length, of Chondroitin Sulfate Chains on Aggrecan Metabolism and Endochondral Ossification

INTRODUCTION

Cartilage consists of a large chondroitin sulfate (CS) proteoglycan, aggrecan, which is thought to contain approximately 100 CS chains. While CS proteoglycan aggrecan is essential for chondrocyte differentiation and maintenance of skeletal development, CS content of aggrecan and length of CS chains gradually decrease with age, resulting in the exacerbation of cartilage degradation [1,2]. Thus, CS can play a leading role in cartilage degradation. Therefore, elucidation of the mechanism of CS biosynthesis and in vivo functions of CS might provide new insights into the development of therapeutic strategies for the prevention of cartilage degradation. However, the mechanism of CS biosynthesis and the in vivo role of CS in cartilage development are not fully understood. CS biosynthesis is catalyzed by a family of CS synthases and occurs in 2 steps: chain initiation and chain elongation. Recently, biochemical characterization has shown that chondroitin sulfate N-acetylglactosaminyltransferase (CSGalNAcT1) and a combination of chondroitin sulfate synthases (CSS) 1 (CSS1) and CSS2 play a pivotal role in the initiation and elongation of CS chains, respectively. Therefore, in this study, we generated and compared Csgalnact1−/− and CSS2−/− mice to investigate the role of these enzymes in CS biosynthesis and the role of CS in cartilage development.

METHODS

Csgalnact1−/− and CSS2−/− mice were generated as follows. A targeting vector harboring Csgalnact1flox and CSS2flox alleles was constructed by flanking exon 1 containing their translational start codon with loxP sites, respectively. This vector was transfected into mouse ES cells. By blastocyst injection of these cells, chimeric mice were obtained. Germline transmission of Csgalnact1flox and CSS2flox alleles was achieved individually by crossing these chimeric mice with C57BL/6 mice. Subsequently, these Csgalnact1flox and CSS2flox mice were crossed with CAG-Cre transgenic mice to obtain CAG-Cre/Csgalnact1−/− and CAG-Cre/CSS2−/− mice. The resultant heterozygotes bearing the null alleles were crossed with each other to generate Csgalnact1−/− and CSS2−/− mice. Sequence analysis of PCR products showed absence of exon 1 in both Csgalnact1 and CSS1 genes in the genomes of the Csgalnact1−/− and CSS2−/− mice, respectively. For skeletal analysis, whole skeletal preparations of four 1-month-old mice were stained with Alcian blue and Alizarin red. The longitudinal lengths of 8 humeri, ulna, femur, and tibia of 2-week-old Csgalnact1−/−, CSS2−/−, and the corresponding wild-type (WT) mice were measured. For histological analysis of the cartilage growth plate, 8 proximal humeri of newborn mice were subjected to hematoxylin and eosin staining, Masson’s trichrome staining, and immunohistochemical staining of aggrecan, collagen II, and link protein 1. Quantitative realtime RT-PCR was performed for the analysis of CSS1, CSS2, CSGlGcAT/ChSy3, Csgalnact1, and Csgalnact2 expression using 3 proximal humeri of newborn mice. For structural analysis, CS was extracted from 3 proximal humeri of newborn mice by using NaOH treatment and DEAE cellulose column elution. The disaccharide composition of CS was analyzed using chondroitinase ABC treatment and fluorescence labeling, and the length of the CS chains was analyzed by 1H labeling and gel filtration. Protein expression was analyzed using western blotting. Statistical analyses were performed using the Student t test. This research was conducted using the protocols approved by the animal ethics committee of Aichi Medical University.

RESULTS

The Csgalnact1−/− and CSS2−/− mice were viable and fertile. Compared with their WT littermates, the Csgalnact1−/− mice exhibited slight dwarfism and a significantly low growth rate (approximately 10% reduction). The Csgalnact1−/− mice also showed approximately 15% decrease in the length of the long bones of the limbs as compared to their WT littermates, in contrast to the observation in the CSS2−/− mice. Histologically, the cartilage growth plate of the Csgalnact1−/− mice comprised short and slightly disorganized chondrocyte columns with a reduced extracellular matrix, principally in the proliferative layer, whereas that of the CSS2−/− mice showed well-organized and similar-sized chondrocyte columns, as compared to their WT littermates. Immunohistochemical analysis showed decreased levels of both aggrecan and link protein-1 in the cartilage of the Csgalnact1−/− mice. Further western blot analysis showed an increase in the amount of processed aggrecan core protein but no change in the expression levels of ADAMTS-1 and 5 in the cartilage. Realtime RT-PCR confirmed no compensation mechanism in both transgenic mice at the transcriptional levels by other CSSs. Biochemically, the cartilage of the Csgalnact1−/− mice showed approximately 50% reduction in the number of CS chains without a clear difference in the CS chain length; on the other hand, the cartilage of the CSS2−/− mice showed a reduction in the CS chain length from approximately 19,000 to 10,000 in molecular weight without a significant difference in the number, as compared to the WT littermates. Disaccharide composition analysis of the CS showed a similar sulfation ratio for CO5A, CO4, and CO3 between the Csgalnact1−/−, CSS2−/−, and the WT littermates. The total amount of CS in the cartilages of the Csgalnact1−/− and CSS2−/− mice was ~55% and ~70%, respectively, of the total amount of CS in the cartilage of their WT littermates.

DISCUSSION

The CS chains are synthesized by a family of CS synthases and exhibit structural diversity in chain length and sulfation patterns. This diversity is important for the specific biological functions of CS in cell homeostasis, morphogenesis, neural network formation, and physical movement. In this study, we showed that CSGalNAcT1 is necessary for normal endochondral ossification, and that the decreased number of CS chains in the cartilage growth plate results in rapid aggrecan catabolism. We also showed that CSS2 is not essential for growth and development, and is only involved in the elongation of CS chains. Taken together, our results indicate that the number, rather than length, of CS chains is highly important for the biological activity of CS proteoglycan aggrecan in the cartilage. In addition, the accelerated aggrecan catabolism and normal level of ADAMTS expression in the Csgalnact1−/− mice strongly suggest that the CS chains in aggrecan modulate the susceptibility of aggrecan to ADAMTS, thus supporting the correlation of age-dependent cartilage degradation with decrease in the number of CS chains with advancing age [1,2]. Previously, we reported that overexpression of CSGalNAcT1 in chondrocytes increased the number of CS chains in aggrecan and in cartilage in vivo [3]. Taken together, the findings of this and the previous study indicate that the increased production of CS chains of aggrecan by CS synthases has a potential to inhibit CS-related abnormal endochondral ossification and reduce cartilage degradation. The next step in our research will be to investigate whether increase in the number of CS chains reduces cartilage degradation in animal model in which the number of CS chains decreased.

SIGNIFICANCE

This is the first study to elucidate the mechanism of CS biosynthesis and the functional role of CS in cartilage development in vivo. Our study provides insights into new therapies for cartilage metabolism diseases and/or degenerative diseases by using CS or CS synthases as targets.

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REFERENCES


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