03 *ABSTRACT*

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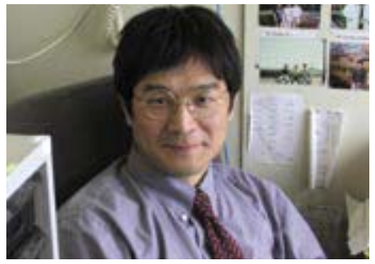
Formation and Function of the Proteoglycan Aggregate



profile

Hideto Watanabe

Hide Watanabe is a molecular pathologist who has worked on functions of proteoglycan aggregates. He obtained M.D. in Kanazawa University School of Medicine in 1985, followed by Ph.D. in Pathology in 1989. For two years in the gradual course, he studied on "purification and characterization of leukocyte elastase and production of its monoclonal antibody" with Prof. Yutaka Nagai at Tokyo Medical and Dental University. After graduation, he studied on activation of matrix metalloproteinases with Dr. Yasunori Okada for three years. From July 1992 to January 2000, he worked with Dr. Yoshihiko Yamada at National Institute of Dental and Craniofacial Research, NIH, U. S. A. His research focused on the function of aggrecan and link protein and their gene regulation. During that period, he identified a causative mutation in aggrecan gene of cmd mice and generated link protein knockout mice. By detailed analyses of these mice, he demonstrated critical roles of the proteoglycan aggregate in cartilage development and maintenance of cartilage tissue. Currently, he is an associate professor in Prof. Koji Kimata's laboratory at Aichi Medical University. He has a variety of research fields including functions of chondroitin sulfate proteoglycans, molecular cloning and characterization of enzymes related to CS synthesis and modification, transcriptional regulation of extracellular matrix genes, and the mechanism of chondrocyte differentiation and cartilage development. However, in short, his research area would be molecular pathology of extracellular matrix.

Key words proteoglycan, aggregate, aggrecan, PG-M/versican, knockout

The extracellular matrix of the cartilage contains two major structures: the fiber structure and the proteoglycan aggregate. Whereas collagen fibers give cartilage its tensile strength, proteoglycan aggregates, composed of aggrecan, hyaluronan (HA), and link protein (LP), contribute to water retention and give cartilage its unique gel-like property and resistance to compression. Aggrecan is a large chondroitin sulfate proteoglycan with a molecular mass of ~2,200 kDa. Its core protein contains three globular domains, G1, G2, and G3, and two glycosaminoglycan-attachment domains, KS and CS. The N-terminal G1 domain binds to both HA and LP, and their interactions are critical for aggregate formation. The G1 domain consists of three looped subdomains of A, B, and B´. Whereas the A loop with immunoglobulin fold interacts with link protein, a segment of B-B´ interacts with HA. The C-terminal G3 domain contains a C-type lectin-like domain that binds to various molecules such as tenascin-C, sulfated glycolipids, and fibulin-1 and -2. The CS domain contains more than a hundred attachment sites for chondroitin sulfate chains, enabling water retention in the cartilage. Aggrecan CS chains undergo age-related structural changes. Compared with fetal and early postnatal ages, the average CS chain size at skeletal maturity is decreased from ~20 kDa to ~8 kDa, and the ratio of 6- to 4-sulfation is increased from ~0.77 to ~23. These age-related changes of CS chains may affect not only its hydrated size but also the interactions with other cartilage molecules, important for cartilage function.

To study the *in vivo* roles of the proteoglycan aggregate, mutant mice of molecules of the aggregate are useful. Mice cartilage matrix deficiency (cmd/cmd) are a natural knockout of aggrecan, characterized by dwarfism, short snout and cleft palate. They die shortly after birth due to respiratory failure, indicating the essential role of aggrecan in cartilage development. The cartilage of homozygotes exhibits tightly packed chondrocytes with little extracellular matrix. Although aggrecan is absent, cartilage of cmd homozygotes contains normal levels of type II collagen and link protein. Cmd heterozygotes show slight dwarfism and develop spinal misalignment and disc herniation with age. Within 19 months of age, they exhibit spastic gait caused by misalignment of the cervical spine and die of starvation. Biochemical analyses and quantitative RT-PCR reveal decreased levels of aggrecan deposition and expression in cmd heterozygotes, indicating that a certain level of aggrecan deposition is critical for the maintenance of disc function. LP-null mice demonstrate milder dwarfism than cmd homozygotes and some survive the perinatal period. Their cartilage shows decreased levels of aggrecan deposition, whereas the level of type II collagen is normal. Although chondrocyte proliferation is unaltered, diminished and altered distributions of signaling molecules such as Indian hedgehog and PTH/PTHrP receptor are found in the growth plate, suggesting that proteoglycan aggregates may store signaling molecules in extracellular matrix and distribute them to the appropriate chondrocytes, and regulate their differentiation. Although LP is expressed in various tissues including the brain, aorta, heart, and skin, LP-null mice show no phenotype in these tissues. We have generated double heterozygotes of cmd and LP-null mice. They show dwarfism more conspicuous than cmd heterozygotes. The correlation of levels of proteoglycan aggregates in the cartilage and phenotype of these mouse models suggests that the proteoglycan aggregate plays important roles in skeletal formation and maintenance.

Members of the aggrecan family such as PG-M/versican, neurocan, and brevican share domain structures similar to aggrecan. Having a homologous G1 domain, these proteoglycans may form aggregates by interacting with both HA and LP. PG-M/versican is expressed in a wide variety of tissues including the central and peripheral nervous system, the luminal surface of grandular epithelia, blood vessels in normal and tumor tissues, dermis, and the proliferative zone of the epidermis, and embryonic tissue. This extracellular proteoglycan is involved in cellular attachment, migration, cell proliferation, and differentiation by interacting with cell surfaces and extracellular matrix molecules. In an early stage of cartilage formation, PG-M/versican is transiently expressed in areas of mesenchymal cell condensation, although its role in chondrocyte differentiation has not been clarified. We have studied interactions of PG-M/versican with HA and LP, using native PG-M/versican purified from brain and recombinant proteins expressed in 293 cells. Immunoprecipitation followed by immunoblotting demonstrates interaction of native PG-M/versican with LP. By HA-transblot assay and a BIAcore™ biosensor system, a minimal segment of the PG-M/versican G1 for HA-binding has been determined to be the B-B´, like aggrecan. The G1 of PG-M/versican and its B-B´segment binds to LP but the A-B and the A subdomains do not, indicating that the minimal segment for LP-interaction is the B-B´. Further analyses using the BIAcore™ system reveal that the B-B´segment of PG-M/versican interacted with both HA and LP to form an aggregate. When we examine tissue localization of PG-M/versican, LP, and HA using specific antibodies and biotinylated HA-binding protein respectively, their co-localization is observed in adventitia of aorta, cardiac cushion, and cartilage. These data suggest that PG-M/versican may form aggregates in these tissues with HA and LP in a different manner from aggrecan, and may provide a clue to the *in vivo* function of the PG-M/versican aggregate and other aggregates possibly formed with other proteoglycans of the aggrecan family.

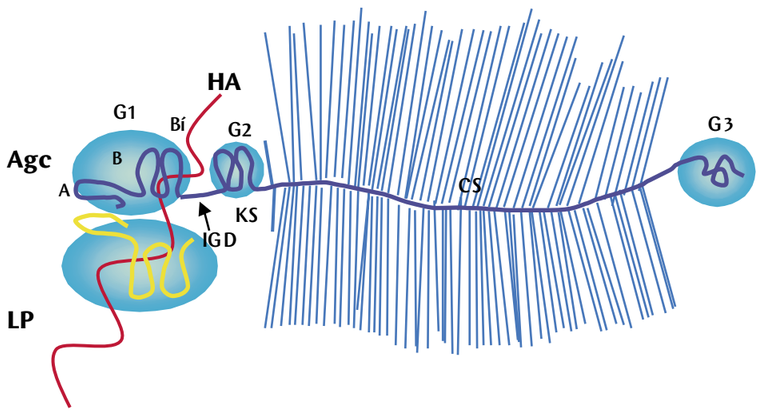


Figure 1. The proteoglycan aggregate composed of aggrecan (Agc), hyaluronan (HA), and link protein (LP). The core protein of Agc contains three globular domains (G1, G2, and G3), two glycosaminoglycan-attachment domains (KS and CS), and the interglobular domain (IGD). The G1 consists of three looped subdomains (A, B, and B´), and binds to both HA and LP.

References

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