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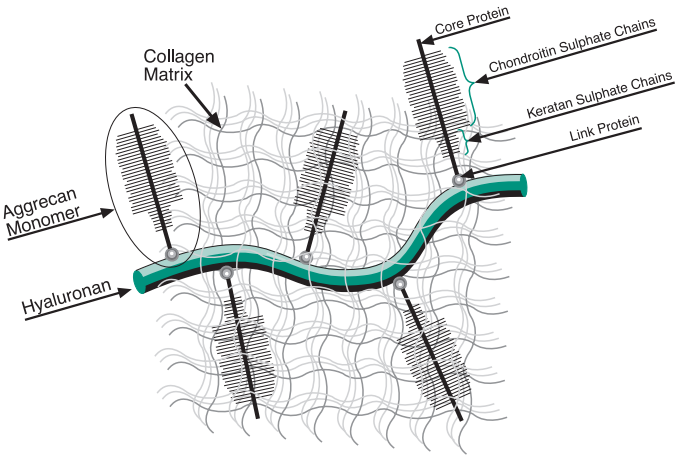
Cartilage, Related Cytokines and Osteoarthritis

Cartilage is a firm and flexible connective tissue, capable of rapid growth and specialized to absorb and resist compression. The tissue is surrounded by a vascularized fibrous layer, the perichondrium, but contains no blood vessels of its own. Therefore, to maintain cartilage vitality, nutrients and waste must be able to diffuse through the tissue. Articular cartilage, the thin layer of smooth hyaline cartilage that covers the joint surfaces of a bone, is unique in that it has no perichondrium. Instead, it is protected by a nutritive and lubricating medium, the synovial fluid of the joint. Cartilage contains relatively few cells, termed chondrocytes, which occupy 10-20% of its volume. The remainder is an extracellular material, which is highly hydrated and contains up to 80% water by weight. The chondrocytes are enclosed within small cavities (called lacunae), often in groups of 2, 4, or 6 cells as a result of mitosis and restricted cellular movement. The extracellular material consists primarily of large hydrated proteoglycan aggregates, entrapped within a matrix of collagen fibrils. The matrix is predominantly made of type II collagen which forms a meshwork of high tensile-strength fibrils. The entrapped proteoglycans (also called mucopolysaccharides), are composed of a core protein that forms a backbone to which many glycosaminoglycan (GAG) chains are covalently attached. The chains extend perpendicularly from the backbone of the protein in a brush-like structure that allows trapping of large amounts of water. The molecular structure of GAGs consists of an unbranched polysaccharide made of a repeating disaccharide unit, which contains an acylated amino sugar moiety (e.g. N-acetylglucosamine) and a sugar acid moiety (e.g. glucuronic acid). The amino sugar moieties in most GAGs are sulfated, hence increasing their anionic character. Hyaluronan (also called hyaluronic acid or HA), is unique among the GAGs in that it does not contain any sulfate and is not involved in covalent attachment to any core protein. The GAGs are highly negatively charged molecules that generate a large osmotic swelling pressure. Their rigid and extended conformation produces a hard, compression-resistant substance that allows diffusion of solutes through the medium. The three major GAGs found in cartilage in order of abundance are chondroitin sulfate (M.W., 20-50 kDa), keratan sulfate (M.W., 5-20 kDa) and hyaluronan (M.W. of up to 6 million Daltons). Numerous chains of chondroitin sulfate (100-150) and keratan sulfate (30-60) covalently linked to a core protein of approximately 250 kDa, constitute the abundant cartilage proteoglycan, the aggrecan monomer. Individual aggrecan monomers interact with hyaluronan, in a molecular ratio of approximately 100:1, to form gigantic hydrated aggregates. The attachment of aggrecan monomers to hyaluronan is stabilized by a 39 kDa protein called link protein, that acts as glue between the entities (see figure below).

The mechanical behavior of articular cartilage bears resemblance to a sponge. During rest, such as lying down or sitting, the osmotic swelling pressure generated by the proteoglycan aggregates fills the tissue with water up to its maximum capacity. This swelling pressure is contained only by the resilient collagen meshwork. Under load, such as while standing up or walking, the weight of the body compresses the cartilage, squeezing water out until the osmotic swelling pressure generates a force equal to the compressive force of the total body



Schematic Presentation of Cartilage Extracellular Matrix.

Most of the bones in the adult skeleton are derived from temporary cartilage templates that are capable of rapid longitudinal growth. The formation of bone in cartilage, or endochondral ossification, involves the destruction and removal of cartilage and formation of bone in the space formally occupied by the cartilage. It is a highly orchestrated process, tightly coordinated in time and space in which the avascular cartilage tissue is replaced by a highly vascularized bone tissue. The process begins in the mesoderm of the embryo, where cartilage progenitor cells accumulate and condense to form a cartilage anlage. Cells in the core of this early cartilage element differentiate into chondrocytes, whereas cells at the periphery form the perichondrium. Initially, all the cells within the anlage proliferate as round immature chondrocytes. As growth proceeds, cells closest to the periarticular ends of the structure are maintained undifferentiated (for future cartilage growth and repair),

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while the others undergo progressive maturation, forming a column of flat proliferating chondrocytes. Proliferating cells synthesize and extrude matrix components, thereby increasing the length and width of the primordial cartilage. They also express functional proteins such as BMP-R and PP-R, the cell membrane receptors that respond, respectively, to bone morphogenetic proteins (BMPs) and to parathyroid hormone-related protein (PTHrP). At a certain growth stage, chondrocytes at the distal end of the cell column start to express increasing levels of PP-R, then go through terminal differentiation and lose their ability to divide. These newly postmitotic cells, termed prehypertrophic chondrocytes, express Indian Hedgehog (Ihh), a signaling protein that plays a central role in coordinating the progression of the process (discussed later). Prehypertrophic chondrocytes gradually grow in size and ultimately (after swelling by more than 500%) turn into round hypertrophic chondrocytes that can no longer express Ihh. Whereas, immature and proliferating chondrocytes express antiangiogenic proteins (e.g., Chondromodulin-1 and Troponin-1), which protect cartilage from vascularization and subsequent calcification, hypertrophic chondrocytes express angiogenic factors such as Transferring and VEGF. They also synthesize unique extracellular components (e.g., Osteopontin and type X collagen), thereby altering their matrix and forming a distinct zone in the center of the growing cartilage element. During hypertrophy, the perichondrium flanking the hypertrophic zone differentiates into periosteum, a fibrous connective tissue that covers the bone and provides attachment sites for tendons and ligaments. Hypertrophy is the final stage in the life of temporal chondrocytes. Upon their apoptosis (programmed cell death), hypertrophic chondrocytes release angiogenic growth factors that diffuse into nearby tissues and trigger a cascade of events that includes: (I) invasion of endothelial cells into the hypertrophic zone, (II) vascularization of the zone, (III) attraction and attachment of osteoprogenitor cells to the hypertrophic matrix, (IV) proliferation and differentiation of the cells into mature bone cells, (V) destruction of the hypertrophic matrix coupled with the formation of new bone matrix, (VI) mineralization of the bone matrix, (VII) bone remodeling associated with the proliferation and differentiation of hematopoetic stem cells. The end result is the replacement of hypertrophic cartilage by a calcified bone complete with functional marrow.

Endochondral ossification continues to proceed from the center to the ends of the skeletal element until the proliferative zones of the cartilage, termed growth plates, are reduced to narrow bands located near the articular ends of the endochondral bone. The extent to which skeletal elements continue to grow depends largely upon the relative rates of proliferation versus hypertrophic differentiation of growth-plate chondrocytes. Accelerated and/or premature onset of terminal differentiation would result in abnormally short or deformed bones. Conversely, delayed hypertrophic differentiation, which allows more chondrocyte proliferation, would lead to longer than normal bones. The molecular mechanisms that govern normal development of endochondral bones are not completely known. However, it has been established that Ihh and PTHrP play major roles in coordinating the transition of growth-plate chondrocytes to hypertrophy, thereby balancing the growth and ossification of skeletal elements .

Ihh belongs to a conserved family of Hedgehog (Hh) proteins, whose members are widely represented throughout the animal kingdom. The rather odd name given to this family is derived from the founding member, Drosophila Hh, which plays an important role in the morphogenesis of the fruit-fly. The biologically active form of Hh molecules is obtained by autocatalytic cleavage of their precursor proteins, and corresponds to approximately the N-terminal one half of the precursor molecule. The three known mammalian Hh proteins, Sonic (Shh), Desert (Dhh) and Ihh are structurally related and share a high degree of amino-acid sequence identity (e.g., Shh and Ihh are 93% identical). Although Hh proteins have unique expression patterns and distinct biological roles within their respective regions of secretion, they use the same signaling pathway, and can substitute for each other in experimental systems. The cell surface receptor for Hh proteins is a twelve-pass membrane protein called Patched1 (Ptc1), which interacts and inhibits a seven-pass membrane protein, called Smoothened (Smo). Smo is the common transducer of all Hh signals. Binding of Hh proteins to Ptc1 relieves the repression of Smo, thereby activating a signal transduction pathway that ultimately results in transcriptional activation of target genes. Among the Ihh target genes are Ptc1 itself, a transcription factor called Gli1, and possibly several BMP genes.

During endochondral bone development, Ihh is expressed exclusively by prehypertrophic chondrocytes, which occupy a distinct domain within the developing skeletal element. This domain is strategically situated between the proliferative zone of growth-plate chondrocytes, and the hypertrophic zone. The developmental processes that take place in these zones are tightly regulated by Ihh. In the hypertrophic zone, Ihh signaling coordinates the development of immature bone cells to osteoblasts and osteoclasts (bone forming and resorbing cells, respectively) . However, it is not clear whether Ihh acts directly on these cells, or if the Ihh response is mediated through secondary signaling molecules (e.g., BMPs). In the growth zone, Ihh signaling regulates two processes that are initiated in the periarticular region. It stimulates immature chondrocyte differentiation to proliferating chondrocytes, and promotes secretion of PTHrP, a potent inhibitor of chondrocyte maturation . PTHrP, a small protein of approximately 9.9 kDa, is expressed by periarticular perichondrial cells, and exerts its biological activity

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on PP-R expressing cells. During endochondral bone development PTHrP plays a major role in suppressing the onset of hypertrophic differentiation. The signaling pathways by which Ihh affects PTHrP expression and cellular differentiation in the distal periarticular region are currently unknown. However, the absence of Ptc1 up-regulation, the hallmark of direct Hh signaling, in the periarticular region suggests an indirect signaling pathway. Vortkamp and colleagues have proposed that Ihh and PTHrP interact through a negative feed-back loop that begins in the Ihh expressing domain. The secreted Ihh diffuses to the adjacent perichondrium, which contains Ptc1-expressing cells. These cells communicate the Ihh signal to the more distant perichondrium located within the periarticular region, thereby inducing PTHrP production. The secreted PTHrP diffuses through the growth-plate and prevents the high PP-R expressing chondrocytes from entering into terminal differentiation. When prehypertrophic chondrocytes turn into hypertrophic chondrocytes, they cease to secrete Ihh, hence the inhibitory effect of Ihh is ultimately removed, and the cells are then free to continue with their maturation program . The range of PTHrP activity in delaying the onset of hypertrophic differentiation depends upon its levels of secretion, which in turn determines the length of the zone between the periarticular region and the Ihh-expressing domain. As most of the longitudinal growth of cartilage occurs in this zone, the Ihh-induced secretion of PTHrP determines the length of the eventual skeletal bones.

Recent studies have indicated that during endochondral bone development Ihh and BMP signaling interact with each other, but may act independently of one another . The BMPs are structurally related signaling proteins that have been implicated in a variety of developmental processes. They constitute a family of more than 30 known members, 15 of which are of mammalian origin. Their mature and functional form, obtained by proteolytic cleavage of biologically inactive precursors, is a disulfide-linked dimer of two 12-16 kDa polypeptide chains. The BMPs exist mostly as homodimers but some are heterodimers. Among their various biological functions, BMPs play important roles in the development, growth and repair of bone and cartilage by stimulating cellular proliferation and differentiation . The expression patterns of the BMPs and their receptors in developing cartilage elements are complex. They include (a partial list) BMP-2, -4, and -7 that are expressed in both the perichondrium adjacent to the Ihh expressing domain and in the periarticular region. BMP-7 (originally called osteogenic protein-1 or OP-1) is also expressed in growth-plate chondrocytes. In addition to facilitating the maturation process of both growth-plate chondrocytes and bone cells, BMP signaling has been shown to slow the transformation of prehypertrophic to hypertrophic chondrocytes. This BMP activity increases not only the size of the Ihh-expressing domain, but also the expression levels of Ihh itself. Targeted blocking of BMP activity in this domain (e.g., with Noggin, a specific natural occurring BMP antagonist) results in decreased Ihh expression and premature onset of hypertrophic differentiation .

Osteoarthritis (OA) is a chronic inflammatory disease characterized by progressive deterioration of articular cartilage in synovial joints. It is the leading cause of disability for individuals over the age of 65, and can become very painful. Risk factors include aging, obesity and overuse or abuse of joints. Although, the exact biological mechanism responsible for the onset of OA is unknown, it is generally accepted that OA, at least in the advanced phase of the desease, results from reactivation of endochondral ossification. After an apparent closure of the ossification process during adulthood, the articular cartilage remains a metabolically active tissue capable of repairing, healing, and remodeling itself in a limited fashion throughout the life of the individual. Biomechanical studies have demonstrated that intermittent compressive pressure facilitates a stable articular cartilage layer, whereas a severe reduction of mechanical stimulation results in dramatic decline of both bone mass density and joint cartilage thickness. The translation of mechanical signals into cellular reaction and tissue remodeling involves signaling molecules capable of responding to mechanical stimuli. Recent studies have shown that the response of chondrocytes to mechanical stress is mediated, at least in part, by Ihh and BMP signaling molecules. Activation of Ihh/BMP signaling pathways can account for both the increase in articular cartilage thickness, and the reactivation of endochondral ossification. The specific outcome likely depends on a number of variables including the loading history and integrity of both the cartilage layer and the subchondral bone. The observations that cartilage injuries often progress to OA, and that during the early phase of OA, mechanisms of cartilage repair rather than degradation are at work (11), suggest that the onset of the pathological process is triggered by overly stimulated chondrocytes. This premise is supported by accumulating data indicating that osteoarthritic chondrocytes are metabolically active, express and respond to BMPs, and synthesize extracellular matrix components reminiscent of early developmental stages (12, 13). The activation of Ihh/BMP signaling pathways by mechanical stimuli occurs via an unknown mechanism. However, the fact that the functional forms of these signaling molecules are generated from inactive precursors, suggests an indirect mechanism that involves activation of precursor processing (e.g., through compression-induced precursor conformational change). The elucidation of the underlying mechanisms that trigger OA should facilitate the development of new pharmacological agents that, when used in combination with specific exercise regimens, may slow or stop the progression of the disease.

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