Best Practice & Research Clinical Rheumatology
Vol. 22, No. 2, pp. 351–384, 2008
doi:10.1016/j.berh.2008.02.001
available online at http://www.sciencedirect.com

11

Cartilage in normal and osteoarthritis conditions

Johanne Martel-Pelletier* PhD
Professor

Christelle Boileau PhD
Doctor

Jean-Pierre Pelletier MD
Professor
Osteoarthritis Research Unit, University of Montreal Hospital Centre, Montreal, Canada

Peter J. Roughley PhD
Doctor
Genetics Unit, Shriner's Hospital for Children, Montreal, Canada

The preservation of articular cartilage depends on keeping the cartilage architecture intact. Cartilage strength and function depend on both the properties of the tissue and on their structural parameters. The main structural macromolecules are collagen and proteoglycans (aggrecan). During life, cartilage matrix turnover is mediated by a multitude of complex autocrine and paracrine anabolic and catabolic factors. These act on the chondrocytes and can lead to repair, remodeling or catabolic processes like those that occur in osteoarthritis. Osteoarthritis is characterized by degradation and loss of articular cartilage, subchondral bone remodeling, and, at the clinical stage of the disease, inflammation of the synovial membrane. The alterations in osteoarthritic cartilage are numerous and involve morphologic and metabolic changes in chondrocytes, as well as biochemical and structural alterations in the extracellular matrix macromolecules.

Key words: anabolic, non-anabolic, and catabolic growth factors; biological markers; cartilage; collagens; cytokines; osteoarthritis; proteases; proteoglycans.

* Corresponding author. Osteoarthritis Research Unit, University of Montreal Hospital Centre, Notre-Dame Hospital, 1560 Sherbrooke Street East, Montreal, Quebec H2L 4M1, Canada. Tel.: +1 514 890 8000x26658; Fax: +1 514 412 7582.
E-mail address: jm@martelpelletier.ca (J. Martel-Pelletier).

INTRODUCTION

Articular cartilage is a specialized, avascular, aneural connective tissue that provides covering for the osseous components of diarthrodial joints. It serves as a load-bearing material, absorbs impact and is capable of sustaining shearing forces. The unique properties of this tissue are related to the composition and structure of its extracellular matrix, which is composed mainly of a high concentration of proteoglycans (aggrecan) entangled in a dense network of collagen fibers and a large amount of water. This tissue allows the frictionless motion of the joint, in which it absorbs and dissipates load. The articular cartilage is composed of a sparse population of cells, named chondrocytes, which are responsible for the synthesis and maintenance of the extracellular matrix.

Osteoarthritis (OA) is characterized by degradation and loss of articular cartilage, hypertrophic bone changes with osteophyte formation, subchondral bone remodeling, and, at the clinical stage of the disease, chronic inflammation of the synovial membrane. Its etiology, although not yet completely understood, appears to result from a complex system of interacting mechanical, biological, biochemical, molecular, and enzymatic feedback loops. The final common pathway of cartilage destruction results from a failure of chondrocytes to maintain a homeostatic balance between matrix synthesis and degradation. As the disease progresses, the degradative process eventually exceeds the anabolic one, leading to a progressive loss of cartilage and eburnation of bone. This appears to occur when the physiologic balance between the synthesis and degradation of the extracellular matrix favors catabolism. At the clinical stage of the disease, an inflammatory reaction involving the synovial membrane is often present. This process favors the synthesis of inflammatory mediators, which impact on cartilage matrix homeostasis by altering chondrocyte metabolism to enhance catabolism and reduce anabolism.

This chapter summarizes the pertinent data related to the molecules and factors involved in cartilage maintenance, remodeling, and during the progression of OA.

CARTILAGE STRUCTURE

Structural organization

The cartilage matrix consists of macromolecules in which collagen and proteoglycans (aggrecan) are the main representatives. These components are highly ordered from the cartilage surface to the deepest layers. Cartilage is divided into four zones with different functions: the superficial, middle or transitional, deep or radial, and calcified cartilage zones. Interestingly, there is no sharp boundary between the first three zones.

The superficial zone, the thinnest of the four, consists of fine collagen fibrils with tangential orientation, low proteoglycan content, and elongated chondrocytes aligned parallel to the surface.[1] This zone is in contact with synovial fluid, and is responsible for most of the tensile properties of cartilage that enable cartilage to resist shear and the tensile and compressive forces imposed by the movement of the articulation.

The middle zone represents 40–60% of the total cartilage height. It is formed by proteoglycans and thicker collagen fibrils organized into radial bundles or layers. The chondrocytes in this zone are at low density and have a round shape.

In the deep zone, the chondrocytes have the same shape as those in the middle zone and are aligned perpendicular to the articular surface, but with low density. This zone contains the largest collagen fibrils in a radial disposition[1], and aggrecan content is at its highest.

The calcified cartilage is divided from the other zones by the tidemark, and separates – physically and mechanically – the hyaline cartilage from the subchondral bone. Its main function seems to be to anchor the cartilage to the bone as collagen fibrils from the radial zone penetrate into the calcified cartilage. In this zone, the cell population is very scarce and chondrocytes are hypertrophic.

The pericellular area surrounding the chondrocytes is made of a thin layer of nonfibrillar material, which most likely represents the synthetic products of the chondrocytes, such as proteoglycans and glycoproteins. Immediately adjacent to the pericellular area is the territorial cartilage matrix, which contains a dense meshwork of thin collagen fibers forming a capsule-like structure around the cells and providing mechanical protection to the chondrocytes.[2]

Collagen, proteoglycans, and other proteins or glycoproteins represent only about 20% of the tissue wet weight; water and inorganic salts represent most of the remaining tissue. The water content of cartilage plays an important role in maintaining the resiliency of the tissue and contributing to the nutrition and lubrication system. In cartilage, the water content is unevenly distributed. The highest concentration (80%) is found near the cartilage surface. Its concentration decreases gradually with increasing depth, to reach about 65% in the deep zone. Most of the water is extracellular. Inorganic salts, such as sodium, calcium, and potassium chloride, are dissolved in the tissue water. The maintenance and flow of water in the tissue relies on its interaction with the macromolecules. The diffusion of water through the cartilage helps move the nutrients from the synovial fluid through the tissue, contributing to the nutrition of chondrocytes.

Collagen represents 50–60% of the cartilage dry weight. Collagen fibrils form a network that provides the shape and form of the tissue. Fibril diameters vary from 20 nm in the superficial zone to 70–120 nm in the deep zone. Type II collagen is specific to cartilage and is the primary collagen in this tissue (90–98% of the total tissue collagen). The most important mechanical properties of collagen fibers are tensile stiffness and strength.

The proteoglycans constitute the second largest portion of the solid phase in articular cartilage, accounting for 5–10% of the tissue wet weight. The proteoglycans in articular cartilage are complex supramolecular aggregates, and consist of a central hyaluronic acid (HA) filament to which multiple monomers are noncovalently attached. Proteoglycan monomers of varying size arise laterally at regular intervals from the opposite side of the HA chain. A low-molecular-weight protein – named link protein – stabilizes the bond between the monomer and the hyaluronate. The chemical structure of the monomer can be compared to a 'test-tube brush'. Proteoglycans in the cartilage matrix are mainly of the large aggregating type (50–85%) and of the large nonaggregating type (10–40%). An aggregating proteoglycan is composed of a monomer formed by a protein backbone, the core protein. Numerous glycosaminoglycan chains radiate at 90° from this core protein, extending stiffly. The glycosaminoglycan molecules are formed of a long chain of repeating polydimeric saccharides: chondroitin-4-sulfate, chondroitin-6-sulfate, and keratan sulfate, of which chondroitin sulfate is the most abundant (60–90%). The average chondroitin sulfate chain consists of 25–30 repeating disaccharide units. Keratan sulfate chains are shorter and consist of only 5 or 6 repeating dimeric units.

Composition

Collagens

Type II collagen. The major macromolecule of articular cartilage is type II collagen. It is composed of three identical polypeptide chains – α1(II) chains – that intertwine along most of their length to form a triple helix. The α-chains are synthesized as pro-α-chains, which possess large propeptides at both their *N* and *C* termini. The propeptides are separated from the central triple-helix-forming region by short telopeptides. The propeptides are required for normal trimer assembly during synthesis within the chondrocyte, but are removed from the resulting procollagen by proteolysis following secretion into the extracellular space, leaving only the telopeptides attached to the triple helix. The *N*-propeptide is removed by a procollagen-*N*-protease (ADAMTS-3, a member of a subgroup of the adamalysin family, is most active on type II collagen[3]) and the *C*-propeptide is removed by procollagen-*C*-protease (BMP1).[4] Two forms of type II procollagen can be generated. Type IIA procollagen, which possesses a variable region, is mostly associated with newly formed cartilage during chondrogenesis, whereas type IIB procollagen is present in all cartilage.

The trimeric collagen molecule does not exist in isolation within the extracellular matrix, but in the form of collagen fibrils. These are formed by the lateral association of collagen molecules in a quarter staggered array. Each collagen molecule is joined to its neighbors via aldimine-derived crosslinks. These crosslinks join the telopeptide of one collagen molecule to the triple helix of another, stabilizing the fibril, preventing thermal or mechanical dissociation, and allowing the fibrils to resist the tensile forces to which the cartilage is exposed *in vivo*. The initially formed aldimine crosslinks are subsequently modified to pyridinoline crosslinks, which can serve as a marker for mature collagen.[5] With increasing age, additional nonaldimine-derived crosslinks (e.g. pentosidine) accumulate in the collagen fibrils[6], making them stronger and more resistant to proteolytic degradation. Indeed, mature collagen fibrils undergo little turnover under normal physiological conditions during the life of an individual.

The triple helical region of the type II collagen molecule is resistant to degradation by most proteases, but can be cleaved by the action of collagenases. Mammalian collagenases act consecutively at a single site in each of the three α-chains to yield fragments that are about three-quarters and one-quarter the length of the intact molecule. Three mammalian collagenases exist and are named metalloprotease (MMP)-1, MMP-8, and MMP-13 – with MMP-13 favoring the cleavage of type II collagen over the other fibrillar collagens.[7] The collagen fragments can be denatured by further cleavage by gelatinases (MMP-2 and MMP-9). However, cleavage of the intact collagen fibril is a slow process, as it might contain hundreds of collagen molecules across its diameter.

The importance of type II collagen in cartilage formation and function is best illustrated by the consequence of type II collagen (*COL2A1*) gene mutations, which give both growth plate and articular cartilage abnormalities.[8] To date, over 100 different mutations have been detected, the most common being missense substitutions in a glycine codon in an exon encoding the helical region of the pro-α-chain. In this region there is an absolute requirement for glycine at every third amino acid, and replacement by any other amino acid perturbs triple helix formation. This results in a dominant negative effect due to both intracellular degradation of the newly synthesized procollagen molecule and perturbation of fibril formation, thus weakening the

cartilage. The site of the mutation determines the severity of the disease. Stickler syndrome is commonly characterized by nonsense mutations and the phenotype is a consequence of haploinsufficiency.[9] The mildest phenotypes are caused by an arginine to cysteine substitution in the triple helical region.[10]

Type XI collagen. Type XI collagen is another fibrillar collagen that is cartilage specific. It is less abundant than type II collagen and its abundance decreases from about 10% of the total collagen in fetal cartilage to around 3% in adult articular cartilage.[11] It is composed of three different α-chains that intertwine to form a heterotrimeric triple helix, which possesses the composition α1(XI).α2(XI).α3(XI). In adult articular cartilage, the α1(V) chain of type V collagen might replace the α1(XI) chain.[12] The α3(XI) chain is derived from the *COL2A1* gene, but differs from the α1(II) chain in its post-translational modification.[12] Type XI collagen is synthesized and secreted as a procollagen in a similar manner to type II collagen, but undergoes proteolytic removal of only its *C*-propeptide. The type XI collagen molecules do not form unique fibrils but occur in heterotypic fibrils in association with type II collagen molecules. The type XI collagen molecules reside at the center of the heterotypic fibril with their *N*-propeptides protruding from its surface and limiting its lateral growth. Aldimine-mediated crosslinking joins type XI collagen molecules within the fibril core. Type XI collagen is most concentrated in the thin collagen fibrils that form the pericellular network. Growth in fibril width might involve proteolytic removal of the N-propeptide of the type XI collagen to permit the accretion of more type II collagen molecules or the fusion of adjacent fibrils.

Mutations in both of the unique type XI collagen genes (*COL11A1* and *COL11A2*) also result in chondrodystrophic phenotypes[8], one of which is the Stickler syndrome, which can result from mutations in either the type II or type XI collagen genes. Early-onset OA is a common feature of these disorders.

Type IX collagen. Type IX collagen is a member of the FACIT (fibril-associated collagen with interrupted triple helix) family; it is also cartilage specific. It does not form a collagen fibril itself but is instead present at the surface of the type II/type XI fibril. Its abundance decreases from about 10% of the total collagen in fetal cartilage to about 1% in adult articular cartilage.[11] It is composed of three different α-chains that form a heterotrimeric molecule possessing the composition α1(IX).α2(IX).α3(IX). The type IX collagen molecule consists of three triple helical domains (COL1-3) bordered by nonhelical domains (NC1-4). Interaction between the type IX collagen molecule and the collagen fibril takes place through the COL1 and COL2 domains, with the COL3 domain and the amino terminal NC4 domain projecting away from the fibril. The type IX collagen molecules are crosslinked to the type II collagen molecules of the fibril via aldimine-derived crosslinks.[13] The cationic NC4 domain of the α1 chain has been suggested to act as a bridge between the collagenous framework and the interspersed extracellular matrix, particularly the anionic glycosaminoglycan chains of the proteoglycans, although this remains to be proven. In some tissues, the cationic NC4 domain is absent due to the use of an alternative promoter in the *COL9A1* gene.[14] Type IX collagen might itself be considered a proteoglycan, as it can be substituted by a chondroitin sulfate chain in the NC3 domain of its α2 chain. The functional significance of such substitution is unclear.

Some mutations have been described in each of the type IX collagen genes (*COL9A1*, *COL9A2*, and *COL9A3*), giving rise to multiple epiphyseal dysplasia.[8] Early-onset OA is again a feature of this disorder. Specific polymorphic changes affecting

tryptophan residues within the *COL9A2* and *COL9A3* genes have been linked to intervertebral disc degeneration[15], but not yet to OA.

Other collagens. As with all connective tissues, articular cartilage also contains type VI collagen, which accounts for about 1% of its total collagen content.[11] Its content is increased and tissue distribution altered in OA cartilage.[16,17] Type VI collagen is a nonfibrillar collagen composed of three distinct α-chains, with each type VI collagen molecule possessing a central triple helical domain flanked by large nonhelical terminal regions. The type VI collagen molecules assemble into a disulfide-bonded filamentous network, which is predominant in the pericellular matrix around the chondrocytes.[16,17] The proteoglycan biglycan, which interacts with the type VI collagen, appears to be important for the assembly of the type VI collagen network. The type VI collagen does not appear to be covalently crosslinked to the other collagen types of the cartilage, but is associated with the fibrillar collagen framework of the tissue via the interaction of matrilin with decorin or biglycan, which might be bound to the collagens. Mutations in any of the type VI collagen genes (*COL6A1*, *COL6A2*, and *COL6A3*) can give rise to congenital muscular dystrophy; it is not clear how cartilage might be affected in these disorders. In the type VI collagen knockout mouse there is no gross abnormality in cartilage function.

Type X collagen is a cartilage-specific collagen that, under normal circumstances, is confined to the hypertrophic zone of the growth plate, where it participates in endochondral ossification. It is a homotrimeric molecule, with one central triple helical domain and nonhelical termini. It is thought to form a hexagonal array mediated via its *C*-terminal NC1 domain. Although type X collagen is not a component of normal articular cartilage, it is present in OA cartilage[18], particularly in the deep zones where clusters of hypertrophic chondrocytes are observed. Type X collagen can be cleaved within its triple helical region by MMP-1. Mutations in the type X collagen gene (*COL10A1*) lead to various forms of metaphyseal dysplasia.[19]

Collagens in osteoarthritis. Whereas changes in the abundance of the various collagen types can occur in OA, the disorder is characterized by the enhanced degradation that occurs in the type II collagen-based fibrils. The collagenases cleave only within the type II collagen triple helix of the heterotypic fibrils, and do not cleave within the triple helices of type IX or type XI collagen.[8] Proteolysis within the nonhelical regions of these collagens is likely to be involved in their degradation. Multiple members of the MMP family acting in unison are responsible for the degradation of the collagen fibrils, which results in the irreversible fibrillation of the articular cartilage in the OA joint. Proteolytic damage to the collagen fibrils during the initial phase of OA probably contributes to the hypertrophy and increased hydration of the articular cartilage, as the weakened collagen fibril network might not adequately resist the swelling properties of the entrapped proteoglycans. All the proteases involved in collagen fibril degradation can be expressed by the chondrocytes. Genetic studies have also indicated that the *COL2A1*, *COL9A1*, and *COL11A2* genes might represent susceptibility genes that predispose to articular cartilage degeneration in some cases of primary idiopathic OA.[20]

Proteoglycans

Aggrecan. Cartilage is characterized by its high content of aggrecan, which exists in association with HA and link protein in the form of proteoglycan aggregates that provide

the osmotic properties needed for articular cartilage to resist compressive loads. Each aggregate is composed of up to 100 aggrecan molecules radiating from a central filament of HA. Proteoglycan aggregates exist in two predominant molecular forms, which differ in their link protein content and probably their functional characteristics, as link protein stabilizes the aggrecan/HA interaction. Proteoglycan aggregate structure is influenced by the length of the HA, the proportion of link protein, and the degree of aggrecan processing; it is not constant throughout life, with decreases in HA length, aggrecan size, and possibly link protein stabilization being evident.

Aggrecan possesses a core protein with three globular regions, termed G1, G2 and G3, which are stabilized by disulfide bonds. The G1 and G2 regions are separated by a short interglobular domain (IGD), and the G2 and G3 regions are separated by a long glycosaminoglycan (GAG)-attachment region. The G1 region is at the amino terminus of the core protein and possesses domains responsible for the interaction with link protein and HA. The function of the G2 region is unknown. The G3 region resides at the carboxy terminus of the core protein and is essential for normal post-translational processing of the aggrecan core protein and subsequent aggrecan secretion.[21] The GAG-attachment region comprises three domains. The keratan sulfate attachment domain is adjacent to the G2 region and is followed by two chondroitin sulfate attachment domains, termed the CS1 and CS2 domains. There is no unique aggrecan structure, as the length, position of sulfation and sites of substitution of the GAG chains can vary throughout life.[22]

In cartilage extracellular matrix, aggrecan exists in a truncated form due to proteolytic processing, with its G3 region and part of the CS2 domain commonly being absent.[23] Each proteolytic cleavage of an intact aggrecan molecule generates one fragment that retains a G1 region, and so remains bound to HA, and a second fragment that is no longer attached. These latter fragments are rapidly lost by diffusion into the synovial fluid. Such proteolysis continues throughout life, further truncating the aggrecan molecules and ultimately yielding proteoglycan aggregates that are enriched in aggrecan G1 regions[24], which are relatively resistant to proteolysis. Aggrecanases and MMPs are associated with aggrecan proteolysis. The aggrecanases show selectivity for aggrecan, and cleave at one site in the IGD domain and four in the CS2 domain.[25]

High concentrations of aggrecan are necessary for the ability of articular cartilage to resist compressive loads. This functional ability depends on the aggrecan molecules possessing a high glycosaminoglycan content and being retained in the extracellular matrix. A decrease in aggrecan glycosaminoglycan content in the extracellular matrix can result in overcompression of the cartilage under load, causing an adverse response by the chondrocytes with increased protease secretion and subsequent tissue degeneration. It has been suggested that aggrecan function could be affected by size polymorphism within the CS1 domain, as those individuals with the shortest core protein length would possess aggrecan with the lowest CS substitution. However, at present it is not clear whether such individuals might be at risk for premature cartilage degeneration.[26]

The essential role of aggrecan in cartilage function is demonstrated by mutations in the *aggrecan* gene that lead to chondrodysplasias.

Link protein. Link proteins have a structure analogous to that of the G1 region of aggrecan, with domains responsible for interaction with the G1 region of aggrecan and HA. Human link protein can exist in three molecular forms: LP1, LP2 and LP3. LP1 and LP2 are intact link proteins; LP3 is formed by proteolytic cleavage in the region between the two oligosaccharide chains. The amino terminal peptide of link protein

that is released during the formation of LP3 can act as a growth factor to stimulate matrix production by the chondrocytes. MMPs have been implicated in such degradation[27], but the link proteins are resistant to aggrecanases. Additional proteolysis does take place *in vivo*, to produce link protein fragments. This type of processing has been mimicked *in vitro* by the action of free radicals. Proteolytic modification of link protein occurs throughout life[28] and the modified link proteins accumulate in the proteoglycan aggregates, but there is currently no evidence that such modified link proteins are functionally impaired.

Link protein serves several functions in the proteoglycan aggregates. It stabilizes the proteoglycan aggregate and prevents its dissociation under physiological conditions, it interacts with newly synthesized aggrecan to produce a conformational change in the G1 region and promote aggregate formation[29], and it forms a protein coat covering the surface of HA, which helps protect the HA from hyaluronidases and free radical mediated degradation.[30]

Hyaluronan. HA is a nonsulfated GAG characterized by its long length. It is synthesized at the plasma membrane of the cell via a hyaluronan synthase (HAS) and extruded directly into the extracellular space where proteoglycan aggregate formation occurs in the pericellular matrix. It is not clear how the proteoglycan aggregates move from this environment to the more remote parts of the extracellular matrix. The HA content in articular cartilage increases with age and its size decreases.[31] The increase in HA content might reflect an accumulation of partially degraded proteoglycan aggregates, and the decrease in size is probably a consequence of degradation. HA degradation might have an adverse metabolic effect on the cells, as HA oligosaccharides can promote cell mediated catabolism.

Small leucine-rich repeat proteoglycans. In common with all connective tissue, cartilage contains a variety of small leucine-rich repeat proteoglycans (SLRPs); decorin, biglycan, fibromodulin, and lumican are the major ones present in articular cartilage. They help to maintain the integrity of the tissue and modulate its metabolism. They can be divided into several subfamilies based on the number of leucine-rich repeats, the type of GAG chain substituent and their gene organization.

In vitro, decorin, biglycan, and fibromodulin have been shown to be degraded by MMPs, and it is likely that such cleavage can occur *in vivo*.[32–34] These changes result in SLRP structure varying throughout life in articular cartilage.

Decorin, fibromodulin, and lumican interact with the fibrillar collagen via their core proteins and in so doing help regulate fibril diameter and fibril–fibril interaction in the extracellular matrix. These interactions might also limit access of the collagenases to their unique cleavage site on each collagen molecule, and therefore help protect the fibrils from proteolytic damage.[35] Fibromodulin and lumican interact with the same region of the collagen molecule, whereas decorin interacts at a distinct site. Molecular modeling predicts that the SLRPs possess a 'horse-shoe' conformation that is able to accommodate a collagen molecule within its concave face. However, X-ray diffraction analysis of decorin indicates that it exists as a dimer with interlocking concave faces[36], and it is not clear how this might influence function within the tissue. As mentioned above, biglycan is able to interact with type VI collagen and so participate in the formation of an organized network. The glycosaminoglycan chains have been associated with the interaction with several growth factors, and they enable the SLRPs to provide a sink for growth factor accumulation within the extracellular matrix, thus regulating growth factor access to the cells.

The functional significance of SLRPs in connective tissues has been demonstrated in both knockout mice and human disorders including skin, bone, cornea, and tendon. One would also expect that the molecular organization of cartilage would be affected in these mice and humans, but any functional consequence awaits future study.

Other proteoglycans. Versican is a ubiquitous proteoglycan related to aggrecan, but is present at a much lower concentration than aggrecan in cartilage. The versican core protein contains two terminal globular regions equivalent to the G1 and G3 regions of aggrecan separated by a large glycosaminoglycan attachment domain. The G1 region permits the formation of proteoglycan aggregates, but it is not clear if versican and aggrecan can reside on the same aggregate. The versican present in the cartilage undergoes extensive proteolytic processing[37], and it is likely that MMPs and aggrecanases participate in its degradation. The glycosaminoglycan attachment region of versican appears to contain only chondroitin sulfate, and bears no structural similarity to that of aggrecan. Its structure can vary, due to alternative splicing, to yield four different versican isoforms, termed V0, V1, V2, and V3. The V1 isoform is ubiquitously expressed and is the major form in cartilage.[37]

Perlecan is present in all basement membranes and in the extracellular matrix of cartilage, which is devoid of basement membranes. Perlecan is present in cartilage and can interact with other cartilage matrix components[38], and influence chondrocyte metabolism via its interaction with fibroblast growth factors and their receptors.[39] Perlecan is essential for normal cartilage development, as knockout mice exhibit growth-plate abnormalities and severe skeletal defects[40] and mutations give rise to the skeletal defects.[41,42]

Proteoglycans in osteoarthritis. Aggrecans are probably among the first cartilage matrix constituents to be affected, as they are progressively depleted in parallel with the severity of the disease. At a certain stage in the evolution of OA, the chondrocytes appear unable to compensate fully for proteoglycan loss even in the presence of increased synthesis, resulting in a net loss of matrix. The structure of the proteoglycan remaining in the cartilage is altered in different ways.[43,44]

Generally, the presence of aggregates appears to reduce the vulnerability of proteoglycans to enzymatic attack. In OA, certain proteases are able to attack the proteoglycan monomer. Such degraded fragments can rapidly diffuse from cartilage, leaving behind normal proteoglycan still capable of aggregation. As soon as the degradation occurs, the products are either further degraded by chondrocyte enzymes or rapidly diffused into the synovial fluid. Alternatively, the reduction in the HA content of OA cartilage, which causes a reduction in the size of the proteoglycan aggregates, could favor a loss of proteoglycan breakdown products from the tissue.

OA is associated with the increased loss of aggrecan fragments that is due to the action of both MMPs and aggrecanases, with predominant cleavage occurring in the interglobular domain (IGD) of aggrecan.[45] Cleavage at this site results in maximal loss of aggrecan function, as it separates the entire glycosaminoglycan attachment region from the G1 region that anchors it in the tissue. In the early phases of OA, MMP-13 is predominantly expressed in the lower intermediate and deep layers of cartilage, whereas aggrecanases are expressed in the superficial cartilage.[46–48] New aggrecan synthesis does occur in OA cartilage, presumably in an attempt by the chondrocytes to initiate repair. The newly synthesized aggrecan forms proteoglycan aggregates that are retained in the cartilage during the early phases of OA, but in later stages they are also lost to the synovial fluid.[49] The newly synthesized aggrecan has a composition

more similar to juvenile cartilage than adult, having a decreased content of keratan sulfate and an increase in chondroitin-4-sulfate relative to chondroitin 6-sulfate.[43]

The tissue content of decorin and biglycan undergoes little change in OA cartilage[50], although there is evidence that loss does occur in the more superficial regions where these proteoglycans are most abundant. The core protein of the SLRPs does become more fragmented in OA[51], compatible with enhanced proteolysis.[33] However, the level of SLRP synthesis is also increased and might compensate for the level of degradation, a situation that does not occur with aggrecan. The rate at which proteoglycan synthesis and degradation occur varies in different regions of the cartilage as a result of differences in the mechanical stress to which it is subjected. Although little is known about the influence of the disease on the other cartilage proteoglycans, increased synthesis and levels of both versican and perlecan have been reported in OA cartilage.[52]

Advanced glycation end products (AGEs)

During the development of arthritic diseases, the physical properties of the extracellular matrix become disrupted and a loss of collagen and aggrecan occurs in cartilage. However, even without any pathologic change, collagen and aggrecan are altered with aging. Collagen crosslinking increases and the collagen framework becomes stiffer and more brittle. The size of the aggrecan molecules and the pattern of sulfation are also altered. It has recently been suggested that, with age, the accumulation of advanced glycation end products (AGEs) in cartilage is responsible for these events, which impair the ability of the cartilage to remodel its extracellular matrix.

The collagen crosslink formation occurs through a process called nonenzymatic glycation, which takes place via the AGE.[53] This collagen crosslink formation via the AGE increases the stiffness of the collagen network and decreases collagen turnover. This type of glycation was also shown to occur with aggrecan in cartilage.[6] In addition, the susceptibility of AGE-modified collagen to proteolytic degradation by MMPs in cartilage is altered[54] and the chondrocyte cellular functions are modified in an environment rich in AGEs in which the proteoglycan and collagen synthesis by chondrocytes is decreased. The capacity of the cartilage to remodel is then limited and degradation is favored. Although RAGE, an AGE receptor, has been identified at the chondrocyte surface, it still remains to be demonstrated that AGEs exert their effect through this receptor.

It has been suggested that the formation of AGEs could be one of the molecular events that causes age to be a major predisposing factor for the development of cartilage damage in OA.[55]

Anabolic factors

Growth factors

Efforts have been devoted to identifying conditions that are favorable to the formation of a durable, functional articular surface, which has led to the study of factors and – more specifically – growth factors that are able to maintain cartilage homeostasis and/or stimulate cartilage repair.

Growth factors are polypeptides that are required for the synthesis and maintenance of an extracellular matrix that is capable of protecting the joint against the damages incurred by repetitive joint movement, injury, or inflammation. The levels of these

factors are normally low in healthy adult cartilage, so as to maintain optimal extracellular matrix quantity and quality. However, although many factors are termed growth factors, some of them are also able to induce the degradative process and are responsible for part of the catabolic process occurring during OA.

The list of growth factors that have a putative effect on the development and proliferation of the articular cartilage has grown considerably. The most characterized in the context of cartilage formation and maintenance include transforming growth factor beta (TGF-β), bone morphogenic proteins (BMPs), cartilage-derived morphogenic proteins (CDMPs), insulin-like growth factors (IGFs), connective-tissue growth factor (CTGF), hepatocyte growth factor (HGF), and fibroblast growth factor (FGF). Although the lectin-related proteins are not identified as growth factors, they can show properties belonging to the growth factors.

TGF-β superfamily. TGF-β is a multifunctional growth factor that regulates cell proliferation and extracellular matrix synthesis. Articular cartilage normally contains TGF-β stored in a latent form and very little active TGF-β. Increased levels of TGF-β have been reported in OA cartilage. TGF-β is capable not only of increasing proteoglycan synthesis, but also of counteracting the interleukin-1β (IL-1β)-mediated suppression of proteoglycan synthesis[56] and other genes.[57] However, the protective effect of TGF-β on articular tissues appears to be age-dependent.[58]

TGF-β has also demonstrated both pro- and anti-inflammatory activities. In animal models, although TGF-β was demonstrated to induce synovial inflammation, it might suppress acute and chronic arthritis. Consistent with its dual role are the effects of TGF-β on some proteolytic enzymes. Whereas its inhibition of the production of MMP-9 and MMP-1 suggests a protective role in tissue destruction, its induction of MMP-13 and the aggrecanase ADAMTS-4 in chondrocytes[47,59] suggests its involvement in macromolecule turnover in normal cartilage, although it might also exacerbate cartilage damage during the OA process. TGF-β also induces the formation of osteophytes.

BMPs are potent stimulators of bone and cartilage formation; they also promote matrix synthesis and the growth of chondrocytes. Like TGF-β, BMPs are normally present in low amounts in healthy cartilage. Several BMPs are expressed in human cartilage, where they are thought to play a role in repair and maintenance of joint integrity. More particularly, BMP-2, BMP-4, BMP-6, BMP-7, BMP-9, and BMP-13 were shown to enhance the synthesis of type II collagen and aggrecan. In OA cartilage, only BMP-2, which is weakly expressed in normal cartilage, has been found to have an increased level.[60,61] BMP-7 (OP-1) has been reported in both normal and OA human cartilage[62], but some researchers[63,64] failed to detect its expression in either normal or OA chondrocytes. BMP-6 was also found to be expressed in human cartilage, but at a similar level in both normal and OA cartilage.[65]

Normal and OA chondrocytes respond to the anabolic signals triggered by BMP-7[63] and BMP-6[65], although an age-related decline in the responsiveness to BMP-6 occurs in normal human chondrocytes.[65] BMP-2 enhances the synthesis of articular proteoglycans *in vivo* but this stimulatory effect is abolished when IL-1 is present.[66] BMP-7 has been reported to counteract the effects of IL-1β-induced MMP-1 and MMP-13 in human chondrocytes and this cytokine decrease in the tissue inhibitor of metalloprotease (TIMP) expression and proteoglycan synthesis. BMP induces the formation of osteophytes, but at different sites from those induced by TGF-β.[67]

BMP antagonists, which block the binding of BMPs to their receptor, are also present in articular cartilage. BMP antagonists comprise a family of structurally unrelated

proteins. Representatives of the BMP antagonist family include follistatin, gremlin, chordin, and noggin, which, with the exception of noggin are expressed and produced in adult cartilage.[68,69] Follistatin, gremlin, and chordin bind BMP-2, BMP-4, and BMP-7; follistatin has a higher affinity for BMP-7, whereas chordin has a higher affinity for BMP-2 and BMP-4. Overexpression of chordin in the embryonic chick-limb system delays chondrocyte maturation and supports a role for chordin as a negative regulator of endochondral ossification. As each antagonist differs in its specificity and affinity for a specific BMP, they were suggested to play different roles, depending on cell and tissue type, in spatial and temporal regulation of BMP activity.

The BMPs and their antagonists constitute a complex dynamic system that is regulated at different levels so as to respond to specific environmental stimuli. In the adult joint, there is an equilibrium between the levels of BMPs and their antagonists. Recent data showed the presence of higher levels of some BMP antagonists in OA cartilage.[68] This might disturb BMP activities, leading to decreased anabolic activities, thus affecting tissue repair and remodeling. Findings also showed that BMP-2 and BMP-4 are favorite candidates for triggering the increase of gremlin expression, and that some growth factors inhibit follistatin expression.[68] In OA, it is suggested that gremlin might first appear at the hypertrophic stage, when the increased level of BMP-2 stimulates its expression. IL-1β, which plays a pivotal role in cartilage degradation, might later upregulate gremlin indirectly by inducing BMP-2.[68] Gremlin would then be present in the early and late stages of OA. Unlike gremlin, follistatin seems to have a stronger link to the inflammatory aspect of OA and might appear later in the OA process, possibly induced by the presence of cytokines involved in inflammation and in severe OA.

CDMPs are the human homologs of the cartilage growth and differentiation factors that also belong to the TGF-β superfamily and are required for cartilage formation and joint development. CDMP-1 and CDMP-2 are expressed in both normal and human OA chondrocytes and cartilage[60,63], and OA chondrocytes are responsive to exogenous CDMPs.[63] CDMP-1 (BMP-14), CDMP-2 (BMP-13), and CDMP-3 are also expressed in osteophytes isolated from human OA joints.[70] Although reports indicate that CDMP-1 can stimulate differentiation of mesenchymal precursors into chondrocytes and promote the differentiation of hypertrophic chondrocytes, and that CDMP-2 was found to support chondrogenesis but not terminal differentiation, their roles in OA are yet to be determined.

Insulin-like growth factors. IGFs are polypeptides that play a part in the growth and development of many tissues. IGF-1 is a major homeostatic factor, controlling matrix synthesis and mitotic activity. It is present in low levels in normal cartilage but its expression is upregulated in OA cartilage.[71] *In vivo*, IGF-1 promotes the synthesis of proteoglycans without significantly affecting inflammation or cartilage breakdown.[72] Transplantation of articular chondrocytes transfected with a plasmid containing human IGF-1 gene onto the surface of articular cartilage explants led to the formation of a new tissue layer containing type II collagen and proteoglycans; transplantation also increased DNA synthesis and synthesis of glycosaminoglycans by the underlying explant cartilage chondrocytes.[73] In a recent study[74], in which human OA chondrocytes were cultured in alginate beads with IGF-1, a prolonged IGF-1 treatment of 21 days stimulated sulfate incorporation without significant accumulation of a proteoglycan matrix. Moreover, when IGF-1 was administered with pentosan polysulphate (an agent that binds growth factors), cartilage damage was protected against by a reduction in MMP levels and an increase in TIMP levels in cartilage.[75] Another mechanism by which IGF-1 could exert its anabolic effect is by its ability to upregulate the type II IL-1

receptor[76], which is downregulated in OA chondrocytes. This type II IL-1 receptor binds to IL-1α and IL-1β, but does not transmit the intracellular signal. In rats, intra-articular injection of IGF-1 results in the production of osteophytes.[77]

OA chondrocytes are hyporesponsive to IGF-1, a phenomenon that appears not to be related to its specific receptor but rather to be due to the higher level of IGF binding proteins (IGFBPs) produced by OA chondrocytes.[78,79] IGFBPs form a complex with IGF-1, preventing its signaling and therefore its anabolic activity. Disturbances in the balance of IGF-1 to IGFBPs have been reported in OA joints.[78,80] Although, to date, several IGFBPs have been cloned and sequenced, disregulation in IGFBP-3 and IGFBP-4 appear to be of the utmost importance in arthritic disorders. It has recently been proposed that the hyporesponsiveness of OA chondrocytes to this factor could be related to the IGF-1 desensitization due to the IL-1-induced suppressor of cytokine signaling 3 (SOCS3), which inhibits IGF-1 signaling.[81]

Connective-tissue growth factor. CTGF is a member of the CCN family of cysteine-rich secreted proteins. It has been reported to stimulate gene expression of aggrecan and types II and X collagens in cultured chondrocytes.[82] CTGF is expressed predominantly by hypertrophic chondrocytes and regulates the proliferation and differentiation of chondrocytes *in vitro*; it is expressed similarly in normal and OA cartilage.[60,83] CTGF binds to vascular endothelial growth factor (VEGF), a strong angiogenic mitogen also produced by hypertrophic chondrocytes. VEGF165 binds to CTGF through a protein–protein interaction so that the angiogenic activity of VEGF is negatively regulated by CTGF in the extracellular environment.[84] VEGF and its receptors are expressed in OA synovial tissue and cartilage.[85,86]

Hepatocyte growth factor. Like several growth factors, HGF is a multifunctional molecule; it induces cell proliferation, motility, and morphogenesis. HGF is found within the deep zone of normal cartilage and is increased in OA.[87] Recently, it was reported that the HGF found in adult cartilage was not produced by the chondrocytes but by the osteoblasts from the subchondral bone plate, and that OA subchondral bone osteoblasts produce five times more HGF than normal.[88] Moreover, although chondrocytes did not express HGF, these cells expressed the two truncated HGF isoforms: NK1 and NK2.[88] Although the exact role of HGF or its isoforms remains to be established, it has been suggested that it might play a role in cartilage homeostasis and repair in early OA. However, HGF was also found to induce MMP-13[89], an enzyme present in OA cartilage predominantly in the lower intermediate and deep layers of the tissue.[47,48]

Fibroblast growth factor. Members of the FGF family include FGF-2, FGF-4, FGF-8, FGF-9, FGF-10, and FGF-18 as well as the FGF receptors FGFR1, FGFR2 and FGFR3. The most studied is the FGF-2 or bFGF, which is reported to stimulate proteoglycan and collagen synthesis, as well as being a potent mitogen for adult articular chondrocytes. Very little is known about the role of FGF-2 in arthritis. Recently, FGF-9 and FGF-18 were found to increase matrix synthesis by chondrocytes[90,91], and FGF-18 promoted cartilage repair in a rat meniscal tear model of OA.[92]

Lectin-related proteins

Human cartilage glycoprotein 39. Human cartilage glycoprotein 39 (HC-gp39), also known as YKL-40 or Chi3L-1, belongs to the family of chi-lectins (chitinase-like

lectins). A unique feature of chi-lectins is their lack of enzymatic activity. Despite the abundance of molecular and structural information on these proteins, evidence for a physiological function has only recently emerged. HC-gp39 is a secreted glycoprotein initially identified in articular chondrocytes and overexpressed in arthritic cartilage, in synovial tissues and in sera from OA.[93,94] Recent work has shown that HC-gp39 can be considered a growth factor because it stimulates proliferation of human cells.[95] This was reinforced by the fact that the guinea-pig ortholog promotes anabolic events, such as the synthesis of the aggrecan in chondrocytes, as well as cell growth.[96] Prominent sites of HC-gp39 production in articular tissues are degenerated cartilage.[93] HC-gp39 was suggested to dampen the cellular response to some inflammatory mediators because it was found to inhibit chondrocyte responses to the inflammatory cytokine IL-1β and tumor necrosis factor alpha (TNFα), as well as the cytokine-induced production of MMP-1, MMP-3, and MMP-13 in chondrocytes.[97] Moreover, HC-gp39 also plays a role in chondrocyte differentiation by initiating SOX-9 and type II collagen expressions in chondrocytes.[98] The ability of HC-gp39 to initiate several signaling cascades in connective tissue cells suggests the existence of a specific receptor for this protein; however, the nature of this receptor remains to be elucidated.

Galectin-3. Galectin-3 is a soluble animal lectin of 30 kDa that preferentially recognizes lactosamine and *N*-acetyllactosamine structures. Galectins, like other lectins, recognize a glycosylation structure with neither enzymatic nor immune activity. Although galectin-3 lacks signal sequence, it is secreted from cells by a novel, incompletely understood mechanism called ectocytosis, which is independent of the classic secretory pathway through the endoplasmic reticulum and Golgi system. Recent work reported the capacity of normal and OA human chondrocytes to synthesize galectin-3, with an increased expression level in human OA articular cartilage.[99] Interestingly, this protein does not seem to have the same function on all cell types, either intracellularly or extracellularly. In articular tissue, although not considered a growth factor, intracellularly galectin-3 plays a protective role in chondrocytes.[100] However, extracellularly, galectin-3 has deleterious effects in both cartilage and subchondral bone tissues[101], inducing joint swelling and lesions in both tissues, and in human OA chondrocytes it induces expression of ADAMTS-5 and MMP-3.

Catabolic factors

Cartilage matrix is known to be turned over throughout life. However, it is believed that the cartilage matrix degradation products could at one point be amplifiers/catalysts of the cartilage degenerative process and lead to the development of OA. Some of these catabolic factors, including proteases, fibronectin, some neuromediators, and inflammatory factors, are found to be involved in the progression of this disorder.

Proteases

Metalloproteases. The metalloproteases comprise one of the foremost classes of enzymes involved in OA. These proteases are broadly divided into groups based on substrate specificity and cellular location: collagenases, stromelysins, gelatinases, membrane-type MMP (MT-MMP) and adamalysin (ADAM and the subgroup ADAMTS).

In OA, the expression and synthesis of three collagenases, MMP-1, MMP-8, and MMP-13, were found at higher levels in OA[7,102]. Although all three collagenases are active on collagen fibrils, the specificity between them for a specific collagen type differs, as does their topographical location, suggesting a selective involvement of each during the disease process. In OA cartilage, MMP-1 and MMP-8 are located predominantly in the superficial and upper layers (superficial zone), whereas MMP-13 is found mostly in the lower intermediate and deep layers (deep zone).[47,48] Data also suggest the involvement of MMP-1 during the inflammatory phase and of MMP-13 in the remodeling phase. The specific role of MMP-8 in OA progression remains to be documented.

Interestingly, in human cartilage, MMP-13 showed another differential feature in which its synthesis pattern could be discriminated into two broad categories of human OA chondrocyte.[103,104] On the one hand, in some OA human chondrocytes, MMP-13 is found, as in normal, in low basal levels and high IL-1β/TGF-β inducibility, whereas others show a high MMP-13 basal level but low IL-1β/TGF-β inducibility; these categories were named low- and high-OA chondrocytes, respectively. The difference in MMP-13 production following the stimulation of OA chondrocytes could be related to the transcription factor JunB, which is abundant in the high-OA chondrocytes and regulates MMP-13 production negatively, thus limiting the induction by proinflammatory cytokines. In addition, a specific site on the MMP-13 promoter has recently been demonstrated to be responsible for the regulation of this enzyme's basal level.[105] This site, named AGRE for AG-rich element, was further shown to be regulated via the transcription factors p130^{cas} and NMP4.[106] Similar findings could not be applied to MMP-1 production.

Three stromelysins have been described in human tissues: MMP-3, MMP-10, and MMP-11. They have a broad substrate specificity that includes proteoglycans, fibronectin, elastin, and laminin. The predominantly expressed stromelysin in normal cartilage is MMP-3, which is considered to be the crucial enzyme in matrix turnover. In cartilage, MMP-3 is upregulated in early OA and downregulated in the late stages.[107] MMP-3 also acts on type IX collagen[108] and is implicated in the enzymatic cascade responsible for the activation of proMMP-1. Interestingly, and although the specific role of MMP-11 in cartilage remains to be elucidated, this protease was also found upregulated in the late stages of OA.[107]

Two gelatinases have been found in articular tissues: MMP-9 and MMP-2. They have a substrate preference for denatured collagen, gelatin, and types IV and V collagen. MMP-9 was reported to be expressed and synthesized in OA but not in normal cartilage.[109] The fact that MMP-9 was found to be selectively expressed in OA fibrillated cartilage is consistent with the possibility that this enzyme could be responsible for progressive articular cartilage degradation in this disease. MMP-2 expression was also shown to be increased in OA cartilage.[107]

To date, six members of the MT-MMP subgroup have been identified and designated MT1-MMP–MT6-MMP. These proteases are characterized by a transmembrane domain at the C-terminus and they act at the cell surface. The relevance of these enzymes in cartilage, excluding MT1-MMP has yet to be determined. Studies have suggested a role for MT1-MMP in the degradation of the extracellular matrix because it possesses collagenolytic and aggrecanase activity. Moreover, MT1-MMP activates MMP-2 and MMP-13.

Analysis of aggrecan fragments in OA tissues showed a proteolytic cleavage of the Glu373–Ala374 bond of the interglobular domain, between the G1 and G2 domains of aggrecan.[110] The enzymes responsible for the cleavage belong to a subgroup of the

ADAM family, the ADAMTS, and were named aggrecanases. Two such enzymes have been reported to be present in cartilage, ADAMTS-4 and ADAMTS-5; recent studies have demonstrated that ADAMTS-5 is the predominant enzyme involved in the OA degradative process.[111,112]

Serine proteases. The urokinase-type (uPA) and tissue-type (tPA) plasminogen activators are proteases that convert plasminogen to plasmin. uPA plays the predominant role in articular tissue. The PA/plasmin proteases have a broad spectrum of activity. Their increased levels in OA joints, as well as their stimulation by IL-1, suggest that they play a role in this disease.[113] Moreover, plasmin has the potential to degrade cartilage proteoglycans as well as to activate latent MMPs.

Several physiologic serine protease inhibitors have also been found in cartilage. In particular, the specific PA inhibitor, PAI, exhibited decreased levels in OA cartilage.[113] This, combined with the increased level of PA, might contribute – at least in part – to the increased level of biologically active MMPs found in OA tissue.

Thiol proteases. The degradation of cartilage extracellular matrix macromolecules often occurs in the perilacunar area, where the matrix pH is in the acid range. Cathepsins are lysosomal enzymes with a maximal activity at acid pH and several require thiol as an activator. Many cathepsins have been found in OA articular joint tissues, including cathepsins B, K, L, and S. Cathepsins B and L cleave collagen types II, IX, and XI, and destroy the crosslinked collagen matrix at low pH. In cartilage, cathepsin B, in addition to having a direct degradative effect on some extracellular macromolecules, might also be an MMP activator.[114] This enzyme was recently suggested as being a marker of the dedifferentiated chondrocyte phenotype.[115] Although cathepsin K is the key enzyme in osteoclastic bone resorption, its expression has also been observed in cartilage. Transgenic mouse models have provided evidence supporting its important role in arthritis. Interestingly, cathepsin K is one of the few noncollagenase enzymes capable of degrading native fibrillar collagen types I and II.[116]

Other catabolic factors

Fibronectin. Fibronectin is an adhesive dimeric glycoprotein found in the extracellular matrix of cartilage. It is a component of normal cartilage matrix, and elevated levels were found in OA cartilage.[117–119] Although fibronectin has no catabolic effect on cartilage, it is readily degraded into fragments by multiple proteases and, once fragmented, acquires catalytic activities. Increased levels of fibronectin fragments of 30–200 kDa have been found in human OA cartilage.[117] In cartilage, fibronectin fragments have been shown to enhance proteoglycan loss; to decrease proteoglycan synthesis[120,121]; and to increase the production of MMP-1, MMP-2, MMP-9, MMP-13, aggrecanase, serine proteases such as uPA[122,123], and NO in association with iNOS induction.[124] Fibronectin can bind several integrins and other cell-surface protein ligands. Evidence suggests that the fibronectin-fragment effects on human chondrocyte MMP production are mediated by the $\alpha 5\beta 1$ integrin.[125,126] Moreover, CD44, a principal hyaluronan receptor, was reported to mediate fibronectin-fragment MMP and NO enhancement.[127] In human chondrocytes, fibronectin fragments were found to activate MMP-13 and to induce NO production in association with the activation of the mitogen-activated protein (MAP) extracellular signal-regulated kinase (ERK), p38, and Jun-N-terminal kinase (JNK).[124,125,128]

Neuromediators. It has been shown that interactions between neuroendocrine systems could contribute to the pathogenesis of arthritic diseases. Among the different endocrine mediators, leptin and some neuropeptides are thought to play a role in OA pathophysiology.

Leptin. Leptin is a 16-kDa nonglycosylated peptide hormone, encoded by the *obese* (*ob*) gene and mainly produced by adipocytes. Under normal conditions, leptin suppresses specific biochemical processes that contribute to the accumulation of lipids and the differentiation of adipocytes. Leptin also stimulates hematopoietic precursor development. Structurally, leptin belongs to the type-I cytokine superfamily. High plasma levels of leptin have been found to be related to increased susceptibility to the development of OA.[129] However, a clear link between circulating leptin levels and OA has yet to be reported and local leptin levels in the joint may be more important that circulating leptin for OA progression.

Leptin levels are increased in OA cartilage, and chondrocytes express both it and its functional receptor.[130,131] Interestingly, injections of leptin into the joints of normal rats can mimic OA features. In chondrocytes, leptin induces, in synergy with INF-α or IL-1β, NO activation via the activation of JAK-2 kinase for the former and PI-3 kinase, MEK-1, and p38 for the latter.[132] Furthermore, leptin is also associated with inflammatory states and with stimulated prostaglandin E_2 and leukotriene production, two factors produced in higher levels in OA cartilage. However, although leptin might be a contributing factor, it might not be sufficient alone, although it *is* necessary, to promote cartilage damage in OA.

Neuropeptides. A number of studies have demonstrated a key role played by some neuropeptides in the production of inflammatory cytokines. In articular cartilage, emerging evidence points to the involvement of the neuropeptides such as substance P in the pathogenesis of OA. Substance P has been shown to potentiate the action of IL-1.[133] Moreover, substance P, as well as others such as gastrin-releasing peptide (GRP), were detected in chondrocytes and found in increased amounts in the damaged zones of human OA cartilage.[134]

Inflammatory factors

There is compelling evidence to suggest that secreted inflammatory mediators impact on the matrix homeostasis of articular tissue cells by altering their metabolism. The inflammatory mediators act on chondrocytes to cause further catabolism by creating a positive amplification loop leading to more protease production, as well as to cytokines and other inflammatory factors. Among these mediators responsible for the progression of the disease, evidence points to the proinflammatory cytokine IL-1β as being the most important factor responsible for the catabolic process in OA. Other proinflammatory cytokines, such as TNF-α, oncostatin M (OSM), IL-6, leukemia inhibitory factor (LIF), IL-17, and IL-18, are also considered to be potential contributing factors in the pathogenesis of OA, although the exact role and importance of each in this disease process is not yet clearly established. In addition to cytokines, other inflammatory mediators also play a major role in the OA pathological process. These include NO, eicosanoids (prostaglandins and leukotrienes), and a newly identified cell membrane receptor family, the protease-activated receptors (PARs), in which an important role for PAR-2 in chronic arthritis has been suggested.

Proinflammatory cytokines. The catabolic effects of IL-1β are multiple. This cytokine can stimulate its own production, increase the synthesis of enzymes (MMPs, PA/plasmin),

inhibit the synthesis of the major physiological inhibitors of these enzymes (TIMPs, PAI-1), inhibit the synthesis of matrix constituents such as collagen and proteoglycans, and stimulate the synthesis and release of some eicosanoids, including prostaglandins and leukotrienes. The action of this proinflammatory cytokine on the enzyme process, combined with the suppression of matrix synthesis, results in severe degradation of articular tissues and the appearance of conditions that we know to be characteristic of OA.

This cytokine also plays important roles in normal physiology, including stimulation of the turnover of extracellular matrix. Hence, control mechanisms exist to limit the extent of cytokine activation and to avoid potential tissue injury. One of these control mechanisms, unique to the IL-1 system, is a physiologic inhibitor of its receptor known as the IL-1 receptor antagonist (IL-1Ra).

Other proinflammatory cytokines include TNF-α, which is present in OA but at a severe stage of the disease. This cytokine exerts diverse effects and its best-studied aspect is its ability to promote inflammation.

Recent studies provide evidence that OSM, a member of the IL-6 family, plays a role in the articular tissue. This cytokine is involved in physiologic as well as pathologic functions.[135,136] OSM is the only member of this cytokine family to cause proteolytic release of proteoglycans and collagen from human articular cartilage. Also of interest is the capacity of OSM to synergize the action of other inflammatory mediators, including IL-1, TNF-α and IL-17.[137,138] In chondrocytes, this striking synergistic effect appears to occur through the induction of the expression of the collagenases, MMP-3, MT1-MMP, and aggrecanases. Among actions relevant to joint degradation, OSM also induces IL-6. However, the role of IL-6 in cartilage pathology remains unclear, as this cytokine can induce the production of TIMP-1, IL-1Ra, and soluble TNF receptor 55, all of which inhibit or block MMP or cytokine activity.

Among the other cytokines, it has been suggested that IL-17 and IL-18 play a role in OA pathophysiology as they share many properties with IL-1. On articular tissue cells, these cytokines also demonstrate effects independent of IL-1β, and both seem to be involved in the early phase of the inflammatory process. However, IL-18 can induce other proinflammatory cytokines such as IL-1, thus creating an amplifying loop. Its potential involvement in OA has been suggested based on its enhanced presence in OA cartilage and synovial membrane.[139,140]

Nitric oxide. In addition to proinflammatory cytokines, other inflammatory mediators could also play major roles in the OA process, the principals being NO and the eicosanoids, prostaglandins and leukotrienes.

NO acts as a mediator in various physiologic and pathophysiologic processes in the human body. NO generated by the inducible NO synthase (iNOS) has regulatory, proinflammatory, and destructive effects. OA cartilage produces a large amount of NO and reactive oxygen species (ROS). NO has been shown to be involved in the promotion of cartilage catabolism in OA through a number of mechanisms. It can inhibit the synthesis of cartilage matrix macromolecules, such as aggrecans, enhance MMP activity, and reduce the synthesis of IL-1Ra by chondrocytes. NO also plays a role in chondrocyte apoptosis and induces the synthesis of cyclo-oxygenase (COX)-2 and PGE_2. It has also been shown that exogenous PGE_2 could sensitize human OA chondrocytes to cell death induced by NO.[141,142] Collectively, NO acts by reducing the major anabolic processes and increasing the catabolic processes.

Eicosanoids. Other major inflammatory factors involved in OA pathophysiology are the prostaglandins and leukotrienes. Prostaglandins are synthesized from arachidonic

acid via the actions of the COX enzymes. The most abundant prostanoid in the human body is PGE_2. In addition to exacerbating joint inflammation, PGE_2 can also potentiate the effects of other mediators of inflammation. It can affect cartilage remodeling directly or function indirectly as an autocrine regulatory factor. PGE_2 can also contribute to cartilage damage by promoting MMP production.

COX-1 and COX-2 activity used to be considered the key step in PGE_2 synthesis. Hence, metabolism of arachidonic acid by COX-1 or COX-2 yields to the unstable intermediary PGH2, which can be further metabolized into prostanoids. The enzyme responsible for the isomerization of PGH2 and the terminal enzyme responsible for prostanoid synthesis was recently identified as PG synthase (PGS). In the case of PGE_2, there are three distinct PGES, named cytosolic PGES (cPGES), microsomal PGES-1 (mPGES-1), and mPGES-2. cPGES is constitutively and ubiquitously expressed and is preferentially coupled with COX-1. The protein expressions of COX-2 and mPGES-1 are concordantly induced by IL-1β. mPGES-2 is constitutively expressed in diverse tissues and has been found to be functionally linked to both COX-1 and COX-2. Accumulating evidence implicates mPGES-1 in the pathogenesis of OA.[143–145]

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) or COX-2 selective inhibitors has shown that PGE_2 inhibition alone does not seem to delay the natural history of progressive OA. In recent years, it has been shown that PGE_2 synthesis is only one part of the arachidonic acid pathway. The precursor, arachidonic acid, is a substrate that gives origin to many other lipid mediators, including leukotrienes. Leukotrienes themselves play a major role in the development and persistence of the inflammatory process, and it is now clear that prostaglandins and leukotrienes have complementary effects. Leukotrienes are produced by the enzyme 5-lipoxygenase (5-LOX). Studies have revealed that, on human OA tissues, leukotriene B4 (LTB4) potently stimulates the release of proinflammatory cytokines such as IL-1β and TNF-α.[146–148] Thus, the failure of NSAIDs to impact OA progression could be due to the fact that inhibiting only the COX pathways leads to a shunt to leukotriene production in these tissues.[149,150] From this concept, it is hypothesized that blocking production of both leukotrienes and prostaglandins could have a synergistic effect in achieving optimal or a wider spectrum of anti-inflammatory activity in articular tissues.

Protease-activated receptors. Some members of the PARs have recently been shown to be involved in inflammatory pathways and, more specifically, an important role for PAR-2 in arthritis has been demonstrated. These receptors belong to a family of seven-transmembrane G-protein-coupled receptors, which are activated through a unique process. Cleavage of their *N*-terminal domains by proteases unmasks a new *N*-terminal sequence that acts as a tethered ligand, binding and activating the receptor itself.[151] The enzymes-activating PARs belong to the serine protease family. Once activated, this process is irreversible. To date, four members of this family have been identified and designated PAR-1–PAR-4. They exhibit differential tissue expression as well as selectivity in activation. More particularly, PAR-2 has recently been reported to be involved in inflammatory conditions including those in rheumatoid arthritis and in OA.[152,153] PAR-2 expression has recently been found to be produced by human chondrocytes[154,155], and was modulated by the proinflammatory cytokines IL-1β and TNF-α, as well as the growth factors bFGF and TGF-β. Activation of this receptor has been found to be associated to NO, COX-2, PGE_2, and MMPs. It is also suggested that its activation induces the production of proinflammatory cytokines, including IL-1β.

SUBCHONDRAL BONE AND CARTILAGE CROSSTALK IN OSTEOARTHRITIS

Although the initiating event responsible for the degradation of cartilage in OA patients remains elusive, the concept of a key role of the subchondral bone tissue in OA is gaining strong support. A plausible hypothesis is that cytokines, growth factors, and eicosanoids produced locally by subchondral bone tissue seep through the bone–cartilage interface and induce changes in the cartilage metabolism. Although it was previously thought that the calcified cartilage layer was an impenetrable structure, the presence of many channels between the subchondral region and the uncalcified cartilage was demonstrated. Moreover, the presence of microcracks in the calcified layer of aging articular cartilage was also shown. These microcracks, and the vascularization in the subchondral bone plate, could facilitate the transfer of humoral information from the subchondral bone region to the basal layer of cartilage.

Several factors produced by subchondral bone cells are known to be capable of inducing cartilage catabolic changes. For instance, TGF-β, which is found at high levels in OA subchondral bone[156], has the capacity to induce MMP-13 synthesis by chondrocytes, and therefore could be responsible for the increased level of this enzyme found in the intermediate and deep layers of the diseased cartilage.[47] Both this enzyme and cathepsin K are synthesized by bone cells, and have been demonstrated to be involved in both endochondral ossification and OA cartilage degradation.[157,158] Moreover, in OA subchondral bone osteoblasts, there is abnormal production of proinflammatory cytokines and PGE_2/LTB4, which have a significant influence on the metabolism of the overlying cartilage and can promote the breakdown of normal cartilage.

HGF could be an important candidate for such crosstalk between the subchondral bone and cartilage.[88] As mentioned earlier, HGF, although not produced by the chondrocytes, is produced at a higher level in OA subchondral bone osteoblasts, and is found in OA cartilage.[88] These data suggest that, following its synthesis by subchondral osteoblasts, HGF can reach the deep layers of articular cartilage via local vascularization and/or channels where it promotes cartilage breakdown and/or enhances matrix remodeling. This is emphasized by the fact that HGF potently induces MMP-13 synthesis by chondrocytes[89], and as mentioned above, *in situ*, MMP-13 is preferentially located in the deep zone of OA cartilage.[47]

CARTILAGE ASSESSMENT

Biological markers of cartilage

When a component of joint tissue is released, measuring its concentration in the joint fluid is theoretically the most reliable approach. However, joint fluid collection is difficult in many OA patients and can be obtained readily only in the knee. Moreover, joint lavage results in significant errors to the concentration of the markers because of uncontrolled sample dilution. The best use of biomarkers in OA is their determination in serum or urine. However, their use in clinical practice has been tempered by the fact that urine and serum biomarkers are affected by age, gender, ethnicity, bone mass index, physical activity, and diurnal variations.[159,160] They are also influenced by the synovial clearance of metabolites into the blood circulation and structural changes in molecules when entering the blood circulation.

The classic approach in OA deals with markers reflecting the synthesis and degradation of cartilage; the principal biomarkers of cartilage are listed below. New

approaches such as genomics, proteomics, and metabolomics have recently been proposed for identifying biomarkers; however, these techniques are still under evaluation and need further validation.

Cartilage synthesis

Products of collagen type II synthesis. *C*- and *N*-propeptides of the IIA procollagen (PIICP, PIIANP) are propeptides that are crosslinked to collagen molecules. Propeptides are released from collagen molecules into biological fluids during maturation and their concentrations are believed to directly reflect the rate of type II collagen synthesis and are consequently an indicator for cartilage formation.[161,162] Their levels are two to four times higher in synovial fluid and 7.6 times higher in cartilage from OA patients than from healthy individuals.[163]

Aggrecan epitope. The epitope 846 is located on the chondroitin sulfate chains of the aggrecan and reflects aggrecan synthesis. It is highly expressed in OA cartilage and elevated in OA synovial fluid. Circulating levels are the highest in OA patients with the longest disease history.[164]

Cartilage-derived retinoic-acid-sensitive protein. Cartilage-derived retinoic-acid-sensitive protein (CD-RAP), also referred to as melanoma-inhibiting activity, was originally isolated from malignant melanoma cells. In physiological conditions, CD-RAP seems to be expressed only by chondrocytes. Two studies reported decreased synovial fluid levels of CD-RAP with increased cartilage degradation in knee OA, suggesting that this marker could reflect the restorative reaction of chondrocytes.[165,166]

Cartilage degradation

Degradation products of type II collagen

C-terminal crosslinking telopeptide of type II collagen

C-terminal crosslinking telopeptide of type II collagen (CTX-II) is a degradation product of the *C*-terminal end of type II collagen. The use of CTX-II as a marker of the disease activity of OA was documented, and a correlation between its levels and the radiographic progression of the disease, or clinical score, as well as the severity of cartilage defect, has been found.[167,168]

C2C and C1,2C. C2C and C1,2C are neoepitopes produced by collagen type II collagenase cleavage.[169] Although they are increased in different animal models[170], their relevance in humans has yet to be documented.

Helix-II. Helix-II is a newly identified specific fragment generated by the degradation of the helical domain of type II collagen. Helix-II has been demonstrated to be increased in the urine of OA patients compared to healthy volunteers.[171]

Cartilage oligomeric matrix protein

Cartilage oligomeric matrix protein (COMP) is a disulfide linked pentameric proteoglycan found mostly in cartilage but also in the other tissues of the joint, including meniscus, tendon, synovial fibroblasts, and osteoblasts.[172,173] COMP is a member of the thrombospondin family and has been shown to stimulate type II collagen fibril

formation.[174] Its level is elevated in the synovial fluid and serum of OA patients, with a positive correlation with the disease activity.[175] Correlation was also found with the radiographic progression of the joint disease, as well as with the clinical evaluation of pain, stiffness and physical function.[176]

Human cartilage glycoprotein 39

HC-gp39 levels are low in normal cartilage but high in the serum of hip OA patients.[177] Although this marker has been suggested to reflect cartilage degradation, some consider it a marker of inflammation, as it was found in increased amounts in other inflammatory pathologies.

Cathepsin K

Cathepsin K levels in rheumatoid arthritis patients have been correlated with radiologic damage.[178] Cathepsin K is presently under investigation as a marker for OA.[179]

Pyridinoline

Pyridinoline (PYD) is a part of the crosslink between adjacent collagen molecules. The measurement of PYD in urine is commonly used for the monitoring of bone, synovium, and cartilage degradation. A glycosylated analog, Glc-Gal-PYD, has recently been identified in abundance in synovium compared to cartilage[180] and is increased in OA patients[181], but no correlation with radiologic change was noted.[182]

Magnetic resonance imaging

A recently described new application of MRI allows precise visualization of the cartilage and its pathological changes. In recent years, there have been advances in the use of optimized MRI acquisition sequences and precise quantification changes in cartilage in individuals.[183,184] This method now allows quantitative assessment of intraindividual cartilage volume/thickness changes over time in healthy individuals and patients with OA. Data showed a mean loss of about 2–3% per year for healthy individuals, and 5–7% per year for patients with OA.[184–187] Recently, this technology has also allowed the discrimination of subgroups of human OA patients, those with slow versus fast progression[184,187], and shown that the major risk factors – in addition to age, gender, and BMI – were meniscal extrusion and tear.[187,188]

CONCLUSION

Cartilage matrix turnover is mediated by a multitude of factors that act on the chondrocytes and could lead to repair, remodeling or catabolic processes. The molecules and factors involved in cartilage repair and maintenance have been the subject of intensive research and significant progress has been made in the understanding of the interactions of the components of this tissue. At a certain point, these processes are altered, resulting in matrix changes including local abnormal biochemical pathways that contribute to cartilage degradation.

OA is a result of alterations of the extracellular matrix that lead to pain, loss of motion, and instability. It has long been suggested that the morphological changes seen early in OA are only age related. We now know that, even if changes associated

with aging and OA appear similar in the early stages, at a certain point they could be discriminatory. However, these processes are not exclusive of each other as the changes occurring during aging could lead to OA pathology.

In OA, the occurrence of alterations in chondrocyte metabolism is well established. Data suggest that they are primarily the result of a disturbance in the remodeling process. During the disease development, cartilage degradation results from several distinct biological processes operating spatially and temporally within the articulation, and include changes in the cell metabolism in other articular tissues. Hence, in this disease, the participation and the role of synovial inflammation is now widely accepted. As synovitis appears to be the result of the synthesis and release of many factors, including microcrystals, abnormal mechanical stress, production of excess amounts of NO and ROS, and the cartilage matrix proteolytic degradation products that produce wear particles and soluble cartilage-specific neoantigens, it is considered to be secondary to the changes in the cartilage. Yet, findings indicate that synovial inflammation could be a component of the early events leading to the clinical stage of OA. In addition, emerging evidence suggests that changes in subchondral bone are closely involved in the disease progression. Data even suggest that these alterations might precede cartilage alterations. Subchondral bone is suggested to be the site causing the most significant pathophysiologic events occurring in cartilage.

The clinical and radiological findings that form the basis for the diagnosis of OA lack sufficient sensitivity to monitor the progression of the disease. Biological markers reflecting quantitative and dynamic changes in joint tissue turnover represent promising adjunct tools. Increasing evidence indicates that the combination of some of these markers might be useful to predict the progression of OA. The usefulness of such markers is evident, and it is hoped that ongoing research will introduce more definite developments in this field.

Plain radiography, the reference technique for assessing the severity of cartilage destruction, provides direct information on bones, but only indirect information on cartilage. The recent development of MRI provides direct information on the cartilage volume/thickness alterations and has proved to be specific, sensitive and reliable in cartilage quantification for human OA progression.

In conclusion, this review concentrates on several interesting advances in the understanding of cartilage in normal and OA conditions. Research has already contributed to, and will continue to play a part in, the development of more specific markers for OA, the improvement of accurate and precise diagnostic systems and new strategies for treatments aimed at specifically and effectively retarding or stopping the progression of this disease. These advances will allow better identification of the target factors that are intimately involved in cartilage degradation.

Practice points

- The clinical efficacy of NSAIDs is related to PGE_2 production inhibition through the inhibition of COX-1 and COX-2 activities. However, these drugs, including the recently developed generation of COXIBs, have shown serious adverse effects. Hence, a selective inhibitor of PGE_2 production targeting only PGE_2 would be of major significance. In this respect, targeting the terminal enzyme responsible for PGE_2 synthesis, mPGES, could prove to be an interesting approach for OA therapeutic development. Moreover, in view of the

concept that prostaglandins and leukotrienes have complementary effects in the persistence of the inflammatory process, and that chronic inhibition of COX might lead to a shunt of the arachidonic acid metabolism towards the leukotriene pathway, blocking both prostaglandin and LTB4 production could have synergistic effects and achieve optimal anti-inflammatory activity.

- Biological markers in serum and urine that can reflect cartilage metabolic or inflammatory processes have been developed. Their use in clinical practice has been tempered by, among other things, circadian and diurnal variations and the fact that systemic levels of markers provide information on the overall metabolism of all joints in the body. Moreover, in advanced OA, there might be little or no cartilage left, making interpretation difficult.
- Plain radiography, the reference technique for assessing the severity of cartilage destruction, provides direct information on bones, but only indirect information on cartilage. The recent development of MRI provides direct information on the cartilage volume/thickness alterations and has proved to be specific, sensitive and reliable in cartilage quantification for human OA progression.
- Until now, almost all OA studies have focused only on symptomatic OA patients, i.e. patients showing clinical parameters such as pain and joint function disability, a stage in which the structural changes of the articular joint tissues, such as cartilage, are already at the moderate to severe range of the disease. Sensitive and accurate methods for the assessment of OA at its onset are crucial in research efforts to find the key players in the initiation and progression of OA, to differentiate between the disease subgroups and to assess the therapeutic efficacy of new treatments. To this end, there has been – in recent years – a series of advances in the use of optimized MRI to precisely quantify changes in cartilage volume and thickness in individuals, and to discriminate subgroups of patients. The use of this technology will be crucial for trials to look at the beginning of the OA process and its progression over time.

Research agenda

- In OA cartilage, in addition to pathological metabolic processes, chondrocyte clustering occurs in the superficial zone; however, it is doubtful whether this mode of tissue repair can mimic normal matrix anabolism. Further research into the collagen type produced and proteoglycan properties is warranted on these cells.
- OA cartilage demonstrates a reversion to a fetal-like phenotype with the re-initiation of fetal skeletal developmental processes occurring mostly in the intermediate and deep layers of the pathological tissue. We do not know all the factors involved in this process.
- Genetic studies have indicated that mutations of some collagen-type genes (e.g. *COL2A1*, *COL9A1*, *COL11A2*) represent susceptibility genes that predispose articular cartilage to degeneration. However, as other collagen types are present in normal cartilage, we do not know whether mutations in these collagen types affect the function of the tissue.

- Although the roles and expression of some growth factors have been studied extensively in OA, the use of growth factors in the treatment of this disease is a challenging avenue, as several problems have yet to be addressed; among them, the formation of osteophytes, the nonresponsiveness of older cells to some growth factors, and their inability to counteract many catabolic actions of proinflammatory molecules.
- Although the initiating event responsible for the degradation of cartilage in OA patients is still elusive, the concept of a key role for the subchondral bone tissue in OA is gaining strong support. This makes it possible to believe that therapies that interfere with bone remodeling could block, or at least attenuate, the progression not only of changes in this tissue, but also in cartilage.
- More clinical trials are needed so that new therapeutic strategies can be brought to clinical practice.

REFERENCES

1. Venn MF. Chemical composition of human femoral and head cartilage: influence of topographical position and fibrillation. *Annals of the Rheumatic Diseases* 1979; **38:** 57–62.
2. Stockwell RA. Morphology of cartilage. In Maroudas A & Keuttner K (eds.). *Methods in cartilage research*. San Diego: Academic Press, 1990, pp. 61–63.
3. Jones GC & Riley GP. ADAMTS proteinases: a multi-domain, multi-functional family with roles in extracellular matrix turnover and arthritis. *Arthritis Research & Therapy* 2005; **7:** 160–169.
4. Prockop DJ, Sieron AL & Li SW. Procollagen N-proteinase and procollagen C-proteinase. Two unusual metalloproteinases that are essential for procollagen processing probably have important roles in development and cell signaling. *Matrix Biology* 1998; **16:** 399–408.
5. Takahashi M, Kushida K, Ohishi T et al. Quantitative analysis of crosslinks pyridinoline and pentosidine in articular cartilage of patients with bone and joint disorders. *Arthritis and Rheumatism* 1994; **37:** 724–728.
6. Verzijl N, DeGroot J, Thorpe SR et al. Effect of collagen turnover on the accumulation of advanced glycation end products. *The Journal of Biological Chemistry* 2000; **275:** 39027–39031.
7. Reboul P, Pelletier JP, Tardif G et al. The new collagenase, collagenase-3, is expressed and synthesized by human chondrocytes but not by synoviocytes: A role in osteoarthritis. *The Journal of Clinical Investigation* 1996; **97:** 2011–2019.
8. Byers PH. Folding defects in fibrillar collagens. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 2001; **356:** 151–157.
9. Ahmad NN, Ala-Kokko L, Knowlton RG et al. Stop codon in the procollagen II gene (COL2A1) in a family with the Stickler syndrome (arthro-ophthalmopathy). *Proceedings of the National Academy of Sciences of the United States of America* 1991; **88:** 6624–6627.
10. Pun YL, Moskowitz RW, Lie S et al. Clinical correlations of osteoarthritis associated with a single-base mutation (arginine519 to cysteine) in type II procollagen gene. A newly defined pathogenesis. *Arthritis and Rheumatism* 1994; **37:** 264–269.
11. Eyre DR, Weis MA & Wu JJ. Articular cartilage collagen: an irreplaceable framework? *European Cells & Materials* 2006; **12:** 57–63.
12. Eyre DR. Collagens and cartilage matrix homeostasis. *Clinical Orthopaedics and Related Research* 2004; (427 Suppl)**:** S118–S122.
13. Wu JJ, Woods PE & Eyre DR. Identification of cross-linking sites in bovine cartilage type IX collagen reveals an antiparallel type II-type IX molecular relationship and type IX to type IX bonding. *The Journal of Biological Chemistry* 1992; **267:** 23007–23014.

14. Muragaki Y, Kimura T, Ninomiya Y & Olsen BR. The complete primary structure of two distinct forms of human alpha 1 (IX) collagen chains. *European Journal of Biochemistry* 1990; **192:** 703–708.
15. Ala-Kokko L. Genetic risk factors for lumbar disc disease. *Annals of Medicine* 2002; **34:** 42–47.
16. Soder S, Hambach L, Lissner R et al. Ultrastructural localization of type VI collagen in normal adult and osteoarthritic human articular cartilage. *Osteoarthritis and Cartilage* 2002; **10:** 464–470.
17. Pullig O, Weseloh G & Swoboda B. Expression of type VI collagen in normal and osteoarthritic human cartilage. *Osteoarthritis and Cartilage* 1999; **7:** 191–202.
18. Boos N, Nerlich AG, Wiest I et al. Immunohistochemical analysis of type-X-collagen expression in osteoarthritis of the hip joint. *Journal of Orthopaedic Research* 1999; **17:** 495–502.
19. Bateman JF. The molecular genetics of inherited cartilage disease. *Osteoarthritis and Cartilage* 2001; **9**(Suppl. A)**:** S141–S149.
20. Bateman J. Genetic aspects of osteoarthritis. *Seminars in Arthritis and Rheumatism* 2005; **34:** 15–18.
21. Chen L, Wu Y, Lee V et al. The folded modules of aggrecan G3 domain exert two separable functions in glycosaminoglycan modification and product secretion. *The Journal of Biological Chemistry* 2002; **277:** 2657–2665.
22. Roughley PJ & White RJ. Age-related changes in the structure of the proteoglycan subunits from human articular cartilage. *The Journal of Biological Chemistry* 1980; **255:** 217–224.
23. Dudhia J, Davidson CM, Wells TM et al. Studies on the G3 domain of aggrecan from human cartilage. *Annals of the New York Academy of Sciences* 1996; **785:** 245–247.
24. Roughley PJ & Mort JS. Ageing and the aggregating proteoglycans of human articular cartilage. *Clinical Science (London, England)* 1986; **71:** 337–344.
25. Tortorella MD, Pratta M, Liu RQ et al. Sites of aggrecan cleavage by recombinant human aggrecanase-1 (ADAMTS-4). *The Journal of Biological Chemistry* 2000; **275:** 18566–18573.
26. Roughley P, Martens D, Rantakokko J et al. The involvement of aggrecan polymorphism in degeneration of human intervertebral disc and articular cartilage. *European Cells & Materials* 2006; **11:** 1–7.
27. Nguyen Q, Murphy G, Hughes CE et al. Matrix metalloproteinases cleave at two distinct sites on human cartilage link protein. *The Biochemical Journal* 1993; **295:** 595–598.
28. Mort JS, Poole AR & Roughley PJ. Age-related changes in the structure of proteoglycan link proteins present in normal human articular cartilage. *The Biochemical Journal* 1983; **214:** 269–272.
29. Melching LI & Roughley PJ. Studies on the interaction of newly secreted proteoglycan subunits with hyaluronate in human articular cartilage. *Biochimica et Biophysica Acta* 1990; **1035:** 20–28.
30. Rodriguez E & Roughley P. Link protein can retard the degradation of hyaluronan in proteoglycan aggregates. *Osteoarthritis and Cartilage* 2006; **14:** 823–829.
31. Holmes MW, Bayliss MT & Muir H. Hyaluronic acid in human articular cartilage. Age-related changes in content and size. *The Biochemical Journal* 1988; **250:** 435–441.
32. Imai K, Hiramatsu A, Fukushima D et al. Degradation of decorin by matrix metalloproteinases: identification of the cleavage sites, kinetic analyses and transforming growth factor-beta1 release. *The Biochemical Journal* 1997; **322:** 809–814.
33. Monfort J, Tardif G, Reboul P et al. Degradation of small leucine-rich repeat proteoglycans by matrix metalloprotease-13: identification of a new biglycan cleavage site. *Arthritis Research & Therapy* 2006; **8:** R26.
34. Heathfield TF, Onnerfjord P, Dahlberg L & Heinegard D. Cleavage of fibromodulin in cartilage explants involves removal of the N-terminal tyrosine sulfate-rich region by proteolysis at a site that is sensitive to matrix metalloproteinase-13. *The Journal of Biological Chemistry* 2004; **279:** 6286–6295.
35. Geng Y, McQuillan D & Roughley PJ. SLRP interaction can protect collagen fibrils from cleavage by collagenases. *Matrix Biology* 2006; **25:** 484–491.
36. Scott PG, McEwan PA, Dodd CM et al. Crystal structure of the dimeric protein core of decorin, the archetypal small leucine-rich repeat proteoglycan. *Proceedings of the National Academy of Sciences of the United States of America* 2004; **101:** 15633–15638.
37. Sztrolovics R, Grover J, Cs-Szabo G et al. The characterization of versican and its message in human articular cartilage and intervertebral disc. *Journal of Orthopaedic Research* 2002; **20:** 257–266.
38. Bengtsson E, Morgelin M, Sasaki T et al. The leucine-rich repeat protein PRELP binds perlecan and collagens and may function as a basement membrane anchor. *The Journal of Biological Chemistry* 2002; **277:** 15061–15068.
39. Chang Z, Meyer K, Rapraeger AC & Friedl A. Differential ability of heparan sulfate proteoglycans to assemble the fibroblast growth factor receptor complex in situ. *The FASEB Journal* 2000; **14:** 137–144.

40. Arikawa-Hirasawa E, Watanabe H, Takami H et al. Perlecan is essential for cartilage and cephalic development. *Nature Genetics* 1999; **23:** 354–358.
41. Arikawa-Hirasawa E, Wilcox WR, Le AH et al. Dyssegmental dysplasia, Silverman-Handmaker type, is caused by functional null mutations of the perlecan gene. *Nature Genetics* 2001; **27:** 431–434.
42. Arikawa-Hirasawa E, Le AH, Nishino I et al. Structural and functional mutations of the perlecan gene cause Schwartz-Jampel syndrome, with myotonic myopathy and chondrodysplasia. *American Journal of Human Genetics* 2002; **70:** 1368–1375.
43. Cs-Szabo G, Roughley PJ, Plaas AH & Glant TT. Large and small proteoglycans of osteoarthritic and rheumatoid articular cartilage. *Arthritis and Rheumatism* 1995; **38:** 660–668.
44. Malemud CJ, Papay RS, Hering TM et al. Phenotypic modulation of newly synthesized proteoglycans in human cartilage and chondrocytes. *Osteoarthritis and Cartilage* 1995; **3:** 227–238.
45. Struglics A, Larsson S, Pratta MA et al. Human osteoarthritis synovial fluid and joint cartilage contain both aggrecanase- and matrix metalloproteinase-generated aggrecan fragments. *Osteoarthritis and Cartilage* 2006; **14:** 101–113.
46. Chambers MG, Cox L, Chong L et al. Matrix metalloproteinases and aggrecanases cleave aggrecan in different zones of normal cartilage but colocalize in the development of osteoarthritic lesions in STR/ort mice. *Arthritis and Rheumatism* 2001; **44:** 1455–1465.
47. Moldovan F, Pelletier JP, Hambor J et al. Collagenase-3 (matrix metalloprotease 13) is preferentially localized in the deep layer of human arthritic cartilage in situ: in vitro mimicking effect by transforming growth factor beta. *Arthritis and Rheumatism* 1997; **40:** 1653–1661.
48. Fernandes JC, Martel-Pelletier J, Lascau-Coman V et al. Collagenase-1 and collagenase-3 synthesis in early experimental osteoarthritic canine cartilage. An immunohistochemical study. *The Journal of Rheumatology* 1998; **25:** 1585–1594.
49. Lohmander LS, Ionescu M, Jugessur H & Poole AR. Changes in joint cartilage aggrecan after knee injury and in osteoarthritis. *Arthritis and Rheumatism* 1999; **42:** 534–544.
50. Poole AR, Rosenberg LC, Reiner A et al. Contents and distributions of the proteoglycans decorin and biglycan in normal and osteoarthritic human articular cartilage. *Journal of Orthopaedic Research* 1996; **14:** 681–689.
51. Witsch-Prehm P, Miehlke R & Kresse H. Presence of small proteoglycan fragments in normal and arthritic human cartilage. *Arthritis and Rheumatism* 1992; **35:** 1042–1052.
52. Tesche F & Miosge N. Perlecan in late stages of osteoarthritis of the human knee joint. *Osteoarthritis and Cartilage* 2004; **12:** 852–862.
53. Chen AC, Temple MM, Ng DM et al. Induction of advanced glycation end products and alterations of the tensile properties of articular cartilage. *Arthritis and Rheumatism* 2002; **46:** 3212–3217.
54. DeGroot J, Verzijl N, Wenting-Van Wijk MJ et al. Age-related decrease in susceptibility of human articular cartilage to matrix metalloproteinase-mediated degradation: the role of advanced glycation end products. *Arthritis and Rheumatism* 2001; **44:** 2562–2571.
55. Verzijl N, DeGroot J, Ben ZC et al. Crosslinking by advanced glycation end products increases the stiffness of the collagen network in human articular cartilage: a possible mechanism through which age is a risk factor for osteoarthritis. *Arthritis and Rheumatism* 2002; **46:** 114–123.
56. Lum ZP, Hakala BE, Mort JS & Recklies AD. Modulation of the catabolic effects of interleukin-1 beta on human articular chondrocytes by transforming growth factor-beta. *Journal of Cellular Physiology* 1996; **166:** 351–359.
57. Takahashi N, Rieneck K, van der Kraan PM et al. Elucidation of IL-1/TGF-beta interactions in mouse chondrocyte cell line by genome-wide gene expression. *Osteoarthritis and Cartilage* 2005; **13:** 426–438.
58. Scharstuhl A, van Beuningen HM, Vitters EL et al. Loss of transforming growth factor counteraction on interleukin 1 mediated effects in cartilage of old mice. *Annals of the Rheumatic Diseases* 2002; **61:** 1095–1098.
59. Moulharat N, Lesur C, Thomas M et al. Effects of transforming growth factor-beta on aggrecanase production and proteoglycan degradation by human chondrocytes in vitro. *Osteoarthritis and Cartilage* 2004; **12:** 296–305.
60. Fukui N, Zhu Y, Maloney WJ et al. Stimulation of BMP-2 expression by pro-inflammatory cytokines IL-1 and TNF-alpha in normal and osteoarthritic chondrocytes. *The Journal of Bone and Joint Surgery. American Volume* 2003; **85-A**(Suppl. 3)**:** 59–66.

61. Nakase T, Miyaji T, Tomita T et al. Localization of bone morphogenetic protein-2 in human osteoarthritic cartilage and osteophyte. *Osteoarthritis and Cartilage* 2003; **11:** 278–284.
62. Chubinskaya S, Merrihew C, Cs-Szabo G et al. Human articular chondrocytes express osteogenic protein-1. *The Journal of Histochemistry and Cytochemistry* 2000; **48:** 239–250.
63. Bobacz K, Gruber R, Soleiman A et al. Cartilage-derived morphogenetic protein-1 and -2 are endogenously expressed in healthy and osteoarthritic human articular chondrocytes and stimulate matrix synthesis. *Osteoarthritis and Cartilage* 2002; **10:** 394–401.
64. Fan Z, Chubinskaya S, Rueger DC et al. Regulation of anabolic and catabolic gene expression in normal and osteoarthritic adult human articular chondrocytes by osteogenic protein-1. *Clinical and Experimental Rheumatology* 2004; **22:** 103–106.
65. Bobacz K, Gruber R, Soleiman A et al. Expression of bone morphogenetic protein 6 in healthy and osteoarthritic human articular chondrocytes and stimulation of matrix synthesis in vitro. *Arthritis and Rheumatism* 2003; **48:** 2501–2508.
66. Glansbeek HL, van Beuningen HM, Vitters EL et al. Bone morphogenetic protein 2 stimulates articular cartilage proteoglycan synthesis in vivo but does not counteract interleukin-1alpha effects on proteoglycan synthesis and content. *Arthritis and Rheumatism* 1997; **40:** 1020–1028.
67. van Beuningen HM, Glansbeek HL, van der Kraan PM & van den Berg WB. Differential effects of local application of BMP-2 or TGF-beta 1 on both articular cartilage composition and osteophyte formation. *Osteoarthritis and Cartilage* 1998; **6:** 306–317.
68. Tardif G, Hum D, Pelletier JP et al. Differential gene expression and regulation of the bone morphogenetic protein antagonists follistatin and gremlin in normal and osteoarthritic human chondrocytes and synovial fibroblasts. *Arthritis and Rheumatism* 2004; **50:** 2521–2530.
69. Tardif G, Pelletier JP, Hum D et al. Differential regulation of the bone morphogenic protein antagonist chordin in human normal and osteoarthritic chondrocytes. *Annals of the Rheumatic Diseases* 2006; **65:** 261–264.
70. Zoricic S, Maric I, Bobinac D & Vukicevic S. Expression of bone morphogenetic proteins and cartilage-derived morphogenetic proteins during osteophyte formation in humans. *Journal of Anatomy* 2003; **202**(Pt3)**:** 269–277.
71. Middleton JF & Tyler JA. Upregulation of insulin-like growth factor-1 gene expression in the lesions of osteoarthritic human articular cartilage. *Annals of the Rheumatic Diseases* 1992; **51:** 440–447.
72. Mi Z, Ghivizzani SC, Lechman ER et al. Adenovirus-mediated gene transfer of insulin-like growth factor 1 stimulates proteoglycan synthesis in rabbit joints. *Arthritis and Rheumatism* 2000; **43:** 2563–2570.
73. Madry H, Zurakowski D & Trippel SB. Overexpression of human insulin-like growth factor-I promotes new tissue formation in an ex vivo model of articular chondrocyte transplantation. *Gene Therapy* 2001; **8:** 1443–1449.
74. Loeser RF, Todd MD & Seely BL. Prolonged treatment of human osteoarthritic chondrocytes with insulin-like growth factor-I stimulates proteoglycan synthesis but not proteoglycan matrix accumulation in alginate cultures. *The Journal of Rheumatology* 2003; **30:** 1565–1570.
75. Rogachefsky RA, Dean DD, Howell DS & Altman RD. Treatment of canine osteoarthritis with sodium pentosan polysulfate and insulin-like growth factor-1. *Annals of the New York Academy of Sciences* 1994; **732:** 392–394.
76. Wang J, Elewaut D, Veys EM & Verbruggen G. Insulin-like growth factor 1-induced interleukin-1 receptor II overrides the activity of interleukin-1 and controls the homeostasis of the extracellular matrix of cartilage. *Arthritis and Rheumatism* 2003; **48:** 1281–1291.
77. Okazaki K, Jingushi S, Ikenoue T et al. Expression of insulin-like growth factor I messenger ribonucleic acid in developing osteophytes in murine experimental osteoarthritis and in rats inoculated with growth hormone-secreting tumor. *Endocrinology* 1999; **140:** 4821–4830.
78. Doré S, Pelletier JP, Di Battista JA et al. Human osteoarthritic chondrocytes possess an increased number of insulin-like growth factor 1 binding sites but are unresponsive to its stimulation. Possible role of IGF-1-binding proteins. *Arthritis and Rheumatism* 1994; **37:** 253–263.
79. Tardif G, Reboul P, Pelletier JP et al. Normal expression of type 1 insulin-like growth factor receptor by human osteoarthritic chondrocytes with increased expression and synthesis of insulin-like growth factor binding proteins. *Arthritis and Rheumatism* 1996; **39:** 968–978.

80. Tavera C, Abribat T, Reboul P et al. IGF and IGF-binding protein system in the synovial fluid of osteoarthritic and rheumatoid arthritic patients. *Osteoarthritis and Cartilage* 1996; **4:** 263–274.
81. Smeets RL, Veenbergen S, Arntz OJ et al. A novel role for suppressor of cytokine signaling 3 in cartilage destruction via induction of chondrocyte desensitization toward insulin-like growth factor. *Arthritis and Rheumatism* 2006; **54:** 1518–1528.
82. Nakanishi T, Nishida T, Shimo T et al. Effects of CTGF/Hcs24, a product of a hypertrophic chondrocyte-specific gene, on the proliferation and differentiation of chondrocytes in culture. *Endocrinology* 2000; **141:** 264–273.
83. Nishida T, Kubota S, Nakanishi T et al. CTGF/Hcs24, a hypertrophic chondrocyte-specific gene product, stimulates proliferation and differentiation, but not hypertrophy of cultured articular chondrocytes. *Journal of Cellular Physiology* 2002; **192:** 55–63.
84. Inoki I, Shiomi T, Hashimoto G et al. Connective tissue growth factor binds vascular endothelial growth factor (VEGF) and inhibits VEGF-induced angiogenesis. *The FASEB Journal* 2002; **16:** 219–221.
85. Enomoto H, Inoki I, Komiya K et al. Vascular endothelial growth factor isoforms and their receptors are expressed in human osteoarthritic cartilage. *The American Journal of Pathology* 2003; **162:** 171–181.
86. Pufe T, Petersen W, Tillmann B & Mentlein R. The splice variants VEGF121 and VEGF189 of the angiogenic peptide vascular endothelial growth factor are expressed in osteoarthritic cartilage. *Arthritis and Rheumatism* 2001; **44:** 1082–1088.
87. Pfander D, Cramer T, Weseloh G et al. Hepatocyte growth factor in human osteoarthritic cartilage. *Osteoarthritis and Cartilage* 1999; **7:** 548–559.
88. Guévremont M, Martel-Pelletier J, Massicotte F et al. Human adult chondrocytes express hepatocyte growth factor (HGF) isoforms but not HGF: potential implication of osteoblasts on the presence of HGF in cartilage. *Journal of Bone and Mineral Research* 2003; **18:** 1073–1081.
89. Reboul P, Pelletier JP, Tardif G et al. Hepatocyte growth factor induction of collagenase 3 production in human osteoarthritic cartilage: involvement of the stress-activated protein kinase/c-Jun N-terminal kinase pathway and a sensitive p38 mitogen-activated protein kinase inhibitor cascade. *Arthritis and Rheumatism* 2001; **44:** 73–84.
90. Shimoaka T, Ogasawara T, Yonamine A et al. Regulation of osteoblast, chondrocyte, and osteoclast functions by fibroblast growth factor (FGF)-18 in comparison with FGF-2 and FGF-10. *The Journal of Biological Chemistry* 2002; **277:** 7493–7500.
91. Au A, Polotsky A, Krzyminski K et al. Evaluation of thermoreversible polymers containing fibroblast growth factor 9 (FGF-9) for chondrocyte culture. *Journal of Biomedical Materials Research. Part A* 2004; **69:** 367–372.
92. Moore EE, Bendele AM, Thompson DL et al. Fibroblast growth factor-18 stimulates chondrogenesis and cartilage repair in a rat model of injury-induced osteoarthritis. *Osteoarthritis and Cartilage* 2005; **13:** 623–631.
93. Hakala BE, White C & Recklies AD. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. *The Journal of Biological Chemistry* 1993; **268:** 25803–25810.
94. Connor JR, Dodds RA, Emery JG et al. Human cartilage glycoprotein 39 (HC gp-39) mRNA expression in adult and fetal chondrocytes, osteoblasts and osteocytes by in-situ hybridization. *Osteoarthritis and Cartilage* 2000; **8:** 87–95.
95. Recklies AD, White C & Ling H. The chitinase 3-like protein human cartilage glycoprotein 39 (HC-gp39) stimulates proliferation of human connective-tissue cells and activates both extracellular signal-regulated kinase- and protein kinase B-mediated signalling pathways. *The Biochemical Journal* 2002; **365:** 119–126.
96. De Ceuninck F, Gaufillier S, Bonnaud A et al. YKL-40 (cartilage gp-39) induces proliferative events in cultured chondrocytes and synoviocytes and increases glycosaminoglycan synthesis in chondrocytes. *Biochemical and Biophysical Research Communications* 2001; **285:** 926–931.
97. Ling H & Recklies AD. The chitinase 3-like protein human cartilage glycoprotein 39 inhibits cellular responses to the inflammatory cytokines interleukin-1 and tumour necrosis factor-alpha. *The Biochemical Journal* 2004; **380:** 651–659.
98. Jacques C, Recklies AD, Levy A & Berenbaum F. HC-gp39 contributes to chondrocyte differentiation by inducing SOX9 and type II collagen expressions. *Osteoarthritis and Cartilage* 2007; **15:** 138–146.

99. Guévremont M, Martel-Pelletier J, Boileau C et al. Galectin-3 surface expression on human adult chondrocytes: a potential substrate for collagenase-3. *Annals of the Rheumatic Diseases* 2004; **63:** 636–643.
100. Boileau C, Poirier F, Pelletier JP et al. Intracellular localization of galectin-3 has a protective role. *Annals of the Rheumatic Diseases* 2008; **67:** 175–181.
101. Janelle-Montcalm A, Boileau C, Poirier F et al. Extracellular localization of galectin-3 has a deleterious role in joint tissues. *Arthritis Research & Therapy* 2007; **9:** R20.
102. Martel-Pelletier J, Tardif G, Fernandes JC & Pelletier JP. Metalloproteases and their modulation as treatment in osteoarthritis. In Tsokos GC (ed.). *Molecular rheumatology.* Totowa, New Jersey: Humana Press Inc., 2000, pp. 499–514.
103. Benderdour M, Tardif G, Pelletier J et al. Interleukin-17 (IL-17) induces collagenase-3 production in human osteoarthritic chondrocytes via an AP-1 dependent activation: Differential activation of AP-1 members by IL-17 and IL-1ß. *The Journal of Rheumatology* 2002; **29:** 1262–1272.
104. Tardif G, Pelletier JP, Dupuis M et al. Collagenase 3 production by human osteoarthritic chondrocytes in response to growth factors and cytokines is a function of the physiological state of the cells. *Arthritis and Rheumatism* 1999; **42:** 1147–1158.
105. Benderdour M, Tardif G, Pelletier J-P et al. A novel negative regulatory element in the human collagenase-3 proximal promoter region. *Biochemical and Biophysical Research Communications* 2002; **291:** 1151–1159.
106. Fan Z, Tardif G, Boileau C et al. Identification in human osteoarthritic chondrocytes of proteins binding to the novel regulatory site AGRE in the human matrix metalloprotease 13 proximal promoter. *Arthritis and Rheumatism* 2006; **54:** 2471–2480.
107. Aigner T, Zien A, Hanisch D & Zimmer R. Gene expression in chondrocytes assessed with use of microarrays. *The Journal of Bone and Joint Surgery. American Volume* 2003; **85-A**(Suppl. 2)**:** 117–123.
108. Okada Y, Konomi H, Yada T et al. Degradation of type IX collagen by matrix metalloproteinase 3 (stromelysin) from human rheumatoid synovial cells. *FEBS Letters* 1989; **244:** 473–476.
109. Mohtai M, Smith RL, Schurman DJ et al. Expression of 92-kD type IV collagenase/gelatinase (gelatinase B) in osteoarthritic cartilage and its induction in normal human articular cartilage by interleukin-1. *The Journal of Clinical Investigation* 1993; **92:** 179–185.
110. Sandy JD, Flannery CR, Neame PJ & Lohmander LS. The structure of aggrecan fragments in human synovial fluid. Evidence for the involvement in osteoarthritis of a novel proteinase which cleaves the Glu 373-Ala 374 bond of the interglobular domain. *The Journal of Clinical Investigation* 1992; **89:** 1512–1516.
111. Glasson SS, Askew R, Sheppard B et al. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature* 2005; **434:** 644–648.
112. Stanton H, Rogerson FM, East CJ et al. ADAMTS5 is the major aggrecanase in mouse cartilage in vivo and in vitro. *Nature* 2005; **434:** 648–652.
113. Martel-Pelletier J, Faure MP, McCollum R et al. Plasmin, plasminogen activators and inhibitor in human osteoarthritic cartilage. *The Journal of Rheumatology* 1991; **18:** 1863–1871.
114. Eeckhout Y & Vaes G. Further studies on the activation of procollagenase, the latent precursor of bone collagenase. Effects of lysosomal cathepsin B, plasmin and kallikrein, and spontaneous activation. *The Biochemical Journal* 1977; **166:** 21–31.
115. Zwicky R & Baici A. Cytoskeletal architecture and cathepsin B trafficking in human articular chondrocytes. *Histochemistry and Cell Biology* 2000; **114:** 363–372.
116. Kafienah W, Bromme D, Buttle DJ et al. Human cathepsin K cleaves native type I and II collagens at the N- terminal end of the triple helix. *The Biochemical Journal* 1998; **331**(Pt 3)**:** 727–732.
117. Homandberg GA, Wen C & Hui F. Cartilage damaging activities of fibronectin fragments derived from cartilage and synovial fluid. *Osteoarthritis and Cartilage* 1998; **6:** 231–244.
118. Brown RA & Jones KL. The synthesis and accumulation of fibronectin by human articular cartilage. *The Journal of Rheumatology* 1990; **17:** 65–72.
119. Wurster NB & Lust G. Synthesis of fibronectin in normal and osteoarthritic articular cartilage. *Biochimica et biophysica acta* 1984; **800:** 52–58.
120. Homandberg GA, Meyers R & Williams JM. Intraarticular injection of fibronectin fragments causes severe depletion of cartilage proteoglycans in vivo. *The Journal of Rheumatology* 1993; **20:** 1378–1382.
121. Xie D, Hui F & Homandberg GA. Fibronectin fragments alter matrix protein synthesis in cartilage tissue cultured in vitro. *Archives of Biochemistry and Biophysics* 1993; **307:** 110–118.

122. Stanton H, Ung L & Fosang AJ. The 45 kDa collagen-binding fragment of fibronectin induces matrix metalloproteinase-13 synthesis by chondrocytes and aggrecan degradation by aggrecanases. *The Biochemical Journal* 2002; **364:** 181–190.
123. Xie D, Hui F, Meyers R & Homandberg GA. Cartilage chondrolysis by fibronectin fragments is associated with release of several proteinases: Stromelysin plays a major role in chondrolysis. *Archives of Biochemistry and Biophysics* 1994; **311:** 205–212.
124. Gemba T, Valbracht J, Alsalameh S & Lotz M. Focal adhesion kinase and mitogen-activated protein kinases are involved in chondrocyte activation by the 29-kDa amino-terminal fibronectin fragment. *The Journal of Biological Chemistry* 2002; **277:** 907–911.
125. Forsyth CB, Pulai J & Loeser RF. Fibronectin fragments and blocking antibodies to alpha2beta1 and alpha5beta1 integrins stimulate mitogen-activated protein kinase signaling and increase collagenase 3 (matrix metalloproteinase 13) production by human articular chondrocytes. *Arthritis and Rheumatism* 2002; **46:** 2368–2376.
126. Homandberg GA, Costa V, Ummadi V & Pichika R. Antisense oligonucleotides to the integrin receptor subunit alpha(5) decrease fibronectin fragment mediated cartilage chondrolysis. *Osteoarthritis and Cartilage* 2002; **10:** 381–393.
127. Yasuda T, Kakinuma T, Julovi SM et al. COOH-terminal heparin-binding fibronectin fragment induces nitric oxide production in rheumatoid cartilage through CD44. *Rheumatology (Oxford, England)* 2004; **43:** 1116–1120.
128. Yasuda T, Julovi SM, Hiramitsu T et al. Requirement of mitogen-activated protein kinase for collagenase production by the fibronectin fragment in human articular chondrocytes in culture. *Modern Rheumatology* 2004; **14:** 54–60.
129. Teichtahl AJ, Wluka AE, Proietto J & Cicuttini FM. Obesity and the female sex, risk factors for knee osteoarthritis that may be attributable to systemic or local leptin biosynthesis and its cellular effects. *Medical Hypotheses* 2005; **65:** 312–315.
130. Dumond H, Presle N, Terlain B et al. Evidence for a key role of leptin in osteoarthritis. *Arthritis and Rheumatism* 2003; **48:** 3118–3129.
131. Figenschau Y, Knutsen G, Shahazeydi S et al. Human articular chondrocytes express functional leptin receptors. *Biochemical and Biophysical Research Communications* 2001; **287:** 190–197.
132. Otero M, Lago R, Lago F et al. Signalling pathway involved in nitric oxide synthase type II activation in chondrocytes: synergistic effect of leptin with interleukin-1. *Arthritis Research & Therapy* 2005; **7:** R581–R591.
133. Kimball ES, Persico FJ & Vaught JL. Substance P, neurokinin A, and neurokinin B induce generation of IL-1- like activity in P388D1 cells. Possible relevance to arthritic disease. *Journal of Immunology* 1988; **141:** 3564–3569.
134. Iannone F & Lapadula G. Neuropeptides and human osteoarthritis. *The Journal of Rheumatology* 1998; **25:** 386–388.
135. Langdon C, Kerr C, Hassen M et al. Murine oncostatin M stimulates mouse synovial fibroblasts in vitro and induces inflammation and destruction in mouse joints in vivo. *The American Journal of Pathology* 2000; **157:** 1187–1196.
136. Plater-Zyberk C, Buckton J, Thompson S et al. Amelioration of arthritis in two murine models using antibodies to oncostatin M. *Arthritis and Rheumatism* 2001; **44:** 2697–2702.
137. Hui W, Barksby HE, Young DA et al. Oncostatin M in combination with tumour necrosis factor {alpha} induces a chondrocyte membrane associated aggrecanase that is distinct from ADAMTS aggrecanase-1 or -2. *Annals of the Rheumatic Diseases* 2005; **64:** 1624–1632.
138. Barksby HE, Hui W, Wappler I et al. Interleukin-1 in combination with oncostatin M up-regulates multiple genes in chondrocytes: implications for cartilage destruction and repair. *Arthritis and Rheumatism* 2006; **54:** 540–550.
139. Saha N, Moldovan F, Tardif G et al. Interleukin-1β-converting enzyme/Caspase-1 in human osteoarthritic tissues: localization and role in the maturation of IL-1β and IL-18. *Arthritis and Rheumatism* 1999; **42:** 1577–1587.
140. Boileau C, Martel-Pelletier J, Moldovan F et al. The in situ up-regulation of chondrocyte interleukin-1-converting enzyme and interleukin-18 levels in experimental osteoarthritis is mediated by nitric oxide. *Arthritis and Rheumatism* 2002; **46:** 2637–2647.

141. Notoya K, Jovanovic DV, Reboul P et al. The induction of cell death in human osteoarthritis chondrocytes by nitric oxide is related to the production of prostaglandin E2 via the induction of cyclooxygenase-2. *Journal of Immunology* 2000; **165:** 3402–3410.
142. Pelletier JP, Jovanovic DV, Lascau-Coman V et al. Selective inhibition of inducible nitric oxide synthase reduces progression of experimental osteoarthritis in vivo: possible link with the reduction in chondrocyte apoptosis and caspase 3 level. *Arthritis and Rheumatism* 2000; **43:** 1290–1299.
143. Kojima F, Naraba H, Miyamoto S et al. Membrane-associated prostaglandin E synthase-1 is upregulated by proinflammatory cytokines in chondrocytes from patients with osteoarthritis. *Arthritis Research & Therapy* 2004; **6:** R355–R365.
144. Masuko-Hongo K, Berenbaum F, Humbert L et al. Up-regulation of microsomal prostaglandin E synthase 1 in osteoarthritic human cartilage: critical roles of the ERK-1/2 and p38 signaling pathways. *Arthritis and Rheumatism* 2004; **50:** 2829–2838.
145. Li X, Afif H, Cheng S et al. Expression and regulation of microsomal prostaglandin E synthase-1 in human osteoarthritic cartilage and chondrocytes. *The Journal of Rheumatology* 2005; **32:** 887–895.
146. Wittenberg RH, Willburger RE, Kleemeyer KS & Peskar BA. In vitro release of prostaglandins and leukotrienes from synovial tissue, cartilage, and bone in degenerative joint diseases. *Arthritis and Rheumatism* 1993; **36:** 1444–1450.
147. Jovanovic DV, Fernandes JC, Martel-Pelletier J et al. The in vivo dual inhibition of cyclooxygenase and lipoxygenase by ML-3000 reduces the progression of experimental osteoarthritis. Suppression of collagenase-1 and interleukin-1beta synthesis. *Arthritis and Rheumatism* 2001; **44:** 2320–2330.
148. He W, Pelletier JP, Martel-Pelletier J et al. The synthesis of interleukin-1beta, tumour necrosis factor-a and interstitial collagenase (MMP-1) is eicosanoid dependent in human OA synovial membrane explants: interactions with anti-inflammatory cytokines. *The Journal of Rheumatology* 2002; **29:** 546–553.
149. Paredes Y, Massicotte F, Pelletier JP et al. Study of the role of leukotriene B4 in abnormal function of human subchondral osteoarthritis osteoblasts: effects of cyclooxygenase and/or 5-lipoxygenase inhibition. *Arthritis and Rheumatism* 2002; **46:** 1804–1812.
150. Celotti F & Durand T. The metabolic effects of inhibitors of 5-lipoxygenase and of cyclooxygenase 1 and 2 are an advancement in the efficacy and safety of anti-inflammatory therapy. *Prostaglandins & Other Lipid Mediators* 2003; **71:** 147–162.
151. Macfarlane SR, Seatter MJ, Kanke T et al. Proteinase-activated receptors. *Pharmacological Reviews* 2001; **53:** 245–282.
152. Lindner JR, Kahn ML, Coughlin SR et al. Delayed onset of inflammation in protease-activated receptor-2-deficient mice. *Journal of Immunology* 2000; **165:** 6504–6510.
153. Ferrell WR, Lockhart JC, Kelso EB et al. Essential role for proteinase-activated receptor-2 in arthritis. *The Journal of Clinical Investigation* 2003; **111:** 35–41.
154. Boileau C, Amiable N, Martel-Pelletier J et al. Activation of proteinase-activated receptor 2 in human osteoarthritic cartilage upregulates catabolic and proinflammatory pathways capable of inducing cartilage degradation: a basic science study. *Arthritis Research and Therapy* 2007; **9:** R121.
155. Xiang Y, Masuko-Hongo K, Sekine T et al. Expression of proteinase-activated receptors (PAR)-2 in articular chondrocytes is modulated by IL-1beta, TNF-alpha and TGF-beta. *Osteoarthritis and Cartilage* 2006; **14:** 1163–1173.
156. Massicotte F, Lajeunesse D, Benderdour M et al. Can altered production of interleukin 1β, interleukin-6, transforming growth factor-ß and prostaglandin E2 by isolated human subchondral osteoblasts identify two subgroups of osteoarthritic patients. *Osteoarthritis and Cartilage* 2002; **10:** 491–500.
157. Konttinen YT, Mandelin J, Li TF et al. Acidic cysteine endoproteinase cathepsin K in the degeneration of the superficial articular hyaline cartilage in osteoarthritis. *Arthritis and Rheumatism* 2002; **46:** 953–960.
158. Nakase T, Kaneko M, Tomita T et al. Immunohistochemical detection of cathepsin D, K, and L in the process of endochondral ossification in the human. *Histochemistry and Cell Biology* 2000; **114:** 21–27.
159. Garnero P & Delmas PD. Biomarkers in osteoarthritis. *Current Opinion in Rheumatology* 2003; **15:** 641–646.

160. Garnero P. Use of biochemical markers to study and follow patients with osteoarthritis. *Current Rheumatology Reports* 2006; **8:** 37–44.
161. Sugiyama S, Itokazu M, Suzuki Y & Shimizu K. Procollagen II C propeptide level in the synovial fluid as a predictor of radiographic progression in early knee osteoarthritis. *Annals of the Rheumatic Diseases* 2003; **62:** 27–32.
162. Rousseau JC, Zhu Y, Miossec P et al. Serum levels of type IIA procollagen amino terminal propeptide (PIIANP) are decreased in patients with knee osteoarthritis and rheumatoid arthritis. *Osteoarthritis and Cartilage* 2004; **12:** 440–447.
163. Lohmander LS, Yoshihara Y, Roos H et al. Procollagen II C-propeptide in joint fluid: changes in concentration with age, time after knee injury, and osteoarthritis. *The Journal of Rheumatology* 1996; **23:** 1765–1769.
164. Poole AR & Dieppe P. Biological markers in rheumatoid arthritis. *Seminars in Arthritis and Rheumatism* 1994; **23:** 17–31.
165. Saito S, Kondo S, Mishima S et al. Analysis of cartilage-derived retinoic-acid-sensitive protein (CD-RAP) in synovial fluid from patients with osteoarthritis and rheumatoid arthritis. *The Journal of Bone and Joint Surgery. British Volume* 2002; **84:** 1066–1069.
166. Schmidt-Rohlfing B, Schneider U, Thomsen M & Bosserhoff AK. Correlation of a novel matrix protein with the degree of cartilage degradation. *Rheumatology International* 2002; **22:** 165–169.
167. Garnero P, Christgau S & Delmas PD. The bisphosphonate zoledronate decreases type II collagen breakdown in patients with Paget's disease of bone. *Bone* 2001; **28:** 461–464.
168. Christgau S & Cloos PAC. Cartilage degradation products as markers for evaluation of patients with rheumatic disease. *Clinical and Applied Immunology Reviews* 2004; **4:** 277–294.
169. Downs JT, Lane CL, Nestor NB et al. Analysis of collagenase-cleavage of type II collagen using a neoepitope ELISA. *Journal of Immunological Methods* 2001; **247:** 25–34.
170. Billinghurst RC, Wu W, Ionescu M et al. Comparison of the degradation of type II collagen and proteoglycan in nasal and articular cartilages induced by interleukin-1 and the selective inhibition of type II collagen cleavage by collagenase. *Arthritis and Rheumatism* 2000; **43:** 664–672.
171. Charni N, Juillet F & Garnero P. Urinary type II collagen helical peptide (HELIX-II) as a new biochemical marker of cartilage degradation in patients with osteoarthritis and rheumatoid arthritis. *Arthritis and Rheumatism* 2005; **52:** 1081–1090.
172. Oldberg A, Antonsson P, Lindblom K & Heinegard D. COMP (cartilage oligomeric matrix protein) is structurally related to the thrombospondins. *The Journal of Biological Chemistry* 1992; **267:** 22346–22350.
173. Di Cesare PE, Fang C, Leslie MP et al. Expression of cartilage oligomeric matrix protein (COMP) by embryonic and adult osteoblasts. *Journal of Orthopaedic Research* 2000; **18:** 713–720.
174. Rosenberg K, Olsson H, Morgelin M & Heinegard D. Cartilage oligomeric matrix protein shows high affinity zinc-dependent interaction with triple helical collagen. *The Journal of Biological Chemistry* 1998; **273:** 20397–20403.
175. Sharif M, Kirwan JR, Elson CJ et al. Suggestion of nonlinear or phasic progression of knee osteoarthritis based on measurements of serum cartilage oligomeric matrix protein levels over five years. *Arthritis and Rheumatism* 2004; **50:** 2479–2488.
176. Wislowska M & Jablonska B. Serum cartilage oligomeric matrix protein (COMP) in rheumatoid arthritis and knee osteoarthritis. *Clinical Rheumatology* 2005; **24:** 278–284.
177. Conrozier T, Carlier MC, Mathieu P et al. Serum levels of YKL-40 and C reactive protein in patients with hip osteoarthritis and healthy subjects: a cross sectional study. *Annals of the Rheumatic Diseases* 2000; **59:** 828–831.
178. Skoumal M, Haberhauer G, Kolarz G et al. Serum cathepsin K levels of patients with longstanding rheumatoid arthritis: correlation with radiological destruction. *Arthritis Research & Therapy* 2005; **7:** R65–R70.
179. Garnero P. Use of biochemical markers to study and follow patients with osteoarthritis. *Current Primary Care Reports* 2007; **2:** 10–17.
180. Gineyts E, Garnero P & Delmas PD. Urinary excretion of glucosyl-galactosyl pyridinoline: a specific biochemical marker of synovium degradation. *Rheumatology (Oxford, England)* 2001; **40:** 315–323.
181. Gineyts E, Mo JA, Ko A et al. Effects of ibuprofen on molecular markers of cartilage and synovium turnover in patients with knee osteoarthritis. *Annals of the Rheumatic Diseases* 2004; **63:** 857–861.

182. Senolt L, Braun M, Olejarova M et al. Increased pentosidine, an advanced glycation end product, in serum and synovial fluid from patients with knee osteoarthritis and its relation with cartilage oligomeric matrix protein. *Annals of the Rheumatic Diseases* 2005; **64:** 886–890.
183. Kauffmann C, Gravel P, Godbout B et al. Computer-aided method for quantification of cartilage thickness and volume changes using MRI: validation study using a synthetic model. *IEEE Transactions on Bio-medical Engineering* 2003; **50:** 978–988.
184. Raynauld JP, Kauffmann C, Beaudoin G et al. Reliability of a quantification imaging system using magnetic resonance images to measure cartilage thickness and volume in human normal and osteoarthritic knees. *Osteoarthritis and Cartilage* 2003; **11:** 351–360.
185. Cicuttini F, Forbes A, Morris K et al. Gender differences in knee cartilage volume as measured by magnetic resonance imaging. *Osteoarthritis and Cartilage* 1999; **7:** 265–271.
186. Jones G, Glisson M, Hynes K & Cicuttini F. Sex and site differences in cartilage development: a possible explanation for variations in knee osteoarthritis in later life. *Arthritis and Rheumatism* 2000; **43:** 2543–2549.
187. Raynauld JP, Martel-Pelletier J, Berthiaume MJ et al. Quantitative magnetic resonance imaging evaluation of knee osteoarthritis progression over two years and correlation with clinical symptoms and radiologic changes. *Arthritis and Rheumatism* 2004; **50:** 476–487.
188. Berthiaume MJ, Raynauld JP, Martel-Pelletier J et al. Meniscal tear and extrusion are strongly associated with the progression of knee osteoarthritis as assessed by quantitative magnetic resonance imaging. *Annals of the Rheumatic Diseases* 2005; **64:** 556–563.