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Recent progress in understanding molecular mechanisms of cartilage degeneration during osteoarthritis

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Abstract

Osteoarthritis (OA) is a highly prevalent disease affecting more than 20% of American adults. Predispositions include joint injury, heredity, obesity, and aging. Biomechanical alterations are commonly involved. However, the molecular mechanisms of this disease are complex, and there is currently no effective disease-modifying treatment. The initiation and progression of OA subtypes is a complex process that at the molecular level probably involves many cell types, signaling pathways, and changes in extracellular matrix. *Ex vivo* studies with tissue derived from OA patients and *in vivo* studies with mutant mice have suggested that pathways involving receptor ligands such as TGF-β1, WNT3a, and Indian hedgehog; signaling molecules such as Smads, β-catenin, and HIF-2a; and peptidases such as MMP13 and ADAMTS4/5 are probably involved to some degree. This review focuses on molecular mechanisms of OA development related to recent findings.

Keywords

osteoarthritis; articular cartilage; growth factors; Mmp13; Adamts5

Normal cartilage degeneration in osteoarthritis

During the past several decades, there has been an explosion of reports describing abnormalities in the gross appearance, material properties, cellular morphologies, biochemical composition, and gene expression in articular cartilages from humans to animals with osteoarthritis (OA)-like joint pathology.[1–7] Notwithstanding this vast research enterprise, the pathogenetic mechanisms involved in the initiation and progression of OA resulting from one or more of the many predisposing factors (e.g., age, injury, genetics, and obesity) remain unclear. A major consideration has been whether the different predispositions translate into a "final common pathway" in the articular cartilage[8] that might be amenable to therapeutic intervention.

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Conflicts of interest
The authors declare no conflicts of interest.

OA is the most common joint disorder worldwide and is a major cause of disability. There are currently no treatments capable of markedly altering its progression. Characteristic features of OA include phenotypic changes in the cells of the superficial layer of the articular cartilage (AC), chondrocyte hypertrophy and apoptosis, progressive fibrillation of the AC, subchondral bone sclerosis, osteophyte formation, and increased remodeling of the periarticular bone.[4,6] The articular cartilage has received much of the attention in OA studies because gross articular cartilage damage is the most obvious pathologic feature leading to joint dysfunction. AC is a smooth, lubricated, reversibly compressible tissue that protects the underlying bones from biomechanical damage during joint loading. About 75% of the wet weight of AC is water, and about 70% of the dry weight is collagen. The principal collagens of adult articular cartilage are type II (often present as a heteropolymer with types IX and XI), type III, and a small amount of types V, VI, and X.[9,10] The noncollagenous matrix (about 20% of the dry weight) is mostly the proteoglycan aggrecan, which is present largely in link-stabilized aggregates with hyaluronan (HA). Full-length aggrecan is itself about 10% (w/w) core protein and 90% (w/w) chondroitin sulfate (CS). Other cartilage proteoglycans, many involved in controlling collagen fibril formation and pericellular matrix organization, include decorin, biglycan, fibromodulin, lumican, epiphycan, and perlecan. However, the relative abundance of these has not been accurately determined. It is important to note that the abundance and composition of all cartilage components can vary with tissue depth,[11] maturation and aging,[12] and diseases such as OA.[13]

The chondrocytic cells of the articular cartilage are organized into three layers—superficial, middle, and deep—where they represent about 2.5%, 2%, and 1.5% of the cartilage volume, respectively.[14] The cartilage collagens form a dense fibrous mesh-work that constrains the highly concentrated aggrecan, which in turn retains water due to the osmotic effect of its negatively charged chondroitin sulfate chains. The chondrocytic cells, which are embedded in these matrix networks, produce and maintain the cartilage by synthesizing and degrading matrix components in response to environmental cues such as growth factors, cytokines, and biomechanical change. Mature articular cartilage is a product of postnatal remodeling of the cartilaginous epiphysis. Development begins with the aggregation of mesenchymal precursors and differentiation of the cells into chondrocytes, as indicated by expression of Sox-5, -6, and -9,[15] and the secretion of matrix components, such as collagens II, VI, IX, and XI; link protein; and the hyaluronan (HA)-binding proteoglycans, aggrecan, and versican. Chondrocytes present in this cartilaginous anlage of the developing skeleton, subsequently organize into zones of quiescence and proliferation. Groups of proliferative cells form proliferating zone columns wherein the cells undergo a differentiation program through prehypertrophy and hypertrophy. Hypertrophic cells are characterized by a high expression of markers such as Runx2, collagen X, and alkaline phosphatase. These changes in expression are accompanied by matrix calcification and the emergence of cells expressing markers such as VEGF and osteocalcin, which in turn results in vascular invasion, chondrocyte apoptosis, and trabecular bone deposition.

In contrast to the proliferative cells, the quiescent chondrocytes of the original cartilage template are the source of the mature articular cartilage. This tissue is characterized by flattened “fibroblastic” cells in the surface zone and small groups of more rounded “chondroid” cells in the mid- and deep zones. The composition and organization of the matrix in each zone is different, indicating that the maintenance of articular cartilage relies on zone-specific programs for the synthesis and turnover of each matrix component. It also appears that the superficial zone[16] and the deep zone[17] are a source of progenitor cells, which are needed to replace chondrocytes lost by apoptosis, necrosis, or autophagy under biomechanical or biologic stress.

Chondrocyte activities related to the induction and/or progression of OA

Many of the foundational studies on mechanisms of cartilage degradation were performed with normal cartilage or chondrocytes. The source of tissue has commonly been animals (lapine, bovine, porcine) or normal human cartilage taken postmortem or at amputation. These analyses identified a range of ligands (e.g., cytokines and growth factors) and receptors (e.g, IL-1R and TGF-βRII) that alter chondrocyte-mediated matrix turnover and the gene expression of effector molecules (e.g., peptidases). Reports have focused on cell responses that are consistent with, but clearly cannot establish alone, a role in OA pathogenesis. Examples of this type of study are described in the series of articles[18] on the effects of mediators on peptidase expression and activity in cartilage explants. Indeed, the discovery of cartilage aggrecanase activity was made in normal cartilage explants.[19–21]

These studies provided an information base to examine changes that have occurred in the OA joint *in vivo* and that can be detected *ex vivo*. In this approach, because chondrocyte and matrix changes result from OA pathology *in vivo*, these data would appear to provide a higher level of confidence in its relevance to important aspects of disease mechanisms. Examples of this type of study are found in the detailed analysis of gene expression in cartilages obtained from different regions of the human OA joint removed at arthroplasty.[22–24] Most recently, studies done *in vivo* with genetically modified mice[25–27] have proven particularly informative in the elucidation of chondrocyte changes, which appear to have high relevance to OA pathogenesis. In the present review, we focus on *in vivo* data of OA animal models. Our objective is to optimize the likelihood that the data reviewed and summarized will have high relevance to the human disease.

TGF-β and OA

The growth factor TGF-β, which strongly inhibits articular chondrocyte hypertrophy and maturation, also represents a potential mechanism in the development of OA.[28] The intracellular signaling initiating by TGF-β is mediated through TGF-β type II and type I transmembrane Ser/Thr kinase receptors. TGF-β first binds to type II receptor, leading to the recruitment of type I receptor. This constitutively active type II receptor phosphorylates the GS domain of the type I receptor. Activated type I receptor phosphorylates R-Smads (Smad2 or Smad3) at a conserved SSXS motif at the C-terminus of Smad2/3. This phosphorylated Smad2/3 thus dissociates from receptor complex and forms a heteromeric complex with the common Smad, Smad4. This heteromeric Smad complex translocates and accumulates into the nucleus and associates with other DNA-binding proteins to regulate gene transcription.

In vivo studies show that loss of TGF-β signaling in mice causes an OA-like phenotype resembling OA in humans. The knee joints of transgenic mice that express the dominant negative type II TGF-β receptor (DNIIR) in skeletal tissue show chondrocyte hypertrophy at an early stage, followed by deceased proteoglycan, articular surface fibrillation and disorganization, as well as chondrocytes clusters in deeper zone of articular cartilage at late stage.[29] Correlating with the DNIIR transgenic mouse model, deletion of the Smad3 gene in mice also results in progressive articular cartilage degeneration resembling human OA.[30] In *Smad3* knockout mice, an abnormal increase in the hypertrophic chondrocyte number was seen at the early stage, followed by progressive loss of the smooth articular cartilage surface that covered with abnormally differentiated chondrocytes. In seven-month-old *Smad3* KO mice, articular surface is fibrillated, accompanied by vertical cleft, and osteophytes that vary in size are developed. Smurf2 is a negative regulator of TGF-β signaling in articular chondrocytes and promotes chondrocyte hypertrophy.[31,32] Smurf2 is highly expressed in human OA cartilage and is not present in normal cartilage. In chondrocyte-specific Smurf2 overexpression transgenic mice, TGF-β signaling is decreased, and expression of

chondrocyte hypertrophic markers (*ColX* and *Mmp13*) is increased, leading to progressive articular cartilage degradation, including reduced cartilage area, fibrillation, clefting, as well as subchondral sclerosis and osteophyte formation.[33] Moreover, we have recently generated chondrocyte-specific *Tgfbr2*-conditional knockout mice (*Tgfbr2*^{Col2CreER}) that also show OA-like features, including chondrocyte hypertrophy at an early stage, progressive cartilage degeneration, and chondrophyte and osteophyte formation (unpublished data). These observations are supported by the recent finding that high frequency of a single nucleotide polymorphism (SNP) of the *Smad3* gene was identified in patients with OA. This study, including a 527 patient cohort, demonstrated that the SNP of human *Smad3* gene is correlated with the incidence of hip and knee OA in patients.[34] The findings suggest that loss of TGF-β signaling represents one of the possible mechanisms in OA development.

Wnt, β-Catenin, and OA

β-catenin is a central molecule in the canonical Wnt signaling pathway, which controls multiple developmental processes in skeletal and joint development[35] and is critical for the progression of OA.[36] When Wnt binds to its receptor Frizzled and the co-receptors LRP5/6, the activity of downstream signaling proteins Dishevelled (Dsh) and Axins 1 and 2 are altered. This leads to the inactivation of Ser/Thr kinase GSK-3β, thus inhibiting the ubiquitination and degradation of β-catenin triggered by GSK-3β. β-catenin is then accumulated in the cytoplasm and translocated to the nucleus and binds to transcription factors LEF-1/TCF to regulate the transcription of downstream target genes. In the absence of Wnt ligands, cytoplasmic β-catenin binds the APC-Axin-GSK-3β degradation complex, and GSK-3β, in this complex, phosphorylates β-catenin. The E3 ubiquitin ligase β-TrCP then targets β-catenin for ubiquitination and proteasome degradation.[37]

β-catenin affects cell fate during early skeletal development. For example, overexpression of constitutively active β-catenin leads to the loss of chondrocyte phenotype characterized by loss of Sox9 and Col2 expression in chick chondrocytes.[35] Conditional inactivation of the β-catenin gene in mouse mesenchymal cells *in vivo* results in the loss of osteoblasts and ectopic chondrocyte formation in bone tissues through intramembranous and endochondral bone formation processes.[38]

During postnatal development, β-catenin plays an important role in chondrocyte proliferation, hypertrophy, and apoptosis. Studies suggest that dysregulation of Wnt/β-catenin signaling represents a possible mechanism of OA. Our recent findings demonstrated that β-catenin was upregulated in articular cartilage tissue derived from patients with OA.[36] In addition, human genetic studies revealed that patients with mutations of the *FrzB* gene have increased susceptibility to hip OA.[39–42] *FrzB* encodes the protein sFRP3, a secreted inhibitor of Wnt signaling. Mutations in *FrzB* cause activation of β-catenin signaling and abnormal chondrocyte hypertrophy.[43,44] Consistent with this finding, *FrzB* knockout mice are susceptible to chemical-induced OA.[45] In *Col2a1-Smurf2* transgenic mice, upregulation of β-catenin signaling in articular chondrocytes was also observed in addition to the reduction of TGF-β signaling. Primary articular chondrocytes isolated from *Col2a1-Smurf2* transgenic mice showed Smurf2-induced GSK-3β ubiquitination and subsequent upregulation of β-catenin protein levels.[33] Furthermore, overexpression of Wnt-induced signaling protein 1 (WISP-1) in the mouse knee joint also leads to cartilage destruction.[46] β-catenin-conditional activation mice also show that overexpression of β-catenin in articular chondrocytes, which causes abnormal articular chondrocyte maturation, results in cartilage degeneration in mice.[36]

It also appears to be relevant that the expression of *Mmp13* is significantly increased in articular cartilage in β-catenin gene-conditional activation mice. Consistent with this, our chondrocyte-specific *Mmp13*-conditional knockout mice have decelerated OA progression

following meniscal-ligamentous injury (MLI). Further, to explore MMP13 inhibition as a therapeutic option for OA treatment, we injected CL82198, an MMP13 inhibitor (unpublished data), into WT mice after MLI surgery. We found that treatment with CL82198 decelerated MLI-induced OA progression, indicating that MMP13 is a critical player in the progression of OA, thus making it an attractive target for OA therapies.

Several reports indicate that a low level of β-catenin is associated with stable differentiated chondrocyte functions and that a high level of β-catenin is associated with loss of function due to dedifferentiation.[47] It remains to be determined whether the upregulation of β-catenin expression in articular cartilage tissue from OA patients[36] is a response to OA or part of the causative cascade.

Hypoxia-inducible factor-2α and OA

Hypoxia-inducible factor (HIF)-2α belongs to the basic helix-loop-helix/Per-ARNT-Sim (bHLH/PAS) domain transcription factor family.[48] HIF-2α is a heterodimeric protein that functions by dimerizing the α-subunit with the β-subunit members. Its activity is regulated by the level of oxygen. Under normoxic conditions, the proline residues on the α-subunits are hydroxylated, recognized by the von Hippel-Lindau (pVHL) tumor suppressor, an E3 ubiquitin ligase, and degraded by proteasome. Under hypoxic conditions, the HIF proteins do not undergo ubiquitination and proteasome degradation. The α-subunits translocate into the nucleus and dimerize with the constitutive β-subunits (also known as ARNT) to regulate HIF responsive genes.[49,50]

Studies show that the expression levels of HIF-2α are significantly increased in both human and mouse osteoarthritic cartilage.[51,52] *In vitro* promoter studies show that HIF-2α is a potent trans-activator of OA marker genes, including Col10a1, MMP13, and VEGF.[51] Overexpression of HIF-2α by intra-articular injection of Ad-*EPAS1,* the gene encodes HIF-2α, leads to spontaneous OA development in knee joints of mice.[52] Moreover, *EPAS1*-heterozygous-deficient mice are resistant to surgery-induced OA development in knee joints of mice.[51,52] Consistent with these findings, a functional SNP study among a Japanese population indicates that SNP of the *EPAS1* promoter is associated with knee OA,[51] suggesting that enhanced transactivation of *EPAS1* in chondrocytes is associated with OA in humans. *In vitro* studies suggest that NF-κB is the upstream inducer of HIF-2α expression and mechanical stress upregulates NF-κB signaling. These observations suggest that the HIF-2α signaling pathway is also involved in OA development.

Indian hedgehog and OA

As an important signaling protein for chondrocyte growth and differentiation, the Indian hedgehog (Ihh) signaling pathway also plays a critical role during OA development. Ihh signaling functions through two transmembrane receptors: patched (Ptch) and smoothened (Smo). In the absence of Ihh ligand, Ptch binds to Smo to inhibit its function. During the activation of Ihh signaling, Ihh binds Ptc leading to the release of Smo. Smo will further activate Gli transcription factors to regulate Ihh responsive genes.

Activation of Ihh signaling is associated with human OA development. The expression of Ihh signaling proteins, including Gli1, Ptch, and hedgehog-interacting protein (HHIP), is highly upregulated in joint tissues of patients with OA accompanied by upregulation of *Adamts5, Col10a1, Runx2,* and*Mmp13*.[53] Furthermore, the expression of Gli1, Ptch, and HHIP is also upregulated in the injury-induced OA mouse model. *In vivo* studies show that transgenic mice with chondrocyte-specific overexpression of the *Gli2* or *Smo* gene have spontaneous OA development accompanied by upregulation of *Adamts5, Col10a1, Mmp13*, as well as aggrecan and Col2 degradation.[53] Histologic and radiographic analyses of these

mice show a progressive worsening of articular cartilage with less Safranin O staining, thinner cartilage layer, or even completely degraded cartilage. Changes in subchondral bone in these mice are similar to those found in surgically induced OA knee joints. In contrast, either deletion of the *Smo* gene or treatment with an Ihh inhibitor attenuates the severity of injury-induced OA in mouse models.[53] Evidence shows that Ihh signaling is activated in the development of OA. Interestingly, it has been reported that Wnt/β-catenin interacts with Ihh signaling, and both β-catenin and Ihh signaling pathways are required for endochondral bone development.[54] However, whether β-catenin is upstream or downstream of Ihh signaling in chondrocytes remains to be determined.

MMP13 and OA

The central event of OA is progressive loss of articular cartilage. Human clinical and animal studies show that MMP13 plays a dominant role in the progression of cartilage degeneration. MMP13 is a collagenase with substrate specificity that targets collagen for degradation. Compared to other MMPs, MMP13 has a more restricted expression pattern in connective tissue.[55] MMP13 preferentially cleaves Col2, which is the most abundant protein in articular cartilage. It also targets aggrecan, types IV and IX collagen, gelatin, osteonectin, and perlecan in cartilage for degradation.[56] MMP13 has a much higher catalytic velocity rate than other MMPs for Col2 and gelatin, which make it the most potent peptidolytic enzyme among collagenases.[57,58]

Clinical investigations revealed that patients with articular cartilage destruction have high MMP13 expression,[59] suggesting that increased MMP13 may be a cause of cartilage degradation. *Mmp13*-deficient mice show no gross phenotypic abnormalities, and the only alteration is in growth plate architecture.[60,61] *Mmp13*-overexpressing transgenic mice developed spontaneous articular cartilage destruction characterized by excessive cleavage of Col2 and loss of aggrecan.[62] These results suggest that *Mmp13* deficiency does not affect articular cartilage development, but abnormally upregulated *Mmp13* can lead to postnatal cartilage destruction. Moreover, the expression of *Mmp13* is significantly increased in articular cartilage in β-catenin-conditional activation mice.[36] Consistent with above findings, we have preliminary data from chondrocyte-specific *Mmp13*-conditional knockout (*Mmp13* cKO) mice showing decelerated OA progression following meniscal-ligamentous injury (MLI) (unpublished data). To explore MMP13 inhibition as a potential therapeutic option for OA treatment, we injected CL82198, a MMP13 inhibitor, into WT mice after MLI surgery. We found that injection of CL82198 decelerated MLI-induced OA progression (unpublished data). These findings implicate that MMP13 is a critical player in the progression of OA that could serve as a molecular target for OA therapies.

ADAMTS and OA

The major contributors causing cartilage degeneration in OA are enzymes targeting collagens and aggrecan for proteolysis. In addition to MMP13, which mainly targets one of the two major structural components, collagens, for degradation; studies show that the aggrecanase Adamts4/5 are the principal enzymes responsible for degradation of the other principal component, aggrecan. Adamts5 is one of the shorter members of the zinc-dependent Adamts enzyme family[63,64] that has two thrombospondin (TS) motifs. Adamts4 is the shortest member of the zinc-dependent Adamts enzyme, containing only one TS motif. Full-length Adamts4 and Adamts5 are proenzymes that are activated by removing their prodomains via furin or furin-like enzymes.[65] Adamts4 and Adamts5 are two of the most active enzymes in aggrecan cleavage. Adamts4 is shown to be active during cartilage degeneration, and its expression is upregulated in degenerative cartilage.[66,67] Adamts5 has been shown to be active in both normal and degenerated cartilage.[68] It was not clear which of the Adamts family members is more important in the development of cartilage

degenerative diseases until both *Adamts4* and *Adamts5* knockout mice were generated. Both *Adamts4* and *Adamts5* knockout mice are normal and have no gross abnormalities.[69–70] However, it was shown that *Adamts5* may play a more important role in OA development than *Adamts4*. Meniscal destabilization experiments were performed in both *Adamts4* and *Admats5* knockout mice showing that deletion of the *Adamts4* gene cannot protect OA progression, while deletion of the *Adamts5* gene alone decelerated cartilage degradation.[71,72]

An important aspect of defining the peptidases that are truly active in OA *in vivo* is the detection of specific cleavage products in the cartilage, synovial fluid, serum, or urine. Measurement of transcript abundance by quantitative PCR and/or peptidase abundance by immunoassay can generate correlative data, but they do not provide the definitive proof obtained with highly specific neo-epitope antibodies. Within this constraint, only a limited number of peptidases can be directly implicated in human OA cartilage pathology. These include one that cleaves in the C-telopeptide region of collagen II,[73] a bona fide collagenase; probably MMP13, which cleaves collagen II at Gly775-Leu/Ile776;[74,75] Cathepsin K, which degrades collagen II at Gly192-Lys193;[76] and an Adamts-aggrecanase, which cleaves aggrecan at Glu373-Ala374.[77] Whereas the expression and/or secretion of many other peptidases, such as MMP3,[78] MMP2, MMP9, and the PA/plasmin system,[79] are also increased in human OA, data to definitively establish *in vivo* activity are not yet available.

Further investigation of the mechanism by which *Adamts5* deletion protects cartilage showed that it may not due to elimination of aggrecanase activity from the cartilage.[26] This finding showed that Adamts5 is not, as previously concluded,[70,71] the major aggrecanase in mouse cartilage. However, a photographic, histologic, and biochemical examination of the "protected" joints in *Adamts5* knockout mice showed that the protection was, in fact, due to an elimination of fibrous overgrowth from the periarticular tissues and deposition of newly synthesized aggrecan in the cartilage.[26] This finding suggests that Adamts5 activity is profibrogenic in cellular responses to joint injury and that deletion of the *Adamts5* gene switches cells to a chondrogenic phenotype. An explanation for these effects of *Adamts5* knockout on collagenous tissues was provided by an analysis of Smad-signaling pathways in wild-type and *Adamts5*-deficient fibroblasts and chondrocytes. This showed that in the presence of the enzyme, TGF-β1–mediated signaling is primarily through Smad2/3, leading to increased expression of fibrogenic genes such as type 1 and type III collagen. Conversely, in the absence of the enzyme and the presence of accumulated pericellular aggrecan, TGF-β1–mediated signaling is primarily through the Smad1/5/8 pathway. Activation of this pathway, which is also activated by BMP7 signaling, activates expression of chondrogenic genes such as aggrecan.[3] The precise mechanism by which a lack of Adamts5 activity promotes TGF-β1–mediated chondrogenesis is not known, but analysis of *Adamts5/CD44* double knockout mice shows that it is dependent on the presence of the hyaluronan receptor, CD44.[80] These data lead to the hypothesis that Adamts5 specifically degrades pericellular aggrecan in OA and that other aggrecanases, such as Adamts4, are responsible for degradation of the bulk of the tissue aggrecan, which resides in the intercellular matrix. If this model is supported by further work, it will directly affect strategies for therapeutic control of human OA.

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