Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis

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Abstract: Chondrogenesis occurs as a result of mesenchymal cell condensation and chondroprogenitor cell differentiation. Following chondrogenesis, the chondrocytes remain as resting cells to form the articular cartilage or undergo proliferation, terminal differentiation to chondrocyte hypertrophy, and apoptosis in a process termed endochondral ossification, whereby the hypertrophic cartilage is replaced by bone. Human adult articular cartilage is a complex tissue of matrix proteins that varies from superficial to deep layers and from loaded to unloaded zones. A major challenge to efforts to repair cartilage by stem cell-based and other tissue-engineering strategies is the inability of the resident chondrocytes to lay down a new matrix with the same properties as it had when it was formed during development. Thus, understanding and comparing the mechanisms of cartilage remodeling during development, osteoarthritis (OA), and aging may lead to more effective strategies for preventing cartilage damage and promoting repair. The pivotal proteinase that marks OA progression is matrix metalloproteinase 13 (MMP-13), the major type II collagen-degrading collagenase, which is regulated by both stress and inflammatory signals. We and other investigators have found that there are common mediators of these processes in human OA cartilage. We also observe temporal and spatial expression of these mediators in early through late stages of OA in mouse models and are analyzing the consequences of knockout or transgenic overexpression of critical genes. Since the chondrocytes in adult human cartilage are normally quiescent and maintain the matrix in a low turnover state, understanding how they undergo phenotypic modulation and promote matrix destruction and abnormal repair in OA may lead to identification of critical targets for therapy to block cartilage damage and promote effective cartilage repair.

Keywords: articular cartilage, chondrogenesis, inflammation, mouse models, osteoarthritis

Introduction

Although osteoarthritis (OA) is considered a disease of the whole joint as an organ, the articular cartilage is altered to some extent in all affected joints with OA. In addition to the development of cartilage changes with aging, cartilage degeneration may occur in response to inappropriate mechanical stress and low-grade systemic inflammation associated with trauma, obesity, and genetic predisposition, which are major risk factors of OA development and progression [Blagojevic *et al.* 2010; Felson *et al.*

2000]. However, strong functional interactions among the cartilage, synovium, and subchondral bone impact on cartilage function in such a way that it is difficult to know where and when pathological changes begin. Nevertheless, the knowledge we have gained from studies of cartilage derived from the clinic and from animal models has uncovered many important biological factors that impinge on chondrocytes, the cellular component of cartilage, in a temporal and spatial manner to produce pathological changes.

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Chondrogenesis and chondrocyte differentiation during development

During skeletal development, chondrocytes arise from mesenchymal progenitors to synthesize the templates, or cartilage anlagen, for the developing bone [Goldring *et al.* 2006]. Chondrogenesis occurs as a result of condensation of mesenchymal cells, which express collagens I, III and V, and chondroprogenitor cell differentiation with expression of cartilage-specific collagens II, IX, and XI. During limb development, the resting chondrocytes form the cartilage at the ends of the opposing bones with the intervening interzones formed during cavitation or they undergo proliferation, cease proliferating, then proceed to terminal differentiation to hypertrophy, and apoptosis to permit endochondral ossification, whereby calcified hypertrophic cartilage is resorbed and replaced by bone. The proliferating chondrocytes express collagen VI and matrilin 1 and are under control of the PTHrP/Ihh axis. The hypertrophic zone is characterized by expression of collagen X and calcification of the matrix. Matrix remodeling involving matrix metalloproteinase (MMP) 9, 13, and 14 and vascularization mediated by vascular endothelial growth factor (VEGF) and VEGF receptors are required to convert the nonvascularized and hypoxic tissue to bone through the actions of osteoclasts and osteoblasts. A similar sequence of events occurs in the postnatal growth plate, leading to rapid growth of the skeleton [Onyekwelu *et al.* 2009]. These processes are subject to complex regulation by interplay of the fibroblast growth factor (FGF), transforming growth factor β (TGF β), bone morphogenetic protein (BMP) and Wnt signaling pathways [Haque *et al.* 2007; Kobayashi *et al.* 2005; Macsai *et al.* 2008; Wu *et al.* 2007; Yoon *et al.* 2006]. Sox9 and Runx2 are two pivotal transcriptional regulators essential for articular cartilage formation and hypertrophic maturation, respectively [Lefebvre and Smits, 2005; Wuelling and Vortkamp, 2011]. Moreover, Runx2 is subject to direct inhibition by Sox9, and TGF β and BMP signals differentially regulate Wnt/ β -catenin signaling through activation of Runx2 [Dong *et al.* 2006; Zhou *et al.* 2006]. Recent studies indicate that epidermal growth factor receptor signaling is involved in regulating endochondral ossification in the developing growth plate [Zhang *et al.* 2011] and its ligand TGF α suppresses articular chondrocyte phenotype through activating Rho/ROCK and MEK/extracellular-regulated kinase (ERK) signaling [Appleton *et al.* 2010].

Composition and structure of articular cartilage

The composition and cellular organization of human adult articular cartilage is complex with qualitative and quantitative differences in matrix constituents between the interterritorial region containing the collagen network of collagens II, IX, and XI and the pericellular matrix, containing collagen VI, fibromodulin, and matrilin 3, but little or no type II collagen. Chondrocytes, which comprise the only cellular component of articular cartilage, have different morphologies ranging from more flattened at the surface to rounder and larger in the deeper zones. At the surface, chondrocytes show distinct spatial patterns in single cells, pairs, clusters or strings depending upon the joint type [Rolaufts *et al.* 2010] and have properties of mesenchymal stem cells [Alsalamah *et al.* 2004; Dowthwaite *et al.* 2004]. Chondrocytes in the superficial zone uniquely produce lubricin, or superficial zone protein, a splice form of PRG4. Lubricin contributes to a boundary layer of lubricants, also including hyaluronic acid [Greene *et al.* 2011], giving cartilage a smooth surface with a very low coefficient of friction that permits efficient gliding motion during joint movement. There are also differences in the arrangement of the collagen fibrillar network ranging from the superficial, where they are parallel to the surface, becoming more random in the middle zone and then radial in the deep zone. Chondrocytes in the middle zone synthesize relatively greater amounts of aggrecan and the relative amounts of small proteoglycans also differ. Under normal, low turnover conditions, chondrocytes are resting in a nonstressed steady state and maintain synthesis of proteoglycans and other noncollagen molecules [Maroudas *et al.* 1998], whereas there is very little turnover of the type II collagen network, which has a half life of 117 years unless it is damaged [Verzijl *et al.* 2000].

The calcified cartilage is the interface between the nonmineralized articular cartilage and the bone. The tidemark is a thin line revealed after hematoxylin staining that marks the mineralization front between the calcified and articular cartilage [Fawns and Landells, 1953; Lane and Bullough, 1980; Lane *et al.* 1977] and is composed of matrix vesicles [Anderson *et al.* 2010]. The calcified cartilage has unique matrix composition, with chondrocytes expressing markers of hypertrophy, as well as vascularization and innervation originating from the subchondral bone in association with advancing age.

Cartilage changes in osteoarthritis

OA is the most common form of arthritis associated with risk factors such as age, gender, prior joint injury, obesity, genetic predisposition, and mechanical factors, including malalignment and abnormal joint shape [Blagojevic *et al.* 2010; Felson *et al.* 2000]. OA is a 'whole joint' disease with pathologic changes in all tissues, including articular cartilage degradation, subchondral bone thickening, osteophyte formation, synovial inflammation, and degeneration of ligaments and, in the knee, the menisci. The earliest changes occur in cartilage appearing at the joint surface in areas where mechanical forces such as shear stress are greatest [Andriacchi *et al.* 2004]. The normally quiescent chondrocytes undergo a phenotypic shift and become 'activated' cells, characterized by cell proliferation, cluster formation, and increased production of both matrix proteins and matrix-degrading enzymes [Goldring and Marcu, 2009]. A major challenge is halting cartilage damage before progression begins because of the inability of the resident chondrocytes to regenerate a matrix with the same properties as that formed during development.

Genomics studies have examined global gene expression profiles in intact human early and late OA samples compared with normal cartilage [Aigner *et al.* 2006; Ijiri *et al.* 2008] and in intact compared with damaged regions of tibial plateau cartilage [Sato *et al.* 2006]. Candidate gene studies and genome-wide linkage analyses have revealed polymorphisms or mutations in genes encoding ECM and signaling molecules that may determine OA susceptibility such as growth and differentiation factor 5, asporin, secreted frizzled-related protein 3, deiodinase 2, and Smad3 [Loughlin, 2011]. The molecules encoded by these genes are associated with the TGF β , BMP and Wnt signaling pathways, and are involved in determining chondrocyte differentiation and maturation during skeletal development. Disruption of these pathways may induce chondrocytes to recapitulate a developmental molecular program, including expression of markers of chondrocyte hypertrophy such as COL10A1, MMP-13, and Runx2. Gene defects associated with congenital cartilage dysplasias that affect the formation of cartilage matrix and patterning of skeletal elements may adversely affect joint alignment and congruity and thus contribute to early onset of OA in these individuals [Kannu *et al.* 2009]. However, these gene defects are rare, potentially defining subsets of patients with OA;

how gene polymorphisms contribute to OA susceptibility in the general population is a goal of large multicenter studies.

Stable changes in patterns of gene expression, not associated with alterations in DNA sequences, have also been observed in OA cartilage. These changes allow the cell to respond rapidly to alterations in the environment, occurring through epigenetic modifications, including DNA methylation, histone modifications, and alterations in chromatin structure, and by microRNA-mediated mechanisms [Barter *et al.* 2012; Goldring and Marcu, 2012]. Specific microRNAs (miRNAs) have been linked to alterations in gene expression in human OA [Iliopoulos *et al.* 2008; Jones *et al.* 2009]. Since miRNAs can have multiple downstream targets, many studies have begun to address whether alterations in the expression of specific miRNAs affect cartilage homeostasis *versus* the OA disease state [Swingler *et al.* 2011].

Much work has been devoted to understanding how the homeostatic balance of healthy cartilage is perturbed and how this leads to disease. Proteolytic degradation of cartilage matrix proteins compromises the remarkable physical properties of cartilage, including its elasticity, compressive resistance, and tensile strength. Two key targets of cartilage degeneration during OA are type II collagen, a major substrate of MMP-13, and aggrecan, a proteoglycan with glycosaminoglycan side chains of chondroitin and keratin sulfate, which is degraded by the aggrecanases of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family, ADAMTS4 and 5 [Cawston and Wilson, 2006; Plaas *et al.* 2007; Wu *et al.* 2002]. In addition, matrix-degrading enzymes found in the OA joint include several serine and cysteine proteinases [Troeborg and Nagase, 2012]. Until the proteoglycan coating is removed, the collagen network is somewhat protected from degradation by the collagenases, MMP-1 and MMP-13. Once the collagen network is degraded, this marks progression to irreversible cartilage degradation.

Articular chondrocytes from patients with OA show 'phenotypic plasticity' comparable to mesenchymal stem cells undergoing chondrogenesis by recapitulating aspects of chondrocyte hypertrophy [Tchetina *et al.* 2005]. Human OA chondrocytes are subject to differential control by the NF κ B-activating inhibitor of κ B kinases (IKK α and IKK β [Olivotto *et al.* 2008], as well as by

MMP-13 itself [Borzi *et al.* 2010]. Phenotypic modifications induced by IKK α and IKK β knockdown are associated with changes in the expression and cellular localization of regulators including Runx2, β -catenin, and Sox9 [Borzi *et al.* 2010]. A loss of chondrocytes expressing high-mobility group box-1 (HMGB2) in the cartilage superficial zone occurs with aging [Taniguchi *et al.* 2009], but there is an increase in the cells expressing the mesenchymal progenitor cell markers, Stro1 and Notch1, in the chondrocyte clusters appearing during OA [Grogan *et al.* 2009].

Inflammation and mechanical stress in osteoarthritis

The role of inflammation in rheumatoid arthritis (RA) is well established given the efficacy of anti-inflammatory therapy. Synovitis is a prominent feature of RA and the junction between the cartilage and overlying synovial pannus is the major site of cartilage destruction by proteinases released primarily by the synovium. Synovitis is also common in OA [Rollin *et al.* 2008], occurring in over 90% of patients with OA [Roemer *et al.* 2011], and inflammation is a major factor associated with the risk of both progression of cartilage loss and signs and symptoms of disease [Sellam and Berenbaum, 2010]. There is evidence that synovitis is associated with pain, disease severity and progression [Baker *et al.* 2010; Benito *et al.* 2005] and that synovitis in OA is a potential target for therapy [Attur *et al.* 2010; Sellam and Berenbaum, 2010]. Current investigations are addressing whether synovitis has an etiopathogenic role, whether it occurs as a consequence of joint failure, and whether it precedes the occurrence of symptomatic OA.

Infiltration of mononuclear cells into the synovial membrane and production of proinflammatory mediators, including interleukin 1 β (IL-1 β), tumor necrosis factor α (TNF α), and chemokines is common in early- and late-stage OA disease [Sellam and Berenbaum, 2010]. MMP-1, -3 and -13 and cathepsins B and S, as well as IL-6, can be detected in OA synovial fluid samples, although at significantly lower levels than in patients with RA [Pozgan *et al.* 2010]. The synovial effusions may be visualized in the joint by magnetic resonance imaging or ultrasound [Hayashi *et al.* 2011]. In patients with traumatic meniscal injury, but no radiographic evidence of

OA, the synovium retrieved during arthroscopic meniscectomy is frequently inflamed and increased inflammation scores are associated with increased pain and dysfunction [Scanzello *et al.* 2011].

There is also a strong relationship between anterior cruciate ligament (ACL) disruption and risk for subsequent development of OA. Following acute ACL injury, biomarkers of inflammation and collagen loss can be detected at higher levels in synovial fluid from the affected knee than in serum [Catterall *et al.* 2010]. A study of the high abundance synovial fluid proteome showed distinct profiles in healthy individuals compared with patients with early OA undergoing arthroscopy after injury of the medial meniscus and patients with late-stage OA undergoing total joint replacement [Gobeze *et al.* 2007]. These studies suggest that low-grade synovitis reflects preclinical disease during the early post-traumatic phase and could impact on the long-term outcome [Lotz and Kraus, 2010].

Abnormal mechanical stress on cartilage induces catabolic and inflammation-related events via intracellular signaling pathways that are similar to those activated by oxidative stress, inflammatory cytokines, and products of matrix damage. Cytokines and their receptors are expressed by chondrocytes and colocalize with MMP-13 and type II collagen cleavage epitopes in regions of matrix depletion in OA cartilage. Inflammatory stimuli can also increase either ADAMTS4 or 5 or both, depending upon the experimental model utilized [Gabay *et al.* 2010; Rogerson *et al.* 2010; Song *et al.* 2007]. In addition to proteolytic enzymes, these stress and inflammatory signals upregulate the expression of genes encoding cyclooxygenase 2, microsomal prostaglandin E synthase 1, soluble phospholipase A2, and inducible nitric oxide (NO) synthase (NOS2). Inflammatory cytokines may also induce chemokines, including IL-8, and other cytokines such as IL-6, leukemia inhibitory factor, IL-17, and IL-18. Many of these factors synergize with one another in promoting chondrocyte catabolic responses. Oncostatin M produces mild catabolic responses in chondrocytes through a gp130 receptor and JAK3, but synergizes strongly with IL-1 β or TNF α . Inflammatory cytokines also suppress the expression of a number of genes associated with the differentiated chondrocyte phenotype, including aggrecan (*ACAN*) and type II collagen (*COL2A1*).

Figure 1. Signaling pathways that converge on matrix metalloproteinase13 (*MMP13*) gene transcription in chondrocytes. Binding of the receptor tyrosine kinase, discoidin domain receptor (DDR) 2, to native type II collagen results in activation of RAS/RAF/MEK/extracellular-regulated kinase (ERK) signaling in a manner independent of integrin- or cytokine-induced signaling. Interleukin (IL)-1, toll-like receptor (TLR) ligand, reactive oxygen species (ROS), advanced glycation endproducts (AGEs) interact with the cell through distinct receptors that transduce phosphorylation events via cytoplasmic interactions initiating various protein kinase cascades. The major pathways involve activation of MAP triple kinases (MTK), MAP kinase kinases (MKKs) 3/6 and 4/7, and p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK), which lead to activation of activator protein 1 (AP-1) (cFos/cJun), Runx2, E Twenty Six (ETS) factors, HIF2 α , and C/EBP β , among other transcription factors; and inhibitor of κ B (I κ B) kinases (IKK) α and β , leading to activation of nuclear factor kappa B (NF κ B) and its translocation to the nucleus. The responses of the target gene, *MMP13*, depend on the presence of DNA sequences within its promoter that bind to the various transcription factors.

Activation of canonical NF κ B (p65/p50) and stress-induced and mitogen-activated protein kinase (MAPK) signaling is required for the chondrocytes to express MMPs, ADAMTSs, and inflammatory cytokines themselves [Goldring *et al.* 2011; Marcu *et al.* 2010; Pulai *et al.* 2005]. NF κ B signaling strongly induces the expression of transcription factors such as HIF2 α [Yang *et al.* 2010] and E1f3 [Otero *et al.* 2012], which in turn bind to and activate *MMP13* and other gene promoters (Figure 1). The stress-induced MAPK pathways, including the ERK, c-Jun N-terminal kinase (JNK) and p38 MAPK cascades, coordinate the induction and activation of gene expression through transcription factors such as activator protein 1 (cFos/cJun), ETS, C/EBP β , and Runx2 [Goldring and Sandell, 2007; Liu *et al.* 2010; Long and Loeser, 2010; Tetsunaga *et al.* 2011;

Tsuchimochi *et al.* 2010]. Induction of both ADAMTS4 and 5 requires Runx2 [Tetsunaga *et al.* 2011], and NF κ B and HIF2 α [Yang *et al.* 2010] mediate ADAMTS4 upregulation, whereas *MMP-13* induction requires all three transcription factors. Recent studies indicate that epigenetic mechanisms also play a role through modulation of the DNA methylation status on promoters driving expression of, for example, *IL1B* and *MMP13* genes [Hashimoto *et al.* 2009] or through dysregulation of the microRNAs that are important for maintenance of homeostasis [Dudek *et al.* 2010; Miyaki *et al.* 2010].

As articular cartilage matrix proteins are degraded, activation of certain receptors stimulates the production of matrix-degrading proteinases and inflammatory cytokines and chemokines, either

as initiating or feedback amplification events. Fragments of matrix proteins are produced which can interact with integrin receptors and stimulate or feedback amplifying further matrix destruction. Fragments found in OA cartilage include fibronectin [Homandberg *et al.* 1998; Zack *et al.* 2006], small leucine-rich proteoglycans [Melrose *et al.* 2008], and collagen [Bank *et al.* 1997]. Fibronectin and collagen fragments can stimulate the production of inflammatory cytokines, chemokines, and MMPs [Fichter *et al.* 2006; Homandberg *et al.* 1998; Pulai *et al.* 2005]. Cartilage matrix degradation products may also activate innate immune responses. Members of the small leucine-rich proteoglycan (SLRP) family such as fibromodulin and decorin may target the classic complement pathway and enhance or inhibit its activation [Heinegard and Saxne, 2011]. COMP, however, is a potent activator of the alternative complement pathway and complexes of COMP and C3b may be found in OA synovial fluids [Happonen *et al.* 2010].

Chondrocytes in OA cartilage, especially those in clonal clusters, also express toll-like receptors (TLRs) and receptor for advanced glycation endproducts (RAGE). TLRs are expressed in chondrocytes and synoviocytes activated by inflammatory stimuli [Geurts *et al.* 2011; Haglund *et al.* 2008; Zhang *et al.* 2008]. Secreted damage-associated molecular patterns or alarmins may act as ligands of TLR or RAGE, in the activation of inflammatory and catabolic events in articular cartilage. Specific peptide ligands that are products of cartilage damage may activate TLR2 and TLR4, leading to increased expression of inflammatory and catabolic genes, including MMP-3, MMP-13 and NOS2, through the cytosolic adaptor myeloid differentiation factor 88 and subsequent N- κ B signaling [Liu-Bryan and Terkeltaub, 2010]. The alarmins, S100A4, A8, A9, and A11, along with HMGB1 [Garcia-Arnandis *et al.* 2010; Heinola *et al.* 2010], also signal through RAGE and TLRs to drive inflammation-associated matrix catabolism and increase reactive oxygen species (ROS) through upregulating cytokines and chemokines [Liu *et al.* 2010; Rasheed *et al.* 2011; Yammani *et al.* 2006; Zreiqat *et al.* 2010]. Proinflammatory cytokines, prostaglandins, ROS, and NO also promote oxidative stress and chondrocyte apoptosis by altering mitochondrial function [Blanco *et al.* 2011].

Chondrocytes also express chemokine receptors including CXCR3, CXCR4, CXCR5, and CCR6

and chemokines including IL-8, macrophage inflammatory protein 1 α , GRO $\alpha\beta\gamma$, monocyte chemotactic protein 1, and RANTES, which may play important roles in activating catabolic pathways and chondrocyte hypertrophy [Chaffier *et al.* 2012; Loeser, 2011; Mazzetti *et al.* 2004; Merz *et al.* 2003; Sandell *et al.* 2008]. Many chemokines are produced in joint tissues of patients with OA and after joint injury [Cuellar *et al.* 2009; Endres *et al.* 2010; Scanzello *et al.* 2011]. The synovium of patients at an early stage of OA has a unique synovial chemokine signature, with expression of CCL19 and its receptor CCR7 associated with increased symptoms [Scanzello *et al.* 2011].

The white adipose tissue is an endocrine organ that secretes adipokines, contributing to a 'low-grade inflammatory state' and modulating both homeostasis and catabolism in cartilage [Conde *et al.* 2011; Pottie *et al.* 2006]. Leptin levels are higher in OA than in normal cartilage, and inflammatory stimuli can induce chondrocytes to express a number of adipokines [Conde *et al.* 2011]. Leptin, adiponectin, or resistin, alone or in combination with other inflammatory cytokines, can increase the expression of cytokines, MMPs, and NOS2 [Kang *et al.* 2010; Koskinen *et al.* 2011; Zhang *et al.* 2010]. Whether adipokines are protective or detrimental *in vivo*, or primary or secondary to the OA development, is still not clear [Chen *et al.* 2006; Griffin *et al.* 2009, 2010].

Lessons from preclinical models

We have learned much about the mediators mentioned above from studies in preclinical models and comparisons of gene and protein expression in clinical material and in cell culture models (Figure 2). Surgical OA models that reflect some aspects of post-traumatic OA in humans have been particularly useful for studying the consequences of knockout or transgenic overexpression of genes of interest in mice [Glasson, 2007; Glasson *et al.* 2007; Little and Fosang, 2010]. As in human genetic disorders, such as chondrodysplasias [Kannu *et al.* 2009], genetic mouse models with abnormal composition and structure of articular cartilage or other joint tissues may develop spontaneous or accelerated OA due to altered biomechanics. Although interpretation of *in vivo* experiments may not be straightforward and mouse models may not mimic all aspects of human disease, they do allow us to study the time course of the disease in a way that is not possible

Figure 2. Strategies for studying mechanisms of osteoarthritis. The upper left panels show Safranin O/ Fast green-stained human cartilage sections from a normal individual and a patient with osteoarthritis (OA) (arrows mark surface fibrillations and duplicated tidemark, derived from the tibial plateau [lower left panel] of a patient who underwent total knee replacement surgery (the medial side on the right is more affected than the lateral side). The upper right panels show Safranin O/ Fast green-stained murine cartilage sections from knee joints left unoperated (control) or subjected to destabilization of the medial meniscus (DMM) surgery. Differentially regulated proteins and genes are identified in both the clinical material and the preclinical model and mechanisms are further studied in cell culture models of isolated chondrocytes in three-dimensional pellet or high-density monolayers.

in humans [Glasson *et al.* 2010; Little and Fosang, 2010]. Gene profiling in experimental OA models has provided additional targets for consideration [Appleton *et al.* 2007; Bernardo *et al.* 2011; Lodewyckx *et al.* 2012; Loeser *et al.* 2012; Yasuhara *et al.* 2011]. Furthermore, certain mediators that determine initiation and progression of cartilage damage are common to all of these models.

The importance of proteoglycan depletion in cartilage erosion was demonstrated in *Adamts5* knockout mice, which are protected against progression of cartilage destruction [Glasson *et al.* 2005; Stanton *et al.* 2005]. However, aggrecan depletion, by itself, does not drive OA progression, as suggested by recent studies in *Mmp13* knockout mice showing that MMP-13 deficiency inhibits cartilage erosion, but not aggrecan depletion [Little *et al.* 2009]. The importance of the stability of the extracellular matrix to cartilage health is also documented in studies of *Timpp3*–/– mice, which each show age-dependent cartilage degeneration similar to that of patients with OA because of loss of this key MMP and ADAMTS inhibitor [Sahebjam *et al.* 2007]. Similarly,

Fgf2–/– mice exhibit accelerated spontaneous and surgically induced OA due to loss of the intrinsic capacity of FGF-2 to inhibit ADAMTS5 [Chia *et al.* 2009].

Mouse models have also taught us about the patterns of receptors on chondrocytes that sense changes in the pericellular matrix. The receptors on the resting chondrocyte are protected from interacting with certain matrix components by the unique structure of the pericellular matrix. But their expression and activation change in response to mechanical or inflammatory stimuli. In *Col9a1* knockout mice and *Col11a1* haploinsufficient mice, the development of OA-like changes can be observed with aging, owing to decreased amounts of the minor collagens that contribute to type II collagen fibril formation [Hu *et al.* 2006; Xu *et al.* 2005]. In these mice, there is little pericellular matrix and the fibrillar collagen bundles can be observed closer to the chondrocytes. This results in exposure of the receptor tyrosine kinase, discoidin domain receptor 2 (DDR2) to its ligand, native type II collagen, and preferential induction and activation of MMP-13. The association of DDR2, MMP-13,

and MMP-specific type II collagen cleavage fragments cannot only be observed in the *Col9a1*^{−/−} and *Col11a1*^{+/−} mice, but also in wild type mice subjected to destabilization of the medial meniscus (DMM) surgery and in human OA cartilage [Xu *et al.* 2007]. Furthermore, the *Ddr2*^{+/−} mice subjected to DMM surgery and the cross between *Ddr2*^{+/−} and *Col11a1*^{+/−} mice show attenuated development of OA progression [Xu *et al.* 2010].

Recent studies in mouse models suggest that mechanical stress may initiate the disruption of the pericellular matrix through the serine proteinase, high-temperature requirement A1 [Polur *et al.* 2010]. The requirement for the pericellular matrix in preventing the activation of DDR2 was also shown in mice with inducible *Comp*-driven overexpression of *Ddr2* [Xu *et al.* 2011]. These events may also affect to activities of other receptors important in homeostasis. Deficiency of syndecan-4, a transmembrane heparan sulfate proteoglycan, results in less severe OA-like cartilage degradation through downregulating MMP-3, an activator of ADAMTS5 [Echtermeyer *et al.* 2009]. The accumulation of fibronectin fragments and type II collagen fragments over time may further increase MMP-13 synthesis through interaction with cell-surface integrins, leading to a positive feedback amplification loop and irreversible destruction of knee joints. However, loss of α 1-integrin disrupts homeostasis during aging of *itga*^{−/−} mice [Zemmyo *et al.* 2003].

A common thread in studies of mouse is that the regulatory factors responsible for initiation and progression of OA-like changes all appear to impact on the control of expression or activities of ADAMTS5, MMP-13, and other proteinases. *Runx2*^{+/−} mice [Kamekura *et al.* 2006], *Epas1*^{+/−} mice with deficiency of HIF2 α [Saito *et al.* 2010; Yang *et al.* 2010], and mice with downregulated *Ihh* signaling [Lin *et al.* 2009] have decreased severity of OA joint disease. The levels of miR140, which downregulates ADAMTS5, are reduced in OA cartilage and also suppressed by exposing chondrocytes to IL-1 β [Miyaki *et al.* 2009]. Mice with miR140 deficiency are predisposed to age-related OA-like changes, while miR140 overexpression is protective against surgically induced OA [Miyaki *et al.* 2010].

Disruption of the normal resting state of chondrocytes may also be viewed as an injury response involving the recapitulation of developmental programs, leading to matrix remodeling,

inappropriate hypertrophy-like maturation, and cartilage calcification [Goldring and Marcu, 2009; Goldring *et al.* 2011]. Targeting chondrocyte hypertrophy with an antibody against syndecan-4 [Echtermeyer *et al.* 2009], a Hedgehog signaling inhibitor [Lin *et al.* 2009], or recombinant human PTH(1-34) (teriparatide) [Sampson *et al.* 2011] reduces the severity of OA in surgically induced OA models. Osteophyte formation is also driven by a process of endochondral ossification involving TGF β and BMP-induced chondrocyte differentiation [van der Kraan and van den Berg, 2007, 2012]. The increased cartilage calcification is associated with tidemark advancement or duplication, and vascular penetration from the subchondral bone [Suri and Walsh, 2011]. The close relationship between the process of cartilage damage and subchondral bone changes is of increasing interest [Goldring and Goldring, 2010] and has been addressed recently in mouse models [Allen *et al.* 2009; Botter *et al.* 2009, 2011; Dreier *et al.* 2008; Wang *et al.* 2008].

BMP, TGF β and Wnt signaling are intertwined during chondrocyte terminal differentiation and may play a role in OA through promoting recapitulation of the developmental program exemplified in expression of markers of chondrocyte hypertrophy such as *Col10a1*, *Runx2*, and *Mmp13* [Luyten *et al.* 2009; van der Kraan and van den Berg, 2012]. Chondrocytes express many Wnt family members and inhibitors of Wnt activation, such as secreted frizzled-related proteins, may be protective [Blom *et al.* 2009]. Wnt-16, Wnt-2B, and Wnt-induced signaling protein 1 (WISP-1) are strongly upregulated in the synovium and cartilage of mice with experimental OA [Blom *et al.* 2009]. Because of the important role of the Wnt pathway in regulating bone formation, alterations in Wnt signaling may be involved in both the cartilage and bone changes observed in OA. Both activation [Zhu *et al.* 2009] and inhibition [Zhu *et al.* 2008] of β -catenin signaling in mice result in the development of an OA-like phenotype through differential effects on the subchondral bone, chondrocyte hypertrophy, and chondrocyte apoptosis. Blockade of the Wnt inhibitor, DKK1, decreases bone loss but leads to osteophyte formation in inflammatory arthritis mouse models and neutralizes the effects of another Wnt inhibitor sclerostin (SOST) [Diarra *et al.* 2007; Heiland *et al.* 2010]. SOST is normally expressed by osteocytes, but a recent study showed upregulated expression of SOST by OA chondrocytes in

regions of cartilage damage, but decreased expression in regions of subchondral bone sclerosis [Chan *et al.* 2011].

Despite the impact of stress- and inflammation-induced signaling on chondrocyte expression of destructive enzymes and the increasing evidence of the contribution of the synovium to the cytokine and chemokine environment of the OA joint [Blom *et al.* 2007b; Bondeson *et al.* 2010; Scanzello *et al.* 2009, 2011], therapies targeting these processes have been elusive. This is partly because more effective biomarker analyses and imaging strategies need to be applied to stratify patients according to criteria that are likely to show structure modification and differentiate the pain response [Kraus *et al.* 2011; Patra and Sandell, 2011; Sellam and Berenbaum, 2010]. For example, efficacy of an aggrecanase-specific inhibitor against cartilage damage has been demonstrated in a rat model of meniscal tear-induced joint instability [Chockalingam *et al.* 2011], although clinical trials have not gone forward. Similarly, treatment of this model with lubricin [Flannery *et al.* 2009] with an inhibitor of LXR β [Li *et al.* 2010], a nuclear receptor transcription factor, or with FGF-18 [Moore *et al.* 2005] reduced or prevented cartilage degeneration. Furthermore, analgesic efficacy of the soluble nerve growth factor receptor, TrkAD5, was demonstrated in murine OA [McNamee *et al.* 2010].

Less severe OA has been reported in IL-1 β knock-out mice subjected to OA [Blom *et al.* 2007a; Glasson *et al.* 2007] and treatment with an inhibitor of IL-1 β -converting enzyme reduces joint damage in OA mouse models [Rudolph *et al.* 2003]. One clinical study indicated that single injections of IL-1 receptor antagonist (IL-1Ra) in OA-affected knees are not likely to be sufficient for controlling catabolic and inflammatory processes [Chevalier *et al.* 2009]. However, IL-1Ra administered within the first month following severe knee injury reduced knee pain and improved function in a proof-of-concept pilot study [Kraus *et al.* 2012]. Studies also suggest a role in OA for innate immune responses triggered by molecular signals of tissue damage, such as hyaluronan and fibronectin, which may act as TLR stimuli [Scanzello *et al.* 2008]. Although TLR4 deficiency reduces disease severity in a mouse model of inflammatory arthritis [Abdollahi-Roodsaz *et al.* 2008], the efficacy of specific TLR deficiency or blockade has not yet been reported in models of OA. A recent study in murine OA models demonstrated that C5- and

C6-deficient mice are partially protected from the development of OA [Wang *et al.* 2011].

Conclusion

We understand a great deal about how cartilage is formed during skeletal development, but very little about how to successfully apply this knowledge to controlling the OA disease process. For that, it will be necessary to obtain further understanding of the complex spatial and temporal expression patterns of these stress- or inflammation-induced signals and how they contribute not only to the initiation phase, whereby chondrocytes in articular cartilage leave their natural growth- and differentiation-arrested state, but also to the irreversible progression of joint damage. Mapping the gene and protein expression patterns at the level of individual chondrocytes at early, middle and late stages of OA disease to elucidate common pathways regulating this complex multifactorial disease will also help to facilitate the identification of specific therapeutic targets linked to cellular stress, inflammatory responses, proliferation, and differentiation. Innovative approaches in preclinical models will be essential before they can be applied for clinical translation. Such studies hold the promise of uncovering common molecular switches leading to OA development that would facilitate the design of rationally targeted therapies to prevent OA onset without unwanted side effects or to aid in restoration of cartilage by tissue engineering approaches.

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