# Knock-Out Mice in Osteoarthritis Research

Aurelia Raducanu and Attila Aszódi*

*Department of Molecular Medicine, Max Planck Institute for Biochemistry, Martinsried, Germany*

**Abstract:** Osteoarthritis (OA) is the most common degenerative disorder of the joints with an etiology involving genetic and environmental factors. Although various animal models have been used to elucidate the pathogenesis of OA, in the past decade gene targeting in mice has become one of the most powerful tools to dissect the molecular mechanisms of the disease. The generation of knockout mice has enormously accelerated the identification of the key genetic players in articular cartilage homeostasis and made a significant contribution to further our understanding of OA pathology. In this review, we will outline the phenotypes of the currently available mouse strains, carrying either engineered or spontaneous gene mutations, which provide insight into the processes of articular cartilage destruction. The analysis of these mice reveals a complex interaction among cytokines, proteases, transcription factors, extracellular matrix, cell surface and signaling molecules during the initiation and progression of OA and, in some cases, suggests new therapeutic interventions for the disease.

**Keywords:** Osteoarthritis, gene targeting, mouse models, extracellular matrix, signaling, proteolysis, cytokines, growth factors.

## INTRODUCTION

Osteoarthritis (OA) is a non-systemic degenerative joint disease affecting millions of people world-wide and is the prevalent cause of disability over 65. Although OA is characterized by the gradual deterioration of the articular cartilage, both destructive and repair processes are activated at certain phases of the disease, suggesting a highly dynamic interplay among catabolic and anabolic molecular cascades. Osteoarthritis is generally accepted as a complex disorder influenced by both environmental and genetic factors [1]. To assess the contribution of genetic factors and to explore the pathogenesis of OA, murine models of human skeletal disorders are important tools. These models include mouse strains carrying spontaneous mutations and induced mutant strains generated by classical transgenesis (transgenic mice) or by gene targeting (knockout mice). Since the publication of OA-like changes in transgenic and knockout mice with mutations in the collagen IX gene in the mid 1990s [2, 3], gene ablation experiments have yielded more than 40 knockout mouse strains which have significantly advanced our knowledge of normal and pathobiology of the articular cartilage. Genetically modified mice not only provide a deeper insight into molecular mechanisms controlling joint development and OA pathogenesis, but also identify potential target molecules for therapeutic interventions against OA. In this review, we summarize the currently available knockout and the most relevant transgenic and spontaneous mouse models for OA research.

## MATRIX TURNOVER AND ECM DEGRADING ENZYMES

Articular cartilage (AC) is composed of a structurally well organized, interactive set of extracellular matrix (ECM) molecules produced by chondrocytes. The two major structural components of the cartilage ECM are the type II collagen fibrils and the proteoglycan (PG) aggrecan. It is generally accepted that in OA the normal turnover of ECM molecules is disturbed, and the imbalance between anabolic and catabolic processes favors proteolytic degradation of aggrecan (aggrecanolysis) and collagens (collagenolysis), which eventually leads to articular cartilage destruction. Several families of proteinases and proteinase inhibitors have been implicated in the initiation and progression of OA. Matrix metalloproteinases (MMPs) are a family of more than 20 zinc- and calcium-dependent proteinases that play crucial roles in various developmental, repair and pathological processes facilitating the turnover and breakdown of most ECM molecules [4-6]. A second family of MMP-related proteinases is composed of the membrane-anchored ADAMs (a disintegrin and metalloproteinases) and the secreted ADAMTSs (ADAM with thrombospondin motifs). Concerning the cartilage, ADAMTS proteins are particularly important because many of them are able to degrade aggrecan (aggrecanases) [7]. The activities of both MMPs and ADAMTSs are tightly regulated at multiple levels including inhibition by tissue inhibitors of metalloproteinases (TIMPs) [8]. Recent expression profiling revealed that most *MMP*, *ADAMTS* and *TIMP* genes are expressed in both normal and osteoarthritic human joints demonstrating the potential importance of these three protein families in OA [9, 10]. Finally, cysteine proteinases such as cathepsins B, L and K and their inhibitor (cystatin C) represent other important molecules that might be involved in AC destruction [11].

### Aggrecanolysis

Proteoglycan depletion is among the first detectable alterations in osteoarthritic cartilage, usually preceding collagen degradation. Aggrecan consists of two N-terminal globular domains (G1 and G2), separated by an interglobular domain (IGD); regions substituted with keratan sulfate (KS) and chondroitin sulfate (CS) glycosaminoglycan (GAG) side chains; and a C-terminal globular domain (G3). Multiple cleavage sites for MMPs and aggrecanases have been identified in aggrecan *in vitro* and the corresponding degradation

*Address correspondence to this author at the Department of Molecular Medicine, Max Planck Institute for Biochemistry, Am Klopferspitz 18, D-82152 Martinsried, Germany; Tel: +49-89-8578-2466; Fax: +49-89-8578-2077; E-mail: aszodi@biochem.mpg.de

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fragments can be detected in human OA synovial fluid and/or cartilage [12]. Two important cleavage sites associated with articular cartilage destruction are located in the IGD: all MMPs expressed in cartilage cleave predominantly between $Asn^{341}$-$Phe^{342}$ generating the G1-$IPEN^{341}$ neoepitope; whereas ADAMTS aggrecanases cleave at $Glu^{373}$-$Ala^{374}$ generating the G1-$TEGE^{373}$ fragment. Since aggrecanase but not MMP inhibitors are able to suppress aggrecan loss from human OA cartilage explants [13], most studies have focused on the identification of the key aggrecanase responsible for destructive aggrecanolysis.

ADAMTS-4 and ADAMTS-5, two aggrecanases possessing high aggrecanolytic activity, are expressed in normal and OA cartilage and their contribution to cartilage pathology was recently dissected in genetically modified mice [14-16]. Surprisingly, neither ADAMTS-4 nor ADAMTS-5 knockout mice exhibit obvious abnormalities indicating that these aggrecanases are dispensable for skeletal development. In unchallenged mutants, the level of G1-$TEGE^{373}$ fragment in cartilage was reduced suggesting that both proteases contribute to physiological aggrecanolysis in mice. However, inflammatory stimulation of aggrecan breakdown in IL-α-treated articular cartilage explants was blocked in ADAMTS-5 knockouts but not in ADAMTS-4 mutant mice. Similarly, only ADAMTS-5 mutant mice were protected from aggrecan loss and articular cartilage erosion in inflammatory or surgically induced *in vivo* models of arthritis. Thus, ADAMTS-5 is the sole enzyme responsible for IGD cleavage and for the initiation of early aggrecan breakdown, however, a recent study showed that some aggrecanolysis still occurs at the CS-rich region in the ADAMTS-5 knockout mice [17]. Furthermore, cartilage explants from ADAMTS-4/ADAMTS-5 double mutant mice release intact or G1 domain-containing aggrecan molecules upon retinoic acid stimulation demonstrating that aggrecan loss is not necessarily coupled to its proteolytic processing [18]. Nevertheless, blocking IGD cleavage by aggrecanase inhibitors might be a useful therapeutic strategy to reduce or even reverse cartilage destruction. Consistent with this assumption, the so-called "Jaffa" mice, in which the aggrecan core protein is rendered resistant to aggrecanase-mediated cleavage, are partially protected against aggrecan loss and cartilage damage progression in surgically and inflammatory-induced experimental models of arthritis [19].

Explant culture experiments revealed that MMPs account for only a small percentage of total aggrecan catabolism in porcine cartilage implying that MMPs are rather involved in basal turnover of aggrecan in the pericellular microenvironment of chondrocytes than in destructive aggrecanolysis [20]. In line with the *in vitro* data, the so-called "Chloe" mice with a mutation resistant to MMP-driven cleavage of aggrecan IGD show normal skeletal development [21] and have no protection against cartilage deterioration in induced models of arthritis [19]. Among the MMPs capable of degrading aggrecan, MMP3 was studied in knockout mice in detail. MMP3 can degrade, in addition to aggrecan, various cartilage ECM components; and is able to activate procollagenases and progelatinases [4]. The incidence of spontaneous OA was recently compared between *Mmp3*-null and wild type mice [22]. While mild OA-like changes were similar in knockout and control 1-year-old mice, the exposure of G1-$IPEN^{341}$ and the deterioration of the AC were significantly less prominent in MMP3-deficient mice at 2 years of age. In inflammatory arthritis models, *Mmp3*-null mice showed normal PG depletion, but did not expose type II collagen-cleavage neoepitopes [23, 24]. Since the findings were accompanied by a lack of significant cartilage destruction, the major function of MMP3 in these arthritis models seems to be the initiation of collagenase-mediated cartilage breakdown by activating procollagenases. In contrast, in a surgically-induced knee joint instability model *Mmp3*-null mice exhibited accelerated OA changes with increased G1-$IPEN^{341}$ and collagenase-cleavage neoepitope staining around the lesion sites [25]. These data imply that MMP3 could be replaced by functionally related MMPs to accelerate OA in some arthritis models, and that MMP3-regulated matrix turnover is important to maintain normal cartilage homeostasis.

**Collagenolysis**

The cleavage of the triple helical domain of type II collagen fibrils by collagenases occurs in both normal and arthritic cartilage. All three collagenases (MMP1, MMP8 and MMP13) cleave at the $Gly^{906}$-$Leu^{907}$ site of each α-chain resulting in the generation of characteristic three-quarter and one-quarter collagen fragments. At the cleavage site, neoepitopes are exposed which can be detected by specific antibodies (C2C or C1,2C) either in the cartilage itself or in body fluids. In addition to collagenases, MMP2 and the membrane-bound MMP14 (MT1-MMP) also cleave native fibrillar collagens. In OA cartilage excessive collagen fragmentation is believed to increase the expression of proteolytic enzymes leading to further degradation of matrix proteins and subsequent cartilage resorption [26, 27].

A transgenic mouse line overexpressing constitutively active human MMP13 in postnatal joint cartilage exhibits deteriorated AC at 5 months of age accompanied by increased C1,2C immunostaining in the pericellular matrix [28]. Loss of Safranin O staining in the AC was also observed demonstrating that the disintegrated collagen network cannot retain proteoglycans in the tissue. In addition, MMP13 transgenic mice exhibit strong collagen X expression above the tidemark in the non-calcified upper and middle zones suggesting that MMP13 overexpression is linked to the reactivation of the normally suppressed differentiation program of AC chondrocytes. Interestingly, mice heterozygous for Runx2, a runt-related transcription factor which may activate both MMP13 and collagen X transcription, show decreased AC degradation along with reduced MMP13 and collagen X expression in a surgically induced OA model [29]. Whether MMP13 is a valuable target for OA treatment is still a question, since articular cartilage was not studied in *Mmp13*-null mice [30].

The emerging view that MMPs are not simply destructive but rather constructive enzymes of the cellular microenvironment [5, 6] is further illustrated by the phenotype of the *Mmp2*$^{-/-}$ and *Mmp14*$^{-/-}$ mice. MMP2-deficiency leads to multiple defects including disrupted long bone and craniofacial development [31]. Arthritic changes of the tibia and femur are visible at 3 months of age and are accompanied by the invasion of fibrous tissue and inflammatory cells into the joints. Ablation of the *Mmp14* gene results in a similar skeletal phenotype and severe, generalized soft tissue fibroses

leading to lethality at 1-3 months of age [32]. Although the pathomechanism of arthritis in *Mmp14*$^{-/-}$ mice was not addressed specifically, it has been suggested that inappropriate remodeling caused by impaired pericellular collagenolysis at the AC-soft tissue interfaces induces AC destruction. On the other hand, MMP14 is a critical activator of other proteases and the conversion of pro-MMP2 to active MMP2 was strongly reduced in *Mmp14*-null mice [33]. This raises the possibility that the common skeletal pathology observed in the two mutant strains are ascribed, at least partially, to diminished MMP2 function.

### Timp3 and Cathepsin K in Articular Cartilage

The balance between MMPs and their endogenous inhibitors TIMPs are critical to prevent excessive ECM degradation in connective tissues. Among the four TIMPs, TIMP3 is uniquely sequestered in the matrix and effectively inhibits, in addition to MMPs, several members of the ADAM and ADAMTS families predicting its role in cartilage homeostasis [8]. Indeed, *Timp3*$^{-/-}$ mice exhibit mild, age-dependent AC degradation characterized by reduced Safranin O staining [34]. Consistent with the inhibitory potential of TIMP3, knockout mice show elevated levels of both aggrecan and type II collagen cleavage products in the AC. Interestingly, the exposure of the G1-IPEN$^{341}$ is more prominent than the exposure of G1-TEGE$^{373}$ implying that TIMP3 modulates proteoglycan turnover primarily *via* the inhibition of MMPs and not aggrecanases.

In addition to the pivotal role of MMPs in cartilage destruction accumulating evidence has suggested that cathepsin K, a lysosomal cysteine proteinase that is able to degrade both aggrecan and fibrillar collagens, is also involved in degenerative cartilage diseases. In normal mice which develop mild OA spontaneously at 9 months of age, cathepsin K is upregulated in close vicinity of the cartilage lesions [35]. Transgenic mice overexpressing cathepsin K under its endogenous promoter display progressive synovitis accompanied by severe destruction of the articular cartilage and subchondral bone [36]. In cathepsin-K-deficient mice (*Ctsk*$^{-/-}$), which develop osteopetrosis due to impaired bone resorption, joints were not investigated [37]. Worth noting, however, is that in the null mice a compensation mechanism is activated resulting in the transcriptional upregulation of several MMPs (including MMP13) and the elevation of collagens I and II degradation products [38, 39].

## CYTOKINES AND GROWTH FACTOR SIGNALING

The permanent communication of chondrocytes with their environment is essentially mediated by soluble growth factors and cytokines. They interact with specific cell surface receptors and control gene expression in order to maintain a balanced ratio between the anabolic and catabolic activities of chondrocytes. To understand the role of cytokine/growth factor-mediated signaling in cartilage biology and OA pathology several models of genetically modified mice have been established.

### Cytokines and Cartilage Homeostasis

Although OA is not defined as a classical inflammatory arthropathy, there is strong evidence for the involvement of catabolic inflammatory cytokines in the evolution of the disease [40]. IL-1 cytokines are probably the most well studied pro-inflammatory cytokines that play important roles in OA [41, 42]. Upon binding to type I IL-1 receptor on chondrocytes, IL-1 activates NF-κB and mitogen-activated protein kinase (MAPK) signaling pathways and stimulates the expression of inducible nitric oxide synthase (iNOS), various cytokines and chemokines, and matrix degrading enzymes such as MMPs and aggecanases. Knockout mice lacking either IL-1β, ICE (IL-1β-converting enzyme) or iNOS develop accelerated OA [25] after knee surgery. This unexpected observation suggests that the primary role of IL-1β is to maintain a balanced metabolism in the AC and IL-1-deficiency might up-regulate the expression of other catabolic enzymes in the knockout mice.

IL-6 is a multifunctional cytokine that mediates joint inflammation, but it also induces the expression of proteinase inhibitors in chondrocytes [43, 44] implicating IL-6 as a protective factor against cartilage destruction. This assumption was supported in a study with IL-6-deficient mice. Aging *IL-6*$^{-/-}$ male mice exhibit severe cartilage erosion, subchondral sclerosis, reduced PG synthesis and bone mineral density with higher incidence than *IL-6*$^{-/-}$ females or wild type mice [45]. In the STR/ort mouse strain, which develop spontaneous OA-like changes more severely in males than in females, young females display increased expression of IL-6 and the chondroprotective IL-4 and TGF-β [46]. It was hypothetized that in *IL-6*$^{-/-}$ females the upregulation of IL-4/TGF-β may compensate for the loss of IL-6, ameliorating OA pathology [45].

### Growth Factors

It is generally accepted that growth factors including the members of the transforming growth factor (TGF)-β superfamily, insulin-like growth factor (IGF)-I and fibroblasts growth factors (FGFs) play pivotal roles in AC biology owing to their ability to modulate chondrocyte differentiation, proliferation and metabolism [42, 47, 48]. IGF-I modulates cartilage homeostasis by promoting PG synthesis and chondrocyte survival, and by opposing the catabolic effects of IL-1 and TNF-α. IGF-I is produced primarily by growth hormone (GH) stimulation and transgenic mice over-expressing bovine GH develop arthritic joints exhibiting AC lesions and osteophyte formation [49, 50]. The tissue degradation correlated with a high TNF-α level, and decreased proliferation and increased apoptosis of the chondrocytes [50].

Numerous *in vitro* studies have shown that mammalian TGF-β isoforms potently induce chondrocyte PG and collagen II biosynthesis and, at the same time, suppress the expression of IL-1-regulated genes [51, 52]. Although many findings imply that TGF-β could be used for treatment of OA, repeated injection of TGF-β into mouse knee joints resulted in osteophyte formation and cartilage lesions suggesting a role for excessive TGF-β in OA development [53]. Transgenic mice expressing a dominant negative form of the human TGF-β type II receptor (*TGFβRII*$^{DN}$) develop progressive joint degeneration characterized by PG depletion, synovial hyperplasia, ectopic hypertrophy and osteophyte formation [54]. Targeted disruption of exon 8 of the *Smad3* (*Smad3*$^{ex8/ex8}$) gene encoding a receptor-activated SMAD which transduces TGF-β signals into the nucleus, leads to a similar phenotype as seen in *TGFβRII*$^{DN}$ transgenics [55]. A recent microarray analysis of *Smad3*$^{ex8/ex8}$ articular chondro-

cytes indicated the enhanced expression of MMPs as well as alterations in the expression of genes responsible for protein synthesis, cellular respiration and growth factor signaling, implying that Smad3-deficiency interferes with several cellular mechanisms which might contribute to the development of OA [56]. LTBP (latent TGF-β binding protein)-3 is a structural component of the ECM which modulates TGF-β availability [57]. Mice lacking LTBP-3 have impaired membranous ossification and develop progressive osteosclerosis and OA associated with ectopic hypertrophic chondrocyte differentiation in the superficial zone of the AC [58]. Collectively, these mouse models demonstrate that TGF-β/Smad-3 signaling is necessary to repress articular chondrocyte differentiation and to maintain normal articular cartilage function.

Bone morphogenetic proteins (BMPs), members of the TGF-β superfamily, are critically involved in the control of multiple aspects of embryogenesis including bone and cartilage formation [47, 59]. The physiological role of BMP signalling in the maintenance of AC was recently addressed by conditional targeting of the gene encoding BMP receptor 1a (BMPR1A) [60]. Since *Bmpr1a*$^{-/-}$ mice die during early embryogenesis, the floxed *Bmpr1a* allele was deleted by expressing Cre recombinase under the control of the joint-specific *Gdf-5* (growth differentiation factor-5) regulatory regions. Young *Gdf5-Cre/Bmpr1a*$^{fl/fl}$ mice showed defective joint development in the ankle, whereas most other joints of the body developed normally. This initially mild phenotype, however, was followed by severe OA-like changes in adult digit and knee joints characterized by PG loss and progressive destruction of the AC. Interestingly, the expression of the *Agn* and *Col2a1* genes was also reduced by the mutant articular chondrocytes but, in contrast to mice with defective TGF-β signalling, the articular cartilage showed no signs of hypertrophic differentiation. These findings reveal that BMPR1A function is required for 1) the formation of specific joints and 2) for the maintenance of adult articular cartilage by stimulating the expression of key ECM molecules. BMP and TGF-β signaling pathways therefore play partially distinct roles in OA pathogenesis.

While the importance of FGF/FGF receptor signalling in limb patterning and skeletal development is well appreciated [61], the role of FGFs in AC was only recently addressed using mice lacking FGF receptor 3 (FGFR-3). *Fgfr-3*$^{-/-}$ mice develop skeletal overgrowth [62], osteopenia [63] and early-onset osteoarthritis [64]. The OA phenotype is characterized by enhanced expression of MMP13 and increased exposure of MMPs-generated aggrecan and collagen II cleavage products accompanied by PG loss and alteration of the biomechanical properties of the articular cartilage. In addition, collagen X is expressed above the tidemark in the mutant AC suggesting that FGFR-3 is not only a critical regulator of AC metabolism but it is also required to suppress terminal differentiation of articular chondrocytes.

*Mitogen-inducible gene 6 (Mig-6)* is an immediate early response gene that can be activated by growth factors [65]. *Mig-6*$^{-/-}$ mice develop severe joint defects and most mice die around 6 month of age due to tempomandibular (TM) joint ankylosis [66]. The phenotype includes early-onset degradation of the AC, synovial hyperplasia, subchondral cyst and osteophyte formation. It was suggested that following mechanical stress Mig-6 suppresses the stimulatory effects of growth factors on the proliferation and differentiation of mesenchymal progenitors required for joint renewal. Consequently, the ablation of *Mig-6* leads to overproliferation of these cells causing bony outgrowths and joint deformities.

## MATRIX INTEGRITY AND SIGNALING

The composition and structural organization of the matrix varies throughout the articular cartilage to fulfill specific mechanical demands [67]. In addition to the structural role, the matrix provides an instructive environment from which extracellular information is transduced to chondrocytes *via* cell surface receptors. The heterodimeric (αβ) integrins are the major class of adhesion molecules that mediate chondrocyte-ECM interactions. Articular cartilage chondrocytes primarily express β1-subunit-containing integrins including receptors for fibrillar collagens (α1β1, α2β1 and α10β1) and fibronectin (α5β1). It has been suggested that ECM degradation products of the AC stimulate MMP13 synthesis through integrin-mediated activation of Erk, p38 and JNK, members of the MAPK family [68, 69]. Type II collagen fibrils also bind to and activate the tyrosine kinase receptor discoidin domain receptor 2 (DDR2), which in turn induces MMP13 expression in human OA cartilage [70, 71].

### The Fibrillar Collagen Network and Collagen Receptors

Collagen fibrils of the cartilage are heterotypic consisting of the abundant type II and the quantitatively minor type IX and XI collagens. Mutations in these collagens lead to a range of heritable disorders primarily affecting the cartilaginous skeleton [72]. In general, the consequence of mutations in the genes encoding collagen IX (*COL9A1*, *COL9A2* and *COL9A3*) are the mildest leading to multiple epiphyseal chondrodysplasia (MED), a heterogenous group of skeletal dysplasias characterized by moderately short stature and early onset osteoarthritis. Some collagen II (*COL2A1*) and collagen XI (*COL11A1* and *COL11A2*) mutations result in Stickler syndrome, a phenotypically and genetically variable disorder associated with ocular defects and OA of the hip and knee joints [73].

Mice with collagen II deficiency in the cartilage matrix as a consequence of engineered (*Col2a1*$^{-/-}$) or spontaneous mutations (*dmm/dmm*; disproportionate micromelia) and transgenic mice expressing high levels of a deleted version of the murine *Col2a1* gene (Del1$^{+/+}$) exhibit severe dwarfism and die at birth from respiratory failure [74]. In contrast, heterozygous animals survive and develop variable degrees of OA. In the Del1$^{+/-}$ mice, OA-like lesions appear at 3 months of age that gradually progress in older mice [75]. Severe AC damage develops slightly slower in *dmm*/+ mice [76]. Whereas Del1$^{+/-}$ and *dmm*/+ mice show early-onset OA, *Col2a1*$^{+/-}$ develop a milder phenotype characterized by a higher prevalence of OA in aged mice in association with a softened articular cartilage [77, 78]. In the Del1$^{+/-}$ mice the onset of cartilage destruction coincides with the upregulation of cathepsin K in chondrocytes, suggesting the involvement of this cysteine protease in the development of OA [35].

The heterotrimeric (α1α2α3) collagen IX and collagen XI molecules are associated with the fibrillar surface or buried in the core of the fibers, respectively. Homozygous *cho/cho* (*chondrodysplasia)* mice harboring a *Col11a1* gene with a naturally mutated premature stop codon develop lethal

chondrodysplasia [79], whereas *cho/+* mice show a normal skeleton at birth but exhibit age-dependent OA-like changes of the knee and TM joints [80-82]. Homozygous transgenic mice expressing a truncated version of the α1(IX) chain or *Col9a1*$^{-/-}$ mice lacking the triple-helical collagen IX molecules survive and develop early-onset OA [2, 3, 82, 83]. A possible molecular mechanism underlying the OA-like phenotype of the *Col9a1*$^{-/-}$ and *cho/+* mice was proposed in a series of recent publications [80-84]. As the result of the structural disorganization of the collagen network, mechanical stress first elevates pericellular PG synthesis and chondrocyte proliferation leading to the formation of cell clusters in the mutant AC by 3 months of age. This repair step is followed by the overproduction of MMP13, PG depletion and collagen degradation by 6 months of age, and severe cartilage erosion over the age of 9-12 months. The key event in the progression of OA is the increasing contacts between type II collagen fibrils and the chondrocyte surface receptor DDR2. The enhanced activation of DDR2 up-regulates the expression of MMP13 as well as the expression of DDR2 itself in the articular cartilage. *In vitro* experiments with mouse chondrocytes revealed that the interaction between native type II collagen and the collagen binding discoidin domain of DDR2 is a prerequisite for the ERK/p38-mediated induction of MMP13 expression. *In vivo*, the MMP13-mediated degradation of the collagen fibrils and fibronectin may further stimulate MMP13 production *via* activation of the integrin receptors α2β1 and α5β1, respectively, resulting in the progressive destruction of the articular cartilage [84].

Chondrocyte integrins act as mechanoreceptors and transduce signals from the pericellular matrix into intracellular pathways regulating cartilage homeostasis [85]. Despite the numerous integrin knockouts described in the literature, the *in vivo* role of integrins in OA pathogenesis is largely unknown. Most mutant lines lacking a particular integrin subunit develop a normal skeleton without reported AC abnormalities [86], whereas the cartilage-specific deletion of the β1 subunit-containing integrins results in severe chondrodysplasia and high mortality rate at birth [87]. To date only α1 integrin-deficiency has been linked to pathological abnormalities of the knee articular cartilage [88]. α1-null mice show precocious PG loss, synovial hyperplasia and cartilage erosion associated with increased MMP2 and MMP3 expression. The mutant AC is also characterized by hypocellularity and increased cell death. Interestingly, a similar, accelerated development of knee OA was observed in mice lacking the membrane-anchored ADAM15 [89]. ADAM15 possesses multiple pericellular functions including the modulation of outside-in signaling of integrins. Altogether, these data implicate collagen-binding integrins as critically involved in the regulation of AC remodeling and chondrocyte survival in response to ECM stimuli.

## Proteoglycans

Aggrecan is the most abundant proteoglycan of the articular cartilage and association of aggrecan gene (*AGC1*) polymorphism with various forms of OA has been demonstrated [90, 91]. More recently, a frameshift mutation in exon 12 of *AGC1* was identified in patients with autosomal dominant spondyloepiphyseal dysplasia type Kiberley (SEDK) characterized by mild short stature and early onset OA [92]. In the *cmd (cartilage matrix deficiency)* mice, a spontaneous mutation leads to a premature stop codon in exon 6 and to the absence of mature aggrecan in the matrix [93]. Homozygous mutants exhibit severe chondrodysplasia and die at birth due to respiratory failure. Heterozygous mice (*cmd/+*) survive and develop mild dwarfism and age-dependent spinal abnormalities [94]. In contrast to SEDK, no OA-like changes were reported in these mice [95]. A possible explanation for this discrepancy is that the SEDK *AGC1* mutation predicts the formation of a relatively large truncated protein, which may be retained in the endoplasmatic reticulum (ER), impairing chondrocyte function in the articular cartilage.

The small leucine-rich proteoglycans (SLRPs) play pivotal roles in connective tissues interacting with various matrix macromolecules and growth factors, thereby modulating both ECM organization and cellular metabolism [96]. Mice lacking SLRPs exhibit a broad range of phenotypes primarily affecting collagen fibrillogenesis in tissues rich in type I collagen such as skin, bone, tendon and cornea [96]. Several SLRPs are expressed in the articular cartilage including biglycan, fibromodulin and lumican. Mice double deficient in biglycan/fibromodulin [97] or lumican/fibromodulin [98] develop premature knee OA characterized by severe erosion of the articular cartilage at 3-6 months of age. The corresponding single knockout mice, except lumican-null, also exhibit OA which is milder and occurs later than in the double mutants [97-99]. The cause of OA pathology in these mice is unclear but it could be secondary to structural and functional defects in tendons and/or ligaments. Biglycan/fibromodulin double knockout mice also develop TM joint OA with significantly later onset and slower progression than knee OA [100]. Biglycan/fibromodulin double knockout mice also develop TM joint OA with significantly later onset and slower progression than knee OA [100]. The overt TM joint OA is preceded by reduced chondrocyte proliferation suggesting that misregulated cell growth could be an important factor in this type of OA. Knockout mouse models demonstrate that SLRPs are involved in the control of cell growth in various tissues via the modulation of TGFb/BMP availability [101], therefore it is possible that biglycan and/or fibromodulin deficiency impairs chondrocyte proliferation by disturbing growth factor signaling.

Perlecan (HSPG2) is a large secreted proteoglycan abundant in basement membranes (BMs) and cartilage. The levels of perlecan protein and mRNA were found to be increased in late stage OA samples from human knee joints which might suggest that perlecan is involved in repair processes activated around the defect areas [102]. The importance of perlecan in skeletogenesis is illustrated by the severe chondrodysplasia developed in human and mice lacking functional perlecan. Patients with dyssegmental dysplasia of the Silverman-Handmaker type (DDSH) and perlecan-null (*Hspg2*$^{-/-}$) mice are characterized by defective endochondral bone formation and embryonic/perinatal lethality [103]. In contrast, mutations reducing the level of perlecan lead to the non-lethal, autosomal-recessive Schartz-Jampel syndrome (SJS) characterized by osteochondrodysplasia and myotonia [104]. Recently a mouse model of SJS with altered *Hspg2* transcription and reduced perlecan matrix deposition was generated [105]. The hypomorphic mutants (C1532Yneo) mimic typical SJS and exhibit rough surface and clefts on the humeral head resembling OA.

**Table 1. Gene Modified Mouse Models Relevant in Osteoarthritis Research**

| Protein/Gene | Model | Articular Cartilage Phenotype | Ref. |
|---|---|---|---|
| ***Proteolytic Enzymes and Related Molecules*** | | | |
| ADAM15 | KO | Accelerated knee OA; modulation of integrin signaling? | [89] |
| ADAMTS-1 | KO | Normal susceptibility to experimentally induced arthritis | [119] |
| ADAMTS-4 | KO | Normal susceptibility to experimentally induced arthritis | [14-16] |
| ADAMTS-5 | KO | Protection from experimental arthritis; major aggrecanase in OA | [14, 16] |
| ADAMTS-4/-5 | KO | Similar to ADAMTS-5 KO, AC releases intact aggrecan | [18, 120] |
| Cathepsin K | TR | Joint destruction associated with sever bone defects | [36] |
| Cathepsin K | KO | Osteopetrosis, OA was not investigated | [37] |
| MMP2 | KO | Age-dependent AC destruction, bone defects | [31] |
| MMP3 | KO | Decreased susceptibility to spontaneous OA and inflammatory arthritis | [22-24] |
| | | Increased susceptibility to surgically-induced OA | [25] |
| MMP9 | KO | Spontaneous OA-like changes in young mice | [22] |
| MMP13 | TR | Accelerated OA, ectopic collagen X expression | [28] |
| MMP14 | KO | Skeletal defects including AC destruction, impaired MMP2 activation | [32, 33] |
| Runx2 (+/-) | KO | Amelioration of surgically induced OA, reduced MMP-13 expression | [29] |
| TIMP3 | KO | Mild OA, increased cleavage of aggrecan and collagen II | [34] |
| ***ECM Molecules and Receptors*** | | | |
| α1 integrin | KO | Accelerated OA, increased MMP2 and MMP3 expression | [88] |
| Aggrecan (*Cmd*/+) | KO | No reported OA-like changes | [95] |
| Aggrecan "Jaffa" | KI | Resistant to aggrecanase-mediated IGD cleavage | [19] |
| | | Partially protected from experimental arthritis | |
| Aggrecan "Chloe" | KI | Resistant to MMP-mediated IGD cleavage | [21] |
| | | Normal susceptibility to experimentally induced arthritis | [19] |
| Biglycan | KO | OA-like changes, ectopic tendon ossification | [97] |
| Fibromodulin | KO | Age-dependent OA-like changes, ectopic tendon ossification | [99] |
| Biglycan/Fibromodulin | dKO | Early onset OA, ectopic tendon ossification, accelerated TMJ OA | [97, 100] |
| Lumican | KO | OA was not reported | [98] |
| Lumican/Fibromodulin | dKO | Early onset OA, ectopic tendon ossification | [98] |
| Collagen II, α1 (+/-) | KO | Higher prevalence of OA in aging mice, softer AC | [77, 78] |
| Collagen II, α1 (*dmm*/+) | NO | Early-onset OA, AC thinning, increased cell density, reduced matrix | [76] |
| Collagen II, α1 (Del/+) | TR | Early-onset OA, up-regulation of cathepsin K | [75] |
| Collagen IX, α1 | KO | Early onset OA, up-regulation of DDR2 and MMP13 expression | [3, 82, 83] |
| Collagen IX, α1 | TR | Early onset OA | [2] |
| Collagen XI, α1 (*cho*/+) | NO | Early onset OA, up-regulation of DDR2 and MMP13 expression | [80-82] |
| Collagen XI, α2 | KO | Mild abnormalities of the AC, no obvious OA | [121] |
| Proteoglycan 4 | KO | Surface changes followed by AC deterioration | [108] |
| Matrilin-3 | KO | No obvious OA in one study; high OA prevalence at 12 months in | [113] |
| | KO | another study | [118] |
| Matrilin-1/Matrilin-3 | dKO | Similar incidence of OA in controls and double mutants at 14 months | [117] |
| Perlecan | KO | OA-like changes on the femoral head | [105] |
| ***Cytokines and Growth Factors*** | | | |
| Growth hormone | TR | OA-like changes of the AC, osteophyte formation | [49, 50] |
| BMPR1A | cKO | Progressive AC degradation | [60] |
| Fgfr3 | KO | Premature OA, increased MMP13 expression | [64] |
| IL-1β | KO | Acceleration of surgically induced OA | [25] |
| IL-6 | KO | Age-dependent OA and subchondral sclerosis in males | [45] |
| ICE | KO | Acceleration of surgically induced OA | [25] |
| Ltbp-3 | KO | Progressive AC degradation, abnormal chondrocyte hypertrophy | [58] |
| Mig-6 | KO | Early-onset OA, osteophyte formation | [66] |
| iNOS | KO | Acceleration of surgically induced OA | [25] |
| Smad3 | KO | Progressive OA, osteophyte formation, increased Col10 expression | [55] |
| TGFβRII | TR | Progressive OA, osteophyte formation, increased Col10 expression | [54] |

Note: KO, knock-out; dKO, double knock-out; cKO, conditional knock-out; KI, knock-in; NO, naturally occurring; TG, transgenic.

Proteoglycan 4 (PRG4) is highly abundant in the synovial fluid and on the surface of AC, and is secreted by both superficial chondrocytes and synoviocytes. Mutations in *PRG4* lead to CACP syndrome characterized by precocious joint failure demonstrating the essential role of PRG4 in maintaining joint integrity [106]. PRG4 levels decrease at early OA stages in surgically induced degenerative AC animal models [107] implicating PRG4 in OA pathology. Mice

lacking proteoglycan 4 (*Prg4*$^{-/-}$) show age-dependent synovial hyperplasia, irregular AC surface with protein deposits, loss of the flattened layer of superficial zone chondrocytes and subsequent AC deterioration [108]. In addition, the mice exhibit abnormal calcification of tendons in the ankle joint. Collectively, these data suggest a protective role for proteoglycan 4 on AC and provide a novel therapeutic target for treatment of OA.

### Glycoproteins Modulating ECM Structure and Function

In hyaline cartilage the collagen-aggrecan networks are interconnected *via* molecular associates containing SLRPs and non-collagenous glycoproteins such as cartilage oligomeric matrix protein (COMP) and the members of the matrilin family [109]. COMP is abundant in both the developing growth plate and the articular cartilage, and mutations in the COMP gene results in MED and pseudoachondrodysplasia [110]. Matrilins, especially matrilin-1 and -3, are highly expressed during cartilage development and matrilin-3 mutations are linked to various skeletal disorders including a distinct group of MED [109]. COMP, matrilin-1 and matrilin-3 levels are upregulated in articular cartilage and/or the synovial fluid during joint diseases; they therefore may serve as markers for disturbed cartilage metabolism. Mice lacking these proteins do not develop chondrodysplasia implying a dominant negative pathomechanism behind the human disorders [111-113]. Similarly, no signs of OA were observed in COMP- and matrilin-1-deficient mice [111, 112]. The role of matrilin-3 in articular cartilage function is more controversial. In humans, the association of matrilin-3 polymorphisms with hand osteoarthritis but not with knee osteoarthritis was demonstrated [114-116]. Matrilin-3-deficient single and double knockout mice (*Matn3*$^{-/-}$ and *Matn1*$^{-/-}$*/Matn3*$^{-/-}$) generated in our laboratory showed no evidence for higher incidence of OA-like changes in the knee joints compared with control mice up to 14 months of age [113, 117]. In contrast, a higher prevalence of severe knee OA at one year of age was reported in a second matrilin-3-deficient mouse line suggesting that matrilin-3 may protect articular cartilage from degradation [118]. The discrepancy between these works might be explained by background or gender specific differences of the investigated mice and call for further, more detailed studies.

## CONCLUSION

During the last decade, the number of genetically modified mice with OA-like changes has dramatically expanded. These mouse models are particularly effective to elucidate the molecular basis of articular cartilage homeostasis under both normal and pathological conditions. The analysis of the phenotypic consequences of gene modifications in transgenic, knockout and knock-in mice has yielded a wealth of information about the complex functional interactions among cytokines, growth factors and ECM structural proteins of the joints and has provided valuable insight to the pathogenesis of cartilage degeneration in osteoarthritis. The genetic mouse models not only help to dissect signal transduction pathways that regulate the catabolic/anabolic balance in AC, they are also useful in identifying OA-candidate genes and to validate potential target molecules to treat or prevent OA. There is no doubt that gene targeting in mice will continue to be an important tool of OA research in the future.

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## REFERENCES

[1] Buckwalter JA, Martin JA. Osteoarthritis. Adv Drug Deliv Rev 2006; 58: 150-67.

[2] Nakata K, Ono K, Miyazaki J, *et al*. Osteoarthritis associated with mild chondrodysplasia in transgenic mice expressing alpha 1(IX) collagen chains with a central deletion. Proc Natl Acad Sci USA 1993; 90: 2870-4.

[3] Fassler R, Schnegelsberg PN, Dausman J, *et al*. Mice lacking alpha 1 (IX) collagen develop noninflammatory degenerative joint disease. Proc Natl Acad Sci USA 1994; 91: 5070-4.

[4] Murphy G, Knauper V, Atkinson S, *et al*. Matrix metalloproteinases in arthritic disease. Arthritis Res 2002; 4 Suppl 3: S39-49.

[5] Mott JD, Werb Z. Regulation of matrix biology by matrix metalloproteinases. Curr Opin Cell Biol 2004; 16: 558-64.

[6] Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodelling. Nat Rev Mol Cell Biol 2007; 8: 221-33.

[7] Jones GC, Riley GP. ADAMTS proteinases: a multi-domain, multifunctional family with roles in extracellular matrix turnover and arthritis. Arthritis Res Ther 2005; 7: 160-9.

[8] Baker AH, Edwards DR, Murphy G. Metalloproteinase inhibitors: biological actions and therapeutic opportunities. J Cell Sci 2002; 115: 3719-27.

[9] Kevorkian L, Young DA, Darrah C, *et al*. Expression profiling of metalloproteinases and their inhibitors in cartilage. Arthritis Rheum 2004; 50: 131-41.

[10] Davidson RK, Waters JG, Kevorkian L, *et al*. Expression profiling of metalloproteinases and their inhibitors in synovium and cartilage. Arthritis Res Ther 2006; 8: R124.

[11] Yasuda Y, Kaleta J, Bromme D. The role of cathepsins in osteoporosis and arthritis: rationale for the design of new therapeutics. Adv Drug Deliv Rev 2005; 57: 973-93.

[12] 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 Cartilage 2006; 14: 101-13.

[13] Malfait AM, Liu RQ, Ijiri K, Komiya S, Tortorella MD. Inhibition of ADAM-TS4 and ADAM-TS5 prevents aggrecan degradation in osteoarthritic cartilage. J Biol Chem 2002; 277: 22201-8.

[14] Glasson SS, Askew R, Sheppard B, *et al*. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. Nature 2005; 434: 644-8.

[15] Glasson SS, Askew R, Sheppard B, *et al*. Characterization of and osteoarthritis susceptibility in ADAMTS-4-knockout mice. Arthritis Rheum 2004; 50: 2547-58.

[16] Stanton H, Rogerson FM, East CJ, *et al*. ADAMTS5 is the major aggrecanase in mouse cartilage *in vivo* and *in vitro*. Nature 2005; 434: 648-52.

[17] East CJ, Stanton H, Golub SB, Rogerson FM, Fosang AJ. ADAMTS-5 deficiency does not block aggrecanolysis at preferred cleavage sites in the the chondroitin sulfate-rich region of aggrecan. J Biol Chem 2007; 282(12): 8632-40. Epub 2007 Jan 25.

[18] Ilic MZ, East CJ, Rogerson FM, Fosang AJ, Handley CJ. Distinguishing aggrecan loss from aggrecan proteolysis in ADAMTS-4 and ADAMTS-5 single and double deficient mice. J Biol Chem 2007; 282: 37420-8.

[19] Little CB, Meeker CT, Golub SB, *et al*. Blocking aggrecanase cleavage in the aggrecan interglobular domain abrogates cartilage erosion and promotes cartilage repair. J Clin Invest 2007; 117: 1627-36.

[20] Fosang AJ, Last K, Stanton H, *et al*. Generation and novel distribution of matrix metalloproteinase-derived aggrecan fragments in porcine cartilage explants. J Biol Chem 2000; 275: 33027-37.

[21] Little CB, Meeker CT, Hembry RM, *et al*. Matrix metalloproteinases are not essential for aggrecan turnover during normal skeletal growth and development. Mol Cell Biol 2005; 25: 3388-99.

[22] Blom AB, van Lent PL, Libregts S, *et al*. Crucial role of macrophages in matrix metalloproteinase-mediated cartilage destruction

during experimental osteoarthritis: involvement of matrix metalloproteinase 3. Arthritis Rheum 2007; 56: 147-57.

[23] van Meurs J, van Lent P, Holthuysen A, *et al*. Active matrix metalloproteinases are present in cartilage during immune complex-mediated arthritis: a pivotal role for stromelysin-1 in cartilage destruction. J Immunol 1999; 163: 5633-9.

[24] van Meurs J, van Lent P, Stoop R, *et al*. Cleavage of aggrecan at the Asn341-Phe342 site coincides with the initiation of collagen damage in murine antigen-induced arthritis: a pivotal role for stromelysin 1 in matrix metalloproteinase activity. Arthritis Rheum 1999; 42: 2074-84.

[25] Clements KM, Price JS, Chambers MG, *et al*. Gene deletion of either interleukin-1beta, interleukin-1beta-converting enzyme, inducible nitric oxide synthase, or stromelysin 1 accelerates the development of knee osteoarthritis in mice after surgical transection of the medial collateral ligament and partial medial meniscectomy. Arthritis Rheum 2003; 48: 3452-63.

[26] Fichter M, Korner U, Schomburg J, *et al*. Collagen degradation products modulate matrix metalloproteinase expression in cultured articular chondrocytes. J Orthop Res 2006; 24: 63-70.

[27] Yasuda T, Tchetina E, Ohsawa K, *et al*. Peptides of type II collagen can induce the cleavage of type II collagen and aggrecan in articular cartilage. Matrix Biol 2006; 25: 419-29.

[28] Neuhold LA, Killar L, Zhao W, *et al*. Postnatal expression in hyaline cartilage of constitutively active human collagenase-3 (MMP-13) induces osteoarthritis in mice. J Clin Invest 2001; 107: 35-44.

[29] Kamekura S, Kawasaki Y, Hoshi K, *et al*. Contribution of runt-related transcription factor 2 to the pathogenesis of osteoarthritis in mice after induction of knee joint instability. Arthritis Rheum 2006; 54: 2462-70.

[30] Stickens D, Behonick DJ, Ortega N, *et al*. Altered endochondral bone development in matrix metalloproteinase 13-deficient mice. Development 2004; 131: 5883-95.

[31] Mosig RA, Dowling O, DiFeo A, *et al*. Loss of MMP-2 disrupts skeletal and craniofacial development and results in decreased bone mineralization, joint erosion and defects in osteoblast and osteoclast growth. Hum Mol Genet 2007; 16: 1113-23.

[32] Holmbeck K, Bianco P, Caterina J, *et al*. MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. Cell 1999; 99: 81-92.

[33] Zhou Z, Apte SS, Soininen R, *et al*. Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase I. Proc Natl Acad Sci USA 2000; 97: 4052-7.

[34] Sahebjam S, Khokha R, Mort JS. Increased collagen and aggrecan degradation with age in the joints of Timp3(-/-) mice. Arthritis Rheum 2007; 56: 905-9.

[35] Morko JP, Soderstrom M, Saamanen AM, Salminen HJ, Vuorio EI. Up regulation of cathepsin K expression in articular chondrocytes in a transgenic mouse model for osteoarthritis. Ann Rheum Dis 2004; 63: 649-55.

[36] Morko J, Kiviranta R, Joronen K, *et al*. Spontaneous development of synovitis and cartilage degeneration in transgenic mice overexpressing cathepsin K. Arthritis Rheum 2005; 52: 3713-7.

[37] Saftig P, Hunziker E, Wehmeyer O, *et al*. Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice. Proc Natl Acad Sci USA 1998; 95: 13453-8.

[38] Kiviranta R, Morko J, Alatalo SL, *et al*. Impaired bone resorption in cathepsin K-deficient mice is partially compensated for by enhanced osteoclastogenesis and increased expression of other proteases *via* an increased RANKL/OPG ratio. Bone 2005; 36: 159-72.

[39] Sondergaard BC, Henriksen K, Wulf H, *et al*. Relative contribution of matrix metalloprotease and cysteine protease activities to cytokine-stimulated articular cartilage degradation. Osteoarthritis Cartilage 2006; 14: 738-48.

[40] Goldring SR, Goldring MB. The role of cytokines in cartilage matrix degeneration in osteoarthritis. Clin Orthop Relat Res 2004; S27-36.

[41] Goldring MB. Update on the biology of the chondrocyte and new approaches to treating cartilage diseases. Best Pract Res Clin Rheumatol 2006; 20: 1003-25.

[42] Aigner T, Soeder S, Haag J. IL-1beta and BMPs--interactive players of cartilage matrix degradation and regeneration. Eur Cell Mater 2006; 12: 49-56.

[43] Fukui N, Zhu Y, Maloney WJ, Clohisy J, Sandell LJ. Stimulation of BMP-2 expression by pro-inflammatory cytokines IL-1 and TNF-alpha in normal and osteoarthritic chondrocytes. J Bone Joint Surg Am 2003; (85-A Suppl 3): 59-66.

[44] Fischer DC, Siebertz B, van de Leur E, *et al*. Induction of alpha1-antitrypsin synthesis in human articular chondrocytes by interleukin-6-type cytokines: evidence for a local acute-phase response in the joint. Arthritis Rheum 1999; 42: 1936-45.

[45] De Hooge AS, van de Loo FA, Bennink MB, *et al*. Male IL-6 gene knock out mice developed more advanced osteoarthritis upon aging. Osteoarthritis Cartilage 2005; 13: 66-73.

[46] Mahr S, Menard J, Krenn V, Muller B. Sexual dimorphism in the osteoarthritis of STR/ort mice may be linked to articular cytokines. Ann Rheum Dis 2003; 62: 1234-7.

[47] Goldring MB, Tsuchimochi K, Ijiri K. The control of chondrogenesis. J Cell Biochem 2006; 97: 33-44.

[48] Blaney Davidson EN, van der Kraan PM, van den Berg WB. TGF-beta and osteoarthritis. Osteoarthritis Cartilage 2007; 15: 597-604.

[49] Ogueta S, Olazabal I, Santos I, Delgado-Baeza E, Garcia-Ruiz JP. Transgenic mice expressing bovine GH develop arthritic disorder and self-antibodies. J Endocrinol 2000; 165: 321-8.

[50] Fernandez-Criado C, Martos-Rodriguez A, Santos-Alvarez I, Garcia-Ruiz JP, Delgado-Baeza E. The fate of chondrocyte in osteoarthritic cartilage of transgenic mice expressing bovine GH. Osteoarthritis Cartilage 2004; 12: 543-51.

[51] Redini F, Galera P, Mauviel A, Loyau G, Pujol JP. Transforming growth factor beta stimulates collagen and glycosaminoglycan biosynthesis in cultured rabbit articular chondrocytes. FEBS Lett 1988; 234: 172-6.

[52] 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 Cartilage 2005; 13: 426-38.

[53] Van Beuningen HM, Glansbeek HL, van der Kraan PM, van den Berg WB. Osteoarthritis-like changes in the murine knee joint resulting from intra-articular transforming growth factor-beta injections. Osteoarthritis Cartilage 2000; 8: 25-33.

[54] Serra R, Johnson M, Filvaroff EH, *et al*. Expression of a truncated, kinase-defective TGF-beta type II receptor in mouse skeletal tissue promotes terminal chondrocyte differentiation and osteoarthritis. J Cell Biol 1997; 139: 541-52.

[55] Yang X, Chen L, Xu X, *et al*. TGF-beta/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. J Cell Biol 2001; 153: 35-46.

[56] Wang H, Zhang J, Sun Q, Yang X. Altered gene expression in articular chondrocytes of Smad3ex8/ex8 mice, revealed by gene profiling using microarrays. J Genet Genomics 2007; 34: 698-708.

[57] Todorovic V, Jurukovski V, Chen Y, *et al*. Latent TGF-beta binding proteins. Int J Biochem Cell Biol 2005; 37: 38-41.

[58] Dabovic B, Chen Y, Colarossi C, *et al*. Bone abnormalities in latent TGF-[beta] binding protein (Ltbp)-3-null mice indicate a role for Ltbp-3 in modulating TGF-[beta] bioavailability. J Cell Biol 2002; 156: 227-32.

[59] Kishigami S, Mishina Y. BMP signaling and early embryonic patterning. Cytokine Growth Factor Rev 2005; 16: 265-78.

[60] Rountree RB, Schoor M, Chen H, *et al*. BMP receptor signaling is required for postnatal maintenance of articular cartilage. PLoS Biol 2004; 2: e355.

[61] Ornitz DM, Marie PJ. FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease. Genes Dev 2002; 16: 1446-65.

[62] Colvin JS, Bohne BA, Harding GW, McEwen DG, Ornitz DM. Skeletal overgrowth and deafness in mice lacking fibroblast growth factor receptor 3. Nat Genet 1996; 12: 390-7.

[63] Valverde-Franco G, Liu H, Davidson D, *et al*. Defective bone mineralization and osteopenia in young adult FGFR3-/- mice. Hum Mol Genet 2004; 13: 271-84.

[64] Valverde-Franco G, Binette JS, Li W, *et al*. Defects in articular cartilage metabolism and early arthritis in fibroblast growth factor receptor 3 deficient mice. Hum Mol Genet 2006; 15: 1783-92.

[65] Zhang YW, Vande Woude GF. Mig-6, signal transduction, stress response and cancer. Cell Cycle 2007; 6: 507-13.

[66] Zhang YW, Su Y, Lanning N, *et al*. Targeted disruption of Mig-6 in the mouse genome leads to early onset degenerative joint disease. Proc Natl Acad Sci USA 2005; 102: 11740-5.

[67] Poole AR, Kojima T, Yasuda T, *et al*. Composition and structure of articular cartilage: a template for tissue repair. Clin Orthop Relat Res 2001; (391 Suppl): S26-33.

[68] 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 Rheum 2002; 46: 2368-76.

[69] Loeser RF, Forsyth CB, Samarel AM, Im HJ. Fibronectin fragment activation of proline-rich tyrosine kinase PYK2 mediates integrin signals regulating collagenase-3 expression by human chondrocytes through a protein kinase C-dependent pathway. J Biol Chem 2003; 278: 24577-85.

[70] Xu L, Peng H, Glasson S, *et al*. Increased expression of the collagen receptor discoidin domain receptor 2 in articular cartilage as a key event in the pathogenesis of osteoarthritis. Arthritis Rheum 2007; 56: 2663-73.

[71] Sunk IG, Bobacz K, Hofstaetter JG, *et al*. Increased expression of discoidin domain receptor 2 is linked to the degree of cartilage damage in human knee joints: a potential role in osteoarthritis pathogenesis. Arthritis Rheum 2007; 56: 3685-92.

[72] Reginato AM, Olsen BR. The role of structural genes in the pathogenesis of osteoarthritic disorders. Arthritis Res 2002; 4: 337-45.

[73] Snead MP, Yates JR. Clinical and Molecular genetics of Stickler syndrome. J Med Genet 1999; 36: 353-9.

[74] Aszodi A, Pfeifer A, Wendel M, Hiripi L, Fassler R. Mouse models for extracellular matrix diseases. J Mol Med 1998; 76: 238-52.

[75] Saamanen AK, Salminen HJ, Dean PB, *et al*. Osteoarthritis-like lesions in transgenic mice harboring a small deletion mutation in type II collagen gene. Osteoarthritis Cartilage 2000; 8: 248-57.

[76] Bomsta BD, Bridgewater LC, Seegmiller RE. Premature osteoarthritis in the Disproportionate micromelia (Dmm) mouse. Osteoarthritis Cartilage 2006; 14: 477-85.

[77] Lapvetelainen T, Hyttinen M, Lindblom J, *et al*. More knee joint osteoarthritis (OA) in mice after inactivation of one allele of type II procollagen gene but less OA after lifelong voluntary wheel running exercise. Osteoarthritis Cartilage 2001; 9: 152-60.

[78] Hyttinen MM, Toyras J, Lapvetelainen T, *et al*. Inactivation of one allele of the type II collagen gene alters the collagen network in murine articular cartilage and makes cartilage softer. Ann Rheum Dis 2001; 60: 262-8.

[79] Li Y, Lacerda DA, Warman ML, *et al*. A fibrillar collagen gene, Col11a1, is essential for skeletal morphogenesis. Cell 1995; 80: 423-30.

[80] Xu L, Flahiff CM, Waldman BA, *et al*. Osteoarthritis-like changes and decreased mechanical function of articular cartilage in the joints of mice with the chondrodysplasia gene (cho). Arthritis Rheum 2003; 48: 2509-18.

[81] Xu L, Peng H, Wu D, *et al*. Activation of the discoidin domain receptor 2 induces expression of matrix metalloproteinase 13 associated with osteoarthritis in mice. J Biol Chem 2005; 280: 548-55.

[82] Lam NP, Li Y, Waldman AB, *et al*. Age-dependent increase of discoidin domain receptor 2 and matrix metalloproteinase 13 expression in temporomandibular joint cartilage of type IX and type XI collagen-deficient mice. Arch Oral Biol 2007; 52: 579-84.

[83] Hu K, Xu L, Cao L, *et al*. Pathogenesis of osteoarthritis-like changes in the joints of mice deficient in type IX collagen. Arthritis Rheum 2006; 54: 2891-900.

[84] Li Y, Xu L, Olsen BR. Lessons from genetic forms of osteoarthritis for the pathogenesis of the disease. Osteoarthritis Cartilage 2007; 15: 1101-5.

[85] Loeser RF. Integrins and cell signaling in chondrocytes. Biorheology 2002; 39: 119-24.

[86] Bouvard D, Brakebusch C, Gustafsson E, *et al*. Functional consequences of integrin gene mutations in mice. Circ Res 2001; 89: 211-23.

[87] Aszodi A, Hunziker EB, Brakebusch C, Fassler R. Beta1 integrins regulate chondrocyte rotation, G1 progression, and cytokinesis. Genes Dev 2003; 17: 2465-79.

[88] Zemmyo M, Meharra EJ, Kuhn K, Creighton-Achermann L, Lotz M. Accelerated, aging-dependent development of osteoarthritis in alpha1 integrin-deficient mice. Arthritis Rheum 2003; 48: 2873-80.

[89] Bohm BB, Aigner T, Roy B, *et al*. Homeostatic effects of the metalloproteinase disintegrin ADAM15 in degenerative cartilage remodeling. Arthritis Rheum 2005; 52: 1100-9.

[90] Horton WE, Jr., Lethbridge-Cejku M, Hochberg MC, *et al*. An association between an aggrecan polymorphic allele and bilateral hand osteoarthritis in elderly white men: data from the Baltimore Longitudinal Study of Aging (BLSA). Osteoarthritis Cartilage 1998; 6: 245-51.

[91] Kirk KM, Doege KJ, Hecht J, Bellamy N, Martin NG. Osteoarthritis of the hands, hips and knees in an Australian twin sample-evidence of association with the aggrecan VNTR polymorphism. Twin Res 2003; 6: 62-6.

[92] Gleghorn L, Ramesar R, Beighton P, Wallis G. A mutation in the variable repeat region of the aggrecan gene (AGC1) causes a form of spondyloepiphyseal dysplasia associated with severe, premature osteoarthritis. Am J Hum Genet 2005; 77: 484-90.

[93] Watanabe H, Kimata K, Line S, *et al*. Mouse cartilage matrix deficiency (cmd) caused by a 7 bp deletion in the aggrecan gene. Nat Genet 1994; 7: 154-7.

[94] Watanabe H, Nakata K, Kimata K, Nakanishi I, Yamada Y. Dwarfism and age-associated spinal degeneration of heterozygote cmd mice defective in aggrecan. Proc Natl Acad Sci USA 1997; 94: 6943-7.

[95] Watanabe H, Yamada Y. Chondrodysplasia of gene knockout mice for aggrecan and link protein. Glycoconj J 2002; 19: 269-73.

[96] Ameye L, Young MF. Mice deficient in small leucine-rich proteoglycans: novel *in vivo* models for osteoporosis, osteoarthritis, Ehlers-Danlos syndrome, muscular dystrophy, and corneal diseases. Glycobiology 2002; 12: 107R-16R.

[97] Ameye L, Aria D, Jepsen K, *et al*. Abnormal collagen fibrils in tendons of biglycan/fibromodulin-deficient mice lead to gait impairment, ectopic ossification, and osteoarthritis. FASEB J 2002; 16: 673-80.

[98] Jepsen KJ, Wu F, Peragallo JH, *et al*. A syndrome of joint laxity and impaired tendon integrity in lumican- and fibromodulin-deficient mice. J Biol Chem 2002; 277: 35532-40.

[99] Gill MR, Oldberg A, Reinholt FP. Fibromodulin-null murine knee joints display increased incidences of osteoarthritis and alterations in tissue biochemistry. Osteoarthritis Cartilage 2002; 10: 751-7.

[100] Wadhwa S, Embree MC, Kilts T, Young MF, Ameye LG. Accelerated osteoarthritis in the temporomandibular joint of biglycan/fibromodulin double-deficient mice. Osteoarthritis Cartilage 2005; 13: 817-27.

[101] Aszodi A, Legate KR, NakchbandiI, Fassler R. What mouse mutants teach us about extracellular matrix function. Annu Rev Cell Dev Biol 2006; 22: 591-621.

[102] Tesche F, Miosge N. Perlecan in late stages of osteoarthritis of the human knee joint. Osteoarthritis Cartilage 2004; 12: 852-62.

[103] Costell M, Gustafsson E, Aszodi A, *et al*. Perlecan maintains the integrity of cartilage and some basement membranes. J Cell Biol 1999; 147: 1109-22.

[104] Nicole S, Davoine CS, Topaloglu H, *et al*. Perlecan, the major proteoglycan of basement membranes, is altered in patients with Schwartz-Jampel syndrome (chondrodystrophic myotonia). Nat Genet 2000; 26: 480-3.

[105] Rodgers KD, Sasaki T, Aszodi A, Jacenko O. Reduced perlecan in mice results in chondrodysplasia resembling Schwartz-Jampel syndrome. Hum Mol Genet 2007; 16: 515-28.

[106] Marcelino J, Carpten JD, Suwairi WM, *et al*. CACP, encoding a secreted proteoglycan, is mutated in camptodactyly-arthropathy-coxa vara-pericarditis syndrome. Nat Genet 1999; 23: 319-22.

[107] Elsaid KA, Jay GD, Warman ML, Rhee DK, Chichester CO. Association of articular cartilage degradation and loss of boundary-lubricating ability of synovial fluid following injury and inflammatory arthritis. Arthritis Rheum 2005; 52: 1746-55.

[108] Rhee DK, Marcelino J, Baker M, *et al*. The secreted glycoprotein lubricin protects cartilage surfaces and inhibits synovial cell overgrowth. J Clin Invest 2005; 115: 622-31.

[109] Wagener R, Ehlen HW, Ko YP, *et al*. The matrilins-adaptor proteins in the extracellular matrix. FEBS Lett 2005; 579: 3323-9.

[110] Briggs MD, Chapman KL. Pseudoachondroplasia and multiple epiphyseal dysplasia: mutation review, molecular interactions, and genotype to phenotype correlations. Hum Mutat 2002; 19: 465-78.

[111] Svensson L, Aszodi A, Heinegard D, *et al*. Cartilage oligomeric matrix protein-deficient mice have normal skeletal development. Mol Cell Biol 2002; 22: 4366-71.

[112] Aszodi A, Bateman JF, Hirsch E, *et al*. Normal skeletal development of mice lacking matrilin 1: redundant function of matrilins in cartilage? Mol Cell Biol 1999; 19: 7841-5.

[113] Ko Y, Kobbe B, Nicolae C, *et al*. Matrilin-3 is dispensable for mouse skeletal growth and development. Mol Cell Biol 2004; 24: 1691-9.

[114] Stefansson SE, Jonsson H, Ingvarsson T, *et al*. Genomewide scan for hand osteoarthritis: a novel mutation in matrilin-3. Am J Hum Genet 2003; 72: 1448-59.

[115] Min JL, Meulenbelt I, Riyazi N, *et al*. Association of matrilin-3 polymorphisms with spinal disc degeneration and osteoarthritis of the first carpometacarpal joint of the hand. Ann Rheum Dis 2006; 65: 1060-6.

[116] Pullig O, Tagariello A, Schweizer A, *et al*. MATN3 (matrilin-3) sequence variation (pT303M) is a risk factor for osteoarthritis of the CMC1 joint of the hand, but not for knee osteoarthritis. Ann Rheum Dis 2007; 66: 279-80.

[117] Nicolae C, Ko YP, Miosge N, *et al*. Abnormal collagen fibrils in cartilage of matrilin-1/matrilin-3-deficient mice. J Biol Chem 2007; 282: 22163-75.

[118] van der Weyden L, Wei L, Luo J, *et al*. Functional knockout of the matrilin-3 gene causes premature chondrocyte maturation to hypertrophy and increases bone mineral density and osteoarthritis. Am J Pathol 2006; 169: 515-27.

[119] Little CB, Mittaz L, Belluoccio D, *et al*. ADAMTS-1-knockout mice do not exhibit abnormalities in aggrecan turnover in vitro and in vivo. Arthritis Rheum 2005; 52: 1461-72.