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Review

Protective effects of the pericellular matrix of chondrocyte on articular cartilage against the development of osteoarthritis

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Summary. Understanding the pathogenesis of osteoarthritis (OA) provides invaluable information in the search of therapeutic targets for the development of disease-modifying OA drugs. Emerging results from investigations demonstrate that the pericellular matrix of chondrocytes plays important roles in protecting articular cartilages from being degraded. Thus, maintaining the structural integrity of the pericellular matrix may be an effective approach to prevent the development of osteoarthritic joints. In this review article, we discuss the consequences of lacking one or more components of the pericellular matrix, and biological effects of the destruction of the pericellular matrix in the development of OA. We believe that more attention should be directed towards the pericellular matrix for the identification of novel biomarkers and therapeutic targets for the prevention and treatment of OA.

Key words: Osteoarthritis, Articular cartilage, Chondron, Pericellular matrix, Animal model

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Introduction

Osteoarthritis (OA) is considered a whole joint disease that involves articular cartilage, subchondral bone, synovial membrane, menisci and ligaments (Loeser et al., 2012). It has been suggested that OA is caused by genetic, biochemical and biomechanical factors. One of the pathological hallmarks in OA is the destruction of articular cartilage. The main cellular events underlying cartilage destruction are type II collagen degradation and loss of proteoglycans, resulting from an imbalance between anabolic and catabolic activities in joints. The immediate pericellular environment of chondrocytes appears to have a critical role in regulating chondrocyte activities. Chondrocytes together with a pericellular environment (matrix) are referred to as chondrons. Much effort has been made to understand the biological and biomechanical functions of the pericellular matrix (PCM). In this review article, we discuss important roles of the PCM in maintaining the homeostasis of articular cartilages and in the development of OA.

Anatomical structure of chondrons

Articular cartilage consists of chondrocytes and an expansive extracellular matrix (ECM), which includes pericellular, territorial and interterritorial matrices. The concept of a chondron has been introduced to

specifically refer to the chondrocyte together with its surrounding. It was originally suggested that chondrons were the primary structural and functional units of articular cartilage (Poole et al., 1988, 1990). Later, a research group completed additional experiments to physically isolate chondrons from cartilage. Results from their study show that chondrons were the primary functional and metabolic unit of articular cartilage (Hunziker et al., 1997; Poole, 1997).

The PCM contains components such as laminin, fibronectin, biglycan, decorin, fibromodulin, matrilins 1 and 3, cartilage oligo matrix protein (COMP), and type VI and IX collagens (Roughley, 2001; Guilak et al., 2006). The pericellular capsule is mostly composed of collagens type VI and IX, and proteoglycans (Li and Xu, 2015). The PCM separates chondrocytes from the adjacent interterritorial or territorial matrices. One of the characteristics in the pericellular matrix is that it lacks type II collagen fibrils in the matrix. Thus, under normal conditions, type II collagen fibrils are not exposed to chondrocytes. It is conceivable that the disruption or disappearance of the PCM would enhance the exposure of chondrocytes to type II collagen fibrils. This, in turn, should elicit the interaction of collagen type II fibrils with cell surface receptors, which leads to the down- or up-regulation of intracellular signaling pathways and the alteration of chondrocyte metabolism (Xu et al., 2011). In fact, Poole's group demonstrates that the structure of the pericellular matrix of chondrocytes is disrupted in human OA cartilage (Poole et al., 1991).

Deletions of the pericellular components leading to early onset osteoarthritis

Results from human and mouse genetic studies indicate the significant roles the components in the PCM play in protecting articular cartilage against the development of OA (Schminke et al., 2016). For example, several clinical studies report that mutations in the genes that encode type IX collagen (*COL9A1, COL9A2* and *COL9A3*) cause multiple epiphyseal dysplasia (MED), which is characterized by mild short stature and early onset OA (Bonnemann et al., 2000; Mustafa et al., 2000; Czarny-Ratajczak et al., 2001; Jackson et al., 2010). Results from mouse genetic studies demonstrate that the deletion of *Col9a1* results in age-dependent OA-like changes in knee and temporomandibular joints (TMJ), characterized by an increase in the degradation of collagen type II and proteoglycans and an increased expression of matrix metalloproteinase 13 (Mmp-13) and discoidin domain receptor 2 (Ddr2) (Hu et al., 2006). Additionally, changes in cartilage mechanical properties have been observed in the femoral and tibial plateaus of *Col9a1*$^{-/-}$ mice. This suggests that the lack of collagen type IX results in early onset OA.

Collagen type VI, primarily in the PCM, has been reported to play a protective role for chondrocytes by maintaining the pericellular microenvironment in OA (Horikawa et al., 2004; Owida et al., 2017). Loss of pericellular type VI collagen has been observed to be associated with abnormal human chondrocytes in non-degenerated cartilage, demonstrating that the lack of collagen type VI could lead to cartilage degeneration (Murray et al., 2010). A study reports that the genetic deletion of collagen type VI in mice alters the mechanical environment surrounding chondrocytes during joint loading, which eventually leads to the development of OA (Alexopoulos et al., 2009; Zelenski et al., 2015).

In humans, mutations in matrilin-3 cause MED and Spondyloepimetaphyseal Dysplasia (SEMD) (Chapman et al., 2001; Borochowitz et al., 2004; Otten et al., 2010). Matrilin-3-deficienct mice reveal early onset OA-like changes in knee joints, including premature hypertrophic chondrocytes, chondrocyte clustering, fibrillation and osteophyte formation (Ko et al., 2004; van der Weyden et al., 2006). In matrilin-3 knockout mice, the level of collagen type II and aggrecan are reduced compared to that in wild-type littermates. This indicates that matrilin-3 plays an important role in maintaining the structural integrity of articular cartilage. It has also been reported that the expression level of matrilin-3 is increased in OA and is correlated with disease severity. Matrilin-3 induces osteoarthritis-associated markers in chondrocytes in a concentration-dependent manner (Muttigi et al., 2016). The function of matrilin-3 shifts from an anabolic to a catabolic effect in articular cartilage. At the concentration of <10 μg/mL, soluble matrilin-3 treatment induces the expression of collagen type II and inhibits the expression of MMP-13. On the other hand, at the concentration of ≥ 20 μg/mL, matrilin-3 increases the expression of MMP13 (Vincourt et al., 2012). A very recent study reports that the genetic deletion of matrilin-1, another component in the PCM, results in severe articular cartilage lesions, induced by destabilization of medial meniscus (DMM), in mouse knee joints (Chen et al., 2016).

Mutations in cartilage oligomeric matrix protein (COMP) are associated with MED. In a case-control study, the serum level of COMP is significantly higher in OA patients than in control individuals (Verma and Dalal, 2013). In an animal model study using rabbits, it has been shown that the elevated levels of COMP and MMP-3 in serum and joint fluid are correlated with OA severity (Chu et al., 2015). Similarly, mutations in Comp result in chondrodysplasia in mice (Pirog-Garcia et al., 2007; Suleman et al., 2012).

The genetic deletion of decorin, biglycan and fibromodulin are also associated with early onset OA in TMJ of mice (Bock et al., 2001; Alexopoulos et al., 2005b; Wadhwa et al., 2005). The deficiency of biglycan and fibromodulin increases the apoptosis of chondrocytes. This, in turn, leads to the premature erosion and degradation of condylar cartilage in TMJ. The data underscores the important role of biglycan and fibromodulin in the maintenance of condylar cartilage integrity of the TMJ (Wadhwa et al., 2005; Embree et al., 2010). However, a very recent study shows that

decorin-deficient mice have significantly less damage of knee joints than their wild-type littermates after the mice were exercised extensively. Results also show the stiffness of articular cartilages is increased in decorin-deficient mice. Thus, the investigators in this study suggest that the protective effect of decorin deficiency may be due to the increased stiffness (Gronau et al., 2017). This is opposed to the current notion that the increased stiffness is considered as a negative factor to normal articular cartilages.

Perlecan, a large heparan sulfate proteoglycan is exclusively localized in the PCM and is co-localized with collagen type VI. The enzymatic removal of heparan sulfate chains from perlecan increases the elastic moduli in the PCM (Wilusz et al., 2012a). Dysfunction of the perlecan gene results in the acceleration of articular cartilage degeneration similar to what is seen in collagen type II knockout mice (Lapvetelainen et al., 2001). This suggests that perlecan is a potential factor in the development of OA.

In summary, the PCM of chondrocytes plays an important role in protecting joints from becoming osteoarthritic. Missing one or more components of the PCM can disrupt the structure of the matrix. One consequence of the disruption of the PCM is that chondrocytes are less protected from mechanical stresses. Another consequence is the exposure of chondrocytes to molecules in the territorial and interterritorial matrices, which would normally be separated by the PCM.

The pericellular matrix as a biomechanical microenvironment in the protection of the chondrocyte from mechanical stress

The mechanical factors that drive the biological responses of chondrocytes and its surrounding environment lead to osteoarthritic progresses in articular cartilage (Khoshgoftar et al., 2018). The mechanical properties of PCM is considered to have a significantly effect on the chondrocytes, suggesting that the PCM plays a biomechanical role in articular cartilage (McLane et al., 2013; Wilusz et al., 2014). During immobilization or destabilization of joints, chondrocytes are exposed to changes of stresses and strains, hydrostatic pressure, interstitial fluid flow, streaming potentials and osmotic pressure (O'Conor et al., 2013). A number of experimental studies suggest that the PCM protects chondrocytes during mechanical loading through an "adaptive water loss from PCM proteoglycans" (Poole, 1997; Haider et al., 2006). Other studies also demonstrate that static compression can stimulate the directional deposition of secreted proteoglycans around chondrocytes, while also inhibiting proteoglycan synthesis (Quinn et al., 1998). Conversely, the deposition and synthesis of the pericellular proteoglycans are stimulated by dynamic compression (Guilak et al., 2006). Evidence shows that the change of osmotic pressure may also regulate chondrocyte response to mechanical loading (Chao et al., 2006; O'Conor et al., 2014). Later studies have reported that the PCM works as a transducer of mechanical and physicochemical signals, possibly through an interaction of collagen type VI with cell surface integrins, hyaluronan and primary cilium (Lee et al., 2000; Muhammad et al., 2012; Zelenski et al., 2015). These findings suggest that cell-matrix interactions may play an important role in the response of chondrocytes to mechanical compression.

The distinct biomechanical properties of the PCM have been measured by techniques such as micropipette aspiration, *in situ* imaging, computational modeling, and atomic force microscopy (AFM). Micropipette aspiration studies have provided direct quantitative measurements of PCM elastic and biphasic properties and have demonstrated the mechanical properties of PCM (Alexopoulos et al., 2003, 2005b). These studies suggest that the presence of PCM significantly affects the micromechanical environment of the chondrocyte in a zone-dependent manner (Alexopoulos et al., 2005a). *In situ* imaging studies have investigated the relative deformations of chondrocytes, PCM and ECM (Choi et al., 2007). These studies indicate that PCM may regulate either amplification or shielding of forces, depending on chondrocyte position within tissue. By combining imaging and application of the computational model, the mechanical properties of PCM can be estimated *in situ* during mechanical loading (Michalek and Iatridis, 2007; Kim et al., 2010). Atomic force microscopy (AFM)-based microindentation has been applied to avoid artificial alterations caused by physical extraction of PCM. By use of AFM-based stiffness mapping, PCM elastic moduli are tested in human cartilage (Darling et al., 2010). AFM with fluorescence microscopy has also been utilized to analyze the biomechanical properties of PCM and the correlation among structural features and biochemical composition (Wilusz et al., 2012b, 2013).

Loss of PCM mechanical properties has been characterized at the macroscale, microscale, and nanoscale in both human OA tissue and experimental animal models. Micropipette aspiration studies reveal that chondrons from human OA cartilage exhibit a 30–40% decrease in Young's moduli, compared with chondrons from healthy tissue (Alexopoulos et al., 2003). A study of elastic mapping indicates a significant loss of mechanical properties of PCM in OA cartilage. The PCM elastic moduli in OA cartilage are reduced by 30% as compared to that in normal cartilage (Wilusz et al., 2012b). This study also demonstrates that in OA cartilage, there is a shallow gradient in the change of modulus outward from PCM to ECM, as compared to the sharper transition observed in normal cartilage.

The biomechanical properties of PCM are influenced by specific PCM components. For example, PCM plays the role of a mechanical transducer during the interaction of type VI collagen with cell surface integrins or hyaluronan (Zelenski et al., 2015). Results from a study of isolated chondrons show that PCM moduli were

reduced by one-third in heterozygous Col6a1$^{+/-}$ mice and by two-thirds in Col6a1$^{-/-}$ mice (Alexopoulos et al., 2009). Knockout of type VI collagen was also shown to increase the extent of cell swelling and osmotically induced TRPV4 (transient receptor potential vanilloid channel 4) signaling in an age-dependent manner, and can accelerate the progression of OA (Zelenski et al., 2015). Another molecule that contributes significantly to PCM properties is perlecan. Perlecan and collagen type VI co-localized regions have lower modulus regions than type VI collagen alone area (Wilusz et al., 2012a). Studies also reported that perlecan may play a role in mechanotransduction to release FGF-2 by mechanical loading (Vincent et al., 2007; Wilusz et al., 2014).

In summary, the aforementioned results from the mechanical studies of PCM demonstrate that the matrix may play a protective role to chondrocytes responding to mechanical stresses. Also, disease-related PCM structure and biomechanical properties may affect the way chondrocytes sense and respond to normal mechanical loading, contributing to further matrix degradation and disease progression.

Pericellular matrix in maintaining homeostasis of chondrocyte metabolism

The disappearance of PCM of chondrocytes exposes chondrocytes to molecules in territorial and interterritorial matrices, particularly collagen type II. Thus, PCM prevents collagen type II from binding to its cognate receptor, discoidin domain receptor 2 (DDR2), in articular cartilages. DDR2 is a cell surface receptor tyrosine kinase (RTK) and is activated by binding of the receptor with collagen type II (Shrivastava et al., 1997; Vogel et al., 1997; Xu et al., 2011). It is conceivable that the activation of DDR2 transduces signaling to turn on/off genes in chondrocytes. This, in turn, changes chondrocyte activities (Goldring, 2012). In fact, in the last ten years, data from our investigations and other studies demonstrate that the activation of DDR2 induces the expression of MMP-13, one of the major extracellular matrix degrading enzymes (Xu et al., 2005, 2007; Vonk et al., 2011). The end result is OA.

DDR2 was originally cloned in 1993 (Di Marco et al., 1993; Karn et al., 1993, Zerlin et al., 1993). Two independent research groups have identified specific amino acids on DDR2 and collagen type II, which are critical for the interaction of the two molecules. A change in any of these amino acids will dramatically affect the affinity of DDR2 with collagen type II (Leitinger et al., 2004, Ichikawa et al., 2007; Konitsiotis et al., 2008). To understand the signaling mechanism responsible for the induction of MMP-13 by the activation of DDR2, a series of experiments were performed (Xu et al., 2005, 2007, 2009; Sunk et al., 2007; Goldring, 2012; Schminke et al., 2014). Results from these experiments showed that the expression of MMP-13 was elevated in chondrocytes cultured on type II collagen. Surprisingly, the expression of DDR2 was also found to be increased in the chondrocytes. This indicated that chondrocytes exposed to native collagen type II were induced the expression of MMP-13 and the DDR2 receptor itself. In addition, when chondrocytes were cultured on the denatured type II collagen, gelatin, the expression of MMP-13 and DDR2 were not induced, suggesting that the chondrocytes responded in a specific manner to triple-helical type II collagen. Moreover, the overexpression of full-length DDR2 cDNA resulted in increased expression of MMP-13, whereas the overexpression of a truncated DDR2 cDNA (lacking the

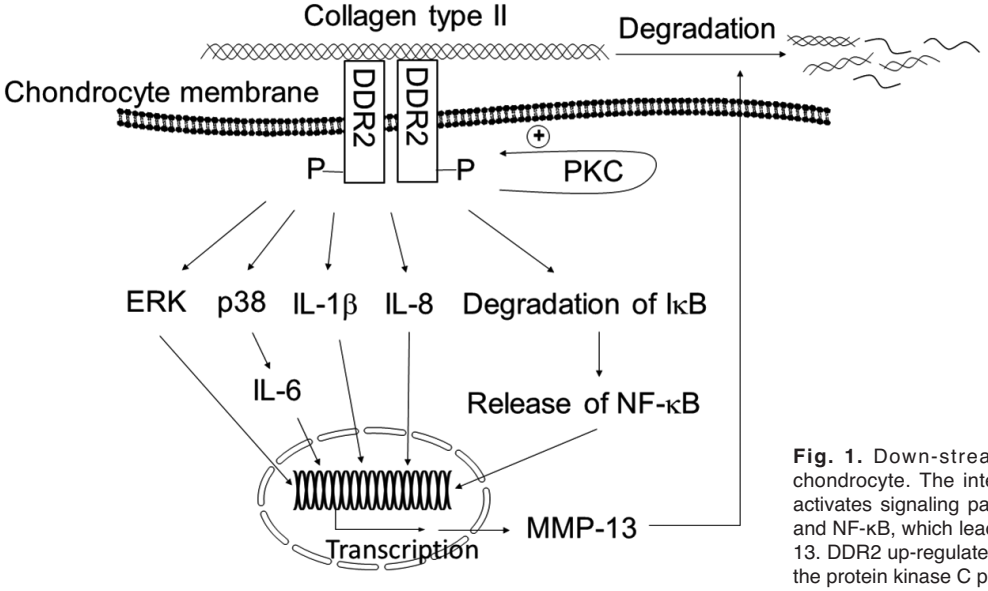

Fig. 1. Down-stream signaling pathways of DDR2 in chondrocyte. The interaction of DDR2 with collagen type II activates signaling pathways including ERK, p38, IL-1β, IL-8 and NF-κB, which leads to the synthesis and release of MMP-13. DDR2 up-regulates the expression of the receptor itself by the protein kinase C pathway.

protein tyrosine kinase) inhibited the increased expression of MMP-13. In addition, DDR2 lacking type II collagen-binding domain (discoidin domain) of the receptor had no effect on the expression of MMP-13 and the receptor itself. These results indicate that increased expression of MMP-13 in chondrocytes requires the interaction of DDR2 with type II collagen. The data also show that Ras/Raf/MEK/ERK and p38 signaling pathways were involved in the induction of MMP-13 in chondrocytes by collagen type II-DDR2 interaction. In addition, another research group reports that DDR2 increases the expression of the receptor itself by a protein kinase C-dependent pathway (Vonk et al., 2011), see Figure 1. Furthermore, transgenic mice were generated that conditionally overexpressed *Ddr2* in mature articular cartilage on mouse knee joints. However, there was no induction of *Mmp-13* in the cartilage (Xu et al., 2011). The result suggests that conditionally overexpressed *Ddr2* in transgenic mice is not activated since the receptor is still separated from collagen type II by PCM. This observation is in agreement with the result from another independent investigation, which demonstrates that *Mmp-13* is not induced in chondron cultured on collagen type II coated plates (Vonk et al., 2011).

On the basis of the findings from *in vitro* experiments, studies were carried out *in vivo* to understand the potential roles of DDR2 in the development of OA. The expression of DDR2 was examined in human osteoarthritic tissues and mouse models of OA. It was found that DDR2 protein was barely detectable in normal articular cartilage. However, the expression of DDR2 was increased in human osteoarthritic tissues and mouse models of OA (Lam et al., 2007; Sunk et al., 2007; Xu et al., 2007). Moreover, DDR2 was co-localized with the increased activity and expression of MMP-13 in degenerative articular cartilage. More importantly, by utilizing the conditional knockout technique, we were able to delete *Ddr2* prior to, and after the onset of, articular cartilage degeneration induced by the destabilization of the medial meniscus (DMM). Our results indicated that articular cartilage was protected under both conditions against the development of OA (Manning et al., 2016). We also conditionally removed *Ddr2* from a genetic form of the mouse model of OA, collagen type XI-haploinsufficient mice ($Col11a1^{+/-}$). We found similar results to what was observed in the DMM model (unpublished data). Moreover the result from another independent research group indicates that the expression of Ddr2 is increased in the articular cartilage of knee joints of a spontaneous mouse model of OA (Holt et al., 2012). A study reports that DDR1 may also play a role in the development of OA in TMJ (Schminke et al., 2014).

In summary, the degradation of PCM enhances the exposure of DDR2 to collagen type II and elicits the interaction of the receptor with collagen type II. This, in turn, activates DDR2 and its down-stream signaling pathways, resulting in the induction of MMP-13 in chondrocytes. MMP-13 degrades collagen type II and proteoglycans, which eventually leads to OA.

Conclusion

OA is the most common form of arthritic disorder. However, there are, currently, no effective drugs to retard the osteoarthritic process once it has initiated. We believe that pericellular matrix is potentially one of the targets for the development of disease-modifying OA drugs. For example, efforts should be made to identify an enzyme(s) responsible for the degradation of pericellular matrix. Biological reagents that inhibit the activity of the enzymes may be developed as drugs to treat OA.

It is worth mentioning that numerous independent research groups, including our group, have reported that a serine protease, high temperature requirement A1 (HTRA1) is highly expressed in human OA cartilage and in mouse models of OA (Hu et al., 1998; Clausen et al., 2002; Grau et al., 2006; Wu et al., 2007; Polur et al., 2010). HTRA1 can degrade the pericellular components. This suggests that HTRA1 may contribute to the development of OA through the degradation of the pericellular network. If so, HTRA1 may be a potential therapeutic target for the development of disease-modifying OA drugs. However, we are aware that results from *in vivo* experiments are needed to demonstrate the causal relationship of the HTRA1 and the development of OA. Another review article has also discussed PCM as a therapeutic target to development DMOADs (Vincent, 2013).

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References

Alexopoulos L.G., Haider M.A., Vail T.P. and Guilak F. (2003). Alterations in the mechanical properties of the human chondrocyte pericellular matrix with osteoarthritis. J. Biomech. Eng. 125, 323-333.

Alexopoulos L.G., Setton L.A. and Guilak F. (2005a). The biomechanical role of the chondrocyte pericellular matrix in articular cartilage. Acta Biomater. 1, 317-325.

Alexopoulos L.G., Williams G.M., Upton M.L., Setton L.A. and Guilak F. (2005b). Osteoarthritic changes in the biphasic mechanical properties of the chondrocyte pericellular matrix in articular cartilage. J. Biomech. 38, 509-517.

Alexopoulos L.G., Youn I., Bonaldo P. and Guilak F. (2009). Developmental and osteoarthritic changes in col6a1-knockout mice: Biomechanics of type VI collagen in the cartilage pericellular matrix. Arthrtr. Rheum. 60, 771-779.

Bock H.C., Michaeli P., Bode C., Schultz W., Kresse H., Herken R. and Miosge N. (2001). The small proteoglycans decorin and biglycan in

human articular cartilage of late-stage osteoarthritis. Osteoarthritis Cartilage 9, 654-663.

Bonnemann C.G., Cox G.F., Shapiro F., Wu J.J., Feener C.A., Thompson T.G., Anthony D.C., Eyre D.R., Darras B.T. and Kunkel L.M. (2000). A mutation in the alpha 3 chain of type ix collagen causes autosomal dominant multiple epiphyseal dysplasia with mild myopathy. Proc. Natl. Acad. Sci. USA 97, 1212-1217.

Borochowitz Z.U., Scheffer D., Adir V., Dagoneau N., Munnich A. and Cormier-Daire V. (2004). Spondylo-epi-metaphyseal dysplasia (semd) matrilin 3 type: Homozygote matrilin 3 mutation in a novel form of semd. J. Med. Genet. 41, 366-372.

Chao P.H., West A.C. and Hung C.T. (2006). Chondrocyte intracellular calcium, cytoskeletal organization, and gene expression responses to dynamic osmotic loading. Am. J. Physiol. Cell Physiol. 291, C718-725.

Chapman K.L., Mortier G.R., Chapman K., Loughlin J., Grant M.E. and Briggs M.D. (2001). Mutations in the region encoding the von willebrand factor a domain of matrilin-3 are associated with multiple epiphyseal dysplasia. Nat. Genet. 28, 393-396.

Chen Y., Cossman J., Jayasuriya C.T., Li X., Guan Y., Fonseca V., Yang K., Charbonneau C., Yu H., Kanbe K., Ma P., Darling E. and Chen Q. (2016). Deficient mechanical activation of anabolic transcripts and post-traumatic cartilage degeneration in matrilin-1 knockout mice. PLoS One 11, e0156676.

Choi J.B., Youn I., Cao L., Leddy H.A., Gilchrist C.L., Setton L.A. and Guilak F. (2007). Zonal changes in the three-dimensional morphology of the chondron under compression: The relationship among cellular, pericellular, and extracellular deformation in articular cartilage. J. Biomech. 40, 2596-2603.

Chu X.Q., Wang J.J., Dou L.D. and Zhao G. (2015). Cartilage oligomeric matrix protein and matrix metalloproteinase-3 expression in the serum and joint fluid of a reversible osteoarthritis rabbit model. Genet. Mol. Res. GMR 14, 14207-14215.

Clausen T., Southan C. and Ehrmann M. (2002). The htra family of proteases: Implications for protein composition and cell fate. Mol. Cell 10, 443-455.

Czarny-Ratajczak M., Lohiniva J., Rogala P., Kozlowski K., Perala M., Carter L., Spector T.D., Kolodziej L., Seppanen U., Glazar R., Krolewski J., Latos-Bielenska A. and Ala-Kokko L. (2001). A mutation in col9a1 causes multiple epiphyseal dysplasia: Further evidence for locus heterogeneity. Am. J. Hum. Genet. 69, 969-980.

Darling E.M., Wilusz R.E., Bolognesi M.P., Zauscher S. and Guilak F. (2010). Spatial mapping of the biomechanical properties of the pericellular matrix of articular cartilage measured *in situ* via atomic force microscopy. Biophys. J. 98, 2848-2856.

Di Marco E., Cutuli N., Guerra L., Cancedda R. and De Luca M. (1993). Molecular cloning of trke, a novel trk-related putative tyrosine kinase receptor isolated from normal human keratinocytes and widely expressed by normal human tissues. J. Biol. Chem. 268, 24290-24295.

Embree M.C., Kilts T.M., Ono M., Inkson C.A., Syed-Picard F., Karsdal M.A., Oldberg A., Bi Y. and Young M.F. (2010). Biglycan and fibromodulin have essential roles in regulating chondrogenesis and extracellular matrix turnover in temporomandibular joint osteoarthritis. Am. J. Pathol. 176, 812-826.

Goldring M.B. (2012). Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis. Ther. Adv. Musculoskelet. Dis. 4, 269-285.

Grau S., Richards P.J., Kerr B., Hughes C., Caterson B., Williams A.S., Junker U., Jones S.A., Clausen T. and Ehrmann M. (2006). The role of human htra1 in arthritic disease. J. Biol. Chem. 281, 6124-6129.

Gronau T., Kruger K., Prein C., Aszodi A., Gronau I., Iozzo R.V., Mooren F.C., Clausen-Schaumann H., Bertrand J., Pap T., Bruckner P. and Dreier R. (2017). Forced exercise-induced osteoarthritis is attenuated in mice lacking the small leucine-rich proteoglycan decorin. Ann. Rheum. Dis. 76, 442-449.

Guilak F., Alexopoulos L.G., Upton M.L., Youn I., Choi J.B., Cao L., Setton L.A. and Haider M.A. (2006). The pericellular matrix as a transducer of biomechanical and biochemical signals in articular cartilage. Ann. NY Acad. Sci. 1068, 498-512.

Haider M.A., Schugart R.C., Setton L.A. and Guilak F. (2006). A mechano-chemical model for the passive swelling response of an isolated chondron under osmotic loading. Biomech. Model. Mechanobiol. 5, 160-171.

Holt D.W., Henderson M.L., Stockdale C.E., Farrell J.T., Kooyman D.L., Bridgewater L.C. and Seegmiller R.E. (2012). Osteoarthritis-like changes in the heterozygous sedc mouse associated with the htra1-ddr2-mmp-13 degradative pathway: A new model of osteoarthritis. Osteoarthritis Cartilage 20, 430-439.

Horikawa O., Nakajima H., Kikuchi T., Ichimura S., Yamada H., Fujikawa K. and Toyama Y. (2004). Distribution of type vi collagen in chondrocyte microenvironment: Study of chondrons isolated from human normal and degenerative articular cartilage and cultured chondrocytes. J. Orthop. Sci. 9, 29-36.

Hu S.I., Carozza M., Klein M., Nantermet P., Luk D. and Crowl R.M. (1998). Human htra, an evolutionarily conserved serine protease identified as a differentially expressed gene product in osteoarthritic cartilage. J. Biol. Chem. 273, 34406-34412.

Hu K., Xu L., Cao L., Flahiff C.M., Brussiau J., Ho K., Setton L.A., Youn I., Guilak F., Olsen B.R. and Li Y. (2006). Pathogenesis of osteoarthritis-like changes in the joints of mice deficient in type ix collagen. Arthritis Rheum. 54, 2891-2900.

Hunziker E.B., Michel M. and Studer D. (1997). Ultrastructure of adult human articular cartilage matrix after cryotechnical processing. Microsc. Res. Tech. 37, 271-284.

Ichikawa O., Osawa M., Nishida N., Goshima N., Nomura N. and Shimada I. (2007). Structural basis of the collagen-binding mode of discoidin domain receptor 2. EMBO J. 26, 4168-4176.

Jackson G.C., Marcus-Soekarman D., Stolte-Dijkstra I., Verrips A., Taylor J.A. and Briggs M.D. (2010). Type IX collagen gene mutations can result in multiple epiphyseal dysplasia that is associated with osteochondritis dissecans and a mild myopathy. Am. Journal Med. Genet. Part A 152a, 863-869.

Karn T., Holtrich U., Brauninger A., Bohme B., Wolf G., Rubsamen-Waigmann H. and Strebhardt K. (1993). Structure, expression and chromosomal mapping of tkt from man and mouse: A new subclass of receptor tyrosine kinases with a factor VIII-like domain. Oncogene 8, 3433-3440.

Khoshgoftar M., Torzilli P.A. and Maher S.A. (2018). Influence of the pericellular and extracellular matrix structural properties on chondrocyte mechanics. J. Orthop. Res. (in press).

Kim E., Guilak F. and Haider M.A. (2010). An axisymmetric boundary element model for determination of articular cartilage pericellular matrix properties *in situ* via inverse analysis of chondron deformation. J. Biomech. Eng. 132, 031011.

Ko Y., Kobbe B., Nicolae C., Miosge N., Paulsson M., Wagener R. and Aszodi A. (2004). Matrilin-3 is dispensable for mouse skeletal growth and development. Mol. Cell. Biol. 24, 1691-1699.

Konitsiotis A.D., Raynal N., Bihan D., Hohenester E., Farndale R.W. and Leitinger B. (2008). Characterization of high affinity binding motifs for the discoidin domain receptor ddr2 in collagen. J. Biol. Chem. 283, 6861-6868.

Lam N.P., Li Y., Waldman A.B., Brussiau J., Lee P.L., Olsen B.R. and Xu L. (2007). 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. 52, 579-584.

Lapvetelainen T., Hyttinen M., Lindblom J., Langsjo T.K., Sironen R., Li S.W., Arita M., Prockop D.J., Puustjarvi K. and Helminen H.J. (2001). 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 9, 152-160.

Lee V., Cao L., Zhang Y., Kiani C., Adams M.E. and Yang B.B. (2000). The roles of matrix molecules in mediating chondrocyte aggregation, attachment, and spreading. J. Cell. Biochem. 79, 322-333.

Leitinger B., Steplewski A. and Fertala A. (2004). The d2 period of collagen II contains a specific binding site for the human discoidin domain receptor, ddr2. J. Mol. Biol. 344, 993-1003.

Li Y. and Xu L. (2015). Advances in understanding cartilage remodeling. F1000Research 4, 642.

Loeser R.F., Goldring S.R., Scanzello C.R. and Goldring M.B. (2012). Osteoarthritis: A disease of the joint as an organ. Arthritis Rheum. 64, 1697-1707.

Manning L.B., Li Y., Chickmagalur N.S., Li X. and Xu L. (2016). Discoidin domain receptor 2 as a potential therapeutic target for development of disease-modifying osteoarthritis drugs. Am. J. Pathol. 186, 3000-3010.

McLane L.T., Chang P., Granqvist A., Boehm H., Kramer A., Scrimgeour J. and Curtis J.E. (2013). Spatial organization and mechanical properties of the pericellular matrix on chondrocytes. Biophys. J. 104, 986-996.

Michalek A.J. and Iatridis J.C. (2007). A numerical study to determine pericellular matrix modulus and evaluate its effects on the micromechanical environment of chondrocytes. J. Biomech. 40, 1405-1409.

Muhammad H., Rais Y., Miosge N. and Ornan E.M. (2012). The primary cilium as a dual sensor of mechanochemical signals in chondrocytes. Cell. Mol. Life Sci. 69, 2101-2107.

Murray D.H., Bush P.G., Brenkel I.J. and Hall A.C. (2010). Abnormal human chondrocyte morphology is related to increased levels of cell-associated il-1beta and disruption to pericellular collagen type VI. J. Orthop. Res. 28, 1507-1514.

Mustafa Z., Chapman K., Irven C., Carr A.J., Clipsham K., Chitnavis J., Sinsheimer J.S., Bloomfield V.A., McCartney M., Cox O., Sykes B. and Loughlin J. (2000). Linkage analysis of candidate genes as susceptibility loci for osteoarthritis-suggestive linkage of col9a1 to female hip osteoarthritis. Rheumatology (Oxford) 39, 299-306.

Muttigi M.S., Han I., Park H.K., Park H. and Lee S.H. (2016). Matrilin-3 role in cartilage development and osteoarthritis. Int. J. Mol. Sci. 17, 590.

O'Conor C.J., Case N. and Guilak F. (2013). Mechanical regulation of chondrogenesis. Stem Cell Res. Ther. 4, 61.

O'Conor C.J., Leddy H.A., Benefield H.C., Liedtke W.B. and Guilak F. (2014). Trpv4-mediated mechanotransduction regulates the metabolic response of chondrocytes to dynamic loading. Proc. Natl. Acad. Sci. USA 111, 1316-1321.

Otten C., Hansen U., Talke A., Wagener R., Paulsson M. and Zaucke F. (2010). A matrilin-3 mutation associated with osteoarthritis does not affect collagen affinity but promotes the formation of wider cartilage collagen fibrils. Hum. Mutat. 31, 254-263.

Owida H.A., De Las Heras Ruiz T., Dhillon A., Yang Y. and Kuiper N.J. (2017). Co-culture of chondrons and mesenchymal stromal cells reduces the loss of collagen vi and improves extracellular matrix production. Histochem. Cell Biol. 148, 625-638.

Pirog-Garcia K.A., Meadows R.S., Knowles L., Heinegard D., Thornton D.J., Kadler K.E., Boot-Handford R.P. and Briggs M.D. (2007). Reduced cell proliferation and increased apoptosis are significant pathological mechanisms in a murine model of mild pseudoachondroplasia resulting from a mutation in the c-terminal domain of comp. Hum. Mol. Genet. 16, 2072-2088.

Polur I., Lee P.L., Servais J.M., Xu L. and Li Y. (2010). Role of htra1, a serine protease, in the progression of articular cartilage degeneration. Histol. Histopathol. 25, 599-608.

Poole C.A. (1997). Articular cartilage chondrons: Form, function and failure. J. Anatomy 191 (Pt 1), 1-13.

Poole C.A., Flint M.H. and Beaumont B.W. (1988). Chondrons extracted from canine tibial cartilage: Preliminary report on their isolation and structure. J. Orthop. Res. 6, 408-419.

Poole C.A., Matsuoka A. and Schofield J.R. (1991). Chondrons from articular cartilage. III. Morphologic changes in the cellular microenvironment of chondrons isolated from osteoarthritic cartilage. Arthritis Rheum. 34, 22-35.

Poole C.A., Honda T., Skinner S.J., Schofield J.R., Hyde K.F. and Shinkai H. (1990). Chondrons from articular cartilage (II): Analysis of the glycosaminoglycans in the cellular microenvironment of isolated canine chondrons. Connect. Tissue Res. 24, 319-330.

Quinn T.M., Grodzinsky A.J., Buschmann M.D., Kim Y.J. and Hunziker E.B. (1998). Mechanical compression alters proteoglycan deposition and matrix deformation around individual cells in cartilage explants. J. Cell Sci. 111, 573-583.

Roughley P.J. (2001). Articular cartilage and changes in arthritis: Noncollagenous proteins and proteoglycans in the extracellular matrix of cartilage. Arthritis Res. 3, 342-347.

Schminke B., Frese J., Bode C., Goldring M.B. and Miosge N. (2016). Laminins and nidogens in the pericellular matrix of chondrocytes: Their role in osteoarthritis and chondrogenic differentiation. Am. J. Pathol. 186, 410-418.

Schminke B., Muhammad H., Bode C., Sadowski B., Gerter R., Gersdorff N., Burgers R., Monsonego-Ornan E., Rosen V. and Miosge N. (2014). A discoidin domain receptor 1 knock-out mouse as a novel model for osteoarthritis of the temporomandibular joint. Cell. Mol. Life Sci. 71, 1081-1096.

Shrivastava A., Radziejewski C., Campbell E., Kovac L., McGlynn M., Ryan T.E., Davis S., Goldfarb M.P., Glass D.J., Lemke G. and Yancopoulos G.D. (1997). An orphan receptor tyrosine kinase family whose members serve as nonintegrin collagen receptors. Mol. Cell 1, 25-34.

Suleman F., Gualeni B., Gregson H.J., Leighton M.P., Pirog K.A., Edwards S., Holden P., Boot-Handford R.P. and Briggs M.D. (2012). A novel form of chondrocyte stress is triggered by a comp mutation causing pseudoachondroplasia. Human Mutat. 33, 218-231.

Sunk I.G., Bobacz K., Hofstaetter J.G., Amoyo L., Soleiman A., Smolen J., Xu L. and Li Y. (2007). 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. 56, 3685-3692.

van der Weyden L., Wei L., Luo J., Yang X., Birk D.E., Adams D.J., Bradley A. and Chen Q. (2006). Functional knockout of the matrilin-3 gene causes premature chondrocyte maturation to hypertrophy and increases bone mineral density and osteoarthritis. Am. J. Pathol. 169, 515-527.

Verma P. and Dalal K. (2013). Serum cartilage oligomeric matrix protein (comp) in knee osteoarthritis: A novel diagnostic and prognostic biomarker. J. Orthop. Res. 31, 999-1006.

Vincent T.L. (2013). Targeting mechanotransduction pathways in osteoarthritis: A focus on the pericellular matrix. Curr. Opin. Pharmacol. 13, 449-454.

Vincent T.L., McLean C.J., Full L.E., Peston D. and Saklatvala J. (2007). Fgf-2 is bound to perlecan in the pericellular matrix of articular cartilage, where it acts as a chondrocyte mechanotransducer. Osteoarthritis Cartilage 15, 752-763.

Vincourt J.B., Etienne S., Grossin L., Cottet J., Bantsimba-Malanda C., Netter P., Mainard D., Libante V., Gillet P. and Magdalou J. (2012). Matrilin-3 switches from anti- to pro-anabolic upon integration to the extracellular matrix. Matrix Biol. 31, 290-298.

Vogel W., Gish G.D., Alves F. and Pawson T. (1997). The discoidin domain receptor tyrosine kinases are activated by collagen. Mol. Cell 1, 13-23.

Vonk L.A., Doulabi B.Z., Huang C., Helder M.N., Everts V. and Bank R.A. (2011). Collagen-induced expression of collagenase-3 by primary chondrocytes is mediated by integrin α1 and discoidin domain receptor 2: A protein kinase c-dependent pathway. Rheumatology (Oxford) 50, 463-472.

Wadhwa S., Embree M., Ameye L. and Young M.F. (2005). Mice deficient in biglycan and fibromodulin as a model for temporomandibular joint osteoarthritis. Cells Tissues Organs 181, 136-143.

Wilusz R.E., Defrate L.E. and Guilak F. (2012a). A biomechanical role for perlecan in the pericellular matrix of articular cartilage. Matrix Biol. 31, 320-327.

Wilusz R.E., DeFrate L.E. and Guilak F. (2012b). Immunofluorescence-guided atomic force microscopy to measure the micromechanical properties of the pericellular matrix of porcine articular cartilage. J. R. Soc. Interface 9, 2997-3007.

Wilusz R.E., Zauscher S. and Guilak F. (2013). Micromechanical mapping of early osteoarthritic changes in the pericellular matrix of human articular cartilage. Osteoarthritis Cartilage 21, 1895-1903.

Wilusz R.E., Sanchez-Adams J. and Guilak F. (2014). The structure and function of the pericellular matrix of articular cartilage. Matrix Biol. 39, 25-32.

Wu J., Liu W., Bemis A., Wang E., Qiu Y., Morris E.A., Flannery C.R. and Yang Z. (2007). Comparative proteomic characterization of articular cartilage tissue from normal donors and patients with osteoarthritis. Arthritis Rheum. 56, 3675-3684.

Xu L., Peng H., Wu D., Hu K., Goldring M.B., Olsen B.R. and Li Y. (2005). Activation of the discoidin domain receptor 2 induces expression of matrix metalloproteinase 13 associated with osteoarthritis in mice. J. Biol. Chem. 280, 548-555.

Xu L., Peng H., Glasson S., Lee P.L., Hu K., Ijiri K., Olsen B.R., Goldring M.B. and Li Y. (2007). Increased expression of the collagen receptor discoidin domain receptor 2 in articular cartilage as a key event in the pathogenesis of osteoarthritis. Arthritis Rheum. 56, 2663-2673.

Xu L., Polur I., Lim C., Servais J.M., Dobeck J., Li Y. and Olsen B.R. (2009). Early-onset osteoarthritis of mouse temporomandibular joint induced by partial discectomy. Osteoarthritis Cartilage 17, 917-922.

Xu L., Polur I., Servais J.M., Hsieh S., Lee P.L., Goldring M.B. and Li Y. (2011). Intact pericellular matrix of articular cartilage is required for unactivated discoidin domain receptor 2 in the mouse model. Am. J. Pathol. 179, 1338-1346.

Zelenski N.A., Leddy H.A., Sanchez-Adams J., Zhang J., Bonaldo P., Liedtke W. and Guilak F. (2015). Type vi collagen regulates pericellular matrix properties, chondrocyte swelling, and mechanotransduction in mouse articular cartilage. Arthritis Rheumatol. 67, 1286-1294.

Zerlin M., Julius M.A. and Goldfarb M. (1993). Nep: A novel receptor-like tyrosine kinase expressed in proliferating neuroepithelia. Oncogene 8, 2731-2739.

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