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Mouse models and new therapeutic targets for OA

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Osteoarthritis (OA) is marked by degeneration of extracellular matrix (ECM) in articular cartilage in the joint. However, the etiology of OA is not very well understood. This is reflected by the deficiency in the development of structure modifying OA drugs (SMOD), in contrast to the availability of efficient drugs to treat osteoporosis. Therefore, it is urgent to identify new therapeutic targets for OA by analyzing causative factors contributing to OA pathogenesis.

Recent literature indicates that at least three factors including two exogenous and one endogenous to cartilage may be involved in causing cartilage degeneration in OA. The first factor is inflammation. In addition to autocrine cytokines and chemokines produced by chondrocytes themselves, articular chondrocytes may also be modulated by cytokines and chemokines in a paracrine manner¹. Articular cartilage is bathed in synovial fluid, which contains cytokines and chemokines produced by synovium.

Furthermore, articular chondrocytes possess receptors for these factors and the intracellular signal transduction pathways to transmit inflammatory signals to the nucleus. The concentrations of these synovial-derived cytokines and chemokines are elevated under inflammatory conditions, which result in heightened inflammatory responses of chondrocytes including synthesis and secretion of matrix metalloproteinases (MMP) and ADAMTS that degrade cartilage ECM. One strategy to intercept these inflammatory signals within chondrocytes is to block common signal transduction pathways that lead to inflammatory responses. Indeed, *in vitro* inhibition of the inflammatory signal transduction such

as the p38 mitogen activated protein (MAP) kinase pathway has been effective in reducing the release of MMPs from cartilage and suppressing chondrocyte death². However, *in vivo* inhibition of p38 MAP kinase or other inflammatory pathways using genetic means in mouse models reveals that these inflammatory signal transduction pathways often play a role in skeletal development and homeostasis in addition to its recognized role in inflammatory responses³. Therefore, the timing and duration of inhibiting inflammatory signal transduction for OA treatment is highly critical *in vivo* and requires further studies.

The second exogenous factor to result in cartilage degeneration is mechanical loading^{4,5}. It has been recognized for a long time that excessive weight bearing from overweight or sports injury is a significant risk factor of OA. Unlike osteocytes that are linked to each other via cellular processes and gap junctions, chondrocytes reside in a capsule of pericellular matrix (chondron), and are completely surrounded by ECM.

Thus, pericellular matrix is at the ideal location to be involved in transmitting mechanical signals from the extracellular microenvironment to a chondrocyte. A major component of the chondrocyte pericellular matrix network is matrilins, a family of non-collagenous ECM proteins that link various pericellular molecules surrounding chondrocytes. We found that changes of the content of matrilins including the use of chondrocytes from matrilin gene knockout mice affect mechanical stimulation of chondrocyte proliferation and gene expression. Thus, normal content of matrilins is essential to optimal activation of chondrocytes by mechanical signals. Our data suggest that the sensitivity of chondrocytes to the changes in the microenvironment can be adjusted by altering the content of proteins such as matrilins in pericellular matrix⁶. This suggests a new strategy to alter the "mechanostat" of cartilage by modifying pericellular matrix through tissue or genetic engineering. Such modification of pericellular matrix architecture may be used to inhibit mechanically-induced OA in the future.

An endogenous factor leading to OA is mutations in a

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gene, which often encodes an ECM molecule in cartilage^{7,8}. For example, mutations in the human gene of matrilin 3 (MATN3) result in a variety of skeletal diseases including multiple epiphyseal dysplasia, spondylo-epi-metaphyseal dysplasia, and hand osteoarthritis. To determine the function of matrilin-3, we examined the consequences of both knocking out MATN3 and over-expressing MATN3 in chondrocytes. In MATN3 KO mice, the hypertrophic zone of the embryonic tibial growth plate was expanded in comparison to its wild-type littermates. Thus, the lack of matrilin-3 leads to mis-regulation of chondrocyte differentiation in the developing epiphyseal growth plate.

By 18 weeks of age MATN3 null mice had a significantly higher total body and knee joint bone mineral density (BMD) than wild-type littermates. Aged MATN3 null mice were much more predisposed to develop severe OA than their wild-type littermates. Conversely, over-expression of MATN3 in embryonic chondrocytes during their transition to hypertrophy led to reduced expression of Col X mRNA, a marker of chondrocyte hypertrophy. Deletion of the cis-acting element in the Col X promoter indicated that the 5' distal region containing BMP responsive elements was necessary and sufficient to mediate the inhibitory effect of MATN3 on Col X. This suggests that MATN3 inhibition of Col X expression is mediated by the BMP pathways. Immunoprecipitation pull-down assay detected BMP-2 in a complex with MATN3 in the conditioned medium of MATN3 transfected cells, suggesting MATN3 may inhibit BMP signaling by interacting and sequestering BMP-2 extracellularly. Interestingly, increased BMP signaling in MATN3 KO mice was indicated by an increase of phosphorylated Smad1 in the growth plate. Our data revealed that MATN3 plays a negative role in regulating chondrocyte hypertrophy by modulating BMP signaling. It suggests a novel pathological mechanism by which matrix defects lead to abnormal BMP signaling that ultimately affects ossification, bone mineral density, and OA pathogenesis.

References

1. Wei L, Sun X, Wang Z, Sun C, Kanbe K, Terek R, Chen Q. Chondrocyte death induced by pathological concentration of chemokine stromal cell-derived factor-1. *J Rheumatol* 2006;33:1818-26.
2. Wei L, Sun X, Wang Z, Chen Q. CD95-induced osteoarthritic chondrocyte apoptosis and necrosis: dependency on p38 mitogen-activated protein kinase. *Arthritis Res Ther* 2006;8:R37.
3. Namdari S, Wei L, Moore D, Chen Q. Genetic inhibition of p38 MAP kinase activity in cartilage reduces limb length and worsens osteoarthritis in adult mice. *Arthritis Rheum* 2008;(in press).
4. Yang X, Vezeridis PS, Nicholas B, Crisco JJ, Moore DC, Chen Q. Differential expression of type X collagen in a mechanically active 3-D chondrocyte culture system: a quantitative study. *J Orthop Surg Res* 2006;1:15.
5. Kanbe K, Inoue K, Xiang C, Chen Q. Identification of clock as mechanosensitive gene by large-scale DNA microarray analysis: downregulation in osteoarthritic cartilage. *Mod Rheumatol* 2006; 16:131-6.
6. Kanbe K, Yang X, Wei L, Sun C, Chen Q. Pericellular matrilins regulate activation of chondrocytes by cyclic load-induced matrix deformation. *J Bone Miner Res* 2007;22:318-28.
7. Pei M, Luo J, Chen Q. Enhancing and maintaining chondrogenesis of synovial fibroblasts by cartilage extracellular matrix protein matrilins, *Osteoarthritis Cartilage* 2008;16:1110-7.
8. Van der Weyden L, Wei L, Luo J, Yang X, Birk DE, Adams DJ, Bradley A, Chen Q. 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.