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Modulating chondrocyte hypertrophy in growth plate and osteoarthritic cartilage

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Osteoarthritis (OA) is a disease of synovial joints, characterised by progressive, focal erosion of articular cartilage, osteophyte formation, subchondral bone erosion and moderate inflammation. Histologically, OA is characterised by cartilage fibrillation, collagen II degeneration, aggrecan loss, blood vessel invasion, chondrocyte hypertrophy and cartilage calcification.

Articular cartilage and growth plate chondrocytes are thought to share a common chondroprogenitor. However, whereas growth plate chondrocytes undergo a maturation process involving proliferation, hypertrophy, terminal differentiation, mineralisation and cell death, normal articular chondrocytes do not progress along this pathway unless they become osteoarthritic[1]. Articular chondrocytes in human OA cartilage often become hypertrophic and die as the cartilage mineralises[1]. Chondrocytes in OA cartilage express several matrix molecules, enzymes and transcription factors involved in growth plate differentiation and maturation including type X collagen[2-4], MMP-13[3,5], MMP-9 and Indian hedgehog[6]. Type I collagen[7], osteocalcin and alkaline phosphatase associated with mineralisation[4] and are also expressed in OA cartilage, as is the apoptotic proteinase, caspase 3[3].

Numerous studies have shown that fragments of matrix molecules are involved in cellular feedback mechanisms in cartilage explant and chondrocyte culture systems. Some fragments induce expression of active proteinases leading to increased matrix degradation[5,8-13]. Other fragments increase matrix synthesis[14,15] and cellular proliferation[16]. Several studies have suggested that type II collagen fragments might influence chondrocyte differentiation in endochondral ossification[5,8,12,13], and we hypothesise that collagen II fragments might also promote cellular hypertrophy when they are over-produced in OA.

Collagen II fragments have been of interest to researchers investigating arthritic disease for some time. Initially they were studied for their utility as biomarkers to monitor collagen synthesis and degradation[17]. Assays for the collagen II N- and C-propeptides have been developed as markers of collagen biosynthesis, whereas assays for peptides derived from the telopeptide and triple helical regions are in development as markers of degradation. More recently, *in vitro* studies have suggested that collagen II fragments might exacerbate osteoarthritis by stimulating induction and activation of matrix metalloproteinases which further degrade the collagen matrix and initiate a positive feedback loop[8,12,13,18]. For example, stimulation of cartilage explants with collagen II fragments generated by *bacterial collagenase* (which cleaves a highly repetitive amino acid sequence in collagen triple helices) causes an increase in gelatinases MMP-2 and -9; once cleaved at the primary cleavage site by collagenases, collagen II is readily degraded by gelatinases[8,18]. In other examples, stimulation of cartilage explants with collagen II fragments generated by *cyanogen bromide*, increases MMP-13 expression and collagen degradation[12,13]. The cyanogen bromide peptides of collagen II also induce chondrocyte hypertrophy, type X collagen expression and apoptosis *in vitro*[12]. Although none of these fragments are physiological in their origin, the results do suggest that type II collagen fragments might have a role in hypertrophy in endochondral ossification. More importantly, the results

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also support earlier studies in which differentiation-related genes were co-localised with focal areas of elevated collagen II degradation in cartilage from osteoarthritis patients[3].

The most compelling evidence that collagen fragments might influence the differentiation and hypertrophy of chondrocytes comes from a study showing that a specific MMP-13 inhibitor prevented the differentiation of prehypertrophic chondrocytes[5]. The inhibitor blocked expression of hypertrophic markers including type X collagen and Cbfa1, which regulates MMP-13 expression. It also blocked mineralisation and inhibited degradation of type II collagen[5].

We have produced and characterised a collagen II knock-in mouse whose type II collagen resists cleavage at the primary collagenase site, at $Gly^{775}\downarrow{}^{776}Leu$[19]. The knock-in mutation that confers resistance to collagenase cleavage is identical to the knock-in mutation made previously in type I collagen[20-22]. Femorotibial joints from collagen II knock-in mice have elongated growth plates and delayed mineralization, suggesting that the program for chondrocyte hypertrophy and differentiation is disturbed. In further studies to identify the underlying mechanism we measured proliferation by *in vivo* incorporation of BrdU and found that whereas cells in the hypertrophic zone of wildtype cartilage stop proliferating, cells in the elongated hypertrophic zone of collagen II knock-in cartilage continue to proliferate. This result suggests that hypertrophic chondrocytes in collagen II knock-in mice are not receiving, or not responding to signals to cease proliferation.

At the bottom of the growth plate, hypertrophic chondrocytes reach terminal differentiation and die. In wildtype mice, these terminally differentiated cells have no nuclei and only sparse cytoplasmic contents. In striking contrast, many hypertrophs at the bottom of collagen II knock-in growth plates retain intact nuclei with well-organized cytoplasm, suggesting that they have failed to enter terminal differentiation and have instead, survived, by reverting to a less-differentiated phenotype. These results strongly suggest that chondrocytes in collagen II knock-in mice respond inappropriately to the signals intended to direct their progression through the proliferative, hypertrophic and terminal stages of differentiation. Hypertrophic chondrocytes undergoing ER stress exhibit similar features of interrupted terminal differentiation; they re-enter the cell cycle and express a more de-differentiated phenotype[23].

Based on the phenotype of the collagen II knock-in mouse, we propose that collagen II fragments in growth plate cartilage are bioactive molecules that provide signals for directing chondrocyte hypertrophy and differentiation. When these signals are absent, hypertrophy and differentiation are interrupted and proceed abnormally. Accordingly, we also propose that collagen II fragments that are abundantly produced in adult OA cartilage provide signals that initiate hypertrophy in an inappropriate context that contributes to the pathology of OA. Because collagen II fragments are absent in the collagen II knock-in mice, these mice provide an opportunity to assess the potential role of collagen II fragments in driving cellular hypertrophy in OA.

References

1. Drissi H, Zuscik M, Rosier R, O'Keefe R. Transcriptional regulation of chondrocyte maturation: potential involvement of transcription factors in OA pathogenesis. Mol Aspects Med 2005;26:169-79.
2. von der Mark K, Kirsch T, Nerlich A, Kuss A, Weseloh G. Type X collagen synthesis in human osteoarthritic cartilage. Indication of chondrocyte hypertrophy. Arthritis Rheum 1992;35:806-11.
3. Tchetina EV, Squires G, Poole AR. Increased type II collagen degradation and very early focal cartilage degeneration is associated with upregulation of chondrocyte differentiation related genes in early human articular cartilage lesions. J Rheumatol 2005;32:876-86.
4. Pullig O, Weseloh G, Ronneberger D, Kakonen S, Swoboda B. Chondrocyte differentiation in human osteoarthritis: expression of osteocalcin in normal and osteoarthritic cartilage and bone. Calcif Tissue Int 2000; 67:230-40.
5. Wu CW, Tchetina EV, Mwale F, Hasty K, Pidoux I, Reiner A, Chen J, Van W, Poole AR. Proteolysis involving matrix metalloproteinase 13 (collagenase-3) is required for chondrocyte differentiation that is associated with matrix mineralization. J Bone Miner Res 2002;17:639-51.
6. Tchetina E, Mwale F, Poole AR. Distinct phases of coordinated early and late gene expression in growth plate chondrocytes in relationship to cell proliferation, matrix assembly, remodeling, and cell differentiation. J Bone Miner Res 2003;18:844-51.
7. Gebhard PM, Gehrsitz A, Bau B, Soder S, Eger W, Aigner T. Quantification of expression levels of cellular differentiation markers does not support a general shift in the cellular phenotype of osteoarthritic chondrocytes. J Orthop Res 2003;21:96-101.
8. Fichter M, Korner U, Schomburg J, Jennings L, Cole AA, Mollenhauer J. Collagen degradation products modulate matrix metalloproteinase expression in cultured articular chondrocytes. J Orthop Res 2006;24:63-70.
9. Yasuda T, Poole AR. A fibronectin fragment induces type II collagen degradation by collagenase through an interleukin-1-mediated pathway. Arthritis Rheum 2002;46:138-48.
10. Stanton H, Ung L, Fosang AJ. The 45 kDa collagen-binding fragment of fibronectin induces matrix metalloproteinase-13 synthesis by chondrocytes and aggrecan degradation by aggrecanases. Biochem J 2002;364:181-90.
11. Xie DL, Hui F, Meyers R, Homandberg GA. Cartilage chondrolysis by fibronectin fragments is associated with release of several proteinases: stromelysin plays a major role in chondrolysis. Arch Biochem Biophys 1994; 311:205-12.
12. Tchetina EV, Kobayashi M, Yasuda T, Meijers T, Pidoux I, Poole AR. Chondrocyte hypertrophy can be

induced by a cryptic sequence of type II collagen and is accompanied by the induction of MMP-13 and collagenase activity: Implications for development and arthritis. Matrix Biol 2007;26:247-58.
13. Yasuda T, Tchetina E, Ohsawa K, Roughley PJ, Wu W, Mousa A, Ionescu M, Pidoux I, Poole AR. Peptides of type II collagen can induce the cleavage of type II collagen and aggrecan in articular cartilage. Matrix Biol 2006;25:419-29.
14. Liu H, McKenna LA, Dean MF. An N-terminal peptide from link protein can stimulate biosynthesis of collagen by human articular cartilage. Arch Biochem Biophys 2000;378:116-22.
15. McKenna LA, Liu H, Sansom PA, Dean MF. An N-terminal peptide from link protein stimulates proteoglycan biosynthesis in human articular cartilage *in vitro*. Arthritis Rheum 1998;41:157-62.
16. Schwartz Z, Carney DH, Crowther RS, Ryaby JT, Boyan BD. Thrombin peptide (TP508) treatment of rat growth plate cartilage cells promotes proliferation and retention of the chondrocytic phenotype while blocking terminal endochondral differentiation. J Cell Physiol 2005;202:336-43.
17. Elsaid KA, Chichester CO. Review: collagen markers in early arthritic diseases. Clin Chim Acta 2006;365:68-77.
18. Jennings L, Wu L, King KB, Hammerle H, Cs-Szabo G, Mollenhauer J. The effects of collagen fragments on the extracellular matrix metabolism of bovine and human chondrocytes. Connect Tissue Res 2001;42:71-86.
19. Gauci SJ, Golub SB, Kurth LM, Little CB, Fosang AJ. Characterisation of a knock-in mouse with type II collagen resistant to collagenase. In: Trans Orthop Res Soc. San Diego: Orthopaedic Research Society; 2007. p. 172.
20. Krane SM, Jaenisch R. Site-directed mutagenesis of type I collagen: effect on susceptibility to collagenase. Matrix Suppl 1992;1:64-7.
21. Liu X, Wu H, Byrne M, Jeffrey J, Krane S, Jaenisch R. 1995. A targeted mutation at the known collagenase cleavage site in mouse type I collagen impairs tissue remodeling. J Cell Biol 1995;130:227-37.
22. Krane SM, Byrne MH, Lemaitre V, Henriet P, Jeffrey JJ, Witter JP, Liu X, Wu H, Jaenisch R, Eeckhout Y. Different collagenase gene products have different roles in degradation of type I collagen. J Biol Chem 1996;271:28509-15.
23. Tsang KY, Chan D, Cheslett D, Chan WC, So CL, Melhado IG, Chan TW, Kwan KM, Hunziker EB, Yamada Y, Bateman JF, Cheung KM, Cheah KS. Surviving endoplasmic reticulum stress is coupled to altered chondrocyte differentiation and function. PLoS Biol 2007;5:e44.