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EDITORIAL

Fibroblast Growth Factor 2: A New Key Player in Osteoarthritis

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Vincent, Saklatvala, and colleagues have taken an interesting approach over the last 5 years by defining molecules that may be of importance in osteoarthritis (OA) through the investigation of proteins that are modulated following damage to articular cartilage. This approach has now paid off in a major way with the discovery that fibroblast growth factor 2 (FGF-2) is a key chondroprotective factor in an OA model, as reported by Chia et al (1) in this issue of *Arthritis & Rheumatism*.

The authors made their original observations regarding FGF-2 through the investigation of ERK activation following explantation or explantation and subsequent cutting of cartilage (2). They showed that as rapidly as 30 minutes after damage, FGF-2 was liberated into the culture medium of cartilage, from which it could be purified. Later studies revealed that FGF-2 is found in a pericellular perlecan-bound pool in normal articular cartilage. Some FGF-2 was hypothesized to be liberated from this pool following mechanical loading, allowing it to stimulate ERK signaling as well as induction of certain matrix metalloproteinase (MMP) genes and tissue inhibitor of metalloproteinases 1 (TIMP-1) (3,4). Chia et al have now pursued these observations in an in vivo model of OA.

Suppression of aggrecan degradation is regarded as a major goal in the prevention of cartilage destruction and loss of function in OA (for review, see ref. 5). Elegant studies in mouse models of arthritis have revealed that ADAMTS-5 is the key enzyme in aggrecan breakdown in this context (6,7). Moreover, biochemical analysis has revealed that human ADAMTS-5 has 1,000-fold greater activity on aggrecan than does ADAMTS-4 (8). Studies of the regulation of aggrecanases have shown that the cytokine interleukin-1 (IL-1) induces ADAMTS-5 to a lesser degree than ADAMTS-4, but given the disparity in aggrecanase activity, this may not reflect a lesser importance for ADAMTS-5 (5). Although metalloproteinase expression levels in end-stage OA showed that ADAMTS-5 was among the proteinases that are repressed at this time point (9), recent genome-wide microarray studies of a surgically induced OA in the rat have revealed that *Adamts5* is up-regulated 4 weeks after surgery (10). These data suggest that ADAMTS-5 is likely to be important in early aggrecan cleavage during the development of OA, but may be repressed, perhaps through a feedback mechanism, during late stages of OA.

Following their previous observations regarding FGF-2 up-regulation in damaged cartilage, Chia et al explored the effect of *Fgf2* gene deletion on cartilage structure and on the development of OA-like characteristics in a surgically induced model of OA. They showed first that *Fgf2*$^{-/-}$ mice had increased development of OA with age as compared with wild-type (WT) mice, with marked effects at ages 6 and 9 months. At age 3 months, no differences were observed between genotypes in terms of cartilage breakdown or expression levels of several key genes, including aggrecan, type II collagen, and *Adamts5*. The authors did not examine the levels of these genes at ages 6 or 9 months, when one might expect to see an up-regulation of *Adamts5* expression and function. Surgical destabilization of the knee was performed at age 10–12 weeks in both WT and *Fgf2*$^{-/-}$ mice, and the authors demonstrated that *Fgf2*$^{-/-}$ mice developed a more severe OA phenotype that was detectable 2, 4, and 8 weeks after surgery. Both cartilage thickness and proteoglycan content (estimated by Safranin O staining) were assessed. Of interest is the fact that

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while the contralateral knees of WT mice showed little cartilage destruction, the contralateral unoperated knees of *Fgf2*$^{-/-}$ mice showed significantly greater damage. The authors suggested that this was due to increased loading on the unoperated side as a consequence of the arthritis that developed more prominently in the operated knees of mice lacking *Fgf2*.

Regulation of aggrecanase expression, including that of ADAMTS-5, has mainly been analyzed in cytokine-treated chondrocytes in which IL-1 often induces both ADAMTS-4 and ADAMTS-5 (for review, see ref. 5). Chia et al found that following surgery, steady-state levels of messenger RNA (mRNA) for *Adamts*5 were elevated and that *Adamts*5 message levels were superinduced in *Fgf2*$^{-/-}$ mice as compared with WT mice, suggesting that FGF-2 repressed the expression of this proteinase. Aggrecanase activity (assessed with an antineoepitope antibody that detects cleavage between the G1 and G2 domains of aggrecan) was also elevated following surgery and appeared prominent in *Fgf2*$^{-/-}$ mice (although not commented on by the authors), concomitant with increased *Adamts*5 expression. Of interest is the fact that the steady-state mRNA levels of other enzymes, including *Adamts4* and *Mmp13*, both of which were elevated after surgery, were not modulated depending on *Fgf2* genotype, indicating a specific effect on *Adamts5* expression. The authors have recently shown in a separate study that FGF-2 represses cartilage destruction induced by IL-1 as well as ADAMTS-4 and ADAMTS-5 expression in human articular chondrocytes (11). In that study, the authors demonstrated that FGF-2 does not suppress IL-1α induction of MMPs 1, 3, and 13. However, a difference was observed in the mouse study, since *Adamts4* was not further elevated when *Fgf2* was deleted. Further studies will reveal whether this difference is due to investigation of isolated chondrocytes or is a true mouse/human divergence of response.

The hypothesis is that FGF-2 normally does not signal until cartilage is damaged, when FGF-2 is liberated from its perlecan-bound pool. However, the mechanisms by which this occurs remain to be established, although Whitelock et al (12) have suggested roles for heparanases or proteinases including MMPs. In the study by Chia et al, *Fgf2* message levels increased in destabilized WT mouse cartilage, which implies that regulation of FGF-2 in this OA model may not only be at the level of release of FGF-2 from the pericellular pool. Another aspect to consider is that ADAMTS-5 has also been shown to be localized in normal cartilage as well as in OA cartilage in a pericellular location (13). Gendron et al (8) have overexpressed human ADAMTS-5 in a chondrosarcoma cell line and have determined that the cysteine-rich domain of ADAMTS-5 is critical for binding to the pericellular matrix. Thus, ADAMTS-5 appears to be present and poised to act should cartilage become damaged. In the study by Chia et al (1), *Fgf2* deletion further enhanced aggrecanase activity. TIMP-3 is the major endogenous inhibitor of aggrecanases known to date (for review, see ref. 5), and *Timp3*$^{-/-}$ mice (age $\geq$6 months) exhibit cartilage destruction (14). In the study by Chia et al, no effects on *Timp3* expression were observed under any conditions, although *Timp1* levels were increased following surgery in either WT or *Fgf2*$^{-/-}$ mice.

These data would suggest that in this case, the traditional "balance" between proteinase and inhibitor tips in favor of aggrecan degradation, although confirmation of the expression of ADAMTS-5 and FGF-2 at the protein level in this model would be of benefit and would currently be possible for FGF-2. It is hoped that this will be achievable for ADAMTS-5 in the future.

A major question raised by the study by Chia et al is, what is the mechanism for FGF-2–mediated ADAMTS-5 repression? Modulation through epigenetic effects could be a possibility, since previous studies have shown that cytokine induction of ADAMTS-4 and ADAMTS-5 as well as other metalloproteinases is repressed by inhibition of histone deacetylases (HDACs) (15). Wang et al (16) have also demonstrated that FGF-2 can suppress constitutive ADAMTS-5 expression in unstimulated human articular chondrocytes in vitro. Neither this constitutive expression nor the FGF-2–mediated repression was modulated by HDAC inhibition (16), suggesting that this mechanism is unlikely to be involved in ADAMTS-5 regulation in this context.

Is there an intermediary repressive molecule induced by FGF-2 that is lost in the absence of FGF-2? Possible candidates could include peroxisome proliferator–activated receptor γ (PPARγ) ligands, since previous studies have demonstrated that such ligands can repress ADAMTS-4 expression in monocytic cells (17) and PPARγ ligands repressed cartilage destruction in a canine model of OA with repression of ADAMTS-5 expression (18). However, the timing of the action of FGF-2 in the human chondrocyte study (11) would suggest that production of an intermediary protein is unlikely.

Since ADAMTS-5 is often, but not always, constitutively expressed (for review, see ref. 5), perhaps a more attractive explanation could be that FGF-2 induces the expression of a microRNA that targets *Adamts5*. Although many microRNA are believed to exert their

actions through blockade of translation, several studies have shown that in certain cases, mRNA levels of target genes are modulated. MicroRNA have already been implicated in chondrocyte biology (19–21). Recently, Iliopoulos et al (22) constructed an OA microRNA gene signature that may reveal possible candidates. Jones et al (23) have demonstrated that microRNA-9 prevents the secretion of MMP-13 and that overexpression of microRNA 9, 98, and 146 reduces IL-1β–induced tumor necrosis factor α production. Thus, future study of microRNA in the surgical OA model in *Fgf2*$^{-/-}$ mice may be of benefit.

An impressive finding by Chia et al is that subcutaneous FGF-2 delivery prevents the development of OA characteristics in operated *Fgf2*$^{-/-}$ mice. It would be of great interest to determine whether *Adamts5* levels in cartilage were restored to WT levels in FGF-2–treated mice. FGF-2 is expressed in both undamaged and damaged articular cartilage and is released rapidly in an in vitro model of cartilage damage (2) or upon mechanical loading of cartilage (3). An aspect that complicates our understanding of the role of FGF-2 in cartilage homeostasis is that this growth factor elevates collagen-degrading MMP-13 in some instances (as the authors have previously demonstrated in human chondrocytes [11]), although in the study by Chia et al, mRNA levels of *Mmp13* were elevated following surgery in both WT and *Fgf2*$^{-/-}$ mice and were not influenced by *Fgf2* mouse genotype. Type II collagen degradation is a later event in cartilage damage, and MMP-13 mRNA levels are elevated in end-stage OA (9), perhaps reflecting a temporal separation between ADAMTS-5 repression and MMP-13 up-regulation. Could FGF-2 be used therapeutically should it become possible to diagnose OA at early stages? Of course, beyond cartilage, FGF-2 can influence the biology of a wide variety of cells, which would complicate its therapeutic use, but perhaps local delivery might limit its potential side effects.

Studies of gene expression in OA, both genome wide (10,24,25) and of specific gene families (9), have revealed important pathways for further study. The article by Chia et al emphasizes the fact that development of OA may well result in modulation of the location and/or function of preexisting proteins in articular cartilage. These data, arising from classic biochemical approaches to an intractable condition, open our eyes to a new way of looking at the development of OA, which, although complicated to unravel, may well result in novel therapeutics in the foreseeable future.

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