IGFBP-5 Metabolism Is Disrupted in the Rat Medial Meniscal Tear Model of Osteoarthritis

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Abstract
Insulin-like growth factor binding protein 5 (IGFBP-5) has been proposed to promote cartilage anabolism through insulin-like growth factor (IGF-1) signaling. A proteolytic activity towards IGFBP-5 has been detected in synovial fluids from human osteoarthritic (OA) joints. The purpose of this study was to determine if protease activity towards IGFBP-5 is present in the rat medial meniscal tear (MMT) model of OA and whether inhibition of this activity would alter disease progression. Sprague-Dawley rats were subject to MMT surgery. Synovial fluid lavages were assessed for the presence of IGFBP-5 proteolytic activity. Treatment animals received intra-articular injections of vehicle or protease inhibitor peptide PB-145. Cartilage lesions were monitored by India ink staining followed by macroscopic measurement of lesion width and depth. The MMT surgery induced a proteolytic activity towards IGFBP-5 that was detectable in joint fluid. This activity was stimulated by calcium and was sensitive to serine protease inhibitors as well as peptide PB-145. Significantly, intra-articular administration of PB-145 after surgery protected cartilage from lesion development. PB-145 treatment also resulted in an increase in cartilage turnover as evidenced by increases in serum levels of procollagen type II C-propeptide (CPII) as well as synovial fluid lavage levels of collagen type II neoepitope (TIINE). IGFBP-5 metabolism is disrupted in the rat MMT model of OA, potentially contributing to cartilage degradation. Inhibition of IGFBP-5 proteolysis protected cartilage from lesion development and enhanced cartilage turnover. These data are consistent with IGFBP-5 playing a positive role in anabolic IGF signaling in cartilage.

Keywords
insulin-like growth factor 1, insulin-like growth factor binding protein 5

Introduction
Cartilage homeostasis is largely controlled by the actions of chondrocytes embedded within the extracellular matrix. Chondrocytes synthesize cartilage-specific matrix proteins such as type II collagen and proteoglycans that are essential for maintaining the structural integrity of cartilage while balancing catabolic forces in order to maintain functional cartilage over long periods of time.1 This homeostasis is disrupted in joint diseases such as osteoarthritis (OA) in which catabolic forces are favored over anabolic signals, resulting in net cartilage degeneration.2 The primary anabolic growth factor for cartilage is insulin-like growth factor (IGF-1), which stimulates proteoglycan synthesis by chondrocytes and also induces chondrocyte proliferation.3,4 Hepatic production provides one source of IGF-1, which is transported to target tissues including cartilage and stimulates growth.5 IGF-1 is also produced by chondrocytes in response to growth hormone (GH) stimulation, and this locally produced IGF-1 is important for stimulating cartilage growth during development.6 It is likely that IGF-1 plays a key role in maintaining cartilage homeostasis during adulthood.

Studies with human articular cartilage indicate that chondrocyte responsiveness to IGF-1 progressively decreases with age.7,8 Additionally, chondrocytes from arthritic cartilage show decreased responsiveness to IGF-1, and this may contribute to cartilage loss during disease.9,10 The mechanism by which chondrocytes lose their responsiveness to IGF-1 is not fully understood. However, several mechanisms have been proposed, including downregulation of IGF-1 receptors, alterations in IGF-1 signaling, and changes in the expression of protease inhibitors that may influence the availability of IGF-1 to its receptors.
IGF-1 is not entirely clear, but IGF-binding proteins (IGFBPs) have been suggested to play a role. IGFBPs are a family of extracellular proteins that interact with and modulate the activities of IGFs including performing an important storage function for IGF-1 in the joint. IGFBPs are synthesized by articular chondrocytes during normal growth as well as during repair after injury. In OA, the levels of IGFBP-3, -4, and -5 are increased in diseased cartilage as are degradation products of IGFBP-3 and -5. IGFBP-3 has been shown to inhibit the stimulatory effect of IGF-1 on proteoglycan synthesis in chondrocytes, suggesting that increased expression of IGFBP-3 in OA may account for decreased IGF-1 responsiveness. Conversely, IGFBP-5 has been proposed to play a positive role in cartilage anabolism through IGF-1 signaling. Overexpression of IGFBP-5 enhanced IGF-1 activation of the PI-3 kinase pathway and promoted IGF-1–enhanced chondrogenic differentiation. These findings, taken together, suggest that under certain situations, IGFBP-5 can act as both a reservoir for IGFs in cartilage and synovial fluid as well as a positive regulator of IGF-1 signaling. Therefore, factors that control the level of intact IGFBP-5 may alter the ability of cartilage to respond to IGF-1.

One variable that regulates the concentration of the intact form of IGFBP-5 is proteolysis. Some studies have reported increased amounts of IGFBP protease activity in joint fluid during the development of arthritis. Significantly, inhibition of proteolytic cleavage of IGFBP-5 was shown to limit the amount of cartilage destruction in a canine model of OA. Inhibition of IGFBP-5 proteolysis was associated with a significant increase in the amount of IGF-1 in the synovial fluid as well as an increase in intact IGFBP-5. The IGFBP-5 protease identified in model canine OA joints and in human OA synovial fluid is the complement protease C1s. C1s is considered highly specific, cleaving the C2 and C4 components of the complement cascade. Aside from IGFBP-5, C1s has been shown to cleave only a few substrates outside the complement cascade, although the physiological relevance is uncertain.

Because IGFBP-5 protease activity has been previously characterized in canine as well as human OA joint fluid, these studies were undertaken to determine if a similar activity was present in a rat model of OA and to test whether inhibition of this activity would have a protective effect on cartilage degeneration. Medial meniscal tear (MMT) in rats results in joint instability and progressive development of OA-like characteristics. The rat MMT model is widely used for characterizing synovial fluid protease activity in the model, as it allows for obtaining synovial fluid lavages at 1, 2, and 3 weeks after surgery. For intra-articular treatment with inhibitory peptide and subsequent analysis of joint cartilage integrity, 15 animals were used per group. Sprague-Dawley male rats (10 weeks old, ~300 g) (Charles River Laboratories, Wilmington, MA) were anesthetized with isoflurane, a skin incision was made over the medial aspect of the right knee, and withdrawal of fluid (typically 25-50 uL). Control lavages were taken from the nonoperated (left) knees for comparison. For analysis of proteolytic activities, lavages were taken 1, 2, and 3 weeks after surgery in untreated animals (n = 6).

**Protease Activity Assays**

IGFBP-5 protease activity in joint fluid lavages was assessed by incubating 150 ng recombinant human IGFBP-5 (Austral...
Biologicals, San Ramon, CA) with 1 uL lavage in a total volume of 20 uL in assay buffer (50 mM Tris, 2 mM CaCl₂, pH 7.4).

After 30 minutes of incubation at 37 °C, samples were electrophoresed on polyacrylamide gels and blotted with a 1:1000 dilution of antisera to IGFBP-5 (AF875, R&D Systems, Minneapolis, MN) in TBS-Tween with 5% milk. Chemiluminescent detection was achieved with a peroxidase-conjugated secondary antibody (#705-036-147, Jackson ImmunoResearch Laboratories Inc., West Grove, PA) and the eCL kit (Amersham Biosciences, Piscataway, NJ). For inhibitor studies, inhibitors from the Protease Inhibitor Set (Roche Applied Science, Penzberg, Germany) were included: antipain (250 μg/mL), E-64 (60 μg/mL), leupeptin (2.5 μg/mL), phosphoramidon (1.65 mg/mL), Pefabloc (5 mg/mL) (Roche Applied Science), EDTA-Na2 (4 mg/mL), and apronin (10 μg/mL). These concentrations were chosen to be 5 times higher than that needed to efficiently inhibit proteases of the relevant classes, as detailed in literature included with the inhibitor set. In the case of phosphoramidon, this concentration is 25 times higher and may have resulted in nontarget class inhibition.

**Treatments and Scoring System**

Treatment animals (15 rats per group) received biweekly intra-articular (IA) injections (0.05 mL) of either vehicle (PBS) or peptide PB-145 (100 or 500 μg in PBS) in the surgery knee beginning 7 days after MMT and continuing for 3 weeks. One day after the last injection, animals were sacrificed and the joints lavaged to obtain synovial fluid. In order to evaluate many potential therapeutic agents for OA, we required a method that was both higher throughput and more time- and cost-effective than histology. Thus, we have employed a quantitative scoring system based on cartilage appearance after India ink staining as well as the thickness of cartilage and depth of lesions. The surgery joints were disarticulated and the tibial plateaus stained with India ink. To quantify the severity of cartilage lesions, a scoring system was devised that included a measure to reflect the apparent thicker cartilage in PB-145–treated joints. The tibial surface was first imaged horizontally using MetaMorph, v6.1 (Molecular Devices Corporation, Downingtown, PA). The area of India ink staining was outlined manually for the total area of lesions. The depth of lesions was evaluated by physical probing. Full-thickness lesions were recorded when the probe hit solid bone. Because the total area of cartilage lesions was not necessarily decreased in PB-145 samples, a new measure designated “skyline” depth or concavity was generated. For this, the medial tibial plateau was viewed from the anterior to posterior direction until a maximum concavity was detected and the image captured. Using this image, a line was drawn from the highest point on the inside (nearest the cruciate ligaments) to the highest point on the outside of the tibial plateau. This represents zero concavity. From this line, a perpendicular line was drawn to the deepest part of the curve, which was recorded as the “skyline depth” in pixels. In order to compare the combined data from all measurements, a total score was calculated that included surface irregularity X area of India ink staining + staining intensity + “skyline” concavity.

**TIINE and CPII Assays**

TIINE (type II collagen neoepitope) was quantitated utilizing BioVeris technology (BioVeris Corporation, Gaithersburg, MD). Synovial lavages were not successfully obtained from all 15 animals in each group, but a minimum of 11 samples from each group were analyzed. Briefly, 25 uL rat synovial fluid lavage, 25 uL streptavidin beads (0.4 mg/mL), 25 uL each antibody at 1 μg/mL (biotin-conjugated mouse 5109 antiprotin and BVTAG-labeled 9A4 antineoepitope), and 100 uL assay buffer (DPBS, 0.1% BSA, 0.05% Tween 20, pH 7.4) were incubated for 2 hours at room temperature before reading on the BioVeris M384 analyzer. Values were calculated from a standard curve prepared from rat 30-mer TIINE peptide (0.313-20 ng/mL).

CPII (procollagen II C-propeptide) was measured by competitive immunoassay (IBEX Technologies Inc., Montreal, Quebec, Canada). Briefly, ELISA plates were coated overnight with bovine CPII. Separately, CPII standards or unknowns (n = 15/treatment group) were mixed with CPII antibody. The mixture was added to the prewashed coated CPII plate and incubated for 2 hours. After washing, goat antirabbit horseradish peroxidase was added for 1 hour. Tetrathenylbenzidine was added and blue color development measured. The OD is inversely proportional to the amount of epitope present.

**Data Analysis**

All values are presented as mean ± standard deviation (except Fig. 7A, mean ± standard error of the mean). Statistical analyses are based on a 1- or 2-sided t test for independent samples as indicated in figure legends.

**Results**

In order to characterize the rat MMT model, we first examined whether a proteolytic activity towards IGFBP-5 would be detectable in joint fluid lavages of rats at various time points after MMT surgery. Samples of MMT synovial fluid lavage were incubated with recombinant IGFBP-5 and proteolysis determined by Western analysis of intact versus fragmented IGFBP-5. As shown in Figure 1, a potent proteolytic activity is present in fluids from surgery knees 2 weeks after MMT that is not present in the contralateral
control knee fluids. Specific proteolytic fragments of IGFBP-5 common only to samples incubated with MMT synovial fluid are evident. This activity was similarly detected in and specific to surgery knee synovial fluid lavages from animals at 1 and 3 weeks after MMT (data not shown).

To further characterize the IGFBP-5 proteolytic activity, samples of MMT synovial fluid lavage were incubated with recombinant IGFBP-5 in the presence of high concentrations of various protease inhibitors. As shown in Figure 2A, the serine protease inhibitors Pefabloc SC (5 mg/mL) (Roche Applied Science) and antipain (250 ug/mL) completely inhibited the proteolytic activity (lanes 9 and 4, respectively). The activity was also inhibited by disodium EDTA (4 mg/mL), indicating a requirement for a divalent cation (Fig. 2A, lane 10). Although there was significant inhibition with a high concentration of phosphoramidon (1.65 mg/mL) (Fig. 2A, lane 8), the activity is not due to a metalloproteinase because 1,10-phenanthroline (1 mM) had no effect (Fig. 2B). The activity was insensitive to E-64, leupeptin, pepstatin, and aprotinin (Fig. 2A, lanes 5, 6, 7, and 11, respectively). To further explore the cation dependence of the proteolytic activity, the reaction was carried out in the absence and presence of calcium. The activity was significantly enhanced in the presence of calcium (Fig. 2C, lane 2). The activity was not enhanced in the presence of divalent zinc, magnesium, or manganese (data not shown). These data are consistent with a calcium-dependent serine protease being responsible for IGFBP-5 proteolysis in rat synovial fluid.

In order to test the hypothesis that inhibition of IGFBP-5 proteolytic activity would alter disease progression in the rat meniscal tear model of OA, we sought out a peptide inhibitor of the activity that would be suitable for intra-articular administration. Peptide PB-145 has been previously described to be an efficient inhibitor of the IGFBP-5 proteolytic activity found in model canine OA joints.20,33 Furthermore, intra-articular injections of PB-145 in the canine ACLT model resulted in decreased disease progression and alterations in IGF-1 metabolism.20 Therefore, we tested the ability of PB-145 to inhibit the IGFBP-5 proteolytic activity derived from rat MMT synovial fluid. As shown in Figure 3, PB-145 inhibits IGFBP-5 proteolysis in the low micromolar range, consistent with its ability to inhibit canine-derived IGFBP-5 proteolytic activity. This result indicated that the inhibitory activity of PB-145 crossed species and that the peptide would be useful as an in vivo tool in the rat.

We next determined if PB-145 would protect cartilage from lesion development in the rat MMT model of OA. Beginning 1 week after MMT, rats were given twice-weekly intra-articular injections of PB-145 or saline as a vehicle control. An additional group of animals did not receive intra-articular injections (no treatment controls). Figure 4A shows India ink staining of an entire tibial plateau 4 weeks after MMT with no treatment. The darkest areas of staining on the medial tibial plateau (left side) indicate deep focal lesions that span nearly the full depth of the cartilage to the underlying subchondral bone. The lighter stained areas represent less severe cartilage degeneration. Note the smooth cartilage and lack of any staining on the lateral tibial plateau (right side), which is typical of this model. Figure 4B depicts the medial aspects of the tibial plateaus from 2 representative animals in the vehicle (saline) treated group. Again, deep focal lesions with an extended area of staining and fibrillation are present. Treatment with PB-145 reduced the depth and severity of lesions. Figure 4C shows medial tibial plateaus from the low-dose PB-145 group (100 ug/injection).
Focal lesions are still present, but there are no full-thickness lesions, and the fibrillated area is shallower in depth, and staining with India ink is less intense. Figure 4D shows medial tibial plateaus from the high-dose PB-145 group (500 ug/injection). Instead of focal lesions, there is a thick layer of cartilage with a rough, slightly fibrillated surface and faint India ink staining.

To quantify the severity of cartilage lesions, a scoring system was devised that included a measure to reflect the apparent thicker cartilage in PB-145–treated joints (see Methods for details). The tibial surface was first imaged horizontally, and the area of India ink staining was outlined manually for the total area of lesions. The depth of lesions was evaluated by physical probing. Because the total area of cartilage lesions was not necessarily decreased in PB-145 samples, a new measure designated “skyline” depth or concavity was generated from viewing the medial tibial plateau from the anterior to posterior direction. A line drawn from the highest point on the inside (nearest the cruciate ligaments) to the highest point on the outside of the tibial plateau represents zero concavity. From this line, a perpendicular line was drawn to the deepest part of the curve, which was recorded as the “skyline” depth in pixels (Fig. 5A). Representative skyline photographs from group 2 (vehicle) (Fig. 5B) and group 3 (PB-145 100 ug) (Fig. 5C) show the reduced skyline depth in PB-145–treated animals. The
skyline data from all animals in groups 1 to 4 (Fig. 5D) indicate a dose response reduction in the depth of lesions with PB-145 treatment. In order to compare the combined data from all measurements, a total score was calculated that included multiplying the surface irregularity score by the area of India ink staining and then adding the staining data from all measurements, a total score was calculated with PB-145 treatment. In order to compare the combined indicate a dose response reduction in the depth of lesions down.35 Four weeks and 1 day after MMT, TIINE levels the synovial fluid or urine is a marker of cartilage break-

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Figure 4. India ink staining of rat tibial plateaus 4 weeks after medial meniscal tear (MMT) surgery. Representative photographs are shown for each group and depict either the entire tibial plateau (group 1) or medial tibial plateaus (groups 2-4). (A) Group 1, no intra-articular treatment. (B) Group 2, intra-articular injections of vehicle. (C) Group 3, intra-articular injections of 100 ug PB-145. (D) Group 4, intra-articular injections of 500 ug PB-145. Injections were given twice weekly for 3 weeks starting 1 week after MMT surgery.

Figure 5. Skyline concavity measurement of the medial tibial surface. (A) Medial tibial plateaus were viewed from anterior to posterior across the tibial surface and photographs taken. The depth of the concavity of the plateaus was measured in pixels and represents the “skyline concavity” measurement or depth. Skyline depth in normal healthy joints is 0 to 30 pixels. (B) Representative skyline photograph of group 2 (vehicle) tibial surface. (C) Representative skyline photograph of group 3 (PB-145 100 ug) tibial surface. (D) Measured skyline depths from all animals in the 4 groups (\(*P = 0.02\) based on a 2-sided t test for independent samples, \(*\ast\ast\ast P = 0.027\) based on a 1-sided t test for independent samples).
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model of OA, IGFBP-3 and -4 were upregulated, and these changes were accompanied by increases in IGF-1. Iwanaga et al. showed increased expression of IGFBP-3, -4, and -5 as well as their degradation products in OA articular cartilage as compared to control. Large increases in the concentrations of IGFBPs in extracellular fluid can limit IGF-1 access to receptors. Consistent with this, pharmacological disruption of the IGF-1 association with IGFBP-3 resulted in improvement in proteoglycan synthesis in human OA cartilage, suggesting that IGFBP-3 was inhibiting IGF-1 actions. However, studies by Duan et al. suggested that IGFBP-3 had an important positive modulatory role on IGF-1 activity in chondrogenesis during development. Kiepe et al. reported that IGFBP-1, -2, -4, and -6 act exclusively as growth inhibitors of chondrocytes in vitro; however, they obtained more complex results with IGFBP-3 showing both potentiation and inhibition of the effects of IGF-1 under different conditions. In contrast to other IGFBPs, IGFBP-5 enhanced IGF-1–stimulated growth plate chondrocyte proliferation, differentiation, and matrix production. It appears that IGFBP-5 plays a positive role in IGF-1 signaling in cartilage and that protecting IGFBP-5 from cleavage should help maintain anabolic responses in disease states such as OA. However, a recent report that ADAM12 cleavage of IGFBP-5 enhances IGF-1–driven chondrocyte proliferation indicates that IGF-1 signaling in chondrocytes may be more complex.

In order to inhibit IGFBP-5 protease activity in the dog ACLT model of OA, Clemmons et al. utilized intra-articular injections of peptide PB-145. PB-145 is a peptide that was originally designed as an inhibitor of complement activation and does indeed inhibit C1s. In order to rule out the possibility that PB-145 may inhibit a more general pathway of cartilage degradation, we determined that PB-145 did not inhibit a panel of matrix metalloproteinases including MMP-1, -2, -3, -9, and -13 as well as the serine protease PACE4 (data not shown). In this study, we showed that PB-145 inhibited the protease activity towards IGFBP-5 from rat MMT synovial fluid (Fig. 3), indicating this peptide would be useful as a tool to determine the consequence of inhibiting IGFBP-5 proteolysis on cartilage degeneration. Intra-articular injection of PB-145 in the rat MMT model of OA resulted in dramatic changes in the appearance of cartilage lesions as well as the thickness of cartilage. Lesions were

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\text{Total Tibial Score} = (\text{Irregularity} \times \text{Area}) + \text{Staining} + \text{Skyline}
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- **Surface Irregularity**
  - 4.00 mostly full thickness cartilage lesions
  - 3.00 mixed lesion depth, some full thickness
  - 2.00 deep, irregular fibrillation of surface
  - 1.00 rough surface
  - 0.00 normal smooth surface

- **Staining Intensity**
  - 3.00 dark
  - 2.00 medium
  - 1.00 faint
  - 0.00 none

- **Skyline Concavity**
  - 4.00 121-150 pixels
  - 3.00 91-120
  - 2.00 61-90
  - 1.00 31-60
  - 0.00 0-30

**Figure 6.** Scoring of joints for cartilage lesion severity. (A) Scoring system. (B) Surface irregularity scores (*P < 0.05). (C) Staining intensity scores (*P < 0.05). (D) Total tibial degeneration scores (*P < 0.05). Total tibial score of a normal healthy joint is zero. Statistical analyses based on a 2-sided t test for independent samples.
more superficial in nature and stained less intensely with India ink. Although the area of each lesion was not necessarily smaller, the cartilage appeared thicker than in untreated or vehicle-treated controls, especially in the high-dose PB-145 group (Fig. 4). In order to capture quantitatively the apparent differences in cartilage thickness, a new measurement was employed that takes advantage of the natural concavity of the medial aspect of the tibial plateau when viewed along the cartilage surface. PB-145 treatment reduced medial tibial plateau concavity in a dose-dependent manner (Fig. 5), suggesting there was increased cartilage thickness in treated animals as compared to control and vehicle-treated animals. Because the cartilage showed signs of being subjected to a mechanically unstable joint (rough surface), yet was thick, having lost its natural concavity, and was protected from deep lesion formation, it is tempting to speculate that PB-145 promoted an anabolic response that was able to counteract the catabolic forces on the cartilage; however, direct evidence for this is still lacking.

Analysis of biomarkers of collagen turnover suggests that degradation of type II collagen (TIINE) was increased in the high-dose PB-145 group (Fig. 7A). In light of the fact that this group of animals showed the highest degree of protection from lesion development and exhibited a thick, yet rough surfaced layer of cartilage, the increase in the level of TIINE can be interpreted as the result of having an increased amount of substrate (cartilage) present at the location where mechanical instability promotes cartilage breakdown. The ability to maintain a layer of cartilage on the medial tibial plateau while catabolic forces result in the continuous degradation of type II collagen suggests that anabolism and new collagen synthesis are playing a role. Because of the limited amount of sample, we were unable to measure the type II collagen synthesis marker CPII directly in the synovial fluid lavages. However, CPII in the serum showed a trend upward with PB-145 treatment. It is likely that the high background of CPII in the serum of growing animals precluded us from getting a statistically significant difference based on events that were occurring in a single joint. Therefore, we cannot conclude definitively that PB-145 is protecting cartilage due to promoting an anabolic response to counteract the catabolic forces of mechanical instability. With respect to inhibiting IGFBP-5 proteolysis, we again were not able to measure the levels of intact IGFBP-5 and IGF-1 in the rat synovial fluid lavages due to dilution from the lavage technique and limited sample volumes. In the canine ACLT model, Clemmons et al. were able to demonstrate increases in both IGFBP-5 and IGF-1 in joint fluids from dogs treated with PB-145. This was associated with improved joint architecture, increased proteoglycan staining, and an increase in the depth of the chondrocyte layers within cartilage, consistent with a robust reparative response.20 In order to more thoroughly understand the action of PB-145 in the rat MMT model, it may be necessary to study the association between levels of IGFBP-5 and IGF-1 and the carefully monitored levels of markers of cartilage synthesis and degradation during the course of OA disease progression.

In summary, our findings support the conclusion that inhibition of IGFBP-5 proteolysis is chondroprotective in experimental animal models of OA. Inhibiting IGFBP-5 proteolysis would be expected to increase the amount of intact IGFBP-5 and IGF-1 in joint fluids from dogs treated with PB-145. This was associated with improved joint architecture, increased proteoglycan staining, and an increase in the depth of the chondrocyte layers within cartilage, consistent with a robust reparative response.20 In order to more thoroughly understand the action of PB-145 in the rat MMT model, it may be necessary to study the association between levels of IGFBP-5 and IGF-1 and the carefully monitored levels of markers of cartilage synthesis and degradation during the course of OA disease progression.

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References

1. Sandell LJ, Adler P. Developmental patterns of cartilage. Front Biosci. 1999;4:D731-42.
2. Sandell LJ, Aigner T. Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. Arthritis Res. 2001;3(2):107-13.
3. van Osch GJ, van den Berg WB, Hunziker EB, Hauelsmann HJ. Differential effects of IGF-1 and TGF beta-2 on the assembly of proteoglycans in pericellular and territorial matrix by cultured bovine articular chondrocytes. Osteoarthritis Cartilage. 1998;6(3):187-95.
4. Hill DJ, Holder AT, Seid J, Preece MA, Tomlinson S, Milner RD. Increased thymidine incorporation into fetal rat cartilage in vitro in the presence of human somatomedin, epidermal growth factor and other growth factors. J Endocrinol. 1983;96(3):489-97.
5. Isaksson OG, Lindahl A, Nilsson A, Isgaard J. Mechanism of the stimulatory effect of growth hormone on longitudinal bone growth. Endocr Rev. 1987;8(4):426-38.
6. Schlechter NL, Russell SM, Spencer EM, Nicoll CS. Evidence suggesting that the direct growth-promoting effect of growth hormone on cartilage in vivo is mediated by local production of somatomedin. Proc Natl Acad Sci U S A. 1986;83(20):7932-4.
7. Trippel SB. Growth factor actions on articular cartilage. J Rheumatol Suppl. 1995;43:129-32.
8. Ashton IK, Matheson JA. Change in response with age of human articular cartilage to plasma somatomedin activity. Calcif Tissue Int. 1979;29(2):89-94.
9. Verschure PJ, Van Noorden CJ, Van Marlen J, Van den Berg WB. Articular cartilage destruction in experimental inflammatory arthritis: insulin-like growth factor-1 regulation of proteoglycan metabolism in chondrocytes. Histochem J. 1996;28(12):835-57.
10. Verschure PJ, Joosten LA, van der Kraan PM, Van den Berg WB. Responsiveness of articular cartilage from normal and inflamed mouse knee joints to various growth factors. Ann Rheum Dis. 1994;53(7):455-60.
11. Martin JA, Ellerbroek SM, Buckwalter JA. Age-related decline in chondrocyte response to insulin-like growth factor-I: the role of growth factor binding proteins. J Orthop Res. 1997;15(4):491-8.
12. Trippel SB. Growth factor inhibition: potential role in the etiopathogenesis of osteoarthritis. Clin Orthop Relat Res. 2004;427 Suppl:S47-52.
13. Duan C, Xu Q. Roles of insulin-like growth factor (IGF) binding proteins in regulating IGF actions. Gen Comp Endocrinol. 2005;142(1-2):44-52.
14. Iwanaga H, Matsumoto T, Enomoto H, Okano K, Hishikawa Y, Shindo H, et al. Enhanced expression of insulin-like growth factor-binding proteins in human osteoarthritic cartilage detected by immunohistochemistry and in situ hybridization. Osteoarthritis Cartilage. 2005;13(5):439-48.
15. Taverna C, Abribat T, Reboul P, Dore S, Brazeau P, Pelletier JP, et al. IGF and IGF-binding protein system in the synovial fluid of osteoarthritic and rheumatoid arthritic patients. Osteoarthritis Cartilage. 1996;4(4):263-74.
16. Olney RC, Wilson DM, Mohtai M, Fielder PJ, Smith RL. Interleukin-1 and tumor necrosis factor-alpha increase insulin-like growth factor-binding protein-3 (IGFBP-3) production and IGFBP-3 protease activity in human articular chondrocytes. J Endocrinol. 1995;146(2):279-86.
17. Tardif G, Reboul P, Pelletier JP, Geng C, Cloutier JM, Martel-Pelletier J. Normal expression of type I insulin-like growth factor receptor by human osteoarthritic chondrocytes with increased expression and synthesis of insulin-like growth factor binding proteins. Arthritis Rheum. 1996;39(6):968-78.
18. Olney RC, Tsuchiya K, Wilson DM, Mohtai M, Maloney WJ, Schurman DJ, et al. Chondrocytes from osteoarthritic cartilage have increased expression of insulin-like growth factor I (IGF-I) and IGF-binding protein-3 (IGFBP-3) and -5, but not IGF-II or IGFBP-4. J Clin Endocrinol Metab. 1996;81(3):1096-103.
19. Kiepe D, Ciaramatori S, Haarmann A, Tonshoff B. Differential expression of IGF system components in proliferating vs. differentiating growth plate chondrocytes: the functional role of IGFBP-5. Am J Physiol Endocrinol Metab. 2006;290(2):E363-71.
20. Clemmons DR, Busby WH Jr, Garmong A, Schultz DR, Howell DS, Altman RD, et al. Inhibition of insulin-like growth factor binding protein 5 proteolysis in articular cartilage and joint fluid results in enhanced concentrations of insulin-like growth factor I and is associated with improved osteoarthritis. Arthritis Rheum. 2002;46(3):694-703.
21. Matsumoto T, Tsurumoto T, Goldring MB, Shindo H. Differential effects of IGF-binding proteins, IGFBP-3 and IGFBP-5, on IGF-I action and binding to cell membranes of immortalized human chondrocytes. J Endocrinol. 2000;166(1):29-37.
22. Rajaram S, Baylink DJ, Mohan S. Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. Endocr Rev. 1997;18(6):801-31.
23. Whellams EJ, Maile LA, Fernihough JK, Billingham ME, Holly JM. Alterations in insulin-like growth factor binding
proteins and complex formation in the arthritic joint. J Endocrinol. 2000;165(3):545-56.
24. Martel-Pelletier J, Di Battista JA, Lajeunesse D, Pelletier JP. IGF/IGFBP axis in cartilage and bone in osteoarthritis pathogenesis. Inflamm Res. 1998;47(3):90-100.
25. Busby WH Jr, Yocum SA, Rowland M, Kellner D, Lazerwith S, Sverdrup F, et al. Complement 1s is the serine protease that cleaves IGFBP-5 in human osteoarthritic joint fluid. Osteoarthritis Cartilage. 2009;17(4):547-55.
26. Busby WH Jr, Yocum SA, Rowland M, Kellner D, Lazerwith S, Sverdrupt F, et al. Complement 1s is the serine protease that cleaves IGFBP-5 in human osteoarthritic joint fluid. Osteoarthritis Cartilage. 2009;17(4):547-55.
27. Kerr FK, O’Brien G, Quinsey NS, Whistock JC, Boyd S, de la Banda MG, et al. Elucidation of the substrate specificity of the Cls protease of the classical complement pathway. J Biol Chem. 2005;280(47):39510-4.
28. Eriksson H, Nissen MH. Proteolysis of the heavy chain of major histocompatibility complex class I antigens by complement component Cls. Biochim Biophys Acta. 1990;1037(2):209-15.
29. Nakagawa K, Sakiyama H, Tsuchida T, Yamaguchi K, Toyoguchi T, Masuda R, et al. Complement Cls activation in degenerating articular cartilage of rheumatoid arthritis patients: immunohistochemical studies with an active form specific antibody. Ann Rheum Dis. 1999;58(3):175-81.
30. Yamaguchi K, Sakiyama H, Matsumoto M, Moriya H, Sakiyama S. Degradation of type I and II collagen by human activated Cls-1. FEBS Lett. 1990;268(1):206-8.
31. Janusz MJ, Bendele AM, Brown KK, Taiwo YO, Hsieh L, Heitmeyer SA. Induction of osteoarthritis in the rat by surgical tear of the meniscus: inhibition of joint damage by a matrix metalloproteinase inhibitor. Osteoarthritis Cartilage. 2002;10(10):785-91.
32. Bendele A, McComb J, Gould T, McAbee T, Sennello G, Chipala E, et al. Animal models of arthritis: relevance to human disease. Toxicol Pathol. 1999;27(1):134-42.
33. Schasteen CS, Levine RP, McLafferty SA, Finn RF, Bullock LD, Mayden JC, et al. Synthetic peptide inhibitors of complement serine proteases. III: significant increase in inhibitor potency provides further support for the functional equivalence hypothesis. Mol Immunol. 1991;28(1-2):17-26.
34. Busby WH Jr, Nam TJ, Moralez A, Smith C, Jennings M, Clemmons DR. The complement component Cls is the protease that accounts for cleavage of insulin-like growth factor-binding protein-5 in fibroblast medium. J Biol Chem. 2000;275(48):37638-44.
35. Elsaid KA, Chichester CO. Review: collagen markers in early arthritic diseases. Clin Chim Acta. 2006;365(1-2):68-77.
36. Hou J, Clemmons DR, Smeekens S. Expression and characterization of a serine protease that preferentially cleaves insulin-like growth factor binding protein-5. J Cell Biochem. 2005;94(3):470-84.
37. Mohan S, Thompson GR, Amaar YG, Hathaway G, Tschesche H, Baylink DJ. ADAM-9 is an insulin-like growth factor binding protein-5 protease produced and secreted by human osteoblasts. Biochemistry. 2002;41(51):15394-403.
38. Loechel F, Fox JW, Murphy G, Albrechtsen R, Wewer UM. ADAM 12-S cleaves IGFBP-3 and IGFBP-5 and is inhibited by TIMP-3. Biochem Biophys Res Commun. 2000;278(3):511-5.
39. Okada A, Mochizuki S, Yatabe T, Kimura T, Shiomi T, Fujita Y, et al. ADAM-12 (meltrin alpha) is involved in chondrocyte proliferation via cleavage of insulin-like growth factor binding protein 5 in osteoarthritic cartilage. Arthritis Rheum. 2008;58(3):778-89.
40. Overgaard MT, Boldt HB, Laursen LS, Sottrup-Jensen L, Conover CA, Oxvig C. Pregnancy-associated plasma protein-A2 (PAPP-A2): a novel insulin-like growth factor-binding protein-5 proteinase. J Biol Chem. 2001;276(24):21849-53.
41. Fowlkes JL, Thraillkill KM, Serra DM, Suzuki K, Nagase H. Matrix metalloproteinases as insulin-like growth factor binding protein-degrading proteinases. Prog Growth Factor Res. 1995;6(2-4):255-63.
42. Grau S, Richards PJ, Kerr B, Hughes C, Caterson B, Williams AS, et al. The role of human HtrA1 in arthritic disease. J Biol Chem. 2006;281(10):6124-9.
43. Tsuchiya A, Yano M, Tocharus K, Kojima H, Fukumoto M, Kawaichi M, et al. Expression of mouse HtrA1 serine protease in normal bone and cartilage and its upregulation in joint cartilage damaged by experimental arthritis. Bone. 2005;37(3):323-36.
44. Villiers CL, Arland GJ, Colomb MG. Domain structure and associated functions of subcomponents C1r and C1s of the first component of human complement. Proc Natl Acad Sci U S A. 1985;82(13):4477-81.
45. Villiers CL, Arland GJ, Painter RH, Colomb MG. Calcium binding properties of the C1 subcomponents C1q, C1r and C1s. FEBS Lett. 1980;117(1):289-94.
46. Andrews JM, Baillie RD. The enzymatic nature of human c1r: a subcomponent of the first component of complement. J Immunol. 1979;123(3):1403-8.
47. Appleton CT, Pitelka V, Henry J, Beier F. Global analyses of gene expression in early experimental osteoarthritis. Arthritis Rheum. 2007;56(6):1854-68.
48. Barve RA, Minnerly JC, Weiss DJ, Meyer DM, Aguier DJ, Sullivan PM, et al. Transcriptional profiling and pathway analysis of monosodium iodoacetate-induced experimental osteoarthritis in rats: relevance to human disease. Osteoarthritis Cartilage. 2007;15(10):1190-8.
49. Rogachevsky RA, Dean DD, Howell DS, Altman RD. Treatment of canine osteoarthritis with insulin-like growth factor-1 (IGF-1) and sodium pentosan polysulfate. Osteoarthritis Cartilage. 1993;1(2):105-14.
50. Tesch GH, Handley CJ, Cornell HJ, Herington AC. Effects of free and bound insulin-like growth factors on proteoglycan
metabolism in articular cartilage explants. J Orthop Res. 1992; 10(1):14-22.

51. Schmidmaier G, Wildemann B, Ostapowicz D, Kandziora F, Stange R, Haas NP, et al. Long-term effects of local growth factor (IGF-I and TGF-beta 1) treatment on fracture healing: a safety study for using growth factors. J Orthop Res. 2004; 22(3):514-9.

52. Wildemann B, Schmidmaier G, Ordel S, Stange R, Haas NP, Raschke M. Cell proliferation and differentiation during fracture healing are influenced by locally applied IGF-I and TGF-beta1: comparison of two proliferation markers, PCNA and BrdU. J Biomed Mater Res B Appl Biomater. 2003;65(1): 150-6.

53. Fortier LA, Mohammed HO, Lust G, Nixon AJ. Insulin-like growth factor-I enhances cell-based repair of articular cartilage. J Bone Joint Surg Br. 2002;84(2):276-88.

54. Madry H, Kaul G, Cucchiarini M, Stein U, Zurakowski D, Remberger K, et al. Enhanced repair of articular cartilage defects in vivo by transplanted chondrocytes overexpressing insulin-like growth factor I (IGF-I). Gene Ther. 2005;12(15): 1171-9.

55. Smith P, Shuler FD, Georgescu HI, Ghivizzani SC, Johnstone B, Niyibizi C, et al. Genetic enhancement of matrix synthesis by articular chondrocytes: comparison of different growth factor genes in the presence and absence of interleukin-1. Arthritis Rheum. 2000;43(5):1156-64.

56. Tyler JA. Insulin-like growth factor 1 can decrease degradation and promote synthesis of proteoglycan in cartilage exposed to cytokines. Biochem J. 1989;260(2):543-8.

57. Loeser RF, Pacione CA, Chubinskaya S. The combination of insulin-like growth factor I and osteogenic protein I promotes increased survival of and matrix synthesis by normal and osteoarthritic human articular chondrocytes. Arthritis Rheum. 2003;48(8):2188-96.

58. Fernihough JK, Billingham ME, Cwyfan-Hughes S, Holly JM. Local disruption of the insulin-like growth factor system in the arthritic joint. Arthritis Rheum. 1996;39(9):1556-65.

59. De Ceuninck F, Caliez A, Dassencourt L, Anract P, Renard P. Pharmacological disruption of insulin-like growth factor I binding to IGF-binding proteins restores anabolic responses in human osteoarthritic chondrocytes. Arthritis Res Ther. 2004; 6(5):R393-403.