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Biological impact of the fibroblast growth factor family on articular cartilage and intervertebral disc homeostasis:

(basic FGF, FGF-18, osteoarthritis, IVD degeneration)

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I. Introduction

Osteoarthritis (OA) and degenerative disc disease (DDD) are prevalent diseases in the United States with staggering socioeconomic effects on today's society. OA is the leading cause of disability among the elderly population (Buckwalter et al., 2004), while DDD is thought to be one mechanism of chronic back pain (Buckwalter, 1995; Andersson, 1999; Freemont et al., 2001), and together OA and DDD constitute a major source of health care costs. At present, the pathogenesis of these two conditions is largely unknown, but both involve the progressive deterioration of cartilaginous tissue. Recent literature has focused on understanding many of the biochemical processes involved in the pathogenesis of OA and DDD with the intention of developing novel therapies aimed at slowing and/or reversing cartilage degradation.

Under normal conditions, both articular chondrocytes and intervertebral disc (IVD) cells maintain a dynamic equilibrium between synthesis and degradation of extracellular matrix (ECM) components, including collagen fibrils that form a network surrounding and restraining large, hydrated aggregates of the proteoglycan (PG) aggrecan (Goldring, 2000; Masuda, 2006). In degenerative states, however, there is a disruption of matrix equilibrium leading to progressive loss of cartilage tissue and clonal expansion of cells in the depleted regions. Chondrocyte metabolism is unbalanced due to excessive production of catabolic factors, including matrix metalloproteases (MMPs), aggrecanases (ADAMTS), and other cytokines and growth factors released by chondrocytes that aid in the destruction of PGs and the ECM (Im et al., 2008; Im et al., 2007; Muddasani et al., 2007). For example, as matrix equilibrium shifts to a pro-catabolic state with advancing degeneration in articular cartilage, collagenase-mediated degradation of type II collagen becomes more prominent (Hollander et al., 1994; Billingham et al., 1997). Among the collagenases, collagenase-3 (MMP-13) has been found to play a significant role in the development of both OA and DDD (Billinghurst et al., 1997; Fernandes et al., 1998; Anderson et al., 2002; Le Maitre et al., 2004). In articular cartilage, MMP-13 is almost exclusively produced by chondrocytes and has a dual role in ECM

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destruction as it degrades both aggrecan and collagen type II (Fosang et al., 1996; Mitchell et al., 1996; Reboul et al., 1996; Fernandes et al., 1998). In the IVD, MMP-13 expression increases with increasing severity of disc degeneration (Le Maitre et al., 2004). Therefore, defining the key factors, receptors, and regulators of MMP-13 expression is important to clearly understand the molecular etiology of OA and DDD.

One particular family of growth factors, the fibroblast growth factor (FGF) family, has been implicated in the regulation of both articular cartilage and IVD homeostasis. This large family of structurally-related proteins binds heparin and heparan sulfate (Friedl et al., 1997) and modulates the growth, differentiation, migration and survival of a wide variety of cell types. Specifically, two particular members of the FGF family, basic fibroblast growth factor (bFGF; also known as FGF-2) and fibroblast growth factor-18 (FGF-18), have been found to play prominent regulatory roles in cartilage matrix homeostasis.

In cartilage, bFGF is produced by chondrocytes, stored in the ECM, and immediately released from the ECM upon cartilage injury (Vincent et al., 2002; Vincent et al., 2004). Several studies have shown a potent mitogenic impact of bFGF in growth plate cartilage (Rosselot et al., 1994) and adult articular cartilage (Osborn et al., 1989; Stewart et al., 2007). However, studies on bFGF from a variety of species have yielded contradictory results with regards to production of ECM in articular cartilage and IVD matrix homeostasis, and the specific role of bFGF on cartilage homeostasis remains controversial. Here, we will review the relevant literature with regards to the role of bFGF in both articular cartilage and IVD metabolism.

In addition, we will review pertinent findings regarding a second member of the FGF family, FGF-18, in articular cartilage homeostasis. In contrast to the controversial role of bFGF in joint and spine disc cartilage, FGF-18 is a well-known anabolic growth factor involved in osteogenesis, chondrogenesis, and articular cartilage repair (Ellsworth et al., 2002; Liu et al., 2002; Ohbayashi et al., 2002; Davidson et al., 2005; Moore et al., 2005), and here we will review its role in joint cartilage. To date, the role of FGF-18 in the IVD has yet to be studied. Additionally, we will examine the specific cell surface receptors utilized by both FGF-18 and bFGF in cartilage tissue as each factor binds to distinct receptors of the tyrosine kinase FGF receptor family (FGFR1-4). We will also review the unique signaling cascades and molecular pathways utilized by bFGF and FGF-18 to exert their biological effects.

II. Basic FGF

(a) Actions of bFGF in articular cartilage

Basic FGF, a well-known member of the FGF family, was originally isolated and identified from bovine brain and pituitary based on its stimulatory activity on fibroblast proliferation (Bohlen et al., 1984; Lobb et al., 1986). It has been extensively studied in the literature and is found to be involved in numerous cellular functions in various cell types, including angiogenesis, tumorigenesis, cell proliferation, differentiation, wound healing, limb formation, and tissue remodeling (Bodo et al., 2002; Bobick et al., 2007; Douwes Dekker et al., 2007; Kakudo et al., 2007; Kanayama et al., 2007; Pratsinis and Kletsas, 2007; Schmal et al., 2007; Choi et al., 2008). In chondrocytes, the role of bFGF as an important cell growth regulator in the growth plate is already well established (Kilkenny and Hill, 1996). However, reports on the action of bFGF in adult articular cartilage are contradictory.

Many studies have implied a potent anabolic effect of bFGF on cartilage homeostasis and suggested its use for cartilage regeneration and repair (Cuevas et al., 1988; Cucchiari et al., 2005; Hiraide et al., 2005; Inoue et al., 2006; Kaul et al., 2006; Deng et al., 2007; Schmal et al., 2007; Stewart et al., 2007). For example, Hiraide et al documented cartilage repair in an *in vivo* model of rabbit knee degeneration using an adeno-associated virus (AAV) to transport

the bFGF gene into knee synovial tissue. Semi-quantitative scores based on macroscopic and histologic repair indicated that the average score was significantly better in the bFGF-transduced group compared to control (AAV plus phosphate-buffered saline), suggesting the potential of bFGF to promote repair using viral vector transduction. More recently, bFGF has been used in scaffold models of cartilage regeneration with promising results (Inoue et al., 2006; Deng et al., 2007; Stewart et al., 2007). Deng et al used gelatin microspheres loaded with bFGF for controlled and sustained release and stimulated repair of knee cartilage defects in rabbits. They found that after 24 weeks, previous cartilaginous defects were filled with hyaline-like cartilage histologically, illustrating the potential of a bFGF scaffold to promote chondrogenesis. Others have reported a positive effect of bFGF on cell differentiation and viability despite a bFGF-mediated downregulation of collagen type II mRNA (Schmal et al., 2007).

One plausible explanation for the success of bFGF in cartilage regeneration is the potent mitogenic impact of this growth factor on cartilage (Osborn et al., 1989; Rosselot et al., 1994; Loeser et al., 2005; Stewart et al., 2007). In bovine adult articular cartilage, bFGF has been associated with a modest stimulation of PG synthesis and cell proliferation (Sah et al., 1994). In lapine articular cartilage, bFGF introduced via gene transfer was found to increase cell proliferation both *in vitro* and *in vivo*, suggesting its potential for repair and regeneration of cartilage defects (Kaul et al., 2006). Due to its proliferative capacity, several studies have identified bFGF as an anabolic factor in chondrogenesis and ECM homeostasis (Thompson et al., 1991; Kaul et al., 2006; Tsai et al., 2007).

In contrast, findings from other groups (Qu et al., 1995; Tchetina et al., 2005; Stewart et al., 2007) and our laboratory (Loeser et al., 2005; Muddasani et al., 2007; Li et al., 2008) suggest that the mitogenic effect of bFGF in human articular chondrocytes or spine disc cells may be a pathologic sign of degeneration rather than regeneration. As an example, although bFGF exerts a potent mitogenic effect on human adult articular cartilage (Loeser et al., 2005) and, more dramatically, on bovine disc cells in a dose-dependent manner (Li et al., 2008), as represented by amount of DNA in alginate bead culture after 21 days, the presence of bFGF failed to demonstrate a simultaneous increase in ECM production. Consequently, stimulation with bFGF results in clustering of cells with little surrounding ECM, a hallmark of arthritic cartilage. Further, Muddasani et al (2008) suggested that the mitogenic effect of bFGF in articular chondrocytes may result from increased turnover of fibroblast-like cells rather than chondrocytes. We found that treatment of human articular chondrocytes with bFGF stimulated an upregulation of both collagen type I and collagen type II mRNA expression, but the ratio of collagen type II:I, which is critical for proper function of mature articular cartilage, decreased. These results suggest that bFGF may promote the formation of fibrocartilage that is a poor substitute for hyaline cartilage (Muddasani et al., 2008). Others have found similar results in ovine meniscal chondrocytes as bFGF mediated a decrease in the ratio of collagen type II relative to collagen type I (Stewart et al., 2007). Poor cartilage quality, in particular in joint cartilage, may accelerate further breakdown of the ECM and generate matrix fragmentation, and these fragmented products may further upregulate cartilage-degrading enzyme production, resulting in a vicious circle that impairs the cartilage repair mechanism.

In addition, a striking antagonistic effect of bFGF has been reported on the well-known cartilage anabolic activity of insulin-like growth factor-1 (IGF-1) and bone morphogenetic protein 7 (BMP7, also known as osteogenic protein-1), as well as a combination of these two growth factors in alginate beads and within cartilage explants (Loeser et al., 2005). Surprisingly, this antagonistic effect of bFGF does not inhibit cellular proliferation mediated by IGF-1 and/or BMP7 as was seen with PG production; rather, bFGF further promoted cellular proliferation when combined with other anabolic growth factors, leading to decreased PG production per cell and clustering of cells. Similarly, others have found bFGF to be a potent

stimulator of cell cluster formation after retroviral transduction of a cDNA library in functional genomic screens for the novel mediators of OA (Daouti et al., 2005; Quintavalla et al., 2005).

Further evidence suggests that bFGF is pathologically associated with joint destruction via upregulation of MMPs and aggrecanases, as well as the stimulation of reactive oxygen species such as nitric oxide and superoxide anion (well-known catabolic factors for articular cartilage and spine discs), revealing the potential catabolic activity of bFGF in articular cartilage (Qu et al., 1995; Tchetina et al., 2005; Im et al., 2007; Muddasani et al., 2007; Muddasani et al., 2008). In patients with degenerative joint disease after traumatic injury, for example, the expression of bFGF is highly upregulated in synovial tissues of arthritic joints compared to normal joints (Cameron et al., 1994; Qu et al., 1995). Manabe et al found that bFGF levels in synovial fluid of human rheumatoid arthritis (RA) joints strongly correlated with Larsen's grade (a measure of bone destruction), demonstrating a pathogenic role of bFGF in progressive joint destruction (Manabe et al., 1999). In both human OA joints (Orito et al., 2003) and rat joints with adjuvant-induced arthritis (Yamashita et al., 2002), a higher concentration of bFGF was correlated with increasing severity of disease. Additionally, bFGF was shown to act as an antagonist for the production of type II collagen and decorin promoted by anabolic growth factors such as IGF-1 and transforming growth factor- β (TGF- β) in porcine articular chondrocytes (Sonal, 2001).

More recently, bFGF has been associated with synovial proliferation and hyperplasia in RA joints, suggesting a role of this growth factor in defective apoptosis. Basic FGF-treated RA synoviocytes were relatively resistant to apoptosis induced by Fas-activated death-domain (FADD) protein transfection compared to apoptosis induced by TNF- α (Kobayashi et al., 2000). The expression of the anti-apoptosis protein Fas-like inhibitory protein (FLIP; also a potential activator of pro-caspase-8) was also augmented in bFGF-treated RA synoviocytes (Kobayashi et al., 2000). Taken together, these results suggest that bFGF has the capacity to dampen Fas-activated apoptosis in RA synovium, and FADD overexpression could not ameliorate apoptosis resistance of bFGF-treated RA synoviocytes *in vitro*, contributing to synovial hyperplasia and cell proliferation associated with RA cartilage.

Results from our laboratory further support potent catabolic and anti-anabolic effects of bFGF on human articular cartilage homeostasis. Stimulation of human articular chondrocytes isolated from knee and ankle cartilage (both normal and OA) with bFGF was shown to upregulate MMP-13 and ADAMTS-4 and -5 expression (aggrecanases), inhibit PG production and synthesis in a time and dose-dependent manner, antagonize the activity of anabolic factors BMP7 and IGF-1, and stimulate pro-inflammatory cytokines such as IL-1 and TNF- α , elucidating the degradative effects of bFGF on articular cartilage homeostasis (Loeser et al., 2005; Im et al., 2008; Im et al., 2007; Muddasani et al., 2007). Others have reported similar findings in cultured human chondrosarcoma cells (Uria et al., 1998) and osteoblasts (Varghese et al., 2000), revealing that treatment with bFGF increases MMP-13 expression in a dose and time-dependent manner.

All of the above studies suggest that the role of bFGF in articular cartilage is still not clearly understood with respect to ECM regulation. Further exploration into the precise role of bFGF and its cognate receptors responsible for the biological effects on articular cartilage may serve to clarify the potential of bFGF agonists/antagonists for use as therapeutic targets in cartilage degenerative diseases.

(b) Actions of bFGF in the IVD

Like knee joint OA, matrix disequilibrium in the intervertebral disc (IVD) leads to tissue degeneration over time. Structurally, the IVD contains a tough outer layer composed mainly of collagen secreted by disc cells, collectively termed the annulus fibrosus (AF), and a

gelatinous inner core containing a high concentration of aggrecan, termed the nucleus pulposus (NP). Disc cells residing in both the AF and NP actively regulate matrix homeostasis through metabolic activities that are modulated by a variety of stimuli, including cytokines and growth factors acting in a paracrine and/or autocrine fashion. Alterations in both anabolic and catabolic processes through these stimuli, which in turn regulate matrix-degrading enzyme production, are thought to play key roles in the onset and progression of IVD degeneration (Iannone and Lapadula, 2003).

Similar to articular cartilage, the role of bFGF in the IVD has yet to be clearly elucidated as the literature has yielded contradictory results. Previously, bFGF has been identified as an anabolic mediator of disc homeostasis via a bFGF-mediated stimulation of PG synthesis in a canine IVD tissue culture system (Thompson et al., 1991) and cell proliferation in rat discs (Nagano et al., 1995) and human discs (Doita et al., 1996). Recently, Tsai et al analyzed the effects of bFGF on bovine NP cell growth and differentiation cultured in monolayer and alginate and found that bFGF stimulated increased sulfated PG synthesis, lower aggrecan turnover, and differentiation of NP cell phenotype by maintaining responsiveness to TGF- β (Tsai et al., 2007).

Findings from our laboratory (Li et al., 2008) and others (Peng et al., 2006), on the other hand, have suggested that bFGF serves primarily as a catabolic rather than anabolic factor in cartilage homeostasis. Peng and his colleagues first demonstrated highly upregulated bFGF and FGFR1 in painful degenerated human spine disc cells compared to normal cells (Peng et al., 2006). In the bovine IVD, we have found that bFGF released by chondrocytes after mechanical injury favors catabolism by stimulating MMP-13 production, inhibiting PG synthesis, and antagonizing the well-known cartilage anabolic activity of BMP7 (Li et al., 2008).

Interestingly, Li et al (2008) found that bFGF stimulates an overall increase in sulfated PG synthesis in bovine NP tissue, similar to results reported by Tsai et al (2007). However, after normalization to cell number, both DMMB and ^{35}S -sulfate incorporation results suggested that, per cell, total PG accumulation and PG synthesis significantly decreased with bFGF stimulation in a dose-dependent manner (Li et al., 2008), suggesting a negative regulatory function of bFGF in spine cartilage homeostasis. In addition, while we (Li et al., 2008) and others (Tsai et al., 2007) have found that bFGF stimulates both collagen type I and II gene expression in the IVD, the ratio of collagen II:collagen I is significantly decreased in bovine IVD tissue (Li et al., 2008), presumably via increased collagen type I synthesis by fibrocyte-like cells leading to the formation of fibrocartilage.

Some have suggested an important role of bFGF in the spontaneous resorption process of degenerative or herniated IVD tissue via stimulation of angiogenesis and/or inflammatory cytokines that aid in cartilage destruction (Minamide et al., 1999; Melrose et al., 2002; Walsh et al., 2004; Tolonen et al., 2006). Minamide et al used a rabbit disc sequestration-type model to emulate IVD herniation *in vivo* and found that epidural injection of bFGF stimulates increased angiogenesis, increased speed of disc resorption, and increased number of inflammatory cells compared to control (saline) (Minamide et al., 1999). Tolonen et al postulated that bFGF contributes to the absorption of herniated disc tissue by regulating matrix-degrading enzyme expression such as collagenase, stromelysin, and plasminogen activator (Tolonen et al., 2006). Melrose et al further emphasized the role of bFGF in the repair process after disc injury. In an ovine annular injury model, immunoreactivity for bFGF and TGF- β was positive in the outer third of the AF (region of trauma) which reached a maximum level 12 months after injury and diminished by 26 months. The presence of bFGF was associated with blood vessel ingrowth and fibroblast infiltration around the plane of the annular defect, and immunoreactivity was strongly associated with regions of the annular lesions undergoing matrix reorganization, consistent with an active repair response mediated in part by bFGF (Melrose et al., 2002). Based on these findings, one could suggest multiple roles of bFGF in

disc homeostasis depending on the stage of degeneration. In normal or recently injured disc tissue, bFGF may act as a catabolic and anti-anabolic mediator, stimulating MMP-13 expression and suppressing PG synthesis. However, these same properties may be beneficial after disc herniation, stimulating degradation of herniated tissue and encouraging spontaneous disc resorption. The expression and role of bFGF in different stages of degeneration should be further analyzed in human disc tissue to gain a better understanding of its pathophysiologic function at each stage.

(c) Extracellular signaling mediated by bFGF

Given the vital and controversial regulatory role of bFGF in both articular and IVD cartilage, a review of the signaling pathways utilized by bFGF may provide a better understanding of its complex mechanisms of action. Extracellular signals from bFGF to the cells are transduced through one of four structurally-related high affinity receptors (FGF receptor 1 – 4) that have intrinsic protein tyrosine kinase activity (Coughlin et al., 1988; Jaye et al., 1992; Johnson and Williams, 1993; Mohammadi et al., 1997; Ornitz, 2000). Using normal human articular chondrocytes (knee or ankle grade 0–1), we observed the basal expression of FGFR1, FGFR2 and FGFR3 with little or no expression of FGFR4 (Muddasani et al., 2008). Specifically, FGFR1 and FGFR3 were most highly expressed in human articular chondrocytes. These results were similar to those reported by Ellsworth et al, who demonstrated the basal expression of FGFR2 and FGFR3 in normal adult articular chondrocytes; however, their findings did not include the study of FGFR1 (Ellsworth et al., 2002).

It is well-established that bFGF interacts with both FGFR1 and FGFR3 in cartilage, and these two receptors play critical yet opposite roles in growth plate cartilage biology. For example, bFGF promotes both proliferation and differentiation of growth plate chondrocytes through interaction with either FGFR1 or FGFR3 (Kilkenny and Hill, 1996; Weksler et al., 1999). Basic FGF binding to FGFR1 has been demonstrated to increase proliferation of growth plate chondrocytes, whereas bFGF binding to FGFR3 inhibits proliferation and promotes differentiation (Kilkenny and Hill, 1996; Weksler et al., 1999; Wang et al., 2001). Compared to the extensive studies of growth plate chondrocytes, however, few studies have examined the FGF receptor responsible for the biological action mediated by bFGF in adult articular chondrocytes and IVD tissue.

In human articular chondrocytes, our findings suggest that FGFR1 is the major FGF receptor that is responsible for the bFGF-mediated biological consequences, such as cellular proliferation and production of MMP-13 in *in vitro* studies (Im et al., 2007b; Muddasani et al., 2008). In bovine NP cartilage, real-time PCR results revealed that the expression of FGFR1, followed respectively by FGFR2, FGFR4 and FGFR3, is the most abundant receptor present (Li et al., 2008). Given that FGFR1 has been associated with increased proliferation in growth plate cartilage, the upregulation of FGFR1 with minimal expression of FGFR3 in the bovine IVD could potentially explain the potent mitogenic effects of bFGF in disc cartilage. Interestingly, Valverde-Franco et al showed that absence of signaling from *fgfr3* in mice results in defective articular cartilage formation characterized by increased MMP-13 expression and increased cleavage product from collagen type II and aggrecan (Valverde-Franco et al., 2006). This result may be attributed to compensatory signaling by bFGF via the FGFR1 receptor, which is upregulated in the absence of *fgfr3* (Valverde-Franco et al., 2006), thus increasing MMP-13 expression and cellular proliferation. Further studies linking pathogenic articular cartilage and disc degeneration with FGF-ligand binding activity to specific FGFRs may provide important information for understanding the potential roles of FGFR1 and FGFR3 in articular cartilage and disc homeostasis.

(d) Intracellular signaling mediated by bFGF

Binding of bFGF to its cognate receptor FGFR1 results in receptor dimerization which, in turn, activates multiple downstream signaling cascades in human articular chondrocytes (Figure 1 & Figure 2) (Im et al., 2007; Muddasani et al., 2007; Muddasani et al., 2008). These include (a) PKC δ , (b) NF κ B, (c) Ras-Raf-MAPK (including all three subgroups: ERK, JNK, and p38), and (d) PI3K/Akt pathways. In the spine, Seguin et al demonstrated that in NP cells, p38, JNK, and NF κ B regulate the induction of MMP-13 (Seguin et al., 2006). Both in human articular chondrocytes and spine discs, it appears that the activation of multiple MAPK pathways (ERK, JNK, and p38) is required for the expression of MMP-13 after stimulation with inflammatory cytokines and growth factors such as IL-1 and bFGF (Im et al., 2007; Muddasani et al., 2007; Li et al., 2008). Clinically, stringent regulation of MMP-13 within the chondrocytic cell signaling network, through a complex interplay of regulatory factors and elements, may be necessary given the potent degrading activity of MMP-13 against a wide spectrum of substrates in the ECM and its pivotal role when present in excess amounts in OA cartilage (Fosang et al., 1996; Mitchell et al., 1996).

The finding that all three MAPK subgroups (ERK, JNK, and p38) must be activated to achieve stimulation of MMP-13 reveals an important mechanistic explanation for the observation that some growth factors, such as IGF-1 (Starkman et al., 2005) or FGF-18 (Muddasani et al., 2008), do not stimulate chondrocyte MMP-13 expression although these growth factors are capable of activating the ERK MAPK subgroup. Similarly, selective inhibition of the individual ERK or p38 MAPK pathway has been shown to alleviate joint disease in an experimental OA model (Pelletier et al., 2003) and IL-1 β -generated cartilage degeneration model using cartilage explants (Radons et al., 2006), supporting the vital role of multiple MAPK pathways in MMP-13 upregulation and development of cartilage degeneration.

Upstream of the three MAPK subgroups and NF κ B pathways, PKC δ plays a key regulatory role in the activation of its downstream effectors via molecular crosstalk, ultimately resulting in MMP-13 expression (Figure 2) (Im et al., 2007). Our data suggest that controlling PKC δ activation could be the principal rate-limiting event for the cellular response to bFGF, because a blockade in PKC δ prevents the activation of multiple downstream MAPK and NF κ B pathways and their ultimate target transcriptional regulatory factors which are required for the biological action of bFGF to stimulate MMP-13 (Im et al., 2007). Moreover, NF κ B appears to be indirectly associated with bFGF-mediated transcriptional stimulation of MMP-13 in which NF κ B controls MMP-13 transcription through the activation of the intermediate regulatory molecule, Elk-1, a critical transcription factor involved in MMP-13 activation in human articular chondrocytes (Muddasani et al., 2007). Although bFGF activates the PI3K/Akt pathway, this pathway was not associated with bFGF-induced activation of Elk-1 and subsequent MMP-13 stimulation, suggesting a pathway-specific stimulation of MMP-13 expression by bFGF (Muddasani et al., 2007). The role of the PI3K/Akt pathway in articular and IVD cartilage is currently unknown, and whether the PI3K/Akt signaling cascades are associated with cellular proliferation and/or the production of other matrix-degrading enzymes such as aggrecanases is not yet clear (Figure 1). Further studies are needed to clarify the potential role of this pathway on articular cartilage and disc homeostasis.

Several studies have already shown that Elk-1 plays a critical transcriptional regulatory role in ECM homeostasis. Carreras et al demonstrated that bFGF-mediated activation of the MAPK-Elk-1 pathway plays a pivotal role in the transcriptional repression of the ECM component elastin in lung fibroblasts (Carreras et al., 2001). Similarly, in human articular chondrocytes, the stimulation of multiple MAPK pathways subsequently activates the downstream transcription factor Elk-1, which then translocates into the nucleus and stimulates MMP-13 gene expression through protein-DNA interaction (Figure 2) (Muddasani et al., 2007). Given that MAPK and NF κ B pathways are critical signaling cascades shared by inflammatory

cytokines and growth factors, and that Elk-1 is a target of these pathways, Elk-1 could be the ultimate downstream transcription effector for inflammatory mediators in cartilage homeostasis.

In summary, after binding to its high affinity cognate receptor FGFR1 on the cell surface of articular chondrocytes, bFGF activates downstream signal transduction pathways including the PKC δ pathway, which subsequently stimulates the MAPK and NF κ B pathways to converge on the transcription factor Elk-1, leading to upregulation of MMP-13 gene expression. Attenuation of the biological activity of bFGF could be beneficial for articular cartilage and disc homeostasis as previous studies have shown that bFGF stored in the adult cartilage matrix is released with mechanical injury or with excessive loading (Vincent et al., 2004, Vincent et al., 2002). This could be achieved by the use of inhibitors of FGFR1, PKC δ , MAPK (ERK, p38, or JNK), NF κ B, or Elk-1, in an attempt to significantly reduce the bFGF-mediated stimulation of MMP-13 and limit progressive articular and IVD cartilage degradation. Future studies are warranted targeting the pathway-specific enzymes involved in the upregulation of matrix-degrading enzymes and the downregulation of PG production in arthritic cartilage and discs.

III. FGF-18

(a) FGF-18 in articular cartilage

In contrast to the controversial role of bFGF in articular and IVD cartilage, FGF-18 has been shown to have significant anabolic effects on chondrocytes in a variety of cartilaginous tissues (Ellsworth et al., 2002; Ohbayashi et al., 2002). Local delivery of adenovirus expressing *Fgf18* into the pinnae of nude mice increased the formation of auricular cartilage, type II collagen formation, PG production, and chondrocyte proliferation (Ellsworth et al., 2002). Systemic delivery of pharmacologic doses of FGF-18 to rats via a single intravenous injection stimulated expansion of various cartilage depots, including the rib-sternum junction, trachea, spine, and articular cartilage within a 2-week period (Ellsworth et al., 2002). Similarly, overexpression of *Fgf18* induced a dramatic enlargement of bronchial cartilage expressing type II collagen in lung tissue (Whitsett et al., 2002). These effects could be due to the direct action of FGF-18 on mature chondrocytes and/or progenitor cells that have undergone FGF-induced differentiation.

In chondrogenesis, FGF-18 regulates chondrocyte proliferation, the onset of hypertrophic chondrocyte differentiation, vascular development in mesenchyme surrounding developing skeletal elements, and vascular invasion of the hypertrophic chondrocyte zone at the growth plate (Liu et al., 2007). In mature cartilage, FGF-18 has been shown to stimulate cell proliferation, ECM production, and PG synthesis in primary porcine and human adult articular chondrocytes (Ellsworth et al., 2002) and the growth of neonatal rat costal chondrocytes (Shimoaka et al., 2002). Further, Moore et al were the first to study the potential for *in vivo* cartilage repair by FGF-18 via intra-articular injection in a rat meniscal tear model of OA (Moore et al., 2005). A series of FGF-18 injections starting 21 days after surgical damage induced a dose-dependent increase in *de novo* cartilage formation and a parallel reduction in cartilage degeneration scores in the tibial plateau of OA rats, demonstrating potent anabolic effects of FGF-18 in an *in vivo* model of OA. To our knowledge, however, these are the only studies revealing the potential anabolic capacity of FGF-18 in an articular cartilage degeneration model, and currently the role of FGF-18 in human tissue degeneration models or IVD homeostasis has yet to be examined.

Interestingly, FGF-18 has been suggested to facilitate the chondrogenic activity of bone morphogenetic proteins (BMPs), well-known anabolic factors, by suppressing the expression of noggin, a naturally occurring inhibitor of BMP signaling (Reinhold et al., 2004). Noggin

expression may play a vital role in helping to explain the opposite roles of FGF-18 and bFGF in human cartilage. Li et al recently found that stimulation of bovine IVD cells with bFGF induced a dose-dependent increase in noggin mRNA expression, suggesting that the increase in noggin may be one mechanism by which bFGF antagonizes the effects of the well-known anabolic factor BMP7 (Li et al., 2008). The stimulation (via bFGF) or repression (via FGF-18) of noggin may serve as one potential mechanism for the contrasting effects mediated by these two growth factors in cartilage homeostasis.

(b) Extracellular signaling mediated by FGF-18

The specific receptors that mediate FGF-18-induced expansion of articular cartilage are currently under investigation. Previous evidence shows an FGF-18-induced activation of the IIIc splice variants of FGFR2 and FGFR3, as well as FGFR4 (Chang et al., 2000; Ellsworth et al., 2002), and FGFR2 and 3 are expressed in chondrocytes of mature articular cartilage (Chang et al., 2000). In mouse pinnae transduced with adeno-*Fgf18*, FGFR2-(IIIc), FGFR3-(IIIc), and FGFR4 mRNAs were detected within proliferating chondrocytes in the expanded perichondrial zone (Ellsworth et al., 2002). While FGFR3 is expressed in proliferating epiphyseal chondrocytes, FGFR1 is expressed in prehypertrophic and hypertrophic chondrocytes in the growth plate (Peters et al., 1992; Peters et al., 1993), suggesting that FGFR1 and FGFR3 maintain very distinct domains of expression with unique functions. Expression of FGFR3 in the reserve and proliferating zone suggests a direct role for FGFR3 in regulating chondrocyte proliferation and possibly differentiation (Peters et al., 1993; Naski et al., 1998). In contrast, the expression of FGFR1 in hypertrophic chondrocytes suggests a role for FGFR1 in regulating cell survival, cell differentiation, ECM production and cell death (Peters et al., 1992). Recently, findings from our laboratory and others suggest that FGF-18 signals selectively through FGFR3 in human articular chondrocytes and mice to suppress cellular proliferation and promote limb mesenchymal cell differentiation (Davidson et al., 2005; Muddasani et al., 2008), as opposed to bFGF-FGFR1 binding that characterizes increased proliferation and catabolic effects seen in articular and IVD cartilage (Figure 3).

Mutation studies have further demonstrated the importance of FGFR3 signaling in chondrogenesis and osteogenesis. Mice lacking *Fgf-18* exhibit malformations in cartilage and bone, including delayed closure of the calvarial sutures, enlargement of the proliferating and hypertrophic zones in the growth plate of long bones, defects in joint development, and delays in osteogenic differentiation (Liu et al., 2002; Ohbayashi et al., 2002). Many of the common forms of dwarfism are caused by activating mutations in *Fgfr3* (Naski et al., 1998), suggesting that in the growth plate of long bones, FGFR3 is a negative regulator of chondrocyte proliferation. However, Iwata et al reported that signaling through FGFR3 can both promote and inhibit chondrocyte proliferation depending on the stage of development (Iwata et al., 2000). FGF-18 signaling through FGFR3 may enhance chondrocyte proliferation in immature committed chondrocytes, even though it is well established that signaling through FGFR3 inhibits chondrocyte proliferation and differentiation in the mature proliferating chondrocyte zone of the growth plate (Ellsworth et al., 2002; Liu et al., 2002). This suggests that signaling through FGFR3 has a biphasic role during chondrocyte development: first, promoting chondrocyte proliferation at early embryonic stages; and later, acting to suppress chondrocyte proliferation. This paradoxical effect on proliferation supports a model in which chondrocytes at different stages of development may switch their cellular responsiveness to FGF-18/FGFR3 signaling from a mitogenic response early in development to a non-mitogenic response later in development (Liu et al., 2002; Liu et al., 2007).

Previous work has already identified *Fgfr3* signaling as a key regulator of chondrocyte function in chondrogenesis, but the role of *Fgfr3* in mature cartilage and cartilage degeneration is largely unknown. Ellsworth et al reported significant anabolic effects of FGF-18 on human articular

cartilage homeostasis via increased chondrocyte proliferation and ECM production both *in vivo* and *in vitro*, and found that proliferation of cells expressing *Fgfr3*-(IIIc) or *Fgfr2*-(IIIc) was increased by incubation with FGF-18 (Ellsworth et al., 2002). The expression of FGF-18, *Fgfr3*-(IIIc) and *Fgfr2*-(IIIc) mRNA was localized to chondrocytes of human articular cartilage by *in situ* hybridization, suggesting a potential role of FGFR2 or FGFR3 in FGF-18-mediated human articular cartilage homeostasis. Valverde-Franco found that the absence of signaling through *fgfr3* in chondrocytes *in vivo* leads to a similar degeneration of articular cartilage in mice as that seen in human OA (Valverde-Franco et al., 2006). In the joints of *Fgfr3*($-/-$) mice, the absence of signaling leads to premature cartilage degeneration and early arthritis, demonstrated by excessive proteolysis of aggrecan and type II collagen, increased expression of MMP-13, cellular hypertrophy, and increased loss of PG at the articular surface compared to control. Their results identified *fgfr3* as a critical anabolic regulator of articular cartilage metabolism and a potential pathway for early intervention in degenerative joint disease.

Similarly, findings from our laboratory reveal that treatment of human adult articular chondrocytes with FGF-18 for 21 days in alginate beads stimulates the activation of FGFR3 rather than FGFR1 and leads to markedly decreased cell proliferation compared to cells treated with bFGF (Muddasani et al., 2008), and similar results were observed using bovine spine disc cells (unpublished data). Collectively, these data suggest that activation of FGFR1 exerts anti-anabolic and catabolic biological effects in human adult articular cartilage, represented by fibroblast-like cell proliferation, inhibition of ECM production, and upregulation of matrix-degrading enzyme production. On the other hand, activation of FGFR3 via, for example, stimulation with FGF-18, most likely exerts anabolic effects in human articular chondrocytes via increased matrix formation and promotion of cell differentiation, leading to dispersed chondrocytes surrounded by abundant ECM instead of clusters of cells seen after stimulation with bFGF (Figure 3).

(c) Intracellular signaling mediated by FGF-18

At present, the intracellular signaling cascades mediated by FGF-18 in adult articular cartilage and IVD tissue are largely unknown. As studies continue to elucidate the precise extracellular signaling pathways and receptors utilized by FGF-18 in cartilage, the underlying molecular mechanisms should be uncovered to provide a greater understanding of the role of FGF-18 in cartilage homeostasis. The anabolic effects of FGF-18 in various cartilaginous tissues suggest that this growth factor along with the receptor FGFR3 could potentially be useful for promoting repair and/or regeneration of damaged cartilage.

IV. Conclusion

Based on the current literature, the role of bFGF is controversial in both articular and IVD cartilage as it has been associated with both anabolic and catabolic events in adult matrix homeostasis. Recent evidence suggests that bFGF selectively activates FGFR1 to exert degradative effects in both human articular chondrocytes and IVD tissue via upregulation of matrix-degrading enzyme activity, inhibition of ECM production, inhibition of PG synthesis, and increased cell proliferation resulting in clustering of cells seen in arthritic states. FGF-18, on the other hand, most likely exerts anabolic effects in human articular chondrocytes by activating FGFR3, increasing ECM formation and cell differentiation while inhibiting cell proliferation, leading to dispersed cells surrounded by abundant ECM instead of clusters of cells seen after stimulation with bFGF. The role of FGF-18 in IVD tissue is currently unknown. The results from these *in vitro* and *in vivo* studies reveal the potential usefulness of bFGF and FGFR1 antagonists, as well as FGF-18 and FGFR3 agonists, as potential therapies to prevent cartilage degeneration and/or promote cartilage regeneration and repair in the future.

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Abbreviations

AAV, adeno-associated virus
 ADAMTS, A Disintegrin-like and Metalloprotease with Thrombospondin motifs
 AF, annulus fibrosus
 bFGF, basic fibroblast growth factor
 BMP, bone morphogenetic protein
 DDD, degenerative disc disease
 DMMB, dimethylethylene blue
 ECM, extracellular matrix
 FADD, Fas-activated death-domain
 FGF, fibroblast growth factor
 FGFR, fibroblast growth factor receptor
 FLIP, Fas-like inhibitory protein
 IGF-1, insulin-like growth factor-1
 IL-1, interleukin-1
 IVD, intervertebral disc
 MAPK, mitogen activated protein kinase
 MMP, matrix metalloprotease
 NP, nucleus pulposus
 OA, osteoarthritis
 PCR, polymerase chain reaction
 PG, proteoglycan
 PKC δ , protein kinase C delta
 RA, rheumatoid arthritis
 TGF- β , transforming growth factor - beta
 TNF- α , tumor necrosis factor - alpha

Figure 1.
Summary of bFGF-activation of multiple signaling pathways associated with articular cartilage degradation.

Figure 2.
The PKCδ pathway acts as an upstream regulator for NFκB and MAPK (ERK, JNK, and p38) pathways involved in the bFGF-mediated phosphorylation of Elk-1, resulting in increased MMP-13 transcription.

Figure 3.
Comparison of bFGF and FGF-18 in adult articular cartilage and/or IVD homeostasis. Basic FGF mainly activates FGFR1 while FGF-18 activates FGFR3 to exert distinctive biological effects in both adult articular cartilage and IVD.