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Basic FGF Stimulates MMP-13 via FGFR1-dependent Activation of MAPK and NFκB That Converge to Activate ELK-1 in Cartilage

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Damage and progressive loss of the articular cartilage is a key feature of many types of arthritis including the most common form of arthritis which is Osteoarthritis (OA). It has become clear, particularly in OA that the chondrocyte is responsible for the destruction of its own matrix through the release of destructive enzymes including matrix metalloproteinase-13 (MMP-13). We show here that the signaling generated by basic fibroblast growth factor (bFGF or FGF-2) favors catabolism by stimulating MMP-13 production in a dose-dependent manner in human articular chondrocytes, and this stimulation may accelerate cartilage destruction. Basic FGF is expressed by chondrocytes and is present in the extracellular matrix of the articular cartilage. Mechanical injury to cartilage releases a factor which activates phosphorylation of Erk^{MAPK} and this factor is identified as bFGF. However, the effects of bFGF on adult articular chondrocytes and the signaling cascades mediated by bFGF to modulate articular cartilage homeostasis are not completely understood. The aim of the present study is to characterize the role of Erk^{MAPK} in the development and progression of human cartilage degeneration after stimulation with bFGF. We established that FGF receptor I is responsible for the biological activity of bFGF in human articular chondrocytes and mediates upregulation of MMP-13 production *via* two major signaling pathways: (i) Ras/Raf/MAP kinase; and (ii) NFκB pathway. Basic FGF activated all three MAP kinases in 5 min and the down-regulation of MMP-13 expression was functionally linked to the specific inhibition of MAP kinase subgroups ERK, p38 and JNK. These results were further supported by co-transient transfection studies of MMP-13 promoter-reporter construct with dominant negative forms of individual MAP kinase expression vectors. Our results suggest that the activation of multiple MAP kinase pathways is required for bFGF-stimulation of MMP-13 in human articular chondrocytes.

Basic FGF acutely phosphorylates IKK within 5 min, which is the key kinase for NFκB activation. Protein-DNA interaction arrays show that bFGF increases NFκB binding to its cognate regulatory element. NFκB inhibitors block MMP-13 induction by bFGF or IL-1β in human articular chondrocytes demonstrating that NFκB is the ultimate target of bFGF signaling. While MMP-13 promoter region contains a putative NFκB recognition motif, deletion of this motif does change neither basal nor bFGF-stimulated levels of MMP-13 promoter activity. We then initiated studies to define mechanism (s) by which NFκB affects bFGF-mediated MMP-13 induction independent of direct promoter binding. The activation of IKK phosphorylation is reduced upon inhibition of distinct MAP kinases, and completely inhibited when JNK signaling is abrogated.

We noted the presence of a putative response element for the Elk-1 transcription factor in the MMP-13 promoter. Elk-1 is a member of Ets domain-containing transcription factors and downstream target gene of MAP kinases. Transient transfection studies using reporter genes fused to serial deletions of the MMP-13 promoter showed that eliminating the Elk-1 binding motif reduced basal promoter activity by 40%. Furthermore, overexpression of Elk-1 increases MMP-13 promoter activity in transient transfection studies

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but not when the MMP-13 promoter segment containing the Elk-1 site was deleted. We find that bFGF induces rapid phosphorylation (2 min) and nuclear localization (5 min) of Elk-1. In addition, bFGF increases the DNA-protein interaction of Elk-1 in gel shift assays with nuclear extracts and this elevation is significantly decreased by individual MAP kinase inhibitors or inhibition of NFκB. Taken together, these results suggest that Elk-1 is downstream target of MAP kinases and NFκB B regulatory factors.

In summary, our results suggest that MMP-13 stimulation by bFGF is mediated by FGFR1 activation which in turn, activates Ras/Raf/MAP kinases and NFκB signaling cascades. These signaling pathways converge *via* molecular cross-talk to activate Elk-1, which may serve as one of the intermediate molecules of NFκB pathway to mediate upregulation of MMP-13 expression in human articular chondrocytes (**Fig. 1**).

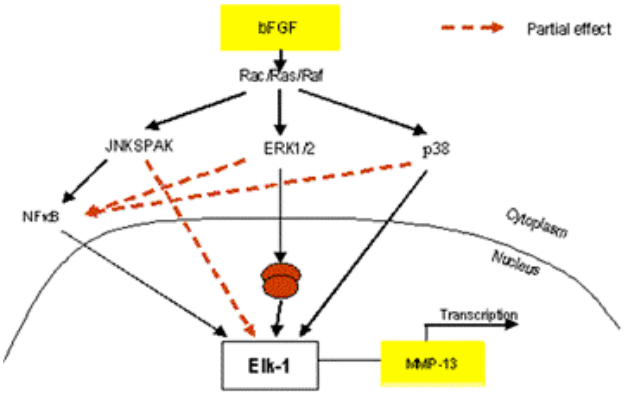

Figure 1 : Schematic diagram of signaling pathways mediated by bFGF in human adult articular chondrocytes. The molecular cross-talks between MAP kinases and NFκB pathway converge to activate Elk-1 leading to upregulation of MMP-13 transcription.

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