 MCB, vol.3, no.4, pp.159-161, 2006

The Pathological Role of the ERK Pathway in Human Adult Articular Cartilage

P. Muddasani [1] and H-J. Im [1]

1 Introduction

Damage and progressive loss of the articular cartilage is a key feature of many types of arthritis including the most common form 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 and other group found that the signaling generated by basic fibroblast growth factor (bFGF or FGF-2) favors catabolism by stimulating MMP-13 production in human articular chondrocytes [1,2], More recently, we reported the striking antagonistic effect of bFGF on the well-known cartilage anabolic activity of IGF-1 and BMP7 [3]. 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 [4]. 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.

2 Materials and Methods

human articular chondrocytes, isolated from normal ankle or knee cartilage obtained from tissue donors were cultured as previously described [3]. Chemical inhibitors for blocking the Erk pathway included PD98059 (20 μM). Immunoblotting was performed by loading equal amount of total protein in the conditioned medium or cell lysates by protein assay (Pierce) on 10% SDS-PAGE gels. Proteoglycan (PG) production was assessed by Dimetylmethylene blue (DMMB) assay and normalized by DNA assay for cell numbers as described previously [3]. Analysis of variance was performed using StatView 5.0 software. p values <0.05 were considered significant.

Departments of Biochemistry, Orthopedic Surgery, Internal Medicine (section of Rheumatology), Rush University Medical Center, Chicago, IL 60612
E-mail: Hee-Jeong_Sampen@rush.edu

3 Results

Erk^{MAPK} pathway is activated by various stimuli with different magnitude. We found strong & sustainee activation of Erk pathway by bFGF, pro-inflammatory cytokine, such as IL-1β. On the other hand, weak/delayed activation or no activation of Erk was noticed by other growth factors and cytokines. These include IGF-1, TGF-β, FGF18 and anti-pro-inflammatory cytokines, such as IL-4 and IL-10. Our studies show that the induction of MMP-13 is corresponding to the activation of Erk. Strong Erk activators induce strong induction of MMP-13 whereas weak or delayed Erk activators did not significantly induce MMP-13. MMP-13 induction by bFGF is diminished in the presence of inhibitor of Erk. MMP-13 induction by bFGF resulted from very specific MAPK pathway product because bFGF-activated PI3K/Akt pathway is not associated with MMP-13 induction.

Apart from Erk, we noticed rapid, strong and sustained activation of Elk-1 after stimulation with bFGF [5]. Elk-1 was rapidly activated by bFGF and the activation was sustained over 1 hr while FGF18 showed no significant activation of Elk-1. These results suggest that perhaps the delayed Erk activation by FGF18 may be not sufficient enough

to activate Elk-1. Previously, it has been reported that bFGF-activation of Elk-1 pathway plays a role in the transcriptional repression of ECM component [1]. We have shown that Elk-1 is a critical regulator of MMP-13 expression after stimulation with bFGF [5].

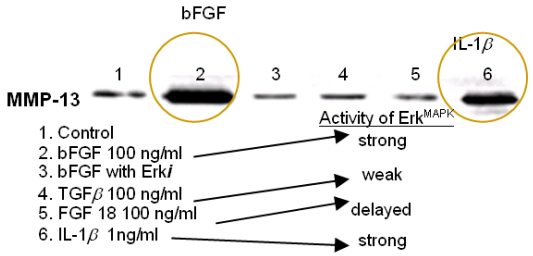

Figure 1 : The induction of MMP-13 is corresponding to the activation of Erk. Strong Erk activators induce strong induction of MMP-13, while weak/delayed Erk activators show no significant induction of MMP-13.

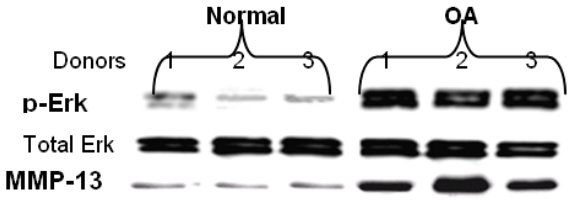

Figure 2 : Basal Erk activation is highly upregulated in OA compared to normal chondrocytes, and so is the corresponding basal expression level of MMP-13.

We have found that bFGF-mediated strong Erk activation is indeed responsible for the inhibition of PG production. bFGF decreases PG production in a dose-dependent manner. The co-incubation with Erk inhibitor not only reversed the bFGF anti-anabolic effect but also further promoted the PG production beyond the control level. Our results from Saranin-O staining using Knee grade 2 cartilage tissues further suggested that bFGF increased PG loss. The addition of Erk inhibitor was able to completely block bFGF-induced PG loss which was closely corresponding to our DMMB assay shown in **Fig. 3.**

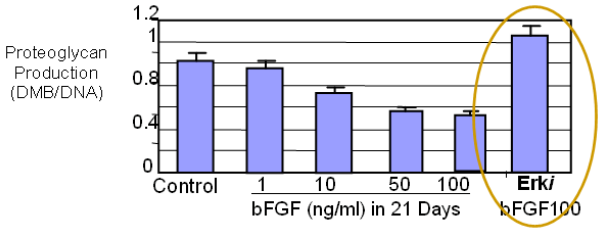

Figure 3 : bFGF decreases proteoglycan production in a dose-dependent manner. The co-incubation with Erk inhibitor reverted bFGF-suppression of proteoglycan in HACs.

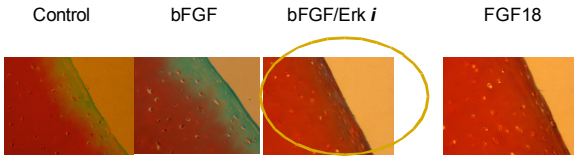

Figure 4 : Safaranin-O staining using Knee grade 2 cartilage tissue. Explants were incubated with/without bFGF, Erk inhibitor (Erki), and FGF18 for 9 days. The addition of Erki completely blocked bFGF-induced proteoglycan loss, which corresponds to the DMMB assay shown in **Fig. 3**.

Our comparison studies showed dramatic differences in the basal expression of MMP-13 between normal and OA. Based on our results, initially, we postulated that the basal Erk activation is highly upregulated in OA compared to normal cells, and thus, so is the corresponding basal expression level of MMP-13. As we expected the basal activation of the Erk pathway in OA was significantly high compared with those in normal. Basal activity of Elk-1 was also highly upregulated in OA compared to normal cell (data not shown here).

4 Conclusion

In summary, our results suggest that the impact of Erk^{MAPK}-Elk-1 pathways on articular cartilage homeostasis is significant. Cartilage injury or trauma stimulates or release bFGF from ECM, which in turn, activate Erk-Elk-1 pathways to stimulate MMP-13, and simultaneously, to decrease PG production. This process, collectively, may contribute to the accelerated human articular cartilage degradation.

Acknowledgements: This work was funded by Falk Foundation, UCR grant from Rush University Medical Center, NIH AR053220, the Arthritis Foundation, and Arthritis National Research Foundation. Thanks to the Gift of Hope Organ and Tissue Donor Network and Dr. Arkady Margulis for donor tissues. We also thankDr. FR Loeser for MMP-13 construct.

References

1. Im, H-J., Carlo, M.D., Pacione, C. and Loeser, R.F. (2004) *Ortho. Trans.* **29**, 143.
2. Wang, X., Manner, P.A., Horner, A., Shum, L., Tuan, R.S., Nuckolls, G.H. (2004) *Osteoarthritis Cartilage* **12,** 963-974.
3. Loeser, R.F., Pacione, C., Chubinskaya, S., Im, H.J. (2005) *Arthritis Rheum.* **52**, 3910-3917.
4. Vincent, T.L., Hermansson, M.A., Bjolton, M., Wait, R., Saklatvala, J. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 8259-8264.
5. Zhao. L-J., Muddasani, P., Rangan, J., Im, H.J . (2005) *Ortho Trans*. **30,** 146.