Mechanisms for Relaxin’s Modulation of MMPs and Matrix Loss in Fibrocartilages

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Our previous studies (Hashem et al., 2006; Kapila, 2009; Naqvi et al., 2005) have shown that the fibrocartilaginous temporomandibular joint (TMJ) disc and pubic symphysis respond to relaxin by upregulation of MMPs and loss of key matrix molecules. These responses to relaxin are enhanced by estrogen, and are also modulated by estrogen alone. Since these fibrocartilaginous tissue are heterogeneous containing fibroblastic, chondrocytic and intermediate cell types, the responses of specific cell types to relaxin are not known. Also the direct effect of specific MMPs induced by relaxin to the loss cartilage matrix in vivo has not been demonstrated. Our purpose was to (1) characterize cell type-specific response(s) to relaxin and estrogen; (2) identify the relaxin receptor and downstream signaling involved in relaxin’s induction of specific MMPs; (3) develop a mouse model for in vivo manipulation of hormones; and (4) identify the contribution of each of these hormones and the MMPs they regulate to in vivo matrix loss.

Two each of fibroblastic and chondrocytic female mouse TMJ disc cell clones immortalized by human telomerase reverse transcriptase were isolated and characterized on the basis of phenotype, growth curves, and fibroblastic and chondroctytic markers, respectively. Chondrocytic cell clones had higher mRNA and protein expression levels of cartilage oligomeric matrix protein (COMP), collagen X, total collagen, collagen II, and collagen II/collagen I mRNA ratios relative to fibroblastic cell clones. Fibroblastic cell clones had higher mRNA and protein expression levels of vimentin and fibroblastic specific protein 1 (FSP1) than the chondrocytic clones. The fibroblastic cell clones expressed higher levels of relaxin receptors RXFP1 (>6) and RXFP2 (>9) while chondrocytic cell clones had higher level of estrogen receptor, ESR2 (>4.5) compared to their counterparts. Consequently the fibroblastic cells showed greater upregulation of MMP-9 (>2.5) and -13 (>9) and relaxin receptors (>2) by relaxin than the chondrocytic cell clones, while the reverse was true for cellular responses to estrogen.

Next using gene overexpression and suppression strategies we found that RXFP1 but not RXFP2 is involved in the upregulation of MMP-9 and -13 in primary TMJ fibrocartilaginous cells (Fig. 1). Studies using chemical inhibitors and siRNAs to signaling molecules revealed that relaxin induces MMP-9 via PI3K, Akt, ERK and PKC-ζ and the transcription factors Elk-1, c-fos and to a lesser extent NF-κB (Ahmed et al., 2012). MMP-9 promoter-luciferase experiments demonstrated the involvement of the elements responsive to these transcription factors, namely Ets/Pea3, AP-1 and NF-κB, respectively, in relaxin’s modulation of MMP-9 (Fig. 2).
We next assessed the contribution of relaxin-induced MMP-9 to matrix loss in vivo. One week following bilateral ovariectomies in 12 week-old female C57BL/6 WT or MMP-9 null mice, osmotic pumps containing PBS or relaxin or estrogen or a combination of these hormones were implanted subcutaneously. Six days later, the mice were euthanized, blood was collected and TMJ discs retrieved. The discs were analyzed for collagen, glycosaminoglycan, and MMPs, while the serum was assayed for the hormones. The administered doses of the hormones resulted in systemic hormone concentrations similar to those in cycling women. In vivo administration of relaxin and/or estrogen contributed to the upregulation of MMP-9 and -13 concomitant with the loss of matrices from disc in WT mice. Unexpectedly, treatment of MMP-9 null mice with estrogen or relaxin also resulted in loss of disc collagen and glycosaminoglycan to a similar extent as that in WT mice. This finding could be explained by our observation that relaxin and estrogen caused significantly greater induction of MMP-13 in MMP-9 null vs. WT mice, suggesting that MMP-13 may be compensating for the absence of MMP-9 in relaxin-mediated matrix loss.

The findings demonstrate that relaxin and estrogen enhance matrix loss in the TMJ disc concomitant with the induction of their respective receptors, MMP-9 and MMP-13, which are cell-type specific responses. Also the similarities between WT and...
MMP-9 null mice in the loss of matrix molecules on treatment with estrogen and/or relaxin suggest that both MMP-9 and MMP-13 may act together in contributing to tissue turnover, or mediators other than MMPs may be involved in the enhanced matrix turnover mediated by these hormones.

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References

