

Matrix Metalloproteinase Induction by Relaxin Causes Cartilage Matrix Degradation in Target Synovial Joints

Receptor Profiles Correlate with Matrix Turnover

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Our long-term goal is to understand the mechanisms by which relaxin and estrogen potentially contribute to joint diseases, particularly those afflicting the fibrocartilaginous temporomandibular joint (TMJ). Previously, we showed that relaxin produces a dose-dependent induction of tissue-degrading enzymes of the matrix metalloproteinase (MMP) family, specifically MMP-1 (collagenase-1), MMP-3 (stromelysin-1), MMP-9 (92-kDa gelatinase), and MMP-13 (collagenase-3) in cell isolates and tissue explants from TMJ fibrocartilage. The induction of these MMPs is accompanied by loss of collagen and glycosaminoglycans (GAGs), which was blocked by a pan-MMP inhibitor. We also found the targeted *in vivo* loss of collagen and GAGs in TMJ discs of ovariectomized rabbits treated with β -estradiol, relaxin, or both hormones together. Progesterone attenuated the induction of MMPs and matrix loss by relaxin and estrogen. The modulation of matrix composition in TMJ fibrocartilage by these hormones was similar to that observed in the pubic symphysis and differed from that of the knee meniscus. The two target tissues showing the greatest modulation of MMPs and matrix loss, namely, the TMJ disc and pubic symphysis, had similar expression profiles of the estrogen receptors α and β , relaxin-1 receptor (RXFP1, LGR7), and insulin-like peptide 3 receptor (RXFP2, LGR8) and these profiles differed from those in cells from the knee meniscus. These findings suggest a novel model for targeted tissue turnover of cartilage of specific joints through hormone-mediated induction of select MMPs.

Key words: degenerative joint disease; fibrocartilage; relaxin; estrogen; progesterone; matrix metalloproteinases; extracellular matrix

Introduction

Degenerative joint diseases (DJDs) of most systemic joints are more prevalent in women than in men and occur primarily in postmenopausal years.¹ However, diseases of the fibrocartilaginous temporomandibular joint (TMJ), which also show a high female-to-male preponderance,² primarily afflict women between 18 and 45 years of age.³ Symptoms of

TMJ-related disorders occur in approximately 6–12% of the adult population, or approximately 10 million individuals in the United States.^{2,4} The reasons for the marked sexual dimorphism and age distribution of TMJ disorders remain unclear. However, a potential role of female reproductive hormones, particularly estrogen in the etiology of these diseases, has been proposed. Both estrogen and progesterone receptors have been localized in the TMJ of humans and other mammals,^{5–8} with some findings suggesting a sexual dimorphism in the presence of estrogen receptors.^{5,8} Despite these studies, until recently no direct evidence existed that linked female reproductive

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hormones to TMJ disease or defined the mechanisms by which these hormones may cause TMJ disease. Additionally, the potential roles of other female hormones such as relaxin in these diseases have been examined only recently. These findings show that relaxin H2, which is found systemically in cycling and pregnant women but not in men, causes the targeted induction of tissue-degrading enzymes of the matrix metalloproteinase (MMP) family in the fibrocartilaginous tissues of the TMJ, potentially predisposing to TMJ diseases.⁹⁻¹¹

MMPs are a family of up to 25 enzymes that are characterized by their extracellular matrix substrate specificity, zinc-dependent activity, inhibition by tissue inhibitors of metalloproteinases, secretion as a zymogen, and sequence similarities. Between them, MMPs can degrade the major matrix macromolecules of cartilage, namely, collagen and proteoglycans, as well as most of the minor proteins in this tissue. Matrix degradation by MMPs is considered to be a primary event in the initiation and progression of joint diseases. The modulation of MMPs by hormones such as relaxin and estrogen in cartilage that may initiate or predispose to a subset of noninflammatory joint diseases has only recently come under scrutiny.^{9,11,12}

Relaxin, β -Estradiol, and Progesterone Modulate MMP Expression and Matrix Content in Various Tissues

Relaxin H2, a 6-kDa polypeptide, is the major stored and circulating form of relaxin in humans¹³ and appears to have long-term effects on connective tissues by altering the turnover of collagen and proteoglycans. In most reproductive tissues, including the cervix, uterus, ovary, breast, and decidua, relaxin mediates changes in matrix composition and organization by modulating the synthesis of matrix macromolecules,¹⁴ altering the expression of matrix-degrading enzymes,¹⁵ or both.¹⁴ In fibrocartilaginous tissues of the pubic symphysis

and TMJ, alterations in matrix composition appear to result from increased degradative responses rather than changes in matrix synthesis.^{11,16,17} Relaxin appears to contribute to tissue degradation by primarily inducing several MMPs, including MMP-1, -3, -9, and -13, in cells of reproductive and nonreproductive tissues, including skin and lung alveolar fibroblasts^{15,18-20} and, as discussed below, of TMJ fibrocartilage.^{9,11}

Estrogens are also capable of modulating degradation of the extracellular matrix by regulating the expression of various MMPs in several cell types.^{21,22} Additionally, β -estradiol is known to potentiate MMP induction and matrix loss by relaxin in cells from reproductive and nonreproductive tissues, including the TMJ fibrocartilage^{9,15,17} (Fig. 1 and 2). In contrast to the effects of estrogen and relaxin in enhancing MMP expression, progesterone downregulates several MMPs, including MMP-1, -2, -3, and -9, in cells from several reproductive tissues,²³⁻²⁶ and as discussed below, of MMP-3, -9, and -13 in mouse TMJ disc cells.

Female Hormones Cause a Targeted Modulation of MMPs in TMJ and Pubic Symphysis, but Not in Knee Meniscus Fibrochondrocytes

Previously we demonstrated that rabbit TMJ disc fibrocartilaginous cells show a dose-dependent induction of MMP-1 and -3, which is potentiated by exposure of the cells to β -estradiol.⁹ More recently we performed studies to assess the MMP responses of TMJ disc cells from young adult cycling female mice to increasing concentrations of relaxin alone or in combination with β -estradiol or progesterone. We also examined if the responses of pubic symphysis and knee meniscus fibrochondrocytes to these hormones are similar to those of TMJ fibrochondrocytes. We found that relaxin produces an induction of 52/55-kDa gelatinolytic enzyme (presumably MMP-13) in TMJ disc

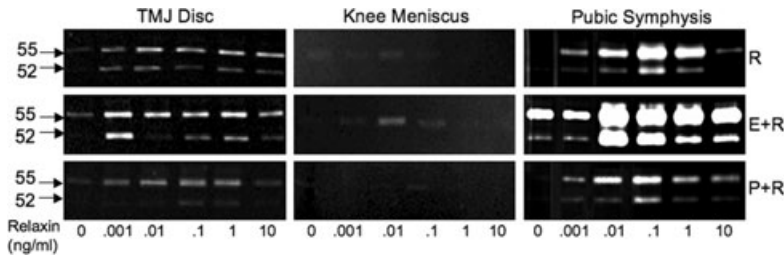


Figure 1. Dose-dependent modulation of 52/55-kDa gelatinolytic proteinase by relaxin (R) in the absence or presence of β -estradiol (E) or progesterone (P) in mouse TMJ disc, knee meniscus, and pubic symphysis fibrochondrocytes. Early passage cells plated at $10^5/\text{cm}^2$ in serum-free medium were exposed to increasing concentrations of relaxin (0–10 ng/mL) in the absence or presence of β -estradiol (0.1 ng/mL) or progesterone (10 ng/mL) for 48 h. The cell-conditioned medium, standardized by total protein, was analyzed by gelatin substrate zymography. Relaxin produced a bimodal dose-dependent increase in expression of the 52/55-kDa gelatinolytic proteinase (presumably MMP-13) in the TMJ disc and pubic symphysis but very minimally in the knee meniscus. The activity of this proteinase band was inhibited in the presence of the MMP inhibitor 1,10-phenanthroline (data not shown) (Sigma-Aldrich, St. Louis, MO, USA), characterizing this enzyme as an MMP. In TMJ disc and pubic symphysis cells the induction of this proteinase by relaxin was potentiated by β -estradiol and attenuated by progesterone.

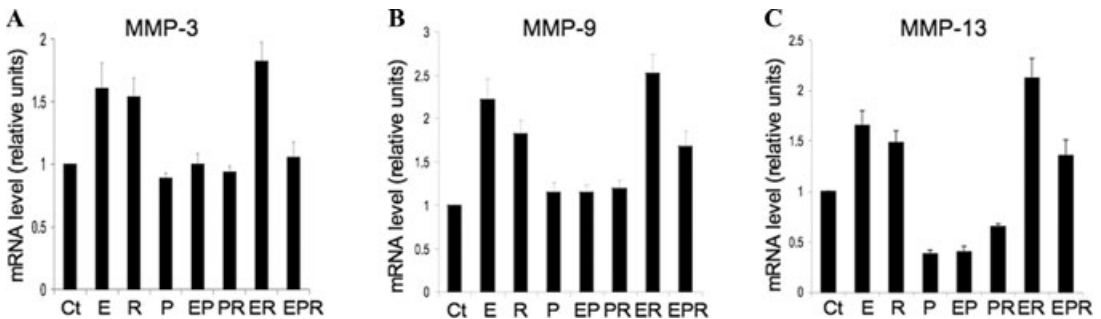


Figure 2. β -estradiol (E), relaxin (R), and progesterone (P) modulate MMP-3 (A), MMP-9 (B), and MMP-13 (C) gene expression in mouse TMJ disc fibrochondrocytes. Early passage fibrochondrocytes were treated as described for Figure 1, except that only a single concentration of relaxin (0.1 ng/mL) was used. Total RNA was extracted and subjected to quantitative reverse transcription-PCR for the MMPs following standardization by total RNA concentration. 18S RNA was used as an internal control. All three MMPs were significantly upregulated by E, R, or E+R treatments. E increased the induction of MMP-9 and -13 by relaxin, while P attenuated the induction of MMP-3, -9, and -13 by relaxin or β -estradiol. Data represent the mean (\pm standard deviation) fold increase relative to controls from three different experiments. Ct, control.

and pubic symphysis cells but minimally in knee meniscus cells (Fig. 1). Relaxin's induction of this proteinase in TMJ disc and pubic symphysis cells was potentiated by exposure to β -estradiol and attenuated by progesterone. Whether used alone or with β -estradiol or progesterone, the peak induction of the 52/55-kDa proteinase by relaxin occurred at relaxin concentrations of 0.01–1 ng/mL, which falls within the physiologic range in cycling and pregnant women.

Next, we assessed the effects of β -estradiol alone or progesterone alone or in various combinations with relaxin on the modulation of MMP-3, -9, and -13 mRNA in mouse TMJ disc cells. β -estradiol or relaxin alone or together produced a significant induction of all three MMPs relative to baseline controls (Fig. 2). Progesterone alone or in combination with β -estradiol or relaxin caused a significant repression of MMP-13 relative to baseline controls and maintained that of MMP-3 and

-9 mRNAs at control levels. More importantly, progesterone caused a significant attenuation of the induction of MMP-3, -9, and -13 by β -estradiol and/or relaxin. Together, these findings show the targeted induction by relaxin and β -estradiol of MMP-3, -9, and -13 in the TMJ disc and pubic symphysis fibrocartilages, but not in the knee meniscus, and the attenuation of this response by progesterone. The divergent regulation of MMPs by estrogen and relaxin on one hand and progesterone on the other suggests that the hormonal cross talk may be an important determinant of the matrix composition and organization of target cartilaginous tissues.

Relaxin, β -Estradiol, and Progesterone's Modulation of MMPs Is Paralleled by Changes in Matrix Content of TMJ Disc and Pubic Symphysis Fibrocartilage

Although the induction of MMPs by relaxin and β -estradiol in TMJ disc cells may be an indicator of enhanced matrix degradative activity, it does not necessarily follow that this results in a net loss of matrix macromolecules. Therefore, we determined whether fibrocartilaginous cells in their native matrix environment show similar responses to relaxin as isolated cells and whether the induction of MMPs is associated with the loss of collagen and glycosaminoglycans (GAGs) in fibrocartilaginous explants.¹¹ We found that relaxin's induction of MMP-1 and MMP-3 in rabbit TMJ disc explants is accompanied by loss of disc collagen and GAGs. The loss of these matrix molecules was prevented when a pan-MMP inhibitor was added to the cultures, demonstrating the contribution of relaxin-induced MMPs to matrix loss.

We have also addressed the question of whether the loss of collagen and GAGs by relaxin with or without β -estradiol observed in TMJ explants occurs *in vivo* and determined the specificity of these effects on TMJ fibrocartilage versus that on the knee meniscus.¹² Addition-

ally, we assessed whether progesterone attenuates the effects of relaxin and β -estradiol in causing the loss of tissue matrices. We found that β -estradiol or relaxin alone or together caused a significant loss of GAGs and collagen from the pubic symphysis and TMJ disc, but not from the knee meniscus. Progesterone prevented the relaxin- or β -estradiol-mediated loss of these molecules. The findings suggest that, by differentially modulating matrix loss from these tissues, female hormones may selectively contribute to diseases of the TMJ. Pertinent to our studies on the fibrocartilaginous TMJ disc, a relationship between peak levels of relaxin during pregnancy and a decrease in total collagen has been demonstrated in the rat pubic symphysis fibrocartilage.¹⁶ Additional evidence for the importance of relaxin to normal tissue turnover is provided by studies on relaxin null mice, which show an increase in collagen density and failure in relaxation of the pubic symphysis during parturition.²⁷ The reasons why relaxin, β -estradiol, and progesterone have targeted effects on matrix-remodeling activities within the TMJ disc and pubic symphysis fibrocartilage are not known but may be associated with the quantity and types of hormone receptors present in these tissues.

Contributions of Known Estrogen and Relaxin Receptors to MMP Induction and Matrix Loss Remain To Be Determined

Although relaxin and estrogen each have two known receptors, relaxin family peptide 1 receptor (RXFP1 or LGR7) and RXFP2 (or LGR8) for relaxin and estrogen receptor alpha (ER- α) and ER- β for estrogen, little is currently known about the contributions that each of these receptors make to the induction of MMPs and to matrix turnover. Relaxin binds with high affinity to the RXFP1 receptor and with low affinity to the RXFP2 receptor.^{28,29} Indirect evidence for the role of RXFP1 in *in vivo* matrix remodeling is provided by the phenotypic

characteristics of female RXFP1 null mice, which have abnormal matrices similar to those found in relaxin-deficient mice.^{27,30} These mice show increased density of collagen of the nipple and of the pubic symphysis, suggesting that relaxin acts on its target tissues via RXFP1 to regulate collagen breakdown and reorganization. In light of the fact that the changes in matrix composition by relaxin parallel its modulation of MMPs in target tissues, it is likely that these changes in matrices result from relaxin's induction of MMPs. However, no evidence is currently available on the involvement of RXFP1 or RXFP2 in relaxin's induction of specific MMPs in any tissues, including those in synovial joints.

While the biological effects of estrogens can be mediated via one of four ER-signaling pathways, evidence indicates that modulation of MMPs by these hormones results from the classical ligand-dependent pathway,^{31–33} in which estrogen-ER complexes regulate gene transcription. Although little is known about the precise contributions of ER- α and ER- β to MMP regulation, the findings that the exposure of endometrial cancer (Ishikawa) cells transfected with an ER- α expression vector to β -estradiol results in their increased invasiveness concomitant with an enhanced expression of MMPs provides indirect evidence for the role of ER- α in the induction of MMPs.³⁴ It is plausible that a similar mechanism may be relevant to estrogen's induction of MMPs in TMJ fibrocartilage, leading to its enhanced degradation. Evidence that estrogen and relaxin likely contribute to the activities of TMJ disc cells was provided by our studies showing that these cells express ER- α , ER- β , RXFP1, and RXFP2.⁸ We also found that the two target tissues showing the greatest induction of MMPs and matrix loss in response to relaxin and β -estradiol, namely, the TMJ disc and pubic symphysis, have similar expression profiles of their receptors which differ from those in cells from the minimally responsive knee meniscus. Although these observations provide important clues for the basis of differential modulation of MMPs

by their respective hormones in different tissues, further studies are required to determine the contribution of each receptor type to these responses.

Conclusion

The initiation of cartilage matrix loss is a critical first step in DJDs and leads to adverse changes in mechanical properties, inability to sustain function, and eventually to disease. Therefore, understanding the key initial events that lead to DJD, possibly including those initiated by relaxin and β -estradiol, may be critical to preventing or reversing these disorders. The findings presented in this chapter offer insights into modulation of joint cartilage turnover by female hormones. However, much work remains to be done to establish a definitive link between the hormone-hormone receptor-MMP axis and joint degeneration.

Acknowledgments

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Conflicts of Interest

The authors declare no conflicts of interest.

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