Detection of Relaxin Receptor in the Dorsoradial Ligament, Synovium, and Articular Cartilage of the Trapeziometacarpal Joint

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ABSTRACT: Basilar thumb osteoarthritis (OA) is postulated to occur due to ligament attenuation of the trapeziometacarpal (TM) joint. Relaxin is a peptide hormone, which loosens ligaments before childbirth, through remodeling of the extracellular matrix via upregulation of matrix metalloproteases (MMPs). We postulated that relaxin family peptide receptor 1 (RXFP-1), the receptor for circulating relaxin, was present in tissues of the TM joint. Ligaments and synovium were sampled from 15 patients during surgery for TM arthritis. We obtained trapezial cartilage from two autopsy donors and four patients. Tissues were fixed, paraffin embedded, and sectioned at 5 μm, then were immunostained for RXFP-1, as well as MMP-1, and MMP-13, using rabbit anti-human polyclonal antibodies. Eight DRL samples showed positive immunostaining for relaxin receptor, with 14/15 positively stained in synovium. Greater staining was seen in specimens obtained from women with more severe TM arthritis. Trapezial cartilage demonstrated receptor staining within chondrocytes in the middle and deep zones. Immunostaining for MMPs co-localized with relaxin receptor staining. Relaxin receptors are present at the ligament, cartilage, and synovium of the TM joint, indicating that it is a potential target for relaxin. This suggests that circulating relaxin may impact joint stability. The role of relaxin in cartilage and synovium may be related to its role in collagen regulation as a possible tissue response to OA. © 2014 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 32:1061–1067, 2014.

Keywords: relaxin; immunohistochemistry; trapeziometacarpal osteoarthritis

Trapeziometacarpal osteoarthritis (TM OA) is a frequent cause of functional disability in the upper extremity, impacting the ability to grasp and pinch in activities of daily living and in occupational use. It is more commonly seen in women. Haara et al. noted the age-adjusted prevalence of radiographic TM osteoarthritis to be 7% in men and 15% in women in an epidemiologic study of 8,000 subjects in Finland, with some correlation to symptoms. The etiology of the progressive joint narrowing and ultimate arthrosis of the TM joint has been attributed to both the high loads placed across the joint and attenuation of the supporting ligaments of the joint. The anterior oblique ligament (AOL) and the dorsoradial ligament (DRL) have both been shown to provide stabilizing restraint to subluxation of the TM joint. Recent evidence has suggested that the DRL may play a more important role in joint stabilization due to its more robust, thicker substance. Therefore, laxity or destruction of this ligament may accelerate abnormal wear at the TM joint and lead to later arthritis.

The peptide hormone relaxin is best known for its role in preparing the uterine ligaments and cervix for parturition by increasing laxity via upregulation of matrix metalloproteases (MMPs) in the extracellular matrix. However, relaxin has been shown to have a variety of physiologic roles outside pregnancy, including renal vasodilation and cardiac function and relaxin knockout mouse data suggest that it is a regulator of collagen metabolism. Relaxin receptors have been shown to be present in the anterior cruciate ligament (ACL), as well as at the anterior oblique ligament of the thumb. Dragoo et al. recently noted higher serum relaxin in a prospectively evaluated cohort of female athletes who sustained ACL tears compared to those who did not have ACL injuries. This suggests a role for relaxin in increasing ligament laxity, thus making ligaments vulnerable to injury.

Relaxin has been shown to induce expression and production of multiple matrix metalloproteases including MMP-1 (collagenase-1), MMP-3 (stromelysin), MMP-9 (gelatinase), and MMP-13 (collagenase-3). MMPs cleave protein substrates, including aggrecan, fibronectin, and types I and II collagen within the extracellular matrix of tissues, and the function of these enzymes is thought to be both physiologic and pathologic. MMPs have been linked to cartilage destruction in osteoarthritis and rheumatoid arthritis. Relaxin potentially may play a direct role in this process, as shown by Naqvi et al. who reported loss of collagen and glycosaminoglycan in temporomandibular synovial explants exposed to relaxin.

To elucidate relaxin’s role in the stability of the TM joint, we studied the DRL in order to learn whether this stouter, potentially stronger ligament is a possible target for circulating relaxin. The purpose of this study was to identify the presence or absence of relaxin receptor (RXFP-1), as well as MMP-1 and MMP-13, in the DRL and tissues of the TM joint from women and men, and evaluate whether a sex difference existed. Our hypotheses were that (i) relaxin receptors would be present in the stabilizing ligament,
articular cartilage, and synovial tissue at the TM joint, and (ii) that women would have a qualitatively greater number of receptors than men. Additionally, we hypothesized that MMP-1 and MMP-13, whose expression is induced by relaxin binding, would be identified in the DRL and articular cartilage.

METHODS

Surgical Specimen Acquisition
Tissue samples were obtained from the DRL during surgery for trapeziometacarpal osteoarthritis, with approval from the institutional review board at our institution. During the approach to the TM joint for trapeziectomy, the DRL was identified and sampled, often with attached synovium. In addition, trapexial cartilage and bone samples (routinely removed during surgery) were also sampled from four patients. Tissue specimens were obtained in a deidentified fashion from 10 women (mean age 59 years, range 44–75) and 5 men (mean age 66 years, range 56–79). The degree of TM osteoarthritis was determined by the operating surgeon using the Eaton staging system.25 All had Eaton Stage II, III, or IV grade osteoarthritis based on preoperative radiographs.

Additionally, two trapeziae, with one with attached DRL, were obtained from organ donors (with tissue bank and family consent) within 24 h of death, a 30-year-old male and an 18-year-old male without history of osteoarthritis of the thumb. Positive ligament controls were derived from uterine round ligament specimens.

Immunohistochemistry
Soft tissues were fixed for 24 h in 4% paraformaldehyde, paraffin embedded, and sectioned at 5 µm. Sections were deparaffinized and rehydrated in a graded series of ethanol. For RXFP-1, antigen retrieval was carried out by HIER with 10 mM sodium citrate buffer solution (pH 6.0) for 40 min at 95°C. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 15 min. To reduce nonspecific binding, sections were treated with protein block for 30 min at room temperature. Sections were incubated with rabbit anti-human polyclonal RXFP-1 for 2.5 h at room temperature. Sections were incubated with rabbit anti-human polyclonal RXFP-1, MMP-1, or MMP-13 overnight at 4°C (1:500, 1:200, and 1:100 dilutions, respectively). Sections were incubated with biotinylated goat anti-rabbit IgG (1:200 dilution, BA-1000, Vector Laboratories, Burlingame, CA) and visualized with DAB. Negative controls were carried out by omitting the primary antibodies. Sections were counterstained with hematoxylin, cleared, and mounted in resinous mounting medium.

The degree of immunohistochemical staining was assessed qualitatively by brightfield microscopy. Additionally, a set of slides was stained with hematoxylin and eosin for gross tissue morphology. Image acquisition was performed using a Q-Imaging Retig 200R camera connected to a Nikon Eclipse 50i microscope (Nikon, Melville, NY).

Statistical Analyses
Relationships among variables were determined by Pearson product-moment correlation coefficient with a significance criterion of p < 0.05 using JMP (SAS Institute, Inc., Cary, NC). Pearson’s chi-squared test was used to examine the relationship between gender and degree of RXFP-1 staining.

RESULTS

A total of 15 samples (10 women, 5 men) from individuals undergoing trapeziometacarpal arthroplasty, as well as the control from the 30-year-old donor, were identified by histological assessment as ligament with attached synovial tissue. Of these, one sample (male) contained no ligament and two samples (female) contained no synovium. RXFP-1 is a membrane receptor; its expression is localized to the cell surface. RXFP-1 was detected in all tissue types examined, but the expression level varied by tissue (Fig. 1A–D). Ligament fibroblast staining was categorized qualitatively in terms of number of immunopositive cells as none, low, or moderate. Overall, the expression of RXFP-1 in the ligamentous fibroblasts was low; seven samples (6 females, 1 male) had no fibroblasts immunopositive for the receptor. Among those samples where receptor was detected, staining was demonstrated in a few to a moderate number of fibroblasts (Fig. 1A). No RXFP-1 staining was seen in the ligament of the 30-year-old male control tissue donor. The highest RXFP-1 expression, both in intensity and amount of signal, was in the cells of the synovial tissue (Fig. 1B–D). Fibroblast-like cells of the synovium stained positively for the receptor. The most abundant staining was seen in macrophage-like cells in all samples where synovium was present (Fig. 1C). The relaxin receptor was also detected in the vascular smooth muscle in all samples (Fig. 1D).

Analyses showed no correlation between amount of RXFP-1 staining and gender (X^2 = 2.86, p = 0.24) or age (r = −0.197, p = 0.50); therefore, we examined the relationships between Eaton score and age, and Eaton score and degree of immunopositive staining, by gender (Table 1). Females underwent trapeziectomy at a younger age and the disease was somewhat less advanced in this cohort. Eaton score was not signifi-
cantly correlated to age in females, whereas males showed a positive correlation with age that approached, but did not reach significance. The relationship between Eaton score and amount of RXFP-1 staining by gender revealed a different picture (Table 1). Among females, there was a robust positive relationship between the Eaton score and amount of RXFP-1 staining \( r = 0.700, \ p = 0.04 \), while males showed a moderately negative correlation, which was not significant.

A total of four trapeziae with associated cartilage (3 females, 1 male, all harvested at surgery for TM OA) were embedded, sectioned and immunostained for RXFP-1, along with trapeziae from the two donor controls. Three of four patient samples were immunopositive for relaxin receptor, while both controls were negative (Fig. 2). Chondrocytes positive for RXFP-1 were restricted to the middle and deep zones of the cartilage. Any blood vessels in the subchondral bone also stained positively.

MMPs are proteolytic enzymes produced in the cytosol by a variety of cell types, including those found in ligament, chondrocytes, and synovium, and secreted into the adjacent extracellular matrix.\(^{24}\) MMP-1 was detected in all patient samples, whereas MMP-13 was detected in all but three. Positive staining for both was seen in all soft tissue cell types. Further, in serial sections, staining for both MMPs was localized to the same regions of the tissues where RXFP-1 expression was observed (Fig. 3). MMP staining was restricted to the cytoplasm of the cells and the adjacent extracellular matrix. In all cases, MMP-1 staining was more abundant than MMP-13. In contrast to ligament and synovium, MMP-13 was more abundant than MMP-1 in the cartilage of the trapezium. MMP-13 was also detected in both donor cartilages; although expression was much lower (Fig. 4). MMP-1 staining was weak or absent in cartilage, but was highly expressed in any synovial soft tissues present in the samples.

DISCUSSION

We demonstrated the presence of relaxin receptors associated with fibroblasts of the TM dorsoradial ligament from arthritic joints, as well as at the articular cartilage of the trapezium and in the synovial lining of the TM joint. Although we were unable to show any qualitative differences in positive staining for either sex or age, there was a correlation between the amount of staining and the severity of osteoarthritis at the TM joint (as measured by the radiographic Eaton classification). These findings suggest a possible role for relaxin in decreasing stability at the TM joint.

The potential contribution of relaxin to ligamentous laxity in the musculoskeletal system has been examined at a number of sites. Relaxin was first investigated for its role in preparing the uterine and pelvic ligaments and softening the cervix for parturition.\(^{8}\) Increased laxity of the ACL, medial collateral ligament and metacarpophalangeal joints of the index finger have also been correlated with pregnancy, when serum relaxin and estrogen levels are highest.\(^{25-27}\) Subsequently, the contribution of relaxin to ligamentous laxity has been investigated in the anterior cruciate

**Table 1.** Correlations Between Age, Eaton Score, and RXFP-1 Staining by Gender

<table>
<thead>
<tr>
<th>Gender (n)</th>
<th>Mean Age (Eaton Score)</th>
<th>Age vs. Eaton, ( r )</th>
<th>RXFP1 Staining</th>
<th>Mean Eaton Score</th>
<th>Eaton vs. Staining, ( r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>F (10)</td>
<td>59 (2.9)</td>
<td>-0.013</td>
<td>None</td>
<td>2.7</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Few</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Moderate</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>M (5)</td>
<td>67 (3.2)</td>
<td>0.73</td>
<td>None</td>
<td>4.0</td>
<td>-0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Few</td>
<td>3.0</td>
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<td></td>
<td></td>
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<td>Moderate</td>
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ligament (ACL) by gender. Relaxin receptors were identified in ACLs of females, although none were detected in males. Similarly, Faryniaz et al. noted that relaxin receptor was present in 4 of 5 female ACL samples compared to 1 of 5 male ACL samples. Exogenous relaxin has been shown to significantly weaken the ACL in a female animal model. A prospective evaluation of 128 female athletes found significantly higher serum relaxin levels in those who sustained ACL tears compared to those who did not. These and other studies have provided evidence that relaxin and estrogen may work synergistically to destabilize the female ACL, increasing the risk of rupture in women. We have previously demonstrated the presence of very low levels of transcripts for RXFP-1 in anterior oblique ligaments sampled at surgery for TM OA; the current study is drawn from a different subset of surgical patients and did not evaluate relaxin receptor transcripts in the DRL, but confirms the presence of receptor protein in this ligament using immunohistochemistry. Additionally, we have shown that circulating relaxin is correlated with the degree of radiographic laxity at the TM joint, although not with generalized laxity. Collectively, these data suggest that relaxin may contribute to pathologic laxity of the stabilizing ligaments at the TM joint.

Recent studies on relaxin and RXFP-1 gene-knockout mice have established relaxin as an important naturally occurring and protective modulator of collagen turnover. The relaxin knockout mouse demonstrates increased collagen deposition and fibrosis in the lung and kidney as well as reproductive tissues. Infusion of relaxin reverses the fibrotic process, suggesting that relaxin acts as a regulator of collagen homeostasis. Similarly, evidence from RXFP-
null mice suggests that relaxin acts on its target tissues via RXFP-1 to regulate collagen breakdown and reorganization. The ability of relaxin to reduce matrix synthesis and increase ECM degradation has important implications in several nonreproductive organs, including ligament, heart, lung, kidney, liver, and skin.11,16,33–35

Relaxin is known to contribute to collagen degradation primarily by inducing the expression of multiple matrix metalloproteases including MMP-1 (collagenase-1), and MMP-13 (collagenase-3) in periodontal ligament and fibrocartilage of the TMJ and pubic symphysis. We noted MMP-1 and -13 immunostaining aligned with RXFP-1 staining in osteoarthritic TMJ joint tissues. These findings are consistent with previously reported roles of MMPs 1 and 13, which have been linked to joint destruction in osteoarthritis. MMP-13 preferentially degrades type II collagen compared to types I and III, and MMP-13 cleaves type II collagen more efficiently than MMP-1. Relaxin potentially may play a direct role in initiating this process, as shown by Naqvi et al. who demonstrated loss of collagen and glycosaminoglycan in temporomandibular synovial explants treated with exogenous relaxin.22 In studies of the rabbit temporomandibular (TMJ) joint, Hashem et al. showed that circulating relaxin caused a significant loss of glycosaminoglycans and collagen from the TMJ disc and articular cartilage. The same investigators then showed that relaxin induced MMP-1 in fibrocartilage of the TMJ in vitro. This suggests a role for relaxin in collagen homeostasis in fibrocartilage. We have demonstrated novel immunohistochemical binding to relaxin receptors within articular cartilage, demonstrating the potential for relaxin to play a significant role in collagen homeostasis in articular cartilage of the TM joint as well.

The abundance of receptor detected in the cells of the synovial lining indicates that synovium is likely a major target for relaxin in the TM joint. The active, circulating form of relaxin (H2-relaxin) is a 6-kDa polypeptide, capable of traversing the synovial membrane. Recently, Takano et al. showed that periodontal ligament fibroblasts subjected to stretch responded with an increase in MMP-1 production, and that subsequent treatment with relaxin enhanced this effect. Similarly, mechanical stretching of synovium results in increased hyaluronic acid secretion by synoviocytes. Mechanical stretching may also result in relaxin-mediated induction of MMP production in the synovium of the TM joint at the thumb. Pellegrini et al. postulated that ligament attenuation was the source of TM joint instability, leading to abnormal loading and ultimately arthritic wear of the cartilage. This hypothesis is supported by the observation of early development of TM joint subluxation and arthritis in young patients with Ehlers–Danlos syndrome. However, given the paucity of receptor in the ligament and the abundance of receptor in the synovium, together with the fact that RXFP-1 binds relaxin with high affinity,44,45 an alternate hypothesis for development of TM joint instability is that excess or continuous ligament stretching may occur as a result of initial damage to the joint; circulating relaxin subsequently binds to its receptor, beginning a cascade of molecular processes that ultimately degrades the ECM, resulting in ligament thinning and attenuation.

This study has a number of limitations. We are limited in our ability to study the anatomic distribution of receptors in the DRL and synovium, as surgical samples varied in size and composition, and therefore may not represent the tissues on the whole. Relaxin levels in the synovial fluid of the TM joint in subjects whose tissue was sampled at surgery were not measured; this information is vital in determining the significance of the amount and distribution of receptor staining. Synchronous expression of RXFP-1 and MMPs-1 and -13 strongly suggests a relationship between relaxin and MMP upregulation in these tissues; however, this is pure correlative, and expres-
sion of MMPs in osteoarthritis is known to be multifactorial. In vitro assays are needed to determine if the cells of the TM joint expressing RXFP-1 are responsive to relaxin, and to quantify the concomitant upregulation of MMPs involved in collagen and articular cartilage degradation. Although the donor tissues were negative for RXFP-1, we were only able to obtain two samples, both young males, due to the rarity of surgery on non-arthritic TM joints. Additional controls are needed, particularly from females, to further investigate the finding that RXFP-1 expression may vary in the TM joint by gender. An additional limitation is that the trapezial saddle was devoid of cartilage in the surgical samples, so it was only possible to examine the cartilage from the proximal articular surface. As we are not able to directly track the changes in RXFP-1 expression with the progression of the disease on the affected articulation, we do not know if the RXFP-1 expression of the saddle cartilage was comparable to the other articular surfaces before it became completely eroded.

In conclusion, detection of relaxin receptor concurrent with MMPs in multiple tissues of the TM joint, together with our previous finding of higher levels of serum relaxin in females in a general population, provides support for the notion that relaxin contributes to the cascade of joint destruction in TM osteoarthritis. The preponderance of RXFP-1 in the synovium suggests that it is the primary target of relaxin in this arthritis. The preponderance of RXFP-1 in the synovium provides support for the notion that relaxin contributes to the cascade of joint destruction in TM osteoarthritis. The DMM surgical instability model of osteoarthritis has been used successfully to challenge mice with gene deletions of potential targets for OA therapies; use of this model with the relaxin knockout mouse may be useful in deciphering the role of relaxin in the etiology of TM OA.

REFERENCES


