Differential expression of matrix metalloproteinases and their tissue inhibitors in leiomyomata: a mechanism for gonadotrophin releasing hormone agonist-induced tumour regression*

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Tissue remodelling involving extracellular matrix (ECM) turnover plays a major role in leiomyoma growth and regression, regulated by the combined action of matrix metalloproteinases (MMPs) and the tissue inhibitors of MMPs (TIMPs). We postulated that leiomyomata express MMP and TIMP mRNA and protein, and their expression is inversely regulated during tumour growth and gonadotrophin releasing hormone agonist (GnRHa)-induced regression. We therefore examined the expression of mRNA and protein for MMPs (interstitial collagenase, MMP-1; gelatinases, MMP-2 and MMP-9; and stromelysin, MMP-3) and TIMPs (TIMP-1 and TIMP-2) in leiomyoma and matched unaffected myometrium from GnRHa (lupron)-treated and untreated patients. Reverse transcription-polymerase chain reaction (RT–PCR) and restriction enzyme analysis revealed that leiomyomata and myometrium expressed MMP-1, -2, -3 and -9, as well as TIMP-1 and -2 mRNA. Quantitative RT–PCR indicated that leiomyomata and myometrium during the secretory phase of the menstrual cycle expressed higher levels of MMP and TIMP mRNA compared to the proliferative phase (P < 0.05), with low to undetectable levels of MMP-1, -2 and -3 mRNA in the tumours. GnRHa therapy induced an overall reduction in MMP and TIMP mRNA expression in both leiomyomata and myometrium, but a significant decrease in TIMP-1, and an increase in MMP mRNA expression compared with untreated tumours (P < 0.05). Immunohistochemically, MMP-1, -2, -3 and -9 and TIMP-1 and -2 proteins were localized in leiomyomata and myometrial smooth muscle cells, arteriole wall and connective tissue fibroblasts, with an overall increase in MMP and a decrease in TIMP staining intensity in GnRHa-treated groups. The results suggest that MMP and TIMP expression in leiomyoma and myometrium are hormonally regulated, and that GnRHa-induced tumour regression is accompanied by an increase in MMP expression with a concomitant decrease in TIMP-1 expression, which may potentially provide an environment favouring ECM degradation.

Key words: GnRH agonist/leiomyoma/metalloproteinases/myometrium/TIMPs

Introduction

Leiomyomata are benign uterine tumours presumably originating from the conversion of normal myometrial smooth muscle cells, which histologically consist mainly of smooth muscle cells and a network of connective tissue (Sullivan and Guzick, 1996). Similar to myometrium, leiomyomata also contain functional oestrogen and progesterone receptors, which are apparently overexpressed in the tumours (Nardelli et al., 1987; Chrapusta et al., 1990; Branden et al., 1993; Bulum et al., 1994; Rein et al., 1995). Because of the ovarian steroid involvement in leiomyoma growth, medical interventions to reverse their growth are centred around the use of gonadotrophin releasing hormone analogues (GnRHa) (Rein et al., 1995; Lemay et al., 1996) and, recently, antiprogestin therapy (Reinsch et al., 1994; Murphy et al., 1995). It appears, however, that the predominant changes in uterine volume due to GnRHa therapy occur in non-leiomyoma tissue, and co-administration of medroxyprogesterone acetate (MPA) reverses the beneficial effect of GnRHa therapy (Carr et al., 1993).

Modulation of mitotic activity, cellular hypertrophy, and excess accumulation of extracellular matrix (ECM) are considered key factors in leiomyoma growth (Kawaguchi et al., 1989; Stewart et al., 1994; Rein et al., 1995). However, the individual contribution of these parameters to tumour growth and regression are poorly characterized. In regard to ECM, leiomyomata and myometrium have been reported to express a similar level of fibronectin mRNA. However, the ratio and the level of collagen type I and III mRNA expression has been shown to be higher in leiomyomata than myometrium, but only in tissues from the proliferative phase of the menstrual cycle (Paistola et al., 1990; Stewart et al., 1994). GnRHa-induced leiomyoma/uterine tissue regression is accompanied by substantial tissue remodelling, presumably involving ECM turnover (Upadhyaya et al., 1990; Gutmann et al., 1994; Rein et al., 1995; Lemay et al., 1996). The ECM turnover is regulated by the rate of synthesis and deposition of various ECM components (Mosher et al., 1992), and a balance between the
action of matrix metalloproteinases (MMPs) which degrade ECM, and their tissue inhibitors (TIMPs) (Matrisian, 1992; Woessner et al., 1994). The MMPs are broadly classified based on their ability to specifically degrade various interstitial matrix and basement membrane components, and are inactivated by TIMPs through binding to the active form of these enzymes in 1:1 ratio (Matrisian, 1992; Overall, 1994; Woessner et al., 1994).

The expression of MMPs and TIMPs has been documented in various normal tissues which undergo tissue remodelling, and their overexpression in certain pathological conditions associated with extensive ECM degradation (Matrisian, 1992; Overall, 1994; Woessner et al., 1994). In female reproductive tract tissues, the expression of MMPs and TIMPs has been demonstrated, and associated with events such as regular menstruation, abnormal uterine bleeding, ovulation, implanta-
The ratios of template to sample band intensities calculated by digitally scanning the photographs shown in Figure 2 after the values were normalized for their molecular weight. The ratio of the band intensity within each lane was determined and then plotted against the copy number of added template RNA standard. The number of template RNA molecules/cell was calculated based on the constant that there is ~26 μg mRNA/cell. The number of message copies (copy/cell) determined where the ratio equals 1. The levels of MMP-1, -2, -3 and -9 and TIMP-1 and -2 expression in myometrium from proliferative (Pro) and secretory (Sec) phases of the menstrual cycle are shown with equations of best fit lines; MMP-1 (Sec): \(Y = 0.418X + 0.365\) with \(r^2 = 0.97\), MMP-2 (Pro): \(Y = 0.560X - 0.351\) with \(r^2 = 0.984\), MMP-2 (Sec): \(Y = 0.496X - 0.478\) with \(r^2 = 0.995\), MMP-3 (Pro): \(Y = 0.862X + 0.891\) with \(r^2 = 1.000\), MMP-3 (Sec): \(Y = 0.533X + 0.102\) with \(r^2 = 0.985\), MMP-9 (Pro): \(Y = 0.378X + 0.055\) with \(r^2 = 0.942\), MMP-9 (Sec): \(Y = 0.525X - 0.550\) with \(r^2 = 0.973\), TIMP-1 (Pro): \(Y = 0.385X - 0.810\) with \(r^2 = 0.959\), TIMP-1 (Sec): \(Y = 0.320X - 0.937\) with \(r^2 = 0.996\), TIMP-2 (Pro): \(Y = 0.467X - 0.670\) with \(r^2 = 0.996\), TIMP-2 (Sec): \(Y = 0.496X - 1.013\) with \(r^2 = 0.996\), β-actin (Pro): \(Y = 0.352X - 1.194\) with \(r^2 = 0.971\), β-actin (Sec): \(Y = 0.399X - 1.297\) with \(r^2 = 0.996\). Only a very weak band was present on the gel for MMP-1 mRNA from proliferative phase myometrium, so that this appears as a single point on the graph. Abbreviations: see Figure 1.

Figure 3. The ratios of template to sample band intensities calculated by digitally scanning the photographs shown in Figure 2 after the values were normalized for their molecular weight. The ratio of the band intensity within each lane was determined and then plotted against the copy number of added template RNA standard. The number of template RNA molecules/cell was calculated based on the constant that there is ~26 μg mRNA/cell. The number of message copies (copy/cell) determined where the ratio equals 1. The levels of MMP-1, -2, -3 and -9 and TIMP-1 and -2 expression in myometrium from proliferative (Pro) and secretory (Sec) phases of the menstrual cycle are shown with equations of best fit lines; MMP-1 (Sec): \(Y = 0.418X - 0.365\) with \(r^2 = 0.97\), MMP-2 (Pro): \(Y = 0.560X - 0.351\) with \(r^2 = 0.984\), MMP-2 (Sec): \(Y = 0.496X - 0.478\) with \(r^2 = 0.995\), MMP-3 (Pro): \(Y = 0.862X + 0.891\) with \(r^2 = 1.000\), MMP-3 (Sec): \(Y = 0.533X + 0.102\) with \(r^2 = 0.985\), MMP-9 (Pro): \(Y = 0.378X + 0.055\) with \(r^2 = 0.942\), MMP-9 (Sec): \(Y = 0.525X - 0.550\) with \(r^2 = 0.973\), TIMP-1 (Pro): \(Y = 0.385X - 0.810\) with \(r^2 = 0.959\), TIMP-1 (Sec): \(Y = 0.320X - 0.937\) with \(r^2 = 0.996\), TIMP-2 (Pro): \(Y = 0.467X - 0.670\) with \(r^2 = 0.996\), TIMP-2 (Sec): \(Y = 0.496X - 1.013\) with \(r^2 = 0.996\), β-actin (Pro): \(Y = 0.352X - 1.194\) with \(r^2 = 0.971\), β-actin (Sec): \(Y = 0.399X - 1.297\) with \(r^2 = 0.996\). Only a very weak band was present on the gel for MMP-1 mRNA from proliferative phase myometrium, so that this appears as a single point on the graph. Abbreviations: see Figure 1.

Materials and methods

All the materials for RT–PCR and immunohistochemistry were purchased from commercial sources as previously described (Chegini...
Abbreviations: see Figure 1.

Histological dating of endometrium and the patients’ last menstrual period, calculated from secretory phase of the menstrual cycle) patients, and individually subjected to standard RT–PCR performed as previously described (Chegini et al., 1994; Dou et al., 1996). Portions of leiomyoma and matched unaffected myometrium from 10 premenopausal women aged 21–47 years were collected immediately following abdominal or vaginal hysterectomies for symptomatic uterine leiomyomata. Based on histological dating of endometrium and the patients’ last menstrual period, three specimens were from mid–late proliferative and seven from early–mid secretory phase of the menstrual cycle. Portions of leiomyoma and matched unaffected myometrium (without any trace of endometrium) were collected from nine patients who had received GnRHα (leuprolide acetate) therapy for symptomatic leiomyomata during the previous 3 months prior to surgery. The tissues were collected at the University of Florida affiliated Shands Hospital with the approval of the Institutional Review Board. The tissues were immediately processed for total cellular RNA isolation and immunohistochemistry as previously described (Chegini et al., 1994; Dou et al., 1996).

**Isolation of cellular RNA and quantitative competitive RT–PCR**

Total cellular RNA was isolated from nine leiomyomata and matched unaffected myometrium from the same subject from GnRHa-treated (n = 10) and GnRHa-treated (n = 9) patients who were collected and fixed in Bouin’s solution, processed and embedded in paraffin (Chegini et al., 1994). Tissue sections were cut and immunostained using monoclonal antibodies to MMP-1, -2, -3 and -9, and TIMP-1 and -2 (Oncogene Sciences, Cambridge, MA, USA) at a concentration of 2.5–5 μg of IgG/ml for 2–3 h at room temperature as previously described (Chegini et al., 1994; Dou et al., 1996), and visualized with vectastain ABC Elite Kit (Vector Laboratories, Burlington, CA, USA). Omission of the primary antibodies, or incubation of tissue sections with non-immune mouse IgG instead of primary antibodies at the same concentration during immunostaining, were used as controls.

**Results**

**Expression of MMP and TIMP mRNA**

Initially the expression of MMPs and TIMPs mRNA in leiomyomata and matched unaffected myometrium was examined using standard RT–PCR. Total cellular RNA isolated from these tissues and subjected to RT–PCR indicated that MMP and TIMPs mRNA were detectable in myometrium and leiomyomata (Figure 1). The specificity of the reactions was confirmed by appropriate restriction enzyme digestion (Figure 1), as well as by amplification of RNA without the RT step to detect the presence of any genomic DNA contamination, and reactions containing all the PCR components except the RT reaction mixture to check for the presence of DNA that may have carried over from a prior reaction.

For competitive quantitative RT–PCR, a synthetic multiprimer external RNA standard was constructed as previously described (Tarnuzzer et al., 1996; Dou et al., 1996). The external RNA standard contains the complementary sequences corresponding to the 3', 5' and internal probe primers for MMP-1, -2, -3 and -9, as well as TIMP-1 and -2 (Tarnuzzer et al., 1996). Briefly, cDNA was synthesized in a series of standard reactions, each containing 2 μg of total cellular RNA prepared from each tissue and several dilutions of competitive external RNA standard (1×10^8 to 1×10^3 copies/reaction), 2.5 μM oligo(dT)_{16}, 1.5 mM MgCl₂, 200 μM of each of dNTPs, 1 U/μl of human placental ribonuclease inhibitor, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, and 200 U/μg RNA Moloney Murine Leukemia Virus reverse transcriptase (MMLV–RT) in a final volume of 100 μl. The reactions were incubated at 25°C for 10 min, 37°C for 60 min and 92°C for 5 min, and DNA amplification was carried out as previously described (Dou et al., 1996). The PCR products were separated on 2% agarose gels containing 2 ng/ml of ethidium bromide, photographed with polaroid film and scanned on a Hewlett-Packard Scanjet 3C, and stored on computer as TIFF files. The band intensities were determined using NIH–Image version 1.54 and their intensity values were normalized for their molecular weight (Dou et al., 1996). The ratio of band intensities within each lane were determined and then plotted against the copy number of added template RNA standard. The number of mRNA molecules/cell was calculated based on the constant that is ~26 pg of mRNA/cell (Dou et al., 1996). The quantity of the target messages was determined where the ratio of template/target band intensity was equal to 1 (Dou et al., 1996; Tarnuzzer et al., 1996; Tang et al., 1997). The data were expressed as mean ± SEM of the band intensity calculated from three separate experiments. The corresponding points on the curves were analysed by Student t-test, and all points on the curves were analysed by ANOVA. P < 0.05 was considered significant.

**Immunohistochemistry**

For immunohistochemical studies small portions of leiomyoma and matched unaffected myometrium from untreated (n = 10) and GnRHa-treated (n = 9) patients were collected and fixed in Bouin’s solution, and processed and embedded in paraffin (Chegini et al., 1994). Tissue sections were cut and immunostained using monoclonal antibodies to MMP-1, -2, -3 and -9, and TIMP-1 and -2 (Oncogene Sciences, Cambridge, MA, USA) at a concentration of 2.5–5 μg of IgG/ml for 2–3 h at room temperature as previously described (Chegini et al., 1994; Dou et al., 1996), and visualized with vectastain ABC Elite Kit (Vector Laboratories, Burlington, CA, USA). Omission of the primary antibodies, or incubation of tissue sections with non-immune mouse IgG instead of primary antibodies at the same concentration during immunostaining, were used as controls.

**Figure 4.** Bar graph representing the level of MMP-1, -2, -3, -9 and TIMP-1 and -2 mRNA expression in myometrium from proliferative and secretory phases of the menstrual cycle, calculated from the bands intensity, where the ratio of template/sample RNA equals 1. The data represent mean ± SEM of mRNA expression (copy/cell) in three tissues from each phase of the menstrual cycle. *Significantly different from proliferative phase (P < 0.05).
expressed levels of MMPs and TIMPs similar to that seen in myometrium from the proliferative phase, which also expressed low levels of MMPs (compare Figures 4 and 7), representing a 10–100-fold increase compared with proliferative phase leiomyoma ($P < 0.05$). The level of MMPs and TIMPs mRNA expression was significantly reduced in leiomyomata and myometrium obtained from GnRHa-treated compared to the untreated group from the secretory (Figures 2 and 7A) and proliferative phase (Figure 7B) of the menstrual cycle ($P < 0.05$). Despite an overall reduction in MMP and TIMP mRNA expression in GnRHa-treated leiomyomata and myometrium, there was a significantly lower TIMP-1, although higher MMP-1, -2, -3 and -9, and minimal alteration in TIMP-2 expression ($P < 0.05$, Figures 5–7). There was no significant difference in the level of β-actin mRNA expression in myometrium and leiomyomata from proliferative and secretory phase of menstrual cycle, or GnRHa-treated and untreated groups (Figures 4 and 7).

**Immunolocalization of MMPs and TIMPs**

Immunohistochemical observations indicate that leiomyomata and myometrium from untreated groups contained immunoreactive MMP-1, MMP-2, TIMP-1 and TIMP-2, but low MMP-3 and MMP-9 protein, associated with the smooth muscle cells, connective tissue fibroblasts and arteriole endothelial and smooth muscle cells (Figure 8). The immunostaining intensity of MMPs and TIMPs was not considerably different in tissues from proliferative compared to secretory phase of the menstrual cycle. However, there was a noticeable increase in MMP and a decrease in TIMP (particularly TIMP-1) immunostaining intensity in leiomyomata (Figure 8) and myometrium (results not shown) from GnRHa-treated com-

**Figure 5.** A representative of quantitative RT–PCR of total cellular RNA isolated from a gonadotrophin releasing hormone (GnRH) agonist-treated and untreated leiomyoma (A) and matched unaffected myometrium (B). The synthetic competitor standard at serial dilutions and total RNA were co-amplified by PCR using primers specific to MMPs and TIMPs for 40 cycles. Top bands are PCR products generated from the specific message in the specimen’s total RNA, and lower bands from serial dilutions of the standard shown from right to left at dilutions corresponding to $10^8$ to $10^3$ copies/reaction. The far left lanes are the DNA markers. Abbreviations: see Figure 1.
The ratios of template to sample band intensities calculated from data shown in Figure 5A after normalization for their molecular weight. The ratio of template to samples was calculated and the log of the ratio was plotted versus the log of the input copy number of the template (10^8 to 10^3 molecules) and the number of message copies (copy/cell) determined where the ratio equals 1. The level of MMP and TIMP mRNA expression in leuprolide-treated (Tr) and untreated (s) leiomyomata are shown with equations of best fit lines; MMP-1 (Un-3Tr): \( Y = 0.788(X) + 0.657 \), with \( r^2 = 1.00 \), MMP-1 (Tr): \( Y = 0.719(X) - 0.176 \) with \( r^2 = 1.00 \), MMP-2 (Un-3Tr): \( Y = 0.376(X) + 0.835 \) with \( r^2 = 0.855 \), MMP-2 (Tr): \( Y = 0.499(X) + 0.038 \) with \( r^2 = 0.986 \), MMP-3 (Un-3Tr): \( Y = 0.559(X) + 0.498 \) with \( r^2 = 0.990 \), MMP-3 (Tr): \( Y = 0.583(X) - 0.002 \) with \( r^2 = 0.993 \), MMP-9 (Un-3Tr): \( Y = 0.510(X) + 0.372 \) with \( r^2 = 0.992 \), MMP-9 (Tr): \( Y = 0.537(X) - 0.380 \) with \( r^2 = 0.975 \), TIMP-1 (Un-3Tr): \( Y = 0.384(X) - 0.610 \) with \( r^2 = 0.995 \), TIMP-1 (Tr): \( Y = 0.348(X) - 0.282 \) with \( r^2 = 0.996 \), TIMP-2 (Un-3Tr): \( Y = 0.431(X) - 0.356 \) with \( r^2 = 0.996 \), TIMP-2 (Tr): \( Y = 0.534(X) + 0.139 \) with \( r^2 = 0.985 \), β-actin (Un-3Tr): \( Y = 0.445(X) - 1.188 \) with \( r^2 = 0.967 \), β-actin (Tr): \( Y = 0.552(X) - 1.521 \) with \( r^2 = 0.997 \). Abbreviations: see Figure 1.

**Discussion**

Leiomyoma growth is considered to be a combination of mitotic activity, ECM accumulation, and cellular hypertrophy (Kawaguchi et al., 1989; Chrapusta et al., 1990; Rein et al., 1995), although the relative individual contribution of each parameter is poorly defined. Due to fibrotic nature of the leiomyoma, ECM turnover, which is regulated by a balance between ECM deposition, and differential expression of MMPs and TIMPs, may play a critical role. In the present study we have demonstrated that (i) myometrium expresses mRNA and protein for MMP-1, -2, -3 and -9 as well as TIMP-1 and TIMP-2, (ii) the myometrial MMPs and TIMPs mRNA expression, but not their immunoreactive proteins, appear to be cycle dependent, with maximal expression occurring during the secretory phase of the menstrual cycle, and (iii) compared with myometrium, leiomyomata from proliferative phase express...
undetectable to low level of MMPs and lower TIMP mRNA. In addition, during the secretory phase, levels of their expression in leiomyoma were comparable to that seen in myometrium of proliferative phase. Collectively the data suggest that MMPs and TIMPs mRNA and protein are expressed in leiomyoma, and at levels lower than myometrium, with maximal mRNA expression occurring during the progesterone-dominated secretory phase.

MMPs, which are classified according to their substrate specificity, degrade collagens I–III (MMP-1; interstitial collagenase), collagens IV, V and fibronectin (MP-2; gelatinase A, or type IV collagenase), collagens III, IV, fibronectin, laminin and proteoglycans (MMP-3; stromelysin 1), and collagens IV, V, and elastin (MMP-9; gelatinase B or type V collagenase), and their proteolytic activities are specifically blocked by TIMPs after binding to the active form of these enzymes (Matrisian, 1992; Overall, 1994; Woessner et al., 1994). The level and content of collagen I and III, but not fibronectin, mRNA expression and protein has been reported to be higher in leiomyoma compared with myometrium from the proliferative, but not the secretory phase, of the menstrual cycle (Puistola et al., 1990; Stewart et al., 1994). This coincides with low levels of MMP-1 expression in leiomyoma, which degrades collagens I and III. Other ECM may also be deposited during the proliferative phase due to the low level of MMP-2, -3 and -9 expression, similar to MMP-1. In addition to that of MMP, TIMP mRNA expression also appears to be cycle dependent, maximally occurring during the secretory phase, at levels significantly higher than that of MMPs. Despite the cyclic variation in MMP and TIMP mRNA expression, their immunoreactive protein levels determined immunohistochemically appeared not to be menstrual cycle dependent, and differed between leiomyoma and myometrium. Immunohistochemistry is qualitative and the approach does not allow determination of the MMP proteolytic activity in these tissues. However, considering the variations in mRNA expression, the data suggest the existence of an environment favouring accumulation of ECM in leiomyomata, particularly during the oestrogen-dominated phase. Further work is needed to determine the enzymatic activity of the MMPs as well as the specific ECM degradation in these tissues.

The importance of ovarian steroids in leiomyoma growth is well established, and medical interventions to reverse the tumour growth have centred around the use of GnRHa or, recently, antiprogestin therapy. Our data further indicate that GnRHa therapy, which results in ovarian suppression, causes an overall reduction in MMP and TIMP mRNA and protein expression in leiomyoma and myometrium. Despite the reduction in the level of MMP and TIMP mRNA expression, there was an inverse relationship between their expression in GnRH-a-treated compared to untreated leiomyomata and myometrium. TIMP-1 has been shown to be secreted as a complex with MMP-9, and specifically inactivates MMP-1, -2, -3 and MMP-9, while TIMP-2, which binds the active form of these enzymes, also binds the latent form of MMP-2 (Woessner et al., 1994; Overall, 1994). The immunohistochemical approach does not allow differentiation between the active and latent forms of MMPs. GnRHa therapy, which often results in leiomyoma regression by reducing the tumour size from 10 to 50% in diameter, is associated with a lack of extensive tissue breakdown. This suggests that the major portion of MMPs in these tissues are in a latent form.

The expression of MMPs and TIMPs has been demonstrated in other reproductive tissues (Sato et al., 1991; Marbaix et al., 1992, 1996; Martelli et al., 1993; Rogers et al., 1993, 1994; Waterhouse et al., 1993; Osteen et al., 1994; Schatz et al., 1994; Bruner et al., 1995; Aston et al., 1996; Hulboy et al., 1997). With the exception of MMP-2, which is constitutively expressed in the endometrium throughout the menstrual cycle, MMP-3, MMP-7 (matrilysin), and MMP-11 (stromelysin-3) are found to be expressed during the proliferative phase and at the onset of menstruation, whereas MMP-1, -9 and -10 are expressed only during menstruation (Rodgers et al., 1993, 1994; Salamonsen and Woolley, 1996; Marbaix et al., 1996; Hulboy et al., 1997). These results, as well as the data obtained from experiments in vitro regarding the expression of MMP-1 and stromelysin in human endometrial stromal cells, implicate progesterone as a negative regulator of MMP expression (Marbaix et al., 1992, 1996; Schatz et al., 1994; Bruner et al., 1995; Salamonsen and Woolley, 1996; Hulboy et al., 1997). However, the ovarian steroids appear to influence TIMP expression in a different manner to that of MMPs (Sato et al., 1991; Waterhouse et al., 1993; Salamonsen and Woolley, 1996). If, similarly to its effect on endometrium, progesterone negatively regulates the expression of MMPs in leiomyomata/myometrium, then one would expect a lack of MMP expression in these tissues. The reason for the differences is unclear. However, unlike myometrium/leiomyoma, the endometrium is a dynamic tissue which undergoes rapid and extensive morphological alterations during the menstrual cycle which requires a differentially regulated and higher MMPs expression. Other differences may be due to the sensitivity of the quantitative RT–PCR technique (estimated to be over 1000-fold higher than Northern blot analysis) used in our study to determine endometrial MMP mRNA expression. This enabled us to...
Figure 8. Immunohistochemical localization of MMP-1, -2, -3, -9 and TIMP-1 and -2 in leiomyoma tissue sections from GnRHa-treated and untreated subjects, associated mainly with the smooth muscle cells (SM) and arteriole wall (arrows). Note an overall higher immunostaining intensity for MMPs and a lower intensity for TIMPs in GnRHa-treated compared to untreated leiomyomata. Small arrow heads point to a group of cells possibly of inflammatory origin with strong immunostaining for MMP-3. In controls, replacement of primary antibodies with non-immune mouse IgG resulted in a considerable reduction in immunostaining intensities. Original magnification ×110.
measure mRNA expression at low copy numbers which would not have been possible using Northern blot analysis.

In addition to ovarian steroids, factors that are locally expressed by leiomyoma and myometrium may also regulate TIMP and MMP expression. These include growth factors and cytokines such as TGF-β, which play a critical role in tissue remodelling and fibrosis (Chegini et al., 1997), as well as possible GnRHα direct action which has been shown to inhibit the rate of DNA synthesis and TGF-β1 production by myometrial smooth muscle cells (Chegini et al., 1994). The effect of TGF-βs on ECM turnover and tissue fibrosis is mediated via its ability to differentially regulate the expression of ECM, TIMPs and MMPs (Woessner, 1994; Overall, 1994; Border et al., 1994). TGF-β1 up-regulates the expression of procollagen-I, fibronectin and TIMP-1, but down-regulates MMP-1 mRNA in endometrial stromal, glandular epithelial cells, and myometrial smooth cells (Tang et al., 1996). Furthermore progesterone-induced suppression of MMP expression in endometrial epithelial cells has been reported to be mediated through TGF-β production (Bruner et al., 1995). We have shown that GnRHα therapy results in down-regulation of TGF-β mRNA and protein expression in leiomyoma and myometrium (Chegini et al., 1994; Dou et al., 1996). Alternatively, GnRHα-induced TGF-β suppression in leiomyoma may in turn alter the balance between the rate of ECM deposition and degradation through differential regulation of MMPs and TIMPs, resulting in leiomyoma regression.

In conclusion, we have shown that leiomyoma/myometrium express MMP and TIMP mRNA and protein, and the patterns of their expression suggest that they may be hormonally regulated during the menstrual cycle and inversely expressed in patients after GnRHα therapy. The data further suggest that GnRHα-induced leiomyoma/uterine reduction in size may be due in part to a mechanism involving MMPs/TIMPs and excess ECM degradation. However, further experiments are required to establish the exact nature of ECM turnover during growth and GnRHα-induced tumour regression.

References


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Received on February 18, 1997; accepted on July 22, 1997