Matrix Metalloproteinase Expression in the Rat Myometrium During Pregnancy, Term Labor, and Postpartum

Tina Tu-Thu Ngoc Nguyen,1 Oksana Shynlova,2,3,5 and Stephen J. Lye3,4,5

1Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada
4Department of Physiology, University of Toronto, Canada
5Department of Obstetrics and Gynecology, University of Toronto, Canada

ABSTRACT

Pregnancy, spontaneous term labor (TL), and postpartum (PP) involution are associated with changes in the cellular and extracellular matrix composition of the uterus. Both the uterine smooth muscle (myometrium) and the infiltrating peripheral blood leukocytes involved in the activation of labor secrete extracellular matrix-degrading enzymes (matrix metalloproteinases, MMPs) that can modulate cellular behavior and barrier function. MMP expression is induced by mechanical stretch in several tissues. We hypothesized that the expression and activity of myometrial MMPs and their tissue inhibitors (TIMPs) are modulated in preparation for TL and PP involution and are regulated by mechanical stretch of uterine walls imposed by the growing fetus. Myometrial tissues were collected from bilaterally and unilaterally pregnant rats across gestation, TL, and PP. Total RNA and proteins were subjected to real-time PCR and immunoblotting, respectively, and tissue localization and activity was examined by immunohistochemistry and in situ zymography. We found that Mmp7, Mmp11, and Mmp12 mRNA levels were upregulated during TL and PP, while Mmp2, Mmp3, Mmp8, Mmp9, Mmp10, and Mmp13 mRNAs were only upregulated during PP. Timp1–Timp4 were stably expressed throughout gestation with some fluctuations PP. Active MMP2 was induced in the empty uterine horn during gestation and in the gravid PP uterus, suggesting negative regulation by biological mechanical stretch. We conclude that specific subsets of uterine MMPs are differentially regulated in the rat myometrium in preparation for two major events: TL and PP uterine involution.

INTRODUCTION

It is well known that uterine smooth muscle cells (SMCs) undergo sequential steps of hyperplasia, hypertrophy, and extracellular matrix (ECM) elaboration in order to adapt to the physiological demands of pregnancy [1–3]. These processes are regulated by both endocrine and mechanical factors [1–3] and are accompanied by extensive remodeling of the uterine ECM [4, 5]. For example, we have previously reported a significant prelabor decrease in the expression of fibrillar collagens (types I and III) and coordinated increases in fibronectin and basement membrane (BM) proteins (laminin and collagen IV) in the rat uterine muscle (myometrium). We found that these in vivo changes in ECM expression are mediated by the ovarian hormone progesterone and by the biological mechanical stretch of the uterus by the growing fetus(es) [2, 5]. We further suggested that phenotypic modulation of myometrial SMCs, focal adhesion proteins, and the biosynthesis of ECM provide cell-matrix stability for forceful labor contractions [6]. ECM biogenesis is accompanied by well-controlled biodegradation by specialized enzymes known as matrix metalloproteinases (MMPs). MMPs are zinc-containing endopeptidases categorized by substrate preference or the organization of their domain constituents, such as collagenases (MMP1, 8 and 13), gelatinases (MMP2 and 9), stromelysins (MMP3, 10, and 11), matrilysin (MMP7), and metalloelastase (MMP12). The expression and activity of MMPs is physiologically controlled by a group of endogenous proteins known as tissue inhibitors of metalloproteinases (TIMPs) that form noncovalent 1:1 complexes with MMPs to either inhibit or promote their enzymatic activity [7]. It is recognized that MMP transcriptional activation can be regulated by several factors, including matrix-integrin receptor signaling, growth factors, and cytokines [8–10]. Typically, MMPs are not expressed by healthy tissues, but they are detected in all diseased, inflamed, or injured tissues [11, 12]. Once activated, MMPs primarily degrade matrix components to modulate the extracellular environment but can also generate chemoattractive peptides and release biological molecules bound to ECM components (i.e., growth factors and cytokines) [13–15].

Several studies investigating the phenotypes of MMP-null mice have concluded that MMPs regulate numerous physiological processes facilitating pregnancy and labor, including ovulation, implantation, cervical ripening, and rupture of fetal membranes [16–18]. MMPs can also promote cellular migration by increasing the permeability between cell-cell junctions [19]. It is well documented that leukocytes utilize both MMPs and TIMPs to infiltrate different compartments of the pregnant uterus near term (myometrium, decidua, and cervix) where they release cytokines and chemokines to produce a sterile inflammatory environment and initiate labor [20–22]. Furthermore, postpartum (PP) uterine involution is also known to be accompanied by extensive ECM remodeling and increased bioavailability of multiple cytokines, chemokines, and growth factors, all modulated by MMP activity [23, 24].
The exact role of specific myometrial MMPs during normal gestation and term labor (TL) has not been well characterized. To date, studies of the potential role of MMPs within the pregnant myometrium are limited by both the number of MMPs and the uterine gestational tissues studied [10, 25, 26]. MMPs may play a direct role in labor initiation because increased MMP expression is associated with preterm labor (PTL), and injection of a broad-spectrum MMP inhibitor GM6001 was able to reduce the rate of PTL in mice [27, 28]. Moreover, the role of mechanical stretch in the regulation of MMP was also demonstrated by increased gelatinase expression and activity in stretched myometrium strips isolated from virgin rats [25].

In the current study we aim to fully characterize a gestational profile of MMP expression and activity within the rat myometrium during pregnancy, TL, and PP. Based on the continuous remodeling of the ECM proteins throughout pregnancy, as well as increased inflammation observed toward TL, we hypothesize that MMPs and TIMPs may play a role in the preparation for labor contractions and PP uterine involution. Using two pregnant rat models, we aim to 1) fully characterize the in vivo myometrial expression profile of major secreted MMPs and TIMPs during pregnancy, TL, and PP; 2) investigate whether there is an increase in MMP expression and/or activity associated with TL and PP involution within the myometrium; and 3) investigate if gravidity is one of the putative mechanisms that regulate their expression and/or activity within the myometrium.

MATERIALS AND METHODS

Animal Models

Bilateral pregnancy. We investigated the in vivo myometrial mRNA expression profile of MMPs and TIMPs during pregnancy, at TL, and PP using the rat. Virgin female Wistar rats (12–15 wk, 225–250 g weight) and male Wistar rats (250–300 g weight) were purchased from Charles River Laboratories and housed in the MaRS Toronto Medical Discovery Tower Animal Research Facility. The research ethics board approved these animal studies (AUP no. 2379). Rats were maintained on standard rat chow and water in a 12L:12D cycle. Male (one per cage) and female rats (one per cage) were housed separately and monogamous pairs were only mated overnight. The following morning, male and female rats were separated, and the day when a vaginal plug was detected was considered Day 1 of gestation. Animals were housed separately and monogamous pairs were only mated overnight. The day when a vaginal plug was detected was considered Day 1 of gestation. Animals were euthanized by carbon dioxide inhalation, and bilaterally pregnant uterine samples were collected on the morning of Gestational Day (GD) 6, 8, 10, 12, 14, 15, 17, 19, 21, 22, 23 (TL), 1 day PP (1PP), and 4PP (n = 4). Labor samples (GD23) were collected during active TL following the delivery of at least one pup.

Unilateral pregnancy. Prior to mating, rats underwent tubal ligation through a flank incision to ensure that they subsequently became pregnant in only one horn (gravid horn) according to the method described in detail by Shynlova et al. [5]. Four sets of uterine tissues from the gravid and empty horns were collected separately on GD 6, 12, 15, 19, 21, 23 (TL), and 1PP (n = 6). Unilateral pregnancy was performed by inserting an expandable dried seaweed stem (laminaria, 2–3% of H2O2 in methanol for 20 min. For MMP7 and MMP11, heat-induced antigen retrieval was performed using a 10 mM sodium citrate solution (pH 9). DAKO) with 0.01% Triton X-100 (Sigma). For MMP3 and MMP12, heat-induced antigen retrieval was performed using a 10 mM sodium citrate solution (pH 6). Slides were then blocked in protein blocking solution (Dako) and incubated with primary antibody for MMP3, MMP7, MMP11, or MMP12 (all 1:200) at 4°C overnight (Supplemental Table S2). Rabbit IgG (IgG) (1:200) was used as the negative control at the same concentration as the primary antibodies. Sections were washed and incubated with a biotinylated secondary anti-rabbit antibody (1:300; Vector Laboratories Inc.) and streptavidin-horseradish peroxidase solution (Dako), developed with a 3,3'-diaminobenzidine kit (DAB, Vector Laboratories Inc.). Slides were counter-

buffer (25 mM bicine, 150 mM NaCl, pH 7.6). For histological analyses, whole uteri were fixed in 10% neutral-buffered formalin (VWR International), 4% paraformaldehyde (Electron Microscopy Sciences) in phosphate-buffered saline (PBS), or zinc-buffered fixative (100 mM Tris buffer pH 7.4, 0.63 mM calcium acetate, 27.3 mM zinc acetate, 36.7 mM zinc chloride) [30] for 24 h, processed, and then embedded in paraffin wax.

Real-Time Quantitative PCR

Total myometrial RNA was reverse transcribed (iScript superscript; Bio-Rad Laboratories Ltd.) to cDNA and subjected to real-time-quantitative PCR analysis (CFX384 system; Bio-Rad Laboratories Ltd.) of specific primers designed using NCBI Primer Blast software and produced by Eurofins Genomics (Eurofins MWG Operon LLC) (Supplemental Table S1; Supplemental Data are available online at www.biolreprod.org). Following real-time-quantitative PCR, a dissociation curve was constructed by increasing the temperature from 65°C to 95°C for detection of PCR product specificity. A cycle threshold (Ct) value was recorded for each sample. The Ct value is defined as the number of amplification cycles required to detect a fluorescent signal (SYBR green) that exceeds threshold level. Ct values are inversely proportional to mRNA levels within each sample. PCR reactions were set up in technical triplicates, and the mean of the three Ct values was calculated by the software (CFX Manager, Bio-Rad). A comparative Ct method (AACt method) was applied to the raw Ct values to find relative gene expression. The mRNA levels of 10 MMPs, that is, collagenases (Mmp1, Mmp8, and Mmp13), gelatinases (Mmp2 and 9), stromelysins (Mmp3, Mmp10, and Mmp11), matrilysin (Mmp7), and metalloelastase (Mmp12), as well as Timp1–Timp4 were detected using SYBR green (Sigma) and normalized to the geometric mean of three housekeeping genes: peptidylprolyl isomerase A (Ppia), platelet derived growth factor alpha (Pdgfa), and TATA box binding protein (Tbp) (Supplemental Table S1). For unilaterally pregnant animals, MMP and TIMP gene expressions were normalized to Ppia, Pdgfa, and insulin-like growth factor 1 receptor (Igf1r) [3]. The chosen housekeeping genes were selected because they quantitatively expressed and did not display significant changes throughout gestation (Supplemental Figs. S1 and S2). Results were displayed as fold change relative to GD6 (bilaterally pregnant rats) or to GD6 in the empty horn (unilaterally pregnant rats). In addition, a no-template control (RNase-free water instead of cDNA) was analyzed for possible contamination in the master mix.

Protein Isolation, Gelatin Zymography, and Western Blot Analysis

Total protein was extracted from myometrial tissue using a bicine lysis buffer (25 mM bicine, 150 mM NaCl, pH 7.6). The homogenate was centrifuged at 14,000 rpm for 15 min at 4°C. The supernatant was collected and protein concentration was determined using a Pierce BCA protein assay kit (Thermo Fisher Scientific Inc.). Total protein was denatured in NuPAGE LDS 4X Novex Sample Buffer (Invitrogen, Thermo Fisher Scientific Inc.) with 10% β-mercaptoethanol solution (Sigma-Aldrich) at 95°C. Thirty micrograms of protein per well was used for Western blot analysis of MMP8 (1:500) while 70 µg of protein was used to analyze MMP7 (1:200), MMP3 and MMP11 (1:500), and MMP12 (1:1000) (Supplemental Table S2). Protein expression levels were normalized to the housekeeping protein calponin (1:1000). Fifty micrograms of each myometrial sample was used to detect protein activity by gelatin zymography using precast Novex 12% tris-glycine gelatin gels (Invitrogen).

Immunohistochemistry

Formalin-fixed (10%) or paraformaldehyde-fixed (4%) paraffin-embedded rat uteri were sectioned at 5 µm thickness, placed on glass slides ( Fisher Scientific), baked at 37°C overnight, deparaffinized in xylene, rehydrated in 100%, 95%, 80%, and 70% ethanol baths for 5 min each, and quenched with 3% H2O2 in methanol for 20 min. For MMP7 and MMP11, heat-induced antigen retrieval was performed using a Target Retrieval Solution (pH 9; DAKO) with 0.01% Triton X-100 (Sigma). For MMP3 and MMP12, heat-induced antigen retrieval was performed using a 10 mM sodium citrate solution (pH 6). Slides were then blocked in protein blocking solution (Dako) and incubated with primary antibody for MMP3, MMP7, MMP11, or MMP12 (all 1:200) at 4°C overnight (Supplemental Table S2). Rabbit IgG (IgG) (1:200) was used as the negative control at the same concentration as the primary antibodies. Sections were washed and incubated with a biotinylated secondary anti-rabbit antibody (1:300; Vector Laboratories Inc.) and streptavidin-horseradish peroxidase solution (Dako), developed with a 3,3'-diaminobenzidine kit (DAB, Vector Laboratories Inc.). Slides were counter-
stained with Gill Accustain hematoxylin (Sigma-Aldrich) and mounted with Surgipath Micromount mounting media (Leica Microsystems Inc.).

In Situ Zymography

Zinc fixation and paraffin embedding was shown to preserve tissue morphology and enzymatic activity better than that of frozen sections [30]. Zinc-fixed and paraffin-embedded sections were sectioned at 5 μm, baked at 37°C for 1 h, deparaffinized in xylene, rehydrated in 100%, 95%, 80%, and 70% ethanol baths for 5 min each. Sections were then incubated with fluorescein-conjugated ECM substrates: DQ gelatin, DQ collagen I, or DQ collagen IV (all from Life Technologies Inc.) diluted 1:50 in a reaction buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM CaCl₂, 0.2 mM sodium azide) for 2 h at 37°C. For a negative control, sections were pre-incubated with 0.02M ethylenediaminetetraacetic acid (EDTA) alone, then incubated for 2 h at 37°C with 0.02 M EDTA plus substrate. Following incubation with the fluorescent substrates, sections were post-fixed in a 4% neutral buffered formalin (in PBS) solution, counterstained with 4’,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) diluted 1:1000 in PBS, and mounted with a coverslip using Immumount (Thermo Fisher Scientific Inc.), which was adapted from the protocol described in detail by Hadler-Olsen et al. [30].

Statistical Analysis

For gestational profiles, data were natural logarithm (ln)-transformed (unless otherwise stated), and statistical analysis was performed using one-way ANOVA with the Newman-Keuls post hoc test for multiple comparisons. For the unilateral pregnant rat model, statistical analysis was performed using two-way ANOVA with Bonferroni post hoc test for multiple comparisons using In-transformed data (unless otherwise stated). Values were considered statistically significant when P < 0.05 after multiple comparisons. Bars that were significantly different from each other after multiple comparisons were indicated by different letters or by *P < 0.05, **P < 0.01, and ***P < 0.001. Significant outliers within each data set were determined by the Grubb test (GraphPad Software, Inc.) and removed from analysis. The Kolmogorov-Smirnov normality test was used due to a small sample size (n = 4–6), revealing that ln-transformed data used for parametric analyses were normally distributed. All statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software, Inc.).

RESULTS

The level of 10 MMP and four TIMP transcripts was assessed in the pregnant, laboring, and PP rat myometrium (Fig. 1). The most significant findings were in the expression of myometrial Mmp7, Mmp11, and Mmp12 genes. For instance, Mmp7 myometrial mRNA levels were low throughout gestation (GD 6–21) but were dramatically upregulated before labor (GD22, 200-fold increase), during TL (GD23, 350-fold increase), and remained elevated until 4PP (150-fold increase), and 4PP (2-fold increase). Mmp11 levels showed a similar expression profile with a significant increase near term and TL (15-fold increase for both) with further increase 1PP (70-fold increase) as compared to early pregnancy (GD6); however, the magnitude of the changes are not as great as for Mmp7. The mRNA levels of Mmp12 were significantly elevated near term (GD22, 155-fold increase) and during the PP period (98-fold increase) compared to early gestation (GD 6–12). Transcript levels of Mmp2 and Mmp9 (gelatinases), Mmp8 and Mmp13 (collagenases), and Mmp3 and Mmp10 (stromelysins) were low throughout gestation and labor with minor fluctuations, but upregulated at 1PP (Fig. 1). Collagenase Mmp1 transcript levels were barely detectable in the rat myometrium. The mRNA expression of Timp1–Timp4 was high and relatively stable throughout gestation and TL. Timp2 mRNA was the most abundant in pregnant myometrium. Timp1, Timp3, and Timp4 expression was relatively unchanged throughout gestation with Timp1 decreasing and Timp3 and Timp4 increasing during the PP period (Fig. 2).

We attempted to assess the role of different MMPs and TIMPs in pregnancy by performing a comparative study (Supplemental Fig. S3). Gene expression was calculated by the ΔΔCt method and normalized to the expression of Mmp10 on GD6 (the lowest among all detected MMPs and TIMPs, a chosen calibrator). Comparative analysis revealed that relative to all other MMPs, the gelatinase Mmp2 was most highly expressed during pregnancy (i.e., 200-fold higher than Mmp10) but did not change with labor onset. During active TL, only Mmp7 displayed a dramatic 550-fold relative increase. Other MMPs showed low mRNA abundance throughout gestation, relative increase near term, and induction at 1PP. For instance, immediately after delivery, stromelysins, collagenases, and gelatinases showed at least a 350-fold increase relative to Mmp10, whereas Mmp7 and Mmp12 showed 150-fold increase. Interestingly, the expression of Timp3 throughout gestation was similar to that of gelatinases Mmp2/9. Further comparison of the Timp family to all Mmps studied (both were standardized to GD6 Mmp10) revealed that Timp mRNAs were expressed relatively higher within the myometrium throughout gestation. Of the four MMP inhibitors, Timp2 transcript levels showed the highest abundance throughout gestation, labor, and PP and along with Timp1 displayed a slight decrease in the PP period. Interestingly, only Timp3 and Timp4 were highly upregulated PP (300- to 450-fold), suggesting a role in promoting uterine involution (Fig. 2 and Supplemental Fig. S3).

The effect of gravidity on the expression of MMPs/TIMPs was assessed in unilateral pregnant rats during gestation and TL. In the gravid uterine horn, MMP transcript levels were similar to that in bilaterally pregnant rats, while expression of all MMP genes in the myometrium of the empty horn was very low. Similar to bilaterally pregnant rats, Mmp7, Mmp11, and Mmp12 exhibited significantly higher transcript levels during active TL in the gravid horn as compared to the empty horn, while other MMPs did not show statistically significant differences in mRNA levels (Fig. 3). Furthermore, Mmp12 gene expression was already upregulated during late gestation (GD 19 and 21) in the gravid horn as compared to the corresponding empty horn. Surprisingly, in the empty uterine horn, we also detected an increase of Mmp7 transcript levels during active labor (GD23) as compared to mid- and late gestation (GD 15–21). Interestingly, Mmp2 gene expression during TL was significantly higher in both uterine horns as compared to GD6 (Fig. 3A). These findings suggest that the transcriptional regulation of MMP genes in the gravid horn may involve mechanisms such as static mechanical stretch, cyclic labor contractions, gestation-related hormonal environment, and/or the presence of the fetal-placental unit.

Therefore, we investigated whether artificial mechanical stretch of the uterine wall, by an expandable material that mimics the effect of gravidity, can regulate MMP gene expression. An intra-uterine expandable tube (IUET) was inserted into the empty horn of unilaterally pregnant rats at late gestation (GD 19–21); myometrial samples were collected 24 h later [29]. As shown in Figure 4, for the majority of MMPs studied, transcript levels in the artificially stretched empty horn were either comparable or higher (i.e., Mmp7, P < 0.05) than in the gravid horn. Importantly, the expression of Mmp2, Timp2, and Timp4 was significantly downregulated by the IUET, which suggests negative regulation by mechanical stretch. It must be noted that the degree of laminaria-induced uterine stretch was comparable, but not equal, to the stretch seen as a result of gravidity and that the speed of stretching was much faster than during gestation.

Specific antibodies were used to check the expression of MMP7, MMP11, and MMP12 proteins. MMP7 protein was detected at 29 kDa, and its expression was increased at late gestation, during TL, and peaked at 1PP as compared to early gestation (Fig. 5, A and B), exhibiting a pattern similar to that
of \textit{Mmp7} gene expression (Figs. 1 and 4). Active MMP11 protein (54 kDa) also displayed a pattern of increased expression at late gestation beginning from GD17 to 1PP (Fig. 5, A and C). The 45kDa proform of MMP12 was highly and relatively stably expressed throughout gestation, but active MMP12 (25 kDa) was not detected (Fig. 5D). The expression of stromelysin MMP3 and collagenase MMP8 proteins was high and stable in the pregnant, laboring, and PP rat myometrium (Supplemental Fig. S4). Next, we compared the expression of active MMP7 and MMP11 proteins in the empty and gravid horns of unilaterally pregnant rats. Although not statistically significant, as with the bilaterally pregnant myometrium, MMP7 was upregulated in the gravid horn during TL compared to levels during pregnancy. However,
MMP11 protein levels in the gravid horn of unilaterally pregnant rats did not display the late increase that was observed in the bilaterally pregnant rat myometrium. We also found no difference in expression of these MMPs between empty and gravid horns (Supplemental Fig. S4, C and D). This inconsistency in protein expression may be due to physiological variability between the two different animal models used.

Spatial localization of MMP7 and MMP11 proteins was assessed in the pregnant rat uterus from midgestation to 1PP by immunohistochemistry (IHC) (Fig. 6). We noticed that MMP7 immunostaining was weak at midgestation (GD15) with an increase within myometrial cells near term (GD21) and was mainly localized to the nuclear and perinuclear regions of SMCs. During TL (GD23) and 1PP, we detected increased expression of MMP7 localized both intracellularly and extracellularly, between SMCs and around the myometrial vasculature. Similarly, MMP11 immunostaining was primarily intracellular but also showed extracellular protein localization. Importantly, both MMP7 and MMP11 immunostaining was also detected in several cells localized around myometrial blood vessels that were morphologically different from myocytes. We speculate these cells might be infiltrating leukocytes that express MMPs (Fig. 6). Similar to protein expression detected by Western Blot analysis, IHC revealed consistent immunostaining of MMP3 and MMP12 throughout gestation and TL primarily in the nuclear and perinuclear region of myocytes, although in the early PP period additional extracellular staining was noticed between SMCs (Supplemental Fig. S5).

The enzymatic activity of MMP2 and MMP9 was also examined by gelatin zymography (Fig. 7A). Pro-MMP2 protein was stably expressed throughout gestation and TL with active-MMP2 protein significantly induced only at 1PP, a profile similar to MMP2 gene expression (Fig. 1). Active-MMP9 protein activity was very low and could not be accurately analyzed. Gelatin zymography using gravid horn myometrium from unilaterally pregnant rats revealed a pro-MMP2 expression profile similar to that in bilateral pregnancy with no differences between the empty and gravid uterine horns (Fig. 7, A and B). In contrast, active MMP2 protein was higher in the empty uterine horn than in the gravid horn from early gestation until TL (Fig. 7B).

Spatial localization of MMP enzymatic activity in the rat uterus was assessed using in situ zymography (ISZ) with different fluorescein-conjugated ECM substrates (DQ-gelatin, DQ-collagen I, and DQ-collagen IV) as described in [31, 32]. We detected higher levels of gelatin and collagen I degradation in the myometrial parenchyma near term (GD22) and during TL (GD23) as compared to GD15; however the highest MMP activity was detected on 1PP as shown by increased fluorescence, depicting extracellular degradation of gelatin, collagen I, and collagen IV (Fig. 8). Similar to immunostaining results, numerous single cells that were morphologically different from SMCs were observed in myometrial tissue in close proximity to blood vessels.

Statistical analysis of each Mmp's gestational profile using one-way ANOVA and the Newman-Keuls multiple comparisons test was performed on the natural log (ln)-transformed normalized expression data and presented as fold changes relative to a corresponding GD6. Values were considered statistically significant when \( P < 0.05 \) after multiple comparisons. Bars with different letters are significantly different from each other.

---

**FIG. 2.** Expression of TIMP mRNA in bilaterally pregnant, laboring, and postpartum rat myometrium. The mRNA expression of tissue inhibitors of metalloproteinases *Timp1* (A), *Timp2* (B), *Timp3* (C), and *Timp4* (D) was normalized to three housekeeping genes (*Ppia*, *Pdgfa*, and *Tbp*). The data from each gestational day (GD) is presented as mean ± SEM (n = 5/GD).
DISCUSSION

Until recently, little was known about the expression and regulation of MMPs/TIMPs within the uterine smooth muscle during pregnancy and TL. Using a rat model of gestation, we present here the first comprehensive study of gene and protein analysis of major MMPs and TIMPs, as well as their tissue localization and activity in the pregnant, laboring, and PP myometrium. Our results indicate that the majority of myometrial MMPs that we studied displayed low expression during gestation; only three enzymes, MMP7 (matrilysin, which differs from most MMP family members because it lacks a conserved C-terminal domain), MMP11 (stromelysin-3), and MMP12 (metalloelastase), exhibited increased expression in the myometrium near term, while other MMPs were significantly upregulated PP.

The role of MMPs in several reproductive processes, that is, ovulation, implantation, cervical ripening, and PP involution, is well-established [17, 18, 33]. However, little is known about
the potential role of MMPs, especially MMP7, MMP11, and MMP12, within the myometrium during gestation and TL. The stromelysins (MMP3, MMP10, and MMP11) and related MMPs (MMP7 and MMP12) are capable of degrading elastin, cell adhesion molecules, proteoglycans, fibronectin, and components of the BM, laminin and collagen type IV [15, 34], suggesting their involvement in modifying the myometrial ECM in preparation for active labor contractions. Our study revealed significant upregulation of Mmp7, Mmp11, and Mmp12 genes before TL with elevated levels sustained during TL and PP. Furthermore, we detected a late increase at the time of TL and PP for MMP7 and MMP11 proteins that resembled the trend observed for Mmp7 and Mmp11. These findings suggest a dual role in promoting both labor and uterine involution.

Throughout gestation, myometrial SMCs transition from a hyperplastic (proliferative) to hypertrophic (or synthetic) phenotype associated with ECM modifications to accommodate the increase in SMC number and size [35]. Williams et al. [36] have previously reported increased α1, α3, and β1 integrin expression localized to myocyte membranes during late gestation and labor, which correlates well with our findings. Because matrix-integrin receptor signaling is known to upregulate MMP transcriptional activation, we speculate this is one of the potential mechanisms in play to regulate myometrial MMP expression [8, 9]. It has been previously reported that MMP-mediated degradation of ECM components can result in the genesis of chemotactic peptides [37]. Elastin- and fibronectin-derived peptides are known to have chemotactic properties for further monocyte recruitment and can stimulate activated macrophages [37, 38]. Our own and other studies have investigated the role of immune cells and inflammatory mediators contributing to events preceding TL. We identified macrophages as the predominant immune cells in the myometrium and decidua during gestation, and this population was increased in parallel with pro-inflammatory cytokine induction during TL and early PP [39–41]. Monocytes/macrophages have been suggested to support active labor

FIG. 4. Artificial mechanical stretching caused by a laminaria regulates MMP gene expression at late gestation (GD 19–21), similar to biological mechanical stretch by growing fetuses. The mRNA expression of collagenase Mmp8 (D); gelatinases Mmp2 (A) and Mmp9 (E); matrilysin Mmp7 (C); stromelysins Mmp3 (B), Mmp10 (F), and Mmp11 (G); metalloelastase Mmp12 (H); and tissue inhibitors of metalloproteinases Timp1–Timp4 (I–L) in the gravid uterine horn (gray bars) compared to the empty horn stretched by laminaria (black bars) was normalized to three housekeeping genes (Pdgfa, Ppia, and Tbp). Laminaria data were expressed as fold-change relative to a corresponding gravid horn and the bars represent mean ± SEM (n = 3). Statistical analysis was performed using the paired t-test with two-tailed P value. A significant difference between the gravid and laminaria-stretched empty horn is indicated by *P < 0.05 and **P < 0.01. The majority of Mmps studied (except Mmp1 that was not detected and Mmp13 that was very low) exhibited comparable or even higher expression in the artificially stretched empty horn than in the gravid horn. Interestingly, the expression of Mmp2, Mmp11, Timp2, and Mmp4 were downregulated by laminaria-induced stretch, whereas Timp1 was upregulated and Timp3 was not changed. It must be noted that the degree of uterine stretch by laminaria was comparable, but not equal, to the stretch seen as a result of gravidity and that the speed of the stretching was much faster than during gestation.
Conclusions: 

In many tissues and cell types, expression of MMPs was shown to be upregulated by mechanical stretch [59, 60]. In particular, mechanical stretch was able to increase gelatinase expression and activity in the virgin rat myometrium [25]. Using a unilaterally pregnant rat model, we observed that during labor, gene expression of Mmp7, Mmp11, and Mmp12 (Fig. 3) was significantly increased in the gravid uterine horn as compared to the empty horn. Furthermore, artificially stretching the empty horn in unilaterally pregnant rats using the IUET rat model significantly increased Mmp7 expression (Fig. 4).

Although uterine MMP7 was first identified in PP tissues [15, 43, 44], we detected a significant increase in MMP7 gene and protein expression near term, which correlates with the increase in myometrial leukocyte recruitment. Apart from promoting chemoattraction, MMP7 has been previously shown to actively cleave vascular endothelial-cadherin, one of the junctional proteins that maintain vascular barrier function [45]. Results from our IHC staining for MMP7 showed particular expression surrounding the myometrial vasculature near term (Fig. 6). We speculate that MMP7 can also promote leukocyte infiltration into the myometrium by upregulating chemotaxis and vascular permeability during late gestation and TL.

Interestingly, the increased expression of MMP7 also correlates with our previous reports of increased IGF1 and IGFBP5 expression in the rat myometrium during TL and PP [3]. The family of insulin-like growth factors (i.e., IGF1, IGF2, IGFBPs) are known to stimulate cell proliferation, collagen synthesis, and muscle growth/regeneration, making their bioavailability important to assist PP uterine involution [46–48]. MMP7-mediated cleavage of IGFBP5 has been shown to liberate IGF2 [49], which also suggests its association in upregulating IGF signaling in the PP myometrium.

The role of MMP11 in the myometrium is not well defined; however, previous studies have indicated that it can regulate BM stability [50, 51]. We and others have previously suggested that remodeling of the ECM increases elasticity of the uterus and supports a change in SMC phenotype in preparation for labor contractions by forming an organized, continuous, and regular BM surrounding individual myocytes [6, 52]. We now speculate that the significant increase in MMP11 levels before and during active labor correlates with changes in ECM composition and may contribute to the increased cellular connectivity for coordinated uterine contractions by modulating the BM surrounding individual SMCs [5, 14, 51]. MMP11 has also been reported to cleave a proteinase inhibitor α1-antitrypsin that predominantly inhibits neutrophil elastase-mediated degradation of ECM components and α2-macroglobulin, a general proteinase inhibitor that induces MMP removal by endocytosis [7, 53, 54]. Therefore, the observed elevation of MMP11 protein levels might also contribute to the maintenance of proteolytic activity of other MMPs by preventing their inhibition during TL and PP.

The metalloelastase MMP12 was first believed to be expressed in vivo solely by macrophages [55] and is important for their migration [14] via direct or indirect induction of elastin degradation by targeting the serine proteinase inhibitor α1-antitrypsin [56, 57]. However, it is now known that macrophages are not the only source of MMP12 expression because trophoblasts and vascular SMCs have been shown to express MMP12 to mediate uterine spiral artery remodeling [58]. It is possible that the increase in MMP12 expression is a result of: 1) the activation of resident tissue macrophages by ECM-derived peptides, 2) infiltrating macrophages secreting MMP12, and 3) resident myometrial SMCs producing MMP12 for elastin remodeling before labor.

In many tissues and cell types, expression of MMPs was shown to be upregulated by mechanical stretch [59, 60]. In particular, mechanical stretch was able to increase gelatinase expression and activity in the virgin rat myometrium [25]. Using a unilaterally pregnant rat model, we observed that during labor, gene expression of Mmp7, Mmp11, and Mmp12 (Fig. 3) was significantly increased in the gravid uterine horn as compared to the empty horn. Furthermore, artificially stretching the empty horn in unilaterally pregnant rats using the IUET significantly increased Mmp7 expression (Fig. 4).

However, protein analysis showed a similar increase for Mmp11 as compared to the empty horn. Furthermore, artificially stretching the empty horn in unilaterally pregnant rats using the IUET significantly increased Mmp7 expression (Fig. 4).

The role of MMP11 in the myometrium is not well defined; however, previous studies have indicated that it can regulate BM stability [50, 51]. We and others have previously suggested that remodeling of the ECM increases elasticity of the uterus and supports a change in SMC phenotype in preparation for labor contractions by forming an organized, continuous, and regular BM surrounding individual myocytes [6, 52]. We now speculate that the significant increase in MMP11 levels before and during active labor correlates with changes in ECM composition and may contribute to the increased cellular connectivity for coordinated uterine contractions by modulating the BM surrounding individual SMCs [5, 14, 51]. MMP11 has also been reported to cleave a proteinase inhibitor α1-antitrypsin that predominantly inhibits neutrophil elastase-mediated degradation of ECM components and α2-macroglobulin, a general proteinase inhibitor that induces MMP removal by endocytosis [7, 53, 54]. Therefore, the observed elevation of MMP11 protein levels might also contribute to the maintenance of proteolytic activity of other MMPs by preventing their inhibition during TL and PP.

The metalloelastase MMP12 was first believed to be expressed in vivo solely by macrophages [55] and is important for their migration [14] via direct or indirect induction of elastin degradation by targeting the serine proteinase inhibitor α1-antitrypsin [56, 57]. However, it is now known that macrophages are not the only source of MMP12 expression because trophoblasts and vascular SMCs have been shown to express MMP12 to mediate uterine spiral artery remodeling [58]. It is possible that the increase in MMP12 expression is a result of: 1) the activation of resident tissue macrophages by ECM-derived peptides, 2) infiltrating macrophages secreting MMP12, and 3) resident myometrial SMCs producing MMP12 for elastin remodeling before labor.

In many tissues and cell types, expression of MMPs was shown to be upregulated by mechanical stretch [59, 60]. In particular, mechanical stretch was able to increase gelatinase expression and activity in the virgin rat myometrium [25]. Using a unilaterally pregnant rat model, we observed that during labor, gene expression of Mmp7, Mmp11, and Mmp12 (Fig. 3) was significantly increased in the gravid uterine horn as compared to the empty horn. Furthermore, artificially stretching the empty horn in unilaterally pregnant rats using the IUET significantly increased Mmp7 expression (Fig. 4).

However, protein analysis showed a similar increase for Mmp11 as compared to the empty horn. Furthermore, artificially stretching the empty horn in unilaterally pregnant rats using the IUET significantly increased Mmp7 expression (Fig. 4).

The role of MMP11 in the myometrium is not well defined; however, previous studies have indicated that it can regulate BM stability [50, 51]. We and others have previously suggested that remodeling of the ECM increases elasticity of the uterus and supports a change in SMC phenotype in preparation for labor contractions by forming an organized, continuous, and regular BM surrounding individual myocytes [6, 52]. We now speculate that the significant increase in MMP11 levels before and during active labor correlates with changes in ECM composition and may contribute to the increased cellular connectivity for coordinated uterine contractions by modulating the BM surrounding individual SMCs [5, 14, 51]. MMP11 has also been reported to cleave a proteinase inhibitor α1-antitrypsin that predominantly inhibits neutrophil elastase-mediated degradation of ECM components and α2-macroglobulin, a general proteinase inhibitor that induces MMP removal by endocytosis [7, 53, 54]. Therefore, the observed elevation of MMP11 protein levels might also contribute to the maintenance of proteolytic activity of other MMPs by preventing their inhibition during TL and PP.
FIG. 6. Spatial and temporal localization of MMP expression in pregnant, laboring, and PP rat myometrium. Tissue sections from GD 15, 21, 23 (laboring), and 1 day PP (1PP) rat uteri were immunostained with anti-MMP7 and anti-MMP11 antibodies. Brown deposition observed inside SMCs in the nuclear and perinuclear area indicates the presence of intracellular proteins in the pregnant myometrium. Both intra- and extracellular immunostaining (between SMCs) was observed for MMP7 and MMP11 proteins during TL at 1PP. It is speculated that positively immunostained single cells around myometrial blood vessels morphologically different from myocytes, are infiltrating leukocytes expressing MMPs. No staining was detected on the negative control slide when primary antibodies were replaced by rabbit IgG at the same concentration (negative control was performed on 1PP). Magnification ×200. Inset magnification ×400.
the gravid horn compared to the gravid horn from early gestation until TL (Fig. 7B). Based on these findings, we speculate that during gestation MMP2 activity in the gravid horn is repressed by the presence of mechanical stretch by the growing fetus and/or paracrine factors produced by the decidua and fetal-placental unit (e.g., inhibin and TGFβ) to prevent uncontrolled gelatinolysis. Previous studies have demonstrated that members of the transforming growth factor beta (TGFβ) superfamily, activin and inhibin, secreted by the decidua and placenta, have the ability to differentially regulate the expression and secretion of human uterine MMPs. Specifically, Jones et al. [63, 64] revealed that activin A promoted decidualization and trophoblast invasion through upregulated production of MMPs by endometrial, decidual, and cytотrophoblast cells.

The major structural components of the uterine ECM are elastin fibers and collagen fibrils consisting of collagen type I and III [5]. Elastin abundance is important within the uterus to accommodate its expansion in pregnancy, while collagen provides tissue strength [5]. Therefore, the major structural component of the uterine ECM, fibrillar collagen, must be constantly reorganized for pregnancy maintenance [5]. It has been reported that myometrial remodeling during pregnancy involves well-controlled collagenolysis, evident by the disruption and partial degradation of collagen fibrils in late pregnancy [52]. Comparative analysis of overall MMP gene expression indicates that among the MMP genes studied, the collagenases (Mmp8 and Mmp13) and gelatinases (Mmp2 and Mmp9) were highly (yet relatively stably) expressed throughout pregnancy. In addition, the activity of pro-MMP2 was also high and stable throughout gestation. We speculate that the collagenases would cleave fibrillar collagens into fragments that would be denatured into gelatin. Furthermore, the gelatinases would then degrade gelatin into small peptide fragments for rapid removal by infiltrating immune cells (possibly macrophages) [52, 65]. This suggests the existence of very well-controlled and spatially restricted collagenolysis to support phenotypic transitions of SMCs from a hyperplastic to hypertrophic phenotype and to increase elasticity of the myometrium to accommodate the growing fetus(es).

Interestingly, IHC and ISZ analysis detected MMP expression and activity in the nuclear and perinuclear region of myometrial SMCs throughout gestation, TL, and PP involution (Figs. 6 and 8, and Supplemental Fig. S5), which suggests intracellular storage and (possible) activity of these proteins. The intracellular compartmentalization and activity of MMPs has been recently demonstrated in several cell types [66]. Although the mechanism of nuclear translocation is poorly characterized, MMPs have been found inside nuclei and their presence has been associated with the regulation of apoptosis and gene expression. For instance, MMP2 has been shown to process two important factors of the DNA repair machinery capable of protecting cardiac myocytes from apoptosis [67]. Also MMP3 acts as a transcription factor preventing transcriptional repression of the connective tissue growth factor (CTFG/CCN2) gene and promoting chondrocytic proliferation and ECM formation [68]. MMP2 has also been shown to regulate myocyte contractile function by cleavage of myosin light chain-1, a component of the contractile machinery [69]. Furthermore, several cell types (i.e., leukocytes and endothelial cells) can store MMPs intracellularly in exocytic vesicles that are readily available for rapid secretion upon cellular activation [66]. These studies correlate well with our findings suggesting additional roles of myometrial MMPs in 1)
FIG. 8. Spatial and temporal localization of MMP activity in the pregnant, laboring, and PP rat myometrium. Representative images from in situ zymography (ISZ) using gelatin and collagens I and IV as substrates. Increased green fluorescence (depicting substrate degradation) was localized intracellularly to the nuclear and perinuclear region of the myometrial cytoplasm during late gestation (GD22) and laboring (GD23), however during 1PP, fluorescence was detected both intra- and extracellularly in the myometrial parenchyma between SMCs. Negative control sections were pre-incubated with EDTA. Magnification ×100.
acting as transcription factors to promote cellular proliferation and ECM formation during gestation and 2) regulating cellular apoptosis and degradation of SMC contractile proteins during PP involution.

Analysis of TIMP expression in the rat myometrium provides a potential mechanism for regulating the expression or activity of MMPs throughout gestation. It is known that TIMP1 and TIMP2 can form a specific complex with MMP9 and MMP2, respectively (via their C-terminus), and further interact with other MMPs via their N-terminus to potentially prevent their activation [70, 71]. Our findings suggest that high, constant expression of major TIMP genes in the rat myometrium throughout gestation ensures an unrestricted formation of inhibitory complexes controlling MMPs activity. Comparative analysis revealed that Timp2 exhibited the highest expression level of all TIMPs while Timp1 and Timp3 have an expression pattern similar to gelatinases. Interestingly, TIMPs are known to have other biological functions that include regulation of cell proliferation, migration, angiogenesis, and apoptosis; specifically, TIMP3 has been shown to have pro-apoptotic function, while TIMP4 is anti-apoptotic [72, 73]. Because we observed induction of both Timp3 and Timp4 expression during PP, this may indicate their involvement in the process of uterine involution and tissue regeneration by stimulation of apoptosis of hypertrophied myometrial SMC and proliferation of smaller postpregnancy cells.

The strength of this comprehensive study is the characterization of a gestational profile for expression, activity, and localization of MMPs and TIMPs within the myometrium of pregnant rat, demonstrating the regulated involvement of these enzymes in promoting labor and PP involution. While the processes of uterine proliferation, growth, contraction, and involution are common to human pregnancy, it remains to be determined whether these matrix remodelling enzymes follow a similar regulation in the human myometrium. Furthermore, the putative role of MMPs in degrading ECM components, liberating chemokines and growth factors, and increasing vascular permeability.

In summary, our results point toward several potentially distinct roles for MMPs within the pregnant, laboring, and PP myometrium. First, we suggest that throughout gestation there is constant remodeling of the ECM by housekeeping MMPs, which are counterbalanced by TIMPs, to accommodate the growing fetus by increasing the elasticity of the uterus [52]. Next, near term and during labor, there is an increase in specific MMPs to promote infiltration of leukocytes, generating sterile physiological inflammation [41]. Following birth, there is a coordinated induction of multiple MMPs/TIMPs to ensure PP uterine tissue regeneration by promoting cellular renewal through well-controlled apoptosis and proliferation, and remodeling of the uterine ECM back to a prepregnant state for the female to remain fertile (Fig. 9).

ACKNOWLEDGMENT

We gratefully thank Mrs. Anna Dorogin for her assistance in collecting and processing of rat tissues and ISZ, and Dr. Caroline Dunk for proofreading the manuscript.

REFERENCES

1. Bokstrom H, Brannstrom M, Alexandersson M, Norstrom A. Leukocyte subpopulations in the human uterine cervical stroma at early and term pregnancy. Hum Reprod 1997; 12:586–590.

2. Shynlova O, Lee YH, Srikhajon K, Lye SJ. Physiologic uterine inflammation and labor onset: integration of endocrine and mechanical signals. Reprod Sci 2013; 20:154–167.

3. Shynlova O, Tsui P, Dorogin A, Langille BL, Lye SJ. Insulin-like growth factors and their binding proteins define specific phases of myometrial differentiation during pregnancy in the rat. Biol Reprod 2007; 76: 571–578.

4. Xu P, Alfaidy N, Challis JR. Expression of matrix metalloproteinase (MMP)-2 and MMP-9 in human placenta and fetal membranes in relation to preterm and term labor. J Clin Endocrinol Metab 2002; 87:1353–1361.

5. Shynlova O, Mitchell JA, Tsampalieros A, Langille BL, Lye SJ. Progesterone and gravidity differentially regulate expression of extracel-
lular matrix components in the pregnant rat myometrium. Biol Reprod 2004; 70:986–992.
6. Shynlova O, Chow M, Lye SJ. Expression and organization of basement membranes and focal adhesion proteins in pregnant myometrium is regulated by uterine stretch. Reprod Sci 2009; 16:960–969.
7. Murphy G, Nagase H. Progress in matrix metalloproteinase research. Mol Aspects Med 2008; 29:290–308.
8. Werb Z, Tremble P, Damsky CH. Regulation of extracellular matrix degradation by cell-extracellular matrix interactions. Cell Differ Dev 1990; 32:299–306.
9. Chakrabarti S, Mandal M, Das S, Mandal A, Chakrabarti T. Regulation of matrix metalloproteinases: an overview. Mol Cell Biochem 2003; 253:269–285.
10. Roh CR, Oh WJ, Yoon BK, Lee JH. Up-regulation of matrix metalloproteinase-9 in human myometrium during labour: a cytokine-mediated process in uterine smooth muscle cells. Mol Hum Reprod 2000; 6:96–102.
11. Parks WC, Wilson CL, Lopez-Boado YS. Matrix metalloproteinases as modulators of inflammation and innate immunity. Nat Rev Immunol 2004; 4:617–629.
12. Sbardella D, Fasciglione GF, Gioia M, Ciacco C, Tundo GR, Marini S, Coletta M. Human matrix metalloproteinases: an ubiquitous class of enzymes involved in several pathological processes. Mol Aspects Med 2012; 33:119–208.
13. Van Lint P, Libert C. Chemokine and cytokine processing by matrix metalloproteinases and its effect on leukocyte migration and inflammation. J Leukoc Biol 2007; 82:1375–1381.
14. Manicone AM, McGuire JK. Matrix metalloproteinases as modulators of inflammation. Semin Cell Dev Biol 2008; 19:34–41.
15. Bonnans C, Chen J, Werb Z. Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol 2014; 15:786–801.
16. Weiss A, Goldman S, Shalev E. The matrix metalloproteinases (MMPs) in the decidua and fetal membranes. Front Biosci 2007; 12:649–659.
17. Fata JE, Ho AT, Leco KJ, Moorehead RA, Khokha R. Cellular turnover and extracellular matrix remodeling in female reproductive tissues: functions of metalloproteinases and their inhibitors. Cell Mol Life Sci 2000; 57:77–95.
18. Hulbey DL, Rudolph LA, Matrisian LM. Matrix metalloproteinases as mediators of reproductive function. Mol Hum Reprod 1997; 3:27–45.
19. Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. Genes Dev 2000; 14:2123–2133.
20. Shynlova O, Tsui P, Dorogin A, Lye SJ. Monocyte chemoattractant protein-1 (CCL-2) integrates mechanical and endothocrine signals that mediate term and preterm labor. J Leukoc Biol 2007; 82:104.e1–104.e11.
21. Osman I, Young A, Ledingham MA, Thomson AJ, Jordan FA, Greer IA, Norman JE. Leukocyte density and pro-inflammatory cytokine expression in human fetal membranes, decidua, cervix and myometrium before and during labour at term. Mol Hum Reprod 2003; 9:61–64.
22. Shynlova O, Li Y, Dorogin A, Lye SJ. Matrix metalloproteinases and TIMPs in the decidua and fetal membranes. Reprod Sci 2009; 16:960–969.
23. Fata JE, Ho AT, Leco KJ, Moorehead RA, Khokha R. Cellular turnover and extracellular matrix remodeling in female reproductive tissues: functions of metalloproteinases and their inhibitors. Cell Mol Life Sci 2000; 57:77–95.
24. Hulbey DL, Rudolph LA, Matrisian LM. Matrix metalloproteinases as mediators of reproductive function. Mol Hum Reprod 1997; 3:27–45.
25. Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. Genes Dev 2000; 14:2123–2133.
26. Shynlova O, Tsui P, Dorogin A, Lye SJ. Monocyte chemoattractant protein-1 (CCL-2) integrates mechanical and endothocrine signals that mediate term and preterm labor. J Leukoc Biol 2007; 82:104.e1–104.e11.
27. Osman I, Young A, Ledingham MA, Thomson AJ, Jordan FA, Greer IA, Norman JE. Leukocyte density and pro-inflammatory cytokine expression in human fetal membranes, decidua, cervix and myometrium before and during labour at term. Mol Hum Reprod 2003; 9:61–64.
28. Shynlova O, Li Y, Dorogin A, Lye SJ. Matrix metalloproteinases and TIMPs in the decidua and fetal membranes. Reprod Sci 2009; 16:960–969.
29. Fata JE, Ho AT, Leco KJ, Moorehead RA, Khokha R. Cellular turnover and extracellular matrix remodeling in female reproductive tissues: functions of metalloproteinases and their inhibitors. Cell Mol Life Sci 2000; 57:77–95.
metalloelastase (MMP-12) by tumor cells in skin cancer. J Invest Dermatol 2000; 114:1113–1119.

56. Owen CA. Roles for proteinases in the pathogenesis of chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 2008; 3:253–268.

57. Hubbard RC, Felis G, Gadek J, Pacholok S, Humes J, Crystal RG. Neutrophil accumulation in the lung in alpha 1-antitrypsin deficiency. Spontaneous release of leukotriene B4 by alveolar macrophages. J Clin Invest 1991; 88:891–897.

58. Harris LK, Smith SD, Keogh RJ, Jones RL, Baker PN, Knofler M, Cartwright JE, Whitney GS, Aplin JD. Trophoblast- and vascular smooth muscle cell-derived MMP-12 mediates elastolysis during uterine spiral artery remodeling. Am J Pathol 2010; 177:2103–2115.

59. Tang Z, Yang L, Zhang J, Wang Y, Chen PC, Sung KL. Differential expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in anterior cruciate ligament and medial collateral ligament fibroblasts after a mechanical injury: involvement of the p65 subunit of NF-kappaB. Wound Repair Regen 2009; 17:709–716.

60. Yoshida M, Sagawa N, Itoh H, Yura S, Takemura M, Wada Y, Sato T, Ito A, Fuji S. Prostaglandin F(2alpha), cytokines and cyclic mechanical stretch augment matrix metalloproteinase-1 secretion from cultured human uterine cervical fibroblast cells. Mol Hum Reprod 2002; 8:681–687.

61. Thompson AJ, Telfer JE, Young A, Campbell S, Stewart CJ, Cameron IT, Greer IA, Norman JE. Leukocytes infiltrate the myometrium during human parturition: further evidence that labour is an inflammatory process. Hum Reprod 1999; 14:229–236.

62. Bowen JM, Chamley L, Keelan JA, Mitchell MD. Cytokines of the placenta and extra-placental membranes: roles and regulation during human pregnancy and parturition. Placenta 2002; 23:257–273.

63. Jones RL, Stoilkos C, Findlay JK, Salamonsen LA. TGF-beta superfamily expression and actions in the endometrium and placenta. Reproduction 2006; 132:217–232.

64. Jones RL, Findlay JK, Farnworth PG, Robertson DM, Wallace E, Salamonsen LA. Activin A and inhibin A differentially regulate human uterine matrix metalloproteinases: potential interactions during decidualization and trophoblast invasion. Endocrinology 2006; 147:724–732.

65. Shimizu K, Maekawa K. Collagen degradation in the mouse uterus during postpartum involution: extracellular pathway. Acta Anat (Basel) 1983; 117:257–260.

66. Hadler-Olsen E, Fadnes B, Sylte I, Uhlin-Hansen L, Winberg JO. Regulation of matrix metalloproteinase activity in health and disease. FEBS J 2011; 278:28–45.

67. Kwan JA, Schulze CJ, Wang W, Leon H, Sariahmetoglu M, Sung M, Sawicka J, Sims DE, Sawicki G, Schulz R. Matrix metalloproteinase-2 (MMP-2) is present in the nucleus of cardiac myocytes and is capable of cleaving poly (ADP-ribose) polymerase (PARP) in vitro. FASEB J 2004; 18:690–692.

68. Eguchi T, Kubota S, Kawata K, Mukudai Y, Uehara J, Ohgawara T, Ibaragi S, Sasaki A, Kuboki T, Takigawa M. Novel transcription-factor-like function of human matrix metalloproteinase 3 regulating the CTGF/CCN2 gene. Mol Cell Biol 2008; 28:2391–2413.

69. Sawicki G, Leon H, Sawicka J, Sariahmetoglu M, Schulze CJ, Scott PG, Szczesna-Cordary D, Schulz R. Degradation of myosin light chain in isolated rat hearts subjected to ischemia-reperfusion injury: a new intracellular target for matrix metalloproteinase-2. Circulation 2005; 112:544–552.

70. Brew K, Nagase H. The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. Biochim Biophys Acta 2010; 1803:55–71.

71. Brew K, Dinakarpandian D, Nagase H. Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biophys Acta 2000; 1477:267–283.

72. Stetler-Stevenson WG. Tissue inhibitors of metalloproteinases in cell signaling: metalloproteinase-independent biological activities. Sci Signal 2008; 1:re6.

73. Baker AH, Zaltsman AB, George SJ, Newby AC. Divergent effects of tissue inhibitor of metalloproteinase-1, -2, or -3 overexpression on rat vascular smooth muscle cell invasion, proliferation, and death in vitro. TIMP-3 promotes apoptosis. J Clin Invest 1998; 101:1478–1487.