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THE ROLE AND REGULATION OF THE UTERINE MATRIX METALLOPROTEINASE SYSTEM IN MENSTRUATING AND NON-MENSTRUATING SPECIES

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1. ABSTRACT

The uterus undergoes dynamic tissue remodeling throughout each reproductive cycle, which is regulated in part by the matrix metalloproteinases (MMP) system. The MMP system is comprised of the proteolytic factors, the MMPs, and their tissue inhibitors (TIMPs), which act in concert to modulate extracellular matrix turnover and cell behaviors and thus play a key role in many physiologic and pathologic conditions throughout the body. MMPs and TIMPs are expressed spatiotemporally in the uterus of various menstruating and non-menstruating species. The balanced function of the MMP system is critical to normal uterine tissue remodeling throughout the cycle as well as during pregnancy, parturition and postpartum uterine involution. The uterine MMP system appears to be under the regulation of not only ovarian steroids but also various autocrine/paracrine factors such as growth factors, cytokines and chemokines. The current review focuses on the expression and regulation of the MMP system within the uterus during the menstrual or estrous cycle, and addresses the roles of various MMPs and TIMPs in uterine biology.

2. INTRODUCTION

The remodeling of the extracellular matrix (ECM) is essential for a variety of cellular processes such as cell proliferation, migration, adhesion and differentiation. Abundant evidence suggests that the matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), together referred to as the MMP system, are involved in ECM remodeling throughout the body. The MMPs are a family of enzymes capable of degrading the structural proteins of the ECM, and the TIMPs act as regulatory factors to control the site and extent of matrix degradation. Recent studies indicate that the MMP system not only participates in ECM remodeling but also modifies cell behavior in many ways. The MMP system plays an essential role in many physiologic and pathologic conditions including angiogenesis, bone growth, periodontal health, wound healing and tumor invasion (1-2). In the female reproductive tract, the MMP system has been postulated to play a key role in the extensive remodeling of the ECM during each menstrual or estrous cycle (3). This review will focus on the expression, regulation and function of the MMP system within the uterus of menstruating and non-menstruating species.

3. THE MMP SYSTEM

The MMP system is composed of both the MMPs and TIMPs. MMPs are a family of structurally related zinc-dependent endopeptidases, which collectively, posses the ability to degrade all components of the ECM (Table 1). Degradation of the ECM by MMPs occurs in normal

Table 1. Substrates and activators of human MMPs

Enzyme	Substrates	Activated by:
Collagenases		
MMP-1 (Collagenase-1)	Collagen I, II, III, VII, VIII, X, aggrecan, serpins, 2-MG,kallikrien, chymase	MMP-3, -7, -10, plasmin
MMP-8 (Collagenase-2)	Collagen I, II, III, aggrecan, serpins, 2-MG	MMP-3, -10, plasmin
MMP-13 (Collagenase-3)	Collagen I, II, III, IV, IX, X, XIV, gelatin, FN, LN, large tenascin, aggrecan, fibrillin, osteonectin, serpins	MMP-2, -3, -10, -14, -15, plasmin
Gelatinases		
MMP-2 (Gelatinase A)	Gelatin, Collagen I, IV, V, VII, X, FN, tenascin, osteonectin, MCP-3	MMP-1, -13, -14, -15, -16, tryptase?
MMP-9 (Gelatinase B)	Gelatin, Collagen IV, V, VII, IX, XIV, elastin, fibrillin, osteonectin 2	MMP-2, -3, -7, -13, plasmin, trypsin, chymotrypsin, cathepsin G
Matrilysins		
MMP-7 (Matrilysin)	Elastin, FN, LN, nidogen, collagen IV, tenascin, versican, 1-PI, E-cadherin, TNF	MMP-3, plasmin
MMP-26 (Matrilysin-2)	Gelatin, 1-PI, TACE substrates	Not determined
Stromelysins		
MMP-3 (Stromelysin-1)	Collagen IV, V, IX, X, FN, elastin, gelatin, aggrecan, nidogen, fibrillin, osteonectin, 1-BI, MBP, OP, E-cadherin	Plasmin, kallikrien, chymase, tryptase
MMP-10 (Stromelysin-2)	Collagen IV, V, IX, X, FN, elastin, gelatin, LN, aggrecan, nidogen, OP, E-cadherin	Elastase, cathepsin-G
Stromelysin-like MMPs		
MMP-11 (Stromelysin-3)	Serine protease inhibitors, 1-PI	Furin
MMP-12 (Metalloelastase)	Collagen IV, gelatin, FN, LN, VN, elastin, fibrillin, 1-PI, MBP, apolipoprotein A	Not determined
Membrane-type MMPs		
MMP-14 (MT1-MMP)	Collagen I, II, III, gelatin, FN, LN, VN, aggrecan, tenascin, nidogen, perlecan, fibrillin, 1-PI, 2-MG, fibrin	Plasmin, furin
MMP-15 (MT2-MMP)	FN, LN, aggrecan, tenascin, nidogen, perlecan	Not determined
MMP-16 (MT3-MMP)	Collagen III, FN, gelatin, casein, cartilage proteoglycans, laminin-1, 2-MG	Not determined
MMP-17 (MT4-MMP)	Fibrin, fibrinogen, TNF precursor	Not determined
MMP-24 (MT5-MMP)	Proteoglycans	Not determined
MMP-25 (MT6-MMP)	Collagen IV, gelatin, FN, fibrin	Not determined
Other MMPs		
MMP-19	Gelatin, aggrecan, COMP, collagen IV, LN, nidogen, large tenascin	Trypsin
MMP-20 (Enamelysin)	Amelogenin, aggrecan, COMP	Not determined
MMP-28	Casein	Not determined

Substrates and activators of MMP-23 have not been identified. Abbreviations used: COMP = cartilage oligomeric matrix protein, TNF = tumor necrosis factor ,TACE = TNF converting enzyme, LN = laminin, FN= fibronectin, , 2-MG = alpha-2-macroglobulin, 1-PI = 1-proteinase inhibitor, OP = osteopontin, VN = Vitronectin

everyday physiological processes such as wound repair, angiogenesis, and various aspects of the reproductive process (1-3).

3.1. The matrix metalloproteinases (MMPs)

To date, 25 vertebrate MMPs and 22 human homologues have been identified (2). Each MMP is identified by either their common name or according to a sequential numeric nomenclature system reserved for vertebrate MMPs (2). MMPs are also classified by subgroups based upon their domain structure (Figure 1) and substrate specificity (Table 1). These substrate-specific classes include collagenases (MMP-1, MMP-8 and MMP-13), gelatinases (MMP-2 and MMP-9), matrilysins (MMP-

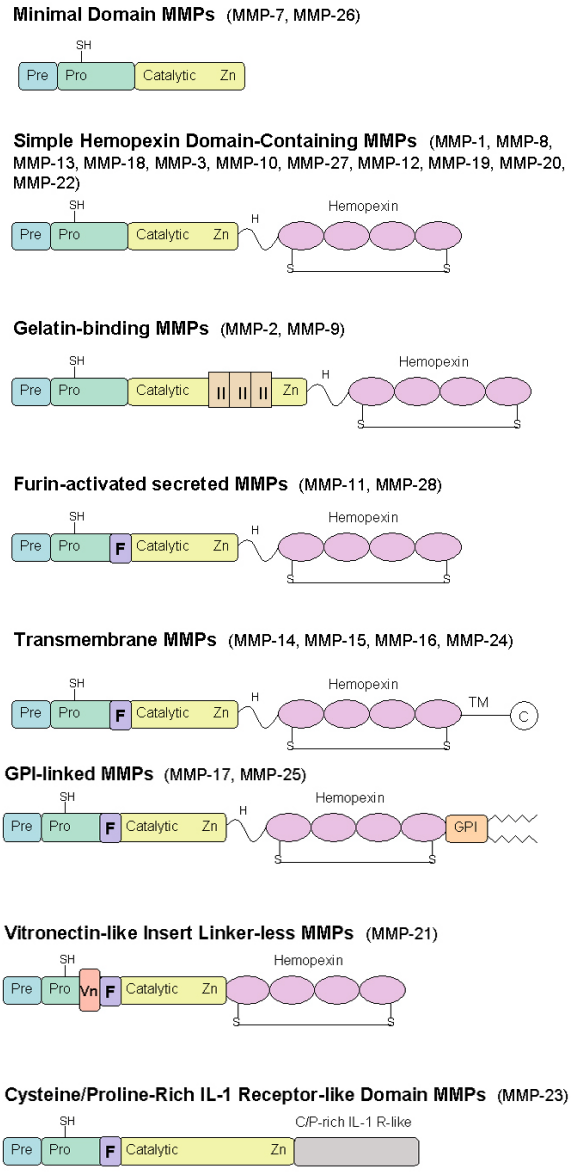

Figure 1. Domain structure of the matrix metalloproteinases (MMPs). Each of the 25 vertebrae MMPs is categorized by domain structure. Abbreviations used: Pre = signal sequence, Pro = propeptide with a free zinc-ligating thiol (SH) group, F = furin-susceptible site, Zn = zinc-binding site, II = collagen-binding fibronectin type II inserts, H = hinge region, TM = Transmembrane domain, C = cytoplasmic tail, GPI = glycophosphatidyl inositol-anchoring domain, C/P = cysteine/proline, IL-1R = interleukin-1 receptor. (Figure adapted from reference 2).

and MMP-26), stromelysins (MMP-3 and MMP-10), stromelysin-like MMPs (MMP-11 and MMP-12), membrane-type (MT) MMPs (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24 and MMP-25) or other MMPs (MMP-19, MMP-20, MMP-23, MMP-28). All MMPs have an N-terminal signal sequence or pre-domain that is cleaved after it directs the MMP's synthesis (Figure 1). As such, the majority of MMPs are secreted proteinases. However there is a class of MMPs that displays transmembrane domains and is expressed on the cell surface. These membrane-type or MT-MMPs can function both as classical proteinases and as co-activators of other MMPs (2).

For MMPs to perform their normal functions, they must be present in the correct pericellular location, at the correct physiological time point, in the appropriate amount, and in an "active" state. For proper MMP function, it is of paramount importance that MMP activity be tightly regulated (Figure 2). This precise regulation of MMP activity is accomplished by: 1) the synthesis of MMPs in an inactive (pre-pro) form, 2) activation of the latent MMP in the extracellular space, and 3) inhibition of MMP activity in the extracellular environment by serum-borne and tissue-derived metalloproteinase inhibitors (TIMPs).

While many MMPs share structural similarities, MMPs also display the ability to recognize and cleave specific ECM components. For example, the collagenases (MMP-1, -8 and -13) cleave both fibrillar and non-fibrillar collagens. Within the fibrillar collagen structure, collagenases cleave the triple helical collagen molecule by making a single "clip" within the collagen strand. This single "clip" changes the stability and solubility of the collagens making them susceptible to a wide array of tissue proteinases, which include the gelatinases (MMP-2, -9) and stromelysins (MMP-3, -10). Gelatinases and stromelysins can also degrade type IV collagen, laminin, fibronectin and tenascin (1 – 3).

In addition to degradation of ECM constituents, many MMPs also exhibit activity towards others MMPs, growth factors, cytokines, adhesion molecules and binding proteins (1, 2). For example, MMPs can also regulate a variety of biological responses by cleavage or activation of growth factors and cytokines including insulin-like growth factor-binding proteins and tumor necrosis factor-alpha (Figure 2). These abilities of MMPs expand their repertoire and allow them to potentially influence cell behavior by cleaving cell to cell adhesion molecules, by releasing bioactive cell surface molecules, by cleaving cell surface receptors, and by either activating or inactivating bioactive proteins. As such, the precise control, appropriate time point of expression and level of MMP activity are of paramount importance for normal physiological processes to ensue. Misexpression, either at the incorrect time or at an inappropriate level, of MMPs can undoubtedly lead to pathophysiological conditions through altered cellular behavior.

3.2. The tissue inhibitors of metalloproteinases (TIMPs)

TIMPs are the major endogenous regulators of the activities of MMPs, and four homologous TIMPs (TIMPs-1 to 4) have been identified to date (5, 6). The TIMPs are a family of 20-29 kDa secreted proteins that bind to and inhibit the active MMPs at molar equivalence. Their inhibitory activity occurs through the N-terminal domain of the TIMP with the catalytic domain and the substrate-binding groove of the MMP (7). The C-terminal

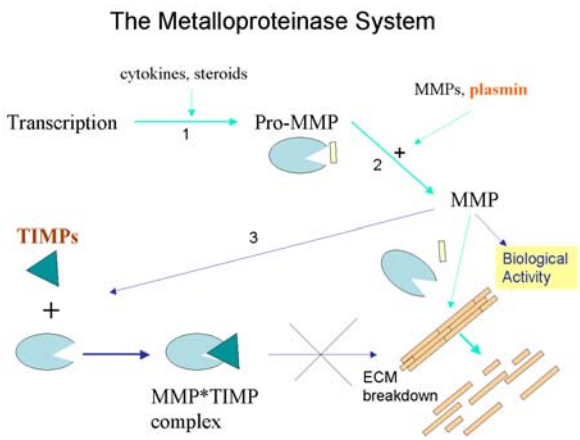

Figure 2. The metalloproteinase system. Matrix metalloproteinase activity is regulated at the level of transcription (1), activation (2) and regulation at the tissue level by tissue inhibitors of metalloproteinases (TIMPs; 3). Once activated and left uninhibited, MMPs can either degrade extracellular matrix (ECM) inducing classical tissue remodeling or regulate numerous biological activities through the modulation of various cell signaling mechanisms.

is involved in protein localization and pro-enzyme complex formation. Individual TIMPs exhibit differential ability to inhibit various MMPs (2). For example, TIMP-1 preferentially binds to MMP-1, -2, -3 and –9, while TIMP-2 has a high affinity for MMP-2 (8). TIMP-3 appears to be a more potent inhibitor of MMP-9 than other TIMPs. TIMP-2 and TIMP-3 inhibit MT1-MMP, while TIMP-1 does not. TIMP-1 and TIMP-3 can also bind to pro-MMP-9 through C-terminal domain inhibiting its activation by stromelysins. TIMP-4 seems to have no remarkable preference in its ability to inhibit MMPs (9). In addition, TIMPs are multifunctional proteins that also participate in the regulation of cell proliferation, apoptosis and differentiation (5, 10-12). Therefore, the TIMPs are important regulators not only in ECM remodeling but also in cellular activities.

4. MMP EXPRESSION WITHIN THE UTERUS

Extensive tissue remodeling occurs within the uterus across each estrus or menstrual cycle. MMPs are spatiotemporally expressed within the uterus and the selective expression, activation or inhibition of specific members of the MMP family is believed to mediate uterine tissue turnover preparing for a successful embryo implantation and pregnancy (3). Numerous investigators have described the patterns of expression and cellular localization for the MMPs within the uterus of several species. This information is described in detail in the paragraphs below and summarized in Table 2.

4.1. MMP expression in the uterus of women and non-human primates

The endometrium of women and female primates undergoes dramatic changes throughout the menstrual cycle. The beginning of each cycle is marked by the sloughing off of the endometrium (menstruation) and the MMP system has been implicated in this process (3). Following the period of menses, the endometrium undergoes a period of cellular regeneration and proliferation (proliferative phase) in response to the actions of estrogen. This proliferative phase proceeds up to the time of ovulation. After the period of ovulation, the endometrium is now under the primary influence of progesterone (as well as estrogen). During the second half of the menstrual cycle (which is referred to as the secretory phase), the endometrium develops the secretory capacity to provide appropriate conditions for the implantation of the fertilized oocyte (embryo implantation). If embryo implantation is successful, pregnancy ensues and is characterized by sustained levels of progesterone. If implantation is not successful, progesterone levels decline and these declining progesterone levels then trigger menstruation and the entire process begins again. Recent studies revealed that the MMP system plays an important role during the destruction and regeneration of the endometrium during the menstrual cycle as well as during pregnancy, parturition and postpartum uterine involution. In human adults, MMPs are expressed in the endometrium in a cyclic, tissue and cell type specific fashion. Their mRNA expression has been determined by in situ hybridization, Northern blot and RT-PCR, and the proteins have been localized by immunohistochemistry. The temporal MMP mRNA expression in human endometrium mainly follows three patterns (13). The first pattern includes MMPs almost exclusively expressed during menstruation such as MMP-1, MMP-3, MMP-8, MMP-9, MMP-10 and MMP-12. The second pattern includes those MMPs expressed throughout the cycle without evident repression such as MMP-2, MMP-19, MT1-MMP and MT2-MMP, among which MT1-MMP mRNA exhibits a significant increase during menstruation. The third pattern includes the MMPs expressed constitutively but with significant variation throughout the cycle such as MMP-7, MMP-11, MMP-26 and MT3-MMP. The mRNA levels of MMP-7 and MMP-11 exhibit a repression at mid-cycle while the levels of MMP-26 and MT3-MMP mRNA are maximal during the proliferative phase and subsequently decline to basal values during the late secretary phase and menstruation. The pattern of uterine MMP expression also exhibits cell-type and menstrual cycle specific features. For example, endometrial epithelial cells produce MMP-7, -9, -26, MT1-MMP and MT2-MMP (14-18). MMP-7 is exclusively produced by epithelial cells, and has been identified focally among glandular structures during the proliferative phase (17). MMP-9 expression has been localized to epithelial, stromal, immune and vascular cells (16, 19-21). There are conflicting reports on the expression of the newly identified MMP-26 in human endometrium. Isaka *et al.* demonstrated that MMP-26 expression is limited to normal epithelial glandular cells (14). However, Chegini and colleagues reported that MMP-26 is present in various types of endometrial cells with the strongest expression in surface and glandular epithelial cells followed by vascular endothelial and endometrial stromal cells, as well as in the inflammatory and immune-related cells (22). The expression of MMP-8, -9 and MT1-MMP has been identified among endometrial leukocytes,

Table 2. Matrix metalloproteinase mRNA and protein expression within the uterus during the female reproductive cycle in various species

Stage of the Menstrual/Estrous Cycle						
Species/MMP			Estrogen-Dominant		Progesterone-Dominant	
Humans		**Menstrual**	**Proliferative**		**Secretory**	
	mRNA	**Protein**	**mRNA**	**Protein**	**mRNA**	**Protein**
MMP-1	+	↑	+/-		-	
MMP-2	++	↑	++	↑	++	↑
MMP-3	++/+	↑	+/-		-	
MMP-7	+++	↑	++	↑	+	↑ (late secretory)
MMP-8	+		-		-	
MMP-9	+	↑	+/-	↑	+/-	↑
MMP-10	++		-		+ (late secretory)	
MMP-11	++		++		+	
MMP-12	+++		-		-	
MT1-MMP	+++		++		++	
MT2-MMP	+		+		+	
MT3-MMP	++		+++		++	
MMP-19	++		++		++	
MMP-26	+	↑	++	↑	++	↑↑
Non-human primates						
MMP-1	++		++		+/-	
MMP-2	++	↑↑	++	↑	+	↑
MMP-3	++	↑	++		+/-	
MMP-7	++	↑↑	++	↑	+/-	↑
MMP-10	++		++		+/-	
MMP-11	++		++		+/-	
MT1-MMP	++		++		+	
Mice/Rats (mRNA only)						
			Proestrus	**Estrus**	**Metestrus**	**Diestrus**
MMP-2			+	+	+	+
MMP-3			++	+	-	++
MMP-7			-	+++	++	+
MMP-9			+	++	+	+
MMP-10			+++	-	-	-
MMP-11			+	+++	+	+
MMP-13			+	+	+	+

Relative levels of mRNA are expressed as – (absent), +/– (minimal), + (focal), ++ (moderate), +++ (intense). ↑ indicates expression of proteins within the tissue while ↑↑ indicates increased expression of proteins compared to lower (↑) levels of expression . Detailed information can be found in Ref. 13-28, 31, 35, 36, and 39.

including neutrophils, eosinophils, macrophages and endometrial granular lymphocyte (18, 19, 23). In contrast to epithelial and immune cells whose ability to produce MMPs are relatively limited, endometrial stromal cells express numerous MMP mRNAs including MMP-1, -2, -3, -8, -9, -10, -11 and MT1-MMP (24, 25). MMP-1 mRNA and protein expression in the endometrium was only detected during the perimenstrual period with either restricted localization at superficial foci of stromal cells or extended expression towards the entire functional layer (26). The prominent expression of MMP-1 at the periphery of shedding fragments and along some arterioles suggests that this MMP may play an important role in the matrix breakdown during menstruation. Mast cells in the uterus, which mainly reside in the myometrium, co-express MMP-1 and mast cell tryptase and the latter is capable of stimulating the MMP cascade (27). The protein of MMP-9 has been identified in endometrial glandular epithelial cells with highest levels during the late proliferative phase and just after ovulation and in glandular secretion and the uterine fluid during the peri-implantation phase. The increase in MMP-9 expression in the endometrium just before and during menstruation has been found to be associated with an influx of polymorphonuclear leucocytes, macrophages and eosinophils. MMP-3 protein expression was identified around stromal cells and limited to microfocal locations at times coincident with stromal edema (days 8-10 and 21-22) during the menstrual cycle (20). The expression of several MMP proteins including MMP-1, -2, -3 and –9 has been demonstrated in the endometrial vascular structures by immunocytochemistry (16). In this survey, MMP-2 was detectable in vessels throughout the cycle. In contrast, MMP-3 was detected in the superficial endometrial vessels

during late-secretory and menstrual phases, while MMP-9 was detected in spiral arteries during the secretory phase and in vascular structures during the midfollicular and menstrual phases. These findings would suggest that MMP-2 and MMP-9 may participate in vascular growth and angiogenesis, whereas MMP-3 may play a role in initiating the breakdown of the vascular wall. MMP-2 and MMP-9 also appear to participate in parturition and postpartum uterine involution since their expression was increased in the cervix at term pregnancy and postpartum compared with nonpregnant state; cervical stromal fibroblasts and smooth muscle cells were identified as main sources of MMP-2, whereas the MMP-9 protein was observed exclusively in invading leukocytes (28). A recent study demonstrated that MMP-26 protein is expressed in the endometrium throughout the menstrual cycle with elevated expression during the early to mid-luteal phase (22). There is little information regarding the expression of MMP-8 in the uterus during the menstrual cycle, but Sennstrom et al. reported that MMP-8 expression was significantly increased in ripened human cervix, primarily in the stromal tissue, suggesting its involvement in parturition (29). Zymographic studies have been used to investigate the activities of MMPs in the uterus. For example, in situ zymography demonstrated that both gelatinase and collagenase activities were increased in menstrual endometrial tissues compared to any other time of the cycle (30).

Non-human primates such as rhesus monkeys and baboons have similar endometrial MMP expression patterns during the menstrual cycle as that of humans, and have been used as experimental models to investigate the mechanism of menstruation. Using ovariectomized rhesus monkeys, Rudolph-Owen and colleagues (31) demonstrated that although all MMPs were up-regulated by progesterone (P4) withdrawal, their expression declined spontaneously after menstruation in the absence of P4. Of 7 MMPs examined, only MMP-7 and MMP-11 were suppressed any further when P4 levels were experimentally re-elevated. MMP expression was confined to the upper functionalis zone during menstruation, but after menstrual breakdown was complete, MMP-7 and TIMP-1 shifted expression from the functionalis to the basalis zone in the absence of both estradiol and P4. It is readily apparent that zone-specific factors greatly influence the pattern and degree of uterine MMP expression. Additional studies have demonstrated that MMP-26 mRNA is expressed in the endometrial compartments of rhesus monkey during early pregnancy with intense signals in the glandular epithelium on day 12 and in the walls of spiral arterioles adjacent to the implantation site on day 26 (32), indicating that MMP-26 perhaps plays a role in the remodeling of glandular epithelium and spiral arteries during pregnancy. Furthermore, a recent study by Li at al. also demonstrated the expression of MMP-28 mRNA and protein in rhesus monkey placenta during early pregnancy (33).

4.2. MMP expression in the uterus of rodents

Rodents and domestic species exhibit much less endometrial tissue remodeling during the estrous cycle compared to menstruating primates. Therefore, it is not surprising that the expression and activity of MMPs in these species are relatively lower. However, much like primates, MMP mRNA expression also exhibits cyclic changes during the reproductive (estrous) cycle in rodents (3, 34). MMP-2, MMP-9, MMP-3, MMP-10, MMP-11, MMP-7 and MMP-13 transcript expression has been reported in the murine uterus during the estrous cycle, and most of them have higher expression levels around the estrous stage (35). Wilson et al. demonstrated that MMP-7 was expressed in the normal cycling, pregnant, and postpartum uterus, with levels of expression highest in the involuting uterus at early time point. The mRNA expression of MMP-7 was confined to epithelial cells lining the lumen and some glandular structures (36). MMP-2, -9 and –1 have been identified in the rat uterus during the implantation period, and the expression of MMP-2 and MMP-9 mRNA was increased during early pregnancy (37, 38). In the mouse uterus, MMP-1 has not been identified whereas MMP-2 and –9 were detected during peri-implantation period (39). A more detailed survey conducted by Das et al. indicated that MMP-2 may participate in the early phase of decidulization and neovascularization required for placentation, and the fine balance between MMP-9 and TIMP-3 may regulate trophoblast invasion in the uterus (40). MMP-13 mRNA levels were very low in virgin and pregnant rat uterus, but were increased transiently postpartum (41). High levels of MMP-8 mRNA have been demonstrated in the postpartum mouse uterus (42). Additionally, a more recent study also reported the expression of MMP-28 mRNA in the murine uterus (43).

4.3. MMP expression in the uterus of domestic animals

The expression of MMPs has also been reported within the uterus of domestic animals including bitches, goats and sheep, although the information in this regard is very limited. MMP-1, -2 and -3 were secreted by cultured ovine endometrial stromal, but not epithelial cells. MMP-1 is produced primarily upon stimulation, whereas MMP-2 production is constitutive (44). MT1-MMP mRNA was expressed in the luminal and glandular epithelial cells of the gestational endometrium of goats (45). Increased activities of MMP-2, -7 and –9 were reported in the endometrium of postpartum bitch (46). Future studies on the expression of the MMPs in the uterus of domestic animals will sure enrich our knowledge on the function of the MMP system in uterine biology.

5. TIMP EXPRESSION WITHIN THE UTERUS

The delicate balance between the expression of MMPs and that of TIMPs is a prerequisite for the normal tissue turnover within the uterus throughout the cycle, as well as during the reproductive processes. The TIMPs serve to precisely regulate the site and extent of ECM degradation, and hence insure the homeostasis of uterine extracellular architecture. Accumulated evidence indicates that the imbalance in the expression of the MMP system within the uterus is associated with various pathological conditions such as endometriosis, abnormal bleeding and endometrial carcinoma. Thus, the importance of understanding the regulation of TIMPs is evident. Several investigators have described the patterns of expression and cellular localization for the TIMPs within the uterus of numerous species. This information is described in detail in the paragraphs below and summarized in Table 3.

Table 3. Tissue inhibitor of metalloproteinase mRNA and protein expression within the uterus during the female reproductive cycle in various species

Stage of Menstrual/Estrous Cycle								
Species/TIMP			Estrogen-Dominant			Progesterone-Dominant		
Human	Menstrual		Proliferative		Secretory		Late Secretory	
	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	protein
TIMP-1	++	↑↑	+	↑	+	↑	++	↑
TIMP-2	++	↑↑	+	↑	+	↑	++/+	↑
TIMP-3	+	↑	+/-	↑	+	↑↑	++	↑
TIMP-4	?	?	?	↑	?	↑↑	?	↑
Non-human primates								
TIMP-1	++	↑↑	++	↑	+	↑		
TIMP-2	-		-		-			
Mice/Rats	(mRNA only)		Proestrus	Estrus		Metestrus	Diestrus	
TIMP-1			++	+		+/-	+/-	
TIMP-2			+	+		+	+	
TIMP-3			++	++		++	+	
TIMP-4			-	-		-	-	

Relative levels of mRNA are expressed as – (absent), +/– (minimal), + (focal), ++ (moderate), +++ (intense). ↑ indicates expression of proteins within the tissue while ↑↑ indicates increased expression of proteins compared to lower (↑) levels of expression . ? indicates that expression patterns have not been determined. Detailed information can be found in Ref. 13-28, 31, 35, 36, and 39.

5.1. TIMP expression in the uterus of women and non-human primates

TIMPs are expressed in a spatiotemporal fashion in the uterus throughout the estrous or menstrual cycle. In human endometrium, TIMP-1, -2, -3 and –4 has each been detected during the menstrual cycle (Table 3). Zhang and Salamonsen reported that TIMP-1, 2 and 3 proteins were present in all tissue compartment of the human endometrium with the most intense immunoreactivity in the luminal epithelium (47). In addition, in vitro data from this study showed that cultured endometrial stromal cells release more TIMP-1 than TIMP-2 or –3 and all were increased following decidulization; epithelial cells in culture produced less TIMPs than stromal cells. Recent investigations have identified the expression of TIMP-4 protein in the human endometrium with highest levels in endometrial epithelial cells, followed by vascular endothelial cells and endometrial stromal cells (48). Furthermore, TIMP-1 and –2 proteins were also detected in vascular structures in the endometrium throughout the cycle, and their intense expression in the vessels delineating necrotic from non-necrotic areas during menses suggests that TIMPs may act to limit tissue damage and allow regeneration of endometrium after menses (16). Expression of TIMPs in the human endometrium varies during the menstrual cycle. Hampton *et al.* reported that both TIMP-1 and TIMP-2 exhibit elevated mRNA expression in menstrual endometrium compared with tissue from the rest of the cycle (49). Similarly, Maatta *et al.* reported that mRNAs for TIMP-1, -2 and –3 were strongly induced in late secretory endometrium (25). More recently, Chegini *et al.* demonstrated that the expression of TIMP-3 and TIMP-4 proteins peaked during the early to mid-luteal phase in normal cycling women (22). Among the TIMPs, TIMP-3 has been found to be the main inhibitor within the uterus that restricts trophoblast invasion during implantation and early pregnancy. For example, Li L *et al.* reported that TIMP-3 expression in human endometrium was elevated during the implantation window phase (50). In rhesus monkeys, TIMP-2 was not detected, while TIMP-1 expression was also found to increase during menstruation in response to progesterone withdrawal (31). Studies using rhesus monkeys also showed that TIMP-3 was specifically expressed in the cells around the spiral arteries and maternal-fetal interface during early pregnancy while TIMP-1 mRNA expression in the maternal deciduas was not localized (51).

5.2. TIMP expression in the uterus of rodents

TIMP-1, -2 and –3 are also expressed in rodent uterus (Table 3). Nothnick (52) reported the expression patterns of mRNA for TIMPs in the murine uterus throughout the estrous cycle. TIMP-1 transcript expression was lowest during diestrus, significantly increased during proestrus and estrus, and then declined again during metestrus. In contrast, TIMP-2 mRNA displayed relatively constant levels of expression across the estrous cycle. The TIMP-3 mRNA expression, which was shown to be the most abundant of the uterine TIMPs, was lowest during diestrus and increased during proestrus through metestrus. TIMP-4 has not been detected in the uterus of rodents. Increased expression of mRNAs for TIMPs has been found

in the rodent uterus during decidulization: Nuttall *et al.* reported that endometrial mRNA levels for TIMP-1, 2 and 3 were increased during oil-induced decidualization *in vivo* (53). Zhao *et al.* demonstrated that mRNAs for TIMP-1, 2 and 3 are expressed in decidulized stromal cells in rat uterus at the implantation site (54). In accord with studies in primates, TIMP-3 mRNA has been found to be strongly expressed in the stroma of the mouse uterus surrounding the embryo, suggesting that this TIMP may regulate trophoblast invasion in the uterus (55).

5.3. TIMP expression in the uterus of domestic animals

The expression of TIMPs has also been investigated in domestic animals such as the ewe. Salamonsen *et al.* demonstrated that mRNAs for TIMP-1 and –2 are expressed in the endometrium of ewes throughout the estrous cycle and early pregnancy (44). TIMP-1 mRNA and protein have been detected correlatively in both the epithelium and stroma of intact endometrium of ewes (56).

6. REGULATION OF UTERINE MMPS AND TIMPS

The expression and activity of the MMP system is tightly regulated in the uterus throughout the menstrual or estrous cycle as well as during pregnancy, parturition and postpartum uterine involution. The precisely controlled regulation of endometrial MMP and TIMP expression is essential for normal tissue growth and remodeling occurring in the uterus during the reproductive processes. The mechanisms whereby the spatiotemporal patterns of the expression of the MMP system are achieved involve not only ovarian steroids but also various autocrine/paracrine factors such as growth factors and cytokines (3, 34). The MMP system is regulated via transcriptional regulation, post-transcriptional regulation, regulation of MMP secretion, activation of latent MMPs and inhibition of active MMPs by their inhibitors (2). Regulation of MMP/TIMP expression usually occurs at the level of transcription, but can also occur due to changes in mRNA stability in response to growth factors and cytokines (57, 58). This review will mainly focus on the regulation of MMP/TIMP expression in the uterus as several excellent review articles regarding general regulation of the MMP activity have recently been published (1-4)

6.1. Ovarian steroids

Ovarian steroids seem to play pivotal roles in regulating the MMP system in the uterus given the reproductive cycle-specific expression patterns of the MMPs and TIMPs. However, the cell-type and tissue-specific features indicate that local regulation by autocrine/paracrine factors is involved. Accumulated knowledge suggests that ovarian steroids regulate the gene expression of the MMP system in the uterus, although the regulation patterns of specific MMP expression by estrogens or P4 are not completely defined yet. Sato and coworkers demonstrated that both estradiol (E2) and P4 down-regulated the mRNA and protein expression of MMP-1 and MMP-3 while simultaneously up-regulated that of TIMP-1 in cultured rabbit uterine cervical fibroblasts (59). Schatz and colleagues reported that E2 alone had no effect on the expression of MMP-3 in primary human endometrial stromal and decidual cells while medroxyprogesterone acetate (MPA) or E2 plus MPA down-regulated MMP-3 expression in these cells (60). A more complicated regulation by estrogens of MMP-2 and MMP-13 expression was reported by Tushaus *et al.*: Estrogens upregulated the mRNA levels of MMP-2 and MMP-13 in the rat uterus through nuclear receptors; in contrast, in the RUCA-1 transplantation endometrial tumor, estrogens down-regulated the mRNA levels of MMP-13 but not MMP-2. The divergent regulation may suggest a varying influence of cell-cell-, cell-ECM interactions and soluble factors (61). P4 appears to down-regulate some MMPs in the uterus. For example, P4 withdrawal induced the expression of MMP-2 in human endometrial stromal cells *in vitro* (62). Interestingly, P4 also inhibited the activation of proMMP-2 by MT1-MMP in cultured endometrial stromal cells (18). In baboon endometrium, P4 suppressed the expression of epithelial MMP-7 but not that of stromal MMP-3 (63), whereas a P4 receptor antagonist augmented the expression of MMP-3 mRNA in the uterine cervix of pregnant rabbits (64).

6.2. Steroid receptors

Changes in the levels of steroid receptors may also contribute to the regulation of the MMP system by ovarian steroids. Immunolocalization of estrogen receptors in ovine endometrial tissue demonstrated marked changes throughout the cycle and in early pregnancy with maximal concentrations during the follicular and very early luteal phases, and the protein synthesis is greater in stromal cells than in epithelial cells (65). MMP-1, which is postulated to be an essential enzyme in the early events leading to menstruation, is focally expressed in stromal cells of the functional layer of the human endometrium when and where steroid receptors disappear, and especially where tissue breakdown is prominent (66). Likewise, Spencer *et al.* demonstrated an inverse correlation between estrogen receptor levels and the activity of MMP-9 in the uterus of pseudopregnant rats (67).

6.3. Gonadotropin-releasing hormone

Another potential regulator of the MMP system in the uterus is gonadotropin-releasing hormone (GnRH). It has been shown that GnRH agonist down-regulates the mRNA and protein expression of TIMP-1 and TIMP-3 in cultured human stromal cells (68) and increases the mRNA expression of MMP-2 and MMP-9 in primary cultures of human decidual stromal cells (69). Of particular interest is the finding that in the human endometrium, GnRH and its receptors are spatiotemporally expressed during the menstrual cycle, suggesting that this peptide may play a role in regulating the remodeling of this dynamic tissue (70).

6.4. Growth factors, cytokines and chemokines

Various growth factors, cytokines and chemokines are potent modulators of the MMP system within the uterus. In general, MMP transcription is induced by many of these factors such as tumor necrosis factor-alpha (TNF-alpha), interleukin-1-beta (IL-1-beta), interleukin-1-alpha (IL-1-alpha), interleukin-6 (IL-6),

epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), platelet-activating factor (PAF), prostaglandin E2 (PGE2), prostaglandin F2-alpha (PGF2-alpha), relaxin, serotonin and nitric oxide. Transforming growth factor-beta (TGF-beta), on the contrary, suppresses the expression of MMPs (71). Specific growth factors and cytokines increase the expression of some but not all MMPs. Both interleukin-1 alpha and tumor necrosis factor-alpha stimulated the secretion of MMP-1, MMP-3, and MMP-9, but not MMP-2 in cultured primary human endometrial stromal cells (72). The same factor may differentially regulate certain MMP within the uterus in different species or under various experimental conditions. For example, relaxin increases the expression of TIMP-1 in porcine uterus (73), but inhibits TIMP-1 expression in human lower uterine segment fibroblasts (74). There are also diverse interactions among individual cytokines, which add to the complexity of the MMP regulation within the uterus. For example, IL-1alpha suppressed the TNF-alpha-induced proMMP-9 production in cultured human uterine cervical fibroblasts (75). Some growth factors and cytokines not only induce MMP transcription but also accelerate their translation. It has been demonstrated that EGF and TGF-alpha augment the translation of proMMP-3 and TIMP-1 mRNA and accelerate their accumulation without modifying their transcripts during the first 1-2 h treatment of human uterine cervical fibroblasts (76). Several factors including relaxin, TGF-alpha, TGF-beta, EGF and IL-1 beta not only regulate MMP expression but also regulate TIMP expression (73, 74, 76). IL-1 decreased TIMP-1 and TIMP-3 mRNA expression, whereas TGF-beta augmented their expression in cultured human endometrial stromal cells. Additionally, MMPs have been reported to degrade and inactivate IL-1-beta therefore providing a negative feedback on MMP transcription (77). MMPs have also been reported to cleave the precursor of TNF-alpha leading to its activation (78) whilst TIMP-3 inhibits TNF-alpha activation (79).

6.5. Interactions among various regulatory factors

Ovarian steroids may interact with locally produced factors such as cytokines and thus coordinate MMP expression within the uterus. P4 exposure prevents MMP-3 stimulation by IL-1alpha in human endometrial stromal cells (80) and suppresses IL-1-alpha- and IL-1-beta -induced MMP-9 mRNA expression in rabbit uterine cervical fibroblasts, suggesting that P4 may preserve tissue integrity during the establishment and maintenance of pregnancy by limiting stimulation of MMPs by inflammatory cytokines such as IL-1 (81). Regulation of MMP system in the uterus also involves complex cell-cell and cell-ECM communications. The numerous leukocytes that infiltrate into the endometrium before menstruation are believed to interact with the endometrial stromal and epithelial cells and co-mediate the expression and activation of MMPs during menstruation (82). A human mast cell line in co-culture with stromal cells stimulated stromal cell proMMP-1 and proMMP-3 and to a lesser extent proMMP-2 production, indicating that the activated mast cells within the endometrium prior to menstruation may stimulate MMP production by endometrial stromal cells (83). Stromal-epithelial interactions play an important role in mediating steroidal regulation of the MMP expression in human endometrium. Epithelium-derived IL-1alpha has been demonstrated to be the key paracrine inducer of MMP-1 in endometrial fibroblasts. Ovarian steroids inhibit the release of IL-1alpha and thus block MMP-1 production, which may explain why MMP-1 expression follows the late-secretory fall in sex steroid plasma concentrations (84). Bruner and colleagues demonstrated that progesterone suppressed endometrial MMP-7 expression by inducing TGF-beta secretion by endometrial stromal cells in human endometrial stromal-epithelial co-cultures (85).

7. FUNTIONAL STUDIES ON UTERINE MMPS AND TIMPS USING ANIMAL MODELS

The exact roles of specific members of the MMP system in uterine biology remain poorly understood. Studies using MMPs/TIMPs-transgenic or knockout mice during the last decade revealed that the MMP system is involved in various aspects of physiology including reproduction. Abnormal phenotypes of mice with the genetic modification of the MMP system (MMP-1, 2, 3, 7, 9, 11, 12 and 14; TIMP 1-3) have been reviewed by Sternlicht and Werb (2). MT-1 MMP null animals exhibit severe skeletal and connective tissue abnormalities and early postnatal lethality, making it hard to examine the impact of MT-1 MMP deficiency on reproductive function (86). Decreased fertility has been reported for both MMP-9 and TIMP-1 knockout mice (52, 87). Mice deficient in MMP-9 have lower breeding efficiency, smaller individual litters and higher percentage of infertile breeding pairs (87). Likewise, TIMP-1 deficient female mice have reduced reproductive life span, lower rate of pregnancy and take longer to achieve pregnancy (Nothnick, unpublished observation). Disruption of the TIMP-1 gene product also results in an altered reproductive cycle characterized by a significant decrease in the length of the estrus and altered uterine morphology (52). Nothnick reported that TIMP-1 gene deficiency in reproductive-age female mice was associated with estrous cycle stage-specific increases in stromelysin messenger RNA expression and activity in the uterus (35). A more recent study by Nothnick and colleagues demonstrated that steroidal modulation of uterine TIMP-3 expression and regulation of wet weight gain/edema were altered in TIMP-1 knockout mice (88). Other MMP/TIMP genetically modified mice appear to have normal fertility, but it would not be surprising if further investigation indicates subtle abnormality in their uterine function considering that the subfertility of MMP-9 or TIMP-1 knockout mice was initially neglected (89 90). One notable issue regarding these studies is that the conclusiveness is often limited by the expression of redundant or compensatory enzymes with overlapping activity. For example, MMP-7 deficient mice exhibit increased expression of MMP-3 and MMP-10 during uterine involution, while MMP-3 deficient mice showed increased MMP-7 and MMP-10 expression (91). These and other similar findings suggest a compensatory mechanism for the loss of a specific MMP, indicating the redundant roles of various MMPs *in vivo*.

The use of broad-spectrum or specific MMP inhibitors also provides approaches for investigating the

role of the MMP system within the uterus. A broad-spectrum MMP inhibitor, doxycycline, and specific MMP-3 inhibitor, N-Isobutyl-N-(4-methoxyphenylsulfonyl)-glycylhydroxamic acid both down-regulated IGF binding protein-1 (IGFBP-1), which is a biochemical marker of primate decidual cells, and up-regulated alpha-smooth muscle actin during decidualization (92). These observations may be interpreted to suggest that MMPs, and particularly MMP-3, may up-regulate IGFBP-1 by disrupting the actin cytoskeleton as a result of ECM degradation. Rechtman and colleagues have also demonstrated that administration of doxycycline during early pregnancy retards decidual development, but does not block implantation (93). The emergence of more specific inhibitors for individual MMPs will allow for the examination of the precise role of these proteases within the uterus in the very near future.

8. SUMMARY AND PERSPECTIVE

The delicate balance between the activity of the MMPs and their inhibitors is key for the normal tissue growth and remodeling that occurs within the uterus during the reproductive processes. Pathological conditions of the uterus such as endometriosis, abnormal bleeding and endometrial carcinoma have been associated with an imbalance in the function of the MMP system. Regulation of this system in the uterus involves ovarian hormones, GnRH, various cytokines and growth factors and complex cell-cell, cell-ECM communications. These regulatory factors orchestrate the expression, activation or inhibition of individual MMPs in the right cell type and pericellular location, and at the right time, to allow for the extensive uterine restructuring while maintaining tissue integrity. Recent studies indicate that the MMP system not only participates in ECM-degradation, but also modulates cell behavior in numerous ways by altering matrix-derived factors, cell surface molecules and paracrine signals. With the careful examination of the reproductive phenotype of MMP and TIMP transgenic and null mice, the roles of these factors within the reproductive tract can be examined. The further gain of knowledge on the mechanisms of the spatiotemporal regulation of the expression of MMPs and TIMPs and the function of individual MMPs within the uterus may lead to a better understanding of the biology and pathology of the uterus as well as new strategies for treating uterine diseases.

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