Complex Mechanisms of Matrix Metalloproteinases Involvement in Endometrial Physiology and Pathology—An Update

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Abstract

Matrix metalloproteinases (MMPs) belong to a multigenic family of proteolytic enzymes with great structural variability which provide a complex intervention in pathophysiological conditions. Our review is focused on both MMPs key role in physiological reproductive events, such as embryo implantation, uterine involution, normal endometrial cycle, and on their role in the main endometrial pathologies. MMPs activity is closely regulated by tissue inhibitors of MMPs (TIMPs). MMP: TIMP imbalance has been incriminated in various pathological conditions, including endometrial cancer and endometriosis. Accumulated data support the involvement of a large spectrum of MMPs and TIMPs in endometrial carcinogenesis. Strong MMP-2 and weak TIMP-2 tissue immunoexpressions have a powerful prognosis value, while MMP-9 high expression suggests its important involvement in endometrial tumor invasiveness. Endometriosis development implies an accumulation of events showing partial overlap with endometrial carcinogenesis and invasion, requiring MMPs involvement. Therefore, increased levels of several MMPs have been detected in peritoneal fluid and/or endometrial tissue of patients diagnosed with endometriosis. Endometriotic mesenchymal stem cells (MSCs) may be involved in the pathogenesis of endometriosis due to their upregulated expression for markers of migration and angiogenesis, such as MMP-2, MMP-3, MMP-9, and VEGF. The hypothesis of therapeutic benefits of synthetic MMPs inhibitors, added to the progesterone or progestins action, has been based on the complex MMPs involvement in

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endometrial pathology. Future research is necessary to elucidate the complex interactions between molecules involved in proliferation, angiogenesis and apoptosis, opening new perspectives in the early diagnosis and treatment of endometrial neoplasia and endometriosis.

Keywords
Matrix metalloproteinases (MMPs) · Tissue inhibitors of MMPs (TIMPs) · Endometrial cycle · Endometrial carcinoma · Endometriosis

1 Introduction

Matrix degrading metalloenzymes, matrix metalloproteinases, matrixins, or metalloproteinases (MMPs) [1] belong to a multigenic family of proteolytic, zinc-dependent enzymes, functioning at neutral pH [2]. MMPs have latent forms, proenzymes, inactive zymogens, or pro-MMPs that require proteolytic activation [3].

Matrix metalloproteinases (MMPs) family consists of endopeptidases that share homologous protein sequences, with conserved domain structures and specific domains related to substrate specificity and recognition of other proteins [2]. MMPs structure consists of a *signal peptide* which directs their secretion from the cell, a *propeptide* which is essential for pro-MMP latent form preservation, a *catalytic domain* which contains the highly conserved Zn^{2+}-binding site, a proline-rich *hinge region* which links the catalytic domain to the C-terminal *hemopexin-like* domain which determines MMPs' substrate specificity and mediates the interactions with endogenous inhibitors [4]. Their possibility of great structural variability provides a complex MMPs intervention in pathophysiological conditions.

MMPs, together with cysteine proteinases, aspartic proteinases, and serine proteinases are mainly involved in extracellular matrix (ECM) and basement membranes (BMs) degradation [5].

MMPs possess a key role in embryogenesis and in physiological activities, such as proliferation, cell motility, remodeling, wound healing, angiogenesis, and reproductive events, such as ovulation, embryo implantation, uterine, breast, and prostate involution, menstruation, and endometrial proliferation [6–8].

MMPs have distinct substrate spectra [3] and their activity is closely regulated by their endogenous inhibitors, TIMPs.

MMP: TIMP imbalance has been incriminated in various pathologic conditions, as tumor invasion, rheumatoid arthritis inflammation, atherosclerosis, aneurysms, nephritis, tissue ulcers, fibrosis, and endometriosis [1, 9].

2 Types of MMPs

Currently, 23 different human MMPs and their codifying genes have been identified [1, 2, 10]. Based on substrate specificity, sequence similarity, and domain organization, vertebrate MMPs are classified into seven groups: collagenases (collagenase-1 or MMP-1, collagenase-2 or MMP-8, and collagenase-3 or MMP-13), gelatinases (gelatinase A or MMP-2 and gelatinase B or MMP-9), stromelysines (stromelysin-1 or MMP-3 and stromelysin-2 or MMP-10), stromelysin-like MMPs (stromelysin-3 or MMP-11 and metalloelastase or MMP-12), matrilysins (matrylisin or MMP-7 and matrylisin-2 or MMP-26), membrane type MMPs (MT1-MMP or MMP-14, MT2-MMP or MMP-15, MT3-MMP or MMP-16, MT4-MMP or MMP-17, MT5-MMP or MMP-24, and MT6-MMP or MMP-25), and other MMPs (MMP-19, MMP-20 or enamelysin, MMP-22, MMP-23, MMP-27, and MMP-28 or epilysin) [1].

3 Mechanisms of MMPs Regulation

MMPs need activation for their in vivo action, thus zymogen forms codified by MMPs genes have to be transformed into active proteinases [11]. MMPs regulation may take place during transcription or secretion by activation or by inhibition of the activated forms.

MMPs can be activated in vitro by proteases, including plasmin, MT-MMPs, and MMP-3, or by treatment with organomercurial compounds. While these enzymes are weakly expressed in normal adult tissues, they become upregulated during normal or pathological remodeling process [7].

MMPs regulation during secretion may be achieved by MMP-7 storage in the secretory component of the exocrine glands and by MMP-8 and MMP-9 storage in secretory granules of eosinophils and neutrophils and their release during active secretion. In vivo expression of MMPs may be induced by exogenous signals, e.g., growth factors, cytokines, which modulate MMP mRNA half-life [12], or altered cell–cell or cell–matrix interactions.

The inhibition of MMPs is mainly attributed to a stoichiometric binding of TIMPs which are able to regulate both the zymogens and the active forms [3].

4 MMPs Physiological Functions

MMPs are involved in embryogenesis and tissue remodeling, due to their capacity of regulation of ECM proteins and variable soluble factors. MMP-1 contributes to keratinocyte migration [7], while MMP-2 and MMP-3 are important in mammary gland branching during morphogenesis [13]. MMP-2 and MMP-9 are involved in lipogenesis [14] and angiogenesis [15], in association with MMP-13 and

MT1-MMP, as demonstrated by inhibition of this process with endostatin (endogenous angiogenic inhibitor) [7].

MMP-9 is involved in endochondral bone formation, being associated to MT1-MMP which regulates skeletal muscle and connective tissue growth [7].

As the reproductive system needs remodelation, MMPs are expected to be involved in the related processes. During postpartum uterus involution, gelatinase A, collagenase-2 and -3, stromelysins, and matrilysin are upregulated [7]. Experimental studies also demonstrate the significant involvement of gelatinase A, stromelysins, and matrilysin in estrous cycle [7]. Moreover, a MMP-9 role has been demonstrated in the implantation process [7].

Experiments in MMPs deficient mutant mice support a functional redundancy between deficient enzymes and the components of the plasminogen system or between different MMPs [7, 16].

5 Endogenous MMP Inhibitors

The extracellular activity of MMPs is strictly controlled by their specific inhibitors and by nonspecific inhibitors, e.g., α2-macroglobulin [12].

5.1 TIMPs

Natural inhibitors of MMPs or TIMPs [17] have N- and C-terminal domains, each containing three conserved disulfide bonds [18]. The N-terminal domain acts as a MMP inhibitor [7].

TIMPs gene family comprises only four members: TIMP-1, TIMP-2, TIMP-3, and TIMP-4.

TIMP-1, expressed by fibroblasts nuclei [19], acts as a broad spectrum MMP inhibitor, except for MT1-MMP and MMP-2 [12]. TIMP-1 has the ability to bind to the hemopexin domain of latent MMP-9 and forms a TIMP-1-pro-MMP-9 complex which blocks the enzyme [20]. Moreover, TIMP-1 binds and consequently inactivates MMP-1, MMP-2, and MMP-3. Several growth factors, cytokines, and phorbol esters may act as TIMP-1 activators by enhancing its expression in cell cultures [20].

TIMP-2 has a constitutive expression, exhibiting the capacity to bind to the hemopexin domain of pro-MMP-2 and to inhibit most MMPs, except MMP-9 [7]. TIMP-2 role is dual, depending on the TIMP-2-MT1-MMP-pro-MMP-2 complex formation. A threshold level of TIMP-2 is required for the trimolecular complex construction, which leaves sufficient MT1-MMP uninhibited to cleave pro-MMP-2. Higher TIMP-2 concentrations are preventing MMP-2 processing by the inhibitory action on free MT1-MMP [21].

TIMP-3 expression is noticed during normal cell cycle progression or as a response to mitogenic stimulation. TIMP-3 may be inhibited by TNF-α in fibroblasts [12]. TIMP-3 may inhibit several MMPs, such as: MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, and MT1-MMP. TIMP-3 has a unique characteristic among the TIMP family as it is bound to the ECM sulfated glycosaminoglycans [20, 21] rather than as a free soluble protein. TIMP-3 has a broader inhibitory spectrum, including members of ADAM (a disintegrin and metalloproteinase domain) and ADAM-TS (aggrecanases—ADAMs with thrombospondin domains) families, as proteases involved in bioactivity of cytokines and growth factors regulation [7, 17]. TIMP-3 shows a better inhibitory effect for ADAM-17 and aggrecanases than that for MMPs, as has been proved by kinetic studies [22]. Supplementary, TIMP-3 shows a proapoptotic activity, either by TNF-α cell receptor 1 stabilization, either by Fas or by TNF-related apoptosis, as has been demonstrated in tumoral cells [22].

TIMP-4 is the most recently identified member of the TIMP family, being expressed in heart but also at the sites of tissue injury, i.e., vascular lesions and dermal wounds. TIMP-4 is able to inhibit MMP-2, MMP-7, MMP-9, and MT1-MMP [17].

Although TIMPs are considered as endogenous inhibitors, wild-type TIMPs could have drawbacks because of multiple MMPs inhibition and TIMP-3 supplementary inhibition of ADAMs and ADAM-TS [12]. As a consequence, a successful therapeutic application would be targeting specific proteinases by development of engineered TIMPs exhibiting altered specificity [12].

In addition to MMPs inhibiting activities, TIMPs have other biological functions. Moreover, TIMP-1 and TIMP-2 exhibit cell growth-promoting activities, antiapoptotic activity, and erythroid-potentiating activity [12]. TIMP-2 is able to inhibit FGF-b induced endothelial growth, in vivo angiogenesis, and in vitro endothelial cell proliferation by means of a MMP-independent mechanism [20, 23]. Likewise, TIMP-3 can also prevent VEGF to VEGF receptor-2 binding, thereby inhibiting downstream signaling and angiogenesis [24].

5.2 Miscellaneous Inhibitors of MMPs

MMPs may be inhibited by several other proteins, as tissue factor pathway inhibitor-2 (a serine protease inhibitor), C-terminal fragment of the procollagen C-terminal proteinase enhancer protein, membrane-bound β-amyloid precursor protein, RECK (reversion-inducing cysteine-rich protein with kazal motifs), a GPI (Glycosylphosphatidylinositol)-anchored glycoprotein [25, 26], chlorotoxin, a scorpion toxin [27], and plasma-macroglobulins (general endopeptidase inhibitors by enzymes trapping within the macroglobulin after proteolysis of the bait region of the inhibitor), as the most active MMP-1 inhibitors [28].

6 MMPs Involvement in Endometrial Physiology

Female genital tract can be interpreted as a highly dynamic environment, coordinated by ovarian steroid hormones, in combination with cytokines and local growth factors. Uterine mucosa is made up of a highly vascularized tissue adapted to create conditions for pregnancy, fetal support, and allows a complex invasive mechanism during implantation and placentation [6].

Although initially only a proteolytic function has been attributed to MMPs, relatively recent studies have shown their interference in the processes of differentiation, proliferation, angiogenesis, and apoptosis, due to their ability to catalyze the hydrolysis of cytokines precursors, growth factors, hormone receptors, serum amyloid A, IGFBP (Insulin-like growth factor-binding protein), proteinase inhibitors, and IL-1β [29]. Thus, complex activities of MMPs and TIMPs are responsible for uterine reshufflings.

Some MMPs, such as MMP-9, are involved in implantation [7], while other MMPs, such as matrilysin, stromelysin, gelatinase A, collagenase-2 and collagenase-3, are responsible for postpartum uterine involution [7].

6.1 MMPs and TIMPs Dynamic Expression in Endometrial Physiology

A dynamic MMPs and TIMPs expression, under hormonal regulation, has been associated to the endometrium physiology, both in common endometrial cycle physiology and in pregnancy, their abnormal levels being responsible of several pregnancy-related conditions.

6.1.1 MMPs Expression in Endometrial Cycle

MMPs expression during various phases of endometrial cycle registers quantifiable variations [6, 30] (Table 1).

Moreover, MMPs may be epithelial-specific, such as MMP-7, stromal or vascular- specific, such as MMP-1, MMP-2, MMP-3, MMP-9, MMP-10, MMP-11, MMP-23, and MT1-MMP, or specifically expressed by resident immune cells (neutrophils, eosinophils, and macrophages), such as MMP-8, MMP-9, and MT1-MMP [30].

Supplementary, a possible compensatory role may be expressed by some MMPs. Thus, although MMP-7 has a key role in achieving endometrial cyclic sequences, as a response to estrogen stimulation, experimental models show that its absence does not result in cycle or fertility abnormalities, due to compensatory role of MMP-3 and MMP-10 [30].

Regarding MMPs expression along the endometrial cycle (Table 1), an increased stromal expression of MMP-1, MMP-2, MMP-3, MMP-10, MMP-11, and MT1-MMP, along with an enhanced MMP-9 expression in developing

Table 1 MMPs expression in endometrial cycle and pregnancy-related conditions

MMP	Proliferative phase	Secretory phase		Menstrual phase	Pregnancy		Childbirth		Uterine involution
		Early	Advanced		Decidua	Trophoblast	Decidua	Trophoblast	
MMP-1	+	–	–	+	+	+	+	–	?
MMP-2	++ (mainly S)	++ (mainly S, V)	++ (S, V)	++ (S, V)	++/?	+/++	?	+	?
MMP-3	+/−	–	–	++ (basal E, S, V)	+	+	+	–	?
MMP-7	++ (only E)	–	+ (only E)	+++ (only E)	–	++/+	–	++	+++
MMP-9	−/+	−/+/++	−/+/++	++ (S and V)	+	++	++	++	?
MMP-10	–	–	+	++	?	−/?	?	?	?
MMP-11	++	–	+	+++	+/−	+	?	?	?
MMP-13	?	?	?	?	?	++/?	?	?	++
MMP-14	?	?	?	?	+/?	+/?	?	?	?
MT1-MMP	?	?	?	?	+/?	++/?	?	?	?
MT2-MMP	?	?	?	?	−/?	+/?	?	?	?

− Absent; + focal; ++ moderate; +++ intense; ? unknown; E (epithelium); S (stroma); V (vessels)

arterioles, and strong MMP-7 epithelial expression are characteristic for the proliferative phase [30].

The secretory phase is characterized by increased stromal and vascular MMP-2 expression and, therefore, this pattern of expression is suggestive for MMP-2 involvement in angiogenesis [30]. The advanced secretory phase is supplemented by epithelial MMP-7 expression, along with MMP-10 and MMP-11 (Table 1).

During menstruation, there is an increased epithelial MMP-7 expression, along with MMP-11 and MMP-2 stromal and vascular expression (Table 1). Moreover, MMP-3 expression in basal epithelium, stroma, and vessels, associated with stromal and vessels MMP-9, MMP-10, MMP-1, and MT1-MMP expression have been demonstrated [30].

In pregnancy, there is an increased expression of MMP-2, MMP-7, MMP-9, MMP-13, MT1-MMP, MMP-11, and MT2-MMP (Table 1).

Enhanced MMP-9 levels, along with MMP-7, MMP-1, MMP-2, and MMP-3 expression, during childbirth has been reported, while MMP-9 exhibits variable involvement in endometrial cycle, without consensus between different reports (Table 1).

6.1.2 Mechanisms of MMP Regulation During Endometrial Cycle

VEGF is intimately associated to MMPs expression in endometrium. A strong interaction between VEGF stromal cell expression, induced by estradiol, in possible association with hypoxia, and MMP-2 increased expression has been demonstrated [31].

Conversely, progesterone increases the expression of stromal cells Angiopoietin-1 (Ang-1), stabilizing vessels and blocking further unlimited angiogenesis [32].

Progesterone also induces tissue factor expression in decidualized stromal cells [31]. Tissue factor has a receptor for coagulation factor VII and its active form, and therefore has the ability to initiate the clotting cascade which promotes hemostasis.

Moreover, EGFR expression is induced by progesterone and ligand binding to EGFR is required for tissue factor expression by stromal cells [31].

Progesterone also induces stromal cells plasminogen activator inhibitor-1 (PAI-1) expression [32] which displays anti-fibrinolytic properties and restrains trophoblast invasion mediated by urokinase-type plasminogen activator [32].

Consequently, the progesterone-dominated midluteal phase has maximal hemostatic, anti-fibrinolytic, and antiproteolytic properties.

Progesterone withdrawal in the perimenstrual period, or progestin withdrawal and/or in vitro treatment with the antiprogestin RU486 result in tissue factor decrease and PAI-1 expression [32], creating a prohemorrhagic environment around endometrial blood vessels and promoting menstrual bleeding, a mechanism incriminated in the failure of conception.

The hemostatic factor expression represents a parallel progestational inhibition of stromal MMP-1, MMP-3, and MMP-9 expression in luteal phase, while progesterone withdrawal or treatment with RU486 enhances MMPs expression in stromal cells [32]. In contrast, neither progestins nor progestational withdrawal is

reflected in MMP-2 or TIMPs expression [32]. Progesterone withdrawal is also associated with upregulation of the neutrophil and macrophage chemoattractants, such as interleukin-8 (CXCL8), and macrophage chemoattractant protein-1 (CCL2) [32].

Thus, luteal phase and gestational endometrium are associated with reduced MMPs activity, resulting in stromal and underlying vascular stabilization in order to impede endometrial hemorrhage during pregnancy.

Conversely, perimenstrual progesterone withdrawal in non-fertile cycles is associated with increased MMPs expression as well as chemokines expression which stimulate leukocyte infiltration, resulting in a proteolytic environment, promoting menstrual bleeding and tissue sloughing [32].

6.1.3 TIMPs Expression in Endometrial Cycle

Co-expression of MMPs and TIMPs demonstrates the importance of their compensatory intervention in the endometrial turnover. The expression of various TIMPs during endometrial cycle phases shows quantifiable variations, with opposed levels of expression registered in pregnancy (MMPs are reduced and TIMPs are amplified) [6, 30] (Table 2).

TIMP-1 and TIMP-3 are expressed in both epithelial and stromal cells and exhibit a strong expression particularly in the luminal compartment of the endometrial epithelium [6], with marked TIMP-1 stromal expression variations, during endometrial cycle.

TIMP-1 and TIMP-2 expressions in endometrial small arterioles and capillaries, in the secretory phase, suggest their intervention in the vascular network stabilization during the endometrial cycle phases and pregnancy [6]. Moreover, TIMP-1 and TIMP-2 also exhibit an antiangiogenic function due to inhibition of VEGF expression [6].

Although TIMP-4 intervention during the endometrial cycle has not been yet demonstrated, it shows a typical expression in term deciduas [6].

6.1.4 MMPs and TIMPs Expression in Menstrual Phase and Regulation Mechanisms

While the endometrial cycle is associated with maximum hemostasis and vascular stability in the midluteal phase, being controlled by progestational induction of tissue factor and PAI-1 expression, inhibition of MMP activity, along with angiogenesis regulation [32], the menstruation is considered a controlled hemorrhage and tissue sloughing in non-fertile cycles. The mechanism involved in menstrual phase is initiated by progesterone withdrawal which diminishes the hemostatic effect and enhances MMP activity, resulting in controlled hemorrhage [32].

Although menstruation has been traditionally attributed to ischemic necrosis, as a consequence of vasospasm of the spiral arterioles, an increasing importance has recently been attributed to proteolysis performed by locally synthesized MMPs, as products of various types of endometrial cells [6, 30]. The loss of progesterone support during menstruation causes focal lysis of reticular fibers and collagen type I

Table 2 TIMPs expression in endometrial cycle and pregnancy-related conditions

TIMP	Proliferative phase	Early secretory phase	Advanced secretory phase	Menstrual phase	Pregnancy		Childbirth		Uterine involution
					Decidua	Trophoblast	Decidua	Trophoblast	
TIMP-1	+ (mainly S, V)	+ (mainly S, V)	++	+++	+/++	++	++	+	++
TIMP-2	+	+	+	++	+/++	−/++	++	+	++
TIMP-3	+/−	+	++	+	++	++	++	+	++
TIMP-4	?	?	?	?	+/++	+/++	++	+	?

− Absent; + focal; ++moderate; +++ intense; ? unknown; S (stroma); V (vessels)

and II, along with an increase in the local expression of different types of MMPs. Thus, MMP-1, MMP-2, and MMP-3 are increased, as a result of autoactivation. Supplementary, pro-MMP-9 is activated by MMP-3, in endothelium. MMP-1, MMP-10, and MMP-11 show an increased expression in stromal fibroblasts, while MMP-7 is enhanced in epithelium, preceding MMPs stromal expression.

MMP-2, MMP-3, and MMP-9 expressions in functional endometrial vessels suggest their involvement in the mechanism of vascular wall damage during menses [30].

A greater hypoxia in the superior regions of the endometrium during menstruation due to spiral arterioles constriction, followed by endometrial tissue shedding are hypothetically induced by an increased expression of MMP-1, MMP-2, and MMP-3. TNF-α seems to play a key role in the induction of apoptosis in the endometrial epithelium and a possible similar action is attributed to IL-1α and IL-1β. Moreover, "LEFTY (left-right determination factor)-A" protein is transitory expressed prior to menstruation [33] and shows an abnormal expression in patients with dysfunctional bleeding.

MT1-MMP and MT2-MMP have been also identified in stromal endometrial cells during the menstrual phase, their mechanism of action being incompletely deciphered.

TIMP-1 reaches its maximum level during menstruation and its vascular co-expression with TIMP-2 in the secretory endometrium and in areas of demarcation between necrotic and viable areas during menses demonstrates their role in vascular stability and in bleeding limitation during menses [30].

Although menstruation is considered as a process of tissue destruction, endometrial repair begins during the first 24 h after initiation of tissue fragmentation, as a mechanism of tissue damage minimization. The transition to the growth phase associated with estrogen secretion is achieved through a complex mechanism, in which VEGF, EGF, IGF, and FGF-b are also involved [33].

VEGF is co-localized with MMPs in newly formed endometrial capillaries, stimulating MMPs expression in vascular smooth muscle. The proinflammatory cytokines produced by epithelial and stromal cells regulate MMPs expression, being supplemented by an influx of lymphomyeloid cells prior to menstruation, co-mediating MMPs activation [33].

6.1.5 MMPs and TIMPs Expression in Pregnancy and Their Involvement in Related Pathology

Receptive endometrium, regulated by ovarian steroids exhibits complex events during pregnancy, such as: blastocyst attachment, implantation, and subsequent development of the placenta. During the last years, researches on MMPs inhibitors were focused mainly on endometrial tissue during implantation and pregnancy. The invasion of trophoblastic cells into the maternal endometrium requires a precisely regulated secretion of specific proteolytic enzymes for the degradation of the endometrial BM and ECM and plays a substantial role during human embryo implantation and placentation. This process is facilitated by MMPs activity regulated by TIMPs.

Thus, the expression of various MMPs during pregnancy registers variable expressions, most of them being quantifiable [6] (Table 1).

The expression of TIMPs in the endometrium also registers quantifiable variations during pregnancy [6] (Table 2). TIMPs decidual levels are increased, mainly that of TIMP-3 [8], while trophoblast levels of both TIMP-3 and TIMP-1 are constantly increased (Table 2). Supplementary, high serum levels of TIMP-1 and TIMP-2 have been detected in ongoing pregnancies [34].

All TIMPs levels are more increased in decidua at childbirth compared to that of trophoblast (Table 2).

Moreover, TIMP-1, TIMP-2, and TIMP-3 are also involved in uterine involution after childbirth (Table 2).

The proinvasive effects of preimplantation factor (PIF), an embryo derived peptide secreted by viable mammalian embryos, are associated with activation of the gelatinase activity of MMP-9 and inhibition of TIMP-1 expression [35].

MMP-9 deficiency results in placental abnormalities in experimental studies, similar to preeclampsia, as MMP-9 activity plays a major role in trophoblast invasion [35]. Thus, MMP: TIMP balance is a component of the mechanism required for PIF induction of trophoblast invasion.

PIF modulates trophoblast invasion via multiple signaling pathways, such as PI3K, MAPK, and JAK-STAT transduction pathways, as have been also identified for other proinvasive factors [35].

PIF upregulates variable proinflammatory cytokines, like IL-6 [35], in human endometrium. Consequently, PIF could promote the extravillous trophoblast invasive activity by modulation of IL-6 placental secretion [35].

It is now considered that MMP: TIMP imbalance and the expression pattern of integrins associated to PIF anomalies could be responsible of pregnancy pathology [35].

The family of IL-1 proinflammatory cytokines modulates MMP expression, facilitating cytotrophoblast endometrial invasion. IL-1α production at the maternal–fetal interface is critical for successful implantation, the exogenous antagonists of IL-1 receptor being able to prevent pregnancy. Thus, IL-1 receptor polymorphism may be associated to a high risk of recurrent miscarriage [33].

Progesterone inhibits MMP-3 and MMP-7 and gradually decreases MMP-9 expression. Thus, progesterone antagonists, such as onapristone, produce an increased MMP-9 expression [9].

Production of IL-1 regulates the expression of leptin (a circulating hormone that regulates food intake) secreted by cytotrophoblast. Leptin exerts a stimulatory effect on MMP-2 and MMP-9 released at the laterobasal domains of the syncytiotrophoblast and is responsible for trophoblast conversion toward an invasive phenotype [33].

The parturition requires inhibition of progesterone, performed also by mifepristone, resulting in cervical infiltration with immune cells and increased expression of MMP-1, MMP-3, MMP-8, MMP-9, TIMP-1, IL-1, IL-8, and TNF-α [6].

Thus, MMP-9 and TIMP-2 or MMP-2-TIMP-2 complex levels are markedly elevated in missed abortions or in mifepristone–misoprostol (steroidian antiprogestative and synthetic prostaglandin E1 combination) medical abortion [36].

Recent researches have shown that aberrant TNF-α increases the expression of MMP-1, MMP-3, and MMP-9 by decidual cells, resulting in abnormal integrin-mediated extravillous trophoblast invasion of the decidua and later onset of preeclampsia [37]. This could be reversed by IFN-γ with restoration of normal stepwise extravillous trophoblast invasion of the deciduas [37]. These findings suggest a mechanism by which IFN-γ derived from decidual NK cells counteracts the shallow trophoblast invasion, a mechanism strongly implicated in impaired decidual vascular remodeling, leading to the later development of preeclampsia [37].

6.2 MMPs and TIMPs Complex Mechanism of Endometrium Regulation

MMPs and TIMPs are regulated by local variations of cytokines and by modulation of gene transcription, by the means of steroid receptors related mechanisms.

As a consequence of exposure to high levels of estrogen, formation of ligand–ER complex results in expression of transcription factors *fos* and *jun* that bind activation element AP-1. Since the most MMPs gene promoters contain AP-1 elements, the mechanism of estrogenic MMPs regulation may be initiated [33].

AP-1 activation also occurs in transactivation of MMPs promoters by phorbol esters and by proinflammatory cytokines, such as IL-1 and TNF-α [33].

Most MMPs promoters contain an A3 amplifier of the polyoma virus, PEA-3, which is connected to the Ets (E-twenty six) transcription factors family. PEA-3 is involved in MMPs regulation by growth factors and cytokines, acting synergistically with AP-1 proteins in activation of MMPs genes transcription [33].

Progesterone stimulates the mechanisms which limit the expression of MMPs, as a consequence of progesterone inhibition of estrogen-induced *c-fos* expression. Thus, *c-jun* and *c-fos* are inhibited during pregnancy. During stromal decidualization, retinoic acid is synthesized, resulting in MMPs inhibitory effects, by sequestration of *fos* and *jun* proteins by retinoic acid receptor binding. Progesterone inhibits the expression of MMP-1 by inhibition of IL-1α release and suppression of this cytokine endometrial action [33].

Androgens inhibit MMPs expression by interacting with Ets proteins. As MMP-2 has no AP-1 site or PEA-3, it is constitutively expressed in the endometrium, being activated by its association with MT-MMPs and TIMP-2 [38].

TGF-β mediates the suppression of MMP-7 in the endometrial epithelium, as a response to progesterone but concomitantly induces the expression of TIMP-1 and TIMP-3 in stromal cells [33].

Among the cytokines that regulate MMPs endometrial expression, there is also a cytokine, LEFTY-A, originally designated as endometrial bleeding associated

factor (EBAF). LEFTY-A is a TGF-β family member secreted as a precursor of 42 kDa. The active form induces MAPK activity, inhibits TGF-β signaling, and induces the expression of pro-MMP-3, pro-MMP-7, and MMP-9 [33].

7 MMPs Involvement in Abnormal Endometrial Bleeding

Currently, the abnormal endometrial bleeding is considered as a disordered process of physiological menstrual cycle, due to impaired endometrial hemostasis and unrestrained aberrant angiogenesis. This abnormal bleeding can be related to bleeding diatheses, long-term progestin-only contraception, uterine leiomyomas, and endometrial polyps [32].

Bleeding diatheses are characterized by menorrhagia onset at menarche and the most common defect associated with menorrhagia is Von Willebrand's disease [32]. By combined hormonal contraceptives treatment applied in this disease, stromal cells are induced to secrete tissue factor and PAI-1 with consequent hemostatic effect [32].

Long-term progestin-only contraceptives induce reductions in endometrial blood flow resulting in hypoxia–reperfusion injury and free radical production. These induce aberrant angiogenesis by VEGF and Ang-2 increased expression and Ang-1 suppression as well as direct damage of blood vessels [32].

Endometrial biopsies demonstrate the presence of enlarged, thin walled blood vessels at bleeding sites despite increased tissue factor expression [32] and a general dramatic increase in immature and partially mature vessel number, area, and density [32], and perivascular extracellular matrix degeneration due to excess MMP-2 activity, culminating in bleeding and collapsed stroma [32].

The most common presentation in leiomyomas is menorrhagia, due to venule ectasia. This is the result of tumor and/or hypoxia-derived angiogenic factors, such as VEGF, FGF-b, PDGF, HBEGF, TGF-β, parathyroid hormone related protein, and prolactin [32]. There is also spiral artery vasodilation due to increased estrogen receptor expression [32]. The most common presentation in endometrial polyps is metrorrhagia, due to increased cyclo-oxygenases and MMPs production [32] and due to enhanced microvascular density by aberrant angiogenesis [32].

In contrast to physiological regulated cycle, anovulatory bleeding is associated with both impaired hemostasis due to an absence of progestational induction of tissue factor and PAI-1 and increased MMP and angiogenesis due to unrestrained estrogenic effects. On the other hand, long-term progestin-only contraceptives-associated bleeding reflects sustained hemostasis with persistently elevated tissue factor expression and vessel damage due to impaired endometrial blood flow with hypoxia-induced unrestrained angiogenesis [32]. Similar defects appear to account

for abnormal bleeding with myomas and endometrial polyps, though in the former condition macrovascular changes lead to menorrhagia while microvascular changes promote metrorrhagia in the latter [32].

8 MMPs Involvement in Endometrial Hyperplasia and Carcinoma

Endometrial carcinoma has the highest incidence among the malignancies of the feminine genital tract [39] and morphologic expressions of precursor lesions (atypical hyperplasia) and carcinoma are correlated to progressive accumulation of genes mutations. The imbalance between MMPs and TIMPs is incriminated in endometrial carcinogenesis, added to hormonal influences, adhesion molecules alteration, and apoptosis deregulation. Endometrial multistep carcinogenesis shows a partial correlation between molecular markers expression and progression of precursors or aggressiveness of carcinomas [39]. Accumulated data support the involvement of a large spectrum of MMPs and TIMPs in endometrial carcinogenesis, such as MMP-1, MMP-2, MMP-3, MMP-9, MT1-MMP, although MMPs real involvement in endometrial pathology is difficult to evaluate considering stromal remodeling during normal menstrual turnover [33, 40].

The progesterone decreased expression seems to be both responsible for an increased production of pro-MMPs [9, 41] and for MMPs activation [9]. Moreover, MMPs genetic polymorphism is considered as a risk factor for endometrial cancer susceptibility [42].

The effects of MMPs and their inhibitors depend on their ratio in local tumor microenvironment, thus individual markers analysis may not reveal the whole picture of the disease. The imbalance in favor of the proteinases is supported by the molar ratio of MMPs to TIMPs which is higher in carcinoma compared to non-neoplastic control tissues [43].

Numerous studies have demonstrated a high MMP-2, MMP-9, and TIMP-2 expression in both endometrial and cervical carcinoma [15, 44–50].

MMP-2 seems to be the main MMP involved in endometrial carcinomas aggressiveness [50], being activated by MT1-MMP and TIMP-2. MMP-2 stimulates cell proliferation and migration of epithelial cells, via fibronectin degradation. Due to pro-MMP-2 stimulation by MT1-MMP, MMP-2-MT1-MMP co-expression in the tumor invasive front has been reported [49]. MMP-2 shows a strong tumor expression, in correlation with histological grade and type, patients' age, and depth of myometrial invasion [39].

High MMP-2 expression is also correlated to a poor survival in endometrial carcinoma [51].

According to the literature reports, MMP-9 expression shows an increasing expression in endometrial hyperplasia and furthermore in carcinoma, in both stromal and epithelial components [49]. MMP-9 effects are attributed to its high affinity to collagen substrates, the capacity to generate fragments of angiostatin

type, the participation in the protection of tumor cells, and the ability to suppress the proliferation of T cells [52]. MMP-9 expression in smooth myocytes may be correlated to myometrial invasion mechanisms of endometrial neoplasia [49].

Although TIMP-2 expression is increased in endometrial carcinoma, it shows a weaker expression in grade 3 endometrial carcinoma when compared to grade 1 or 2, exhibiting an analogous pattern to that previously reported in breast, colorectal, hepatocellular, and gallbladder carcinomas [44].

Due to their synthesis and translocation from stromal to epithelial cells, MMP-2 and MMP-9 mRNAs have been located in endometrial stroma, including in endothelial cells, macrophages and fibroblasts, mainly in areas within or surrounding tumor aggregates, and show variable expression in epithelial tumor cells [39].

Increased co-expression of MMP-2 and MMP-9 is also correlated to the stage transition and the depth of myometrial invasion [53].

The significance of MMP-2 and TIMP-2 expression is different in terms of prognosis significance. Thus, TIMP-2 overexpression is associated to a favorable prognosis and overall survival in endometrial carcinoma patients but it is still debatable which of the two, MMP-2 or TIMP-2, has superior effect on the prognosis [54]. Moreover, MMP-2 seems to be superior to TIMP-2 in determining the prognosis in endometrial cancer if used separately [55]. Associated strong MMP-2 and weak TIMP-2 expressions have a powerful value of poor prognosis when compared to MMP-2 or TIMP-2 alone.

MMP-2 and TIMP-2 expression patterns are different in the two main types of endometrial cancer [55]. Type II endometrial tumors which consist mainly of serous and clear cell carcinomas show a positive immunoreaction for MMP-2 and negative for TIMP-2 and carry a higher mortality than type I tumors (endometrioid adenocarcinomas) in which MMP-2 immunostaining negativity is associated to a favorable prognosis [56]. Negative MMP-2 and TIMP-2 immunostainings in adenoacanthomas render a prognosis which resembles the prognosis of endometrioid adenocarcinoma [55]. In contrast, histologic types correlated with MMP-2 positivity and TIMP-2 negativity are associated to a poor prognosis.

MMP-9 and Bcl-2 are overexpressed, unlike steroid receptors and CD44-v6 variant, in carcinoma compared to atypical hyperplasia [57]. KAI1 (a metastasis suppressor protein) as well as ER and PR might modulate MMP-2 and MMP-9 expressions in endometrial cancer. The overlapping expression of these biomarkers suggests their possible cooperation, even at early stages of endometrial cancer growth, modulating the speed of tumor cell dissemination [58].

MMP-9 immunoexpression in the areas containing tumor-infiltrating CD3 lymphocytes suggests a possible role of these lymphocytes in mediating the endometrial cancer microenvironment [59].

MMP-9 overexpression is associated to LCN-2 (lipocalin-2) increased expression in several cancers, including high-grade endometrial cancer [60]. Accordingly, the immunopositivity of LCN-2 and MMP-9 are correlated with shorter survival in patients with high-grade endometrial carcinoma, LCN-2 overexpression being

associated with shorter overall and disease-free survival. Moreover, LCN2 immunopositivity has been associated with expression of the angiogenesis marker, VEGF-A, but not with several EMT (epithelial–mesenchymal transition)-related markers, like E-cadherin, N-cadherin, P-cadherin, β-catenin, nor with vascular invasion [61]. Furthermore, LCN2 is significantly associated with distant tumor recurrences, as well as with the S100A family of metastasis related genes, thereby this marker being associated with aggressive features and poor prognosis in endometrial cancer [61].

MMPs immunolocation in endometrial carcinoma reveals correlations between MMP-1 and TIMP-1 expressions and histologic stage, depth of infiltration, histologic type, and patients' age [39].

TIMP-1 slightly decreases in expression in hyperplasia and endometrial carcinoma, in both endometrial components, suggesting a MMP-9: TIMP-1 imbalance in endometrial carcinogenesis [49].

MMP-26 (matrilysin-2 or endometase) promotes matrix destruction in estrogen-dependent tumors, like type I endometrial carcinomas, contributing to malignant progression, by inactivating α1-antitrypsin serpin. Moreover, the transactivation of MMP-26 promoter activity and the enhancing of the endogenous MMP-26 expression by estrogen may represent one of the mechanisms involved in endometrial carcinogenesis [62].

A low constitutive expression of the ECM metalloproteinase inducer (Emmprin) or CD147, a member of the immunoglobulin superfamily [63], has been identified in most cells, being involved in different physiologic processes. High Emmprin expression is observed during remodeling processes associated to embryonic development, wound healing, inflammatory processes [63], and in malignancies. Emmprin has been identified as a modulator of tumor–stromal interaction and of a wide spectrum of molecular events, such as acquisition of anchorage-independent growth capacities, invasive abilities, and tumor angiogenesis [64]. Consequently, Emmprin overexpression in human cancers is correlated with aggressive behavior and poor prognosis. Emmprin modality of action implies stimulation of peritumoral fibroblasts or even tumor cells to produce large amounts of MMPs which facilitate tumor invasion and metastasis processes [65]. Emmprin promotes invasion via activation of urokinase-type plasminogen activator, nuclear factor kappa B (NF-κB), and c-Jun N-terminal kinase (JNK). Moreover, Emmprin stimulates tumor angiogenesis via VEGF [66].

High levels of Emmprin expression have a significant correlation with endometrial carcinoma recurrence [67]. Moreover, increased Emmprin expression is correlated to clinicopathological parameters (histology, depth of myometrial invasion, FIGO stage, cervical involvement, lymphatic vessels involvement, lymph node metastasis, and peritoneal cytology) [68]. Supplementary, strong Emmprin expression is significantly associated with disease-free and overall survival [68]. These findings suggest that low Emmprin expression might have the predictor value of a favorable prognosis in endometrial cancer [68].

EMT occurs during cancer progression, being responsible of tumor cells acquirement of migration, invasion, and metastasizing abilities [69] by induction of E-cadherin transcription repressors, such as Slug, Snail, Smad-interacting protein 1 (SIP1), and Twist [70].

An important role in mesenchymal phenotype promotion is attributed to NF-κB involvement in the transcription of several mesenchymal genes encoding MMP-2, MMP-9, VEGF, and Vimentin [68].

The inhibitory effect of Emmprin on cell proliferation, migration, and invasion in endometrial carcinoma is achieved by NF-κB, TGF-β, EGF, VEGF, MMP-2, and MMP-9 expression and results in increased expression of E-cadherin and reduced levels of Vimentin and Snail, suggesting Emmprin identification as a potential therapeutic target [68].

9 MMPs Involvement in Endometriosis and Updated Therapeutic Approach

Endometriosis development requires accumulation of events represented by the increased capacity of the migrated endometrial tissue to prevent apoptosis, to adhere to and to invade the host tissue, to proliferate, and to stimulate the host angiogenic activity [71]. These may be achieved by alterations in the apoptotic pathways, with upregulation of Bcl-2 and downregulation of Bax, and an increased expression of αvβ3 integrin receptors, as markers of neovascularization and cell adhesion to the extracellular matrix [71]. A recent study using tissue microarrays in endometriotic and normal endometrial tissues has found higher expression of survivin in endometriotic tissues compared to normal endometrium, with a higher glandular expression in non-ovarian than in ovarian endometriotic tissues and lower expression in stromal components [72].

Showing a similar pattern to that previously detected in cancer [73], an increased MMP activity [74] correlated to plasminogen and cathepsin D expression is detected in endometriosis. Moreover, β-catenin mutations result in intercellular adhesion anomalies, associated with E-cadherin mutations, both in endometriosis [75–77] and in female genital malignancies [78]. Consequently, endometriosis management could be oriented to catenin and cadherin signalization, along with MMP selective inhibitors.

In order to perform extracellular matrix degradation and to stimulate the sprouting of new vessels from preexisting capillaries, MMPs, VEGF, IL-8, and COX2 are required [71]. Therefore, increased levels of several MMPs have been detected in peritoneal fluid of patients diagnosed with endometriosis, such as: MMP-1, MMP-2, MMP-7, and MMP-9 or in endometrial tissue, such as MMP-2 and MMP-9 [79]. Moreover, the assessment of MMP-2 and MMP-9 levels in the follicular fluid of infertile patients demonstrates the direct correlation between pelvic endometriosis severity and MMP-2 higher serum levels [80]. Furthermore, MMPs involvement in endometriosis invasiveness is attributed to MMP-2 and

MT1-MMP, in correlation with TIMP-2, feature that underscores its possible key regulatory role [80].

MMPs seem to be activated by IL-1 and TNF-α, in endometriosis. TNF-α exhibits an inhibitory action on TIMP-2 expression in vitro, increasing the MMP-2/MMP-9/TIMP-1: TIMP-2 ratio imbalance [81]. Taking into account the regulatory role of MMP-1, MMP-3, pro-MMP-9, or MMP-9, low TIMP-1 levels may be considered as a result of characteristic MMP-TIMP-1 complexes detected in the endometriotic peritoneal fluid [20]. Supplementary, MMP hemopexin domain is probably recognized by T-like autoantibodies, except MMP-7 [82], as an important pathway of the characteristic immunologic pattern of endometriosis.

A specific MMP, MMP-27, is expressed in a subset of endometrial macrophages (CD45+, CD163+, and CD206+) related to both menstruation and endometriotic lesions, with different patterns of expression in ovarian and peritoneal locations [83].

New data have demonstrated that selected MMPs confer a degree of genetic susceptibility for endometriosis, as haplotypes of angiotensin I-converting enzyme (ACE) and MMP-2 genes are not associated with endometriosis, while those of MMP-1, MMP-3, and MMP-9 genes are related to a high risk for the disease [84]. Moreover, a comparative analysis of the allelic polymorphism of MMPs gene family, which included MMP-3 (rs3025058), MMP-7 (rs11568818), MMP-9 (rs17576, rs2250889), MMP-12 (rs2276109), and MMP-13 (rs2252070), has found important differences in the incidence of particular MMP-3 and MMP-9 allelic combinations in patients with endometriosis [85]. Supplementary, a recent study points out that DNA methylation at the promoter region of MMP-9 gene can enhance the expression of MMP-9 in ectopic endometrial stromal cells [86].

A comparative analysis of MSCs phenotypes, differentiation potential, gene expression for pattern recognition receptors (PRRs) and proinflammatory cytokine release, along with markers of migration and angiogenesis, between eutopic and ectopic locations, showed that TGF-β exhibits significant downregulation, while IL-10 exhibits a significant increase in endometriotic MSCs. Moreover, these cells show an upregulated expression for markers of migration and angiogenesis such as MMP-2, MMP-3, MMP-9, and VEGF, respectively. By exhibiting this distinct immune phenotype, the endometriotic MSCs may be responsible for the reduced immunosuppressive host reaction [87].

Relatively recent data show that Lipoxin A4 (LXA4), a member of the lipid-derived mediators generated at sites of vascular and mucosal inflammation [88], suppresses the development of endometriosis, by anti-inflammatory, anti-proliferative and anti-invasive effects on ectopic endometrial tissue, by p38 MAPK (mitogen-activated protein kinase) downregulation mediated by ALX receptors (LXA4 receptors). LXA4 also decreases the invasive activity of endometriotic stromal cells by suppressing the expression and activity of MMP-9 [89, 90].

The results of a prospective experimental study suggested that resveratrol is a potential agent for the treatment of endometriosis and may be an alternative to leuprolide acetate (LA). This possible therapy has been supported by significantly

reduced immunoreactivity to MMP-2, MMP-9, and VEGF of surgically induced endometriotic implants by administration of resveratrol, LA or both, correlated with decreased plasmatic and peritoneal levels of IL-6, IL-8, and TNF-α, in experimental models [91].

10 MMPs Inhibitors Spectrum and Potential Utility in Endometrium

The hypothesis of therapeutic benefit of MMP inhibition has been based on the complex MMPs involvement in endometrial pathology.

Added to TIMPs, as previously mentioned, several natural inhibitors or compounds obtained from natural sources have been identified, such as tissue factor pathway inhibitor-2, membrane-bound β-amyloid precursor protein (MMP-2 inhibitor), RECK (inhibition of MMP-9, MMP-2, and MT1-MMP), chlorotoxin (MMP-2 inhibitor), α-macroglobulins (inhibitors of the most MMPs), added to hydroxamates (BE16627B and matlystatin B), and BE16627B (broad spectrum MMPs inhibitor) [48, 92].

In the early 80s, the first synthetic MMP inhibitor was produced. Consequently, several MMPs inhibitors have been tested in experimental models, such as: batimastat, CDP-845 (inhibitor of stromelysin-1), RO32-3555 (hydroxamate-based selective gelatinase inhibitor), hydroxamate inhibitors (GI168 and GI173) [93], GI173 (gelatinases inhibitor), GI168 (stromelysin-1 inhibitor), ilomastat or GM6001 (related batimastat compound) [93], marimastat [94], chelating agents (EDTA, 1,10-phenanthroline, or EGTA—inhibitory action on matrixins), cysteine and dithiothreitol (MMP-3 inhibition), phosphonamidate compounds (MMP-3 inhibitors), nonpeptide hydroxamate inhibitors (MMP-3 inhibitors), pseudopeptide-hydroxamate compounds (MMP-3 inhibitors) [95], and SC-44463 (broad range MMP inhibitor and of pro-MMP-3 activation in cultures) [96].

The mechanisms of action had been thought to be: fibrotic changes, angiogenesis inhibition, constriction of invasive growth resulting in an increased interstitial pressure, compression of blood vessels, followed by ischemia and subsequent necrosis, inhibition of collagen degradation, and collagen biosynthesis stimulation ("encapsulation" of primary or secondary tumors) [93, 94].

However, in cancer models, synthetic MMP inhibitors are applied when the tumor diameter is small and they lack stromal tissue, the source of most MMP activity. Therefore, the clinical effects may be amplified.

Considering the local environment, Progesterone may be considered an endometrial MMP inhibitor, acting by negative control of the production of MMP-1 (interstitial collagenase) and MMP-3 (stromelysin-1), in experimental models [97]. Withdrawal of progesterone results in increased stromal cell MMP-1, MMP-2, and MMP-3 production [97], in downregulation of TIMP-2 [98], without any change in expression or secretion of TIMP-1 and TIMP-3 [97].

Progestins are able to continuously inhibit the production of MMP-1 and MMP-3 in cultures that have been designed to mimic the control of long-acting progestogenic contraceptives on endometrium. Although progesterone has the potential to inhibit MMP production by stromal cells within the short term, it may lose the capacity to maintain this inhibition in the longer term administration.

Endometrial stromal cells show variable immunopositivity for endothelin-1 and TIMPs in cultures [99], corresponding to focal endometrial tissue breakdown at menstruation due to local, rather than endocrine regulation. Endothelin-1, TNF-α and IL-1 stimulate MMP-1 and MMP-3 production in short-term culture [100]. Endometrial paracrine actions of both IL-1 [101] and TGF-β [102] may modify the production of certain endometrial MMPs, while mast cells mediators modulate both MMPs production and activation by stromal cells [103]. In slow release progestins users, the endometrium shows an increased number of macrophages, neutrophils, and eosinophils and their products are stimuli for local MMP expression, overriding the inhibitory effect of the progestins on MMP production.

Progestins responses variability could be accounted to endometrial PR subtypes in normal cycling women, compared with those using progestogenic steroids. Surprisingly, women receiving slow release progestins show increased total immunoreactive endometrial PR and PR mRNA [104]. PR B is a stronger activator of target genes while PR A can act as a dominant repressor of PR B and other hormone receptors.

ARs have been demonstrated as cycle dependent in normal endometrium [105], showing a B subtype dominance [106]. Some of the synthetic progestins may also exhibit androgenic activity and regulate MMPs expression by ARs activation [107]. As a consequence, the identification of endometrial ARs and their subtypes could be relevant in administration of synthetic progestins and may offer important information related to their mechanism of endometrial MMPs regulation. In this regard, it is assumed that androgens have a significant role in MMP-1 regulation. The secretion and production of MMP-1 is inhibited by testosterone by specific ARs binding, in the same manner to that noticed for progesterone, in human endometrial stromal cells cultures [37].

11 Conclusions

MMPs are involved in many biological processes, mainly due to their ability of ECM proteolysis and/or capacity to initiate unrevealed functions. Recent progresses were made in understanding biochemical and structural aspects of MMPs, and their molecular complexes with TIMPs allow them to play a complex role in endometrial physiology and pathology by facilitating both turnover and invasion mechanisms.

MMPs: TIMPs ratio imbalances are involved in hormonal disorders and furthermore in hyperplasia, as steps along endometrial malignant transformation. Partially using the mechanisms already identified in carcinogenesis, an increased MMP activity is also detected in endometriosis.

Future studies are necessary to elucidate the complex interactions between molecules involved in proliferation, angiogenesis, and apoptosis, as part of EMT mechanism, opening new perspectives in the early diagnosis and treatment of endometrial neoplasia.

The design of potent specific inhibitors for MMPs represents a challenge for scientists, not only for gaining insights into the biological roles of MMPs but also for the development of new targeted therapies in endometrial neoplasia and endometriosis.

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