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Review

Extracellular matrix metalloproteinases in the etiopathogenesis of endometriosis: a systematic review and critical appraisal

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Summary

Despite extensive research in the field, the etiopathogenesis of endometriosis remains an unresolved enigma. The possible role of different enzymes of the extracellular matrix, in particular the role of the matrix metalloproteases or metalloproteinases (MMPs) in the etiopathogenesis and the mechanisms involved in the processes of benign dissemination of endometriosis have been widely investigated in recent years. Various members of the enzymatic system of the MMPs, as well as their inhibitors, termed tissue inhibitors of metalloproteinases (TIMPs) and their inducer, termed extracellular matrix metalloproteinase inducer (EMMPRIN), have been implicated in the mechanisms involved in endometriosis formation, progression, and maintenance. The aim of the present paper was to provide an overview and critical evaluation of existing experimental evidence on this issue. For this purpose the authors have conducted a systematic review of the literature and evaluated relevant papers regarding experimental animal models, in vitro experiments, and analyses in human samples and studies regarding genetic polymorphisms in humans. In conclusion, members of the system of matrix MMPs, their inhibitors and inducers could be useful as novel diagnostic and prognostic biomarkers in determining the severity of endometriosis and response to therapy. Furthermore, in depth knowledge in this field could possibly lead to the development of more efficient treatment modalities. Future research should focus on the systematic investigation of the entire MMPs system in endometriosis, as well as on the interaction between its members.

Key words: Endometriosis; Etiopathogenesis; Extracellular matrix metalloproteases; Metalloproteinases; Tissue Inhibitors of metalloproteases; Extracellular matrix metalloproteinase inducer.

Introduction

Endometriosis is a common problem in women of reproductive age. In particular, it is a frequent cause of chronic pelvic pain and/or infertility. By definition, endometriosis is the presence of functional ectopic endometrium, including endometrial glands and stroma outside of the uterus. It is usually confined to the pelvis and only rarely it might be found in more distant sites [1, 2]. Despite extensive research in the field, the etiopathogenesis of endometriosis remains enigmatic. The most widely accepted theory on its pathogenesis is the theory of ectopic endometrial implantation through retrograde menstruation, making endometriosis a paradigm of benign disease dissemination and metastasis [3-5].

Although endometriosis is not a neoplastic disease, it shares common characteristics with cancer, including cellular motility, adhesion and invasion, immunological factors, maintenance of the initial tissue architecture in ectopic sites, and angiogenesis [3, 4]. Furthermore, an increased risk of certain subtypes of ovarian cancer in women with endometriosis has been described in epidemiological studies [6]. Hence, endometriosis may be regarded as a unique model in order to closely investigate molecular and genetic mechanisms possibly involved in the metastatic cascade [3, 4].

Numerous studies have shown that different enzymes of the extracellular matrix are active in endometriotic lesions and/or the eutopic endometrium of women with endometriosis. Their action leads to self-destruction of the extracellular matrix, which in turn facilitates the invasion of endometriotic epithelial cells into deeper tissue layers, a process necessary for the local formation and dissemination of endometriotic lesions. Among various such enzymatic systems, the system of the extracellular matrix metalloproteases or metalloproteinases (MMPs) is probably the most extensively studied in endometriosis. This system consists of an enzymatic, the MMPs, an inhibitory element, the tissue inhibitors of the metalloproteinases (TIMPS), and the extracellular matrix metalloproteinase inducer (EMMPRIN) or basigin [7]. The aim of the present article is to systematically review the role of the system of matrix metalloproteinases in endometriosis and critically evaluate the existing experimental evidence on this issue.

Materials and Methods

An extensive search of the literature was conducted in the bibliographic database Medline for articles ever published in English until December 31, 2018, by using the search terms combinations

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Table 1. — *Overview of studies in experimental animal models regarding the matrix metalloproteinase (MMP) system.*

Publication	Animal model	MMP-system member(s)	Other factors	Correlation of MMP member with endometriosis
Paul *et al.* [8]	Mouse	proMMP-9, TIMP-1	Effect of Melatonin	Yes / Yes
Paul *et al.* [9]	Mouse	MMP-3, MMP-9, TIMP-3	uPA; effect of Melatonin	Yes / Yes / Yes
Machado *et al.* [10]	Rat	MMP-9	VEGF, Flk-1	Yes
Swarmakar and Paul [11]	Mouse	MMP-9	Effect of curcumin	Yes
Chen *et al.* [12]	Mouse	MMP-2 and MMP-9	None	Yes / Yes
Lu *et al.* [13]	Mouse	MMP-2	VEGF	Yes
Wang and Ma [14]	Nude mouse	MMP-2, TIMP-2	Effects of E2 and progestin	Yes / Yes
Bruner-Tran *et al.* [15]	Nude mouse	MMP-3, MMP-7	Effects of progesterone	Yes / Yes
Bruner-Tran *et al.* [16]	Mouse	MMP-3, MMP-7	Effect of tanaproget	Yes / Yes
Nothnick and Soloway [17]	Mouse	TIMP-1	Activated macrophages	Yes
Stilley *et al.* [18]	Rat	TIMP-1	None	Yes
Braundmeier *et al.* [19]	Baboon	EMMPRIN, MMPs	None	Yes / Yes

uPA = urokinase-type plasminogen activator; VEGF = vascular endothelial growth factor.

Table 2. — *Overview of in vitro studies and studies in human samples regarding MMP-9.*

Publication	Type of sample	MMP-system member(s)	Other factors	Correlation with endometriosis
Chung *et al.* [20]	ET	MMP-9, TIMP-3	None	Yes / Yes
Collete *et al.* [21]	Cell culture	MMP-9, MMP-2, TIMP-1	None	Yes / No / Yes
Szymanowski *et al.* [22]	ET, serum	MMP-9, MMP-2, TIMP-1	TGF-β2	No / No / No
Collette *et al.* [23]	ET	MMP-9, TIMP-1	None	Yes / Yes
Liu *et al.* [24]	Serum, PF, ET	MMP-9	None	Yes
Liu *et al.* [25]	Plasma, PF	MMP-9	None	Yes
Szamatowitcz *et al.* [26]	PF	MMP-9, TIMP-1	None	Yes / Yes
Wu *et al.* [27]	PF	MMP-9, TIMP-1, TIMP-2	Effects of PGE2	Yes / Yes / Yes
De Sanctis *et al.* [28]	Peripheral blood	MMP-9, MMP-3	VEGF-A	No / Yes
Salata *et al.* [29]	Serum	MMP-9, MMP-2, TIMP-1, TIMP-2	None	No /No / No/ No
Yang *et al.* [30]	Cell culture	MMP-9	Osteopontin, E2	Yes
Zhang *et al.* [31]	Cell culture	MMP-9	Wnt signaling, E2	Yes

ET = endometrial tissue; PF = peritoneal fluid; PGE2 = prostaglandin E2; VEGF = vascular endothelial growth factor.

Table 3. — *Overview of in vitro studies and studies in human samples regarding various members of the matrix metalloproteinase MMP-system other than MMP-9.*

Publication	Type of Sample	MMP-system member(s)	Other factors	Correlation with endometriosis
Hudelist *et al.* [32]	ET	MMP-1	Interleukin-1	Yes
Hudelist *et al.* [33]	ET	MMP-1	ER-alpha, ER-beta	Yes
Juhasz-Böss *et al.* [34]	CAM	MMP-1, MMP-2	None	Yes / Yes
Huang *et al.* [35]	PF and serum	MMP-2	E2 and progesterone	Yes
Kim *et al.* [36]	ET	MMP-2	VEGF, CD44, Ki-67	Yes
Jana *et al.* [37]	ET	MMP-2	VEGF, VEGFR-2, COX-2, vW	Yes
Ramón *et al.* [38]	ET	MMP-3	uPA	Yes
Gilabert-Estellés *et al.* [39]	ET	MMP-3, TIMP-1	uPA, PAI-1	Yes / Yes
Gilabert-Estellés *et al.* [40]	PF	MMP-3	VEGF, uPA	Yes
Matsuzaki *et al.* [41]	ET	MMP-7	None	Yes
Laudanski *et al.* [42]	PF	MMP-13, MMP-14, TIMP-2	None	Yes / Yes / No
Gaetje *et al.* [43]	ET	MMP-24/25	None	Yes
Sharpe-Timms *et al.* [44]	PF, serum	TIMP-1	Effect of GnRH-agonists	Yes
Smedts *et al.* [45]	ET	EMMPRIN	None	Yes

ET = endometrial tissue; CAM = chorioallantoic membrane; PF = peritoneal fluid, ER = estrogen receptor; VEGF = vascular endothelial growth factor; vW = von Willebrand factor, uPA = urokinase-type plasminogen activator; PAI-1= plasminogen activator inhibitor type 1.

"metalloproteinases" or "metalloproteases" and "endometriosis", "TIMP" and "endometriosis", and "EMMPRIN" and "endometriosis" in the field "Title". There were no other limits or filters, including no limits for year of publication. The titles of identified studies were screened first and irrelevant publications were excluded. Then, the abstracts of the remaining studies were evaluated. The next step was the evaluation of the content of the main text of identified studies. Finally, the lists of references of relevant studies were screened in order to possibly identify additional relevant articles.

Results

Thirty-two papers were initially found by the Medline search (24 by using the search terms "metalloproteinases" or "metalloproteases" and "endometriosis", eight by using the terms "TIMP" and "endometriosis", and none by using the terms "EMMPRIN" and "endometriosis"). After evaluating the titles, abstracts and/or the full-texts of these studies, 45 relevant studies were identified. The studies were then classified into three major categories: a) studies in experimental animal models (Table 1) [8-19], b) in vitro studies and studies in human samples (Table 2 and 3) [20-45], and c) studies on genetic polymorphisms in humans (Table 4) [46-51].

Discussion

The role of melatonin was evaluated in an experimental mouse model [8]: Melatonin downregulated proMMP-9 activity and expression and upregulated TIMP-1 expression, causing remission of peritoneal endometriotic lesions. Consistently, in a further study in mice [9], melatonin led to regression of endometriosis, possibly through suppression of MMP-3 activity and amplification of apoptosis. Furthermore, in the early phase of endometriosis MMP-3, but not MMP-9, was increased and TIMP-3 and uPA (urokinase plasminogen activator) were both involved in MMP-3 regulation. In another animal model in mice, curcumin reversed MMP-9 activity and led to regression of endometriotic lesions [10].

In an experimental endometriosis model in rats [11], MMP-9 was significantly higher in endometriotic lesions than in eutopic endometrium. Moreover, MMP-9 was found to increase overtime, together with MMP-2, in an induced endometriosis model in mice [12]. MMP-2 was also found to be stronger expressed in endometrial transplants from endometriosis patients than controls in an experimental mouse model [13] and similar findings regarding MMP-2 were also found in human samples of peritoneal, rectovaginal and ovarian endometriosis [37]. Furthermore, in a nude mouse model, MMP-2 expression was higher after treatment with estrogen alone and estrogen+progestin than with progestin alone and control, whereas the reverse was found for TIMP-2 [14]. In another experimental endometriosis model in nude mice involving administration of progesterone [15] and a progesterone receptor agonist [16] MMP-3 and MMP-7 were also implicated in the pathogenesis of endometriosis.

In an experimental mouse model [17] activated macrophages in the peritoneum were implicated in the development of endometriosis through TIMP-1 expression. TIMP1 was also found to be increased in the peritoneal fluid of female rats with surgically induced endometriosis [18]. Finally, in an animal model in primates, [19] the extracellular matrix metalloproteinase inducer (EMMPRIN) and multiple MMPs were regulated by ovarian hormones and dysregulated in animals with endometriosis. Taken together, these studies suggest that MMPs are involved in endometriosis formation and maintenance, whereas TIMPs exert the reverse actions. An overview of experimental studies in animal models is presented in Table 1.

MMP-9 expression and the MMP-9/TIMP-3 ratio were found to be higher in ectopic rather than the eutopic endometrium from women with endometriosis, and the reverse association was found for TIMP-3 [20]. Furthermore, MMP-9 secretion was elevated in vitro in the eutopic endometrium from women with endometriosis compared with normal women [21]. However, in two other studies there was no difference in eutopic endometrial expression of MMP-2 [22], and MMP-9, and TIMP-1 [22, 23] between endometriosis patients and healthy women, but the ratio of MMP-9/TIMP-1 expression was significantly higher in women with endometriosis [23]. In two other studies, MMP-9 levels were progressively higher with advancement of disease stage and higher in plasma, serum, and peritoneal fluid from endometriosis patients than controls [24, 25]. Likewise, MMP-9 concentrations were higher, whereas TIMP-1 concentrations were lower in the peritoneal fluid of endometriosis patients [26]. Moreover, treatment of macrophages with peritoneal fluid from patients with severe endometriosis inhibited MMP-9 expression and gelatinase activity [27]. However, in two other studies, there was no difference regarding MMP-9 in peripheral blood samples between patients with endometriosis and healthy subjects [28, 29]; likewise, there was no correlation regarding MMP-2, TIMP-1, and TIMP-2 [29], while only MMP-3 could be possibly used as a biomarker in peripheral blood [28]. Finally, two functional studies showed that up-regulation of MMP-9 may correlate with the migration of endometrial epithelial cells in patients with endometriosis [30] and that the MMP-9 gene and protein expression are upregulated by the Wnt signaling pathway under the regulation of E2, and this mechanism may contribute to the pathophysiology of endometriosis [31]. Taken together, these studies show that MMP-9 might be involved in the etiopathogenesis of endometriosis and disease progression. However, there are contradicting findings in different studies suggesting that the role of MMP-9 in endometriosis is rather complex. An overview of studies regarding MMP-9 in endometriosis is presented in Table 2.

In Table 3 an overview of in vitro studies and studies in human samples regarding members of the MMP enzymatic system (including TIMPs and EMMPRIN) other than MMP-9 is presented. In detail, MMP-1 was found to be increased in the ectopic endometrium of patients with endometriosis, suggesting that it is involved in the pathogenetic mechanisms leading to local invasion and tissue destruction [32, 33]. Consistently, a study using endometrial grafts in chicken chorioallantoic membrane showed that MMP-1 and MMP-2 might be involved in the processes of

Table 4. — *Overview of studies regarding genetic polymorphisms of the MMP-system in humans.*

Publication	MMP-system member(s)	Correlation with endometriosis
Ferrari *et al.* [46]	MMP-1 and MMP-3 promoters	No / No
Borghese *et al.* [47]	MMP-12 and MMP-13 genes	Yes, in combination, in superficial lesions
Han *et al.* [48]	MMP-9 haplotypes and SNPs	Yes for two haplotypes / No for 4 SNPs
Kang *et al.* [49]	MMP-2, TIMP-2	No / Yes, protective role of TIMP-2
Cho *et al.* [50]	MMP-2, TIMP-2 (10 SNPs)	Yes, with advanced endometriosis
Ye *et al.* [51]	MMP-1, MMP-2, MMP-3, MMP-9	Yes, for one MMP-1 polymorphism

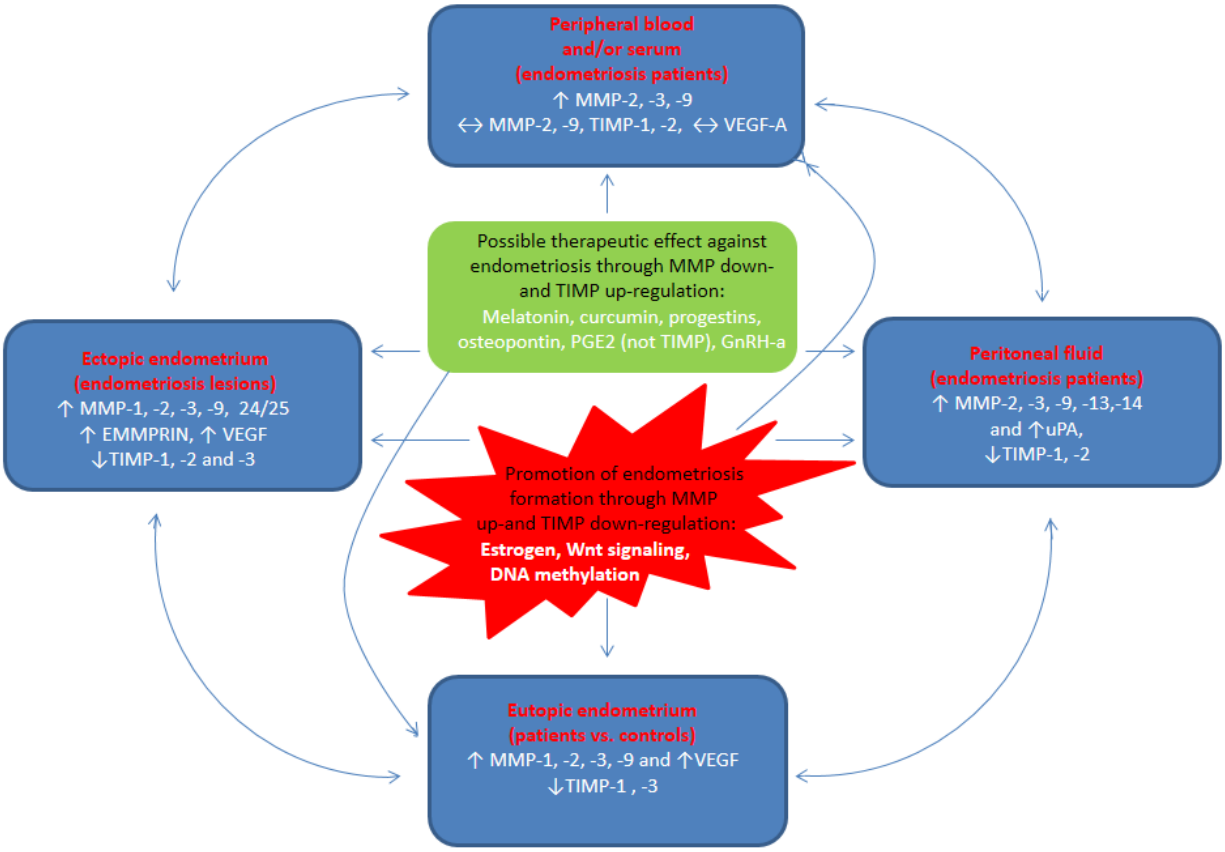

Figure 1. — Diagrammatic representation of the role of different members of the matrix metalloproteases (MMP) enzymatic system in endometriosis formation, maintenance, and progression.

invasion and vascularization in the pathogenesis of endometriosis [34]. Furthermore, MMP-2 concentrations in peritoneal fluid and serum were higher in endometriosis patients compared to women without endometriosis [35], its expression was higher in advanced stages of endometriosis [36], and its activity was significantly elevated in ectopic ovarian endometrioma in proportion to disease severity [37].

As mentioned earlier, MMP-3 could be possibly used as a biomarker in peripheral blood in order to discriminate between patients with endometriosis and healthy subjects [25]. MMP-3 was also found to be increased in the endometrium of women with endometriosis than healthy controls [38, 39], and in the peritoneal fluid of endometriosis patients [40]. MMP-3 expression also appeared to be closely associated with angiogenesis and the uPA/PAI-2 enzymatic system [38-40]. In addition, ovarian endometriotic tissue expressed higher levels of TIMP-1 compared to normal endometrium [39].

The expression of MMP-7 was found to vary significantly among the different forms of endometriosis; MMP-7 levels were significantly higher in endometrial epithelial cells from patients with deep infiltrating endometriosis than controls, and significantly higher in red peritoneal lesions compared to that of deep infiltrating endometriosis, ovarian endometriosis, and black peritoneal lesions [41]. In another

study, MMP-13 and MMP-14 (or membrane-type 1 metalloproteinase; MT1-MMP) levels were significantly decreased in the peritoneal fluid of women with endometriosis, while TIMP-2 levels did not differ significantly [42]. MMP-24 (or membrane-type 5 metalloproteinase; MT5-MMP) levels were elevated in peritoneal endometriosis lesions [43]. In another study, TIMP-1 levels were significantly lower in peritoneal fluid and sera of women with endometriosis compared with disease-free women [44]. Finally, extracellular matrix metalloproteinase inducer or basigin (EMMPRIN) exhibited differential expression patterns in the human ovary and ovarian endometriosis suggesting a possible role in normal ovarian function and in the dysregulation of proteolytic MMPs in endometriosis [45]. Taken together, these studies show that MMP-1, -2, -3, -7, -13,- 14, -24/25, as well as TIMP-1 and -2, and EMMPRIN are all differentially expressed and all seem to be involved in the etiopathogenesis of endometriosis. In particular, up-regulation of MMPs and EMMPRIN, and down-regulation of TIMPs seem to promote the formation, maintenance and progression of endometriosis.

Studies on MMP genetic polymorphisms in humans

The role of two MMP polymorphisms in the promoter regions of MMP-1 and MMP-3 was studied in an Italian population, but the authors concluded that they do not constitute an important factor for the genetic predisposition to endometriosis and its invasive behavior [46]. In contrast, the combination of two polymorphisms of MMP12 (82 A/G) and MMP13 (77 A/G) in superficial endometriosis and their absence in deep infiltrating endometriosis, suggested that this combination might protect from deeper penetration of tissues [47], and two different MMP-9 haplotypes may correlate with the progression of endometriosis [48]. Furthermore, a TIMP-2 polymorphism (418C/C homozygote) may be a protective factor against the development of endometriosis [49], while certain MMP-2 and TIMP-2 polymorphisms were found to be associated with advanced-stage endometriosis [50]. Finally, in a systematic review regarding different gene polymorphisms, a significant association between an MMP-1 polymorphism ((1607 1G/2G) and the susceptibility of endometriosis and adenomyosis was reported, while there was no association regarding certain polymorphisms of MMP-2, 3 and -9. In summary, evidence regarding the role of MMP gene polymorphisms in the pathogenesis of endometriosis is at present scarce. An overview of these studies is presented in Table 4 [46-51].

Conclusions

The possible role of MMPs, TIMPS, and EMMPRIN in the pathogenesis and pathophysiology of endometriosis has been the subject of extensive research in recent years. However, it seems that we are still far from getting the complete picture in sight. Most animal studies regarding the role of MMPs and TIMPs have been conducted in rodents and most data have been generated regarding MMP-9 and MMP-2. In animal models, melatonin, curcumin, estradiol, and progestins seem to regulate the expression of different members of the MMP system [8-19]. In human samples and in vitro experiments, MMP-9 is the most studied member of the MMP system involved in endometriosis; TIMP-1, -2, and -3 have all been implicated in the regulation of MMP-9 in endometriosis, and MMP-9 expression in endometriosis seems to be affected by osteopontin, estradiol, prostaglandin E2, and Wtn signaling [20-31]. MMP-1, -2, -3, -7, -13,- 14, -24/25, as well as TIMP-1 and -2, and EMMPRIN are all differentially expressed and seem all to be involved in the pathogenesis of endometriosis [32-45]. MMP-1 expression seems to be regulated by different mechanisms including interleukin-alpha and estrogen receptor-beta, MMP-2 and MMP-3 expression are closely associated with angiogenesis and the uPA/PAI-2 enzymatic system [32-45], and more recently DNA-methylation changes may be the underlying cause to the changes of MMP expression in endometriosis [52]. Data regarding the role of MMP gene polymorphisms in the pathogenesis of endometriosis remains scarce [46-51]. In general, up-regulation of MMPs and EMMPRIN, and down-regulation of TIMPs seem to promote the formation, maintenance, and progression of endometriosis, while the reverse may lead to disease regression (Figure 1). Future studies should focus on a holistic, large scale, systematic investigation of the entire MMP enzymatic system in endometriosis, and investigate the interplay between its members and associations with other enzymatic systems.

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