

THE RELEVANCY OF THE MATRIX METALLOPROTEINASE SYSTEM TO THE PATHOPHYSIOLOGY OF ENDOMETRIOSIS

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1. ABSTRACT

The matrix metalloproteinase (MMP) system is composed of the enzymatic component, the MMPs, and the enzyme inhibitory component, the tissue inhibitors of metalloproteinases or TIMPs. It is well established that the MMP system plays a critical role during the normal development and growth of the endometrium as well as many other physiological processes. Because of the necessity for the balance between MMP and TIMP, it is not surprising that aberrant expression of MMPs and TIMPs is associated with the pathophysiology of many diseases. Included in this list is the female disease endometriosis, a disease in which endometrial tissue grows outside of the uterus usually within the pelvic cavity. Both endometriotic (ectopic) endometrial tissue as well as the eutopic endometrium from women with the disease exhibit altered patterns of MMP and TIMP expression which favor tissue invasion/remodeling by the endometriotic tissue. As such, it has been proposed that successful modulation of the MMP system to limit or prevent the invasive events necessary for endometriosis development and/or progression may open new avenues to the medical management of endometriosis. This review will present general knowledge of the MMP system relative to the pathophysiology of the endometriosis as well as address its potential value in relation to the treatment of the disease.

2. INTRODUCTION

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases whose primary function is to degrade extracellular matrix (ECM) as well as regulate biological activity of a variety of proteases, growth factors, cytokines and their respective receptors. Within the endometrium, the MMP system is regulated by steroid hormones, growth factors and cytokines under normal physiological conditions (1). Altered expression of MMPs is associated with disease processes which include the female disease endometriosis. Endometriosis is defined as the growth of ectopic endometrial tissue and altered

expression of MMPs has been associated with the disease. Further studies have demonstrated a functional role for the MMPs in the development of endometriosis. These observations might suggest that the MMP system may be an attractive target for future treatment of endometriosis. This review will focus on the MMP system, how it relates to the disease endometriosis and how this system may be targeted as a future therapy for the disease.

3. THE MATRIX METALLOPROTEINASE SYSTEM

The matrix metalloproteinase (MMP) system is composed of the enzymatic component, the MMPs and the enzyme inhibitory component, the tissue inhibitors of metalloproteinases or TIMPs. MMPs are a family of structurally related zinc-dependent endopeptidases, which collectively, possess the ability to degrade all components of the extracellular matrix. These components include, among others, collagens, gelatin, fibronectin and laminin. Degradation of the ECM by MMPs occurs in normal everyday physiological processes such as wound repair, angiogenesis, and various aspects of the reproductive process (1-3). The activity of these enzymes is controlled at the tissue or cellular level by the TIMPs

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. MMPs are also classified by subgroups based upon their structure and substrate specificity. These classes include collagenases (MMP-1, MMP-8 and MMP-13), gelatinases (MMP-2 and MMP-9), matrilysins (MMP-7 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 which is cleaved after it directs the MMP's synthesis. As such, the majority of MMPs are secreted proteinases. However there are a class of MMPs which display transmembrane domains and are 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. 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).

At the tissue or cellular level, the TIMPs are the major endogenous regulators of the activities of MMPs, and four homologous TIMPs (TIMPs-1 to 4) have been identified to date (1). The TIMPs are a family of 20-29 kDa secreted proteins that bind to and inhibit the active MMPs at molar equivalence. Individual TIMPs exhibit differential ability to inhibit various MMPs (2). In addition, TIMPs are multifunctional proteins that also participate in the regulation of cell proliferation, apoptosis and differentiation (3). Therefore, the TIMPs are important regulators not only in ECM remodeling but also in cellular activities.

While many MMP 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 (3). 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 is 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.

4. THE PATHOPHYSIOLOGY OF ENDOMETRIOSIS

Endometriosis is a disease that occurs in menstruating females and is characterized by such symptoms as pelvic pain, dysmenorrhea and infertility. It is estimated that endometriosis affects as many as 10 to 15 % of all women of reproductive age and as many as 40 to 50% of all women with infertility. Classically defined as the presence of ectopic endometrial stromal and glandular tissue, the disease is thought to develop via reverse menstruation of viable endometrial tissue into the peritoneal cavity. However, because almost all women of reproductive age exhibit some degree of retrograde menstruation (4,5), it is postulated that some other factors must contribute to the development and progression of endometriosis. Numerous investigators have suggested that there is an association between the presence of endometriosis and an altered immune system/inflammatory reaction.

An increase in peritoneal inflammation as evidenced by elevated peritoneal fluid cytokine levels, is well established in women with endometriosis (6). While it is uncertain if the elevated cytokines levels/inflammation is a cause for or a result of the disease, it is clear that these cytokines may have profound effects which can lead to the establishment and further progression of the disease. For example, cytokines can stimulate endometrial cell adhesion to peritoneal mesothelial cell monolayers *in vitro* (7) as well as to specific extracellular matrix proteins (8). The role of adhesion molecules in the establishment of the early developmental stages of endometriosis is just becoming apparent (9-12) and appear to play a very important role in the initial stages of endometriosis development. Once the endometrial cells/tissue adheres to the peritoneum or other pelvic surfaces with which they come into contact, the next proposed step in the development/progression of endometriosis involves the invasion of the underlying tissue/cell layer. Both *in vitro* (13) and *in vivo* (14,15) studies indicate that endometriosis is an invasive disease.

One of the major groups of proteases postulated to play an important role in these invasive processes and hence the etiology and pathophysiology of endometriosis is the matrix metalloproteinases (MMPs). MMPs are produced by endometrial tissue and this pattern of MMP expression is altered compared to eutopic endometrium. Considerable evidence indicates that MMPs play an active role in the establishment and progression of endometriosis.

5. THE ROLE OF THE MMP SYSTEM IN THE PATHOPHYSIOLOGY OF ENDOMETRIOSIS

Alterations in the patterns of expression for several members of the MMP system have been reported in eutopic and ectopic endometrial tissues obtained from women with endometriosis. The human endometrial implant expresses aberrant or elevated levels of MMP-1 (16,17), MMP-2 (18), MMP-3 (19,20), MMP-7 (21) and

MMP-9 (22) as well as reduced levels of the MMP inhibitors, TIMP-1 (16,20) and TIMP-2 (16). Together, these data may be interpreted to suggest that a net elevation in MMP expression, whether it be due to increased MMP expression or reduced TIMP expression, would indicate an invasive nature of the endometriotic implant.

In addition to associational data which suggests a role of MMPs in the pathophysiology of endometriosis, functional studies also suggest a role of MMPs in the development of endometriosis. Using a nude mouse model for endometriosis, Bruner and colleagues (23-25) demonstrated that MMP expression by the human endometrial tissue paralleled the ability of this tissue to develop into ectopic lesions. If MMP expression and activity was blocked by either progesterone treatment of the tissue prior to injection or if the tissue was injected into the mice concurrently with the MMP inhibitor, TIMP-1, the development of endometriosis was blocked. Collectively, the patterns of MMP expression by the endometriotic tissue, the known invasive nature of the disease and action of these MMPs, and the functional blockage of MMP action and inhibition of endometriosis development, strongly suggest that MMPs play an active role in the development and progression of the disease endometriosis.

To further understand the role of MMPs in the etiology of endometriosis, it is of paramount importance to decipher the mechanisms which lead to the aberrant expression and action of these proteases. These mechanisms may include innate anomalies in the eutopic endometrium from women with endometriosis, alterations in the resident immune and peritoneal cells which juxtapose the endometriotic cells and/or a contribution by the numerous cytokines and growth factors which are elevated in the peritoneal fluid of women with the disease.

It is well established that women with endometriosis have elevated peritoneal fluid cytokine concentrations compared to women free of the disease (6). Of the dozens of cytokines examined, one cytokine in particular has been thoroughly examined with respect to the contribution to MMP regulation within endometrial/endometriotic tissue and the development of endometriosis. Tumor necrosis factor- α (TNF- α) is a pleiotrophic cytokine with profound effects on cellular growth, differentiation and migration and plays an important role in endometrial physiology (26). *In vitro*, TNF- α stimulates MMP expression by endometrial tissue as well as suppress endogenous TIMPs (16,27). The stimulation of MMPs by TNF- α appears to be augmented in endometrium from women with endometriosis (28). Functional studies which incorporate animal models for endometriosis also suggest a role for TNF- α in the development of endometriosis. Using a TNF-binding protein, independent studies by D'Antonio and colleagues (29) and D'Hooghe and coworkers (30) demonstrated that blockage of TNF action reduced the development of endometriosis. Taken together, these data may be interpreted to suggest that TNF- α may play a role in the development of endometriosis and that the mechanism by

which this cytokine does so is through regulation of MMP expression.

6. POTENTIAL TARGETS IN DEVELOPING NEW TREATMENTS FOR ENDOMETRIOSIS

6.1. Current treatments for endometriosis

Current treatment modalities of endometriosis may be classified as either medical or surgical and in some cases both medical and surgical treatment may be combined. Surgical treatment is usually used in cases of severe disease. Current medical modalities for endometriosis include estrogen-progesterone combinations, progestogens, anti-progestogens, danazol, and gonadotropin-releasing hormone agonists (GnRH-a; 31). While primarily considered to act at the level of the hypothalamic-pituitary axis, the mechanisms by which these agents prevent endometriotic implant growth action may actually be at multiple levels. Both steroidal and GnRH α therapies induce a decrease in gonadotropin release and subsequent suppression of ovarian steroidogenesis. The suppression of ovarian steroids and the induction of a hypoestrogenic state is then thought to be the primary mechanism by which these modalities suppress endometriotic implant growth. However, progesterone is a potent immunosuppressive agent capable of blocking both cytokine release and action (32-34).

Similarly, both danazol (35-37) and GnRH- α analogues (38,39) have been postulated to perhaps act at the level of immune cells which may in turn lead to the suppression of cytokine levels. Cytokines and growth factors derived from peritoneal immune cells have been implicated as factors which may regulate endometriotic implant growth (40). GnRH peptide and receptors in both eutopic endometrium (41,42) and endometriotic tissue (43) suggests that GnRH agonists may exert anti-proliferative effects on endometrial tissue as well. Borroni and colleagues (43) have demonstrated that the GnRH agonist, leuprolide acetate can inhibit endometriotic cell proliferation. Thus, it is uncertain if current therapies elicit their inhibitory effect on endometriotic implant growth at the level of the pituitary-ovarian axis by inhibiting the subsequent production of estradiol, on the cells of the immune system by inhibiting cytokine release, on the endometrial tissue itself or at all three levels.

While GnRH agonists are one of the most common medical therapies to treat endometriosis their use is associated with some disadvantages and side effects. Treatment of endometriosis with GnRH agonists is limited to 6 months because of possible adverse effects on bone metabolism (44) as well as other consequences of the hypoestrogenic state which ensues from their use. This adverse effect on bone density can be overcome by hormone add-back therapy but add-back therapy may reduce the efficacy of GnRH α (45). In addition, it is very well established that use of GnRH α is associated with physical side effects such as vasomotor instability, headache, hot flashes (46,47) as well as psychiatric side effects which include depressive mood symptoms (48,49).

In all, it may seem counter-productive on one hand to use GnRH agonists to suppress steroid production to reduce the disease and the disease-associated symptoms while on the other hand have to add-back the steroids to overcome the detrimental effects of their absence. Additionally, one must consider the variability in therapeutic efficacy of the different brands of GnRHa (Leuprolide, Nafarelin, Buserelin, Goserelin, etc.) coupled with the different doses, durations and forms of the “added back” steroids. For the most part, current data suggests that GnRHa therapy coupled with steroid add-back provides effective suppression of endometriosis and endometriosis-associated symptoms (such as pelvic pain) while protecting against detrimental effects of GnRHa such as bone loss. One can only wonder thought that in this day and age of medicine why more specific treatments for endometriosis have not been developed. Ideally, such a treatment would induce a regression of the disease and its’ associated symptoms, but not have the detrimental effects associated with a hypoestrogenic state (such as bone loss). As it is well-established that: 1) endometriosis is an invasive disease in which MMPs are thought to play an active role in its establishment, 2) elevated cytokine levels are associated with the disease, and 3) these cytokines contribute to the development of endometriosis, possibly by stimulating MMP expression, anti-MMP or anti-TNF alpha therapies may be viable candidates for use in the treatment of endometriosis.

6.2. The use of anti-MMP therapies for endometriosis treatment

Collectively, current evidence suggests that endometriotic implants either express elevated or inappropriately expressed levels of MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9. It is postulated that these MMPs play a role in the development/progression of endometriosis by first allowing for the initial invasion of the endometriotic tissue into the peritoneum (or whatever tissue surface the retrogradely shed endometrial tissue comes into contact with. Adhesion of the endometrial tissue may also further stimulate MMP production by the endometriotic tissue. MMPs further allow for the progression of endometriosis by continuing to degrade the ECM and also by stimulating angiogenesis and the establishment of the blood supply for the endometriotic tissue. As such, the ability to successfully block the action of these MMPs may prove beneficial in treating endometriosis or even preventing its initial establishment. Surprisingly, little information exists on the use of MMP inhibitors in the treatment of endometriosis. The pioneering work of Bruner and colleagues was the first to demonstrate that blockage of MMP activity using the endogenous MMP inhibitor, TIMP-1 could prevent establishment of ectopic endometrial tissue growth (23-25).

While little emphasis has been placed upon the use of MMP inhibitors in the treatment or management of endometriosis, a wide variety of MMP inhibitors are currently in clinical use to combat diseases associated with MMP overexpression. Several types of MMP inhibitors have been used in the treatment of breast, gastric, ovarian, prostate and renal cancer. These MMP inhibitors include synthetic peptides or non-peptidic molecules, chemically

modified tetracyclines, bisphosphonates or natural MMP inhibitors (such as TIMP-1). Clearly more information is needed on which MMPs play an active role in the development and progression of endometriosis and how these molecules do so. In-situ hybridization and immunohistochemical localization studies would suggest that MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9 may be leading candidates, but functional data is lacking. Identification of which of these MMPs play functional roles in endometriosis may allow for the targeting of specific individual MMPs or individual classes of MMPs.

6.3. Blocking MMP action with anti-tumor necrosis factor- α therapies

While the management of endometriosis today is almost exclusively accomplished through the use of GnRH-agonists and steroidogenic compounds, the use of anti-TNF- α agents to treat endometriosis-associated infertility and suppress endometriotic implant growth has been suggested (50-52). One potential mechanism by which anti-TNF- α therapies may elicit their effect is through the inhibition of MMP transcription. TNF- α is a potent stimulator of MMP expression in endometrial and endometriotic tissue (28). It is postulated that cytokines such as TNF- α , which are elevated in the peritoneal fluid of women with endometriosis, stimulate MMP expression by retrogradely shed endometrial tissue and allow for the development and progression of ectopic endometrial tissue growth/endometriosis. Thus, by blocking the production or action of this cytokine, subsequent induction and actions of MMPs would be inhibited. Potential anti-TNF- α therapies may include pentoxifylline, leflunomide, Etanercept, Infliximab and recombinant human TNF binding protein-1. The potential use of anti-TNF- α therapies in endometriosis has recently been reviewed (53) and with the continuation of studies in animal models may someday be herald as the next wave of endometriosis treatment.

7. SUMMARY AND PERSPECTIVE

Endometriosis remains one of the most perplexing diseases from the standpoint of the researcher, the clinician and the patient. The difficulty in coping with, treating and understanding this disease is derived from the poor understanding of how and why the disease develops. While the majority of current therapies are successful in treating the major symptoms associated with the disease, they are not ideal and their side effects are not well-tolerated by many patients who use them. This problem primarily arises in that current therapy induces a systemic response to suppress the disease and in doing so also suppresses or alters many other physiological pathways within the body. Identification and targeting of more specific mediators in the development and/or progression of endometriosis may lead to development of more desirable and effective treatment regimes. One potential target area is the MMP system as these proteases have been postulated to play a role in the establishment and progression of the disease. For the successful targeting and development of anti-MMP therapies for endometriosis treatment, research will have to: 1) utilize human study subjects to further characterize the MMPs associated with

the disease, and 2) incorporate animal models for endometriosis to better understand how these proteases function in the pathophysiology of endometriosis. Together, these avenues will allow for a greater understanding on the mechanisms by which endometriosis may develop and how MMPs may be a viable target for future therapies.

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