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Regulation of uterine matrix metalloproteinase-9 and the role of microRNAs

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Abstract

Controlled expression of matrix metalloproteinase-9 (MMP9) is vital for normal uterine physiology while abnormal expression of MMP9 is associated with uterine disease. Despite the importance of MMP9 within the uterus, its regulation is poorly understood. In this review, the complex regulation of MMP9 within the uterus is highlighted and the potential novel role of microRNAs in the regulation of this protease is discussed. Emphasis is placed upon the necessity to enhance our understanding on the regulation of MMP9 expression which may lead to the establishment of novel approaches to counteract mis-expression of this protease and the diseases associated with it.

Keywords

MMP9; uterus; estrogen; miRNA

MATRIX METALLOPROTEINASE-9

Matrix metalloproteinase-9 (MMP9) is one member of the MMP family. MMPs are a large family of zinc-dependent endopeptidases that includes the collagenases, gelatinases, stromelysins, metalloelastases and membrane-type MMPs.¹ All MMPs possess the ability to degrade various components of the extracellular matrix (ECM) as well as non-ECM components which include cytokines, growth factors, growth factor binding proteins and cell surface receptors.² The proteolytic activities of these enzymes are precisely regulated at multiple levels: production and secretion of proenzymes, activation from proenzymes and inhibition by inhibitors of MMPs.³ Tissue inhibitors of metalloproteinases (TIMPs) are the major endogenous MMP inhibitors, which inhibit MMP activity by formation of 1:1 complexes. Four homologous TIMPs (TIMPs-1 to –4) have been identified to date.^{4,5} The MMP system comprising MMPs and TIMPs participates in ECM remodeling throughout the body and modifies cell behavior in many ways. MMP9 (also referred to as gelatinase B, type IV collagenase), is usually secreted as a 92-kDa (105 kDa in mice) proenzyme. Activation of the proenzyme involves the proteolytic removal of the N-terminal prodomain which then results in an 82-kDa (95 kDa in mice) active enzyme. MMP9 degrades the denatured collagens (gelatin) with high specific activity. It can also cleave native type IV, V, and XI collagens; elastin; and a variety of non-ECM molecules.^{1,3} MMP9 plays a role in physiological processes which include angiogenesis, cell migration, and wound healing.⁶

MATRIX METALLOPROTEINASE-9 AND UTERINE FUNCTION

Within the uterus, MMP9 is expressed during the reproductive cycle^{7,8} as well as during early pregnancy and decidualization^{7,9} and has been localized to epithelial, stromal, immune and vascular cells.^{10–13} MMP9 protein has been detected in endometrial glandular epithelial cells with highest levels during the late proliferative phase and just after ovulation as well as in glandular secretions and uterine fluid during the peri-implantation phase. The increase in MMP9 expression in the endometrium just before and during menstruation is associated with an influx of polymorphonuclear leukocytes, macrophages and eosinophils.¹² MMP9 is also detected in spiral arteries during the secretory phase and in vascular structures during the midfollicular and menstrual phases, suggesting MMP9 involvement in uterine vascular growth and angiogenesis.¹⁰ Uterine MMP9 is proposed to play a role in menstrual breakdown of the human endometrium as its expression and activity are significantly increased at menstruation and because it is capable of degrading basement membrane components.¹⁴ MMP9 also appears to participate in parturition and postpartum uterine involution since its expression is increased in the cervix at term pregnancy and postpartum.¹⁵

In addition to its role within the uterus under normal physiological states, altered expression of MMP9 has also been associated with uterine pathologies such as dysfunctional uterine bleeding or breakthrough bleeding,^{16–19} endometriosis,^{20–23} infertility,^{21,24} leiomyoma²⁵ and endometrial cancer.^{26–30} A functional role for MMP9 in cancer development was strengthened by Chantrain and colleagues³¹ who demonstrated that after grafting of cancer cells, mice deficient in MMP9 show markedly reduced angiogenesis. Further, production of MMP9 by infiltrating inflammatory cells is increased in carcinoma tissues.³² Thus, MMP9 appears to play an important role in several forms of cancer including that of the uterus.

REGULATION OF UTERINE MATRIX METALLOPROTEINASE-9

Despite a rather thorough investigation of MMP9 within the uterus, the regulation of this protease is poorly understood. The reproductive cycle-specific expression pattern of MMP9 suggests that this protease may be regulated by ovarian steroids. However, there are conflicting reports on this topic. MMP9 was detected in benign postmenopausal endometrium, and its expression was reduced in patients undertaking hormone replacement therapy.¹⁷ In human endometrial explants, MMP9 activity increases *in vitro* independent of steroid administration.^{33,34} Further at the transcript level, ovarian steroids slightly decreased MMP9 mRNA expression but have no consistent effect on MMP9 release.³⁴ MMP9 transcript is significantly increased in rodent uterine horns undergoing oil-induced decidualization but not in pregnant uteri. MMP9 protein, however, is detected in uteri both during pregnancy and in oil-induced decidualization.⁹ Many of these conflicting results may be due to the nature of the study as well as the duration of exposure to steroids.

A detailed review on matrix metalloproteinase-9 and its regulation is provided by Van den Steen and colleagues³⁵ and only salient points are summarized in this section. Like most if not all MMPs, MMP9 activity is regulated at multiple levels (Fig. 1). In the majority of systems, MMP9 expression is induced or repressed at the transcriptional level. Translation

then parallels transcript induction or repression. In those instances in which MMP9 transcript is induced and translated, the MMP9 protein is secreted as a pro-enzyme (pro-MMP9) incapable of cleaving substrate. Pro-MMP9 must be activated by cleavage of the pro-peptide. At the tissue level, MMP9 activity is further regulated by tissue inhibitors of metalloproteinases (TIMPs). Additional post-translational regulation of MMP9 activity occurs via glycosylation of the MMP9 protein. The literature suggests that MMP9 transcript expression parallels translation; factors that increase transcript expression lead to subsequent increased translation of protein while factors that suppress MMP9 transcript expression result in decreased protein synthesis. Recent findings from our laboratory indicate that within the uterus there is a disparity in MMP9 transcript expression and subsequent translation of MMP9 protein (Fig. 2). To our knowledge, this is the first description of a unique expression pattern of MMP9 in any organ or cell type. The majority of published work in the MMP field is under the assumption that the pattern of protein expression/translation parallels that of transcript expression. Based upon our MMP9 data, studies which may be limited to assessing only mRNA expression which then postulate a similar change in protein expression may inadvertently make an incorrect conclusion. This is of paramount importance when one considers the limitations of human-based studies in which conclusions must be drawn from *in-situ* analysis of human specimens or from *in vitro* cell or explant cultures. We have recently demonstrated that steroid regulation of uterine MMP9 in the mouse appears to occur at multiple levels.³⁶ Interestingly, we also noted that there is an inverse relationship in transcript and protein/activity expression during the early phase of estrogen treatment. Figure 2 is a summary of our observation over the course of several studies in which MMP9 transcript and protein/activity were examined. This observation would suggest that MMP9 transcript may be present in high levels but translation may be repressed preventing protein and subsequent MMP9 activity. Based upon what we know about microRNAs (miRNAs), we propose that these factors may play a role in this mechanism of estrogen regulation of uterine MMP9 post-transcription.

MICRORNAS REGULATE TRANSCRIPT EXPRESSION AND TRANSLATION

miRNAs are small RNAs which form RNA-protein complexes and effect post-transcriptional regulation of gene expression. To date, over 500 mRNAs have been identified which are capable of repressing protein synthesis and/or increasing mRNA degradation. miRNAs are expressed in a tissue/cell-specific manner and are proposed to play vital roles in mediation of basic biological processes such as cell proliferation, differentiation and death.³⁷⁻⁴⁰ Not surprisingly, altered expression of miRNAs has been linked to various pathologies with the most prominent being the development of cancer.⁴¹⁻⁴³ As such, miRNAs have been proposed as potential novel targets for anti-cancer therapy.⁴⁴ Thus, it is clear and concise that a thorough understanding on the regulation and function of miRNAs is of paramount importance in normal and pathological states.

MICRORNAS AND UTERINE MMP9

To determine if miRNAs may play a role in the unique pattern of uterine MMP9 transcript and protein expression, we performed the following series of experiments. Mature female mice were ovariectomized, rested two weeks and treated s.c. with either estradiol 17- β (E₂;

10 µg/kg BW) or sesame seed oil vehicle and sacrificed 8 h later. Uteri were removed and stored in RNAlater (Ambion) until processed for RNA isolation. Total RNA was isolated from uteri pooled from 2 to 3 mice using miRNEasy columns according to the recommendations of the manufacturer (Qiagen; Valencia, CA). Small RNA isolation and hybridization were performed by LC Sciences (Houston, TX) using 5 to 10 µg of total RNA. Briefly, small RNA species were isolated from total RNA by column exclusion. The concentrated small RNAs were 3'-polyadenylated with poly(A) polymerase. A nucleotide tag was then ligated to the poly(A) tails. The tagged RNAs were hybridized to a µParaflo superfluidic array chip (miRBase version 10.0) containing probes for 568 murine microRNA sequences as well as controls. Tagged RNA was subsequently labeled in a second hybridization reaction with Cy5 dendrimer dye. After overnight hybridization, arrays were stringently washed and scanned on an Axon GenePix 4000B laser scanner (Molecular Devices, Sunnyvale, CA). Data extraction, imaging and statistical analysis were performed by LC Sciences using ArrayPro software (Media Cybernetics; Bethesda, MD). After signal amplification, the background was subtracted and normalized using LOWESS (locally weighted regression) method. For a transcript to be listed as detectable, it had to meet the following criteria: signal intensity higher than 3x (background SD), spot coefficient of variation < 0.5 (coefficient of variation = SD/signal intensity), and signals from at least 50% of the repeating probes above detection level. The array output was received in Excel spreadsheets as lists of raw data and also as "simple detectable" data, which were the average of 6 signal values for each microRNA on the array. For each set at each time point analyzed, significant differences (probability values) among groups for a given detectable microRNA signal were calculated. Those with $P \leq 0.05$ were analyzed using gene hierarchical clustering of the $\log_2$ value of each signal.

Analysis of uterine miRNA expression (N=3 separate observations/array analysis) revealed that of the 568 murine miRNAs analyzed, 123 were detectable in uterine tissue. The most abundant miRNAs were let-7 (a,b,c,d,e,f,g,i), miR-106a, miR-10b, miR-144, miR-195, miR-199a, miR-21, miR-23a, miR-23b, miR-26a, miR-26b, and miR-690. In response to E₂ treatment, (at 8 h) 49 miRNAs displayed significant changes ($P < 0.01$) from 0 h values. Nineteen miRNAs were reduced by E₂ treatment, while the expression of 30 miRNAs was increased. Of those miRNAs which displayed significant changes, only those that showed a 2-fold change or greater ($\log_2$ value $+ 1.0$ or $- 1.0$) are displayed in Table 1. Of these miRNAs, one has been proposed to bind MMP9 transcript (miR-705). The others have yet to be characterized for binding of MMP9 but are proposed to bind to numerous transcripts associated with cellular responses (see the Sanger miRNA database for specific information). The potential role of these miRNAs within the uterus is currently being examined. Collectively, these data demonstrate for the first time that estrogen regulates miRNA expression within the uterus and that in particular, miR-705 may play a role in the estrogen regulation of MMP9 translation.

CONCLUSIONS

MMP9 expression and activity is controlled at multiple levels. Within the uterus, MMP9 expression appears to be unique as estrogen increases MMP9 protein expression and activity while concurrently reducing MMP9 mRNA. We propose that estrogen regulation of uterine

MMP9 may involve miRNA post-transcriptional regulation. Our working model is depicted in Figure 3 which implies that in addition to conventional regulatory mechanisms (summarized in Figure 1), MMP9 may also be regulated by miRNAs which provide yet another layer of complexity in the regulation of this unique protease.

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Figure 1.

General mechanisms for regulation of MMP-9 expression and activity. Induction and regulation of MMP9 is controlled at multiple levels which begin with induction of MMP9 transcript. MMP9 is translated as a pro-MMP which is incapable of eliciting biological responses. Pro-MMP9 is subsequently converted to the active form via cleavage of the pro-peptide. Active MMP9 is further regulated by endogenous inhibitors such as the tissue inhibitors of metalloproteinases (Timp). When the levels of Timp exceed that of MMP9, MMP9 activity is suppressed. In contrast, when levels of active MMP9 protein exceed levels of endogenous Timp, MMP9 is capable of inducing various biological responses.

MMP-9 (relative expression)

Figure 2.

Disparity in MMP9 transcript, protein and active enzyme expression in response to early phase estrogen treatment. Figure 1 represents a summary of the pattern of uterine MMP9 transcript (mRNA) and protein/activity (protein) expression in response to estrogen treatment. In untreated, ovariectomized mice, MMP9 transcript expression is high but protein/activity is very low to undetectable. In response to estrogen administration, MMP9 mRNA expression decreases reaching nadir levels at 8 h post estrogen administration. This decrease in MMP9 transcript expression is coupled with an increase in MMP9 protein/

activity. At 24 hours post estrogen administration, both MMP9 mRNA and protein/activity are elevated.

Figure 3.

Working model of estrogen regulation of uterine miRNAs and subsequent repression of MMP9 translation. Based upon the data in the literature and our preliminary findings, we propose that in the absence of estrogen (1), specific miRNA(s) are bound to the 3' UTR of MMP9. This stabilizes MMP9 transcript, but represses translation. In response to estrogen treatment, the early response (2) leads to removal of bound miRNA(s) from the 3' UTR of MMP9 transcript which in turn relaxes translation repression. MMP9 transcript can then be translated to MMP9 protein and undergo subsequent transcript degradation. A late response to estrogen (3) may also occur (for example at 24 h post estrogen treatment and/or in chronic estrogen treatment) such that miRNA(s) expression is suppressed allowing for induction of MMP9 transcript expression and subsequent translation without repression.

Table 1

Uterine miRNAs differentially regulated by estrogen.

Probe ID	Log2 (8 h Estrogen/0 h Estrogen)
mmu-miR-29c	+ 2.54
mmu-miR-29b	+ 1.90
mmu-miR-424	+1.83
mmu-miR-15a	+ 1.65
mmu-miR-203	+ 1.45
mmu-miR-196b	+ 1.40
mmu-miR-30b	+ 1.17
mmu-miR-497	+ 1.12
mmu-miR-451	+ 1.07
mmu-miR-689	– 3.06
mmu-miR-671	– 2.10
mmu-miR-720	– 1.48
mmu-miR-705	– 1.35
mmu-miR-709	– 1.00
mmu-miR-320	– 1.00