Macrophage Colony-Stimulating Factor Modulates Matrix Metalloproteinase 2 and 9 Expression in Endometrial Epithelial Cells

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Abstract

Objective: To investigate m-Colony Stimulating Factor (CSF-1) effect on the expression of Matrix Metalloproteinases (MMP) 2 &-9 by primary endometrial epithelial cells (EECs) and Endometrial Stromal Cells (ESCs).

Design: In vitro study.

Main Outcome measure: the expression of MMP 2 &-9 by EECs and ESCs.

Results: MMP-2 expression and activity of primary epithelial cells significantly decreased in CSF-1 treated EECs compared to control vehicle (P Value<0.02). A non-significant decrease in MMP-9 RNA expression and activity was observed in EECs due to CSF-1 treatment. MMP-9 activity was significantly higher in ESCs than in EECs (P Value<0.004). 26122013.

Conclusion: This study suggests that CSF-1 modulates MMP-2 expression and activity in EECs and may regulate MMP-9 via transcriptional and post-transcriptional mechanisms. Future studies are needed to further address the complex regulation of MMP expression in endometriotic lesions.

Keywords: Macrophage Colony-stimulating factor (m-CSF); Matrix metalloproteinase (MMP); Endometrial Epithelial cells (EEC); Endometriosis

Introduction

Establishment of endometriotic lesions requires endometrial cells to adhere and invade the peritoneal mesothelium [1]. Matrix Metalloproteinases (MMPs) which mediate tissue remodeling, may contribute to these pathologic processes by degrading Extracellular Matrix (ECM). MMPs can also affect basic cellular functions (proliferation, differentiation, motility, apoptosis, etc.) by regulating the ECM proteins with which cells interact [2]. The peritoneal microenvironment had been previously suggested to play a critical role for the formation of endometriotic peritoneal lesions [3]. Several studies demonstrated that Macrophage-Colony Stimulating Factor (CSF-1) plays an important role in establishing early endometriotic lesions [4-6]. CSF-1 is secreted by eukaryotic cells including or as well as endometrial cells [7,8]. We have shown previously that CSF-1 exerts a direct effect on enhancing Endometrial Epithelial Cell (EEC) proliferation, attachment to Peritoneal Mesothelial Cells (PMCs), and invasion [5].

Digestion of Extracellular Matrix (ECM) components such as collagen by Matrix Metalloproteinases (MMPs) has been shown to play an important role in the remodeling of the ECM [9,10]. In addition, MMPs may release active growth factors and cytokines from inactive precursors bound to the ECM. The activity of these factors may then contribute to the development of endometriosis [3].

In vitro studies have shown a higher expression of MMPs 1, 2, 3, 7, and 9 in ectopic endometrium when compared with eutopic endometriotic lesions of healthy women [11]. Endometrial fragments growing in the peritoneal cavity exhibit high level of MMP-2, which may be responsible for the adhesive growth of endometrial cells [11]. The increase of MMP activity induced by peritoneal cytokines can enhance the invasive potential of the endometriotic lesions. Moreover, suppression of metalloproteinases activity inhibits the formation of ectopic lesions in nude mice [12].

In this study, we examined whether CSF-1 is involved in regulating MMP expression and activity, which would be a possible mechanism for CSF-1 induced invasion by EECs. We have specifically investigated CSF-1 effects on the expression of MMP-2 &-9 by primary EECs and ESCs.

Materials and Methods

Cell culture

Eutopic EECs from proliferative phase endometrium were obtained from an IRB-approved endometriosis tissue repository in the Department of Obstetrics and Gynecology at the University of Texas Health Science Center at San Antonio. Primary EEC’s from the proliferative phase were purified and maintained in culture as described previously EECs were grown in monolayer cultures in an enriched medium containing (volumes per liter of solution) [13].

mCDB 131 (Sigma-Aldrich, St-Louis, MO, USA, 330 ml), Medium 199 (Sigma, 335 ml), Minimal Essential Medium-alpha modification (JRH Biosciences, Lenexa, KS, USA, 222 ml), antibiotics and antimycotics (10 ml), 10 µg/ml insulin (1 ml), D-glucose 0.3 µg/ml (667 µl) and FBS (100 ml). To eliminate the effects of steroid hormones and growth factors present in FBS in the media, cells were washed with phenol red-free Hanks buffer salt solution (Invitrogen) and incubated in phenol red-free RPMI media containing charcoal-stripped (C-S), heat-inactivated (H-I) FBS (10%) for 24-48 h before treatment. CSF-1 was used at a concentration of 10 ng/ml based on our previous studies [14].

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The growth media was then replaced with the same (containing 10% C-S, H-I FBS) and treatments added. The cells lysate was used for RNA expression experiments and the cells supernatant was used for Zymography.

RNA expression studies

RNA was isolated using the Biofilin RNAeasy plus (Boston, MA, USA) with a genomic DNA removal step as per manufacturer’s protocol. Reverse Transcription (RT) was carried out using the Applied Biosystems kit (Foster City, CA, USA). Real-time PCR and subsequent analyses were carried out using Smartmix PCR beads (Cepheid, Sunnyvale, CA, USA) with 0.25% Sybr Green in the Cepheid Smart-Cycler to detect MMP-2, 9 and the housekeeping gene β-actin transcripts. MMP2 Human Forward Primer: TTGACGGAATTGACGAGCTC, (sequences, 5’ to 3’). MMP2 Human Reverse Primer: AACTCCGGAATTCCTCCATCG, MMP9 Human Forward Primer: CTTGAGGATTCGACGTG, MMP9 Human Reverse Primer: TCCACCGTGTCAACTCCAC. All primers were acquired from SIGMA Life Sciences.

Melt curve analysis was performed after each real-time PCR to ascertain PCR product specificity. PCR reactions using MMP-2, MMP-9 and β-actin primer sets gave unique melt peaks, indicative of discrete amplification products, at 87.7°C, 76.3°C, and 90.2°C, respectively. Relative expression was determined using the formula $2^{-\Delta\Delta Ct}$. Real-time PCR assays were performed in duplicate and repeated at least three times.

Zymography

Cell culture supernatants were collected and centrifuged at 800 g for 3 min to remove cells or cell debris. The culture supernatant was used to determine the enzymatic activity of Matrix Metalloproteinase 2 (MMP2) and MMP9 by using SDS-PAGE gelatin zymography as described previously using Novex Zymogram Gels (Invitrogen) [15]. Recombinant MMP2 and MMP9 were purchased from R&D systems and used as positive controls [16].

Gelatin Zymography was performed using the supernatants as described by Kleiner with slight modification [17]. Briefly, the supernatant was mixed with Novex Tris-Glycine SDS Sample Buffer (2x) 56 loading buffer and then electrophoresed (95 V; 120 min in (2x) 56 loading buffer and then electrophoresed (95 V; 120 min in) in 10% polyacrylamide containing 0.1% gelatin. The gel was stained in 0.1% Coomassie blue in 40% ethanol, 10% acetic acid (3 h at room temperature) and repeatedly destained in 10% ethanol, 7.5% acetic acid (30 min, room temperature) until visualizing digested bands in the gelatin matrix. Individual bands were quantitated by scanning densitometry using Alphalmager 2200 [18]. Both RNA expression experiments and the Zymography were repeated twice.

Statistical analysis

Given normal distribution, the data are presented as mean ± standard error of the mean (SEM). Differences between treatments were assessed by one-way ANOVA with Bonferroni correction. P<0.05 was considered statistically significant. We used the Data Add-on package for Microsoft Office EXCEL.

Results

We first examined the expression of MMP-2 and MMP-9 RNA of EEC and ESC cells treated with CSF-1 or vehicle. Little effect was observed for MMP-2 expression in primary EECs due to CSF-1 treatment compared to control vehicle (Figure 1A). MMP-2 activity was also examined by zymography gel electrophoresis. The data in Figure 1B shows a significant decrease of MMP-2 activity due to CSF-1 treatment of EECs compared to controls (P<0.02). MMP-2 expression and activity in ESCs, on the other hand, were only slightly affected by CSF-1 treatment (Figure 1).

We also examined the expression levels and activity of MMP-9 in EECs and ESCs after CSF-1 treatment. No change in MMP-9 RNA expression and activity was observed in EECs due to CSF-1 treatment (Figure 2A and B). Although a strong increase in MMP-9 RNA expression was observed in ESCs, a change in its activity in
MMP2 activity are demonstrated by Zymography in Figure 1, panel C.

Discussion

Previously we have shown that CSF has a role in establishing early endometriotic lesions including invasion in a murine model and enhancing invasiveness of endometrial cells [4,5]. Because of the involvement of MMP2 and MMP9 in invasion we examined the possibility that CSF-1 may affect MMP-2 and MMP-9 expression and activity as a way to enhance endometrial cell invasion [19]. Surprisingly, CSF-1 had a significant effect in reducing the activity of MMP-2 and to a lesser extent MMP-9 in EECs. This suggests that EECs may not be a major source for MMP-2 and MMP-9 and that CSF-1 induced invasiveness by EECs may depend on other factors during endometriotic lesion development.

Several factors and mechanisms have been suggested to play a role for endometrial cell invasion and eventual establishment of endometriotic lesions, including PMCs mediated invasion of ESCs, activated macrophages, reciprocal communication between macrophages and ESC, and recently Raf-1 kinase pathway through affecting attachment to PMC & trans-mesothelial invasion by primary EEC and ESC, or through TGFβ induced trans-mesothelial invasion [3,20,21].

While differential expression has been detected for the expression of MMPs in ectopic compared to eutopic endometrial tissue, little has been done to examine MMP activity [11,22]. Nonetheless, these differences suggest that MMP expression may be under different regulatory mechanisms in early stages during establishment of endometriotic lesions compared to later stages of established endometriotic lesions. Our data showing that MMP9 activity did not follow the increase in RNA expression may be explained by earlier studies that MMP9 RNA expression was limited to the menstrual phase but the protein was found throughout the cycle phases [23-28]. This could suggest that post transcriptional or posttranslational processes may be involved in regulating MMP-9 protein and activity levels. Future studies are needed to further examine whether potential perturbation in MMP2 and MMP9 activity are relevant in endometriosis lesion formation.

In summary, our data suggest that CSF-1 modulates MMP-2 expression and to a larger extent activity in EECs. Future studies are needed to further address the regulation of MMPs at different stages of the menstrual cycle and endometriotic lesion formation.

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