Analysis of matrix metalloproteinase-7 expression in eutopic and ectopic endometrium samples from patients with different forms of endometriosis†

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BACKGROUND: The objective of the present study was to expand our understanding of the role of matrix metalloproteinase-7 (MMP-7) in the pathophysiology of endometriosis.

METHODS: Expression levels of MMP-7 mRNA and protein in the eutopic endometrium and ectopic endometrium of patients with different forms of endometriosis were measured with immunohistochemistry and real-time RT–PCR. Endometrial tissues from patients with uterine myomas and those with macroscopically normal pelvic cavities were included as comparison groups. The real-time RT–PCR utilized endometrial cells isolated by laser capture microdissection. MMP-7 immunostained cells were quantified using a computerized image analysis system.

RESULTS: MMP-7 expression levels were significantly higher in the endometrial epithelial cells from patients with deep infiltrating endometriosis compared with those isolated from the endometria of patients with only superficial peritoneal endometriosis, uterine myomas or normal endometrium, in the proliferative, late secretory and menstrual phases. MMP-7 protein expression was detected in the ectopic endometrial epithelial cells of 13 samples of deep infiltrating endometriosis (24.5%), 11 samples of ovarian endometriosis (28.6%), 23 samples of black peritoneal lesions (76.7%) and 24 samples of red peritoneal lesions (100%). MMP-7 protein expression in epithelial cells was significantly higher in red peritoneal lesions compared with that of deep infiltrating endometriosis, ovarian endometriosis and black peritoneal lesions, in all phases of the menstrual cycle.

CONCLUSION: These findings suggest that MMP-7 expression levels vary significantly among the different forms of endometriosis.

Key words: endometriosis / endometrium / deep infiltrating endometriosis / superficial peritoneal endometriosis / MMP-7

Introduction

Endometriosis, a common cause of infertility and pelvic pain, is defined as the presence of endometrial glands and stroma within extrauterine sites (Clemeny, 1994). It affects approximately 10% of women of reproductive age (Eskenazi and Warner, 1997). However, despite extensive studies its etiology and pathogenesis are not fully understood.

In prior studies, to clarify the pathophysiology of endometriosis, we identified differentially expressed genes between samples of ectopic and eutopic endometria, as well as between eutopic endometrium from affected patients and endometrium from healthy fertile women (Matsuzaki et al., 2004a, 2005). To ensure the specificity of our cDNA microarray analysis, laser capture microdissection (LCM) was used to isolate endometrial cells from each of the samples (Matsuzaki et al., 2004a, 2005). Using this method, we identified up-regulation of matrix metalloproteinase-7 (MMP-7) mRNA during the proliferative phase in the eutopic endometrial epithelial cells obtained from patients with deep infiltrating endometriosis (Matsuzaki et al., 2005). This was in comparison with control endometrium of fertile women with macroscopically normal pelvic cavities (Matsuzaki et al., 2005). However, we did not identify MMP-7 as a differentially expressed gene in the endometrial epithelial cells of patients with deep infiltrating endometriosis compared with control endometrial tissue.
MMP-7 expression and endometriosis

Materials and Methods

Patients

Patients undergoing laparoscopy for endometriosis were recruited at the Polyclinique de l’Hôtel Dieu, CHU Clermont-Ferrand, France. As control samples, endometrial tissues were obtained from healthy fertile women with macroscopically normal pelvic cavities who underwent laparoscopic tubal ligation or reversal of tubal sterilization, as well as from patients with uterine myomas who underwent laparoscopic myomectomy. None of the women in either group had received hormonal treatments such as gonadotrophin-releasing hormone agonists or sex steroids, and none had used intrauterine contraception, for at least 6 months prior to surgery. Recruited patients had regular menstrual cycles (between 26 and 32 days) with confirmation of their menstrual history. Published endometrial dating criteria (Noyes et al., 1950) and menstrual history as well as serum progesterone levels were utilized to assess the menstrual cycle phase. Endometrial sampling was performed independently by C.D. and an independent pathologist. All patients, independent of group, were selected for the present study on the basis of consistent histological findings, menstrual history and serum 17β-estradiol and progesterone levels. Endometrial biopsies were classified into one of the four groups: proliferative (P) (Days 5–14), early- and mid-secretory (E&MS) (Days 15–23), late secretory (LS) (Days 24–28), or menstrual (M) (Days 1–3).

Samples from 137 patients who had histological evidence of pelvic endometriosis, samples from 34 patients with uterine myomas without endometriosis, and samples from 50 fertile women with macroscopically normal pelvic cavities (distribution, P: n = 10; E&MS: n = 10; LS: n = 10; M: n = 4) were used for the present analysis. Of the 137 patients with endometriosis, 53 patients had deep infiltrating endometriosis without ovarian endometriosis (with or without superficial peritoneal endometriosis) (distribution, P: n = 19; E&MS: n = 18; LS: n = 13; M: n = 3), 42 patients had ovarian endometriosis without deep infiltrating endometriosis (with or without superficial peritoneal endometriosis) (distribution, P: n = 18; E&MS: n = 12; LS: n = 9; M: n = 3) and 41 patients had only superficial peritoneal endometriosis (distribution, P: n = 15; E&MS: n = 12; LS: n = 11; M: n = 3). Samples of tissue representing deep endometriotic lesions, ovarian endometriosis or superficial peritoneal endometriosis (ectopic endometrium) were paired with eutopic endometrial samples from the same patient and analyzed. Deep infiltrating endometriosis was defined as endometriosis located 5 mm under the peritoneal surface. Deep infiltrating endometriosis was localized in rectovaginal septum (n = 29), uterosacral ligament (n = 14), bladder wall (n = 6) and bowel wall (n = 5). We also included patients with endometriotic ovarian cysts of more than 3 cm in diameter. Superficial peritoneal endometriosis was defined as endometriosis located on the peritoneal surface. Superficial peritoneal endometriotic lesions were further categorized as red, black or white according to the latest version of the American Society for Reproductive Medicine classification (sARSM) (American Society for Reproductive Medicine, 1997). There were 24 samples categorized as red lesions (P: n = 10; E&MS: n = 5; LS: n = 7; M: n = 2) and the remaining 30 samples were classified as black lesions (P: n = 12; E&MS: n = 8; LS: n = 7; M: n = 3). We excluded patients in whom the myomas had distorted the endometrial cavity. All of the patients with myomas in the present study had intramural and/or subserosal myomas. Clinical characteristics of the patients are shown in Table I.

All of the non-menstrual endometrial samples were used for both mRNA expression analysis and protein expression analysis by immunohistochemistry. Endometrial samples from the menstrual phase were used only for the immunohistochemical analysis, because of technical difficulties in performing LCM.

All the endometriotic samples were used for immunohistochemical analysis. Of these, 10 samples of deep infiltrating endometriosis (P: n = 5, E & MS: n = 3, LS: n = 2) and 10 samples of ovarian endometriosis (P: n = 5, E & MS: n = 3, LS: n = 2) were also used for mRNA expression analysis using LCM and real-time RT–PCR.

Endometrial tissue biopsies were performed just prior to surgery using an endometrial suction catheter (Pipelle, Laboratoire CCD, Paris, France). Samples of endometrial and endometriotic tissue, with the exception of the superficial peritoneal samples, were divided into two portions. The first tissue portion was fixed in 10% formalin–acetic acid and embedded in paraffin. The second portion was immediately collected in RNAlater (Ambion, Cambridgeshire, UK) and stored at −20°C until analyzed. Samples of superficial peritoneal endometriosis were fixed in 10% formalin–acetic acid and embedded in paraffin.

All tissue samples were obtained with full and informed patient consent. The research protocol was approved by the Consultative Committee for Protection of Persons in Biomedical Research (CCPPRB) of the Auvergne (France) region.

LCM and RNA extraction

From each fresh frozen tissue sample, 10-μm thick frozen sections were prepared. The sections were mounted on positively charged slides
(Super Frost Plus, Menzel GmbH, Braunschweig, Germany). Hematoxylin and eosin (H&E) staining on frozen sections was performed using the National Cancer Institute protocol (http://cgap-mf.nih.gov/Protocols/index.html) with some minor modifications as previously described (Matsuzaki et al., 2004a, 2005). Glandular epithelial cells and stromal cells were isolated from the slides using the PixCell II LCM System (Arcturus, Plaisir, France) according to the manufacturer’s instructions. Microdissected tissues were collected on optically transparent LCM Macro caps (Arcturus).

After LCM, RNA extraction was performed using the PicoPure RNA extraction kit (Arcturus) as previously described (Matsuzaki et al., 2004a, 2005). Glandular epithelial cells and stromal cells were isolated from the slides using the PixCell II LCM System (Arcturus, Plaisir, France) according to the manufacturer’s instructions. Microdissected tissues were collected on optically transparent LCM Macro caps (Arcturus).

**Examination of RNA yield and integrity**

RNA yield and integrity were analyzed using the RNA 6000 Pico kit and the Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). The Agilent 2100 bioanalyzer, a bio-analytical device based on a combination of microfluidics, microcapillary electrophoresis and fluorescence detection, provides a platform to record the size distribution of molecules, RNA, DNA and protein, in a digital format (Schroeder et al., 2006). The RNA 6000 Pico kit allows the determination of the integrity of very small amounts of RNA as well as the estimation of the quantity of the isolated RNA, which has a linear range of 200–5000 pg/μl.

The RIN (RNA integrity number) value was greater than six in all the samples prior to use.

**Quantitative real-time RT–PCR**

Quantitative real-time RT–PCR with a Light Cycler was performed on total RNA from microdissected tissues as previously described (Matsuzaki et al., 2004a, 2005). Total RNA (50 ng) was subjected to an RT reaction using Superscript II Reverse Transcriptase (Invitrogen). Quantitative real-time PCR was performed in a Light Cycler System using the FastStart DNA Master SYBR Green I kit as recommended by the manufacturer (Roche, Mannheim, Germany). In a total volume of 20 μl, each reaction contained 2 μl SYBR Green I reaction mix (consisting of Taq DNA-polymerase reaction buffer, dNTP mix, SYBR Green I, MgCl₂ and Taq DNA polymerase), 0.5 μM of primer (MMP-7: forward 5’-GGT TAC CCA AAC TCA AGG -3’, reverse 5’-CTT TGA CTA CAC TAA TCG -3’, GAPDH: forward 5’-TGC ACC ACA CTG CTT TAG AAG CCA AAC TCA AGG -3’, reverse 5’-CTT TGA CTA CAC TAA TCG ATC CAC -3’, GAPDH: forward 5’-TGC ACC ACA CTG CTT TAG AAG CCA AAC TCA AGG -3’, reverse 5’-CTT TGA CTA CAC TAA TCG ATC CAC -3’), 4 mM MgCl₂ and 2 μl cDNA, and standard or nuclease free water as a negative control. Quantification of the targets in the unknown samples was performed using a relative quantification method with external standards. The target concentration was expressed relative to the concentration of a reference housekeeping gene, GAPDH. After each run, a melting curve analysis was performed to verify the specificity of the PCR reaction. The procedure was repeated three times independently to ensure the reproducibility of the results. All of the samples with a cycle threshold coefficient of variation value higher than 5% were retested.

**Immunohistochemistry**

We conducted a preliminary study to perform a comparative analysis of two different antibodies against MMP-7 (mouse monoclonal antibody, Thermo Scientific, Illkirch, France; rabbit polyclonal antibody, Chemicon International Inc, Temecula, CA) in selected samples. Using these two different antibodies, consistent results were obtained.

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**Table I Clinical characteristics of patients**

<table>
<thead>
<tr>
<th></th>
<th>Endometriosis</th>
<th>Uterine fibroma</th>
<th>Healthy fertile women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DE</td>
<td>OE</td>
<td>SE</td>
</tr>
<tr>
<td>Number of cases</td>
<td>54</td>
<td>42</td>
<td>41</td>
</tr>
<tr>
<td>Agea</td>
<td>31.5 (21–39)</td>
<td>30.5 (20–39)</td>
<td>31.0 (22–39)</td>
</tr>
<tr>
<td>Parityb</td>
<td>0 (0–1)</td>
<td>0 (0–1)</td>
<td>0 (0–1)</td>
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<tr>
<td>rASRM stageb</td>
<td></td>
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<td></td>
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<tr>
<td>Stage I (n = 15)</td>
<td></td>
<td></td>
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<tr>
<td>Stage III (n = 19)</td>
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<td>Stage IV (n = 10)</td>
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<tr>
<td>Stage III (n = 14)</td>
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<td></td>
</tr>
<tr>
<td>Stage IV (n = 15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of fibromaa</td>
<td></td>
<td></td>
<td>1 (1–5)</td>
</tr>
<tr>
<td>Dominant fibroma diameter (cm)b</td>
<td></td>
<td></td>
<td>5.1 (2.1–8.5)</td>
</tr>
<tr>
<td>Location of dominant fibroma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Fundus</td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Posterior</td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Type of dominant myoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intramural</td>
<td></td>
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<td>27</td>
</tr>
<tr>
<td>Subserosal</td>
<td></td>
<td></td>
<td>7</td>
</tr>
</tbody>
</table>

DE, patients with deep infiltrating endometriosis without ovarian endometriosis; OE, patients with ovarian endometriosis without deep infiltrating endometriosis; SE, patients with only superficial peritoneal endometriosis.

a Median (range).
b Revised American Society for Reproductive Medicine classification (rASRM) (American Society for Reproductive Medicine, 1997).
In the present study, immunohistochemical staining was performed on paraffin sections with mouse monoclonal antibodies directed against MMP-7 (Thermo Scientific) by using the following protocol. Sections were deparaffinized and antigen retrieval was performed in 0.01 M sodium citrate (pH 6.0) for 3 min at full pressure using a pressure cooker. Sections were then rinsed in distilled water and treated with 3% hydrogen peroxide solution for 5 min to inhibit endogenous peroxidase activity. After rinsing in 0.01 M phosphate-buffered saline (PBS, pH 7.2), sections were incubated overnight at 4°C with the primary antibody (diluted 1:100 with 3% bovine serum albumin). Negative controls were performed by replacing primary antibodies with normal mouse IgG or IgG2b (DAKO) diluted to the same concentration. After rinsing in PBS, sections were incubated with peroxidase-labeled anti-mouse IgG (DAKO EnVision, DAKO Corp.) for 30 min. Sections were then washed with PBS, colored with aminocarbazole substrate, counterstained with Mayer’s hematoxylin and mounted.

**Quantification of MMP-7 immunostained cells**

To quantify immunostained cells objectively, we utilized a computerized image analysis system consisting of a light microscope (Leica, Lyon, France) (×40 objective, ×10 ocular) and a color charge coupling device camera (Sony, Paris, France) connected to a SAMBA 2005 computer analysis system (Alcatel-TITN, Grenoble, France). Several parameters per sample were computed: the percentage of immunostained surface (compared with the counterstained surface) (Matsuzaki et al., 2004b), the mean staining intensity and an immunostained score (percentage of immunostained surface × mean staining intensity). In all the samples of endometrial tissue, 10 non-overlapped fields were analyzed. Because of the tissue heterogeneity in endometriosis, we analyzed all glandular and stromal cells within each sample. The number of areas analyzed in the endometriotic tissues varied from 6 to 26 areas per sample.

**Statistical analysis**

The Statview 4.5 program (Abacus Concepts, Inc., Berkeley, CA, USA) was used for statistical analysis. Comparisons were made using the one-way analysis of variance (ANOVA) following Scheffé’s method or the Wilcoxon sign rank test. Statistical significance was defined as $P < 0.05$.

**Results**

**Endometrial samples**

**Proliferative phase**

MMP-7 mRNA and protein expression levels were significantly higher in the epithelial cells of patients with deep infiltrating endometriosis in comparison with patients with superficial peritoneal endometriosis, uterine myomas, or macroscopically normal pelvic cavities (Tables II and III, one-way ANOVA, Fig. 1). There were no significant differences in either the mRNA or protein expression levels between the epithelial cells isolated from patients with deep infiltrating endometriosis and those from patients with ovarian endometriosis (Tables II and III, one-way ANOVA). There were no significant differences in the mRNA and protein expression levels of stromal cells among the different groups (Tables II and III, one-way ANOVA).

**Early–mid-secretory phases**

MMP-7 mRNA and protein expression levels were significantly higher in the epithelial cells from patients with deep infiltrating endometriosis

| Table II Results of quantitative real-time RT–PCR for MMP-7 mRNA expression in microdissected endometrial epithelial and stromal cells from patients with and without endometriosis |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Endometriosis   | Uterine fibroma | Healthy control |
| Endometrial cycle | DE              | SE              | E               | SE              | NA              |
| P                | $0.19$, $1.0(19)$ | $0.16$, $0.5(18)$ | $0.16$, $1.3(18)$ | $0.16$, $0.6(12)$ | $0.07$, $0.4(12)$ |
| E-MS             | $1.7$, $0.0(15)$     | $1.7$, $0.2(15)$     | $1.7$, $0.0(15)$     | $1.7$, $0.0(15)$     | $1.7$, $0.0(15)$     |
| LS               | $2.2$, $0.0(18)$     | $2.2$, $0.0(18)$     | $2.2$, $0.0(18)$     | $2.2$, $0.0(18)$     | $2.2$, $0.0(18)$     |
| M                | $0.15$, $0.4(13)$    | $0.15$, $0.4(13)$    | $0.15$, $0.4(13)$    | $0.15$, $0.4(13)$    | $0.15$, $0.4(13)$    |
| NA               | $0.15$, $0.4(13)$    | $0.15$, $0.4(13)$    | $0.15$, $0.4(13)$    | $0.15$, $0.4(13)$    | $0.15$, $0.4(13)$    |

Expression levels of MMP-7 mRNA are given relative to the expression levels of the reference gene, GAPDH. All data are expressed as means ± SEM.

Values in parentheses indicate the number of samples examined for MMP-7 mRNA expression. NA, not available. P, proliferative phase; E-MS, early mid-secretory phase; M, menstrual phase; DE, deep infiltrating endometriosis; OE, ovarian endometriosis; SE, superficial peritoneal endometriosis; E, epithelial cells; S, stromal cells.
Table III Immunostained score for MMP-7 in endometrium of patients with and without endometriosis

<table>
<thead>
<tr>
<th>Menstrual cycle</th>
<th>Endometriosis</th>
<th>Uterine fibroma</th>
<th>Healthy control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DE</td>
<td>OE</td>
<td>SE</td>
</tr>
<tr>
<td>P</td>
<td>3.79 ± 2.9a</td>
<td>0.1 ± 0.05 (19)</td>
<td>29.6 ± 2.1 (18)</td>
</tr>
<tr>
<td>E-MS</td>
<td>6.1 ± 1.5b</td>
<td>0.07 ± 0.1 (18)</td>
<td>2.4 ± 1.3 (12)</td>
</tr>
<tr>
<td>LS</td>
<td>31.5 ± 1.8a</td>
<td>0.4 ± 0.3 (13)</td>
<td>14.1 ± 1.3 (9)</td>
</tr>
<tr>
<td>M</td>
<td>38.9 ± 1.7a</td>
<td>0.4 ± 0.4 (3)</td>
<td>14.7 ± 1.7 (3)</td>
</tr>
</tbody>
</table>

All data are expressed as mean ± SEM.
Values in parentheses indicate the number of samples examined for MMP-7 protein expression.

*P* < 0.0001 versus SE, uterine fibroma and healthy control.

**Ectopic versus matched eutopic endometrium**

We detected no MMP-7 mRNA expression in the microdissected endometrial epithelial cells from 10 samples of deep infiltrating endometriosis or from 10 samples of ovarian endometriosis. MMP-7 protein expression was detected in the non-epithelial cell of the P phase (P < 0.02). E-MS, SE, and M (Table IV, one-way ANOVA). There were no significant differences in the MMP-7 protein expression levels of the epithelial cells in the deep infiltrating endometriosis compared with those in patients with ovarian endometriosis and those with macroscopically normal pelvic endometriotic epithelial cells (Table IV, one-way ANOVA). There were no significant differences in the stromal cells among the different groups (Tables II and III, one-way ANOVA). There were no significant differences in the mRNA or protein expression levels of the stromal cells in the struma among the different groups (Tables I and III, one-way ANOVA). There were no significant differences in the RNA or protein expression levels of the epithelial cells in the deep infiltrating endometriosis compared with those in patients with ovarian endometriosis (Table II, and III, one-way ANOVA). There were no significant differences in the mRNA or protein expression levels of the epithelial cells in the deep infiltrating endometriosis compared with those in patients with ovarian endometriosis (Table II, and III, one-way ANOVA). There were no significant differences in the mRNA or protein expression levels of the epithelial cells in the deep infiltrating endometriosis compared with those in patients with ovarian endometriosis (Table II, and III, one-way ANOVA). There were no significant differences in the mRNA or protein expression levels of the epithelial cells in the deep infiltrating endometriosis compared with those in patients with ovarian endometriosis (Table II, and III, one-way ANOVA).
MMP-7 expression and endometriosis

Discussion

The implantation theory, first proposed by Sampson (1927), for the development of pelvic endometriosis, has been tested in animal models. MMP-7 has been shown to be the dominant metalloproteinase during the initial development of endometriosis in a baboon model (Fazleabas et al., 2002). In addition, a recent study clearly demonstrated that MMP-7 mRNA was identified in host peritoneal tissues during the development of endometriosis in a nude mouse model (Hull et al., 2008). If the implantation theory is correct, the present findings might provide some new insights into the pathophysiology of endometriosis.

At least, three processes appear to be critical to the establishment of endometriosis, according to the implantation theory: invasiveness, tissue remodeling and interactions between the ectopic endometrium and the surrounding peritoneal tissues (Giudice and Kao, 2004; Hull et al., 2008). First, with regard to the invasion of endometrial cells into the mesothelial cell layer, elevated expression levels of MMP-7 in eutopic endometrial epithelial cells might be involved in the initial development of deep infiltrating endometriosis. The present study demonstrated significantly higher levels of MMP-7 expression in endometrial epithelial cells from patients with deep infiltrating endometriosis in comparison with patients with only superficial peritoneal endometriosis or uterine myomas, and healthy fertile controls in all phases of the menstrual cycle. Invasion appears to be critical for the development of endometriosis. Indeed, the invasiveness of endometriotic cells has been shown to be comparable to that of a metastatic bladder carcinoma cell line in an in vitro study (Gaetje et al., 1995). In contrast, it has been shown that cells from the eutopic endometrium do not invade (Gaetje et al., 1995), although these studies did not account for differences based on the type of endometriosis. Further in vitro studies are necessary to investigate whether endometrial epithelial cells from patients with deep infiltrating endometriosis are more invasive than those from patients with only superficial peritoneal endometriosis.

When cells invade, tissue remodeling is often triggered. This is the case with superficial red remodeling, which undergo some active tissue remodeling and also exhibit some invasiveness (Zeitvogel et al., 2008)
et al., 2001), whereas it is not clear if already established deep infiltrating endometriosis and ovarian endometriosis might have a similar nature as red peritoneal lesions. In the present study, we detected only very low or no MMP-7 protein expression in the samples of deep infiltrating endometriosis and ovarian endometriosis. In contrast, MMP-7 expression was elevated in the red peritoneal lesions. Elevated MMP-7 expression was detected not only in the stromal and endothelial cells, but also in the fibromuscular tissues surrounding the red superficial peritoneal lesions. MMP-7 is overexpressed in a variety of cancers, and is important for tumor progression (Yamamoto et al., 1999; Crawford et al., 2002; Zeng et al., 2002; Jiang et al., 2005). MMP-7 promotes the in vitro invasiveness of cancer cells (Wang et al., 2005, 2006), and its expression correlates with the in vivo invasiveness of cancer tissues (Adachi et al., 2001; Masakiet al., 2001; Gu et al., 2005). However, MMP-7 is not in itself responsible for tissue remodeling and invasiveness (Stamenkovic, 2003; Wolf and Friedl, 2006) and it is likely that other factors play a role in deep infiltrating and ovarian endometriosis. Further studies are needed to identify these factors as this will aid in the development of treatments customized for each type of endometriosis. In addition, further studies will be necessary to investigate the activity of MMP-7 as well as expression of other MMPs and tissue inhibitors of metalloproteinases in eutopic and ectopic endometria of patients with different forms of endometriosis.

The third factor in the establishment of endometriosis is the interactions between endometrial cells and the surrounding peritoneal tissues (Hull et al., 2008). In contrast, black lesions had significantly lower expression of MMP-7. There is growing evidence that black and red peritoneal lesions may be different stages of the spontaneous evolution of endometriotic implants, with red lesions being the first stage (Nisolle and Donnez, 1997; Fazleabas et al., 2002). Thus, the present findings suggest that MMP-7 protein expression may be down-regulated during the evolution of peritoneal endometriotic implants, as active red lesions transition into inactive black lesions. This conclusion, however, does not explain why MMP-7 expression is nearly undetectable in deep infiltrating endometriosis and ovarian endometriosis. A possible explanation might be that MMP-7 expression in endometriosis is modulated depending on the microenvironment as the lesion evolves. An alternate explanation is that the implantation theory might not be applied in these types of endometriosis. Elevated MMP-7 expression in endometrial epithelial cells of patients with deep infiltrating endometriosis may actually be a consequence of the presence of deep infiltrating endometriosis, as shown in baboon and mouse models for endometriosis (Gashaw et al., 2006; Kim et al., 2007; Lee et al., 2009; Winterhager et al., 2009). Further studies both in vitro and in vivo will be required to describe the interactions between endometriotic cells and their microenvironment as the disease evolves.

The present findings differ from those of previous studies. Bruner-Tran et al. (2002) demonstrated abundant MMP-7 mRNA expression in ovarian endometriosis by in situ hybridization. Although we investigated only 10 samples of ovarian endometriosis for MMP-7 mRNA, we were unable to replicate this result using the more sensitive combined method of LCM and real-time RT-PCR. In addition, we confirmed very low or no MMP protein expression in a large number of

![Figure 2](image-url)
ovarian endometriosis samples, which was not investigated in the previous study (Bruner-Tran et al., 2002). Nap et al. (2004) demonstrated that the expression pattern of MMP-7 protein appeared to be similar between rectovaginal nodules and the menstrual endometrium. However, they investigated only five archival samples of rectovaginal nodules and differences in the immunohistochemical staining technique between their study and ours were likely present. Unlike previous studies which quantified immunostained cells subjectively (Mizumoto et al., 2002; Nap et al., 2004), we applied a computerized image analysis system to objectively quantify immunostained cells.

Though we were unable to completely describe the role of MMP-7 in all forms of endometriosis, we were able to establish an association between superficial endometriosis and MMP-7 expression. Our findings suggest that treatments targeting MMP-7 may be effective in red peritoneal lesions, but may have little effect on black peritoneal lesions, ovarian endometriosis or deep infiltrating endometriosis. This finding may be corroborated by what has been shown in animal models of endometriosis (Falconer et al., 2006). A baboon study demonstrated that the main effect of anti-TNF-mAb (cSN) treatment appears to be a significant decrease in number, surface area and volume of preferentially red lesions (Falconer et al., 2006). An in vitro study has shown that MMP-7 expression is reduced by the recombinant human TNF receptor in an endometriotic epithelial cell line derived from red peritoneal lesions (Grund et al., 2008). These findings and our present findings suggest that TNF-mAb (cSN) may preferentially target red lesions over the other forms of endometriosis given its mechanism of action.

Our findings as well as those published by Klemmt et al. (2007) suggest that biological characteristics of endometriotic cells might differ among the different forms of endometriosis. These findings and the present findings imply that a single universal treatment may not apply to all of the different forms. Additionally, when one interprets the results of in vitro studies, or animal studies involving novel therapeutic strategies, one should consider the endometriosis form tested. While animal or cell culture models are invaluable for preliminary investigations into the pathophysiology of endometriosis or novel therapeutic strategies for it, they likely fall short of duplicating the microenvironment surrounding a developing endometriotic lesion in its natural state. An additional limitation of the current animal models is that most approximate only peritoneal endometriosis (Fazleabas et al., 2002; Grümmer, 2006).

In conclusion, MMP-7 expression levels were significantly higher in the endometrial epithelial cells of patients with deep infiltrating endometriosis compared with those of patients with only superficial peritoneal endometriosis, uterine myomas or macroscopically normal pelvic cavities analyzed in the P, LS and M phases. MMP-7 protein expression, however, was nearly undetectable in deep infiltrating endometriosis and ovarian endometriosis. In contrast, significantly higher MMP-7 protein levels were detected in red peritoneal lesions. These findings suggest that the pathophysiology might differ among the different types of endometriosis, thus necessitating the development of type-specific treatments.

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