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Thesis :

The Role of MMP-7 (PUMP-1, Matrilysin) in the Pathogenesis of Adenomyosis Uteri

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ABSTRACT **We are presently investigating the involvement of matrix metalloproteinases (MMPs), MMP-7 (Pump-1, matrilysin) in particular, in the development of adenomyosis uteri. To examine whether progression of adenomyosis uteri might correlate with an expression of MMP enzyme activity, we initially utilized an animal model for adenomyosis uteri induced in pituitary-grafted mice. Gelatin zymography identified three distinct enzyme activities of MMP (20-30 kDa, 50 kDa, and 70 kDa) in extracts obtained from a uterus with adenomyosis, whereas no enzyme activities of MMP were demonstrated in those obtained from normal uterus. Since MMP enzyme activity of 20-30 kDa corresponds to the reported low molecular weight MMP-7 in an activated form (19 kDa) and an inactive form (28-29 kDa), the expression of MMP-7 mRNA was investigated by RT-PCR combined with Southern blot analysis. The result showed that a band of 440 bp PCR product was demonstrated in comparable amounts in both normal mouse uterus and mouse adenomyosis uteri, suggesting that an enhanced enzyme activity of MMP-7 observed in adenomyosis uteri may not be controlled solely at mRNA level. This suggestion was supported by subsequent human studies, the result of which showed a band of 440 bp PCR product in comparable amounts in both adenomyosis uteri and normal uterine endometrium. To examine the expression of MMP-7 protein in human adenomyosis uteri, Western blot analysis of the immunoreactive MMP-7 was performed in the same samples with those used in the study on the expression of the MMP-7 mRNA. Higher amounts of immunoreactive MMP-7 were observed in the epithelial cells of uterine adenomyosis than in those of normal uterine endometrium. The semi-quantitative scoring for immunoreactive MMP-7 staining showed that MMP-7 expression observed in adenomyosis uteri was menstrual cycle-independent and significantly higher than that in normal uterine endometrium, in which MMP-7 expression was menstrual cycle-dependent with its higher expression during the proliferation phase than during the early to mid secretory phase of the menstrual cycle. The data, taken together, are consistent with an idea that epithelial synthesis of MMP-7 in uterine adenomyosis contributes to its invasion into the myometrium.**

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Key words : Adenomyosis uteri, Matrix metalloproteinases (MMPs), MMP-7 (PUMP-1, Matrilysin), Pituitary-grafted mice

1. Introduction

Adenomyosis uteri is characterized by the presence and growth of functioning ectopic endometrial tissue that consists of endometrial glands and stroma within the myometrium (direct endometriosis, endometriosis interna). The pathogenesis of adenomyosis uteri is not known. The complications associated with adenomyosis uteri include pain, dysmenorrhea, and infertility[1]. Thus, it is worthwhile to elucidate the mechanisms responsible for the development of adenomyosis uteri for the treatment and prophy-

laxis of this lesion. Histopathology of adenomyosis shows an invasive growth of endometrial glands and stroma into the myometrium, causing its derangement and disintegration. Since the invasion of adenomyosis uteri into the myometrium is likely to be associated with the remodeling of the myometrium involved. Many biological activities including tissue remodeling are affected profoundly by the extracellular matrix, a complex and dynamic meshwork of proteins and proteoglycans that provides structural support to organisms. Tissue remodeling, which occurs in both physiological and pathological processes, such as embryonic development[2], wound healing[3], bone remodeling[4], and tumor invasion[5-14], has been reported to involve matrix metalloproteinases (MMPs), a multi-gene family of enzymes whose substrates are extracellular matrix components. The expression of MMPs has been reported in the normal endometrium in association with tissue remodeling or breakdown in the menstrual cycles, and in the postpartum uterus, in which a rapid degradation of the extracellular matrix occurs in association with uterine involution[15-19]. So far as adenomyosis uteri is concerned, however, it is not precisely known whether the induction and activation of MMPs are involved in its development. The purpose of this study was to examine whether MMPs might be involved in the pathogenesis of adenomyosis uteri. To identify MMPs associated with the development of adenomyosis uteri, we initially used a mouse model for adenomyosis uteri that had been induced by intrauterine pituitary grafting. The result showed that the expression of MMP-7 enzyme activity is significantly higher in mouse adenomyosis uteri than normal uterine endometrium. We subsequently investigated expression of MMP-7 enzyme activity, MMP-7 mRNA and immunoreactive MMP-7 in human adenomyosis uteri.

2. Materials and method

2. 1 Materials

2. 1. 1 Mice

Female mice of SHN strain were used in this study. They were housed in plastic cages with wood shavings in a temperature-monitored and light-controlled (12 h/day) room, and were fed with commercial diet and tap water *ad libitum*.

2. 1. 2 Patients and tissue specimens for immunohistochemistry

Both tissues of adenomyosis uteri and intact endometrium were obtained from 40 women undergoing surgical hysterectomy (Table 1). They had not undergone hormonal therapy before surgical hysterectomy. Sections from intact endometrium were stained with hematoxylin and eosin, and dated for menstrual cycle.

2. 1. 3 Antibodies

For obtaining anti-MMP-7 polyclonal antibody, rabbits were immunized by standard procedures with a peptide synthesized from MMP-7 cDNA. Monoclonal antibody for MMP-7 was purchased from Fuji pharmaceutical Co., Tokyo, Japan.

2. 2 Methods

2. 2. 1 Induction of adenomyosis uteri

Induction of adenomyosis uteri was performed according to the method reported by Mori T. *et al*[20,21]. In brief, a single pituitary obtained from an age-matched male mouse of SHN strain was trans-

Table 1 *The cases examined*

Phases in the menstrual cycle	No. of cases
Proliferative phase	23
Early to mid secretory phase	17

planted into the right uterine horn of a female mouse of SHN strain at the age of 5-6 weeks old. Three months after the transplantation, the recipient mice were sacrificed and the uteruses were obtained. A part of the right horn of the bicornous uterus was used for histological study, and the remaining right uterus horn was kept at −80°C for measurement of MMP activity and RNA preparation.

2. 2. 2 Gelatin zymography

Gelatinase activity was measured by zymography[22]. In brief, Uterine extracts (20 to 40 mg protein) were subjected to electrophoresis in 10% polyacrylamide gels containing 1 mg/m*l* gelatin. Samples were diluted in nonreducing sample buffer [final concentration, 1% sodium dodecyl sulfate (SDS) and 5% glycerol] and electrophoresed for 3 h at a constant current of 10 mA. The gels were washes for 2 h in 2.5% Triton X-100 to remove SDS, rinsed three times with distilled water, and then incubated for 20 h in 50 mM Tris-HCl at pH 8.0 with 5 mM $CaCl_2$. After Coomassie blue staining, the density of the digested band was measured with a scanning densitometer.

2. 2. 3 RT-PCR

Total RNAs were extracted from frozen tissues by acid guanidinium thiocyanate-phenol-chloroform extraction[23]. Messenger RNAs were purified from total RNA by a mRNA purification kit (BIOMAG). Randomly primed cDNAs were prepared by Super Script II reverse transcriptase (GIBCO, USA) following PCR amplification. MMP-7 cDNA was amplified using the following primer pairs[24]: (sense primer) 5' AAC TCC CGC GTC ATA GAA ATA ATG (antisense primer) 3' TGG GTT TCT TAC CGG TTC AAG TAC. PCR amplification was done in the following conditions: 1 cycle of denatured at 92°C for 2 min, annealed at 52°C for 1 min, extended at 72°C for 1 min. And there were a total of 38 amplification cycles consisting of 30s at 92°C, 1 min at 52°C, 2 min at 72°C, and 30s at 92°C, 1 min at 52°C, a final extension time of 10 min at 72°C was used in all cases.

Following amplification, the products are analyzed on a 1.0% agarose gel with appropriate DNA markers. Bands were visualized after staining with ethidium bromide.

2. 2. 4 Southern blotting hybridization analysis of RT-PCR product with mouse MMP-7 oligonucleotide probe

The PCR products were electrophoresed on 1.0% agarose gel, and blotted to positively charged nylon membrane in a solution of 0.4 M NaOH. Blotted membranes were hybridized with cDNA probe in 50% formamide/50 mM sodium phosphate buffer pH 7.0/5×SSC/2% blocking reagent/0.1% Lauroylsarcosine/10 mg/m*l* salmon sperm DNA/ 7% SDS at 42°C and washed in 2×SSC/0.1% SDS at room temperatture for 5 min 2 times and 0.1×SSC/0.1% SDS at 68°C for 15 min 2 times. The probe for mouse MMP-7 was partial cDNAs obtained from PCR product of mouse postpartum uterus, amplified in pMOSblue and XbaI, purified in HPLC, and digoxigenin-labeled[25]. Hybridized membranes were reacted in solution containing anti digoxigenin antibody (1 : 10000) for 30 min and washed for 15 min twice. Films were exposed for 30 min.

2. 2. 5 Polyclonal antibody production and Western blot analysis

For obtaining anti-MMP-7 polyclonal antibody, rabbits were immunized by standard procedures with a peptide synthesized from MMP-7 cDNA. The electrophoresed gel was transferred to a nitrocellulose membrane; the blot was incubated for 1 h with a 1 : 200 dilution of the anti-MMP-7 polyclonal antibody. A goat anti-rabbit immunoglobulin was used as the secondary antibody. The immunoreactive MMP-7 was detected by ECL Western blotting detection reagents. (Amersham, USA)

2. 2. 6 Immunohistochemical staining

Tissues were fixed with 20% formaldehyde and embedded in paraffin blocks. Paraffin sections (5 μm) were reacted with a 1 : 300 dilution of the anti-MMP-7 polyclonal antibody for 2 h. A goat anti-rabbit Immunoglobulin was used as the secondary antibody. Avidin-biotin-HRP complex solution was

used for the detection of the immunoreactive MMP-7. Just the same sections were reacted with a 1 : 200 dilution of the anti-MMP-7 IgG for 2 h. Biotinated anti-mouse secondary antibody and avidin-biotin peroxidase complex were reacted by using Nichirei Histofine SAB-PO(R) kit (NICHIREI, Tokyo, Japan). Slide were then reacted in a mixture of diaminobenzidine and hydrogen peroxide, which yielded a brown reaction product. Negative controls were prepared by substituting non-immune mouse serum at equivalent protein concentrations for the primary antibody.

2. 2. 7 Evaluation of staining

Evaluation of staining was performed in five non-overlapping fields of view per specimen in a systematic random sampling pattern. The intensity of staining for MMP-7 expression was scored as follows: 3 for intense staining; 2 for moderate staining; 1 for weak staining; 0 for staining not significantly greater than that of the control slide.

2. 2. 8 Statistical analysis

The scores for the intensity of staining were compared using the Mann-Whitney U test for nonparametric data.

3. Results

3. 1 Induction of adenomyosis uteri in pituitary-grafted mice

Three months after the intrauterine pituitary grafting, mice were sacrificed, and the right uterine horn was used for histological study. Seven out of the ten grafted mice developed adenomyosis uteri. Three mice had highly invasive adenomyosis uteri that invaded not only the inner layer but also the outer layer of the myometrium and even protruded into the pelvic cavity, whereas the remaining four mice had focal adenomyosis that invaded only the inner layer of the myometrium, leaving the outer layer intact, as is shown in Figs. 1 and 2.

3. 2 Expression of MMP enzyme activity in the mouse adenomyosis uteri

Gelatin zymography was used to investigate whether enzyme activities of MMP might be involved in adenomyosis uteri in pituitary-grafted mice. The representative results are shown in Fig. 3. Three bands of gelatinase activities (the bands of 20-30 kDa, 50 kDa, and 70 kDa) were present in extracts from uterus with the highly invasive adenomyosis uteri (lane 1), whereas no gelatinase activity was present in extracts from normal uterus (lane 2). These gelatinase activities disappeared in the presence of EDTA, suggesting that MMP activities of 20-30 kDa, 50 kDa, and 70 kDa are expressed in mouse

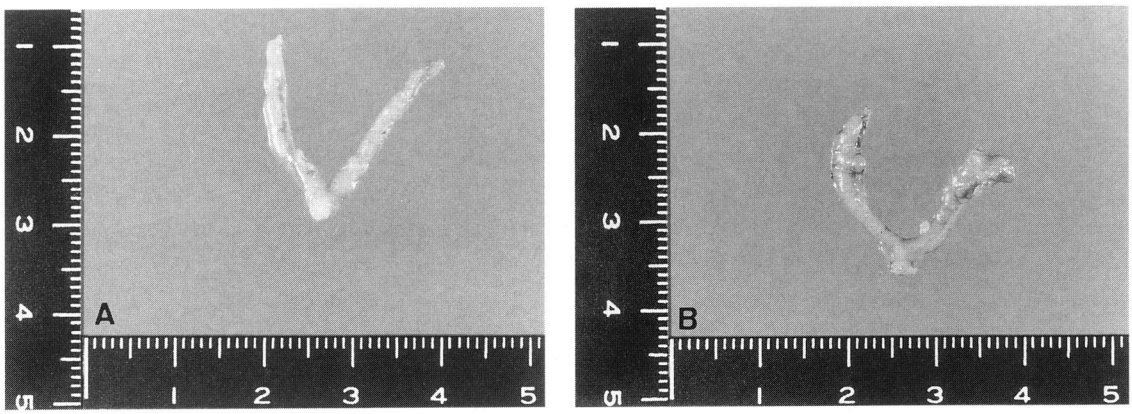

Fig. 1 Macroscopy of adenomyosis uteri induced in pituitary-grafted mice. (A) Normal mouse uterus. (B) Mouse uterus with adenomyosis uteri. Tumorous protrusions of adenomyosis uteri are visible.

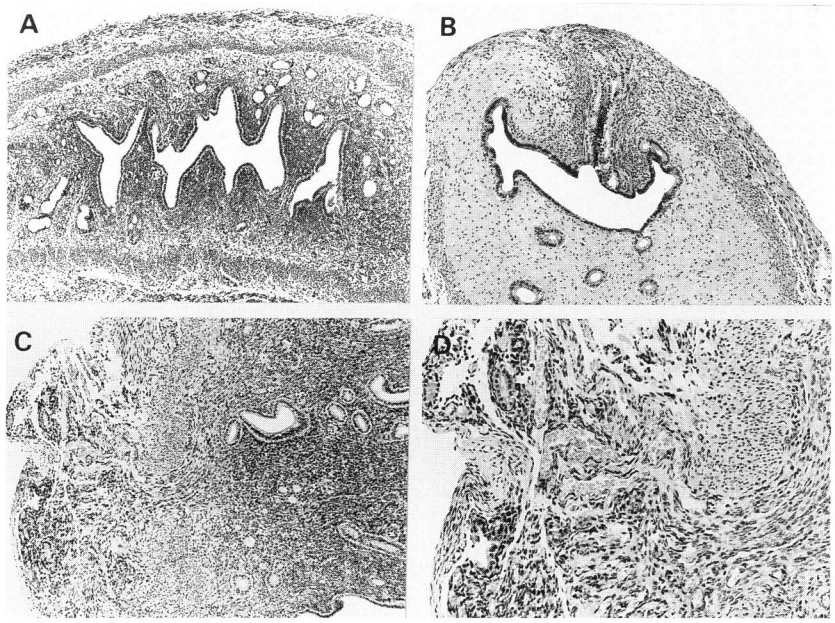

Fig. 2 Microscopy of adenomyosis uteri induced in pituitary-grafted mice. Staining was with hematoxylin and eosin. (A) Normal uterus with endometrium and double (inner and outer) layers of the myometrium. ×20; (B) Uterus with focal adenomyosis, which invades only the inner muscle layer of the myometrium, leaving the outer layer intact. ×20; (C) Uterus with highly invasive adenomyosis, which invades both the inner and the outer layers of the myometrium. ×20. Extrusion of adenomyosis uteri into the pelvic cavity is visible; (D) Uterus with highly invasive adenomyosis, ×40. Invasion of adenomyosis through the double layers of myometrium is visible.

adenomyosis uteri.

3. 3 Detection of MMP-7 mRNA in mouse adenomyosis uteri by RT-PCR and Southern blotting hybridization analysis

Considering the reported molecular weight of MMP-7 in an activated form (19 kDa) and an inactive form (28-29 kDa)[19], the enzyme activity of MMP identified in the band of 20-30 kDa in gelatin zymography may possibly be identical with the MMP-7. Accordingly we next examined whether MMP-7 mRNA might be expressed in the mouse adenomyosis uteri by RT-PCR, as is shown in Fig. 4A. The result showed that the band of 440 bp PCR product was present in normal mouse uterus (lane 1), and mouse adenomyosis uteri (lane 3). Mouse postpartum uterus (lane 2) and mouse kidney (lane 4) were used as positive controls for the gene expression.[19,26,27].

The band of 440 bp PCR product was hybridized with mouse oligonucleotide MMP-7 probe by

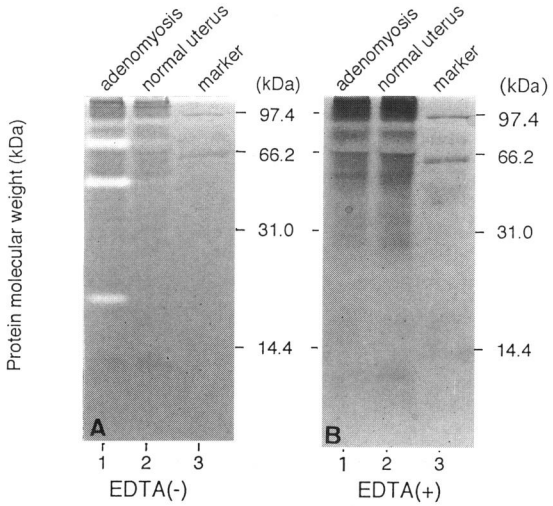

Fig. 3 Gelatinase activity in mouse adenomyosis uteri. The enzyme activities were measured in the absence or in the presence of EDTA in extracts from uterus with highly invasive adenomyosis (lane 1), and those from normal uterus (lane 2).

Southern blotting hybridization (Fig. 4B). MMP-7 mRNA was clearly detected in comparable amounts in normal mouse uterus (lane 1) and uterus with adenomyosis uteri (lane 3). The results suggest that the overexpression of enzyme activity observed in mouse adenomyosis uteri may not be controlled solely at mRNA level.

3. 4 Detection of MMP-7 mRNA in human adenomyosis uteri by RT-PCR

The involvement of MMP, MMP-7 in particular, in the development of adenomyosis uteri in the mice adenomyosis uteri led us to investigate expression of MMP-7 in human adenomyosis uteri. The expression of MMP-7 mRNA in adenomyosis uteri was examined by RT-PCR, as is shown in Fig. 5. Colon cancer was used as positive control for the gene expression[13,28]. The result showed that a band of 440 bp PCR product was present in normal uterine endometrium (lane 1), adenomyosis uteri (lanes 2 and 3), uterine adenocarcinoma (lane 4). Thus, the expression of MMP-7 mRNA is present in human adenomyosis uteri as well as in human normal endometrium.

3. 5 Western blot analysis of MMP-7 expression in human adenomyosis uteri

The comparable expression of MMP-7 mRNA demonstrated in adenomyosis uteri and normal uterine endometrium led us to examine whether the activity of MMP-7 might be regulated at post-transcriptional levels. Accordingly we performed Western blot analysis in the same samples with those used in the study on the expression of the MMP-7 mRNA. The representative results are shown in Fig. 6. Larger amounts of immunoreactive MMP-7 (both 28 kDa and 20 kDa bands) were detected in human adenomyosis uteri (lanes 2 and 3) in comparison with those in normal uterine endometrium (lane 1). The data suggest that an enhanced expression of MMP-7 protein in human adenomyosis uteri may be ascribed to the up-regulation at post-transcriptional levels.

3. 6 Effect of menstrual cycle on the expression of MMP-7 in human adenomyosis uteri

MMP-7 is specifically expressed only in the glandular cells epithelium of the endometrium. In the normal endometrium MMP-7 has been reported to be expressed during proliferative (cycle day 3-15), late secretory (cycle day 25-27) phases and menstrual period (cycle day 0-2), but be absent in the early to mid secretory phase (cycle day 16-24)[17]. Therefore, we used immunohistochemistry to localize expression of MMP-7 in cells of adenomyosis uteri during proliferative and early to mid secretory phases. Of the total 40 cases examined by the histological dating of menstrual cycle phase, 23 cases were in proliferative phase, 17 cases in early to mid secretory phase (Table 1). The MMP-7 expression of the epithelial cells of adenomyosis uteri was compared with that of intact uterine endometrium obtained from the same case. Representative results are shown in Fig. 7. In the intact endometrium, the epithelial expression of MMP-7 was faint in proliferative phase (Fig. 7A left), and almost undetectable in mid secretory phase (Fig. 7B left). The expression of MMP-7 was greatly enhanced, however, in the epithelial cells of human adenomyosis uteri, whether in proliferative phase (Fig. 7A right) or in mid secretory phase (Fig. 7B right).

The effect of menstrual cycle on the expression of immunoreactive MMP-7 was examined semi-quantitatively by scoring for the intensity of the staining, as is shown in Fig. 8. MMP-7 expression in the adenomyosis uteri was significantly higher than that in the intact endometrium, whether during in the proliferative phase (mean$\pm$SD, 2.2 ± 0.2 versus 1.2 ± 0.3, $P<0.01$) or in the early to mid secretory phase (mean$\pm$SD, 2.1 ± 0.3 versus 0.1 ± 0.2, $P<0.001$). The result indicates that MMP-7 in adenomyosis uteri is constantly overexpressed during the menstrual cycle, whereas that in the intact endometrium is regulated during the menstrual cycle.

4. Discussion

The present study demonstrated the overexpression of MMP-7 protein in both experimental

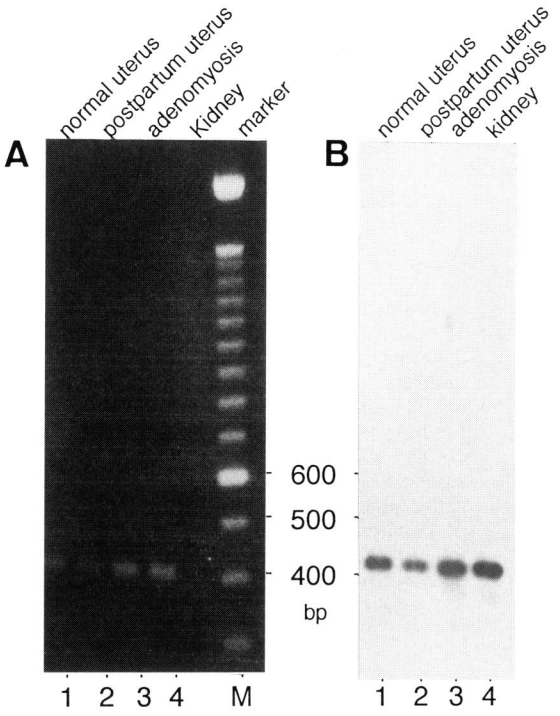

Fig. 4 Expression of MMP-7 mRNA in mouse adenomyosis uteri and normal mouse endometrium by RT-PCR and Southern blotting hybridization analysis. Mouse postpartum uterus and mouse kidney were used as positive controls for the gene expression. (A) The expression of MMP-7 mRNA by RT-PCR was examined in normal mouse uterus (lane 1), mouse postpartum uterus (lane 2), mouse adenomyosis uteri (lane 3), and mouse kidney (lane 4). (B) Southern blot hybridization analysis of each RT-PCR product with mouse MMP-7 oligonucleotide probe. The mouse MMP-7 probe was used to hybridize with each 440 bp PCR product in normal mouse uterus (lane 1), mouse postpartum uterus (lane 2), mouse adenomyosis uteri (lane 3), and mouse kidney (lane 4).

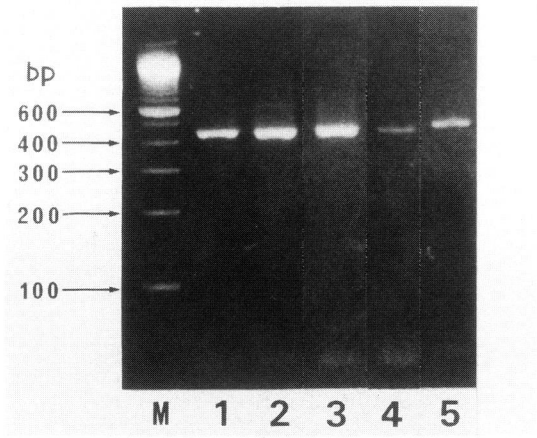

Fig. 5 Expression of MMP-7 mRNA in human samples by RT-PCR. Human colon cancer tissue was used as positive control for the gene expression. Sample used are human uterine endometrium (lane 1), human uterine adenomyosis (lanes 2 and 3), human uterine adenocarcinoma (lane 4), and human colon cancer (lane 5).

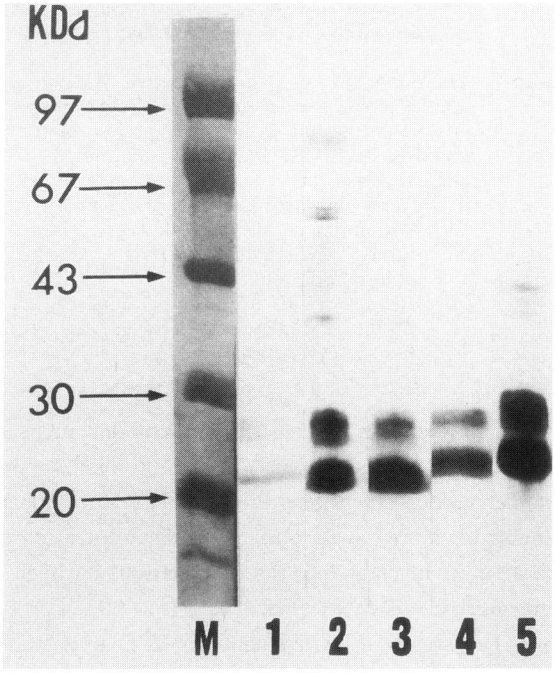

Fig. 6 Western blot analysis of human samples. Samples used are the same with those giving the results in Fig. 5: normal human endometrium (lane 1), human adenomyosis uteri (lanes 2 and 3), human uterine adenocarcinoma (lane 4), and human colon cancer (5).

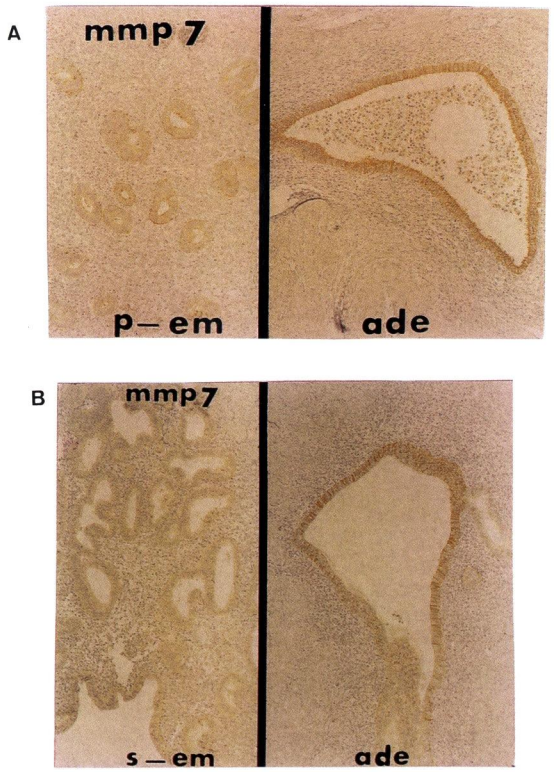

Fig. 7 The expression of immunoreactive MMP-7 in human adenomyosis uteri in defferent phases of the menstrual cycle. Expression of immunoreactive MMP-7 was examined in adenomyosis uteri and intact uterine endometrium obtained from the same cases.
(A) Adenomyosis uteri (right) and intact uterine endometrium (left) in proliferative phase; ×20.
(B) Adenomyosis uteri (right) and intact uterine endometrium (left) in early to mid secretory phase. ×20.

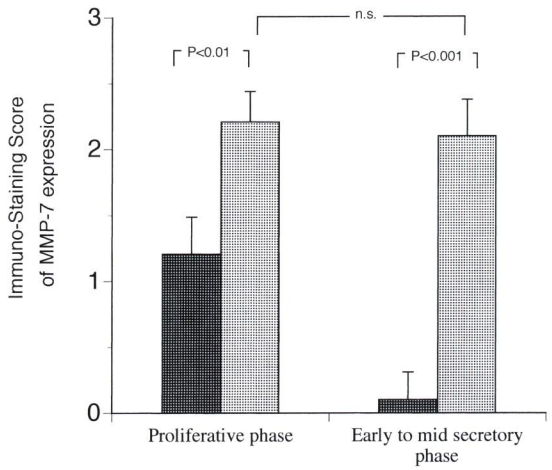

Fig. 8 Semi-quantitative scoring for the expression of MMP-7 in human adenomyosis uteri in defferent phases of the menstrual cycle. The intensity of staining for immunoreactive MMP-7 expression was scored as follows: 3 for intense staining; 2 for moderate staining; 1 for weak staining; 0 for staining not significantly greater than that of the control slide. Each bar represents mean scores ± SD of 23 cases in proliferative phase and 17 cases in early to mid secretory phase. ▩, intact endometrium; ▦, adenomyosis.

adenomyosis uteri in mice and human adenomyosis uteri by gelatin zymography and Western blot analysis. MMP-7 protein was constantly overexpressed during menstrual cycle, whereas the intact endometrium obtained from the same case with adenomyosis uteri is regulated during menstrual cycle. As far as we know, our report on the demonstration of MMP enzyme activity in adenomyosis uteri was the first that investigated the correlation between MMP enzyme activities and the development of adenomyosis uteri.

To assess the involvement of MMP activity in the development of adenomyosis, we took advantage of a mouse model for adenomyosis uteri induced in pituitary-grafted mice[20,21,29,30]. This model is a well-established animal model for adenomyosis uteri, in which a rapid and frequent induction of adenomyosis uteri permits to study the origin of this lesion. In fact, seven out of the ten grafted mice developed adenomyosis uteri, thus providing enough tissue material for the present studies.

Of the three MMP activities (20-30 kDa, 50 kDa, and 70 kDa) demonstrated by gelatin zymography in highly invasive adenomyosis uteri (Fig. 3), we are most interested in the enzyme activity of 20-30

kDa, because this lower molecular weight band was the constant band observed in uterine extracts from examined all the mice with the invasive adenomyosis uteri. No MMP enzyme activity was demonstrated in the extracts obtained from either uterus with less invasive adenomyosis or histologically normal uterus. The association of the MMP enzyme activity of 20-30 kDa with the highly invasive adenomyosis uteri suggests that this MMP activity may have an important role on its development. As far as the molecular weight is concerned, MMP enzyme activity of 20-30 kDa corresponds to the reported low molecular weight MMP-7, which has the molecular weight of 28-29 kDa in an inactive form and 19 kDa in an activated form[19]. The subsequent demonstration of MMP-mRNA in mouse adenomyosis uteri indicate that the MMP enzyme activity of 20-30 kDa is MMP-7.

MMP-7 (Pump-1, matrilysin) is a zymogen, which is converted to an active form to display catalytic activity against a broad range of extracellular matrix substrates including proteoglycans, gelatin, fibronectin, laminin and elastin[19,27,28]. The expression of MMP-7 mRNA in adenomyosis uteri was comparable to that in the normal endometrium (Figs. 4 and 5). Thus, the enzyme activity of MMP-7 is likely to be regulated at secretion levels and by means of enzyme activation machinery. Total MMP activity is determined by the amount of zymogens produced, the extent to which the zymogens are converted to active forms, and the effect of the tissue inhibitors of matrix metalloproteinases (TIMPs)[10,33]. Mechanism responsible for MMP-7 activation has been reported to be similar to that for other MMPs, and involve an activator proteinase such as serine proteinase[34].

Progesterone has been reported to inhibit the expression of endometrial MMP-7[18,35]. Overexpression of MMP-7 protein in adenomyosis uteri irrespective of the menstrual phases (Fig. 8) suggests that adenomyosis uteri may be insensitive to the inhibitory effect of progesterone. In fact, clinical experiences show that adenomyosis uteri do not respond to progesterone therapy. Inhibitory effect of progesterone on the expression of endometrial MMP-7 has been shown to be mediated by stromal-derived growth factor beta (TGF-beta)[35]. MMP-7 promoter contains sequences with a high homology to the TGF-beta inhibitory element originally identified in the rat stromelysin promotor[36]. On the other hand, epidermal growth factor (EGF) has been shown to up-regulate MMP-7 and other MMPs[36]. Histological studies on the adenomyosis uteri in the mouse model have shown that the invasion of endometrial parenchyma into the myometrium is preceded by the invasion of endometrial stroma proceeding through loose connective tissues in the myometrium[20,21]. Thus, defective stromal-epithelial interactions may be involved in the pathogenesis of the overexpression of MMP-7 in adenomyosis uteri. Since the expression of MMP-7 has been reported to be decreased by all-trans retinoic acid (ATRA)[13], ATRA as anti-MMP-7 therapeutic agent may possibly be effective in the treatment of human adenomyosis uteri.

In conclusion, the data are consistent with an idea that constantly enhanced synthesis of MMP-7 in the epithelial cells of adenomyosis uteri contributes to its invasion into the myometrium.

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References

1. Emge LA. The elusive adenomyosis of the uterus: Its historical past and its present state of recognition. Am J Obstet Gynecol 1962, 83: 1541-1563.
2. Tienari J, Pertovaara L, Saksela O, Lehtonen E, Vartio T. Increased expression of the matrix

metalloproteinase 2 in differentiating Tera 2 human embryonal carcinoma cells. Int J Cancer 1994, 56 : 219-223.
3. Salo T, Makela M, Kylmaniemi M, Harmainen AH, Larjava H. Expression of matrix metalloproteinase-2 and -9 during early human wound healing. Lab Invest 1994, 70 : 176-182.
4. Wossner JF. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J 1991, 5 : 2145-2153.
5. Goldberg GI, Eisen AZ. Extracellular matrix metalloproteinases in tumor invasion and metastasis. Cancer Treat Res. 1991, 53 : 421-440.
6. Cockett MI, Birch ML, Murphy G, Hart IR, Docherty AJ. Metalloproteinase domain structure, cellular invasion and metastasis. Biochem Soc Trans 1994, 22 : 55-57.
7. McDonnell S, Wright JH, Gaire M, Matrisian LM. Expression and regulation of stromelysin and matrilysin by growth factors and oncogenes. Biochem Soc Trans 1994,22 : 58-63.
8. Kohn EC, Liotta LA. Molecular insights into cancer invasion : strategies for prevention and intervention. Cancer Res 1995, 55 : 1856-62.
9. Stetler-Stevenson WG, Aznavoorian S, Liotta LA. Tumor cell interactions with the extracellular matrix during invasion and metastasis. Annu Rev Cell Biol 1993, 9 : 541-573.
10. Liotta LA, Stee PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis : An imbalance of positive and negative regulation. Cell 1991, 64 : 327-336.
11. Pajouh MS, Nagel RB, Breathnach R, Finch JS, Brawer MK, Bowden GT. Expression of metalloproteinase genes in human prostate cancer. J Cancer Res Clin Oncol 1991, 117 : 144-150.
12. Bsset P, Bellcq JP, Wolf C, Stoll I, Hutin P, Limacher JM, Podhajcer JM, Chenard OL, Chenard MP, Rio MC, Chambon P. A novel metalloproteinase gene specifically expressed in stroma cells of breast carcinomas. Nature 1990, 348 : 699-704.
13. Yamamoto H, Itoh F, Hinoda Y, Imai K. Suppression of matrilysin inhibits colon cancer cell invasion *in vitro*. Int J Cancer 1995, 61 : 218-222.
14. Senota A, Itoh F, Yamamoto H, Adachi Y, Hinoda Y, Imai K. Relation of matrilysin messenger RNA expression with invasive activity in human gastric cancer. Clin Exp Metastasis 1998, 16 : 313-321.
15. Marbaix E, Donnez J, Courtoy PJ, Eeckhout Y. Progesterone regulates the activity of collagenase and related gelatinases A and B in human endometrial explants. Proc Natl Acad Sci USA 1992, 89 : 11789-11793.
16. Martelli M, Campana A, Bischof P. Secretion of matrix metalloproteinases by human endometrial cells in vitro. J Reprod Fertil 1993, 98 : 67-76.
17. Rodgers WH, Osteen KG, Matrisian LM, Navre M, Giudice LC, Gorstein F. Expression and localization of matrilysin, a matrix metalloproteinase, in human endometrium during the reproductive cycle. Am J Obstet Gynecol 1993, 168 : 253-260.
18. Osteen KG, Rodgers WH, Gaire M, Hargrove JT, Gorstein F, Matrisian LM. Stromal-epithelial interaction mediates steroidal regulation of metalloproteinase expression in human endometrium. Proc Natl Acad Sci USA 1994, 91 : 10129-10133.
19. Wossner JF, Taplin CJ. Purification and properties of a small latent matrix metalloproteinase of the rat uterus. J Biol Chem 1988, 263 : 16918-16925.
20. Mori T, Nagasawa H. Mechanisms of development of prolactin-induced adenomyosis in mice. Acta Anat 1983, 116 : 46-54.
21. Ohta Y, Mori T, Nagasawa H. Ultrastructural changes of endometrium and myometrium during development of adenomyosis in mice with ectopic pituitary transplants. Zool Sci 1985, 2 : 239-247.
22. Endo T, Aten RF, Wang F, Behrman HR. Coordinate induction and activation of metalloproteinase and ascorbate depletion in structural luteolysis. Endocrinology 1993, 133 : 690-698.
23. Chomczynsky P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987, 162 : 156-159.
24. Muller D, Quantin B, Gesnei MC, Millon-Collard R, Abecassis J, Breathnach R. The collagenase gene family in humans consists of at least four members. Biochem J 1988, 253 : 187-192.
25. Feinberg AP, Vogelstein B. A technique for radiorabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 1983, 132 : 6-13.
26. Marcotte PA, Kozan IM, Dorwin SA, Ryan JM. The matrix metalloproteinase Pump-1 catal-

yzes formation of low molecular weight (pro) urokinase in cultures of normal human kidney cells. J Biol Chem 1992, 267 : 13803-13804.
27. Marti HP, McNeil L, Thomas G, Davies M, Lovett DH. Molecular characterization of a low-molecular-mass matrix metalloproteinase secreted by glomerular mesangeal cells as Pump-1. Biochem J 1992, 285 : 899-905.
28. Miyazaki K, Hattori Y, Umenishi F, Yasumitsu H, Umeda M. Purification and characterization of extracellular matrix-degrading metalloproteinase, matrilysin (Pump-1), secreted from human rectal carcinoma cell line. Cancer Res 1990, 50 : 7758-7764.
29. Mori T, Singtripop T, Kawashima S. Animal model of uterine adenomyosis : Is prolactin a potent inducer of adenomyosis in mice? Am J Obstet Gynecol 1991, 165 : 232-234.
30. Iizuka A, Nakabachi A, Mori T, Park MK, Fujii Y, Nagasawa H. Changes in citrate concentration in the mouse uterus with experimentally-induced adenomyosis. Life Sci 1996, 58 : 397-403.
31. Murphy G, Cockett MI, Ward RV, Docherty AJP. Matrix metalloproteinase degradation of elastin, type IV collagen and proteoglycan : A quantitative comparison of the activities of 95kDa and 72 kDa gelatinases, stromelysins-1 and -2 and punctuated metalloproteinase (PUMP). Biochem J 1991, 277 : 277-279.
32. Quantin B, Murphy G, Breathnach R. Pump-1 cDNA codes for a protein with characteristics similar to those of classical collagenase family members. Biochemistry 1989, 28 : 5327-5334.
33. Matrisian LM. Metalloproteinases and their inhibitors in matrix remodeling. Trends Genet 1990, 6 : 121-125.
34. Koshikawa N, Yasumitsu H, Umeda M, Miyazaki K. Multiple secretion of matrix serine proteinases by human gastric carcinoma cell lines. Cancer Res 1992, 52 : 5046-5053.
35. Bruner KL, Rodgers WH, Gold LI, Korc M, Hargrove JT, Matrisian LM, Osteen KG. Transforming growth factor beta mediates the progesterone suppression of an epithelial metalloproteinase by adjacent stroma in the endometrium. Proc Natl Acad Sci USA 1995,92 : 7362-7366.
36. Gaire M, Magbanua Z, McDonell S, McNeil L, Lovett DH, Matrisian LM. Structure and expression of the human gene for the matrix metalloproteinase matrilysin. J Biol Chem 1994, 269 : 2032-2040.

子宮腺筋症の浸潤におけるMMP-7（PUMP-1, Matrilysin）の関与形態について

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子宮腺筋症は，子宮内膜腺，間質細胞からなる基本構造を備えた組織が，子宮筋層内に発育し増殖する良性疾患である．子宮腺筋症の病理組織所見より，筋層に浸潤していくその組織形態は腫瘍的性質をもっていると考えられ，発生及び進展の機序に癌の浸潤・転移に類似した機構の存在が示唆される．今回，マウス子宮腺筋症モデルを作成し，マウス子宮腺筋症の発生及び進展とMMPとの関係を中心に解析を行った．さらに，ヒト子宮腺筋症についても同様な解析を行い，MMPの組織的局在についても検討を加え，以下の結果を得た．マウス子宮腺筋症組織を用いたgelatin zymographyにおいて，正常子宮組織では認められなかった20～30 kDaの低分子量gelatinase活性を認め，EDTAにて抑制されたことより分子量20～30 kDaのMMPの関与が明らかとなった．この新たに確認されたMMPをMMP-7と推定し，RT-PCR，southen blot hybridizationにより検討した結果，子宮腺筋症，正常子宮筋組織共にMMP-7のmRNA由来のcDNAの増幅を認めた．また，ヒト子宮腺筋症組織における検討でも同様な結果を得，さらにwestern blot hybridizationにおいても子宮腺筋症，正常子宮筋組織共にMMP-7の検出が認められた．ヒトの免疫組織学的な検討では，MMP-7は，正常子宮内膜，子宮腺筋症の異所性内膜ともに，腺細胞にのみ発現し，正常子宮内膜では増殖期のみに発現しているのに反し，異所性内膜腺細胞では正常子宮内膜腺細胞に比べ強い発現が月経周期にかかわらず認められた．以上より，マウス，ヒト子宮腺筋症においてMMP-7が関与しており，MMP-7は，月経周期にかかわらず，腺細胞にのみ，正常を逸脱し強く発現していた．また，MMP-7は，正常子宮内膜，子宮腺筋症異所性内膜ともにmRNAレベルで発現しているものの，子宮腺筋症のみにおいて特異的に活性を有していることが明らかとなった．この活性化が子宮腺筋症の異所性内膜が子宮筋層内への浸潤，増殖過程に関与している可能性が示唆された．