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Metalloproteinase-2, -7 and -9 and tissue inhibitor of metalloproteinase-1 and -2 expression in normal, hyperplastic and neoplastic endometrium: a clinical-pathological correlation study

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Background: Matrix metalloproteinases (MMPs) and their inhibitors are key-players in extracellular matrix and basement membrane degradation, and are involved in both physiological and malignant processes. The aim of this study was to examine MMP-2, -7 and -9 and TIMP-1 and -2 expression in normal, hyperplastic and malignant endometrium, and their relation to clinical and histological prognostic factors.

Materials and methods: We performed qualitative and semi-quantitative immunohistochemical analysis of 20 samples of normal endometrium (10 in the proliferative phase, 10 in the secretory phase), 39 samples of hyperplastic endometrium (17 without atypia and 22 with atypia) and 38 samples of endometrioid carcinoma, by using specific monoclonal antibodies.

Results: In normal endometrium, epithelial expression of MMP-2 ($P = 0.0007$), MMP-7 ($P = 0.0002$) and TIMP-2 ($P = 0.0004$) was increased during the proliferative phase of the menstrual cycle. MMP-2 expression correlated negatively with TIMP-2 expression ($P = 0.001$, $\rho = 0.702$). Endometrial stromal cells in the secretory phase showed strong MMP-2 expression ($P = 0.004$) and weak MMP-7 ($P = 0.001$) and TIMP-1 expression ($P = 0.01$). In hyperplastic endometrium, the presence of atypia was associated with lower TIMP-2 expression ($P = 0.005$) and was also associated with a trend towards higher MMP-2 expression. Endometrial stromal cell expression of MMP-2, -7 and -9 and TIMP-1 and -2 did not differ between hyperplastic endometrium with and without atypia. A gradient of MMP-2 and -9 expression was observed from hyperplastic endometrium to endometrial carcinomas. In endometrial carcinomas, MMP-2 expression increased ($P = 0.0004$) and TIMP-2 expression decreased ($P = 0.0005$) with the histological grade. TIMP-2 expression correlated with myometrial invasion ($P = 0.005$), lymphovascular space involvement ($P = 0.008$) and lymph node involvement ($P = 0.007$).

Conclusion: These results support the involvement of MMPs and TIMPs in endometrial carcinogenesis. Strong MMP-2 and weak TIMP-2 expression were the most potent markers of endometrial malignancies with a high risk of local and distant spread.

Key words: metalloproteinases, metalloproteinase inhibitors, endometrial cancer, hyperplasia, normal endometrium, immunohistochemistry

introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteinases known for their ability to degrade the extracellular matrix [1, 2]. Over 20 human MMPs have been described including collagenases that cleave native fibrillar collagen types I, II and III and stromelysin 1 and 2 that have broad substrate specificity [3–7]. Gelatinases A (MMP-2) and B (MMP-9) degrade denatured collagens and basement membrane components [8, 9]. MMPs and TIMPs are thought to be essential for the proliferative, invasive and metastatic properties of various carcinomas [10–13].

Endometrial carcinoma is the most common malignancy of the female genital tract, and affects mostly menopausal women [14]. Prognostic factors include the histological grade, disease stage, myometrial invasion, vascular space involvement and lymph node metastasis [15].

Endometrial hyperplasia with atypia (EH) is considered to be a precursor of endometrial carcinoma (EC) [16]. Precursor

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lesions of EC are monoclonal and recent studies suggest a multistep process involving hormonal regulation [17], gene mutation [18–20], adhesion molecules [21], apoptosis [22–24], metalloproteinases (MMP) and tissue inhibitors of metalloproteinases (TIMP) [25].

Several molecular studies have shown the involvement of MMPs and TIMPs in matrix remodelling of normal endometrium throughout the menstrual cycle [26, 27]. In contrast, few data are available on their expression in hyperplastic endometrium. Soini et al. [28], analysing the messenger RNA of MMP-9 by *in situ* hybridisation, found that MMP-9 was expressed in hyperplastic endometrium, contrary to non-neoplastic endometrium, suggesting a role in the malignant phenotype. In a small study using *in situ* hybridisation, Määtä et al. [29] found no difference in TIMP-1, -2 or -3 mRNA expression between normal and hyperplastic endometrium. In contrast, previous studies using immunohistochemistry, zymography or *in situ* hybridisation have shown a correlation between MMP-2 and/or -9 expression and the histological grade and disease stage of endometrial carcinoma [30–32]. Moreover, in a previous immunohistochemical study, we found that MMP-2, TIMP-1 and -2 expressions were correlated with the invasiveness of endometriotic lesions [33]. However, the prognostic value of MMP and TIMP expression in endometrial carcinoma is controversial [34, 35].

Therefore, the aims of this study were (a) to analyse MMP-2, -7 and -9 and TIMP-1 and -2 expression in normal, hyperplastic endometrium (with and without atypia) and endometrioid carcinomas, (b) to compare their expression according to the histological type and (c) to evaluate their prognostic value in endometrial carcinoma.

materials and methods

From 1999 to 2004, tissue samples from 97 women, comprising 20 samples of normal endometrium, 39 samples of hyperplastic endometrium and 38 samples of endometrioid carcinomas were obtained in the Gynaecology Departments of Tenon Hospital, Paris and Institut Mère Enfant Alix de Champagne, Reims, France. Two pathologists reviewed all tissues samples to confirm the diagnosis and histological characteristics. None of the 38 patients with endometrioid carcinoma received chemotherapy or radiotherapy before surgery, which consisted of peritoneal cytology, total hysterectomy, bilateral salpingo-oophorectomy, and pelvic and para-aortic lymph node sampling when necessary.

Formalin-fixed, paraffin embedded samples of 20 normal endometria (10 obtained in the proliferative phase and 10 in the secretory phase of the menstrual cycle), 39 samples of hyperplastic endometria (17 without atypia and 22 with atypia) and 38 samples of endometrioid carcinoma were studied. The histological grade of endometrioid carcinoma was assessed according to the International Federation of Gynaecology and Obstetrics (FIGO) guidelines [36]. Histological and clinical prognostic factors, including the depth of myometrial invasion, lymphovascular space involvement, lymph node metastasis, the FIGO clinical stage, recurrence and death from disease, were recorded. The histological characteristics of the 38 endometrioid carcinomas are summarised in Table 1. In accordance with FIGO recommendations, women with stage I grade 1 endometrial cancer did not routinely undergo lymphadenectomy.

Table 1. Clinical and histological characteristics of the 38 patients with endometrial cancers (endometrioid type)

Endometrial cancers	Number of patients (%)
Grades	
1	17 (44.7%)
2	13 (34.2%)
3	8 (21.1%)
Stages	
I	23 (60.5%)
II	6 (15.8%)
III	7 (18.4%)
IV	2 (5.3%)
Depth of myometrial invasion	
M0 (no invasion)	5 (13.1%)
M1 (<50%)	15 (39.5%)
M2 (>50%)	18 (47.4%)
Lymph node metastasis	
Yes	6 (15.8%)
No	20 (52.6%)
Vascular/lymphatic invasion	
Yes	14 (36.8%)
No	24 (63.2%)
Recurrence	
Yes	3 (7.9%)
No	35 (92.1%)
Death (from disease)	2 (5.2%)

immunohistochemistry

antibodies. Purified mouse monoclonal antibodies against human MMP-2 (gelatinase-A, Ab-2, clone 17B11, Novocastra Laboratories Ltd, Newcastle, UK), human MMP-7 (matrilysin, Ab-3, clone ID2, Calbiochem, Oncogene Research Products, Cambridge, UK), human MMP-9 (gelatinase-B, Ab-3, clone 56 2A4, Calbiochem, Oncogene Research Products), human TIMP-1 (Ab-4, clone 3A4, Novocastra Laboratories Ltd) and human TIMP-2 (Ab-2, clone 6F6a, Novocastra Laboratories Ltd) were used as primary antibodies. The immunogenic component was a recombinant protein corresponding to 132 amino acids of the C-terminal part of human MMP-2; recombinant human MMP-7; a synthetic peptide corresponding to amino acids 626–644 of human MMP-9; a prokaryotic recombinant protein corresponding to a 135-amino-acid region of human TIMP-1; and a synthetic peptide corresponding to a specific sequence located in the N-terminal region of TIMP-2. Anti-MMP-2, anti-MMP-7 and anti-MMP-9 recognise both latent and active forms of MMP.

immunohistochemical technique. Tissues were immediately fixed in formalin (10%) and then processed as paraffin blocks. Sections of formalin-fixed tissues, 4 μm thick, were deparaffinated in xylene and rehydrated through a graded series of ethanol solutions. Sections were immunostained using the Ventana Nexes automated immunohistochemistry system (Ventana Medical Systems, Tucson, Arizona). Prior to MMP-2, MMP-9, TIMP-1 and TIMP-2 immunoreaction, an antigen retrieval step was used, combined with a high-temperature antigen-unmasking technique (Dako® Target Retrieval Solution, Glostrup, Denmark; 100°C, 30 min). For MMP-7, antigen unmasking was achieved with proteinase K. The automated procedure was based on an indirect biotin-avidin system with a universal biotinylated immunoglobulin as secondary antibody, diaminobenzidine as substrate and hematoxylin as counterstain. For MMP-7 and MMP-9, in addition to the automated procedure, a Ventana amplification kit was used (Ventana Medical Systems®). Anti-MMP-2 and anti-MMP-9 antibodies were used at

a dilution of 1/10 (both 10 μg/ml), anti-MMP-7 and anti-TIMP-1 were used at a dilution of 1/50 (4 μg/ml and 2 μg/ml, respectively), and anti-TIMP-2 was used at a dilution of 1/15 (6.5 μg/ml).

Positive controls for MMP-2, -7, -9, TIMP-1 and -2 were sections of bronchial tissue, ovarian cancer, breast cancer and placenta, respectively. For negative control, the primary antibody was replaced by an irrelevant non-immune mouse antibody of the same IgG subtype (Dako Laboratories, Glostrup, Denmark).

analysis of immunohistochemistry

Immunoreaction of endometrial epithelial cells was assessed qualitatively and semi-quantitatively. For semi-quantitative analysis of tumour cells, the percentage of positive cells was noted for approximatively 1000 neoplastic cells per slide, subdivided into 10 selected fields at ×400 magnification.

Immunoreaction of endometrial stromal cells in normal and hyperplastic endometrium was assessed qualitatively and semi-quantitatively. Semi-quantitative analysis was based on the following four classes: less than 10% of cells labelled, between 10% and <25%, between 25% and <50%, and 50% or more. Immunoreaction was assessed by two independent observers. Variations between the two observers were below 5%.

statistical analysis

We used the chi-square test for categorical variables and the Kruskal–Wallis and Mann–Whitney tests for continuous variables. Correlations with MMP and TIMP expression were evaluated by using Spearman's test. *P* values below 0.05 were considered significant.

results

MMP and TIMP expression in normal endometrium

Endometrial cell cytoplasm immunoreaction was positive for MMP-2, -7 and -9 and TIMP-1 and -2. No MMP-2 (Figure 1A) immunoreaction was observed in samples from the secretory phase, while all samples from the proliferative phase were positive ($P = 0.0007$). Whatever the phase of the menstrual cycle, endometrial epithelial cells were positive for MMP-7 and -9 and TIMP-1 (Figure 1B) and TIMP-2.

Semi-quantitative immunoreaction of endometrial epithelial cells for MMP-2, -7 and -9 and TIMP-1 and -2 is shown in Table 2 according to the phase of menstrual cycle. Expression was higher in the proliferative phase than in the secretory phase for MMP-2 ($P = 0.0007$), MMP-7 ($P = 0.0002$) and TIMP-2 ($P = 0.0004$). MMP-9 and TIMP-1 expression did not vary with the phase of the menstrual cycle. A negative correlation was observed between MMP-2 and TIMP-2 expression ($P = 0.001$, $\rho = 0.702$) (Figure 2).

In endometrial stromal cells, qualitative expression of MMP-2, -7 and -9 and TIMP-1 and -2 did not differ according to the phase of the menstrual cycle.

Semi-quantitative analysis of endometrial stromal cell immunoreaction showed that MMP-2 expression increased in the secretory phase ($P = 0.004$) (Table 3). In contrast, MMP-7 ($P = 0.001$) and TIMP-1 ($P = 0.01$) expression decreased in the secretory phase (Table 3). MMP-9 and TIMP-2 expression did not vary with the phase of the menstrual cycle.

MMP and TIMP expression in hyperplastic endometrium

The cytoplasm of endometrial epithelial cells in hyperplastic endometrium reacted positively for MMP-2, -7 and -9 and TIMP-1 (Figure 1C, D) and TIMP-2. No qualitative difference in immunoreaction for MMP-2, -7 and -9 and TIMP-1 and -2 was observed between hyperplastic endometrium with and without atypia.

Table 4 shows semi-quantitative MMP-2, -7 and -9 and TIMP-1 and -2 immunoreaction values for endometrial epithelial cells in hyperplastic endometrium with and without atypia. TIMP-2 expression was lower in hyperplastic endometrium with rather than without atypia ($P = 0.005$). A trend towards higher MMP-2 expression was observed in hyperplastic endometrium with atypia compared with samples without atypia ($P = 0.1$). No difference in MMP-7, MMP-9 or TIMP-1 expression was found between samples with and without atypia.

Qualitative and semi-quantitative expression of MMP-2, -7 and -9 and TIMP-1 and -2 by endometrial stromal cells did not differ between hyperplastic endometrium with and without atypia.

MMP and TIMP expression in endometrial carcinoma

In the epithelial compartment, all 38 samples of endometrial carcinoma (Figure 1E, F) showed positive cytoplasmic immunoreaction for MMP-7, MMP-9 and TIMP-1. All but one of the samples were positive for MMP-2 (Figure 1G, H). Four samples were negative for TIMP-2.

The mean percentage ± SD (median, range) of cells reacting positively for MMP-2, -7 and -9 and TIMP-1 and -2 in endometrial carcinomas were 53.3 ± 29.9 (55, 0–90), 74.3 ± 18.8 (80, 30–100), 93.8 ± 7.3 (95, 70–100), 64.9 ± 22.6 (70, 10–92) and 46.9 ± 26.5 (50, 0–90), respectively. A trend towards a negative correlation was observed between MMP-2 and TIMP-2 expression ($P = 0.05$, $\rho = 0.314$).

Quantitative analysis of the immunoreaction in the endometrial stromal compartment of endometrial carcinomas was not possible because of the paucity of the stromal reaction, particularly in grade 2 and 3 carcinomas. In the endometrial stromal compartment, immunoreaction was stronger close to endometrial cancer cells. Tumour fibroblasts and endothelial cells reacted positively for MMP-2. MMP-7 was not detected in stromal cells. MMP-9 was predominantly expressed by inflammatory cells of the stroma. TIMP-1 and TIMP-2 showed variable stromal expression.

MMP and TIMP expression according to the diagnosis

MMP-2, -7 and -9 and TIMP-1 and -2 expression in proliferative and secretory endometrium with hyperplastic endometrium with and without atypia is compared in Table 5.

Stronger expression of MMP-2 ($P = 0.002$) and MMP-9 ($P < 0.0001$) was observed in endometrial carcinomas than in hyperplastic endometrium with atypia (Table 6). With a cut-off of 80% of positive cells, MMP-9 positivity had a sensitivity, specificity and positive and negative predictive values of 100%, 50%, 78% and 100%, respectively, for endometrial carcinoma. MMP-7 expression was lower in endometrial carcinomas than in hyperplastic endometrium with atypia ($P = 0.04$), while TIMP-1 and -2 expression was similar.

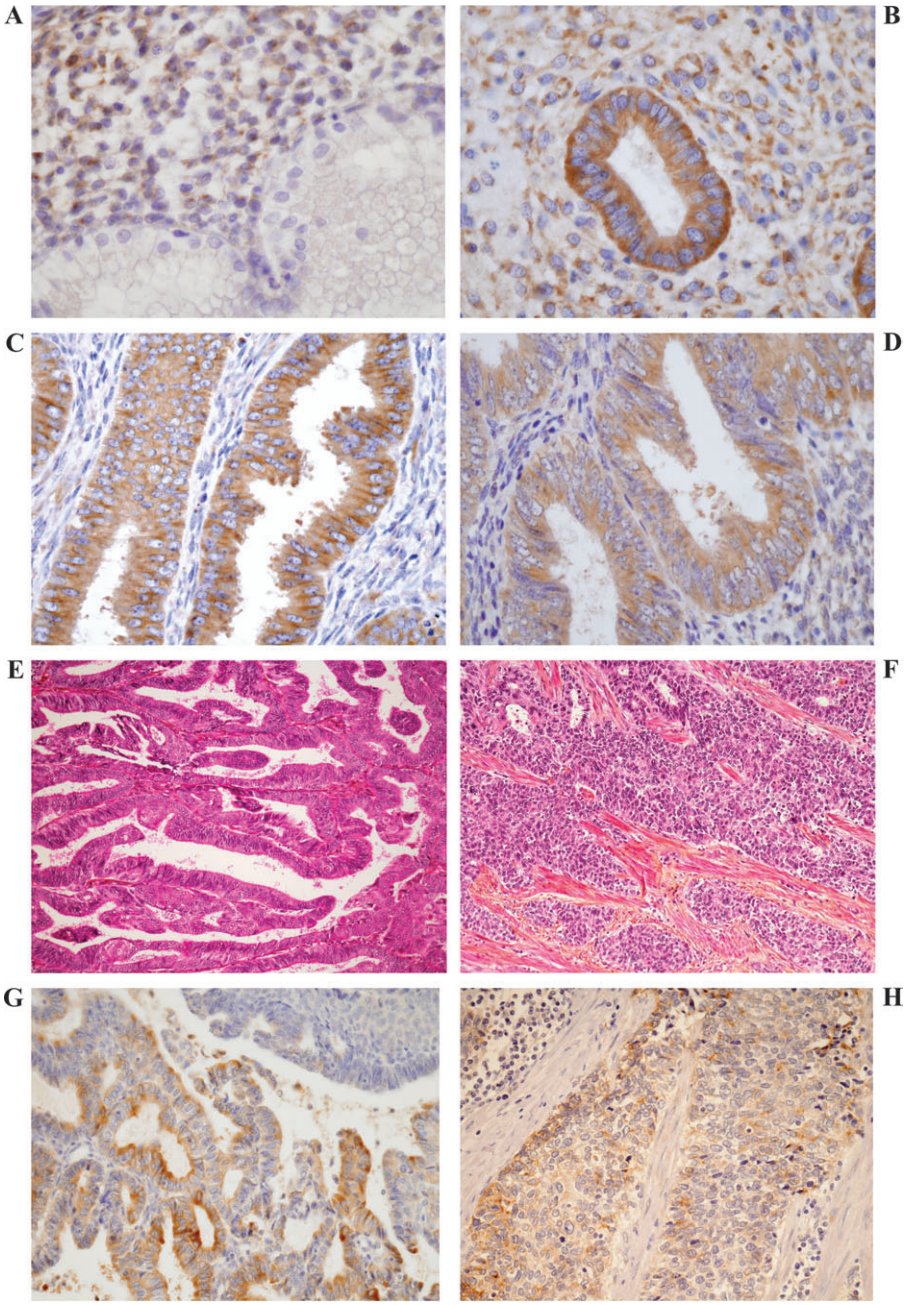

Figure 1. Representative samples of normal endometrium showing no cytoplasmic immunoreaction for MMP-2 in endometrial epithelial cells from secretory phase with a marked reaction in endometrial stromal cells (**A**), and a positive immunoreaction for TIMP-1 in endometrial epithelial cells from proliferative phase (**B**). TIMP-1 immunoreaction in endometrial epithelial cells from hyperplasia without atypia (**C**) and TIMP-1 immunoreaction in both endometrial epithelial and stromal cells from hyperplasia with atypia (**D**). Endometrioid carcinomas of the uterus (hematoxylin-eosin), grade 1 (**E**) and grade 3 (**F**). Representative samples of endometrioid carcinomas showing a low cytoplasmic immunoreaction for MMP-2 in a grade 1 (**G**), and a high immunoreaction in a grade 3 (**H**) carcinomas.

MMP and TIMP expression in endometrioid cancers according to clinical and histological characteristics

Histological grade correlated with semi-quantitative MMP-2 and TIMP-2 expression. MMP-2 expression increased with histological grade ($P = 0.0004$). The difference was significant between grade 1 and 2 ($P < 0.0001$) and between grade 1 and 3 ($P = 0.003$), but not between grade 2 and 3 (Table 7). TIMP-2 expression declined with histological grade ($P = 0.0005$). The difference was significant between grade 1 and 3 ($P < 0.0001$) and between grade 2 and 3 ($P = 0.002$), while a trend towards a difference was noted between grade 1 and 2 ($P = 0.08$).

The mean percentage ± SD of TIMP-2-positive cells in tumour samples from patients with no myometrial invasion, invasion involving less than 50% of the myometrium, and

Table 2. Semi-quantitative values of immunostaining for endometrial epithelial cells in normal endometrium according to the phase of menstrual cycle for MMP-2, -7 and -9 and TIMP-1 and -2

	Proliferative phase Mean value ± SD Median (range)	Secretory phase Mean value ± SD Median (range)	*P* value
MMP-2	42.0 ± 26.6 40 (0–70)	0 ± 0 0 (0)	0.0007
MMP-7	99.0 ± 2.1 100 (95–100)	62.0 ± 31.2 72.5 (15–90)	0.0002
MMP-9	87.0 ± 9.2 90 (70–95)	90.6 ± 1.7 90 (90–95)	NS
TIMP-1	91.0 ± 5.2 90 (85–100)	84.0 ± 11.7 87.5 (60–95)	NS
TIMP-2	85.8 ± 9.9 90 (60–95)	23.4 ± 33.8 40.5 (0–80)	0.0004

SD, standard deviation; NS, not significant.

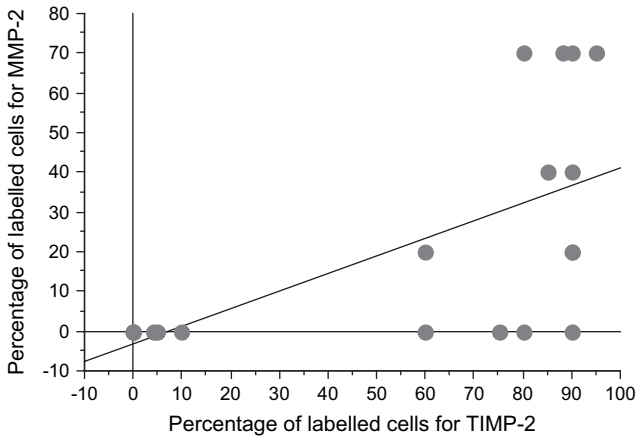

Figure 2. Negative correlation between MMP-2 expression and TIMP-2 expression in normal endometrium.

Table 3. Immunoreaction results for MMP-2, -7 and TIMP-1 in endometrial stromal cells of normal endometrium according to the phase of menstrual cycle

	Proliferative phase	Secretory phase	*P* value
MMP-2			
<10%	4	1	0.004
>10%, <25%	2	0	
>25%, <50%	3	0	
>50%	1	9	
MMP-7			
<10%	0	0	0.001
>10%, <25%	0	0	
>25%, <50%	0	7	
>50%	10	3	
TIMP-1			
<10%	0	0	0.01
>10%, <25%	0	3	
>25%, <50%	3	6	
>50%	7	1	

invasion involving more than 50% of the myometrium was 62.0 ± 14.8, 57.3 ± 26.6 and 34.2 ± 23.6, respectively ($P = 0.005$). The difference was significant between patients with no myometrial invasion and more than 50% of myometrial invasion ($P = 0.02$), and between patients with less than 50% and more than 50% invasion ($P = 0.01$), but not between patients with no invasion and patients with less than 50% invasion.

Table 4. Immunoreaction results for MMP-2, -7 and -9 and TIMP-1 and -2 in endometrial epithelial cells from hyperplastic endometrium with and without atypia

	Hyperplasia without atypia Mean value ± SD Median (range)	Hyperplasia with atypia Mean value ± SD Median (range)	*P* value
MMP-2	15.4 ± 18.4 5 (0–50)	28.0 ± 27.9 15 (0–80)	0.1
MMP-7	77.4 ± 23.9 90 (0–90)	82.9 ± 8.8 87.5 (60–90)	NS
MMP-9	78.8 ± 22.9 85 (10–100)	74.3 ± 15.7 77.5 (40–95)	NS
TIMP-1	60.9 ± 10.6 60 (40–80)	58.4 ± 12.2 60 (30–75)	NS
TIMP-2	70.9 ± 19.7 80 (20–90)	50.7 ± 22.4 50 (0–90)	0.005

SD, standard deviation; NS, not significant.

The mean percentage ± SD of TIMP-2-positive cells in patients with or without lymphovascular space involvement was 32.7 ± 22.4 and 55.2 ± 25.9, respectively ($P = 0.008$). The mean percentage ± SD of TIMP-2-positive cells in patients with and without lymph node involvement was 26.7 ± 19.9 and 58.8 ± 22.8, respectively ($P = 0.007$). No relation was found between TIMP-2 expression and the FIGO stage, recurrence, or death from disease.

The mean percentage ± SD of TIMP-1-positive cells in samples from patients with and without lymphovascular space involvement was 57.2 ± 20.2 and 68.8 ± 23.5, respectively ($P = 0.04$). The mean percentage ± SD of TIMP-1-positive cells in patients with and without recurrence was 28.3 ± 10.4 and 68.1 ± 20.5, respectively ($P = 0.01$). No relation was found between TIMP-1 expression and the FIGO stage, the depth of myometrial invasion, lymph node metastasis, or death from disease.

A trend towards stronger MMP-2 expression was found in samples from women with myometrial invasion. In the same way, a trend towards stronger MMP-7 expression was found in samples from women with lymphovascular space involvement. In contrast, no relation was found between MMP-9 expression and the FIGO stage, the depth of myometrial invasion, lymphovascular space involvement, lymph node metastasis, recurrence, or death from disease.

discussion

We observed a continuum of MMP and TIMP expression from hyperplastic endometrium without atypia to hyperplastic endometrium with atypia and endometrial carcinomas. MMP and TIMP expression correlated with clinical and histological prognostic factors of endometrial carcinoma.

Few reports have focused on MMP and TIMP expression in hyperplastic endometrium. Lower TIMP-2 expression was observed in endometrial epithelial cells in hyperplastic endometrium with atypia than in hyperplastic endometrium without atypia. Moreover, MMP-2 expression tended to be stronger in hyperplastic endometrium with atypia. These results

Table 5. Comparison between immunoreaction results for MMP-2, -7 and -9 and TIMP-1 and -2 in endometrial epithelial cells from normal endometrium and hyperplastic endometrium with and without atypia

	MMP-2 *P* value	MMP-7 *P* value	MMP-9 *P* value	TIMP-1 *P* value	TIMP-2 *P* value
Proliferative endometrium versus atypical hyperplasia	NS	<0.0001	0.02	<0.0001	<0.0001
Proliferative endometrium versus simple hyperplasia	0.005	<0.009	NS	<0.0001	0.03
Atypical hyperplasia versus simple hyperplasia	NS	NS	NS	NS	0.006
Secretory endometrium versus atypical hyperplasia	0.004	0.006	0.004	0.0001	0.01
Secretory endometrium versus simple hyperplasia	0.01	NS	NS	0.0001	0.0001

NS, not significant.

Table 6. Immunoreaction results for MMP-2, -7 and -9 and TIMP-1 and -2 in endometrial epithelial cells from hyperplastic endometrium with atypia and in carcinoma cells

	Hyperplasia with atypia Mean value ± SD	Endometrioid carcinoma Mean value ± SD	*P* value
MMP-2	28.0 ± 27.9	53.3 ± 29.9	0.002
MMP-7	82.9 ± 8.8	74.3 ± 18.8	0.04
MMP-9	74.3 ± 15.7	93.8 ± 7.3	< 0.0001
TIMP-1	58.4 ± 12.2	64.9 ± 22.6	NS
TIMP-2	50.7 ± 22.4	46.9 ± 26.5	NS

SD, standard deviation; NS, not significant.

Table 7. Relation between clinical and pathological prognostic factors and MMPs and TIMPs expression in endometrial carcinomas from endometrioid type

Clinicopathologic features	MMP-2	MMP-7	MMP-9	TIMP-1	TIMP-2
Grade	0.0004	NS	NS	NS	0.0005
Stage (FIGO)	NS	NS	NS	NS	NS
Depth of myometrial invasion	*P* = 0.06	NS	NS	NS	*P* = 0.005
M0 (no invasion) versus M1 (<50%)					NS
M0 versus M2 (>50%)					*P* = 0.02
M1 versus M2					*P* = 0.01
Vascular/lymphatic invasion					
Presence versus absence	NS	*P* = 0.05	NS	*P* = 0.04	*P* = 0.008
Lymph node metastasis					
Presence versus absence	NS	NS	NS	NS	*P* = 0.007
Recurrence					
Yes versus no	NS	NS	NS	*P* = 0.01	NS
Death (from disease)	NS	NS	NS	NS	NS

NS, not significant.

support the involvement of MMP and TIMP in premalignant endometrial lesions. Our results are in keeping with those of Uzan et al. [33] showing that MMP-2 immunohistochemical expression increased with the aggressiveness of endometriotic lesions. In contrast, they disagree with the results of Määtä et al. [29], who found no difference in MMP and TIMP expression between hyperplastic endometrium with and without atypia. However, these authors using *in situ* hybridisation (ISH) analysed only six samples of hyperplastic endometrium. Very few data are available on MMP and TIMP expression by endometrial stromal cells in hyperplastic endometrium. Soini et al. [28] observed variable MMP-2 mRNA expression in endometrial stromal cells of hyperplastic and neoplastic endometrium, but data were insufficient to determine the role of MMP in endometrial physiopathology. TIMP-2 expression by endometrial stromal cells in hyperplastic endometrium has not previously been studied. We found lower TIMP-2 expression in endometrial stromal cells from hyperplastic endometrium with atypia than in hyperplastic endometrium without atypia.

Semi-quantitative TIMP-2 expression distinguished hyperplastic endometrium with atypia from hyperplastic endometrium without atypia, while MMP-2, -7 and -9 expression distinguished hyperplastic endometrium with atypia from endometrial carcinoma. These results support the existence of a continuum of MMP and TIMP expression from hyperplastic to malignant endometrium. Our data are in keeping with previous findings suggesting that MMP-2 and MMP-9 mRNA expression is associated with the malignant phenotype of endometrial lesions [28]. Malignant transformation of endometrial precancers occurs by accumulation of sufficient genetic damage to permit invasion of the adjacent stroma [20] and increased expression of matrix-metalloproteinases could participate in this invasive process.

We found a positive relation between MMP-2 expression and the histological grade of endometrial carcinomas. A trend towards higher MMP-2 expression was also noted in women with myometrial invasion. These results are in line with previous zymographic or immunohistochemical studies suggesting that MMP-2 is the main metalloproteinase involved in the malignant behaviour of endometrial cancer [37, 38] and that MMP-2 expression is increased in regions adjacent to tumours, particularly at the invasive front, where it is co-expressed with MT1-MMP [29]. No relation was noted between MMP-2 expression and the FIGO stage, vascular/lymphatic invasion or disease-free survival, as previously reported in immunohistochemical studies [30, 31, 34]. We also noted a trend towards a relation between lymphovascular space

involvement and strong MMP-7 expression. Only one previous study has focused on MMP-7 expression in endometrial cancer, using immunohistochemistry, zymography and *in situ* hybridization techniques, and it also showed a relation between strong MMP-7 expression and lymph node metastasis [39]. These results are consistent with the work of Wang et al. [40], who demonstrated that MMP-7 can also activate progelatinases (proMMP-2 and proMMP-9) and thereby facilitate tumour invasion and metastasis.

No relation between MMP-9 expression and clinical or histological prognostic factors was observed in our study. These results are in keeping with those of Inoue et al. [41] but not with previous immunohistochemical and zymographic studies suggesting a relation between MMP-9 expression and FIGO stage and/or histological grade [30, 31, 42]. This discrepancy could be explained by the sample size and by the use of immunostaining or zymography, which only takes into account active forms of endoproteases. However, immunohistochemical analysis is particularly valuable because it can be applied to formalin-fixed samples, thereby offering better reproducibility in diagnostic histopathology and precise localisation of MMPs. Standardisation of immunohistochemical staining procedures and their evaluation is necessary to clarify the complex interactions between MMPs and TIMPs involved in local and distant progression of malignant diseases [43, 44]. The relatively small size of our sample could explain why no relation was found between clinical parameters and the expression of some MMPs and TIMPs. However, it should be noted that we used a reliable automated procedure (Nexes, Ventana).

TIMP-2 expression was lower in grade 3 endometrial cancers than in grade 1 and 2 samples. Moreover, low TIMP-2 expression correlated with the depth of myometrial invasion, lymphovascular space involvement and lymph node metastasis. A combination of low TIMP-2 and high MMP-2 expression identified a subgroup of women at high risk of aggressive endometrial carcinoma. These results are in line with those of a recent microarray gene expression profiling study of endometrial cancers, showing that TIMP-2 is down-regulated in high-grade tumours [45]. We also found a correlation between low TIMP-1 expression and both lymphovascular space involvement and recurrence. The few available data on TIMP-2 and TIMP-1 expression in endometrial cancers show an increase in TIMP mRNA or protein expression with histological grade [29, 32, 46]. Nevertheless, our results are in keeping with those observed in hepatocellular [47, 48], gallbladder [49], colorectal [50] and breast cancers [51], showing that TIMP-2 expression declines as tumour invasiveness increases. Our results also confirm those of Moser et al. [34] showing a negative correlation between MMP-2 and TIMP-2 expression in an immunohistochemical study. Together, these data show that the effects of MMPs and their inhibitors depend on their relative proportions in local microenvironments [52, 53]. However, TIMPs may directly modulate cell growth in an MMP-independent fashion [54]. It should also be noted that TIMP-2 has other roles, such as MMP-2 inhibition. Low levels of TIMP-2 activate MMP-2 through MT1-MMP, whereas high levels of TIMP-2 inhibit MMP-2 [55].

In normal endometrium, MMP-2 was expressed throughout the menstrual cycle, with strong epithelial cell expression during the proliferative phase and strong stromal cell expression during the secretory phase. These results confirm the differential expression of MMPs and TIMPs during the menstrual cycle, pointing to hormonal regulation [26–28]. In contrast to MMP-2, relatively few data are available on epithelial MMP-7 expression in normal endometrium. We observed strong epithelial MMP-7 expression in the proliferative phase, in keeping with the role of MMP-7 in degrading luminal debris and facilitating tissue remodelling during the early and mid-proliferative phases, and progesterone control of MMP-7 expression [26, 56, 57]. Bruner-Tran et al. [58] suggested that the ability of progesterone to inhibit endometrial MMP-7 expression required the local action of TGFβ. In addition to growth factors, MMP activity is regulated by cytokines and angiogenic factors [7, 52, 56]. We found no variation in epithelial MMP-9 expression between the proliferative and secretory phases. No attempt was made to evaluate MMP-9 expression during menstruation. Our results are partly in line with those of Goffin et al. [26] showing the pivotal role of MMP-9, with an increase in mRNA levels during menstruation but low expression in both the proliferative and secretory phases.

We found no variation in TIMP-1 expression by endometrial epithelial cells during the menstrual cycle. TIMP-1 expression by endometrial stromal cells was higher in the proliferative phase than in the secretory phase. Stronger epithelial TIMP-2 expression was noted in the proliferative phase, while stromal expression did not vary. Therefore, our results support sequential MMP and TIMP expression during the menstrual cycle.

In addition to the MMPs and TIMPs examined here, recent studies underline the role of other MMPs such as MMP-26 in tumour progression. By cleaving and inactivating the serpin α1-antitrypsin, MMP-26 promotes matrix destruction in estrogen-dependent tumours and thus contributes to malignant progression [59, 60].

Finally, interaction and co-operation between the stromal and epithelial compartments seem to be crucial for the invasion process [52]. Indeed, *in vitro*, MMPs have been shown to be synthesised and then translocated from stromal cells to epithelial cells under paracrine control involving integrins [38].

In conclusion, our results support the involvement of MMPs and TIMPs in endometrial carcinogenesis, as their expression profiles differ in hyperplastic endometrium with and without atypia, and in endometrial carcinomas. Moreover, MMP-2 and TIMP-2 expression profiles define a subgroup of endometrial cancers with a high risk of local and distant spread, suggesting they may have prognostic potential.

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