

Expression of metalloproteinases endometrial stromal sarcoma: immunohistochemical study using image analysis

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Abstract

Aim—To investigate the expression of matrix metalloproteinases (MMP), a group of proteolytic enzymes with a central role in extracellular matrix invasion and degradation, in stromal sarcomas.

Methods—11 endometrial stromal sarcomas (four low grade tumours, seven high grade) were stained for MMP-2, MMP-3, and MMP-9 using immunohistochemical stains. The surgical material consisted of nine hysterectomy specimens and two pelvic recurrences. Three hysterectomy specimens, removed for leiomyomas, were studied as controls. Staining area was evaluated using image analysis.

Results—Age at the time of diagnosis ranged from 21 to 67 years. Four of the 11 patients (three with high grade tumours and one with a low grade tumour) died of the disease, six remained free of disease, and one was lost to follow up. Staining for MMP-2, MMP-3, and MMP-9 was more diffuse in high grade tumours than in low grade tumours and controls. Staining for MMP-3 and MMP-9 was more pronounced in high grade than in low grade tumours ($p = 0.04$; $p = 0.05$). Staining for MMP-9 was significantly greater in all stromal sarcomas than in controls ($p < 0.001$ for high grade tumours *v* controls; $p < 0.01$ for low grade tumours *v* controls). Diffuse staining for MMP-2, exceeding 90% of the tumour area, was observed in three of seven high grade tumours but in no low grade tumours. There was no apparent correlation between staining for any of the three enzymes and survival.

Conclusions—Both low and high grade endometrial stromal tumours express matrix metalloproteinases. MMP-3 and MMP-9 are expressed more diffusely in high grade than in low grade tumours. In the individual case, diffuse staining for MMP-2 appears to best characterise the high grade tumours. Thus staining for MMP-2 may aid in differentiating high grade from low grade tumours, and MMP-9 in differentiating normal endometrial stroma from low and high grade endometrial stromal sarcomas. MMP expression does not appear to predict disease outcome in endometrial stromal sarcoma.

Keywords: metalloproteinases; gelatinases; stromelysins; endometrial stromal sarcoma; image analysis

Tumours of endometrial stromal origin are rare, comprising 10–15% of malignant mesenchymal uterine neoplasms.¹ These tumours contain areas resembling other sarcomas, as well as epithelium-like areas, and variably stain for both epithelial and mesenchymal markers.^{2–6} Tumour margins and mitotic counts were described by Norris and Taylor as key markers for their classification into stromal nodules (pushing margins) and low and high grade stromal sarcomas (infiltrative margins, with less than or more than 10 mitoses/10 high power fields, respectively).⁷ The role of mitotic counts in segregating these tumours into low and high grade sarcomas and therefore in predicting their clinical behaviour was questioned by Evans⁸ and by Chang and coworkers.⁹

A correlation between patient survival and several morphological and clinical indices has been shown in various studies. These included tumour size,^{10–11} tumour grade,^{10–11} involvement of surgical margins by tumour,¹¹ patient age¹² and menopausal status,¹¹ flow cytometry criteria and chromosomal aberrations,^{12–15} and tumour stage.^{9–12} The role of some of these variables in predicting disease outcome has not been supported by other studies.^{2–9–11}

Matrix metalloproteinases (MMP), a family of zinc and calcium dependent enzymes, appear to play a central role in the process of basement membrane and extracellular matrix degradation involved in tumour invasion and metastasis.^{16–21} More than 15 MMP are known to date, subgrouped according to their substrate specificity into interstitial collagenases, stromelysins, gelatinases, elastases, and membrane-type MMP.²² MMP expression has been studied extensively in tumours of diverse origins,^{16–17–23} but few studies have investigated their role in sarcomas. MMP expression has been reported in rat sarcomas,^{24–25} as well as in cell lines of human fibrosarcoma,^{26–27} osteosarcoma,^{26–28} malignant fibrous histiocytoma,²⁶ granulocytic sarcoma,²⁹ and Kaposi sarcoma.³⁰ In a case report of metastatic endometrial stromal sarcoma, MT-MMP was not expressed³¹.

We have now studied the expression of MMP-2, MMP-3, and MMP-9 in 11 stromal sarcomas (four low grade, seven high grade) and three controls.

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Table 1 Clinical data and metalloproteinase (MMP) staining results in the study group

No	Age (years)	Stage	LN†	Survival	Diagnosis‡	Specimen	MMP-2	MMP-3	MMP-9
1	44	–	–	NED	Control	Hysterectomy	8 (2)§	36 (22)	15 (5)
2	56	–	–	NED	Control	Hysterectomy	16 (2)	87 (8)	18 (8)
3	42	–	–	NED	Control	Hysterectomy	13 (8)	20 (4)	4 (1)
4	32	III	P+, PA–	NED	Low grade	Hysterectomy	22 (4)	44 (9)	44 (4)
5	50	I	ns	LFU	Low grade	Hysterectomy	59 (6)	46 (8)	81 (4)
6	48	I	ns	NED	Low grade	Hysterectomy	8 (1)	2 (1)	46 (7)
7	63	III	ns	DOD	Low grade	Hysterectomy	1 (0)	17 (8)	53 (17)
8	56	IV	ns	DOD	High grade	Hysterectomy	14 (3)	92 (3)	93 (4)
9	21	IV	ns	DOD	High grade	Hysterectomy	92 (2)	47 (8)	96 (2)
10	44	I	ns	NED	High grade	Pelvic mass	97 (3)	36 (5)	93 (7)
11	41	III	P+, PA–	NED	High grade	Hysterectomy	41 (8)	93 (2)	93 (2)
12	23	I	PA+ (late)	DOD	High grade	Pelvic mass	1 (0)	0 (0)	77 (9)
13	67	I	ns	NED	High grade	Hysterectomy	7 (9)	87 (2)	28 (8)
14	40	I	ns	NED	High grade	Hysterectomy	91 (3)	76 (2)	81 (3)

†Lymph nod evaluation (ns, not sampled; P, pelvic nodes; PA, periaortic nodes). In cases 7, 8, and 9, lymph node biopsy was not performed owing to preoperative or intraoperative evidence of metastatic disease (case 7 to ovary; case 8 to brain; case 9 to omentum).

‡Low/high grade endometrial stromal sarcoma.

§Mean (SD) percent stained area in six fields ($\times 60$ magnification).

DOD, died of disease; LFU, lost to follow up; NED, no evidence of disease.

Methods

CLINICAL DATA

The study population consisted of 11 patients, diagnosed with endometrial stromal sarcoma of the uterine corpus, treated and followed in our division of gynaecological oncology between 1978 and 1996. Follow up ranged from

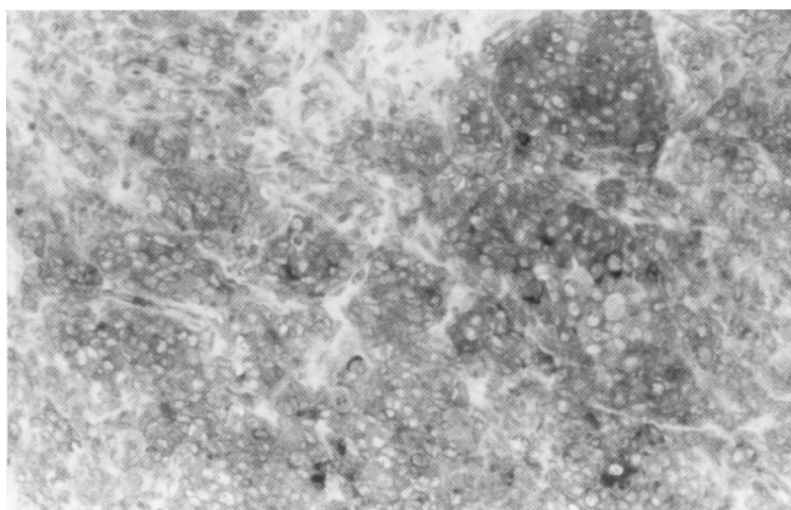


Figure 1 Low grade endometrial stromal sarcoma. Negative staining for MMP3. Image analysis revealed 2% staining, owing to staining of occasional lymphocytes (labelled avidin-biotin).

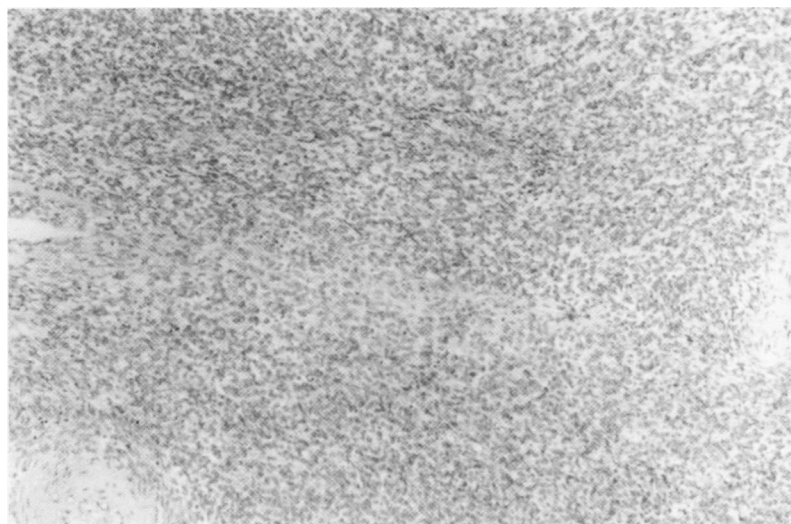


Figure 2 High grade endometrial stromal sarcoma. Intense positive staining for MMP9 (labelled avidin-biotin).

one to 17 years (mean five years). The surgical material consisted of nine hysterectomy specimens and two tumour recurrences, presenting as pelvic masses. Three hysterectomy specimens, removed for leiomyomas, were chosen as controls. Histological diagnosis of endometrial stromal sarcoma and segregation into low and high grade tumours was made according to established criteria.⁷ Diagnosis was made only after evaluation of the slides independently by two experienced senior pathologists (JK and IR).

Follow up surveillance included physical examination every three months for the first year, every four months for the second year, and every six months thereafter. Patients underwent yearly chest x rays and blood analyses, as well as additional examinations based on physical findings or specific complaints, as clinically indicated.

ANTIBODIES

Primary antibodies against the MMP used in immunohistochemical staining were anti-MMP-2 clone 42-5D11, anti-MMP-3 clone 55-2AH, and anti-MMP-9 clone 56-2AH. All three antibodies were obtained from Calbiochem.

IMMUNOHISTOCHEMICAL ANALYSIS

Immunohistochemical staining with the above antibodies was performed in all cases. Formalin fixed, paraffin embedded sections, 4 μ m thick, were thaw mounted onto Fisherbrand super frost/plus slides. After air drying at 37°C for 16 hours and incubation for 30 minutes at 60°C, slides were deparaffinised and rehydrated. No antigen exposure procedure of any kind was performed. Staining was performed after incubation with the antibodies at 4°C for 16 hours. Staining was performed with labelled avidin-biotin (LAB).^{32,33} All cases were stained simultaneously.

IMAGE ANALYSIS

Quantitative computerised image analysis was performed with Cis-2 computerised analysis system (Galai-Electro-Optical Inspection and Diagnosis Laboratories, Israel). The system software was designed as a joint venture

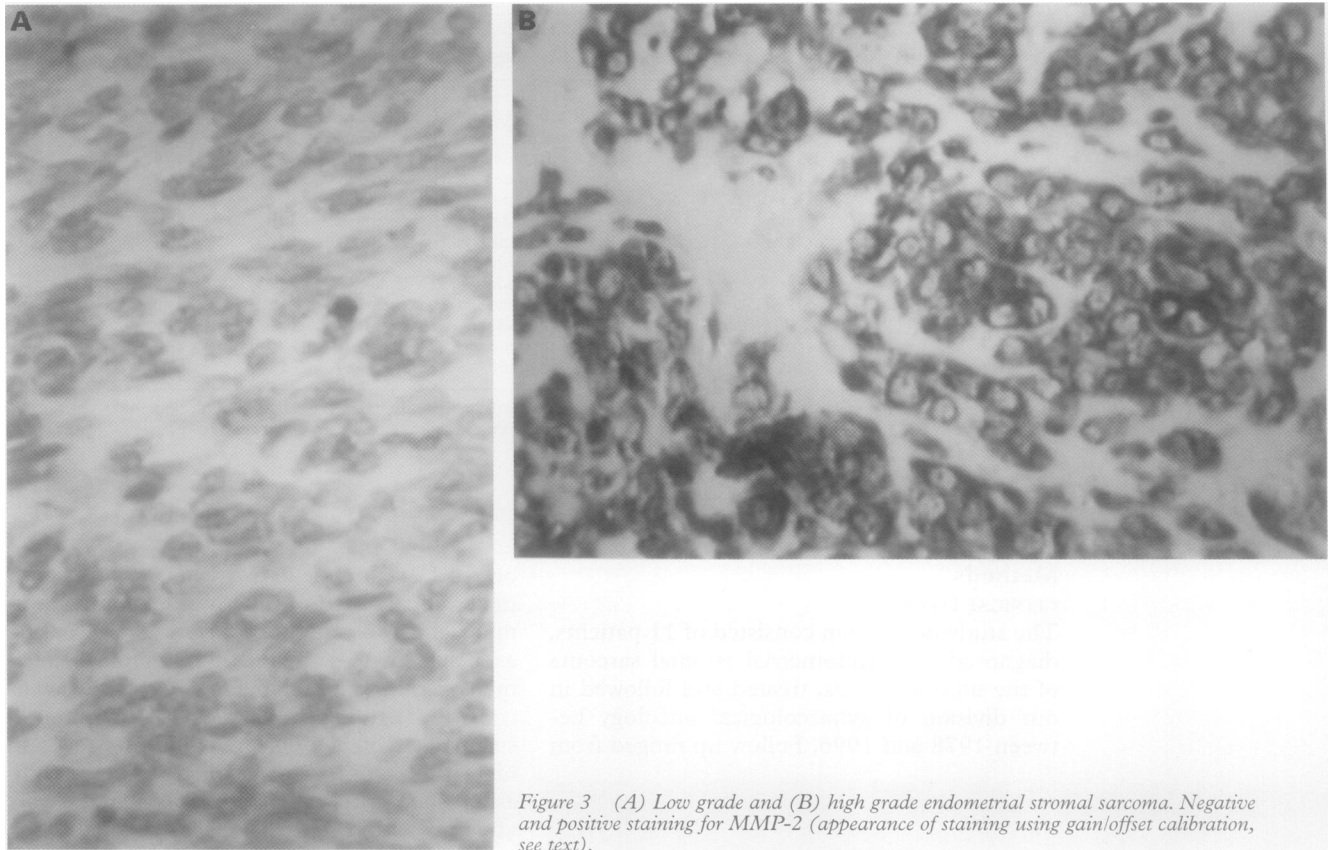


Figure 3 (A) Low grade and (B) high grade endometrial stromal sarcoma. Negative and positive staining for MMP-2 (appearance of staining using gain/offset calibration, see text).

between Olympus (Japan) and Galai Laboratories. The system included a high resolution CCD camera (M-852 Microtechnica; Sony) for image acquisition, and a positioning and autofocusing motorised system comprised of a remote controlled and automatically moved specimen holder table, a digitising card in-

stalled in a PC-AT type computer (MPC-486; Amigo) and a colour monitor (Trinitron; Sony). The monitor enabled acquisition and continuous display of the pictures—512×512 pixels with 256 grey levels of each of the red, green, and blue colours (data arrays). Gain/offset calibration was adjusted to distinguish

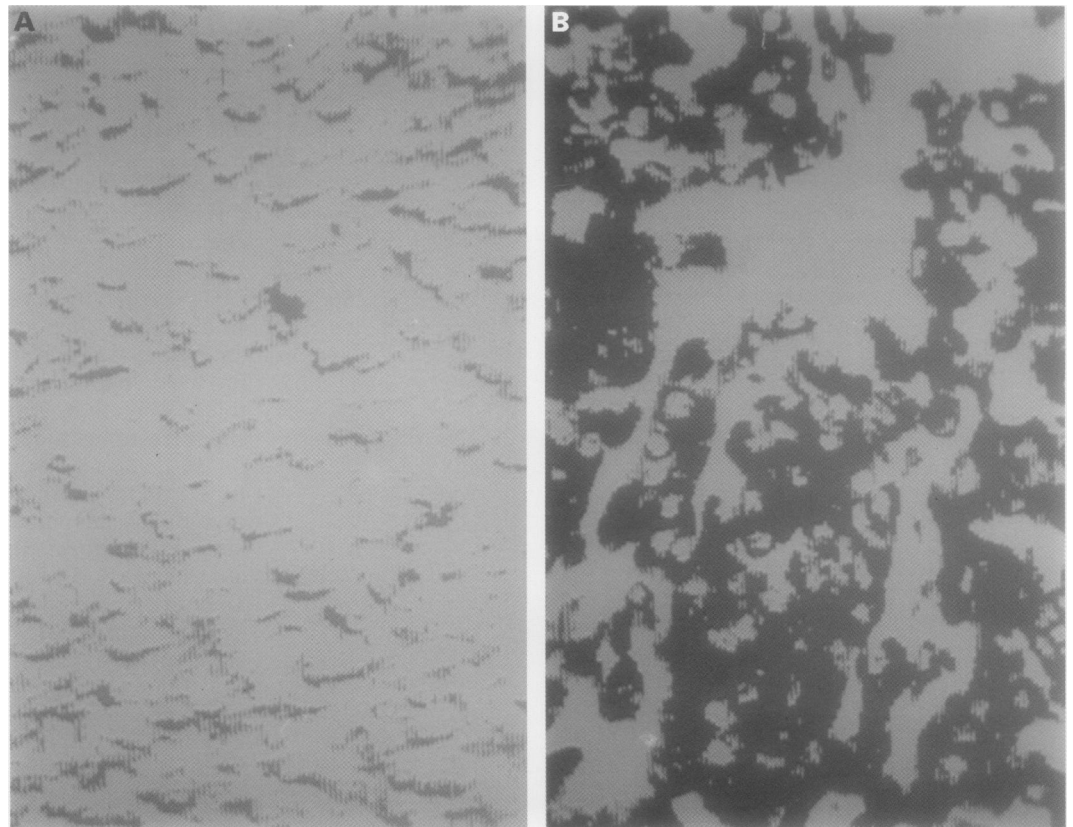


Figure 4 Appearance of MMP9 immunostaining for low grade (A) and high grade (B) sarcomas by image analysis.

Table 2 Metalloproteinase (MMP) staining results according to diagnostic group

Diagnosis	No of cases	MMP-2	MMP-3	MMP-9
Control	3	12.2 (3.9)†	47.6 (34.7)	12.6 (7.4)
Low grade ESS	4	22.3 (26.0)	27.2 (21.5)	56.0 (17.4)
High grade ESS	7	49.1 (43.2)	61.8 (35.3)	78.4 (23.2)

†Mean (SD) percent stained area for all cases in the diagnostic group.
ESS, endometrial stromal sarcoma.

the immunohistochemical staining from the background colours. Staining localisation and amount were analysed by a Fugitech computer (80486 DX processor).

Slides were scanned in a BH2 Olympus light microscope for six consecutive fields, chosen randomly and consisting of tumour cells exclusively, in $\times 60$ magnification. Areas at the margins of the tissue sections were not scanned. Six fields of benign endometrial stroma were scanned in the control sections. Colour ranges were adjusted to all sections.

Statistical analysis was performed using the one tailed *t* test.

Results

The patients' age at the time of diagnosis ranged from 21 to 67 years. Four patients died of disease (one with low grade and three with high grade endometrial stromal sarcoma), six remained free of disease, and one was lost to follow up (table 1). Tumour stage and node status (when available) are shown in table 1.

Positive staining for MMP-2 and MMP-9 was detected in all sarcomas, as well as in normal endometrial stroma. Staining for MMP-3 was detected in 10 of the 11 cases, being negative in one high grade tumour. Staining intensity appeared similar in all areas of a given tumour, and epithelioid elements in low grade tumours, when present, showed the same staining pattern as stromal elements. However, staining pattern varied widely among tumours, ranging from 1% to 97% (table 1, figs 1-4). Staining for MMP-2, MMP-3, and MMP-9 was more diffuse in high grade tumours as compared to low grade tumours and controls. Similarly, staining for MMP-2 and MMP-9 was more diffuse in low grade tumours when compared to controls (table 2).

Significant differences were observed between low grade and high grade tumours for MMP-3 ($p = 0.04$) and MMP-9 ($p = 0.05$). In addition, MMP-9 staining in both low and high grade tumours was more diffuse than in control specimens ($p < 0.001$ for high grade tumours *v* controls; $p < 0.01$ for low grade tumours *v* controls). Diffuse staining for MMP-2, exceeding 90% of the tumour area, was observed in three of seven high grade tumours but in none of their low grade counterparts. Staining for both MMP-2 and MMP-9 was consistently less than 20% in controls. Staining results did not correlate with survival ($p > 0.05$).

Discussion

In this study, we examined matrix metalloproteinase expression in uterine endometrial stromal sarcoma using immunohistochemistry. Image analysis was employed to evaluate staining.

MMP expression was detected in the majority of cases studied. As both low and high grade endometrial stromal sarcomas are capable of local uterine wall and vascular invasion, these enzymes may play a role in the tumour's propensity to invade locally and—mainly in the case of high grade tumours—to metastasise to distant organs. Nevertheless, the pattern of staining varied considerably for the three enzymes—staining area for both MMP-2 and MMP-3 was less than 10% in some tumours, both low and high grade. This staining appeared to be localised mainly to endothelial cells of small vessels and to inflammatory cells in the vicinity of tumour cells. Conversely, neoplastic cases consistently showed more diffuse staining, at least partly owing to the contribution of tumour cells. Although MMP-2 and MMP-9 share similar substrate specificity,²² MMP-9 (and MMP-3) have been shown to degrade collagen IV more efficiently than MMP-2,²¹ possibly reflecting their contribution to vascular basement membrane degradation that characterises the endometrial stromal sarcoma. This would be in agreement with our finding of raised MMP-9 values in both low grade and high grade tumours compared with controls. It is noteworthy that only MMP-9 did not show any overlap between control and tumour values.

The central role of MMP in tumour invasion and metastasis has been studied extensively in recent years. A correlation between MMP expression and tumour spread or unfavourable disease outcome has been shown in carcinomas of the cervix and of the ovary,³⁴⁻³⁵ as well as in various non-gynaecological tumours.³⁶⁻³⁸ In this study, we did not detect any correlation between staining for any of these enzymes and clinical outcome. Expression of all MMP varied considerably, both in the group of surviving patients and in those who died of the disease. This can be attributed to the number of tumours analysed, but may also be associated with the partly sarcomatous nature of endometrial stromal sarcomas. As cell lines were investigated in previous reports of MMP expression in sarcomas,²⁶⁻³⁰ this finding needs to be analysed further.

Distinction of low from high grade endometrial stromal sarcoma cannot be made on the basis of the absence or presence of staining for MMP-2, MMP-3, or MMP-9. However, for MMP-2 and MMP-9, a diffuse pattern of staining, exceeding 90% of tumour area, was seen exclusively in high grade endometrial stromal sarcoma. This distinction was more pronounced for MMP-2. As all the high grade tumours that were studied showed marked cellular atypia and pleomorphism, often accompanied by increased mitotic counts ($> 10/10$ high power fields) and abnormal mitotic figures, diffuse staining for these enzymes may serve as an additional guide to morphological criteria in some cases. Further investigation will be necessary to validate these findings.

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