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Expression of matrix metalloproteinases, tissue inhibitors of metalloproteinase, collagens, and Ki67 antigen in pleural malignant mesothelioma: an immunohistochemical and electron microscopic study

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Abstract Because matrix metalloproteinases (MMPs) degrade extracellular matrix, including basement membrane, and because tissue inhibitors of MMP (TIMPs) suppress MMP activities, MMPs and TIMPs are considered to play important roles in invasion and metastasis in many malignancies. We examined immunohistochemically the expression of MMPs (MMP-1, -2, -3, -7, and -9), TIMPs (TIMP-1 and -2), and collagens (types I, III, and IV) in 16 patients with pleural malignant mesothelioma (PMM; 8 with the epithelial, 4 with the sarcomatous, and 4 with the biphasic type). Electron microscopy revealed that the tumor cells in all types possessed the characteristics of malignant mesotheliomas, including numerous microvilli and moderate amounts of intermediate filaments. Basement lamina was present only focally. The proliferative Ki67 index was at a high level, compared with values reported in various other malignancies. Positive staining for MMP-1 was observed in most tumor cells in all 16 patients (100%). MMP-2 was expressed in most tumor cells in 2 patients (13%). In contrast, MMP-3, -7, and -9 were not detected in any PMM. TIMP-1 and TIMP-2 were expressed in 3 patients (19%) and 2 patients (13%), respectively. The stromal cells were simultaneously positive for MMPs or TIMPs in the patients whose tumor parenchymal cells were positive for each enzyme. These results indicate that the expression of MMP-1 and MMP-2 may be related to PMM invasion and spread. In particular, as MMP-1 was overexpressed in contrast to the lower expression of TIMP-1, MMP-1 is strongly suggested to play an important role in PMM invasion by degrading the tumor stroma. In spite of general agreement that epithelial-type PMM has a better prognosis than other types, there was no significant difference in the Ki67 index among the histological types of PMM.

Key words Pleural malignant mesothelioma · Immunohistochemistry · Metalloproteinase (MMP) · Tissue inhibitors of metalloproteinase (TIMP) · Collagenase · Ki67 index

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Introduction

Epidemiological and experimental studies have shown a close association between asbestos exposure and pleural malignant mesothelioma (PMM).[1] Although asbestos use is now restricted, there is still a high incidence of PMM in populations exposed to asbestos, because the disease has a long latent period of 30 to 40 years after asbestos exposure.[2] PMM, usually classified into epithelial, sarcomatous, and biphasic types of histology, is aggressive, with a very poor prognosis.[3] Especially, the sarcomatous and biphasic types are known to have a poorer prognosis.[3] PMM characteristically spreads rapidly along the serosal surfaces of the pleural cavity, encasing the lung with diffusely thickened pleura. PMM frequently accompanies pleural effusion, which may contribute to the dissemination and reimplantation of tumor cells in the thoracic cavity.[3,4] Matrix substances, including hyaluronic acid secreted from PMM, may participate in the highly invasive behavior of PMM.[5,6] In particular, proteolytic degradation of the extracellular matrix (ECM) appears to facilitate PMM invasion and spread. Because therapies for patients with PMM remain disappointing, the elucidation of the invasive mechanism of PMM may provide a novel clue for treatment options for PMM.

Recently, a number of studies have demonstrated that the overexpression of matrix metalloproteinases (MMPs), whose activity is dependent on metal ions (Zn^{2+} and Ca^{2+}),

is related to tumor invasion and metastasis through the degradation of ECM in many malignancies.[7–11] MMPs are currently classified into five subgroups (see "Discussion"): interstitial collagenase (MMP-1); gelatinases known as type IV collagenases, including gelatinase A (MMP-2) and gelatinase B (MMP-9); stromelysins (STR), which consist of four members, STR-1 (MMP-3), STR-2 (MMP-10), STR-3 (MMP-11), and matrilysin (MMP-7); elastases; and membrane-type MMPs (MT-MMPs). On the other hand, tissue inhibitors of matrix metalloproteinase (TIMPs), a family of natural inhibitors of MMP activity, may suppress the tumor invasion.[12] There was no report on the expression of MMPs and TIMPs in malignant mesothelioma until that of Liu et al.,[13] who very recently examined the expression of the mRNA of MMPs and TIMPs in MM cell lines, using reverse a transcriptase-polymerization configurations reaction method. They found that the mRNAs for MMP-1, -2, -3, and -9, and those for TIMP-1, -2, and -3 were expressed in all cell lines, that for MMP-7 in 6 cell lines, and that for MMP-10 in 3 cell lines, while MMP-11 mRNA was expressed in none of the cell lines. The production of these MMPs was also confirmed. The present study was intended to clarify whether MMPs and TIMPs were expressed in PMM, and if so, how their expression correlated with histological types of PMM and proliferative activity.

Patients and methods

Patients

Surgically resected tumor tissues obtained from 16 patients with PMM (8 with the epithelial, 4 with the sarcomatous, and 4 with the biphasic type), treated between 1978 and 1988 at the Hyogo Medical Center for Adults (Hyogo, Japan), were used for the present study (Table 1). The average age of the patients (13 men and 3 women) at the time of diagnosis was 62.6 years, ranging from 50 to 75 years. Of the 16 patients, 8 had a history of asbestos exposure for more than 10 years. The remaining patients denied a history of asbestos exposure. The chief complaints of the patients were symptoms of respiratory distress, such as chest pain, cough, and dyspnea. Ten patients were available for prognostic follow-up surveillance. Eight of the 10 patients died within 1 year after the onset of symptoms. One patient (case 4) who presented with the epithelial type of PMM was alive for more than 1 year after diagnosis.

Electron microscopy

Ten specimens of PMM (3 epithelial type, 3 biphasic type, and 4 sarcomatous type) were processed for electron microscopy. Small pieces of the tumors were immersed in 3% glutaraldehyde buffered with pH 7.3, 0.067 M phosphate, followed by fixation with 2% osmium tetroxide. Sections cut from Epon-embedded blocks and exhibiting light gold interference color were counterstained with uranyl acetate and lead citrate, and viewed at 75 kv with a JEOL 100SX electron microscope (JEOL, Tokyo, Japan).

Immunohistochemistry

Monoclonal antibodies against MMP-1, -2, -3, -7, and -9, those against TIMP-1 and -2, and those against types I, III, and IV collagen were purchased from Fuji Chemical (Toyama, Japan). Antibodies against MMP-2 and TIMP-1 were diluted 1:100, while the other antibodies were used at a dilution of 1:500. A monoclonal antibody against Ki67 (clone Mib1; Immunotech, Marseilles, France) was used at a dilution of 1:50.

Sections 4-μm-thick, cut from formalin-fixed, paraffin-embedded blocks, were subjected to immunohistochemical staining, using an LSAB kit (Dako, Kyoto, Japan). Endog-

Table 1. Clinical data of patients with pleural malignant mesothelioma

Patient	Age	Sex	Asbestos history (years)	Symptoms	Prognosis
Epithelial type					
1	67	M	15	Dyspnea	Death after 6 months
2	75	M	25	Cough	Unknown
3	67	M	Denied	Chest pain	Death after 1 year
4	50	M	Denied	Chest pain	Alive more than 1 year
5	64	M	Denied	Chest pain	Unknown
6	70	M	36	Cough	Unknown
7	67	M	20	Cough	Unknown
8	60	M	Denied	Chest pain	Unknown
Sarcomatous type					
9	67	F	16	Back pain	Death after 1 month
10	52	M	Denied	Dyspnea	Death after 4 months
11	68	M	Denied	Chest pain	Death after 1 month
12	68	F	Denied	Cough	Death after 11 months
Biphasic type					
13	68	M	10	Cough	Death after 4 months
14	55	M	15	Cough	Unknown
15	49	M	20	Chest pain	Death after 4 months
16	55	F	Denied	Back pain	Death after 1 months

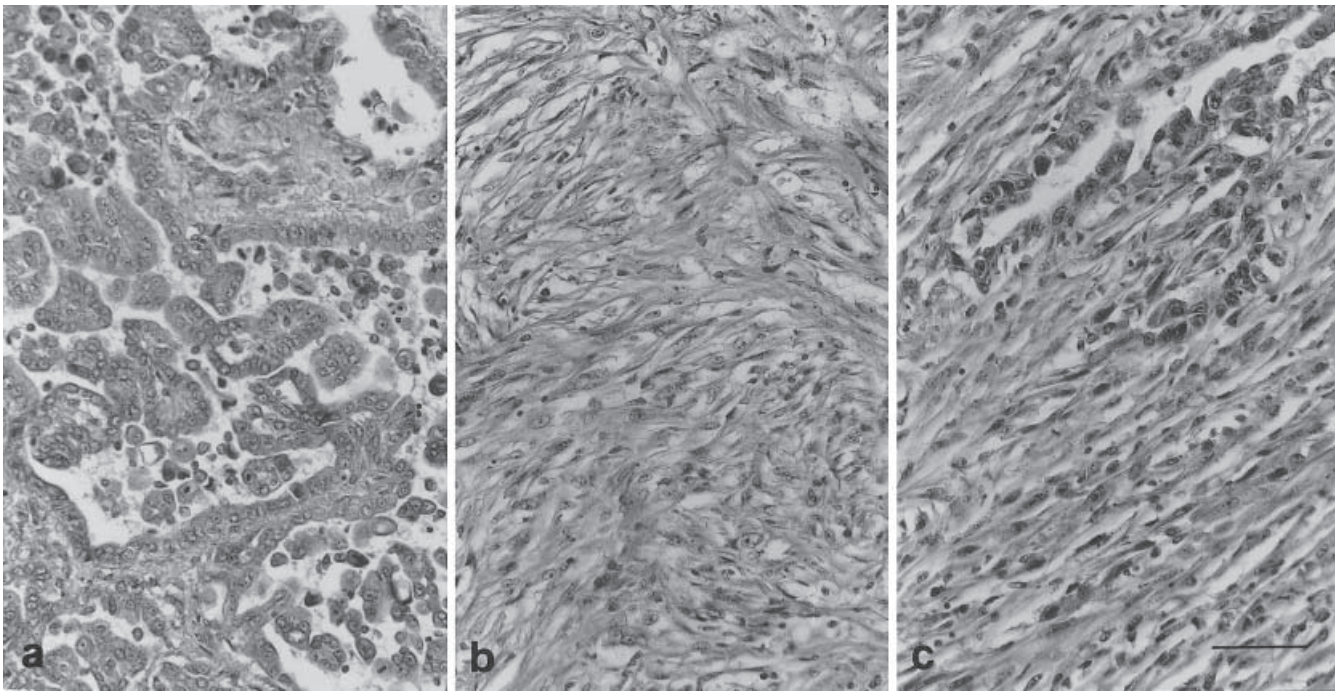

Fig. 1a–c. Microscopic appearance, showing **a** epithelial, **b** sarcomatous, and **c** biphasic types of pleural malignant mesothelioma (PMM) H & E; *bar*, 50 μm for **a**, **b**, **c**

enous peroxidase activity was blocked with 0.3% H_2O_2 in methanol for 10 min. Before incubation with primary antibodies against collagen types I, III, and IV, and Ki67, sections were incubated with 0.05% protease (Protease, type XXIV; Sigma Chemical, St. Louis, MO, USA) at 37°C for 30 min, and boiled twice, for 5 min each time, in 0.01% citrate buffer (pH 6.0). After incubation with primary antibodies at 4°C overnight, a secondary antibody was applied for 30 min, followed by the application of 0.02%, 3,3′-diaminobenzidine tetrahydrochloride in 50 mM Tris-aminomethane buffer, pH 7.6, containing 0.01% H_2O_2, for 5 min. The sections were counterstained with hematoxylin. Staining controls were carried out by replacing the primary antibodies with normal goat serum.

The Ki67 labeling index was expressed as the percentage of Ki67-positive cells among 500 tumor cells. In the biphasic type of PMM, Ki67-labeled cells were separately counted in epithelial and sarcomatous lesions. The results of immunostaining for MMPs and TIMPs were evaluated and graded as follows, irrespective of staining intensity: no positive cells, (−); <5% of the tumor cells staining positive, (+); 5%–50% of the tumor cells staining positive, (2+); and >50% of the tumor cells staining positive, (3+).

Statistical analysis

Statistical comparisons were performed using the two-tailed Student's *t*-test. We considered a P value of <0.05 to be significant.

Results

Light microscopy

Three types of PMM were examined (Fig. 1). The first was the epithelial type, in which cuboidal or flattened neoplastic cells were arranged in solid nests or tubulovesicular structures. The tumor cells had a moderate amount of acidophilic cytoplasm and uniform vesicular nuclei with prominent nucleoli. The second type was sarcomatous PMM. Spindle-shaped cells with slender nuclei and scanty cytoplasm proliferated in a storiform or intervening pattern. In biphasic PMM, tubular patterns of epithelioid cells were seen in the sarcomatous tissue, the latter being predominant over the epithelial components.

Electron microscopy

Electron microscopy revealed the ultrastructural characteristics of the tumors. In some mesotheliomas of the epithelial type, loosely apposed cells were joined in places by desmosomes and interdigitations, while other cells were closely apposed, with a narrow intercellular space (Fig. 2). The cytoplasm contained a moderate amount of intermediate filaments, clusters of mitochondria, and cisternae of rough endoplasmic reticulum. The intermediate filaments mostly occurred in densely aggregated tonofilments, and partly in more dispersed arrangements. In most mesotheliomas, microvilli without rootlets were prominent, not only on luminal surfaces but also on the adluminal surfaces of the cells (Fig. 3a, b). Mesotheliomas of the sarcomatous type showed

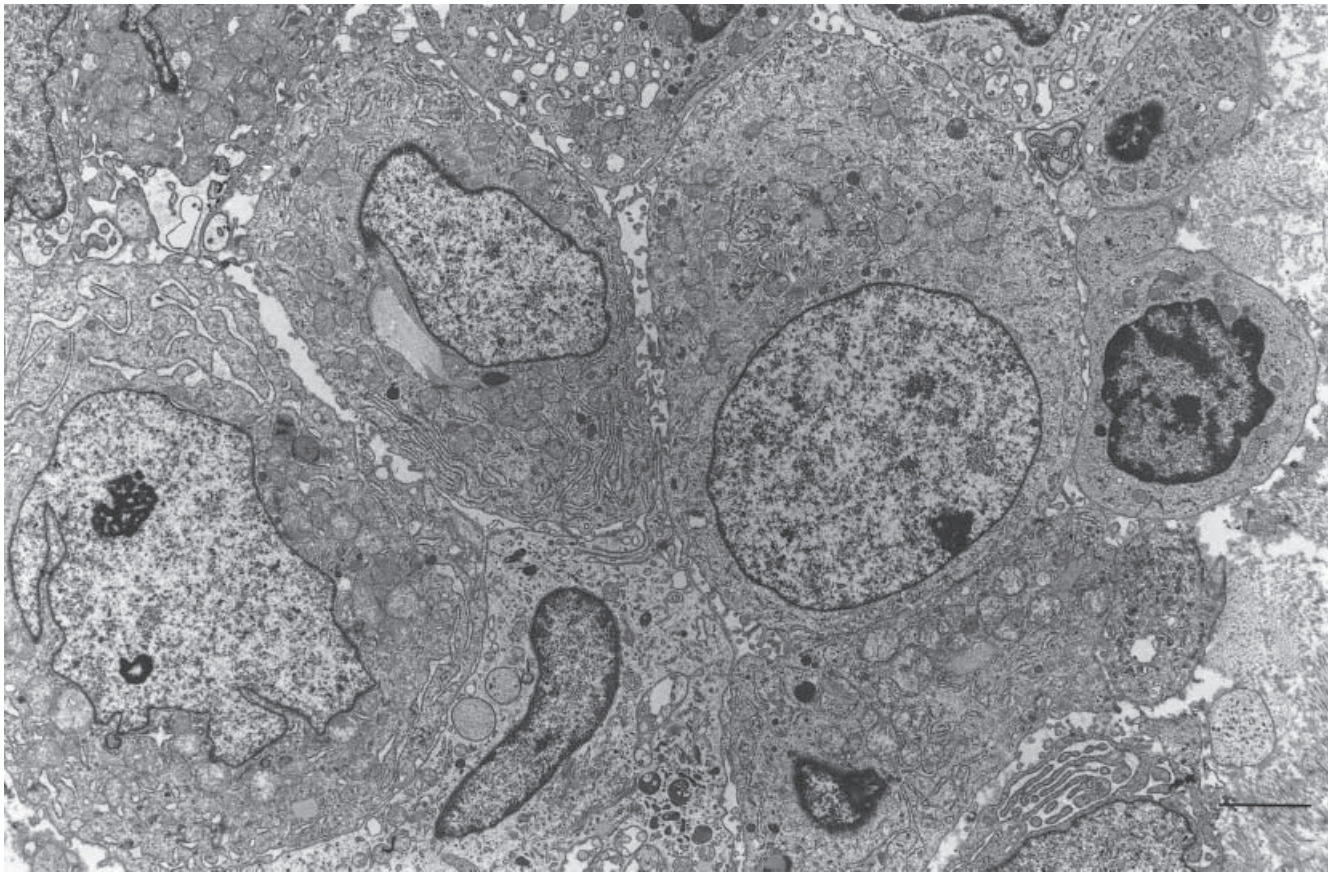

Fig. 2. Low-power electron micrograph, showing epithelial type of PMM. Loosely apposed tumor cells are round in shape, and contain clusters of mitochondria and cisternae of rough endoplasmic reticulum. Short microvilli are present on the cell surface, except for regions with a narrow intercellular space. *Bar*, 5 μm

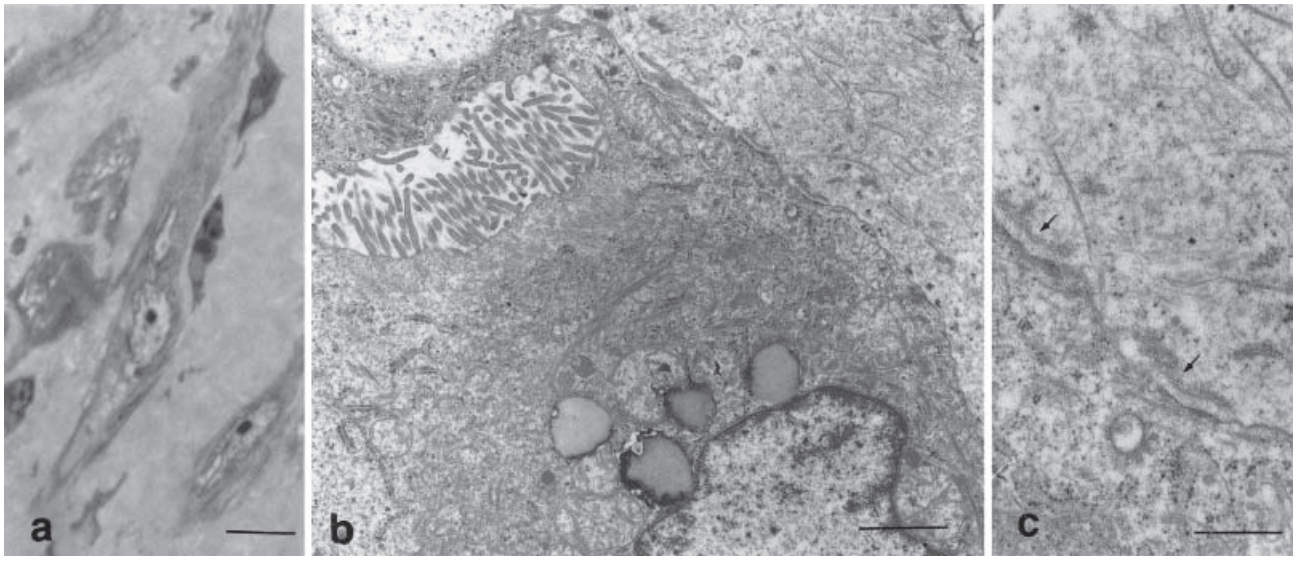

Fig. 3. **a** High-power view of sarcomatous type of PMM. Elongated tumor cells are scattered in the ample extracellular matrix. Epon-embedded, toluidine blue stain; *bar*, 100 μm. **b** Electron micrograph of sarcomatous type. Well developed intermediate filaments run in random directions. Numerous microvilli occur in the intercellular space sealed by desmosomes between neighboring tumor cells. Basal lamina-like amorphous materials are present focally. *Bar*, 3.3 μm. **c** High-power view of *upper right part of* ***b***. *Arrows* indicate lamina-like amorphous materials. *Bar*, 1.2 μm

Table 2. Results of immunohistochemical staining for MMPs and TIMPs in pleural malignant mesothelioma

Patient		MMP-1	MMP-2	MMP-3	MMP-7	MMP-9	TIMP-1	TIMP-2	Ki67 (%)
Epithelial type									
1		3+	−	−	−	−	−	−	20.9
2		3+	−	−	−	−	−	−	14.5
3		3+	3+	−	−	−	−	−	12.1
4		3+	−	−	−	−	1+	3+	16.1
5		3+	−	−	−	−	−	−	32.3
6		3+	−	−	−	−	1+	−	31.2
7		3+	−	−	−	−	−	−	23.3
8		3+	−	−	−	−	−	−	15.5
Sarcomatous type									
9		3+	−	−	−	−	−	−	20.1
10		3+	3+	−	−	−	−	−	12.2
11		3+	−	−	−	−	−	1+	18.8
12		3+	−	−	−	−	−	−	21.9
Biphasic type									
13	Epi	3+	−	−	−	−	−	−	35.5
	Sar	3+	−	−	−	−	−	−	38.5
14	Epi	3+	−	−	−	−	−	−	16.5
	Sar	3+	−	−	−	−	−	−	20.3
15	Epi	3+	−	−	−	−	3+	−	26.6
	Sar	3+	−	−	−	−	2+	−	20.4
16	Epi	3+	−	−	−	−	−	−	15.6
	Sar	3+	−	−	−	−	−	−	13.2

MMP, metalloproteinase; TIMP, tissue inhibitors of MMP; Epi, epithelial tumor cells; Sar, sarcomatous tumor cells
The results of immunohistochemical staining were graded as follows: no immunoreaction, (−); <5% of tumor cells staining positive, (+); 5%–50% of the tumor cells staining positive, (2+); >50% of the tumor cells staining positive, (3+)

features essentially identical to those of the epithelial type. The intermediate filaments tended to be localized in the peripheral cytoplasm. Amorphous material, probably representing the basal lamina, was present only focally in both types (Fig. 3b,c).

Immunohistochemistry of MMPs and TIMPs

The immunohistochemical results for MMPs and TIMPs in patients with PMM are summarized in Table 2. MMP-1 was intensely expressed in the cytoplasm of most tumor parenchymal cells in the specimens of all 16 patients with PMM (Fig. 4a,b). Stromal cells (fibroblasts and endothelial cells) were occasionally positive for MMP-1 in the specimens of all patients. MMP-2 was positive in most of the tumor parenchymal cells and a small number of neighboring stromal cells in the specimens of 2 patients (13%), 1 with the epithelial and 1 with the sarcomatous type (Fig. 4c,d). MMP-3, -7, and -9 were not expressed in any specimens of patients with PMM.

TIMP-1 was expressed in the cytoplasm of the tumor cells in the specimens of three patients (19%), two with the epithelial type and one with the biphasic type, with a range of (+) to (3+) (Fig. 4e). TIMP-2 immunoreactivity was present in the cytoplasm of the tumor cells in the specimens of two patients (13%), one with the epithelial type and one with the sarcomatous type, with degrees of (3+) and (+) respectively (Fig. 4f). TIMP-1 or TIMP-2 was also expressed in the stromal cells in specimens from patients in whom tumor parenchymal cells were positive for each enzyme. Collagenous fibers which formed the tumor stroma showed densely positive reactivity for types I and III collagen (Fig. 5a,b), but were negative for type IV collagen, except for the basement membrane of blood vessels within the tumor mass.

Ki67 labeling index

The Ki67 index (Table 2) was 20.7 ± 7.7% (mean ± SD) in the epithelial type ($n = 8$) and 18.3 ± 4.2% in the sarcomatous type ($n = 4$). In the biphasic type, it was 23.6 ± 9.4% in the epithelial lesion ($n = 4$) and 23.1 ± 10.8% in the sarcomatous lesion ($n = 4$). There was no significant difference in the proliferative index between the epithelial and sarcomatous types, or between the epithelial and sarcomatous lesions in the biphasic type. The average Ki67 index of the patients who died within 1 year was 19.8 ± 7.3% ($n = 8$), while one surviving patient had a Ki67 index of 16.1%.

Discussion

Electron microscopy revealed that the tumors examined in the present study possessed the characteristics of mesotheliomas reported elsewhere.[14–16] Microvilli and intracytoplasmic intermediate filaments were prominent, not only in the epithelial type but also in the sarcomatous type. Basal lamina, reported to be one of the characteristics of mesotheliomas, was seen only focally, even in the epithelial type, in the present study. Fern-like crystalline structures, or scroll-like structures, which are reported to be produced by hyalu-

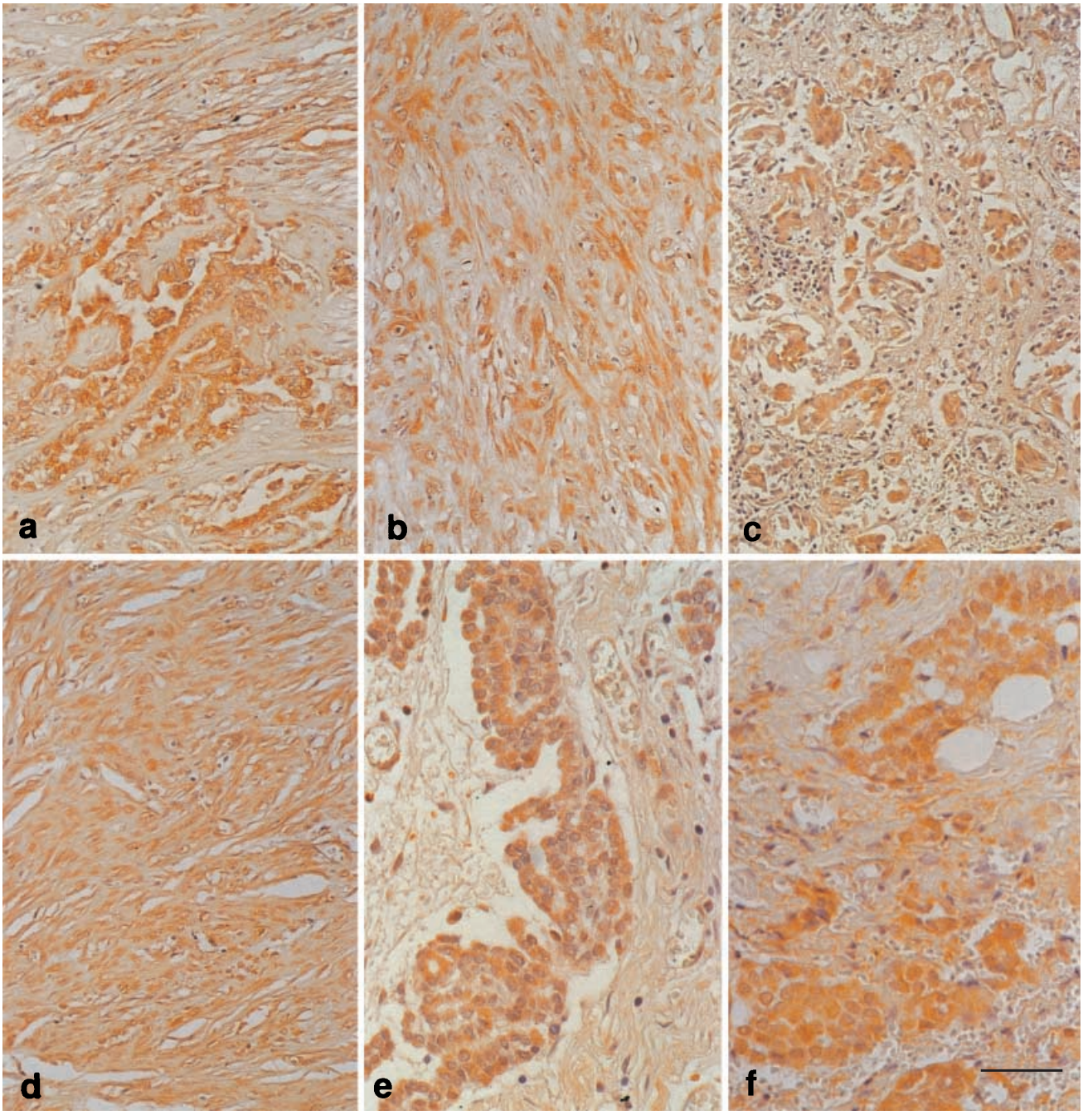

Fig. 4a–f. Immunohistochemical staining showing **a** matrix metalloproteinase (MMP)-1 in epithelial type, **b** MMP-1 in sarcomatous type, **c** MMP-2 in epithelial type, **d** MMP-2 in sarcomatous type, **e** tissue inhibitor of matrix metalloproteinase (TIMP)-1 in epithelial type, and **f** TIMP-2 in epithelial type. MMPs and TIMPs were expressed in the cytoplasm of tumor cells, as well as stromal cells, (spindle-shaped cells, endothelial cells, and lymphocytes). *Bar*, 25 μm, for **a–f**

ronic acid and/or proteoglycans, were not observed in the present study.[14]

Among the proliferative markers of tumors, the Ki67 index is widely accepted as one of the most reliable markers for estimating the malignancy grade and prognosis of various tumors.[17] This study showed that PMM had a high Ki67 labeling index, reflecting aggressive tumor behavior, compared with values reported in various other malignancies.[6] A survival study reported that epithelial-type PMM had a better prognosis than the sarcomatous and biphasic types.[3] However, no significant difference in the Ki67 index between the epithelial and sarcomatous types, or between the epithelial and sarcomatous lesions of the biphasic type, was shown in the present study. Also, the Ki67 labeling index value of a surviving patient with PMM was not lower than that of patients who died within 1 year after the onset of

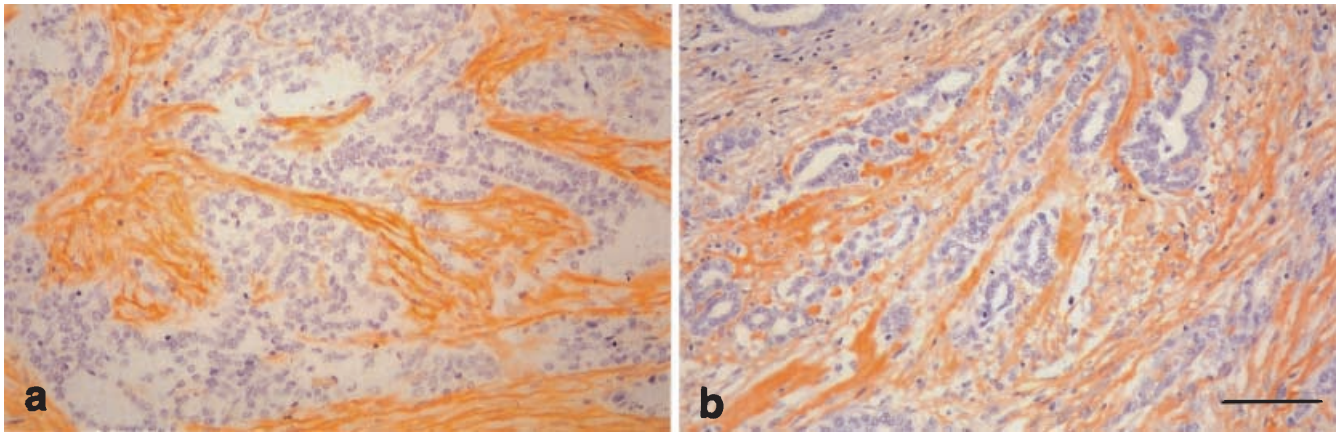

Fig. 5a–b. Immunohistochemical staining, showing **a** type I collagen and **b** type III collagen. Both types of collagen are densely present in the tumor stroma. *Bar*, 25 μm for **a** and **b**

symptoms. Therefore, other factors seem to be required to explain the prognostic difference in patients with histologically different types of PMM.

PMMs of all types had considerably abundant stroma, where types I and III collagen, but not type IV collagen, were shown to be localized in the present study by immunohistochemistry. MMP-1, which degrades collagens by cleaving the α-chain of types I and III collagen,[18] was widely distributed in the tumor parenchymal and stromal cells of all PMMs in the present study. This suggests that MMP-1 plays a key role in PMM invasion and spread, irrespective of the PMM type. To date, MMP-1 immunohistochemical expression has been examined in various malignancies, including cancers of the esophagus, colon, and pancreas.[19–21] Several studies reported that the expression of MMP-1 was associated with more rapid progression of carcinomas. In esophageal cancers, all patients in whom MMP-1 was expressed (11/46; 24%) died within 29 months, while nine of the patients (20%) whose tumors were negative for MMP-1 were alive at 60 months.[19] The median survival in patients with colorectal cancer was 11 months when the tumors were positive for MMP-1 (8/10; 80%), while it was 46 months when MMP-1 was negative (27/54; 50%).[19] Patients bearing pancreatic cancer with MMP-1 positivity in the primary site (33/46; 72%) had a significantly poorer prognosis than patients who were negative for MMP-1.[21] MMP-1 was expressed in 87% of 47 breast cancers, with a tendency toward a higher expression in the scirrhous type, in which the prognosis is known to be poorer than that for other types.[22] These findings imply that MMP-1 is important for facilitating the invasion and spread of some carcinomas. The expression of MMP-1 in the PMMs in the present study was extremely frequent and intense compared with that in the other malignancies cited above. Therefore, the poor prognosis of patients with PMM seems to be accounted for, at least in part, by the overexpression of MMP-1.

When epithelial malignancies grow and spread, tumor cells must break the basement membrane encircling cell nests. Components of the basement membrane, such as type IV collagen, laminin, and fibronectin, are degraded mainly by gelatinases (MMP-2 and MMP-9).[18] A positive correlation between gelatinase expression and tumor metastasis or the prognosis of patients has been described in various cancers of the digestive tract, breast, urogenital system, thyroid, and others.[19,22–30] In our PMMs, however, MMP-2 was expressed in only 2 of the 16 patients, and expression of MMP-9 was negative in all patients. The less frequent expression of gelatinases in PMMs may be accounted for by the poor development of basement membrane in PMMs. Normal mesothelial cells rest on the basement membrane with a submesothelial layer of connective tissue. When transformed mesothelial cells initially invade the submesothelial layer, degradation of the basement membrane is necessary. But, in general, the basement membrane is poorly developed or lacking in PMM, even in the epithelial type, as compared with carcinomas. This was shown in the present electron microscopy examination. Therefore, it appears that PMM, once established, no longer requires gelatinases for tumor growth and invasion.

The activities of MMPs are controlled by several factors. MMPs are secreted initially in an inactive form, and are then activated by the removal of the first 80 amino acids from the N-terminal by serine proteases, except for MMP-2, which is activated by cell-surface activators (MT-MMPs). On the other hand, MMP activity is inhibited by TIMPs, of which there are four members.[12] TIMP-1 inhibits the action of MMP-1, and TIMP-2 is an inhibitor of MMP-2 by the competitive inhibition of MT-MMP activation.[4,11,12,31] Examining laryngeal carcinoma using immunohistochemistry and in situ hybridization, Sawatsubashi et al.[32] found that positivity for MMP-1 and negativity for TIMP-1 tended to be associated with a sparse pattern of type I collagen, and negativity for MMP-1 and positivity for TIMP-1 with a dense or moderate pattern.[32] In our PMMs, type I collagen was dense in the specimens of all patients, without a difference among histological types, although MMP-1 positivity was intense in all PMMs. TIMP-1 was expressed in only 3 of the 16 patients' specimens, among which we could not find

any difference in the staining of type I and type III collagens or in the survival period, compared with findings in TIMP-1-negative patients.

In this context, the findings of Iwata et al.[22] are noteworthy. They reported that when TIMP-1 levels were measured in the media of primary breast tumor cultures by sandwich enzyme immunoassay, carcinomas produced significantly less TIMP-1 than fibroadenomas. However, immunohistochemistry did not indicate decreased TIMP-1 production in the carcinoma tissue. They speculated on the reasons for this discrepancy. One possibility was thought to be that secreted TIMP-1 might be trapped by active MMPs in the extracellular milieu of the carcinoma tissues, hindering TIMP-1 release in the culture media. The second possibility was that the actions of MMPs in the breast cancer tissues may have been pericellular. It may be that MMPs secreted from PMM in the present study acted only locally to bore a hole large enough for the through passage of tumor cells, so that the dense collagens remained almost unchanged, irrespective of the presence or absence of TIMP-1 expression.

Because there was no significant variation in the immunohistochemical staining of MMPs and TIMPs between the epithelial and sarcomatous types of MMP in this study, the prognostic difference in patients with histologically different types of PMM should be further explained by other factors, probably by clinical stages. This remains to be clarified in future studies.

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References

1. Battifora H, McCaughey WTE (1995) Tumors of the serosal membranes. Atlas of tumor pathology, 3rd series. Armed Forces Institute of Pathology, Washington, DC
2. Mossman BT, Kamp DW, Weitzman SA (1996) Mechanisms of carcinogenesis and clinical features of asbestos-associated cancers. Cancer Invest 14:466–480
3. Hillerdal G (1983) Malignant mesothelioma 1982: review of 4710 published cases. Br J Dis Chest 77:321–343
4. Attanoos RL, Gibbs AR (1997) Pathology of malignant mesothelioma. Histopathology 30:403–418
5. Rennard SI, Jaurand MC, Bignon J, Kawanami O, Ferrans VJ, Davidson J, Crystal RG (1984) Role of pleural mesothelial cells in the production of the submesothelial connective tissue matrix of lung. Am Rev Respir Dis 130:267–274
6. Kominek J, Robert KH, Sundqvist KG (1993) Chemotaxis and haptotaxis of human malignant mesothelioma cells: effects of fibronectin, laminin, type IV collagen, and an autocrine motility factor-like substance. Cancer Res 53:4376–4382
7. MacDougall JR, Matrisian LM (1995) Contributions of tumor and stromal matrix metalloproteinases to tumor progression, invasion and metastasis. Cancer Metast Rev 14:351–362
8. Aznavoorian S, Murphy AN, Stetler-Stevenson WG, Liotta LA (1993) Molecular aspects of tumor cell invasion and metastasis. Cancer 71:1368–1383
9. Crawford HC, Matrisian LN (1995) Tumor and stromal expression of matrix metalloproteinases and their role in tumor progression. Invasion Metastasis 14:234–245
10. Jones JL, Walker RA (1997) Control of matrix metalloproteinase activity in cancer. J Pathol 183:377–379
11. Stetler-Stevenson WG, Aznavoorian S, Liotta LA (1993) Tumor cell interactions with the extracellular matrix during invasion and metastasis. Annu Rev Cell Biol 9:541–573
12. Henriet P, Blavier L, Declerck YA (1999) Tissue inhibitors of metalloproteinase (TIMP) in invasion and proliferation. APMIS 107:111–119
13. Liu Z, Ivanoff A, Klominek J (2001) Expression and activity of matrix metalloproteinases in human malignant mesothelioma cell lines. Int J Cancer 91:638–643
14. Cook DS, Attanoos RL, Jalloh SS, Gibbs AR (2000) 'Mucin-positive' epithelial mesothelioma of the peritoneum: an unusual diagnostic pitfall. Histopathology 37:33–36
15. Ordonez NG, Mackay B (1998) Electron microscopy in tumor diagnosis: indications for its use in the immunohistochemical era. Hum Pathol 29:1403–1411
16. Oury TD, Hammar SP, Roggli VL (1998) Ultrastructural features of diffuse malignant mesotheliomas. Hum Pathol 29:1382–1392
17. Gerdes J, Schwab U, Lemke H, Stein H (1983) Production of a mouse monoclonal antibody reactive with a nuclear antigen associated with cell proliferation. Int J Cancer 31:13–20
18. Emonard H, Grimaud JA (1990) Matrix metalloproteinases. Cell Mol Biol 36:131–153
19. Murray GI, Duncan ME, O'Neil P, McKay JA, Melvin WT, Fothergill JE (1998) Matrix metalloproteinase-1 is associated with poor prognosis in oesophageal cancer. J Pathol 185:256–261
20. Murray GI, Duncan ME, O'Neil P, Melvin WT, Fothergill JE (1996) Matrix metalloproteinase-1 is associated with poor prognosis in colorectal cancer. Nature Med 2:461–462
21. Ito T, Ito M, Shiozawa J, Naito S, Kanematsu T, Sekine I (1999) Expression of the MMP-1 in human pancreatic carcinoma: relationship with prognostic factor. Mod Pathol 12:669–674
22. Iwata H, Kobayashi S, Iwase H, Masaoka A, Fujimoto N, Okada Y (1996) Production of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human breast carcinomas. Jpn J Cancer Res 87:602–611
23. Migita T, Sato E, Saito K, Mizoi T, Shiba K, Matsuno S, Nagura H, Ohtani H (1999) Differing expression of MMPs-1 and -9 and urokinase receptor between diffuse- and intestinal-type gastric carcinoma. Int J Cancer 84:74–79
24. Shima I, Sasaguri Y, Kusukawa J, Yamana H, Fujita H, Kakegawa T, Morimatsu M (1992) Production of matrix metalloproteinase-2 and metalloproteinase-3 related to malignant behavior of esophageal carcinoma. Cancer 70:2747–2753
25. Levy AT, Cioce V, Sobel ME, Garbisa S, Grigioni WF, Liotta LA, Stetler-Stevenson WG (1991) Increased expression of the Mr 72000 type IV collagenase in human colonic adenocarcinoma. Cancer Res 51:439–444
26. D'Errico A, Garbisa S, Liotta LA, Castronovo V, Stetler-Stevenson WG, Grigioni WF (1991) Augmentation of type IV collagenase, laminin receptor, and Ki67 proliferation antigen associated with human colon, gastric, and breast carcinoma progression. Mod Pathol 4:239–246
27. Garzetti GG, Ciavattini A, Lucarini G, Goteri G, De Nictolis M, Biagini G (1996) Microinvasive cervical carcinoma and cervical intraepithelial neoplasia: biologic significance and clinical implications of 72-kDa metalloproteinase immunostaining. Gynecol Oncol 61:197–203
28. Hoyhtya M, Fridman R, Komarek D, Porter-Jordan K, Stetler-Stevenson WG, Liotta LA, Liang CM (1994) Immunohistochemical localization of matrix metalloproteinase 2 and its specific inhibitor TIMP-2 in neoplastic tissues with monoclonal antibodies. Int J Cancer 56:500–505
29. Stearns ME, Wang M (1993) Type IV collagenase (Mr 72000) expression in human prostate: benign and malignant tissue. Cancer Res 53:878–883
30. Ozdemir E, Kakehi Y, Okuno H, Yoshida O (1999) Role of matrix metalloproteinase-9 in the basement membrane destruction of superficial urothelial carcinomas. J Urol 161:1359–1363
31. Strongin AY, Marmer BL, Grant GA, Goldberg GI (1993) Plasma membrane-dependent activation of the 72-kDa type IV collagenase is prevented by complex formation with TIMP-2. J Biol Chem 268:14033–14039
32. Sawatsubashi M, Mizokami H, Tokunaga O, Shin T (1998) Expression of MMP-1, TIMP-1, and Type 1 collagen in laryngeal carcinoma. Mod Pathol 11:878–885