Clinical role of pleural effusion MMP-3 levels in malignant pleural mesothelioma

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Abstract. Malignant pleural mesothelioma (MPM) is an aggressive malignant tumor of mesothelial origin associated with asbestos exposure. MPM exhibits a limited response to conventional chemotherapy and radiotherapy. This, early diagnosis of MPM is essential. Malignant tumor progression requires the destruction of the basement membrane, which is constructed from extracellular matrix (ECM) materials. Various types of human tumor cells are reported to produce ECM-degrading proteases that are important in tumor progression. Among this group of proteolytic enzymes, matrix metalloproteinases (MMPs) are thought to be important due to their wide degrading function. We investigated the pleural effusion MMP-3 levels of patients with MPM and compared them with those of a population with non-malignant pleuritis or lung cancer involving malignant pleural effusion. The pleural effusion MMP-3 concentrations of 52 MPM patients and 67 non-MPM patients were measured. The results showed that the MPM patients had significantly higher pleural effusion MMP-3 levels than the population with non-malignant pleuritis. The overall survival of the MPM patients with lower pleural effusion MMP-3 levels was longer than that of patients with higher pleural effusion MMP-3 levels. Our data therefore suggest a clinical role of pleural effusion MMP-3 levels in malignant pleural mesothelioma.

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Abbreviations: AUC, area under the ROC curve; BM, basement membrane; CI, confidence interval; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; MMP, matrix metalloproteinase; MPM, malignant pleural mesothelioma; PDGF, platelet-derived growth factor; ROC, receiver operating characteristic; TGF, transforming growth factor

Key words: asbestos-related lung diseases, malignant mesothelioma, tumor marker, diagnosis, prognosis

Introduction

Malignant pleural mesothelioma (MPM) is an aggressive malignant tumor of mesothelial origin associated with asbestos exposure (1-3). The lifetime risk of MPM is associated with a history of occupational and/or environmental asbestos exposure (4). Due to the long latency period (typically over 30 years) between the first asbestos exposure and the onset of the disease, MPM remains a universally fatal disease of increasing incidence worldwide (1,2,5), although asbestos usage has recently decreased in Western countries and Japan.

Malignant tumor progression requires the destruction of the basement membrane (BM), which is constructed from extracellular matrix (ECM) materials. Various human tumor cells are reported to produce ECM-degrading proteases that are important in tumor progression (6). Among this group of proteolytic enzymes, matrix metalloproteinases (MMPs) are thought to be important due to their wide degrading function. MMPs are zinc-dependent endopeptidases, whose activities are targeted to all components of the ECM (7).

MMP-3 is known to be involved in tumor cell invasion and metastasis (8). The increased expression of MMP-3 has been reported in several malignant tumors, including esophageal cancer (9), breast cancer (10) and glioma (11). Moreover, a correlation between a higher MMP-3 expression and disease progression has been reported in patients with gastric cancer (12), hepatocellular carcinoma (13) and bladder cancer (14). However, the clinical importance of MMP-3 in MPM patients has not been fully investigated, although MMP-3 expression has been reported in certain MPM cells (15,16). In this study, we evaluated the clinical role of the pleural effusion MMP-3 concentration as a biomarker in MPM.

Materials and methods

Patients and pleural effusion samples. The MMP-3 levels in pleural effusion samples collected from 119 individuals presenting at the Department of Respiratory Medicine of Hyogo College of Medicine between 2005 and 2009 were examined. The pleural effusions were obtained by thoracocentesis. All cases were diagnosed by pathologists, and it was confirmed that their clinical course matched their diagnosis. Fifty-two individuals had MPM involving a documented asbestos exposure history. These cases were diagnosed by patholo-

gists skilled in the diagnosis of MPM using histopathological samples. The patients were classified using the staging system of the International Mesothelioma Interest Group (IMIG) (17). Patients with MPM were treated according to our therapeutic guidelines: combination chemotherapy including the multi-target anti-folate pemetrexed was performed for patients with performance status (PS) 0-1 who were aged <70, and the best supportive care was selected for the remaining patients. Surgical treatment was not performed in any patient in the present study. Thirty-three individuals, including 8 cases with benign asbestos pleurisy, had non-malignant pleural effusion. Thirty-four individuals had lung cancer involving malignant pleural effusion without asbestos exposure. We verified the asbestos exposure by interview. The study was approved by our ethics committee in accordance with the 1975 Declaration of Helsinki. Informed consent was obtained from all patients. Fresh pleural effusion samples were collected prior to treatment and centrifuged for 10 min at 2000 x g at 4˚C, and the resultant supernatants were immediately frozen in liquid nitrogen and stored at -80˚C until use.

Measurement of MMP-3. The MMP-3 concentrations of the pleural effusions were measured using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Oxford, UK) according to the manufacturer's instructions.

Statistical analysis. The non-parametric Mann-Whitney U test was used to compare three groups of samples. In all tests, $p<0.05$ was considered to indicate a statistically significant result. To estimate the significance of the pleural effusion MMP-3 values, receiver operating characteristic (ROC) curves, the area under the ROC curve (AUC) and their 95% confidence intervals (95% CI) were calculated using standard techniques. To examine the cut-off values for pleural effusion MMP-3 levels, we calculated the total sensitivity and specificity for each cut-off value and then selected the cut-off values that maximized each factor. Estimates of the probability of survival were calculated by the Kaplan-Meier method and compared using the log-rank test to evaluate the prognostic significance of MMP-3 with regard to the survival of patients with MPM.

Results

MMP-3 pleural effusion levels in MPM and non-MPM patients. We recruited a total of 119 subjects presenting with pleural effusion. Of the 119 patients, 52 had confirmed MPM, 33 had non-malignant pleural effusion, and 34 had lung cancer involving malignant pleural effusion. Their characteristics are shown in Table I. Of the 52 patients with MPM, 40 were of epithelioid histology, 10 sarcomatoid and 2 biphasic.

The ROC curves for pleural effusion MMP-3 levels (Fig. 1A) reveal that the patients with MPM had an AUC of 0.651 in comparison with those with non-malignant pleural effusion (95% CI 0.555-0.747). At the optimal cut-off value of 50 ng/ml, the diagnostic sensitivity was 30.8% and the specificity was 97.0%. The mean pleural effusion MMP-3 concentration of the patients with MPM was significantly higher (49.1±59.1 ng/ml) than that of the patients with non-malignant pleural effusion (19.8±13.8 ng/ml; p=0.028; Fig. 1B). Although the mean pleural effusion MMP-3 concentrations of the patients with MPM was higher than that of the patients with lung cancer involving malignant pleural effusion (33.7±22.4 ng/ml), no statistically significant difference was found between them (p=0.909; Fig. 1B). No statistically significant differences were observed between the pleural effusion MMP-3 levels of the MPM histological groups (epithelioid, 54.2±62.9 ng/ml; non-epithelioid, 32.1±45.5 ng/ml) or the different disease stages (stage I, 33.1±30.2 ng/ml; stage II, 34.0±32.2 ng/ml; stage III, 84.6±120.3 ng/ml; and stage IV, 49.9±58.8 ng/ml) and there were no significant differences between the pleural

Table I. Characteristics of the MPM and non-MPM patients.

Patient group	Cases (%)	Total
MPM		52
Age, years (mean ± SD)	69.1±10.3	
Gender		
Male	39 (75.0)	
Female	13 (25.0)	
Histology		
Epithelioid	40 (76.9)	
Sarcomatoid	10 (19.2)	
Biphasic	2 (3.9)	
Stage		
I	8 (15.4)	
II	5 (9.6)	
III	8 (15.4)	
IV	31 (59.6)	
Non-malignant		33
Age, years (mean ± SD)	70.6±11.1	
Gender		
Male	28 (84.8)	
Female	5 (15.2)	
Histology		
Benign asbestos pleurisy	8 (24.1)	
Tuberculous (Tb) pleurisy	9 (27.3)	
Infectious (non-Tb) pleurisy	9 (27.3)	
Empysema	2 (6.1)	
Heart failure	2 (6.1)	
Hepatic failure	1 (3.0)	
Renal failure	2 (6.1)	
Lung cancer		34
Age, years (mean ± SD)	67.6±11.1	
Gender		
Male	23 (67.6)	
Female	11 (32.4)	
Histology		
Adenocarcinoma	31 (91.2)	
Squamous cell carcinoma	2 (5.9)	
Small cell carcinoma	1 (2.9)	

MPM, malignant pleural mesothelioma.

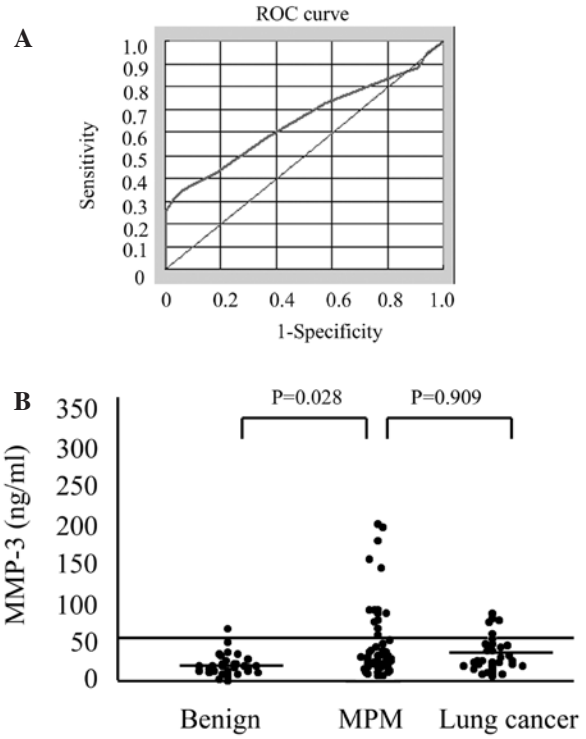

Figure 1. Pleural effusion MMP-3 levels in patients with MPM and patients with non-MPM. (A) An analysis that included 52 MPM and 33 non-malignant pleural effusion patients revealed an area under the curve (AUC) of 0.651 (95% CI 0.555-0.747). At the optimal cut-off value of 50 ng/ml, the diagnostic sensitivity was 30.8% and the specificity was 97.0%. (B) The pleural effusion MMP-3 levels of the patients with MPM versus those of the patients with non-malignant pleural effusion or lung cancer involving malignant pleural effusion were measured as described in Materials and methods. The non-parametric Mann-Whitney U test was used, and $p<0.05$ was considered to indicate a statistically significant result. The horizontal bars are the mean for each group. The cut-off value is shown as a horizontal line. ROC, receiver operating characteristic; MPM, malignant pleural mesothelioma; MMP-3, matrix metalloproteinase-3; CI, confidence interval.

effusion MMP-3 levels of the subjects with benign asbestos pleurisy and those with benign pleurisy without a history of asbestos exposure (14.4±9.0 ng/ml and 20.3±14.2 ng/ml, respectively).

Correlation between pleural effusion MMP-3 levels and overall survival. Among the 52 MPM patients, we were able to follow 47 patients closely for up to 1,700 days. Twenty patients had died by the end of the follow-up. Five patients were lost due to lack of information following transfer to other hospitals. These 5 subjects were homogenously distributed in the two groups compared (3 subjects were >50 ng/ml and 2 subjects were ≤50 ng/ml).

To study the correlation between pleural effusion MMP-3 levels and patient clinical courses, we separated the patients according to their pleural effusion MMP-3 levels at the time of the first measurement. The first group included patients with pleural effusion MMP-3 levels lower than 50 ng/ml, the cut-off value that we selected. In this group of 34 patients, the mean MMP-3 value was 18.2 ng/ml (interquartile range, 10.5-26.2). The other group included the remaining 13 patients, who had pleural effusion MMP-3 levels higher than 50 ng/ml and whose mean MMP-3 pleural effusion concentration was 109.5 ng/ml (interquartile range, 71.8-139.1). The difference in overall survival between the groups with lower and higher pleural effusion MMP-3 concentrations than the cut-off point of 50 ng/ml was not statistically significant ($p=0.51$; Fig. 2). However, there was a tendency for the survival of the patients with lower pleural effusion MMP-3 levels to be longer than that of the patients with higher pleural effusion MMP-3 levels.

Discussion

MPM shows a limited response to conventional chemotherapy and radiotherapy. Although the multi-target anti-folate pemetrexed has recently been approved as a first-line agent for use in combination with cisplatin for the treatment of MPM, survival of patients remains extremely poor (18), with a median survival duration of 8-18 months (19). Although in advanced cases, resection of the tumor only prolongs survival by approximately 3 months, it has been reported that patients with stage IA disease survive for five or more years following total resection of the tumor (20). Moreover, in several centers, potentially curative surgery combined with some form of adjuvant therapy has been performed. Early diagnosis may provide an opportunity for early treatment using new treat-

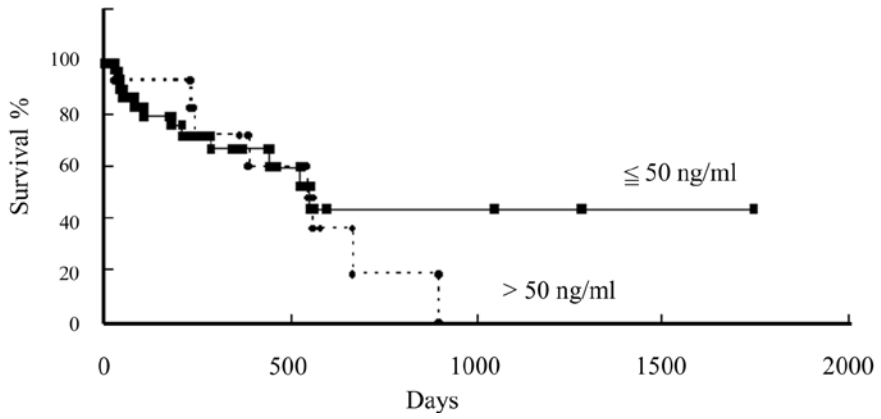

Figure 2. Survival of MPM subjects according to pleural effusion MMP-3 levels. Estimates of the probability of survival were calculated using the Kaplan-Meier method and compared using the log-rank test. MPM, malignant pleural mesothelioma; MMP-3, matrix metalloproteinase-3.

ment regimens, although whether early intervention results in good prognosis has yet be confirmed.

Due to the difficulty of diagnosing MPM by radiological and/or histological examinations, efficient and practical pleural effusion biomarkers are required to aid the diagnosis of MPM. Several cytokines, including interleukin (IL)-6 (21), transforming growth factor (TGF)-β1 (22-24), platelet-derived growth factor (PDGF) (25), TGF-α (26), and IL-8 (27), are significant in the development of MPM. Pleural effusion biomarkers for MPM, including hyaluronic acid and CYFRA 21-1, have also been reported and used to assist the diagnosis of MPM (20,28-32). The level of mesothelin-related protein (SMRP), the soluble form of mesothelin, has been reported to be a useful pleural effusion marker in MPM (33). However, little is known about their biological functions or effects on MPM cells.

A number of studies have focused on the expression of MMPs, including MMP-2 and MMP-9 in MPM cells, or in patients with MPM or lung cancer (34,35). In the present study, we examined the serum and pleural effusion MMP-2 levels of MPM patients and found no significant differences between samples from patients with MPM and those from non-MPM patients (data not shown). MMP-2 and MMP-9, which are also known as gelatinase A and B, respectively, cleave type IV collagen. However, MMP-3 degrades several components of the ECM, including fibronectin, laminin and collagen type IV (36). MMP-3 is also involved in tumor cell invasion and metastasis (8).

In this study, we evaluated the clinical role of the pleural effusion MMP-3 concentration as a biomarker in MPM, and demonstrated that the pleural effusion MMP-3 concentrations of patients with MPM were significantly higher than those of patients with non-malignant pleural effusion. At the optimal cut-off value of 50 ng/ml, the diagnostic sensitivity of the MMP-3 pleural effusion concentration was low (30.8%), and its negative predictive value (non-MPM patients/all patients with pleural effusion MMP-3 levels of <50 ng/ml) was not high (47.1%), suggesting that the pleural effusion MMP-3 concentration cannot be used to select MPM patients from individuals with lower pleural effusion MMP-3 levels. However, its specificity was high (97.0%) and its positive predictive value of 94.1% (MPM patients/all patients with pleural effusion MMP-3 levels of >50 ng/ml), suggests that the MMP-3 concentration could be used to differentiate MPM patients from patients with higher MMP-3 pleural effusion levels. Although the difference in overall survival between the groups with lower and higher pleural effusion MMP-3 values than the cut-off point (50 ng/ml) was not statistically significant, the survival of patients with higher pleural effusion MMP-3 levels showed a tendency to be shorter than that of the patients with lower pleural effusion MMP-3 levels. This observation is compatible with the previous studies demonstrating a correlation between higher MMP-3 expression and disease progression in patients with certain malignancies (12-14). It is well known that MPM patients with higher stage and/or non-epithelioid tumor have a poor prognosis. In the present study, however, there were no significant differences in pleural effusion MMP-3 levels among disease stages as well as histological types (epithelioid versus non-epithelioid). We consider that the prognostic impact of pleural effusion MMP-3 levels needs further evaluation. From these findings, patients with high pleural effusion MMP-3 levels may be suspected of having MPM and a poor prognosis.

We evaluated the clinical role of the pleural effusion MMP-3 concentration as a biomarker in MPM, and demonstrated that patients with MPM had significantly higher pleural effusion MMP-3 levels than a population with non-malignant pleuritis involving benign asbestos pleurisy, suggesting MMP-3 to be a useful diagnostic marker of MPM. The Kaplan-Meier method revealed that the survival of the patients with higher pleural effusion MMP-3 levels showed a tendency to be shorter than that of the patients with lower pleural effusion MMP-3 levels, indicating the usefulness of MMP-3 as a prognostic marker.

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