Matrix Metalloproteinase-2 Status in Stromal Fibroblasts, Not in Tumor Cells, Is a Significant Prognostic Factor in Non–Small-Cell Lung Cancer

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ABSTRACT

Purpose: **The purpose is to assess clinical significance of matrix metalloproteinase (MMP)-2 and MMP-9 status, especially MMP-2 status, in stromal cells in non–small-cell lung cancer (NSCLC) because experimental studies have revealed that stromal MMP-2 plays important roles in progression of malignant tumors, but most clinical studies focused on tumoral MMP-2 expression, not stromal MMP-2 expression.**

Experimental Design: **We conducted a retrospective study on MMP-2 and MMP-9 expression as evaluated immunohistochemically in a total of 218 consecutive patients with completely resected pathological stage I–IIIA, NSCLC.**

Results: **Strong MMP-2 expression in tumor cells and stromal fibroblasts were documented in 54 (24.8%) and 132 (60.6%) patients, respectively. Strong MMP-2 expression in stromal fibroblasts was more frequently seen in squamous cell carcinoma (72.7%) than in adenocarcinoma (54.9%; $P = 0.016$). Tumors showing strong MMP-2 expression in stromal fibroblasts showed a significantly higher intratumoral microvessel density (IMVD) than weak stromal MMP-2 tumors (mean intratumoral microvessel density, 50.9 *versus* 32.4, $P = 0.003$). In addition, postoperative prognosis of strong stromal MMP-2 patients was significantly poorer than that of weak stromal MMP-2 patients (5-year survival rate, 77.5 *versus* 60.2%, $P = 0.032$), and the prognostic significance was enhanced in squamous cell carcinoma patients but disappeared in adenocarcinoma patients. Multivariate analyses confirmed that strong stromal MMP-2 expression was a significant factor to predict a poor prognosis in squamous cell carcinoma patients, not in adenocarcinoma patients. In contrast, MMP-2 or MMP-9 status in tumor cells was not a significant prognostic factor.**

Conclusions: **MMP-2 status in stromal fibroblasts, not in tumor cells, was a significant prognostic factor associated with angiogenesis in NSCLC.**

INTRODUCTION

Primary lung cancer is the most common cause of cancer-related deaths in most industrialized countries, and non–small-cell lung cancers (NSCLCs) account for ~80% of primary lung cancer (1). Therefore, it is necessary to establish clinical markers, other than the tumor-node-metastasis staging system, that may predict the prognosis and response toward a specific therapy. Although experimental studies have revealed many biological markers that may be correlated with development and progression of malignant tumors, including NSCLC, no biological markers have been established as a clinical marker in the diagnosis or therapy (2).

Recent experimental studies have revealed that degradation of the extracellular matrix (ECM) by matrix metalloproteinases (MMPs) is a critical process in progression of malignant tumors, including NSCLC, because degradation of the ECM is required in tumor angiogenesis, as well as tumor invasion and metastases (3–7). Among many MMPs that have been identified, MMP-2 (Gelatinase-A) and MMP-9 (Gelatinase-B) are thought to be key enzymes as they degrade type IV collagen, the main component of ECM (6, 7). After these experimental studies, many clinical studies on MMP-2 and/or MMP-9 expression in malignant tumors, including NSCLC, have been conducted (8–13), but the clinical significance remains controversial (6). In addition, in most clinical studies, MMP expression only in tumor cells was assessed, whereas experimental studies have revealed that stromal tumor cells as well as tumor cells do express MMPs, especially MMP-2, and stromal fibroblast expression MMP-2 plays an important role in tumor progression (6, 14). Thus, we conducted a large-scale clinical study on MMP-2 and MMP-9 expression in tumor cells and in stromal fibroblasts as evaluated immunohistochemically to clarify the clinical significance in resected NSCLC.

PATIENTS AND METHODS

Patients and Tissue Preparation. A total of 237 consecutive patients with pathological stage I–IIIA NSCLC, who

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underwent complete resection without any preoperative therapy at Kyoto University Hospital from January 1, 1985, through December 31, 1990, was retrospectively reviewed. One patient was excluded from the study due to operation-related death, and 18 patients in evaluation of MMP-2 and 23 patients in evaluation of MMP-9 were excluded from the study due to lack of surgical specimens, and finally a total of 218 patients in evaluation of MMP-2 and 213 patients in evaluation of MMP-9 was evaluated (Table 1; refs. 15–17). p-stage and histologic type were reevaluated and determined with the current tumor-node-metastasis classification as revised in 1997 (18) and the current classification by WHO as revised in 1999 (19), respectively.

For all these patients, the inpatient medical records, chest X-ray films, whole-body computed tomography films, bone scanning data, and records of surgery were reviewed. Intraoperative therapy was not performed in any patient. As postoperative adjuvant therapy, cisplatin-based chemotherapy, radiation, and oral administration of tegafur (a fluorouracil-derivative drug) were prescribed for 55, 35, and 58 patients, respectively (15). Follow-up of the postoperative clinical course was conducted by outpatient medical records and by inquiries by telephone or letter.

All primary tumor specimens were immediately fixed in 10% (v/v) formalin and then embedded in paraffin. Serial 4-μm sections were prepared from each sample and served for H&E staining, immunohistochemical staining, and the terminal deoxynucleotidyltransferase-mediated nick end labeling staining. Results of immunohistochemical staining and terminal deoxynucleotidyltransferase-mediated nick end labeling staining were evaluated by two authors independently (S. Ishikawa and F. Tanaka) without knowledge of any clinical data. In case of a discordant evaluation after reevaluation, the slides were evaluated by another author (K. Takenaka). This study has been approved by the Ethics Committee, Graduate School and Faculty of Medicine, Kyoto University.

Evaluation of MMP-2 and MMP-9 Expression. Expression of MMP-2 and MMP-9 was assessed immunohistochemically using a standard streptavidin-biotinylated horseradish peroxidase complex method (LSAB+ kit/HRP; Dako, Kyoto, Japan). For antigen retrieval, sections were autoclaved at 121°C for 5 minutes in 0.01 mol/L citrate buffer (pH 6.0), and then sections were incubated in methanol containing 0.03% H_2O_2 (Nakalai Tesque, Kyoto, Japan) for 30 minutes. After incubation in a nonspecific staining blocking agent (BlockAce; Dainihon Seiyaku, Osaka, Japan), sections were incubated overnight at 4°C with each primary antibody as follows: an antihuman MMP-2 monoclonal antibody (mAb) (500 μg/mL mouse IgG1/κ, F-68; Daiichi Fine Chemical Co. Ltd., Tokyo, Japan) diluted at 1/200 and an antihuman MMP-9 monoclonal antibody (500 μg/mL mouse IgG1/κ, F-69; Daiichi Fine Chemical Co. Ltd.) diluted at 1/250. As a negative control, each section was treated without the primary antibody.

MMP-2 or MMP-9 expression in tumor cells and MMP-2 expression in stromal fibroblasts were classified according to the following grading system. MMP-9 expression in stromal cells was not assessed because the expression was negative or faint. A percentage score was defined as follows: score 0 if the percentage positive staining cells was ≤25%, score 1 if the percentage was >25 and ≤50%, and score 2 if the percentage was >50%; an intensity score was defined as follows: score 0 if no staining was documented, score 1 if the staining intensity was weak, score 2 if the intensity was moderate, and score 3 if the intensity was high. Each section was finally classified based on the sum of the percentage score and the intensity score as

Table 1 Expression of MMP-2 in stromal fibroblasts and tumor cells in non-small cell lung cancer

	Expression of MMP-2					
	Stromal fibroblasts			Tumor cells		
	Weak	Strong	*P*	Weak	Strong	*P*
All Patients	86 (39.4%)	132 (60.6%)		164 (75.2%)	54 (24.8%)	
Age (mean in y)	61.5	63.1	0.228	63.5	59.5	0.006
Lower*	45 (41.7%)	63 (58.3%)	0.580	75 (69.4%)	33 (30.6%)	0.060
Higher*	41 (37.3%)	69 (62.7%)		89 (80.9%)	21 (19.1%)	
Gender						
Male	58 (36.9%)	99 (63.1%)	0.280	120 (76.4%)	37 (23.6%)	0.600
Female	28 (45.9%)	33 (54.1%)		44 (75.2%)	17 (27.9%)	
Performance status						
0	79 (41.4%)	112 (58.6%)		144 (75.4%)	47 (24.6%)	
1	7 (28.0%)	18 (72.0%)	0.227	19 (76.0%)	6 (24.0%)	0.707
2	0 (0.0%)	2 (100%)		1 (50.0%)	1 (50.0%)	
Histologic type						
Squamous cell carcinoma	21 (27.3%)	56 (72.7%)	0.016†	67 (87.0%)	10 (13.0%)	0.004†
Adenocarcinoma	55 (45.1%)	67 (54.9%)		84 (68.9%)	38 (31.1%)	
Large-cell cacinoma	4 (36.4%)	7 (63.6%)		8 (72.7%)	3 (27.3%)	
Others	6 (75.0%)	2 (25.0%)		5 (62.5%)	3 (37.5%)	
Tumor differentiation						
I	53 (42.4%)	72 (57.6%)		91 (72.8%)	34 (27.2%)	
II	10 (41.7%)	14 (58.3%)	0.453	21 (87.5%)	3 (12.5%)	0.311
IIIA	23 (33.3%)	46 (66.7%)		52 (75.4%)	17 (24.6%)	

NOTE. Each figure shows the number of patients, and the percentage is shown in parentheses.
* Lower, age < 64 years; Higher, age ≥ 64 years.
† Comparison between squamous cell carcinoma and adenocarcinoma.

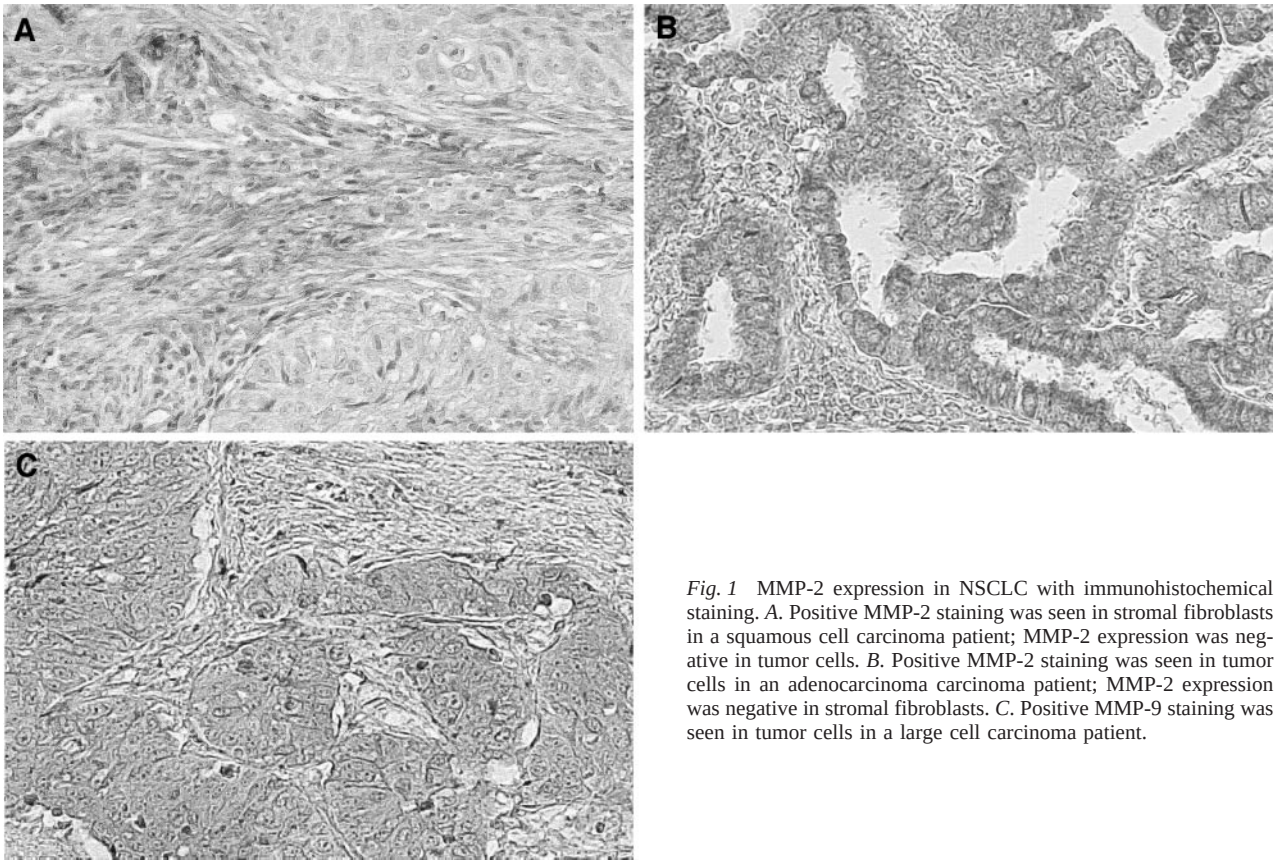

Fig. 1 MMP-2 expression in NSCLC with immunohistochemical staining. *A*. Positive MMP-2 staining was seen in stromal fibroblasts in a squamous cell carcinoma patient; MMP-2 expression was negative in tumor cells. *B*. Positive MMP-2 staining was seen in tumor cells in an adenocarcinoma carcinoma patient; MMP-2 expression was negative in stromal fibroblasts. *C*. Positive MMP-9 staining was seen in tumor cells in a large cell carcinoma patient.

follows: weak expression when the sum was ≤3 and strong expression when the sum was 4 or 5.

Expression of vascular endothelial growth factor (VEGF) was also evaluated immunohistochemically as described previously (16, 17). Briefly, an anti-VEGF polyclonal antibody A-20 (200 μg/mL rabbit IgG; Santa Cruz Biotechnology, Santa Cruz, CA) diluted at 1/50 was used as the primary antibody. VEGF expression was also evaluated according to the same scoring system and was finally classified into weak or strong expression based on the score (16, 17).

Quantification of Angiogenesis [Intratumoral Microvessel Density (IMVD)]. IMVD, a measurement of tumor angiogenesis, was evaluated immunohistochemically as described in previous studies (16, 17). Briefly, immunohistochemical staining for CD34 (a pan-endothelial marker) and CD 105 (a proliferation-related endothelial marker) to highlight endothelial cells was performed using a sensitive streptavidin-biotinylated horseradish peroxidase complex system (TSA-Indirect kit; NEN Life Science Products, Boston, MA). Primary antibodies used were an anti-CD34 mAb QBEnd10 (50 μg/mL mouse IgG1/κ; Dako), diluted at 1/50, and an anti-CD105 mAb SN6 h (366 μg/mL mouse IgG1/κ; Dako), diluted at 1/100. The 10 most vascular areas within a section were selected for evaluation of angiogenesis, and vessels labeled with the anti-CD34 mAb or the anti-CD105 mAb were counted under light microscopy with a 200-fold magnification. The average counts were recorded as theCD34-IMVD or CD105-IMVD for each case.

Evaluation of Cell Proliferation, Apoptotic Cell Death, and p53 Status. Proliferative activity of tumor cells was evaluated by immunohistochemical staining using a mAb against proliferative cell nuclear antigen (clone PC-10, 400 μg/mL mouse IgG2a/κ; Dako) as described previously (15). A total of 1000 tumor cells was counted for positive staining, and the proliferative activity was represented as the percentage of proliferative cell nuclear antigen-positive tumors cells.

The terminal deoxynucleotidyltransferase-mediated nick end labeling staining to detect apoptotic cells was performed using *In Situ* Death Detection kit POD (Boehringer Mannheim, Mannheim, Germany) as described previously (15). In each case, a total of 10,000 tumor cells was evaluated, and apoptotic index was defined as the number of apoptotic cells per 1000 tumor cells.

Evaluation of p53 status was performed by immunohistochemical staining using an antihuman p53 mAb DO-7 (250 μg/mL mouse IgG2b/κ; Dako) diluted at 1:50 as described previously (15). When the percentages of positive cells exceed 5%, each section was judged to exhibit aberrant p53 expression.

Statistical Methods. The χ^2 was used to compare counts. Continuous data were compared using Student's *t* test, if the distribution of samples was normal, or the Mann-Whitney *U* test, if the sample distribution was asymmetrical. Postoperative survival was analyzed by the Kaplan-Meier method, and the difference was assessed by the log-rank test. A multivariate analysis of prognostic factors was performed using a Cox's

regression model. Differences were considered significant when $P < 0.05$. All statistical manipulations were performed using the SPSS for Windows software system (SPSS, Inc., Chicago, IL).

RESULTS

Expression of MMP-2 and MMP-9 in NSCLC. Expression of MMP-2 and MMP-9 was seen mainly in the cytoplasm of tumor cells (Fig. 1), and strong MMP-2 and MMP-9 expression in tumor cells were seen in 54 (24.8%) and 98 (46.0%) patients, respectively (Table 1). Strong MMP-2 expression in tumor cells was more frequent in adenocarcinoma patients (38 of 122, 31.1%) than in squamous cell carcinoma patients (10 of 77, 13.0%, $P = 0.004$), and the mean age of patients with strong tumoral MMP-2 expression was significantly lower than that of patients with weak tumoral MMP-2 expression (59.5 and 63.5years, respectively, $P = 0.006$; Table 1). There was no significant correlation between tumoral MMP-9 status and any patient characteristic (data not shown).

Strong MMP-2 expression in the stromal fibroblasts was also seen in 132 (60.6%) of all patients (Fig. 1 and Table 1), whereas no definite MMP-9 expression was seen in any patient. In contrast to tumoral MMP-2 expression, strong MMP-2 expression in stromal fibroblasts was more frequent in squamous cell carcinoma patients (56 of 77, 72.7%) than in adenocarcinoma patients (67 of 122, 54.9%, $P = 0.016$; Table 1). No significant correlation between stromal MMP-2 status and other patient characteristics was documented (Table 1).

MMP-2 Status and Other Biomarkers. The mean VEGF score for strong tumoral MMP-2 tumor (4.15) was significantly higher than that for weak tumoral MMP-2 tumor (3.58, $P = 0.030$). No significant difference in the mean VEGF score according to the stromal MMP-2 status (Table 2).

The mean CD105-IMVD for strong stromal MMP-2 tumor (50.9) was significantly higher than that for weak stromal MMP-2 tumor (32.4. $P = 0.003$), whereas no significant difference in the mean CD34-IMVD was documented (Table 2). The difference in the mean CD105 according to the stromal MMP-2 status was marked in squamous cell carci-

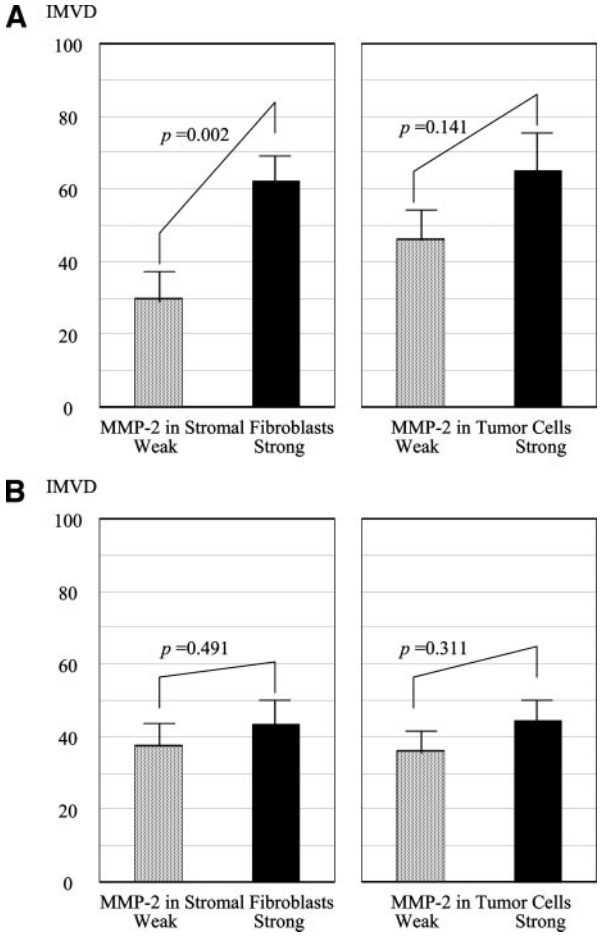

Fig. 2 *A.* IMVD in squamous cell carcinoma according to the status of MMP-2 expression in stromal fibroblasts and MMP-2 expression in tumor cells. IMVD was determined with an anti-CD105 antibody. (Each *column* shows the mean IMVD value, and the *error bars* show the SE.) *B.* IMVD in adenocarcinoma according the status of MMP-2 expression in stromal fibroblasts and MMP-2 expression in tumor cells. IMVD was determined with an anti-CD105 antibody. (Each *column* shows the mean IMVD value, and the *error bars* show the SE.)

Table 2 Biomarkers according to expression of MMP-2

	Expression of MMP-2					
	Stromal fibroblasts			Tumor cells		
	Weak	Strong	*P*	Weak	Strong	*P*
Expression of VEGF						
Mean score	3.56	3.83	0.264	3.58	4.15	0.030
IMVD						
CD34*	168.1	187.6	0.138	180.2	179.2	0.949
CD105*	32.4	50.9	0.003	38.6	48.6	0.185
Proliferative index						
Mean	39.3	50.6	0.004	47.5	42.1	0.191
Apoptotic index						
Mean	15.6	18.6	0.280	17.3	17.6	0.940
Aberrant expression of p53						
Negative	50 (40.3%)	74 (59.7%)	0.781	97 (78.2%)	27 (28.7%)	0.269
Positive	36 (38.3%)	58 (61.7%)		67 (71.3%)	27 (28.7%)	

NOTE. Each figure shows the number of patients, and the percentage is shown in parentheses.
* CD34, IMVD evaluated with an anti-CD34 antibody; CD105, IMVD evaluated with an anti-CD105 antibody.

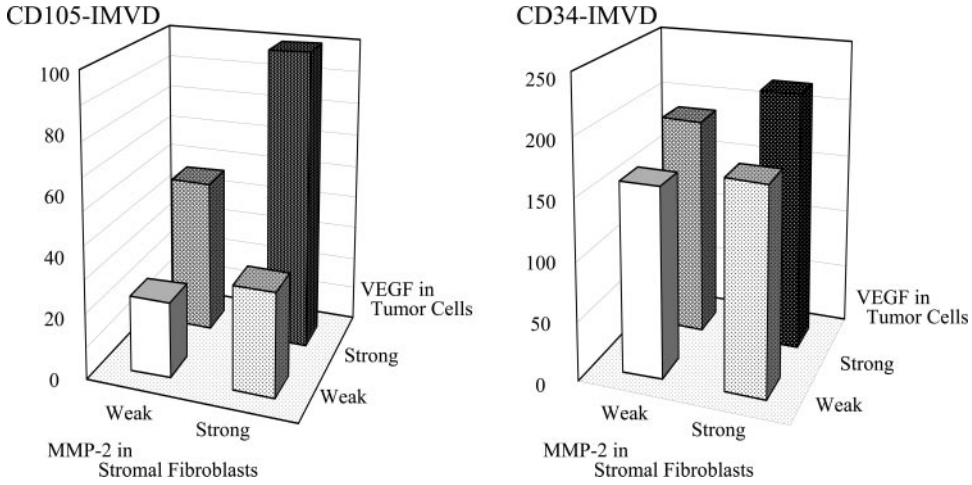

Fig. 3 IMVD in NSCLC according to the status of MMP-2 expression in stromal fibroblasts in combination with the status of VEGF expression in tumor cells. IMVD was determined with an anti-CD105 antibody (CD105-IMVD; *left*) or with an anti-CD34 antibody (CD34-IMVD; *right*).

noma ($P = 0.002$; Fig. 2*A*) and was not significant in adenocarcinoma ($P = 0.491$; Fig. 2*B*). When combined with VEGF status in tumor cells, the effect of stromal MMP-2 status on CD105-IMVD was enhanced (Fig. 3); tumor with strong tumoral VEGF expression and strong stromal MMP-2 expression showed the highest CD105-IMVD, and tumor with weak tumoral VEGF expression and weak stromal MMP-2 expression showed the lowest CD105-IMVD. There was no difference in the mean CD105-IMVD or CD34-IMVD according to the tumoral MMP-9 status (data not shown).

Strong stromal MMP-2 tumor showed a significantly higher proliferative index (50.6) than weak stromal MMP-2 tumor (39.3, $P = 0.004$; Table 2). There was no significant difference in other biomarker according to the stromal MMP-2 status (Table 2).

MMP-2 and MMP-9 Status and Postoperative Survival. Five-year survival rates of the patients with weak MMP-2 expression and strong MMP-2 expression in stromal fibroblasts were 77.5 and 60.2%, showing that weak stromal MMP-2 patients showed a significantly favorable postoperative survival ($P = 0.032$; Table 3 and Fig. 4). Subset analyses revealed that the prognostic significance of MMP-2 status in stromal fibroblasts was evident in squamous cell carcinoma patients, especially pathological stage I squamous cell carcinoma patients, but disappeared in adenocarcinoma patients (Table 3). Multivariate analyses showed that MMP-2 status in stromal fibroblasts was a marginal prognostic predictor for all NSCLC patients [$P = 0.064$; relative hazard, 1.666, 95% confidence interval (0.971–2.856)]; stromal MMP-2 status was an independent and significant prognostic predictor for squamous cell carcinoma patients ($P = 0.022$; relative hazard, 9.828, 95% confidence interval (1.414–22.911)] but not for adenocarcinoma patients ($P = 0.745$; relative hazard, 1.119, 95% confidence interval (0.568–2.206)].

There was no significant difference in the postoperative survival according to MMP-2 status or MMP-9 status in tumor cells. (Tables 3 and 4).

Table 3 Postoperative survival according to expression of MMP-2

	Expression of MMP-2					
	Stromal fibroblasts			Tumor cells		
	Weak	Strong	*P*	Weak	Strong	*P*
All patients	77.5%	60.2%	0.032	66.7%	67.1%	0.715
Stratified with pathologic stage						
p-Stage I	85.8%	71.4%	0.118	77.4%	76.2%	0.817
p-Stage II	76.2%	69.6%	0.921	68.6%	100%	0.843
p-Stage IIIA	55.0%	36.5%	0.214	43.4%	40.0%	0.694
Stratified with histologic type and p-stage						
Squamous cell carcinoma all p-stages	95.0%	58.4%	0.007	70.1%	54.0%	0.419
p-Stage I	100%	62.1%	0.013	81.3%	39.0%	0.065
p-Stage II	100%	66.7%	0.545	71.4%	ND	
p-Stage IIIA	80.0%	48.6%	0.413	49.8%	0.0%	0.550
Adenocarcinoma all p-stages	71.1%	61.8%	0.624	64.6%	68.6%	0.821
p-Stage I	83.1%	70.0%	0.855	78.1%	84.7%	0.951
p-Stage II	68.6%	66.7%	0.896	58.9%	100%	0.506
p-Stage IIIA	41.7%	27.1%	0.568	32.0%	33.3%	0.950

NOTE. Each figure shows the 5-year survival rate of the each patient-group.
Abbreviations: p-, pathological; ND, not determined due to lack of patients.

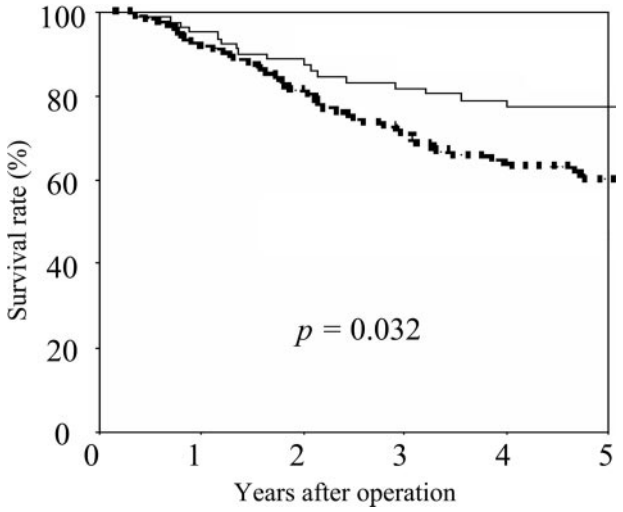

Fig. 4 Postoperative survival of completely resected pathological stage I–IIIA, NSCLC. Comparison according to the status of MMP-2 expression in stromal fibroblasts. Weak MMP-2 expression in stromal fibroblasts 5-year survival: 77.5%. Strong MMP-2 expression in stromal fibroblasts 5-year survival: 60.2%.

DISCUSSION

In the present study, we revealed that MMP-2 status in stromal fibroblasts was a significant prognostic factor in NSCLC, especially in squamous cell carcinoma, which was the first study that documented the clinical importance of MMP status in stromal cells, not in tumor cells. Although it had been initially believed that MMPs derived from tumor cells played principal roles in tumor progression, recent experimental studies have revealed that stromal cells also express MMPs, especially MMP-2, and that MMPs derived from stromal cells play equally or more important roles (6). Despite these experimental results, no clinical studies have focused on MMP expression in stromal cells; in a few studies assessing stromal MMP-2 expression, little has been reported on correlation between stromal MMP-2 status and clinicopathological features (20–22). In a clinical study on MMPs in squamous cell carcinoma of the lung, weak MMP-2 expression in tumor cells and enhanced MMP-2 expression in stromal fibroblasts were documented (21), which was in accordance with the results documented in the present study. In contrast to MMP-2, MMP-9 expression in stromal fibroblasts was negative in most patients and faint in some cases, which was same as results documented in other studies (20, 21). These results strongly suggested that MMP-2, not MMP-9, derived from stromal fibroblasts might play critical roles in tumor progression of NSCLC, especially squamous cell carcinoma. Most postoperative recurrence occurred not as local recurrence but as distant recurrence, and a difference in the metastatic sites according to the status of MMP-2 expression should be investigated in future studies.

In contrast to stromal MMP-2 status, MMP-2 or MMP-9 status in tumor cells did not provide a prognostic significance in the present study, although many clinical studies showed that enhanced MMP-2 and/or MMP-9 expression in tumor cells was a significant factor to predict a poor prognosis (8–13). In some clinical studies, it has been reported that enhanced expression of MMPs such as MMP-9 may be associated with reduced metastatic proclivity and favorable prognosis (6, 23, 24). These discrepancies may suggest limits of such retrospective studies. Thus, to assess and establish the prognostic significance of MMP-2 and MMP-9 status in tumor cells, as well as MMP-2 status in stromal fibroblasts, prospective clinical studies should be conducted. In addition, recent experimental studies have revealed that proteolytic degradation of ECM barriers by MMPs and other proteolytic enzymes is not essential for tumor cell migration and/or invasion (25). These results suggest that tumor progression and prognosis may not be predicted by the status of MMPs expression.

Many experimental studies have revealed that MMPs, especially MMP-2 and MMP-9, play important roles in tumor angiogenesis because MMPs degrade the ECM and provide a microenvironment for the development of new vessels (3–6), but only a few clinical studies documented correlations between MMPs expression and tumor angiogenesis where enhanced expression of MMP-9, not MMP-2, in tumor cells was correlated with elevated IMVD (26–28). We documented a significantly higher CD105-IMVD in tumor with strong MMP-2 expression in stromal fibroblasts, and no significant difference in IMVD according to tumoral MMP-2 or MMP-9 status in the present study. CD 105 (endoglin) is a M_r 180,000 homodimetric membrane glycoprotein expressed on endothelial cells that can bind transforming growth factor β1 and transforming growth factor β3, and experimental studies have revealed that CD105 is a marker of proliferating endothelial cells; anti-CD 105 antibodies have greater affinity for activated endothelial cells and preferentially bind to activated endothelial cells in tissues participating in angiogenesis (29). Thus, in contrast to antibodies against

Table 4 Multivariate analysis of prognostic factors in NSCLC

Prognostic factors	β	*P*	Relative hazard (95% confidence interval)
Age	0.021	0.189	1.021 (0.990–1.053)
Sex (male/female)	−0.359	0.247	0.698 (0.380–1.283)
Performance status (0/1/2)	0.138	0.653	1.148 (0.630–2.090)
Histologic type (Nonadenocarcinoma/adenocarcinoma)	−0.054	0.152	0.948 (0.881–1.020)
Pathologic stage (I, II, IIIa)	0.621	<0.001	1.860 (1.449–2.388)
MMP-2 in stromal fibroblasts (weak/strong)	0.510	0.064	1.666 (0.971–2.856)
MMP-2 in tumor cells (weak/strong)	0.058	0.843	1.060 (0.595–1.889)
MMP-9 in tumor cells (weak/strong)	0.189	0.457	1.208 (0.734–1.989)

pan-endothelial cells such as anti-CD34 antibodies, anti-CD105 antibodies preferentially react with endothelial cells of all angiogenic tissues, including tumors, but weakly or not at all with those of most normal tissues. In clinical studies, we reported that increased CD105-IMVD, not CD34-IMVD, was significantly correlated with poor postoperative survival, as well as lower incidence of apoptotic cell death in NSCLC (16, 30), which was consistent with the results in breast cancer reported by Kumar *et al.* (31). The validity of use of CD105 a marker of angiogenesis along with the correlation between MMPs status and angiogenesis should be in future prospective.

In conclusion, enhanced MMP-2 expression in stromal cells, not in tumor cells, was a significant factor to predict a poor postoperative survival in NSCLC, especially squamous cell carcinoma, which might be correlated with active tumor angiogenesis. These results added a new insight into tumor angiogenesis and clinical outcomes in NSCLC and warrant a prospective study to confirm the clinical significance of MMPs status in stromal cells.

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