Expression of matrix metalloproteinases in recurred stage IB non-small cell lung cancer

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Expression of matrix metalloproteinases in recurred stage IB non-small cell lung cancer

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Abstract

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We aimed to clarify the prime role of matrix metalloproteinases (MMPs) in recurrence of stage IB non-small cell lung cancer (NSCLC). Ten stage IB NSCLC patients presenting recurrence were selected and compared with the other ten stage IB NSCLC patients without recurrence since biopsied from August, 1999 to December, 2001. Semiquantitative RT-PCR and real-time PCR were performed subsequently to evaluate the validity of meaningful molecules identified by 0.12K cDNA microarray experiments. The protein levels of these entities were also evaluated by immunohistiochemistry of archival slides. On cDNA microarray data analysis using 6 pairs of recurred and non-recurred tumor tissues, the most frequently upregulated genes in recurred lung cancer were MMP-10 (5/6 cases) and MMP-12 (2/6 cases). The other genes, such as MMP-11, MMP-14, MMP-15, fos, cyclin E2, E2F3 and TGF-α, are upregulated in only one recurred case. To confirm these findings, MMP-10 and MMP-12 transcripts were checked by semi-quantitative RT-PCR and real-time RT-PCR analysis. The results obtained were consistent with cDNA microarray data. By immunohistochemistry, MMP-10 showed more intense immunoreactivity in recurred stage IB compared to non-recurred cases. Our approach revealed that MMP-10 might play an important role in the pathogenesis of the recurrence of stage IB NSCLC, irrespective of the histological types.

Key Words: NSCLC, MMP-10, MMP-12, cDNA microarray, real-time RT-PCR

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I. INTRODUCTION

The prognosis of stage IB non-small cell lung cancer (NSCLC) depends on the complete surgical removal of the tumors. The 5-year survival rate is no more than 80% and the remaining patients dying succumbed to the recurrence[1-3]. Approximately 40% of patients who undergo surgical resection of NSCLC without overt metastases (pT1-2, N0, M0, R0) relapse within 24 months after surgery[4]. It has been found that tumor cells are present in some distant organs such as bone marrow and lymph nodes in a significant proportion of lung cancer patients, even if any distant metastasis cannot be detected by conventional clinical examinations[5, 6]. Recent studies also showed worse postoperative outcomes of such patients and their trend toward recurrence after curative operation[5, 6]. Therefore, molecular markers of tumor aggressiveness are necessary to predict the different prognosis among the stage IB NSCLC and to improve therapeutic planning. If a reliable prognostic marker could be found, such patients with high risk of poor prognosis could be administered aggressive chemotherapy, resulting in improvement of survival. Angiogenesis and micrometastasis highly contribute to the development and progression of lung cancer. MMPs are known to play important role in angiogenesis and micrometastasis and believed to be the main tissue specific physiologically relevant mediators of matrix degradation[7-9]. The majority of studies, however, have estimated mRNA expression by RT-PCR, or mRNA in situ hybridization of several MMPs in NSCLC without stage limitation. In addition, the protein activity by gelatin or casein zymography of tumor tissue homogenates is limited to specific MMPs. This study aimed to identify the novel genes importantly upregulated in recurred compared to non-recurred stage IB NSCLC using a cDNA microarray containing the MMP superfamily and cell-cycle-related genes. Furthermore, we confirmed the gene upregulation by RT-PCR, real-time RT-PCR and

immunohistochemistry.

II. MATERIALS AND METHODS

1. Case selection

Twenty cases of stage IB non-small cell lung carcinoma were selected from 1999 to 2001 at the tissue bank of the Department of Pathology, Yonsei University College of Medicine. All archived paraffin-embedded slides were re-examined and re-classified. Clinical records were reviewed retrospectively. Clinical factors such as sex, age, pathology, recurrence, disease free time, and survival time were compared between recurred and non-recurred group. Recurrence was confirmed by follow up computed tomographic scan, whole body bone scan and abdominal ultrasonogram, i.e., metastasis to contra-lateral lung, lymph nodes, or some other organs. Disease free time was the period measured from the date of operation until the first evidence of recurrence from primary NSCLC or the last date of follow-up for patients who remained alive without recurrence.

2. RNA extraction

Total cellular RNA was extracted from 20 fresh frozen tumor tissues. The validity of the fresh frozen tissues was checked by the examination of the corresponding tissue slides. The total RNA was prepared using Ultraspec reagent (Biotec Lab, TX, USA) according to the manufacturer's chloroform extraction, isopropanol purification and ethanol elution protocol. Quality and integrity of RNA extracted were confirmed by an Agilent 2100 bioanalyzer (Agilent, Palo Alto, CA, USA) and spectrophotometry. Six pairs of the twenty samples from recurred and non-recurred patients with a 28S to 18S RNA ratio of more than 1.0 were chosen for the next procedure.

3. Microarray hybridization

cDNA microarray with 0.12K genes (Digital Genomics, Seoul, Korea; http://www.digital-Genomics.co.kr), containing the MMP superfamily and cell-cycle-related genes, were used for the study. These chips contained MMP1, 2, 7, 9, 10, 11, 12, 13, 14, 15, 16, and 19 and tissue inhibitor of MMP (TIMP)- 1, and 3. In addition to the MMP superfamily, the array contained cell cycle related genes and human proto-oncogenes. Total RNA (approximately 5 μg) was converted into cDNA

and labeled with aminoallyl dUTP using an Amino Allyl cDNA labeling kit (Ambion, Austin, TX, USA). The RNA samples of recurred lung cancer were labeled with Cy3-dUTP (Amersham, Uppsala, Sweden) and those of non-recurred lung cancer with Cy5-dUTP (Amersham, Uppsala, Sweden). The two color probes were then mixed, precipitated with ethanol, and dissolved in 45 μl DMSO. Hybridization and washes were performed according to the manufacturer's instructions (Digital Genomics, Seoul, Korea). Hybridization (hybridization buffer: Formamide 25%, SSC 5X, SDS 0.1%, polyA 0.5 mg/ml, Cot-1 DNA 0.5 mg/ml) was performed in a hybridization oven at 42℃ for 16 hours. After washing (2XSSC/0.1% SDS for 5 min at 42℃, 0.1X SSC/0.1% SDS for 10 min at RT, 0.1X SSC for 1 min at RT), the slides were scanned with a DNAchip scanner (Scanarray lite; GSI Lumonics, Ottawa, Canada) at two wavelengths to detect emissions from both Cy3 (green) and Cy5 (red). Overall intensities were normalized using a correction coefficient obtained from the ratios of 5 endogenous housekeeping genes.

4. Microarray data analysis

To avoid misinterpretation of the results that could result from variation in the hybridizations, any two compared filters were normalized using 5 internal control genes (ACTB, PDK2, UBC, YWHAZ, RPL13A), and a normalization coefficient calculated for each comparison was used to correct the signal intensities. In addition, if the intensity of any signal failed to be at least three times above the background value, it was discarded from our analysis. Our analysis of any two hybridizations obtained from a given sample showed no difference using a ratio of 1.5 for over-expression and 0.75 for under-expression. However, when the threshold values were stricter as 2.0 for over-expression and 0.5 for under-expression, differentially expressed several genes were identified.

5. RT-PCR

Two hundreds nanograms of RNA were transcribed to cDNA in a final volume of 20 μl. Primers for MMP-10, and MMP-12 were selected using the ENTREZ computer analysis package (Table 3). Homologies with other human proteinases were excluded. The PCR products were sequenced and showed no homology with other known gene sequences. PCR was performed using the primers in a volume of 100 μl containing first strand cDNA, primer, buffer, $MgCl_2$, and T_{aq} polymerase. MMP-10 and MMP-12 were amplified using 35 cycles of following condition: denaturation at

94°C for 60s, annealing at 55°C and 57.5°C for 60s, extension at 72°C for 60s, and a final extension of 10 minutes at 72°C. To determine the gene expression level, ß-globin was used as an internal standard in accordance with the manufacturer's (Invitrogen, Carlsbad, CA, USA) instructions.

6. Real time RT-PCR

Primer and the TaqMan probe were designed using Primer Express software, Ver 2.0 (Applied Biosystems) as shown in Table 3. Primers for MMP-10, and MMP-12 and 6-carboxyfluorescein (FAM)-labeled probes were obtained from MWG-Biotec. To normalize the results, we used a VIC-labeled glyceraldehyde 3-phosphate dehydrogenase (GAPDH) TaqMan PDAR endogenous control reagent set (Applied Biosystems). Each of the probes was quenched by 6-carboxy-tetramethylrhodamine (TAMRA) at the 3'-end. TaqMan PCR assays were performed on an ABI Prism 7900 HT Sequence Detection System (Perkin Elmer Applied Biosystems). To quantify each ABC transporter gene, we prepared a master mixture containing 10 µl of 2X TaqMan Universal PCR Master Mix, 1µl of gene-specific forward and 1 µl of reverse primer (each at 18 mM), 1µl of the gene specific probe (5 mM), and 2 µl of sterile water, and aliquoted this mixture into the wells of a 384-well optical plate. A master mixture for the endogenous control GAPDH, contained 10 µl of 2X TaqMan Universal PCR Master Mix, 1 µl of predeveloped TaqMan assay reagents (PDAR) from the endogenous control reagent set, and 4 µl of sterile water, was treated similarly. Finally, triplicates of cDNA templates were added to a final volume of 20 µl. The thermal cycling conditions used were; 2 min at 50°C and 10 min at 95°C, followed by 45 cycles of 15s at 95°C and 1 min at 60°C. Sequence Detector Software SDS 2.0 (Applied Biosystems) was used for the data analysis. The first step involved generating an amplification plot for every sample, which showed a Rn on the y-axis against the cycle number, displayed on the x-axis. From each amplification plot, a threshold cycle (Ct) value was calculated (defined as the cycle at which a statistically significant increase in Rn is first detected), and this was displayed in the graph as the intercept point of the amplification curve with the threshold. The threshold was calculated automatically by SDS at the 10-fold SD of Rn in the first 15 cycles. The Ct values obtained were then exported to a Microsoft Excel spreadsheet for further analysis. The next step involved the construction of calibration curve plots, using Microsoft Excel, as recommended in Bulletin 2 of the ABI Prism 7700 Sequence Detection System (Applied Biosystems), showing Ct values on the y-axis and the

logarithm of the amount of cDNA inputted (equivalent to the amount of total RNA) on the x-axis. To construct calibration curves for relative quantification, we performed real-time amplification of several cDNA dilutions with ABC transporter genes and a reference gene. We compared the expressions of each ABC transporter in the tissue panel. Therefore, for each ABC transporter, the normalized amount of expression in the tissues that showed the lowest expression was used as a calibrator and the remaining tissue samples were displayed as fold changes.

7. Immunohistochemistry

Four μm pulmonary archival tissue sections corresponding to the fresh frozen tissues were cut from the 20 formalin-fixed, paraffin-embedded tissue blocks. Sections were dewaxed in xylene, rehydrated through graded ethanol solutions, rinsed in PBS for 5 min, and immersed in 0.3% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase activity. The slides were then rinsed in PBS for 5 min, blocked with a solution of 10% normal rabbit serum in PBS at room temperature for 10 min and incubated at 4℃ overnight with the primary antibodies of MMP-10/stromelysin-2 (NeoMarkers, Fremont, CA, USA) which contain an epitope of the at the hinge region of MMP-10 at a dilution ratio of 1:200 without any pretreatment. Nonspecific mouse immunoglobulin G1 monoclonal antibody (03001 D, PharMingen, 1mg/ml) was used as the negative control. Tissues were incubated with biotinylated horse anti-mouse secondary antibody, at a 1:500 dilution (Vector Laboratories, Burlingame, CA, USA), and washed extensively with avidin-biotin peroxidase complex, at a 1:25 dilution. Diaminobenzene was used as the chromogen, and hematoxylin as the nuclear counterstain. Cytoplasmic immunoreactivities were classified as a continuum extending from the undetected level (0%) to diffuse and homogeneous strong staining (100%), using a semi-quantitative three-tier grading system based on the percentage of tumor cells staining positively in the entire tumor boundary (0, 1:minimal <5%, 2: moderately stained <50%, 3: markedly stained >50%).

8. Statistical analysis

The standard χ^2-test, Fisher's exact test and *t* test were used for comparative analysis. The survival rates were estimated by the Kaplan-Meier method, and the statistical analysis was performed by the log-rank test. The level of significance was p less than 0.05.

III. RESULTS

1. Clinical Records

20 samples of stage I lung cancer were classified as recurred or non-recurred(Table 1). The primary histological types of the recurred carcinoma were 4 squamous cell carcinomas, 4 adenocarcinomas, 1 adenosquamous cell carcinoma and 1 large cell carcinoma, whereas the histological types of the non-recurred carcinoma were 4 squamous cell carcinomas, 4 adenocarcinomas, 1 large cell carcinoma and 1 adenoid cystic carcinoma.

Table 1. Clinical summary of patients

	Recurrence (n=10)	**No recurrence (n=10)**	***p*-Value**
Sex			NS[a]
Male	8	7	
Female	2	3	
Age (Mean ± SD: year)	66.7±4.69	61.7±11.21	NS[b]
Pathologic type			NS[a]
Squamous cell carcinoma	4	4	
Adenocarcinoma	4	4	
Large cell carcinoma	1	1	
Adenosquamous carcinoma	1		
Adenoid cystic carcinoma		1	
Tumor size (Mean ± SD: cm)	5.0±1.31	5.6±2.51	NS[b]
Invasion to visceral pleura	5/10 (50%)	1/10 (10%)	NS[a]
Follow up time (month)			
range	5.9 41.2	26.8 41.6	
Mean ±SD	19.9±11.60	34.9±4.33	
Disease Free Time (Mean ± SD)	9.0±5.09	34.9±4.33	
Survival	4/10 (40%)	10/10(100%)	
Median Survial Time(±SE) (month)	17.1±7.98	All survive	0.002[c]

[a] Fisher's exact or χ^2 test; [b] *t* test for equality of means; [c] log-rank test

The recurred group manifested metastasis in the contra-lateral lung (3 cases), bilateral lungs (1), bone (1), liver (1), brain (1), brain and bone (1), liver and bone (1) and ipsilateral scalene node (1).

Disease free time (DFT) of recurred group was 9 months on average. The mean

follow up time was 34.9 months on average (26.8- 41.6 months) for non-recurred group and 19.9 months on average (5.9-41.2 months) for recurred group. All patients of non-recurred group have survived. Meanwhile, median survival time of recurred group was 17.1 months, which was significantly shorter than that of non-recurred group (p=0.002)(Figure 1).

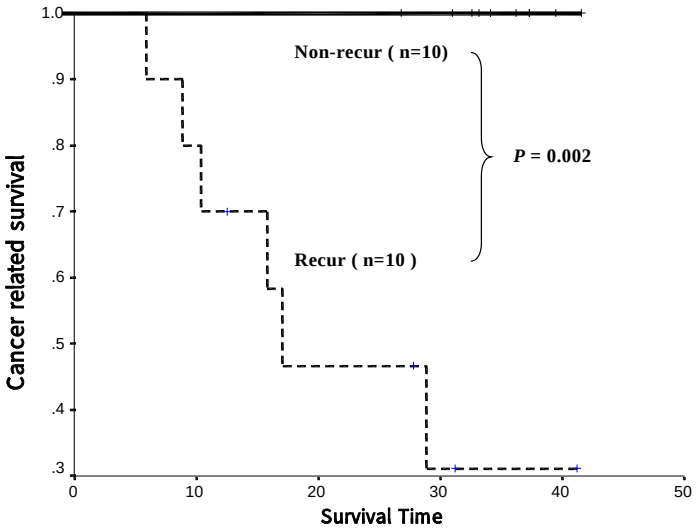


Figure 1. The Kaplan-Meier survival curve for 20 stage IB non-small cell lung cancer patients; The survival rate of the recurred group was significantly lower than that of the non-recurred group (*p*=0.002, log rank test).

2. Differential Expression by cDNA microarray

We investigated altered gene expression profiles in 6 pairs for the 'recurred' and 'non-recurred' stage IB NSCLCs. The expressions of MMP-10, -11, -12, -14, -15, -16, -19 with fos, cyclin E2, E2F3, TGF-α were upregulated in recurred stage IB NSCLC group as compared with the non-recurred stage IB NSCLC group (Figure 2) . cDNA microarray data of representative two pairs of NSCLC samples (cDNA of recurred lung cancer labeled with Cy3-dUTP and equivalent cDNA of non-recurred lung cancer with Cy5-dUTP) are shown in Table 2.

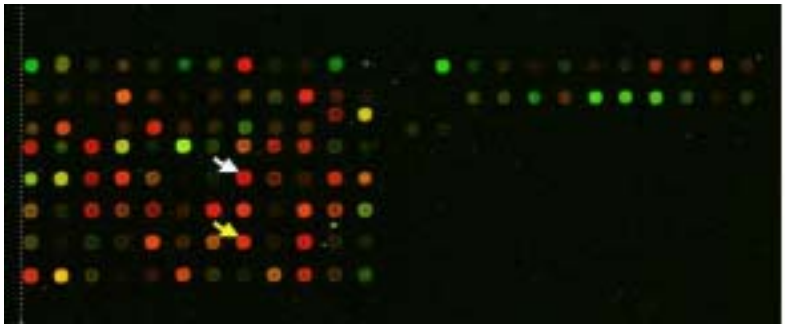


Figure 2. Image displaying a cDNA microarray comprised of 120 genes; upregulated genes in the recurred samples are seen in red fluorescence (Cy-3) and down-regulated genes in green fluorescence (Cy-5). MMP-10 (white arrow) and MMP-12 (yellow arrow) spots are recognized as the red fluorescence, which means both are upregulated in the recurred samples when compared with the non-recurred samples

Table 2. cDNA array data of the representative NSCLC samples

Item	Normalized Ratio
MMP1	0.540848889
MMP2	0.713426395
MMP7	0.577443544
MMP9	0.542648001
MMP-10	0.557597408
MMP-10	26.71548239
MMP11	4.129027705
MMP11	0.549721958
MMP-12	4.025507961
MMP13	0.851018891
MMP14	5.750816866
MMP15	12.77765412
MMP16	0.642203194
MMP16	2.13169071
MMP19	2.938037442
TIMP1	0.584378709
TIMP3	0.620869302

The blank filled with red-color represents upregulated genes more than 2.0 of threshold value in the recurred cancer. MMP-10 was the most frequently upregulated in 5 out of 6 recurred cases (p=0.015) and MMP-12 was upregulated in 2 out of 6 recurred cases (p>0.05) compared with non-recurred cases. The other genes, except MMP-10 or MMP-12, showed an upregulated expression in only one recurred case, respectively.

3. Transcriptional levels of MMP- 10 and MMP-12 by RT-PCR

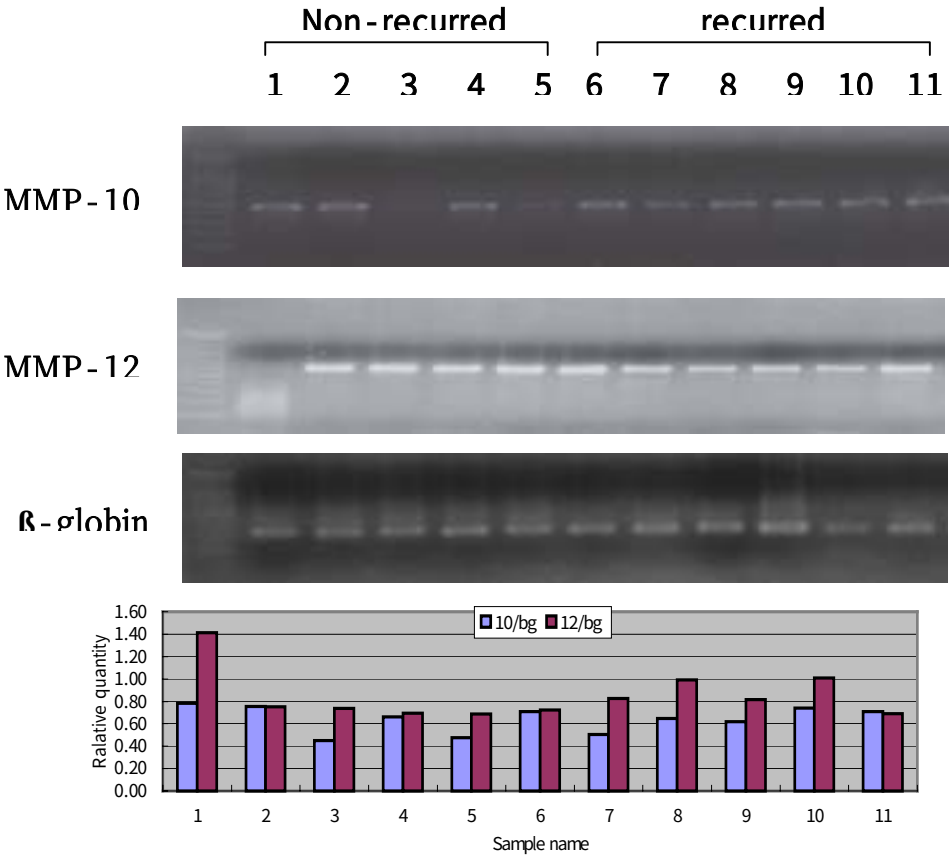


Figure 3. Quantitative RT-PCR results for MMP-10 and MMP-12. Note the downregulated expression of MMP-10 mRNA in the non-recurred samples (lanes 1-5) versus the recurred samples (lane 6-11). In lanes 3 and 5, non-recurred cases show relatively weaker signals than those in other lanes, especially right lanes from 6-11 lanes (recurred). MMP-12 mRNA was expressed at similar levels in recurred and non-recurred samples

Assessments of MMP-10 and MMP-12 transcripts were performed in 20 early lung cancers. MMP-10 mRNA (163bp) revealed relatively weaker expression in the non-recurred samples than in all recurred samples by semi-quantitative RT-PCR, whereas MMP-12 mRNA (594bp) was constitutively expressed regardless of the histological type (Figure 3)

4. Expression analysis of MMP-10 and MMP-12 using real-time RT-PCR

After collecting the correct consensus sequences of all human ABC transporter mRNAs by GenBank screening, we used Primer Express Software to design optimum specific primer and probe combinations (Table 3).

Table 3. Primer sequences of MMP-10 and MMP-12 and gene-specific probes, 5'-3' for TaqMan analysis.

A. Primer

MMP-10(5'→3')	FW	ACC TGG GCT TTA TGGAGA TAT TC
	RV	GAA GCC AGA AAT CAA GTT
MMP-12(5'→3')	FW	TCA TAT GCA GCA TCC AAA TAT GAT G
	RV	TCA AGC AAG AAT GGA CAA

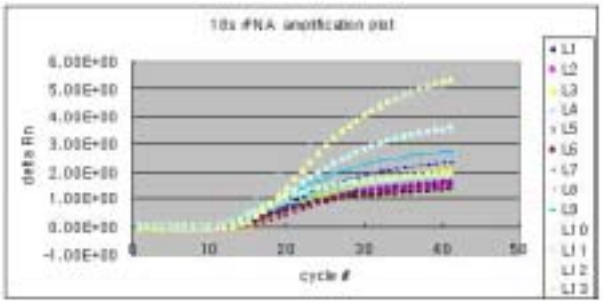
B. Probe

Assay ID	Gene symbol	NCBI Gene Reference	Context Sequence
Hs00233987_ml	MMP-10	NM_002425	ACAGGCATTTGGATTTTTCTACTTC
Hs00159178_ml	MMP-12	NM_002426	TTCAGGAGGCACAAACTTGTTCCTC

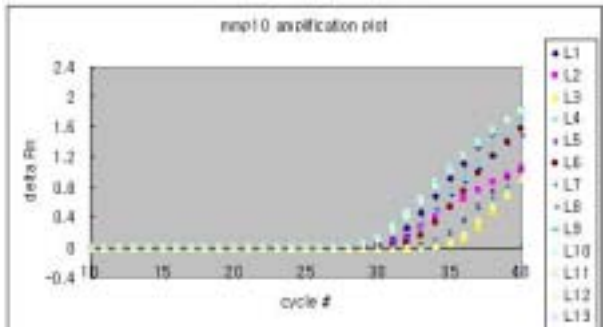
Figure 4 shows representative examples of MMP-10 and MMP-12. The Ct values plotted vs. the log concentrations of these calibration samples showed an inverse linear correlation; the correlation coefficient for each calibrator thus should be close to one. The ratios of MMP-10 or MMP-12 to 18s mRNA was converted to a bar graph in an end-point of cycles (Figure 5). The increased ratios of 18s mRNA of MMP-10 or MMP-12 were compatible with those upregulated in cDNA microarray

5. Immunohistochemistry

MMP-10 was variably expressed in tumor cells as well as faintly expressed in normal bronchial epithelial cells. MMP-10 was relatively intense in the recurred group as compared with the non-recurred group (Fig. 5), the difference of which was statistically significant (p=0.031). There was no difference of MMP-10 expression according to histologic type (p=0.847).



A



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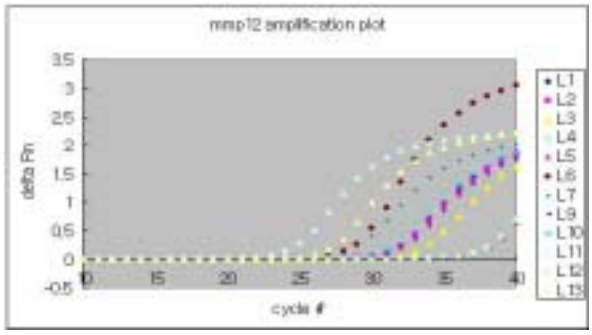

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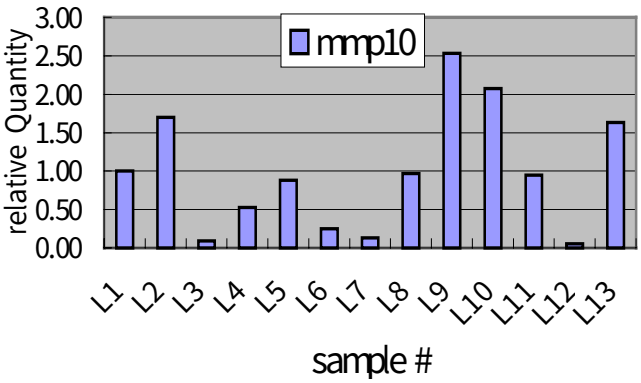
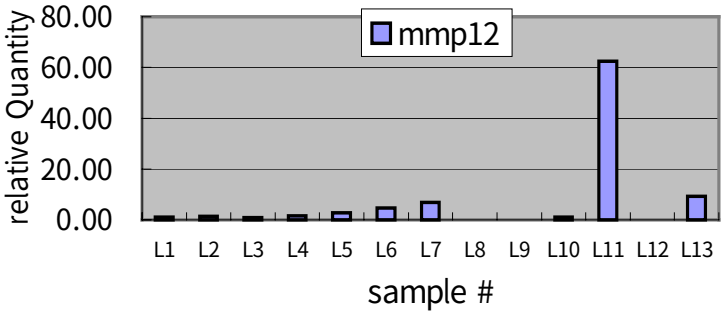


D



E.

Fig. 4. Results of TaqMan assay to determine optimum primer and TaqMan probe concentrations. Normalized fluorescence values (Δ Rn) on the x-axis. A) amplification plot for 18s RNA is shown as a calibration curve, depicting normalized fluorescence value (Δ Rn) plotted against the number of amplification cycles. The curves represent triplicate measurements at each sample dilution. B) amplification plot for MMP-10 is shown. Lane 1-5 show a tendency toward higher Ct and lower Δ Rn than lanes 6-11. (lanes 1-5: the non-recurred samples, lanes 6-11: recurred samples). C) An amplification plot for MMP-12 is shown. Lanes 1-5 show a tendency toward higher Ct and lower Δ Rn than lanes 6-11. (lanes 1-5: the non-recurred samples, lanes 6-11: recurred samples). Two bar graphs are diagram of Real-time RT-PCR of MMP-10 and MMP-12. The non-recurred samples (L1-L7) showed relatively lower MMP-10 expression than recurred samples (L8-L13). D) Note the high expression end-point ratio of MMP-10 in L9 (MMP-10/18s ratio: 22) and L10 (MMP-10/18s ratio: 18). E) In all but one case, lane 11, recurred samples, showed comparatively high expressions of MMP-12 mRNA.

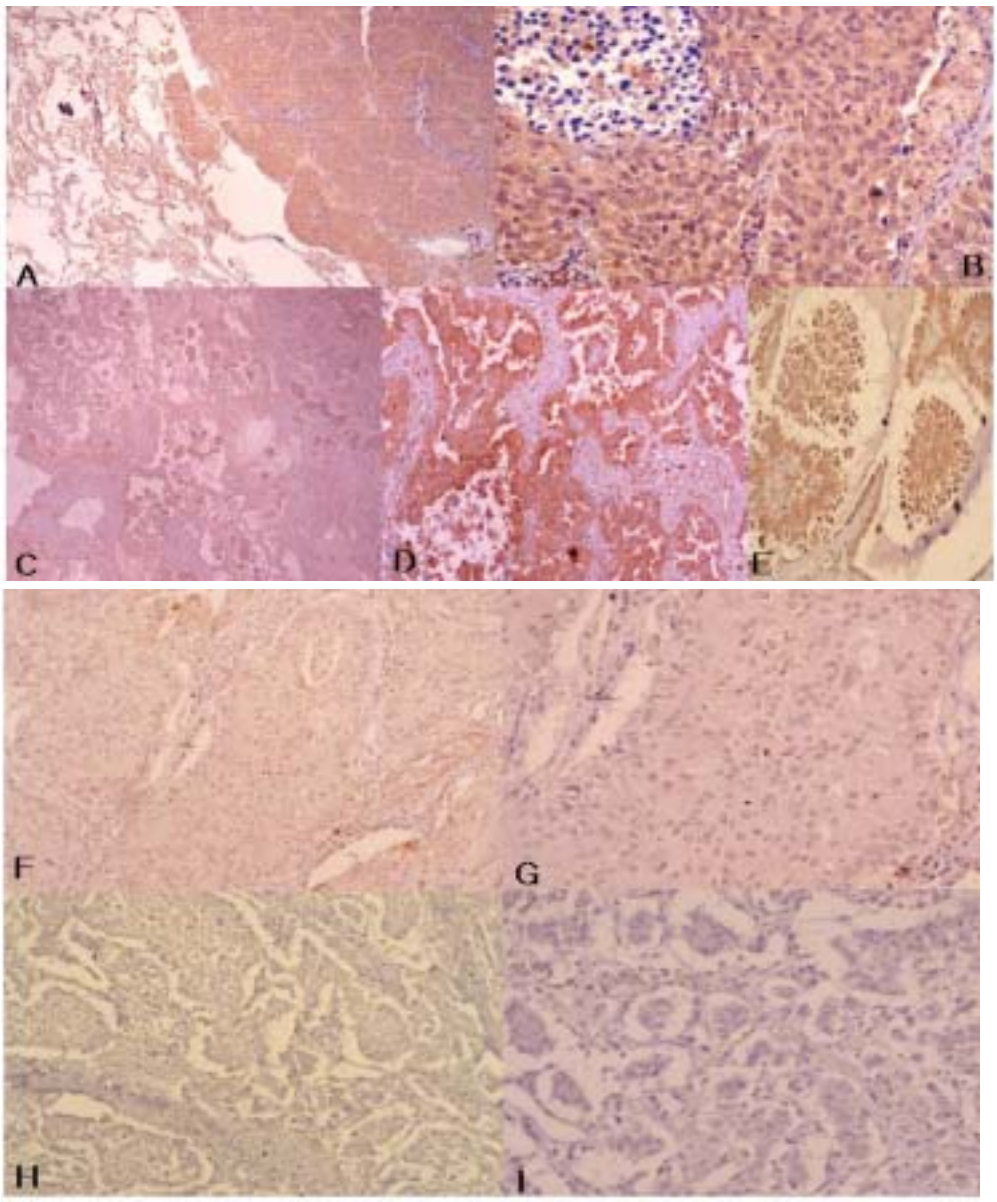


Fig.5. Immunohistochemical results of MMP-10 expression in lung cancer and normal tissue. MMP-10 was diffusely expressed in recurred stage I squamous cell carcinoma (A-B), and well-differentiated adenocarcinomas (C-E), compared with negative immunoreactivity in non-recurred stage I squamous cell carcinoma (F-G) and adenocarcinoma (H-I). MMP-10 was selectively stained in tumor cells, not in normal inflammatory cells or stromal cells. (A: x40, B: x200, C: X40, D: x100, E: x200, F: x100, G: x200, H: x40, I: x100, diaminobenzene)

IV. Discussion

We have explored the gene expression profiles of 6 pairs of representative of recurred and non-recurred early NSCLC using a 0.12K array containing the MMP superfamily, cell-cycle related genes, and oncogenes critical in the malignancy. We validated the expression results obtained using the cDNA microarray by semi-quantitative RT-PCR and real-time RT-PCR on all tissue samples, and performed immunohistochemistry on all sample slides. MMP-10 and MMP-12 genes were found to be upregulated in more than two tumors. In addition, MMP-14, and MMP-15 with fos, cyclin E2, E2F3, TGF-α were upregulated in 'recurred' stage IB NSCLC less frequently than MMP-10.

MMPs are believed to be the main physiologically relevant mediators of matrix degradation, and are tissue specific[7-9]. Previous reports regarding MMPs and lung cancer have focused on MMP-2 (gelatinase A)[10-12], MMP-9 (gelatinase B)[10,13], MMP-7 (matrilysin)[14], MMP-11 (stromelysin-3)[15-17], MMP-14 (MT1-MMP)[18] and rarely on MMP-10 (stromelysin-2)[14]. However, the majority of studies, have estimated the mRNA expression by RT-PCR, or by using mRNA in situ hybridization, or by determining protein activity by gelatin or casein zymography of tumor tissue homogenates, and only rarely by immunohistochemisty. Other investigator have suggested that certain MMPs might predict a poor prognosis when they are expressed at high levels, for example, MMP-2 expression was found to be correlated with nodal invasion [18], MMP-14 with advanced stage disease[19], and TIMP-1 with poor survival[20]. Cellular localization studies by in situ hybridization and immunohistochemisty in NSCLCs have been focused on MMP-2 and MMP-11[21, 22]. MMP-11 was found to be expressed in the stromal fibroblasts of tumors and to correlate with tumor size and nodal metastasis[22]. We utilized a cDNA microarray including almost all known MMPs and cell-cycle-related genes in order to detect the genes most related to the recurrence of NSCLCs.

The present study suggests that MMP-10 could play an important role in the recurrence of stage IB NSCLC. In addition, we found that MMP-12 could play an important role in the pathogenesis of some cases of recurred stage IB NSCLC. We confirmed the over-expressions of MMP-10 and MMP-12 in recurred NSCLC by RT-PCR, by the more specific real-time RT-PCR, and by immunohistochemistry. In particular, real-time RT-PCR analysis has several advantages over previous, mostly

gel-based RT-PCR methods[23]. Thus, TaqMan PCR is a closed homogenous system that does not require post-PCR processing and which has a low contamination risk. Moreover, real-time RT-PCR allows precise and reproducible quantification because it is based on Ct values rather than end-point detection, when the PCR components are rate limiting. The results obtained from the real-time RT-PCR assay are consistent with those obtained using the cDNA microarray and by semi-quantitative RT-PCR, suggesting that real-time RT-PCR offers a reliable method of quantifying ABC transporter gene expression.

MMP-10 (stromelysin-2), which is located on chromosome 11q22-q23, consists of a propeptide (82 amino acids), a catalytic domain (165 aa); a proline-rich hinge region (25 aa); and a C-terminal domain (188 aa)[24]. MMP-10 degrades in vitro several protein components relevant to wound repair such as collagens III and IV, gelatin, nidogen, laminin-1, proteoglycans, and elastin. MMP-10 activates other matrix metalloproteinases such as MMP-1, MMP-8 and MMP-9.

The biological role of MMP-10 has been little studied, but it is suggested to be linked with reproduction, such as, endometrial shedding during menstruation, and wound healing[24,25]. Although the exact role of MMP-10 in tumor progression is not clearly understood, transformed rat embryo cell lines with high metastatic potential were found to produce higher levels of MMP-10 than non-metastatic lines[26]. Bodey and colleagues reported MMP-3 and MMP-10 are implicated in the pathogenesis of breast carcinoma[27]. Mathew and colleagues reported that significant associations were observed between Stromelysin-2 overexpression and tumor size, local invasiveness of the tumor and distant organ metastasis in human esophageal squamous cell carcinoma[28]. Overexpression of Stromelysin-2 was also found to be associated with NSCLC. Bodey and colleagues also reported stromelysin-1 and-2 are involved in the generalized growth and expansion of the neoplastic cell mass in NSCLC, while MMP-2, -9 and -13 are involved in the neoangiogenic and focal clonal selection and expansion phenomena associated with in situ tumor progression, invasion of the microvasculature, and metastasis[29]. These studies suggest a correlation between invasive behavior of tumor cells and the expression of stromelysins.

MMP-12 (macrophage elastase) incorporates the principal cellular source of this enzyme and it is able to degrade insoluble elastin[30]. Because macrophages can express several elastolytic proteinases other than macrophage elastase, i.e., cystein proteinases, metalloproteinases, particularly MMP-9, MMP-7, and perhaps MMP-3[30]. MMP-12

most closely resembles the stromelysins, MMP-3 and MMP-10 and matrilysin (MMP-7) with respect to their ability to hydrolyze a broad variety of extracellular matrix components[30]. MMP-12 is unique with respect to its predominantly macrophage-specific pattern of expression and perhaps its ability to readily shed its C-terminal domain on processing[24, 30]. Recently, MMP-12 has been shown to play a central role in the pathogenesis of pulmonary emphysema[31] and of atherosclerotic lesions[32]. MMP-12 is nearly undetectable in normal alveolar macrophages, but is expressed in cigarette smokers[30]. Unfortunately, we were not able to demonstrate the tissue localization of MMP-12, because it is not commercially available. The molecular bases for the MMP-12 expressions of macrophages are unknown.

V. Conclusion

We conclude that MMP-10 is mainly upregulated during the process of recurrence of Stage IB NSCLC at the transcriptional level and protein level, and that MMP-12 is partly upregulated. Furthermore, the determination of MMP-10 may provide a clue to the pre-selection of patients with NSCLC in clinical trials to investigate the benefits of MMP-inhibitory therapy.

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<국문요약>

재발한 1B병기 비소세포 폐암에서 Matrix Metalloproteinases의 발현

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1B병기 비소세포 폐암의 수술 후 재발에 있어서 Matrix Metalloproteinases의 역할을 알아보기 위해 1999년 8월부터 2001년 12월까지 수술한 환자 중 재발한 10례와 재발하지 않은 10례를 대상으로 0.12K cDNA microarray 실험결과 의미 있게 확인된 molecule에 대하여 semi-quantitative RT-PCR and real-time RT-PCR을 통하여 검증하였다. 각 molecule에 대한 조직 내 단백질 level은 면역조직화학검사를 통해 검사하였다. 재발한 폐암 조직과 재발하지 않은 폐암 조직 6쌍의 cDNA microarray 실험결과 재발한 폐암 조직에서 가장 높은 빈도로 발현된 gene은 MMP-10(5예)와 MMp-12(2예)이었다. 그 외에 MMP-11, MMP-14, MMP-15, fos, cyclin E2, E2F3 그리고 TGF-α등의 gene은 각각 1 예의 재발한 폐암 조직에서 높게 발현되었다. 결과를 확증하기 위해 semi-quantitative RT-PCR과 real time RT-PCR을 실시하였으며 실험결과는 cDNA microarray결과와 일치하였다. 면역조직화학검사상 재발한 원발폐암조직에서 MMP-10이 재발하지 않은 원발폐암조직보다 더욱 강한 면역반응을 보였다. 본 연구 결과 폐암 조직의 유형에 관계없이 MMP-10이 1B병기 비소세포 폐암의 재발에 중요한 역할을 담당할 것으로 생각된다.

핵심되는말 : 1B병기 비소세포 폐암, MMP-10, MMP-12, cDNA microarray, real-time RT-PCR