ADAM8 as a Novel Serological and Histochemical Marker for Lung Cancer

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ABSTRACT

Purpose and Experimental Design: We have been investigating genes involved in pulmonary carcinogenesis by examining gene expression profiles of non–small-cell lung cancers to identify molecules that might serve as diagnostic markers or targets for development of new molecular therapies. A gene encoding ADAM8, a disintegrin and metalloproteinase domain-8, was selected as a candidate for such molecule. Tumor tissue microarray was applied to examine expression of ADAM8 protein in archival lung cancer samples from 363 patients. Serum ADAM8 levels of 105 lung cancer patients and 72 controls were also measured by ELISA. A role of ADAM8 in cellular motility was examined by Matrigel assays.

Results: ADAM8 was abundantly expressed in the great majority of lung cancers examined. A high level of ADAM8 expression was significantly more common in advanced-stage IIIB/IV adenocarcinomas than in adenocarcinomas at stages I–IIIA. Serum levels of ADAM8 were significantly higher in lung cancer patients than in healthy controls. The proportion of the serum ADAM8-positive cases defined by our criteria was 63% and that for carcinoembryonic antigen was 57%, indicating equivalent diagnostic power of these two markers. A combined assay using both ADAM8 and carcinoembryonic antigen increased sensitivity because 80% of the lung cancer patients were then diagnosed as positive, whereas only 11% of 72 healthy volunteers were falsely diagnosed as positive. In addition, exogenous expression of ADAM8 increased the migratory activity of mammalian cells, an indication that ADAM8 may play a significant role in progression of lung cancer.

Conclusions: Our data suggest that ADAM8 should be useful as a diagnostic marker and probably as a therapeutic target.

INTRODUCTION

Lung cancer is one of the most common cancers in the world, and non–small-cell lung cancer (NSCLC) accounts for ~80% of those cases (1). Because the prognosis of advanced lung cancer remains poor, development of novel therapeutic and diagnostic strategies is an urgent goal (2). Tumor markers that are currently available for lung cancer, such as carcinoembryonic antigen (CEA), serum cytokeratin 19 fragment (CYFRA 21-1), and progastrin-releasing peptide (pro-GRP), are not satisfactory for diagnosis at an early stage or for monitoring the disease because of their relatively low sensitivity and specificity in detecting the presence of cancer cells (3–5). Although the precise pathways involved in lung tumorigenesis remain unclear, some evidence indicates that tumor cells express cell surface markers unique to each histologic type at particular stages of differentiation. Because cell surface proteins are considered more accessible to immune mechanisms and drug delivery systems, identification of cancer-specific cell surface and secretory proteins is likely to be an effective approach to development of novel diagnostic markers and therapeutic strategies.

We have been screening genes encoding transmembrane/secretory proteins that are up-regulated in lung cancers, with cDNA microarrays and tumor cells purified by laser-capture microdissection (6, 7). To verify the biological and clinicopathological significance of the respective gene products, we have been performing tumor tissue microarray analysis of clinical lung cancer materials. This systematic approach revealed that a disintegrin and metalloproteinase domain-8 (ADAM8), a cell surface disintegrin and metalloproteinase domain-8 molecule, was frequently transactivated in primary lung cancers.

ADAM family members are implicated to be involved in the proteolytic processing of membrane-bound precursors, and they modulate cell-cell and cell-matrix interactions. ADAM8 encodes a protein of 824 amino acids with a COOH-terminal transmembrane domain and potential extracellular adhesion and protease domains (8, 9). This molecule, localized to the plasma membrane, is processed by autocatalysis into two forms; one is derived by removal of a prodomain and the other is a remnant protein composed of the extracellular region with a disintegrin domain at the NH_2 terminus (10). ADAM8 behaves as an active metalloprotease *in vitro*, hydrolyzing myelin basic protein and a variety of peptide substrates based on the cleavage sites of membrane-bound cytokines, growth factors, and receptors (11–

14). Other studies have demonstrated overexpression of some ADAM family proteins in a variety of human tumors (15, 16), but involvement of ADAM8 in human cancer was not indicated previously.

We report here the identification of ADAM8 as a novel diagnostic marker and a potential target for therapeutic agents/antibodies and also provide evidence for its possible role in human pulmonary carcinogenesis.

MATERIALS AND METHODS

Cell Lines and Clinical Samples. The 23 human lung cancer cell lines used in this study included nine adenocarcinomas (A427, A549, LC319, NCI-H1373, PC-3, PC-9, PC-14, NCI-H1666, and NCI-H1781), nine squamous cell carcinomas (EBC-1, LU61, NCI-H520, NCI-H1703, NCI-H2170, RERF-LC-AI, SK-MES-1, NCI-H226, and NCI-H647), one large-cell carcinoma (LX1), and four small-cell lung cancers (SCLCs; DMS114, DMS273, SBC-3, and SBC-5). All cells were grown in monolayers in appropriate media supplemented with 10% FCS and were maintained at 37°C in an atmosphere of humidified air with 5% CO_2.

Surgically resected primary NSCLC samples had been obtained earlier with informed consent (6). A total of 363 formalin-fixed primary tumors (206 adenocarcinomas, 118 squamous cell carcinomas, 25 large-cell carcinomas, and 14 SCLCs) and adjacent normal lung tissue samples from 336 patients undergoing surgery at Saitama Cancer Center (Saitama, Japan) and from postmortem materials (27 individuals) obtained from Hiroshima University (Hiroshima, Japan) were also used in this study.

Serum samples were obtained with informed consent from 72 healthy individuals as controls (40 males and 32 females; median age, 61.5 ± 9.1 SD; range, 42–89 years). The healthy individuals showed no abnormalities in complete blood cell counts, C-reactive proteins, erythrocyte sedimentation rates, liver function tests, renal function tests, urinalyses, fecal examinations, chest X-rays, or electrocardiograms. Serum samples were also obtained with informed consent from 105 lung cancer patients admitted to Hiroshima University Hospital (78 males and 27 females; median age, 68.0 ± 10.8 SD; range, 30–84 years). Samples were selected for the study on the basis of the following criteria: (*a*) patients were newly diagnosed and previously untreated and (*b*) their tumors were pathologically diagnosed as lung cancers (stages I–IV). These 105 cases included 62 adenocarcinomas, 25 squamous cell carcinomas, and 18 SCLCs. Clinicopathological records were fully documented. Serum was obtained at the time of diagnosis and stored at −80°C. Disease staging in all 105 cases was supported by a computed tomography scan of the chest and abdomen, bone scintigraphy, and magnetic resonance imaging of the head.

Semiquantitative Reverse Transcription-PCR Analysis. Total RNA was extracted from cultured cells and clinical tissues with Trizol reagent (Life Technologies, Inc., Gaithersburg, MD), according to the manufacturer's protocol. Extracted RNAs and normal human tissue polyA RNAs were treated with DNase I (Roche Diagnostics, Basel, Switzerland) and then reverse transcribed with oligo(dT)$_{12–18}$ primer and SuperScript II reverse transcriptase (Life Technologies, Inc.). Semiquantitative reverse transcription-PCR experiments were carried out with synthesized *ADAM8* gene-specific primers (5′-GTGTGTGTA-CGTGTCTCCAGGT-3′ and 5′-CAGACAAGATAGCTGAC-TCTCCC-3′), or with β-actin (*ACTB*)-specific primers (5′-AT-CAAGATCATTGCTCCTCCT-3′ and 5′-CTGCGCAAGTT-AGGTTTTGT-3′) as an internal control. All PCR reactions involved initial denaturation at 94°C for 2 minutes followed by 22 (for *ACTB*) or 28 cycles (for *ADAM8*) of 94°C 30 s, 54°C to 60°C for 30 seconds, and 72°C for 60 seconds on a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA).

Northern Blot Analysis. Human multiple tissue blots (BD Biosciences, Palo Alto, CA) were hybridized with ^{32}P-labeled PCR products. Prehybridization, hybridization, and washing were done according to the supplier's recommendations. The blots were autoradiographed with intensifying screens at −80°C for 1 week.

Immunohistochemistry and Tissue Microarray. Tumor tissue microarrays were constructed with 363 formalin-fixed primary lung cancers, as published previously (17–19). The tissue area for sampling was selected based on visual alignment with the corresponding H&E-stained section on a slide. Three, four, or five tissue cores (diameter, 0.6 mm; height, 3–4 mm) taken from a donor tumor block were placed into a recipient paraffin block with a tissue microarrayer (Beecher Instruments, Sun Prairie, WI). A core of normal tissue was punched from each case, and 5-μm sections of the resulting microarray block were used for immunohistochemical analysis.

Three independent investigators assessed ADAM8 positivity semiquantitatively, recording staining intensity as absent (scored as 0), weak (scored as 1+), or strongly positive (scored as 2+), without prior knowledge of clinicopathological data. Cases were accepted as strongly positive only if reviewers independently defined them as such. Contingency tables were used to analyze the relationship of ADAM8 expression in NSCLCs to clinicopathological data.

To investigate the presence of ADAM8 protein in clinical samples that had been embedded in paraffin blocks, we stained the sections in the following manner. Briefly, 50 μg/mL goat polyclonal antihuman ADAM8 antibody (R&D Systems, Inc., Minneapolis, MN) were added after blocking of endogenous peroxidase and proteins, and the sections were incubated with horseradish peroxidase-labeled antigoat IgG (Histofine Simple Stain MAX PO (G), Nichirei, Tokyo, Japan) as the secondary antibody. Substrate-chromogen was added, and the specimens were counterstained with hematoxylin.

Flow Cytometric Analysis. Lung cancer cells (1×10^6 cells) were incubated with a mouse monoclonal antihuman ADAM8 antibody for detecting the ectodomain of the protein (0.34 mg/mL; R&D Systems, Inc.) or control mouse IgG (0.34 mg/mL; Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C for 1 hour. The cells were washed in PBS and then incubated with AlexaFluor 488-conjugated antimouse IgG (Molecular Probes, Eugene, OR) at 4°C for 30 minutes. The cells were washed in PBS and analyzed on a FACScan flow cytometer (Becton Dickinson Labware, Bedford, MA) and analyzed by ModFit software (Verity Software House, Inc., Topsham, ME). Mean fluorescence intensity was calculated as a relative signal-intensity value, *i.e.,* of cells treated with antihuman ADAM8 antibody/cells treated with control mouse IgG.

ELISA. Serum levels of ADAM8 were measured by ELISA using a commercially available enzyme test kit (R&D Systems, Inc.). In brief, 3-fold diluted sera were added to a 96-well microplate precoated with monoclonal antibody specific for ADAM8 and incubated for 2 hours at room temperature. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for ADAM8 was added to the wells and incubated for 2 hours at room temperature. After a wash to remove any unbound antibody-enzyme reagent, a substrate solution (R&D Systems, Inc.) was added to the wells and allowed to react for 30 minutes. The reaction was stopped by adding 100 μL of 2 N sulfuric acid. Color intensity was determined by a photometer at a wavelength of 492 nm, with a reference wavelength of 630 nm. Levels of CEA in serum were measured by ELISA with a commercially available enzyme test kit (HOPE Laboratories, Belmont, CA), according to the same protocol as above. Differences in the levels of ADAM8 and CEA between tumor groups and a healthy control group were analyzed by Mann-Whitney U tests. The levels of ADAM8 and CEA were additionally evaluated by receiver-operating characteristic curve analysis to determine cutoff levels with optimal diagnostic accuracy and likelihood ratios. The correlation coefficients for these two markers were calculated with Pearson's correlation coefficient. Significance was defined as $P < 0.05$.

Matrigel Invasion Assay. NIH3T3 and COS-7 cells transfected either with plasmids expressing ADAM8 or with mock plasmids were grown to near confluence in DMEM containing 10% FCS. The cells were harvested by trypsinization, washed in DMEM without addition of serum or proteinase inhibitor, and suspended in DMEM at 1×10^5 cells/mL. Before preparing the cell suspension, the dried layer of Matrigel matrix (Becton Dickinson Labware) was rehydrated with DMEM for 2 hours at room temperature. DMEM (0.75 mL) containing 10% FCS was added to each lower chamber in 24-well Matrigel invasion chambers, and 0.5 mL (5×10^4 cells) of cell suspension were added to each insert of the upper chamber. The plates of inserts were incubated for 22 hours at 37°C. After incubation, the chambers were processed; cells invading through the Matrigel were fixed and stained by Giemsa as directed by the supplier (Becton Dickinson Labware).

RESULTS

ADAM8 Expression in Lung Tumors, Cell Lines, and Normal Tissues. To search for novel molecules to serve as diagnostic markers and/or targets for development of therapeutic agents for lung cancer, we had applied cDNA microarray analysis to identify genes that were transactivated in the majority of NSCLCs examined. Among 23,040 genes screened, we identified the *ADAM8* transcript, indicating 2-fold or higher expression (mean fold expression, 5.6 ± 4.5 SD; range, 2.2–16.4) in cancer cells than in normal lung cells (control) in 63% of the NSCLC samples examined, and confirmed its transactivation by semiquantitative reverse transcription-PCR experiments in 8 of 10 additional NSCLC tissues and in 11 of 23 lung cancer cell lines (Fig. 1*A*). Northern blot analysis with human cDNA as a probe detected a 3.5-kb transcript but at a very low level and only in leukocytes, lymph node, and bone marrow among 16 normal tissues examined (data not shown).

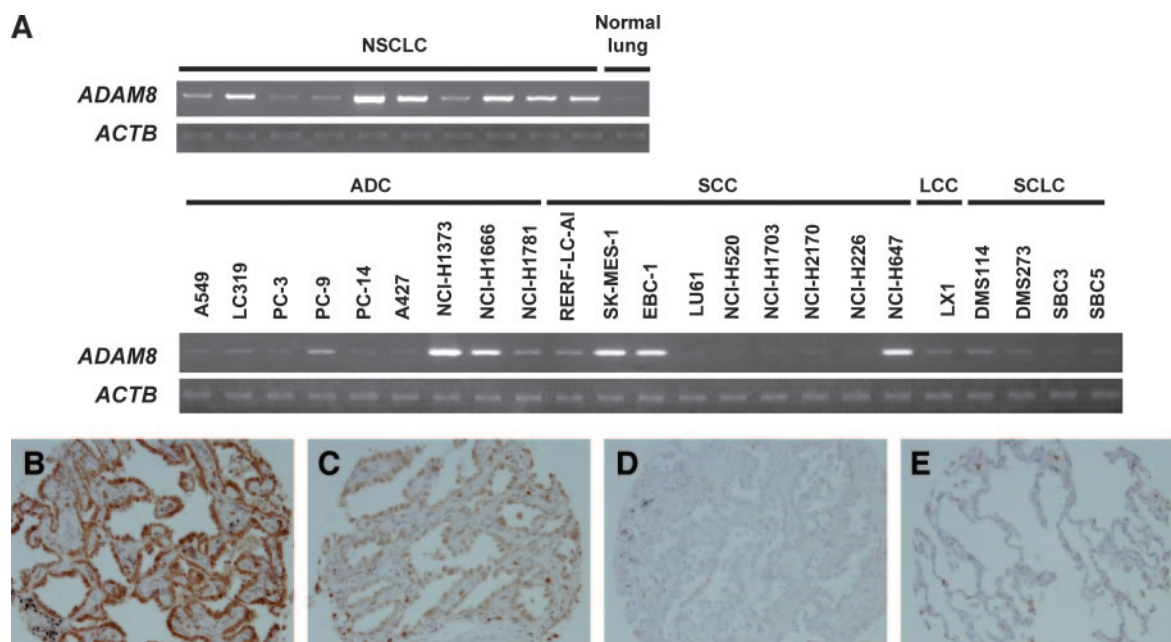We also examined expression of ADAM8 protein in clin-

Fig. 1 Validation of ADAM8 expression in lung tumors and cell lines. *A*, expression of *ADAM8* in a normal lung tissue and 10 clinical NSCLC samples (*top panel*) and 23 lung cancer cell lines (*bottom panel*) detected by semiquantitative reverse transcription-PCR analysis. ADC, adenocarcinoma; SCC, squamous cell carcinoma; LCC, large-cell carcinoma; SCLC, small-cell lung cancer. *B–E*, representative images after immunohistochemical staining of lung tissues, with anti-ADAM8 antibody on tumor tissue microarrays (×100). Examples are shown of strong (*B*), weak (*C*), and absent (*D*) ADAM8 expression in ADCs and of no expression in normal lung (*E*).
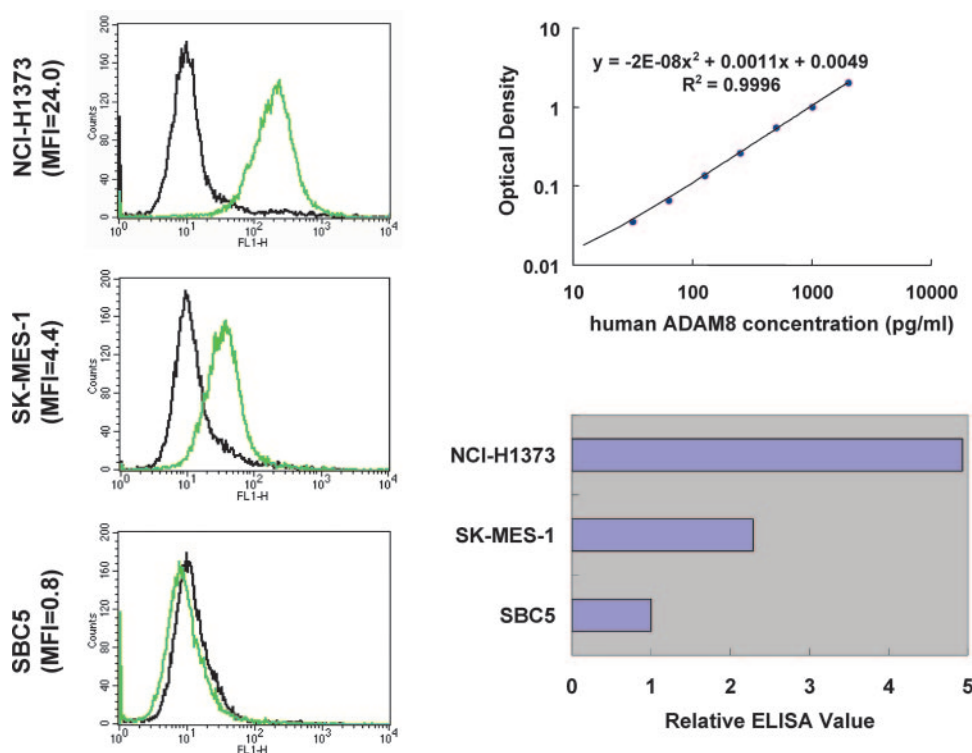
Fig. 2 Cellular localization and secretion of ADAM8 in lung cancer cells. *A*, expression of ADAM8 on cell surfaces in lung cancer lines NCI-H1373, SK-MES-1, and SBC-5 evaluated by flow cytometric analysis. Mean fluorescence intensity (MFI) was calculated as relative signal intensity values of cells treated with antihuman ADAM8 antibody (*green*)/cells treated with mouse IgG (control; *black*). *B*, standard curve of sandwich ELISA with anti-ADAM8 antibody (*X* axis, ADAM8 concentration; *Y* axis, absorbance). *C*, specific detection of ADAM8 with ELISA in conditioned medium from ADAM8-expressing NCI-H1373 and SK-MES-1 cells and nonexpressing SBC-5 cells. The *X* axis indicates the relative ELISA values (concentration of secreted ADAM8 from NCI-H1373 or SK-MES-1/concentration of secreted ADAM8 from SBC-5 cells).

ical lung cancers by tissue arrays with anti-ADAM8 antibody. ADAM8 localized at the plasma membrane, as well as in the cytoplasm of tumor cells, but was hardly detectable in surrounding normal tissues (Fig. 1*B–E*). Positive staining was observed in 158 (77%) of 206 adenocarcinomas cases examined, 55 (47%) of 118 squamous cell carcinomas, 16 (64%) of 25 large-cell carcinomas, and 11 (79%) of 14 SCLCs, whereas no staining was observed in any of the normal portions of the same tissues. We classified a pattern of ADAM8 expression on the tissue array ranging from absent/weak (scored as $0 \sim 1+$) to strong (scored as $2+$). Expression levels of ADAM8 were not associated with any of the clinicopathological factors in squamous cell carcinomas. However in adenocarcinomas, strong ADAM8 staining was significantly more common in stages IIIB–IV tumors (22 of 60; 37%) than in stages I–IIIA tumors (34 of 146, 23%; $P = 0.049$; χ^2 test). The sample sizes of large-cell carcinomas and SCLCs were too small to be evaluated additionally.

Secretion of ADAM8 in Lung Cancer Cells. We then validated ADAM8 expression on the surfaces of lung cancer cells with flow cytometry and anti-ADAM8 monoclonal antibody. This analysis indicated that the antibody bound to NCI-H1373 and SK-MES-1 cells in which ADAM8 transcript had been detected at a high level but not to SBC-5 cells, which did not express ADAM8 (Fig. 2*A*).

Because the extracellular domain of ADAM8 protein is thought to be secreted (10), we applied ELISA method to examine its presence in the culture media of these cell lines. High levels of ADAM8 protein were detected in media of NCI-H1373 and SK-MES-1 cultures but not in the medium of SBC-5 cells (Fig. 2, *B* and *C*). The amounts of detectable ADAM8 in the culture media accorded well with the expression levels of ADAM8 on the cell surfaces detected with flow cytometric analysis.

Serum Levels of ADAM8 in Lung Cancer Patients. Because the *in vitro* findings had suggested a possibility for development of a novel tumor maker for lung cancer, we investigated whether the extracellular domain of ADAM8 is secreted into sera of patients with lung cancer. ELISA experiments detected ADAM8 in serologic samples from lung cancer patients and also from normal individuals; serum levels of ADAM8 in lung cancer patients were 431 ± 249 pg/mL (mean $\pm$ SD) and those in healthy individuals were 267 ± 56 pg/mL. The difference was significant with P of <0.001 (Mann-Whitney U test). When classified according to histologic type, the serum levels of ADAM8 were 427 ± 286 pg/mL in adenocarcinomas patients, 467 ± 210 pg/mL in squamous cell carcinomas patients, and 400 ± 112 pg/mL in SCLC patients (Fig. 3*A*); the differences among the three histologic types were not significant. High levels of serum
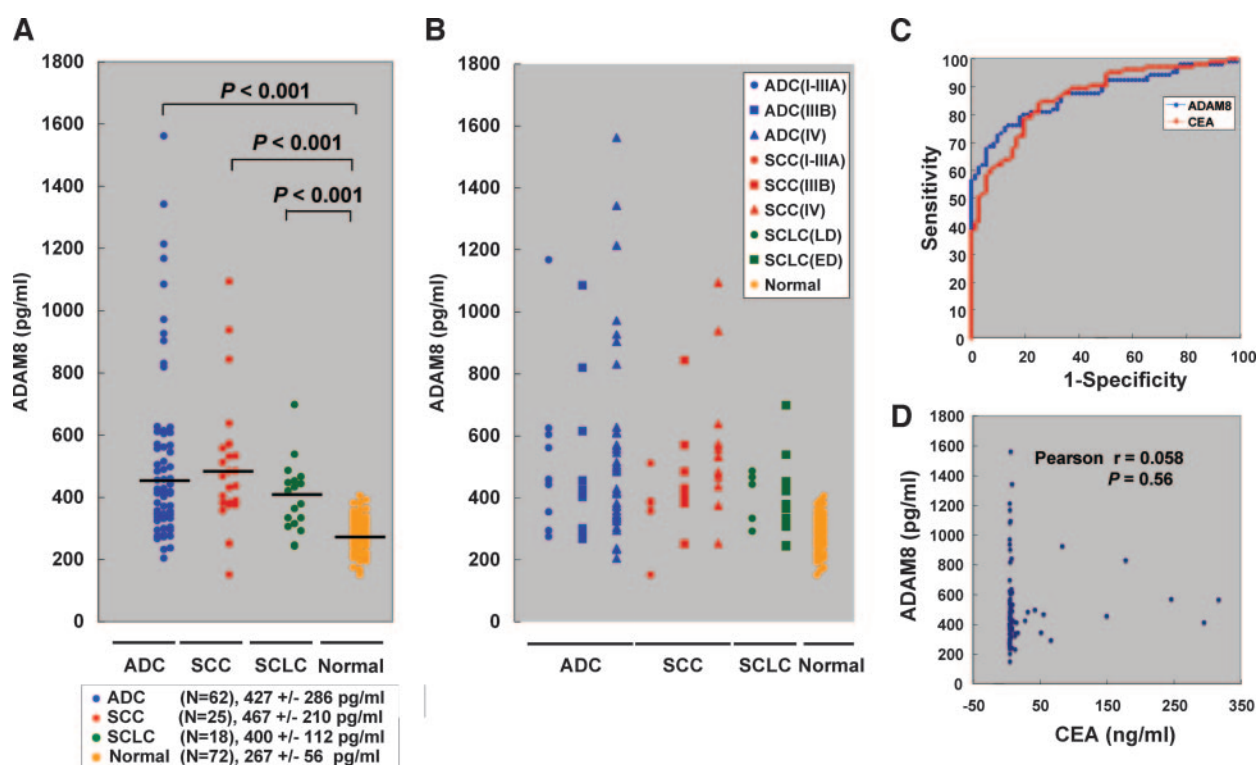
Fig. 3 Serologic concentration of ADAM8 determined by ELISA in patients with lung cancers and in healthy controls. *A*, distribution of ADAM8 in sera from patients with lung adenocarcinoma (ADC), lung squamous cell carcinoma (SCC), or SCLC. Averaged serum levels are shown as *black lines*. Differences were significant between ADC patients and healthy individuals ($P < 0.001$, Mann-Whitney U test), between SCC patients and healthy individuals ($P < 0.001$) and between SCLC patients and healthy individuals ($P < 0.001$). *B*, distribution of ADAM8 in sera from patients at various clinical stages of lung cancers. LD indicates limited disease; ED, extensive disease. *C*, receiver-operating characteristic curve analysis of ADAM8 (*blue*) and CEA (*red*) as serum markers for lung cancer (*X* axis, 1-specificity; *Y* axis, sensitivity). *D*, relationship between serum levels of ADAM8 and CEA (*X* axis, CEA concentration; *Y* axis, ADAM8 concentration).

ADAM8 were detected even in patients with earlier-stage tumors (stages I–IIIA; Fig. 3*B*). We also found no significant association between the serum ADAM8 level and age or gender (Table 1).

Comparison of ADAM8 and CEA as Tumor Markers. To evaluate the feasibility of using serum ADAM8 level as a tumor detection marker, we also measured by ELISA serum levels of CEA, a conventional tumor marker, in the same patients and controls. ADAM8 and CEA were additionally analyzed by drawing receiver-operating characteristic curves to determine their cutoff levels (Fig. 3*C*). The sum of the area under the receiver-operating characteristic curve for serum ADAM8 value was slightly larger than that for serum CEA, suggesting slightly better specificity and likelihood for ADAM8 as diagnostic marker for lung cancer. Cutoff levels in this assay were set to result in optimal diagnostic accuracy and likelihood ratios for ADAM8 and CEA, *i.e.,* 379 pg/mL for ADAM8 and 5.3 ng/mL for CEA. As shown in Fig. 3*D*, the correlation coefficient between serum ADAM8 and CEA values was not significant (Pearson's correlation: r = 0.058, $P = 0.56$), indicating that measuring both markers in serum can improve overall sensitivity for detection of NSCLC to 80% (for diagnosing NSCLC, the sensitivity of CEA alone is 57% and that of ADAM8 is 63%.). False-positive results for

either of the two tumor markers among 72 normal volunteers (control group) amounted to 11% (8 of 72), whereas the false-positive rates for CEA and ADAM8 in the same control group were 5.5% (4 of 72) each.

Table 1 Correlation of serum ADAM8 level with age or gender

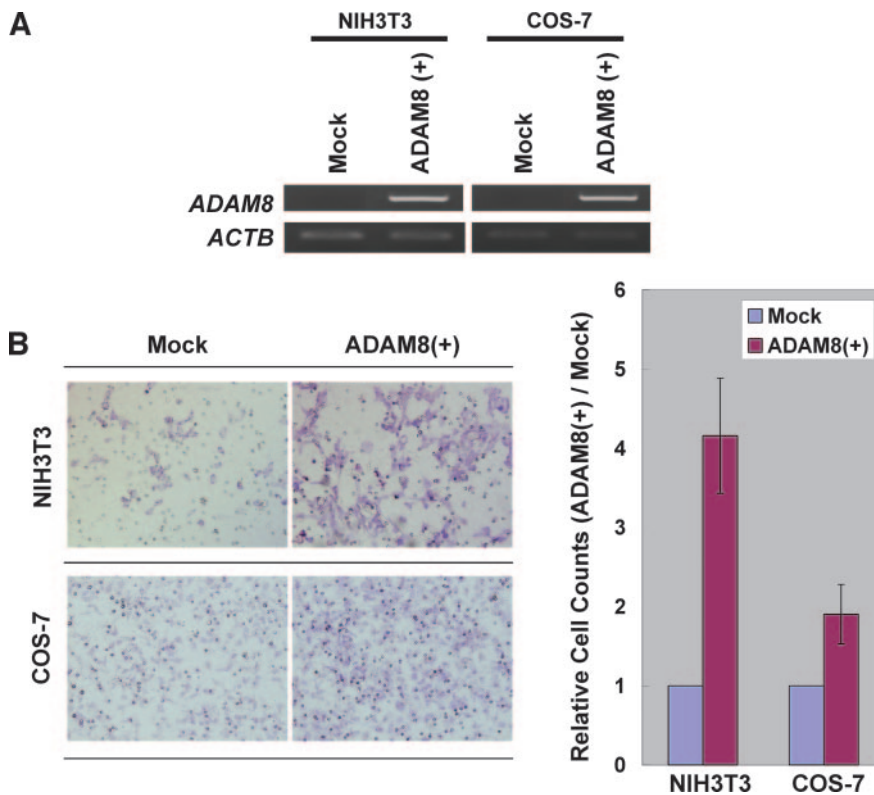|	Serum ADAM8			
Factors	Percentage (%)	No. of patients	Mean ± SD	P
Lung cancer patients				
Gender				
Male	74	78	442 ± 232	0.54*
Female	26	27	418 ± 299	
Age (y)				
≤65	43	45	413 ± 288	0.26*
>65	57	60	445 ± 218	
Normal individuals				
Gender				
Male	56	40	267 ± 52	0.65*
Female	44	32	273 ± 62	
Age (y)				
≤65	71	51	266 ± 53	0.08*
>65	29	21	308 ± 60	

* Mann-Whitney U test.

Fig. 4 Promotion of invasiveness of mammalian cells transfected with ADAM8-expressing plasmids. *A,* transient expression of *ADAM8* in NIH3T3 and COS-7 cells, detected by semiquantitative reverse transcription-PCR analysis. *B,* assay demonstrating the invasive nature of NIH3T3 and COS-7 cells in Matrigel matrix after transfection with expression plasmids for human *ADAM8.* *Left panel* shows Giemsa staining (×200), and *right panel* represents the relative number of cells migrating through the Matrigel-coated filters (cells with ADAM8-expressing plasmids/cells with mock plasmids). Assays were performed three times and in triplicate wells.

Activation of Cellular Migration/Invasive Activity by ADAM8. ADAM8 protein contains conserved ectodomains that are considered important for cell adhesion and protease activities; other ADAM family members are known to be associated with cellular motility and invasion (9). Hence, we examined a possible role of ADAM8 in cellular motility and invasion in Matrigel assays, with NIH3T3 and COS-7 cells. As shown in Fig. 4, transfection of ADAM8 cDNA into either cell line significantly enhanced its invasive activity through Matrigel, compared with cells transfected with mock vector.

DISCUSSION

Despite many advances in diagnostic imaging of tumors, combination chemotherapy, and radiation therapy, little improvement has been achieved within the last decade in terms of prognosis and quality of life for patients with lung cancer. Given the frequent failure of conventional treatment strategies, many cancer-related molecules have been characterized toward the goal of developing novel anticancer therapies such as molecular-targeted drugs and antibodies or cancer vaccines (20, 21). Molecular-targeted therapies are expected to be highly specific to malignant cells, with minimal adverse effects due to their well-defined mechanisms of action. Equally desirable in prospect are minimally invasive, highly sensitive, and specific new diagnostic methods that would adapt readily to clinical settings. From these points of view, tumor-specific transmembrane/secretory proteins should have significant advantages because they are presented either on the cell surface or within the extracellular space and/or in serum, making them easily acces-

sible as molecular markers and therapeutic targets. Some tumor-specific markers already available, such as CYFRA or Pro-GRP, are transmembrane/secretory proteins (4, 5); the example of rituximab (Rituxan), a humanized monoclonal antibody against CD20-positive lymphomas, provides proof that targeting specific cell surface proteins can result in significant clinical benefits (22). As an approach to identifying novel cancer-specific cell surface or secretory proteins, we have been exploiting the power of genome-wide expression analysis to select genes that are overexpressed in cancer cells. Analysis of candidate molecules revealed ADAM8 as a potential target for development of novel tools for diagnosis and treatment of lung cancer.

ADAM8 protein is homologous to a snake disintegrin, Reprolysin (M12B), a zinc metalloprotease (9). Members of the ADAM family are cell surface proteins with a unique structure combining potential adhesion and protease domains. A published report has suggested that the ADAM8 ectodomain is cleaved by ADAM8 itself (10). Because various matrix metalloproteinases and ADAM family proteins had been described as being overexpressed in human cancers (23), ADAM8 seemed likely to have a potential role in tumor development or progression. In this study, we demonstrated that induction of exogenous expression of ADAM8 enhanced the cellular migration/invasive activity of mammalian cells. Concordantly, the strong ADAM8 staining in primary NSCLC tissues detected by tissue microarray analyses correlated with disease progression; overexpression of ADAM8 protein was significantly more common in tumors from patients with locally advanced lung cancer (stage IIIB) or distant organ metastases (stage IV) than in those with earlier

stage diseases (stages I–IIIA). The former patients are generally not candidates for curative resection (24). Although the precise mechanism of ADAM8 in lung carcinogenesis is unknown and the processes of cancer-cell invasion to adjacent tissues and distant metastasis consist of a complex series of sequential step, these results indicate that ADAM8 expression and its cleavage could promote dissemination of tumors by stimulating cell migration. ADAM8 has been described as a shedding enzyme of the low-affinity IgE receptor CD23 (13); it also appears to play an important role in physiologic and pathological cell interactions by specifically releasing a functional form of a neural-cell adhesion molecule, a homologue of L1 (CHL1), from cell surfaces (14). However, when we analyzed mRNA expression of *ADAM8*, *CD23*, and *CHL1* in lung cancer cell lines and cancer tissues by semiquantitative reverse transcription-PCR, no expression of *CD23* was detectable in most of the lung cancer samples examined, and the expression pattern of *CHL1* was not concordant with that of *ADAM8* (data not shown). Additional studies to identify unknown substrates of ADAM8 in lung cancers may contribute not only to identification of novel tumor markers and therapeutic targets but also should yield new understanding of the signaling pathway mediated by ADAM8 expression.

We also found high levels of ADAM8 protein in serologic samples from lung cancer patients. Because most of the serum samples used for were derived from patients with advanced cancers (stages IIIB–IV), we evaluated ADAM8 as a tumor marker for early diagnosis of this disease. Tissue microarray analyses of NSCLCs at stages I–IV documented ADAM8 positivity even in early-stage tumors; ADAM8 staining was observed in 176 (64%) of 274 cases at stages I–IIIA, including 111 (76%) of 146 adenocarcinomas cases, whereas as mentioned above, strong ADAM8 staining was observed more frequently in adenocarcinomas cases at advanced stages IIIB–IV than in cases at stages I–IIIA. These results indicated that ADAM8 should be useful for diagnosis of even early-stage lung cancers and that the high level of ADAM8 expression could indicate increased malignant potential of ADAM8-positive tumors.

To validate the feasibility of applying ADMA8 as the diagnostic tool, we compared serum levels of ADAM8 with those of CEA, a conventional diagnostic marker for NSCLCs, in terms of sensitivity and specificity for diagnosis. The proportions of positive cases among the same serum samples were 57% for CEA and 63% for ADAM8, whereas the false-positive rate for ADAM8 (5.5%) was the same as that of CEA, indicating equivalent or better diagnostic power of ADAM8. Furthermore, an assay combining both markers increased the sensitivity such that 80% of the patients with lung cancer were diagnosed as positive, whereas 11% of 72 healthy volunteers were falsely diagnosed as positive. Although additional validation with a larger set of serum samples covering various clinical stages will be necessary, the data presented here sufficiently demonstrate a potential clinical application of ADAM8 itself as a serologic/histochemical marker for lung cancers. We should mention also that we observed activation of *ADAM8* in nearly half of a group of pancreatic cancers, which have a significantly invasive phenotype (data not shown). This suggests that overexpression of *ADAM8* might play a significant role in progression of pancreatic cancer and could warrant investigation of serum levels of ADAM8 in patients with other types of invasive cancer as well.

In conclusion, we have identified ADAM8 as a potential marker for diagnosis of lung cancers. This molecule is also a likely candidate for development of therapeutic approaches such as antibody therapy. ADAM8 was specifically overexpressed in most lung cancer tissues we examined and was elevated in the sera of a large proportion of patients with lung cancer. ADAM8, combined with other tumor markers, could significantly improve the sensitivity of lung cancer diagnosis.

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