Japan) (21). The Lu-130, Lu-135 and PC-13 cell lines were gifts from Dr Y. Dobashi (Jichi Medical University, Omiya Medical Center Hospital, Omiya, Japan). A549, ABC-1, H460, LC-2/ad, VMRC-LCD, RERF-LC-MS, REAF-LCKJ and TKB-2 were gifts from Dr T. Niki (Jichi Medical University, Shimotsuke, Japan). TMK-1 was a gift from the Department of Genetics, National Cancer Center (Tokyo, Japan). SAEC was purchased from Clontech (San Diego, CA). WI-38, H358, H820, H2087, H1299, H526, H1688, HCT116, HT-29, DLD-1 and AGS were obtained from ATCC (Manassas, VA, USA). PC-3, LU65, KATO3, MKN-1, MKN-28, MKN-45, MKN-74, A431, HSC-2, HSC-3 and HSC-4 were obtained from the Health Science Research Resources Bank (Osaka, Japan). HLC-1 was obtained from RIKEN Cell Bank (Ibaraki, Japan).

Clinical samples. The subjects were selected from among patients of Hamamatsu University School of Medicine and Mikatahara Seirei General Hospital. Written informed consent to participate in this study was obtained and the entire study design was approved by the Institutional Review Boards (IRB) of Hamamatsu University School of Medicine (18-4,18-5) and Mikatahara Seirei General Hospital. Lifestyle information such as smoking habits was obtained by professional interviewers. Histopathological classification was performed according to the WHO classification (2004) (22). Stages of the clinical samples according to the TNM classification system (http://www.uicc.org/) are shown in Table I.

Table I. Clinicopathological characteristics of the patients.

No of patients, N	73
Average age (range), years	64.7 (39-83)
Gender, n (%)	
Male	41 (56.2)
Female	32 (43.8)
Histology, n (%)	
Adenocarcinoma	50 (68.3)
Squamous cell carcinoma	17 (23.3)
Small cell carcinoma	2 (2.7)
Large cell carcinoma	4 (5.5)
Brinkman index, n (%)	
BI = 0	24 (32.9)
0<BI≤400	4 (5.5)
400<BI	36 (49.3)
Unknown	9 (12.3)
TNM stage, n (%)	
I	47 (64.4)
II	6 (8.2)
III	13 (17.8)
IV	3 (4.1)
Unknown	4 (5.5)

3'-Rapid amplification of cDNA end. 3'-RACE was performed with the 3' RACE System for Rapid Amplification of cDNA Ends (Invitrogen). Briefly, the first strand cDNA was reverse transcribed from 1 μg of total RNA using SuperScript II RT (Invitrogen) and the adapter primer; 1 μl of the first strand cDNA was then amplified using an *EPHA7* gene-specific forward primer (EphA7 RACE 5'-CACCATACGTTGCATG CACA-3') and the Universal Amplification Primer. In the polymerase chain reactions, after initial denaturation at 95°C for 15 min, 35 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, and elongation at 72°C for 45 sec were used, followed by a final elongation step at 72°C for 5 min.

Sequencing of the RACE product and validating sequencing of the secreted form of EPHA7 (EPHA7-S). We sequenced the band of ~600 bp generated by the RACE procedure with an *EPHA7* gene-specific forward sequencing primer, and identified it as *EPHA7*-S. We searched for EPHA7-S in lung cancer cell lines by sequencing the cDNAs synthesized from mRNAs. Gene-specific primer pairs were designed to cover the region from exon 4 to intron 5 of *EPHA7*. Primers for PCR were 5'-CATCTGACCCACCATACGTTGC-3' (*EPHA7* exon 4) and 5'-GCTGGAAGAATCAAGCTCTGTG-3' (*EPHA7* intron 5). PCR was carried out in reaction mixtures containing cDNA, 1X HotStar Taq buffer, 0.25 mmol/l deoxynucleotide triphosphate mixture, 0.05 U of HotStar Taq (Qiagen, Dusseldorf, Germany), and 0.5 mmol/l of forward and reverse primers in a volume of 20 μl. PCR cycling parameters were one cycle of 95°C for 15 min; 40 cycles of 95°C for 30 sec, 58°C for 30 sec, and 75°C for 45 sec; followed by one cycle of 72°C for 5 min. The PCR products were purified with a PCR purification kit (Qiagen) and directly sequenced with a Big Dye Terminator Cycle Sequencing Reaction Kit and the ABI 3100 Genetic Analyzer (Applied Biosystems Incorporated, Tokyo, Japan). Sequencing reactions were done in both forward and reverse directions with two primers for PCR.

Statistical analysis. χ^2 analysis and the Cochran-Armitage trend test were conducted to compare EPHA7-S expression with various clinical features (sex, smoking history, histological type, and TNM stages). A $p<0.05$ was considered significant. Statistical analyses were performed using the SAS (Statistical Analysis System) program (SAS Institute Japan, Tokyo, Japan).

Results

Detection of EPHA7-S in cell lines. First, we attempted to identify a downstream sequence, expecting a fusion partner of EPHA7. We adopted the 3'-RACE method using the primer corresponding to exon 4 of *EPHA7* and the RACE specific 3' primer for the RNAs from PC-13 and H82 cell lines, both of which express EPHA7 (data not shown). The RERF-LC-MS cell line, which has no EPHA7 expression, was used as a negative control. By the 3'-RACE method, we detected amplified fragments in both PC-13 and H82 (Fig. 1a).

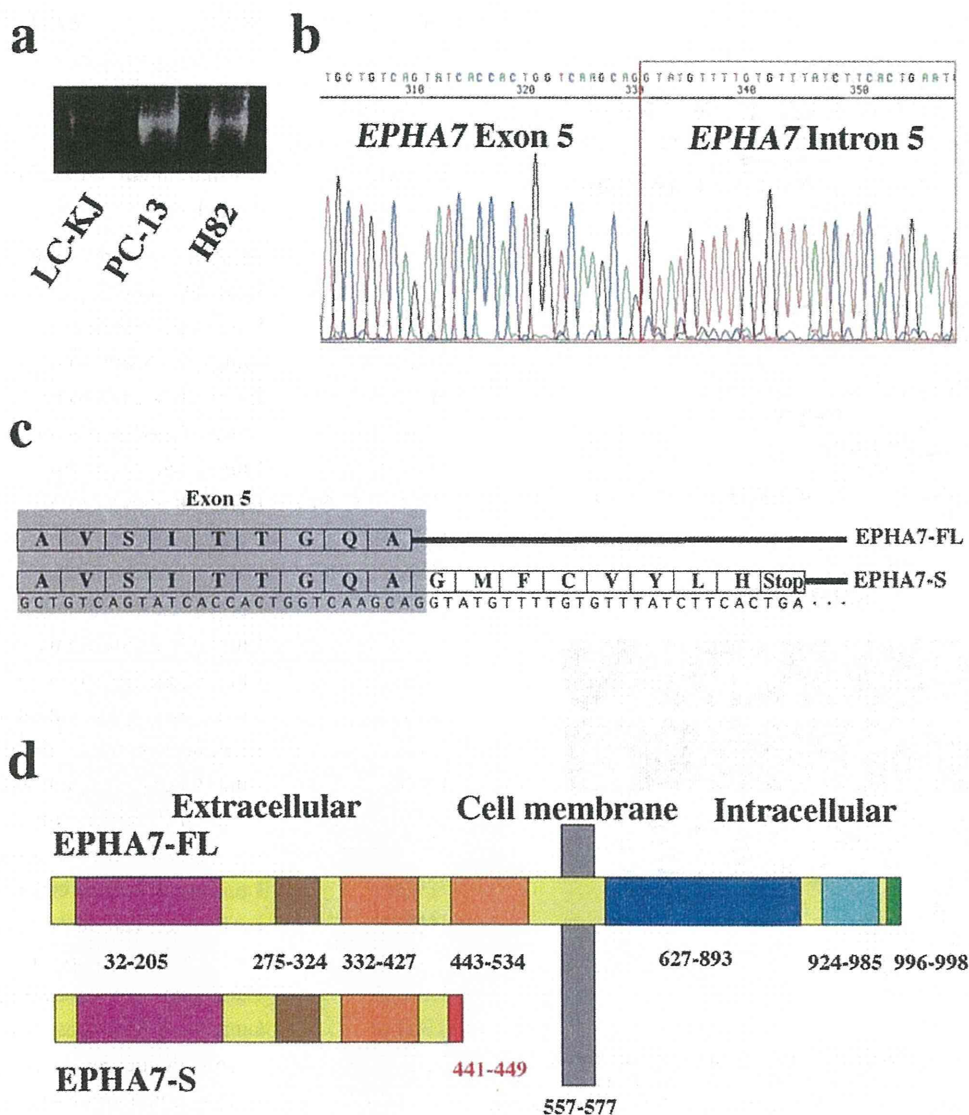

Figure 1. Detection of the secreted form of EPHA7 (EPHA7-S) in lung cancer cell lines. (a) 3'-RACE method showing amplification of EPHA7-S in PC-13 and H82 but not in RERF-LC-KJ. (b) Sequencing analysis of the EPHA7-S gene transcript of PC-13. The red box indicates intron 5 of the *EPHA7* gene. (c) Nucleotide sequence and predicted amino acid sequence of full-length EPHA7 (EPHA7-FL) at the end of exon 5 and for the EPHA7-S based on sequencing of a cDNA from the PC-13 cell line. Shaded region is exon 5 of the *EPHA7* gene. (d) Schematic representation of EPHA7-FL and EPHA7-S. 32-205, Ephrin receptor ligand binding domain; 275-324, TNF receptor domain; 332-427 and 443-534, Fibronectin type 3 domain; 557-577, transmenbrane domain; 627-893, tyrosine kinase domain; 924-985, SAM domain; 996-998, PDZ binding motif; 441-449, intron 5 (EPHA7-S).

Sequencing analysis of these fragments disclosed that they were not fusion partners, while the sequence derived from EPHA7 itself had its intron 5 (Fig. 1b and c). DNase treatment and the cDNA minus negative control excluded the possibility of mis-amplification of the genomic sequence. This cDNA contained the exon 5 sequence directed to the intron 5 sequence of *EPHA7* (Fig. 1c), and it had a stop codon after coding 8 amino acids, generating the structure lacking the transmembrane domain of the authentic EPHA7 (Fig. 1d). Given these structural features, we consider this form to be the human counterpart of EphA7-S reported in murine lymphocytes by Dawson *et al* (23). In addition, they demonstrated human tonsillar lymphocytes to also express an EPHA7-S protein of consistent size by Western blotting, though the exact message was not shown (23). This structure lacks a cytoplasmic domain, which would include a kinase domain, indicating that the product would be secreted outside the cells.

EPHA7-S expression in lung cell lines. We examined cell lines, including those of lung cancers, gastrointestinal cancers and immortalized bronchial epithelium, for detection of EPHA7-S. We used the primers in the *EPHA7* exon 4 (forward) and intron 5 (reverse) for reverse transcription PCR. This primer set discriminates the contaminated genomic amplified product (1677 bp) from the target product (475 bp) (Fig. 2a). EPHA7-S was detected mainly in lung cancer cell lines, rarely in those from other organs (16/21 in lung cancer cell lines vs. 1/15 gastrointestinal cancer cell lines) (Fig. 2b and Table II), that is, EPHA7-S is a variant occurring mainly in lung cancer cells.

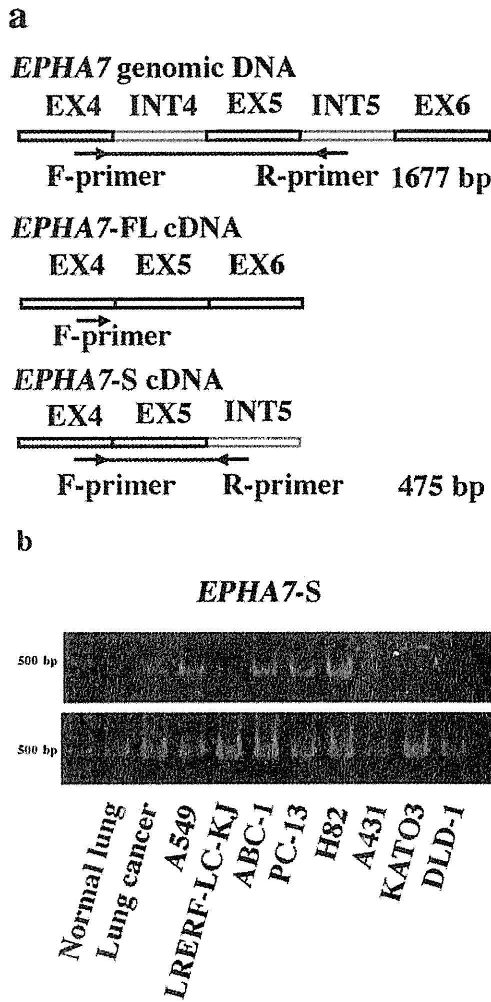

Figure 2. Secreted form of EPHA7 (EPHA7-S) in various cell lines. (a) PCR primer design and the structure of EPHA7-S. The amplified structure of genomic *EPHA7* (1677 bp) is shown above, EPHA7-FL cDNA (no amplification) is the middle, and EPHA7-S cDNA (475 bp) below. (b) EPHA7-S cDNA (475 bp) was detected in some of these cell lines. The bands in the upper panel are EPHA7-S and those in the lower panel sare GAPDH. The sources of the normal lung and lung cancer lanes are clinical samples from patient no. 15 (squamous cell carcinoma and the corresponding non-tumor lung portion). A549, LRERF-LC-KJ, ABC-1, PC-13 and H82 are lung cancer cell lines, A431 is an esophageal cancer cell line, KATO3 is a gastric cancer cell line, and DLD-1 is a colon cancer cell line. The left-most lane indicates size markers, a 100-bp DNA ladder.

Some clinical lung cancer specimens express EPHA7-S. The tendency for EPHA7 to be expressed by lung cancer cell lines prompted us to look for it in primary lung cancer tissues. A substantial proportion of human primary lung cancers expressed EPHA7-S; 2 out of 50 cases with lung adenocarcinoma and 8 of 23 cases with non-adenocarcinoma of the lung. Among the non-adenocarcinoma cases, 6 of 17 squamous cell carcinoma cases, one of the two cases with small cell carcinoma, and one of four large cell carcinoma cases were positive for EPHA7-S (Table III). In 2 cases, EPHA7-S was detected in non-tumor lung tissue adjacent to the lung cancer (data not shown).

The prevalence of EPHA7-S was significantly greater in non-adenocarcinoma than in adenocarcinoma (Table IV). No

Table II. Secreted form of *EPHA7* (*EPHA7-S*) in various cell lines.

Material		EphA7-S
16HBE14o-	Normal human bronchial epithelium	+
SAEC	Small airway epithelial	+
WI-38	Lung fibroblast	-
H358	Lung adenocarcinoma	+
H820	Lung adenocarcinoma	+
H2087	Lung adenocarcinoma	-
A549	Lung adenocarcinoma	+
HLC-1	Lung adenocarcinoma	+
RERF-LC-MS	Lung adenocarcinoma	+
LC-2/ad	Lung adenocarcinoma	-
VMRC-LCD	Lung adenocarcinoma	+
PC-3	Lung adenocarcinoma	+
RERF-LC-KJ	Lung adenocarcinoma	-
ABC-1	Lung squamous cell carcinoma	+
H460	Lung large cell carcinoma	-
H1299	Lung large cell carcinoma	+
LU65	Lung large cell carcinoma	+
PC-13	Lung large cell carcinoma	+
H82	Lung small cell carcinoma	+
H526	Lung small cell carcinoma	+
H1688	Lung small cell carcinoma	+
Lu-130	Lung small cell carcinoma	+
Lu-135	Lung small cell carcinoma	+
TKB-2	Lung small cell carcinoma	-
DLD-1	Colon adenocarcinoma	-
HCT-116	Colon adenocarcinoma	-
HT29	Colon adenocarcinoma	-
AGS	Gastric adenocarcinoma	-
HSC-39	Gastric adenocarcinoma	+
KATO3	Gastric adenocarcinoma	-
MKN-1	Gastric adenocarcinoma	-
MKN28	Gastric adenocarcinoma	-
MKN-45	Gastric adenocarcinoma	-
MKN-74	Gastric adenocarcinoma	-
TMK-1	Gastric adenocarcinoma	-
A431	Esophageal squamous cell carcinoma	-
HSC-2	Esophageal squamous cell carcinoma	-
HSC-3	Esophageal squamous cell carcinoma	-
HSC-4	Esophageal squamous cell carcinoma	-

+, EPHA7-S detectable, -, not detectable.

Table III. Clinicopathological characteristics of 10 patients with *EPHA7-S*-positive lung cancer.

Patient no.	Sex	Age	Histology	Stage	BI
9	F	77	SCC	3b	0
15	M	62	SQCC	3a	1260
18	M	61	SQCC	2b	1200
24	M	69	SQCC	1a	740
31	M	39	SQCC	1b	920
34	M	66	LCC (LCNEC)	1b	1350
42	M	65	ADC	1a	1350
47	F	82	ADC	1a	0
55	F	54	SQCC	3b	720
63	F	67	SQCC	1a	0

ADC, adenocarcinoma; LCC, large cell carcinoma; LCNEC, large cell neuroendocrine carcinoma; SCC, small cell carcinoma; SQCC, squamous cell carcinoma; BI, Brinkman index.

Table IV. Associations between *EPHA7*-S and clinicopathological features.

	EPHA7-S+	*EPHA7*-S-	P-value
Gender			
Male	6	35	0.7924[a]
Female	4	28	
Histology			
Adenocarcinoma	2	48	0.0004[a]
Other carcinomas	8	15	
Brinkman index			
0≤BI<400	3	25	0.557[a]
400≤BI	7	29	
TNM stage			
I	6	43	0.3548[b]
II	1	4	
III	3	10	
IV	0	3	

[a]χ^2 test. [b]Cochran-Armitage trend test.

other clinicopathological factors were related to EPHA7-S expression.

Discussion

While pursuing the possibility of EPHA7 fusion partners, we incidentally isolated EPHA7-S in lung cancer cell lines and tissues. Intriguingly, EPHA7-S tends to be expressed in lung but not other cancer cell lines. Furthermore, this EPHA7-S expression in lung cancers was seen mainly in non-adenocarcinomas. These findings may facilitate diagnosing of the primary site of such cancers, especially non-adenocarcinoma types.

Lung carcinogenesis probably constitutes heterogeneous steps according to its histological subtype and/or environmental effects such as smoking. Classically, non-adenocarcinomas are believed to have a stronger association with smoking than adenocarcinoma of the lung (24), such that the greater representation of EPHA7-S in non-adenocarcinoma than adenocarcinoma may suggest this difference to reflect EPHA7-S involvement in smoking related carcinogenesis. The amount of tobacco did not, however, correlate with the EPHA7-S expressions in the tumors of our subjects. Thus, the significance of this tendency must be carefully interpreted and further accumulation of cases is warranted. Recently, expression of EPHA2, another EPHA family member, was reported to be associated with smoking history (25). EPHA7-S is possibly another member of the EPH family of genes related to all form of smoking. The prevalences of EPHA7-S in lung cancer cell lines of different histological origin did not, however, differ between adenocarcinomas and non-adenocarcinomas. Again, the significance of the apparent difference in primary lung tumors according to subtype remains an open question. There is only one report documenting EPHA7-S expression in human tumors. Dawson *et al* reported EPHA7-S in germinal center lymphocytes (23). They speculated that hypermethylation of the EPHA7 promoter and the secreted form of EphA7 interacted in lymphomatogenesis. In fact, the finding of frequent promoter methylation in *EPHA7* in colorectal cancer raised the possibility that *EPHA7* is one of the tumor suppressor genes inactivated in colorectal carcinogenesis (8). The EPHA7 expression profile in human lung cancers was quite different from those in colorectal, gastric, prostate, and brain cancers (8,13,14,26). EPHA7 was expressed mainly in the tumor portion (data not shown). In this context, EPHA7 may be an oncogene rather than a suppressor gene in lung carcinogenesis. A larger clinical study is needed to corroborate this view of EPHA7 as an oncogene. This paradoxical situation (oncogene in one and suppressor gene in an other organ) has been described in several organs and settings in which the *EPH* family of genes play roles (27). EPHA7-S is also overexpressed in some lung cancers, meaning that the role of EPHA7-S in lung cancer may not be consistent with that assumed in lymphoma, as previously reported (23). We detected EPHA7-S in two non-tumor lung tissues. We do not know the exact reason, but environmental stress may induce various forms of aberrant splicing (28). Since we also detected EPHA7 in SAEC and 16HBE14o- cell lines, both of which are from non-tumorigenic bronchogenic epithelium, the same microenvironment as in lung cancer tissue may exist in the non-tumor portion of the lung. In addition, there was an exception: HSC-39, a gastric cancer cell line, expresses EPHA7-S. The biological and pathological situations triggering EPHA7-S expression clearly require further study. In conclusion, we have, for the first time, described the secreted form of EPHA7 in a subset of human lung cancers.

Acknowledgments

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References

1. Eph nomenclature and committee: unified nomenclature for Eph family receptors and their ligands, the ephrins. Eph Nomenclature Committee. Cell 90: 403-404, 1997.
2. Pasquale EB: Eph-ephrin bidirectional signaling in physiology and disease. Cell 133: 38-52, 2008.
3. Nakamoto M and Bergemann AD: Diverse roles for the Eph family of receptor tyrosine kinases in carcinogenesis. Microsc Res Tech 59: 58-67, 2002.
4. Noren NK, Lu M, Freeman AL, Koolpe M and Pasquale EB: Interplay between EphB4 on tumor cells and vascular ephrin-B2 regulates tumor growth. Proc Natl Acad Sci USA 101: 5583-5588, 2004.
5. Campbell TN, Attwell S, Arcellana-Panlilio M and Robbins SM: Ephrin A5 expression promotes invasion and transformation of murine fibroblasts. Biochem Biophys Res Commun 350: 623-628, 2006.
6. Tanaka M, Sasaki K, Kamata R and Sakai R: The C-terminus of ephrin-B1 regulates metalloproteinase secretion and invasion of cancer cells. J Cell Sci 120: 2179-2189, 2007.
7. Nakamura R, Kataoka H, Sato N, *et al*: EPHA2/EFNA1 expression in human gastric cancer. Cancer Sci 96: 42-47, 2005.
8. Wang J, Kataoka H, Suzuki M, *et al*: Downregulation of EphA7 by hypermethylation in colorectal cancer. Oncogene 24: 5637-5647, 2005.
9. Taddei ML, Parri M, Angelucci A, *et al*: Kinase-dependent and -independent roles of EphA2 in the regulation of prostate cancer invasion and metastasis. Am J Pathol 174: 1492-1503, 2009.
10. Kataoka H, Igarashi H, Kanamori M, *et al*: Correlation of EPHA2 overexpression with high microvessel count in human primary colorectal cancer. Cancer Sci 95: 136-141, 2004.
11. Kataoka H, Tanaka M, Kanamori M, *et al*: Expression profile of EFNB1, EFNB2, two ligands of EPHB2 in human gastric cancer. J Cancer Res Clin Oncol 128: 343-348, 2002.
12. Ding L, Getz G, Wheeler DA, *et al*: Somatic mutations affect key pathways in lung adenocarcinoma. Nature 455: 1069-1075, 2008.
13. Guan M, Xu C, Zhang F and Ye C: Aberrant methylation of EphA7 in human prostate cancer and its relation to clinicopathologic features. Int J Cancer 124: 88-94, 2009.
14. Wang J, Li G, Ma H, *et al*: Differential expression of EphA7 receptor tyrosine kinase in gastric carcinoma. Hum Pathol 38: 1649-1656, 2007.
15. Foster RE, Abdulrahman M, Morris MR, *et al*: Characterization of a 3;6 translocation associated with renal cell carcinoma. Genes Chromosomes Cancer 46: 311-317, 2007.
16. Tognon C, Knezevich SR, Huntsman D, *et al*: Expression of the ETV6-NTRK3 gene fusion as a primary event in human secretory breast carcinoma. Cancer Cell 2: 367-376, 2002.
17. Tomlins SA, Laxman B, Dhanasekaran SM, *et al*: Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. Nature 448: 595-599, 2007.
18. Soda M, Choi YL, Enomoto M, *et al*: Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 448: 561-566, 2007.
19. Takeuchi K, Choi YL, Togashi Y, *et al*: KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. Clin Cancer Res 15: 3143-3149, 2009.
20. Rikova K, Guo A, Zeng Q, *et al*: Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 131: 1190-1203, 2007.
21. Okudela K, Suzuki M, Kageyama S, *et al*: PIK3CA mutation and amplification in human lung cancer. Pathol Int 57: 664-671, 2007.
22. Kleihues Pand L and Sobin H: (eds.) Tumours of the lung, pleura, thymus and heart. IARC Press, Lyon, 2004.
23. Dawson DW, Hong JS, Shen RR, *et al*: Global DNA methylation profiling reveals silencing of a secreted form of Epha7 in mouse and human germinal center B-cell lymphomas. Oncogene 26: 4243-4252, 2007.
24. Kreyberg L: Relationship of different histological lung tumour groups to tobacco smoking. Br J Cancer 15: 51-53, 1961.
25. Brannan JM, Dong W, Prudkin L, *et al*: Expression of the receptor tyrosine kinase EphA2 is increased in smokers and predicts poor survival in non-small cell lung cancer. Clin Cancer Res 15: 4423-4430, 2009.
26. Wang LF, Fokas E, Juricko J, *et al*: Increased expression of EphA7 correlates with adverse outcome in primary and recurrent glioblastoma multiforme patients. BMC Cancer 8: 79, 2008.
27. Noren NK and Pasquale EB: Paradoxes of the EphB4 receptor in cancer. Cancer Res 67: 3994-3997, 2007.
28. Korner M and Miller LJ: Alternative splicing of pre-mRNA in cancer: focus on G protein-coupled peptide hormone receptors. Am J Pathol 175: 461-472, 2009.

INTERNAL MEDICINE

□ CASE REPORT □

IgG4-related Lung Disease in a Worker Occupationally Exposed to Asbestos

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Abstract

A case of IgG4-related lung disease in a worker who had been exposed to asbestos is described. The patient had nocturnal cough and wheeze that responded to inhaled corticosteroid, and the radioallergosorbent test was positive against common allergens, suggesting an association with atopic asthma. IgE elevation is reported in asbestos-exposed workers, and asbestos exposure may cause atopic conditions. Predominance of Th2 cytokines and up-regulation of regulatory T lymphocytes have been reported in IgG4-related disease. IgG4-related disease may occur from hypersensitivity of the regulatory immune system to atopic conditions. Asbestos exposure may be a causal factor of IgG4-related disease.

Key words: IgG4-related systemic sclerosing disease, asbestos, bronchial asthma

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Introduction

IgG4-positive plasma cell infiltration can occur in almost all major organs, including the lungs. Some of these IgG4-related diseases include autoimmune pancreatitis, sclerosing sialadenitis, and sclerosing cholangitis. These conditions share clinical and pathological characteristics, such as high serum IgG4 concentrations, sclerosing inflammation with many IgG4-positive plasma cells, and effectiveness of corticosteroid therapy, and this is recognized as IgG4-related systemic sclerosing disease (1, 2). However, the pathogenesis of IgG4-related systemic sclerosing disease has not been fully clarified. A case of IgG4-related lung disease in a worker who was occupationally exposed to asbestos is described, and a possible relationship between IgG4-related systemic sclerosing disease and asbestos exposure is discussed.

Case Report

A 57-year-old man complaining of nocturnal cough, wheeze, and exertional dyspnea of 2 years' duration was referred to our hospital. He had a history of smoking (30 cigarettes per day for 30 years), and he had been occupationally exposed to asbestos released during building demolition for 10 years. Physical examination revealed swelling of bilateral cervical and submandibular lymph nodes, as well as the right subauricular lymph nodes. Chest auscultation revealed bilateral fine crackles in both lungs.

A chest radiograph showed bilateral reticular shadows and emphysema (Fig. 1a). HRCT imaging of the lung revealed multiple consolidations (Fig. 1b), nodules, and a subpleural curvilinear shadow (Fig. 1c), as well as ground-glass opacities and emphysema. A pleural plaque and hilar and mediastinal lymphadenopathy were also observed (Fig. 1d). Gallium-67 scintigraphy imaging showed uptake in bilateral cervical, submandibular, and hilar lymph nodes, in the right subauricular lymph nodes, and in bilateral lower lung fields. Pancreatic uptake was not observed, and swelling of the pancreas was not seen on abdominal CT scan.

Laboratory results included: C-reactive protein, 5.9 mg/dL; erythrocyte sedimentation rate, 119 mm/hr; total protein, 12.4 g/dL; albumin, 1.4 g/dL; γ-globulin, 69.1%; IgG, 8,396

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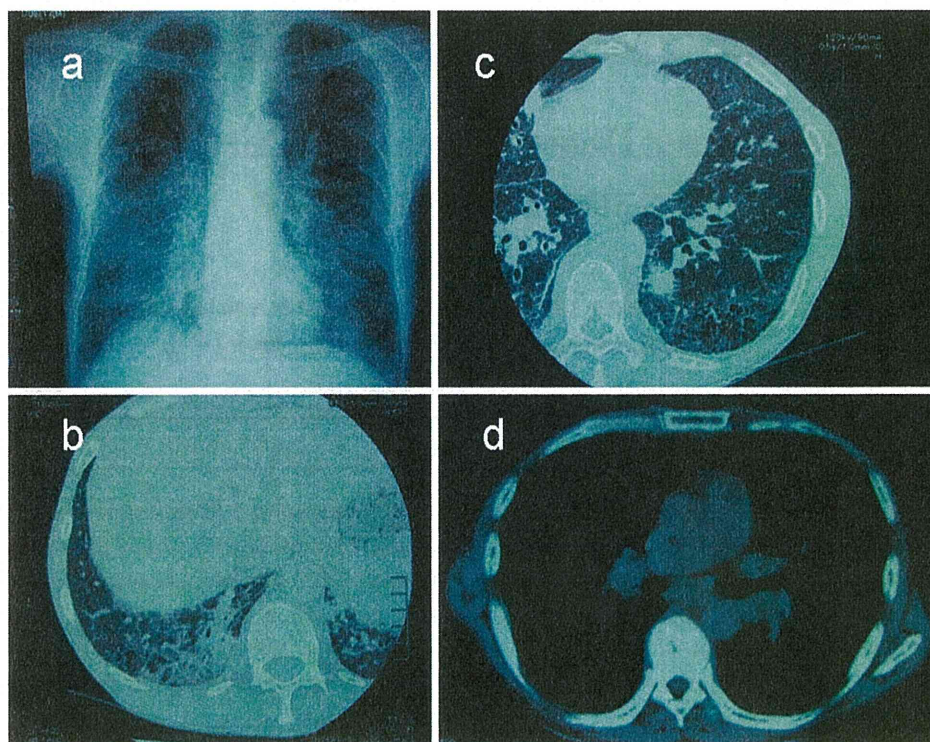

Figure 1. **A chest radiograph shows hyperinflation and reticular and linear shadows in bilateral lung fields (a). High-resolution computed tomography of the chest demonstrates multiple consolidations (b), nodules (c), and a subpleural curvilinear shadow (c), in addition to ground-glass opacities and emphysema. A pleural plaque and swelling of hilar and mediastinal lymph nodes are also observed (d).**

mg/dL; IgG4, 3,520 mg/dL; IgE, 4,940 IU/ml; and KL-6, 711 U/mL. Serum electrolytes, amylase, renal function, and liver function were normal. M-protein was not detected. Radioallergosorbent testing (RAST) for common allergens revealed: dermatophagoides, score 4; moth, score 4; cockroach, score 3; dog scurf, score 2. Respiratory function testing showed a vital capacity of 3.94 L (113.5% predicted) and a forced expiratory volume in one second of 2.44 L (89.4% predicted). Bronchial reversibility after inhalation of procaterol hydrochloride with ultrasonic nebulizer was 1.6%. Arterial blood gas analysis results on room air were: pH, 7.442; PaO_2, 65.4 mmHg; $PaCO_2$, 36.4 mmHg. On the sputum smear, numerous eosinophils were observed.

Transbronchial lung biopsy yielded no significant findings. Video-assisted thoracoscopy was performed, and a nodule at left S^{10} was resected. The surgical specimen showed interstitial and perivascular hyaline sclerosing-type fibrosis associated with lymphoplasmacytic infiltration (Fig. 2a). Immunostaining for IgG and IgG4 was performed using mouse monoclonal antibodies against human IgG (Dako Cytomation, Glostrup, Denmark) and human IgG4 (ZYMED Laboratory, San Francisco, CA, USA). Immunostaining revealed infiltration of numerous IgG4-positive plasma cells in the interstitium (Fig. 2b). IgG4-positive plasma cells accounted for 55.7% of the IgG-positive plasma cells. Thus, the patient was diagnosed as having IgG4-related lung disease and pulmonary emphysema.

Exertional dyspnea improved with tiotropium bromide hydrate, but nocturnal cough and wheeze continued. However, the nocturnal cough and wheeze disappeared after fluticasone propionate (500 μg) and salmeterol xinafoate (50 μg) twice daily were started. Systemic corticosteroid therapy was not given because his respiratory symptoms improved, and no involvement of other organs was observed. His IgG 4-related lung disease and lymphadenopathy have remained stable for more than 2 years after his initial presentation.

Discussion

This report describes a case of IgG4-related lung disease in a worker exposed occupationally to asbestos. The history of asbestos exposure is circumstantial because quantification of asbestos in lung tissue was not performed in this case. However, this patient appears to have certainly been exposed to asbestos because the chest CT scan revealed a pleural plaque, which is only observed in asbestos-exposed individuals in Japan (3). The pathogenesis of IgG4-related systemic sclerosing disease is unclear. However, Zen et al proposed a possible relationship between IgG4-related lung disease and atopic conditions. They reported a predominance of Th2 cytokines and up-regulation of regulatory T lymphocytes in IgG4-related sclerosing pancreatitis and cholangitis, and they proposed that the up-regulated functions of regulatory T lymphocytes in atopic conditions might play a role in this disorder (4). Miura et al also speculated that asbestos

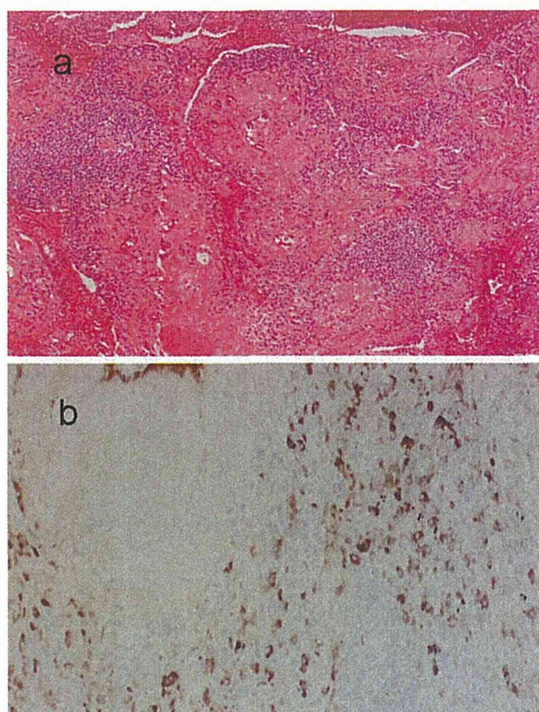

Figure 2. Surgical lung biopsies show interstitial and perivascular hyaline sclerosing-type fibrosis with associated lymphoplasmacytic infiltration (a). Immunostaining of the lung biopsy specimen shows infiltration of numerous IgG4-positive plasma cells in the interstitium (b).

might up-regulate functions of regulatory T lymphocytes in a human T-cell leukemia virus type-1-immortalized human polyclonal T cell line (5).

In the present case, RAST was positive to some common allergens, and numerous eosinophils were observed in the sputum smear. The nocturnal cough and wheeze disappeared with inhaled corticosteroid therapy, suggesting the presence of bronchial asthma, even though bronchial reversibility was not detected. Although the possibility that he had an atopic condition before asbestos exposure cannot be completely excluded, his atopy appears to have developed after asbestos exposure, because he did not have childhood asthma, and his asthmatic symptoms appeared after asbestos exposure. Elevated IgE levels have been reported in asbestos-exposed workers, and asbestos-exposed subjects may develop an atopic environment and, therefore, become more sensitive to allergic stimuli than nonexposed people (6, 7). However, we could not find any studies indicating a relationship between asbestos exposure and bronchial asthma. IgG4 is known to be a blocking antibody to IgE (8). In addition, asbestos exposure has been proposed as a causal factor for both pleural and retroperitoneal fibrosis (9-11), which is known to also be an IgG4-related condition (12). An association between lymphoplasmacytic pancreatitis and bronchial asthma has also been reported (13). Practically, it is thought that IgG4-related systemic sclerosing disease often accompanies atopic diseases such as bronchial asthma (14).

Based on these previous reports, the present patient's exposure to asbestos may have resulted in bronchial asthma, and up-regulated functions of regulatory T lymphocytes to atopic conditions may have induced infiltration of IgG4-positive plasma cells into the lungs, though the mechanisms of the development of atopic conditions in asbestos-exposed subjects remain unknown. However, our hypothesis is only speculative, with no supporting evidence.

In the present case, systemic corticosteroid therapy was not given because the respiratory symptoms disappeared with inhaled corticosteroid and bronchodilator therapy, and no involvement of other organs was observed. Zen et al reported that patients without respiratory symptoms were not treated with systemic corticosteroid in their cases of IgG4-related lung disease (15). There is no consensus report regarding the treatment of IgG4-related lung disease. To establish a treatment guideline for IgG4-related lung disease, further studies involving a large number of patients will be needed.

In conclusion, IgG4-related sclerosing disease may occur as a result of up-regulated functions of regulatory T lymphocytes to atopic conditions. To clarify this point, further investigations on allergic and environmental conditions of IgG 4-related sclerosing disease will be needed.

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References

1. Kamisawa T, Funata N, Hayashi Y, et al. A new clinicopathological entity of IgG4-related autoimmune disease. J Gastroenterol **38**: 982-984, 2003.

2. Kamisawa T, Okamoto A. IgG4-related sclerosing disease. World J Gastroenterol **14**: 3948-3955, 2008.

3. Kishimoto T. Health impairment induced by asbestos exposure. Saishin Igaku (The Medical Frontline) **62**: 7-13, 2007 (in Japanese).

4. Zen Y, Fujii T, Harada K, et al. Th2 and regulatory immune reactions are increased in immunoglobulin G4-related sclerosing pancreatitis and cholangitis. Hepatol **45**: 1538-1546, 2007.

5. Miura Y, Nishimura Y, Katsuyama H, et al. Involvement of IL-10 and Bcl-2 in resistance against an asbestos-induced apoptosis of T cells. Apoptosis **11**: 1825-1835, 2006.

6. Rosenthal GJ, Simeonova P, Corsini E. Asbestos toxicity: an immunologic perspective. Rev Environ Health **14**: 11-20, 1999.

7. Lavska S, Jahnova E, Tulinska J, et al. Immunological monitoring in workers occupationally exposed to asbestos. Toxicol **206**: 299-308, 2005.

8. Hussain R, Poindexter RW, Ottesen EA. Control of allergic reac-

tivity in human filariasis: predominant localization of blocking antibody to the IgG4 subclass. J Immunol **148**: 2731-2737, 1992.
9. Uibu T, Oksa P, Auvinen A, et al. Asbestos exposure as a risk factor for retroperitoneal fibrosis. Lancet **363**: 1422-1426, 2004.
10. Boulard JC, Hanslik T, Doleris LM, Prinseau J, Baglin A. Asbestosis and idiopathic retroperitoneal fibrosis. Lancet **345**: 1379, 1995.
11. Sauni R, Oksa P, Larvenpaa R, Parker JE, Roto P. Asbestos exposure: a potential cause of retroperitoneal fibrosis. Am J Ind Med **33**: 418-421, 1998.
12. Neild GH, Rodriguez-Justo M, Wall C, Connolly JO. Hyper-IgG4 diseases: report and characterization of a new disease. BMC Med **4**: 23, 2006.
13. Roggin KK, Rudloff U, Klimstra DS, Russell LA, Blumgart LH. Adult-onset asthma and periocular xanthogranulomas in a patient with lymphoplasmacytic sclerosing pancreatitis. Pancreas **34**: 157-160, 2007.
14. Nakanuma Y, Zen Y. Etiopathogenesis of IgG4-related sclerosing disease. Byouri to Rinsho (Pathology and Clinical Medicine) **27**: 17-24, 2009 (in Japanese).
15. Zen Y, Inoue D, Kitao A, et al. IgG4-related lung and pleural disease: a clinicopathologic study of 21 cases. Am J Surg Pathol **33**: 1886-1893, 2009.

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SHORT REPORT Open Access

Identification of 5 novel germline *APC* mutations and characterization of clinical phenotypes in Japanese patients with classical and attenuated familial adenomatous polyposis

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Abstract

Background: Familial adenomatous polyposis (FAP) is an autosomal dominant hereditary disease characterized by multiple colorectal adenomatous polyps and frequent extracolonic manifestations. An attenuated form of FAP (AFAP) is diagnosed based on a milder colorectal phenotype, and the colorectal phenotype of (A)FAP has been linked to germline *APC* mutations. The relationships between the spectrum of mutations and extracolonic manifestations are quite well known, but they need to be further defined.

Findings: Nine germline *APC* mutations, but no large deletions, were identified in the *APC* locus of 8 (A)FAP patients, and 5 of the mutations, c.446A > T (p.Asp149Val), c.448A > T (p.Lys150X), c.454_457insAGAA (p. Glu152ArgfsX17), c.497insA (p.Thr166AsnfsX2), and c.1958G > C (p.Arg653Ser), were novel mutations. In one patient the p.Asp149Val mutation and p.Lys150X mutation were detected in the same *APC* allele. The c.1958G > C mutation was located in the last nucleotide of exon 14, and RT-PCR analysis revealed that the mutation resulted in abnormal splicing. The above findings meant that a nonsense mutation, a frameshift mutation, or an exonic mutation leading to abnormal splicing was found in every patient. The following phenotypes, especially extracolonic manifestations, were observed in our (A)FAP patients: (1) multiple gastroduodenal adenomas and early-onset gastric carcinoma in AFAP patients with an exon 4 mutation; (2) a desmoid tumor in two FAP patients with a germline *APC* mutation outside the region between codons 1403 and 1578, which was previously reported to be associated with the development of desmoid tumors in FAP patients; (3) multiple myeloma in an AFAP patient with an exon 4 mutation.

Conclusions: Nine germline *APC* mutations, 5 of them were novel, were identified in 8 Japanese (A)FAP patients, and some associations between germline *APC* mutations and extracolonic manifestations were demonstrated. These findings should contribute to establishing relationships between germline *APC* mutations and the extracolonic manifestations of (A)FAP patients in the future.

Background

Familial adenomatous polyposis (FAP) is an autosomal dominant familial cancer syndrome characterized by the early onset of large numbers of adenomatous polyps throughout the entire colon and a nearly 100% lifetime risk of colorectal cancer (CRC) if the colon is not removed [1]. A small proportion of familial colorectal polyposis cases were recently found to be associated with biallelic germline mutations of the *MutYH* gene [2]. However most FAP cases are caused by germline mutations of the tumor suppressor gene *adenomatous polyposis coli* (*APC*), which encodes a 2843-amino-acid protein that contains a variety of functional domains involved in cell cycle control, differentiation, transcription, migration, and apoptosis [3]. More than 1000 pathogenetic mutations have been detected throughout

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