39. Varlotto JM, Recht A, Flickinger JC, Medford-Davis LN, Dyer AM, Decamp MM. Factors associated with local and distant recurrence and survival in patients with resected nonsmall cell lung cancer. *Cancer* 2009;115:1059–69.
40. Yokoyama A, Kondo K, Nakajima M, Matsushima T, Takahashi T, Nishimura M, Bando M, Sugiyama Y, Totani Y, Ishizaki T, Ichiyasu H, Suga M, et al. Prognostic value of circulating KL-6 in idiopathic pulmonary fibrosis. *Respirology* 2006;11: 164–8.
41. Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer* 2004;4: 45–60.
42. Tsutsumida H, Goto M, Kitajima S, Kubota I, Hirotsu Y, Yonezawa S. Combined status of MUC1 mucin and surfactant apoprotein A expression can predict the outcome of patients with small-size lung adenocarcinoma. *Histopathology* 2004;44:147–55.
43. Ohgami A, Tsuda T, Osaki T, Mitsudomi T, Morimoto Y, Higashi T, Yasumoto K. MUC1 mucin mRNA expression in stage I lung adenocarcinoma and its association with early recurrence. *Ann Thorac Surg* 1999;67:810–4.

Original Paper

Pathobiology

Pathobiology 2012;79:24–33
DOI: 10.1159/000331230

Received: April 5, 2011
Accepted after revision: July 26, 2011
Published online: January 12, 2012

Interferon (Alpha, Beta and Omega) Receptor 2 Is a Prognostic Biomarker for Lung Cancer

Sonosuke Tanaka[a] Noboru Hattori[a] Nobuhisa Ishikawa[a] Yasushi Horimasu[a] Naoko Deguchi[a] Atsushi Takano[a] Yoshitaka Tomoda[a] Koji Yoshioka[a] Kazunori Fujitaka[a] Koji Arihiro[b] Morihito Okada[c] Akihito Yokoyama[d] Nobuoki Kohno[a]

Departments of [a]Molecular and Internal Medicine, [b]Anatomical Pathology and [c]Surgical Oncology, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, and [d]Department of Hematology and Respiratory Medicine, Kochi Medical School, Kochi University, Nankoku City, Japan

Key Words
Interferon (alpha, beta and omega) receptor 2 · Cell surface antigen · Biomarker · Lung cancer

Abstract
Objectives: It has been reported that the type I interferon receptor subunit, interferon (alpha, beta and omega) receptor 2 (IFNAR2), is overexpressed in several malignancies, primarily adenocarcinomas (ADCs); however, the biological significance of IFNAR2 in human lung cancer has not yet been studied. ***Methods:*** Immunohistochemical analysis of 113 surgically resected lung specimens was performed, and the results were evaluated in association with clinical variables, including survival. Serum concentrations of IFNAR2 were also determined by an enzyme-linked immunosorbent assay in 157 lung cancer patients and 164 healthy volunteers. ***Results:*** IFNAR2 overexpression was observed in all histological types of lung cancer examined. Furthermore, strong IFNAR2 expression was associated with shorter progression-free survival (PFS) and overall survival (OS) ($p < 0.0001$ and $p = 0.0110$, respectively) in non-small cell lung cancer patients. Multivariate analyses confirmed its independent prognostic value for PFS and OS ($p < 0.0001$ and $p = 0.0222$, respectively). IFNAR2 serum levels were also significantly higher in lung cancer patients than in healthy volunteers ($p < 0.0001$). ***Conclusions:*** IFNAR2 overexpression was observed in various histological types of lung cancer, and appears to be associated with lung cancers that behave aggressively. The results of this study strongly support the potential of IFNAR2 to be a prognostic biomarker for lung cancer.

Introduction

Lung cancer is the leading cause of cancer death in the world. Among various histological types of lung cancer, approximately 80% of the cases are non-small cell lung cancer (NSCLC), whereas small cell lung cancer (SCLC) accounts for approximately 15% [1]. Regardless of histological subtype, the 5-year survival rate of lung cancer patients is around 10–15% [2]. About 30% of NSCLC patients can undergo curative resection, and the remaining patients are mainly treated with chemotherapy alone or in combination with radiation therapy. Novel therapeutic

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–2008/12/0791–0024$38.00/0

Accessible online at:
www.karger.com/pat

Noboru Hattori, MD, PhD
Department of Molecular and Internal Medicine
Graduate School of Biomedical Sciences, Hiroshima University
1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551 (Japan)
Tel. +81 82 257 5196, E-Mail nhattori@hiroshima-u.ac.jp

strategies based on the biological characteristics of tumor cells in addition to TNM staging are expected to improve survival in lung cancer patients [3, 4].

Because cell surface proteins are considered more accessible to host immune response and drug intervention, the identification of cancer-specific cell surface and/or secretory proteins is likely to be an effective approach for discovering novel diagnostic markers and developing therapeutic strategies [5]. Furthermore, if cancer-specific proteins are in patient sera, they become easily accessible molecular markers. We and other researchers have been screening for biomarkers, mainly concentrating on cell surface and/or secretory proteins that are upregulated in lung cancer. In the process, we have identified various diagnostic and/or prognostic biomarkers for lung cancer [5–9].

The type I interferons (IFNs), which have many biological functions, are mediated by the type I IFN receptor [10–13]. This cell surface receptor is composed of two functional transmembrane proteins, IFN [alpha, beta and omega receptor 1 (IFNAR1)] and IFNAR2. IFNAR2 is the major binding receptor and IFNAR1 is necessary for tight binding. Overexpression of IFNAR2 has been reported in several types of malignancies, primarily adenocarcinomas (ADCs) [14–25], but the biological significance of IFNAR2 in human lung cancer has not yet been studied.

We hypothesized that IFNAR2 overexpression in lung cancer might be associated with malignant potential. IFNAR2 expression was examined in 113 human lung cancer tissues and the results were evaluated in association with clinical variables, including survival. IFNAR2 serum levels were determined in samples from 157 lung cancer patients and 164 healthy volunteers, and the results were also evaluated in association with clinical variables.

Material and Methods

Clinical Tissue Samples

In this study, lung cancer tissue samples were obtained from 113 patients with NSCLC who underwent curative surgery at Hiroshima University (Hiroshima, Japan) between 2000 and 2008. Patient inclusion criteria were similar to those described in previous studies [7–9]. In brief, they were as follows: (1) age ≥20 years, (2) newly diagnosed, untreated NSCLC, (3) patients undergoing curative surgery, (4) no significant abnormalities in liver or kidney function and (5) absence of hepatitis C virus infection. The final diagnosis of NSCLC was made histologically on surgically excised tissues using WHO criteria [26]. Postsurgical pathologic TNM staging was performed according to the guidelines of the American Joint Committee on Cancer [27]. For postoperative adjuvant chemotherapy, oral administration of tegafur and uracil was chosen for patients with pathologic stage IB ADC, and platinum-based chemotherapy was chosen for patients with histologically confirmed lymph node metastases. Written informed patient consent and approval by the research ethics committees from participating hospitals were obtained for this study.

Serum Samples and Patient Characteristics

Serum samples from 157 lung cancer patients and 164 healthy volunteers admitted to Hiroshima University Hospital and its affiliated hospitals between 2000 and 2008 were obtained at the time of diagnosis and stored at –80°C. Disease staging in all 157 cases included computed tomography scans of the chest and abdomen, bone scintigraphy, or ^{18}F fluorodeoxyglucose positron emission tomography and magnetic resonance imaging of the head. The healthy volunteers had no abnormalities in routine laboratory tests and examinations, which included complete blood cell counts, C-reactive protein level, erythrocyte sedimentation rate, liver function tests, renal function tests, urinalysis, fecal examination, chest X-rays and an electrocardiogram.

Immunohistochemistry

Immunohistochemical analysis of IFNAR2 expression was performed on tissue sections from paraffin blocks of primary lung tumors and normal human tissues as previously described [28–32]. Antigens were retrieved by heating the sections in Target Retrieval Solution, Citrate pH 6 (DAKO Japan, Tokyo, Japan). ENVISION+ kits/horseradish peroxidase (DAKO Japan) were used for immunostaining according to the manufacturer's instructions. Mouse monoclonal anti-IFNAR2 antibodies (kindly provided by Otsuka Pharmaceuticals, Tokushima, Japan) or mouse isotype control (Zymed Laboratories, San Francisco, Calif., USA) were added to each slide after endogenous peroxidase and proteins had been blocked. The sections were then incubated with HRP-labeled anti-mouse IgG (DAKO Japan) followed by the reaction with a DAB substrate-chromogen solution (DAKO Japan) for 10 min, and were counterstained with hematoxylin. Three independent investigators assessed IFNAR2 staining patterns without prior knowledge of the clinicopathological data. The expression of IFNAR2 in cancer cells was certified when the staining of cytoplasm and/or plasma membrane was observed. When less than 10% of the cancer cells were stained, the expression of IFNAR2 was considered to be absent (score 0). When 10–75% and more than 75% of cancer cells were stained, the expression of IFNAR2 was recognized as weakly positive (score 1+) and strongly positive (score 2+), respectively. The unmatched or equivocal cases were reexamined, and the consensus category was decided by the three investigators.

ELISA

Serum levels of IFNAR2 were measured by a sandwich ELISA from a kit kindly provided by Otsuka Pharmaceuticals [24, 25, 33].

Statistical Analysis

The data were analyzed using a statistical software package (SPSS for Windows version 15.0, SPSS Inc., Chicago, Ill., USA). Contingency tables were used to analyze the relationship between IFNAR2 expression levels and clinicopathological variables. Progression-free survival (PFS) was defined as the interval starting from the date of the operation or the blood sampling and ending

at the date of documented disease progression, death from any cause or the last follow-up. Overall survival (OS) was defined as the interval starting from the date of the operation or the blood sampling and ending at the date of death from any cause or the last follow-up. Survival data were analyzed, 95% confidence intervals (CIs) were estimated by the Kaplan-Meier method and differences between groups were compared by a log-rank test. Risk factors associated with patient prognosis were evaluated using the Cox proportional hazards regression model with a stepdown procedure. Univariate and multivariate analyses were performed with the Cox proportional hazards regression model to determine associations between clinicopathological variables and cancer-related mortality. First, the associations between death and possible prognostic factors, including age, gender, histological type, pT and pN classification, were analyzed, taking into consideration one factor at a time. Second, multivariate Cox analysis was applied in backward stepwise procedures that forced IFNAR2 expression into the model, along with any and all variables that satisfied an entry level of $p < 0.05$. As factors were added to the model, independent factors did not exceed an exit level of $p < 0.05$.

Results

IFNAR2 Expression in Lung Cancer Tissues and Its Association with Clinical Characteristics

To determine whether IFNAR2 is overexpressed in NSCLC tissues and whether IFNAR2 expression is associated with clinical characteristics in NSCLC patients, an immunohistochemical analysis of IFNAR2 expression was performed on surgically resected tissues from 113 NSCLC cases. As shown in figure 1a, the strong staining in the cytoplasm and the weak staining in the plasma membrane were observed in cancer cells. In addition, a weak expression of IFNAR2 was also detected in both the lymphocytes and stromal cells around the cancer tissue (fig. 1b). There were 21 (18.6%), 40 (35.4%) and 52 (46.0%) cases scored as 0, 1 and 2, respectively (table 1). Positive staining (scored as 1 and 2) was observed in 74.7% (62 of 83) of ADC cases examined, 100% (21 of 21) of squamous cell carcinomas (SCCs), 100% (1 of 1) of large-cell carcinomas and 100% (9 of 9) of adenosquamous cell carcinomas, while no staining was observed in any of the normal epithelial portions of the same tissues. The association between IFNAR2 status and clinicopathological variables was evaluated. Histological type (other histology vs. ADC; $p = 0.0027$ by the Fisher exact test) and pT classification (pT1 vs. pT2 + 3; $p = 0.0245$) were significantly associated with IFNAR2 status.

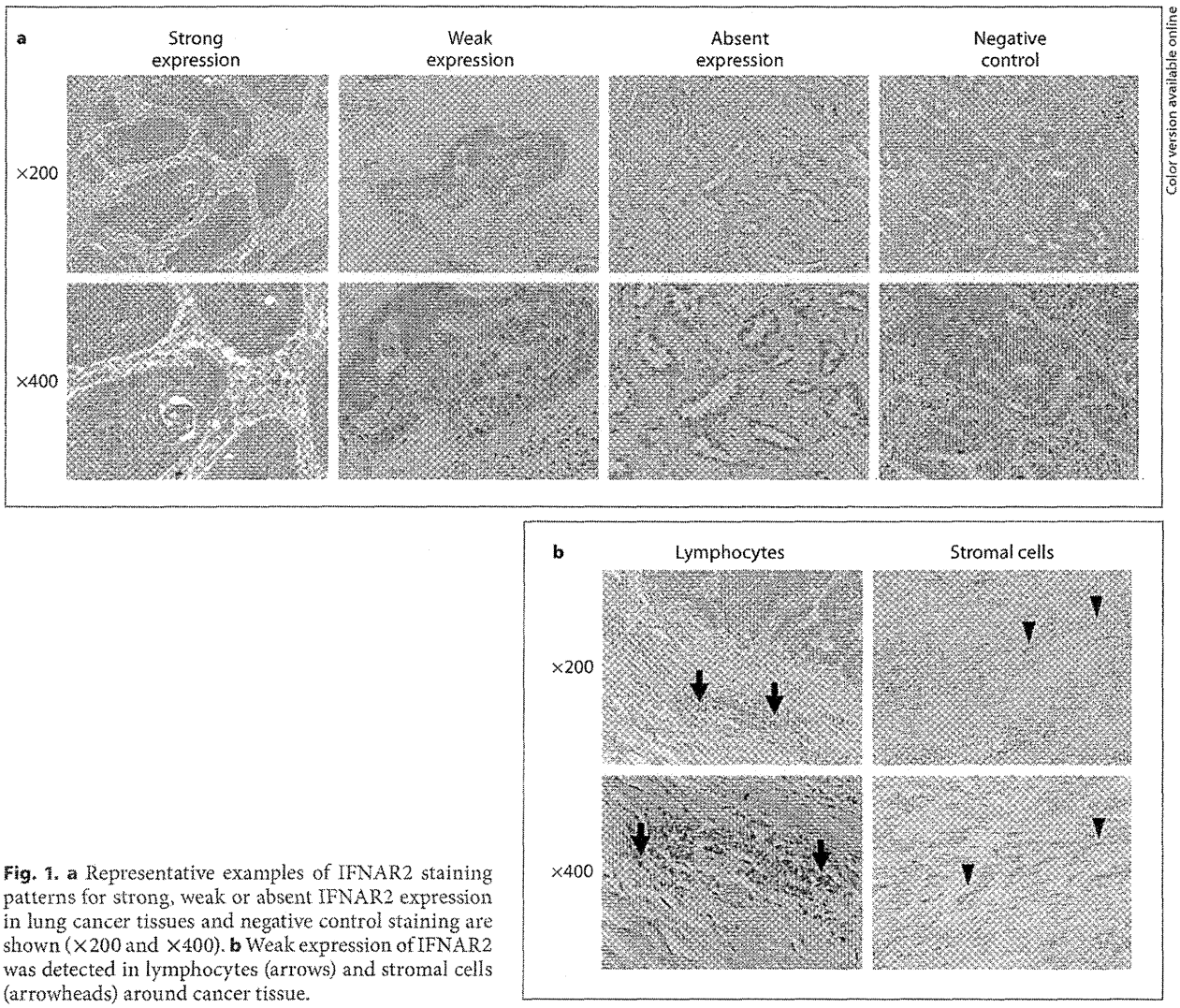
Table 1. Association between characteristics and IFNAR2 positivity in patients with surgically resected NSCLC

	Patients (total) n = 113	IFNAR strongly positive n = 52	IFNAR weakly positive n = 40	IFNAR absent n = 21	p value (strong vs. weak/absent)
Gender					
Male	74	37	27	10	0.3211
Female	39	15	13	11	
Age					
<65 years	49	22	18	9	0.8513
≥65 years	64	30	22	12	
Histological type					
ADC	83	31	31	21	0.0027[a, *]
SCC	20	13	7	0	
Others	10	8	2	0	
pT factor					
T1	61	22	22	17	0.0245*
T2 + 3	52	30	18	4	
pN factor					
N0 + 1	102	46	37	19	0.7518
N2	11	6	3	2	
Smoking history					
Never	38	16	13	9	0.6898[b]
Former	34	16	11	7	
Current	41	20	16	5	

* $p < 0.05$ (by Fisher exact test). Others = Large-cell carcinoma plus adenosquamous cell carcinoma; NS = no significance.
[a] ADC versus other histology.
[b] Never versus others.

Association of Strong IFNAR2 Expression with Poor Clinical Outcomes for Patients with NSCLC

The PFS and OS of patients with strong INFNAR2 expression were significantly poorer than seen in the cases with absent/weak IFNAR2 expression ($p < 0.0001$ and $p = 0.0110$, respectively, by a log-rank test) (fig. 2a, b). Therefore, in order to determine the prognostic importance of the clinical characteristics and IFNAR2 expression status, a Cox proportional hazards regression analysis of the parameters listed in tables 2 and 3 was performed. Univariate analyses revealed that strong IFNAR2 expression (odds ratio 3.933, 95% CI 1.937–7.986, $p = 0.0002$), histological type (odds ratio 1.434, 95% CI 1.028–2.000, $p = 0.0339$), pT stage (odds ratio 2.271, 95% CI 1.166–4.421, $p = 0.0158$) and pN stage (odds ratio 9.140, 95% CI 4.111–20.319, $p < 0.0001$) were significant prognostic factors for PFS. Strong IFNAR2 expression (odds ratio 3.286, 95% CI 1.244–8.679, $p = 0.0164$), histological type (odds ratio 1.589, 95% CI 1.007–2.509, $p = 0.0466$) and pN stage (odds ratio 11.743, 95% CI 3.963–34.791, $p < 0.0001$) were significant prognostic factors for OS (table 4). A multivariate analysis for PFS in NSCLC patients



Color version available online

Fig. 1. a Representative examples of IFNAR2 staining patterns for strong, weak or absent IFNAR2 expression in lung cancer tissues and negative control staining are shown (×200 and ×400). **b** Weak expression of IFNAR2 was detected in lymphocytes (arrows) and stromal cells (arrowheads) around cancer tissue.

demonstrated that strong IFNAR2 expression (odds ratio 4.659, 95% CI 2.208–9.830, $p < 0.0001$) and pN stage (odds ratio 11.800, 95% CI 4.701–29.623, $p < 0.0001$) were independent prognostic factors. Another multivariate analysis revealed that strong IFNAR2 expression (odds ratio 3.143, 95% CI 1.178–8.387, $p = 0.0222$) and pN stage (odds ratio 12.357, 95% CI 4.134–36.933, $p < 0.0001$) were independent prognostic factors for OS in these patients.

To further evaluate the prognostic value of IFNAR2 expression in cancer tissue, we conducted subgroup analyses based on tumor stage and histological type. As shown in figure 2c and d, strong IFNAR2 expression was found to associate with shorter PFS than absent/weak IFNAR2 expression regardless of the tumor stage (stage IA $p = 0.003$, stage IB–IIIA $p = 0.017$, respectively). Strong IFNAR2 expression was also associated with shorter PFS than absent/weak IFNAR2 expression in the patients with ADC ($p < 0.0001$) but not in the patients with non-ADC (fig. 2e, f).

Serum Levels of IFNAR2 in Patients with Lung Cancer

Whether or not IFNAR2 was secreted into the sera of patients with lung cancer was examined by IFNAR2 ELISAs of samples from 157 patients and 164 healthy volunteers (fig. 3a); the mean serum level of IFNAR2 in the

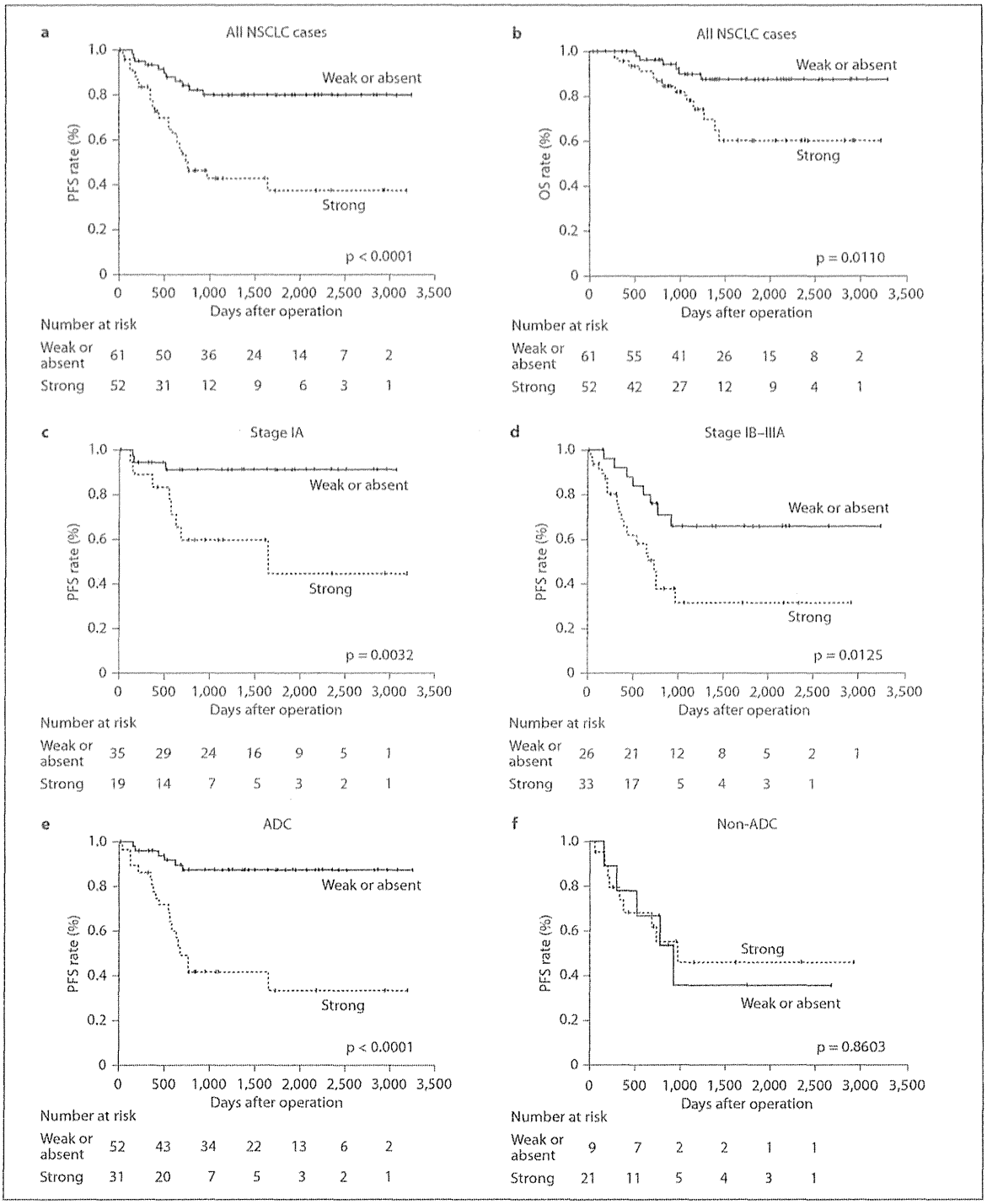


Fig. 2. Association between IFNAR2 expression in cancer tissue and prognosis in surgically resected NSCLC patients. PFS (**a**) and OS (**b**) in the 113 NSCLC patients, and PFS analyzed in the subgroup restricted to the patients with stage IA (**c**), stage IB–IIIA (**d**), ADC (**e**), or non-ADC (**f**) are shown. Differences between the 2 groups were evaluated using a log-rank test. Number at risk is the number of patients whose follow-up continued at each time point.

Table 2. Cox proportional hazards model analysis of PFS in patients with surgically resected NSCLC

Variables	Hazard ratio	95% CI	Unfavorable/favorable	p value
Univariate analysis				
IFNAR2	3.933	1.937–7.986	strong/weak or absent	0.0002*
Age (years)	0.986	0.514–1.890	≥65/<65	0.9652
Gender	1.095	0.776–1.545	male/female	0.6069
Histological type	1.434	1.028–2.000	others/ADC	0.0339*
pT factor	2.271	1.166–4.421	pT2 + 3/T1	0.0158*
pN factor	9.140	4.111–20.319	pN2/N0 + 1	<0.0001*
Smoking history	1.060	0.751–1.495	smoker/never-smoker	0.7418
Multivariate analysis				
IFNAR2	4.659	2.208–9.830	strong/weak or absent	<0.0001*
Histological type	1.045	0.741–1.473	others/ADC	0.8025
pT factor	1.360	0.660–2.804	pT2 + 3/T1	0.4051
pN factor	11.800	4.701–29.623	pN2/N0 + 1	<0.0001*

* $p < 0.05$.

Table 3. Cox proportional hazards model analysis of OS in patients with surgically resected NSCLC

Variables	Hazard ratio	95% CI	Unfavorable/favorable	p value
Univariate analysis				
IFNAR2	3.286	1.244–8.679	strong/weak or absent	0.0164*
Age (years)	1.473	0.579–3.747	≥65/<65	0.4165
Gender	1.173	0.722–1.905	male/female	0.5194
Histological type	1.589	1.007–2.509	others/ADC	0.0466*
pT factor	1.891	0.757–4.722	pT2 + 3/T1	0.1723
pN factor	11.743	3.963–34.791	pN2/N0 + 1	<0.0001*
Smoking history	1.125	0.693–1.826	smoker/never-smoker	0.6348
Multivariate analysis				
IFNAR2	3.143	1.178–8.387	strong/weak or absent	0.0222*
Histological type	1.366	0.861–2.168	others/ADC	0.1854
pN factor	12.357	4.134–36.933	pN2/N0 + 1	<0.0001*

* $p < 0.05$.

patients was 1.55 ± 0.72 ng/ml (mean ± SD) and in healthy volunteers 1.09 ± 0.44 ng/ml ($p < 0.0001$, by the Mann-Whitney U test). When classified according to histological type, the serum levels of IFNAR2 were 1.54 ± 0.70 ng/ml in ADC patients, 1.54 ± 0.79 ng/ml in SCC patients and 1.59 ± 0.71 ng/ml in SCLC patients (fig. 3a). Differences were significant between ADC patients and healthy volunteers ($p < 0.0001$, Mann-Whitney U test), between SCC patients and healthy volunteers ($p = 0.0025$), and between SCLC patients and volunteers ($p = 0.0009$).

Receiver-operating characteristic curve analysis of the IFNAR2 data from these 157 lung cancer patients and 164 healthy volunteers was performed to determine the ELISA cutoff level that provided optimal diagnostic accuracy and likelihood ratios (minimal false-negative and false-positive results). A value of 1.81 ng/ml had a sensitivity of 25.4% (40 of 157 lung cancer patients) and a specificity of 93.3% (11 of 164 healthy volunteers). To evaluate the potential of serum IFNAR2 as a cancer-specific biomarker, the relationships between serum IFNAR2 positivity and patient characteristics were examined. As shown in table 4, serum IFNAR2 positivity was significantly associated with age and distant organ metastases in lung cancer patients ($p = 0.0296$ and $p = 0.0107$, respectively, by the Fisher exact test).

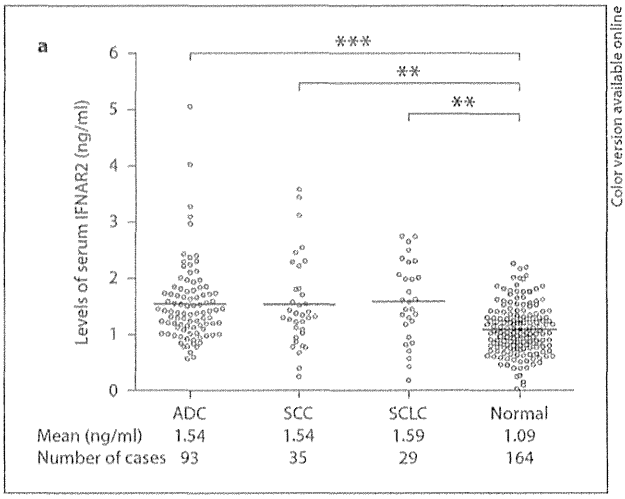
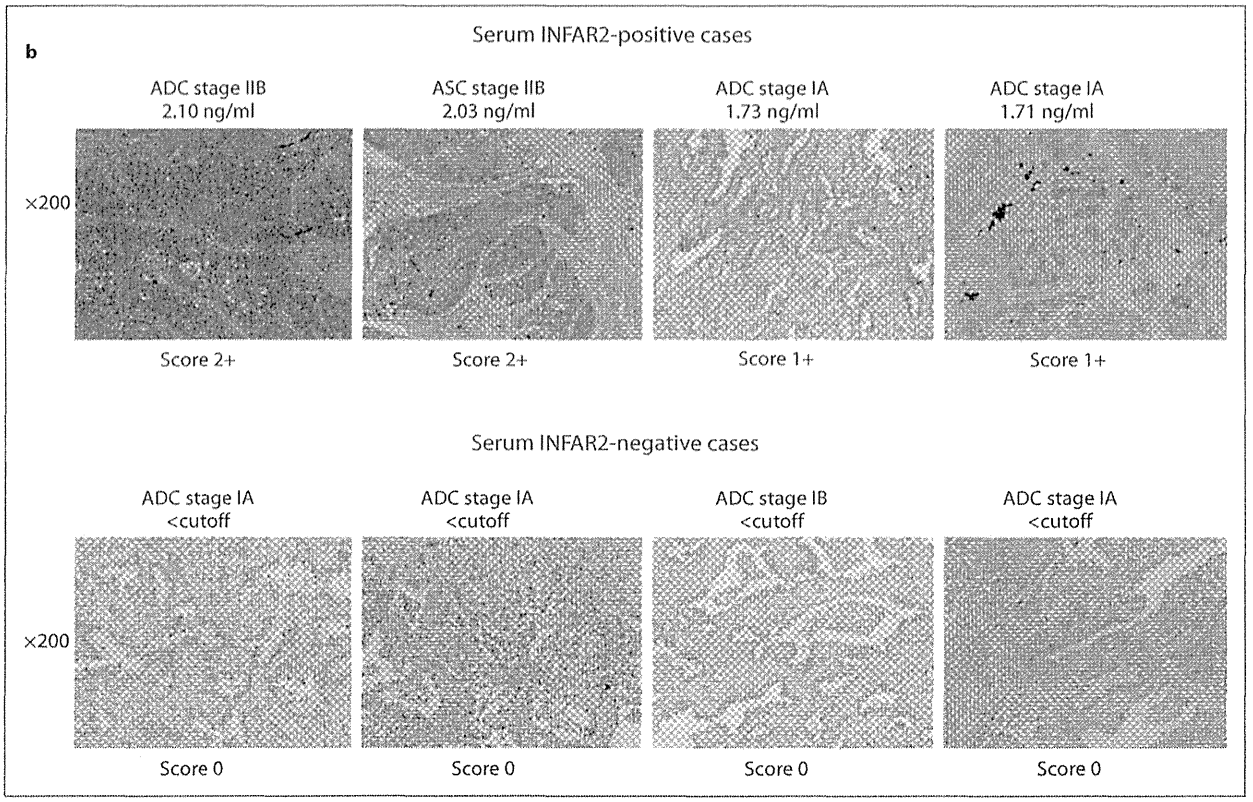


Fig. 3. a Serum levels of IFNAR2 were determined by ELISA in lung cancer patients and in healthy volunteers (normal). Distribution of IFNAR2 in sera from patients with lung ADC, SCC and SCLC. Averaged serum levels are shown as black lines. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (vs. normal volunteers, Mann-Whitney U test). **b** Comparison between the serum IFNAR2 level (ng/ml) and the expression of IFNAR2 in the tumor tissues from patients with ADC and adenosquamous cell carcinoma (ASC).



To determine whether there was a relationship between the serum and expression levels of IFNAR2 in a tumor, the two levels were compared in 8 NSCLC patients whose sera had been collected before surgery (4 patients with IFNAR2-positive tumors and 4 with IFNAR2-negative tumors). Although the analysis was performed in a limited number of patients, the serum levels of IFNAR2 showed a trend of correlating with the expression levels of IFNAR2 in the tumors (fig. 3b).

Table 4. Association between serum IFNAR2 levels and clinical characteristics in patients with NSCLC

Variables	Patients (total) n = 157	Circulating IFNAR2, pg/ml		p value
		high level (≥1.81 ng/ml) n = 41	low level (<1.81 ng/ml) n = 116	
Age				0.0296*
<65 years	69	12	57	
≥65 years	88	29	59	
Gender				0.8456
Male	107	29	78	
Female	50	12	38	
Histological type				0.2683[a]
ADC	93	21	72	
SCC	35	9	26	
SCLC	29	11	18	
pT factor				0.3627
T1 + 2	84	19	65	
T3 + 4	73	22	51	
pN factor				0.9999
N0 + 1	63	16	47	
N2 + 3	94	25	69	
pM factor				0.0107*
M0	85	15	70	
M1	72	26	46	
Smoking history				0.4200[b]
Never	43	9	34	
Former	28	8	20	
Current	86	24	34	

* $p < 0.05$ (by Fisher exact test).
[a] ADC versus others.
[b] Never versus others.

Discussion

In this study, we found that IFNAR2 was detectable by immunohistochemistry in all histological types of lung cancer specimens. Strong IFNAR2 expression in lung cancer tissue was shown to correlate with poor prognosis. In addition, serum levels of IFNAR2 were significantly higher in patients with lung cancer than in healthy volunteers. These results support the hypothesis that IFNAR2 can serve as a prognostic biomarker in lung cancer.

IFNAR2 is a type I membrane protein that forms one of the two chains of the receptor for alpha and beta interferons. When IFN binds to the receptors, IFNAR-associated JAK tyrosine kinases are activated, followed by the phosphorylation of signal transducer and activation of transcription factors (STATs) 1, 2 and 3. Recent studies have revealed that mutant epidermal growth factor receptor proteins selectively activate Akt and STAT pathways, which are important in NSCLC cell survival [34]. Furthermore, it was recently reported that nearly 50% of early-stage NSCLCs exhibit phosphorylated STAT3 expression, which approximates the percentage of patients experiencing clinical benefit from epidermal growth factor receptor tyrosine kinase inhibitors [35]. Considered along with these results, JAK-STAT pathways that are activated via IFNAR2 may play an important role in the pathogenesis of lung cancer.

Expression of IFNAR2 has been studied in cancers including hepatocellular and renal cell carcinoma, chronic myelogenous leukemia, and pancreatic cancer [14–25]; however, the significance of IFNAR2 expression in the clinical outcomes of these cancers was variable. In addition, the association between the IFNAR2 expression levels and the response to IFN therapy is still controversial [18–21, 24, 33]. In this study, immunohistochemical analysis demonstrated that IFNAR2 expression was higher in all histological types of lung cancer compared with normal tissues. With regard to the localization of IFNAR2 in cancer cells, strong staining in the cytoplasm and weak staining in the plasma membrane were observed. This observation is consistent with the result of a previous study that demonstrated the localization of INFAR2 in the hepatocytes of chronic hepatitis C patients which utilized the same monoclonal antibody as our study [13]. Furthermore, strong IFNAR2 expression in NSCLC tissues was found to correlate with shorter cancer-specific survival than seen in patients with tumors with weak or absent IFNAR2 expression. Even when the survival analysis was restricted to those NSCLC patients with stage IA, strong IFNAR2 expression was found to correlate with shorter PFS than absent/weak IFNAR2 expression. This result suggests that IFNAR2 expression levels in NSCLC tissues may indicate the aggressiveness of disease and be useful for selecting patients who should receive adjuvant chemotherapy. In addition, strong IFNAR2 expression was associated with shorter PFS than absent/weak IFNAR2 expression in the patients with ADC but not in the patients with non-ADC. Taking all these observations into consideration, an association between INFAR2 expression and disease progression in lung cancer, particularly in patients with ADC, is suggested. To our best knowledge, this is the first report indicating that high IFNAR2 expression in human lung cancer has a prognostic value.

Increased serum levels of IFNAR2 have been reported in many malignancies and chronic viral infections, al-

though the biological significance of IFNAR2 in these diseases remains unclear [22–25, 33]. In this study, the serum levels of IFNAR2 in patients with lung cancer were significantly higher than in the healthy volunteers. Although the analysis was performed in a limited number of patients, the IFNAR2 serum levels showed a trend of correlating with the expression levels in the tumor tissues. Furthermore, the serum levels of IFNAR2 in patients with distant organ metastases (stage IV) were significantly higher than in patients with stage I–III disease. These results suggest that IFNAR2 may accelerate metastasis development in patients with lung cancer. However, the sensitivity of IFNAR2 for the detection of lung cancer was lower than that of conventional serum biomarkers, such as carcinoembryonic antigen and the cytokeratin 19 fragment (data not shown). It is possible that the significant difference between lung cancer patients and healthy volunteers was observed because of the extremely high serum levels of IFNAR2 in some of the patients. Furthermore, serum levels of IFNAR2 reflect the presence of inflammation and/or comorbidities. These results suggest that serum IFNAR2 may not be valuable as a diagnostic biomarker in lung cancer.

Inflammation plays an important role in various types of carcinogenesis [36]. IFNs are important inflammatory cytokines and can stimulate the proliferation, migration and differentiation or apoptosis of various cancer cells [37]. In this study, we found that IFNAR2 was also weakly expressed in both lymphocytes and stromal cells around cancer tissue. A similar observation was reported in a previous publication [14] that studied the expression of IFNAR2 in a large series of liver tissues obtained from patients with chronic hepatitis C. These results suggest that IFNAR2 expression might be upregulated at the site of inflammation. Additional studies investigating the signaling pathways in cancer cells mediated by IFNAR2 should provide important information on the association between inflammation and carcinogenesis.

Although promising results were obtained, we are aware that this study has a number of limitations. First, we could not evaluate the association between IFNAR2 expression and inflammatory markers such as high-sensitivity C-reactive protein. Second, only Asian patients were studied. Considering that ethnicity has an effect on lung cancer treatment efficacy, we should carefully interpret the results when this monitoring system is applied to non-Asian lung cancer patients. Third, the association between IFNAR2 and JAK-STAT pathway (including pAkt or pSTAT) expression status was not evaluated. To confirm the linkage of IFNAR2 expression to this signal transduction pathway in cancer cells, another immunohistochemical study would be required. In addition, large validation studies investigating the association between IFNAR2 expression patterns in tumors and circulating IFNAR2 levels are also needed to confirm the usefulness of IFNAR2 as a prognostic biomarker.

In conclusion, we observed an elevated expression of IFNAR2 in lung cancer compared with normal tissues, and our results indicated that this was associated with aggressive lung cancer characteristics. The data reported here strongly support the potential of IFNAR2 to be a diagnostic and prognostic marker for lung cancer.

Acknowledgements

We thank Ryohei Nishino, Shigeo Kawase, Ken Masuda and Shin Akita for their support. This work was partly supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1 Jemal A, Siegel R, Xu J, Ward E: Cancer statistics, 2010. CA Cancer J Clin 2010;60:277–300.
2 Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA: Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. Mayo Clin Proc 2008;83:584–594.
3 Pérez-Soler R: Individualized therapy in non-small-cell lung cancer: future versus current clinical practice. Oncogene 2009; 28:S38–S45.
4 Amelung JT, Bührens R, Beshay M, Reymond MA: Key genes in lung cancer translational research: a meta-analysis. Pathobiology 2010;77:53–63.
5 Daigo Y, Nakamura Y: From cancer genomics to thoracic oncology: discovery of new biomarkers and therapeutic targets for lung and esophageal carcinomas. Gen Thorac Cardiovasc Surg 2008;56:43–53.
6 Kohno N, Akiyama M, Kyoizumi S, Hakoda M, Kobuke K, Yamakido M: Detection of soluble tumor-associated antigens in sera and effusions using novel monoclonal antibodies, KL-3 and KL-6, against lung adenocarcinoma. Jpn J Clin Oncol 1988;18:203–216.
7 Inata J, Hattori N, Yokoyama A, Ohshimo S, Doi M, Ishikawa N, Hamada H, Kohno N: Circulating KL-6/MUC1 mucin carrying sialyl Lewisa oligosaccharide is an independent prognostic factor in patients with lung adenocarcinoma. Int J Cancer 2007;120: 2643–2649.
8 Ishikawa N, Hattori N, Yokoyama A, Tanaka S, Nishino R, Yoshioka K, Ohshimo S, Fujitaka K, Ohnishi H, Hamada H, Arihiro K, Kohno N: Usefulness of monitoring the circulating Krebs von den Lungen-6 levels to predict the clinical outcome of patients with advanced non-small cell lung cancer treated with epidermal growth factor receptor tyrosine kinase inhibitors. Int J Cancer 2008;122: 2612–2620.

9 Tanaka S, Hattori N, Ishikawa N, Shoda H, Takano A, Nishino R, Okada M, Arihiro K, Inai K, Hamada H, Yokoyama A, Kohno N: Krebs von den Lungen-6 (KL-6) is a prognostic biomarker in patients with surgically resected non-small cell lung cancer. Int J Cancer 2011, E-pub ahead of print.
10 Pitha PM: Introduction: interferon's connection to cancer. Semin Cancer Biol 2000;10:69–72.
11 Borden EC: Review: Milstein Award lecture: interferons and cancer: where from here? J Interferon Cytokine Res 2005;25:511–527.
12 Borden EC, Sen GC, Uze G, Silverman RH, Ransohoff RM, Foster GR, Stark GR: Interferons at age 50: past, current and future impact on biomedicine. Nat Rev Drug Discov 2007;6:975–990.
13 Theofilopoulos AN, Baccala R, Beutler B, Kono DH: Type I interferons (alpha/beta) in immunity and autoimmunity. Annu Rev Immunol 2005;23:307–336.
14 Yatsuhashi H, Fujino T, Matsumoto T, Inoue O, Koga M, Yano M: Immunohistochemical analysis of hepatic interferon alpha-beta receptor level: relationship between receptor expression and response to interferon therapy in patients with chronic hepatitis C. J Hepatol 1999;30:995–1003.
15 Fujiwara D, Hino K, Yamaguchi Y, Ren F, Satoh Y, Korenaga M, Okuda M, Okita K: Hepatic expression of type I interferon receptor for predicting response to interferon therapy in chronic hepatitis C patients: a comparison of immunohistochemical method versus competitive polymerase chain reaction assay. Hepatol Res 2003;25:377–384.
16 Takemoto Y, Yano H, Momosaki S, Ogasawara S, Nishida N, Kojiro S, Kamura T, Kojiro M: Antiproliferative effects of interferon-alphaCon1 on ovarian clear cell adenocarcinoma in vitro and in vivo. Clin Cancer Res 2004;10:7418–7426.
17 Eguchi H, Nagano H, Yamamoto H, Miyamoto A, Kondo M, Dono K, Nakamori S, Umeshita K, Sakon M, Monden M: Augmentation of antitumor activity of 5-fluorouracil by interferon alpha is associated with upregulation of p27Kip1 in human hepatocellular carcinoma cells. Clin Cancer Res 2000;6:2881–2890.
18 Kondo M, Nagano H, Wada H, Damdinsuren B, Yamamoto H, Hiraoka N, Eguchi H, Miyamoto A, Yamamoto T, Ota H, Nakamura M, Marubashi S, Dono K, Umeshita K, Nakamori S, Sakon M, Monden M: Combination of IFN-alpha and 5-fluorouracil induces apoptosis through IFN-alpha/beta receptor in human hepatocellular carcinoma cells. Clin Cancer Res 2005;11:1277–1286.
19 Ota H, Nagano H, Sakon M, Eguchi H, Kondo M, Yamamoto T, Nakamura M, Damdinsuren B, Wada H, Marubashi S, Miyamoto A, Dono K, Umeshita K, Nakamori S, Wakasa K, Monden M: Treatment of hepatocellular carcinoma with major portal vein thrombosis by combined therapy with subcutaneous interferon-alpha and intra-arterial 5-fluorouracil; role of type 1 interferon receptor expression. Br J Cancer 2005;93:557–564.
20 Ota H, Nagano H, Doki Y, Sekimoto M, Kondo M, Wada H, Nakamura M, Noda T, Damdinsuren B, Marubashi S, Miyamoto A, Takeda Y, Dono K, Umeshita K, Nakamori S, Wakasa K, Sakon M, Monden M: Expression of type I interferon receptor as a predictor of clinical response to interferon-alpha therapy of gastrointestinal cancers. Oncol Rep 2006;16:249–255.
21 Kamai T, Yanai Y, Arai K, Abe H, Yamanishi T, Kurimoto M, Yoshida K: Increased interferon alpha receptor 2 mRNA levels is associated with renal cell carcinoma metastasis. BMC Cancer 2007;7:159.
22 Hardy MP, Owczarek CM, Trajanovska S, Liu X, Kola I, Hertzog PJ: The soluble murine type I interferon receptor Ifnar-2 is present in serum, is independently regulated, and has both agonistic and antagonistic properties. Blood 2001;97:473–482.
23 Ambrus JL Sr, Dembinski W, Ambrus JL Jr, Sykes DE, Akhter S, Kulaylat MN, Islam A, Chadha KC: Free interferon-alpha/beta receptors in the circulation of patients with adenocarcinoma. Cancer 2003;98:2730–2733.
24 Ito K, Tanaka H, Ito T, Sultana TA, Kyo T, Imanaka F, Ohmoto Y, Kimura A: Initial expression of interferon alpha receptor 2 (IFNAR2) on CD34-positive cells and its down-regulation correlate with clinical response to interferon therapy in chronic myelogenous leukemia. Eur J Haematol 2004;73:191–205.
25 Miyake K, Imura S, Nishioka M, Batmunkh E, Sugimoto K, Ohmoto Y, Shimada M: Serum evaluation of soluble interferon-alpha/beta receptor and high-sensitivity C-reactive protein for diagnosis of the patients with gastrointestinal and hepatobiliary-pancreatic cancer. Cytokine 2010;49:251–255.
26 Travis WD, Colby TV, Corrin B, Shimosato Y, Brambilla E: Histological Typing of Lung and Pleural Tumors: World Health Organization International Histological Classification of Tumors, ed 3. Berlin, Springer, 1999.
27 Sobin LH, Wittekind CH (eds): UICC TNM Classification of Malignant Tumors, ed 5. New York, Wiley, 1997.
28 Ishikawa N, Daigo Y, Yasui W, Inai K, Nishimura H, Tsuchiya E, Kohno N, Nakamura Y: ADAM8 as a novel serological and histochemical marker for lung cancer. Clin Cancer Res 2004;10:8638–8670.
29 Ishikawa N, Daigo Y, Takano A, Taniwaki M, Kato T, Hayama S, Murakami H, Takeshima Y, Inai K, Nishimura H, Tsuchiya E, Kohno N, Nakamura Y: Increases of amphiregulin and transforming growth factor-alpha in serum as predictors of poor response to gefitinib among patients with advanced non-small cell lung cancers. Cancer Res 2005;65:9176–9184.
30 Ishikawa N, Daigo Y, Takano A, Taniwaki M, Kato T, Tanaka S, Yasui W, Takeshima Y, Inai K, Nishimura H, Tsuchiya E, Kohno N, Nakamura Y: Characterization of SEZ6L2 cell-surface protein as a novel histochemical and prognostic marker for lung cancer. Cancer Sci 2006;97:737–745.
31 Ishikawa N, Takano A, Yasui W, Inai K, Nishimura H, Ito H, Miyagi Y, Nakayama H, Fujita M, Hosokawa M, Tsuchiya E, Kohno N, Nakamura Y, Daigo Y: Cancer-testis antigen lymphocyte antigen 6 complex locus K is a serologic biomarker and a therapeutic target for lung and esophageal carcinomas. Cancer Res 2007;67:11601–11611.
32 Takano A, Ishikawa N, Nishino R, Masuda K, Yasui W, Inai K, Nishimura H, Ito H, Nakayama H, Miyagi Y, Tsuchiya E, Kohno N, Nakamura Y, Daigo Y: Identification of nectin-4 oncoprotein as a diagnostic and therapeutic target for lung cancer. Cancer Res 2009;69:6694–6703.
33 Mizukoshi E, Kaneko S, Kaji K, Terasaki S, Matsushita E, Muraguchi M, Ohmoto Y, Kobayashi K: Serum levels of soluble interferon alpha/beta receptor as an inhibitory factor of interferon in the patients with chronic hepatitis C. Hepatology 1999;30:1325–1331.
34 Sordella R, Bell DW, Haber DA, Settleman J: Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science 2004;305:1163–1167.
35 Haura EB, Zheng Z, Song L, Cantor A, Bepler G: Activated epidermal growth factor receptor-Stat-3 signaling promotes tumor survival in vivo in non-small cell lung cancer. Clin Cancer Res 2005;11:8288–8294.
36 Balkwill F, Mantovani A: Inflammation and cancer: back to Virchow? Lancet 2001;357:539–545.
37 Seruga B, Zhang H, Bernstein LJ, Tannock IF: Cytokines and their relationship to the symptoms and outcome of cancer. Nat Rev Cancer 2008;8:887–899.

Contents lists available at SciVerse ScienceDirect

Respiratory Investigation

journal homepage: www.elsevier.com/locate/resinv

Review

Utility of KL-6/MUC1 in the clinical management of interstitial lung diseases

Nobuhisa Ishikawa[a], *Noboru Hattori*[a,*], *Akihito Yokoyama*[b], *Nobuoki Kohno*[a]

[a]*Department of Molecular and Internal Medicine, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan*

[b]*Department of Hematology and Respiratory Medicine, Kochi Medical School, Kochi University, Kohasu Oko-cho, Nankoku-City, Kochi 783-8505, Japan*

ARTICLE INFO

Article history:
Received 27 December 2011
Received in revised form
3 February 2012
Accepted 8 February 2012
Available online 8 March 2012

Keywords:
KL-6
MUC1
Serum biomarker
Interstitial lung disease
Ethnic differences

ABSTRACT

Interstitial lung diseases (ILDs) are a diverse group of pulmonary disorders characterized by various patterns of inflammation and fibrosis in the interstitium of the lung. Because injury and/or regeneration of type II pneumocytes are prominent histological features of ILDs, substances derived from type II pneumocytes have been the focus of research investigating potential biomarkers for ILD. One important biomarker for ILD is the high-molecular-weight glycoprotein, Krebs von den Lungen-6 (KL-6). KL-6 is now classified as a human MUC1 mucin protein, and regenerating type II pneumocytes are the primary cellular source of KL-6/MUC1 in the affected lungs of patients with ILD. KL-6/MUC1 is detectable in the serum of patients with ILD, and extensive investigations performed primarily in Japan have revealed that serum KL-6/MUC1 is elevated in 70–100% of patients with various ILDs, including idiopathic interstitial pneumonias, collagen vascular disease-associated interstitial pneumonia, hypersensitivity pneumonia, radiation pneumonitis, drug-induced ILDs, acute respiratory distress syndrome, pulmonary sarcoidosis, and pulmonary alveolar proteinosis. The results from these various studies have supported the utility of KL-6/MUC1 as a serum biomarker for detecting these various ILDs. Moreover, KL-6/MUC1 serum levels have been demonstrated to be useful for evaluating disease activity and predicting the clinical outcomes of various ILD types. Based on these observations, we believe that KL-6/MUC1 is currently one of the best and most reliable serum biomarkers available for ILD management.

Abbreviations: ILDs, interstitial lung diseases; IIPs, idiopathic interstitial pneumonias; CVD-IP, collagen vascular disease-associated interstitial pneumonia; HP, hypersensitivity pneumonia; RP, radiation pneumonitis; D-ILDs, drug-induced ILDs; ARDS, acute respiratory distress syndrome; IPF, idiopathic pulmonary fibrosis; UIP, usual interstitial pneumonia; NSIP, nonspecific interstitial pneumonia; HRCT, high-resolution computed tomography; SLB, surgical lung biopsy; mAb, monoclonal antibody; KL-6, Krebs von den Lungen-6; CEA, carcinoembryonic antigen; ELISA, enzyme-linked immunosorbent assay; CLIEA, chemiluminescent enzyme immunoassay; VNTR, variable number tandem repeat; TACE, TNF-α converting enzyme; ADAM17, disintegrin and metalloproteinase 17; ELF, epithelial lining fluid; ECM, extracellular matrix; PAP, pulmonary alveolar proteinosis; ROC, receiver operating characteristic; SSc, systemic sclerosis; PM/DM, polymyositis/dermatomyositis; EAA, extrinsic allergic alveolitis; FLD, farmer's lung disease; NSCLC, non-small cell lung cancer; EGFR-TKIs, epidermal growth factor receptor tyrosine kinase inhibitors; SBRT, stereotactic body radiotherapy; DAD, diffuse alveolar damage; CIP, chronic interstitial pneumonia; BALF, bronchoalveolar lavage fluid; ALI, acute lung injury; DIC, disseminated intravascular coagulation; AUC, area under the curve

*Corresponding author. Tel.: +81 82 257 5196; fax: +81 82 255 7360.
E-mail address: nhattori@hiroshima-u.ac.jp (N. Hattori).

2212-5345/$ - see front matter © 2012 The Japanese Respiratory Society. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.resinv.2012.02.001

Contents

1. Introduction

Interstitial lung diseases (ILDs) are a diverse group of pulmonary disorders characterized by various patterns of inflammation and fibrosis in the interstitium of the lung, including idiopathic interstitial pneumonias (IIPs), collagen vascular disease-associated interstitial pneumonia (CVD-IP), hypersensitivity pneumonia (HP), radiation pneumonitis (RP), drug-induced ILDs (D-ILDs), acute respiratory distress syndrome (ARDS), and sarcoidosis [1–4]. Moreover, based on histological features, IIPs have been further classified into several types, including idiopathic pulmonary fibrosis (IPF) with the histopathology of usual interstitial pneumonia (UIP) and nonspecific interstitial pneumonia (NSIP).

High-resolution computed tomography (HRCT), bronchoscopic examination, and/or surgical lung biopsy (SLB) are fundamental steps required to make a definite diagnosis of various ILDs, including IIPs [4–6]. Furthermore, serial lung function testing is generally used to monitor disease activity and/or predict the prognosis in patients with ILDs [7]. However, these examinations require specific medical facilities and may result in considerable discomfort to patients. Thus, the identification of serum biomarkers for ILDs would greatly improve current diagnostic methods. Serum biomarkers offer several advantages over other methods, including being generally easy to perform, inexpensive, reproducible, and less invasive. To date, various serum biomarkers have been tested for their use in ILDs [8–13]. Among these, biomarkers derived from type II pneumocytes have been of particular interest, because ILDs show a common pathophysiological development, i.e., type II pneumocyte injury or remodeling [8]. The most widely used biomarkers for ILDs derived from type II pneumocytes are KL-6 and 2 surfactant proteins, SP-A and SP-D. These 3 biomarkers have been studied independently by 2 Japanese research groups (Hiroshima University and Sapporo Medical University) and are currently in wide clinical use in Japan. Lactate dehydrogenase (LDH) has also been used as a biomarker for ILDs in Japan; however, LDH serum levels are not specific for lung damage and have been superseded by KL-6, SP-A, and SP-D [8]. This review, from the research group that discovered KL-6, discusses the clinical application of KL-6 as one of the most promising serum biomarkers for patients with various types of ILDs.

2. From discovery to clinical application: the novel glycoprotein, KL-6

A murine IgG1 monoclonal antibody (mAb) was developed to recognize a sialylated sugar chain, designated as Krebs von den Lungen-6 (KL-6), by immunizing a mouse with the human lung adenocarcinoma cell line VMRC-LCR [14]. KL-6 was first suggested as a serum tumor biomarker for pulmonary, breast, and pancreatic cancers. However, the diagnostic accuracy of KL-6 as a tumor marker was found to be inferior to that of carcinoembryonic antigen (CEA) based on the high rate of false positive cases in patients with pulmonary fibrosis. Further investigations in our laboratory revealed the possibility of KL-6 as a biomarker for ILDs, because patients with benign noninterstitial lung disease did not show a significant elevation in the serum levels of KL-6 [15]. A cooperative study on KL-6 as a serum biomarker was initiated with the diagnostic division of Eidia Co., Ltd. (Tokyo, Japan) in 1992. The findings of this study led to the development of an enzyme-linked immunosorbent assay (ELISA) that enabled the determination of the absolute amount of KL-6 in samples collected in clinical practice. KL-6 has been approved by Japan's Health Insurance Program as a diagnostic marker for ILDs since 1999, and KL-6 levels are examined in more than 2,000,000 samples per year in Japan. A chemiluminescent enzyme immunoassay (CLEIA) system has now been developed that can measure serum KL-6 levels within 1 h in ordinary Japanese clinical settings. However, the measurement of KL-6 is currently not possible for clinical practices in

most countries. For instance, the KL-6 ELISA kit, available from SCETI Bioscience Export Co., Ltd. (Tokyo, Japan), under contract with Edia Co., Ltd., is available for research purposes only.

3. Biochemical and biological properties of KL-6

As described above, we developed an mAb to recognize an undefined high-molecular-weight (200 kDa) glycoprotein designated KL-6. Using this anti-KL-6 mAb, we purified KL-6 from the culture medium of human breast cancer YMB-S cells. Because sialidase digestion or periodate oxidation of KL-6 reduced the binding of anti-KL-6 mAb to KL-6, the epitope on KL-6 was suggested to be a carbohydrate-containing sialic acid [14]. Subsequently, KL-6 was classified as "Cluster 9 (MUC1)" at the Third World International Workshop of the International Association for the Study of Lung Cancer on lung tumor and differentiation antigens according to the results of immunohistochemical and flow cytometry studies, although the precise epitope structure recognized by the anti-KL-6 mAb was unclear [16,17]. A previous study from our laboratory clearly demonstrated that KL-6 was a submolecule of MUC1 based on the results of a carbohydrate composition analysis [18]. In accordance with these different observations, KL-6/MUC1 is commonly used to denote the KL-6 molecule. Recently, the possible carbohydrate epitopes of the anti-KL-6 mAb have been reported to be novel O-linked glycans containing 6'sulfo-Gal/GalNAc of MUC1 [19].

MUC1 (episialin, polymorphic epithelial mucin) is a large glycoprotein containing 3 domains: (1) a cytoplasmic tail, (2) a single transmembrane region, and (3) an extracellular domain (Fig. 1a). The extracellular region of MUC1 contains sites of O- and N-linked glycosylation and a variable number tandem repeat (VNTR) domain with 20–100 repeats of a 20-amino acid sequence [20,21]. MUC1 has an extended, rigid structure protruding 200–500 nm above the plasma membrane and is found on the apical surface of normal glandular epithelial cells. The MUC1 extracellular domain can be shed into the pulmonary epithelial lining fluid (ELF) through the action of TNF-α converting enzyme (TACE; also called a disintegrin and metalloproteinase 17 [ADAM17]) and potentially ADAM9 [21,22]. In addition, some soluble MUC1 may result from alternative splicing. Transfection studies have revealed that MUC1 reduces cell–cell and cell–extracellular matrix (ECM) interactions, decreases cell aggregation, and prevents E-cadherin-mediated cell–cell adhesion and integrin-mediated cell–ECM adhesion. Previous studies have demonstrated that E-cadherin can be functionally suppressed by MUC1 overexpression [23] and that anti-KL-6/MUC1 mAb induces the capping of MUC1 and facilitates E-cadherin-mediated cell–cell interactions [24].

As described below in this review, the clinical importance of KL-6/MUC1 in the management of ILD has been established. However, very little is known about the pathophysiological role of KL-6/MUC1 in patients with ILDs. Previous studies from our laboratory demonstrated that purified KL-6/MUC1 has chemotactic and anti-apoptotic effects on fibroblasts and that the proliferative and anti-apoptotic effects of KL-6/MUC1 are additive to those of transforming growth factor-β [18,25]. These results support the hypothesis that KL-6/MUC1 is one of the key molecules involved in the intra-alveolar fibrotic process and pulmonary fibrosis. Moreover, these results indicate that KL-6/MUC1 may become a promising molecular target for the treatment of pulmonary fibrosis.

4. Expression of KL-6/MUC1 in tissues

Several studies have evaluated the expression of KL-6/MUC1 using immunohistochemistry (Table 1) [14,15,26]. KL-6/MUC1 is moderately expressed in type II pneumocytes and respiratory bronchiolar epithelial cells and only weakly expressed in basal cells of the terminal bronchiolar epithelium of normal lung tissues. On the other hand, type I pneumocytes, goblet cells, and mucous cells of the bronchial glands do not express KL-6/MUC1. Furthermore, KL-6/MUC1 is not expressed by the epithelial cells of the stomach, small intestine, or large intestine, with the exception of the fundic gland cells in the stomach. In addition to strong expression in lung, pancreatic, and breast cancer tissues, KL-6/MUC1 is strongly expressed by atypical and/or regenerating type II pneumocytes in tissue sections obtained from patients with ILDs [14,27,28]. Ohtsuki et al. reported linear and continuous staining for KL-6/MUC1 on the cell surface of regenerating type II pneumocytes in patients with IPF or NSIP, but only discontinuous staining in normal lung tissues (Fig. 1b) [29,30]. KL-6/MUC1 is also strongly expressed in areas of destruction in the pulmonary structures, loose stroma, and endothelial cells of lymph vessels, as well as the contents of these regions [31]. Weak to moderate expression was also observed in several cancer tissues, such as stomach, colon, and hepatocellular tumors [32–34]. KL-6/MUC1 is also expressed in the premature lung during the early weeks of pregnancy, and its expression persists even after lung maturation [35,36].

5. Positive rates of KL-6/MUC1 serum levels in various diseases

A clinical cut-off value of 500 U/mL has been established for distinguishing patients with ILDs from healthy subjects and patients with lung diseases other than ILDs [37]. KL-6/MUC1 serum levels higher than the cut-off value have been observed in more than 70% of patients with ILDs, including IIPs, CVD-IP, HP, RP, D-ILDs, ARDS, pulmonary sarcoidosis, and pulmonary alveolar proteinosis (PAP, Table 2) [15,38–50]. Interestingly, less than 10% of patients with alveolar pneumonia tested positive for KL-6/MUC1. Meanwhile, 28% of patients with active pulmonary tuberculosis and 2.6% of patients with inactive pulmonary tuberculosis test positive for KL-6/MUC1, with most positive patients showing widespread involvement of the lungs [51]. Patients with advanced stages of lung, pancreatic, and breast cancers showed an almost 50% positive rate [14,27,52–54]. However, the positive rate was low for gastric, hepatocellular, colon, and rectal cancers, and for hepatitis, liver cirrhosis, and pancreatitis.

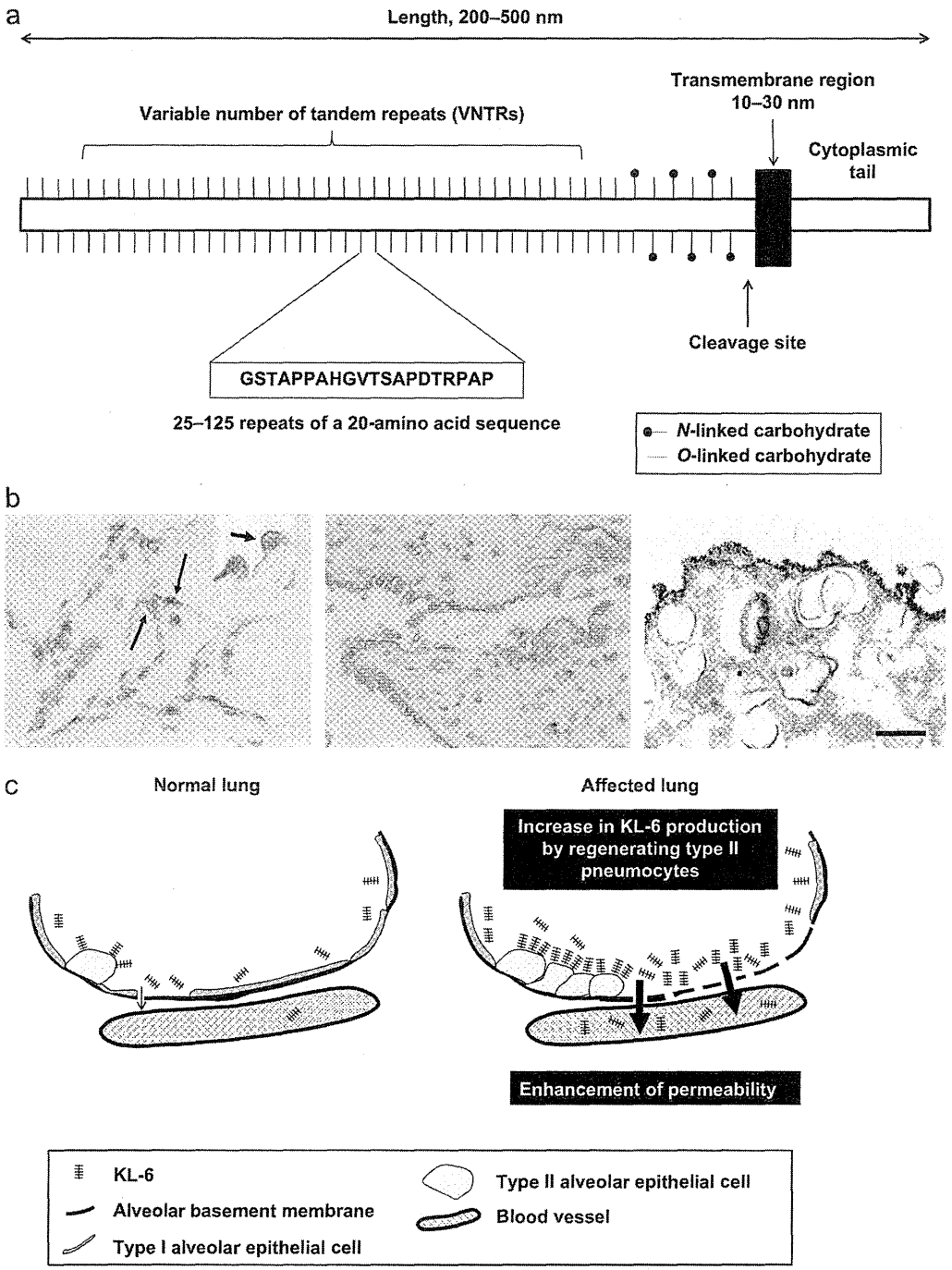


Fig. 1 – (a) Structure of MUC1. MUC1 is a large glycoprotein that contains 3 domains: (1) a cytoplasmic tail, (2) a single transmembrane region, and (3) an extracellular domain. The extracellular region contains sites of O- and N-linked glycosylation and a variable number tandem repeat (VNTR) domain of 20–100 repeats of a 20-amino acid sequence, (b) KL-6/MUC1 expression on the surface of type II pneumocytes. A discontinuous positive reaction (arrows) with anti-KL-6 antibody was observed in presumably normal lung tissue from a case of pneumothorax (left panel; magnification, ×400). Note the distinct dome-shaped positivity of the type II alveolar cells on staining with KL-6 antibody (inset at left panel; magnification, ×800). Linear and continuous staining for KL-6/MUC1 was observed on the cell surface of regenerating type II pneumocytes in patients with IPF (middle panel; magnification, ×400). Immunoelectron microscopic findings revealed that the reaction with anti-KL-6 antibody exhibits a linear pattern on the cell surface of type II pneumocytes in a patient with NSIP (right panel; magnification, ×400). Note that positive surface granular structures are approximately 100–200 nm in diameter. Scale bar = 0.5 μm. Modified from [29], with permission from the publisher. (c) Mechanism for the blood uptake of KL-6/MUC1. The increased serum levels of KL-6 in patients with ILDs may be due to an increase in KL-6 production by regenerating alveolar type II pneumocytes and/or enhanced permeability following the destruction of alveolar capillaries in the affected lung.

Table 1 – KL-6 expression in various tissues. Modified from

	Negative	Positive		
		Weak	Moderate	Strong
Normal				
Lung	Type I pneumocytes	Basal cells of terminal bronchi	Type II pneumocytes	
	Ciliated bronchial cells		Respiratory bronchi epithelial cells	
	Goblet cells Mucous cells of the bronchial gland		Serous cells of the bronchial glands	
Others	Surface mucous cells of the stomach Pyloric cells of the stomach		Fundic gland cells of the stomach Ductal epithelial cells of the mammary gland	
	Epithelial cells of the duodenum Epithelial cells of the rectum Epithelial cells of the colon Acinar cells of the pancreas Leukocytes Red blood cells		Ductal epithelial cells of the pancreas	
Interstitial lung disease	Granuloma Giant cells			Regenerating type II pneumocytes
Malignant cells	Some malignant cells	Most malignant cells		Lung cancer Pancreatic cancer Breast cancer

Table 2 – Positive rate of KL-6 in various diseases. Modified from

	Positive rate			
	0–10%	10–30%	30–70%	70–100%
Benign disease				
Lung	Alveolar pneumonia	Pulmonary tuberculosis (total)	Diffuse panbronchitis	Idiopathic interstitial pneumonias
	Bronchial asthma	Pneumoconiosis	Sarcoidosis	Collagen vascular disease-associated interstitial pneumonitis
	COPD		Pulmonary tuberculosis with wide-spread involvement of the lung field	Hypersensitivity pneumonitis
	Bronchiectasis Pneumoconiosis			Radiation pneumonitis Drug-induced pneumonitis Acute respiratory distress syndrome Pulmonary sarcoidosis pulmonary alveolar proteinosis
Others	Hepatitis Liver cirrhosis Pancreatitis Cholecystitis			
Malignancies				
	Gastric cancer Colon cancer Rectal cancer Hepatic cancer		Lung cancer Pancreatic cancer Breast cancer	

6. Clinical evaluation of serum KL-6/MUC1 levels

More than 50 papers investigating the clinical significance of KL-6 in various types of ILDs have been published from our research groups, and more than 350 papers on KL-6/MUC1 can be found in PubMed, with an increasing number of reports from international groups of researchers [8]. The data from these reports suggest that KL-6/MUC1 serum levels are useful for (1) detecting the presence of disease, (2) evaluating disease activity, and (3) predicting clinical outcomes in various types of ILDs. The clinical utility of KL-6/MUC1 in various types of ILDs is summarized in Table 3.

6.1. *IIPs*

The first report from our laboratory describing KL-6/MUC1 serum levels demonstrated that KL-6/MUC1 serum levels in patients with various types of ILDs were significantly higher than those of healthy control subjects [15]. KL-6/MUC1 serum levels were found to be particularly high in IIP patients with a positive uptake of ^{67}Ga-citrate in their diseased lung. Furthermore, a significant positive correlation was observed between changes in KL-6/MUC1 serum levels and the subjective and objective signs of disease activity in patients whose clinical courses were followed. In another study, the KL-6/MUC1 serum levels in 33 patients with ILDs (21 patients with IPF and 12 with CVD-IP) were compared to 82 control subjects (70 healthy controls and 12 patients with bacterial pneumonia) [46]. A receiver operating characteristic (ROC) curve drawn in this study revealed the superiority of KL-6/MUC1 to SP-A, SP-D, and MCP-1 as a diagnostic marker of ILDs, as revealed by the diagnostic accuracy, sensitivity, specificity, and likelihood ratios. Based on these data, KL-6/MUC1 is thought to be useful for distinguishing most ILDs from other benign lung diseases, such as alveolar pneumonia. However, KL-6/MUC1 serum levels are elevated in 70–100% of patients with ILDs and therefore cannot be used to differentiate patients with IPF from those with NSIPs [55]. A detailed analysis of the relationship between KL-6/MUC1 serum levels and disease extent on HRCT in patients with NSIP revealed that KL-6/MUC1 serum levels are significantly correlated with the extent of interstitial disease [25,56,57]. Follow-up CT and changes in KL-6/MUC1 serum levels after treatment showed that the percent change in disease extent is reflected in the levels of KL-6/MUC1. Together, these observations indicate that KL-6/MUC1 serum levels may reflect the presence of fibrotic lung lesions accompanied by regenerating epithelial cells.

Acute exacerbation is a critical prognostic factor that shortens the survival period of patients with IPF. A small study followed 14 patients with rapidly progressive IPF who received weekly high-dose corticosteroid pulse therapy for at least 3 weeks [43]. KL-6/MUC1 serum levels significantly decreased in survivors, but tended to increase in nonsurvivors, suggesting that changes in KL-6/MUC1 serum levels can predict the efficacy of high-dose corticosteroid pulse therapy. However, this study was conducted prior to the 2002 ATS/ERS classification of ILDs; therefore, the diagnosis of some of these patients may be different from what is now accepted.

Elevated serum KL-6/MUC1 (KL-6/MUC1 levels $\geq$1000 U/mL) in IPF patients at the initial visit were associated with increased mortality [48]. Satoh et al. also reported that the progression of the disease was significantly faster in patients with ILDs whose KL-6/MUC1 levels were 1000 U/mL or more at the initial measurement than in patients whose KL-6/MUC1 levels were less than 1000 U/mL [58].

6.2. *CVD-IP*

ILDs are common manifestations in patients with collagen vascular disease (CVD), with an overall incidence estimated at 15% [59]. In a study conducted in our laboratory, KL-6/MUC1 serum levels were shown to be elevated in patients with CVD-IP compared to those of control subjects (healthy subjects and patients with bacterial pneumonia) [46]. In another study from our laboratory, the serum levels of KL-6/MUC1 were measured in 177 patients with rheumatoid arthritis. The results showed that an increase in KL-6/MUC1 serum levels was correlated with the presence of active CVD-IP [60]. Nakajima et al. evaluated the serum levels of KL-6/MUC1 in patients with CVD with or without ILDs and demonstrated that KL-6/MUC1 serum levels are useful markers in the diagnosis and evaluation of CVD-IP disease activity [61]. The utility of KL-6/MUC1 as a serum biomarker to detect ILDs and evaluate disease activity in patients with systemic sclerosis

Table 3 – Clinical utility of KL-6 in various types of ILDs.

Disease	Detection of disease		Evaluation of disease activity		Prediction of the prognosis	
	Utility	References	Utility	References	Utility	References
IIPs	++	[15,41,46,55]	++	[41,43]	++	[48,58]
CVD-IP	++	[41,46,60–64,66–69]	++	[41,61–69]	NE	
HP	++	[15,41,78,80,81]	++	[41,78,79]	NE	
RP	+	[15,38,82,83]	++	[38,39,82,83]	+	[84]
D-ILDs	+	[47,86]	++	[47,86]	++	[47,86]
ARDS	++	[49,87,92]	++	[49,87,92]	++	[50,87,88]

IIPs: idiopathic interstitial pneumonias; CVD-IP: collagen vascular disease-associated interstitial pneumonia; HP: hypersensitivity pneumonia; RP: radiation pneumonitis; D-ILDs: drug-induced interstitial lung diseases; ARDS: acute respiratory distress syndrome; ++: high utility, +: moderate utility NE: not evaluated.

(SSc) and polymyositis/dermatomyositis (PM/DM) has also been reported in several publications [62–69]. Furthermore, the serum levels of KL-6/MUC1 in patients with SSc are correlated with functional lung impairment, as expressed by diffusing capacity for carbon monoxide (DL_{CO}) reduction [69].

Glucocorticosteroids, immunosuppressants, and biological agents are widely used for the treatment of CVD, particularly in rheumatoid arthritis [70,71]. However, these therapies sometimes cause adverse effects, such as the occurrence of opportunistic infection. One such infection is *Pneumocystis jiroveci* pneumonia (PCP). This infection results in the appearance of ground-glass opacity on HRCT imaging and resembles acute exacerbation of ILD. Several reports demonstrate that KL-6/MUC1 serum levels are elevated in patients with PCP [72–75]. However, β-D-glucan may be more reliable as a serum diagnostic marker for PCP than KL-6/MUC1, since KL-6/MUC1 is too sensitive for underlying ILDs. Therefore, KL-6/MUC1 can be used to determine the extent of damaged alveolar epithelium and alveolar–capillary permeability, whereas β-D-glucan is a marker for fungal volume.

6.3. *HP*

HP, also known as extrinsic allergic alveolitis (EAA), is an immunologically mediated lung disease induced by the inhalation of antigens present in various systemic organs [76]. The clinical manifestation of HP can be divided into acute, subacute, or chronic types [77]. In patients with summer-type acute HP, KL-6/MUC1 serum levels are elevated [15,41]. Takahashi et al. evaluated KL-6/MUC1 serum levels in 272 farmers in a daily farming community and showed that KL-6/MUC1 serum levels were significantly higher in patients with farmer's lung disease (FLD) compared to farmers without FLD. In patients with FLD, KL-6/MUC1 serum levels were correlated with the activity of the disease [78]. Several reports demonstrate that KL-6/MUC1 serum levels are useful for evaluating disease activity in patients with HP caused by spores of the Japanese mushroom [79,80]. Inase et al. reported that KL-6/MUC1 serum levels were also elevated in cases of chronic HP with the potential to develop end-stage lung fibrosis similar to IPF [81].

6.4. *RP*

RP is a common complication that restricts the use of radiotherapy against lung cancer and sometimes leads to progressive respiratory failure or even death. The utility of KL-6/MUC1 in distinguishing RP from lung cancer is limited. However, the serum levels of KL-6/MUC1 are useful for the early diagnosis of RP in patients with lung cancer who receive radiation therapy [38,39]. Goto et al. monitored KL-6/MUC1 serum levels in patients with lung cancer at multiple time points after the start of radiation therapy and showed a correlation between the changes in serum KL-6/MUC1 levels and the clinical course of RP [82]. Furthermore, patients whose KL-6/MUC1 serum levels rose more than 1.5 times higher than baseline levels showed a trend toward the development of severe life-threatening RP [83,84]. Yamashita et al. retrospectively evaluated the incidence rate and risk factors of severe RP after stereotactic body radiotherapy (SBRT) for 117 patients with lung cancers. Grade 4–5 RP was observed in 9 patients (7.7%), and a correlation was found between the incidence of grade 4–5 RP and higher serum KL-6/MUC1 levels [84].

6.5. *D-ILDs*

Various agents can cause pulmonary toxicity, including ILDs, which often results in a fatal outcome. Moreover, a high incidence of ILDs is reported in patients with advanced non-small cell lung cancer (NSCLC) treated with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), particularly in Japanese populations [85]. The serum levels of KL-6/MUC1 were examined in 30 patients with D-ILDs who were classified into 4 different HRCT patterns [47]. The absolute KL-6/MUC1 serum levels at the onset of D-ILDs increased only in life-threatening disease types, such as those ILDs displaying diffuse alveolar damage (DAD) and chronic interstitial pneumonia (CIP) patterns. Serum KL-6/MUC1 levels increased or decreased in accordance with the clinical outcome of the disease in patients with the DAD and CIP patterns. Recently, the clinical records and radiographs of 341 patients with advanced NSCLCs who were treated with EGFR-TKIs were retrospectively reviewed, and changes in serum KL-6/MUC1 levels were also monitored in patients who developed D-ILDs [86]. Although absolute KL-6/MUC1 serum levels could not discriminate between life-threatening and non-life-threatening D-ILDs at either the baseline reading or the onset of D-ILDs, the ratio of the serum KL-6/MUC1 level at the onset of D-ILDs to that at the baseline could clearly discriminate between the 2 outcomes.

6.6. *ARDS*

ARDS is characterized by the influx of protein-rich edema fluid into air spaces, with the influx resulting from the increased permeability of the alveolar–capillary barrier. A previous study examined KL-6/MUC1 levels in the serum and pulmonary ELF or bronchoalveolar lavage fluid (BALF) of patients with ARDS or acute lung injury (ALI) [87,88]. These studies reported that the KL-6/MUC1 levels in these samples were significantly higher in nonsurvivors than in survivors. A recent study from our laboratory evaluated the levels of KL-6/MUC1 in ELF and serum obtained at multiple time points from patients with ARDS [50]. A comparison of the kinetics of KL-6/MUC1 levels in ELF and serum between survivors and nonsurvivors revealed that only the KL-6/MUC1 levels in ELF on days 0–3 after the diagnosis of ARDS were significantly higher in nonsurvivors than in survivors. In another study from our laboratory, KL-6/MUC1 serum levels, serially measured in patients with ARDS, along with the indices of respiratory failure, inflammation, coagulation, fibrinolysis, and multiple organ dysfunctions were shown to be associated with the development of disseminated intravascular coagulation (DIC) [49].

The expression of MUC1-associated sialyl Lewis^a has been demonstrated in previous studies [89–91], and studies from our laboratory have also found the presence of sialyl Lewis^a on KL-6/MUC1 [54,92]. This KL-6/MUC1 molecule containing sialyl Lewis^a was designated as SLAK, and an ELISA system using both anti-sialyl Lewis^a and anti-KL-6 antibodies was developed to measure SLAK levels in samples. The serum

levels of SLAK in patients with ARDS were found to be useful in predicting future development of DIC [92].

6.7. *Pulmonary sarcoidosis*

Sarcoidosis is a chronic systemic disorder characterized by noncaseating epithelioid cell granulomas and the accumulation of T-lymphocytes and macrophages in multiple organs [93]. The serum levels of KL-6/MUC1 in patients with sarcoidosis are increased and significantly influenced by the severity of lung involvement and the positive uptake of ^{67}Ga-citrate in the diseased lung [42]. Janssen et al. evaluated the ability of serum KL-6/MUC1, SP-D, and Clara cell 16 (CC16) levels to discriminate between patients with sarcoidosis and control subjects and concluded that KL-6/MUC1 was the best discriminative biomarker [94]. The investigators also observed a trend in which the serum KL-6/MUC1 levels were associated with pulmonary disease outcomes in the patients with sarcoidosis. In another study evaluating the significance of various biomarkers in patients with pulmonary sarcoidosis, Miyoshi et al. measured the serum levels of KL-6/MUC1, serum amyloid A, soluble interleukin 2 receptor, lysozyme, and angiotensin-converting enzyme [95]. These researchers demonstrated that KL-6/MUC1 serum levels were significantly correlated with the number of the total cells, lymphocytes, and $CD4^+$ T lymphocytes in BALF and were the single indicator of increased parenchymal infiltration in chest radiographs.

7. Mechanism for blood uptake of KL-6/MUC1

The primary cellular source of KL-6/MUC1 in the affected lungs of patients with ILDs is regenerating type II pneumocytes [15,29], and KL-6/MUC1 is present at high concentrations in BALF [40]. KL-6/MUC1 levels in BALF were significantly correlated with the total cell number, lymphocytes, neutrophils, and albumin concentrations in BALF and with serum KL-6/MUC1 levels in patients with ILDs. A correlation between KL-6/MUC1 serum levels and albumin levels in BALF was also found in patients with chronic beryllium disease, suggesting the utility of serum KL-6/MUC1 levels as a marker for the permeability of the air–blood barrier [96]. Both the destruction of the alveolar–capillary barrier and the enhancement of alveolar-capillary permeability are thought to be necessary for the leakage of KL-6/MUC1 into systemic circulation, since KL-6/MUC1 is a high-molecular-weight glycoprotein. As shown in Fig. 1c, the increase in serum KL-6/MUC1 levels in patients with ILDs results from an increase in KL-6/MUC1 production by regeneration of alveolar type II pneumocytes and/or enhancement of permeability following destruction of the alveolar–capillary barrier in the affected lung.

Simultaneous measurement of the serum levels of KL-6/MUC1, SP-A, and SP-D in patients with ILDs sometimes reveals a discrepancy between these serum markers. For instance, a transient increase in the serum levels of SP-A and SP-D following mild lung injury is frequently observed, while serum KL-6/MUC1 levels remain unchanged [8]. This discrepancy suggests that increases in serum KL-6/MUC1 levels do not reflect the intensity of inflammation, but rather indicate the extent of damaged alveolar epithelium and alveolar-capillary permeability (Table 4).

8. Association between serum KL-6/MUC1 levels and genetic variants in the MUC1 gene

Measurements of serum KL-6/MUC1 levels have been performed primarily in Japanese populations, and therefore, the data for non-Japanese populations are rather limited. We recently found that the levels of circulating KL-6/MUC1 were higher in European populations than in Japanese populations [97–100]. Another recent study evaluating the relationship between the functional A-to-G polymorphism at nucleotide position 568 (exon 2; rs4072037) in the MUC1 gene and serum KL-6/MUC1 levels in Caucasian subjects revealed that the genotype of this polymorphism affected serum KL-6/MUC1 levels. KL-6/MUC1 levels were highest for the GG genotype, lowest for the AA genotype, and in an intermediate range for the AG genotype [100]. The HapMap data (http://hapmap.ncbi.nlm.nih.gov/) indicate that the distributions of the AA, AG, and GG genotypes differ between European subjects (30.1%, 55.8%, and 14.2%, respectively) and Japanese subjects (69.8%, 25.6%, and 4.7%, respectively). These observations suggest an ethnic difference in the serum levels of KL-6/MUC1 and imply that a different cut-off level for KL-6/MUC1 is needed in Caucasians to discriminate between patients with ILDs and healthy subjects.

Table 4 – Acute phase reactant and serum KL-6 levels as serum indicators for the activity of interstitial lung diseases.

		KL-6	
		Normal range	Increased
Acute phase reactant	Normal range	Inactive	Inflammation (–) Insufficient repair of damaged alveoli • Progressing alveolar remodeling
	Increased	Inflammation (± ~ +) Epithelial barrier damage (–) • Alveolar remodeling (–) • Mild to severe respiratory dysfunction	Inflammation (± ~ +) Presence of alveolar damage • Rapid progression of alveolar remodeling • Severe clinical manifestation

9. Conclusions and future directions

In this review, we summarized the utility of KL-6/MUC1 in the clinical management of patients with various types of ILDs. Based on the results from a number of reports investigating KL-6/MUC1, the serum levels of KL-6/MUC1 are thought to be useful for (1) detecting the presence of disease, (2) evaluating disease activity, and (3) predicting outcomes in various types of ILDs. Because the measurement of serum KL-6/MUC1 levels is rapid, inexpensive, reproducible, less invasive, and easier to perform than SLB, HRCT, BAL, and pulmonary function tests, we believe that this biomarker would provide a significant benefit to the clinical management of patients with ILDs.

In Japan, KL-6/MUC1 has been used in clinical practice for more than 10 years; however, evidence from clinical trials validating the clinical efficacy of KL-6/MUC1 remains limited. In addition, we are aware of ethnic differences in the prevalence of pulmonary diseases such as D-ILDs and cystic fibrosis [85,101,102] and in the serum levels of KL-6/MUC1 [97–100]. In order to establish KL-6/MUC1 as an internationally useful serum biomarker, further prospective and international studies to determine the clinical efficacy of KL-6/MUC1 in the management of patients with ILDs are necessary.

Conflict of interest

Nobuoki Kohno received patent royalties/licensing fees from Eisai Co., Ltd.

Nobuhisa Ishikawa, Noboru Hattori, Akihito Yokoyama, they have no potential conflict of interest.

Acknowledgments

This work was supported by the Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. The authors appreciate insightful suggestions from Y. Ohtsuki (Matsuyama-Shimin Hospital, Matsuyama, Japan) regarding the figures.

REFERENCES

[1] American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med 2000;161:646–64.

[2] American Thoracic Society. European Respiratory Society. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. Am J Respir Crit Care Med 2002;165:277–304.

[3] Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011;183:788–824.

[4] Kinnula VL, Ishikawa N, Bergmann U, et al. Proteomic approaches for studying human parenchymal lung diseases. Expert Rev Proteomics 2009;6:619–29.

[5] King Jr. TE. Clinical advances in the diagnosis and therapy of the interstitial lung diseases. Am J Respir Crit Care Med 2005;172:268–79.

[6] Kim R, Meyer KC. Therapies for interstitial lung disease: past, present and future. Ther Adv Respir Dis 2008;2:319–38.

[7] British Thoracic Society recommendation. The diagnosis, assessment and treatment of diffuse parenchymal lung disease in adults. Thorax 1999;54(Suppl 1):S1–14.

[8] Nukiwa T. The role of biomarkers in management of interstitial lung disease: implication of biomarkers derived from type II pneumocytes. In: Du Bois RM, Richeldi L, editors. The European Respiratory Monograph 46 Interstitial lung disease. Leeds: Maney Publishing; 2009. p. 47–66.

[9] Tzouvelekis A, Kouliatsis G, Anevlavis S, et al. Serum biomarkers in interstitial lung diseases. Respir Res 2005;6:78.

[10] Prasse A, Müller-Quernheim J. Non-invasive biomarkers in pulmonary fibrosis. Respirology 2009;14:788–95.

[11] Thomeer M, Grutters JC, Wuyts WA, et al. Clinical use of biomarkers of survival in pulmonary fibrosis. Respir Res 2010;11:89.

[12] van den Blink B, Wijsenbeek MS, Hoogsteden HC. Serum biomarkers in idiopathic pulmonary fibrosis. Pulm Pharmacol Ther 2010;23:515–20.

[13] Barlo NP, van Moorsel CH, van den Bosch JM, et al. Predicting prognosis in idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis 2010;27:85–95.

[14] Kohno N, Akiyama M, Kyoizumi S, et al. Detection of soluble tumor-associated antigens in sera and effusions using novel monoclonal antibodies, KL-3 and KL-6, against lung adenocarcinoma. Jpn J Clin Oncol 1988;18:203–16.

[15] Kohno N, Kyoizumi S, Awaya Y, et al. New serum indicator of interstitial pneumonitis activity. Sialylated carbohydrate antigen KL-6. Chest 1989;96:68–73.

[16] Kohno N, Inoue Y, Hamada H, et al. Difference in serodiagnostic values among KL-6-associated mucins classified as cluster 9. Int J Cancer Suppl 1994;8:81–3.

[17] Stahel RA, Gilks WR, Lehmann HP, et al. Third International Workshop on Lung Tumor and Differentiation Antigens: overview of the results of the central data analysis. Int J Cancer 1994;8:6–26.

[18] Hirasawa Y, Kohno N, Yokoyama A, et al. KL-6, a human MUC1 mucin, is chemotactic for human fibroblasts. Am J Respir Cell Mol Biol 1997;17:501–7.

[19] Seko A, Ohkura T, Ideo H, et al. Novel O-linked glycans containing 6'sulfo-Gal/GalNAc of MUC1 secreted from human breast cancer YMBS cells: possible carbohydrate epitopes of KL-6(MUC1) monoclonal antibody. Glycobiology 2012;22:181–95.

[20] Singh PK, Hollingsworth MA. Cell surface-associated mucins in signal transduction. Trends Cell Biol 2006;16:467–76.

[21] Hattrup CL, Gendler SJ. Structure and function of the cell surface (tethered) mucins. Annu Rev Physiol 2008;70:431–57.

[22] Thathiah A, Blobel CP, Carson DD. Tumor necrosis factor-alpha converting enzyme/ADAM 17 mediates MUC1 shedding. J Biol Chem 2003;278:3386–94.

[23] Kondo K, Kohno N, Yokoyama A, et al. Decreased MUC1 expression induces E-cadherin-mediated cell adhesion of breast cancer cell lines. Cancer Res 1998;58:2014–19.

[24] Doi M, Yokoyama A, Kondo K, et al. Anti-tumor effect of the anti-KL-6/MUC1 monoclonal antibody through exposure of surface molecules by MUC1 capping. Cancer Sci 2006;97:420–9.

[25] Ohshimo S, Yokoyama A, Hattori N, et al. KL-6, a human MUC1 mucin, promotes proliferation and survival of lung fibroblasts. Biochem Biophys Res Commun 2005;338:1845–52.