Src Kinase-associated Phosphoprotein2 Expression Is Associated with Poor Prognosis in Non-small Cell Lung Cancer

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Abstract. *Background/Aim: Non-small cell lung cancer (NSCLC) is among the leading causes of cancer-related deaths worldwide. In certain human cancer types, Src is associated with cancer progression and refractory cancer. To improve the prognoses of NSCLC patients, we evaluated Src kinase-associated phosphoprotein 2 (SKAP2), a factor associated with integrin-stimulated cytoskeletal rearrangement, as a new therapeutic target. Materials and Methods: We performed immunohistochemistry for SKAP2 in 99 NSCLC samples and evaluated the relationship between SKAP2 expression, clinicopathological factors and prognosis. Results: Higher SKAP2 expression was detected in cancerous tissues and was predominantly expressed in the cytoplasm. Elevated SKAP2 expression levels were associated with poor prognosis (p=0.007) and shorter survival time after recurrence (p=0.035). High SKAP2 expression was an independent prognostic factor in NSCLC patients (p=0.027). Conclusion: High SKAP2 expression levels in NSCLC tissues could be a powerful biomarker of poor prognosis. Therefore, SKAP2 is a promising candidate molecular target for NSCLC treatment.*

Non-small cell lung cancer (NSCLC) represents approximately 85% of lung cancer cases and is among the leading causes of cancer-related deaths worldwide (1). Despite recent advances in cancer therapy, it is difficult to completely cure NSCLC. Studying potential biomarkers of NSCLC will be the key to monitoring cancer recurrence, providing information on the need for adjuvant therapy.

Src, is the first identified oncogene, associated with cancer progression and refractory cancer (2). In the present study, we focused on Src kinase-associated phosphoprotein 2 (SKAP2, also known as SCAP2, SKAP55R, SKAP-HOM and RA70), a reported Src substrate that is phosphorylated by Src family kinases (3). SKAP2, which self-dimerizes using a coiled-coil domain at its amino terminus, is associated with hematopoietic cell function by regulating integrin signaling and actin remodeling, mechanisms known to regulate cancer progression and chemoresistance (4). SKAP2-mediated integrin signaling participates in cell adhesion and migration (5). However, no previous studies have addressed whether SKAP2 regulates NSCLC development.

The purpose of the present study was to clarify the clinical significance of the SKAP2 in NSCLC. We examined the expression of SKAP2 in clinical NSCLC samples by using immunohistochemistry to evaluate whether the level of SKAP2 expression in cancerous tissues can be used as a prognostic marker in NSCLC patients.

Materials and Methods

Clinical samples. We analyzed tumor specimens from 99 patients with lung cancer who underwent excision surgery for a primary tumor between September 1999 and March 2006 at the Department of General Surgical Science of Gunma University School of Medicine. The patients included 68 men and 31 women with a median age of 65.7 years (range=32-84 years) at the time of surgery. Sixty-six patients were former/current smokers with a median Brinkman index (number of cigarettes per day times years) of 1.052.1. The remaining 33 patients had never smoked. Sixty-nine patients had adenocarcinomas, 18 had squamous cell carcinomas and 12 had other carcinoma types. Fifty-seven patients had stage I, 10 had stage II, 29 had stage III and 3 had stage IV lung cancer at the time of surgery. All patients signed written informed consent forms as required by our institutional guidelines.

Immunohistochemistry. Resected surgical specimens were fixed with 10% formaldehyde, embedded in paraffin blocks, cut into 4-μm-thick sections and mounted on glass slides. The staining procedure was performed using the standard methods described previously (6). The sections were then incubated overnight at 4°C and at room temperature for 30 min with rabbit polyclonal anti-SKAP2 antibody (Proteintech Group Inc., Chicago, IL, USA) at a dilution of 1:200 in phosphate-buffered saline containing 1% bovine serum albumin. The sections were lightly counterstained in Mayer's hematoxylin and mounted on glass slides.

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Table I. *Relationship between SKAP2 expression and clinicopathological features.*

Factors	SKAP2 expression		
	Low expression	High expression	
	n=66	n=33	*p*-Value
Age			
	65.4+10.8	66.4+10.4	0.66
Sex			
Male	42	26	0.16
Female	24	7	
Brinkman index			
	602.2+603.5	899.7+871.6	0.05
Histological type			
Adenocarcinoma	47	22	0.9
Adenosquamous	1	0	
LA	5	3	
LCNEC	1	0	
Squamous	11	7	
Carcionoid	1	1	
Tumor size			
	33.4+20.1	35.1+17.6	0.68
T factor			
T1	29	13	0.83
T2, 3, 4	37	20	
N factor			
Negative	41	20	1
Positive	25	13	
Recurrence			0.2
Negative	43	17	
Positive	23	16	
Stage			
I, II	46	21	0.65
III, IV	20	12	

LA; Large cell carcinoma. LCNEC; large cell neuroendocrine carcinoma.

The level of SKAP2 immunoreactivity was defined as follows: score 0, no staining or weak complete cytoplasmic staining in <10% of tumor cells; score 1+, weak complete cytoplasmic staining in >10% of tumor cells or intermediate cytoplasmic staining in <10% of tumor cells; score 2+, intermediate cytoplasmic staining in >10% of tumor cells or strong cytoplasmic staining in <30% of tumor cells; and score 3+, strong cytoplasmic staining in >30% of tumor cells. Immunohistochemically stained slides were examined and evaluated independently by two experienced researchers. Each score was classified into high (1+; 2+; 3+) or low (0) expression groups.

Statistical analysis. Differences between two groups were estimated using the Student's *t*-test, Chi-square analysis and analysis of variance. Survival curves were generated according to the Kaplan-Meier method. The differences between survival curves were examined by using the Wilcoxon test. In addition, univariate and multivariate survival analyses were performed using the Cox proportional hazards model. A result was considered statistically significant when the relevant *p*-value was <0.05. All statistical analyses were performed using the JMP 5.0 software (SAS Institute Inc., Cary, NC, USA).

Results

Immunohistochemical analysis of SKAP2 expression in NSCLC tissues. The cytoplasmic expression of SKAP2 in 99 NSCLC specimens was investigated immunohistochemically. SKAP2 expression was greater in cancerous tissues than in corresponding non-cancerous tissues and found predominant expression in the cytoplasm. Thirty-three NSCLC specimens were assigned to the high-SKAP2 expression group and 66 were assigned to the low-SKAP2 expression group. Representative results of the immunohistochemistry experiments are shown in Figure 1.

Association between SKAP2 expression and clinicopathological factors in clinical NSCLC samples. The correlations between SKAP2 expression in the NSCLC specimens and 8 clinicopathological characteristics of the patients (age, sex, Brinkman index, histological type, tumor size, T factor, N factor, recurrence and stage) are shown in Table I. No significant differences were observed with respect to age, sex, Brinkman index, histological type, T factor (including tumor size), N factor, recurrence and stage.

Prognostic significance of SKAP2 expression in NSCLC patients. The overall survival rates of patients with high-SKAP2-expressing tumors were significantly lower than those of with low-SKAP2-expressing tumors (p=0.007) (Figure 2a). The overall survival rates of 16 patients after recurrence with high-SKAP2-expressing tumors treated with adjuvant therapies was significantly shorter than those in the other group (p=0.035; Figure 2b). In patients with lung adenocarcinoma, the high-SKAP2 expression group had poorer prognoses than the low-SKAP2 expression group (p=0.021) (Figure 2c); however, the overall survival rates of the high-expression group, excluding adenocarcinoma, were not significantly shorter than the low-expression group (p=0.17) (Figure 2d). In multivariate analyses, high expression of SKAP2 in NSCLC tissues was an independent prognostic factor for poor survival, as was T factor and lymph node metastasis (p=0.027) (Table II).

Discussion

In this study, we showed that SKAP2 expression levels in NSCLC tissues were not associated with clinicopathological factors; however, high SKAP2 expression was associated with poor prognoses and shorter survival times after recurrence. Moreover, its expression was an independent prognostic factor in NSCLC patients.

We found higher SKAP2 expression in NSCLC than in non-cancerous lung tissues. The regulatory mechanism of SKAP2 expression has been reported previously. Transcriptional regulation of *SKAP2* by HSF4b is related to actin remodeling and focal adhesion (7). HSF4b expression is regulated by

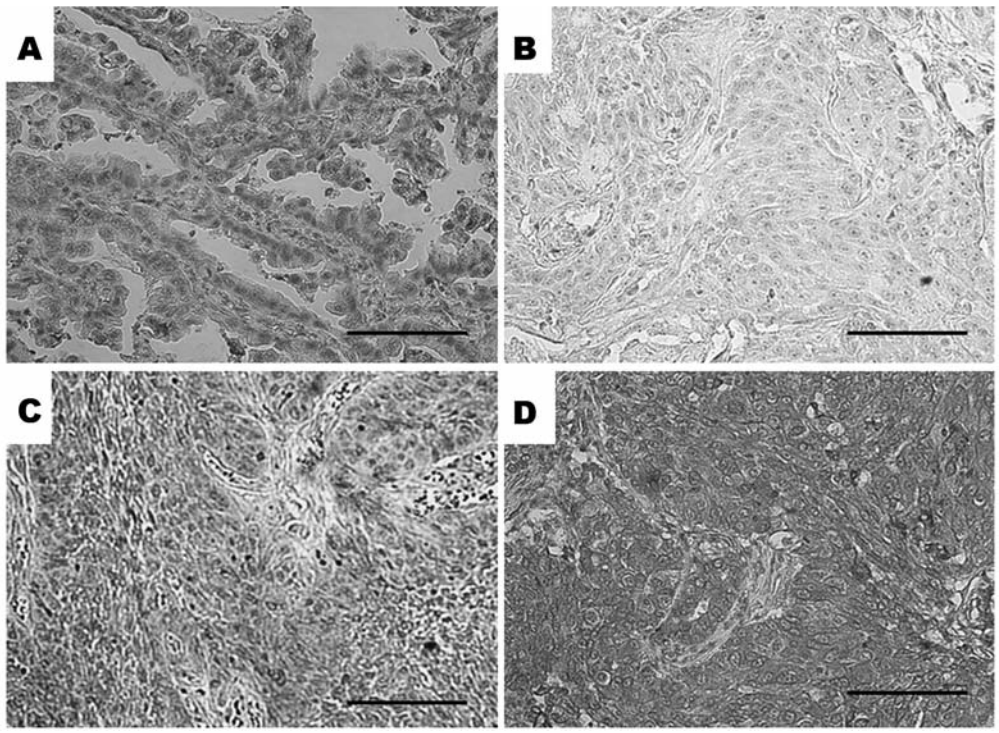

Figure 1. *Immunohistochemical analysis of SKAP2 expression in representative non-small cell lung cancer (NSCLC) tissue samples. (a-d) Example of (a) no, (b) low, (c) moderately-high and (d) high SKAP2 expression in primary NSCLC specimens (×100 magnification; scale bar, 100 μm).*

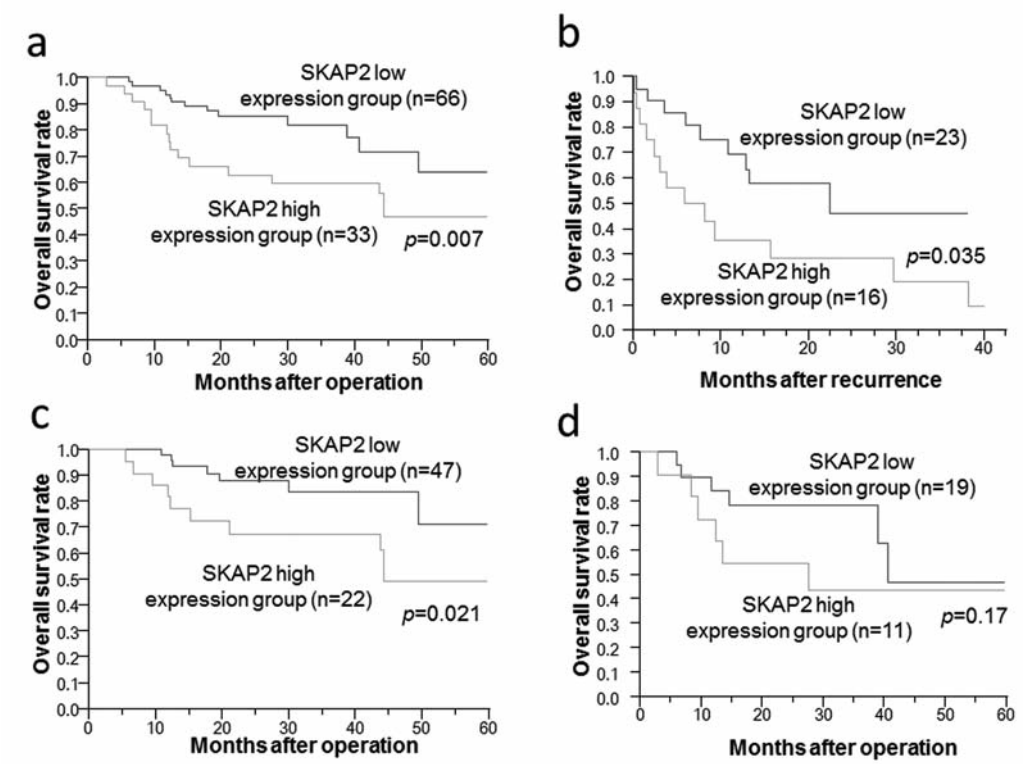

Figure 2. *The relationships between SKAP2 expression in NSCLC samples and survival time. (a) Overall survival curves of NSCLC patients according to SKAP2 expression (p=0.007). (b) Overall survival rate after recurrence of NSCLC patients according to SKAP2 expression (p=0.035). (c, d) Overall survival curves of patients (c) with and (d) without lung adenocarcinoma according to SKAP2 expression (p=0.021 and 0.17, respectively).*

Table II. *Univariate and multivariate analyses of survival in 99 patients with NSCLC.*

Clinicopathologic variable	Univariate analysis			Multivariate analysis		
	RR	95%CI	*p*-Value	RR	95%CI	*p*-Value
Age (<65/≥65)	1.08	0.52-2.35	0.84	-	-	-
Gender (Male/Female)	0.61	0.37-0.93	0.021	0.84	0.49-1.33	0.47
T factor (T1/T2, 3, 4)	4.69	1.95-13.9	0.0003	3.63	1.43-11.26	0.0054
Lymph node metastasis (Negative/Positive)	1.92	1.33-2.88	0.0005	1.67	1.15-2.51	0.0072
SKAP2 expression (Low/High)	2.36	1.14-5.02	0.02	2.34	1.10-5.11	0.027

RR; Relative risk. CI; confidence interval.

fibroblast growth factor 2 (FGF2), a factor known to be associated with NSCLC proliferation and anchorage-independent growth (8). Therefore, SKAP2 regulation by a FGF2/HSF4b mechanism might be important in NSCLC development. Harada *et al*. (9) performed a DNA copy number analysis in micro-dissected clinical pancreatic ductal adenocarcinoma and suggested that *SKAP2* genomic amplification is associated with pancreatic cancer development. In NSCLC cells, DNA amplification of chromosome 7q, which harbors *SKAP2*, was reported (10, 11). In clinical practice, V-Erb-B2 Avian Erythroblastic Leukemia Viral Oncogene Homolog 2 (*ERBB2*) and *MET* proto-oncogene, which are amplified in the genome, have received attention as candidates of therapeutic molecular targets (12, 13). Therefore, SKAP2, which is controlled by genomic copy number variation, might be promising as a NSCLC therapeutic target.

Whether SKAP2 positively regulates cancer cell migration and proliferation remains a controversial issue (3, 5, 14). Ayoub *et al*. (15) reported that cells with suppressed SKAP2 have reduced migratory ability; however, this ability is enhanced in *SKAP2* SH3 domain mutant cells that have higher SKAP2 tyrosine phosphorylation levels. Moreover, SKAP2 reportedly activates migration and proliferation in melanoma cells (14) and has an important role in regulating integrin-related actin remodeling, which is known to function in the metastatic processes of cancer cells (5). Targeting SKAP2 in NSCLC might suppress migration and metastasis by controlling integrin activation and actin remodeling.

SKAP2-related signal transduction is important for interleukin-10 (IL-10) production in neutrophils (16). IL-10 reportedly promotes aggressiveness in lung cancer tumors by transcriptionally activating *CIP2A* (17) and is associated with chemoresistance in B-cell lymphoma by suppressing the BCL2 family (18). In the present study, we clarified that low SKAP2 expression in NSCLC was related to good survival intervals after recurrence. On the basis of these results, we speculate that SKAP2 suppression might be a useful strategy to treat NSCLC, not only by regulating integrin and actin remodeling but also by suppressing IL-10.

Previous findings and our presented data suggest that SKAP2 is a very promising candidate target. Notably, however, SKAP2 is reported to be important in migration and normal immune reactions of several hematopoietic cells (19, 20, 21). Therefore, we should consider the side-effects in the host, including hematotoxicity and tumor immune reaction failure, if SKAP2-targeting therapy is investigated in animal experiments and clinical trials. Further studies should involve examination of tumor inoculation data and antitumor drug administration in *SKAP2* knockout mice.

In conclusion, we showed that SKAP2 expression in NSCLC tissues is associated with poor prognosis and shorter survival time after recurrence, despite the fact that no association with clinicopathological factor progression exists. Inhibition of SKAP2 function may be an effective therapeutic approach for NSCLC by overcoming chemoresistance after recurrence. Further studies are required to evaluate the role of SKAP2 and its clinical application for NSCLC treatment.

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