c-Met activation in lung adenocarcinoma tissues: An immunohistochemical analysis

Yu Nakamura,[1] Toshiro Niki,[1,4] Akiteru Goto,[1] Teppei Morikawa,[1] Keiji Miyazawa,[2] Jun Nakajima[3] and Masashi Fukayama[1]

[1]Department of Human Pathology; [2]Department of Molecular Pathology, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033; [3]Department of Cardiothoracic Surgery, Tokyo University Hospital, Tokyo 113-8655; [4]Department of Integrative Pathology, Jichi Medical University, Tochigi 329-0498, Japan

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c-Met is often overexpressed in non-small cell lung cancer, but it remains unsolved whether its overexpression leads to its activation. We used an antibody specific to phospho-c-Met (Tyr1235) to investigate c-Met activation immunohistochemically in 130 surgically resected lung adenocarcinomas. The expression of c-Met and hepatocyte growth factor (HGF) was also investigated. Phospho-c-Met was positive in 21.5% (28/130) of cases. c-Met was positive in 74.6% of cases (97/130) and was expressed at high levels in 36.1% of cases (47/130). HGF was expressed at high levels in 31.5% of cases (41/130). Phospho-c-Met was correlated with high levels of HGF (P = 0.0010) and high levels c-Met expression (P = 0.0303), but it was also found to be positive in 12 cases with little to no HGF expression. Phospho-c-Met expression was significantly associated with tumor differentiation (P = 0.0023) and papillary histology (P = 0.0011), but not with pathological stage, lymph node metastasis or survival. High levels of c-Met and HGF were also associated with papillary histology (P = 0.0056 and P = 0.0396, respectively), but not with tumor differentiation. Phospho-c-Met was correlated with phospho-Akt (P = 0.0381), but not with phospho-Erk or phospho-Stat3. Phospho-Akt expression was marginally correlated with the expression of phospho-epidermal growth factor receptor (EGFR) (P = 0.0533) and, importantly, it was strongly correlated with the expression of either phospho-c-Met or phospho-EGFR (P = 0.0013). The data suggest that in lung adenocarcinoma tissue, c-Met activation may take place either ligand-dependently or ligand-independently via c-Met overexpression. c-Met activation may play special roles in the papillary subtype and in well differentiated lung adenocarcinomas. (*Cancer Sci* 2007; 98: 1006–1013)

Lung cancer is the leading cause of cancer death in many developed countries, including the USA and Japan.[1,2] Lung adenocarcinoma, one of the major histological subtypes, is rising in incidence.[3] Patients with lung carcinoma have a poor prognosis: those with stage I disease have a 5-year survival of only 70%.[4] Molecular-targeted therapy provides a new therapeutic modality in this setting and is now under intense investigation. Among the various molecular targets, receptor-type tyrosine kinases such as epidermal growth factor receptor (EGFR) and c-Met show promise for therapeutic intervention.[5]

The *c-Met* gene was originally identified as a cellular counterpart of the chemically induced oncogene *tpr-met* isolated from a human osteosarcoma cell line.[6] The *c-Met* gene encodes a high-affinity receptor for hepatocyte growth factor (HGF).[7] HGF binding augments the intrinsic tyrosine kinase activity of c-Met, resulting in autophosphorylation of several tyrosine residues within the intracellular region.[8,9] The phosphorylation of each tyrosine residue initiates distinct signal transduction cascades involving signaling molecules such as Erk, Akt and Stat3.[8,9]

Several studies have documented overexpression and/or amplification of c-Met in various cancers[8,9] including those of the stomach, colon, liver, prostate, ovary and lung.[10–12] Addition of HGF stimulates cell proliferation, motility and invasion of carcinoma cells.[8,9] Moreover, the abrogation of c-Met signaling by ribozymes, small interfering RNA (siRNA) and pharmacological inhibitors decreases the invasion and metastasis of tumor cells.[8,9] Thus, c-Met appears to be a promising molecular target for the treatment of cancer patients, including those with lung cancer.[8,9]

Although culture studies indicate that c-Met overexpression induces a ligand-independent activation of c-Met,[13] it remains to be seen whether c-Met overexpression leads to c-Met phosphorylation and activation in primary tumor tissues.[14] Inoue *et al*. recently examined c-Met expression and activation in normal and cancerous gastric tissues.[14] To do so they used a newly developed rabbit polyclonal antiphospho-c-Met antibody that specifically recognizes the phosphorylation of Tyr1235, a tyrosine residue known to play a crucial role in the kinase-induced activation of c-Met.[15] Although c-Met was expressed in both normal gastric mucosa and gastric cancer cells, Inoue *et al*. detected phospho-c-Met expression only in the latter.[14]

In the present study we used this same antibody to determine the prevalence of c-Met activation in a series of surgically resected lung adenocarcinomas. We then examined the expression of c-Met and HGF by immunohistochemistry, and analyzed the correlations among these three parameters and their correlations with clinicopathological variables. Lastly, we examined the expression of phospho-Stat3, phospho-Akt and phospho-Erk, which are downstream signaling molecules that mediate the biological actions of the HGF/c-Met system[9] and investigated their correlation with phospho-c-Met.

Materials and Methods

Lung adenocarcinoma patients and tissues. We examined a consecutive series of 130 primary lung adenocarcinomas resected at the University of Tokyo Hospital, Tokyo, Japan, between 1999 and 2003. The patients included 82 men and 48 women, ranging in age from 36 to 81 years (average 65.4 years). The observation period ranged from 0.8 to 57.3 months, with a median follow-up period of 31.4 months. The adenocarcinomas were staged according to the tumor-node-metastasis system adopted by the American Joint Committee on Cancer and the International Union Against Cancer.[16] Ninety-one were stage I (59 stage IA, 32 stage IB), 15 were stage II (four stage IIA, 11 stage IIB), and 24 were stage III (16 stage IIIA, eight stage IIIB). No stage IV cases were found. The adenocarcinomas were subtyped histologically according to the World Health Organization (WHO) classification of tumors.[17] Because most lung adenocarcinomas consist of heterogeneous histological components, dominant subtypes were mainly documented;[18,19] tumors were classified into mixed subtypes only when more than one subtype was distinctly present within the same tumor. Thus, 62 were papillary, five were acinar, 21 were solid, 15 were bronchiolo-alveolar, and 27 were mixed subtype. Histological differentiation of tumors was graded as

[5]To whom correspondence should be addressed. E-mail: tniki@jichi.ac.jp

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described previously.[20] The adenocarcinomas were also histologically evaluated for lymph node metastasis, pleural infiltration, blood vessel invasion and lymphatic vessel invasion. Pleural infiltration and blood vessel invasion were evaluated by staining the sections routinely with elastica van Gieson to identify the elastic fibers of pleura and blood vessels.

Antibodies. Rabbit polyclonal anti-HGF-α and rabbit polyclonal antic-Met antibodies were purchased from IBL (Gunma, Japan). Rabbit polyclonal phospho-c-Met (Tyr1235) has been described previously.[14] Rabbit polyclonal anti-phospho-Erk (Thr202/Tyr204, #9101), anti-phospho-Stat3 (Ser727, #9134), and anti-phospho-Akt (Ser473, #9277) antibodies were from Cell Signaling (Danvers, MA, USA).

Immunohistochemical staining for carcinoma specimens. Immunohistochemical staining was carried out on formalin-fixed, paraffin-embedded tissue sections of lung adenocarcinoma specimens. Sections (5-μm thick) were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol. The antigens were retrieved by 20 min of heating at 95°C in 0.01 M citrate buffer (pH 6.0) in a waterbath, followed by 20 min of cooling. After blocking the endogenous peroxidase activity with a 3% aqueous H_2O_2 solution for 5 min, the sections were reacted with primary antibodies (anti-HGF-α antibody, 1/50 dilution; anti c-Met antibody, 1/50 dilution; anti-phospho-c-Met [Tyr1235] antibody, 1 μg/mL; anti-phospho-Erk antibody, 1/50 dilution; anti-phospho-Akt antibody, 1/50 dilution; and anti-phospho-Stat3 antibody, 1/50 dilution) for 1 h at room temperature, washed with Tris-buffered saline (TBS), treated with an appropriate secondary antibody for 1 h at room temperature, and allowed to react for 30 min with the avidin–biotin–peroxidase complex (ABC) using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA). The DAB (3,3′-diaminobenzidine tetrahydrochloride) Liquid System (DakoCytomation, Glostrup, Denmark) was used to detect immunostaining. Omission of primary antibodies served as negative controls. Immunoreactivity was evaluated independently by two investigators (Y. N. and A. G.).

Evaluation of immunohistochemical results. c-Met immunoreactivities were evaluated as follows in accordance with the method of Tsao *et al*. with slight modifications:[11] –, complete absence of staining or only focal weak staining; 1+, weak to moderate staining in less than 40% of cancer cells; 2+, weak to moderate staining in at least 40% of cancer cells; 3+, strong staining in at least 10% of cancer cells, among the specimens with weak to moderate staining in at least 40% of cancer cells. Weak to moderate staining was defined as staining similar to or weaker than the staining of normal bronchial epithelium; strong staining was defined as staining clearly more intense than the staining of normal bronchial epithelium. Cases were then divided into two groups, either c-Met low (– or 1+) or c-Met high (2+ or 3+) when correlations with other parameters were analyzed. Immunoreactivities for phospho-Erk, phospho-Akt and phospho-Stat3 were judged as positive when more than 5% of cancer cells exhibited cytoplasmic or nuclear staining, according to the criteria of Han *et al*.[21] Phospho-c-Met immunoreactivities were evaluated as either positive or negative, with a cut-off value of 5% of positively stained cancer cells.[21] HGF immunoreactivities were evaluated as either high or low, with a cut-off value of 50% of positively stained cancer cells, according to the method of Masuya *et al*.[12]

Statistical analysis. Associations were determined by the χ^2-test. The Kaplan–Meier method was used to analyze patient survival. The Wilcoxon method was used to evaluate the statistical significance of the results. A *P*-value of less than 0.05 was considered to indicate statistical significance.

Results

Immunohistocytochemical analysis of HGF, c-Met and phospho-c-Met in human lung adenocarcinoma specimens. Immunoreactivity for phospho-c-Met was consistently absent in non-tumorous lung tissue with a normal appearance (Fig. 1a). In contrast, faint c-Met immunoreactivity was observed in bronchial epithelia, bronchiolar epithelia, and type II pneumocytes. Immunoreactivities were generally observed in the apical cytoplasm of these cells. The staining intensity was weak irrespective of the tumor size or distance from the tumor (Fig. 1b).

Immunoreactivity for phospho-c-Met was found exclusively in the adenocarcinoma cells. Phospho-c-Met was positive in 28 of 130 cases (21.5%) overall. Two patterns of phospho-c-Met immunolocalization were noted in the 28 cases positive for phospho-c-Met. The first was localization of phospho-c-Met mainly in the apical cell membrane or cytoplasm (13 cases) and apparent lamellipodial protrusion into the alveolar space (Fig. 1c). Intense membranous staining was observed occasionally in cancer cells that appeared to be floating in alveoli. The second pattern of phospho-c-Met immunolocalization was granular cytoplasmic staining (15 cases) (Fig. 1d).

c-Met was positive (1+, 2+ or 3+) in 97 cases (74.6%), strongly expressed (2+ or 3+) in 47 cases (36.1%), and weakly expressed (1+) in 50 cases (38.5%). c-Met immunoreactivity was found in the cytoplasm and membranes of the cancer cells (Fig. 1e). Comparison between phospho-c-Met and c-Met revealed distinct staining patterns in some cases. c-Met was localized mainly in the basolateral aspect of the cytoplasm, whereas phospho-c-Met was localized in the apical cytoplasm (Fig. 2a,b). There was considerable intratumoral heterogeneity with regard to phospho-c-Met expression. The relationship between intratumoral distribution of phospho-c-Met-positive cells and histological pattern was not evident, however, except for the overall correlation between papillary morphology and phospho-c-Met expression (see below). Interestingly, phospho-c-Met stained micropapillary clusters preferentially in one case, but this characteristic localization was not observed in c-Met staining (Fig. 2c,d). c-Met was also positive in the stromal fibroblasts of some cases. Staining for HGF in separate experiments revealed high levels of HGF expression in the cytoplasm of cancer cells in 41 of 130 cases (31.5%) (Fig. 1f). Immunoreactivity for HGF was additionally observed in the stromal cells in four cases, although HGF expression was found predominantly in the cancer cells.

Phospho-c-Met, c-Met and HGF expression and clinicopathological correlations. We next examined whether the expression of phospho-c-Met, c-Met and HGF was correlated with the clinicopathological parameters. To do so, we categorized the cases into two groups for each parameter: phospho-c-Met positive or negative, c-Met high (2+ or 3+) or low (– or 1+), and HGF high or low. The results are shown in Table 1. Interestingly, phospho-c-Met expression correlated positively with the papillary subtype of the WHO classification ($P = 0.0011$). Phospho-c-Met expression was also correlated with higher differentiation ($P = 0.0023$) and an absence of vascular invasion ($P = 0.0266$). No correlations were found between phospho-c-Met expression and age, pathological stage, or lymph node metastasis. As with phospho-c-Met, strong c-Met expression was significantly correlated with the papillary subtype of the WHO classification ($P = 0.0056$). Unlike phospho-c-Met, however, it had no correlation with tumor differentiation. In keeping with the results of previous studies, high levels of c-Met expression correlated with higher pathological stage (≥IIIA) ($P = 0.0060$) and the presence of nodal metastasis ($P = 0.0010$).[22] There was no correlation between papillary histology and tumor differentiation ($P = 0.2320$).

Correlations between phospho-c-Met, c-Met and HGF expression. The overall relationships among phospho-c-Met, c-Met and HGF expression are shown in Fig. 3. High levels of HGF expression and high levels of c-Met expression were both correlated with phospho-c-Met expression ($P = 0.0010$ and $P = 0.0303$, respectively). Notably, 12 cases with little to no HGF expression were positive for phospho-c-Met.

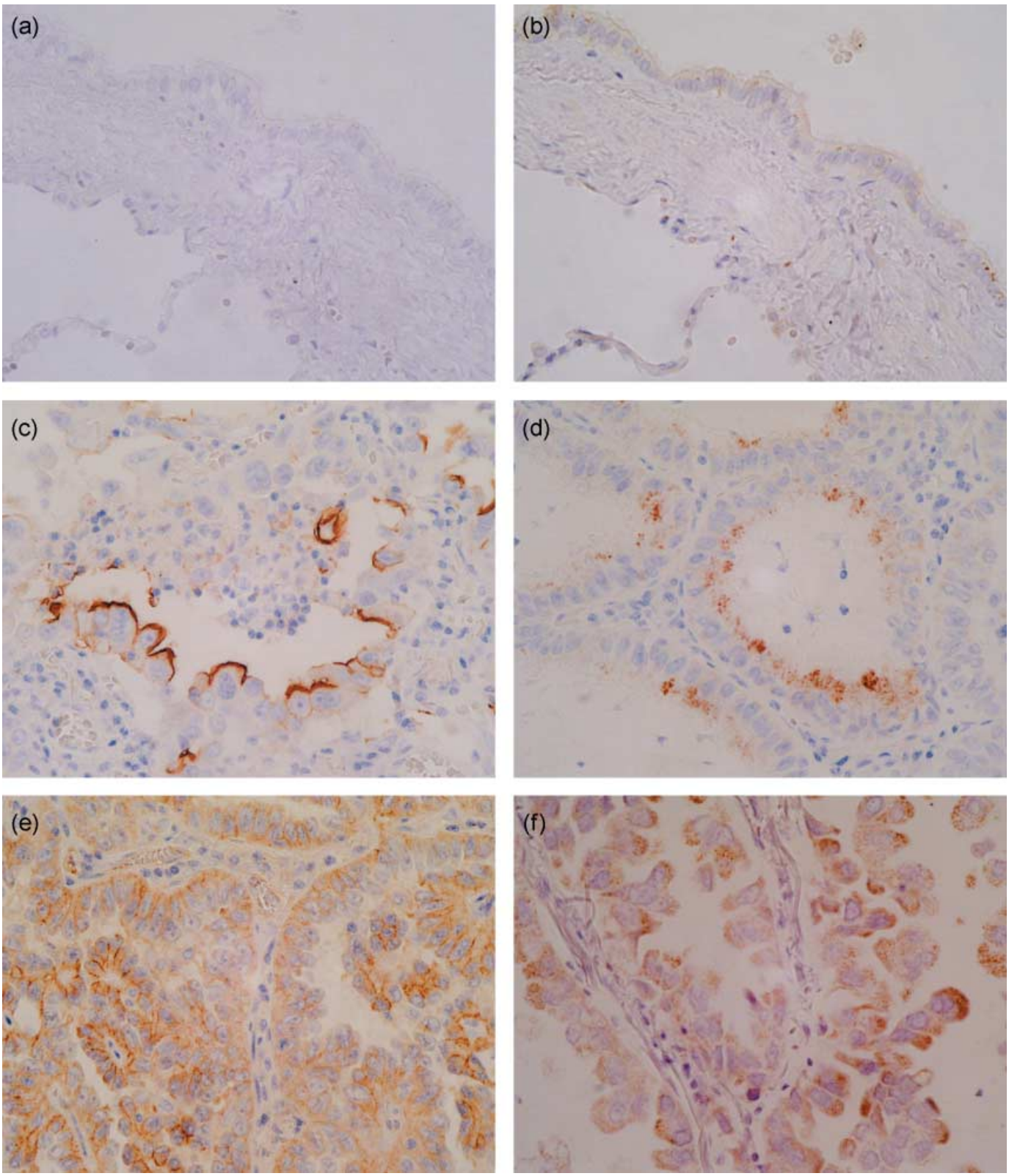

Fig. 1. Expression of phospho-c-Met, c-Met, and hepatocyte growth factor (HGF) in normal and cancerous lung tissues. Phospho-c-Met expression was absent in bronchiolar epithelium and type II pneumocytes in normal lung (a), while c-Met was faintly positive in these cells (b). Adenocarcinoma cells showing positive staining for phospho-c-Met (c and d), c-Met (e), and HGF (f). Both membranous and granular cytoplasmic staining patterns were observed for phospho-c-Met (c and d). c-Met staining was found in the cytoplasms and membranes of cancer cells (e). HGF staining was mainly found in the cytoplasms of cancer cells (f).

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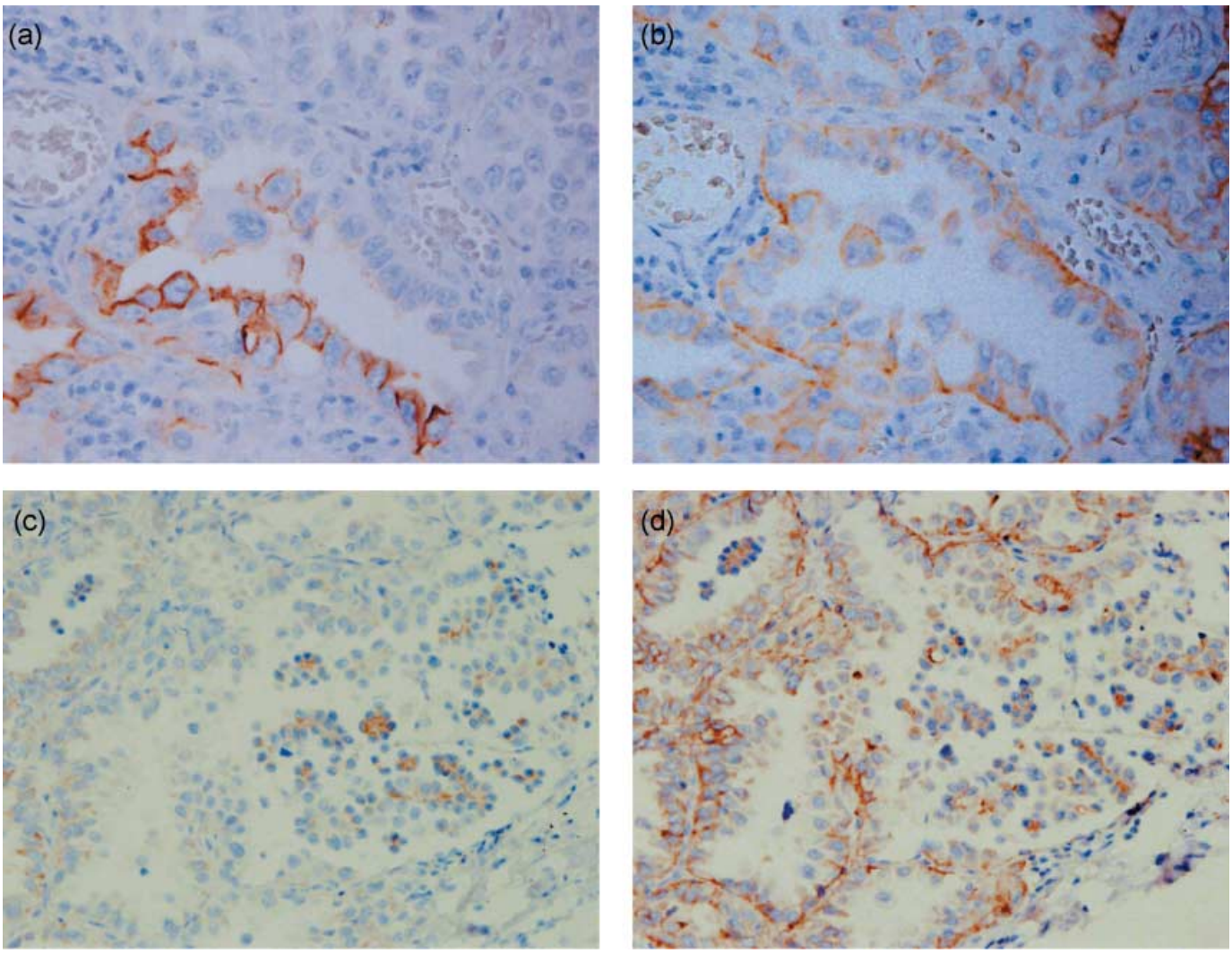

Fig. 2. Comparison of the staining patterns of phospho-c-Met and c-Met. Serial sections stained for phospho-c-Met (a, c) and c-Met (b, d). A distinct staining pattern was observed: phospho-c-Met staining was mainly seen in the membrane facing the alveolar space (a), whereas c-Met staining was concentrated in the basolateral aspects (b). In one case, phospho-c-Met preferentially stained micropapillary clusters (c), but such localization was not found in c-Met staining (d).

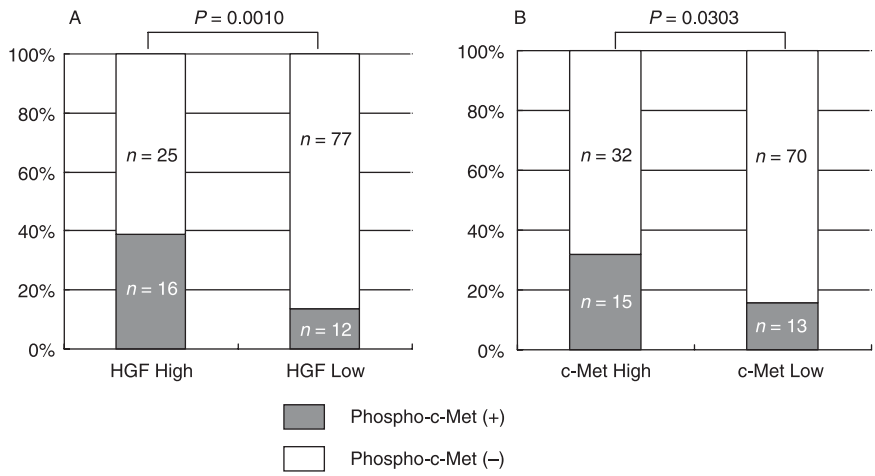

Fig. 3. The overall relationships between phospho-c-Met, c-Met, and hepatocyte growth factor (HGF) expression. High levels of HGF expression correlated with phospho-c-Met expression ($P = 0.0010$). High levels of c-Met expression also correlated with phospho-c-Met expression ($P = 0.0303$).

Table 1. Clinicopathological correlations with phospho-c-Met, c-Met and hepatocyte growth factor (HGF) expression

	Phospho-c-Met		*P*-value	c-Met		*P*-value	HGF		*P*-value
	+	–		High	Low		High	Low	
Age (years)									
<65	9	40	0.4939	25	24	**0.0061**	19	30	0.1672
≥65	19	62		22	59	22	59		
Sex									
Male	17	65	0.7699	29	53	0.8069	27	55	0.6561
Female	11	37		18	30	14	34		
Differentiation									
Well	24	55	**0.0023**	27	52	0.5593	28	51	0.2331
Moderate–poor	4	47		20	31	13	38		
Histological subtype									
Papillary	21	41	**0.0011**	30	32	**0.0056**	25	37	*0.0396*
Non-papillary	7	61		17	51	16	52		
P-stage									
≤IIB	21	70	0.5145	26	65	**0.0060**	32	59	0.1741
≥IIIA	7	32		21	18	9	30		
Size (cm)									
<3	17	61	0.9306	27	51	0.6548	27	51	0.3551
≥3	11	41		20	32	14	38		
(v)									
Positive	4	37	**0.0266**	15	26	0.9446	7	34	*0.0160*
Negative	24	65		32	57	34	55		
(ly)									
Positive	6	28	0.5207	15	19	0.2607	9	25	0.4593
Negative	22	74		32	64	32	64		
(p)									
Positive	11	49	0.4105	24	36	0.3981	16	44	0.2684
Negative	17	53		23	47	25	45		
(pm)									
Positive	5	11	0.3129	11	5	0.0740	4	12	0.5478
Negative	23	91		23	91	37	77		
(LN)									
Positive	5	24	0.5231	18	11	**0.0010**	8	21	0.6033
Negative	23	78		29	72		33	68	

LN, lymph node metastasis; ly, lymphatic permeation; p, pleural invasion; pm, pulmonary metastasis; v, vessel invasion. Statistically significant values are in bold type.

Table 2. Correlations of phospho-c-Met and phospho-epidermal growth factor receptor (EGFR) with phospho-Erk, phospho-Akt and phospho-Stat3 expression

		phospho-Erk			phospho-Akt			phospho-Stat3		
		+	–		+	–		+	–	
phospho-c-Met	+	13	8	*P* = 0.1717	19	2	*P* = 0.0381	14	7	*P* = 0.3089
	–	38	46		57	27		65	19	
phospho-EGFR	+	45	40	*P* = 0.0648	65	20	*P* = 0.0533	67	18	*P* = 0.0793
	–	6	14		11	9		12	8	
phospho-c-Met OR	+	47	46	*P* = 0.0618	72	21	***P* = 0.0013**	69	24	*P* = 0.4900
phospho-EGFR	–	4	8		4	8		10	2	

Statistically significant values are in bold type.

Correlations of phospho-c-Met and phospho-EGFR with phospho-Erk, phospho-Akt and phospho-Stat3 expression. Next, we investigated the expression of phospho-Erk (Thr202/Tyr204), phospho-Akt (Ser473) and phospho-Stat3 (Ser727), the three downstream signaling molecules that mediate the biological effects of receptor-type tyrosine kinase. We also investigated the expression of phospho-EGFR (Tyr1085) for comparison, because it has been shown that activation of EGFR leads to phosphorylation of these three signaling molecules.[23] Although not all cases were available for further immunohistochemical analysis, we analyzed a total of 105 cases for phospho-Erk, phospho-Akt, phospho-Stat3 and phospho-EGFR. The results are shown in Fig. 4. Cytoplasmic staining was mainly observed for phospho-Erk (48.6%, 51/105 cases), phospho-Akt (72.4%, 76/105 cases) and phospho-Stat3 (75.2%, 79/105 cases) (Fig. 4a–c). In addition, nuclear staining was observed for phospho-Erk and phospho-Stat3 in some cases.[21] Phospho-EGFR was positive in 95 of 105 cases (88.0%). Staining for phospho-EGFR was predominantly cytoplasmic (Fig. 4d). Table 2 shows the correlations of phospho-c-Met and phospho-EGFR with phospho-Erk, phospho-Akt

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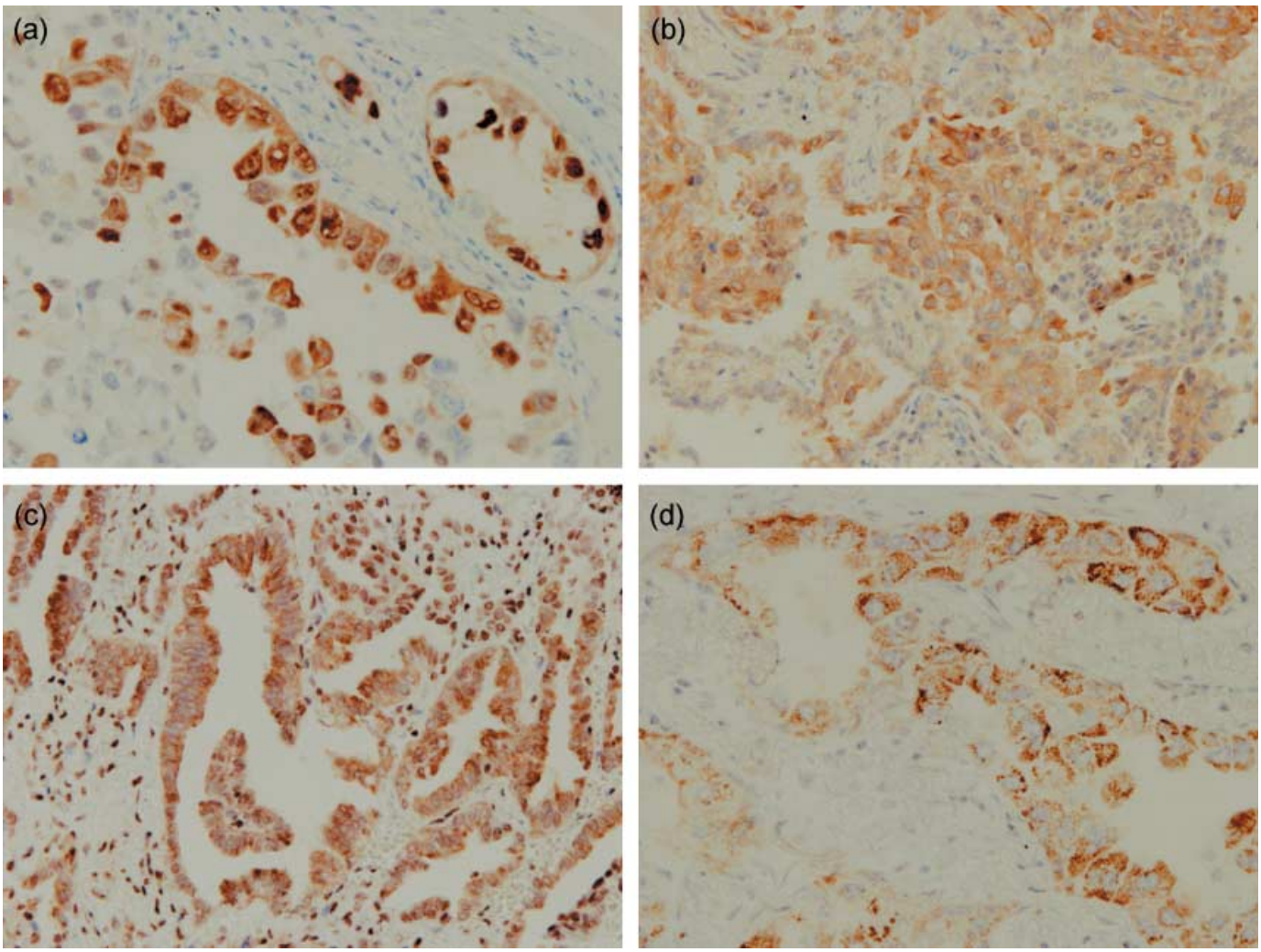

Fig. 4. Expressions of phospho-Erk, phospho-Akt, phospho-Stat3, and phospho-epidermal growth factor receptor (EGFR) in lung adenocarcinomas. Cytoplasmic staining was mainly observed for phospho-Erk (a), phospho-Akt (b), and phospho-Stat3 (c). Nuclear staining was additionally observed in some cases (a). Staining for phospho-EGFR was predominantly cytoplasmic (d).

and phospho-Stat3. Phospho-c-Met was not correlated with phospho-Erk ($P = 0.1717$) or phospho-Stat3 ($P = 0.3089$), but it was marginally correlated with phospho-Akt ($P = 0.0381$). Phospho-EGFR (Y1085) was marginally correlated with phospho-Erk ($P = 0.0648$), phospho-Akt ($P = 0.0533$) and phospho-Stat3 ($P = 0.0793$). Further analysis showed that phosphorylation of either c-Met or EGFR was strongly correlated with the expression of phospho-Akt ($P = 0.0013$), suggesting that EGFR and c-Met play complementary roles in activating the Akt pathway. Phospho-c-Met expression was not correlated with that of phospho-EGFR (data not shown).

Patient survival according to the expression of HGF, c-Met and phospho-c-Met. Survival analysis using the Kaplan–Meier method was carried out to assess the prognostic significance of HGF, c-Met and phospho-c-Met expression in lung adenocarcinoma patients. As shown in Fig. 5, there were no significant differences in survival among cases grouped according to their expression of HGF, c-Met and phospho-c-Met.

Discussion

Several studies have reported the overexpression of HGF or c-Met in lung cancer and have investigated the correlations between this overexpression and clinicopathological parameters.[10–12,22,24,25] Tsao *et al*., for instance, observed the overexpression of c-Met mRNA and protein in 35% of lung adenocarcinomas and confirmed a correlation between c-Met overexpression and higher tumor differentiation.[11] Takanami *et al*. reported an association between c-Met overexpression and shorter patient survival.[22] At the present time, however, there is no direct evidence that c-Met overexpression actually leads to receptor activation. Ma *et al*. recently examined c-Met activation immunohistochemically in a limited number of lung adenocarcinomas using a commercially available antiphospho-c-Met antibody.[26] Their results confirmed c-Met activation in some of the adenocarcinomas, as well as in other types of lung cancers investigated in their study. The number of cases analyzed was rather limited, however, and no analyses were undertaken to identify the clinicopathological features of the tumors with c-Met activation.[26]

In the present study we carried out a series of immunohistochemical analyses of lung adenocarcinoma tissues using an antiphospho-c-Met (Tyr1235) antibody recently developed by Inoue *et al*.[14] Using this antibody, phospho-c-Met was positive in 28 of the 130 cases (21.5%) included in our study, and phospho-c-Met expression correlated with HGF expression ($P = 0.0010$). We know that some HGF immunoreactivity may represent the

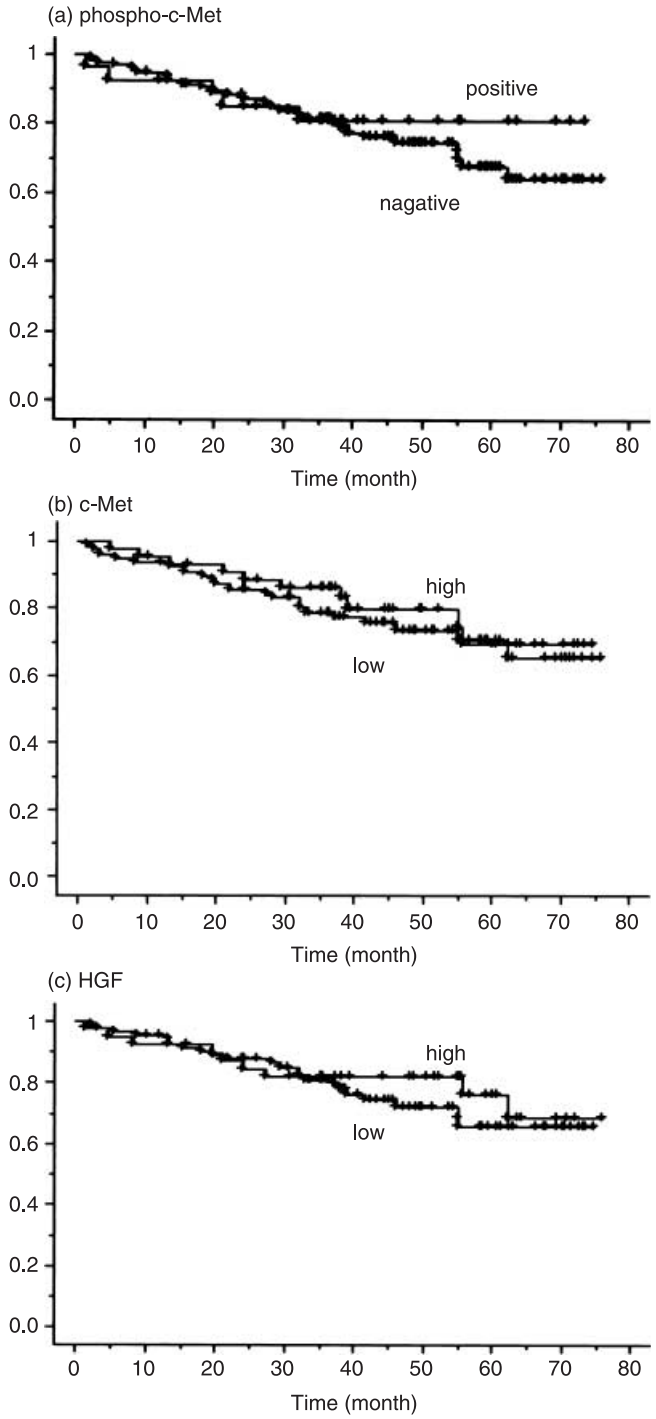

Fig. 5. Survival analysis. Patient survival according to the expressions of phospho-c-Met expression (a), c-Met (b), and hepatocyte growth factor (c). There were no significant differences in survival.

inactive form of HGF, given that HGF is secreted as an inactive precursor that requires extracellular activation by proteolytic cleavage.[27] Overall, however, the correlation between phospho-c-Met and HGF expression supports the notion that the HGF/c-Met loop may be activated in an autocrine fashion in cancer cells.[28,29] We were interested to note that phospho-c-Met expression also correlated with high levels of c-Met expression ($P = 0.0303$), and that 12 cases (9.2%) were positive for phospho-c-Met among tumors with little to no HGF expression. This suggests that some tumors may acquire mechanisms for the ligand-independent activation of c-Met in the same manner as observed in cell lines,[13] probably via c-Met overexpression. Thus, c-Met activation may occur in either a ligand-dependent (autocrine) or a ligand-independent manner in lung adenocarcinoma tissues. This conclusion is supported by our *in vitro* experiments that demonstrate the constitutive activation of c-Met by a ligand-independent mechanism in a significant proportion of lung adenocarcinoma cell lines (Nakamura Y *et al*., unpublished data). The findings of this study have important clinical implications from a therapeutic standpoint. Investigators have envisioned approaches to disrupt HGF/c-Met signaling using strategies such as anti-HGF antibody, an NK4 antagonist, ribozymes, a dominant negative c-Met mutant, pharmacological inhibitors and siRNA.[8,9] Among these strategies, the first two may be of only limited effect against tumors with ligand-independent activation of c-Met.

The ligand-independent activation of the c-Met receptor may occur by gene mutation (usually missense mutation) and c-Met overexpression with or without gene amplification.[8,9] Amplification of the *c-Met* gene has been described for gastric and colonic adenocarcinomas.[8,9] Missense mutations of *c-Met* have been found in the kinase domains of papillary-type renal cell carcinoma, ovarian cancer, head and neck squamous cell carcinoma, and childhood hepatocellular carcinomas.[9] Ma *et al*. recently identified missense mutations in the juxtamembrane and extracellular (Sema) domains of c-Met in small-cell carcinoma and adenocarcinomas of the lung.[26,30] A goal for further studies will be to determine the frequencies of mutation and amplification of the c-Met gene in lung adenocarcinoma.

It is of interest that c-Met and phospho-c-Met overexpression were correlated with histological features of adenocarcinoma, such as papillary histology. In thyroid carcinoma, c-Met expression occurs more frequently in papillary carcinoma than in follicular and undifferentiated carcinoma.[31] Germline and somatic activating c-Met mutations are causally linked with hereditary and sporadic forms of papillary renal cell carcinomas, respectively.[8,9] These observations (including ours) strongly suggest that activation of c-Met leads to formation of papillary structures in carcinomas of diverse origins. Because c-Met is involved in branching tubulogenesis of the lung[32] and other organs[8] it is interesting to investigate whether excessive or inappropriate activation of c-Met converts tubules into papillary structure. c-Met activation was also associated with higher differentiation and absence of vascular invasion. Both facts might seem paradoxical, in view of the role of c-Met in cell growth, invasion and metastasis.[8,9] Interestingly, Belfiore *et al*. reported similar findings for thyroid carcinomas;[31] high c-Met expression was inversely associated with vascular invasion and negative or low c-Met expression was associated with distant metastasis. One explanation would be that in poorly differentiated tumors, molecular abnormalities other than c-Met overexpression and activation may be responsible for the malignant properties of cancer, such as invasion and metastasis. Alternatively, activation of c-Met may require integrity of cell–cell or cell–matrix adhesion, and therefore may occur less efficiently in poorly differentiated tumors.[33–35] c-Met expression was correlated with higher pathological stage and lymph node metastasis, but it had no influence on patient survival in this study. This somewhat puzzling result could be explained if we assume that the association is not causally linked. In fact, the prognostic impact of c-Met expression was shown only by univariate analysis in previous studies.[12,22]

c-Met and phospho-c-Met showed distinct cellular localization in some cases. c-Met was often preferentially localized in basolateral aspects of the cells, whereas phospho-c-Met tended to localize in the apical portion. In this setting, it is interesting that Vadnais *et al*. observed a localization of phosphorylated c-Met in lamellipodial protrusions in MDCK cells transformed by c-Met overexpression.[36] Trusolino *et al*. showed that constitutively active c-Met selectively merged with with α_4 integrin to form a

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complex essential for cell migration.[37] This tempts us to speculate that cancer cells stained positively for phospho-c-Met in apical cell membrane represent HGF-stimulated cells with lamellipodial protrusions.

Also interesting was the finding that phosphorylation of either c-Met or EGFR was strongly correlated with expression of phospho-Akt ($P = 0.0013$). This suggests that EGFR and c-Met play complementary roles in activating the Akt pathway. Activation of Akt is causally related to antiapoptotic characteristics of tumors.[23] It is therefore conceivable that inhibition of the c-Met pathway may provide an alternative therapeutic approach in lung adenocarcinomas with resistance to EGFR inhibitors.

In summary, the results of the present study suggest that the c-Met activation in lung adenocarcinomas may be induced via ligand-dependent (autocrine) and ligand-independent mechanisms, the latter possibly through c-Met overexpression. c-Met activation may play special roles in the papillary subtype and in well differentiated lung adenocarcinomas. c-Met may be an alternative pathway to EGFR in the activation of Akt in lung adenocarcinomas. Further studies on the mechanisms underlying the ligand-independent activation of c-Met are warranted.

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