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High *MET* copy number and MET overexpression: Poor outcome in non-small cell lung cancer patients

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Summary. The aim of this study was to evaluate the prevalence and prognostic role of increased gene copy number and protein expression of *MET* and *EGFR* in non-small cell lung cancer (NSCLC) patients. Samples were collected from 380 patients with surgically resected NSCLC, and fluorescence in situ hybridisation (FISH) and immunohistochemistry (IHC) were performed. *EGFR* amplification and high polysomy (*EGFR* FISH-positive) were observed in 9.7% and 17.4% of the patients, respectively. EGFR was overexpressed (EGFR IHC-positive) in 19.2% of the patients. Neither *EGFR* FISH-positive nor EGFR IHC-positive status affected survival after resection. Increased *MET* copy number (*MET* FISH-positive by University of Colorado Cancer Center criteria) was observed in 11.1% of the patients (high polysomy, 8.7%; gene amplification, 2.4%). According to the Cappuzzo system, 7.1% of the patients were *MET* FISH-positive. *MET* FISH positivity was a negative prognostic factor, especially in patients with adenocarcinoma histology (p=0.040), female gender (p=0.010), old age (p=0.084), and *EGFR* FISH negativity (p=0.020) at the univariate level but not at the multivariate level. MET was overexpressed (MET IHC-positive) in 13.7% of the patients and associated with shorter overall and disease-free survival (p=0.010 and p=0.056, respectively). Multivariate analysis revealed that MET IHC-positive patients had a significantly increased risk of death (hazard ratio, 1.618; 95% confidence interval, 1.066-2.456; p=0.024). Increased *MET* copy number and MET overexpression are negative prognostic factors for surgically resected NSCLCs.

Key words: MET, EGFR, Non-small cell lung cancer, FISH, Gene copy number

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Introduction

Novel targeted agents that act on specific signalling molecules of cellular growth pathways have emerged as effective agents for treating non-small cell lung cancer (NSCLC). Patients with NSCLC with mutations in the epidermal growth factor receptor (EGFR) tyrosine kinase domain achieve response rates exceeding 70% and superior progression-free survival when treated with an EGFR tyrosine kinase inhibitor (TKI) compared with standard chemotherapy (Ramalingam et al., 2011). The identification of patients most likely to derive clinical benefit from these drugs is of paramount importance. The discovery of reliable markers that will enable clinicians to proactively select patients who will gain the most from targeted therapy should provide a substantial clinical advantage for patients with NSCLCs. Some randomised trials of EGFR TKIs have evaluated survival benefits on the basis of EGFR expression, *EGFR* copy number, and *EGFR* mutation status (Bell et al., 2005; Eberhard et al., 2005; Herbst et al., 2005; Tsao et al., 2005; Hirsch et al., 2006; Yoshida et al., 2010). At the same time, the therapeutic inactivation of essential kinases creates selective pressures for the development

of resistance mechanisms. Patients initially sensitive to anti-EGFR strategies invariably become resistant. Several mechanisms, including the T790M mutation, are responsible for primary and acquired resistance to EGFR TKIs, with *MET* amplification emerging as one of the most critical events responsible for acquired resistance (Engelman et al., 2007; Nguyen et al., 2009; Suda et al., 2010). *MET* is a proto-oncogene that encodes a receptor tyrosine kinase for hepatocyte growth factor (HGF)/scatter factor (SF). MET is known to be overexpressed in various cancer tissues and associated with disease progression (Ma et al., 2003). The number of pharmaceutical and biotechnology companies that have announced drug development programs targeting MET TK has grown. The relevance of MET as a mechanism of escape of NSCLC from anti-EGFR therapy needs to be investigated in preclinical and clinical studies (Arteaga, 2007; Engelman et al., 2007; Cappuzzo et al., 2009). Previous studies reported *MET* amplification in up to 10% of gastric cancers, in 4% of oesophageal cancers, and in endometrial cancer. The rate of MET amplification in NSCLC remains controversial, and ranges from 3% to up to 10% depending on the detection technique and cut-off criteria (Cappuzzo et al., 2009; Go et al., 2010; Toschi and Capuzzo, 2010). Only a few studies evaluated whether *MET* copy number influenced patient survival (Okuda et al., 2008; Cappuzzo et al., 2009; Go et al., 2010). Furthermore, MET overexpression in NSCLC is variable, ranging from 5% to 75%, and only a few reports have correlated biological findings with prognosis (Nakamura et al., 2007; Ma et al., 2008). Most of the studies reported thus far have consistently indicated a negative prognostic impact of MET overexpression on survival in lung cancer, providing the rationale for the development of strategies aimed at disrupting MET (Toschi and Capuzzo, 2010).

There are few large-scale studies of the clinical and biological features and prognostic significance of *MET* copy number and protein expression in the same cohort of NSCLC patients from a prognostic viewpoint. Accordingly, we investigated the prevalence and prognostic role of increased gene copy number and protein expression for *MET* and *EGFR* in NSCLC patients.

Materials and methods

Study population

We reviewed H&E slides, clinical charts, and pathological reports of surgically resected primary lung masses from 380 NSCLC patients seen between September 1994 and December 2001 at Samsung Medical Center. No patients received EGFR-targeted anticancer therapy during the follow-up period. Appropriate chemotherapy and radiotherapy were performed as indicated. The World Health Organization Classification of Tumors was used to determine histological classification (Travis, 2004).

TNM classification and staging were performed adequately in all patients. The follow-up period ranged from 1 to 167 months (mean [S.D.], 42.2[32.1]). Clinical evidence of tumour recurrence, metastasis, and death due to the disease were regarded as failures. The institutional review board at Samsung Medical Center approved the pathological review and study of archived tumour tissues.

Tissue array methods

Two representative 2-mm-diameter cores were taken from a paraffin-embedded donor tissue block per case, and these were arranged in new recipient paraffin blocks using a trephine apparatus (ISU abxis, Seoul, Korea). In cases with variable histologic features, we selected the most predominant area to construct the tissue microarray. Serial sections from tissue microarray blocks were subjected to FISH and IHC. Accordingly, the target areas of FISH and IHC were nearly identical for each tumor. An adequate case was defined as the tumor occupying more than 50% of the core area.

Immunohistochemistry (IHC)

EGFR expression was evaluated by IHC using modified methods and assessment criteria described previously (Dziadziuszko et al., 2006; Hirsch et al., 2007) with mouse monoclonal anti-EGFR antibody (NCL-EGFR-384; Novocastra Laboratories, Newcastle, UK) at a 1:100 dilution. MET was detected by IHC using rabbit polyclonal anti-MET antibody (3D4;Zymed Laboratories, San Francisco, CA, USA) at a 1:100 dilution according to the manufacturer's protocol. Semi-quantitative assessment of the IHC stain results was performed by 2 pathologists (Y.L.C and S.P) who were unaware of the clinicopatholoigcal details and the fluorescence in situ hybridisation (FISH) results. Only membranous staining was defined as positive. The IHC pattern was relatively homogenous, and thus the score was determined by predominant intensity. The expression was scored on the basis of the intensity and fraction of positive cells. The intensity score was defined as follows: 0=no appreciable staining in the tumour cells, 1=faint/barely perceptible partial membrane staining, 2=weak to moderate staining of the entire membrane, and 3=strong staining of the entire membrane. The fraction score was defined as follows: 0=less than 5%, 1=from 5% to 25%, 2=from 26% to 50%, 3=from 51% to 75%, and 4=more than 75%. The total score was calculated by multiplying the intensity score and the fraction score, producing a total range of 0-12. For statistical analyses, scores of 0-3 were considered negative, and scores of 4-12 were considered positive.

FISH Analyses

EGFR and *MET* copy numbers per cell were investigated on formalin-fixed paraffin-embedded

specimens using Dual-color Vysis kits (Vysis, Downers Grove, IL for *EGFR* and Kreatech Diagnostics, Amsterdam, The Netherlands for *MET*). Briefly, representative 4 μm sections of microarray blocks were deparaffinized, dehydrated, immersed in 0.2N HCl, boiled in a microwave in citrate buffer (pH 6.0), and incubated in 1M NaSCN for 35minutes at 80°C. Sections were then immersed in pepsin solution, and the tissues were fixed in 10% neutral-buffered formalin. The probe was applied and the sections were appropriately covered and sealed. The slides were incubated in a humidified atmosphere (Hybrite, Vysis) at 73°C for 5 minutes and at 37°C for 19 hours followed by immersion in 0.4 x SSC/0.3% NP-40 at room temperature and at 73°C for 5 minutes. After drying, nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI). FISH signals for each locus-specific FISH probe were assessed under an Olympus BX51TRF microscope (Olympus, Tokyo, Japan) equipped with a triple-pass filter (DAPI/Green/Orange; Vysis, Downers Grove, IL). The entire area of the tissue microarray core was evaluated in each case, and as many non-overlapping nuclei as possible were assessed for orange (marker) and green (reference) signals by two pathologists (S.P., Y.L.C.) blinded to any information about the patients. By using the University of Colorado Cancer Center (UCCC) criteria, *EGFR* and *MET* status was classified into six categories according to the frequency of tumor cells with specific numbers of copies as described elsewhere (Hirsch et al., 2003; Cappuzzo et al., 2005): disomy (≤2 copies in >90% of cells); low trisomy (≤2 copies in ≥40% of cells, 3 copies in 10-40% of cells, and ≥4 copies in <10% of cells); high trisomy (≤2 copies in ≥40% of cells, 3 copies in ≥40% of cells, and ≥4 copies in <10% of cells); low polysomy (≥4 copies in 10-40% of cells); high polysomy (≥4 copies in ≥40% of cells); and gene amplification (presence of tight gene clusters and a ratio of the *EGFR* or *MET* to chromosome 7 of ≥2 or 15≥ copies of *EGFR* or *MET* per cell in ≥10% of cells). Patients were further classified into two groups based on *EGFR* or *MET* status: negative (disomy, low trisomy, high trisomy, and low polysomy) and positive (high polysomy and gene amplification). Alternatively, by using the Cappuzzo scoring system, cases were classified into those with a mean of more than or equal to 5 copies per cell and with a mean of less than 5 copies per cell (Cappuzzo et al., 2009).

Statistical analysis

Pearson's χ^2 testor Fisher's exact test was used to examine the relationships between groups. Each *p*-value was corrected by Bonferroni's method for multiple testing. Median age was compared by Wilcoxon two-sample test between squamous cell carcinoma (SCC) and adenocarcinoma (ADC) patients. Overall survival (OS) and disease-free survival (DFS) were determined and plotted using the Kaplan-Meier method with the log-rank test for comparison between groups. The Cox proportional hazards model was used to calculate the relative risks regarding the DFS and OS rates. The results were adjusted for age group, sex, smoking, histology, stage, and *EGFR* or *MET* copy status and protein expression. SPSS software (version 16.0; SPSS Inc., Chicago, IL) was used for statistical analyses. All statistical tests were2-sided, and *p*-values less than 0.05 were considered significant.

Results

Patient characteristics

The clinicopathological features of the patients are summarised according to histological types in Table 1. A total of 380 surgically resected NSCLC patients were included in this study. The majority of the patients were male (81.1%), and the median age was 62.0 years. All patients received radical surgery, with evidence of pathological stages III and IV in 24.2% and 2.1% of the patients, respectively. The proportions of SCC and ADC including bronchioloalveolar carcinoma were 64.5% (n=245) and 35.5% (n=135), respectively. The majority of SCC patients (95.9%) were male, whereas the male-to-female ratio among ADC patients was 1.17:1.

Correlation of EGFR copy number and EGFR expression in NSCLC patients

EGFR copy number was evaluated by FISH, and 103 patients (27.1%) were considered FISH-positive according to previously described criteria (Varella-Garcia, 2006). Among them, *EGFR* disomy, low trisomy, high trisomy, low polysomy, high polysomy, and gene amplification were present in 125 (32.9%), 54 (14.2%), 11 (2.9%), 87 (22.9%), 66 (17.4%), and 37 patients (9.7%), respectively. Overall, *EGFR* FISH positivity as represented by high polysomy and gene amplification was observed in 27.1% of the patients. *EGFR* FISH positivity was more common in ADC patients than in SCC patients (44/135[32.6%] vs. 59/245 [24.1%]; p=0.049). The difference in median OS between the *EGFR* FISH-positive and FISH-negative groups was not statistically significant (31.73 vs.39.26 months; p=0.3). Overall, there were no significant differences in *EGFR* status (*EGFR* FISH-positive vs. FISH-negative) based on most clinicopathological characteristics, including histology, age, sex, smoking status, and stage. *EGFR* FISH positivity was more frequent in *MET* FISH-positive patients than in FISH-negative patients (18/42[42.9%] vs. 85/338[25.1%]; p=0.026). According to IHC data, EGFR protein overexpression was detected in 73 patients (19.2%) (Fig.1A). EGFR IHC positivity was more frequent in SCC patients than in ADC patients (60/245[24.5%] vs. 13/135[9.6%]; p<0.01). There were no significant differences in EGFR expression status (EGFR IHC-positive versus IHC-negative) based on other clinicopathological characteristics including age, sex,

smoking status, and stage. The difference in survival between the EGFR IHC-positive and IHC-negative groups was not statistically significant. EGFR overexpression was significantly more frequent in *EGFR* FISH-positive patients than in EGFR FISH-negative patients (47/103[45.6%] vs. 26/277[9.4%]; $p<0.01$).

Correlation of MET copy number and MET expression in NSCLC patients

MET copy number was evaluated by FISH, and 42 cases (11.1%) were considered FISH-positive according to the criteria used for *EGFR* evaluation (Fig.2) (Varella-Garcia, 2006). MET disomy, low trisomy, high trisomy, low polysomy, high polysomy, and gene amplification were present in 238 (62.6%), 33 (8.7%), 8 (2.1%), 59 (15.5%), 33 (8.7%), and 9 patients (2.4%), respectively. Overall, *MET* FISH positivity as represented by high polysomy and gene amplification was observed in 11.1% of the patients (SCC: 30/245[12.2%]; ADC, 12/135[8.9%]). *MET* FISH positivity was significantly associated with advanced stage ($p=0.011$), EGFR FISH positivity ($p=0.029$), and MET IHC positivity ($p<0.001$) (Table 2). *MET* FISH-positive patients exhibited worse OS and DFS than *MET* FISH-negative patients, and the difference was statistically significant in patients with ADC histology ($p=0.04$), female gender ($p=0.01$), and *EGFR* FISH negativity ($p=0.02$), as well as in all NSCLC patients ($p=0.048$) (Fig. 3). Among the *MET* FISH-positive NSCLCs, patients with gene amplification according to University of Colorado Cancer Center (UCCC) criteria exhibited worse OS than those with high polysomy. When *MET* FISH positivity was defined according to the criteria of Cappuzzo, the patients with high *MET* copy number (≥5 per cell) tended to have shorter OS and DFS than those with low *MET* copy number (<5 per cell) (Fig. 3A).

According to IHC data, MET protein overexpression was observed in 52 patients (13.7%) (Fig. 1B). MET IHC positivity was significantly associated with younger age ($p=0.009$), female gender ($p=0.012$), no smoking history ($p=0.004$), ADC histology ($p<0.001$), and *EGFR* FISH negativity ($p=0.028$) (Table 2). The difference in survival between the MET IHC-positive and IHC-negative groups was statistically significant ($p=0.01$, OS; $p=0.056$, DFS) (Fig. 4A,B). MET IHC-positive patients had significantly shorter OS and DFS than MET IHC-negative patients, and the difference was statistically significant in patients with *EGFR* FISH negativity ($p=0.013$), no smoking history ($p=0.015$), ADC histology ($p=0.008$), and female gender ($p<0.001$) (Fig. 4C-F). Among 52 MET IHC-positive patients, 12 patients (by Cappuzzo criteria) and 15 patients (by UCCC criteria) exhibited *MET* FISH positivity. Among

Table 1. Clinical characteristics of patients according to histologic subtype.

Feature	NSCLC histologic type			*P*-value
	All patients (n=380), n (%)	SCC (n=245), n (%)	ADC (n=135), n (%)	
Age				
Median(yr) (interquartile range)	62.0 (54.0-67.0)	63.0 (55.0-68.0)	60.0 (53.0-65.0)	0.0072*
Sex				
Male	308 (81.1)	235 (95.9)	73 (54.1)	<0.001
Female	72 (18.9)	10 (4.1)	62 (45.9)	
Smoking				
Never smoker	101 (26.6)	22 (11.0)	74 (54.8)	<0.001
Smoker	279 (73.4)	218 (89.0)	61 (45.2)	
T stage				
T1	63 (16.6)	22 (9.0)	41 (30.4)	<0.001
T2	243 (63.92)	170 (69.4)	73 (54.1)	
T3	37 (9.7)	28 (11.4)	9 (6.7)	
T4	37 (9.7)	25 (10.2)	12 (8.9)	
Lymph node				
Negative	235 (61.8)	145 (59.2)	90 (66.7)	0.227
Positive	145 (38.2)	100 (40.8)	45 (33.4)	
Metastasis				
M0	373 (98.2)	240 (98.0)	133 (98.5)	0.520
M1	7 (1.8)	5 (2.0)	2 (1.5)	
Stage				
I	192 (50.0)	115 (46.9)	77 (57.0)	0.285
II	88 (23.2)	62 (25.3)	26 (19.3)	
III	92 (24.2)	63 (25.7)	29 (21.5)	
IV	8 (2.1)	5 (2.0)	3 (2.2)	

NSCLC, non-small cell lung cancer; SCC, squamous cell carcinoma; ADC, adenocarcinoma; T: tumor status; *: Median age was compared by Wilcoxon two-sample test between SCC and ADC patients.

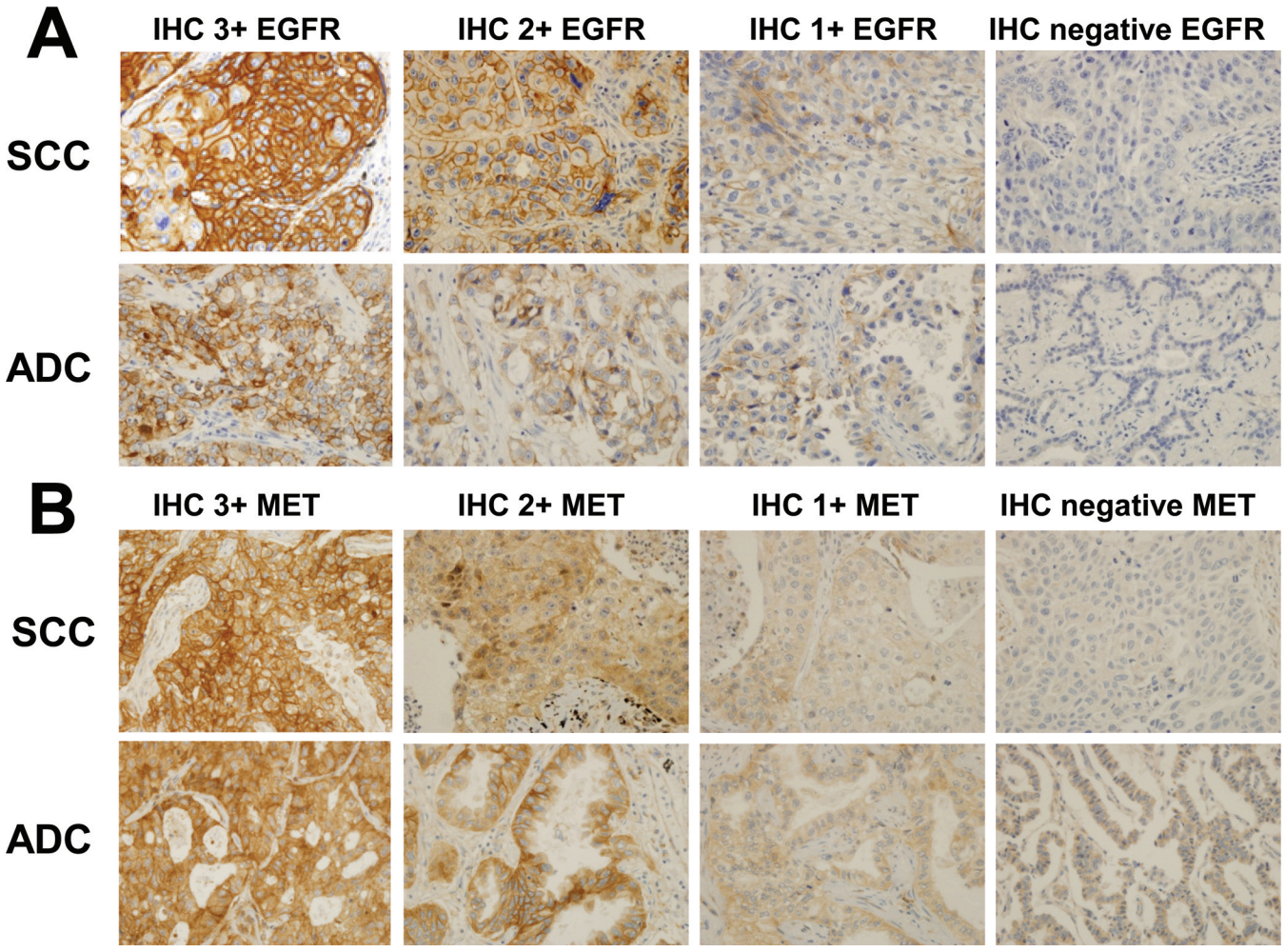

Fig. 1 EGFR **(A)** and MET **(B)** expression by IHC according to histological subtype. SCC: squamous cell carcinoma; ADC: adenocarcinoma. x 200

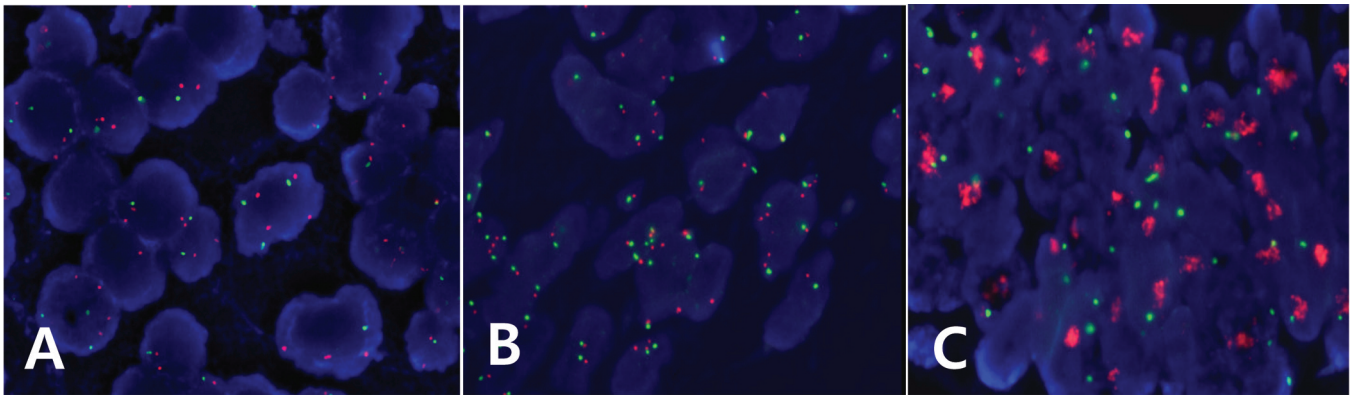

Fig. 2. Representative cases of *MET* FISH: low polysomy **(A),** high polysomy **(B)**, and gene amplification **(C)**. x 1000

Table 2. Clinicopathologic features of NSCLC patients according to the *MET* gene copy status based on the UCCC criteria and Cappuzzo scoring systems.

	UCCC[a]			Cappuzzo[b]			MET IHC		
	MET FISH- (n=338)	MET FISH+ (n=42)	p-value	MET<5 (n=353)	MET≥5 (n=27)	p-value	Negative	Positive	p-value
Age, (n)									
<65 (237)	210(88.6)	27(11.4)	1.0	224(94.5)	13(5.5)	0.227	196(82.7)	41(17.3)	0.009
>65 (143)	128(89.5)	15(10.5)		129(90.2)	14(9.8)		132(92.3)	11(7.7)	
Sex,(n)									
Male (308)	274(88.9)	34(11.1)	1.0	285(92.5)	23(7.5)	1.0	273(88.6)	35(11.4)	0.012
Female (72)	64(88.9)	8(11.1)		68(94.4)	4(5.6)		55(76.4)	17(23.6)	
Smoking, (n)									
Ever (279)	250(89.6)	29(10.4)	0.992	259(92.8)	20(7.2)	1.0	250(89.6)	29(10.4)	0.004
Never (101)	88(87.1)	13(12.9)		94(93.1)	7(6.9)		78(77.2)	23(22.8)	
Histology, (n)									
SCC (245)	215(87.8)	30(12.2)	0.636	226(92.2)	19(7.8)	1.0	225(91.8)	20(8.2)	<0.001
ADC (135)	123(91.1)	12(8.9)		127(94.1)	8(5.9)		103(76.3)	32(23.7)	
Stage, (n)									
I, II (280)	256(91.4)	24(8.6)	0.011	266(95.0)	14(5.0)	0.015	245(87.5)	35(12.5)	0.309
III, IV (100)	82(82.0)	18(18.0)		87(87.0)	13(13.0)		83(83.0)	17(17.0)	
EGFR FISH, (n)									
Negative (277)	253(91.3)	24(8.7)	0.029	261(94.2)	16(5.8)	0.196	246(88.8)	31(11.2)	0.028
Positive (103)	85(82.5)	18(17.5)		92(89.3)	11(10.7)		82(79.6)	21(20.4)	
EGFR IHC, (n)									
Negative (307)	277(90.2)	30(9.8)	0.205	288(93.8)	19(6.2)	0.301	265(86.3)	42(13.7)	1.0
Positive (73)	61(83.6)	12(16.4)		65(89.0)	8(11.0)		63(86.3)	10(13.7)	
MET IHC, (n)									
Negative (328)	302(92.1)	26(7.9)	<0.001	313(95.4)	15(4.6)	<0.001			
Positive (52)	36(69.2)	16(30.8)		40(76.9)	12(23.1)				

[a]: The University of Colorado Cancer Center (UCCC) criteria; [b]: MET FISH-positive group was defined mean *MET* gene copy number ≥5 copies per cell; SCC: squamous cell carcinoma; ADC: adenocarcinoma; FISH: Fluorescence *in situ* hybridization; IHC: immunohistochemistry.

Table 3. Univariate and multivariate analysis of prognostic factors for overall survival.

Variable	Category	Univarite			Multivariate		
		HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age group	≥65 yr/<65 yr	1.462	1.082-1.975	0.013	1.741	1.278-2.380	<0.001
Sex	Male/Female	2.026	1.304-3.148	0.002	1.677	0.903 -3.115	0.102
Smoking	Smoker/Never smoker	1.504	1.052-2.150	0.025	1.430	0.878-2.327	0.150
Histology	SCC/ADC	1.037	0.765-1.406	0.813	1.592	1.099-2.306	0.014
Stage	III+IV/I+II	2.660	1.957-3.615	<0.001	2.714	1.953-3.771	<0.001
MET IHC	Positive/ Negative	1.636	1.123-2.411	0.010	1.618	1.066-2.456	0.024
MET FISH	Positive/ Negative	1.480	0.995-2.269	0.052	1.105	0.700-1.757	0.675
EGFR IHC	Positive/ Negative	1.281	0.892-1.839	0.180	1.306	0.863-1.977	0.206
EGFR FISH	Positive/ Negative	1.238	0.894-1.715	0.198	1.214	0.832-1.770	0.315

HR: hazard ratio; FISH: fluorescence *in situ* hybridization; SCC: squamous cell carcinoma; ADC: adenocarcinoma.

Table 4. Multivariate analysis of prognostic factors for overall survival by histologic type.

Variable	Category	SCC			ADC		
		HR	95% CI	P-value	HR	95% CI	P-value
Age group	≥65 yr/<65 yr	1.807	1.149-2.841	0.003	1.56	0.825-2.862	0.122
Sex	Male/Female	2.139	0.398-9.053	0.311	1.724	0.665 -4.473	0.200
Smoking	Smoker/Never smoker	1.191	0.554-2.563	0.608	1.560	0.681-3.572	0.230
Stage	III+IV/I+II	2.625	1.635-4.215	<0.001	3.133	1.636-6.002	<0.001
MET IHC	Positive/ Negative	1.466	0.660-3.254	0.283	1.706	0.908-3.207	0.057
MET FISH	Positive/ Negative	1.001	0.447-1.828	0.748	1.560	0.660-3.691	0.247
EGFR IHC	Positive/ Negative	1.419	0.830-2.427	0.144	1.021	0.317-2.857	0.919
EGFR FISH	Positive/ Negative	1.178	0.651-2.134	0.536	1.350	0.718-2.536	0.287

HR: hazard ratio; FISH: fluorescence *in situ* hybridization; SCC: squamous cell carcinoma; ADC: adenocarcinoma.

the 9 patients with true amplification (by UCCC criteria), 6 patients exhibited MET IHCpositivity.

Univariate and multivariate survival analyses

The results of the univariate and multivariate survival analyses using Cox's proportional hazard model, including age group; sex; smoking history; histological type; stage; and MET IHC, *MET* FISH, EGFR IHC, and *EGFR* FISH data, are summarised in Table 3. In the univariate analysis, sex, age group, smoking history, and stage were significantly associated with a higher risk of death. MET IHC positivity or *MET* FISH positivity increased the risk of death (hazard ratio [HR], 1.636; 95% confidence interval [95% CI], 1.123-2.411, p=0.011 and HR, 1.480; 95% CI, 1.101-2.252, p=0.048, respectively). In the multivariate analysis, however, patients with older age, stage III+IV tumours, and MET IHC positivity had a higher risk of death than patients with younger age, stage I+II tumours, and MET IHC-negative tumours.

The multivariate analysis restricted to both

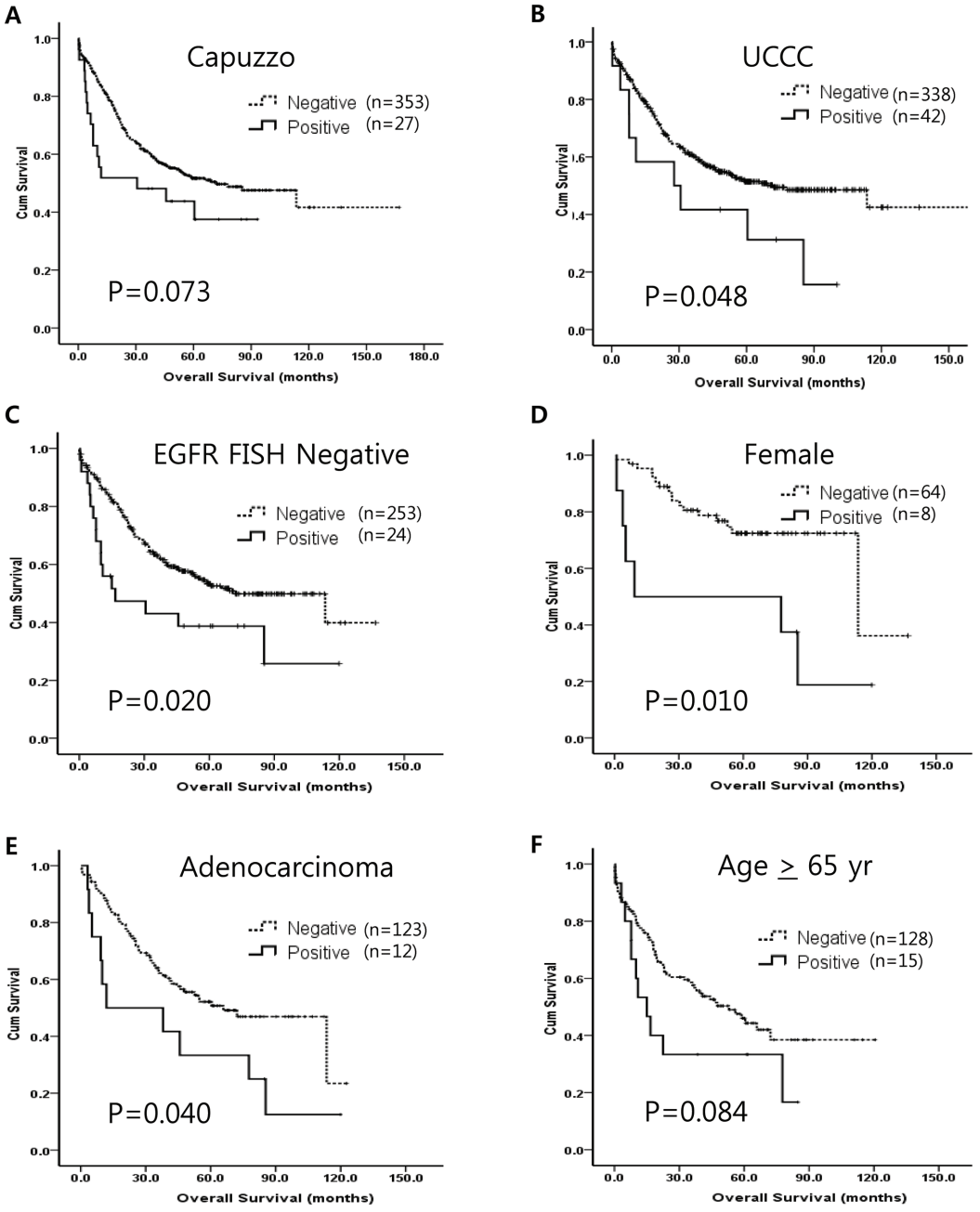

Fig. 3. Kaplan-Meier curves, using the log-rank test, showing the OS of all NSCLC patients by *MET* FISH status (**A, B**). NSCLC patients with *EGFR* FISH negativity (**C**), NSCLC patients with female gender (**D**), ADC patients (**E**), and NSCLC patients older than 65 years old (**F**) based on the UCCC criteria. NSCLC, non-small cell lung cancer; FISH, fluorescence *in situ* hybridisation; ADC, adenocarcinoma; UCCC, the University of Colorado Cancer Center; CNG, copy number gain.

histological types showed that age group and stage were significantly associated with a higher risk of death in SCC group. OS tended to be shorter in MET IHC-positive patients in ADC group (Table 4).

Discussion

This study has 2 important characteristics: all patients were Korean, and all of the tissues in our study were obtained from surgically resected primary lung tumours. Asians are known to have a higher frequency of *EGFR* mutations, which are associated with increased responsiveness to EGFR TKIs. This study was designed to investigate the association of EGFR and MET in the prognosis of resected NSCLCs and to help optimise patient selection for EGFR- or MET-targeted therapy. We demonstrated that *MET* FISH-positive and MET IHC-positive patients had significantly shorter survival.

Some studies have found that the overexpression of MET or HGF in lung cancer correlates with clinico-

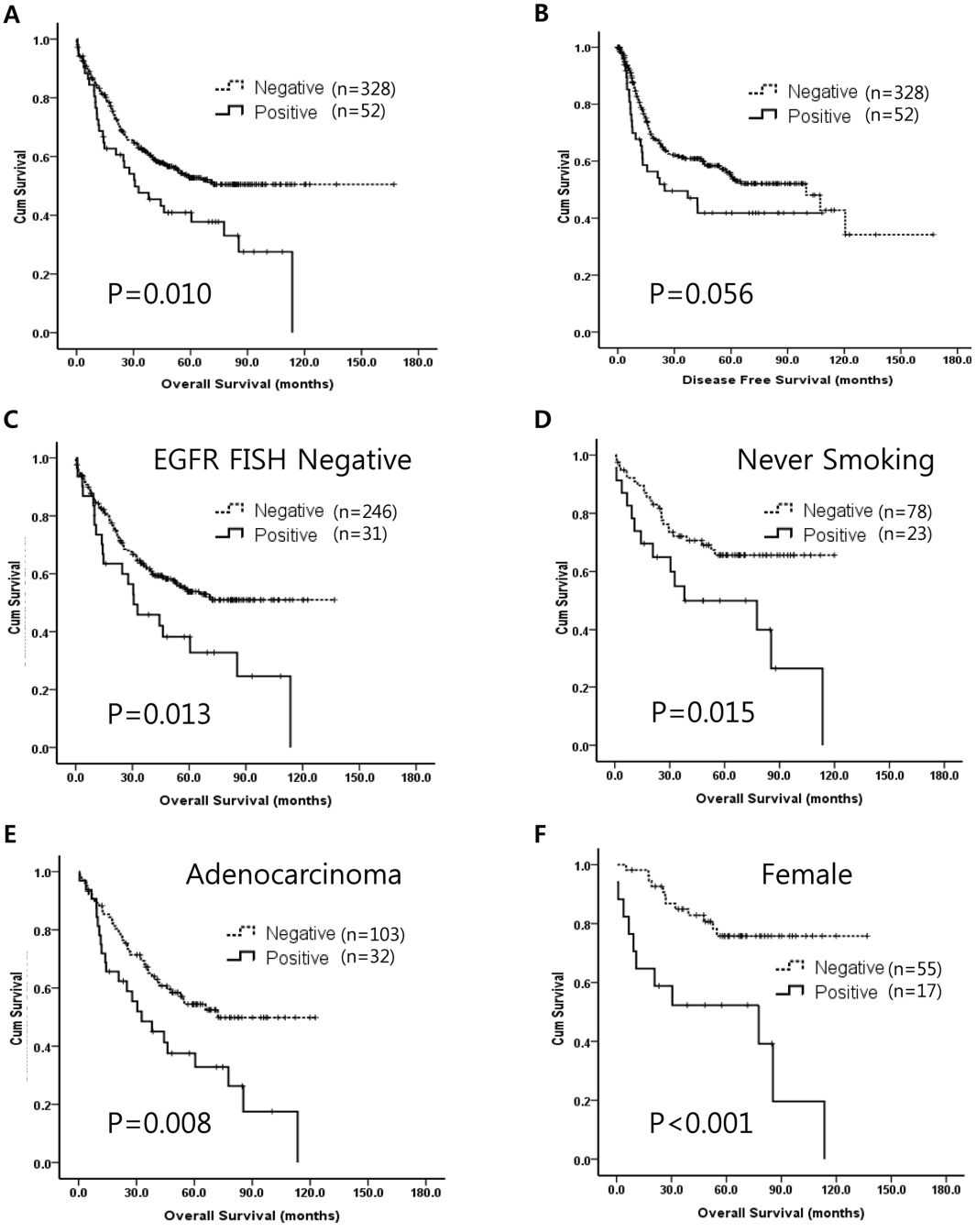

Fig. 4. Kaplan-Meier curves, using the log-rank test, showing the OS and DFS of all NSCLC patients by MET IHC status (**A, B**). NSCLC patients with *EGFR* FISH negativity (**C**), NSCLC patients with no smoking history (**D**), ADC patients (**E**), and female gender (**F**) according to the MET IHC status. NSCLC, non-small cell lung cancer; FISH, fluorescence *in situ* hybridisation; ADC, adenocarcinoma.

pathological parameters and prognosis (Ichimura et al., 1996; Cappuzzo et al., 2009; Kanteti et al., 2009). MET and its ligand, HGF/SF, have become leading molecular targets in cancer therapy, and several anti-MET drugs are under evaluation in clinical trials. The general indication for treatment with kinase and signalling pathway inhibitors is based on their presence in cancer cells, and when possible, knowledge of their role in expression and mutational status (Knudsen and Vande Woude, 2008). However, the measurement of MET expression in tissues is problematic. Unlike HER-2, the expression of which is proportional to its activity, the relationship between MET expression and activation is variable. Instead, anti-phospho-MET antibodies have been suggested as immunohistochemical markers for the examination of MET activation (Ma et al., 2005). Because of the difficulties of measuring MET by IHC, gene amplification and mutational analysis have been employed as markers of tumour dependence (McDermott et al., 2007; Nakamura et al., 2008). Moreover, some studies have revealed that *MET* amplification is responsible for acquired resistance to EGFR TKIs in approximately 20% of patients (Engelman et al., 2007). Recently, Cappuzzo et al. reported that *MET* copy number is an independent negative prognostic factor for surgically resected NSCLC (Cappuzzo et al., 2009) based on a definition of *MET* FISH positivity as a mean of ≥5 copies per cell. Using this cut-off, 11.1% of their patients were *MET* FISH-positive, including 4.1% of those with true gene amplification. *MET* amplification has been reported variably, ranging from 5.6% to 21% of patients, but has consistently exhibited an association with worse prognosis (Beau-Faller et al., 2008; Okuda et al., 2008; Cappuzzo et al., 2009; Go et al., 2010). Our study demonstrated that increased *MET* copy number was observed in 7.1% of the patients as estimated by the Cappuzzo system, and the *MET* FISH-positive rate was 11.1% according to UCCC criteria, which is similar to previously published data (Cappuzzo et al., 2009; Go et al., 2010). Our study also provides similar evidence that MET is a negative prognostic factor, further supporting anti-MET strategies. The clinicopathological correlation of MET amplification in NSCLC is conflicting in several studies. Go et al. reported that MET copy numbers were significantly associated with poorer survival in SCC patients rather than ADC patients. However, Beau-Faller et al. demonstrated that ADC patients with MET amplification trended towards poor prognosis, which was not observed in SCC patients (Beau-Faller et al., 2008). Furthermore, Go et al. reported that MET copy numbers were significantly associated with poor survival in patients with stage III-IV but not with stage I-II cancer. However, Cappuzzo et al. reported that *MET* FISH-positive patients had statistically significant shorter survival than FISH-negative patients only in stage I-II but not in stage III-IV, which contrasts with the data reported by Go et al. In our study, *MET* FISH status was not significant in subgroup analysis with stage. Cappuzzo did not mention the survival association in both histological groups but reported that *MET* FISH-positive status was significantly associated with grade 3 (p=0.016) and advanced stage (p=0.01). In our data, the MET FISH positivity was statistically higher in advanced stage (p=0.011), which was not observed in Go's data. MET FISH positivity was a negative prognostic factor, especially in patients with adenocarcinoma histology (p=0.040), female gender (p=0.010), old age (p=0.084), and EGFR FISH negativity (p=0.020) but this correlation was found only at the univariate level but not at the multivariate level.

Interestingly, the difference in survival between the MET IHC-positive and IHC-negative groups was statistically significant in this study, even though we used an anti-MET antibody instead of an anti-phospho-MET antibody. MET immunoreactivity was observed in both the cytoplasm and cell membranes of carcinoma cells, and we used a scoring system based on membrane staining.

When patients were divided according to *EGFR* FISH results, MET positivity had prognostic implications only among *EGFR* FISH-negative patients. This finding has been consistently reported in recent studies (Beau-Faller et al., 2008; Cappuzzo et al., 2009; Go et al., 2010). These findings suggested that anti-MET drugs might be beneficial for EGFR FISH-negative NSCLC patients who are not initially selected for EGFR TKI treatment. As illustrated in Figures 1 and 2, *MET* FISH positivity based on the UCCC criteria and MET IHC positivity were significantly associated with a shorter overall survival among females, ADC patients and older patients. MET status may be helpful in identifying a group of NSCLC patients eligible for MET-targeted therapy. However, it remains to be determined which system is more effective for selecting patients for MET-targeted therapy.

In conclusion, we found that *MET* FISH-positive and MET IHC-positive patients had significantly shorter survival than *MET* FISH-negative and MET IHC-negative patients. With the advent of anti-EGFR and anti-MET drugs for NSCLC, EGFR and MET gene and protein status will be important to select patients who will derive the greatest clinical benefit from targeted therapy for EGFR or MET. The prognostic implications of MET gene and protein expression should be analysed in the context of *EGFR* copy number status and clinicopathological variables in NSCLC.

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