

Met gene copy number predicts the prognosis for completely resected non-small cell lung cancer

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(Received May 7, 2008/Revised June 16, 2008/Accepted July 2, 2008/Online publication October 23, 2008)

The *Met* oncogene encodes the tyrosine kinase receptor for hepatocyte growth factor (HGF). Uncontrolled activation of *Met* is oncogenic and has been implicated in the growth, invasion and metastasis in a variety of tumors. Several distinct mechanisms including amplification, translocation or mutation of *Met* may underlie uncontrolled *Met* activation. In several solid tumors, amplification and mutation of *Met* were reported to be associated with tumorigenesis, invasion and metastasis. The present study evaluated the amplification and mutation of *Met* in a large number of non-small cell lung cancer (NSCLC). Among 213 NSCLC patients, increased *Met* copy number was identified in 12 patients (5.6%) and associated with a worse prognosis ($P = 0.0414$). The mutation of *Met* in 534 NSCLC patients was also evaluated. In these patients there were no previously reported mutations within the juxtamembrane (JM) domain (R988C, T1010I, S1058P and G1085X). However, a somatic exon 14 deleting splice variant in 3 (1.7%) of 178 NSCLC samples was identified for which sequencing was performed. *Met* amplification and mutation were rare in Japanese NSCLC. However, the results support a critical role of *Met* gene dose in NSCLC, suggesting that *Met* may be a specific molecular therapeutic target in selected NSCLC patients with increased *Met* copy number. (*Cancer Sci* 2008; 99: 2280–2285)

Lung cancer is a leading cause of cancer death worldwide. The most effective therapy to cure the non-small-cell lung cancer (NSCLC) is complete resection; but 20–30% of patients with pathological stage I die within 5 years post operatively, and this percentage is higher in advanced cases.^(1,2) Recent clinical success of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) in relapsed, advanced NSCLC has raised hopes for individual tailored targeting therapy. EGFR inhibitors are more effective in patients with *EGFR* mutation.^(3–5) *EGFR* mutation is identified in about 20% of NSCLC patients, more frequently in Asian, female, non-smoker and adenocarcinoma patients. Recently, *Met* gene amplification leads to gefitinib resistance in NSCLC by activating ERBB3 signaling.⁽⁶⁾

Met is a proto-oncogene that encodes a tyrosine kinase membrane receptor for hepatocyte growth factor/scatter factor (HGF/SF). Alteration of the *Met* gene, including amplification, overexpression and mutations, have been described in a number of solid tumors such as papillary renal cancer and gastric cancer.^(7–9)

Met-receptor tyrosine kinase is activated by its cognate ligand HGF and receptor phosphorylation activates downstream pathways of mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase and phospholipase C γ .^(10,11) Signaling mediated by HGF/*Met* promotes biological activities such as proliferation, motility, invasion and angiogenesis.^(12–15) Dysregulation of these activities leads to uncontrolled cell proliferation and oncogenesis.⁽¹⁶⁾

In the present study, we studied a large number of lung cancer specimens to identify *Met* mutations and amplification, and their association with the clinical characteristics and survival of NSCLC patients.

Materials and Methods

Patients and genomic DNA. NSCLC tissues were obtained by surgical excision from 534 patients between 1997 and 2007 at Nagoya City University Hospital. The research was approved by the Institutional Review Board of the hospital. All the patients consented to the use of their tissues for the present analysis. Genomic DNA was extracted using the Wizard SV Genomic DNA purification system (Promega) according to the manufacturer's instructions. The *Met* copy number was analyzed in 213 NSCLC patients. The clinical and pathologic characteristics of the 213 lung cancer patients were as follows: 95 cases at stage I; 35 at stage II; 76 at stage III; and 7 at stage IV. The median age was 66 years (range, 38–88). Among the 213 lung cancer patients, 136 (64%) were diagnosed as having adenocarcinoma, 56 (26%) squamous cell carcinoma, 14 (7%) adenosquamous cell carcinoma and 7 (3%) large cell carcinoma (Table 1). We also analyzed the *Met* gene alterations that were previously reported at the semaphorin domain (N375S) and juxtamembrane domain (R988C, T1010I, S1058P and G1085X) using TaqMan PCR assay for 534 NSCLC patients and confirmed the results by direct sequencing of complementary DNA (cDNA) in 178 of these patients.

Met DNA amplification. The *Met* gene copy number was analyzed for 213 NSCLC patients by quantitative real-time PCR, performed on a PRISM 7500 sequence detector (Applied Biosystems) by using a QuantiTect SYBR Green kit (Qiagen, Valencia, CA, USA). We quantified each tumor DNA by comparing the target locus to the reference *Line-1*, a repetitive element for which copy numbers per haploid genome are similar among all of the human normal and neoplastic cells. Quantification is based on standard curves from a serial dilution of human normal genomic DNA. The relative *Met* copy number was also normalized to the normal human genomic DNA as calibrator. Copy number change of *Met* gene relative to the *Line-1* and the calibrator were determined by using the formula $(T_{Met}/T_{Line-1})/(C_{Met}/C_{Line-1})$, where T_{Met} and T_{Line-1} are quantities from tumor DNA by using *Met* and *Line-1*, and C_{Met} and C_{Line-1} are quantities from calibrator by using *Met* and *Line-1*. PCR for each primer set were performed in triplicate at least, and means were reported. Conditions for quantitative PCR reaction were as follows: one cycle of 50°C for 2 min; one cycle of 95°C for 15 min; 40 cycles of 95°C for 15 s; 56°C for 30 s; and 72°C for 34 s. At the end of the PCR reaction, samples were subjected to a melting analysis to confirm specificity of the amplicon. Primers for *Met* gene were designed using Primers 3⁽¹²⁾ to span a 100–150 bp non-repetitive region at exon 19 and were synthesized by Invitrogen (Carlsbad, CA, USA). Primer sequences used in the present study for the *Met* gene are as follows: forward, TCACATCTCTCACCTCATCTG; and reverse, GAAGGCAGGCATTTCTGTA.

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Table 1. Characteristics and comparison of 213 patients

Characteristic		Met amplification			P-value
		n	(-) n = 204	(+) n = 12	
Age (years)	High (>60)	144	136	8	0.9431
	Low (≤60)	69	65	4	
Sex	Male	148	136	12	0.0181
	Female	65	65	0	
Pathologic subtypes	Adenocarcinoma	136	130	6	0.3040
	Others	77	71	6	
Differentiation	Well	93	90	3	0.0969
	Moderately or poorly	100	91	9	
Nodal metastasis	(+)	82	79	3	0.3226
	(-)	131	122	9	
Smoking status	Never smoker	70	70	0	0.0126
	Smoker	143	131	12	
Pathological stage	II-IV	118	112	6	0.6985
	I	95	89	6	
EGFR mutations	(+)	59	56	3	0.9096
	(-)	146	138	8	

EGFR, epidermal growth factor receptor.

Table 2. Sequence of the mutation-specific TaqMan probes and PCR primers

No	Nucleotide	Amino acid	Primer sequence (forward)	TaqMan probe	Primer sequence (reverse)
1	c1124A > G	N375S	TGTGTGCATTCCCT ATCAAATATGTC	VIC-ACCTTCTCAACAAGATC FAM-TTCTTCAGCAAGATC	GCTCATGATTGGGT CCGTAAA
2	c2692C > T	R988C	GCCTATCCAAATGA GGAGTGTGT	VIC-ATCGTAGCGAACTAAT FAM-ATCGTAGCAAATAAT	TCTGTTTAAAGATC TGGGCAGTGA
3	c3029C > T	T1010I	AGGCTTGTAAATGCC CGAAGT	VIC-TAAGCCCAACTACAGAAA FAM-TAAGCCCAATTACAGAAA	CTGGAAAAGTAGCT CGGTAGTCTACAG
4	c3172T > C	S1058P	CAATGTGGACAGTA TTTTGCAGTAATG	VIC-ACTGGATATATCAGAGTCC FAM-CTGGATATATCAGGGTCC	ACATGTCCCCCATC CTAACTAGTG
5	c3307G > T	G1085X	CCAGTAGCCTGATT GTGCATTTTC	VIC-ATGAAGTCATAGGAAGAGGTA FAM-ATGAAGTCATATGAAGAGGTA	AATCTGCAAAGGCC AAAGATAAAA

Met amplification (increased Met copy number) was defined as more than three copies.⁽¹⁷⁾

Genotyping by the TaqMan PCR assay. The primers and TaqMan Minor Groove Binder (MGB) probes for the Met gene were designed with Primer Express 2.0 software (Applied Biosystems). The sequences of the allele-specific probes and primers used in the TaqMan PCR assay are shown in Table 2. TaqMan PCR and genotyping analysis were performed on Applied Biosystems 7500 Real Time PCR System (Applied Biosystems) using the manufacturer's instructions. The reaction mixtures were amplified in 1 µL of genomic DNA (10 ng/µL) or 1 µL of 100-fold-diluted PCR products, 5 µL of 2 × TaqMan Universal Master Mix (Applied Biosystems), 0.5 µL of 20 × primer/probe mix (each final concentration of primer and probe is 9 µM and 2 µM) and 3.5 µL of H₂O in a volume of 10 µL. PCR cycling conditions were as follows: one cycle at 95°C for 10 min; and 40 cycles at 95°C for 15 s and 58°C for 1 min. The results were analyzed on Applied Biosystems 7500 Real Time PCR System using allelic discrimination assay program.

Direct sequencing. One-hundred and seventy-eight cDNA samples were amplified by PCR reaction to confirm the results of the TaqMan assay for c-Met exon 14–15. RNA samples were extracted using Isogen Kit (Nippongen), and reverse transcription (RT) was performed using First Strand cDNA synthesis Kit (Roche) according to the manufacturer's instructions. PCR of cDNA was carried out using TaKaRa LA Taq. PCR primers were as follows: forward, CTCAACCGTCCTTGGAAAA; and reverse,

GATGATTCCCTCGGTCAGAA. Thermal cycling conditions were as follows: initial denaturation at 94°C for 5 min; followed by 30 cycles of 94°C for 30 s; 60°C for 30 s; and 72°C for 45 s. The final extension was for 5 min at 72°C. Met exon 14 was PCR amplified from genomic DNA about the three cases (tumor and normal tissues) carried an exon 14 splice variant of the cDNA Met gene. PCR primers were as follows: forward, GCCGTCTTTAACAAGCTCTTTC; and reverse, TGTCACAACCC ACTGAGGTAT. Thermal cycling conditions were as follows: initial denaturation at 95°C for 5 min; followed by 40 cycles of 95°C for 30 s; 56°C for 30 s; and 72°C for 30 s. The final extension was for 7 min at 72°C. The PCR products were purified using MiniElute PCR purification kit (Qiagen) and then directly sequenced by ABI PRISM 3100 Genetic Analyzer and analyzed by ABI PRISM SeqScape Software Version 2.1.1.

Met immunohistochemistry. Met protein expression was evaluated by immunohistochemistry (IHC) using an anti-Met antibody (rabbit, SPRING BIOSCIENCE). We used a standard protocol for immunostaining samples. Four µm sections were made from paraffin tissue blocks of NSCLC tumors. The slides were treated with xylenes and then dehydrated in alcohol. For epitope retrieval, specimens were exposed to 10 mM citrate buffer (pH 6.0) and heated to about 120°C for 15 min in the autoclave. Endogenous peroxidase activity was blocked with H₂O₂ in methanol. Sections were incubated with the blocking solution (10% Block Ace) and then reacted with anti-Met polyclonal antibody (1:50) overnight at 4°C. After the excess antibody had been

Table 3. Increased *Met* copy number cases in non-small cell lung cancer (NSCLC) patients

Sample number	Age	Sex	BI	Pathology	Differentiation	pStage	EGFR	Copy number
1	67	Male	1500	AD	Moderately	III	del1a	4.08
2	60	Male	2400	SCC	Well	III	WT	10.23
3	76	Male	1800	SCC	Well	I	WT	5.33
4	68	Male	720	SCC	Moderately	I	WT	4.31
5	61	Male	1600	AD	Moderately	I	WT	3.59
6	64	Male	1200	SCC	Poorly	III	del1a	3.72
7	47	Male	540	AD	Poorly	I	WT	4.21
8	50	Male	900	AD	Poorly	III	WT	6.16
9	72	Male	600	AD	Well	I	L858R	8.26
10	59	Male	900	AS	Poorly	I	WT	3.38
11	63	Male	600	AS	Poorly	III	WT	8.44
12	70	Male	1000	AD	Moderately	II	WT	3.20

Abbreviations: BI, Brinkman Index; AD, adenocarcinoma; SCC, squamous cell carcinoma; AS, adenosquamous cell carcinoma; EGFR, epidermal growth factor receptor; del1a, E746-A750 deletion.

washed out with phosphate-buffered saline (PBS), samples were incubated with peroxidase-conjugated antirabbit immunoglobulin G (IgG) antibody for 45 min. After the excess antibody had been washed out with PBS, 3,3-diaminobenzidine (DAB) substrate (10 min) was used to visualize the antibody binding, and the sections were counterstained with hematoxylin. *c-Met* staining was evaluated under the light microscope at $\times 400$ magnification. Tumor cytoplasmic and cell membrane immunohistochemical staining intensity and extent of *c-Met* was classified using the three-scale scoring system: weak (0), medium (1+), and strong (2+). Cases of strongly expressed *c-Met* were evaluated as positive.

Analysis of EGFR mutation and K-ras codon 12 status. EGFR mutation statuses of NSCLC resected samples were analyzed by the TaqMan PCR assay⁽¹⁸⁾ and direct sequencing.⁽¹⁹⁾ The primers and TaqMan MGB probes were designed with Primer Express 2.0 software (Applied Biosystems). We used 13 sets of specific TaqMan probes as previously reported.⁽¹⁸⁾ TaqMan PCR and genotyping analysis were performed on a 7500 Real Time PCR system (Applied Biosystems) according to the manufacturer's instructions. *K-ras* codon 12 mutation status was analyzed by genotyping using a Light Cycler.⁽²⁰⁾

Statistical analysis. Survival curves were generated using the Kaplan–Meier method and the log rank test was used to assess the statistical significance of the difference between groups. Cox proportional hazards model was used to identify statistical significance in the difference in survival and to estimate the hazard ratios and 95% confidence intervals (CI). Prognostic variables identified by the univariate analysis were further analyzed in a multivariate Cox model. The two-sided significance level was at $P < 0.05$. All analyses were carried out using Stat View (version 5, SAS Institute, Cary, NC, USA) software.

Results

Analysis of *Met* DNA copy number. The *Met* gene amplification in 213 NSCLC samples was analyzed by quantitative real-time PCR. At first, we divided the level of *Met* copy number into ≤ 2 , $2 < (\text{copy number}) \leq 3$ and > 3 . The prognosis was almost the same between ≤ 2 and $2 < (\text{copy number}) \leq 3$ cases (Fig. 1). And the cases of > 3 were significantly worse than $2 < (\text{copy number}) \leq 3$ cases ($P = 0.0425$). We determined the cut-off line of *Met* amplification as > 3 (Fig. 2). Twelve of 213 cases were found to have an increased *Met* copy number. Relationships between the *Met* copy number and the clinicopathologic factors are shown in Tables 1 and 3. All of the cases with an increased *Met* copy number were male smokers; *Met* copy number was significantly correlated with sex ($P = 0.0181$) and smoking status

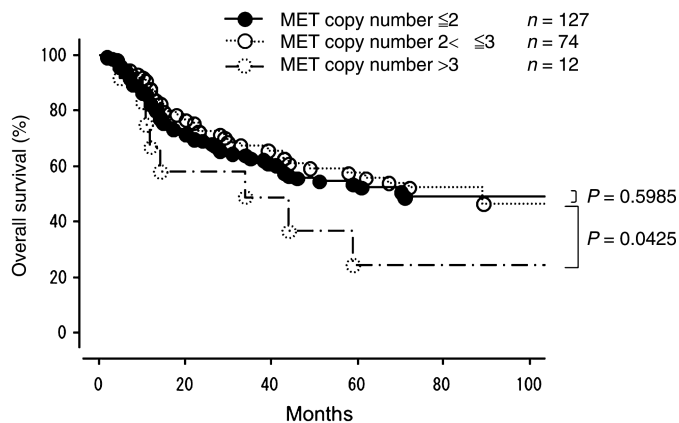


Fig. 1. Kaplan–Meier estimates of overall survival according to the *Met* copy number (divided by ≤ 2 , $2 < \leq 3$, and > 3). The prognosis was almost the same between ≤ 2 and $2 < (\text{copy number}) \leq 3$ cases.

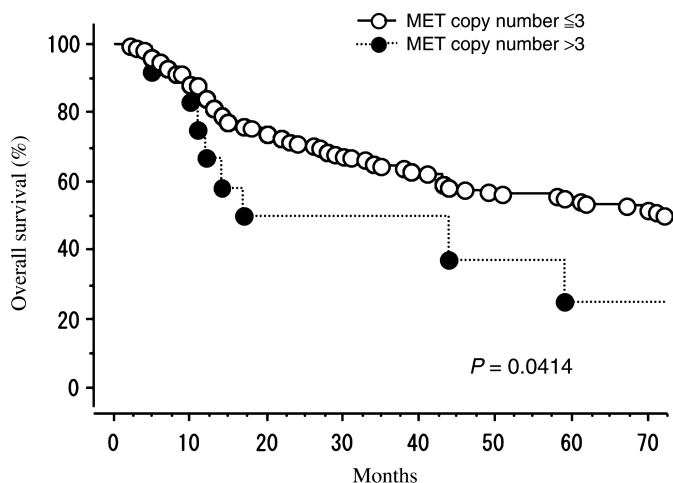


Fig. 2. Kaplan–Meier estimates of overall survival according to the *Met* copy number (divided by ≤ 3 and > 3). The cases of > 3 were significantly worse than $2 < (\text{copy number}) \leq 3$ cases ($p = 0.0425$). The case of > 3 were significantly worse than ≤ 3 cases ($p = 0.0414$).

Table 4. Factors associated with overall survival of 213 patients

Factor	5-year survival			Hazard ratio [‡]	
	<i>n</i>	(%)	Log-rank [†]	(95% confidence interval)	
Age (years)	High (>60)	144	53.7	0 = 0.5247	NT
	Low (≤60)	69	53.1		
Sex	Male	148	46.9	0 = 0.0058	1.451 (0.757–2.781) <i>P</i> = 0.2621
	Female	65	68.4		
Pathologic subtypes	Others	77	47.4	0 = 0.1043	NT
	Adenocarcinoma	136	56.9		
Differentiation	Moderately or poorly	100	42.9	0 = 0.0095	1.262 (0.798–1.995) <i>P</i> = 0.3198
	Well	93	63.4		
Smoking status	Smoker	143	46.9	0 = 0.0083	1.003 (0.520–1.993) <i>P</i> = 0.9934
	Never smoker	70	66.8		
Pathological stage	II–IV	118	37.1	0 < 0.0001	2.423 (1.537–3.821) <i>P</i> = 0.0001
	I	95	73.9		
EGFR mutations	(–)	146	48.2	0 = 0.0557	NT
	(+)	59	63.4		
Met copy number	>3	12	25.0	0 = 0.0414	1.877 (0.925–3.808) <i>P</i> = 0.0811
	≤3	201	55.0		

[†]Univariate analysis using log-rank test. [‡]Multivariate analysis using Cox proportional hazards model. NT: not tested. EGFR, epidermal growth factor receptor.

(*P* = 0.0126). The *Met* copy number was not correlated with age, pathologic subtypes, differentiation, nodal metastasis, pathological stage or the presence of *EGFR* mutation (Table 1). In the 12 cases with increased *Met* copy number, three had an *EGFR* mutation. Two were del1a (E746-A750 deletion at exon 19) mutations and one was an L858R mutation.⁽¹⁸⁾ One other case had a *K-ras* codon 12 mutation. The association between the patient characteristics and overall survival (OS) is shown in Table 4. In the univariate analysis, sex (0.0058), differentiation (*P* = 0.0095), smoking status (*P* = 0.0083), pathological stage (*P* < 0.0001) and *Met* copy number (*P* = 0.0414) were prognostic factors. The Kaplan–Meier curves of OS according to *Met* copy number in 213 patients are shown in Fig. 2. When analysis was restricted to those at stages II to IV, increased *Met* copy number was associated with significantly worse prognosis (*P* = 0.0349). No patient with increased *Met* copy number survived for 5 years. Multivariate Cox proportional hazards model was applied and we found that pathological stage (*P* = 0.0001) was the only independent prognostic factor. Although it was not significant, there was a tendency towards worse prognosis in cases with an increased *Met* copy number (*P* = 0.0811). We treated two patients with *Met* amplification by gefitinib. One patient with an L858R mutation of *EGFR* (No9, Table 3) showed a good response to gefitinib but became resistant when the tumor relapsed in the mediastinal lymph node in 2 years; the other with wild type *EGFR* (No11, Table 3) did not.

TaqMan PCR assay for *Met* mutation. We subjected 534 NSCLC samples to TaqMan PCR assay to detect previously reported *Met* alterations (Sema domain N375S, and JM domain R988C, T1010I, S1058P and G1085X). We designed five sets of specific TaqMan probes (Table 2). Using this analysis, 49 cases were detected to have a N375S alteration. In 24 of these cases, the non-neoplastic tissues from the same individual showed the same alteration, indicating a polymorphism. We did not find other mutations by the TaqMan PCR assay in any other cases studied. N375S polymorphism was not associated with clinicopathologic status and prognosis (data not shown).

Sequencing of the exons 14 and 15 of the *Met* gene. One-hundred and seventy-eight NSCLC tumor samples were subjected to conventional cDNA sequencing in exon 14 and 15, corresponding to the JM domain of *Met*. No sample carried previously reported

mutations, such as R988C, T1010I, S1058P or G1085X. Three of 178 carried a splice variant that deleted the exon 14 of the *Met* gene (Fig. 3a). In two of these, cDNA from adjacent normal lung tissues was available. The sequencing of the cDNA from these showed the wild type sequence (Fig. 3b) and suggested that this splicing occurred only in the tumor. All patients had wild type *EGFR*, wild type *K-ras* codon 12. All three tumors were at pathological stage I. From the genomic DNA sequencing of the three patients carrying a splice variant, we found the intron deletion and the intron point mutation only in the tumor tissues but not in the normal ones. One of the cases is 10 bp deletion (99432–99441, TCTCTGTTTT) at the intronic region upstream of the 5' exon 14 and another is the point mutation (99586, G→A) at the intronic region downstream of the 3' exon 14 (Fig. 3(c)). We could not detect any intronic change at other case.

Immunohistochemistry for c-*Met*. c-*Met* protein expression was evaluated using IHC in 49 NSCLC samples. These included 10 samples with an increased *Met* copy number and two samples with exon 14 deleting splice variant. Seven of the 10 cases with an increased *Met* copy number were IHC positive; 12 of the 39 cases with the normal copy number of *Met* were IHC positive. There were significantly association between *Met* copy number and c-*Met* IHC (*P* = 0.023). For cases with exon 14-deleting splice variant, one was positive and the other was negative.

Discussion

In the present study, we investigated *Met* amplification, mutation and *Met* protein expression for completely resected NSCLC. *Met* gene was amplified in 12 of 213 (5.6%) tumors of primary NSCLC. Our data showed that NSCLC patients with *Met* amplification had a poor prognosis. However, in the multivariate Cox proportional hazard model, *Met* amplification was not independent prognostic factor. One of the reasons for this is that the amplification cases were too small (12/213, 5.6%). Second, *Met* copy number was not so much high in original NSCLC samples. In support of our clinical data, in several solid tumors including NSCLC, the activation of *Met* has been reported to promote cell growth, survival, motility, invasion and angiogenesis.⁽¹⁶⁾ We also report that the patients with *Met* amplification were all male and smokers.

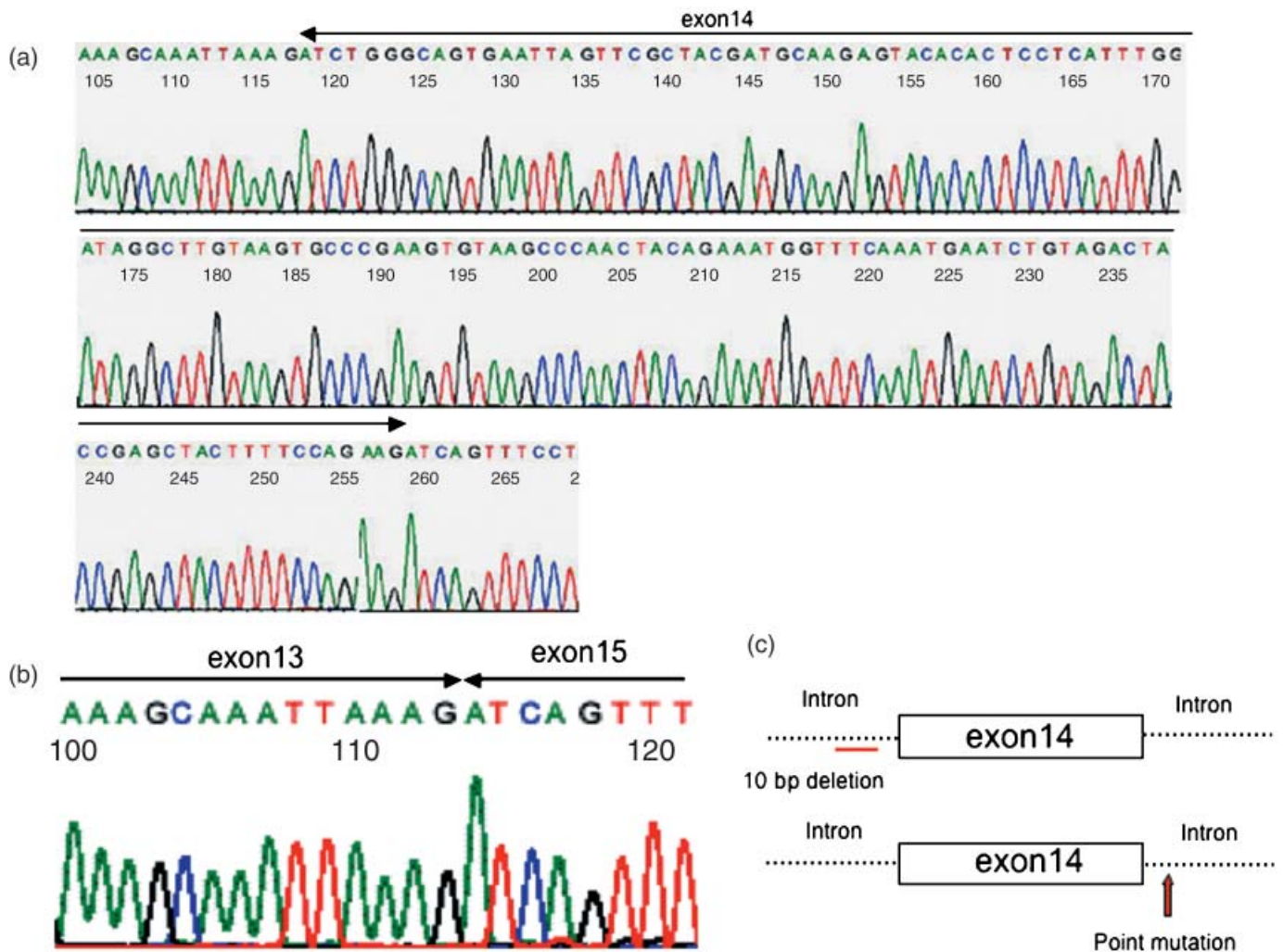


Fig. 3. One-hundred and seventy-eight NSCLC tumor samples were subjected to conventional cDNA sequencing in exon 14 and 15, corresponding to JM domain of *Met*. (a) Data from direct sequencing showing a normal *Met* exon 14–15. (b) Data from direct sequencing showing *Met* exon 14 (47 amino acids) deletion. (c) Identification of tumor-specific, intronic mutations in *Met* leading to exon 14 splicing.

Chromosomal copy number alterations can lead to activation of oncogenes and inactivation of tumor suppressor genes in human cancers.⁽²⁰⁾ High-resolution, genome-wide approaches to characterize copy-number alterations in primary NSCLC have been reported.^(21,22) *Met* amplification has been identified in 5% to 10% of gastric cancers,^(23–25) 4% of esophageal cancers⁽²⁶⁾ and 3–4% of lung cancers.^(22,27) *Met* amplification as well as *Met* and HGF overexpression have also been correlated with poor clinical outcome in a variety of human cancers.^(11,28)

Recently *Met* amplification has been identified as one of the mechanisms of acquired resistance to the EGFR kinase inhibitors, gefitinib and erlotinib.^(6,27) *Met* amplification was found not only in tumors with acquired resistance to EGFR kinase inhibitor, but also in primary tumors in untreated NSCLC.⁽²⁷⁾ *Met* activation can have profound effects on cell growth, survival, motility, invasion and angiogenesis.⁽¹¹⁾ *In vitro*, cell lines with *Met* amplification are suppressed by *Met* inhibitors.^(8,27) These results suggest that patients with *Met* amplification may be good candidates for *Met* TKI. We had already investigated the ERBB3 protein expression by the immunohistochemistry about NSCLC patients,⁽²⁹⁾ but no relationships were detected between *Met* amplification and ERBB3. It is thought that activation of ERBB3 is related with acquisition of resistance to gefitinib in lung cancer, and ERBB3

signaling is not activated by *Met* amplification in primary lung cancer.

In the present study, there was no relationship between the *Met* copy number and *EGFR* mutation in the primary NSCLC. We found three cases with both *Met* amplification and *EGFR* mutation. One of these patients with L858R mutation of *EGFR* originally showed a good response to gefitinib. We only studied the specimen before gefitinib treatment. However, our data may suggest that *Met* amplification is an early event.

We have used TaqMan PCR assay as a rapid and sensitive method of detection of previously reported mutations.⁽¹⁸⁾ In the present study, we studied the *Met* mutation using TaqMan PCR assay. We could not find any previously reported *Met* mutation within the JM domain. Interestingly, we found exon 14-deleting splice variant in 3/178 NSCLC samples. All the tumors with *Met* splice variant were without *EGFR*, *K-ras* or *B-raf* mutation (data not shown), consistent with a previous report.⁽³⁰⁾ All cases were stage I and completely resected by operation; no patient received adjuvant therapy and has not relapsed. We found that the deletions were tumor specific, suggesting that this deletion may have played a role in tumorigenesis. We also investigated the genomic DNA of three cases with exon 14 splicing variant to detect the mechanism of the alternative splicing. Consistent

with previous reports, we detect the intronic deletion and point mutation around the exon 14.⁽³⁰⁾

In the present study, *Met* amplification was significantly correlated with *Met* protein expression as studied by IHC. However, there was no relationship between protein expression and the exon 14 deleted splice variant. More cases with the splice variant need to be studied to clarify its relationship with the protein expression and the function of *Met*.

In conclusion, we have reported a minor (5.6%) subset of NSCLC patients with *Met* amplification who may be good candidates for *Met* tyrosine kinase inhibitors.

Acknowledgments

The authors would like to thank Mrs Emi Sugiyama and Mariko Nishio for their excellent technical assistance.

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