Activation of MET by Gene Amplification or by Splice Mutations Deleting the Juxtamembrane Domain in Primary Resected Lung Cancers

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Introduction: MET (Met proto-oncogene) activation either by gene amplification or mutation is implicated in various types of human cancers. For lung cancer, *MET* gene amplification is reported to occur in a subset of adenocarcinomas. Although somatic mutations of *MET* in lung adenocarcinomas are rare, all but one of those reported so far entail a splice mutation deleting the juxtamembrane domain for binding the c-Cbl E3-ligase; normally such binding leads to ubiquitination and receptor degradation, and loss of this domain leads to MET activation. The purpose of this study was to clarify in the role of MET activation in lung carcinogenesis.

Materials and Methods: *MET* gene copy number was determined by real-time quantitative polymerase chain reaction in 187 of the patients with lung cancer and the *MET* gene splice mutation deleting the juxtamembrane domain was examined by direct sequencing in 262. The results were correlated with various clinical and pathologic features including mutations of the epidermal growth factor receptor, *KRAS*, and *HER2* genes.

Results: All the instances of MET activation occurred in patients with adenocarcinomas. The prevalences of *MET* gene amplification and splice mutations were 1.4% (2 of 148) and 3.3% (7 of 211), respectively. We identified four different intronic mutations that disrupted a splice consensus sequence in genomic DNA. Activation of MET and mutations of the epidermal growth factor receptor, *KRAS*, and *HER2* genes had strict mutual exclusionary relationships.

Conclusions: About 5% of pulmonary adenocarcinomas in this cohort of Japanese patients were driven by activated MET by gene amplification or splice mutations. Such patients would be candidates for targeted therapy against MET.

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MET (met proto-oncogene) is a receptor tyrosine kinase that phosphorylates several tyrosine residues after binding its specific ligand, hepatocyte growth factor. Receptor phosphorylation activates downstream signals, including phosphatidyl inositol 3-kinase, mitogen-activated protein kinase, and phospholipase C-γ and leads to epithelial-mesenchymal transition, cell scattering, angiogenesis, proliferation, enhanced cell motility, invasion, and metastasis.[1,2]

Accumulating evidence suggests that MET plays an important role in the pathogenesis of human lung cancer. Amplification and overexpression of the *MET* gene (located at 7q31) is reported to occur in a subset of patients with pulmonary adenocarcinomas that do not harbor mutation of the *EGFR* (epidermal growth factor receptor) gene.[3] Lung and gastric cancer cell lines with *MET* gene amplification has been reported to lead to enhanced phosphorylation of the *MET* gene.[4,5] Furthermore, *MET* gene amplification could be detected in about 20% of patients with lung adenocarcinomas who developed acquired resistance to EGFR TKI.[6,7]

In Caucasian patients with lung adenocarcinoma, mutations of the *MET* gene have been reported to occur in 7.1% (9 of 127)[8] and 12.1% (4 of 33).[9] When confined to somatic mutations, the prevalences have been reported to occur in 1.6% (2 of 127)[8] and 6.0% (2 of 33).[9] Of these 4 somatic mutations, 3 were splice mutations that resulted in deletion of exon 14 coding for the juxtamembrane domain. Another was a point mutation in an extracellular semaphorin domain (L229F). Mutations in the kinase domain were not detected in any lung adenocarcinomas (0 of 160).[8,9] On the contrary, all the mutations found in sporadic papillary-type renal cell carcinomas (17 of 129; 13%), childhood hepatocellular carcinomas (3 of10; 30%), and head and neck squamous cell carcinomas (4 of 15; 27%) occurred within the tyrosine kinase domain.[10–13] This fact contrasts strongly with other types of cancer.

Tyrosine 1003 in the juxtamembrane domain of MET is a binding site for c-Cbl, an ubiquitin protein ligase (E3), which causes ubiquitination, receptor endocytosis, and degradation of MET.[14] Therefore, deletion of the juxtamembrane domain (exon

14) is one mechanism for MET activation, as shown by Kong-Beltran et al.[9] The mutant *MET* exhibits decreased ubiquitination and delayed down-regulation correlating with elevated, distinct MET production.[9] Therefore, phospho-MET levels and downstream mitogen-activated protein kinase activation are sustained following ligand stimulation.[9]

Because the mutational frequencies of the *EGFR* or *KRAS* genes differ markedly between Japanese and Western patients, it would be of interest to determine the prevalence of MET activation by gene amplification or mutation in a Japanese cohort. Therefore, we decided to search for *MET* amplification and for mutations deleting the juxtamembrane domain, and evaluated their clinicopathological significance including any associations with *EGFR* and *KRAS* mutations. Additionally, we performed the mutational search of the *HER2* gene.

PATIENTS AND METHODS

Patients

We studied 262 patients with lung cancer who underwent potentially curative pulmonary resection at the Department of Thoracic Surgery, Aichi Cancer Center Hospital, from May 2000 through 2002. Tumor samples were frozen rapidly in liquid nitrogen, after obtaining the appropriate approval from the Institutional Review Board and the patients' written informed consent. All the patients were Japanese; 149 were men and 113 were women, with ages at diagnosis ranging from 26 to 89 years (median 64 years). One hundred forty-nine patients had stage I disease, 33 had stage II, 75 had stage III, and 5 had stage IV. There were 211 adenocarcinomas, 33 squamous cell carcinomas, 6 adenosquamous carcinomas, 10 large cell carcinomas, and 2 small cell carcinomas. One hundred and seven patients had never smoked and 155 were current or former smokers. We had previously determined the *EGFR*, and *KRAS* mutational status in this cohort.[15–17]

Cell Lines

Twenty-two lung cancer cell lines were available for this study. These comprised 9 adenocarcinomas, A549, ACC-LC-319, NCI-H358, NCI-H838, NCI-H1666, NCI-H1993, NCI-H2009, NCI-H2882, RERF-LC-MT; 5 squamous cell carcinomas, Calu1, PC-1, PC-10, SK-MES-1 and RERF-LC-AI; 3 large cell carcinomas, Calu6, NCI-H460 and SK-LC-6; one non-small cell lung carcinoma (NSCLC), NCI-H1299; 4 small cell carcinomas, ACC-LC-48, ACC-LC-49, ACC-LC-80, and SK-LC-2. Two gastric cancer cell lines, KATOIII and MKN45 were also used. NCI-H358, H460, H838, H1299, H1666, H1993, H2009, and H2882 were gifts from Dr. Adi F. Gazdar. KATOIII and MKN45 were obtained from the Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer (Tohoku University, Sendai, Japan). The derivation of other cell lines has been described previously.[18,19] All cell lines were cultured in RPMI-1640 (Sigma-Aldrich, Irvine, UK) with supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) and 1×antibiotic-antimycotic (Invitrogen) at 37°C in a humidified incubator with 5% CO_2. NCI-H1993[4,7] and MKN45[5] cell lines have amplification of the *MET* gene, while KATO III does not.[5]

RNA and DNA Extraction

Frozen tumor tissue sections were grossly dissected by a surgical pathologist (Y. Y.) to enrich tumor cells as much as possible. Total RNAs and genomic DNAs contained at least 20% of tumor content in our cohort. Total RNA was isolated using the RNeasy Kit (Qiagen, Valencia, CA) in 262 cases. For extraction of genomic DNA, tissues were incubated with 1×PCR (polymerase chain reaction) buffer containing 100 μg/ml proteinase K for 1 hour at 54°C. Next, the solution was incubated for 3 minutes at 95°C. Genomic DNA was extracted in 187 unselected patients for whom tumor blocks were available.

Analysis of *MET* Gene Amplification

The copy number of the *MET* gene relative to a LINE-1 repetitive element was determined by quantitative real-time PCR using the SYBR Green Method (QuantiTect SYBR Green PCR Kit; Qiagen) using an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster city, CA) according to Engelman et al.[7] The copy number of the LINE-1 is reported to be similar between normal and cancerous cells.[20] The standard curve method was used to calculate *MET* gene copy number in the cell line or tumor DNA sample relative to the Line-1 repetitive element.[20] Quantification was based on standard curves from a serial dilution of Calu6 genomic DNA. Calu6 was selected as a standard sample, because amount of *MET* gene relation to *Line-1* was almost 1.00. Primer sequences for the MET gene were 5′-TAGAAGAGCCCAGCCAGTGT-3′ (forward), 5′-CGAATGCAATGGATGATCTG-3′ (reverse), and for LINE-1 were 5′-AAAGCCGCTCAACTACATGG-3′ (forward), 5′-TGCTTTGAATGCGTCCCAGAG-3′(reverse). All the specimens were analyzed in triplicate using 20 ng of genomic DNA. We defined that amplification was present when the copy number was two or more.

Analysis of the Splice Mutation of the *MET* Gene Around the Juxtamembrane Domain

The cDNA sequence of the *MET* gene was obtained from GenBank (accession number NM 000245.2). The exon 14 that codes for the juxtamembrane domain of the *MET* gene was amplified with primer F (5′-TGAAATTGAACAGC-GAGCTAAAT-3′) and R (5′- TTGAAATGCACAATCAG-GCTAC-3′), in an one-step reverse transcription (RT)-PCR setup with Qiagen OneStep Reverse Transcription-PCR kits (Qiagen) using 4 ng of total RNA. The conditions for RT-PCR were one cycle of 50°C for 30 minutes, 95°C for 15 minutes, 45 cycles of 94°C for 40 seconds, 62°C for 40 seconds, 72°C for 1 minute, and one cycle of 72°C for 10 minutes. After RT-PCR, free nucleotides and excess primer were removed using PCR Purification kit (QIAGEN). PCR products were diluted and cycle sequenced using the BigDye Terminator Cycle Sequencing Kit v. 3.1/1.1 (Applied Biosystems). Sequencing reaction products were separated electrophoretically on an ABI PRISM 3100 apparatus (Applied Biosystems). Both the forward and reverse sequences obtained were analyzed with BLAST and by manual review.

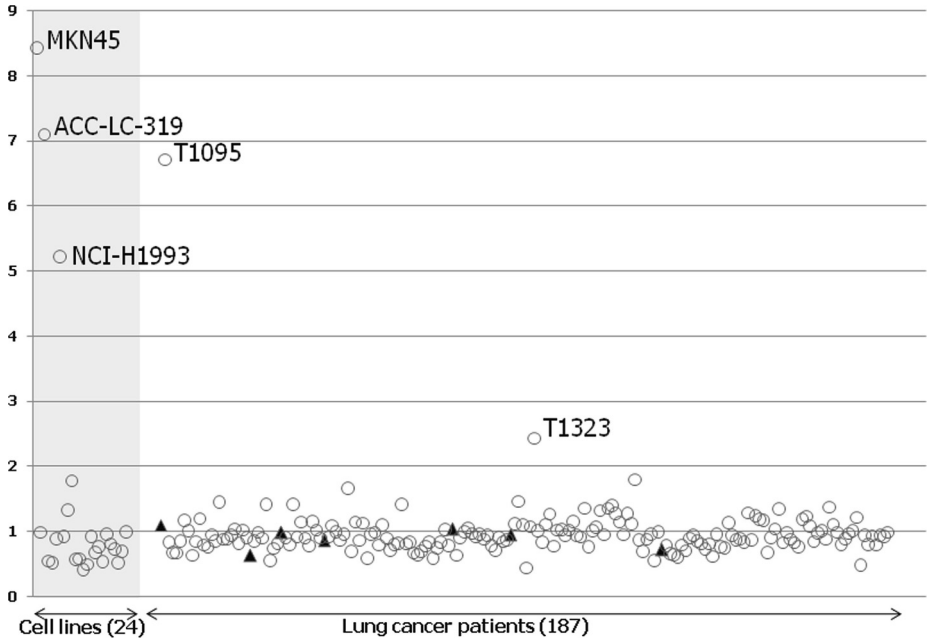

FIGURE 1. Relative copy number of the *MET* gene determined by real-time quantitative PCR (polymerase chain reaction) in 24 cell lines and 187 lung cancer specimens. MKN45 and NCI-H1993 cell lines showing *MET* amplification served as positive controls. Solid triangles indicate samples that had *MET* splice mutations in the following experiments.

For cases with *MET* mutations identified by sequencing of the RT-PCR product, we also analyzed 100 ng aliquots of genomic DNA to examine the mechanism for alternative splicing. The genomic DNA sequence of the *MET* gene was obtained from GenBank (accession number NC 000007.12). PCR of genomic DNA was carried out using AmpliTaq Gold (Applied Biosystems) for introns 13 and 14. PCR primers (encompassing from the 5′ splice site of intron 13 to the 5′ splice site of intron 14; nucleotide numbers of genomic DNA from 99615 to 99728) were as follows: 5′-GATTGCTGGTGT-TGTCTCAATATC-3′ (forward) and 5′-TGTCAAATACT-TACTTGGCAGAGG-3′ (reverse). The PCR condition was: one cycle of 95°C for 10 minutes, 40 cycles of 94°C for 30 seconds, 62°C for 30 seconds, 72°C for 40 seconds, and one cycle of 72°C for 10 minutes. Sequence analysis was carried out as for the RNA analysis.

Analysis of the *HER2* Gene Mutation

We sequenced exon 20 of the tyrosine kinase domain of the *HER2* gene where all the mutations are reported.[21–23] The cDNA sequence of the *HER2* gene was obtained from GenBank (accession number NM 004448). Primer sequences were 5′-ACAGTCTACAAGGGCATCTGGA-3′ (forward), and 5′-AACTCCACACATCACTCTGGTG-3′ (reverse). The RT-PCR conditions were one cycle of 50°C for 30 minutes, 95°C for 15 minutes, 40 cycles of 94°C for 40 seconds, 62°C for 40 seconds, 72°C for 1 minute, and one cycle of 72°C for 10 minutes.

Statistical Analysis

The χ^2 test was used to compare proportions. The two-sided significance level was set at $p < 0.05$. All analyses were carried out using StatView software (version 5; SAS Institute, Cary, NC).

RESULTS

MET Gene Amplification in Lung Cancer Specimens

First, we searched for amplification of the *MET* gene in 22 lung cancer cell lines and 2 gastric cancer cell lines (Figure 1). *MET* amplifications were detected in the ACC-LC-319 adenocarcinoma cell line. Two of the cell lines (NCI-H1993, lung cancer and MKN45, gastric cancer) have been reported to harbor *MET* gene amplifications[4,5,7] and we confirmed this findings. Relative copy numbers of the *MET* gene for the ACC-LC-319, NCI-H1993, and MKN-45 cell lines were 7.26, 5.22, and 8.43, respectively.

In 187 clinical specimens (148 adenocarcinomas, 28 squamous carcinomas, 4 adenosquamous carcinomas, 6 large cell carcinomas, and 1 small cell carcinoma), we detected only 2 *MET* gene amplifications (Figure 1). The *MET* copy numbers of these 2 patients were 6.72 (T1095) and 2.43 (T1323). The prevalence of *MET* gene amplification in adenocarcinomas was thus 1.4% (2 of 148).

MET Gene Splice Mutations Deleting the Juxtamembrane Domain

We searched for splice mutations of the *MET* gene deleting the juxtamembrane domain using 22 lung cancer cell lines, however, mutation was not detected. Next, we performed mutational search in a cohort of 262 lung cancers that included those examined for gene amplification. We identified 7 *MET* mutations in 262 lung cancer specimens (2.7%; Table 1, Figure 2*A*). All 7 mutations were confirmed by a second independent PCR. All the mutations were deletions of nucleotides 3075 to 3215 (according to NM 000245.2) corresponding with the sequence of exon 14. This would result in a 47 amino-acid

TABLE 1. Clinicopathological Features of the Patients with the *MET* Amplification or Splice Mutation in Lung Cancer Patients

	Patient Number	Age	Sex	Histology (differentiation)	Stage	Smoking Status	Prognosis (d)
Amplification	T1095	64	Male	AD(M)	IB	S	1094:DOD
	T1323	71	Male	AD (P)	IB	S	516:DOD
Mutation	T1021	62	Male	AD (P)	IA	S	2732: NED
	T1148	74	Female	AD(M)	IB	NS	2029: NED
	T1165	76	Male	AD(M)	IA	S	1611:DOD
	T1181	67	Male	AD(W)	IA	S	1953: NED
	T1241	60	Female	AD(M)	IIB	NS	2241: NED
	T1307	75	Male	AD (P)	IB	S	2101: NED
	T1384	56	Female	AD(M)	IA	NS	1872: NED

AD, adenocarcinoma; M, moderately differentiated; P, poorly differentiated; W, well differentiated; S, smoker; N, never-smoker; DOD, dead of disease; NED, no evidence of disease.

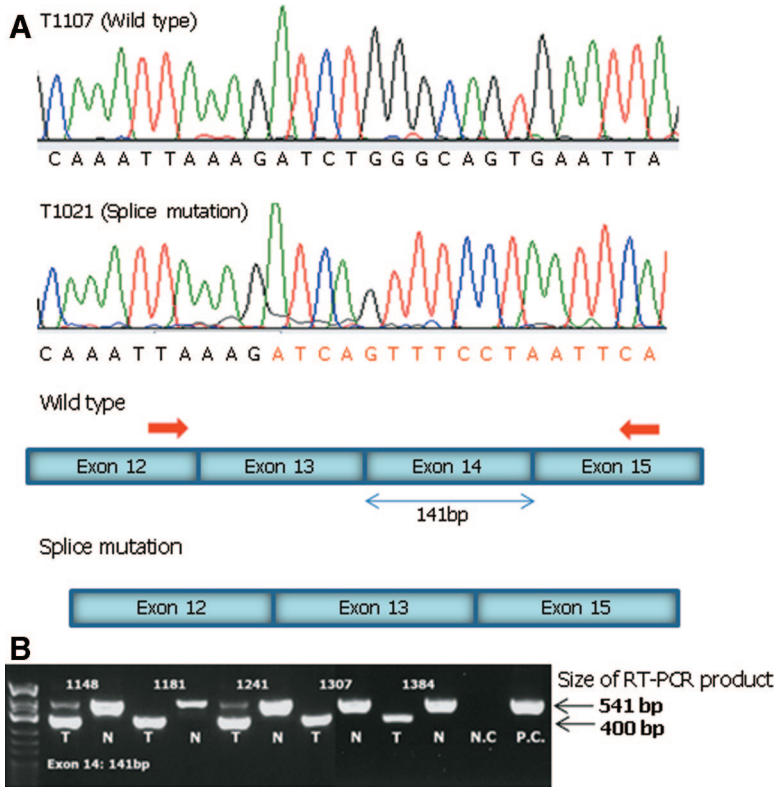

FIGURE 2. *A*, Examples of cDNA sequencing chromatograms of the *MET* gene. In patient T1021, exon 13 was spliced directly to exon 15, skipping exon 14. The chromatogram for the wild type sequence is shown in the upper panel for comparison. Red characters represent a splice mutation. Red arrows indicate the primer position for reverse transcription-polymerase chain reaction (RT-PCR). *B*, Agarose gel electrophoresis of the RT-PCR product encompassing exon 14 of the *MET* gene of the paired tumor and normal samples. T, tumor; N, normal lung tissue; N.C, negative control; P.C, positive control. The difference between the larger and smaller bands appears to correspond with length of the exon 14 (141 bp).

deletion of the exon 14 (L964 through D1010 in the juxtamembrane domain), identical with that reported.[8,9]

RNAs from matched tumor and normal lung tissues were available for 5 patients with *MET* mutations (except T1021 and T1065). Deletion of exon 14 was only present in tumor samples by RT-PCR and sequencing experiments indicated that this was a somatic event (Figure 2*B*). In addition, we noticed that bands for the mutant allele (shorter) were always stronger than bands for the wild type allele (Figure 2*B*). In 3 tumors (patients T1181, T1307, and T1384), bands for the wild type allele were almost invisible. However, none of seven tumors with *MET* mutations harbored *MET* amplification in the preceding experiments.

Mechanisms for the Deletion of Exon 14 of the *MET* Gene

To investigate the mechanism for the deletion of exon 14 of the *MET* gene in cDNA, we sequenced introns 13 and14 for any sequencing alterations, using genomic DNA. We identified four intronic mutations that would affect RNA splicing by disrupting consensus sequences.[24,25] Patient T1021 had a point mutation at the 5′ splice site of intron 14. Patients T1148, T1165, T1307 had deletions either involving the 3′ splice site, a branch site, or the polypyrimidine tract of intron 13 (Figure 3*A*, *B*), respectively.[24] Contrary to the cDNA sequencing, these

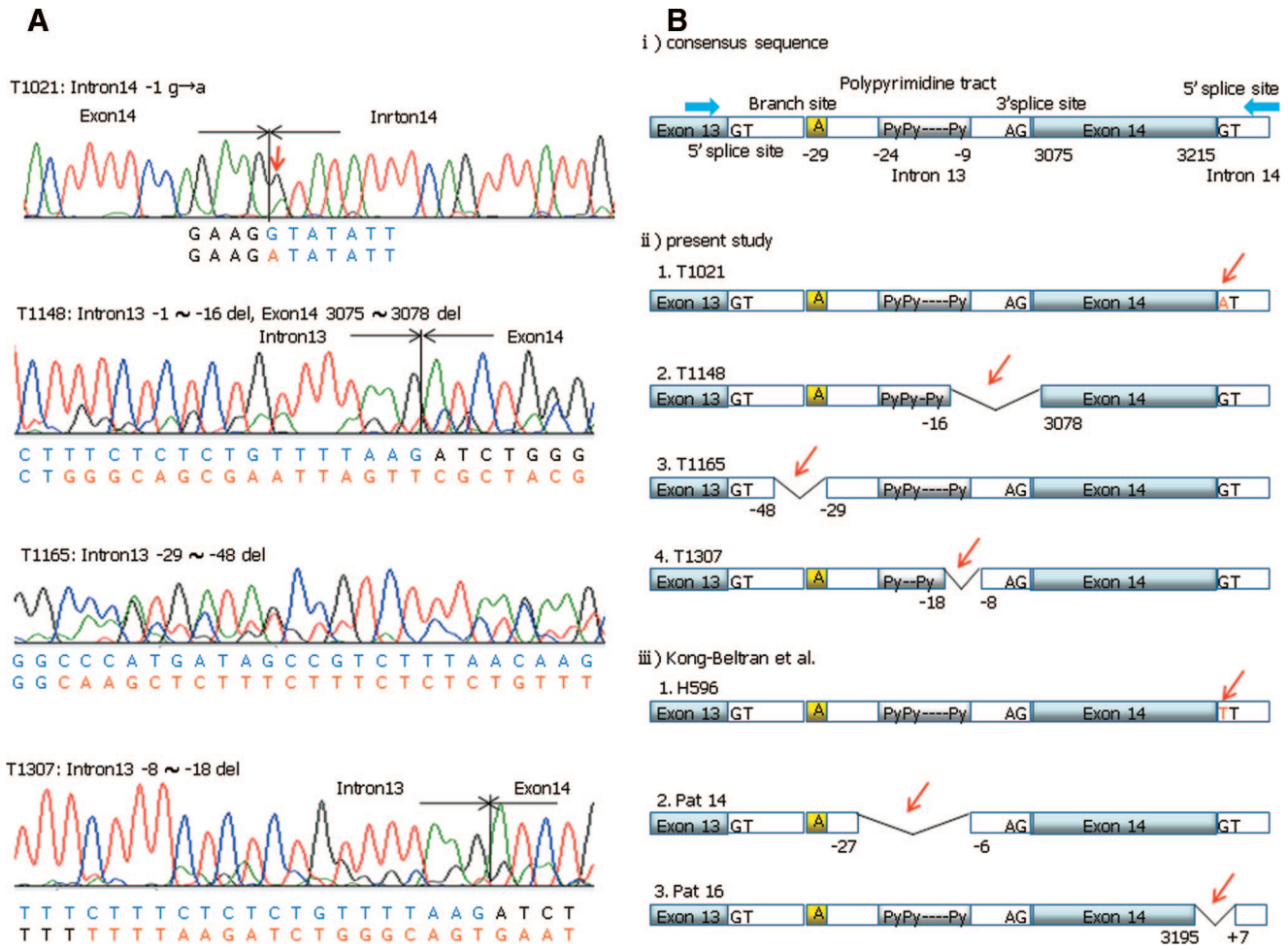

FIGURE 3. *A*, Sequencing chromatograms of genomic DNA with intronic mutation of the *MET* gene: Red characters represent deletions or point mutations in genomic DNA. Four intronic mutations were detected in this analysis. *B*, Diagrammatic representations of the mechanisms for eliminating exon 14 of the *MET* gene: (i) The 5′ splice site, 3′ splice site, branch site, and polypyrimidine tract are indicated as consensus motifs at the top of figure.[24,25,28] Blue arrows indicated primers for polymerase chain reaction (PCR). (ii) Diagrammatic representations of Figure 3A. (iii) Three intronic mutations reported previously by Kong-Beltran et al.[9] All intronic mutations detected in this analysis were different from those reported.[9] Each mutation is indicated by a red arrow. Py, pyrimidine.

mutations were heterozygous (Figure 3*A*). Matched normal lung of these four patients had wild-type sequences (data not shown).

HER2 Mutation in Lung Cancers

We identified 6 *HER2* mutations in the 262 lung cancer specimens (2.3%). Five mutations were a 12-bp duplication/insertion of the amino acids YVMA at codon 776 and one mutation was a 9-bp insertion of the amino acids VGS at codon 779. All patients with the *HER2* mutation, except for one with an adenosquamous carcinoma, had adenocarcinomas. Five patients were women and one was a man. Four patients had stage IA tumors, one had stage IB, and one had stage IIIA. Four patients were never smokers and two were smokers.

Relationships Between Patients with *MET* Gene Amplification or Splice Mutation and Clinicopathological Backgrounds Indicating Mutations of the *EGFR*, *KRAS*, and *HER2* Genes

Table 1 shows the clinicopathological backgrounds for 9 patients with *MET* gene amplification or splice mutation. All the patients had adenocarcinomas. Two patients with amplification were male smokers. Four patients with the *MET* mutation were male smokers and three were female never-smokers. All except for T1241 had stage I tumors. There were no significant differences among clinicopathological factors according to *MET* mutation status (Table 2).

EGFR, *KRAS*, and *HER2* mutations were identified in 103 (48.8%), 29 (13.7%), and 5 (2.4%) of 211 lung adenocarcinomas, respectively, and these 3 mutations were mutually exclusionary as reported.[15,26] *MET* gene activation by

TABLE 2. Characteristic Clinicopathological Factors According to *MET* Mutation Status in 211 Patients with Lung Adenocarcinomas

Characteristic	Mutated	Wild Type	P (univariate)	P (multivariate)
Sex				
Male	4 (57%)	98 (48%)	0.714	0.552
Female	3 (43%)	106 (52%)		
Age				
≤64	2 (29%)	104 (51%)	0.279	0.736
>64	5 (71%)	100 (49%)		
Stage				
I–II	7 (100%)	140 (69%)	0.104	0.981
III–IV	0 (0%)	64 (31%)		
Smoking status				
Never-smoker	3 (43%)	102 (50%)	>0.999	0.644
Smoker	4 (57%)	102 (50%)		
Differentiation				
W-M	5 (71%)	139 (68%)	>0.999	0.365
P	2 (29%)	65 (32%)		
EGFR				
Mutated	0 (0%)	103 (50%)	0.014	0.976
Wild type	7 (100%)	101 (50%)		
KRAS				
Mutated	0 (0%)	29 (14%)	0.597	0.987
Wild type	7 (100%)	175 (86%)		
HER2				
Mutated	0 (0%)	5 (2%)	>0.999	0.995
Wild type	7 (100%)	199 (98%)		

M, moderately differentiated; P, poorly differentiated; W, well differentiated; EGFR, epidermal growth factor receptor.

amplification or mutation occurred selectively in these adenocarcinomas without *EGFR*, *KRAS* or *HER2* mutations with a statistical significance ($p < 0.0001$; Figure 4).

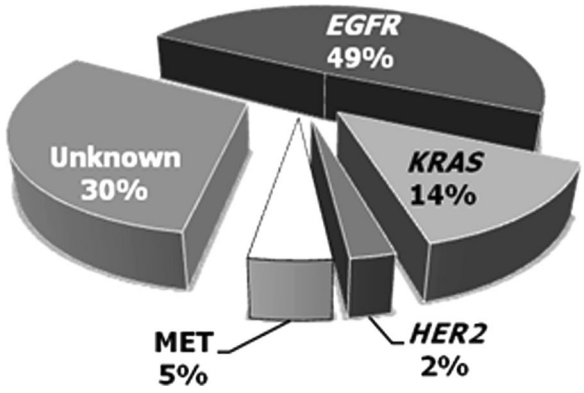

FIGURE 4. Frequencies of MET activation by gene amplification or splice mutation and the mutations of each gene in 211 Japanese patients with lung adenocarcinomas. Thirty percent of the specimens had no mutations of the epidermal growth factor receptor (*EGFR*), *KRAS* or *HER2* genes and did not show MET activation. Each instance of MET activation detected in this series was mutually exclusionary with these gene mutations. *FGFR4* mutation and *EML4-ALK* fusion gene have been reported to occur in similar exclusionary fashion and be present in 1 of 158 (0.6%) and 5 of 149 (3.4%) of lung adenocarcinomas, respectively, these may be encompassed in the part of Unknown.[29,30]

DISCUSSION

We found that about 5% of lung adenocarcinomas had MET activation either by gene amplification (2 of 148 patients, 1.4%) or splice mutations deleting the juxtamembrane domain (7 of 211 patients, 3.3%). Unlike *EGFR* or *HER2* mutations that mainly target female, Asian and nonsmoking patients, and unlike *KRAS* mutations that mainly target male, Caucasian and smoking patients,[15–17,21,26] there was no such relationship for MET activation.

MET gene amplification was detected in 22% (4 of 18) of NSCLCs that had developed acquired resistance to EGFR tyrosine kinase inhibitors (TKIs).[7] The prevalence of *MET* amplification in primary lung cancer patients unexposed to such inhibitors had not been elucidated until recently. However, Bean et al.[6] reported that *MET* amplification was present only in 2 of 62 EGFR-TKI-untreated patients, but that it was present in 9 of 43 patients with acquired resistance. This is in agreement with our results, suggesting that there is no ethnic difference in this clinical feature and that gene amplification does not play a major role for development of pulmonary adenocarcinomas without EGFR-TKI treatment.

There was also no significant difference in the prevalence of *MET* somatic mutation between our study (7 of 211, 3.3%) and previous studies from Western countries (2 of 127, 1.6%[8] or 2 of 33, 6%[9]). We were able to identify cis-acting intronic mutations of the *MET* gene that disrupted splice consensus sequence in four of seven patients. However, in the remaining three patients, the mechanisms for abnormal splicing could not be determined. Other possible mechanisms included mutations and nongenetic alterations of factors required for constitutive or alternative splicing or formation of the fusion proteins involving splice factors resulting from cancer-associated chromosomal translocations.[24] Interestingly, mechanisms leading to deletion of exon 14 in 7 cases including those reported by Kong-Beltran et al. (Figure 3*B*) were all different. This also suggest the notion that deletion of exon 14 plays an important role in the development of lung adenocarcinomas, conferring various advantages to the tumor cells and that *MET* splice mutations are ''driver mutations'' and not just ''passenger mutations.''[27]

Although a *MET* splice mutation did not occur in tumors with *MET* amplification, we noticed that mutant alleles were transcribed preferentially in RT-PCR or sequencing experiments. Kong-Beltran et al.[9] reported similarly that the deleted form of the MET receptor is expressed predominantly despite their tumor samples being heterozygous for exon 14, suggesting preferential expression of the variant transcript.

Shibata et al.[3] reported that *MET* amplification occur in tumors without *EGFR* mutations. Kong-Beltran et al.[9] found that tumors harboring these intronic mutations were wild type for *KRAS*, *BRAF*, *EGFR*, and *HER2.* In the present study, we were able to confirm and extend these findings. MET activation by amplification or splice mutation was present only in tumors without any mutations of the *EGFR*, *KRAS*, or *HER2*

genes. This again suggests that MET activation plays an important role that is equivalent to mutations of the *EGFR*, *KRAS* or *HER2* genes. Altogether, lung adenocarcinomas containing the MET activation seem to form a novel and independent subclass of such tumors.

In the present study, 30% of Japanese patients with adenocarcinomas of lung did not harbor any mutations of *EGFR*, *KRAS* or *HER2* and MET activation by gene amplifications or splice mutations (Figure 4). To generate a therapeutic strategy for these patients, it will be important to discover activated genes that have complementary roles.

Tumor cell lines with activated MET either by gene amplification or splice mutations are highly sensitive to MET targeted therapy.[4,5,9] A dramatic reduction in tumor cell numbers was observed using the MET TKI, PHA665752, in gastric cancer cell lines with a *MET* amplification including the MKN45 cells used in the present study.[5] Short hairpin RNA-mediated MET knockdown induced significant growth inhibition, G1/S cell cycle arrest, and apoptosis in EBC-1 and H1993 cells showing MET amplification, whereas it had little or no effect on cell lines not exhibiting MET amplification.[4] Similarly, a lung cancer cell line (NCI-H596) harboring a splice variant of *MET* was sensitive to an anti-MET OA-5D5 antibody.[9] Altogether, these results strongly suggest that MET activation identifies a subset of NSCLCs that is likely to respond to new molecular therapies targeting MET.

In conclusion, we found that about 5% of pulmonary adenocarcinomas were driven by activated MET by gene amplifications or splice mutations. Although this prevalence seems low, a considerable number of patients with lung cancers would benefit from anti-MET strategy, considering that over one million deaths are caused by lung cancer worldwide annually.

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