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MET exon 14 skipping mutation in triple-negative pulmonary adenocarcinomas and pleomorphic carcinomas: An analysis of intratumoral MET status heterogeneity and clinicopathological characteristics

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ABSTRACT

Objectives: *MET* mutations leading to exon 14 skipping rarely occur in non-small cell lung cancer (NSCLC). Recently, small molecule inhibitors targeting *MET* mutations showed clinical benefit. However, the clinicopathological characteristics of NSCLC harboring *MET* mutations, and the correlation among mutations, protein expression, and gene copy number of MET in NSCLC remain unclear. Therefore, we address these issues.

Materials and methods: *MET* exon 14 skipping mutations were evaluated using real-time quantitative reverse-transcription-PCR (qRT-PCR) in 102 triple-negative (i.e., *EGFR* mutation (−)/*ALK* translocation (−)/*KRAS* mutation (−)) pulmonary adenocarcinomas, and 45 pleomorphic carcinomas. *MET* mutation and gene copy were also examined in microdissected tissues obtained from tumor areas with heterogeneous MET immunohistochemical expression.

Results: *MET* mutations were detected in 8.8% (9/102) of triple-negative adenocarcinomas and 20% (9/45) of pleomorphic carcinomas of the lung. Patients with *MET*-mutated adenocarcinomas was significantly older than those without *MET* mutations ($P = 0.015$). The male to female and ever-to never-smoker ratios were 3:6 and 2:7, respectively, among patients with *MET*-mutated adenocarcinomas. All (9/9) of the *MET*-mutated adenocarcinomas showed acinar predominant histology with associated lepidic patterns. In contrast, the male to female and ever- to never-smoker ratios were 8:1 and 7:1, respectively, among patients with *MET*-mutated pleomorphic carcinomas. The carcinoma component of *MET*-mutated pleomorphic carcinomas was mostly adenocarcinoma of acinar pattern (8/9). *MET* mutation was detected by qRT-PCR in all samples with heterogeneous MET expression microdissected from five cases with *MET*-mutated adenocarcinoma, while *MET* gene amplification was detected in tumor areas expressing high MET protein levels among *MET*-mutated adenocarcinomas.

Conclusion: *MET*-mutated NSCLC is characterized by older age in patients with adenocarcinoma and by an acinar histology and variable MET expression in patients with adenocarcinoma and pleomorphic carcinomas. Moreover, *MET* gene amplification might occur in the tumor cells harboring the *MET* mutation.

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1. Introduction

Tyrosine kinase inhibitors (TKIs) targeting *EGFR* mutations and *ALK* translocations have significantly improved the clinical outcomes of patients with non-small cell carcinoma (NSCLC). Therefore, this therapy has been established as a primary treatment modality for patients with advanced NSCLC and *EGFR* mutations or *ALK* translocations [1,2]. In recent years, targeted therapies against rare genetic alterations in the *MET*, *ROS1*, *RET*, *BRAF*, and *HER2* genes have demonstrated promising results [3].

MET is a receptor tyrosine kinase that is activated upon binding to the hepatocyte growth factor (HGF)/scatter factor (SF) ligand. It plays an important role in both cancer development and progression by promoting cell survival, proliferation, angiogenesis, invasion and metastasis [4]. MET alterations (e.g., by protein overexpression and gene amplification) have been observed in certain types of cancer and have been associated with aggressive behavior of NSCLC [4–6]. Therefore, MET has been considered as a potential therapeutic target for NSCLC, and several therapies (e.g., HGF antagonists, anti-MET monoclonal antibodies, and MET TKIs) have already been developed [7]. Of these treatment, crizotinib was considered an effective reagent for patients with NSCLC and *MET* amplification [8,9]. On the other hand, MET protein overexpression was also assumed to be a potential predictive biomarker for anti-MET antibody therapy in patients with NSCLC [10]. However, recent phase III clinical trials with anti-MET antibody failed [11]. More research on predictive biomarkers for MET-targeted therapy is required.

The *MET* mutations found in patients with NSCLC usually involve the splicing sites adjacent to exon 14, which precipitate exon 14 skipping. *MET* exon 14 skipping leads to impaired c-Cbl-mediated ubiquitination and degradation of MET, resulting in sustained activation of MET [12–15]. In previous preclinical studies, it has been demonstrated that NSCLC cells with *MET* mutations are dependent on MET signaling for survival and proliferation; thus, as anticipated, MET TKI suppresses tumor growth [13,14,16]. Furthermore, in clinical studies, patients with NSCLC and the *MET* exon 14 skipping mutation responded to various MET TKIs including crizotinib, cabozantinib, and capmatinib [8,16–22]. Therefore, the *MET* exon 14 skipping mutation is a promising therapeutic target for patients with NSCLC.

The prevalence of the *MET* exon 14 skipping mutation is found in approximately 3% of pulmonary adenocarcinomas, which is exclusive to *EGFR*, *KRAS* and *ALK* mutations [23–26]. Recently, it was reported that 20–30% of pulmonary sarcomatoid carcinomas show the *MET* exon 14 skipping [20,25]. To date, the clinicopathological features of patients with NSCLC harboring *MET* mutations remain to be elucidated. Since a very small percentage of patients with NSCLC actually harbor a *MET* mutation, efficient screening strategies are challenging from the perspective of clinical practice. In our previous study using a tissue microarray, MET immunohistochemistry was not helpful for prescreening NSCLC harboring the *MET* mutation [27]. The relationship among *MET* amplification, MET protein overexpression, and *MET* mutations in patients with NSCLC has been controversial [21,25–27]. In this study, we hypothesized that *MET* gene copy number, mutation, and protein expression statuses might be complex and heterogeneous in NSCLCs harboring a *MET* mutation.

Thus, this study was intended 1) to elucidate the clinicopathological characteristics of patients with NSCLC harboring the *MET* mutation; 2) to evaluate both protein expression and gene amplification in *MET*-mutated NSCLC; and 3) to examine the intratumoral heterogeneity of MET alterations in *MET*-mutated NSCLC. Considering the low prevalence of *MET* mutations in NSCLC, we collected and examined adenocarcinomas of triple-negative genotype (i.e., *EGFR* mutation (−)/*KRAS* mutation (−)/*ALK* translocation (−)) and pleomorphic carcinomas (PCs) to address these issues.

2. Material and methods

2.1. Patients and samples

Of all patients who underwent surgical resection for pulmonary adenocarcinoma from February 2012 to March 2015 and were submitted to genetic tests for *EGFR*, *KRAS* and *ALK* at Seoul National University Hospital (SNUH) as previously reported [28], total 102 patients without an *EGFR* mutation, *KRAS* mutation, or *ALK* translocation (i.e., triple-negative) were selected. Forty-five patients who underwent surgical resection for PCs from February 2001 to August 2015 at SNUH irrespective of genotypes were also selected for the study. All patients were Korean descent and no patients received neoadjuvant chemotherapy prior to surgery. Clinical data were obtained from medical records. Pathological tumor-node-metastasis (TNM) stage followed the 7th American Joint Committee on Cancer. This study was conducted in accordance with the recommendations of the World Medical Association Declaration of Helsinki and approved by the institutional review board of SNUH (IRB approval number H-1407-142-597).

2.2. MET *mutation analysis using real-time quantitative reverse-transcription PCR (qRT-PCR)*

Hematoxylin and eosin (H&E)-stained slides of resected tumor were reviewed. Representative whole formalin-fixed paraffin-embedded (FFPE) tissue blocks were cut, deparaffinized, and extracted for RNA using the RecoverAll™ Total Nucleic Acid Isolation Kit (Ambion; Life Technologies, Carlsbad, CA, USA). A quantitative qRT-PCR kit for detection of the c-MET exon 14 deletion mutation in the juxtamembrane domain was used in accordance with the manufacturer's instructions (Cat# Met-001, Custom Diagnostics, Irvine, CA, USA; kindly provided by James G. Christensen, Mirati Therapeutics, San Diego, CA, USA), as reported previously [21,27]. In addition, to assess the intratumoral heterogeneity of the *MET* mutation, whole FFPE tissue blocks from *MET*-mutated cases were sliced, placed on glass slides, and analyzed; each section with multifocal areas and heterogeneous MET expression was microdissected and analyzed by qRT-PCR to detect *MET* mutations. The appropriate positive and negative control samples included in the kit were evaluated simultaneously in each experiment.

2.3. Immunohistochemistry

MET expression was analyzed by immunohistochemistry (IHC) using a rabbit monoclonal anti-c-Met (SP44) antibody (Ventana Medical Systems, Tucson, AZ, USA). IHC was performed using the Benchmark XT autostainer (Ventana Medical Systems), with antigen retrieval achieved using CC1 buffer and a 1:50 antibody dilution, according to the manufacturer's instructions. MET expression was evaluated based on membranous and/or cytoplasmic staining and given intensity scores of 0 (none), 1 (weak), 2 (moderate), or 3 (strong) and the proportion of staining. H scores was calculated according to the following formula: (1 × percentage of cells with weak staining) + (2 × percentage of cells with moderate staining) + (3 × percentage of cells with strong staining).

2.4. Fluorescence in situ hybridization (FISH)

To determine the copy number of *MET*, FISH was performed using Vysis LSI MET SpectrumRed and Vysis CEP7 (D7Z1) SpectrumGreen probes (Abbott Molecular Inc., Chicago, IL, USA), according

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