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MET amplification, protein expression, and mutations in pulmonary adenocarcinoma

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ABSTRACT

Objectives: *MET* amplification, protein expression, and splice mutations at exon 14 are known to cause dysregulation of the MET/HGF pathway. Our study aimed to confirm the relationship among *MET* amplification, protein expression, and mutations in pulmonary adenocarcinoma.
Materials and methods: MET protein expression by immunohistochemistry (IHC) and *MET* amplification by fluorescence in situ hybridization (FISH) were evaluated in 316 surgically resected lung adenocarcinomas. Patients were divided into 4 groups (IHC-negative/FISH-negative, IHC-negative/FISH-positive, IHC-positive/FISH-negative, and IHC-positive/FISH-positive), and 15–20 tumors in each group were randomly selected for mutation analyses to find splice mutations at exon 14.
Results: An IHC score of 0–3 was found in 168 (53.2%), 71 (22.5%), 59 (18.7%), and 18 (5.7%) tumors, respectively. The mean gene copy number (GCN) was 3.56; *MET* FISH positivity was detected in 123 (38.9%) samples, and 26 (8.2%) of them were gene amplifications. *MET* amplification were significantly associated with the IHC score ($P < 0.001$, χ^2 test). Splice mutations were identified in only 2 (2.9%) of 70 cases. One had a MET IHC score of 2 and negative FISH without amplification; The other had a MET IHC score of 0 and positive FISH without amplification. MET IHC or FISH results were not prognostic indicators of overall survival in multivariate analysis.
Conclusion: There is a significant relationship between *MET* amplification and protein expression, and selection of tumors with amplification using IHC was effective. However, because of its rarity, a selection strategy for mutated tumors is implausible using IHC or FISH.

1. Introduction

Because the epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) tyrosine kinase inhibitors (TKIs) have shown benefits, other potential therapeutic target genes like *MET*, *ROS1*, *BRAF* and *HER2* have being actively investigated in non-small cell lung cancer (NSCLC) [1]. MET is a heterodimeric receptor tyrosine kinase composed of extracellular, transmembrane, juxtamembrane, and kinase domains [2,3]. Binding of hepatocyte growth factor (HGF) to MET induces phosphorylation of the docking site and stimulates downstream signal pathways such as the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase-Akt (PI3K)/protein kinase B pathways [3]. These pathways are known to involve cell growth, migration, angiogenesis, and survival [4].

Overexpression of HGF or MET, amplification, or mutation of *MET* has been identified as a cause of MET pathway dysregulation. In addition to NSCLC, breast cancer, colon cancer, kidney cancer, and stomach cancer have demonstrated overexpression of MET [5–8]. *MET* amplification has been discovered in colon cancer, esophageal cancer, and stomach cancer [9].

One of the activating mechanisms of *MET* is gene amplification or increased gene copy number (GCN). In an *in vitro* study, the level of tyrosine phosphorylation was greater in a *MET*-amplified cell

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line than in a non-amplified one, and the knockdown of *MET* in the amplified cell line caused growth inhibition, cell cycle arrest, and apoptosis [10]. The prevalence of high *MET* GCN and amplification were 10.6–20.8% and 2.1–4.4% in previous studies, and they were associated with poor prognosis in NSCLC patients [11–16]. In addition to this *de novo* mechanism, *MET* amplification has been identified as the mechanism resulting in EGFR-TKI resistance in about 20% of resistant tumors [17,18].

MET protein is expressed in 22.2–74.6% of NSCLC, and it has been associated with poor prognosis in several studies [19–21]. Some of those studies also reported that MET expression is more common in adenocarcinoma than in other histologic types [19,22]. Increased MET protein expression is associated with phosphor-MET expression, and this suggests that MET overexpression may be related to activation of the MET pathway [21]. The prognostic value of MET GCN and protein expression is controversial, although one meta-analysis has documented that both of them are significantly associated with poor overall survival (OS) in surgically resected NSCLC [23].

The semaphorin domain and juxtamembrane domain are the key sites of *MET* mutations in NSCLC. *MET* can have splice mutations in the juxtamembrane region, which is the binding site of Cbl E3-ligase, and these mutations lead to exon 14 deletion. These somatic mutations are associated with ligand-mediated proliferation and tumor growth by decreased ubiquitination and delayed down-regulation of receptors, and are known to be important activating mechanisms of the MET pathway [24,25].

Among the many kinds of alterations, amplification, protein expression, and splice mutations at the juxtamembrane domain of *MET* have been extensively studied in NSCLC. However, the proper target of a MET inhibitor has not been established. The randomized phase II trial of the MET inhibitor, onartuzumab, in combination with erlotinib, has reported a benefit for OS and progression-free survival (PFS) in MET immunohistochemistry (IHC)-positive patients [26]. However, the phase III trial using the same criteria for IHC did not confirm the efficacy of onartuzumab [27]. A phase I trial for another MET inhibitor, crizotinib, used *MET* amplification as a target, and reported promising results in the interim analysis [28].

In order to define an adequate target population and selection strategy for treatment with MET inhibitors, it is essential to first understand the associations of *MET* alterations. The purpose of our study was to determine the relationship among *MET* amplification, protein expression, and mutations in pulmonary adenocarcinoma.

2. Materials and methods

2.1. Patient selection

The records of patients who underwent pulmonary resection between 2004 and 2011 at the Seoul National University Hospital were reviewed, and patients with adenocarcinoma whose surgical tissues were available for evaluation were included in the analysis. To perform our study in a homogeneous setting, histologic types other than adenocarcinoma, and patients who had received chemotherapy or TKI treatment before surgery were excluded. A total of 316 patients were enrolled, and clinical data were collected from the medical records. Survival data of the enrolled patients were obtained through the Korean civil registry. The median follow-up time was 73 months (range 2–153 months), and 104 patients (32.8%) died during the follow-up period. The study was approved by the Institutional Review Board at the Seoul National University Hospital (IRB No. H-1407-142-597).

2.2. *Immunohistochemistry and fluorescence* in situ *hybridization*

A core tissue of 2 mm in diameter was taken from each representative tissue block, and tissue microarrays were created for evaluation. Sections with 4 μm thickness from each tissue microarray were cut for IHC and fluorescence *in situ* hybridization (FISH) analysis. MET protein expression was evaluated by IHC using a rabbit monoclonal antibody against c-MET (SP44, catalog 7904430, Ventana Medical Systems, Tucson, AZ, USA) and the Benchmark XT autostainer from Ventana Medical Systems. IHC score was defined by the modified criteria used in the clinical trial for the MET inhibitor as follows: 0, absence of staining or any intensity staining in less than 50% of tumor cells; 1, weak to moderate intensity staining in more than 50% of tumor cells; 2, moderate to strong intensity staining (comparable to that in bronchial epithelium) in more than 50% of tumor cells; 3, strong intensity staining in more than 50% of tumor cells [26]. An IHC score of 2 or 3 was defined as positivity.

MET GCN and amplification was estimated using and LSI MET SpectrumRed/CEP7SpectrumGreen probe (Abbott Molecular, Des Plaines, IL, USA), and was counted in at least 100 tumor nuclei. Gene amplification (*MET* to CEP7 ratio ≥2; >15 copies of the *MET* signals in >10% of the tumor cells; small gene cluster [4–10 copies] or innumerable tight gene cluster in >10% of the tumor cells) and high polysomy (≥40% of cells displaying ≥4 copies of the *MET* signal) were defined as FISH positivity according to University of Colorado Cancer Center (UCCC) criteria [12].

2.3. *Reverse transcription polymerase chain reaction and direct sequencing*

Patients were divided into four groups (IHC-negative/FISH-negative, IHC-negative/FISH-positive, IHC-positive/FISH-negative, and IHC-positive/FISH-positive). Then, 15–20 patients were randomly selected from each group, and reverse transcription polymerase chain reaction (RT-PCR) was performed to detect splice mutations in the juxtamembrane domain. Direct sequencing was also performed at exon-intron 13 and 14 to identify the mutations.

For the selected cases, a pathologist reviewed representative hematoxylin and eosin-stained slides and manually microdissected tumor regions from consecutive formalin-fixed, paraffin-embedded sections. After deparaffinization, genomic RNA was extracted, and RT-PCR for detection of a *MET* exon 14 deletion was performed using a qualitative kit (catalog MET-001, Custom Diagnostics, Irvine, CA, USA). The protocol for the RT-PCR was one cycle of 45 °C for 30 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s and 60 °C for 30 s. DNA was also extracted and subjected to nested-PCR amplification of *MET* exon-intron 13 and 14. PCR products were visualized on a 2% agarose gel, purified, and subsequently subjected to direct Sanger sequencing using an ABI-PRISM 3100 DNA Analyzer (Applied Biosystems, Vernon Hills, IL, USA).

2.4. Statistical analysis

To analyze the relationship between clinicopathological factors and IHC or FISH groups, a χ^2 test or Fisher's exact probability test was used. The χ^2 test was also used to compare IHC score with positive FISH or amplification. The mean *MET* GCN was compared among IHC score groups with an analysis of variance (ANOVA) test, and the trend of GCN was identified with the Jonckheere-Terpstra test. A Cox proportional hazards model was used for survival analysis. A *P*-value less than 0.05 was considered significant. Analyses were performed using SPSS for Windows, version 20.0 (IBM Corporation, Armonk, NY, USA).