

## Oncogenic Function of a KIF5B-MET Fusion Variant in Non-Small Cell Lung Cancer

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### Abstract

A kinesin family member 5b (*KIF5B*)-*MET* proto-oncogene, receptor tyrosine kinase (*MET*) rearrangement was reported in patients with lung adenocarcinoma but its oncogenic function was not fully evaluated. We used one-step reverse transcription-polymerase chain reaction for RNA samples to screen for the *KIF5B-MET* fusion in 206 lung adenocarcinoma and 28 pulmonary sarcomatoid carcinoma patients. Genomic breakpoints of *KIF5B-MET* were determined by targeted next-generation sequencing. Soft agar colony formation assays, proliferation assays, and a xenograft mouse model were used to investigate its oncogenic activity. In addition, specific *MET* inhibitors were administered to evaluate their anti-tumor activities. A *KIF5B-MET* fusion variant in a patient with a mixed-type adenocarcinoma and sarcomatoid tumor was identified, and another case was found in a pulmonary sarcomatoid carcinoma patient. Both cases carried the same chimeric gene, a fusion between exons 1–24 of *KIF5B* and exons 15–21 of *MET*. *KIF5B-MET*-overexpressing cells exhibited significantly increased proliferation and colony-forming ability. Xenograft tumors harboring the fusion gene demonstrated significantly elevated tumor growth. Ectopic expression of the fusion gene stimulated the phosphorylation of *KIF5B-MET* as well as downstream *STAT3*, *AKT*, and *ERK1/2* signaling pathways. The *MET* inhibitors significantly repressed cell proliferation; phosphorylation of downstream *STAT3*, *AKT*, and *ERK1/2*; and xenograft tumorigenicity. In conclusion, the *KIF5B-MET* variant was demonstrated to have an oncogenic function in cancer cells. These findings have immediate clinical implications for the targeted therapy of subgroups of non-small cell lung cancer patients.

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Abbreviations: ADC, adenocarcinoma; ALK, anaplastic lymphoma kinase; CT, computed tomography; HGF, hepatocyte growth factor; IHC, immunohistochemical; *KIF5B*, kinesin family member 5b; *MET*, *MET* proto-oncogene, receptor tyrosine kinase; NSCLC, non-small cell lung cancer; PTK, protein tyrosine kinase; *RET*, *RET* proto-oncogene; TKI, tyrosine kinase inhibitor; TTF-1, thyroid transcription factor-1.

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## Introduction

In non-small cell lung cancer (NSCLC), a considerable proportion of patients present extensive genomic instability within their tumors. This instability occurs at different levels, ranging from simple nucleotide changes, gene amplifications, chromosomal and structural rearrangements to gains or losses of entire chromosomes [1]. Structural chromosome rearrangements have been shown to result in gene fusions and can be a major driving force for tumorigenesis [2]. Originating from chromosomal translocations, oncogenic fusion proteins are frequently observed in lung adenocarcinoma (ADC); these are typically composed of an N-terminal dimerization domain provided by the fusion partner protein fused to the kinase domain of a tyrosine kinase. These fusion proteins often lead to kinase domain activation and provide ideal targets for the development of anti-cancer therapies [3].

More than 80 chromosome rearrangements have been reported in NSCLC [4]. Anaplastic lymphoma kinase (*ALK*), ROS proto-oncogene tyrosine-protein kinase (*ROS1*) and RET proto-oncogene (*RET*) fusions are the most common chromosome translocations involving a kinase domain [5,6]. Kinesin family member 5b (*KIF5B*) is the second frequent fusion partner of *ALK* [4,7], and also serves as a fusion donor for *RET* [8]. The *KIF5B* gene is located on chromosome 10p11.22 and encodes the KIF5B protein. It has been suggested that KIF5B-protein tyrosine kinase (PTK) fusion proteins result in aberrant kinase activation of the PTK [6,7,9]. These fusion genes usually contain at least the N-terminal 1–15 exons of *KIF5B*, which encode a protein comprising the kinesin motor and coiled-coil domains that mediate homodimerization [7]. In lung tissue, KIF5B is ubiquitously and constitutively expressed, and its active promoter might consequently drive the activation of ALK/RET tyrosine kinases and subsequently enhance their downstream oncogenic effects [10,11].

Recently, another *KIF5B*-PTK fusion transcript, *KIF5B-MET*, has been discovered in lung ADC patients; this consisted of a chimeric fusion of exons 1–24 of *KIF5B* to either exons 14–21 [12,13] or exons 15–21 [14] of the *MET* proto-oncogene, receptor tyrosine kinase (*MET*), which was denoted as K24;M14 or K24;M15 based on the last *KIF5B* and first *MET* exons in the fusion, respectively. This is a remarkable finding given that *MET* is a known oncogenic driver of lung cancer. Given that *MET* gene alterations were not uncommon among lung ADC and pulmonary sarcomatoid carcinoma [15–17], we sought to screen *KIF5B-MET* fusions in such two histologic types of NSCLC in Taiwanese patients. We identified two patients with same *KIF5B-MET* fusion—K24;M15 variant. We further performed *in vivo* functional assays and applied a mouse xenograft model to corroborate its oncogenic activity, as well as its responsiveness to specific MET tyrosine kinase inhibitors.

## Material and Methods

### Sample Collection

A total of 206 diagnostic lung ADC and 28 pulmonary sarcomatoid carcinoma patient specimens, which had been identified as negative for *EGFR*, *ALK*, *KRAS*, *HER2*, or *RET* mutations in previous cohort studies [15,18,19], were included. Specimens were obtained from primary/metastatic surgical excisions, computed tomography-guided biopsies, and malignant pleural effusions at the National Taiwan University Hospital (Taipei, Taiwan). The histology of the lung cancer tissue was determined through pathological examination using immunohistochemical (IHC) stain-

ing for thyroid transcription factor-1 (TTF-1), CK7, CK5/6, p40, vimentin, and hematoxylin and eosin. Tumor specimen collection, preparation, and RNA extraction were performed as previously described [15]. Informed, written consent was obtained from all study participants prior to specimen collection. The institutional review board of the hospital approved the study.

### Hematoxylin–Eosin and Immunohistochemical Staining

Tissue sections (4  $\mu$ m thick) were dewaxed and rehydrated. For hematoxylin–eosin staining, sections were reacted with hemalum, which was followed by counterstaining with eosin. For IHC staining, slides were subjected to antigen retrieval and allowed to react with an anti-human c-MET C-terminus antibody (Spring Bioscience Corp., Pleasanton, CA; clone SP44, 1:50 dilution). The incubation procedure, counterstaining with hematoxylin, and negative controls were performed as described previously [15].

### Reverse Transcription-Polymerase Chain Reaction Analysis of *KIF5B-MET* Transcript

The RT-PCR conditions were based on the manufacturer's protocol. Briefly, 50–100 ng of total RNA was used as template and the following components were added: (1) 10 ml 5 $\times$  reaction buffer, (2) 2 ml dNTP mix (10 mM each), (3) 3 ml of 10 mM forward and reverse primer each, (4) 2 ml QIAGEN OneStep RT-PCR enzyme mix and (5) RNase-free water to reach a total volume of 50 ml. The RT-PCR reaction was initiated at 50  $^{\circ}$ C for 30 minutes, heated to 95  $^{\circ}$ C for 15 minutes, then followed by 40 cycles of denaturation at 94  $^{\circ}$ C for 50 seconds, annealing at 60  $^{\circ}$ C for 50 seconds, extension at 72  $^{\circ}$ C for 1 minutes, and a final extension at 72  $^{\circ}$ C for 10 minutes. The primers were forward primers for *KIF5B* exon 15 (*KIF5B*-E15F) (5'-TAAGGAAATGACCAAC CACCAG-3'), *KIF5B* exon 20 (*KIF5B*-E20F) (5'-AGCCACAGAT CAGGAAAAGA-3'), or *KIF5B* exon 24 (*KIF5B*-E24F) (5'-ATCG CAAACGCTATCAGCAAGA-3'), and a reverse primer for *MET* exon 15 (*MET*-E15R) (5'-TGCACAATCAGGCTACTGGGCC-3'). The resulting products were directly sequenced in both directions using the primers *KIF5B*-E15F/*KIF5B*-E20F/*KIF5B*-E24F and *MET*-E15R. The National Center for Biotechnology Information (NCBI) *KIF5B* (NM\_004521.2; 963 amino acids) and *MET* (NM\_000245.3; 1390 amino acids) were used as reference sequences.

### Mapping the Translocation Breakpoint by Targeted Next Generation Sequencing

DNA was extracted from fresh frozen cell pellets using QIAamp tissue DNA extraction kits (Qiagen, Valencia, CA). Based on human genome 19, NCBI build GRCh37, 94 probes for the targeted region (32,304,368–32,306,347 in chromosome 10 for *KIF5B* and 116,411,501–116,415,300 in chromosome 7 for *MET*) and four internal controls in chromosomes 6, 10, 16, and 17 were used for targeted capture reactions. In total, 2  $\mu$ g of genomic DNA was sonicated into fragment sizes of approximately 800 base pairs, and used for library construction. We next performed target enrichment using the double capture protocol (Roche NimbleGen Inc., Madison, WI) following the manufacturer's instruction. Read sequences were analyzed using the Illumina Miseq system (Illumina, San Diego, CA) and with different tools (bwa.0.7.4, picard-tools-1.90, Genome Analysis-TK-2.5-2, and IGV2.1.16). The genomic *KIF5B-MET* translocation breakpoints were further confirmed by PCR using a forward primer specific for *KIF5B* intron 24, (5'-GGAACCTGG GAAGTGGAGAT-3') and a reverse primer for *MET* intron 14 (5'-

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