Case Report

SEC31A-ALK Fusion Gene in Lung Adenocarcinoma

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Anaplastic lymphoma kinase (*ALK*) fusion is a common mechanism underlying pathogenesis of non-small cell lung carcinoma (NSCLC) where these rearrangements represent important diagnostic and therapeutic targets. In this study, we found a new *ALK* fusion gene, *SEC31A-ALK*, in lung carcinoma from a 53-year-old Korean man. The conjoined region in the fusion transcript was generated by the fusion of *SEC31A* exon 21 and *ALK* exon 20 by genomic rearrangement, which contributed to generation of an intact, in-frame open reading frame. *SEC31A-ALK* encodes a predicted fusion protein of 1,438 amino acids comprising the WD40 domain of SEC31A at the N-terminus and ALK kinase domain at the C-terminus. Fluorescence *in situ* hybridization studies suggested that *SEC31A-ALK* was generated by an unbalanced genomic rearrangement associated with loss of the 3'-end of *SEC31A*. This is the first report of *SEC31A-ALK* fusion transcript in clinical NSCLC, which could be a novel diagnostic and therapeutic target for patients with NSCLC.

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Key words

Introduction

Point mutation and gene amplification events may enhance oncogenic activity of anaplastic lymphoma kinase (ALK); however, the most common genetic alteration involving *ALK* in tumors is genomic rearrangement [1]. Thus far, various translocations and inversions involving the 2p23 chromosomal locus harboring the *ALK* gene have been reported. These rearrangements result in fusion genes involving fusion of the 3′-end of *ALK* to the 5′-end of various partners [1]. The NPM-ALK chimeric gene, originally identified in patients with anaplastic large cell lymphoma, was the first reported fusion involving the ALK tyrosine kinase domain. Various other ALK chimeric proteins have recently been identified in several tumor types.

Interestingly, most oncogenic *ALK* fusion genes share common features. First, expression of the *ALK* fusion gene is regulated by the promoter of the 5′-end partner gene, which usually encodes a protein widely expressed in healthy tissues [1]. Second, most N-terminal fusion partners contain domains that could be involved in oligomer formation, which is essential for the oncogenic activation of the ALK fusion protein [1]. Such oligomerization domains likely allow ligand-independent dimerization of ALK kinase domain and lead to constitutive kinase activation and aberrant activation of downstream signaling pathways for carcinogenesis [2,3].

Recent studies have demonstrated the important role of ALK in the pathogenesis of non-small cell lung cancer (NSCLC), where various *ALK* fusion genes have been identified. In particular, EML4-ALK [4] appeared as an oncogenic target in 6%-7% of NSCLC patients. In addition, kinesin family member 5B (KIF5B)–ALK, *TRK*-fused gene (TFG)–ALK, kinesin light chain 1 (KLC1)–ALK, protein tyrosine phosphatase and non-receptor type 3 (PTPN3)–ALK, huntingtin interacting protein 1 (HIP1)–ALK [5], and translocated promoter region (TPR)–ALK [6] have been found in lung adenocarcinoma by several research groups worldwide, including our group.

The oncogenic role of SEC31A-ALK fusion protein was well established in rare cases of ALK-positive large B-cell lymphomas [7,8] and inflammatory myofibroblastic tumor [9]; however, its occurrence in NSCLC has not been previously reported. Here, we report on the first case of *SEC31A-ALK* as an oncogenic target in NSCLC.

Case Report

A 53-year-old Korean man was diagnosed with NSCLC.

A computed tomography (CT) scan of the chest was performed, and an unusual mass measuring 1.5×1.0 cm² was identified in the lower lobe of the right lung (Fig. 1A). Microscopic evaluation of the lesion showed a poorly differentiated adenocarcinoma (Fig. 1B and D). Immunohistochemistry for ALK (1:50, clone 5A4, Novocastra, Newcastle upon Tyne, UK) showed diffuse cytoplasmic and granular staining (Fig. 1E and F). Fluorescence *in situ* hybridization (FISH) analysis for *ALK* using the Vysis ALK FISH Break-apart Probe Kit (Abbott Molecular, Abbott Park, IL) showed clear splitting between the 5′- and 3′-probe signals (Fig. 1C). Fifteen months after lobectomy of the tumor mass, CT scans did not show any evidence of recurrence or metastatic disease.

During screening for *ALK* fusion genes in the lung adenocarcinoma tumor using a single-tube multiplexed assay previously developed by our group [10], we found that the tumor tissue showed elevated expression of an *ALK* fusion transcript. Using Anchored Multiplex polymerase chain reaction (PCR) for *ALK*, *RET*, and *ROS1* (Enzymatics Inc., Boulder, CO), followed by next generation sequencing on MiSeq (Illumina, San Diego, CA) and reverse transcription polymerase chain reaction, we found that the tumor expressed a novel *SEC31A-ALK* rearrangement bearing a fusion junction between *SEC31A* exon 21 and *ALK* exon 20, which could contribute to production of an intact, in-frame open reading frame (Fig. 2). Further genomic PCR showed that *SEC31A-ALK* occurred via genomic recombination between nucleotide 83764951 (nucleotide 664 downstream of the *SEC31A* exon 21) on chromosome 4 and nucleotide 29447585 (nucleotide 1167 upstream of the *ALK* exon 20) on chromosome 2 (Fig. 2).

FISH with *SEC31A* break-apart probes [7] showed one co-localized red and green signal and one red signal alone in neoplastic cells (Supplementary Fig. S1). This pattern indicated loss of the 3′-end of *SEC31A* covered by BAC clones labeled in SpectrumGreen (RP11-251L03/RP11-450H10/RP11-791G16/RP11-331E09) (Supplementary Fig. S2). The *SEC31A-ALK* fusion gene was eventually demonstrated using a combination of the SpectrumOrange-labeled 5′-end probes of *SEC31A* (RP11-163O17/RP11-93E17/RP11-66E15/RP11-353M17) and the SpectrumGreen-labeled 3′-end of *ALK* (P1 clone 1111H1) [11], which were co-localized in neoplastic cells (Supplementary Fig. S3). These FISH findings suggest that the *SEC31A-ALK* rearrangement was generated not by a reciprocal t(2;4)(p23;q21), but by complex and/or imbalanced genomic rearrangement, like those in the previously reported cases [7-9].

The new *SEC31A-ALK* fusion transcript described in this study encodes a predicted protein of 1,438 amino acids with a WD40 domain encoded by the 5′-end of *SEC31A* and a tyrosine kinase domain encoded by the 3′-end of *ALK*
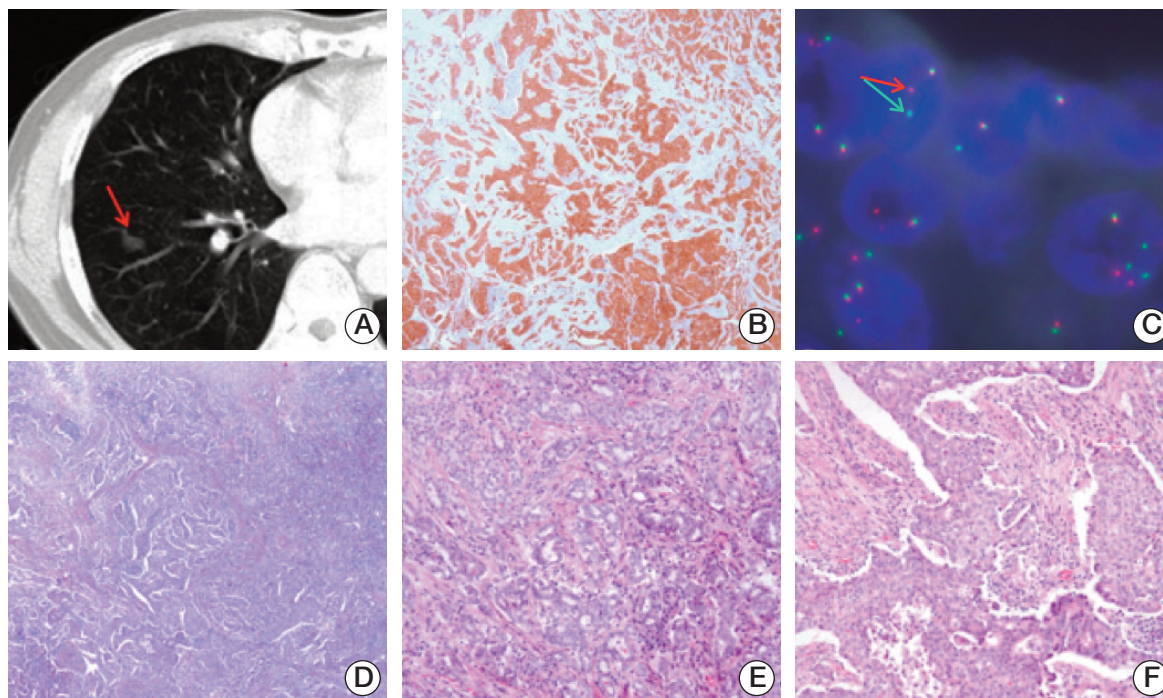
Fig. 1. (A) Computed tomography scan of the chest showing a solid nodule with a speculated margin (arrow) measuring 1.5-cm in size in the lower lobe of the right lung. (B) Anaplastic lymphoma kinase (ALK) immunohistochemical staining showing cytoplasmic staining of tumor cells (×100). (C) Fluorescence *in situ* hybridization assay of *ALK* showing *ALK* genomic rearrangement by split 5′- and 3′-probe signals (arrows). (D-F) Hematoxylin and eosin staining showing adenocarcinoma (D, ×100), and a mixture of acinar pattern (E, ×200) and solid pattern (F, ×200).

(Fig. 2A). Although this type of fusion gene was previously identified in large B-cell lymphoma and inflammatory myofibroblastic tumor (IMT), to the best of our knowledge, for the first time we discovered the *SEC31A-ALK* fusion gene in NSCLC. In contrast to the intact in-frame fusion between *SEC31A* exon 24 and *ALK* exon 20 in the previous fusion transcript identified in ALK-positive large B-cell lymphoma [7,8], the *SEC31A-ALK* fusion gene found in the reported case was formed by intact in-frame fusion between *SEC31A* exon 21 and *ALK* exon 20 at the fusion transcript level. However, all of these fusions harbored the same two intact functional domains (WD40 and tyrosine kinase), strongly suggesting that they play similar oncogenic roles [7,8]. We deposited the cDNA sequence for the new fusion transcript spanning the conjoined region at the transcript level into the NCBI Genbank under accession number KJ495955.

Discussion

Lung cancer is the leading cause of cancer-related deaths in the world. *ALK* has recently emerged as a novel molecular target for therapeutic strategies in lung adenocarcinoma. Tumors with translocations involving *ALK* usually express chimeric transcripts containing the 3′-end region of *ALK* encoding tyrosine kinase domain and the 5′-end region of various partner genes [12]. The known *ALK* partner genes in lung adenocarcinoma include *EML4* [4], *TFG*, *KIF5B*, *KLC1*, and *PTPN3* [13]. Our research group recently reported two novel fusion genes, *HIP1-ALK* and *TPR-ALK*, in lung adenocarcinoma [5,6].

We report here on a new *SEC31-ALK* fusion variant identified in a case of ALK-positive lung adenocarcinoma. Our FISH assay with break-apart probes for *ALK* and *SEC31A* suggested that the chimeric gene was not generated by a reciprocal t(2;4)(p23.2;q21.22), but by an unbalanced rearrangement associated with loss of 3′ *SEC31A*.

The *SEC31A* gene, which is known to be ubiquitously expressed in human cells, encodes for a SEC31A (*alias* SEC31L1) protein found within vesicular structures, which are distributed throughout the cell and are especially concentrated at the perinuclear region. SEC31A is a component of the coat protein complexes involved in mediating transport from the endoplasmic reticulum to the Golgi
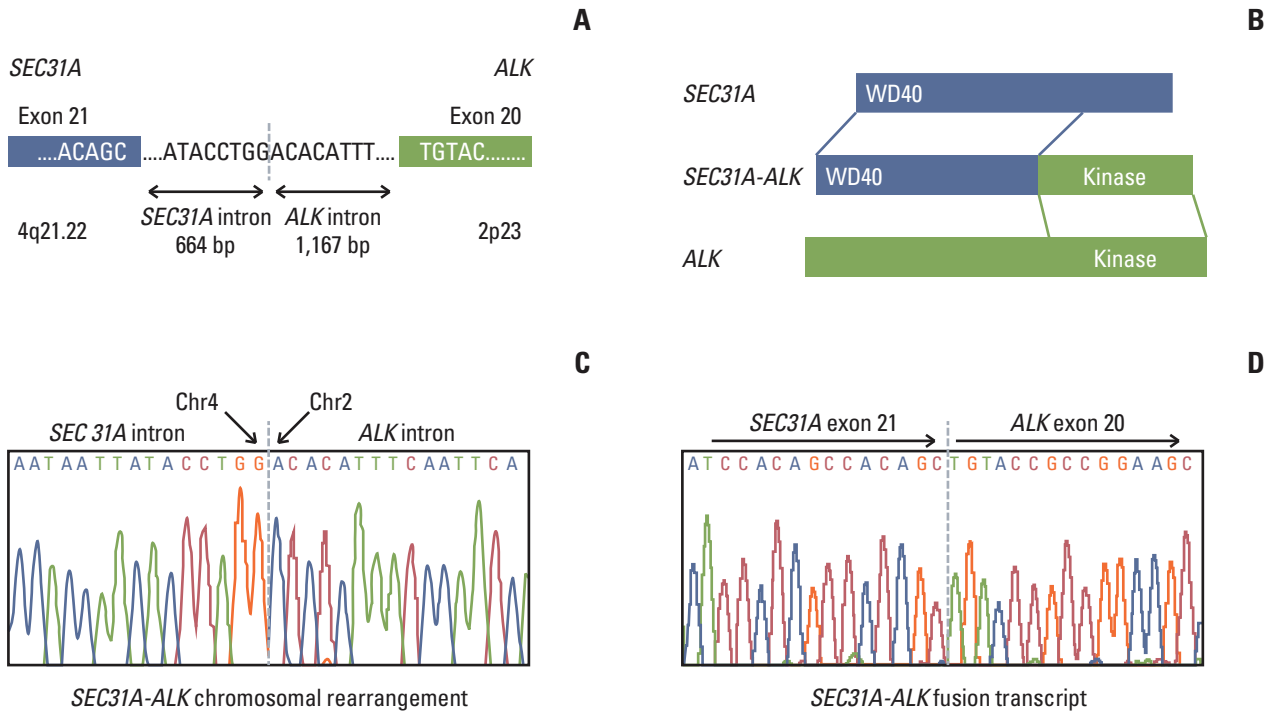
Fig. 2. Schematic (A) showing the intron containing and surrounding the conjoined region for genomic rearrangement for the *SEC31A-ALK* fusion gene encoding the fusion protein (B) harboring the WD and tyrosine kinase functional domains as well as the adjacent exons. (C, D) DNA sequence chromatograms showing the conjoined regions at the genomic DNA sequence level (C) and cDNA sequence level (D) of the *SEC31A-ALK* fusion gene.

complex [14]. Such cellular localization of SEC31A may explain a diffuse cytoplasmic and granular expression pattern of SEC31A-ALK observed in the current case of lung adenocarcinoma.

Oncogenic potential of SEC31A-ALK was previously demonstrated *in vitro* by Van Roosbroeck et al. [7], who showed that the fusion transforms interleukin 3 (IL-3)–dependent Ba/F3 cells to growth factor-independence and that kinase activity of SEC31A-ALK and its downstream signaling activity are reduced by NVP-TAE-684, the selective ALK inhibitor. In addition, suppression of Ba/F3 cell growth by NVP-TAE-684 was inhibited by treatment with IL3, thus reverting cell growth back to an IL-3 dependent pathway. The oncogenic tyrosine kinase activity of the SEC31A-ALK fusion protein is probably induced by the WD40 domain located at the N-terminus, which interacts with SEC13 and other proteins [9,14,15]. In the case of the *SEC31L1-ALK* variant identified in IMT, its fusion protein structure contained the same functional domains (WD40 and tyrosine kinase) [9].

SEC31A-ALK is a new *ALK* fusion gene in NSCLC. As previously demonstrated, the fusion harboring two intact functional domains, WD40 and ALK tyrosine kinase, is oncogenic and sensitive to NVP-TAE-684, an inhibitor of the ALK kinase [7]. Therefore, the *SEC31A-ALK* fusion gene is a potential driving rearrangement and diagnostic and therapeutic target for patients with NSCLC. In addition, our study confirms that NSCLC and ALK-positive lymphomas and IMT share the same *ALK* rearrangements.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).

Conflicts of Interest

M. Lira and M. Mao are current and former employees of Pfizer, Inc., respectively. D. Mann, J. Stahl, and A. Licon are employees of Enzymatics Inc.

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