

HIP1–ALK, a Novel Fusion Protein Identified in Lung Adenocarcinoma

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Introduction: The most common mechanism underlying overexpression and activation of anaplastic lymphoma kinase (ALK) in non-small-cell lung carcinoma could be attributed to the formation of a fusion protein. To date, five fusion partners of ALK have been reported, namely, echinoderm microtubule associated protein like 4, tropomyosin-related kinase-fused gene, kinesin family member 5B, kinesin light chain 1, and protein tyrosine phosphatase, nonreceptor type 3.

Methods: In this article, we report a novel fusion gene huntingtin interacting protein 1 (*HIP1*)–*ALK*, which is conjoined between the huntingtin-interacting protein 1 gene *HIP1* and *ALK*. Reverse-transcriptase polymerase chain reaction and immunohistochemical analysis were used to detect this fusion gene's transcript and protein expression, respectively. We had amplified the full-length cDNA sequence of this novel fusion gene by using 5'-rapid amplification of cDNA ends. The causative genomic translocation t(2;7)(p23;q11.23) for generating this novel fusion gene was verified by using genomic sequencing.

Results: The examined adenocarcinoma showed predominant acinar pattern, and ALK immunostaining was localized to the cytoplasm, with intense staining in the submembrane region. In break-apart, fluorescence in situ hybridization analysis for *ALK*, split of the 5' and 3' probe signals, and isolated 3' signals were observed. Reverse-transcriptase polymerase chain reaction revealed that the tumor harbored a novel fusion transcript in which exon 21 of *HIP1* was fused to exon 20 of *ALK* in-frame.

Conclusion: The novel fusion gene and its protein HIP1–ALK harboring epsin N-terminal homology, coiled-coil, juxtamembrane, and kinase domains, which could play a role in carcinogenesis, could become diagnostic and therapeutic target of the lung adenocarcinoma and deserve a further study in the future.

Key Words: Lung cancer, Anaplastic lymphoma kinase, HIP1, Translocation.

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Anaplastic lymphoma kinase (ALK)-positive non-small-cell lung carcinoma (NSCLC) is highly sensitive to ALK kinase inhibitors such as crizotinib.¹ More than 20 variants of the *EML4*–*ALK* fusion gene have been identified along with diversity in the breakpoint and length of *EML4*.² In addition, four more fusion partner genes of *ALK* have been identified: TRK-fused gene, kinesin family member 5B (*KIF5B*), kinesin light chain 1, and protein tyrosine phosphatase, nonreceptor type 3.^{3–6} In the current study, we identified the aberrant expression of huntingtin interacting protein 1 (*HIP1*)–*ALK* and the chromosomal translocation for generating this fusion gene in NSCLCs.

CASE DESCRIPTION

A 38-year-old Korean woman with a nonsmoking history underwent lobectomy because of an irregular mass on the lingual division of the upper-left lobe (Fig. 1A). The tumor area measured 2.5 × 2.5 cm² and microscopic examination revealed an adenocarcinoma with predominant acinar pattern (Fig. 1C). Metastasis of one of nine lymph nodes was noticed. Immunohistochemistry showed that ALK (dilution 1:50; clone 5A4, Novocastra, Newcastle, United Kingdom) localized to the cytoplasm, with higher density in the submembrane region of tumor cells (Fig. 1D and E). In break-apart, fluorescence in situ hybridization analysis for *ALK* (Abbott Molecular, Abbott Park, IL), split of the 5' and 3' probe signals and isolated 3' signals were observed (Fig. 1B), confirming the chromosomal rearrangement. The tumor did not harbor either epidermal growth factor receptor (*EGFR*) or Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations.

By using total RNA extracted from tumor tissue obtained from the patient, we had prepared a first-strand cDNA library by reverse-transcriptase polymerase chain reaction (PCR) to screen fusion transcripts involving *ALK* as a fusion partner. Then, we amplified a double-strand cDNA fragment for the fusion region of *HIP1*–*ALK* transcript by PCR using specific primers and the first-strand cDNA library. The SMARTer 5'-rapid amplification of cDNA ends cDNA Amplification Kit (Clontech Laboratories, Inc., Mountain View, CA) was used to amplify the full-length cDNA of this novel fusion gene *HIP1*–*ALK*. Then, the PCR products were cloned into the pCR4-TOPO vector using the TOPO TA Cloning Kit (Invitrogen,

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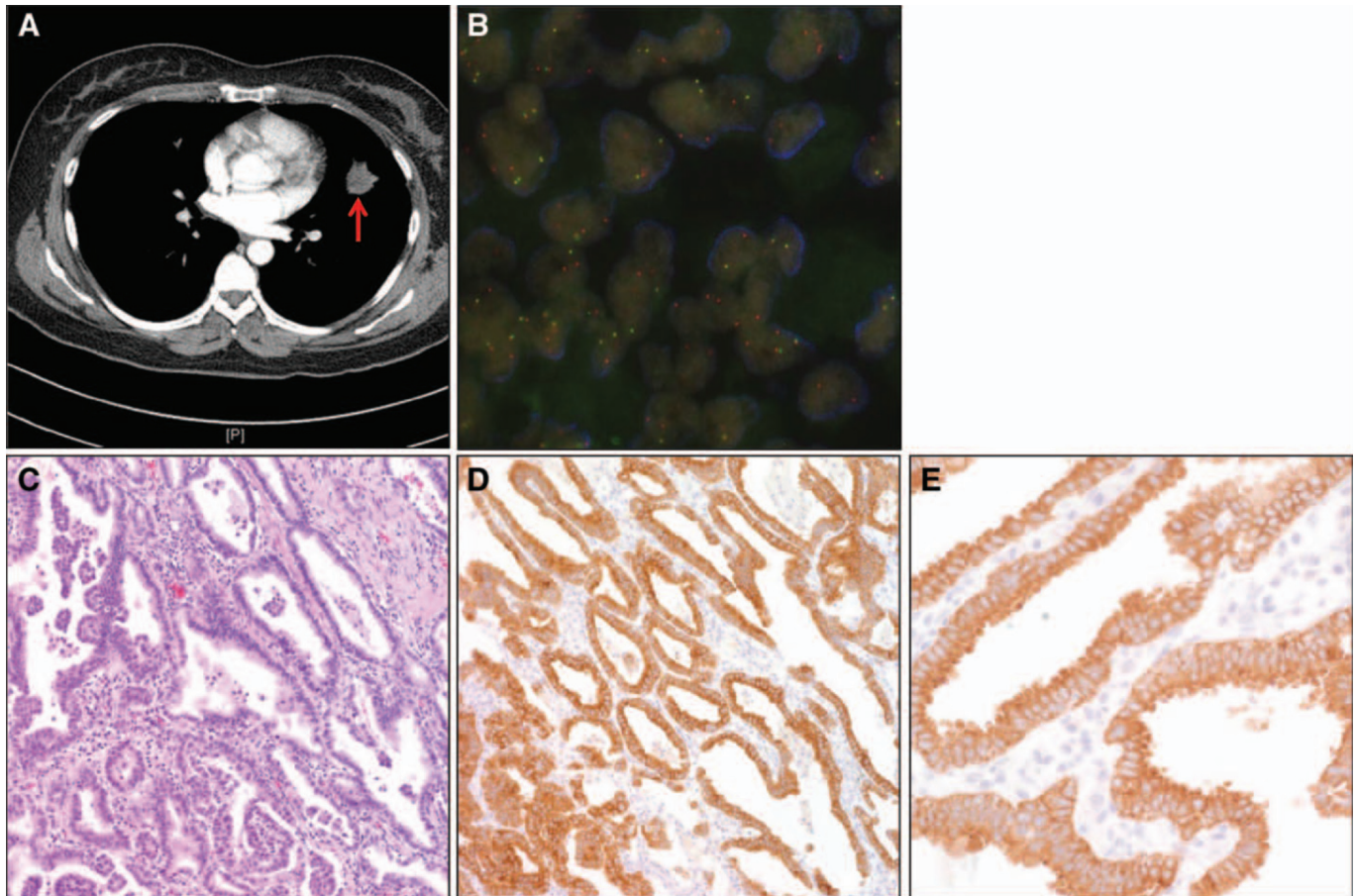


FIGURE 1. A, An irregular-shaped mass (red arrow) adjacent to the pleura revealed by chest computed tomography. B, Fluorescence in situ hybridization assay of *ALK* rearrangement revealed individual 3'-signals, indicating a chromosomal translocation event. C, Histology of *HIP1-ALK*-positive lung adenocarcinoma. Hematoxylin and eosin staining shows adenocarcinoma with predominant acinar pattern. D, E On *ALK* immunohistochemistry, the tumor cells were stained in the cytoplasm, showing densely stained submembrane region (D, $\times 100$, E, $\times 200$). *ALK*, anaplastic lymphoma kinase; *HIP1*, huntingtin interacting protein 1.

Carlsbad, CA) for DNA sequencing. The sequence analysis of *HIP1-ALK* cDNA showed that exon 21 of *HIP1* was fused to exon 20 of *ALK* in-frame, generating a fusion mRNA harboring an intact 3762-base pair open reading frame encoding a deduced 1253-amino acid protein sequence that contains epsin N-terminal homology, coiled-coil, juxtamembrane, and kinase domains (Fig. 2A and B). The cDNA sequence of the novel fusion gene *HIP1-ALK* has been deposited in the National Center for Biotechnology Information database (accession number KF360988). Direct sequencing of genomic DNA also revealed that the genomic breakpoint occurred between 79th nucleotide of intron 21 of *HIP1* and 1017th nucleotide upstream from the nucleotide before the beginning nucleotide of the exon 20 of *ALK*, thereby resulting in a chromosomal translocation $t(2;7)(p23;q11.23)$ (Fig. 2A). Noticeably, the beginning nucleotide of this novel fusion gene's 5'UTR was 127,263-base pair far away from the beginning nucleotide of 5'UTR of reference gene *HIP1* (NM_001243198.1) in the University of California, Santa Cruz (UCSC) Genome Browser. To check whether the 5'UTR of this novel fusion gene, which had been identified by our 5'-rapid amplification

of cDNA ends in this study, could be very close to another promoter which is different from the previously known one of the *HIP1* reference gene (NM_001243198.1), we had searched ChIP-Seq database in the UCSC Genome Browser. Very interestingly, H3K4me3 signal, which could provide strong evidence for existence of a promoter region, appeared exactly around the beginning part of the 5'UTR of the fusion gene (Fig. 3A), confirming that *HIP1-ALK* could be regulated by not the conventional promoter of *HIP1* but a novel promoter. The existence of this novel promoter could have been substantiated once more by our discovery in this study of a new *HIP1* transcript variant (in submission process to National Center for Biotechnology Information) whose transcription starts from the same genomic nucleotide position as the first 5' nucleotide position of the *HIP1-ALK* and ends at the 3'UTR of the *HIP1* (Fig. 3B), implying that *HIP1-ALK* and the new *HIP1* variant gene could be regulated by the novel promoter.

The binding site (755–783 in *HIP1-ALK* amino acid sequence) for crizotinib, which is an adenosine triphosphate (ATP)-binding site in a normal condition, resides behind the breakpoint just after 691th amino acid,

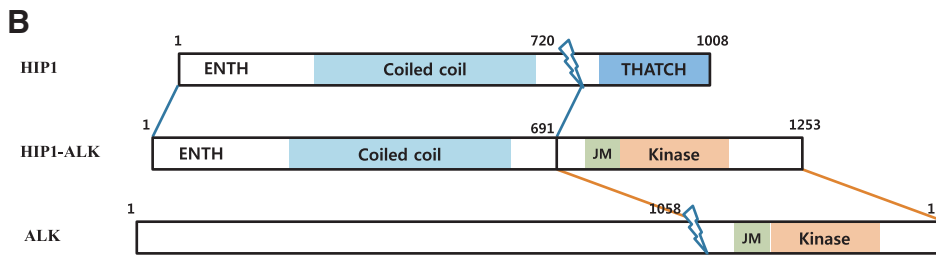
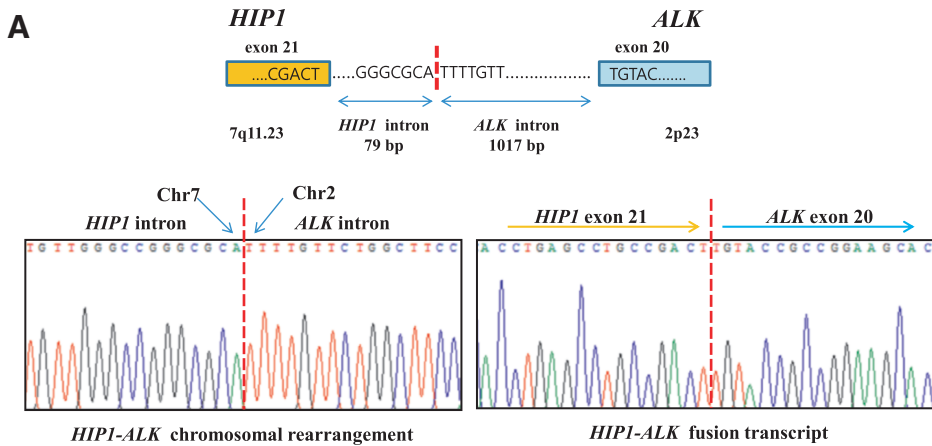


FIGURE 2. A, Sequence analysis of the region fused between *HIP1* and *ALK* in genome and transcript level. B, Functional domain analysis of *HIP1*, *ALK*, and *HIP1-ALK* protein sequences. *ALK*, anaplastic lymphoma kinase; *ENTH*, epsin N-terminal homology; *HIP1*, huntingtin interacting protein 1; *JM*, juxtamembrane.

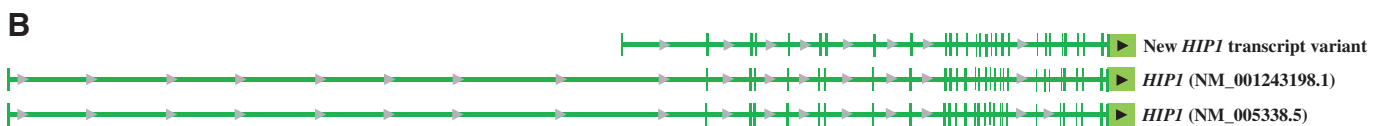
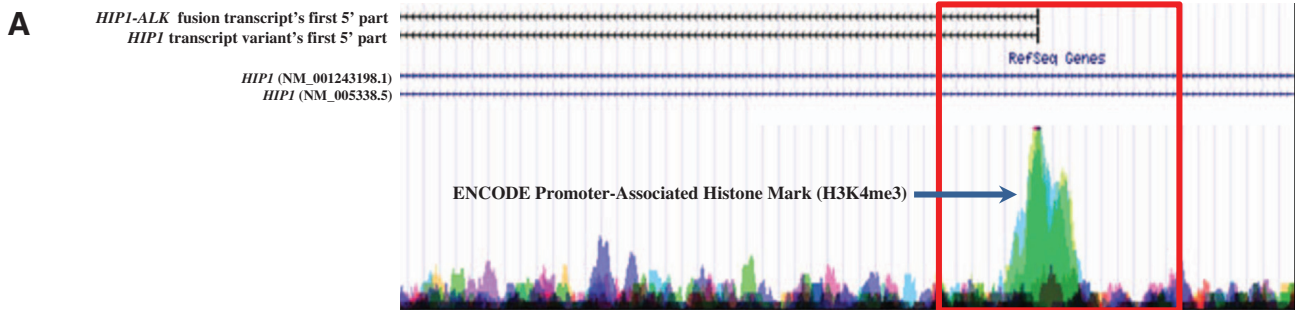


FIGURE 3. A, A novel promoter region (indicated by green mountain signal) for the novel fusion gene *HIP1-ALK* and a new *HIP1* transcript variant revealed by an ENCODE promoter-associated ChIP-Seq mark (H3K4me3) in the UCSC genome database. The different colors indicate that this promoter-associated histone mark landscape (for H3K4me3) had been established in the following cells; Gm12878 cells (red), H1 cells (orange), HepG2 cells (light green), HMEC cells (dense green), HSMC cells (light blue), HUVEC cells (blue), K562 cells (purple), NHEK cells (dense pink) and NHLF cells (pink). The higher the height of the peak is, the higher a probability of the peak site to become an H3K4me3 site (in other word, a potential promoter region) is. When the fusion gene *HIP1-ALK* and *HIP1* novel variant had been transcribed, a novel exonization (for the first exon of *HIP1-ALK* and *HIP1* novel variant) occurred, creating a novel exon with a length of 37 base pair that is not present in *HIP1* transcript. B, The new *HIP1* gene variant lacking a majority of first intron sequence compared with those of the two *HIP1* reference genes. *ALK*, anaplastic lymphoma kinase. ENCODE, The Encyclopedia of DNA Elements; *HIP1*, huntingtin interacting protein 1; HMEC, Human Mammary Epithelial Cells; HSMC, Human Skeletal Muscle Myoblasts; HUVEC, Human Umbilical Vein Endothelial Cells; NHEK, epidermal keratinocytes; NHLF, Normal Human Lung Fibroblasts; UCSC, University of California, Santa Cruz.

participating in *ALK* part in the fusion protein *HIP1-ALK* and also strongly suggesting that *HIP1-ALK* activity could be inhibited by crizotinib.

The patient was treated with crizotinib according to the National Comprehensive Cancer Network guideline,

which recommends crizotinib as a first-line therapy for locally advanced or metastatic *ALK*-positive NSCLC. After 15 months of follow-up, computed tomography scans did not reveal any evidence of recurrence or metastasis in the patient.

DISCUSSION

This is the first report of a novel *HIP1-ALK* fusion gene in NSCLC. Kalchman et al.⁷ reported that HIP1 is essential for the membrane-cytoskeletal integrity in the brain. HIP1 plays an important role in clathrin trafficking and cell survival. The epsin N-terminal homology domain-containing protein binds to polyphosphoinositide-signaling lipids and has been found in cofactors of clathrin-mediated trafficking.⁸ HIP1 is overexpressed in various human cancer cell lines, which indicates that HIP1 could provide a selective growth advantage to cancer cells. Among them, mRNA level of *HIP1* increased in prostate and colon cancer cell lines compared with that in normal epithelial cell lines of prostate and colon.⁹ The first description of chromosomal translocation of *HIP1* in the pathogenesis had come out from chronic myelomonocytic leukemia with platelet-derived growth factor β receptor. HIP1-platelet-derived growth factor β receptor fusion with t(5;7)(q33;q11.2) translocation may contribute to leukemogenesis and lead to transformation of hematopoietic cells.¹⁰ The HIP1-ALK fusion protein comprises the coiled-coil domain of HIP1 and the juxtamembrane intracellular region of ALK, which plays an important role in maintaining ALK kinase activity (Fig. 2B). Together with dimerization through the coiled-coil domain, the ALK tyrosine kinase activity of the fusion protein may be activated aberrantly, thus facilitating oncogenesis in the lung.^{11,12} Although further studies are needed to ascertain the transforming properties of this HIP1-ALK fusion protein in cellular and mouse models, it is likely that this novel fusion protein also may harbor transforming activities from its chimeric protein structure. Takeuchi et al.⁴ found a novel ALK fusion protein KIF5B-ALK in lung cancer using an immunohistochemistry-based diagnostic system. They suggested that the subcellular localization of ALK fusion proteins probably depends on the fusion partner. Whereas nucleophosmin-ALK exhibits both nuclear and cytoplasmic staining, EML4-ALK stains in the cytoplasm, but not in the nucleus. The perinuclear halo pattern observed in KIF5B-ALK may indicate the accumulation of fusion protein at the periphery of the cytoplasm. In this case, the HIP1-ALK staining pattern is similar to that of KIF5B-ALK, which resembles submembrane granular staining.

CONCLUSION

To the best of our knowledge, HIP1-ALK is the first novel fusion transcript reported in ALK-positive lung cancer.

Given that the HIP1 protein contains coiled-coil domains, the fusion protein possibly dimerizes constitutively and, thereby, could possess a strong transforming potential.¹² Patients with *HIP1-ALK* translocation may therefore respond to treatment with ALK inhibitors. This case report could provide novel diagnostic and therapeutic candidate target for patients with NSCLC.

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