KIF5B-ALK, a Novel Fusion Oncokinase Identified by an Immunohistochemistry-based Diagnostic System for ALK-positive Lung Cancer

Kengo Takeuchi,1 Young Lim Choi,3 Yuki Togashi,1 Manabu Soda,3 Satoko Hatano,1 Kentaro Inamura,1 Shuji Takada,3 Toshihide Ueno,3 Yoshihiro Yamashita,3 Yukitoshi Satoh,2 Sakae Okumura,2 Ken Nakagawa,2 Yuichi Ishikawa,1 and Hiroyuki Mano3,4

Abstract

Purpose: EML4-ALK is a transforming fusion tyrosine kinase, several isoforms of which have been identified in lung cancer. Immunohistochemical detection of EML4-ALK has proved difficult, however, likely as a result of low transcriptional activity conferred by the promoter-enhancer region of EML4. The sensitivity of EML4-ALK detection by immunohistochemistry should be increased adequately.

Experimental Design: We developed an intercalated antibody-enhanced polymer (iAEP) method that incorporates an intercalating antibody between the primary antibody to ALK and the dextran polymer-based detection reagents.

Results: Our iAEP method discriminated between tumors positive or negative for EML4-ALK in a test set of specimens. Four tumors were also found to be positive for ALK in an archive of lung adenocarcinoma (n = 130) and another 4 among fresh cases analyzed in a diagnostic laboratory. These 8 tumors were found to include 1 with EML4-ALK variant 1, 1 with variant 2, 3 with variant 3, and 2 with previously unidentified variants (designated variants 6 and 7). Inverse reverse transcription-PCR analysis revealed that the remaining tumor harbored a novel fusion in which intron 24 of KIF5B was ligated to intron 19 of ALK. Multiplex reverse transcription-PCR analysis of additional archival tumor specimens identified another case of lung adenocarcinoma positive for KIF5B-ALK.

Conclusions: The iAEP method should prove suitable for immunohistochemical screening of tumors positive for ALK or ALK fusion proteins among pathologic archives. Coupling of PCR-based detection to the iAEP method should further facilitate the rapid identification of novel ALK fusion genes such as KIF5B-ALK.

Gene fusion is a major mechanism of carcinogenesis in hematologic malignancies and sarcomas (1). Identification of the BCR-ABL fusion kinase, which is generated as a result of the balanced chromosome anomaly t(9;22)(q34;q11) in chronic myelogenous leukemia (2), has thus been followed by the discovery of many fusion-type oncogenes (3). In contrast, it has remained unclear whether such translocation-dependent fusion-type oncogenes also play a major role in the pathogenesis of epithelial tumors. Recently, however, almost 50% of prostate cancer cases have been suggested to harbor gene fusions involving ETS transcription factor loci (4), and we have discovered a recurrent chromosome translocation, inv(2)(p21p23), in non–small cell lung cancer (NSCLC) that results in the production of an EML4-ALK fusion-type protein tyrosine kinase (PTK; refs. 5–8).

Forced expression of EML4-ALK in lung epithelial cells induced the rapid development of hundreds of lung cancer nodules in mice, and peroral administration of inhibitors of the PTK activity of EML4-ALK was shown to clear such tumors from the lungs, demonstrating the pivotal role of EML4-ALK in the pathogenesis of NSCLC positive for this fusion kinase (9). This latter observation also supports the clinical application of ALK

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Current address of Y. Satoh: Department of Thoracic Surgery, Kitasato University School of Medicine, Kanagawa 228-8520, Japan.

Requests for reprints: Kengo Takeuchi, Division of Pathology, The Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan; and Hiroyuki Mano, Division of Functional Genomics, Jichi Medical University, Tochigi, Japan.

Received 12/15/08; revised 1/23/09; accepted 2/1/09; published OnlineFirst 4/21/09.

Grant support: Supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan as well as by grants from the Japan Society for the Promotion of Science; the Ministry of Health, Labor, and Welfare of Japan; the National Institute of Biomedical Innovation of Japan; the Smoking Research Foundation of Japan; and the Vehicle Racing Commemorative Foundation of Japan.
inhibitors (6, 10) to treat EML4-ALK–positive lung cancer in humans. It should be noted, however, that multiple isoforms of EML4-ALK, generated mainly as a result of diversity in the breakpoint-fusionpoint within EML4 (6,8,11,12), have been identified in NSCLC specimens. The accurate diagnosis of EML4-ALK–positive tumors will therefore require detection of all in-frame fusions between EML4 and ALK cDNAs, as exemplified by our multiplex reverse transcription– and PCR-based detection system for EML4-ALK (8).

Given that, in many pathology laboratories, most specimens submitted for histopathologic diagnosis are stored as formalin-fixed, paraffin-embedded tissue, the DNA or RNA of which may be substantially degraded, it is desirable to develop a suitable and sensitive means to detect EML4-ALK in such samples. An immunohistochemistry-based diagnostic system is one potential approach to such screening. In contrast to the efficient detection of NPM-ALK fusion proteins in anaplastic large cell lymphoma specimens with such an approach (13), however, many researchers have encountered difficulty in detecting ALK fusion proteins in lung tissue by immunohistochemical analysis (14), possibly as a result of weak transcriptional activity of the promoter-enhancer region of EML4 that drives the expression of EML4-ALK compared with that of the NPM promoter. We have now attempted to establish a sensitive screening system for ALK fusion protein–positive tumors with an immunohistochemical approach. Furthermore, with such an approach, we unexpectedly discovered a novel ALK fusion gene, KIF5B-ALK, in NSCLC.

**Materials and Methods**

**Samples.** As a test set of samples for the development of sensitive immunohistochemical detection of EML4-ALK, we examined specimens from 11 patients with NSCLC positive for EML4-ALK (previously analyzed in ref. 8) and 10 patients with NSCLC negative for the fusion
gene. The former cohort comprised 3 cases each for EML4-ALK variants 1, 2, and 3 as well as one case each for variants 4 and 5. As a validation set of samples, we examined specimens from 130 consecutive patients with lung adenocarcinoma, from whom written informed consent was obtained. All specimens were collected with the approval of the ethics committee at the Cancer Institute Hospital (Tokyo, Japan), and the study was approved by the institutional review board of the Japanese Foundation for Cancer Research. Surgically removed cancer specimens were routinely fixed in 20% neutralized formalin and embedded in paraffin. Total RNA was extracted from the corresponding snap-frozen specimens and purified with the use of an RNeasy Mini kit (Qiagen).

**Intercalated antibody-enhanced polymer method.** Formalin-fixed, paraffin-embedded tissue was sliced at a thickness of 4 μm, and the sections were placed on silane-coated slides. Five antibody preparations specific for the intracellular region of ALK (ALK1 from Dako, 5A4 and SP8 from Abcam, ZAL4 from Zymed, and p80 from Nichirei) were evaluated for immunohistochemical staining according to standard protocols with the use of a dextran polymer reagent (anti-rabbit or anti-mouse immunoglobulin EnVision+DAB system; Dako). On the basis of their reactivity in such experiments, three antibodies (ALK1, 5A4, and SP8) were selected for development of the intercalated antibody-enhanced polymer (iAEP) method as follows. For antigen retrieval, the slides were heated for 40 min at 97°C in Target Retrieval Solution (pH 9.0; Dako). They were then incubated at room temperature first with Protein Block Serum-free Ready-to-Use solution (Dako) for 10 min and then with antibodies to ALK for 30 min. To increase the sensitivity of detection, we included an incubation step of 15 min at room temperature with rabbit polyclonal antibodies to mouse immunoglobulin (Dako) or mouse antibodies to rabbit immunoglobulin (Dako), as appropriate. The immune complexes were then detected with the dextran polymer reagent and an AutoStainer instrument (Dako).

Detection of EML4-ALK and KIF5B-ALK cDNAs and characterization of their protein products is described in Supplementary Methods.

### Results

**Development of the iAEP method.** A specimen of NPM-ALK–positive anaplastic large cell lymphoma was subjected to immunohistochemical staining with 5 different antibody preparations specific for ALK (ALK1 at 1:20 dilution, 5A4 at 1:50, SP8 at 1:100, ZAL4 at 1:200, or p80 at 1:100) by the EnVision+DAB polymer method. All antibody preparations stained both the nucleus and cytoplasm of the lymphoma cells, whereas ZAL4 also reacted with normal mesenchymal cells (data not shown). In addition, the staining intensity with p80 was relatively low. We therefore selected ALK1, 5A4, and SP8 for initial development of a detection system for EML4-ALK.

Immunohistochemical analysis of a test set of samples (11 specimens of EML4-ALK–positive NSCLC and 10 specimens of EML4-ALK–negative NSCLC) with these 3 antibody preparations revealed negative to marginally positive reactivity with EML4-ALK by a conventional staining protocol based on the EnVision+DAB system (Supplementary Fig. S1; Table 1). We therefore incorporated an intercalating antibody before the EnVision+DAB system and applied this iAEP method to the same set of specimens. All three antibody preparations detected EML4-ALK in all EML4-ALK–positive cases in the test cohort (Supplementary Fig. S1; Table 1). However, SP8 also reacted with most of the EML4-ALK–negative specimens (Supplementary Fig. S2; Table 1), rendering it unsuitable for large-scale screening. Furthermore, a low level of nonspecific background staining of nontumor cells was apparent in all sections stained with ALK1.

We selected ALK1 and 5A4 for examination of a validation set of samples (a consecutive series of 130 lung adenocarcinoma...
specimens). Four cases of this cohort were positive for staining with both antibodies by the iAEP method. Again, most of the other specimens also showed a low level of background staining with ALK1, whereas only a few did so with 5A4. We therefore selected 5A4 to detect EML4-ALK with the iAEP method and included this approach in our routine diagnostic service at the pathology division of The Cancer Institute during the study period, thereby identifying four additional cases of lung adenocarcinoma positive for staining with 5A4.

Identification of variants 6 and 7 of EML4-ALK. The four specimens recognized by 5A4 in the validation set (IDs #24461, #24704, #26020, and #26422) were examined for the presence of EML4-ALK transcripts with our multiplex reverse transcription-PCR (RT-PCR) screening system, which was designed to capture all possible in-frame fusions between EML4 and ALK at the cDNA level (8). Three cases (#24461, #26020, and #26422) were positive for EML4-ALK cDNAs (Fig. 1A), and nucleotide sequencing of the PCR products revealed that #24461 and #26422 tumors harbored variants 1 and 3 of EML4-ALK, respectively. The cDNA derived from tumor #26020, however, contained exon 13 of EML4 as well as a portion of intron 19 and exon 20 of ALK, corresponding to a previously unidentified fusion variant (designated variant 6) of EML4 and ALK (Supplementary Fig. S3A; Fig. 1B). The fusion of exon 13 of EML4 to a position 69 bp upstream of exon 20 of ALK in this fusion cDNA would be expected to constitute an in-frame fusion between the two genes. Although there were no reported mRNAs or expressed sequence tags containing intron 19 of ALK in the sequence databases, the genomic sequence surrounding the fusion point in this intron is AG-GA (Fig. 1B), which conforms to the consensus sequence for a splicing acceptor site, suggesting that this position of intron 19 may act as a cryptic acceptor site for RNA splicing.

Similar analysis by multiplex RT-PCR and sequence determination revealed that the additional 4 ALK-positive cases identified by our routine pathologic diagnostic service comprised one case (tumor ID #27265) with variant 2 of EML4-ALK, 2 cases (#26813 and #26953) with variant 3, and 1 case (#27998) with another novel variant (designated variant 7), in which exon 14 of EML4 is fused to nucleotide 13 of exon 20 of ALK (Supplementary Fig. S3B; Fig. 1C and D). Genomic PCR analysis of the specimens positive for variants 6 and 7 of EML4-ALK cDNA confirmed the presence of genomic rearrangements responsible for the fusion events detected at the cDNA level (data not shown).

Identification of KIF5B-ALK as a novel ALK fusion gene. Whereas tumor #24704 of the validation cohort was strongly positive for ALK immunostaining by the iAEP method, multiplex RT-PCR analysis failed to amplify a specific product from this sample. We therefore examined the possibility that this tumor might harbor an ALK fusion gene other than EML4-ALK. We subjected the sample to an inverse RT-PCR analysis and obtained a PCR product containing both exon 24 of KIF5B and exon 20 of ALK. KIF5B is located on the short arm of human chromosome 10 and encodes member 5B of the kinesin family of proteins. To confirm the presence of a KIF5B-ALK fusion gene in this tumor, we directly amplified the fusion point of the KIF5B-ALK cDNA by RT-PCR with one primer targeted to exon 24 of KIF5B and the other to exon 22 of ALK. A single PCR product with the expected size of 546 bp was obtained (Fig. 2A). Nucleotide sequencing of the product further confirmed the fusion point of KIF5B-ALK at the cDNA level (data not shown).

KIF5B is a component of a motor protein complex that is associated with microtubules and mediates the transport of organelles within eukaryotic cells (15). It consists of an amino terminal motor domain followed by a neck region and a stalk region, the latter of which directly mediates homodimerization of KIF5B (Fig. 2B). Fusion of exons 1 to 24 of KIF5B to exon 20 of ALK would be expected to result in the production of a fusion protein consisting of almost the entire KIF5B sequence ligated to the intracellular region of ALK. It might therefore also be expected that KIF5B-ALK would undergo homodimerization mediated by the stalk region of KIF5B, with consequent activation of the kinase function of ALK, similar to the case of EML4-ALK, in which homo-oligomerization and activation are mediated by the amino terminal coiled–coil domain of EML4 (5, 8).

We next modified our multiplex RT-PCR method so that it could detect both EML4-ALK and KIF5B-ALK fusion mRNAs. In addition to a forward primer targeted to the boundary of exons 23 and 24 of KIF5B (to amplify the identified KIF5B-ALK fusion point), we included another forward primer targeted to exon 10 of KIF5B to detect potential novel fusion cDNAs for
KIF5B-ALK proteins containing a partial stalk region of KIF5B (given that the stalk region contains seven coiled-coil domains), a partial stalk region may still allow homodimerization of KIF5B-ALK). This newly designed multiplex RT-PCR assay was then applied both to the eight specimens found in this study to harbor EML4-ALK (7 cases) or KIF5B-ALK (#24704) and to the panel of cancer specimens including 253 samples of lung adenocarcinoma, 111 samples of other types of lung cancer, and 292 samples of tumors from 10 other organs, which was studied previously (8). Our modified multiplex RT-PCR method detected all 8 cases shown to be positive for EML4-ALK or KIF5B-ALK in the present study as well as 11 cases known to harbor various EML4-ALK fusion genes in the previous cohort (data not shown). The modified multiplex RT-PCR assay also identified one case (#2524) of lung adenocarcinoma harboring KIF5B-ALK among the previous cohort. We thus identified two cases positive for KIF5B-ALK among a total of 383 cases of lung adenocarcinoma (2 of 383 = 0.52%). Genomic rearrangement responsible for the identified KIF5B-ALK cDNAs was also confirmed in these two cases by genomic PCR analysis. The PCR products differed between the 2 cases, indicative of distinct breakpoints and fusion points within intron 24 of KIF5B and intron 19 of ALK (Fig. 2C).

**Histopathology of KIF5B-ALK-positive lung adenocarcinoma.** Histopathologic analysis of the two cases of KIF5B-ALK-positive lung adenocarcinoma revealed papillary structures, whereas the acinar pattern with prominent mucin production typically apparent in EML4-ALK-positive cases (7) was rarely observed. The individual cancer cells contained abundant eosinophilic cytoplasm and a large vesicular nucleus with one or two prominent nucleoli, and they were generally larger than those observed in EML4-ALK-positive cases (Supplementary Fig. S4A; Fig. 3A and C). Lymphatic invasion was prominent in tumor #24704, and the tumor cells in the lymphatic vessels contained an eccentric nucleus and a perinuclear eosinophilic globule (Supplementary Fig. S4A). Immunohistochemical detection of KIF5B-ALK with 5A4 by the IAEP method revealed a diffuse cytoplasmic staining in all of the cancer cells. Some cells manifested an uneven staining profile, with a perinuclear halo (Supplementary Fig. S4B; Fig. 3D) or macroglobular spots (Fig. 3B), neither of which was observed in tumors positive for EML4-ALK (8).

**Fluorescence in situ hybridization analysis of KIF5B-ALK-positive tumors.** To confirm further the genomic rearrangement in the two tumors positive for KIF5B-ALK, we did three fluorescence in situ hybridization assays: an ALK split assay, a KIF5B split assay, and a KIF5B-ALK fusion assay. The results for all three assays were consistent with the presence of a t(2;10)(p23;p11) responsible for the KIF5B-ALK fusion gene. Neither an isolated 5'-ALK signal and one isolated 5'-KIF5B signal in the ALK split assay and the KIF5B split assay, respectively, whereas they manifested one merged signal in the KIF5B-ALK fusion assay. Cancer cells of tumor #24704 exhibited at least two merged signals, indicative of possible amplification of the fusion gene. Neither an isolated 3'-KIF5B signal nor an isolated 5'-ALK signal was detected in the split assays for either case, suggesting that the derivative chromosome 2 harboring the ALK-KIF5B fusion gene may have been deleted after the balanced translocation between chromosomes 2 and 10.

**Transforming activities of EML4-ALK variants 6 and 7 and of KIF5B-ALK.** To isolate full-length cDNAs for the new variants of EML4-ALK, we did RT-PCR analysis with a forward primer targeted to the 5' untranslated region of EML4 cDNA and a reverse primer targeted to the 3' untranslated region of ALK cDNA as described previously (6, 8). From oligo(dT)-primed cDNA preparations of tumor IDs #26020 or #27998, we isolated cDNAs of 3365 and 3435 bp, corresponding to variants 6 and 7 of EML4-ALK, respectively (data not shown). Similarly, a full-length cDNA of 4479 bp for KIF5B-ALK was obtained by RT-PCR analysis from tumor ID #2524. Nucleotide sequencing of these cDNAs confirmed that each of them would be expected to produce a functional PTK, with a predicted molecular size of 119,380 Da for EML4-ALK variant 6 (Supplementary Fig. S3A),

![Fig. 3. Histopathology of KIF5B-ALK-positive lung adenocarcinoma. Sections of tumors #24704 (A and B) and #2524 (C and D) were stained with H&E (A and C) or subjected to immunohistochemical analysis with 5A4 by the IAEP method (B and D). Some cancer cells of tumor #24704 contained intracytoplasmic macroglobular spots strongly positive for KIF5B-ALK (C). Some tumor cells showed a perinuclear halo positive for KIF5B-ALK (D). Scale bars, 100 μm.](image-url)
Recombinant retroviruses encoding each of these fusion PTKs were generated and used to infect cultured 3T3 fibroblasts. Infection with the viruses encoding EML-ALK variant 6, EML4-ALK variant 7, or KIF5B-ALK, but not that with the empty virus, resulted in the formation of dozens of transformed foci in vitro (Fig. 5). As positive controls for focus formation, EML4-ALK variant 1 and NPM-ALK each yielded a similar number of transformed foci.

The same set of 3T3 cells was injected into nude mice for an in vivo tumorigenicity assay. All fusion PTKs induced s.c. tumors at all injection sites within an observation period of 20 days (Fig. 5), confirming the transforming potential of the novel variants of EML4-ALK as well as that of KIF5B-ALK.

**Discussion**

Immunohistochemical detection of ALK fusion proteins has been applied successfully to analysis of anaplastic large cell lymphoma and inflammatory myofibroblastic tumors, with the mouse monoclonal antibody ALK1 being most widely used for this purpose. However, many researchers were not able to reliably detect EML4-ALK in NSCLC specimens with this same immunohistochemical technique (14). Even if NSCLC specimens were positive for such staining, its intensity was usually low and varied substantially among sections of the same tumor, rendering the current standard technique unsuitable for screening of NSCLC specimens. This low sensitivity for the detection of EML4-ALK may be attributable to the low level of EML4 transcriptional activity (see, for example, a public database for serial analysis of gene expression) or to instability of EML4-ALK in cells.

However, given that immunohistochemical analysis is a convenient means to detect a protein of interest in pathology laboratories, it is desirable to establish a sensitive and accurate screening system for ALK fusion proteins based on this approach. Several candidate techniques with improved sensitivity, such as tyramide signal amplification (16), have been recently proposed. These techniques generally require multiple steps, however, which can compromise reproducibility and render them unsuitable for screening in routine pathologic diagnosis.

We have now achieved a moderate increase in the sensitivity of immunohistochemical detection of ALK fusion proteins by including antibodies to mouse or rabbit immunoglobulin as an intercalating reagent between the primary antibody and the EnVision+DAB polymer detection system. This iAEP method allowed the detection of EML4-ALK fusion proteins in all 11 specimens positive for EML4-ALK in our test cohort. This simple method can be readily done in ordinary diagnostic pathology laboratories. Although we selected the mouse monoclonal antibody 5A4 for immunohistochemistry by the iAEP method, other antibodies may be more suitable for routine diagnostic analysis with a modified version of this approach.

All antibodies used in the present study are specific for the intracellular region of ALK and so would be expected to detect both EML4-ALK and wild-type ALK. It is therefore possible that positive staining with 5A4 by the iAEP method does not reflect only the presence of ALK fusion proteins. To address this issue, we determined the amount of ALK mRNA with primers targeted to the 5’ or 3’ regions of ALK cDNA separately (whereas the latter would be expected to amplify cDNAs for both wild-type ALK and ALK fusion genes, the former would be expected to amplify only that of wild-type ALK). None of the EML4-ALK–positive specimens in the test set of samples yielded a substantial amount of wild-type ALK cDNA (although tumor #9968 may express the wild-type gene at a low level), suggesting that our iAEP method with 5A4 detected EML4-ALK proteins rather than wild-type ALK in the positive specimens. For ALK-rich tissues such as the brain or spinal cord (17), however, it would be important to determine which proteins are recognized by 5A4 in iAEP analysis.

We identified 8 tumors positive for staining with 5A4 by the iAEP method among the validation set of samples (n = 130) and the fresh cases subjected to routine diagnostic testing. Although 5 of these specimens harbored known EML4-ALK variants, the remaining three were found to express novel ALK fusion proteins, including EML4-ALK variants 6 (#26020) and 7 (#27998) and KIF5B-ALK (#24704). These results thus showed that sensitive immunohistochemical analysis was superior to PCR-based methods for detecting novel ALK fusion constructs among tumor specimens. This conclusion was further supported by the fact that neither EML4-ALK nor KIF5B-ALK was identified in the iAEP-negative cases by our modified multiplex RT-PCR assay (data not shown).

![Figure 4. Fluorescence in situ hybridization analysis of KIF5B-ALK. Sections of tumors positive for KIF5B-ALK (IDs #24704 and #2524) were subjected to fluorescence in situ hybridization with an ALK split assay (left), a KIF5B split assay (middle), or a KIF5B-ALK fusion assay (right). Bottom, the color of fluorescence for the BAC clones in each hybridization. Nuclei are stained blue with 4',6-diamidino-2-phenylindole.](image-url)
A fusion protein containing most of KIF5B and the intracellular (kinase) domain of the platelet-derived growth factor receptor A has been detected in idiopathic hypereosinophilic syndrome (18). The genome of some patients with this condition exhibits a chromosomal translocation, t(4;10)(q12;p11), which results in the production of a KIF5B-PDGFRα fusion mRNA in which exon 23 of KIF5B is ligated to exon 12 of PDGFRα. Given that the KIF5B portion of KIF5B–platelet-derived growth factor receptor A contains six of the seven coiled-coil domains within the stalk region, the fusion protein likely dimerizes constitutively and thereby possesses transforming potential. KIF5B-ALK is thus the second example of an oncogenic KIF5B fusion to a PTK.

The subcellular localization of ALK fusion proteins likely depends on the fusion partner. For instance, whereas NPM-ALK, which is associated with anaplastic large cell lymphoma, is present in both the nucleus and cytoplasm, nuclear localization has not been detected for other ALK fusion proteins including CLT-C-ALK, TPM3-ALK, TFG-ALK, ATIC-ALK (19), and EML4-ALK (5). The pattern of immunohistochemical staining for KIF5B-ALK did not resemble that of any of these other ALK fusion proteins. The observed perinuclear halo of KIF5B-ALK staining may indicate accumulation of the fusion protein at the periphery of the cytoplasm (subcell membrane region), possibly reflecting transport of KIF5B-ALK along microtubules. Signaling downstream of KIF5B-ALK may thus differ substantially from that of other ALK fusion proteins, as exemplified by the differential phosphorylation of STAT proteins associated with these fusion proteins (19).

In conclusion, we have developed a modified immunohistochemical staining procedure for the detection of ALK and ALK fusion proteins in lung cancer that may prove suitable for screening purposes in pathology laboratories. Our identification of a second ALK fusion gene, KIF5B-ALK, in NSCLC further supports the clinical relevance of ALK in the pathogenesis of this disease. Given the recent development of several ALK inhibitors and their potential therapeutic efficacy for tumors positive for ALK fusion proteins (6, 10, 20), accurate diagnosis of tumors expressing activated ALK or ALK fusion proteins (5, 21, 22) will be essential to identify subgroups of patients who are suitable for treatment with such drugs.

Disclosure of Potential Conflicts of Interest

K.T. serves as a consultant to Dako.

Acknowledgments

We thank Motoyoishi Iwakoshi, Keiko Shiozawa, Tomoyo Kakita and Reimi Asaka for their technical assistance. The nucleotide sequences of the cDNAs for EML4-ALK variants 6 and 7 and for KIF5B-ALK have been deposited in the DDBJ/European Molecular Biology Laboratory/Genbank databases under the accession numbers AB462411, AB462412, and AB462413, respectively.

References

KIF5B-ALK, a Novel Fusion Oncokinase Identified by an Immunohistochemistry-based Diagnostic System for ALK-positive Lung Cancer

Kengo Takeuchi, Young Lim Choi, Yuki Togashi, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/15/9/3143

Supplementary Material
Access the most recent supplemental material at:
http://clincencerres.aacrjournals.org/content/suppl/2009/05/20/1078-0432.CCR-08-3248.DC1

Cited articles
This article cites 22 articles, 7 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/15/9/3143.html#ref-list-1

Citing articles
This article has been cited by 85 HighWire-hosted articles. Access the articles at:
/content/15/9/3143.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.