SPANDIDOS PUBLICATIONS

EXPERIMENTAL AND THERAPEUTIC MEDICINE 1: 271-275, 2010

EML4-ALK fusion transcripts in immunohistochemically ALK-positive non-small cell lung carcinomas

KAZUYA SHINMURA[1], SHINJI KAGEYAMA[1], HISAKI IGARASHI[1], TAKAHARU KAMO[1], TAKAHIRO MOCHIZUKI[2], KAZUYA SUZUKI[2], MASAYUKI TANAHASHI[3], HIROSHI NIWA[3], HIROSHI OGAWA[4] and HARUHIKO SUGIMURA[1]

[1]First Department of Pathology, and [2]First Department of Surgery, Hamamatsu University School of Medicine, Hamamatsu 431-3192; [3]Division of Thoracic Surgery, Respiratory Disease Center, and [4]Division of Pathology, Seirei Mikatahara General Hospital, Hamamatsu 433-8558, Japan

Received October 12, 2009; Accepted November 27, 2009

DOI: 10.3892/etm_00000042

Abstract. EML4-ALK fusion transcripts have been found in a subset of non-small cell lung carcinomas (NSCLCs); however, their protein expression status has not yet been fully elucidated. In this study we investigated ALK protein expression in 302 NSCLCs and 291 gastric carcinomas by means of immunohistochemical analysis. Twelve (4.0%) NSCLCs, but none of the gastric carcinomas, were found to be positive for ALK. The ALK signal was detected in the cytoplasm of cancer cells. Subsequent RNA analysis of 10 RNA-available, immunohistochemically ALK-positive tumors revealed that three tumors had EML4-ALK variant 1, three tumors had variant 2, three tumors had variants 3a and 3b, and one tumor had a novel variant in which exon 14 of EML4 is connected to the nucleotide at position 53 of exon 20 of ALK by a 2-bp insertion. These results suggest that immunohistochemical ALK detection is a useful way to screen NSCLCs for tumors containing ALK fusions.

Introduction

Structural chromosome aberrations that result in the production of fusion oncogenes are one of the most common causes of oncogenesis. In the past they have been reported in many classes of hematological malignancies and mesenchymal tumors (1,2), and recently in a few types of epithelial carcinomas (3-5). A fusion gene comprising portions of the *EML4* gene and the *ALK* gene that resulted from a small inversion in chromosome 2p was recently discovered in a subset of non-small cell lung carcinomas (NSCLCs) (4). The fused mRNA based on the gene fusion encodes the N-terminal portion of EML4 ligated to the intracellular region of the receptor-type protein tyrosine kinase ALK. EML4-ALK oligomerizes constitutively in cells through the coiled-coil domain within the EML4 region and becomes activated to exert oncogenicity both *in vitro* and *in vivo* (4,6). Several types of EML4-ALK variants have been found in NSCLCs (4,6-18), and although one NSCLC containing KIF5B-ALK and another NSCLC containing TFG-ALK have been found (13,15), all of the other ALK fusions detected in NSCLCs have been EML4-ALK fusions.

Notably, recent studies have shown that ALK inhibitors have potential therapeutic efficacy for NSCLCs that are positive for ALK fusion proteins (4,6,16,19). Thus, the development of a diagnostic system for NSCLCs expressing ALK fusion proteins will be essential to identifying subgroups of NSCLC patients for treatment with ALK inhibitors. Immunohistochemical analysis of paraffin-embedded sections during routine pathologic diagnosis is a convenient means of examining the level of protein expression when the analytical condition is determined. Takeuchi *et al* recently reported an effective means of immunohistochemical detection of EML4-ALK by the intercalated antibody-enhanced polymer (iAEP) method (13). However, another group reported difficulty detecting EML4-ALK immunohistochemically (14), and it is speculated that the low expression level of EML4-ALK protein may be attributable to a low level of EML4 transcriptional activity or to instability of EML4-ALK in cells (13). Moreover, based on the results of a fluorescence *in situ* hybridization (FISH) analysis, Perner *et al* reported finding that only a subset of tumor cells contains the 2p rearrangement that leads to the formation of EML4-ALK (10). Thus, a system for immunohistochemical detection of ALK in NSCLCs would need to be established in order to diagnose tumors containing ALK fusions and elucidate the expression status of ALK fusion proteins. We also believe that immunohistochemical screening for ALK fusions may lead to the identification of novel EML4-ALK variants or novel fusions with ALK in addition to known EML4-ALK variants. Moreover, although the only carcinomas in

Correspondence to: Dr Kazuya Shinmura, First Department of Pathology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi Ward, Hamamatsu 431-3192, Japan
E-mail: kzshinmu@hama-med.ac.jp

Key words: EML4, ALK, non-small cell lung carcinoma, fusion transcript, immunohistochemistry

which ALK fusions have been found thus far are NSCLCs, ALK fusions may be present in other types of carcinomas. However, no studies using the iAEP method, except a study by Takeuchi *et al* (13), have been published. Therefore, in the present study, we immunohistochemically evaluated a total of 302 NSCLCs and 291 gastric carcinomas for ALK expression using the iAEP method and then investigated RNA-available, immunohistochemically ALK-positive tumors for expression of EML4-, KIF5B- and TFG-ALK fusions.

Materials and methods

Surgical specimens. Samples of surgical specimens from 302 NSCLC and 291 gastric carcinoma patients who underwent surgery for their cancer at Hamamatsu University School of Medicine, University Hospital or Mikatahara Seirei General Hospital were obtained. The mean age of the 302 NSCLC patients was 63.9 years [standard deviation (SD) 10.7], and they consisted of 168 men and 134 women. The NSCLC tumors were histologically classified as adenocarcinoma in 184 cases, squamous cell carcinoma in 98 cases, large-cell carcinoma in 9 cases and adenosquamous carcinoma in 11 cases. The mean age of the 291 gastric carcinoma patients was 65.4 years (SD 11.8), and they consisted of 206 men and 85 women. The gastric tumors were histologically classified as intestinal-type adenocarcinoma in 151 cases, diffuse-type adenocarcinoma in 138 cases and adenosquamous carcinoma in 2 cases. This study was approved by the Institutional Review Board (IRB) of Hamamatsu University School of Medicine and the IRB of Mikatahara Seirei General Hospital.

Immunohistochemical staining. Immunostaining for ALK using the iAEP method was performed as described previously (13) with slight modifications. In brief, paraffin-embedded tissue sections were deparaffinized, rehydrated and boiled at 96˚C for 40 min in Target Retrieval Solution (pH 9.0) (Dako, Kyoto, Japan) for antigen retrieval. Endogenous peroxidase activity was blocked by incubation for 5 min in a 3% hydrogen peroxide solution. Next, the sections were incubated with a Protein Block, Serum-free (Dako) for 10 min at room temperature (RT) and then with a mouse anti-ALK monoclonal antibody (clone 5A4; Abcam, Cambridge, UK) at a dilution of 1:50 for 30 min at RT. To increase the sensitivity of detection, the sections were incubated with polyclonal rabbit anti-mouse immunoglobulin at a dilution of 1:500 for 15 min at RT. After washing, the sections were incubated for 30 min at RT with an amino acid polymer conjugated with goat anti-rabbit IgG and horseradish peroxidase (Histofine Simple Stain MAX-PO Kit; Nichirei, Tokyo, Japan). The antigen-antibody complex was visualized with 3,3'-diaminobenzidine tetrahydrochloride, and the sections were counterstained with hematoxylin. The staining was performed with a Dako autostainer (Dako) (20).

Reverse transcription (RT)-polymerase chain reaction (PCR). Total RNA was extracted from lung tissue samples with an RNeasy Kit (Qiagen, Valencia, CA, USA) and converted to first-strand cDNA with a SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA) by following the supplier's protocol. PCR was performed in 20-μl reaction mixtures containing HotStarTaq DNA polymerase (Qiagen) under the following conditions: 30 sec at 94˚C, 30 sec at 61˚C and 90 sec at 72˚C for 45 cycles. A total of five different PCR primer pairs for EML4-ALK, three PCR primer pairs for KIF5B-ALK and one PCR primer pair for TFG-ALK were used for the RT-PCR. The forward PCR primers were: 5'-GCC TCA GTG AAA AAA TCA GTC TCA AG-3' for the sequence on exon 2 of EML4, 5'-ACA AAT TCG AGC ATC ACC TTC TCC-3' for the sequence on exon 4 of EML4, 5'-GTG CAG TGT TTA GCA TTC TTG GGG-3' for the sequence on exon 13 of EML4, 5'-CTG TGG GAT CAT GAT CTG AAT CCT G-3' for the sequence on exon 14 of EML4, 5'-CTT CCT GGC TGT AGG ATC TCA TGA C-3' for the sequence on exon 19 of EML4, 5'-CAC TAT TGT AAT TTG CTG CTC TCC ATC ATC-3' for the sequence on exon 10 of KIF5B, 5'-AAT CTG TCG ATG CCC TCA GTG AAG-3' for the sequence on exon 17 of KIF5B, 5'-TGA TCG CAA ACG CTA TCA GCA AG-3' for the sequence on exon 24 of KIF5B and 5'-TCG TTT ATT GGA TAG CTT GGA ACC AC-3' for the sequence on exon 4 of TFG. The reverse PCR primer used was the same, i.e., 5'-GAG GTC TTG CCA GCA AAG CAG TAG-3' for the sequence on exon 20 of ALK. The PCR products were fractionated by electrophoresis on an agarose gel and stained with ethidium bromide. The PCR-amplified products were purified with a PCR purification kit (Qiagen) and directly sequenced with a BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, Tokyo, Japan) and the ABI 3100 Genetic Analyzer (Applied Biosystems) as described previously (7). The reference sequences for the *ALK*, *EML4*, *KIF5B* and *TFG* genes are accession numbers NM_004304, NM_019063, NM_004521 and NM_006070, respectively.

Statistical analysis. Statistical comparisons were performed by the two-tailed Student's t-test with Excel software (Microsoft Corp., Redmond, WA, USA).

Results

Immunohistochemical detection of ALK-positive NSCLCs. Samples of 302 NSCLCs and 291 gastric carcinomas were immunohistochemically stained for ALK with 5A4 anti-ALK monoclonal antibody using the iAEP method, and 12 (4.0%) of the NSCLCs and none (0%) of the gastric carcinomas were positive for ALK expression (Table I). ALK staining was observed in the cytoplasm of the cancer cells in all 12 NSCLCs, but not in any of the non-cancerous cells (Fig. 1). The mean age of the NSCLC patients whose tumors were positive for ALK was 57.3 years (SD 15.7) and significantly lower than the mean age of the NSCLC patients whose tumors were negative for ALK (64.2 years of age, SD 10.4) (p=0.027). The NSCLC patients whose tumors were positive for ALK consisted of 6 men and 6 women, and the ALK-positive NSCLC tumors were classified histologically as adenocarcinoma in 10 cases, adenosquamous carcinoma in 1 case and squamous cell carcinoma in 1 case (Table I).

Detection of various EML4-ALK fusion transcripts in NSCLCs. Next, 10 RNA-available, ALK-positive NSCLCs were investigated for expression of EML4-, KIF5B- and TFG-ALK fusion transcripts by RT-PCR and subsequent sequencing

Table I. Clinicopathological information and EML4-ALK fusions detected in immunohistochemically ALK-positive NSCLCs.

No.	Age	Gender	Histopathological diagnosis	EML4-ALK transcript
1	48	Female	Adenocarcinoma	Variant 1
2	49	Male	Adenocarcinoma	Variant 1
3	66	Male	Adenocarcinoma	Variant 1
4	46	Female	Adenocarcinoma	Variant 2
5	57	Male	Adenocarcinoma	Variant 2
6	79	Male	Adenocarcinoma	Variant 2
7	33	Female	Adenosquamous carcinoma	Variants 3a and 3b
8	63	Female	Adenocarcinoma	Variants 3a and 3b
9	83	Male	Adenocarcinoma	Variants 3a and 3b
10	58	Male	Adenocarcinoma	A novel variant[a]
11	36	Female	Adenocarcinoma	Not examined[b]
12	69	Female	Squamous cell carcinoma	Not examined[b]

[a]Exon 14 of EML4 is connected to a 2-bp fragment that in turn is ligated to the nucleotide at position 53 of exon 20 of ALK. [b]Due to the absence of an RNA sample, RT-PCR analysis for ALK fusions was not performed.

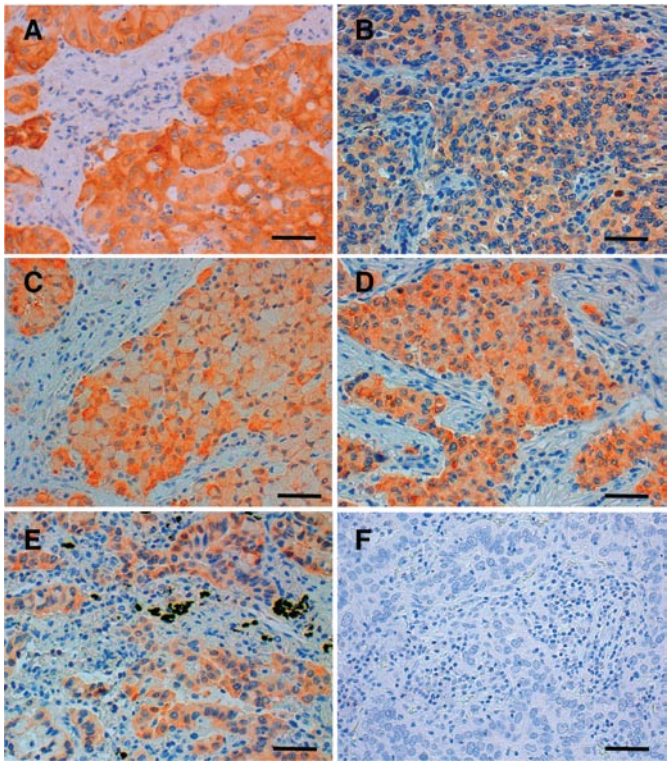

Figure 1. Representative results of immunohistochemical staining for ALK in non-small cell lung carcinomas. ALK protein expression was detected with 5A4 anti-ALK monoclonal antibody by the intercalated antibody-enhanced polymer method. A, B, C, D and E are the adenocarcinomas in cases No. 2, 5, 6, 8 and 10, respectively. F is the adenocarcinoma which showed no ALK expression. Bar, 50 μm.

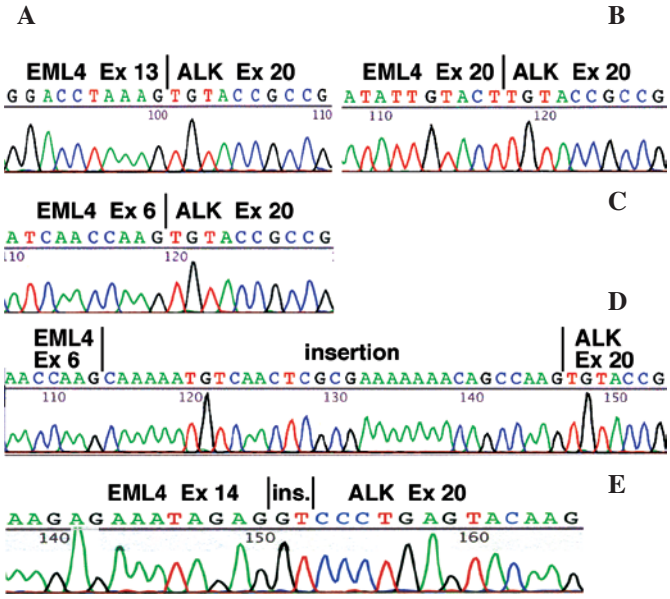

Figure 2. Detection of EML4-ALK fusion transcripts in non-small cell lung carcinomas. (A) EML4-ALK variant 1 transcript detected in case No. 1. (B) EML4-ALK variant 2 transcript detected in case No. 4. (C and D) EML4-ALK variants 3a (C) and 3b (D) transcripts detected in case No. 7. (E) Novel EML4-ALK transcript variant detected in case No. 10.

analyses. As a negative control, we also performed an RT-PCR analysis of 30 randomly selected, immunohistochemically ALK-negative NSCLCs. No expression of KIF5B-ALK or TFG-ALK fusion transcripts was detected in any of the carcinomas, but EML4-ALK fusion transcripts were detected in all 10 RNA-available, ALK-positive NSCLCs (Table I). As expected, no RT-PCR products were detected in any of the 30 immunohistochemically ALK-negative NSCLCs. Regarding the type of the EML4-ALK transcript, in 3 cases (No. 1-3) the fusion was variant 1, a fusion between exon 13 of EML4 and exon 20 of ALK (Fig. 2A), and in 3 cases (No. 4-6) it was variant 2, a fusion between exon 20 of EML4 and exon 20 of ALK (Fig. 2B). In 3 other cases (No. 7-9) the RT-PCR analysis yielded two bands, and they corresponded to variant 3a, a fusion between exon 6 of EML4 and exon 20 of ALK (Fig. 2C), and variant 3b, a fusion containing an additional 33-bp sequence derived from intron 6 of EML4 between exon 6 of EML4 and exon 20 of ALK (Fig. 2D). Notably, in case No. 10,

sequencing of the RT-PCR product revealed that exon 14 of EML4 was connected to an unidentified 2-bp fragment that was in turn ligated to the nucleotide at position 53 of exon 20 of ALK (Fig. 2E). The EML4-ALK sequence detected in case No. 10 allows an in-frame connection between the two genes and is a novel variant. The mean age of the 10 NSCLC patients whose tumors contained EML4-ALK transcripts was 58.2 years (SD 15.3), and they consisted of 6 men and 4 women. The NSCLC tumors containing EML4-ALK transcripts were histologically classified as adenocarcinoma in 9 cases and adenosquamous carcinoma in 1 case. These findings suggest that the iAEP method is useful for screening paraffin-embedded tissue sections for NSCLCs containing ALK fusion transcripts.

Discussion

Immunohistochemical screening for ALK expression using the iAEP method in the present study revealed an immunohistochemical ALK signal in 12 (4.0%) of 302 NSCLCs but not in any of the 291 gastric carcinomas. The ALK signal was detected in the cytoplasm of the cancer cells in all of the ALK-positive NSCLCs. RT-PCR and subsequent sequencing analyses of RNA from the 10 RNA-available, ALK-positive NSCLCs revealed the EML4-ALK variant 1 in 3 cases, variant 2 in 3 cases, both variants 3a and 3b in 3 cases, and a novel variant consisting of a fusion between exon 14 of EML4 and a nucleotide within exon 20 of ALK in 1 case. These results suggest that the immunohistochemistry-based system is useful for screening NSCLCs for ALK fusions, and identification of a novel EML4-ALK variant would be helpful in diagnosing NSCLCs containing ALK fusions in the future.

The proportion of immunohistochemically ALK-positive NSCLCs in this study (4.0%) is almost the same as the proportions of NSCLCs containing ALK fusion transcripts reported in previous studies (4,6-18), and in the present study EML4-ALK variants were detected in all RNA-available, immunohistochemically ALK-positive NSCLCs. These results suggest that our immunohistochemical analysis was performed properly and that the iAEP method with 5A4 anti-ALK antibody is a useful diagnostic tool for screening for NSCLCs containing ALK fusion proteins.

The histopathological diagnosis of 10 of the 12 immunohistochemically ALK-positive NSCLCs and 9 of the 10 EML4-ALK-positive NSCLCs in this study was adenocarcinoma. The predominance of adenocarcinomas among EML4-ALK-positive NSCLCs is consistent with the results of previous studies (6,12). This finding is also consistent with the recent finding of the growth of hundreds of adenocarcinoma nodules in transgenic mice in which EML4-ALK mRNA was transcribed specifically in lung epithelial cells (21). In the present study the mean age of the patients with immunohistochemically ALK-positive NSCLCs was significantly lower than that of the patients with ALK-negative NSCLCs. Although the mechanism responsible for the age difference is unknown, early onset may be a characteristic of ALK fusion-positive NSCLCs.

A novel EML4-ALK variant was found in this study. In this novel variant, exon 14 of EML4 was connected to a 2-bp fragment that was in turn ligated to the nucleotide at position 53 of exon 20 of ALK. Notably, the connection in each of two EML4-ALK variants, 4 and 7, is known to be between exon 14 of EML4 and a nucleotide within exon 20 of ALK (11,13). In variant 4, exon 14 of EML4 is connected to an unidentified 11-bp cDNA fragment that in turn is ligated to the nucleotide at position 50 of exon 20 of ALK (11), while in variant 7, exon 14 of EML4 is connected to the nucleotide at position 13 of exon 20 of ALK (13). Thus, the variant identified in this study is the third variant with a connection between exon 14 of EML4 and a nucleotide within exon 20 of ALK. Connections located within, rather that at the 5' terminus of, exon 20 of ALK have also been reported in MSN-ALK and MYH9-ALK, both of which have been detected in anaplastic large-cell lymphoma (22,23). Since a systemic understanding of the ALK fusions is important to correctly diagnose NSCLCs containing ALK fusions, our identification of a novel EML4-ALK variant should contribute to establishing a practical and accurate diagnostic system in the future.

Since the intracellular region of ALK was used as the antigen to produce the 5A4 anti-ALK antibody used in this study, both EML4-ALK and wild-type ALK should have been detected by the antibody. Takeuchi *et al* attempted to determine whether both transcripts are expressed by quantitatively analyzing the amount of mRNA specific for wild-type ALK and ALK fusion transcript separately, and found that none of the EML4-ALK-positive tumors yielded a substantial amount of wild-type ALK mRNA (13). Thus, immunohistochemical staining with the 5A4 antibody using the iAEP method appears to detect ALK fusion proteins and not wild-type ALK in NSCLCs. Our results for detection of EML4-ALK variants in all of the RNA-available, immunohistochemically ALK-positive NSCLCs support this view.

Our immunohistochemical analysis did not detect ALK protein expression in any of the gastric carcinomas. This was the first search for ALK fusion proteins in gastric carcinomas, and the results clearly demonstrated the absence of ALK fusion in gastric carcinomas. Since previous RNA analyses showed no EML4-ALK transcripts in 96 gastric carcinomas (8) and 33 gastric carcinomas (11), our results are consistent. The only human carcinomas in which ALK fusions have ever been found are NSCLCs. However, since it is unknown whether ALK fusions are involved in the genesis and development of other types of carcinoma, it may be worth investigating various types of carcinomas for expression of ALK fusion proteins in the future.

Acknowledgements

We are grateful to Dr K. Takeuchi (Cancer Inst, JFCR) for his technical advice regarding the iAEP method. This study was supported, in part, by a Grant-in-Aid from the Ministry of Health, Labour and Welfare for the Comprehensive 10-Year Strategy for Cancer Control (19-19), by a Grant-in-Aid from the Japan Society for the Promotion of Science for Scientific Research (no. 19790286), by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan on Priority Area (no. 18014009), by the 21st century COE program 'Medical Photonics' and by the Smoking Research Foundation.

References

1. Look AT: Oncogenic transcription factors in the human acute leukemias. Science 278: 1059-1064, 1997.
2. Lucansky V, Sobotkova E, Tachezy R, Duskova M and Vonka V: DNA vaccination against bcr-abl-positive cells in mice. Int J Oncol 35: 941-951, 2009.
3. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, Lee C, Montie JE, Shah RB, Pienta KJ, Rubin MA and Chinnaiyan AM: Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 310: 644-648, 2005.
4. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, Fujiwara S, Watanabe H, Kurashina K, Hatanaka H, Bando M, Ohno S, Ishikawa Y, Aburatani H, Niki T, Sohara Y, Sugiyama Y and Mano H: Identification of the transforming EML4-ALK fusion gene in non-small cell lung cancer. Nature 448: 561-566, 2007.
5. Verdorfer I, Fehr A, Bullerdiek J, Scholz N, Brunner A, Krugmann J, Hager M, Haufe H, Mikuz G and Scholtz A: Chromosomal imbalances, 11q21 rearrangement and MECT1-MAML2 fusion transcript in mucoepidermoid carcinomas of the salivary gland. Oncol Rep 22: 305-311, 2009.
6. Mano H: Non-solid oncogenes in solid tumors: EML4-ALK fusion genes in lung cancer. Cancer Sci 99: 2349-2355, 2008.
7. Shinmura K, Kageyama S, Tao H, Bunai T, Suzuki M, Kamo T, Takamochi K, Suzuki K, Tanahashi M, Niwa H, Ogawa H and Sugimura H: EML4-ALK fusion transcripts, but no NPM-, TPM3-, CLTC-, ATIC-, or TFG-ALK fusion transcripts, in non-small cell lung carcinomas. Lung Cancer 61: 163-169, 2008.
8. Fukuyoshi Y, Inoue H, Kita Y, Utsunomiya T, Ishida T and Mori M: EML4-ALK fusion transcript is not found in gastrointestinal and breast cancers. Br J Cancer 98: 1536-1539, 2008.
9. Choi YL, Takeuchi K, Soda M, Inamura K, Togashi Y, Hatano S, Enomoto M, Hamada T, Haruta H, Watanabe H, Kurashina K, Hatanaka H, Ueno T, Takada S, Yamashita Y, Sugiyama Y, Ishikawa Y and Mano H: Identification of novel isoforms of the EML4-ALK transforming gene in non-small cell lung cancer. Cancer Res 68: 4971-4976, 2008.
10. Perner S, Wagner PL, Demichelis F, Mehra R, Lafargue CJ, Moss BJ, Arbogast S, Soltermann A, Weder W, Giordano TJ, Beer DG, Rickman DS, Chinnaiyan AM, Moch H and Rubin MA: EML4-ALK fusion lung cancer: a rare acquired event. Neoplasia 10: 298-302, 2008.
11. Takeuchi K, Choi YL, Soda M, Inamura K, Togashi Y, Hatano S, Enomoto M, Takada S, Yamashita Y, Satoh Y, Okumura S, Nakagawa K, Ishikawa Y and Mano H: Multiplex reverse transcription-PCR screening for EML4-ALK fusion transcripts. Clin Cancer Res 14: 6618-6624, 2008.
12. Inamura K, Takeuchi K, Togashi Y, Hatano S, Ninomiya H, Motoi N, Mun MY, Sakao Y, Okumura S, Nakagawa K, Soda M, Choi YL, Mano H and Ishikawa Y: EML4-ALK lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology and young onset. Mod Pathol 22: 508-515, 2009.
13. Takeuchi K, Choi YL, Togashi Y, Soda M, Hatano S, Inamura K, Takada S, Ueno T, Yamashita Y, Satoh Y, Okumura S, Nakagawa K, Ishikawa Y and Mano H: KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. Clin Cancer Res 15: 3143-3149, 2009.
14. Martelli MP, Sozzi G, Hernandez L, Pettirossi V, Navarro A, Conte D, Gasparini P, Perrone F, Modena P, Pastorino U, Carbone A, Fabbri A, Sidoni A, Nakamura S, Gambacorta M, Fernández PL, Ramirez J, Chan JK, Grigioni WF, Campo E, Pileri SA and Falini B: EML4-ALK rearrangement in non-small cell lung cancer and non-tumor lung tissues. Am J Pathol 174: 661-670, 2009.
15. Rikova K, Guo A, Zeng Q, Possemato A, Yu J, Haack H, Nardone J, Lee K, Reeves C, Li Y, Hu Y, Tan Z, Stokes M, Sullivan L, Mitchell J, Wetzel R, Macneill J, Ren JM, Yuan J, Bakalarski CE, Villen J, Kornhauser JM, Smith B, Li D, Zhou X, Gygi SP, Gu TL, Polakiewicz RD, Rush J and Comb MJ: Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 131: 1190-1203, 2007.
16. Koivunen JP, Mermel C, Zejnullahu K, Murphy C, Lifshits E, Holmes AJ, Choi HG, Kim J, Chiang D, Thomas R, Lee J, Richards WG, Sugarbaker DJ, Ducko C, Lindeman N, Marcoux JP, Engelman JA, Gray NS, Lee C, Meyerson M and Jänne PA: EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin Cancer Res 14: 4275-4283, 2008.
17. Horn L and Pao W: EML4-ALK: honing in on a new target in non-small cell lung cancer. J Clin Oncol 27: 4232-4235, 2009.
18. Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, Solomon B, Stubbs H, Admane S, McDermott U, Settleman J, Kobayashi S, Mark EJ, Rodig SJ, Chirieac LR, Kwak EL, Lynch TJ and Iafrate AJ: Clinical features and outcome of patients with non-small cell lung cancer who harbor EML4-ALK. J Clin Oncol 27: 4247-4253, 2009.
19. McDermott U, Iafrate AJ, Gray NS, Shioda T, Classon M, Maheswaran S, Zhou W, Choi HG, Smith SL, Dowell L, Ulkus LE, Kuhlmann G, Greninger P, Christensen JG, Haber DA and Settleman J: Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase inhibitors. Cancer Res 68: 3389-3395, 2008.
20. Shinmura K, Iwaizumi M, Igarashi H, Nagura K, Yamada H, Suzuki M, Fukasawa K and Sugimura H: Induction of centrosome amplification and chromosome instability in p53-deficient lung cancer cells exposed to benzo[a]pyrene diol epoxide (B[a]PDE). J Pathol 216: 365-374, 2008.
21. Soda M, Takada S, Takeuchi K, Choi YL, Enomoto M, Ueno T, Haruta H, Hamada T, Yamashita Y, Ishikawa Y, Sugiyama Y and Mano H: A mouse model for EML4-ALK-positive lung cancer. Proc Natl Acad Sci USA 105: 19893-19897, 2008.
22. Tort F, Pinyol M, Pulford K, Roncador G, Hernandez L, Nayach I, Kluin-Nelemans HC, Kluin P, Touriol C, Delsol G, Mason D and Campo E: Molecular characterization of a new ALK translocation involving moesin (MSN-ALK) in anaplastic large cell lymphoma. Lab Invest 81: 419-426, 2001.
23. Lamant L, Gascoyne RD, Duplantier MM, Armstrong F, Raghab A, Chhanabhai M, Rajcan-Separovic E, Raghab J, Delsol G and Espinos E: Non-muscle myosin heavy chain (MYH9): a new partner fused to ALK in anaplastic large cell lymphoma. Genes Chromosomes Cancer 37: 427-432, 2003.