Anaplastic Thyroid Cancers Harbor Novel Oncogenic Mutations of the ALK Gene

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Abstract

Thyroid cancer is the most common endocrine cancer, and targeted approaches to treat it pose considerable interest. In this study, we report the discovery of ALK gene mutations in thyroid cancer that may rationalize clinical evaluation of anaplastic lymphoma kinase (ALK) inhibitors in this setting. In undifferentiated anaplastic thyroid cancer (ATC), we identified two novel point mutations, C3592T and G3602A, in exon 23 of the ALK gene, with a prevalence of 11.11%, but found no mutations in the matched normal tissues or in well-differentiated thyroid cancers. These two mutations, resulting in L1198F and G1201E amino acid changes, respectively, both reside within the ALK tyrosine kinase domain where they dramatically increased tyrosine kinase activities. Similarly, these mutations heightened the ability of ALK to activate the phosphatidylinositol 3-kinase (PI3K)/Akt and mitogen-activated protein (MAP) kinase pathways in established mouse cells. Further investigations showed that these two ALK mutants strongly promoted cell focus formation, anchorage-independent growth, and cell invasion. Similar oncogenic properties were observed in the neuroblastoma-associated ALK mutants K1062M and F1174L but not in wild-type ALK. Overall, our results reveal two novel gain-of-function mutations of ALK in certain ATCs, and they suggest efforts to clinically evaluate the use of ALK kinase inhibitors to treat patients who harbor ATCs with these mutations. Cancer Res; 71(13); 4403-11. ©2011 AACR.

Introduction

Anaplastic lymphoma kinase (ALK) is a member of the insulin receptor subfamily of receptor tyrosine kinases (RTKs), with its encoding gene located on the short arm of chromosome 2 (1, 2). ALK was initially identified as part of an oncogenic fusion gene, NPM1-ALK (also known as NPM-ALK), in anaplastic large-cell non-Hodgkin’s lymphomas (ALCL; 3). It is also part of the fusion gene EML4-ALK in non-small-cell lung cancer (NSCLC; 4). There are a few other ALK fusion genes, such as TMP3/4-ALK and RANBP2-ALK, in inflammatory myofibroblastic tumors (IMT; 5). The tyrosine kinase activities of these fusion ALK proteins are aberrantly activated and promote cell proliferation and survival (6, 7). ALK fusion proteins have also been shown to activate various signaling pathways, among which are the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and the Ras → Raf → MEK → extracellular signal regulated kinase (ERK)/mitogen-activated protein (MAP) kinase pathway with multiple interaction points to mediate the ALK signaling (8, 9).

Recently, ALK mutations were found in 6% to 14% of sporadic neuroblastomas (10–14). ALK mutations were also reported in familial neuroblastomas (13, 14). Moreover, genetic amplification of the ALK gene could also occur in neuroblastomas or cell lines derived from this tumor (10, 11, 13, 15). Except for occasional mutations in the juxtamembrane domain, most ALK mutations identified so far are within the tyrosine kinase domain of ALK. ALK mutations and/or copy gain were found particularly in advanced and metastatic neuroblastomas, and patients with ALK mutations had a worse prognosis (11, 12, 14). Several common ALK mutations were shown to be functional. For example, siRNA-mediated knockdown of the ALK expression in cell lines harboring ALK mutants F1174L or R1275Q caused cell apoptosis and suppression of cell proliferation (12–14). The F1174L and another mutant ALK, K1062M, were shown to display increased tyrosine kinase activity and promote cell focus formation, cell transformation, and xenograft tumorigenicity in nude mice (10). The oncogenicity of ALK F1174L and R1275Q was also shown in another study (12). Genetic copy gain of the ALK is also functionally important, as suggested by the demonstration that inhibition of ALK in neuroblastoma cell lines harboring ALK copy gain induced cell apoptosis through reduced signaling of the PI3K/Akt and MAP kinase pathways (15).

Mutations of the ALK gene have not been reported in human cancers other than neuroblastomas. In the present study, we investigated the mutation status of the ALK gene in various thyroid cancers, including well-differentiated papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC) and undifferentiated anaplastic thyroid cancer (ATC). Prompted
by our finding of ALK mutations in ATC, a rapidly aggressive and deadly human cancer (16), we also examined melanoma and colon carcinoma for ALK mutation.

Materials and Methods

Tumor samples, cell lines, and DNA isolation
A large number of human tumor samples, as indicated in Results, and human thyroid cancer cell lines K1, BCPAP, K5, FTC133, OCUT-1, OCUT-2, FB-1, SW1736, HTh7, HTh74, KAT18, and C643 (with the later 8 cell lines being derived from ATC), melanoma cell lines M14, A375, and UACC62, and colon carcinoma cell lines T84, RKO, and HT-29 were used for mutational analysis of the ALK gene. The original sources of the cell lines used in this study were as follows: K1 and K5 from Dr. David Wynford-Thomas (University of Wales College of Medicine, Cardiff, UK); BCPAP from Dr. Massimo Santoro (University of Federico II, Naples, Italy); FTC133 from Dr. Georg Brabant (University of Manchester, Manchester, UK); OCUT-1 and OCUT-2 from Dr. Naoyoshi Onoda (Osaka City University Graduate School of Medicine, Osaka, Japan); KAT18 from Dr. Kenneth B. Ain (University of Kentucky Medical Center, Lexington, KY); FB-1 from Dr. Riccardo Giannini (Department of Surgery, University of Pisa, Pisa, Italy); SW1736, HTh7, HTh74, and C643 from Dr. N. E. Heldin (University of Uppsala, Uppsala, Sweden). These cell lines have recently been tested and authenticated to be distinct thyroid cancer cell lines (17). The melanoma cell lines UACC62 and M14 were obtained from the cell bank of National Cancer Institute (Bethesda, MD), and the melanoma cell line A375 and the colon cancer cell lines T84, RKO, and HT-29 were obtained from American Type Culture Collection (ATCC). Use of tumor samples was Institutional Review Board approved as reported previously (18). Except for FTC133 cells cultured in DMEM/Ham’s F-12 medium, all tumor cell lines were cultured in RPMI-1640 medium, supplemented with 10% FBS, 2 mmol/L L-glutamine, streptomycin (100 µg/mL), and penicillin (100 U/mL). Genomic DNA (from cell lines and tumors) was isolated by phenol–chloroform extraction, using MaxXtract high-density gel tubes (Qiagen) as described previously (19).

PCR amplification and sequencing
PCR amplification of exons 23, 24, and 25 of the ALK gene was conducted using the primers and conditions as described previously (10). The amplified PCR products were directly sequenced using a BigDye terminator v3.1 cycle sequencing ready reaction kit (Applied Biosystems) and an ABI PRISM 3730 automated next generation genetic analyzer (Applied Biosystems). GenBank accession number for ALK is NM_004304.3.

Multiple amino acid sequence alignment
Original amino acid sequences of ALK of various species were obtained from NCBI database (http://www.ncbi.nlm.nih.gov/protein/) as follows: H_sapiens (NP_004295.2), C_lupus (XP_540136.2), B_taurus (XP_616782.3), M_musculus (NP_031465.2), G_gallus (XP_419964.2), and D_rerio (XP_691964.2). These amino acid sequences were compared using a computer-based multiple sequence alignment program (http://pir.georgetown.edu/pirww/search/multialn.shtml).

Expression vectors and site-directed mutagenesis
The expression vector pcDNA3 carrying wild-type ALK and mutant ALKs (K1062M and F1174L) are a kind gift from Profs. Yasuhide Hayashi and Seishi Ogawa at The University of Tokyo, Tokyo, Japan (10). The expression vector pcDNA3 carrying wild-type ALK was used to generate the novel ALK mutants L1198F and G1201E discovered in the present study with a Quick Change XL II Site-Directed mutagenesis kit (Stratagene) according to the instructions of the manufacturer. The primers were designed using a template-specific mutagenic primer design program. The primer sequences are as follows: for L1198F: sense, ALK-C3592T_F 5'-CAT-CCTGCTGGAGTTCTATGGCCCGGGG-3' and antisense, ALK-C3-592T_R 5’-CCCCTGCCATGAACTCCAGGATG-3’; for G1201E: sense, ALK-G3602A_F 5’-GAAGCTCAGGCGGAGGAGAACCTCAAG-3’ and antisense, ALK-G3602A_R 5’-CCGGTCTCCCCTCCGCGCAGCCTG-3’. The mutations were confirmed in the vectors by sequencing with the primer ALK-VEC_F 5’-TCTCGCTTGTGGTACCTCCTG-3’. Plasmid DNAs for the transfection experiments were purified using a mini prep kit (catalogue no. K2100-11; Invitrogen).

Cell culture, transfection, and pooled stable expression
NIH3T3 cells (ATCC) were grown in DMEM and supplemented with fetal calf serum and plated (8.0 × 10^5 cells/well) on 6-well plates. Twenty-four hours later, cells were transfected using the Lipofectamine 2000 Transfection Reagent (Invitrogen) with equal amount of empty vector or vector containing wild-type ALK or mutant ALK DNA per manufacturer’s instructions. Cells were selected using 800 µg/mL G418 (Stratagene) 48 hours after transfection. Medium was changed after every 3 days. After 14 days of selection, stably formed clones were pooled and integration of the plasmid was checked by PCR and expression of the ALK protein was confirmed by Western blotting. Stably transfected pooled clones were used for functional studies.

ALK kinase assay
The ALK tyrosine kinase assay was a nonradioactive solid-phase ELISA, which was conducted as described previously (10) using the Universal Tyrosine Kinase Assay Kit (catalogue no. MK410; Takara Bio Inc.). Briefly, cells stably transfected with empty vector, wild-type ALK, the 2 novel ALK mutants (L1198F and G1201E), and the 2 positive ALK mutant controls (K1062M and F1174L) were lysed and lysates were centrifuged at 12,000 × g for 10 minutes at 4°C. The supernatants were collected, and protein concentration was measured using a DC protein assay kit (Bio-Rad Laboratories). For each enzymatic reaction, an equal volume of cell lysate containing the same amount of protein was added to the kinase reaction buffer and the mixture was transferred to the microplate supplemented with the kit containing the substrate [immobilized peptide poly(Glu-Tyr)]. The kinase reaction was initiated by adding 40 mmol/L ATP-2Na solution, followed by incubation for 20 minutes at room temperature.
Western blotting

Western blotting was done as described previously (20). Briefly, 15 μg of cell lysate proteins prepared for ALK kinase assay was separated on SDS-PAGE and transferred to polyvinylidene difluoride membrane (Millipore Co.). After transfer, the membrane was blocked with 5% skim-milk/PBS containing 0.1% Tween 20 (PBST) for 1 hour at room temperature and the membrane was sliced on the basis of the molecular weight and incubated with primary antibodies. Membranes were incubated overnight at 4°C with anti-Flag (catalogue no. 2368; Cell Signaling Technology), anti-phospho-Akt (catalogue no. sc-7985; Santa Cruz Biotechnology), or anti-phospho-ERK (catalogue no. sc-7383; Santa Cruz Biotechnology) primary antibodies. Membranes were incubated with anti-Flag (catalogue no. sc-8312), anti-ERK (catalogue no. sc-94), or anti-β-actin (catalogue no. sc-1616; Santa Cruz Biotechnology) primary antibodies for 1 hour at room temperature. After washing 4 times with PBST, blots were incubated with respective horseradish peroxidase–conjugated secondary antibodies (catalogue nos. sc-2004 and sc-2005; Santa Cruz Biotechnology) for 1 hour at room temperature. After washing with PBST, protein bands on the membrane were detected with enhanced chemiluminescence reaction and exposure to X-ray films.

Cell focus formation assay

Cell focus formation assay was conducted as described previously (21). Briefly, NIH3T3 cells were transfected with equal amount of empty vector, wild-type, or each of mutant ALK expression vectors, using the Lipofectamine 2000 transfection reagent following the manufacturer’s instructions (Invitrogen Life Technologies). Twenty-four hours after the transfection, cells were selected using G418 (800 μg/mL) for 7 days. Selected cells were trypsinized, pooled, and plated (5 × 10^5 cells) on 6-well plates. Medium was changed every 3 to 4 days. After 14 days, the number of morphologically transformed foci was counted and photographed (Zeiss Axiovert 200M).

Soft agar colony formation assay

Soft agar colony formation assay was conducted as previously described (10). Briefly, NIH3T3 cells stably expressing vector, wild-type, or mutant ALKs were seeded (1.0 × 10^4 cells) on 6-well plates (Costar; Corning) in 0.3% agar (catalogue no. 214010; BD Biosciences) over a bottom layer of 0.6% agar. After 4 weeks, the colonies of greater than 0.1 mm diameter were counted and photographed (Zeiss Axiovert 200M; Carl Zeiss).

Invasion assay

Cell invasion assay was conducted as described previously (20). Briefly, the assay was conducted using Matrigel invasion chambers consisting of BD Falcon cell culture inserts containing a polyethylene terephthalate (PET) membrane with 8-μm pores coated with Matrigel matrix (BD BioCoat Matrigel Invasion Chamber; BD Biosciences). Cells expressing the vector, wild-type, or mutant ALK were completely serum starved for 7 to 8 hours and then collected and resuspended (5 × 10^5 cells) in 500 μL of serum-free DMEM with 0.1% bovine serum albumin. Culture inserts were placed in the wells of a BD Falcon 24-well multiwell companion plate, and 750 μL of DMEM containing 1% serum was added to the lower compartment of each well. Cell suspensions were added to each culture inserts. After a 22-hour incubation at 37°C with 5% CO₂, the noninvading cells on top of the Matrigel were removed using cotton swab and invaded cells on the lower side of the membrane were fixed with 70% ethanol and stained with Coomassie Brilliant Blue. Invading cells were counted and photographed under a microscope with 10× magnification (Nikon Eclipse ME-600-DS-5M-L1). All the data of assays presented represent at least 2 similar experiments.

Results

Identification of novel somatic ALK mutations in ATC

We analyzed the tyrosine kinase domain exons 23 and 25 of the ALK gene for mutation in 12 thyroid cancer cell lines (including 8 ATC cell lines), 36 FTCs (including 12 cases of each conventional, follicular variant, and tall cell PTC), 20 FTCs, and 18 ATC tumor samples. These exons were chosen for analysis because they contained the hot spots for ALK mutations found in neuroblastomas. No mutation was found in the thyroid cancer cell lines as well as in the PTC and FTC tumor samples. However, we found 2 novel missense heterozygous ALK point mutations in ATC tumor samples. As shown in Figure 1A, a mutation found in a case of ATC represented a C>T transition in nucleotide position 3,592. This mutation changed codon 1,198 from CTC to TTC, resulting in the amino acid change from lysine to phenylalanine (L1198F) of ALK. As shown also in Figure 1A, the other mutation found in another case of ATC represented a G>A transition in nucleotide position 3,602. This mutation changed codon 1,201 from GGG to GAG, resulting in the amino acid change of glycine to glutamic acid (G1201E) of ALK. Reverse sequencing using antisense primers confirmed these 2 mutations (Fig. 1A). The matched normal tissues in these 2 cases showed the wild-type ALK gene (Fig. 1A), suggesting that the novel ALK mutations identified in the ATC samples were somatic mutations. We did not find these mutations in COSMIC Catalog Of Somatic Mutations In Cancer, a database of Sanger Institute, UK (http://www.sanger.ac.uk/genetics/CGP/cosmic/), and in the literature. We also checked the databases of single-nucleotide polymorphism, including the major Web sites of Ensembl (http://uswest.ensembl.org) and NCBI (http://www.ncbi.nlm.nih.gov/projects/SNP/), and did not find germ line variations that represented these ALK mutations. Therefore, this is the first report of these somatic mutations of the ALK gene in human cancers. We also examined exon 24 of the ALK gene in the ATC samples but did not find any mutations. In these ATC tumors, we did not find ALK mutations that had been previously reported in neuroblastomas. The prevalence of ALK mutations in ATC in the present study was 11.11% (2 of 18).

We also analyzed exons 23 and 25 of the ALK gene in 3 melanoma cell lines, 3 colon carcinoma cell lines, 44 melanomas, and 47 colon carcinoma samples. We did not find ALK mutation in any of these samples, suggesting that ALK mutation is not a common event in these cancers. We found a few silent mutations in these cancers (data not shown).
Increased tyrosine kinase activities of novel somatic ALK mutants L1198F and G1201E and their activation of the PI3K/Akt and MAP kinase pathways

As shown in Figure 1B, the somatically mutated amino acid residues L1198F and G1201E are located within the tyrosine kinase domain (amino acids 1,057–1,383) of the ALK protein. Moreover, as shown in Figure 1C, alignment comparison of amino acid sequences of ALK proteins from 6 different species revealed that the L1198 and G1201 residues are evolutionarily conserved residues of the ALK proteins among various species. We therefore speculated that these novel somatic ALK mutations likely altered the tyrosine kinase activity of ALK. To test this idea, we conducted in vitro mutagenesis to generate these 2 mutations and tested their tyrosine kinase activities.

To this end, NIH3T3 cells stably expressing vector, wild-type, and each mutant ALK were lysed and assayed for in vitro tyrosine kinase activities. As shown in Figure 2A, the novel ALK mutants L1198F and G1201E displayed dramatically increased tyrosine kinase activities compared with the wild-type ALK. The neuroblastoma-associated ALK mutants K1062M and F1174L, as positive controls in the assay, also displayed high activities as expected (Fig. 2A). Western blot analysis confirmed the corresponding protein expression of the expression vector constructs (Fig. 2B, top). These results showed that the 2 novel ALK mutations found in ATC are gain-of-function mutations.
As oncogenic fusion ALK proteins were previously shown to activate the PI3K/Akt and MAP kinase pathways (8, 9), we next tested whether the novel ALK mutants discovered in this study had any impact on the signaling of these 2 pathways. As shown in Figure 2B, compared with wild-type ALK, phosphorylation of both Akt and ERK was elevated in NIH3T3 cells expressing the mutant L1198F or G1201E, similar to ALK mutants K1062M and F1174L tested here as positive controls. This was consistent with high immunostaining scores for the phosphorylation levels of Akt and ERK in the 2 cases of ATC harboring the ALK mutations, which were revealed in a previous study of ours (19). Thus, the role of the 2 novel ALK mutations in the tumorigenesis of ATC likely involves aberrant activation of the PI3K/Akt and MAP kinase pathways.

**Novel somatic ALK mutants L1198F and G1201E promoted cell transformation and invasion**

Given the known cell-transforming abilities of fusion ALK proteins and neuroblastoma-associated ALK mutants (10), we examined the transforming abilities of the 2 novel ALK mutants L1198F and G1201E to functionally test their oncogenic potential. To this end, we transfected NIH3T3 cells with empty vector, wild-type ALK, and various ALK mutants and examined their ability to form cell focus and anchorage-independent colonies. As shown in Figure 3A, cells expressing ALK mutants L1198F and G1201E lost cell contact–mediated growth inhibition and grew foci of multilayers of cells whereas control cells (vector and wild-type ALK) exhibited contact inhibition and grew in monolayer of cells. Moreover, ALK mutants L1198F and G1201E were also associated with a much larger number of foci of multilayers of cells than the wild-type ALK (Fig. 3B). As a positive control, the previously well-characterized neuroblastoma-associated ALK mutants K1062M and F1174L showed similar cell focus–forming abilities (Fig. 3A and B).

Anchorage-independent growth on soft agar also reflects a transformation property of cells. As shown in Figure 3C, like the positive control ALK mutants K1062M and F1174L, the novel ALK mutants L1198F and F1174L induced the formation of much larger cell colonies on soft agar than that by vector and wild-type ALK. The number of large cell colonies induced by these mutants was also much bigger than that of the vector and the wild-type ALK (Fig. 3D).

An earlier study showed cell invasion–promoting properties of various fusion ALK proteins (22). We tested such properties of the ALK mutants L1198F and G1201E in the present studies. As shown in Figure 4A, like ALK K1062M and F1174L, NIH3T3 cells transfected with the 2 novel ALK mutants were much more invasive on Matrigel matrix–coated membranes than vector or wild-type ALK-transfected cells. The number of invading cells was much bigger with the ALK mutants than with the vector and wild-type ALK (Fig. 4B).
Discussion

We for the first time analyzed the ALK gene for mutations in tumors other than neuroblastomas and identified 2 somatic novel missense ALK point mutations, C3592T and G3602A, in ATC. The 2 mutations caused amino acid change from lysine to phenylalanine (L1198F) and from glycine to glutamic acid (G1201E) of ALK, respectively. They are both in exon 23 and only 3 codons apart, with a relatively high ALK mutation prevalence of 11% in ATC, similar to that in sporadic neuroblastomas (10–12, 14). The novel mutations L1198F and G1201E are located in the tyrosine kinase domain of the ALK. More specifically, they are in the hinge region of this domain. It has been shown that ALK binds ADP and staurosporine at the hinge region of the interlobe cleft, suggesting important specific functions of this region (23). Therefore, it may be expected that amino acid changes in this region may result in conformational change of the ALK protein, with significant functional consequences to the tyrosine kinase activity of ALK. Indeed, both mutations were shown to be gain-of-function mutations, conferring that ALK dramatically increased tyrosine kinase activities. We also showed that, like several previously characterized ALK mutants in neuroblastomas, the 2 novel ALK mutants found in ATC strongly promoted cell focus formation, anchorage-independent growth, and cell invasion, effectively showing their oncogenic functions. We thus report ALK mutations in ATC with a comparable prevalence and oncogenic power with those in

Figure 3. Focus-formation and anchorage-independent growth of cells promoted by ALK mutants. A, cell focus–forming activities of ALK mutants. Shown are images of adherent growth of NIH3T3 cells transfected with Flag-tagged vector, wild-type ALK (wt-ALK), and each of the ALK mutants indicated. Cells were cultured in regular medium with 10% fetal calf serum under standard conditions. Images of cell foci were photographed with 10× magnification after appropriate culture of cells as described in Materials and Methods. B, number of cell foci formed with the indicated transfections. The number of transformed foci was counted 14 days after cell transfection. Results represent mean ± SD of 3 independent experiments. C, anchorage-independent cell growth of ALK mutants on soft agar. NIH3T3 cells stably transfected with Flag-tagged vector, wild-type ALK, and each of the ALK mutants indicated were seeded in soft agar, and colonies formed 4 weeks later were photographed with 40× magnification. D, analyses of number of colonies. The number of cell colonies corresponding to C that were greater than 0.1 mm in diameter was counted. Results represent mean ± SD of 3 independent experiments.
Expression of ALK seems to be restricted to tissues originating from neuroectodermal developmental origin (24). Thyroid cancer cells were specifically shown to express ALK (24). Thus, the mutant ALK gene can be expected to be expressed in thyroid cells, making such mutations relevant for thyroid tumorigenesis.

ATC is the most aggressive type of thyroid cancer; in fact, it is one of the most rapidly developing lethal human cancers (16). With the current available treatments, patients with ATC usually die within 5 to 6 months after initial diagnosis. The finding of the ALK mutations in ATC, but not in generally indolent differentiated PTC and FTC, suggests that activating genetic alterations of the ALK gene play a role in the aggressiveness of a subgroup of ATC. This is interestingly consistent with previous observations that ALK mutations were also associated with advanced disease stages and poor outcomes of neuroblastoma (11, 12, 14).

The molecular signaling pathways mediating the oncogenic role of ALK mutations in ATC involved the PI3K/Akt and MAP kinase pathways, as suggested by our demonstration of the coupling of the 2 novel ALK mutants to increased phosphorylation of Akt and ERK in both ALK-transfected cell lines and

Figure 4. Cell invasion promoted by ALK mutants. A, in vitro invasion assay of NIH3T3 cells with various transfections. Cells transfected with Flag-tagged vector, wild-type ALK (wt-ALK), and each construct of the indicated ALK mutants. Cell invasion assay was conducted as described in Materials and Methods. Shown are the cells that invaded on the Matrigel matrix-coated polyethylene terephthalate (PET) membrane after removal of the noninvasive cells. B, number of invasive cells with the indicated transfections. Results of each column represent the mean ± SD of the numbers of invasive cells from 3 independent experiments.
ATC tumor tissues. This is not surprising, as tyrosine kinases, particularly RTK, typically activate these 2 pathways. Interestingly, in neuroblastoma, ALK mutations were also shown to be coupled to PI3K/Akt and MAP kinase signalings (8, 9). Thus, it seems that coupling to these pathways is a common mechanism involved in the oncogenic role of genetic alterations of the ALK gene in human cancer. The PI3K/Akt and MAP kinase pathways play a fundamental role in thyroid tumorigenesis (25, 26). Dual activation of the 2 pathways driven by genetic alterations is a fundamental mechanism for the pathogenesis of ATC (19, 27). ALK mutations discovered in the present study add a novel set of genetic alterations that contribute to this mechanism.

Development of inhibitors targeting ALK is an exciting current research area as a novel therapeutic strategy for neuroblastomas harboring ALK mutations, ALCI, NPM1-ALK, NSCLC harboring EML4-ALK, and IMT harboring TMP3/4-ALK and RANBP2-ALK fusion genes (1, 2). It has been recently reported that most of the patients with NSCLC harboring EML4-ALK responded effectively to the treatment with an anti-ALK agent, crizotinib (28), which has also been interesting to examine the effects of this ALK inhibitor on harboring recently reported that most of the patients with NSCLC harboring ALK mutations respond effectively to the treatment. This will not only test the in vivo animal tumor studies. This will not only test the dependence of cells or tumors on these ALK mutants but also test the therapeutic potentials of targeting these novel ALK mutations in human cancers, particularly ATC. It is therefore highly attractive to speculate that the subset of patients with ATC harboring ALK mutations may be similarly and effectively treated with this and other ALK inhibitors.

In summary, in the present study, we report novel ALK mutations in ATC with a comparable prevalence and oncogenic power to those in neuroblastomas. This is the first report on ALK mutations in nonneuroblastoma human cancers. These are gain-of-function mutations that cause dual activation of the PI3K/Akt and MAP kinase pathways in ATC. They may represent a novel therapeutic target in ATC for the recently developed ALK inhibitors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


27. Xing M. Genetic alterations in the phosphatidylinositol-3 kinase/Akt pathway in thyroid cancer. Thyroid 2010;20:697–706.


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