Unique Clinicopathologic Features Characterize *ALK*-Rearranged Lung Adenocarcinoma in the Western Population

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Abstract

Purpose: The anaplastic large cell kinase gene (*ALK*) is rearranged in ~5% of lung adenocarcinomas within the Asian population. We evaluated the incidence and the characteristics of *ALK*-rearranged lung adenocarcinomas within the western population and the optimal diagnostic modality to detect *ALK* rearrangements in routine clinical practice.

Experimental Design: We tested 358 lung adenocarcinomas from three institutions for *ALK* rearrangements by fluorescent *in situ* hybridization (FISH) and immunohistochemistry with and without tyramide amplification. The clinicopathologic characteristics of tumors with and without *ALK* rearrangements were compared.

Results: We identified 20 (5.6%) lung adenocarcinomas with *ALK* rearrangements within our cohort of western patients. *ALK* rearrangement was associated with younger age ($P = 0.0002$), never smoking ($P < 0.0001$), advanced clinical stage ($P = 0.0001$), and a solid histology with signet-ring cells ($P < 0.0001$). *ALK* rearrangement was identified by FISH in 95% of cases and immunohistochemistry with and without tyramide amplification in 80% and 40% of cases, respectively, but neither FISH nor immunohistochemistry alone detected all cases with *ALK* rearrangement on initial screening. None of the *ALK*-rearranged tumors harbored coexisting *EGFR* mutations.

Conclusions: Lung adenocarcinomas with *ALK* rearrangements are uncommon in the western population and represent a distinct entity of carcinomas with unique characteristics. For suspected cases, dual diagnostic testing, with FISH and immunohistochemistry, should be considered to accurately identify lung adenocarcinomas with *ALK* rearrangement. (Clin Cancer Res 2009;15(16):5216–23)

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Lung cancer is the leading cause of death from cancer in both men and women (1). Despite advances in treatment, the 5-year overall survival rate is ~15.7% (2). Non–small cell lung cancer (NSCLC) accounts for ~85% of lung cancer cases (3, 4). Currently, pathologic stage is the most important system to predict survival in patients with NSCLC and to define groups with similar treatment strategies (5, 6). However, the discovery of novel molecular alterations may help identify therapeutic targets that are more effective and with fewer side effects than current treatments.

The current classification of lung adenocarcinoma by the WHO recognizes several distinct morphologic subtypes of adenocarcinoma: papillary, acinar, solid, and bronchioloalveolar (7). However, the majority of lung adenocarcinomas exhibit combinations of morphologic patterns and are categorized as mixed subtype (7–9). Although the biological basis for the histologic subtypes remains an area of active investigation (9), there is evidence that some subtypes may be associated with specific molecular alterations (9–14) or a better outcome (15–17).

Anaplastic large cell kinase gene (*ALK*) was originally identified through cloning of the t(2;5) (p23;q35) translocation found in a subset of anaplastic large cell lymphomas (ALCL), a tumor

Translational Relevance

With the recent discovery that a subset of lung adenocarcinomas derived from the Asian populations harbor anaplastic large cell kinase gene (*ALK*) gene rearrangements, we sought the characteristics of tumors with this genetic abnormality within a large cohort of western patients from three institutions. We show that lung adenocarcinomas with *ALK* rearrangements are very rare among patients amenable to surgical resection (prevalence 0.45%) but significantly higher among young nonsmoking patients with advanced clinical stage (prevalence 15%). The majority of the *ALK*-rearranged lung adenocarcinomas we identified have unique histopathologic features characterized by solid growth and signet-ring cell morphology. Detection of *ALK* rearrangements in lung adenocarcinomas by either fluorescent *in situ* hybridization or immunohistochemical assays poses technical and interpretive challenges and suggests dual testing to ensure accurate diagnosis. These findings show that *ALK*-rearranged lung adenocarcinomas are rare and have distinct clinicopathologic characteristics that can provide guidance to health-care professionals for considering the optimal treatment options.

of T-cell lineage (18, 19). *ALK* encodes a tyrosine kinase receptor that is normally expressed only in select neuronal cell types. In *ALK*-rearranged ALCLs, the intracytoplasmic portion of *ALK* is fused to the NH_2-terminal portion of nucleophosmin resulting in a chimeric protein with constitutive kinase activity. Several other balanced translocations involving *ALK* have been discovered in ALCLs; however, the various resulting chimeric proteins all retain the *ALK* kinase domain (20). The importance of the kinase activity is exemplified by *ALK*-rearranged ALCL cell lines, which are dependent on ALK enzymatic activity for growth and survival.

Recently, *ALK* rearrangements were identified in rare NSCLC cell lines and in isolated primary adenocarcinomas from Japanese and Chinese populations (21, 22). The majority of the *ALK* rearrangements within NSCLCs result from an interstitial deletion and inversion in chromosome 2p and result in the *EML4-ALK* fusion gene product (21, 22). *EML4* encodes echinoderm microtubule-associated protein-like 4, a protein that may function in microtubule assembly. Transgenic mice expressing EML4-ALK within the lung epithelium develop numerous tumors, thereby confirming the oncogenic nature of the mutant protein (23). Murine tumors and human cell lines expressing EML-ALK are sensitive to inhibitors of ALK kinase activity (23, 24). Together, these data indicate that, like epidermal growth factor receptor (EGFR), ALK is an important molecular target in lung carcinoma. Thus, it will be critical to efficiently and accurately identify those lung adenocarcinomas that harbor *ALK* rearrangements in routine practice to guide the appropriate clinical therapy.

In this study, we evaluated 358 lung adenocarcinomas from three institutions and identified 20 harboring an *ALK* rearrangement. None of *ALK*-rearranged adenocarcinomas in our study showed coexistent mutations in *EGFR*. We found that *ALK*-rearranged adenocarcinomas are more likely to present in younger patients with a history of never-smoking and at higher stage relative to those without *ALK* rearrangements (*ALK* germ-line). The majority of *ALK*-rearranged adenocarcinomas had a distinct histology represented by solid tumor growth and frequent signet-ring cells with abundant intracellular mucin. Finally, we illustrate that the routine screening for *ALK* rearrangements in lung adenocarcinomas is challenging and suggest that screening by both fluorescent *in situ* hybridization (FISH) and immunohistochemistry will accurately identify patients with this uncommon molecular abnormality.

Materials and Methods

Case selection. Three hundred fifty-eight cases of lung adenocarcinoma from three participating institutions were examined (Supplementary Table S1). The first group consists of 116 consecutive lung adenocarcinoma patients treated with surgery with or without postoperative adjuvant chemotherapy at Brigham & Women's Hospital (BWH) between March 1997 and December 1999 (Supplementary Table S1). Each resection specimen was evaluated with standard pathologic methods as described in the Surgical Pathology Dissection Manual of the Department of Pathology (25). The cases were reviewed and staged according to the sixth edition of the American Joint Committee on Cancer manual (5). Patients were selected for study with the following inclusion criteria: lung adenocarcinoma; first treatment by surgery alone, with or without postoperative adjuvant treatment; no other malignant tumors in 5 years before the diagnosis of lung adenocarcinoma, except squamous cell or basal cell carcinoma of the skin or carcinoma *in situ* of the uterine cervix; and no deaths in the perioperative period <30 days after surgery.

The second group consists of 111 consecutive surgically resected adenocarcinomas from the University of Pittsburgh (UP) that were subjected to *EGFR* and *KRAS* mutational analysis between February 2005 and July 2007 using the same inclusion criteria as for the BWH group.

The third group consists of 131 patients with lung adenocarcinoma who were referred for treatment at Massachusetts General Hospital (MGH). These patients were screened for an *ALK* translocation by FISH as part of routine clinical care along with *EGFR* mutation analysis. The cohort was enriched for clinical characteristics associated with EGFR mutation including young age and never or light smoking history (Supplementary Table S1). The pathologic characteristics were tumor size, pathologic stage, tumor, and lymph node status (Supplementary Table S1). Protocol reviews and approvals were obtained from the Dana-Farber Harvard Cancer Center, UP, and MGH institutional review boards.

Histologic analysis. For each case, multiple slides corresponding to whole tissue sections were reviewed simultaneously by at least two pathologists and classified according to WHO criteria (7). In mixed-subtype adenocarcinomas, we assessed the percentage of each histologic pattern (acinar, papillary, solid, and bronchioloalveolar) in 10% increments and recorded the predominant histologic pattern (9). Cases with differences between the two reviewers were reevaluated and a consensus interpretation was rendered. Poorly differentiated adenocarcinomas with a pure solid growth pattern were confirmed by a positive mucicarmine stain and negative p63 immunohistochemical stain.

Tissue microarrays used for FISH and immunohistochemistry analyses were constructed from a representative block from formalin-fixed, paraffin-embedded archival tissue specimens from the BWH and UP cohorts as described previously (8). Three tumor samples (0.6 mm cores) from each case were included into paraffin-recipient blocks using a manual arrayer (Beecher Instruments). The three cored areas on each donor block were randomly selected from different parts of the tumor tissue based on a histologic characterization of the H&E-stained slide.

Table 1. Clinicopathologic characteristics of *ALK*-rearranged and *ALK* germ-line NSCLC

Characteristics	*ALK* rearrangement	*ALK* germ-line	*P*
No. cases	20 (6)	338 (94)	
Sex, *n* (%)			
Male	11 (55)	127 (38)	0.16
Female	9 (45)	211 (62)	
Age (y), median (range)	51 (29-76)	66 (29-90)	0.0002
Smoking status, *n* (%)			
Never smoker	14 (70)	71 (21)	<0.0001
Smoker	6 (30)	237 (70)	
Unknown		30 (9)	
Tumor status,* *n* (%)			
pT_1	4 (20)	103 (30)	0.15
pT_2	2 (10)	119 (35)	
pT_3	0 (0)	10 (3)	
pT_4	0 (0)	37 (11)	
Not evaluated	14 (70)	69 (20)	
Lymph node status,* *n* (%)			
Negative	4 (20)	176 (52)	0.13
Positive	6 (30)	81 (24)	
Not evaluated	10 (50)	81 (24)	
Stage,* *n* (%)			
I	4 (15)	165 (49)	0.0001
II	0 (0)	29 (9)	
III	0 (0)	67 (20)	
IV	16 (85)	77 (23)	

NOTE: Due to rounding not all percentages total 100.
*Tumor and node status were based on pathology evaluation (pStage). Stage was based on both radiology and pathology evaluation (cStage).

Immunohistochemistry. Immunohistochemistry was done on 4-μm-thick, formalin-fixed, paraffin-embedded tissue sections as described (26). Briefly, slides were deparaffinized and pretreated with 1 mmol/L EDTA (pH 8.0; Zymed) and heat-mediated antigen retrieval in a steam pressure cooker (Decloaking Chamber; BioCare Medical). All further steps were done at room temperature in a hydrated chamber. Endogenous peroxidase activity was quenched with Peroxidase Block (DAKO USA) for 5 min and slides were preincubated in 20% normal goat serum in 50 mmol/L Tris-HCl (pH 7.4). Mouse monoclonal anti-human CD246, *ALK* culture supernatant (clone ALK1; DAKO USA), was applied at 1:2 in DAKO diluent overnight (for unamplified detection), washed in Tris-HCl, and detected with horseradish peroxidase–conjugated anti-mouse Envision+ kit (DAKO).

Alternatively, for tyramide amplification, anti-CD246 culture supernatant (DAKO) was applied at 1:10 in DAKO diluent for 1 h and then further amplified using the catalytic activity of horseradish peroxidase to bind biotin-labeled tyramide (Perkin-Elmer) diluted 1:250 for 10 min (26, 27). Chromogenic visualization of amplified slides was accomplished through the use of horseradish peroxidase–conjugated streptavidin (DAKO), followed by DAB. All slides were then counterstained with hematoxylin.

All cases were evaluated and scored as either positive or negative for ALK expression by two pathologists (S.J.R. and L.R.C.).

FISH. FISH was done on formalin-fixed, paraffin-embedded tumor tissues using a break-apart probe to the *ALK* gene (Vysis LSI *ALK* Dual Color, Break Apart Rearrangement Probe; Abbott Molecular) per manufacturer's instructions. FISH-positive cases were defined as >15% split signals in tumor cells.

Statistical analysis. Fisher's exact test was used to compare categorical data for clinicopathologic characteristics between *ALK*-rearranged and *ALK* germ-line subgroups, and Wilcoxon rank-sum test was used to compare differences in the distribution of continuous data. All *P* values are based on a two-sided hypothesis test.

Results

ALK *rearrangements are rare among surgically resected lung adenocarcinomas.* The vast majority of *ALK*-rearranged NSCLCs identified in the Asian population to date have been adenocarcinomas, with rare cases exhibiting squamous components (21, 28). To establish the prevalence of *ALK*-rearranged adenocarcinomas in the western population, we screened 227 consecutive, unselected patients with lung adenocarcinoma from the archives of two institutions in the United States (BWH and UP Hospital). The two cohorts showed similar clinicopathologic characteristics (Supplementary Table S1). The average patient age and proportion with a positive smoking history in these groups is comparable with that observed nationally (compared with Surveillance, Epidemiology and End Results statistics; data not shown; refs. 1, 2). However, because both cohorts consisted of surgically resected tumor specimens, these cases were also enriched for low stage (74% stages I and II) without involvement of regional lymph nodes (66% node-negative).

Each case was tested for *ALK* rearrangements by immunohistochemistry and FISH. Both techniques independently identified the same single positive case from the BWH group and no positive cases from the UP group (1 of 227 cases; 0.45%). The patient with an *ALK* rearranged adenocarcinoma was a 62-year-old woman with a history of 1.5 pack-year smoking.

Table 2. Histologic characteristics of lung adenocarcinomas with *ALK* rearrangement

Characteristics	Total samples	*ALK* rearrangement	*ALK* germ-line	*P*
Signet-ring cells in tumor, *n* (%)	342	17 (100)	325 (100)	<0.0001
None	295	3 (18)	292 (90)	
≤10%	21	2 (12)	19 (6)	
>10%	26	12 (71)	14 (4)	
Dominant histologic pattern, *n* (%)*	326	16 (100)	310 (100)	0.11
Bronchioloalveolar	22	1 (6)	21 (7)	
Acinar	124	4 (25)	120 (39)	
Papillary	46	0 (0)	46 (15)	
Solid	134	11 (69)	123 (40)	
Solid pattern and >10% signet-ring cells, *n* (%)*		9 (56)	9 (3)	<0.0001

NOTE: Due to rounding not all percentages total 100.
*Tumor architecture was not evaluated in 16 cases where the diagnosis was made only by cytology.

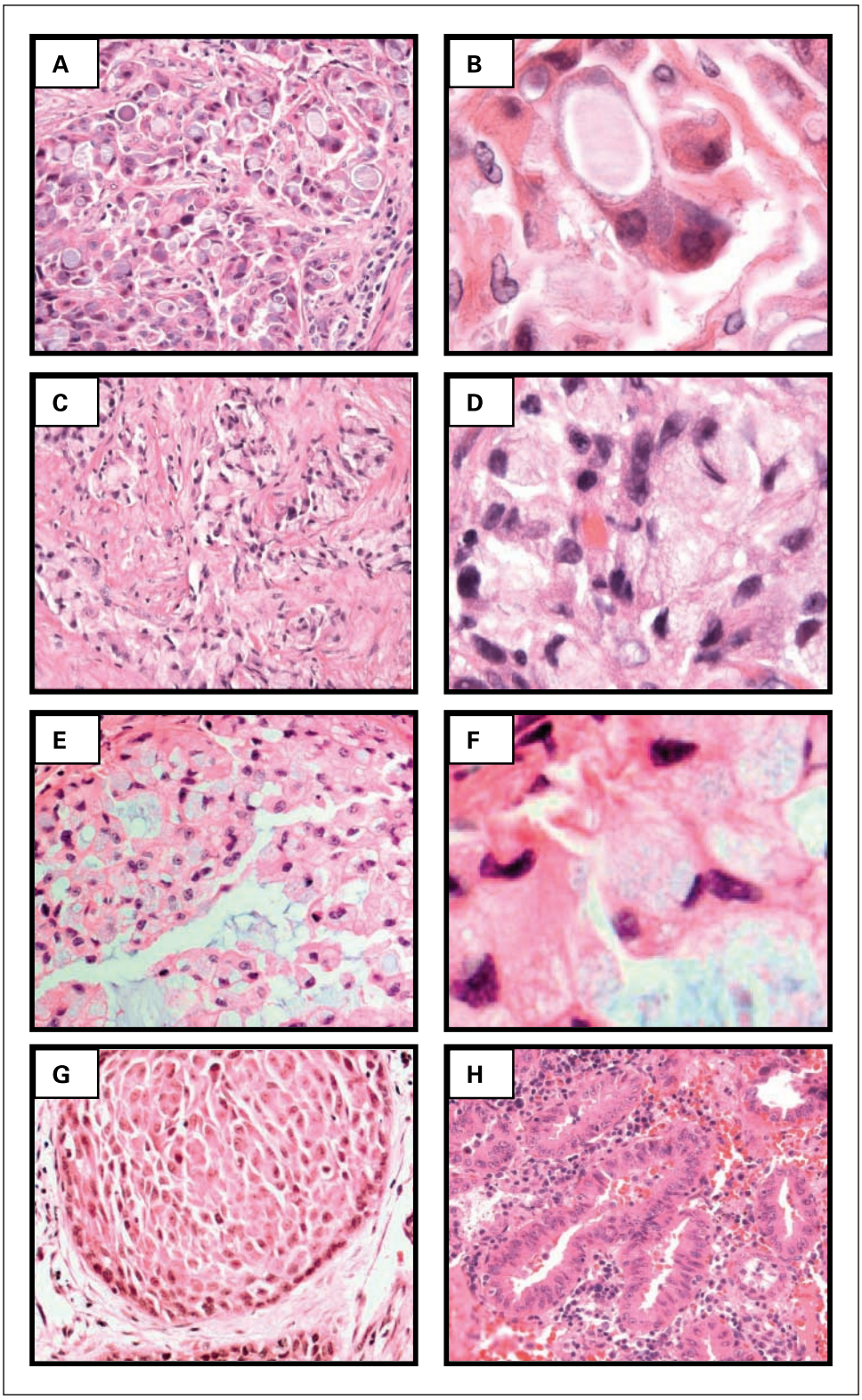

Fig. 1. *ALK*-rearranged NSCLC have distinct histologic characteristics. Representative examples of *ALK*-rearranged NSCLC showing the distinct solid growth pattern (*A*, *C*, and *E*; ×400 original magnification) and >10% signet-ring cells (*B*, *D*, and *F*; ×1,000 original magnification). An *ALK*-rearranged adenocarcinoma with focal squamous differentiation (*G*; ×400 original magnification) and an *ALK*-rearranged adenocarcinoma with the typical acinar histologic features (*H*; ×400 original magnification).

ALK ***rearrangements are enriched among adenocarcinomas from a selected patient population.*** We next examined a group of cases collected and analyzed simultaneously for *EGFR* mutations as part of a clinical trial at a third institution based on case referral to a thoracic oncology practice (MGH; ref. 29). This group consists of patients (predominantly stage IV 64% versus 4% from BWH and UP; Supplementary Table S1) as expected from an oncology clinic population. In addition, the MGH patients were younger at diagnosis (mean age, 63 years) and more likely to have had a history of never-smoking (40% for MGH versus 15% for BWH and UP).

Among 131 MGH cases examined by FISH analysis, 19 (14%) cases were positive for an *ALK* rearrangement. A subset of these cases was confirmed to express ALK protein by immunohistochemistry (see below; Table 3). The difference in the incidence of *ALK*-rearranged tumors between the combined unselected BWH and UP cohorts and the MGH cohort is significant ($P < 0.001$) and suggests differences between the selection criteria.

Characteristics of lung adenocarcinomas with* ALK *rearrangements. The clinicopathologic characteristics of patients with tumors with *ALK* rearrangement are illustrated in Table 1. Compared with national statistics (Surveillance, Epidemiology and End Results database; data not shown) and with the patients with *ALK* germ-line (Table 1), patients with lung adenocarcinomas with an *ALK* rearrangement are younger at diagnosis and have a history of never-smoking. Strikingly, *ALK*-rearranged tumors presented at higher stage, most commonly at stage IV, relative to *ALK* germ-line tumors (Table 1).

The characteristics of NSCLC with *ALK* rearrangements in our study are very similar to those for NSCLC harboring mutations in *EGFR* (30, 31). Specifically, patients with NSCLC with *ALK* rearrangements are young patients without a significant smoking history. We found that the MGH cohort included 28 (21%) cases of NSCLC with *EGFR* mutations. Of the 19 MGH *ALK*-rearranged NSCLCs, we found that none showed coexisting *EGFR* mutations. The mutual exclusivity of *ALK* rearrangements and *EGFR* mutations is significant ($P = 0.013$; 0 of 19 in *ALK*-rearranged versus 28 of 112 in *ALK* germ-line). The details of this association and its significance are reported elsewhere (29). Of note, the MGH group includes a percentage of *EGFR* mutant cases comparable with that found within the UP cohort (22 cases, 20%; data not shown).

Morphologic profile of* ALK*-rearranged adenocarcinomas. The morphologic characteristics of *ALK*-rearranged tumors are illustrated in Table 2. Among cases with sufficient tissue for adequate analysis, the majority (11 of 16 cases, 69%) showed tumor cells with a solid or sheet-like pattern easily distinguishable from the acinar, papillary, or bronchioloalveolar patterns. Of the remaining five cases, four showed a

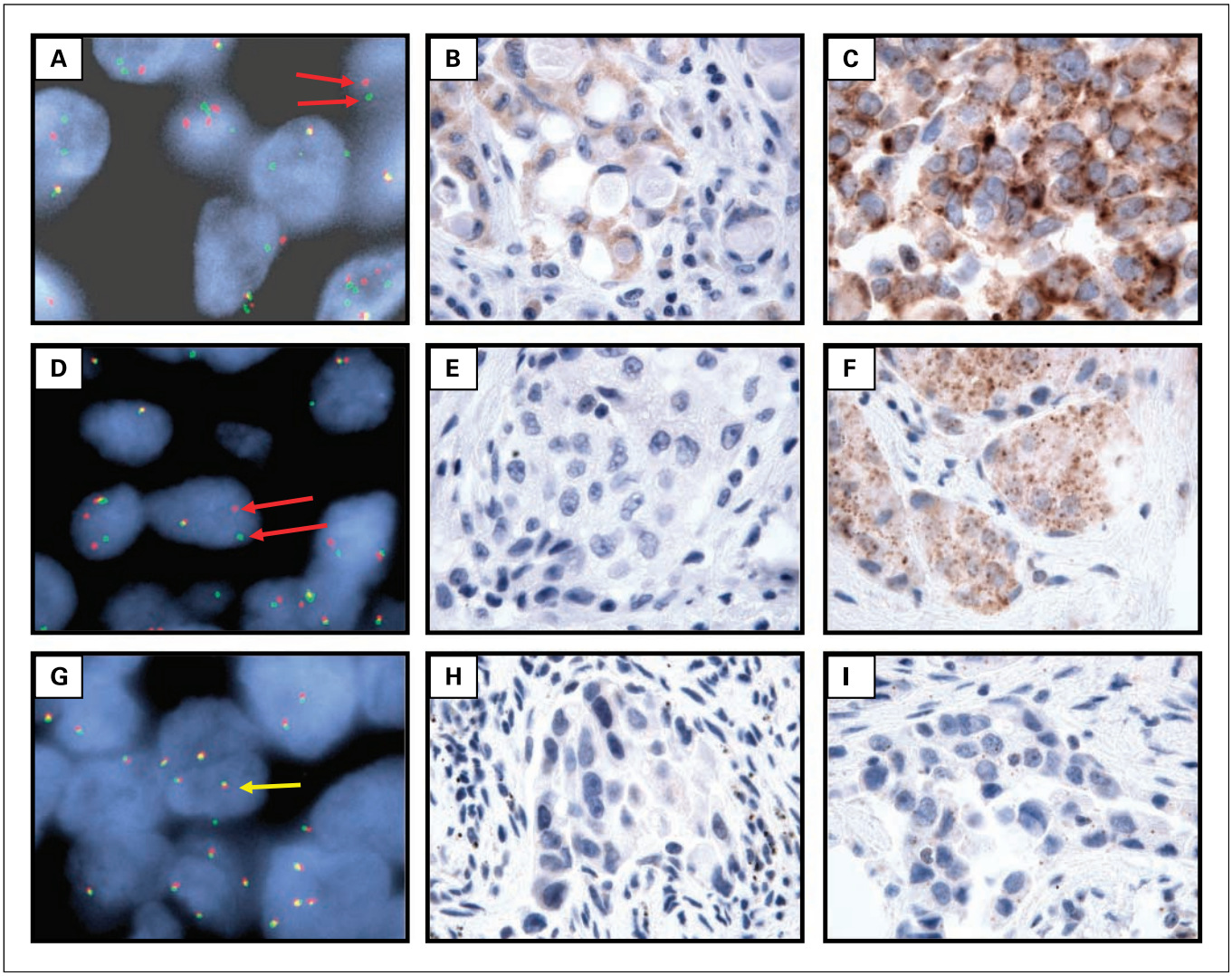

Fig. 2. Standard diagnostic techniques are not optimal for the routine detection of *ALK*-rearranged NSCLC. Representative *ALK*-rearranged (*A-F*) and *ALK* germ-line (*G-I*) tumors analyzed by FISH using probes flanking the *ALK* gene (*A, D,* and *G*), standard immunohistochemical staining for ALK protein (*B, E,* and *H*), and tyramide-amplified immunohistochemical staining for ALK protein (*C, F,* and *I*). *Red arrows,* split FISH probes characteristic of an *ALK* rearrangement; *yellow arrow,* nonsplit FISH probes characteristic of *ALK* germ-line.

Table 3. Correlation of FISH and immunohistochemistry results

Immunohistochemistry	FISH test result	
	ALK rearrangement	*ALK* germ-line
Without tyramide amplification		
Positive	4	0
Negative	6	233
With tyramide amplification		
Positive	8	0
Negative	2	233

NOTE: Ten of 20 cases with *ALK* rearrangement by FISH had available tissue for immunohistochemistry analysis.

predominantly acinar pattern and one showed a predominantly bronchioloalveolar pattern.

Among tumors without *ALK* rearrangements only a minority (123 of 310 cases, 40%) showed a solid growth as the predominant pattern, which is significantly different from *ALK*-rearranged tumors ($P = 0.034$). The remaining cases showed a variety of histologic patterns predominated by acinar, papillary, or bronchioloalveolar morphology (data not shown).

Even more striking were the cytologic features of tumors harboring ALK rearrangements. *ALK*-rearranged tumors (82%) and showed, at least focally, tumor cells with abundant intracellular mucin and small, marginalized nuclei (Fig. 1; Table 2). In a majority of cases (71%), cells with abundant intracellular mucin comprised >10% of the overall tumor cellularity. In contrast, the majority of *ALK* germ-line tumors (90%) showed no tumor cells with this morphology. This distinct cytologic characteristic, unusual for lung carcinoma, is reminiscent of the "signet-ring" cells more commonly seen in gastric, colonic, and breast adenocarcinomas.

The majority of ALK-rearranged tumors (56%) show a solid growth pattern with >10% signet-ring cells (Fig. 1; Table 2). In contrast, only a small minority of ALK-germ-line tumors (3%) show a solid growth pattern with >10% signet ring cells ($P < 0.0001$). Importantly, we identified two *ALK*-rearranged tumors with a predominantly acinar growth pattern and no signet-ring cells. The morphology of these two cases is, to us, indistinguishable from tumors without *ALK* rearrangement (data not shown). Intriguingly, we also observed a single *ALK*-rearranged tumor with morphologic evidence for focal squamous differentiation (Fig. 1G). This sample arose as a recurrence of a primary tumor originally classified as an adenocarcinoma. Unfortunately, material from the original tumor was unavailable for complete pathologic rereview.

Detection of* ALK *rearrangements in lung adenocarcinoma. Among cases with an *ALK* rearrangement verified by FISH, only a subset (4 of 10 cases, 40%) showed detectable ALK protein expression by standard immunohistochemistry techniques with monoclonal antibody clone ALK1 (DAKO; Fig. 2; Table 3). In contrast, ALK protein was readily detectable in an *ALK*-rearranged ALCL (data not shown). For the additional *ALK*-rearranged lung adenocarcinomas (10 cases), there was not sufficient tissue available to us for immunohistochemistry analysis (missing cases are a combination of cytology and core biopsy specimens and cases referred for FISH analysis only). In an attempt to increase the sensitivity of our immunohistochemistry assay, we explored a wide variety of antibody concentrations and antigen retrieval methods (data not shown). Of the methods tested, we found that diluting the primary antibody and secondarily amplifying the signal with a tyramide-biotin–based protocol resulted in the highest sensitivity for detecting *ALK* protein without excessive loss of specificity (Fig. 2; refs. 26, 27). With this method, we were able to detect *ALK* protein in 8 of 10 FISH-positive cases (sensitivity = 80%; Table 3). Because of the modest sensitivity of tyramide-amplified immunohistochemistry for *ALK*-rearranged adenocarcinomas, we considered FISH analysis as a more reliable diagnostic test. However, during the course of the study, we discovered one case originally classified as *ALK* negative by FISH but consistently positive for ALK protein by both unamplified and tyramide-biotin–based immunohistochemistry (data not shown). This tumor was further examined because it showed abundant signet-ring cells and a solid growth pattern characteristic of *ALK*-rearranged tumors. Subsequent reassessment of the FISH analysis from this patient confirmed that the case was indeed *ALK*-rearranged. This case shows that the FISH finding for rearrangement of the *ALK* locus can manifest as a modest, split signal that is subtle and can be misinterpreted as normal. Thus, immunohistochemistry staining for ALK serves as a useful ancillary test for detecting *ALK* rearrangements despite a lower overall sensitivity than FISH analysis.

Discussion

The *EML4-ALK* fusion was recently identified as a novel molecular abnormality in 5% of lung adenocarcinomas within Asian populations (21, 22). The clinical and histopathologic characteristics of lung cancers with the *EML4-ALK* fusion gene and the relationship with ALK protein expression have not been established in the western population in detail (28, 32).

In this study, we assessed the prevalence of *ALK* rearrangement in a group of patients from three institutions and found a total of 20 *ALK*-rearranged adenocarcinomas. We found that the incidence of *ALK*-rearranged tumors in our surgically resected specimens from unselected groups of patients is 0.45%, which is lower that that reported for the Asian cohorts also consisting of surgically resected cases (28, 33). Importantly, however, the incidence of *ALK*-rearranged tumors in biopsy specimens from the selected cohort of patients (generally younger, with a history of never-smoking, and with high-stage disease) is 14%, much higher than that of the surgical cohorts (generally older, with a smoking history, and early-stage disease). Indeed, we found that young age, history of never-smoking, and high-stage disease are all statistically different between patients with *ALK*-rearranged and *ALK* germ-line lung adenocarcinomas (Table 1). Despite this general difference, we did identify cases of older patients (oldest age 76 years) with a smoking history with *ALK*-rearranged tumors. Therefore, the clinical characteristics alone are not sufficient to predict the genetic aberration with absolute certainty.

Given that a younger age at presentation and a lack of smoking history are characteristic of patients with tumors harboring *EGFR* mutations, we screened our group of *ALK*-rearranged tumors for coexisting *EGFR*. Within the group of 20 *ALK*-rearranged tumors, we found no cases with coexisting mutations. Furthermore, no patient with an *ALK*-rearranged tumor treated with an EGFR inhibitor showed clinical response.[6] These findings suggest that

ALK rearrangements may be mutually exclusive with *EGFR* mutations and will require a distinct group of tyrosine kinase inhibitors that specifically target ALK enzymatic activity.

One of the striking findings of this study is the unique histopathologic characteristics of the *ALK*-rearranged NSCLC in our cohort. The tumors in a majority of cases (56%) showed a solid pattern of growth and a significant (≥10%) component of signet-ring cells. Although this cytologic pattern is a well-recognized variant of adenocarcinomas of the stomach, colon, and breast, it is reported to be only rarely observed in lung adenocarcinoma (34–37). In agreement with published findings, we found that, within the same group of patients (MGH), a solid growth pattern with signet-ring cells was found in only a minority of cases (6 of 113 cases, 5%) of *ALK* germ-line NSCLC.

Among the cases without the unique histologic pattern, that is, a solid growth with signet-ring cells, we did not find overt features distinguishing *ALK*-rearranged from nonrearranged NSCLC. These cases included lung adenocarcinomas, mixed subtype, with acinar, papillary, and bronchioloalveolar patterns. Interestingly, one case showed the coexistence of adenocarcinoma and focal squamous cell carcinoma with *ALK* rearrangement also present in the squamous cell component. The combined squamous and glandular morphology has been reported in rare *ALK*-rearranged cases (38). Despite the distinct histologic pattern found in the majority of *ALK*-rearranged NSCLC, our finding that *ALK* rearrangements can occur in adenocarcinomas with a variety of histologic patterns suggests that, in isolation, histologic characteristics alone are not sufficient for selecting individual cases for further testing for an *ALK* rearrangement. However, if prominent signet ring cells are observed in a lung adenocarcinoma, especially a nonsmoker, ALK testing will yield a high rate of positivity.

Intriguingly, our histopathologic findings differ from those reported for a group of 11 *ALK*-rearranged NSCLCs from Japan, in which either acinar or papillary histologic patterns were the predominant histology (33). Whether regional or ethnic differences account for this discrepancy, whether it is attributed to the difference in clinical presentation (early stages versus stage IV), or whether it is a reflection of the very small sample size (11 cases) in this prior report compared with ours remains to be determined.

With the emergence of molecularly targeted therapies, it is reasonable to assume that many, and perhaps all, NSCLCs will be screened for *ALK* rearrangements. Given the tremendous number of cases of NSCLC and the relative rarity of this genetic alteration, any screening procedure will need to be highly sensitive, specific, reproducible, and cost-effective. Our experience suggests the need for more effective diagnostic procedures than those that currently exist. We screened all of our cases for an *ALK* rearrangement by FISH using a commercially available set of probes. However, given that the *ALK* rearrangements in lung cancer involve target loci relatively close to one another and on the same chromosome, interpretation of a positive rearrangement is more difficult than in other *ALK*-rearranged tumors, such as ALCL or inflammatory myofibroblastic tumor, where the target loci are on different chromosomes. One possible solution is to add an additional fluorescent probe targeting the deleted portion of chromosome 2 in *ALK*-rearranged NSCLC. This type of three-probe combination has been successfully developed for detecting the *TMPRSS2-ERG* rearrangement in prostate cancer (39, 40).

For the majority of cases, we also screened for *ALK* rearrangement by immunohistochemistry. Using a commonly used commercially available antibody for *ALK* and standard techniques, we found immunohistochemistry to be specific but not sensitive for detecting *ALK* rearrangements among FISH-confirmed, *ALK*-rearranged cases (considered as the "gold standard" for this analysis). Furthermore, the level of ALK protein expression was significantly lower in the *ALK*-rearranged NSCLC cases than in the *ALK*-rearranged ALCLs that we used as positive controls. In attempts to improve detection of ALK, we found that tyramide signal amplification, a technique developed several years ago, was sufficient to increase the sensitivity without decreasing specificity for detecting *ALK* (27). This modified method could improve the detection of ALK protein from 40% to 80% among FISH-confirmed, *ALK*-rearranged cases tested, but the sensitivity of 80% may not be sufficient to justify the ALK immunohistochemistry to be a sole modality for detecting *ALK* rearrangements. Additional antibodies recognizing *ALK* are commercially available, and although we have not tested all of them, it has been reported that none are sufficient to detect *ALK* in all cases of *ALK*-rearranged adenocarcinomas (38, 41). It is notable that the *ALK* antibody used in this study is the standard reagent used by pathology laboratories worldwide and one used in a prior study of a cohort of Japanese patients with lung adenocarcinoma (33). Although our results may suggest that FISH testing is the preferred method of screening and considered to be the "gold standard" in our evaluation of immunohistochemistry, we encountered one case that was originally interpreted as non-*ALK*-rearranged by FISH, but the subsequent FISH analysis prompted by concurrent, positive ALK protein expression by immunohistochemistry and the characteristic tumor morphology revealed *ALK* rearrangement. Therefore, given the current limitations of both FISH and immunohistochemistry, we believe that both methods should be employed to facilitate the detection of *ALK*-rearranged lung adenocarcinomas. Given that immunohistochemistry is a routine methodology in most pathology laboratories around the world, better antibodies recognizing ALK are likely needed to facilitate the large-scale screening of NSCLC in the future. An alternative and complementary approach in screening NSCLC is through a multiplexed RT-PCR–based analysis to detect the various ALK-fusion transcripts. This approach is currently under investigation in our laboratory as well as others (38). It is be important to determine whether RT-PCR–based screening can detect ALK-rearranged tumors missed by both immunohistochemistry and FISH.

Since their discovery in ALCL, *ALK* rearrangements or mutations have been identified in inflammatory myofibroblastic tumors, a subset of diffuse large B-cell lymphomas (42–45), and a subset of neuroblastomas (46, 47). These findings have prompted the development of inhibitors of ALK enzymatic activity for therapeutic use (48). With the discovery of *ALK* rearrangements in NSLCL (21), the number of potential patients who might benefit from such drugs has increased dramatically. Importantly, our findings indicate that *ALK*-rearranged NSCLC comprise a unique subgroup of adenocarcinoma with distinct clinicopathologic characteristics. Compared with non-*ALK*-rearranged NSCLC, this group is significantly enriched for young, nonsmoking patients with tumors that show distinct solid growth pattern and signet-ring cell histology. These

[6] Data reported by Shaw et al., in press.

patients typically present in late stages not amenable to surgical resection and are therefore candidates for aggressive and novel chemotherapeutic regimens that target the mutated ALK protein. Thus, it is important to raise a suspicion about the possibility of *ALK* rearrangements based on the unique clinicopathologic characteristics and identify *ALK*-rearranged tumors by dual testing with immunohistochemistry and FISH.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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