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Histologic Features of ALK-Expressing Adenocarciomas of the Lung

Purpose: This study was designed to define the specific histologic features of anaplastic lymphoma kinase (ALK)-expressing pulmonary adenocarcinoma. **Materials and Methods:** Of the 580 pulmonary adenocarcinomas diagnosed between March 2010 and February 2011, immunohistochemical staining for ALK was performed in 269 cases showing any suspicious histologic features in previous reports. The subtype according to the World Health Organization classification and the characteristic histologic features were re-evaluated in ALK-expressing cases. **Results:** A total of 46 cases (7.9% of the 580 adenocarcinomas, 17.1% of the 269 studied cases) were positive for ALK. Among the 46 cases showing ALK positivity, 35 cases (76%) showed intra- and/or extra-cytoplasmic mucin. The most well-known characteristic finding associated with ALK, signet ring cells, was found in 18 cases (39.1%). Cribriform pattern with extracytoplasmic mucin was identified in five cases. In six cases, all three features were found. On the other hand, there were three other cases that did not show any of the aforementioned histologic features. In 12 lobectomy specimens, the most common histologic pattern was a solid pattern (five cases, 41.6%). **Conclusion:** Intra- and/or extra-cytoplasmic mucin, including signet ring cell appearance and a cribriform pattern with extracytoplasmic mucin, are characteristic features of ALK-expressing non-small cell lung cancer. **(J Lung Cancer 2011;10(1):32−36)**

Key Words: Non-small cell lung carcinoma, Anaplastic lymphoma kinase, Immunohistochemistry, Adenocarcinoma

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INTRODUCTION

Lung cancer is the leading cause of morbidity and mortality worldwide, despite evolving medical technology (1). Recently, the discovery of molecular alterations associated with tyrosine kinase receptors such as epidermal growth factor receptor (EGFR) has improved the prognosis in lung cancer (2,3). The fusion of the anaplastic lymphoma kinase (ALK) with the echinoderm microtubule-associated protein-like 4 (EML4) was identified in 2007 in Japanese non-small cell lung cancer (NSCLC) (4). The ALK inhibitor is a definite candidate for the latest specific molecular targeting treatment in NSCLC (5,6).

Morphologic classification on hematoxylin and eosin-stained slides remains the gold standard for the diagnosis of lung cancer, but the detection of molecular alterations is also critical (7). ALK fluorescent in situ hybridization (FISH) is a standard method for detection of genetic alterations and ALK immunohistochemistry (IHC) correlates well with FISH results (8). A variety of morphologic features of pulmonary adenocarcinoma including solid, acinar, papillary, cribriform, mucin production, and signet ring cells have been reported in ALK-expressing lung cancer (9-14). If the specific histologic and cellular details with ALK-expressing lung cancer can be clarified, they could be powerful diagnostic findings for pathologists suspicious of underlying ALK rearrangement of a tumor.

In this study, we evaluated the histologic features of ALK-expressing pulmonary adenocarcinomas in Korean patients, with the aim of identifying specific histologic features.

MATERIALS AND METHODS

1) Patient selection

Samples from 580 Korean patients diagnosed with pulmonary adenocarcinoma at Samsung Medical Center between March 2010 and February 2011 were acquired by various procedures including biopsy, wedge resection, metastasectomy, and lobectomy. Specimens of 269 cases showed suspicious histologic patterns (9-14) and were selected as candidates for ALK-positive adenocarcinomas.

2) Histological analysis

All specimens were fixed in 10% buffered formalin. The fixed biopsy specimens were entirely embedded in paraffin, or representatively if it was a surgical specimen larger than a wedge resection. Hematoxylin and eosin (H&E)-stained slides were reviewed by two independent board-certified pathologists. Histologic classification with subtype grouping was made according to the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory So-

Table 1. Characteristics of Patients and Specimens

Age (median), yr	25～77 (52)	
Sex	Male	20 (43.5)
	Female	26 (56.5)
Procedure	Lung biopsy	22 (47.8)
	Lobectomy	12 (26.1)
	LN biopsy	10 (21.7)
	Wedge resection	1 (2.2)
	Metastasectomy	1 (2.2)

Values are presented as number (%) unless otherwise indicated.

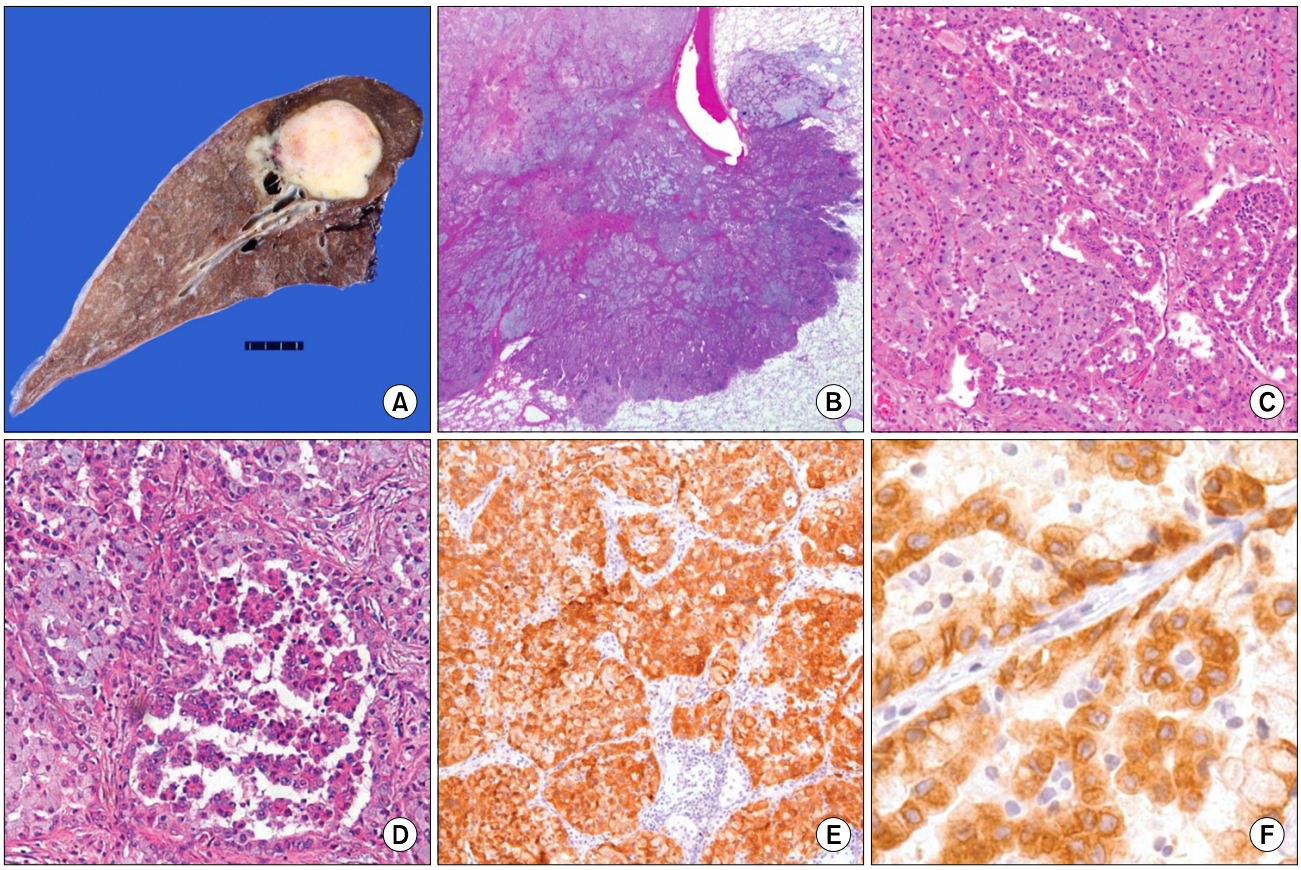

Fig. 1. Representative example of ALK-expressing pulmonary adenocarcinoma. (A) The cut surface in the lobectomy specimen shows a well-demarcated pale yellow solid mass. (B～D) The specimen is consisted of mixed histologic patterns including solid, acinar, and papillary pattern. Intra- and/or extra-cytoplasmic mucin with signet ring cells were frequently identified (hematoxylin-eosin stain, ×16, ×100, and ×200 respectively). (E, F) ALK antibody stains cytoplasm of tumor cells diffusely with slightly granular pattern (ABC method, ×100 and ×400 respectively).

ciety (IASLC/ATS/ERS) International Multidisciplinary Classification of Lung Adenocarcinoma (15) and characteristic histologic features were re-evaluated in cases showing ALK expression. The presence of intra- and/or extra-cytoplasmic mucin, cribriform pattern with extracytoplasmic mucin, and signet ring cells was checked in every case, despite the proportion of features in the entire specimen.

3) Immunohistochemical (IHC) analysis

IHC for ALK (NCL-ALK [clone 5A4], 1 : 40, Novocastra, UK) were performed using a biotin-avidin peroxidase complex method on a BOND-MAX autostainer (Leica, Wetzlar, Germany) after retrieval with ER2 solution. Samples showing diffuse strong ALK-positivity in cytoplasm were regarded as positive cases. For such samples, the medical records were reviewed for age, gender, diagnostic procedure, and results of other mutational studies.

4) Mutation analysis for EGFR and KRAS

In some cases, molecular analysis of EGFR exons 18, 19, 20 and 21 and KRAS exons 12 and 13 were performed. The extracted DNA from formalin-fixed and paraffin-embedded tissue were analyzed by direct-sequencing polymerase chain reaction in both the forward and reverse directions.

RESULTS

1) Characteristics of patients and specimens

The findings are presented in Table 1 and Fig. 1. Forty six cases (7.9% of the 580 adenocarcinomas, 17.1% of the 269 studied cases) were ALK-positive by IHC (Fig. 1E, F). The age of patients showing ALK expression ranged from 25～77 years (median, 52 years) and 56.5% of patients were female. Almost half of the specimens were obtained by lung needle biopsies. One surgical case taken from the brain was confirmed as metastatic carcinoma from lung by clinical history of the 3.4-cm-sized pulmonary mass and immunoreactivity for thyroid transcription factor-1 (TTF-1). Two cases of lobectomy underwent concurrent chemoradiation therapy or chemotherapy before surgical intervention, but both showed tumor regression of less than 10% of the entire tumor volume. In 25 cases, EGFR and K-ras mutations were studied by direct sequencing. All 25 cases revealed no EGFR or K-ras mutation in four EGFR hot spots and three KRAS hot spots.

Table 2. Pathologic Features in Immunohistochemically ALK-positive NSCLC

Dominant pattern (lobectomy cases)		
Solid		5 (41.7)
Papillary		3 (25.0)
Acinar		4 (33.3)
Characteristic features		
Intra- and/or extra-cytoplasmic mucin	(+)	35 (76.1)
	(−)	11 (23.9)
Cribriform with extracytoplasmic mucin	(+)	7 (15.2)
	(−)	39 (84.8)
Signet ring cells	(+)	18 (39.1)
	(−)	28 (60.9)

Values are presented as number (%).

2) Findings of histopathologic features

The results are presented in Table 2 and Fig. 1. When subtype or predominant pattern was evaluated in 12 lobectomy cases, the most common subtype was a solid pattern (five cases, 41.7%). But, variable histologic patterns were also evident including acinar pattern (four cases, 33.3%) and papillary pattern (three cases, 25%) (Fig. 1B～D). More than 75% of the specimens displayed intra- and/or extra-cytoplasmic mucin (Fig. 1B～D). In 23 cases (50%), only 'intra- and/or extra-cytoplasmic mucin' was identified without signet ring cells or cribriform pattern. In some cases, the amount of mucin was small, which hampered identification. The signet ring cell feature, which is the most well-known characteristic associated with ALK, was apparent in 18 cases (39.1%). A cribriform pattern with extracytoplasmic mucin was also identified in seven cases. In six cases, all three features were found. On the other hand, there were three cases that did not show any of these aforementioned histologic features; these cases represented metastasized adenocarcinoma from lung (n=1), solid pattern (n=1), and papillary pattern (n=1).

DISCUSSION AND CONCLUSION

The therapeutic efficacy of the inhibitor of an ALK tyrosine kinase is currently being evaluated in a clinical trial, and inhibition of ALK in ALK-expressing NSCLC has resulted in tumor shrinkage or stable disease in most patients (6). ALK FISH or PCR-based analyses are standard methods for detection

of genetic alteration. But, the PCR-based method is too complicated because of variable translocation of ALK and a high false-positive rate, and FISH is relatively expensive and its subtle signals are sometimes difficult to interpret (16). IHC for ALK suffers from low sensitivity despite its high specificity (17). Nonetheless, technological advances are making ALK IHC amenable as a screening test (9,18,19). Moreover, IHC correlates well with FISH (8,20). But, it is not easy to perform IHC for ALK in every case in daily practice. Thus, being aware of common histologic features of ALK-expressing adenocarcinoma is important for a pathologist.

The characteristic microscopic finding of ALK-expressing NSCLC is a solid pattern with signet-ring cells (12,17,21). On the other hand, signet-ring cell appearance alone lacks diagnostic significance, and intra- and/or extra-cytoplasmic mucin and a cribriform pattern with excessive extracytoplasmic mucin are useful histologic features suggesting ALK rearrangement (13).

The concept of characteristic histologic features described in the present study is different from grouping of each tumor into separate categories or naming tumors by the criterion of proportion. The purpose of describing these features is to predict underlying ALK-rearrangement. Presently, all three histologic features overlapped each other. Cases with cribriform pattern with extracytoplasmic mucin are always counted as having intra- and/or extra-cytoplasmic mucin, and cases with signet ring cells as having intracytoplasmic mucin.

In the present study, many cases showed intra- and/or extra-cytoplasmic mucin as the only characteristic feature, without signet ring cells or the cribriform pattern. This suggests that a pathologist should undertake further evaluation, such as ALK IHC or FISH, when encountering intra- and/or extra-cytoplasmic mucin with no signet ring cells or a cribriform pattern. As for the subtype or histologic pattern, the present cases showed variable patterns including solid, acinar, and papillary.

We could not perform ALK IHC on all the adenocarcinoma cases, and a certain number of ALK-false negative cases are bound to exist. Thus, the present findings are not conclusive and further studies will be needed for validation.

In conclusion, intra- and/or extra-cytoplasmic mucin, including signet ring cell appearance and cribriform pattern with extracytoplasmic mucin, are characteristic features of ALK-expressing pulmonary adenocarcinoma.

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