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Isolation of PPFIBP1–ALK fusion

To obtain cDNA fragments corresponding to a novel *ALK* fusion gene, we used a 5′-RACE method with the SMART RACE cDNA Amplification Kit (Clontech) according to the manufacturer's instructions, with a minor modification: the ALK2458R primer (5′-GTAGTTGGGGTTGTAGTCGGT-CATGATGGT-3′) was used as the gene-specific reverse primer.

From the oligo(dT)-primed cDNA obtained from case 2 RNA, a 471bp cDNA fragment containing the fusion point was specifically amplified with the primers PPFIBP1-592F (5′-AGAGACACAGAGGGGCTGATT-3′) and ALK3078RR (5′-ATCCAGTTCGTCCTGTTCAGAGC-3′).

PCR analysis of genomic DNA for *PPFIBP1–ALK* in case 2 was carried out with a pair of primers flanking the putative fusion point, PPFIBP1-607F (5′-CTGATTCAGGAGATCA-ATGATTTGAGGT-3′) and Fusion-RT-AS (5′-TCTTGCCAG-CAAAGCAGTAGTTGG-3′).

From the cDNA, a full-length cDNA for *PPFIBP1–ALK* was amplified by PCR with the PA-w-cDNA-in-S primer (5′-TATCTGGGTTGGAATTTGCCCCTG-3′) and the KA-w-cDNA-in-AS primer (5′-TGAGTGTGCGACCGAGCTCAGG-3′) and PrimeSTAR HS DNA polymerase (TakaraBio).

FISH

FISH analysis of gene fusion was carried out with bacterial artificial chromosome (BAC) clone-derived DNA probes for *ALK* and *PPFIBP1*. Unstained sections (4 μm thick) were subjected to hybridization with an *ALK*-split probe set (Abbott) or BAC clone-derived probes for *ALK* (RP11–984I21, RP11–62B19) and *PPFIBP1* (RP11–1060J15). Hybridized slides were then stained with DAPI and examined with the fluorescence microscope BX51 (Olympus).

Transformation assay for ALK fusion proteins

Analysis of the transforming activity of PPFIBP1–ALK was carried out as described previously (20, 37, 38). Briefly, the pMXS-based expression plasmid for PPFIBP1–ALK, EML4–ALK variant 1, or NPM–ALK was used to generate recombinant ecotropic retrovirus, followed by individual infection of mouse 3T3 fibroblasts (39). Formation of the transformed foci was evaluated after culturing the cells for 14 days. The same set of 3T3 cells was subcutaneously injected into nu/nu mice, and tumor formation was examined after 20 days. The animal experiments were approved by the animal ethics committee of Jichi Medical University.

Results

Morphology and immunophenotype of PPFIBP1–ALK-positive IMT

Histopathologic analysis of the 2 IMT cases revealed a marked proliferation of cells composed of somewhat histiocytoid spindle cells showing a fascicular or storiform pattern. The tumor cells were uniform and had pale eosinophilic cytoplasm and an oval vesicular nucleus, within which a small nucleolus was centrally located. Mild inflammatory infiltrate containing lymphocytes, plasma cells, foamy histiocytes, and multinucleated giant cells was observed (Fig. 1A and 1D). The immunophenotype of the 2 cases was negative for smooth muscle actin,

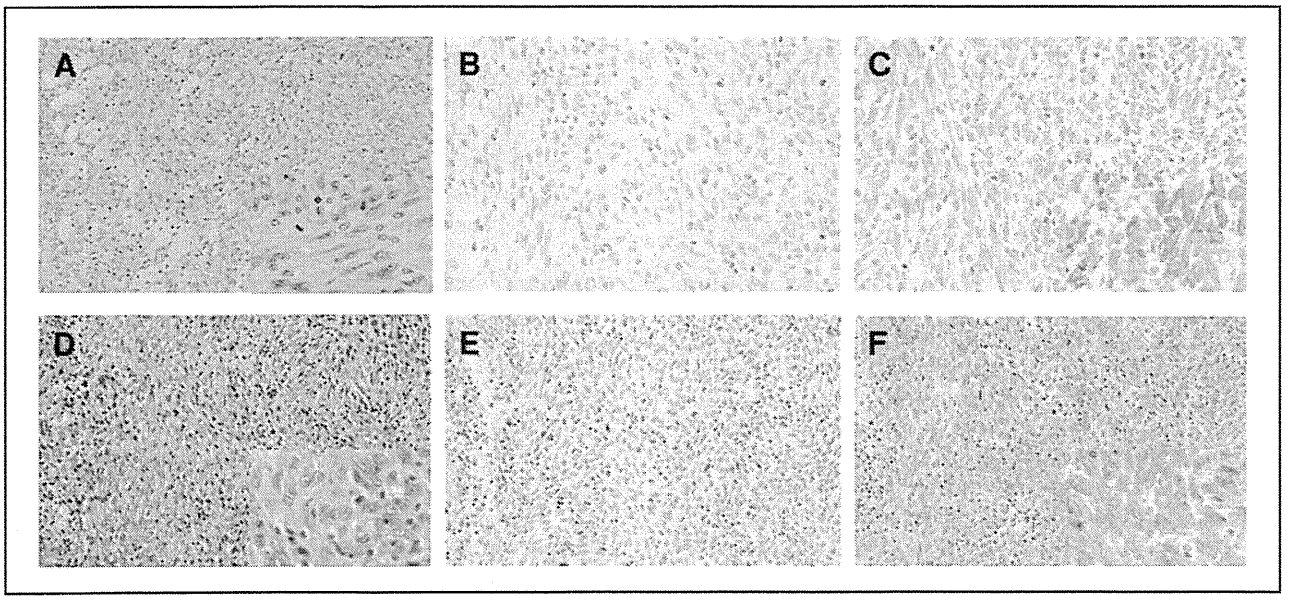

Figure 1. Histopathology of PPFIBP1–ALK-positive IMT. Diffuse proliferation of histiocytoid spindle cells showing a fascicular or storiform pattern. The tumor cells were uniform and had pale eosinophilic cytoplasm and an oval vesicular nucleus, within which a small nucleolus was centrally located. Mild inflammatory infiltrate containing lymphocytes, plasma cells, and foamy histiocytes is observed (A and D). The tumor cells were negative for ALK with conventional anti-ALK immunohistochemistry (B and E), but were clearly positive for ALK when the iAEP method was used. The staining pattern is diffuse cytoplasmic (C and F). Case 1 (A–C), Case 2 (D–F).

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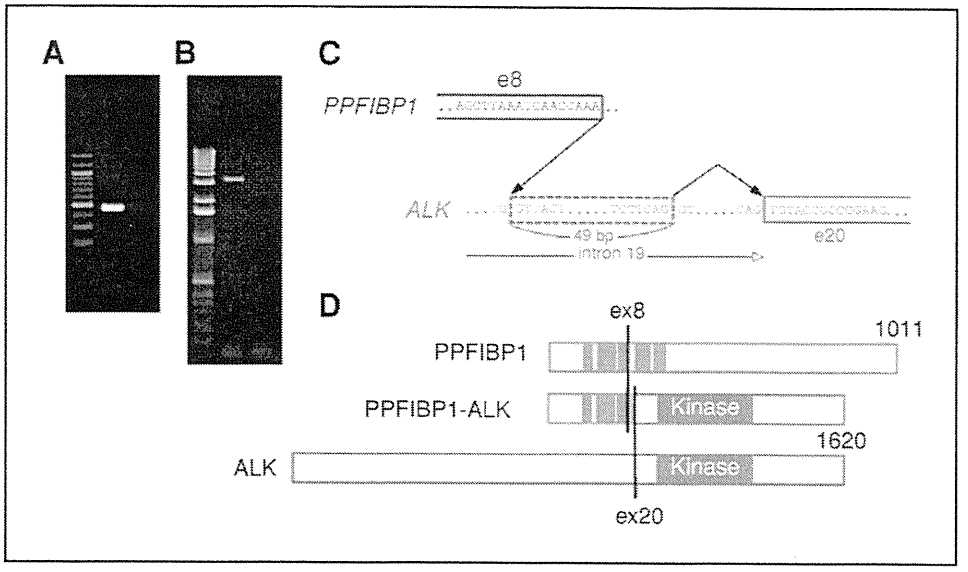

Figure 2. Identification of PPFIBP1–ALK: a PCR product of 471 bp covering the fusion point of *PPFIBP1–ALK* cDNA was specifically amplified from the tumor cells of case 2. The left lane contains DNA size standards (100 bp ladder). The right lane represents no template control (A). A PCR product of approximately 3 kbp covering the genomic fusion point of *PPFIBP1–ALK* was specifically amplified from the tumor cells of case 2. The left lane contains DNA size standards (1 kbp ladder). The right lane represents no template control (B). In our 5′-RACE products, exon 8 of *PPFIBP1* cDNA was fused to a 49 bp sequence in intron 19 of *ALK*, followed by exon 20 of *ALK* (C). PPFIBP1 contains 5 coiled-coil domains. A chromosome translocation, t(2;10)(p23;p11), generates a fusion protein in which the top 3 coiled-coil domains of PPFIBP1 and the intracellular region of ALK (containing the tyrosine kinase domain) are conserved. Numbers indicate amino acid positions of each protein (D).

HHF35, CD34, AE1/AE3, and S100. Desmin was focally positive in case 1, but was negative in case 2.

Identification of PPFIBP1–ALK as a novel ALK fusion gene

We conducted anti-ALK immunohistochemistry on 2 morphologically typical pulmonary IMT cases, originally diagnosed as fibrous histiocytoma. Immunostaining for ALK with the conventional polymer method led to the revised diagnosis of "ALK-negative" IMT (Fig. 1B and E). In the present study, anti-ALK immunohistochemistry with the iAEP method, however, showed a diffuse positive cytoplasmic staining (Fig. 1C and F), indicating the possibility of ALK fusion to a novel partner gene, the expression level of which is modest. To address this issue, in case 2 we conducted 5′-RACE assay for the isolation of an upstream cDNA to the ALK kinase domain cDNA, for which snap-frozen material was available.

Interestingly, we isolated a cDNA fragment containing exon 8 of *PPFIBP1* followed by a 49 bp-sequence within intron 19 of *ALK* and coupled to exon 20 of *ALK* (Fig. 2), suggesting the presence of a novel fusion between *PPFIBP1* and *ALK* genes. Because insertion of the intronic 49 bp allows an in-frame fusion between the 2 genes, this rearrangement likely produces a novel fusion-type tyrosine kinase. To confirm the genomic rearrangement responsible for the *PPFIBP1–ALK* fusion, a genomic PCR assay (Fig. 2B) and both *ALK* split and *PPFIBP1–ALK* fusion FISH assays (Fig. 3) were carried out. All results were consistent with the presence of t(2;12)(p23;p11) leading to the generation of *PPFIBP1–ALK*. Owing to the limited material available in case 1, only the FISH analyses were carried out. Surprisingly, these results also indicate the presence of *PPFIBP1–ALK* (Fig. 3, Supplementary Fig. 2A–C).

Transforming activities of PPFIBP1–ALK

To prove that the t(2;12)(p23;p11) rearrangement leads to the production of PPFIBP1–ALK kinase, in case 2 we attempted to amplify from the cDNA a full-length cDNA encoding the protein. By using a sense primer at the 5′-untranslated region of *PPFIBP1* mRNA (GenBank accession no. NM_003622) and an antisense primer at

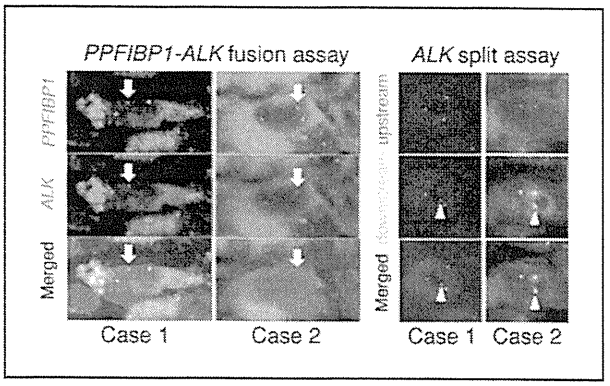

Figure 3. FISH analyses for *PPFIBP1–ALK*: sections of tumors positive for PPFIBP1–ALK were subjected to FISH analyses. In *PPFIBP1–ALK* fusion assays (left) the fusion genes are indicated by arrows. In *ALK* split assays (right) the 3′-sides of *ALK* are indicated by arrowheads. The color of fluorescence for the BAC clones and the case numbers in each hybridization are indicated. Nuclei are stained blue with DAPI.

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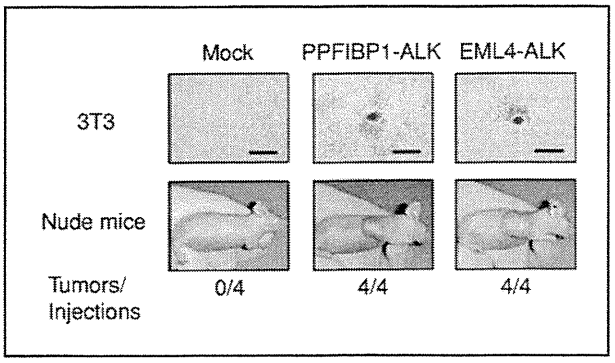

Figure 4. Transforming potential of PPFIBP1–ALK. Top, mouse 3T3 fibroblasts were infected with retroviruses encoding PPFIBP1–ALK or EML4–ALK or with the corresponding empty virus (Mock). The cells were photographed after 14 days of culture. Scale bars, 400 μm. Bottom, Nude mice were injected subcutaneously with the corresponding 3T3 cells, and tumor formation was examined after 14 days. The number of tumors formed per 4 injections is indicated at the bottom.

the 3′-untranslated region of *ALK* mRNA (GenBank accession no. NM_004304), a full-length *PPFIBP1–ALK* cDNA of 2488 bp was successfully amplified, which should have produced a fusion kinase of 811 amino acids with a predicted molecular weight of 90,740 Da (Supplementary Fig. 1).

To examine the transforming potential of PPFIBP1–ALK, a recombinant ecotropic retrovirus was generated to express PPFIBP1–ALK, which was used to infect mouse 3T3 fibroblasts. As shown in Figure 4, PPFIBP1–ALK produced hundreds of transformed foci over 14 days of culture, which was comparable with the observation with EML4–ALK. Furthermore, subcutaneous injection of the infected 3T3 cells into the shoulder of nude mice revealed that those expressing either PPFIBP1–ALK or EML4–ALK formed large tumors *in vivo*.

Discussion

Since their discovery in 1994, appropriate diagnosis of ALK fusion-positive tumors with conventional anti-ALK immunohistochemistry methods has been accepted. However, EML4–ALK in lung adenocarcinoma, identified in 2007, did not stain positive for ALK with conventional immunohistochemistry methods (21, 35). We developed a sensitive immunohistochemistry method, the iAEP method, and successfully stained EML4–ALK with ordinary anti-ALK mouse monoclonal antibodies (21, 31–33). Such observation further indicates a possibility that staining cancer specimens with sensitive immunohistochemical methods (such as iAEP) may detect novel ALK fusions in the "ALK-negative" tumors defined by conventional anti-ALK immunohistochemistry methods. On the basis of this hypothesis, we have identified a novel ALK fusion in "ALK-negative" IMT.

Caution is needed in practical settings. For example, rhabdomyosarcoma, especially of the alveolar type, often expresses wild-type ALK at a detectable level with conventional anti-ALK immunohistochemistry (40). Moreover, in our experience, a small portion of small cell carcinoma and large cell endocrine carcinoma of the lung, and some sarcomas, may be positive for ALK by iAEP immunohistochemistry, expressing wild-type ALK. Therefore, in order to specifically detect ALK fusions with sensitive anti-ALK immunohistochemistry, a confirmatory test by using FISH, RT-PCR, or similar is usually required. If a tumor is positive for a confirmatory test and the suspected partner gene is not a reported one, 5′-RACE or inverse reverse transcriptase PCR methods can be used for the identification of the suspected partner. Even if overexpressed, wild-type ALK may not be oncogenic (20, 21, 37, 38), although some investigators have suggested that wild-type ALK overexpression above a certain threshold level drives the growth of neuroblastoma (41). Further investigation will be required to clarify if wild-type ALK overexpression is a target for ALK inhibitor therapy.

IMT is a rare mesenchymal tumor that has been referred to as inflammatory pseudotumor, plasma cell granuloma, fibroxanthoma, fibrous histiocytoma, pseudosarcomatous myofibroblastic tumor, and invasive fibrous tumor of the tracheobronchial tree (42). It occurs in the soft tissues as well as in the viscera and the lung, and is more likely to occur in children and young adults. Histologically, IMT is composed of a variable admixture of bland, spindle-shaped myofibroblast-like cells and an inflammatory component of lymphocytes, eosinophils, plasma cells, and macrophages. Recent genetic studies have elucidated clonal chromosomal abnormality involving 2p23, at which ALK is located, in a subset of IMT. The expression of ALK fusion proteins is detected by anti-ALK immunohistochemistry in approximately 50% of IMT cases (42), in which various ALK fusion genes have been reported (Table 1). Collectively, these lines of evidence support ALK-positive IMT being a distinct neoplastic entity. However, the other 50% of IMT cases are negative for anti-ALK immunohistochemistry, and thus in terms of pathogenesis it remains unknown whether these ALK-negative IMTs should be included in the same entity or not. In fact, 1 ALK-negative IMT case did not respond to crizotinib therapy (29). However, we have detected a novel ALK-fusion in "ALK-negative" IMT that subsequently proved positive for ALK with the iAEP immunohistochemistry method. Therefore, unexpectedly lowly expressed ALK fusions may explain the pathogenesis of a portion of "ALK-negative" IMT cases. PPFIBP1–ALK represents such an ALK fusion, although we do not yet know what proportion of "ALK-negative" IMTs can be attributed to this novel subtype. "ALK-negative" IMT warrants screening with the iAEP method to detect this fusion or other, unrecognized, ALK fusions.

PPFIBP1 codes liprin beta 1 (also called PTPRF-interacting protein-binding protein 1). This 114 kDa protein is a member of the leukocyte common antigen–related (LAR) transmembrane tyrosine phosphatase-interacting protein family that may regulate LAR protein properties via interaction with another member of the family, liprin alpha1 (43). Liprin beta 1 expresses in intestinal lymphatic endothelial cells *in vitro* and lymphatic vasculature *in vivo*,

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Table 1. ALK fusion partners in well-documented IMT cases

Partner	Locus	Age	Sex	Site	Year, First author
TPM3	1p23	30	F	Lung	2000, Lawrence
		23	F	Abdomen	2000, Lawrence
		4	M	Lung	2006, Yamamoto
		29	F	Ileum	2006, Milne
		4	M	Lung	2007, Kinoshita
TPM4	19p13	1	M	Abdomen	2000, Lawrence
		6	M	Mesentery	2003, Hisaoka
		25	M	Prostate	2003, Hisaoka
		5	M	Mesentery	2006, Yamamoto
		5	F	Urinary bladder	2006, Yamamoto
CLTC	17q23	3	F	Neck	2001, Bridge
		37	M	Pelvis	2001, Bridge
		2	M	Thoracic cavity	2006, Yamamoto
		6	M	Mesentery	2006, Yamamoto
		0	F	Mediastinum	2007, Patel
CARS	11p15	0	M	Abdomen	2002, Cools
		10	M	Neck	2003, Debelenko
RANBP2	2q13	7	M	Abdomen	2003, Ma
		0	M	Abdomen	2003, Ma
		2	M	Abdomen	2007, Patel
		34	M	Liver	2008, Chen
		44	M	Abdomen	2010, Butrynski
ATIC	2q35	46	M	Urinary bladder	2003, Debiec-Rychter
SEC31L1	4q21	23	M	Abdomen	2006, Panagopoulos
PPFIBP1	12p11	45	M	Lung	Present case 1
		34	F	Lung	Present case 2

and plays an important role in the maintenance of lymphatic vessel integrity in *Xenopus* tadpoles (44). PPFIBP1 has 5 coiled-coil domains in exons 5 through 12, and the upper 3 domains are conserved in fusion form with ALK (Fig. 2D). The coiled-coil domain is shared in all ALK fusion partners (except for NPM, MSN, and SQSTM1), with which the ALK fusion proteins homodimerize leading to constitutive activation of ALK kinase domains (8, 19). As expected, in the present study, the oncogenicity of PPFIBP1–ALK was clearly confirmed with an *in vitro* focus formation assay and an *in vivo* tumorigenicity assay.

The difference in subcellular localization has contributed to the discovery/identification of various ALK fusions. Likewise, the difference in the expression level found is here proved important in the accurate detection of fusion proteins. Sensitive immunohistochemical methods such as iAEP will broaden the potential value of immunohistochemistry, which is a simple and long-established histopathologic technique in the fields of research and diagnosis. The ALK positivity rate (approximately 50%) in IMT should be reassessed with these more sensitive methods, possibly leading to the identification of novel ALK fusions and more candidates for ALK inhibitor therapy. A novel ALK fusion, VCL–ALK, has recently been identified in renal cancers (45, 46). In addition to IMT, therefore, a reassessment of diverse "ALK-negative" human cancers may be required in the forthcoming era of ALK inhibitor therapy.

Disclosure of Potential Conflicts of Interest

K. Takeuchi, scientific advisor for developing an anti-ALK iAEP immunohistochemistry kit (ALK Detection Kit, Nichirei Bioscience, Japan) and in charge of pathology screening for ALK fusions using the immunohistochemistry kit and an original probe set for ALK split FISH assay in a clinical trial of an ALK inhibitor (AF802, Chugai, Japan). The other authors disclosed no potential conflicts of interest.

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Clinicopathological findings of non-small-cell lung cancer with high serum progastrin-releasing peptide concentrations

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ABSTRACT

Although progastrin-releasing peptide (proGRP) is used as a serum tumor marker for small cell lung cancer (SCLC), high serum pro-GRP concentrations are observed in some non-small-cell lung cancers (NSCLCs). The characteristics of these NSCLCs are not well known. To determine the clinicopathological features of NSCLC in patients with elevated serum proGRP concentrations, serum proGRP values were assessed in 654 advanced lung cancer patients, and positive (>46 pg/mL) NSCLC specimens were subjected to cytological and histopathological reevaluation. Serum proGRP concentrations were positive in 34 of 421 NSCLC patients (8.1%) and 186 of 233 SCLC patients (80%). Histological subtypes of the 34 NSCLC patients at diagnosis were 20 adenocarcinomas, 5 squamous cell carcinomas, 4 large cell carcinomas, and 5 large cell neuroendocrine carcinomas. Six of 27 cytology specimens contained characteristic neuroendocrine morphology. Immunohistochemical analysis showed that 11 of 17 tumors were positive for neuroendocrine markers (64.7%). Twenty of 34 serum proGRP-positive NSCLC patients received platinum-based chemotherapy, and the response rate was 55.0%. These results suggest that serum proGRP-positive NSCLCs may have neuroendocrine differentiation. In addition, serum proGRP-positive NSCLCs may have clinical characteristics that are different from other NSCLCs.

1. Introduction

Lung cancer is the leading cause of cancer death worldwide. In 2005, the number of deaths due to lung cancer in Japan exceeded 60,000 [1]. Conventionally, lung cancer is classified into small cell lung cancer (SCLC) and non-small-cell carcinoma (NSCLC). Because SCLC has neuroendocrine features, it has a poorer prognosis and shows greater sensitivity to chemotherapy than NSCLC. Although NSCLC is subclassified into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, some NSCLCs have neuroendocrine differentiation. In 1999, the World Health Organization categorized large cell neuroendocrine carcinoma (LCNEC) as a variant of large cell carcinoma [2]. LCNEC has been reported to have a poor prognosis, even for early-stage disease [3,4]. Different types of NSCLCs differ in their clinical behavior according to the presence or absence of neuroendocrine differentiation. Neuroendocrine differentiation in a tumor is generally determined by immunohistochemistry and/or electron microscopy, which reveal the characteristic neuroendocrine morphology [2,5]. However, it is difficult to obtain sufficient tissue by biopsy, and limited tumor tissue sampling may make it difficult to diagnose neuroendocrine differentiation in NSCLC. Therefore, the development of a sensitive serum marker for the detection of neuroendocrine differentiation is greatly desired to facilitate the diagnosis of NSCLCs and neuroendocrine tumors.

Progastrin-releasing peptide (proGRP) is a signal peptide that is produced by small cell lung cancer cells (SCLC). Serum proGRP is considered to be a sensitive tumor marker for SCLC. The sensitivity and specificity of serum proGRP as a tumor marker for SCLC is 60–70% and 96%, respectively [6]. Elevated serum proGRP concentrations have been observed in some NSCLC patients, especially LCNEC patients [6,7], suggesting that serum proGRP is a potentially good marker not only for SCLC but also for NSCLC with neuroendocrine features. However, the clinical and pathological characteristics of NSCLCs with elevated serum proGRP concentrations have not been well studied. In the present study, serum proGRP levels were measured in 654 lung cancer patients and the clinical characteristics of serum proGRP-positive NSCLC patients

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were analyzed; the histopathology of the surgical or biopsy specimens of the positive patients were also evaluated.

2. Patients and methods

Serum proGRP concentrations were measured in 654 patients who were diagnosed with lung cancer by histology or cytology at the Cancer Institute Hospital of the Japanese Foundation for Cancer Research between April 1998 and April 2006.

An enzyme-linked immunosorbent assay (ELISA) kit (serumlabo ProGRP; Fujirebio Diagnostics Inc., Tokyo, Japan) was used to determine serum proGRP concentrations, and samples were considered positive when their values exceeded 46 pg/mL [8].

The clinical characteristics of serum proGRP-positive NSCLC patients were retrospectively analyzed, including age at diagnosis, gender, smoking history, and TNM stage. Response to platinum-based chemotherapy in serum proGRP-positive NSCLC patients was determined according to RECIST criteria (without confirmation).

In addition, the cytological and histological findings of the surgical or biopsy specimens of these patients were reevaluated. Immunohistochemical (IHC) staining was used to evaluate neuroendocrine differentiation in the tumors. Formalin-fixed paraffin-embedded sections were stained for a panel of epithelial markers, including thyroid transcription factor-1 (TTF-1; Dako EnVision+, Saitama, Japan) and carcinoembryonic antigen (CEA; Nichirei, Tokyo, Japan), and neuroendocrine markers, including chromogranin A (CGA) (Dako EnVision+, Saitama, Japan), synaptophysin (Dako EnVision+, Saitama, Japan), CD56 (neural cell adhesion molecule [NCAM]) (Clone 1B6; Novocastra, and proGRP (Advanced Life Science Institute Inc., Saitama, Japan). IHC staining was performed according to standard protocols with EnVision kits (Dako EnVision+, Saitama, Japan). IHC results were grouped into 3 categories – strongly positive, weakly positive, or negative – by well-trained pathologists (WH and NM).

Statistical calculations were performed using StatView version 5.0 for Windows XP (SAS Institute, Cary, NC). Associations between categorical variables and serum proGRP concentrations were evaluated using Student's *t* test. Survival was measured from the start of chemotherapy to the last follow-up evaluation or death, and survival rates were estimated using the Kaplan–Meier method.

3. Results

3.1. Patient characteristics

Of a total of 654 patients, 421 were diagnosed with NSCLC and 233 with SCLC. Serum proGRP samples were positive in 220 of 654 patients, of which 34 (8.1%) had NSCLC and 186 (80%) had SCLC.

The clinical characteristics of serum proGRP-positive and negative NSCLC patients are shown in Table 1. There were no significant differences in the clinical characteristics between the serum proGRP-positive and -negative NSCLC patients.

In serum ProGRP-positive NSCLC patients, the median age of these patients was 67 years (range, 49–77). There were 22 males and 12 females, and 65% of the patients were heavy smokers (smoking index > 400). Most of the patients (94%) had advanced NSCLC. Serum creatinine concentrations were less than 1.6 mg/dL in all 34 serum proGRP-positive NSCLC patients.

The histological subtypes of the 34 serum proGRP-positive NSCLCs at diagnosis were as follows: 20 adenocarcinomas, 5 squamous cell carcinomas, 4 large cell carcinomas, and 5 LCNECs. The rates of positive serum proGRP in each histological subtype were as follows: 7.7% in 260 adenocarcinomas, 5.9% in 85 squamous cell carcinomas, 9.3% in 43 large cell carcinomas, and 44.4% in 11 LCNECs.

Table 1
Clinical characteristics of NSCLC patients.

Characteristics	ProGRP-positive NSCLC patients	ProGRP-negative NSCLC patients
Total no. of patients	34	387
Age, years		
Median (range)	67 (49–77)	62 (29–87)
Sex		
Male/female	22/12	261/126
Smoking index		
Mean (range)	807.5 (0–1400)	661 (0–3000)
Never/≤400/>400	10/2/22	121/29/237
Histological subtype at diagnosis		
Adenocarcinoma	20	240
Squamous cell carcinoma	5	80
Large cell carcinoma	4	48
LCNEC	5	6
Adenosquamous	0	2
Other	0	11
Stage		
I/II	2	56
IIIA	7	63
IIIB	6	106
IV	16	144
Recurrent	3	18

LCNEC: large cell neuroendocrine carcinoma, ProGRP: progastrin-releasing peptide, NSCLC: non-small-cell lung cancer.

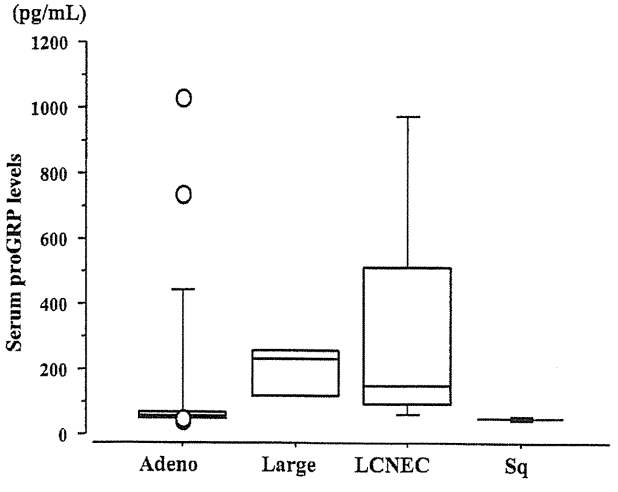

Fig. 1. Serum progastrin-releasing peptide (proGRP) concentrations of 34 non-small-cell lung cancer patients with elevated proGRP. Adeno: adenocarcinoma, Large: large cell carcinoma, LCNEC: large cell neuroendocrine carcinoma, Sq: squamous cell carcinoma.

The median serum proGRP concentration of the 34 NSCLC patients was 60.7 pg/mL and the range was 46.0–973.0 pg/mL. The serum proGRP concentrations in these 34 NSCLC patients were significantly lower than the serum concentrations in proGRP-positive SCLC patients (median, 469 pg/mL; range, 47.1–344,000 pg/mL) ($P < 0.05$).

Fig. 1 shows the serum proGRP concentrations for each histological subtype of serum proGRP-positive NSCLC. The mean serum proGRP concentration in LCNECs was 147 pg/mL and the range was 78.6–973 pg/mL. These concentrations are relatively high compared to other NSCLCs. On the other hand, serum proGRP concentrations were relatively low, even in serum proGRP-positive squamous cell carcinoma patients (median, 47.4 pg/mL; range, 46–56.7 pg/mL).

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Table 2
Immunohistochemistry results of non-small-cell lung cancer with elevated serum progastrin-releasing peptide concentrations.

	Serum proGRP (pg/dL)	Histological subtype at diagnosis	Specimen	Stage	IHC					
					TTF-1	CEA	CGA	Synapto-phisin	NCAM	proGRP
1	973	Large	TBLB	IV	–	++	++	++	+	–
2	363	LCNEC	TBLB	IV	++	–	++	++	++	++
3	269	LCNEC	TBLB	IV	++	++	++	++	++	+
4	229	Large	TBLB	IV	–	–	–	–	–	–
5	78.6	Large	TBLB	IV	–	–	–	–	–	–
6	60.3	Adeno	TBLB	IV	++	–	–	–	–	–
7	56.7	Sq	Surgery	Rec	–	+	–	+	++	–
8	56.0	Adeno	Surgery	Rec	++	–	–	+	–	–
9	55.3	Adeno	TBLB	IIIB	++	–	–	+	+	–
10	54.7	Adeno	TBLB	IV	++	–	–	+	–	–
11	53.1	Adeno	Surgery	IB	++	–		+	–	–
12	51.8	Adeno	TBLB	IV	++	+	++	++	++	+
13	51.4	Adeno	Surgery	IB	++	++	++	++	++	++
14	48.3	Sq	TBLB	IB	–	++	–	–	–	–
15	47.4	Sq	Surgery	IIIA	–	++	–	–	–	–
16	47.3	Sq	TBLB	IIIA	–	–	–	+	–	–
17	46	Sq	TBLB	IIIB	–	++	–	–	–	–

IHC: immunohistochemistry, proGRP: progastrin-releasing peptide, TTF-1: thyroid transcription factor 1, CEA: carcinoembryonic antigen, CGA: chromogranin A, NCAM: neural cell adhesion molecule, Adeno: adenocarcinoma, Sq: squamous cell carcinoma, Large: large cell carcinoma, LCNEC: large cell neuroendocrine carcinoma, TBLB: transbronchial lung biopsy, –: negative, +: weakly positive, ++: strongly positive.

3.2. Cytological and histological examination

Cytological specimens corresponding to 27 of the 34 serum proGRP-positive NSCLCs were reevaluated. All 5 cytology specimens diagnosed as LCNEC contained typical neuroendocrine features such as rosette-like and palisading patterns. A rosette-like formation was found in only 1 cytology specimen diagnosed as squamous cell carcinoma, and the other 21 specimens did not contain the typical cytological features of neuroendocrine differentiation.

IHC staining was performed on 17 histological specimens of the 34 serum proGRP-positive NSCLCs (7 adenocarcinomas, 5 squamous cell carcinomas, 3 large cell carcinomas, and 2 LCNECs) to examine neuroendocrine differentiation (Table 2). Four of 17 specimens (24%) showed positive staining (2 weakly and 2 strongly positive) for proGRP, and some neuroendocrine markers were positive in 11 of 17 specimens (64.7%). In particular, 2 of 7 adenocarcinomas, 1 of 3 large cell carcinomas, and 2 of 2 LCNECs showed strongly positive staining for at least 2 out of the 3 neuroendocrine markers CGA, synaptophysin, and NCAM. None of the squamous cell carcinomas showed strongly positive staining for at least 2 out of the 3 neuroendocrine markers CGA, synaptophysin, and NCAM. One of 5 squamous cell carcinomas showed strongly positive staining for NCAM. One of 5 squamous cell carcinomas showed strongly positive staining for NCAM. There was no significant relationship between serum proGRP concentrations and proGRP immunoreactivity.

3.3. Response to chemotherapy

Twenty of 34 serum proGRP-positive NSCLC patients received platinum-based chemotherapy (Table 3). There were 11 partial responses, 4 stable diseases, and no responses observed in the 5 patients. The objective response rate was 55.0%. The median survival of the 20 patients was 11 months, and the 1-year survival rate was 48%.

On the other hand, 232 of 387 serum proGRP-negative NSCLC patients received platinum-based chemotherapy. There were 82 partial responses, 97 stable diseases, and no complete responses observed in the 53 patients. The objective response rate was 35.0%, the median survival was 11.5 months, and the 1-year survival rate was 49.1%.

Table 3
Platinum doublet regimens administered to serum proGRP-positive and -negative patients.

Regimens	ProGRP-positive NSCLC patients ($n=20$)		ProGRP-negative NSCLC patients ($n=232$)	
	n	%	n	%
CBDCA/PTX	9	45	141	60.7
CBDCA/GEM	0	0	16	7
CBDCA/VP16	1	5	5	2
CDDP/DOC	3	15	53	23
CDDP/S-1	2	10	4	1.8
CDDP/VNR	2	10	1	0.5
CDDP/VP16	1	5	0	0
CDDP/CPT11	2	10	2	1
CDDP/GEM	0	0	10	4

CBDCA: carboplatin, CDDP: cisplatin, PTX: paclitaxel, GEM: gemcitabine, VP16: etoposide, DOC: docetaxel, VNR: vinorelbine, CPT11: irinotecan, ProGRP: progastrin-releasing peptide.

4. Discussion

In the present study, the positive rate of serum proGRP concentration in NSCLC patients was 8.1% (34/421), and histological neuroendocrine features were detected in 11 of 17 (64.7%) serum proGRP-positive NSCLC specimens. Several studies have examined serum proGRP concentrations in lung cancer, and all of these studies reported that serum proGRP was a specific tumor marker for SCLC [6,9]. The sensitivity and specificity for SCLCs were around 70% and 99%, respectively, and serum proGRP was superior to NSE [10].

Although increases in serum ProGRP concentration have been observed in some NSCLC patients in previous studies, the reported positive rates of serum proGRP in NSCLCs demonstrated a wide range of variability (3–30%) [6,11]. The 8% positive rate found in the present study was relatively higher than in previous studies, with the exception of 2 reports (the Takada study used a lower cutoff for positive, at 34 pg/mL, and the Molina study included a higher proportion of renal failure patients) [11,12]. Although several studies have reported that serum proGRP values were elevated in NSCLC patients, there have been few studies examining the clinicopathological characteristics of serum proGRP-positive NSCLC patients [13]. Only 1 study examined the clinicopathological

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characteristics of 24 NSCLC patients with elevated serum proGRP concentrations. Positive IHC staining for neuroendocrine differentiation was reported in only 4 of 24 serum proGRP-positive NSCLCs, and a small-cell component or neuroendocrine differentiation was detected in all 4 of those patients [13].

In the present study, 27 cytological specimens were reevaluated and IHC staining was performed on 17 histological specimens out of 34 serum proGRP-positive NSCLCs. The cytology results showed the presence of neuroendocrine features such as rosette-like formations in the analysis of 1 squamous cell carcinoma and 5 LCNECs. Histologic examination also identified 2 cases with neuroendocrine morphology (i.e., LCNEC). In addition, weakly or strongly positive staining for some neuroendocrine markers was observed in 12 of 17 histological specimens.

Previous reports have suggested that renal failure can be a source of false-positive proGRP results [11,14], and serum proGRP concentrations were elevated in patients who had serum creatinine levels greater than 1.6 mg/dL [7,13,15]. Therefore, the elevation of serum proGRP detected in present study could be associated with renal dysfunction in the patients analyzed. However, the serum creatinine levels were less than 1.2 mg/dL (median, 0.7 mg/dL) in all 34 serum proGRP-positive NSCLC patients Furthermore, no correlation was observed between serum creatinine and serum proGRP concentrations (data not shown). These results indicate that renal dysfunction could not have accounted for the serum proGRP elevations seen in the present study.

Recently, several reports have suggested that the clinical features of NSCLC with neuroendocrine differentiation, such as LCNEC, may be different from other types of NSCLC [3,16]. The clinical characteristics of serum proGRP-positive NSCLCs were similar to those of SCLCs, as suggested by data showing that the majority of patients were male and heavy smokers. The response rate to platinum-doublet chemotherapy in the present study was 55.0%. This response rate seems higher than the response rates to platinum-doublet chemotherapy reported for nonselected NSCLC [17,18] and serum ProGRP-negative NSCLC in this study, but they were similar to the previously reported response rates to platinum-doublet chemotherapy in LCNEC [16,19]. These results suggest that the sensitivity to chemotherapy in serum proGRP-positive NSCLC may be different than that of other types of NSCLC.

In the present study, IHC staining and a morphologic description were not performed in NSCLC patients with non-elevated proGRP. Therefore, the number of false-negative results and the real performance of the proGRP assay in the detection of neuroendocrine differentiation remain unknown. However, the difficulty in obtaining sufficient tissue by biopsy, and limited tumor tissue sampling make the accurate diagnosis of neuroendocrine differentiation in NSCLC complicated. Sensitive and simple methods for the detection of neuroendocrine differentiation of NSCLC are greatly desired.

In conclusion, serum proGRP-positive NSCLCs may contain manifest neuroendocrine differentiation. In addition, serum proGRP-positive NSCLC patients may have different clinical characteristics compared to other NSCLC patients

Conflict of interest statement

The authors declare no conflicts of interest.

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International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma

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Introduction: Adenocarcinoma is the most common histologic type of lung cancer. To address advances in oncology, molecular biology, pathology, radiology, and surgery of lung adenocarcinoma, an international multidisciplinary classification was sponsored by the International Association for the Study of Lung Cancer, American Thoracic Society, and European Respiratory Society. This new adenocarcinoma classification is needed to provide uniform terminology and diagnostic criteria, especially for bronchioloalveolar carcinoma (BAC), the overall approach to small nonresection cancer specimens, and for multidisciplinary strategic management of tissue for molecular and immunohistochemical studies.

Methods: An international core panel of experts representing all three societies was formed with oncologists/pulmonologists, pathologists, radiologists, molecular biologists, and thoracic surgeons. A systematic review was performed under the guidance of the American Thoracic Society Documents Development and Implementation Committee. The search strategy identified 11,368 citations of which 312 articles met specified eligibility criteria and were retrieved for full text review. A series of meetings were held to discuss the development of the new classification, to develop the recommendations, and to write the current document. Recommendations for key questions were graded by strength and quality of the evidence according to the Grades of Recommendation, Assessment, Development, and Evaluation approach.

Results: The classification addresses both resection specimens, and small biopsies and cytology. The terms BAC and mixed subtype adenocarcinoma are no longer used. For resection specimens, new concepts are introduced such as adenocarcinoma in situ (AIS) and minimally invasive adenocarcinoma (MIA) for small solitary adenocarcinomas with either pure lepidic growth (AIS) or predominant lepidic growth with ≤5 mm invasion (MIA) to define patients who, if they undergo complete resection, will have 100% or near 100% disease-specific survival, respectively. AIS and MIA are usually nonmucinous but rarely may be mucinous. Invasive adenocarcinomas are classified by predominant pattern after using comprehensive histologic subtyping with lepidic (formerly most mixed subtype tumors with nonmucinous BAC), acinar, papillary, and solid patterns; micropapillary is added as a new histologic subtype. Variants include invasive mucinous adenocarcinoma (formerly mucinous BAC), colloid, fetal, and enteric adenocarcinoma. This classification provides guidance for small biopsies and cytology specimens, as approximately 70% of lung cancers are diagnosed in such samples. Non-small cell lung carcinomas (NSCLCs), in patients with advanced-stage disease, are to be classified into more specific types such as adenocarcinoma or squamous cell carcinoma,

Affiliations are listed in the appendix.

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whenever possible for several reasons: (1) adenocarcinoma or NSCLC not otherwise specified should be tested for epidermal growth factor receptor (*EGFR*) mutations as the presence of these mutations is predictive of responsiveness to EGFR tyrosine kinase inhibitors, (2) adenocarcinoma histology is a strong predictor for improved outcome with pemetrexed therapy compared with squamous cell carcinoma, and (3) potential life-threatening hemorrhage may occur in patients with squamous cell carcinoma who receive bevacizumab. If the tumor cannot be classified based on light microscopy alone, special studies such as immunohistochemistry and/or mucin stains should be applied to classify the tumor further. Use of the term NSCLC not otherwise specified should be minimized.

Conclusions: This new classification strategy is based on a multidisciplinary approach to diagnosis of lung adenocarcinoma that incorporates clinical, molecular, radiologic, and surgical issues, but it is primarily based on histology. This classification is intended to support clinical practice, and research investigation and clinical trials. As *EGFR* mutation is a validated predictive marker for response and progression-free survival with EGFR tyrosine kinase inhibitors in advanced lung adenocarcinoma, we recommend that patients with advanced adenocarcinomas be tested for *EGFR* mutation. This has implications for strategic management of tissue, particularly for small biopsies and cytology samples, to maximize high-quality tissue available for molecular studies. Potential impact for tumor, node, and metastasis staging include adjustment of the size T factor according to only the invasive component (1) pathologically in invasive tumors with lepidic areas or (2) radiologically by measuring the solid component of part-solid nodules.

Key Words: Lung, Adenocarcinoma, Classification, Histologic, Pathology, Oncology, Pulmonary, Radiology, Computed tomography, Molecular, *EGFR*, *KRAS*, *EML4-ALK*, Gene profiling, Gene amplification, Surgery, Limited resection, Bronchioloalveolar carcinoma, Lepidic, Acinar, Papillary, Micropapillary, Solid, Adenocarcinoma in situ, Minimally invasive adenocarcinoma, Colloid, Mucinous cystadenocarcinoma, Enteric, Fetal, Signet ring, Clear cell, Frozen section, TTF-1, p63.

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RATIONALE FOR A CHANGE IN THE APPROACH TO CLASSIFICATION OF LUNG ADENOCARCINOMA

Lung cancer is the most frequent cause of major cancer incidence and mortality worldwide.[1,2] Adenocarcinoma is the most common histologic subtype of lung cancer in most countries, accounting for almost half of all lung cancers.[3] A widely divergent clinical, radiologic, molecular, and pathologic spectrum exists within lung adenocarcinoma. As a result, confusion exists, and studies are difficult to compare. Despite remarkable advances in understanding of this tumor in the past decade, there remains a need for universally accepted criteria for adenocarcinoma subtypes, in particular tumors formerly classified as bronchioloalveolar carcinoma (BAC).[4,5] As enormous resources are being spent on trials involving molecular and therapeutic aspects of adenocarcinoma of the lung, the development of standardized criteria is of great importance and should help advance the field, increasing the impact of research, and improving patient care. This classification is needed to assist in determining patient therapy and predicting outcome.

NEED FOR A MULTIDISCIPLINARY APPROACH TO DIAGNOSIS OF LUNG ADENOCARCINOMA

One of the major outcomes of this project is the recognition that the diagnosis of lung adenocarcinoma requires a multidisciplinary approach. The classifications of lung cancer published by the World Health Organization (WHO) in 1967, 1981, and 1999 were written primarily by pathologists for pathologists.[5–7] Only in the 2004 revision, relevant genetics and clinical information were introduced.[4] Nevertheless, because of remarkable advances over the last 6 years in our understanding of lung adenocarcinoma, particularly in area of medical oncology, molecular biology, and radiology, there is a pressing need for a revised classification, based not on pathology alone, but rather on an integrated multidisciplinary platform. In particular, there are two major areas of interaction between specialties that are driving the need for our multidisciplinary approach to classification of lung adenocarcinoma: (1) in patients with advanced non-small cell lung cancer, recent progress in molecular biology and oncology has led to (a) discovery of epidermal growth factor receptor (*EGFR*) mutation and its prediction of response to EGFR tyrosine kinase inhibitors (TKIs) in adenocarcinoma patients[8–11] and (b) the requirement to exclude a diagnosis of squamous cell carcinoma to determine eligibility patients for treatment with pemetrexed, (because of improved efficacy)[12–15] or bevacizumab (because of toxicity)[16,17] and (2) the emergence of radiologic-pathologic correlations between ground-glass versus solid or mixed opacities seen by computed tomography (CT) and BAC versus invasive growth by pathology have opened new opportunities for imaging studies to be used by radiologists, pulmonologists, and surgeons for predicting the histologic subtype of adenocarcinomas,[18–21] patient prognosis,[18–23] and improve preoperative assessment for choice of timing and type of surgical intervention.[18–26]

Although histologic criteria remain the foundation of this new classification, this document has been developed by pathologists in collaboration with clinical, radiology, molecular, and surgical colleagues. This effort has led to the development of terminology and criteria that not only define pathologic entities but also communicate critical information that is relevant to patient management (Tables 1 and 2). The classification also provides recommendations on strategic handling of specimens to optimize the amount of information to be gleaned. The goal is not only longer to solely provide the most accurate diagnosis but also to manage the tissue in a way that immunohistochemical and/or molecular studies can be performed to obtain predictive and prognostic data that will lead to improvement in patient outcomes.

For the first time, this classification addresses an approach to small biopsies and cytology in lung cancer diagnosis (Table 2). Recent data regarding *EGFR* mutation predicting responsiveness to EGFR-TKIs,[8–11] toxicities,[16] and therapeutic efficacy[12–15] have established the importance of distinguishing squamous cell carcinoma from adenocarcinoma and non-small cell lung carcinoma (NSCLC) not otherwise specified (NOS) in patients with advanced lung cancer. Approximately 70% of lung cancers are diagnosed and

TABLE 1. IASLC/ATS/ERS Classification of Lung Adenocarcinoma in Resection Specimens

Preinvasive lesions
Atypical adenomatous hyperplasia
Adenocarcinoma in situ (≤3 cm formerly BAC)
Nonmucinous
Mucinous
Mixed mucinous/nonmucinous
Minimally invasive adenocarcinoma (≤3 cm lepidic predominant tumor with ≤5 mm invasion)
Nonmucinous
Mucinous
Mixed mucinous/nonmucinous
Invasive adenocarcinoma
Lepidic predominant (formerly nonmucinous BAC pattern, with >5 mm invasion)
Acinar predominant
Papillary predominant
Micropapillary predominant
Solid predominant with mucin production
Variants of invasive adenocarcinoma
Invasive mucinous adenocarcinoma (formerly mucinous BAC)
Colloid
Fetal (low and high grade)
Enteric

BAC, bronchioloalveolar carcinoma; IASLC, International Association for the Study of Lung Cancer; ATS, American Thoracic Society; ERS, European Respiratory Society.

staged by small biopsies or cytology rather than surgical resection specimens, with increasing use of transbronchial needle aspiration (TBNA), endobronchial ultrasound-guided TBNA and esophageal ultrasound-guided needle aspiration.[27] Within the NSCLC group, most pathologists can identify well- or moderately differentiated squamous cell carcinomas or adenocarcinomas, but specific diagnoses are more difficult with poorly differentiated tumors. Nevertheless, in small biopsies and/or cytology specimens, 10 to 30% of specimens continue to be diagnosed as NSCLC-NOS.[13,28,29]

Proposed terminology to be used in small biopsies is summarized in Table 2. Pathologists need to minimize the use of the term NSCLC or NSCLC-NOS on small samples and aspiration and exfoliative cytology, providing as specific a histologic classification as possible to facilitate the treatment approach of medical oncologists.[30]

Unlike previous WHO classifications where the primary diagnostic criteria for as many tumor types as possible were based on hematoxylin and eosin (H&E) examination, this classification emphasizes the use and integration of immunohistochemical (i.e., thyroid transcription factor [TTF-1]/p63 staining), histochemical (i.e., mucin staining), and molecular studies, as specific therapies are driven histologic subtyping. Although these techniques should be used whenever possible, it is recognized that this may not always be possible, and thus, a simpler approach is also provided when only H&E-stained slides are available, so this classification may be applicable even in a low resource setting.

METHODOLOGY

Objectives

This international multidisciplinary classification has been produced as a collaborative effort by the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS), and the European Respiratory Society. The purpose is to provide an integrated clinical, radiologic, molecular, and pathologic approach to classification of the various types of lung adenocarcinoma that will help to define categories that have distinct clinical, radiologic, molecular, and pathologic characteristics. The goal is to identify prognostic and predictive factors and therapeutic targets.

Participants

Panel members included thoracic medical oncologists, pulmonologists, radiologists, molecular biologists, thoracic surgeons, and pathologists. The supporting associations nominated panel members. The cochairs were selected by the IASLC. Panel members were selected because of special interest and expertise in lung adenocarcinoma and to provide an international and multidisciplinary representation. The panel consisted of a core group (author list) and a reviewer group (Appendix 1, see **Supplemental Digital Content 1** available at http://links.lww.com/JTO/A59, affiliations for coauthors are listed in appendix).

Evidence

The panel performed a systematic review with guidance by members of the ATS Documents Development and Implementation Committee. Key questions for this project were generated by each specialty group, and a search strategy was developed (Appendix 2, see **Supplemental Digital Content 2** available at http://links.lww.com/JTO/A60). Searches were performed in June 2008 with an update in June 2009 resulting in 11,368 citations. These were reviewed to exclude articles that did not have any relevance to the topic of lung adenocarcinoma classification. The remaining articles were evaluated by two observers who rated them by a predetermined set of eligibility criteria using an electronic web-based survey program (www.surveymonkey.com) to collect responses.[31] This process narrowed the total number of articles to 312 that were reviewed in detail for a total of 141 specific features, including 17 study characteristics, 35 clinical, 48 pathologic, 16 radiologic, 16 molecular, and nine surgical (Appendix 2). These 141 features were summarized in an electronic database that was distributed to members of the core panel, including the writing committee. Articles chosen for specific data summaries were reviewed, and based on analysis of tables from this systematic review, recommendations were made according to the Grades of Recommendation, Assessment, Development, and Evaluation (GRADE).[32–37] Throughout the rest of the document, the term GRADE (spelled in capital letters) must be distinguished from histologic grade, which is a measure of pathologic tumor differentiation. The GRADE system has two major components: (1) grading the strength of the recommendation and (2) evaluating the quality of the evidence.[32] The strength of recommendations is based on weighing estimates of benefits versus downsides. Evidence was rated as high, moderate, or low or very low.[32] The

TABLE 2. Proposed IASLC/ATS/ERS Classification for Small Biopsies/Cytology

2004 WHO Classification	SMALL BIOPSY/CYTOLOGY: IASLC/ATS/ERS
ADENOCARCINOMA Mixed subtype Acinar Papillary Solid	*Morphologic adenocarcinoma patterns clearly present:* Adenocarcinoma, describe identifiable patterns present (including micropapillary pattern not included in 2004 WHO classification) Comment: If pure lepidic growth – mention an invasive component cannot be excluded in this small specimen
Bronchioloalveolar carcinoma (nonmucinous)	Adenocarcinoma with lepidic pattern (if pure, add note: an invasive component cannot be excluded)
Bronchioloalveolar carcinoma (mucinous)	Mucinous adenocarcinoma (describe patterns present)
Fetal	Adenocarcinoma with fetal pattern
Mucinous (colloid)	Adenocarcinoma with colloid pattern
Signet ring	Adenocarcinoma with (describe patterns present) and signet ring features
Clear cell	Adenocarcinoma with (describe patterns present) and clear cell features
No 2004 WHO counterpart – most will be solid adenocarcinomas	*Morphologic adenocarcinoma patterns not present (supported by special stains):* Non-small cell carcinoma, favor adenocarcinoma
SQUAMOUS CELL CARCINOMA Papillary Clear cell Small cell Basaloid	*Morphologic squamous cell patterns clearly present:* Squamous cell carcinoma
No 2004 WHO counterpart	*Morphologic squamous cell patterns not present (supported by stains):* Non-small cell carcinoma, favor squamous cell carcinoma
SMALL CELL CARCINOMA	Small cell carcinoma
LARGE CELL CARCINOMA	Non-small cell carcinoma, not otherwise specified (NOS)
Large cell neuroendocrine carcinoma (LCNEC)	Non-small cell carcinoma with neuroendocrine (NE) morphology (positive NE markers), possible LCNEC
Large cell carcinoma with NE morphology (LCNEM)	Non-small cell carcinoma with NE morphology (negative NE markers) – see comment Comment: This is a non-small cell carcinoma where LCNEC is suspected, but stains failed to demonstrate NE differentiation.
ADENOSQUAMOUS CARCINOMA	*Morphologic squamous cell and adenocarcinoma patterns present:* Non-small cell carcinoma, with squamous cell and adenocarcinoma patterns Comment: this could represent adenosquamous carcinoma.
No counterpart in 2004 WHO classification	*Morphologic squamous cell or adenocarcinoma patterns not present but immunostains favor separate glandular and adenocarcinoma components* Non-small cell carcinoma, NOS, (specify the results of the immunohistochemical stains and the interpretation) Comment: this could represent adenosquamous carcinoma.
Sarcomatoid carcinoma	Poorly differentiated NSCLC with spindle and/or giant cell carcinoma (mention if adenocarcinoma or squamous carcinoma are present)

IASLC, International Association for the Study of Lung Cancer; ATS, American Thoracic Society; ERS, European Respiratory Society; WHO, World Health Organization; NSCLC, non-small cell lung cancer; IHC, immunohistochemistry; TTF, thyroid transcription factor.

quality of the evidence expresses the confidence in an estimate of effect or an association and whether it is adequate to support a recommendation. After review of all articles, a writing committee met to develop the recommendations with each specialty group proposing the recommendations, votes for or against the recommendation, and modifications were conducted after multidisciplinary discussion. If randomized trials were available, we started by assuming high quality but down-graded the quality when there were serious methodological limitations, indirectness in population, inconsistency in results, imprecision in estimates, or a strong suspicion of publication bias. If well-done observational studies were available, low-quality evidence was assumed, but the quality was upgraded when there was a large treatment effect or a large association, all plausible residual confounders would diminish the effects, or if there was a dose-response gradient.[36] We developed considerations for good practice related to interventions that usually represent necessary and standard procedures of health care system—such as history taking and physical examination helping patients to make informed decisions, obtaining written consent, or the importance of good communication—when we considered them helpful. In that case, we did not perform a grading of the quality of evidence or strength of the recommendations.[38]

Meetings

Between March 2008 and December 2009, a series of meetings were held, mostly at Memorial Sloan Kettering Cancer Center, in New York, NY, to discuss issues related to

lung adenocarcinoma classification and to formulate this document. The core group established a uniform and consistent approach to the proposed types of lung adenocarcinoma.

Validation

Separate projects were initiated by individuals involved with this classification effort in an attempt to develop data to test the proposed system. These included projects on small biopsies,[39,40] histologic grading,[41–43] stage I adenocarcinomas,[44] small adenocarcinomas from Japan, international multiple pathologist project on reproducibility of recognizing major histologic patterns of lung adenocarcinoma,[45] molecular-histologic correlations, and radiologic-pathologic correlation focused on adenocarcinoma in situ (AIS), and minimally invasive adenocarcinoma (MIA).

The new proposals in this classification are based on the best available evidence at the time of writing this document. Nevertheless, because of the lack of universal diagnostic criteria in the literature, there is a need for future validation studies based on these standardized pathologic criteria with clinical, molecular, radiologic, and surgical correlations.

PATHOLOGIC CLASSIFICATION

Histopathology is the backbone of this classification, but lung cancer diagnosis is a multidisciplinary process requiring correlation with clinical, radiologic, molecular, and surgical information. Because of the multidisciplinary approach in developing this classification, we are recommending significant changes that should improve the diagnosis and classification of lung adenocarcinoma, resulting in therapeutic benefits.

Even after publication of the 1999 and 2004 WHO classifications,[4,5] the former term BAC continues to be used for a broad spectrum of tumors including (1) solitary small noninvasive peripheral lung tumors with a 100% 5-year survival,[46] (2) invasive adenocarcinomas with minimal invasion that have approximately 100% 5-year survival,[47,48] (3) mixed subtype invasive adenocarcinomas,[49–53] (4) mucinous and nonmucinous subtypes of tumors formerly known as BAC,[50–52,54,55] and (5) widespread advanced disease with a very low survival rate.[4,5] The consequences of confusion from the multiple uses of the former BAC term in the clinical and research arenas have been the subject of many reviews and editorials and are addressed throughout this document.[55–61]

Pathology Recommendation 1

We recommend discontinuing the use of the term "BAC." Strong recommendation, low-quality evidence.

Throughout this article, the term BAC (applicable to multiple places in the new classification, Table 3), will be referred to as "former BAC." We understand this will be a major adjustment and suggest initially that when the new proposed terms are used, it will be accompanied in parentheses by "(formerly BAC)." This transition will impact not only clinical practice and research but also cancer registries future analyses of registry data.

CLASSIFICATION FOR RESECTION SPECIMENS

Multiple studies have shown that patients with small solitary peripheral adenocarcinomas with pure lepidic growth may have 100% 5-year disease-free survival.[46,62–68] In addition, a growing number of articles suggest that patients with lepidic predominant adenocarcinomas (LPAs) with minimal invasion may also have excellent survival.[47,48] Recent work has demonstrated that more than 90% of lung adenocarcinomas fall into the mixed subtype according to the 2004 WHO classification, so it has been proposed to use comprehensive histologic subtyping to make a semiquantitative assessment of the percentages of the various histologic components: acinar, papillary, micropapillary, lepidic, and solid and to classify tumors according to the predominant histologic subtype.[69] This has demonstrated an improved ability to address the complex histologic heterogeneity of lung adenocarcinomas and to improve molecular and prognostic correlations.[69]

The new proposed lung adenocarcinoma classification for resected tumors is summarized in Table 1.

TABLE 3. Categories of New Adenocarcinoma Classification Where Former BAC Concept was Used

1. Adenocarcinoma in situ (AIS), which can be nonmucinous and rarely mucinous
2. Minimally invasive adenocarcinoma (MIA), which can be nonmucinous and rarely mucinous
3. Lepidic predominant adenocarcinoma (nonmucinous)
4. Adenocarcinoma, predominantly invasive with some nonmucinous lepidic component (includes some resected tumors, formerly classified as mixed subtype, and some clinically advanced adenocarcinomas formerly classified as nonmucinous BAC)
5. Invasive mucinous adenocarcinoma (formerly mucinous BAC)

BAC, bronchioloalveolar carcinoma.

Preinvasive Lesions

In the 1999 and 2004 WHO classifications, atypical adenomatous hyperplasia (AAH) was recognized as a preinvasive lesion for lung adenocarcinoma. This is based on multiple studies documenting these lesions as incidental findings in the adjacent lung parenchyma in 5 to 23% of resected lung adenocarcinomas[70–74] and a variety of molecular findings that demonstrate a relationship to lung adenocarcinoma including clonality,[75,76] *KRAS* mutation,[77,78] *KRAS* polymorphism,[79] *EGFR* mutation,[80] p53 expression,[81] loss of heterozygosity,[82] methylation,[83] telomerase overexpression,[84] eukaryotic initiation factor 4E expression,[85] epigenetic alterations in the *Wnt* pathway,[86] and FHIT expression.[87] Depending on the extensiveness of the search, AAH may be multiple in up to 7% of resected lung adenocarcinomas.[71,88]

A major change in this classification is the official recognition of AIS, as a second preinvasive lesion for lung adenocarcinoma in addition to AAH. In the category of preinvasive lesions, AAH is the counterpart to squamous dysplasia and AIS the counterpart to squamous cell carcinoma in situ.

Atypical Adenomatous Hyperplasia

AAH is a localized, small (usually 0.5 cm or less) proliferation of mildly to moderately atypical type II pneumocytes and/or Clara cells lining alveolar walls and sometimes, respiratory bronchioles (Figures 1*A, B*).[4,89,90] Gaps are

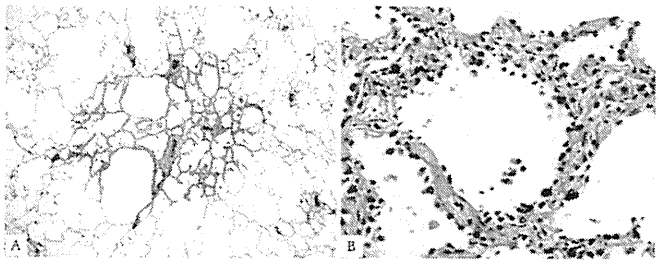

FIGURE 1. Atypical adenomatous hyperplasia. *A*, This 3-mm nodular lesion consists of atypical pneumocytes proliferating along preexisting alveolar walls. There is no invasive component. *B*, The slightly atypical pneumocytes are cuboidal and show gaps between the cells. Nuclei are hyperchromatic, and a few show nuclear enlargement and multinucleation.

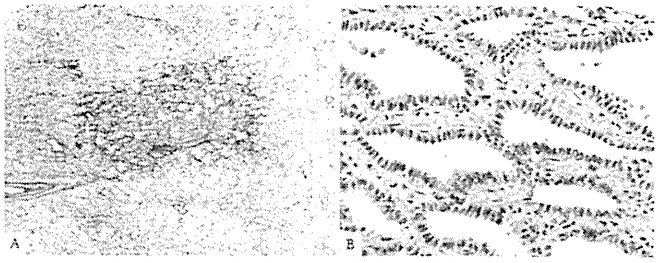

FIGURE 2. Nonmucinous adenocarcinoma in situ. *A*, This circumscribed nonmucinous tumor grows purely with a lepidic pattern. No foci of invasion or scarring are seen. *B*, The tumor shows atypical pneumocytes proliferating along the slightly thickened, but preserved, alveolar walls.

usually seen between the cells, which consist of rounded, cuboidal, low columnar, or "peg" cells with round to oval nuclei (Figure 1*B*). Intranuclear inclusions are frequent. There is a continuum of morphologic changes between AAH and AIS.[4,89,90] A spectrum of cellularity and atypia occurs in AAH. Although some have classified AAH into low- and high-grade types,[84,91] grading is not recommended.[4] Distinction between more cellular and atypical AAH and AIS can be difficult histologically and impossible cytologically.

AIS, Nonmucinous, and/or Mucinous

AIS (one of the lesions formerly known as BAC) is a localized small (≤3 cm) adenocarcinoma with growth restricted to neoplastic cells along preexisting alveolar structures (lepidic growth), lacking stromal, vascular, or pleural invasion. Papillary or micropapillary patterns and intraalveolar tumor cells are absent. AIS is subdivided into nonmucinous and mucinous variants. Virtually, all cases of AIS are nonmucinous, consisting of type II pneumocytes and/or Clara cells (Figures 2*A, B*). There is no recognized clinical significance to the distinction between type II or Clara cells, so this morphologic separation is not recommended. The rare cases of mucinous AIS consist of tall columnar cells with basal nuclei and abundant cytoplasmic mucin; sometimes they resemble goblet cells (Figures 3*A, B*). Nuclear atypia is absent or inconspicuous in both nonmucinous and mucinous

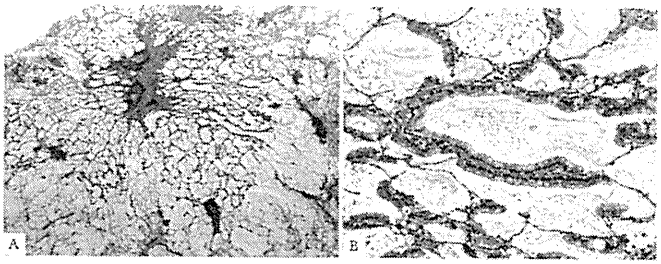

FIGURE 3. Mucinous adenocarcinoma in situ. *A*, This mucinous AIS consists of a nodular proliferation of mucinous columnar cells growing in a purely lepidic pattern. Although there is a small central scar, no stromal or vascular invasion is seen. *B*, The tumor cells consist of cuboidal to columnar cells with abundant apical mucin and small basally oriented nuclei. AIS, adenocarcinoma in situ.

AIS (Figures 2*B* and 3*B*). Septal widening with sclerosis is common in AIS, particularly the nonmucinous variant.

Tumors that meet criteria for AIS have formerly been classified as BAC according to the strict definition of the 1999 and 2004 WHO classifications and type A and type B adenocarcinoma according to the 1995 Noguchi classification.[4,46] Multiple observational studies on solitary lung adenocarcinomas with pure lepidic growth, smaller than either 2 or 3 cm have documented 100% disease-free survival.[46,62–68] Although most of these tumors are nonmucinous, 2 of the 28 tumors reported by Noguchi as types A and B in the 1995 study were mucinous.[46] Small size (≤3 cm) and a discrete circumscribed border are important to exclude cases with miliary spread into adjacent lung parenchyma and/or lobar consolidation, particularly for mucinous AIS.

Pathology Recommendation 2

For small (≤3 cm), solitary adenocarcinomas with pure lepidic growth, we recommend the term "Adenocarcinoma in situ" that defines patients who should have 100% disease-specific survival, if the lesion is completely resected (strong recommendation, moderate quality evidence).

Remark: Most AIS are nonmucinous, rarely are they mucinous.

MIA, Nonmucinous, and/or Mucinous

MIA is a small, solitary adenocarcinoma (≤3 cm), with a predominantly lepidic pattern and ≤5 mm invasion in greatest dimension in any one focus.[47,48,92] MIA is usually nonmucinous (Figures 4*A–C*) but rarely may be mucinous (Figures 5*A, B*).[44] MIA is, by definition, solitary and discrete. The criteria for MIA can be applied in the setting of multiple tumors only if the other tumors are regarded as synchronous primaries rather than intrapulmonary metastases.

The invasive component to be measured in MIA is defined as follows: (1) histological subtypes other than a lepidic pattern (i.e., acinar, papillary, micropapillary, and/or solid) or (2) tumor cells infiltrating myofibroblastic stroma. MIA is excluded if the tumor (1) invades lymphatics, blood vessels, or pleura or (2) contains tumor necrosis. If multiple microinvasive areas are found in one tumor, the size of the largest invasive area should be measured in the largest dimension, and it should be ≤5 mm

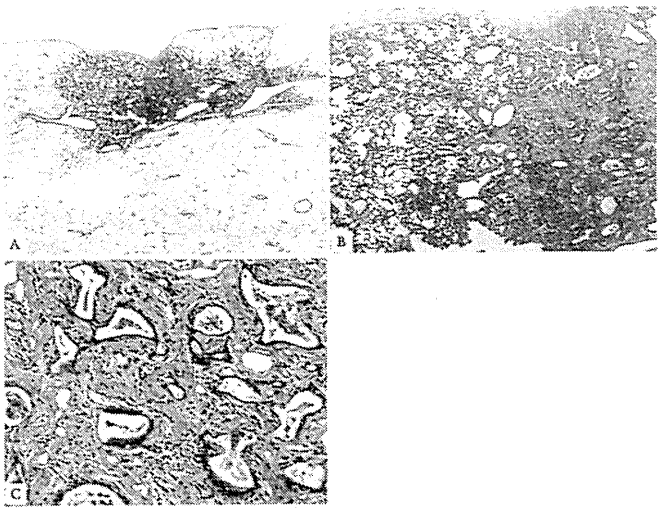

FIGURE 4. Nonmucinous minimally invasive adenocarcinoma. *A,* This subpleural adenocarcinoma tumor consists primarily of lepidic growth with a small (<0.5 cm) central area of invasion. *B,* To the left is the lepidic pattern and on the right is an area of acinar invasion. *C,* These acinar glands are invading in the fibrous stroma.

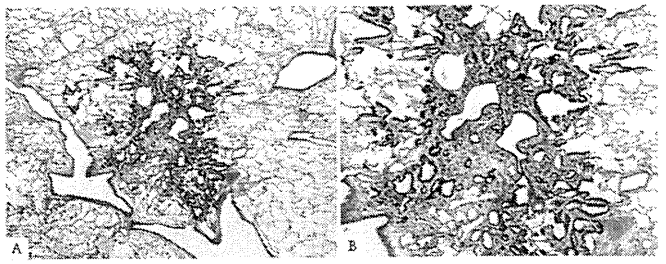

FIGURE 5. Mucinous minimally invasive adenocarcinoma. *A,* This mucinous MIA consists of a tumor showing lepidic growth and a small (<0.5 cm) area of invasion. *B,* The tumor cells consist of mucinous columnar cells growing mostly in a lepidic pattern along the surface of alveolar walls. The tumor invades the areas of stromal fibrosis in an acinar pattern. MIA, minimally invasive adenocarcinoma.

in size. The size of invasion is not the summation of all such foci, if more than one occurs. If the manner of histologic sectioning of the tumor makes it impossible to measure the size of invasion, an estimate of invasive size can be made by multiplying the total percentage of the invasive (nonlepidic) components times the total tumor size.

Evidence for a category of MIA with 100% disease-free survival can be found in the 1995 article by Noguchi et al., where vascular or pleural invasion was found in 10% of the small solitary lung adenocarcinomas that otherwise met the former definition of pure BAC. Even these focally invasive tumors also showed 100% disease-free survival.[46] Subsequent articles by Suzuki et al. and Sakurai et al.[19,21] defined subsets of small lung adenocarcinomas with 100% disease-free survival using scar size less than 5 mm and stromal invasion in the area of bronchioloalveolar growth, respectively. More recently, articles by Yim et al., Borczuk et al., and Maeshima et al.[47,48,92] have described patients with MIA defined similar to the above criteria, and these have demonstrated near 100% disease specific or very favorable overall survival. There is very limited data regarding mucinous MIA; however, this seems to exist. A mucinous MIA with a minor mixture of a nonmucinous component is being reported.[44] The recent report by Sawada et al.[93] of localized mucinous BAC may have included a few cases of mucinous AIS or MIA, but details of the pathology are not specific enough to be certain. A recent series of surgically resected solitary mucinous BAC did not document histologically whether focal invasion was present or not, so AIS versus MIA status cannot be determined, but all eight patients with tumors measuring ≤3 cm had 100% overall 5-year survival rates.[94] Presentation as a solitary mass, small size, and a discrete circumscribed border is important to exclude cases of miliary involvement of adjacent lung parenchyma and/or lobar consolidation, particularly for mucinous AIS.

Pathology Recommendation 3

For small (≤3 cm), solitary, adenocarcinomas with predominant lepidic growth and small foci of invasion measuring ≤0.5 cm, we recommend a new concept of "Minimally invasive adenocarcinoma" to define patients who have near 100%, disease-specific survival, if completely resected (strong recommendation, low-quality evidence).

Remark: Most MIA are nonmucinous, rarely are they mucinous.

Tumor Size and Specimen Processing Issues for AIS and MIA

The diagnosis of AIS or MIA cannot be firmly established without entire histologic sampling of the tumor. If tumor procurement is performed, it should be done strategically as discussed in the molecular section.

Because most of the literature on the topic of AIS and MIA deal with tumors 2.0 or 3.0 cm or less, there is insufficient evidence to support that 100% disease-free survival can occur in completely resected, solitary tumors suspected to be AIS or MIA that are larger than 3.0 cm. Until data validate 100% disease-free survival for completely resected, solitary, adenocarcinomas larger than 3.0 cm suspected to be AIS or MIA after complete sampling, the term "lepidic predominant adenocarcinoma, suspect AIS or MIA" is suggested. In such a tumor larger than 3.0 cm, particularly if it has not been completely sampled, the term "lepidic predominant adenocarcinoma" is best applied with a comment that the clinical behavior is uncertain and/or that an invasive component cannot be excluded.

Invasive Adenocarcinoma

As invasive adenocarcinomas represent more than 70 to 90% of surgically resected lung cases, one of the most important aspects of this classification is to present a practical way to address these tumors that are composed of a complex heterogeneous mixture of histologic subtypes. This complex mixture of histologic subtypes has presented one of the greatest challenges to classification of invasive lung adenocarcinomas. In recent years, multiple independent research groups have begun to classify lung adenocarcinomas according to the most predominant subtype.[43,44,69,95–102] This approach provides better stratifi-

cation of the "mixed subtype" lung adenocarcinomas according to the 1999/2004 WHO Classifications and has allowed for novel correlations between histologic subtypes and both molecular and clinical features.[43,44,69,95–102]

In the revised classification, the term "predominant" is appended to all categories of invasive adenocarcinoma, as most of these tumors consist of mixtures of the histologic subtypes (Figures 6*A–C*). This replaces the use of the term adenocarcinoma, mixed subtype. Semiquantitative recording of the patterns in 5% increments encourages the observer to identify all patterns that may be present, rather than focusing on a single pattern (i.e., lepidic growth). This method provides a basis for choosing the predominant pattern. Although most previous studies on this topic used 10% increments, using 5% allows for greater flexibility in choosing a predominant subtype when tumors have two patterns with relatively similar percentages; it also avoids the need to use 10% for small amounts of components that may be prognostically important such as micropapillary or solid patterns. Recording of these percentages also makes it clear to the reader of a report when a tumor has relatively even mixtures of several patterns versus a single dominant pattern. In addition, it provides a way to compare the histology of multiple adenocarcinomas (see later).[102] This approach may also provide a basis for architectural grading of lung adenocarcinomas.[43] A recent reproducibility study of classical and difficult selected images of the major lung adenocarcinoma subtypes circulated among a panel of 26 expert lung cancer pathologists documented kappa values of 0.77 ± 0.07 and 0.38 ± 0.14, respectively.[45] This study did not test recognition of predominant subtype.

Pathology Recommendation 4

For invasive adenocarcinomas, we suggest comprehensive histologic subtyping be used to assess histologic patterns semiquantitatively in 5% increments, choosing a single predominant pattern. Individual tumors are then classified according to the predominant pattern and the percentages of the subtypes are also reported (weak recommendation, low-quality evidence).

Histologic Comparison of Multiple Adenocarcinomas and Impact on Staging

Comprehensive histologic subtyping can be useful in comparing multiple lung adenocarcinomas to distinguish multiple primary tumors from intrapulmonary metastases. This has a great impact on staging for patients with multiple lung adenocarcinomas. Recording the percentages of the various histologic types in 5% increments, not just the most predominant type, allows these data to be used to compare multiple adenocarcinomas, particularly if the slides of a previous tumor are not available at the time of review of the additional lung tumors.[102] In addition to comprehensive histologic subtyping, other histologic features of the tumors such as cytologic (clear cell or signet ring features) or stromal (desmoplasia or inflammation) characteristics may be helpful to compare multiple tumors.[102]

Pathology Recommendation 5

In patients with multiple lung adenocarcinomas, we suggest comprehensive histologic subtyping may facilitate in the comparison of the complex, heterogeneous mixtures of histologic patterns to determine whether the tumors are metastases or separate synchronous or metachronous primaries (weak recommendation, low-quality evidence).

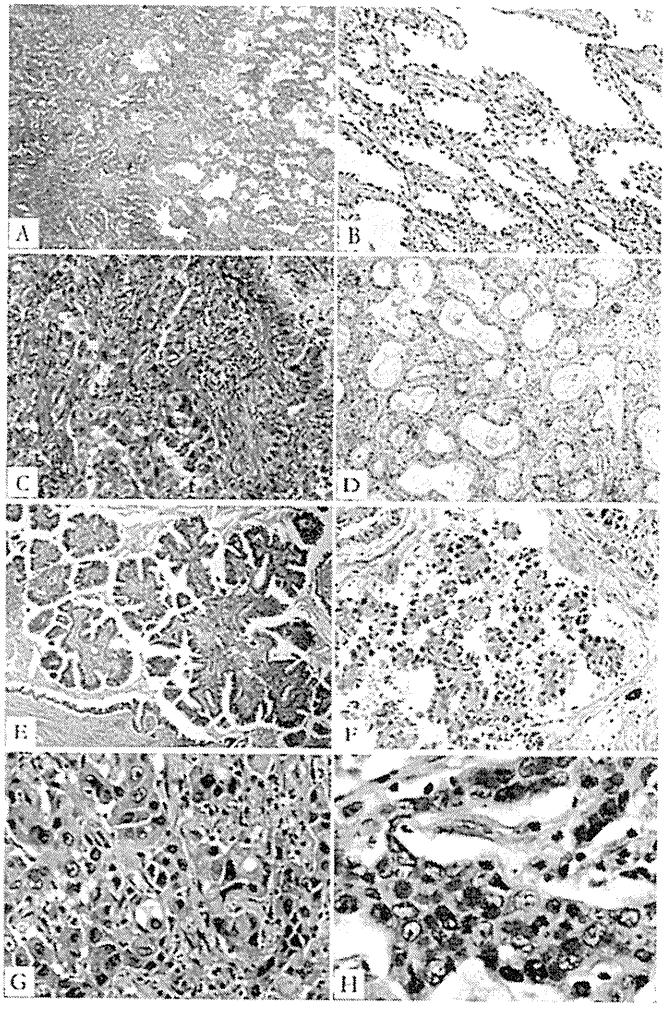

FIGURE 6. Major histologic patterns of invasive adenocarcinoma. *A*, Lepidic predominant pattern with mostly lepidic growth (right) and a smaller area of invasive acinar adenocarcinoma (left). *B*, Lepidic pattern consists of a proliferation type II pneumocytes and Clara cells along the surface alveolar walls. *C*, Area of invasive acinar adenocarcinoma (same tumor as in *A* and *B*). *D*, Acinar adenocarcinoma consists of round to oval-shaped malignant glands invading a fibrous stroma. *E*, Papillary adenocarcinoma consists of malignant cuboidal to columnar tumor cells growing on the surface of fibrovascular cores. *F*, Micropapillary adenocarcinoma consists of small papillary clusters of glandular cells growing within this airspace, most of which do not show fibrovascular cores. *G*, Solid adenocarcinoma with mucin consisting of sheets of tumor cells with abundant cytoplasm and mostly vesicular nuclei with several conspicuous nucleoli. No acinar, papillary, or lepidic patterns are seen, but multiple cells have intracytoplasmic basophilic globules that suggest intracytoplasmic mucin. *H*, Solid adenocarcinoma with mucin. Numerous intracytoplasmic droplets of mucin are highlighted with this DPAS stain. DPAS, diastase-periodic acid Schiff.

LPA typically consists of bland pneumocytic cells (type II pneumocytes or Clara cells) growing along the surface of alveolar walls similar to the morphology defined in the above section on AIS and MIA (Figures 6*A*, *B*). Invasive adenocarcinoma is present in at least one focus measuring more than 5 mm in greatest dimension. Invasion is defined as (1) histological subtypes other than a lepidic pattern (i.e., acinar, papillary, micropapillary, and/or solid) or (2) myofibroblastic stroma associated with invasive tumor cells (Figure 6*C*). The diagnosis of LPA rather than MIA is made if the tumor (1) invades lymphatics, blood vessels, or pleura or (2) contains tumor necrosis. It is understood that lepidic growth can occur in metastatic tumors and invasive mucinous adenocarcinomas. Nevertheless, the specific term "Lepidic predominant adenocarcinoma (LPA)" in this classification defines a nonmucinous adenocarcinoma that has lepidic growth as its predominant component, and these tumors are now separated from invasive mucinous adenocarcinoma. The term LPA should not be used in the context of invasive mucinous adenocarcinoma with predominant lepidic growth.

In the categories of mixed subtype in the 1999/2004 WHO classifications and type C in the Noguchi classification,[4,46] there was no assessment of the percentage of lepidic growth (former BAC pattern), so in series diagnosed according to these classification systems, most of the LPAs are buried among a heterogeneous group of tumors that include predominantly invasive adenocarcinomas. Nevertheless, several studies have shown lepidic growth to be associated with more favorable survival in small solitary resected lung adenocarcinomas with an invasive component.[47,64,103–105] One recent study of stage I adenocarcinomas using this approach demonstrated 90% 5-year recurrence free survival.[44]

Pathology Recommendation 6

For nonmucinous adenocarcinomas previously classified as mixed subtype where the predominant subtype consists of the former nonmucinous BAC, we recommend use of the term LPA and discontinuing the term "mixed subtype" (strong recommendation, low-quality evidence).

Acinar predominant adenocarcinoma shows a majority component of glands, which are round to oval shaped with a central luminal space surrounded by tumor cells (Figure 6*D*).[4] The neoplastic cells and glandular spaces may contain mucin. Acinar structures also may consist of rounded aggregates of tumor cells with peripheral nuclear polarization with central cytoplasm without a clear lumen. AIS with collapse may be difficult to distinguish from the acinar pattern. Nevertheless, when the alveolar architecture is lost and/or myofibroblastic stroma is present, invasive acinar adenocarcinoma is considered present. Cribriform arrangements are regarded as a pattern of acinar adenocarcinoma.[106]

Papillary predominant adenocarcinoma shows a major component of a growth of glandular cells along central fibrovascular cores (Figure 6*E*).[4] This should be distinguished from tangential sectioning of alveolar walls in AIS. If a tumor has lepidic growth, but the alveolar spaces are filled with papillary structures, the tumor is classified as papillary adenocarcinoma. Myofibroblastic stroma is not needed to diagnose this pattern.

Micropapillary predominant adenocarcinoma has tumor cells growing in papillary tufts, which lack fibrovascular cores (Figure 6*F*).[4] These may appear detached and/or connected to alveolar walls. The tumor cells are usually small and cuboidal with minimal nuclear atypia. Ring-like glandular structures may "float" within alveolar spaces. Vascular invasion and stromal invasion are frequent. Psammoma bodies may be seen.

The micropapillary pattern of lung adenocarcinoma was cited in the 2004 WHO classification in the discussion,[4] but there were too few publications on this topic to introduce it as a formal histologic subtype.[107–109] Although most of the studies have used a very low threshold for classification of adenocarcinomas as micropapillary, including as low as 1 to 5%,[108,109] it has recently been demonstrated that tumors classified as micropapillary according to the predominant subtype also have a poor prognosis similar to adenocarcinomas with a predominant solid subtype.[44] All articles on the topic of micropapillary lung adenocarcinoma in early-stage patients have reported data indicating that this is a poor prognostic subtype.[95,108–119] Additional evidence for the aggressive behavior of this histologic pattern is the overrepresentation of the micropapillary pattern in metastases compared with the primary tumors, where it sometimes comprises only a small percentage of the overall tumor.[43]

Pathology Recommendation 7

In patients with early-stage adenocarcinoma, we recommend the addition of "micropapillary predominant adenocarcinoma," when applicable, as a major histologic subtype due to its association with poor prognosis (strong recommendation, low-quality evidence).

Solid predominant adenocarcinoma with mucin production shows a major component of polygonal tumor cells forming sheets, which lack recognizable patterns of adenocarcinoma, i.e., acinar, papillary, micropapillary, or lepidic growth (Figure 6*G*).[4] If the tumor is 100% solid, intracellular mucin should be present in at least five tumor cells in each of two high-power fields, confirmed with histochemical stains for mucin (Figure 6*H*).[4] Solid adenocarcinoma must be distinguished from squamous cell carcinomas and large cell carcinomas both of which may show rare cells with intracellular mucin.

Variants

Rationale for Changes in Adenocarcinoma Histologic Variants

Rationale for separation of invasive mucinous adenocarcinoma (formerly mucinous BAC) from nonmucinous adenocarcinomas. Multiple studies indicate that tumors formerly classified as mucinous BAC have major clinical, radiologic, pathologic, and genetic differences from the tumors formerly classified as nonmucinous BAC (Table 4).[55,77,120,121,125–127,136,145–148] In particular, these tumors show a very strong correlation with *KRAS* mutation, whereas nonmucinous adenocarcinomas are more likely to show *EGFR* mutation and only occasionally *KRAS* mutation (Table 4). Therefore, in

TABLE 4. Difference between Invasive Mucinous Adenocarcinoma and Nonmucinous Adenocarcinoma In Situ/Minimally Invasive Adenocarcinoma/Lepidic Predominant Adenocarcinoma

	Invasive Mucinous Adenocarcinoma (Formerly Mucinous BAC)	Nonmucinous AIS/MIA/LPA (Formerly Nonmucinous BAC)
Female	49/84 (58%)[52,120–123]	101/140 (72%)[52,120–123]
Smoker	39/87 (45%)[52,120–122,124]	75/164 (46%)[52,120–122,124]
Radiographic appearance	Majority consolidation; air bronchogram[125] Frequent multifocal and multilobar presentation[56,125–128]	Majority ground-glass attenuation[23,56,58,103,129–134]
Cell type	Mucin-filled, columnar, and/or goblet[50–52,125,135]	Type II pneumocyte and/or Clara cell[50–52,125,135]
Phenotype		
CK7	Mostly positive (~88%)[a54,55,136–139]	Positive (~98%)[a54,55,136–139]
CK20	Positive (~54%)[a54,55,136–139]	Negative (~5%)[a54,55,136–139]
TTF-1	Mostly negative (~17%)1[a54,55,120,137–139]	Positive (~67%)[a54,55,120,137–139]
Genotype		
KRAS mutation	Frequent (~76%)[a55,94,121,127,140–144]	Some (~13%)[a55,121,127,140–144]
EGFR mutation	Almost none (~3)[a55,121,127,140–142]	Frequent (~45%)[a55,121,127,140–142]

[a] Numbers represent the percentage of cases that are reported to be positive.

BAC, bronchioloalveolar carcinoma; AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; LPA, lepidic predominant adenocarcinoma; EGFR, epidermal growth factor receptor; TTF, thyroid transcription factor.

the new classification, these tumors are now separated into different categories (Table 1). The neoplasms formerly termed mucinous BAC, now recognized to have invasive components in the majority of cases, are classified as invasive mucinous adenocarcinoma (formerly mucinous BAC).[149]

Rationale for including mucinous cystadenocarcinoma in colloid adenocarcinoma. Tumors formerly classified as "Mucinous cystadenocarcinoma" are very rare, and they probably represent a spectrum of colloid adenocarcinoma. Therefore, we suggest that these adenocarcinomas that consist of uni- or oligolocular cystic structures by imaging and/or gross examination be included in the category of colloid adenocarcinoma.[150] For such tumors, a comment could be made that the tumor resembles that formerly classified as mucinous cystadenocarcinoma.

Rationale for removing clear cell and signet ring carcinoma as adenocarcinoma subtypes. Clear cell and signet ring cell features are now regarded as cytologic changes that may occur in association with multiple histologic patterns.[151,152] Thus, their presence and extent should be recorded, but data are not available that show a clinical significance beyond a strong association with the solid subtype. They are not considered to be specific histologic subtypes, although associations with molecular features are possible such as the recent observation of a solid pattern with more than 10% signet ring cell features in up to 56% of tumors from patients with echinoderm microtubule-associated protein-like 4 (*EML4*) and anaplastic lymphoma kinase (ALK) gene fusions (*EML4-ALK*).[153]

Rationale for adding enteric adenocarcinoma. Enteric adenocarcinoma is added to the classification to draw attention to this rare histologic type of primary lung adenocarcinoma that can share some morphologic and immunohistochemical features with colorectal adenocarcinoma.[154] Because of these similarities, clinical evaluation is needed to exclude a gastrointestinal primary. It is not known whether there are any distinctive clinical or molecular features.

Histologic Features

Invasive mucinous adenocarcinoma (formerly mucinous BAC) has a distinctive histologic appearance with tumor cells having a goblet or columnar cell morphology with abundant intracytoplasmic mucin (Figures 7*A, B*). Cytologic atypia is usually inconspicuous or absent. Alveolar spaces often contain mucin. These tumors may show the same heterogeneous mixture of lepidic, acinar, papillary, micropapillary, and solid growth as in nonmucinous tumors. The clinical significance of reporting semiquantitative estimates of subtype percentages and the predominant histologic subtype similar to nonmucinous adenocarcinomas is not certain. When stromal invasion is seen, the malignant cells may show less cytoplasmic mucin and more atypia. These tumors differ from mucinous AIS and MIA by one or more of the following criteria: size (>3 cm), amount of invasion (>0.5 cm), mul-

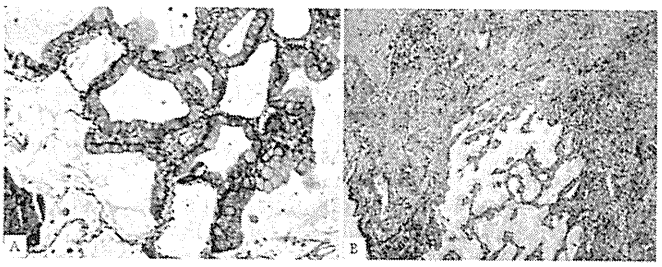

FIGURE 7. Invasive mucinous adenocarcinoma. *A*, This area of invasive mucinous adenocarcinoma demonstrates a pure lepidic growth. The tumor consists of columnar cells filled with abundant mucin in the apical cytoplasm and shows small basal oriented nuclei. *B*, Nevertheless, elsewhere this tumor demonstrated invasion associated with desmoplastic stroma and an acinar pattern.