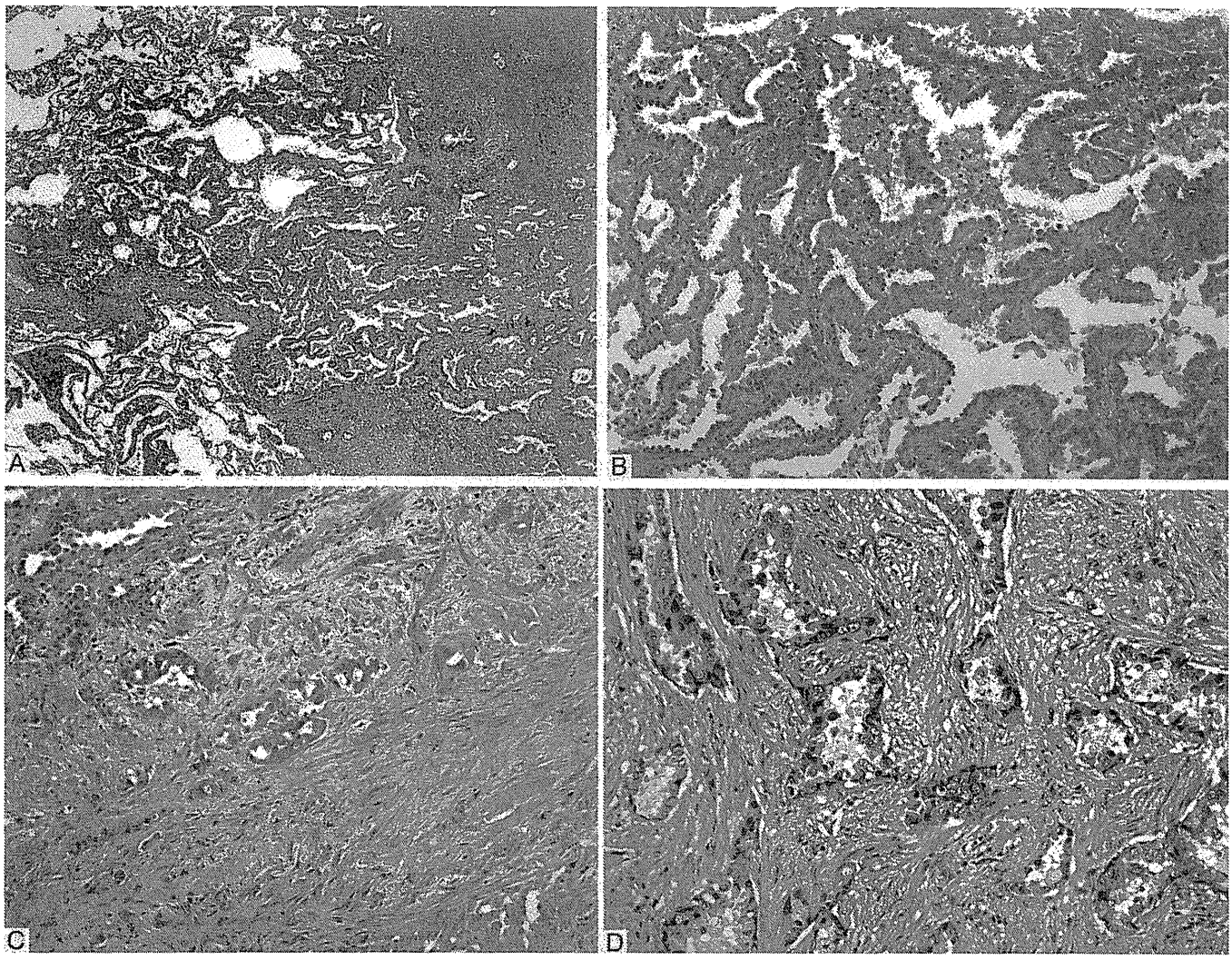

Figure 6. *Lepidic-predominant and acinar adenocarcinoma. A, Lepidic-predominant pattern with mostly lepidic growth (right) and a smaller area of invasive acinar adenocarcinoma (left). B, Lepidic pattern consists of a proliferation of type II pneumocytes and Clara cells along the surface of alveolar walls. C, Area of invasive acinar adenocarcinoma (same tumor as in A and B). D, Acinar adenocarcinoma consists of round to oval malignant glands invading a fibrous stroma (hematoxylin-eosin, original magnifications ×4 [A], ×20 [B and D], and ×10 [C].*

patient in order 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.[21] 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.[21]

Pathology Recommendation 5.—In patients with multiple lung adenocarcinomas, we suggest that comprehensive histologic subtyping may facilitate comparison of the complex, heterogeneous mixtures of histologic patterns for determining if the tumors are metastases or separate synchronous or metachronous primary tumors (weak recommendation, low-quality evidence).

Lepidic-predominant adenocarcinoma typically consists of bland pneumocytic cells (type II pneumocytes or Clara cells) growing along the surface of alveolar walls with morphology similar to that defined in the above section on AIS and MIA (Figure 6, A and B). Invasive adenocarcinoma is present in at least 1 focus, measuring more than 5 mm in greatest dimension. Invasion is defined as (1) histologic subtypes other than a lepidic pattern (ie, acinar, papillary, micropapillary, and/or solid) and/or (2) myofibroblastic stroma associated with invasive tumor cells (Figure 6, C). The diagnosis of lepidic-predominant adenocarcinoma rather than MIA is made if the cancer (1) invades lymphatics, blood vessels, or pleura or (2) contains tumor necrosis. It is understood that lepidic growth can occur in metastatic tumors as well as in invasive mucinous adenocarcinomas. However, the specific term *lepidic-predominant adenocarcinoma* 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 *lepidic-predominant adenocarcinoma* should not be used in the

context of invasive mucinous adenocarcinoma with predominant lepidic growth.

Lepidic growth may also be composed of neoplastic cells with nuclear atypia resembling that of the adjacent invasive patterns. Whether there is any clinical implication is unknown, that is, it is not established if this is in situ or invasive carcinoma. This point is made in the classification as a research recommendation to encourage further investigation of this issue (Table 5).

In the categories of mixed subtype in the 1999[2]/2004[3] WHO classifications and of type C in the Noguchi classification,[5] respectively, there was no assessment of the percentage of lepidic growth (former BAC pattern); therefore, in published series diagnosed according to these classification systems, most of the lepidic-predominant adenocarcinomas are buried among a heterogeneous group of tumors that include predominantly invasive adenocarcinomas. However, several studies[25,63,91–93] have shown that lepidic growth is associated with more favorable survival in cases of small solitary resected lung adenocarcinomas with an invasive component. Using this approach, several recent studies of early stage adenocarcinomas[8,20,84] demonstrated excellent outcome for these patients, with as high as 86% to 90% 5-year recurrence-free survival.

Pathology Recommendation 6.—For nonmucinous adenocarcinomas previously classified as mixed subtype, for which the predominant subtype consists of the former nonmucinous BAC, we recommend use of the term *lepidic-predominant adenocarcinoma* and discontinuation of the term *mixed subtype* (strong recommendation, low-quality evidence).

Acinar-predominant adenocarcinoma shows a majority component of glands, which are round to oval with a central luminal space surrounded by tumor cells (Figure 6, D).[3] The neoplastic cells and/or 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. Adenocarcinoma in situ with collapse may be difficult to distinguish from the acinar pattern. However, 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.[94]

Papillary-predominant adenocarcinoma shows a major component of a growth of glandular cells along central fibrovascular cores (Figure 7, A and B).[3] This should be distinguished from tangential sectioning of alveolar walls in an area of lepidic adenocarcinoma. 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 (florets that lack fibrovascular cores; Figure 7, C and D).[3] These may appear detached and/or connected to alveolar walls. The tumor cells are usually small and cuboidal with minimal nuclear atypia. Ringlike glandular structures may "float" within alveolar spaces. Vascular and stromal invasion is frequent. Psammoma bodies may be seen.

The micropapillary pattern of lung adenocarcinoma was cited in the 2004 WHO classification[3] in the discussion, but there were too few publications on this topic to introduce it as a formal histologic subtype.[9,10,95] While most of the studies have used a very low threshold for classification of adenocarcinomas as micropapillary, including as low as 1% to 5%,[9,10] recent reports[8,20,84,87] have demonstrated that tumors classified as micropapillary, according to the predominant subtype, also have a poor prognosis similar to adenocarcinomas with a predominant solid subtype. All articles on the topic of micropapillary lung adenocarcinoma in patients with early-stage disease have reported data indicating this is a poor prognostic subtype.[8–10,75,78,96–103]

Additional evidence for the aggressive behavior of this histologic pattern is the overrepresentation of the micropapillary pattern in metastases compared to the primary tumors, where it sometimes comprises only a small percentage of the overall tumor.[78] The clinical significance of minor micropapillary components in primary lung adenocarcinomas that are not micropapillary predominant needs further study.

Pathology Recommendation 7.—For patients with early-stage adenocarcinoma, we recommend the addition of "micropapillary-predominant adenocarcinoma," when applicable, as a major histologic subtype owing to its association with poor prognosis in early-stage disease (strong recommendation, low-quality evidence).

Solid-predominant adenocarcinoma with mucin production shows a major component of polygonal tumor cells forming sheets that lack recognizable patterns of adenocarcinoma, that is, acinar, papillary, micropapillary, or lepidic growth (Figure 8, A through C).[3] If the tumor is 100% solid, intracellular mucin should be present in at least 5 tumor cells in each of 2 high-power fields, confirmed with histochemical stains for mucin (Figure 8, B).[3] Solid adenocarcinoma must be distinguished from squamous cell carcinomas and large cell carcinomas, both of which may show rare cells with intracellular mucin. Some solid adenocarcinomas have dense eosinophilic cytoplasm that resembles that of squamous cell carcinoma with a "pseudosquamous" morphology. Even in resection specimens, in poorly differentiated tumors that have a suggestion of squamous morphology (Figure 8, A) but lack clear squamous morphology, such as keratinization, pearls, or bridges, immunohistochemistry may be indicated with an adenocarcinoma marker such as thyroid transcription factor-1 (TTF-1) (Figure 8, C) and a squamous marker, such as p63 or the recently described p40, which is an isomer of p63 with greater specificity for squamous cell carcinoma.[104]

Neuroendocrine immunohistochemical markers should only be used in cases for which there is suspected neuroendocrine morphology. If neuroendocrine morphology is not suspected, neuroendocrine markers should not be used.

VARIANTS

Rationale for Changes in Adenocarcinoma Histologic Variants

Rationale for Separation of Invasive Mucinous Adenocarcinoma (Formerly Mucinous BAC) from Nonmucinous Adenocarcinomas.—Multiple studies[11,13–15,17,105–109] indicate that tumors formerly classified as mucinous BAC have major

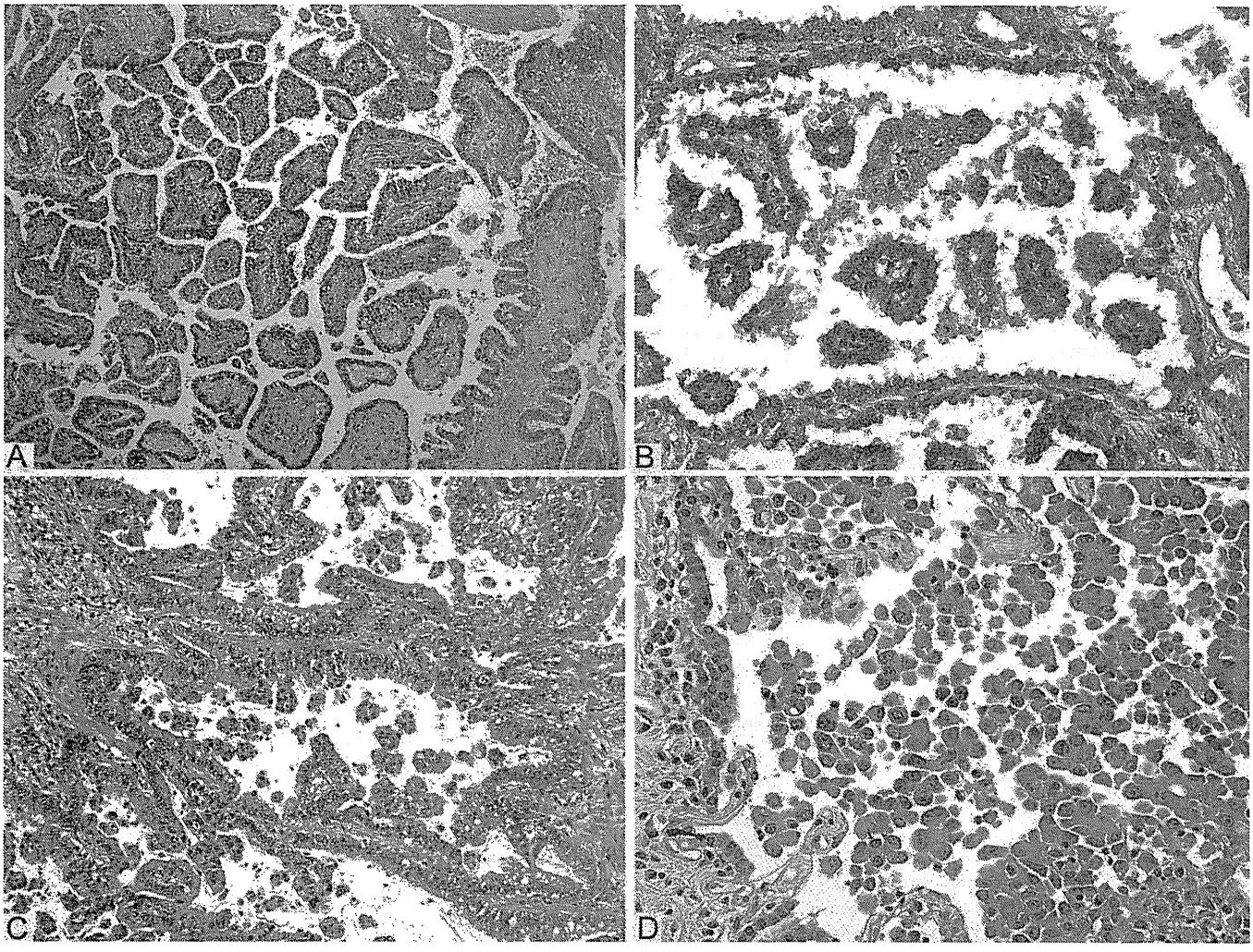

Figure 7. *Papillary and micropapillary adenocarcinoma. A, Papillary adenocarcinoma consists of malignant cuboidal to columnar tumor cells growing on the surface of fibrovascular cores. B, Papillary adenocarcinoma consisting of intra-alveolar papillary structures with fibrovascular cores. Although there is a cuboidal lining of tumor cells around the airspace in a lepidic fashion, this pattern should be classified as papillary adenocarcinoma. C, Micropapillary adenocarcinoma. Within the airspaces the tumor is growing in papillary structures lacking fibrovascular cores. Although there are some true papillary areas with fibrovascular cores, and some tumor cells growing in a lepidic pattern along the surfaces of the airspaces, most of this pattern should be regarded as micropapillary. D, Micropapillary adenocarcinoma. This tumor is spreading through the alveolar space with a spectrum of small papillary structures lacking fibrovascular cores to single dyscohesive cells (hematoxylin-eosin, original magnifications ×4 [A], ×20 [B and C], and ×40 [D]).*

clinical, radiologic, pathologic, and genetic differences from the tumors formerly classified as nonmucinous BAC (Table 6). In particular, these tumors show a very strong correlation with *KRAS* mutation and lack of *EGFR* mutation, while nonmucinous adenocarcinomas are more likely to show *EGFR* mutation and only occasionally *KRAS* mutation (Table 6). Therefore, in the new classification, these tumors are now separated into different categories (Table 1). The neoplasms formerly termed *mucinous bronchioloalveolar carcinoma* (*mucinous BAC*), are now recognized as having invasive components in most cases, and are classified as "invasive mucinous adenocarcinoma (formerly mucinous BAC)," or mucinous AIS or MIA if they meet the criteria outlined in Tables 2 and 3.

Rationale for Including Mucinous Cystadenocarcinoma in Colloid Adenocarcinoma.—Tumors formerly classified as "mucinous cystadenocarcinoma" are very rare and they probably represent part of the spectrum of colloid adenocarcinoma. Therefore, we suggest that these adenocarcinomas, which consist of unilocular or oligolocular cystic structures by imaging and/or gross examination, be included in the category of colloid adenocarcinoma.[110] 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.[111,112] 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 greater 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

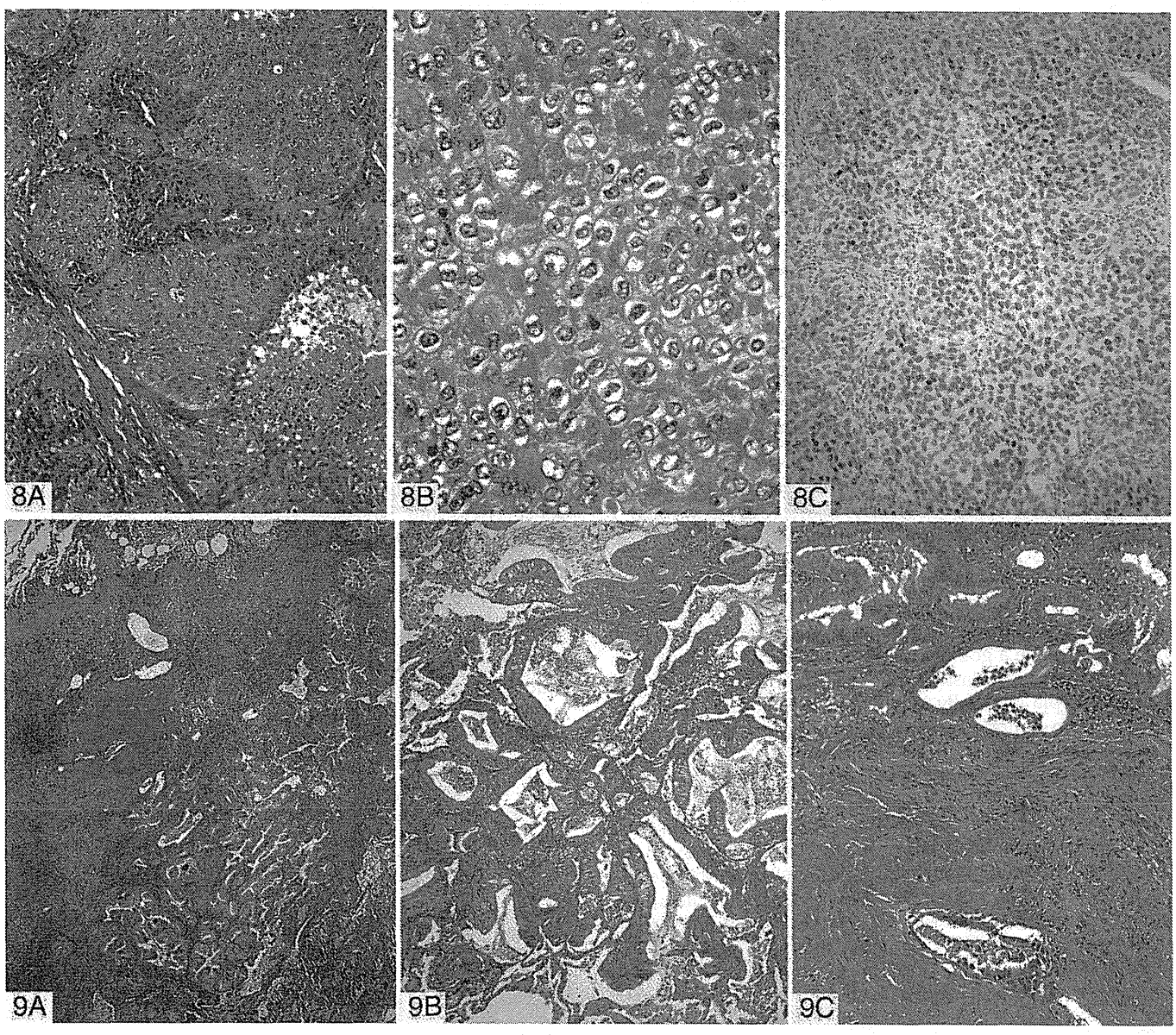

Figure 8. *Solid adenocarcinoma with mucin and pseudosquamous morphology. A, This tumor consists of sheets of tumor cells with abundant cytoplasm and mostly vesicular nuclei with several conspicuous nucleoli. Because this tumor had prominent eosinophilic cytoplasm, it was originally classified as squamous cell carcinoma. No acinar, papillary, or lepidic patterns are seen and there was no suggestion of mucin in tumor cell cytoplasm. B, The tumor showed foci of strong staining for intracytoplasmic mucin in numerous tumor cells. C, Thyroid transcription factor-1 (TTF-1) was diffusely and strongly positive. This tumor had an EGFR exon 19 deletion (hematoxylin-eosin, original magnification ×10 [A]; mucicarmine, original magnification ×40 [B]; TTF-1, original magnification ×20 [C]).*

Figure 9. *Invasive mucinous adenocarcinoma. A, This area of invasive mucinous adenocarcinoma demonstrates areas with lepidic, acinar, and papillary patterns. In addition, there is a fibrotic focus that contains invasive tumor with a desmoplastic stroma. B, The tumor consists of columnar cells filled with abundant mucin in the apical cytoplasm and shows small, basally oriented nuclei. This area shows mostly lepidic growth but also areas suggesting an acinar pattern. C, This photomicrograph highlights a focus of invasion with desmoplastic stroma from A. The invasive area shows an acinar pattern and the tumor cells show less cytoplasmic mucin (hematoxylin-eosin, original magnifications ×4 [A], ×10 [B], and ×20 [C]).*

(EML4-ALK).[113] Rather than diminishing the recognition of these features, this approach will now record any percentage (even <5%) of clear cell or signet ring features, whereas in the previous WHO classifications, the amount needed to be substantial, at the level of a histologic subtype, before it would be included in the diagnosis.

Rationale for Adding Enteric Adenocarcinoma.—Enteric adenocarcinoma is added to the classification to draw attention to this rare histologic type of primary lung adenocarcinoma, which can share some morphologic and immunohistochemical features with colorectal adenocarcinoma.[114] Owing to these similarities, clinical evaluation is needed to exclude a gastrointestinal primary tumor. It is not known if there are any distinctive clinical or molecular features.

Rationale for Maintaining Fetal Adenocarcinoma.—Fetal adenocarcinomas are maintained in this classification with the recognition that low-grade fetal adenocarcinomas are most commonly seen in the fourth decade of life with a slight female

Table 6. Difference Between Invasive Mucinous Adenocarcinoma and Nonmucinous Adenocarcinoma In Situ (AIS)/ Minimally Invasive Adenocarcinoma (MIA)/Lepidic-Predominant Adenocarcinoma (LPA)

Characteristics	Invasive Mucinous Adenocarcinoma (Formerly Mucinous BAC)	Nonmucinous AIS/MIA/LPA (Formerly Nonmucinous BAC)
Female, No. (%)	49/84 (58)[11,17,30,142,143]	101/140 (72)[11,17,30,142,143]
Smoker, No. (%)	39/87 (45)[11,17,30,142,144]	75/164 (46)[11,17,30,142,144]
Radiographic appearance	Majority consolidation; air bronchogram;[13] frequent multifocal and multilobar presentation[12–14,106,108,145,146]	Majority ground-glass attenuation[13,14,37,147–151]
Cell type	Mucin filled, columnar, and/or goblet[13,27,28,30,152]	Type II pneumocyte and/or Clara cell[13,27,28,30,152]
Immunophenotype		
CK7	Mostly positive[a] (+, ≈88%)[14,16,153–156]	Positive[a] (+, ≈98%)[14,16,153–156]
CK20	Positive[a] (+, ≈54%)[14,16,153–156]	Mostly negative[a] (+, ≈5%)[14,16,153–156]
TTF-1	Mostly negative[a] (+, ≈17%)[14,16,17,153,154,156,157]	Positive[a] (+, ≈67%)[14,16,17,153,154,156,157]
Genotype		
KRAS mutation	Frequent[a] (+, ≈76%)[14,15,106,158–160]	Uncommon[a] (+, ≈13%)[11,14,15,106,158–161]
EGFR mutation	Almost none[a] (+, ≈3%)[11,14,15,106,158–161]	Frequent[a] (+, ≈45%)[11,14,15,106,158–161]

Abbreviations: BAC, bronchioloalveolar carcinoma; CK, cytokeratin; TTF-1, thyroid transcription factor-1.

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

preponderance, whereas high-grade fetal adenocarcinomas are most commonly seen in elderly males, suggesting the 2 subtypes may have different oncogenic pathways.[115–117]

Histologic Features of Variant Subtypes

Invasive mucinous adenocarcinoma (formerly mucinous BAC) has a distinctive histologic appearance in which the tumor cells have a goblet or columnar cell morphology with abundant intracytoplasmic mucin (Figure 9, A through C). Nuclear 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 (Figure 9, B and C). 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 (Figure 9, C). These tumors differ from mucinous AIS and MIA by 1 or more of the following criteria: size (>3 cm), amount of invasion (>0.5 cm), multiple nodules, or lack of a circumscribed border with miliary spread into adjacent lung parenchyma.

There is a strong tendency for multicentric, multilobar, and bilateral lung involvement, which may reflect aerogenous spread. Mixtures of mucinous and nonmucinous tumors may rarely occur; if so, the percentage of invasive mucinous adenocarcinoma should be recorded in a comment. If there is at least 10% of each component, it should be classified as "mixed invasive mucinous and nonmucinous adenocarcinoma" with a description of the various components that comprise the tumor.

Invasive mucinous adenocarcinomas (formerly mucinous BAC) need to be distinguished from adenocarcinomas that produce mucin but lack the characteristic goblet cell or columnar cell morphology of the tumors that have historically been classified as mucinous BAC. When mucin is identified by light microscopy or mucin stains in adenocarcinomas that do not meet the above criteria, this feature should be reported in a comment after classifying the tumor according to the appropriate terminology and criteria proposed in this classification. This can be done by adding a descriptive phrase such as "with mucin production" or "with mucinous features," rather than the term *invasive mucinous adenocarcinoma*. Because of the multiple ways mucin can be expressed in lung adenocarcinomas, the specific wording "invasive mucinous adenocarcinoma" is important for this diagnosis.

Metastatic mucinous adenocarcinomas from sites such as the pancreas and ovary can appear morphologically identical to pulmonary invasive mucinous adenocarcinomas; therefore, clinical and radiologic correlation should be made to exclude primary tumors in these locations. Pancreatic mucinous adenocarcinomas are more likely to express cytokeratin (CK) 20 and mucin 2 (MUC2).[118] Metastatic colorectal adenocarcinomas often express caudal-related homeobox 2 (CDX-2) and CK20 with lack of CK7.

Pathology Recommendation 8.—For adenocarcinomas formerly classified as mucinous BAC, we recommend they be separated from the adenocarcinomas formerly classified as nonmucinous BAC and, depending on the extent of lepidic versus invasive growth, that they be classified as mucinous AIS, mucinous MIA, or for overtly invasive tumors, as "invasive mucinous adenocarcinoma" (weak recommendation, low-quality evidence).

Colloid adenocarcinoma shows abundant extracellular mucin in pools, which distend alveolar spaces and destroy their walls (Figure 10, A and B). The mucin pools contain clusters of mucin-secreting tumor cells, which may comprise only a small percentage of the total tumor volume (or area) and thus be inconspicuous (Figure 10, A).[119,120] The tumor cells may consist of goblet cells or other mucin-secreting cells and may form a single layer along fibrous septa (Figure 10, B). Colloid adenocarcinoma is found more often as a mixture with other adenocarcinoma histologic subtypes rather than as a pure pattern. A tumor is classified as a colloid adenocarcinoma when it is the predominant component; the percentages of other components should be recorded.[110] Cystic gross and or histologic features are included in the spectrum of colloid adenocarcinoma, but in most cases this is a focal feature. Cases previously reported as mucinous cystadenocarcinoma are extremely rare and now these should be classified as colloid adenocarcinoma with cystic changes. The cysts are filled with mucin, and lined by goblet or other mucin-secreting cells. The lining epithelium may be discontinuous and replaced with inflammation including a granulomatous reaction or gran-

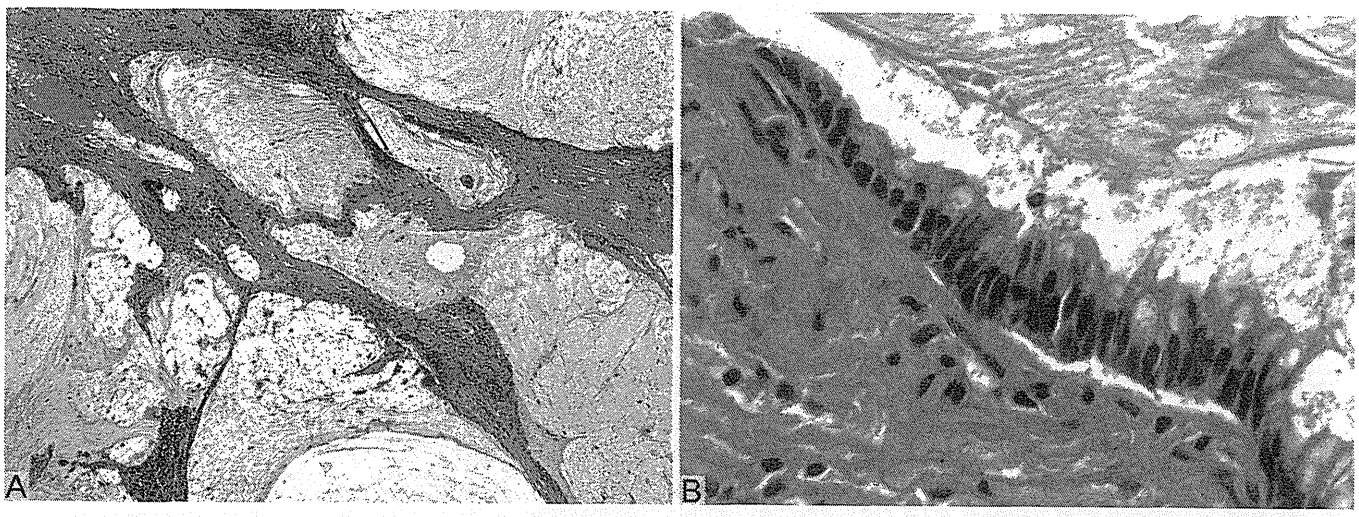

Figure 10. *Colloid adenocarcinoma. A,This tumor consists of abundant pools of mucin growing within and distending airspaces. Focally well-differentiated mucinous glandular epithelium grows along the surface of fibrous septa and within the pools of mucin. Tumor cells may be very inconspicuous. B, The surface of the fibrous wall is lined by well-differentiated cuboidal or columnar mucinous epithelium (hematoxylin-eosin, original magnifications ×10 [A] and ×40 [B]).*

ulation tissue. Nuclear atypia of the neoplastic epithelium is usually minimal.[121] Morphologically, this tumor may be fully indistinguishable from metastases from the appendix; clinical history should be very helpful.[122]

Fetal adenocarcinoma consists of glandular elements with tubules composed of glycogen-rich, nonciliated cells that resemble fetal lung tubules (Figure 11).[3] Subnuclear vacuoles are common and characteristic. Squamoid morules may be in the lumens. Most are low grade with a favorable outcome; high-grade tumors occur. When mixtures occur with other histologic subtypes, a feature that occurs more often in the high-grade tumors, the tumor should be classified according to the predominant component.[115] This tumor typically occurs in younger patients than do other adenocarcinomas. Uniquely, the low-grade fetal adenocarcinomas appear to be driven by mutations in the β-catenin gene and the epithelial cells express aberrant nuclear and cytoplasmic staining with this antibody by immunohistochemistry.[116,117,123] Nakatani et al[116,117] and Sekine et al[123] have suggested that upregulation of components in the WNT signaling pathway, such as β-catenin, are important in low-grade fetal adenocarcinomas, as well as in biphasic pulmonary blastomas. This is in contrast to the high-grade fetal adenocarcinomas, which appear to be distinct from the low-grade tumors.[116,117,123]

Enteric differentiation can occur in lung adenocarcinoma and when this component exceeds 50%, the tumor is classified as pulmonary adenocarcinoma with enteric differentiation. The enteric pattern shares morphologic and immunohistochemical features with colorectal adenocarcinoma.[114] In contrast to metastatic colorectal adenocarcinoma, these tumors are histologically heterogeneous with some component that resembles primary lung adenocarcinoma such as lepidic growth. Recording of the percentages of these other components may be useful. The enteric pattern consists of glandular and/or papillary structures, sometimes with a cribriform pattern (Figure 12, A), lined by tumor cells that are mostly tall columnar with nuclear pseudostratification, luminal necrosis, and prominent nuclear debris.[114] Poorly differentiated tumors may have a more solid pattern. These tumors show at least 1 immunohistologic marker of enteric differentiation (CDX-2, CK20, or MUC2) (Figure 12, B). Consistent positivity for CK7 and expression of TTF-1 in approximately half the cases help in the distinction from metastatic colorectal adenocarcinoma.[114,124] Cytokeratin 7–negative cases may occur.[125] CDX-2 is reduced or absent in most poorly differentiated colorectal carcinomas and more than half show the high-frequency microsatellite instability phenotype.[126] Although this type of tumor will rarely metastasize to the lung, since immunohistochemical detection of mismatch repair protein

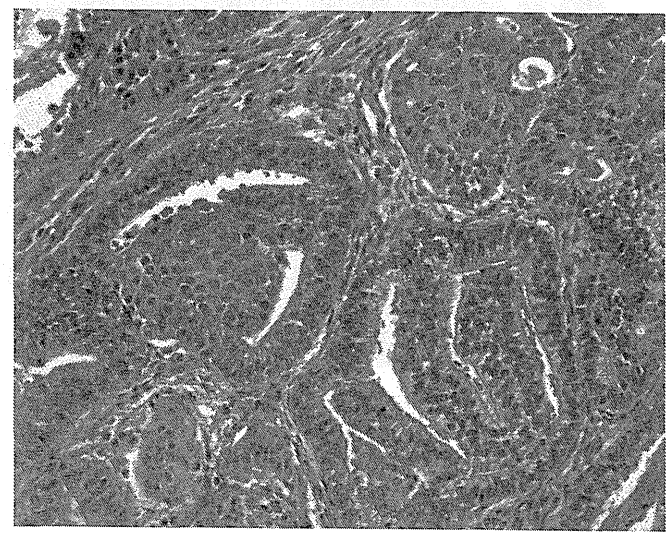

Figure 11. *Fetal adenocarcinoma consists of malignant glandular cells growing in tubules and papillary structures with endometrioid morphology. Some tumor cells have prominent clear cytoplasm, and squamoid morules are present (hematoxylin-eosin, original magnification ×20).*

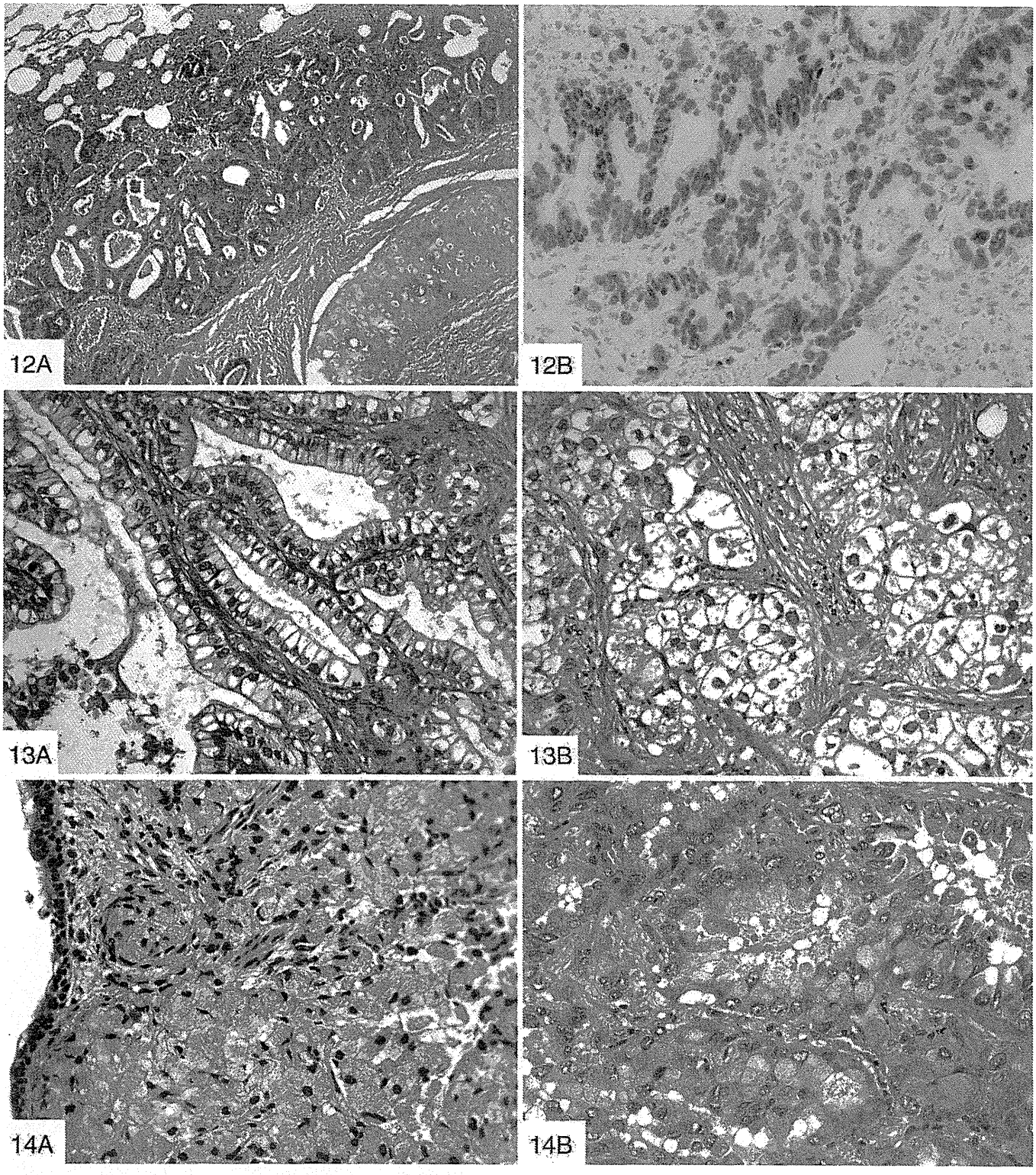

Figure 12. *Enteric adenocarcinoma. A, This tumor consists of an adenocarcinoma that morphologically resembles colonic adenocarcinoma with back-to-back angulated acinar structures. The tumor cells are cuboidal to columnar with nuclear pseudostratification. B, The tumor stains strongly for CDX-2 (hematoxylin-eosin, original magnification ×10 [A]; original magnification ×40 [B].*

Figure 13. *A, Acinar adenocarcinoma with clear cells. The cytoplasm in these tumor cells shows prominent clear cell features. B, Solid adenocarcinoma with clear cell features. The tumor cells in these solid nests have abundant clear cytoplasm (hematoxylin-eosin, original magnifications ×40 [A and B]).*

Figure 14. *Clear cell features: Signet ring features. A, Solid adenocarcinoma with signet ring features. This tumor consists of a uniform population of tumor cells with cytoplasm distended with abundant mucin, with many showing signet ring features. B, Acinar adenocarcinoma with signet ring features. Many tumor cells in this acinar pattern of adenocarcinoma have signet ring morphology (hematoxylin-eosin, original magnifications ×40 [A and B]).*

with antibodies for mutL homolog-1 (*MLH1*), mutS homologs 2 and 6 (*MSH2*, *MSH6*), and postmeiotic segregation increased 2 (*PMS2*) gives a predictive value that is virtually equivalent to microsatellite instability testing, this may be worth testing in selected cases as microsatellite instability in primary lung adenocarcinomas is extremely rare.[127] Primary lung adenocarcinomas that histologically resemble colorectal adenocarcinoma, but lack immunohistochemical markers of enteric differentiation, are probably better regarded as lung adenocarcinomas with *enteric morphology* rather than pulmonary adenocarcinoma with enteric differentiation.[128]

Signet Ring and Clear Cell Features

Both clear cell (Figure 13, A and B) and signet ring (Figure 14, A and B) features are regarded as cytologic features rather than primary histologic subtypes. They both occur most commonly in the solid component of lung adenocarcinomas (Figures 13, B, and 14, A), but they can also be seen in other patterns such as acinar (Figures 13, A, and 14, B), papillary, and micropapillary adenocarcinoma.[111,112] Therefore, these features should not be included in predominant subtype or the summary of percentages for comprehensive histologic subtyping, but rather they can be mentioned at the end of the diagnosis as "with signet ring features" or "with clear cell features," along with the estimated percentage of this cytologic change.

HISTOLOGIC GRADING

Unlike carcinomas of organs such as the breast, prostate, and kidney, there is no established grading system for lung adenocarcinoma in resection specimens. Since the effort to develop this new classification was begun, several studies have examined both architectural and nuclear approaches. While certain histologic subtypes are associated with favorable (lepidic pattern)[5] or unfavorable (solid, micropapillary)[6,9,10,129] prognosis, few articles have addressed grading across all histologic subtypes. Two major studies have addressed architectural grading using the single most predominant pattern[8] or the 2 most prominent patterns.[78] Both approaches have identified prognostically important subsets of lung adenocarcinoma. Nuclear grading has been evaluated in 3 studies, with two suggesting that nuclear size[130,131] and the other that cytologic atypia[129] were predictive of survival. A recent study[132] evaluated both nuclear and architectural grading in stage I lung adenocarcinomas. Although nuclear diameter, nuclear atypia, mitotic count, and atypical mitoses were significant predictors of recurrence in univariate analysis, in multivariate analysis only mitotic count had a significant independent association with risk of recurrence. Increased risk of recurrence was best predicted by a combined high architectural/mitotic grade after adjusting for clinical factors.[132] The impact of adding mitotic counts was greatest in tumors with intermediate architectural grade.[132] While these studies are promising, more validation studies are needed before a final grading system can be recommended for lung adenocarcinoma.

MOLECULAR-HISTOLOGIC CORRELATIONS

The molecular issues in lung adenocarcinoma are addressed in detail in the master classification document.[1] However, because of the importance of molecular histologic correlations, a few brief comments will be addressed. Unlike the specific genetic alterations seen in sarcomas, lymphomas, and leukemias, in lung cancer there are no histologic molecular correlations that are totally specific. Overall molecular, radiologic, and gene pathway correlations with adenocarcinoma subtypes are summarized in Table 7. The most robust histologic molecular correlation is with invasive mucinous adenocarcinoma, since a high percentage of these tumors have *KRAS* mutations and lack of *EGFR* mutations (Table 6). *EGFR* and *KRAS* mutations, as well as *ALK* rearrangement, can be seen in most of the invasive adenocarcinoma histologic subtypes. However, *EGFR* mutations are most often seen in association with nonmucinous adenocarcinomas that are lepidic or papillary predominant, and some report an association with a micropapillary pattern (Table 7). *KRAS* mutations are reported most often in tumors with a solid or micropapillary pattern and can be present in tumors producing extracellular mucin (Table 7). *ALK* rearrangement has been mostly associated with an acinar pattern, including a cribriform morphology, and with signet ring cell features, particularly those with TTF-1 and p63 coexpression.[133–136] Another point of interest is that nonsmoker-associated gene mutations, including *EGFR, EML4-ALK, BRAF,* and human epidermal growth factor receptor 2 (*HER2/neu*), are involved in a subset of adenocarcinomas with TTF-1 expression.[137]

With the emerging importance of molecular diagnostics to guide therapy, a multidisciplinary approach is needed to set a consistent strategy for obtaining and preserving tissue samples optimized to perform studies such as DNA sequence analysis, fluorescence in situ hybridization, and, in some settings, RNA-based studies. It is not yet possible to provide specific guidelines on how to do this in the current document because of the wide variation in infrastructure and expertise from one institution to another. If a portion of a sampled tumor is snap frozen for molecular studies, a few considerations exist for resection specimens. As most critical molecular studies can be performed from formalin-fixed, paraffin-embedded tissue, there is a need for frozen samples only for certain techniques such as comparative genomic hybridization and gene expression profiling. If frozen tissue is being obtained from tumors with lepidic-predominant tumors, for which AIS or MIA is in the differential diagnosis, efforts should be made to ascertain whether this frozen piece has an invasive component. The CT and gross appearance of the lesion should be considered to ensure a solid component is sampled in a tumor that appears part solid on CT. One approach is to perform a frozen section from the tissue saved for storage in a freezer. It is important to have a pathologist confirm the presence of tumor cells before performing molecular studies in the frozen tumor tissue samples.

RADIOLOGIC-HISTOLOGIC CORRELATIONS

There are settings in which pathologic assessment of lung adenocarcinomas can be greatly improved by correlation with radiologic findings. While review of CT reports may be informative, it is also helpful to have access to primary CT images in the frozen section and gross rooms, where pathology specimens are initially processed, and also at the time of review of histologic sections. Review of CT images may be valuable because they may give a better impression about the gross pathologic findings, which can be difficult to appreciate if tumors are removed by the surgeon in several pieces or if the tumor is difficult to identify on gross examination. In this sense, the CT is an extension of the

Table 7. Adenocarcinoma Histologic Subtypes, Molecular and Radiologic Associations

Histologic Subtype Predominant	Molecular Features	CT Scan Appearance	Gene Pathways Associated	Sources
Nonmucinous adenocarcinoma in situ and minimally invasive adenocarcinoma	TTF-1+ (100%); *EGFR* mutation never-smokers: 10%–30%; *KRAS* mutation smokers: 10%–30%	Ground-glass nodule, part-solid nodule	Not known	160, 162, 163, 164, 165
Lepidic (nonmucinous)	TTF-1+ (100%); *EGFR* mutation never-smokers: 10%–30%; *EGFR* amplification: 20%–50%; *KRAS* mutation smokers: 10%; *BRAF* mutations: 5%	Part-solid nodule; ground-glass nodule or solid nodule	Low cell cycle stimulatory; high *WNT*	6, 163, 164, 165, 166, 167, 168, 169, 170, 171
Papillary	TTF-1+ (90%–100%); *EGFR* mutation: 10%–30%; *EGFR* amplification: 20%–50%; *KRAS* mutation: 3% (lack of KRAS); *ERBB2* mutations: 3%; *TP53* mutations: 30%; *BRAF* mutations: 5%	Solid nodule	Low cell cycle stimulatory; high *EGFR*; high Notch	6, 79, 163, 166, 167, 168, 170, 172, 173, 174, 175
Acinar	TTF-1+ or –; *KRAS* mutation in smokers: 20%; *EGFR* mutations: <10% nonsmokers; *EGFR* amplification: 10%; *EML4/ALK* translocation: >5%; *TP53* mutations: 40%	Solid nodule	High *PDGF*; low *EGFR*; low angiogenesis	6, 79, 166, 176, 177
Micropapillary	*KRAS* mutations: 33%; *EGFR* mutations: 20%; *BRAF* mutations: 20%	Unknown	Unknown	6, 75, 171
Solid	TTF-1 (70%); MUC1 positive; *KRAS* mutation smokers: 10%–30%; *EGFR* mutation never-smokers: 10%–30%; *EGFR* amplification: 20%–50%; *EML4/ALK* translocation: >5%; *TP53* mutation: 50%; *LRP1B* mutations; *INHBA* mutations	Solid	High cell cycle stimulatory; high angiogenesis; high *JAK-STAT*; low Notch	6, 79, 166, 176, 177, 178, 179
Invasive mucinous adenocarcinoma	TTF-1 (0%–33% positive); *KRAS* mutation: 80%–100%; no *EGFR* mutation; MUC5+ MUC6+ MUC2+	Consolidation, air bronchograms; less often, ground-glass opacity	Not known	15, 91, 105, 153, 157, 159, 160, 175, 178, 180, 181

Abbreviations: CT, computed tomography; TTF-1, thyroid transcription factor-1.

gross pathologic assessment. There are 2 primary settings in which radiologic pathologic correlation is helpful: (1) in lepidic-predominant tumors (see "Radiologic-Pathologic Correlation for Tumor Size Assessment" above) and (2) if there are multiple tumors. In processing specimens with multiple nodules, review of the CT scan can also be helpful to be sure that each nodule is sampled.

IMPLICATIONS OF THIS CLASSIFICATION FOR TNM STAGING

There are several important implications of this new adenocarcinoma classification for staging that need to be considered for the next revision of the TNM classification. Importantly, we are not making official recommendations, as this can only be done by the International Union Against Cancer/American Joint Committee on Cancer TNM committees. However, we hope to stimulate investigators to study their case material with the intention of providing data that will allow these committees to determine whether official changes should be made in the 8th edition of the TNM classification. The changes relating to the concepts of AIS, MIA, and lepidic-predominant adenocarcinoma parallel classification criteria and terminology currently used in breast cancer,[138] but they would not be applicable to other histologic types of lung cancer. In addition, the comprehensive histologic subtyping approach to assessing invasive adenocarcinomas in this classification provides a useful approach to staging multiple adenocarcinomas.

Adenocarcinoma in situ would be classified as Tis. However, because carcinoma in situ (CIS) can occur with both lung squamous cell carcinoma and adenocarcinoma, these should be specified as Tis (squamous) or Tis (adenocarcinoma), similar to breast cancer where there is Tis (DCIS) for ductal CIS and Tis (LCIS) for lobular CIS.

Minimally invasive adenocarcinoma would be classified as T1mi, similar to microinvasive breast cancer, which is defined as an invasive carcinoma with no focus measuring greater than 1 mm; however, the size for MIA is not greater than 5 mm.

Also, similar to breast cancer, the size T factor for adenocarcinomas with an in situ or lepidic component may best predict prognosis according to the size of the invasive component only rather than the way it is currently practiced by including total tumor size inclusive of both the invasive and the lepidic or in situ components. In early-stage tumors, the tumor size T factor may need to be adjusted from total tumor size to the size of the invasive component only. Several publications in the literature[8,20,23] suggest invasive tumor size is an independent prognostic factor, and it may be a better predictor of prognosis than overall tumor size in lepidic predominant tumors. This needs to be tested radiologically and pathologically by comparing survival according to analysis of total tumor size (ground-glass opacity plus solid components by CT and invasive versus in situ/lepidic components by pathology) compared to only by the size of the solid or invasive component by CT and pathology examinations, respectively.

In addition, for multiple lung adenocarcinomas, comprehensive histologic subtyping can help in distinguishing intrapulmonary metastasis from synchronous or metachro-

nous primary tumors.[21] However, comprehensive histologic subtyping is only 1 tool that should be used to compare tumors, because valuable information can be obtained to address this problem from tumor cytologic characteristics and tumor stroma.[21] The role of molecular testing in this setting is promising, but needs further study.[22,139–141]

These concepts need to be tested vigorously in the next 5 years in both early- and advanced-stage lung adenocarcinoma to determine whether they are sufficiently robust to warrant changes in the 8th edition TNM classification.

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