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Updates in grading and invasion assessment in lung adenocarcinoma

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The pathologic evaluation of lung adenocarcinoma, because of greater understanding of disease progression and prognosis, has become more complex. It is clear that histologic growth patterns reflect indolent and aggressive disease, resulting in clearer morphologic groups that can be the underpinning of a grading system. In addition, the progression of adenocarcinoma from a tumor that preserves alveolar architecture to one that remodels and effaces lung structure has led to criteria that reflect invasive rather than in-situ growth. While some of these are based on tumor cell growth pattern, aspects of this remodeling from desmoplasia to artifacts of lung collapse and sectioning, can lead to difficult to interpret patterns with lower reproducibility between observers. Such scenarios are examined to provide updates on new histologic concepts and to highlight ongoing problem areas.

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INTRODUCTION

Adenocarcinoma of the lung is a multifaceted disease with diversity in histology, imaging, causation, age of onset, and molecular pathogenesis. In addition, it is the most common type of lung cancer and as such advances in adenocarcinoma characterization have a significant impact on patient care. In this review the focus will be on aspects of histopathological evaluation of patterns important for grading and those that influence measurement of invasion for staging.

Precursors of invasive adenocarcinoma

The understanding of pulmonary adenocarcinoma begins in part with precursor lesions. Neoplastic lesions known as atypical adenomatous hyperplasia (AAH) are found in patients with resection for lung cancer, although not exclusively so[1]. These proliferations of type 2 cells or club cells are associated with driver mutations similar to those seen in lung adenocarcinoma which is the main basis for their status as non-mucinous adenocarcinoma precursors[2–4]; however BRAF and KRAS mutations seem to outnumber EGFR mutations. These lesions are usually less than 0.5 cm and the proliferations are varied histologically with an admixture of type 2 or club cells of various shapes and sizes alongside residual type 1 cells (Fig. 1A, B). These are usually incidental lesions and are generally not an explanation for the target lesion of a fine needle aspirate, biopsy, or resection. Bronchiolar adenoma, which can also be an incidental finding, is usually small airway localized and associated with mucous cells, ciliated cells and double layered architecture[5]; its relationship to invasive adenocarcinoma remains relatively unexplored. One unanswered question is whether AAH is an obligate precursor to adenocarcinoma; their incidental and often multifocal presence in lung resections as well as some differences in mutation spectrum suggests they are either non-obligate precursors or have a long latency to progression. The time of latency of these lesions and thus their frequency to progression is unknown.

However, tumors in which these neoplastic epithelial cells proliferate result in a uniform replacement of the alveolar lining with neoplastic cells and at that point are designated adenocarcinoma-in-situ (AIS; Fig. 1C) and when non-mucinous, are generally of low nuclear grade with a cuboidal morphology, sometimes referred to as hobnail shape. These are single layers of cells, although a variety of histologic scenarios and artifacts can lead to foci that may appear to be double layered (Fig. 1D). Further stratification that is, greater than 2 cells thick, should not be multifocally present. The alveolar walls can become thickened, but retention of alveolar architecture despite sclerosis and lack of desmoplasia is critical to the belief that these tumors are non-invasive[6].

The imaging characteristics of AIS are also worth mentioning. While invasive adenocarcinomas have an imaging density described as solid, AIS, likely as result of their retention of alveolar architecture, retain air density. This appearance has been described as ground glass and in this setting is considered ground glass nodule rather than a diffuse ground glass appearance[7]. Subsequent loss of alveolar architecture with invasion results in replacement of alveolar architecture with tumor cells, fibroblasts, collagen, and inflammation that converts this ground glass appearance to one of a developing solid component, an appearance that is described as part solid[8–10].

While the association between imaging and histology is not perfect, it is this transition to invasion from a background of an AIS that guides clinical management and underlies many of the histologic questions that follow. While it is likely that not all adenocarcinomas arise from this sequence of AIS[11,12], the invasive patterns to follow are relevant regardless of origin.

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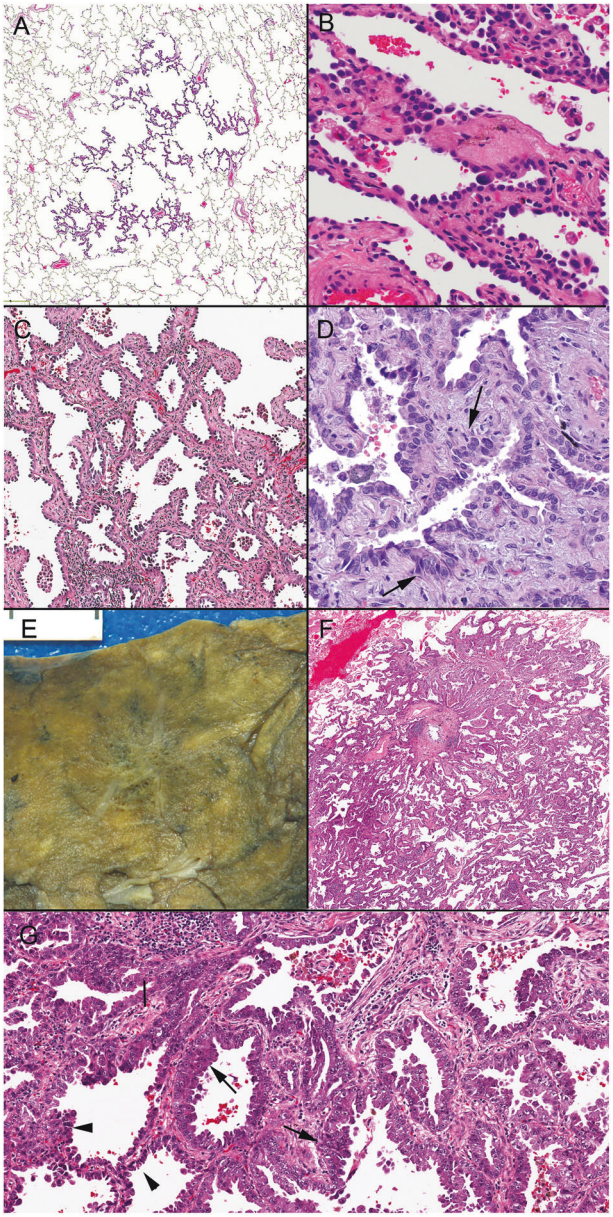

Fig. 1 Precursors of invasive adenocarcinoma. A Atypical adenomatous hyperplasia can be identified at low magnification by the increase in lining cell size and sharp demarcation with adjacent lung. **B** Alveolar walls can be thickened, and the lining with both type 2 pneumocytes of variable shape and type 1 pneumocytes. **C** Adenocarcinoma-in-situ, non-mucinous contain areas of alveolar spaces more uniformly replaced by type 2 pneumocytes, often low grade, without the gaps of residual type 1 cells. **D** While adenocarcinoma-in-situ can consist of high cuboidal cells or columnal cells with greater atypia, they are usually single layers with occasional folds or tufts of 2 layers (arrow). **E** Macroscopic image of a minimally invasive adenocarcinoma, grossly indistinct but with area of central distortion. **F** At low magnification, the alveolar growth pattern predominates with an area of greater density and distortion. **G** At high magnification, proliferation includes multi-layered epithelium along with angulated glandular profiles.

Evolution to invasive adenocarcinoma

The first category which emerges from this proposed sequence is that of minimally invasive adenocarcinoma (MIA)[13], defined as having an invasive focus less than or equal to 5.0 mm in an overall macroscopic tumor less than or equal to 3.0 cm (Figure E–G). In addition to features of invasion that follow (e.g. pleural, large airway, or vascular), MIA lack necrosis or spread through airspaces. This type of early invasive adenocarcinoma shares with AIS a favorable prognosis and high rate of progression free survival. It may be that tumors that are over 3.0 cm with such small focus of invasion also have favorable prognosis, but at this point are not classified as MIA, but instead lepidic predominant adenocarcinoma. It is likely that non-invasive and minimally invasive tumors over 3.0 cm have the same outcome as those below 3.0 cm[14,15]. While based on outcome AIS and MIA may be thought of together, they are given distinct histologic and T categories. The MIA category is important in the avoidance of underutilization of the AIS category and more importantly, inadvertent widening of the spectrum of lepidic predominant adenocarcinoma to include tumors with biologically insignificant invasive foci less than or equal to 5.0 mm.

Lepidic predominant adenocarcinoma is the category in which quantitation of the invasive component becomes most important. Again, with focus on the concept of transition from AIS to invasion and ground glass to solid and part solid nodules, this tumor type represents a mixture of pre-invasive and invasive components. The pattern of growth that retains alveolar architecture is designated as lepidic and the invasive components assigned a pattern of acinar, papillary, solid, or micropapillary. While it may be controversial in some cases as to whether non-mucinous lepidic pattern may represent the invasive tumor spreading along alveolar walls, which is described as outgrowth in the current World Health organization classification, the majority of cases in which the lepidic pattern is predominant correspond to clinical nodules with a ground glass component (alongside a solid component, or part solid) and therefore the lepidic pattern is considered the non-invasive part of a mixed invasive-non-invasive tumor. With this concept in mind the invasive component needs definition[6,16].

Acinar

In most series, this is the most common invasive pattern (Fig. 2A). It is a gland-forming pattern but must be distinguished from distorted alveolar architecture. The glands are often lined by a single layer of cells, often with cuboidal to columnar cells, however stratification is often seen. The glandular profiles can be angulated or with convex shapes with oval or round contour. The stromal response can be varied, from fibroelastosis to frank desmoplasia. In some instances, nuclear grade is intermediate or high, but in others relatively low-grade nuclei can be seen. In cases with low nuclear grade there can be overlap with lepidic pattern especially when sclerosis or alveolar wall thickening is present. Infiltration of vessels, airway or pleura can be especially useful in acinar pattern. Fusion in acinar pattern is a cribriform feature which blends with solid pattern in morphology and outcome.

Solid

The solid pattern represents a confluence of neoplastic cells (Fig. 2B). In three dimensions this likely represents a ball of cells, and when sectioned this looks like a solid nest of cells. This pattern may have mucinous cells. It can be confused with non-keratinizing squamous carcinoma and even some non-epithelial tumors such as melanoma. Some cases will have occasional spaces in such a pattern and as such have overlap with cribriform features.

Papillary

This pattern is characterized by neoplastic epithelial proliferation lining arborizing structures with a fibrovascular core (Fig. 2C). Such structures are often clover-like and protrude into epithelial lined spaces. The epithelial cells are high cuboidal to columnar and possibly show some stratification as well.

Micropapillary

This pattern is the most histologically diverse. In some cases, finger-like projections within a space that would otherwise look acinar has

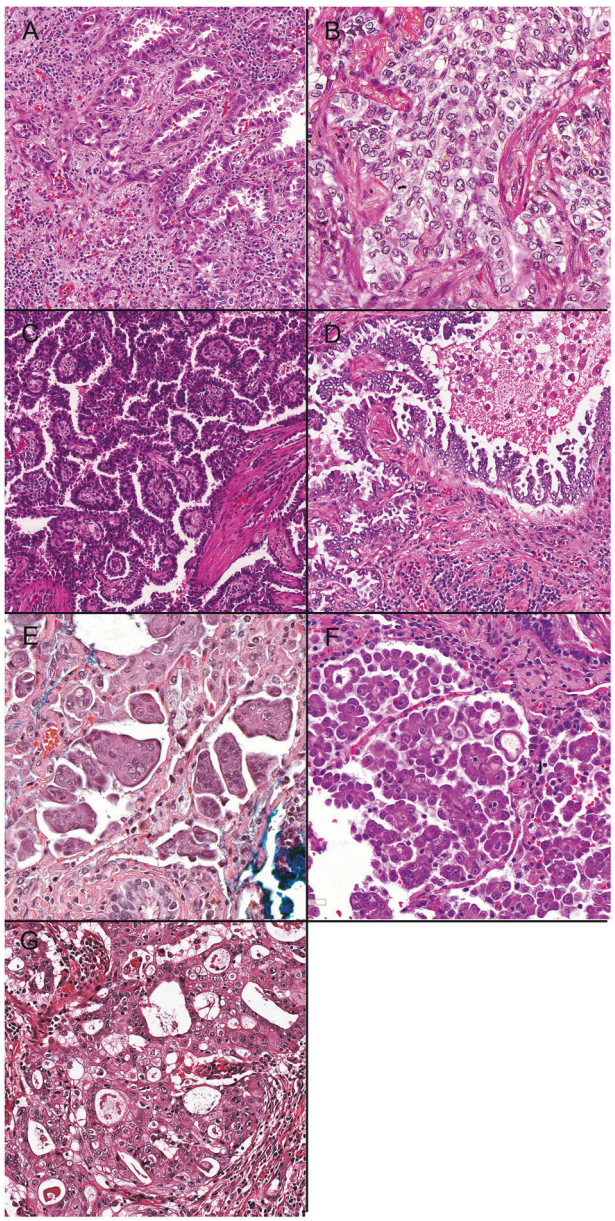

Fig. 2 Invasive patterns of adenocarcinoma. A The acinar pattern is gland forming with large and small gland, with stromal change including desmoplasia. **B** The solid pattern is a confluence of cells without lumen formation. **C** Papillary adenocarcinoma is composed of arborizing structures with fibrovascular cores, with clover-like formations. **D** Micropapillary pattern of finger like stratifications without fibrovascular cores. **E** Micropapillary structures can be invasive with small clusters within spaces (retraction artefact). **F** Accumulations of small ring-like or radially oriented nuclei are one pattern of micropapillary. **G** Aggregates of cells with small lumen formation represent the complex glandular formation of cribriform pattern.

been described as the filagree pattern, and in fact is reported in a variety of organ systems (e.g. breast) as micropapillary without subclassification. Within a glandular space, a finger or lace like stack of cells greater or equal to 3 cells is considered micropapillary; the width of such a lesion is not defined. Other examples represent small clusters of cells with flower like arrangements in the stroma with clear space around the epithelium described as retraction, a stromal invasive pattern (Fig. 2D–F).

What these histologic discussions establish is that invasion designation can be a manifestation of a proliferative pattern alone (stratification or solid cellularity) or, in the case of micropapillary, combination of proliferation in some descriptions and evidence of stromal remodeling in others.

Additional histologic features

Cribriform patterns, those of complex glandular architecture or "cookie cutter" spaces in a solid background has been increasingly described in lung adenocarcinoma with an adverse prognosis (Fig. 2G). The series vary in their case selection, some with acinar pattern with complex growth and others solid patterns with glandular foci[17–19]. The mutational profile of these adenocarcinomas appears to be enriched in KRAS mutation, a feature associated with solid pattern. However, whether a variant of solid or a poorly differentiated acinar adenocarcinoma, this appear to be a high-grade growth pattern. In addition, there is some suggestion that cribriform tumors with signet ring cells are enriched for tumors with ALK rearrangements and ROS rearrangements.

Signet ring features in the lung are somewhat distinct from those of gastric adenocarcinoma. In gastric tumors, dyscohesion and single cell infiltration are characteristic of signet ring type; in lung the signet ring cells tend to be more cohesive forming cribriform or solid clusters[20,21].

Clear cell histology can be seen in most carcinoma subtypes in the lung and as a result some adenocarcinomas will have clear cell features. When present, it is not usually exclusive in that there are both clear and non-clear cell components.

There are several unusual histologic adenocarcinoma subtypes. Well differentiated fetal adenocarcinoma, a histology of some beta-catenin mutation associated lung adenocarcinoma, have subnuclear vacuoles and distinct gland formation; some authors state a resemblance to fetal lung or secretory endometrium. Enteric-type adenocarcinoma resemble intestinal type tumors but often have non-enteric areas in the same tumor.

Tumors with mucinous histology are also encountered. Mucinous adenocarcinomas, characterized by cells with prominent apical mucin vacuoles (resembling gastric type mucosa) can be in-situ but are more often multifocal and invasive. Some mucinous adenocarcinoma will have a mixture of gastric type mucin and goblet cells. Some tumors will have abundant extracellular mucin, and these are classified as colloid type adenocarcinoma.

Patterns and adenocarcinoma grading

An agreed upon grading system for adenocarcinoma has been lacking. However, many prior studies examining predominant pattern and survival as well as predominant pattern and risk for lymph node metastasis noted three tiers of tumor—suggesting that Grade 1 could comprise tumors that are lepidic predominant, Grade 2 acinar and papillary and Grade 3 as solid or micropapillary predominant. While such a system has validity, it is also recognized that predominant pattern can have a wide range and that heterogeneity in adenocarcinomas is common. In addition, some patterns such as micropapillary may be relevant as a minor pattern. With these observations in mind, a recently proposed grading system would follow predominant pattern, with the caveat that a tumor with greater than 20% micropapillary, solid or complex glandular/cribriform would be considered grade 3 or poorly differentiated. Using such a system, the prediction of recurrence free and overall survival is better stratified than using predominant pattern alone. The adoption of this grading system awaits wider validation, but it is a starting point for establishing a pulmonary adenocarcinoma grading system[22].

Why do patterns have prognostic significance?

The relationship between growth patterns and prognosis is complex. Cellular proliferation, increased cell survival, changes in intercellular adhesion and adhesion to stroma (e.g. basement membrane) all contribute to these growth patterns, and are linked

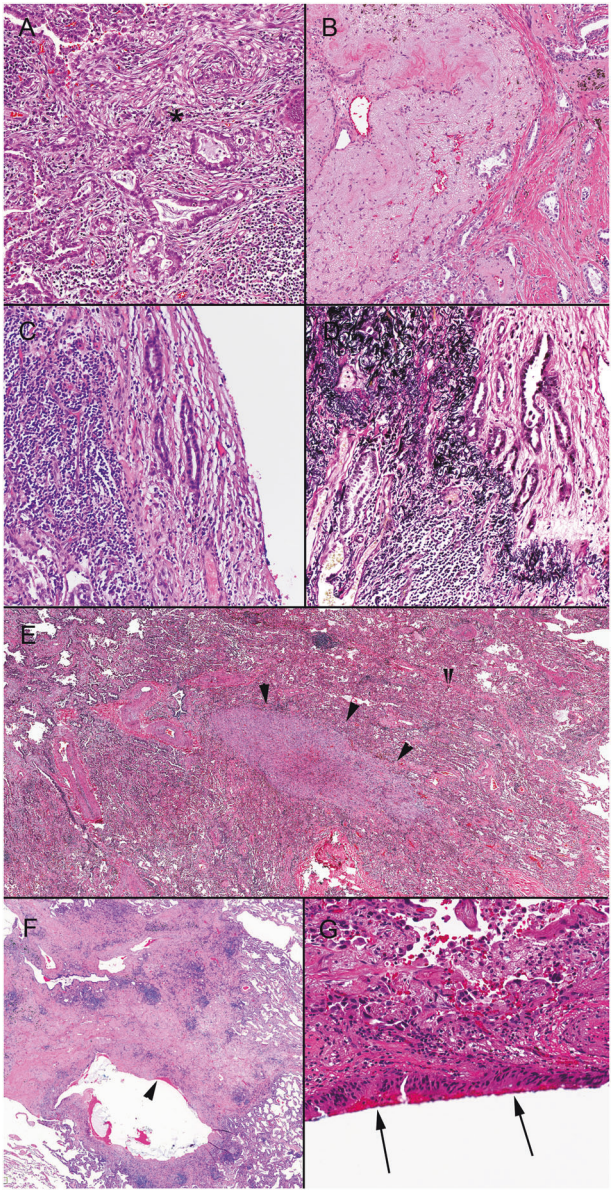

Fig. 3 Stromal changes associated with invasion. A While an acinar pattern is a tumor cell pattern of invasion, the presence of desmoplasia (asterisk) is also a key observation. **B** While vessels can become hyalinized, vascular invasion when recognized is proof of invasion. **C** Pleural reaction can be seen without pleural invasion, but (**D**) elastin stain can help delineate the edge of the pleura, with glands outside that edge. **E** Needle tracts are linear are while fibroblastic (arrowheads), can be distinguished from desmoplasia. **F** Needle tracts can also be recognized as spaces (arrowhead) in the tissue with histiocytic reaction seen in (**G**) at arrows.

to motility, stromal remodeling and immune response. The cellularity of a solid pattern tumor is higher than that of a lepidic tumor and may reflect proliferation rate or a decrease in cell death/apoptosis. A solid pattern must demonstrate different properties of cell adhesion at the periphery where cells interact with stroma than at the center where tumor cells interact with other tumor cells. Finger-like projections of a micropapillary pattern incorporate proliferation, differential adhesion, cell survival, and likely motility, but how this alone translates into invasion and metastasis remain complex. For lepidic pattern, the single layer with attachment to the alveolar wall may reflect maintenance of cohesion and cellular organization with lack of stromal remodeling. Therefore, while some of these observations may explain patterns and prognosis, the evidence is observational and descriptive[23,24] and these biologic associations, while potentially interesting, less clear.

Patterns and interobserver agreement

Several studies have examined the reproducibility of the pattern approach. Predominant pattern has a good interobserver reproducibility, with solid and lepidic shows better agreement than papillary or micropapillary[25]. The agreement with difficult patterns drops, and the use of a secondary pattern may also decrease reproducibility. Ultimately the reproducibility of the proposed grading system is subject to the primary pattern and high-grade pattern recognition, and so how this evolves will contribute to the adoption of the grading system.

Invasion assessment

The AJCC 8th edition established the T categories for AIS, MIA as well as the guidance that tumor size should be invasive size only. This guidance results in a need to correct the gross size of the tumor for its invasive size only.

However, the criteria for invasion and their consequence require some comment as they incorporate tumor cell components such as growth pattern and stromal response such as desmoplasia. When we observe tumor cells crossing microanatomic boundaries such as pleura, vessels or airways, or when unequivocal vascular or lymphatic invasion is seen, there criteria are clear and well understood. When single or small nests of tumor cells are seen in between other structures, pathologists equate such patterns with invasion. The presence of desmoplasia is also well accepted as a manifestation of invasion (Fig. 3A). However the concept of a mucosal based tumor invading into lamina propria or submucosa, as can be demonstrated in tumors of the gastrointestinal tract or squamous mucosa of head and neck or cervix (or for that matter large bronchus) is harder to apply to the periphery of the lung as identification of such architectural breakdown is more challenging. As a result, we combine criteria of loss of alveolar architecture, invasion of vessels, airway or pleura and certain patterns of growth as these patterns have been associated with recurrence or nodal metastasis (Fig. 3B–D). Tissue distortion from needle biopsy can cause fibroblastic or even histiocytic/giant cell tissue response and needs to be distinguished from true desmoplasia (Fig. 3E–G). Pre-existing fibrosis, such as from an interstitial lung disease, can be a particular challenge in the examination of stromal criteria in assessment of invasion.

Of course, not every case with, for example, pleural invasion, recurs or has nodal metastasis, but these criteria are in a sense an assessment of risk through conveying these criteria. The connection between patterns of growth and invasion was discussed in the grading section, but again apply here as in the correlation of pattern to prognosis. When cells pile up as in micropapillary tumors, or when they grow into alveolar spaces such as in spread through airspaces pattern, they are likely demonstrating a form of non-adherent growth. Tumor cells may also survive by resisting pathways of apoptosis, but also through mechanisms of immune cell evasion. These factors contribute to the morphologic spectrum of tumor patterns; these morphologic observations also have links to stromal remodeling and motility that is part of invasion. In fact, some of the more clear-cut examples of invasion are reflected in the stromal response rather than the tumor growth pattern—changes in collagen or elastic tissue, myofibroblastic proliferation, and neovasculature. Collagen deposition or desmoplasia may reflect these stromal responses; we can show elastic tissue damage (e.g. pleural invasion) using elastic von Gieson stain.

Our understanding of invasion in lung adenocarcinoma stem from a long history of clinicopathologic studies. In a landmark study published in 1980, Shimosato et al.[26] demonstrated that the presence of central fibrosis could be graded and the presence of

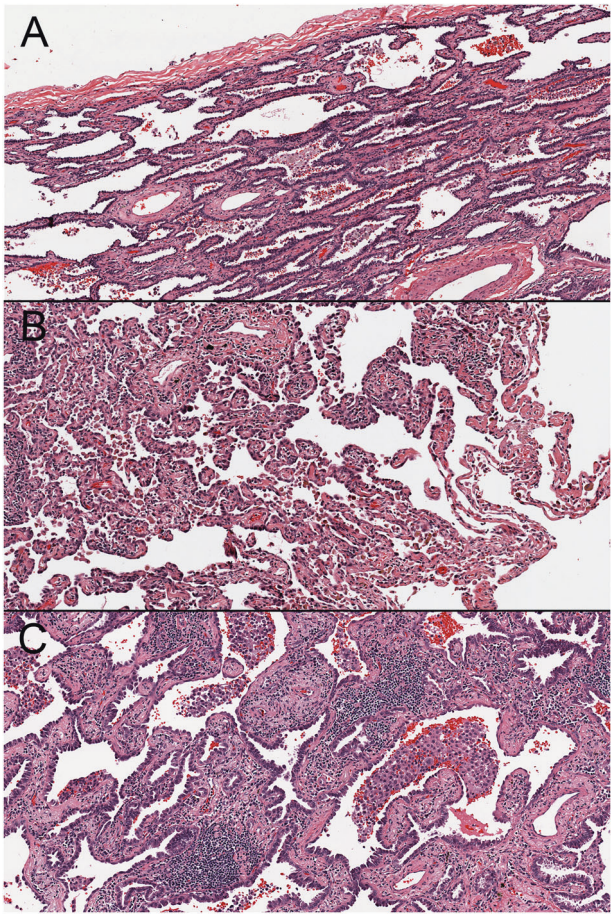

Fig. 4 Challenges in distinction of invasive from lepidic pattern—collapse. A When alveolar spaces are oriented with parallel long axis, changes of collapse can distort a lepidic pattern. **B** Collapse can produce complex profiles mimicking complex architectures (**C**) with can be made more complicated with stromal fibrous and inflammatory reaction.

increasing desmoplasia was related to poorer survival. Noguchi et al.[27] further supported this view and proposed a letter system which in many ways, is the underpinning of the current adenocarcinoma classification. Tumors with loss of alveolar architecture, development of central scarring, and then particular morphologic subtypes had prognostic implications. In a later series, measuring the diameter of central fibrosis had prognostic impact with strata at 5.0 and 15.0 mm[28]. Other studies focused on measurement of invasive size[15,29]. In a retrospective-prospective study of consecutive cases with 10-year follow-up and blinded to outcome, invasive size as measured linearly to adjust for non-invasive growth was a better predictor of overall survival than gross size measurement[15]. In addition, a retrospective series of over 1000 Stage 1 patients[30] showed that invasive size correction moved over 10% of patients into a more favorable recurrence group, including 4% with a change form over 3.0 cm to under 3.0 cm. Therefore over 10% of patients were overstaged using gross size, and recurrence rate was reflective of the invasive, rather than the gross, size.

Thus, the reason for this AJCC staging guidance represents the outcome of the background and history of the pre-invasive adenocarcinoma and its favorable survival. What these studies have in common is that invasive size was a better predictor of outcome than the gross size in tumors with a non-invasive component which is the lepidic pattern that preserves alveolar architecture.

The paradox in this topic is that while individual patterns have good reproducibility for straightforward patterns[25] measurement of invasion does not. This may in part be explained by the observation that difficult patterns have lower reproducibility and that within an individual tumor, straightforward and difficult patterns may co-exist. It may also reflect the reality that pattern reproducibility needs to be combined with evidence of stromal response, and these two evaluations are not the same. The reproducibility of lepidic pattern and linear measurement in small tumors was shown to have a moderate level of agreement 82.4 and 63%[31] while underscoring invasive size measurement still overestimates invasion based on the favorable outcome reported in the cases at the cusp of MIA and invasive cancer. As such, defining these difficult patterns require more effort than defining straightforward ones and stromal features may need to be added to the analysis to achieve invasion reproducibility. With these thoughts in mind, what are the situations in which difficult assessment of patterns leads to discrepancy?

Potential problem areas in invasion assessment

Lepidic versus acinar. When tumor cells line pre-existing alveolar spaces, and those spaces have relatively normal architecture, the lepidic pattern is straightforward and reproducible. Even with collapse of alveoli, many cases will show parallel long axis of collapse, alveolar macrophages, and single-cell lining of a non-mucinous lepidic pattern (Fig. 4A). However, it is recognized that alveolar walls can become thickened in lepidic patterns, and this includes AIS, MIA, and LPA. This thickening can be due to fibrous tissue and inflammation, which can be lymphocytic, lymphohistiocytic, or non-necrotizing granulomas. In addition, the alveolar architecture can be distorted by chronic disease, such as emphysema or interstitial fibrosis. This results in a fairly wide spectrum of alveolar shapes and sizes that can represent preserved architecture. To make these situations even more complex, alveoli that are collapsed can mimic complex architectures, including angulated profiles of acinar pattern (Fig. 4B, C). Finally, cells in lepidic tumors can become detached from the alveolar wall and in this setting become harder to recognize as lepidic[32].

There are glandular profiles that are convincing as acinar structures. When these profiles are consistently smaller than alveolar pattern and as such not just from tangential sectioning, such confluent foci become strong candidates for an acinar pattern. When these small glands are convex shaped, they are less likely to be due to collapse (Fig. 5A). The presence of desmoplasia around glands is a definitive feature of invasion, but examples of small glands invading pleura can be found that do not have obvious desmoplasia, and such glands would still be considered invasive, previously seen in the panels of Fig. 3. In some instances, the putative acinar foci are lined by cells with stratification of more than 2 layers and this cellularity would move the observer towards acinar pattern (Fig. 5B). In addition, a transition from a lepidic to an acinar pattern within a tumor can at times be associated with increases in nuclear size and atypia, and often with changes in cellular morphology from the hobnail cuboidal cell to one with a higher cuboidal cell or one with less obvious polarity (Fig. 5C).

Papillary versus lepidic

The assessment of adenocarcinoma as collapsed alveolar growth and as part of lepidic involvement in emphysema are situations in which papillary architecture can be confused with lepidic pattern. In these settings, the fibrovascular core of a papillary structure must be distinguish from an alveolar capillary of a thickened alveolar wall (Fig. 6A). Here again, the alteration of the alveolar wall thickness in a lepidic tumor can contribute to the problem[33]. True papillary structures are arborizing structures which have clover-leaf branches and subbranches. The cells lining papillary adenocarcinoma can be stratified, but even when single layered are not described as hobnail cells (Fig. 6B). In a lepidic pattern, collapse can cause in-folding, but these should be lined by the

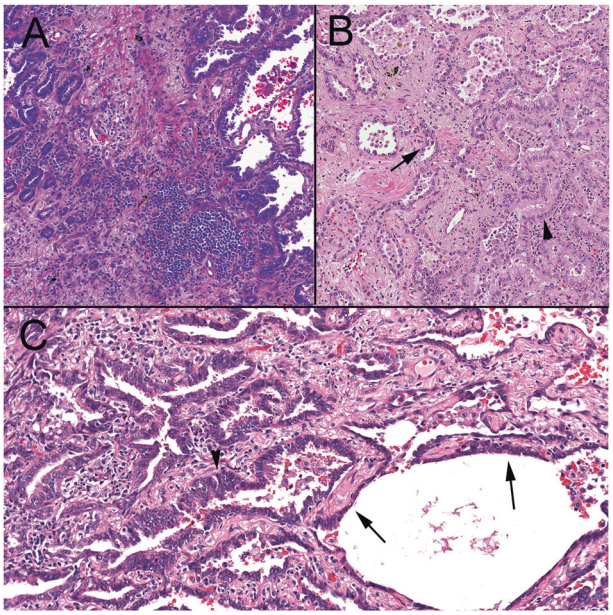

Fig. 5 Challenges in distinction of acinar from lepidic pattern. A Small glandular profiles on the left alongside lepidic spaces support pattern change to invasion. **B** While both showing collapse and stromal change, increase in nuclear grade, cell shape and size from the area of the arrowhead to the arrow may support an acinar over lepidic pattern. **C** In addition to atypia, areas of transition to invasion often show greater stratification (arrowhead) than the lepidic pattern (arrows).

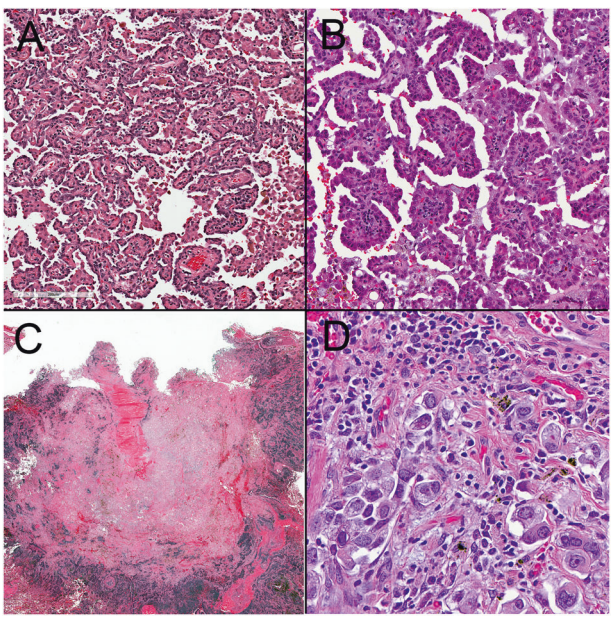

Fig. 6 Challenges in the distinction of papillary from lepidic pattern and assessment of scar. A Folding of collapsed alveolar can mimic a papillary pattern, but the lining is cuboidal and single layered which can be compared to lepidic areas without collapse. **B** Papillary structures have more complex arborization and cells high cuboidal to columnar. **C** Scarred foci can appear paucicellular at low power but invasive tumor cells as in (**D**) may be present and require high magnification to detect. Cytokeratin can also be helpful in this setting, if needed.

same cells as the collapsed lepidic pattern, without stratification or change in morphology of the cells. A background of collapsed alveoli should raise this potentially confounding issue. Lepidic growth on the broken septal structures of emphysema is subject to similar observations regarding the morphology of the lining neoplastic cells; additionally, the comparison of the architecture to the adjacent non-neoplastic lung can be informative in assessing whether alveolar architecture is potentially preserved.

Assessment of scar

The inclusion of paucicellular scarred areas, which are very common in adenocarcinoma of the lung, varies by observer and contributes to the problem of reproducibility in invasion measurement[34]. The presence of a centrally scarred focus, often fibroelastotic, devoid of neoplastic glands but surrounded by a lepidic pattern is especially difficult—is this AIS or does the presence of any scar preclude this characterization (Fig. 6C)? If there are neoplastic glands within the scarred focus, it is clearer that such areas should be included in invasive measurement; this is also made clearer if these glands have desmoplasia. However, if very bland such glands may be considered entrapment of pneumocytes rather than invasion, so here cytomorphologic features may provide guidance. While the origin of central fibroelastotic scar remains controversial in that some cases may represent pre-existing scar while others develop in the setting of a radiologic ground glass lesion, the presence of neoplastic glands in the scar is needed to assign this an invasive pattern, and as such, fibroelastosis without glands should not be included in invasive measurement. It is acknowledged that in some instances, careful high magnification assessment of the scar is needed and can highlight single tumor cells or cluster, changing the impression to that of invasion (Fig. 6D). If needed, a cytokeratin immunohistochemistry stain can highlight such clusters.

Lepidic, acinar and micropapillary

While the distinction of acinar from micropapillary does not by itself impact invasive size as these are both invasive patterns, criteria that would absolutely designate a pattern as micropapillary could help resolve a decision between lepidic and acinar raised in the prior section. Foci of stratification that lead to finger like or filagree pattern of micropapillary are defined as 3 cells high[35,36], but when stratification occurs more diffusely in that it does not resemble micropapillary fingers or tufts, observers may have a more difficult time deciding between collapsed lepidic with tangential sectioning and acinar. There does appear to be a level of cellularity and atypia, that even in the absence of desmoplasia or definitive invasion of other structures, moves the classification away from a non-invasive to an invasive pattern. Such transition zones require a more precise definition to convert this impression into a consistent interobserver method of invasive measurement.

Technical and procedural considerations in invasive size measurement

There are important considerations in the evaluation of lung resections to optimize histological examination of adenocarcinomas. Imaging findings can help determine tumor size and importantly the proportion of ground glass and solid component. With this information in hand, the examination of the gross specimen can include are more reliable assessment of the boundaries of the tumor; the lepidic component of an adenocarcinoma is often at the periphery and more subtle. Invasive portions of an adenocarcinoma are often depressed and at the center of the tumor, rendering an umbilicated gross appearance. If the tumor is under 2.0 cm, the entire cut surface of the tumor can be placed into a full-face section. It is not ideal to cut across the umbilicated center as that would limit the ability of a continuous

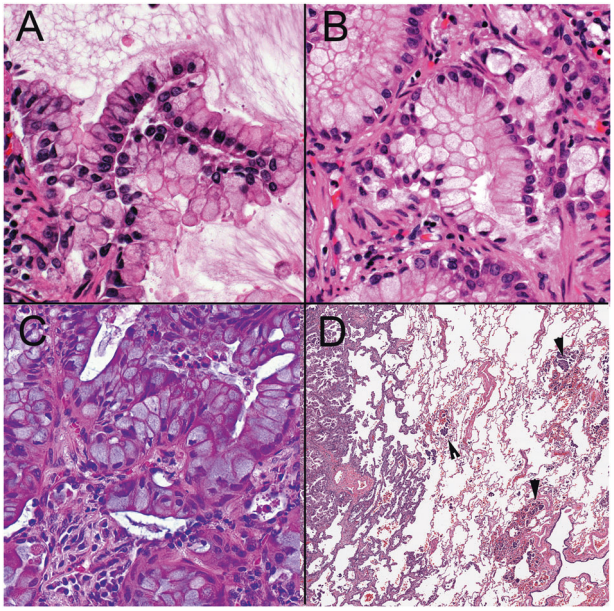

Fig. 7 Adenocarcinoma features in the consideration of invasion. **A** Invasive mucinous adenocarcinoma is characterized by growth along pre-existing alveoli, but back to back glands and stromal reaction should be considered invasive as seen in (**B**). **C** While many tumors have an apical mucin droplet resembling gastric mucosa, some have a more goblet cell morphology. **D** While not associated with stromal reaction, nests of tumor cells away from the main mass and showing histologic features of the invasive tumor pattern are a form of invasion known as spread through air spaces, but does not add to invasive size.

linear measurement. If bigger than 2.0 cm it may be difficult to get a full-face section; one approach that can be tried is to divide the full face into two consecutive cassettes and align them during microscopic review. However, for some tumors it is difficult to assess one linear measurement,

There are two main methods of measuring invasive size. Both require agreement as to what constitutes invasion. It is often possible to outline the proposed invasive focus and measure across its longest extent. Alternatively, it is proposed that after estimating the overall percent of invasive patterns to multiply this by the diameter of the tumor. While these two methods can result in a similar measurement, in some instances it can be quite different. In such circumstances it may be of utility to correlate the result with the diameter of the solid component on CT imaging. Once determined, the invasive size should be used for T staging, not the macroscopic size.

Mucinous adenocarcinoma

This subtype of adenocarcinoma consists of columnar cells with apical mucin resembling gastric type epithelium. The cells of this tumor type can be very bland, but rows of mucinous cells without intervening squamous metaplasia or ciliated cells are diagnostic of mucinous adenocarcinoma. These tumors frequently grow along pre-existing alveolar architecture, but more confluent growth with gland formation can be seen (Fig. 7A, B). One challenge is that such tumors elicit less fibrosis and desmoplasia than non-mucinous tumors. While complete alveolar growth constitutes mucinous AIS, such tumors are fairly rare and most mucinous adenocarcinomas are invasive. In some tumors, the mucinous cells are goblet cells rather than gastric-like (Fig. 7C). These tumors often form non-contiguous nodules and intralobar spread is very common[37,38].

Spread through airspaces

The concept of dyscohesion and non-adherent growth are central to cases in which aeroginous spread occurs, sometimes as spread through airspaces and other times as separate nodules in the same lobe (Fig. 7D). The presence of such tufts of tumor cells away from the main mass is a feature that is associated with intralobar spread and is of particular interest in the assessment of margin in limited resections[38,39]. While there are important scenarios in which such cells are more likely technical artifacts, it is increasingly apparent that tumor stratification and budding, associated with survival of such buds, is associated with recurrence. Whether this feature is by itself invasion and how to incorporate this feature into invasive size remain challenging as these foci may be more relevant to R factor staging than to T staging.

Future directions

It may be that molecular markers of tumor cell biology such as EGFR amplification or p53 mutation can be incorporated into prognostic considerations of pattern or in invasion assessment. Stromal changes that reflect remodeling or immune response may herald or coincide with invasion. It is also possible that more precise prognostication will come from distinction of invasion as a local tumor phenomenon from intravasation/metastasis; this may be enhanced by the addition of blood-based biomarkers.

CONCLUSION

The heterogeneity of adenocarcinoma has revealed subgroups of tumor with favorable and unfavorable outcome based on the histologic patterns of growth, and these observations incorporated into a grading system based on predominant pattern with correction for non-predominant high-grade patterns over 20% of the tumor. The measurement of invasive size remains a more difficult assessment but important to prevent overstaging; it may require integration of tumor growth pattern and stromal effects. Particular areas that are known difficult patterns with lower reproducibility need to be the focus for improvement.

REFERENCES

1. Chapman, A. D. & Kerr, K. M. The association between atypical adenomatous hyperplasia and primary lung cancer. *Br. J. Cancer* **83**, 632–636 (2000).
2. Hu, X. et al. Multi-region exome sequencing reveals genomic evolution from preneoplasia to lung adenocarcinoma. *Nat. Commun.* **10**, 2978 (2019).
3. Sakamoto, H. et al. Disproportionate representation of KRAS gene mutation in atypical adenomatous hyperplasia, but even distribution of EGFR gene mutation from preinvasive to invasive adenocarcinomas. *J. Pathol.* **212**, 287–294 (2007).
4. Sivakumar, S. et al. Genomic landscape of atypical adenomatous hyperplasia reveals divergent modes to lung adenocarcinoma. *Cancer Res.* **77**, 6119–6130 (2017).
5. Chang, J. C. et al. Bronchiolar adenoma: expansion of the concept of ciliated muconodular papillary tumors with proposal for revised terminology based on morphologic, immunophenotypic, and genomic analysis of 25 cases. *Am. J. Surg. Pathol.* **42**, 1010–1026 (2018).
6. WHO Classification of Tumours Thoracic Tumors. 5th Edition ed. (International Agency for Research on Cancer, 2021).
7. Suzuki, K., Asamura, H., Kusumoto, M., Kondo, H. & Tsuchiya, R. "Early" peripheral lung cancer: prognostic significance of ground glass opacity on thin-section computed tomographic scan. *Ann. Thorac. Surg.* **74**, 1635–1639 (2002).
8. Austin, J. H. et al. Radiologic implications of the 2011 classification of adenocarcinoma of the lung. *Radiology* **266**, 62–71 (2013).
9. Azour, L., Ko, J. P., Naidich, D. P. & Moore, W. H. Shades of gray: subsolid nodule considerations and management. *Chest* **159**, 2072–2089 (2021).
10. Takahashi, M. et al. Tumor invasiveness as defined by the newly proposed IASLC/ATS/ERS classification has prognostic significance for pathologic stage IA lung adenocarcinoma and can be predicted by radiologic parameters. *J. Thorac. Cardiovasc. Surg.* **147**, 54–59 (2014).
11. Chirieac, L. R. & Flieder, D. B. High-resolution computed tomography screening for lung cancer: unexpected findings and new controversies regarding adenocarcinogenesis. *Arch. Pathol. Lab. Med.* **134**, 41–48 (2010).
12. Yatabe, Y., Borczuk, A. C. & Powell, C. A. Do all lung adenocarcinomas follow a stepwise progression? *Lung Cancer* **74**, 7–11 (2011).
13. Borczuk, A. C. Assessment of invasion in lung adenocarcinoma classification, including adenocarcinoma in situ and minimally invasive adenocarcinoma. *Mod. Pathol.* **25**, S1–S10 (2012).

14. Inafuku, K. et al. Should pathologically noninvasive lung adenocarcinoma larger than 3 cm be classified as T1a? *Ann. Thorac. Surg.* **108**, 1678–1684 (2019).
15. Borczuk, A. C. et al. Invasive size is an independent predictor of survival in pulmonary adenocarcinoma. *Am. J. Surg. Pathol.* **33**, 462–469 (2009).
16. Travis, W. D. et al. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. *J. Thorac. Oncol.* **6**, 244–285 (2011).
17. Kadota, K. et al. Cribriform subtype is an independent predictor of recurrence and survival after adjustment for the eighth edition of TNM staging system in patients with resected lung adenocarcinoma. *J. Thorac. Oncol.* **14**, 245–254 (2019).
18. Moreira, A. L., Joubert, P., Downey, R. J. & Rekhtman, N. Cribriform and fused glands are patterns of high-grade pulmonary adenocarcinoma. *Hum. Pathol.* **45**, 213–220 (2014).
19. Warth, A. et al. Prognostic impact and clinicopathological correlations of the cribriform pattern in pulmonary adenocarcinoma. *J. Thorac. Oncol.* **10**, 638–644 (2015).
20. Mescam-Mancini, L. et al. On the relevance of a testing algorithm for the detection of ROS1-rearranged lung adenocarcinomas. *Lung Cancer* **83**, 168–173 (2014).
21. Popat, S. et al. ALK translocation is associated with ALK immunoreactivity and extensive signet-ring morphology in primary lung adenocarcinoma. *Lung Cancer* **75**, 300–305 (2012).
22. Moreira, A. L. et al. A grading system for invasive pulmonary adenocarcinoma: a proposal from the international association for the study of lung cancer pathology committee. *J. Thorac. Oncol.* **15**, 1599–1610 (2020).
23. Yoshizawa, A. et al. Impact of proposed IASLC/ATS/ERS classification of lung adenocarcinoma: prognostic subgroups and implications for further revision of staging based on analysis of 514 stage I cases. *Mod. Pathol.* **24**, 653–664 (2011).
24. Borczuk, A. C. Prognostic considerations of the new World Health Organization classification of lung adenocarcinoma. *Eur. Respir. Rev.* **25**, 364–371 (2016).
25. Thunnissen, E. et al. Reproducibility of histopathological subtypes and invasion in pulmonary adenocarcinoma. *Int. Interobserver Study Mod. Pathol.* **25**, 1574–1583 (2012).
26. Shimosato, Y. et al. Prognostic implications of fibrotic focus (scar) in small peripheral lung cancers. *Am. J. Surg. Pathol.* **4**, 365–373 (1980).
27. Noguchi, M. et al. Small adenocarcinoma of the lung. Histologic characteristics and prognosis. *Cancer* **75**, 2844–2852 (1995).
28. Suzuki, K. et al. Prognostic significance of the size of central fibrosis in peripheral adenocarcinoma of the lung. *Ann. Thorac. Surg.* **69**, 893–897 (2000).
29. Yim, J. et al. Histologic features are important prognostic indicators in early stages lung adenocarcinomas. *Mod. Pathol.* **20**, 233–241 (2007).
30. Kadota, K. et al. Prognostic significance of adenocarcinoma in situ, minimally invasive adenocarcinoma, and nonmucinous lepidic predominant invasive adenocarcinoma of the lung in patients with stage I disease. *Am. J. Surg. Pathol.* **38**, 448–460 (2014).
31. Boland, J. M. et al. Adenocarcinoma in situ, minimally invasive adenocarcinoma, and invasive pulmonary adenocarcinoma-analysis of interobserver agreement, survival, radiographic characteristics, and gross pathology in 296 nodules. *Hum. Pathol.* **51**, 41–50 (2016).
32. Blaauwgeers, H. et al. A prospective study of loose tissue fragments in non-small cell lung cancer resection specimens: an alternative view to "spread through air spaces". *Am. J. Surg. Pathol.* **41**, 1226–1230 (2017).
33. Thunnissen, E. et al. In compressed lung tissue microscopic sections of adenocarcinoma in situ may mimic papillary adenocarcinoma. *Arch. Pathol. Lab. Med.* **137**, 1792–1797 (2013).
34. Trejo-Bittar, H. et al. Prognostic significance of microscopic size in peripherally located scar-associated clinical stage I lung carcinomas. *Lung Cancer* **143**, 12–18 (2020).
35. Thunnissen, E. & Flieder, D. Morphologic logic: "filigree" and "classical" micropapillary pattern are orientation-dependent views of the same lesion. *J. Thorac. Oncol.* **15**, e120–e121 (2020).
36. Emoto, K., Tan, K. S., Rekhtman, N., Adusumilli, P. S. & Travis, W. D. The newly described filigree pattern is an expansion of the micropapillary adenocarcinoma concept rather than a proposed new subtype. *J. Thorac. Oncol.* **15**, e121–e124 (2020).
37. Boland, J. M. et al. Pulmonary invasive mucinous adenocarcinoma and mixed invasive mucinous/nonmucinous adenocarcinoma-a clinicopathological and molecular genetic study with survival analysis. *Hum. Pathol.* **71**, 8–19 (2018).
38. Sugano, M. et al. HNF4alpha as a marker for invasive mucinous adenocarcinoma of the lung. *Am. J. Surg. Pathol.* **37**, 211–218 (2013).
39. Kadota, K. et al. Tumor spread through air spaces is an important pattern of invasion and impacts the frequency and location of recurrences after limited resection for small stage I lung adenocarcinomas. *J. Thorac. Oncol.* **10**, 806–814 (2015).

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COMPETING INTERESTS

The author declares no competing interests.

ETHICS APPROVAL/CONSENT TO PARTICIPATE

This is a review article. No unpublished data is provided.

ADDITIONAL INFORMATION

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