

Contemporary Pathological Classification of Primary Lung Tumors: Integration of Morphology, Immunohistochemistry, and Molecular Pathology

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Received: 1 June 2026; **Accepted:** 16 September 2026

Abstract

This review summarizes the current World Health Organization (WHO) classification of lung tumors, emphasizing key morphological, immunohistochemical, and molecular features relevant to diagnosis and management. **Review.** Primary pulmonary tumors comprise a heterogeneous spectrum of neoplasms with distinct morphological, immunophenotypic, molecular, and clinical characteristics. The current WHO classification integrates traditional histopathological assessment with immunohistochemical and molecular findings, reflecting advances in our understanding of tumor biology and the growing importance of precision medicine. This review summarizes the major categories of primary pulmonary tumors, including adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma, large cell carcinoma, sarcomatoid carcinomas, neuroendocrine neoplasms, salivary gland-type tumors, and mesenchymal tumors specific to the lung, as well as selected rare pulmonary neoplasms. Particular attention is given to the defining morphological characteristics and diagnostic criteria of individual tumor entities, relevant immunohistochemical profiles, and recurrent molecular alterations with diagnostic, prognostic, and therapeutic implications. The challenges of diagnosing pulmonary tumors in small biopsies and cytological specimens are also addressed, with an emphasis on the judicious use of immunohistochemistry and preservation of tissues for molecular testing. **Conclusion.** Accurate classification of primary pulmonary tumors requires the integration of morphological, immunohistochemical, and molecular findings within the framework of the current WHO Classification of Thoracic Tumors. Recognition of the characteristic morphological and immunophenotypic features, together with appropriate molecular characterization, is essential for precise diagnosis and optimal patient management. Continued advances in molecular pathology are expected to further refine pulmonary tumor classification and contribute to increasingly personalized therapeutic strategies.

Key Words: Lung Carcinoma ▪ Morphology ▪ Molecular Pathology ▪ WHO Classification ▪ Biomarkers.

Introduction

Lung cancer is a leading cause of cancer-related death, with 1.8 million deaths in 2022 (1). Advances in the understanding of genomic alterations in lung cancer and the development of targeted therapies for some of these tumors have undoubtedly contributed to the classification of lung cancer.

Histological classification remains the most important factor in the diagnosis of lung cancer; however, in many cases, molecular and biomarker testing is crucial for treatment. The latest 2021 World Health Organization (WHO) Classification

of Thoracic Tumors (2) included some changes and new entities. Lung cancer comprises numerous histomorphological subtypes; however, there are two main types of lung cancer: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC).

Over the years, the proportions of the histological types have changed significantly. The most notable change is in the proportion of adenocarcinoma, which now accounts for more than 50% of all lung cancer cases, whereas the proportion of SCLC has decreased to 13%. A significant decrease was also recorded in the proportion of large cell carcinoma (LCC), from 8% to 1% of all lung

cancers (2). These changes can be partially attributed to the broader use of immunohistochemical staining and better determination of subtypes; however, the influence of changes in smoking habits and other carcinogenic factors should not be excluded. However, precise histological classification remains the primary objective, as it underpins personalized therapy and has significant prognostic and therapeutic implications.

This review provides an overview of the current pathological classification of lung tumors according to the latest World Health Organization (WHO) Classification of Thoracic Tumors, with particular emphasis on morphological, immunohistochemical, and molecular features relevant to diagnosis and clinical management.

Materials and Methods

This narrative review was based on a literature search of PubMed/MEDLINE, Scopus, and Web of Science, focusing on recent publications relevant to the classification and diagnosis of primary pulmonary tumors. The search included combinations of terms such as “lung tumors,” “pulmonary tumors,” “lung carcinoma,” “adenocarcinoma,” “squamous cell carcinoma,” “neuroendocrine neoplasm,” “sarcomatoid carcinoma,” “salivary gland-type tumors,” “mesenchymal tumors,” “immunohistochemistry,” “molecular pathology,” and “WHO classification.” Particular emphasis was placed on the current WHO Classification of Thoracic Tumors, with priority given to recent studies, consensus recommendations, guidelines, and key publications addressing morphological, immunohistochemical, and molecular features. Seminal earlier studies were also included when relevant to established diagnostic criteria or tumor classification.

This review is primarily intended for practicing pathologists and pathology trainees, with an emphasis on practical diagnostic challenges, including the evaluation of small biopsies and cytological specimens, appropriate use of immunohistochemistry, tissue stewardship, and integration of morphological and molecular findings. Practical diagnostic algorithms are included to

illustrate a stepwise approach to pulmonary tumor classification and differential diagnosis.

Diagnostic Approach to Small Biopsy and Cytology Specimens

The diagnosis of lung cancer often relies on minimally invasive specimens, including bronchoscopic and transthoracic biopsies, bronchoalveolar lavage (BAL), and cytological samples. These specimens may provide sufficient material for morphologic diagnosis and immunohistochemical classification, while also serving as substrates for molecular testing. However, limited tissue quantity, crush artifacts, necrosis, and suboptimal preservation may complicate diagnosis and restrict the availability of material for ancillary studies. Therefore, careful specimen handling and tissue stewardship are essential, particularly when molecular profiling is required to guide targeted or immunotherapeutic treatment.

Careful assessment of tumor morphology should be followed by a tissue-sparing immunohistochemical approach. In tumors showing clear glandular differentiation, adenocarcinoma may be diagnosed morphologically and supported by markers such as thyroid transcription factor 1 (TTF-1) or Napsin A. When morphology does not allow definitive classification, a limited panel with TTF-1 and p40 can distinguish most adenocarcinoma-spectrum and squamous cell carcinoma cases. A diagnosis of NSCLC, not otherwise specified (NSCLC, NOS) should be reserved only for cases in which a more specific classification cannot be established after appropriate morphological and immunohistochemical evaluation. Extensive immunohistochemical testing should be avoided because it may exhaust the specimen and compromise subsequent molecular analysis.

Cell blocks prepared from cytological specimens can provide suitable material for histological evaluation, immunohistochemistry, and, when adequately cellular, molecular testing. Cytological specimens may represent an important diagnostic and predictive-testing resource, particularly when tissue biopsy is limited or unavailable.

World Health Organization Classification of Lung Cancer (2021)

Basics of Lung Tumor Classification

The 5th WHO Classification of Thoracic Tumors remains based on the histological origin of the tumors. Lung cancers are broadly categorized into epithelial tumors, neuroendocrine neoplasms, ectopic

tissue tumors, mesenchymal tumors specific to the lung, and hematolymphoid tumors (Table 1). In this review, we focus on the key morphological, immunohistochemical, and molecular features of malignant lung tumors. Epithelial tumors, the largest group, consist of nine main types and more than 20 different tumor subtypes. This group also includes precursor lesions for the main tumor types.

Table 1. Categories of Lung Tumors in the 5th WHO Classification of Lung Tumors

Main Category	Subcategory	Tumor / Lesion
Epithelial tumors	Papillomas	Bronchial papillomas
—	Adenomas	Sclerosing pneumocytoma
—	—	Alveolar adenoma
—	—	Papillary adenoma of the lung
—	—	Bronchiolar adenoma / ciliated muconodular papillary tumor
—	—	Mucinous cystadenoma of the lung
—	—	Mucous gland adenoma of the lung
—	Precursor glandular lesions	Atypical adenomatous hyperplasia of the lung
—	—	Adenocarcinoma in situ of the lung
—	—	Minimally invasive adenocarcinoma of the lung
—	—	Invasive non-mucinous adenocarcinoma of the lung
—	—	Invasive mucinous adenocarcinoma of the lung
—	—	Colloid adenocarcinoma of the lung
—	—	Fetal adenocarcinoma of the lung
—	—	Enteric-type adenocarcinoma of the lung
—	Squamous precursor lesions	Squamous dysplasia and carcinoma in situ of the lung
—	Squamous cell carcinomas	Squamous cell carcinoma of the lung
—	—	Lymphoepithelial carcinoma of the lung
—	—	Large cell carcinoma of the lung
—	—	Adenosquamous carcinoma of the lung
—	—	Pleomorphic carcinoma of the lung
—	—	Pulmonary blastoma
—	—	Carcinosarcoma of the lung
—	Other epithelial tumors	NUT carcinoma of the lung
—	—	Thoracic SMARCA4-deficient undifferentiated tumor
—	Salivary gland-type tumors	Pleomorphic adenoma of the lung
—	—	Adenoid cystic carcinoma of the lung
—	—	Epithelial-myoepithelial carcinoma of the lung
—	—	Mucoepidermoid carcinoma of the lung
—	—	Hyalinizing clear cell carcinoma of the lung
—	—	Myoepithelioma and myoepithelial carcinoma of the lung
Lung neuroendocrine neoplasms	Precursor lesion	Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia

Continuation of Table 1.

Main Category	Subcategory	Tumor / Lesion
—	Neuroendocrine tumors	Carcinoid / neuroendocrine tumor of the lung
—	Neuroendocrine carcinomas	Small cell lung carcinoma
—	—	Large cell neuroendocrine carcinoma of the lung
Tumors of ectopic tissues	—	Melanoma of the lung
—	—	Meningioma of the lung
Mesenchymal tumors specific to the lung	—	Pulmonary hamartoma
—	—	Pulmonary chondroma
—	—	Diffuse pulmonary lymphangiomatosis
—	—	Pleuropulmonary blastoma
—	—	Pulmonary artery intimal sarcoma
—	—	Congenital peribronchial myofibroblastic tumor
—	—	Primary pulmonary myxoid sarcoma with EWSR1-CREB1 fusion
PEComatous tumors	—	Lymphangioleiomyomatosis of the lung
—	—	PEComa of the lung
Haematolymphoid tumors	—	MALT lymphoma of the lung
—	—	Pulmonary diffuse large B-cell lymphoma
—	—	Lymphomatoid granulomatosis of the lung
—	—	Intravascular large B-cell lymphoma of the lung
—	—	Pulmonary Langerhans cell histiocytosis
—	—	Pulmonary Erdheim-Chester disease

Adenocarcinoma and Precursor Lesions

Adenocarcinoma

Lung adenocarcinoma, the most common type of lung cancer, is further classified into minimally invasive adenocarcinoma (MIA), invasive non-mucinous adenocarcinoma, invasive mucinous adenocarcinoma (IMA), colloid adenocarcinoma, fetal adenocarcinoma, and enteric-type adenocarcinoma.

In addition, the current classification recognizes two precursor glandular lesions: atypical adenomatous hyperplasia (AAH) and adenocarcinoma in situ (AIS). The main morphological difference between these lesions is their size, with small lesions (usually ≤ 5 mm) designated as AAH and larger lesions (≤ 30 mm) designated as AIS (2, 3). Radiologically, AAH and AIS most commonly present as pure ground-glass nodules and are primarily differentiated based on lesion size. However,

Pan et al. reported that additional radiological features, particularly the vacuole sign, occur predominantly in AIS rather than AAH and may aid in their differentiation (4). Microscopically, both lesions show proliferation of atypical type II pneumocytes in a pure lepidic growth pattern, with complete absence of invasive growth and necrosis. As illustrated in Figure 1, tumor cells show mild to moderate atypia and proliferate along the alveolar walls without evidence of papillary or micropapillary patterns (2). The majority of AIS are of non-mucinous differentiation, with extremely rare cases of mucinous AIS. Immunohistochemical expression of TTF-1 and Napsin A is present in both precursor lesions. They also share similar molecular profiles. Epidermal Growth Factor Receptor (EGFR) mutations have been detected in up to one-third of AAH cases and almost half of AIS cases, suggesting a multistep progression model from AAH through AIS to invasive adenocarcinoma (2).

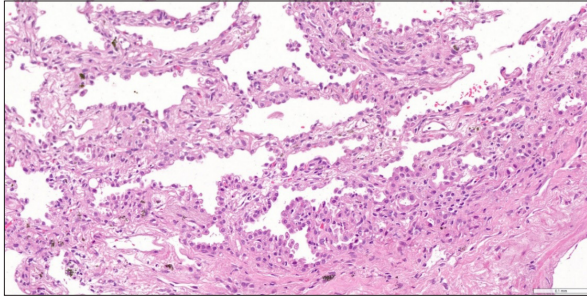


Figure 1. Atypical adenomatoid hyperplasia – Microscopy shows proliferation of atypical pneumocytes along intact alveolar spaces without significant architectural distortion, fibrosis, or inflammation (hematoxylin and eosin (H&E) staining at $\times 20$ magnification).

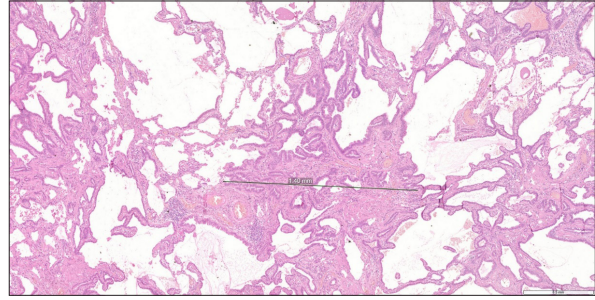


Figure 2. Minimally invasive adenocarcinoma consists predominantly of a lepidic growth pattern with a small focus of invasion (1.4 mm in extent) characterized by irregular glandular structures accompanied by desmoplastic stroma (H&E, $\times 10$ magnification).

Minimally Invasive Adenocarcinoma

The category of minimally invasive adenocarcinoma (MIA) was formally included in the 2015 WHO Classification of Thoracic Tumors as a small (≤ 30 mm), solitary adenocarcinoma with a predominantly lepidic pattern and an invasive component of ≤ 5 mm (2). The characteristic histopathological features of MIA are illustrated in Figure 2. In contrast, tumors with a predominant lepidic pattern that are >3 cm in total size and/or contain an invasive component >5 mm are classified as lepidic-predominant adenocarcinomas (2). Rare cases of tumors >3 cm exhibiting pure lepidic growth without an identifiable invasive component have also been reported (5). According to the WHO Classification of Thoracic Tumors, such tumors should be classified as lepidic-predominant adenocarcinomas rather than adenocarcinoma in situ (AIS), as AIS is restricted to tumors measuring ≤ 3 cm.

The definitive diagnosis of AIS and MIA requires complete surgical resection and complete histological examination of the entire tumor and cannot be reliably established on small diagnostic specimens. Although the lepidic pattern can be readily recognized, morphological assessment of invasive patterns in adenocarcinoma has shown poor interobserver reproducibility (6,7). Therefore, a modified classification has recently been recommended that accounts for iatrogenic collapse and tangential sectioning and incorporates the use of

cytokeratin 7 (CK7) and elastin stains to identify the true invasive component (8).

Similar to other adenocarcinoma categories, MIA can be divided into three morphological subtypes: mucinous, non-mucinous, and mixed. Non-mucinous MIA expresses TTF-1 and Napsin A, immunohistochemical markers, whereas mucinous MIA is typically negative.

Additionally, MIA has some genetic alterations similar to those of invasive adenocarcinoma, usually *EGFR* alterations, but also tumor protein p53 (*TP53*), Kirsten rat sarcoma virus oncogene homologue (*KRAS*), and neurofibromatosis 1 gene (*NF1*) alterations (2). The main clinical significance of MIA is reflected in the fact that it shows almost 100% disease-specific or recurrence-free survival after complete resection, similar to AIS (9), and that these patients are practically cured with resection.

Invasive Non-Mucinous Adenocarcinoma

Most lung adenocarcinomas are invasive, non-mucinous adenocarcinomas with morphologic characteristics or immunohistochemical evidence of glandular differentiation.

Morphologically, non-mucinous adenocarcinomas usually consist of admixed architectural patterns and are subtyped according to the predominant pattern into lepidic, acinar, papillary, micropapillary, and solid. Representative histopathological features of the lepidic, acinar,

papillary, and solid patterns are shown in Figure 3A–D, respectively. In resected specimens, a single predominant component should be selected, and any other patterns should be estimated in 5–10% increments (2). Assessment of the predominant histological pattern allows tumors to be stratified into prognostic groups, with lepidic-predominant tumors having the most favorable prognosis, acinar- and papillary-predominant tumors having an intermediate prognosis, and solid- and micropapillary-predominant tumors having the poorest prognosis (10).

The latest WHO Classification of Thoracic Tumors incorporates a three-tiered histological grading system for lung adenocarcinoma (Table 2), based on the proportions of architectural patterns, including lepidic, acinar, papillary, micropapillary, and solid patterns, as well as the presence of cribriform and complex glandular patterns characterized by fused glands or single cells infiltrating a desmoplastic stroma.

Table 2. Grading System for Lung Adenocarcinomas

Grade 1 (well differentiated)	Lepidic predominant with no or <20% high-grade pattern (solid, micropapillary, cribriform, or complex glandular pattern)
Grade 2 (moderately differentiated)	Acinar or papillary predominant with no or <20% high-grade pattern
Grade 3 (poorly differentiated)	Any tumor with $\geq 20\%$ high-grade pattern

The International Association for the Study of Lung Cancer (IASLC) grading system stratifies invasive pulmonary adenocarcinomas according to the predominant histological pattern and the proportion of high-grade patterns. Grade 1 includes lepidic-predominant adenocarcinomas with less than 20% high-grade patterns, whereas Grade 2 includes acinar- or papillary-predominant adenocarcinomas with less than 20% high-grade patterns. Grade 3 comprises adenocarcinomas with $\geq 20\%$ high-grade patterns, regardless of the predominant pattern. High-grade patterns include

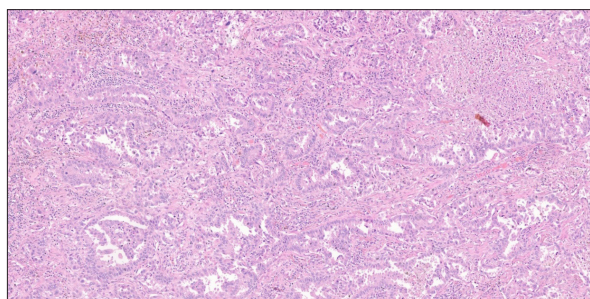


Figure 3. Different patterns of lung adenocarcinoma. 3A – Acinar pattern showing irregularly shaped gland formation surrounded by fibrous stroma (H&E, $\times 20$ magnification).

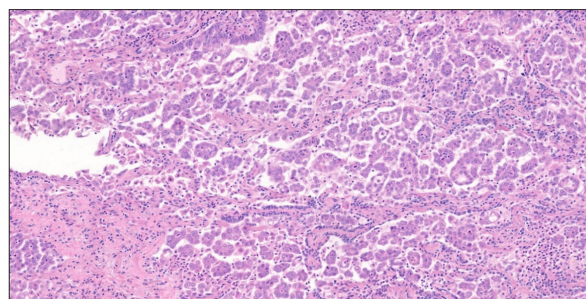


Figure 3C. Micropapillary adenocarcinoma showing tufts of tumor cells without fibrovascular cores, characteristic of the micropapillary pattern (H&E, $\times 20$ magnification).

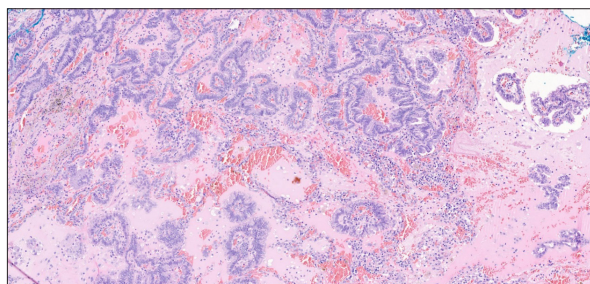


Figure 3B. Papillary adenocarcinoma showing papillary structures with true fibrovascular cores, characteristic of the papillary growth pattern (H&E, $\times 20$ magnification).

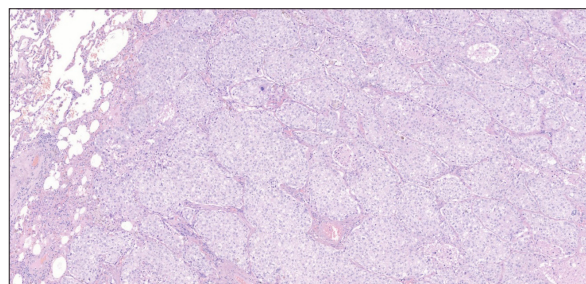


Figure 3D. Solid pattern showing sheets of neoplastic cells lacking glandular differentiation (H&E, $\times 20$ magnification).

solid, micropapillary, and complex glandular patterns.

A higher tumor grade is associated with an increased risk of recurrence and poorer disease-specific outcomes. Therefore, accurate recognition and quantification of high-grade patterns are important components of the pathological assessment of resected pulmonary adenocarcinomas and should be reported as part of the integrated histological evaluation.

Lepidic-predominant adenocarcinoma is characterized by the proliferation of type II pneumocytes or club cells along the alveolar surface and the presence of an invasive component >5 mm in the greatest dimension. The invasive component includes any other histological pattern or the presence of myofibroblastic stroma associated with invasive tumor cells (2). In addition to the size of the invasive component, several additional features are used to differentiate lepidic-predominant adenocarcinoma from MIA, including lymphovascular or pleural invasion, tumor necrosis, and spread through air spaces (STAS).

STAS is a pattern of tumor spread characterized by the presence of tumor cells, clusters, or micropapillary structures within air spaces beyond the edge of the main tumor. It is an important adverse prognostic factor associated with an increased risk of local recurrence and poorer survival, particularly after limited surgical resection, such as wedge resection or segmentectomy (11).

The acinar pattern is characterized by neoplastic glands invading the myofibroblastic stroma, destroying the alveolar architecture of the lung (Figure 3a). Although cribriform growth, characterized by back-to-back fused glands, is considered a subtype of acinar adenocarcinoma, it is recognized as a high-grade architectural pattern.

The papillary pattern (Figure 3b) is characterized by papillary structures composed of glandular tumor cells that grow along the surfaces of fibrovascular cores. In contrast, the micropapillary pattern (Figure 3c) consists of tumor cells growing in papillary tufts or florets that lack fibrovascular cores and are either detached from or connected to the alveolar walls. The micropapillary pattern also

includes the so-called filigree pattern, which consists of tumor cells arranged in delicate, lace-like, narrow strands of three or more cells without fibrovascular cores.

Adenocarcinoma with a solid pattern (Figure 3d) consists of polygonal cells arranged in solid sheets. Glandular differentiation must be confirmed by immunohistochemical expression of a pneumocyte marker (TTF-1 or Napsin A) or by the histochemical presence of intracellular mucin in ≥ 5 tumor cells in each of two high-power fields (~ 0.4 mm²) (2).

Immunohistochemistry plays an important role in confirming the diagnosis of primary lung adenocarcinoma versus metastatic carcinoma and in distinguishing solid-predominant adenocarcinoma from other non-small cell lung carcinoma subtypes, particularly when evaluating small biopsy or cytological specimens. TTF-1 and Napsin A are the most reliable pneumocytic markers, and even focal TTF-1 expression may be sufficient to support glandular differentiation. In the present cases, the tumor cells showed positive TTF-1 immunoreactivity (Figure 4). However, its interpretation should take into account the antibody clone, staining localization and pattern, tumor morphology, and clinical context. TTF-1 demonstrates high sensitivity (94.1%) and specificity (86.0%), whereas Napsin A shows slightly lower sensitivity (87.4%) but higher specificity (97.8%) for pulmonary adenocarcinoma (12).

The molecular profile of lung adenocarcinoma has been extensively studied, and several oncogenic driver gene alterations have been identified, which are often mutually exclusive, although

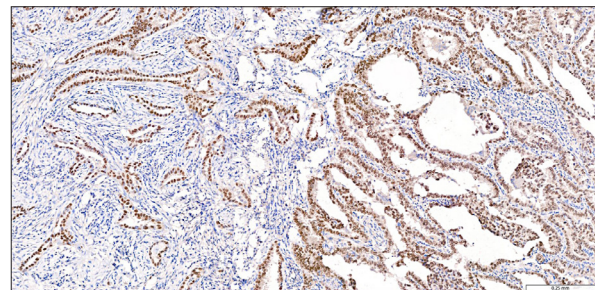


Figure 4. Immunohistochemical staining for TTF-1 showing strong nuclear positivity in tumor cells, supporting pulmonary adenocarcinoma (H&E, $\times 20$ magnification).

clinically relevant co-alterations may occur. Some of the most common include alterations or rearrangements involving *EGFR*, *KRAS*, *anaplastic lymphoma kinase (ALK)*, *proto-oncogene ROS1*, *rearranged during transfection (RET)*, *neurotrophic receptor tyrosine (NTRK1–3)*, *proto-oncogene MET*, *human epidermal growth factor receptor 2 (ERBB2)/ (HER2)*, *MAP2K1 (MEK1)*, *neuroblastoma RAS viral oncogene homolog (NRAS)*, and *neu-regulin 1 (NRG1)*.

Although strong histological–molecular correlations are generally lacking in lung adenocarcinoma, certain histological patterns have been linked to specific genetic alterations. *KRAS* alterations are most common in invasive mucinous and solid-predominant adenocarcinomas; *EGFR* alterations are more often observed in non-mucinous lepidic and papillary tumors; and *ALK* and *ROS1* rearrangements are often associated with cribriform architecture and signet-ring cell morphology (13–15).

Targeted therapies are currently available for many of these alterations, including *EGFR*, *ALK*, *ROS1*, *B-Raf proto-oncogene, serine/threonine kinase (BRAF)*, *MET*, *RET*, *NTRK*-family gene fusions, and certain *KRAS* alterations. The evaluation of tumors for clinically relevant molecular alterations is included as a desirable criterion in the 2021 WHO Classification of Thoracic Tumors, reflecting the growing importance of molecular findings in the classification framework.

Invasive Mucinous Adenocarcinoma

Lung adenocarcinoma composed of tumor cells exhibiting goblet or columnar cell morphology with abundant intracytoplasmic mucin is classified as invasive mucinous adenocarcinoma (IMA). It accounts for approximately 3–10% of all lung adenocarcinomas. Morphologically, IMAs are most often lepidic-predominant, with variable amounts of invasive components exhibiting other architectural patterns (2). Immunohistochemically, IMAs typically lack expression of the pneumocytic markers TTF-1 and Napsin A, while showing strong CK7 positivity and occasional focal co-expression of

cytokeratin 20 (CK20) and caudal type homeobox 2 (CDX2). Although IMAs can harbor many of the same driver alterations as non-mucinous adenocarcinomas, the most common molecular abnormalities are *KRAS* alterations, particularly the *p.G12D* and *p.G12V* variants, which are identified in approximately 70% of cases (16).

Colloid Adenocarcinoma

Colloid adenocarcinoma is a rare subtype of invasive adenocarcinoma characterized by extensive extracellular mucin pools, alveolar space distention, and alveolar wall destruction. As shown in Figure 5, tumor cells typically float within mucin pools or line the residual alveolar walls and usually exhibit mucin-laden cuboidal, tall columnar, or signet-ring cell morphology. The immunohistochemical features resemble those of IMA: tumor cells are CK7-positive and, in most cases, TTF1- and Napsin A-negative.

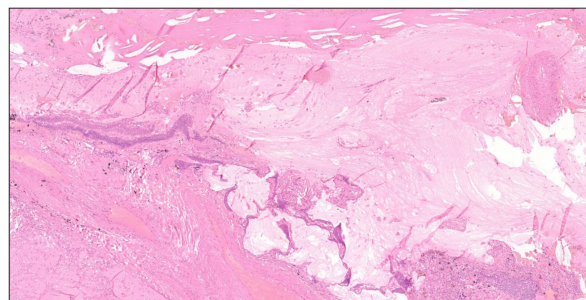


Figure 5. Colloid adenocarcinoma is characterized by abundant extracellular mucin infiltrating the lung parenchyma, with scattered columnar and cuboidal tumor cells focally lining residual alveolar structures (H&E, $\times 20$ magnification).

Fetal Adenocarcinoma

Fetal adenocarcinoma of the lung is a rare subtype with a morphology resembling that of the developing fetal lung, displaying complex glandular, papillotubular, or cribriform structures composed of pseudostratified columnar cells with glycogen-rich, clear cytoplasm that forms subnuclear vacuoles (2). It is classified as either low- or high-grade based on the degree of nuclear atypia, presence

or absence of morule formation, and presence of necrosis.

Enteric-Type Adenocarcinoma of the Lung

Another rare subtype is enteric-type adenocarcinoma, a primary pulmonary adenocarcinoma that resembles colorectal adenocarcinoma in its morphological, immunohistochemical, and molecular features. Given its characteristic enteric morphology, expression of enteric markers, CDX2, CK20, hepatocyte nuclear factor 4 alpha (HNF4α), and/or mucin 2 (MUC2), absence of TTF-1 expression in most cases, and frequent *KRAS* alterations, the diagnosis of pulmonary enteric adenocarcinoma requires clinical exclusion of a metastatic gastrointestinal primary tumor.

In diagnostically challenging cases, advanced genomic and epigenetic profiling may provide valuable support for distinguishing primary pulmonary enteric adenocarcinoma (PEAC) from metastatic gastrointestinal (GI) adenocarcinoma. Whole-exome sequencing can identify lineage-associated alterations, with *EGFR* alterations and *ERBB2* amplification favoring a pulmonary origin, whereas *adenomatous polyposis coli* gene (*APC*) mutations are more characteristic of colorectal adenocarcinoma (17). DNA methylation profiling may further aid in determining the tissue of origin through tissue-specific epigenetic patterns. In addition, genome-wide copy number alterations (CNAs), including changes involving 13q, 14q, and 18p, may provide complementary molecular evidence for distinguishing PEAC from metastatic colorectal carcinoma (18).

Squamous Cell Carcinoma

Squamous cell carcinoma (SCC) is the second most prevalent type of lung cancer, representing 20% of all cases, and is strongly associated with smoking (19). It arises from the malignant transformation of epithelial cells lining the airways and, therefore, most commonly occurs in the central regions of the lung or within the major bronchi. According to the WHO Classification of Thoracic Tumors,

the diagnostic criteria for SCC include keratinization, intercellular bridges, and/or immunohistochemical evidence of squamous differentiation. The characteristic histopathological features, including keratinization, are illustrated in Figure 6A. Preinvasive lesions of squamous cell carcinoma (SCC) include squamous dysplasia and squamous carcinoma in situ. Squamous dysplasia is graded as low-grade (mild and moderate dysplasia) or high-grade (severe dysplasia) based on cellular size and maturation, nuclear features, cell orientation, and extent of epithelial involvement (2).

SCC comprises three main subtypes: keratinizing, non-keratinizing, and basaloid. Keratinizing SCC is morphologically characterized by keratinization, pearl formation, and/or intercellular bridges. Non-keratinizing SCC lacks keratinization, and these tumors require immunohistochemical confirmation of squamous cell differentiation. Basaloid SCC consists of small to intermediate-sized cells with peripheral palisading, without squamous morphology, and with immunohistochemical expression of the squamous markers. It is a highly proliferative tumor with a high mitotic count and a very high Ki-67 index (50–80%). Most SCCs diffusely express squamous cell markers, with p40 being the most specific marker for SCCs, while high-molecular-weight cytokeratins (CK5/6, 34βE12) and p63 are less specific. Across different studies, p40 demonstrates a sensitivity approaching 100% and a specificity ranging from 90% to 100%, whereas p63 shows a sensitivity ranging from 80% to 100% and a specificity of approximately 96% (20, 21). Representative p40 immunoreactivity in SCC is shown in Figure 6B.

Genomic characterization of SCC revealed complex genomic alterations, and commonly mutated genes include: *tumor protein p53* (*TP53*), *cyclin-dependent kinase inhibitor 2A* (*CDKN2A*), *phosphatase and tensin homolog* (*PTEN*), *phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha* (*PIK3CA*), *Kelch-like ECH-associated protein 1* (*KEAP1*), *lysine methyltransferase 2D* (*KMT2D* (*MLL2*)), *major histocompatibility complex, class I, A* (*HLA-A*), *nuclear factor, erythroid 2-like 2* (*NFE2L2*), *notch receptor 1* (*NOTCH1*),

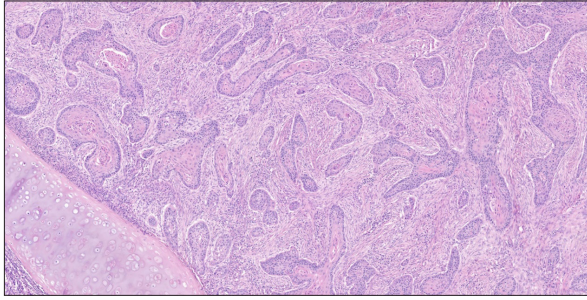


Figure 6 (A). Squamous cell carcinoma of the lung. H&E staining (×20 magnification) showing the morphological features of squamous cell carcinoma.

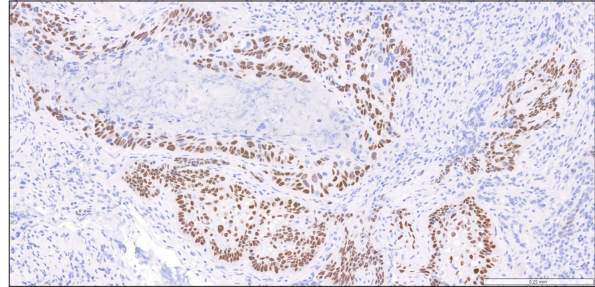


Figure 6 (B). Immunohistochemical staining for p40 showing strong, diffuse nuclear positivity in tumor cells, supporting squamous differentiation (×20 magnification).

and *retinoblastoma transcriptional corepressor 1* (*RB1*). Although uncommon, targetable driver alterations, such as *EGFR* alterations or *ALK* rearrangements, can be found in lung SCC, particularly in young, never-smoking patients (22, 23). Targeted therapy remains unavailable for most patients with squamous cell carcinoma; however, they may benefit from anti-programmed cell death protein 1 (PD-1) or programmed death ligand 1 (PD-L1) therapy, either alone or in combination with chemotherapy (24).

Lymphoepithelial Carcinoma of the Lung

Lymphoepithelial carcinoma is classified as a type of poorly differentiated SCC admixed with variable amounts of lymphoplasmacytic infiltrate and is frequently associated with Epstein-Barr virus (EBV). Immunohistochemically, tumor cells show diffuse staining for pancytokeratin, CK5/6, p40, and p63, whereas the presence of EBV is usually demonstrated by in situ hybridization for EBER1.

Adenosquamous Carcinoma

Adenosquamous carcinoma is composed of a mixture of adenocarcinoma and SCC components, with each component comprising at least 10% of the tumor (2). Each component exhibits characteristic morphological and immunohistochemical features, whereas the molecular profile is intermediate between those of adenocarcinoma and squamous cell carcinoma and may harbor common,

targetable driver mutations. For a definitive diagnosis, adenosquamous carcinoma requires a resection specimen with adequate sampling to demonstrate both glandular and squamous components, each comprising at least 10% of the tumor, and cannot be reliably diagnosed on small biopsy or cytological specimens.

Large Cell Carcinoma of the Lung

Large cell carcinoma (LCC) is an undifferentiated non-small cell lung carcinoma that lacks the morphological or immunohistochemical features of other lung cancer types. It is composed of sheets of large polygonal cells with vesicular nucleoli and moderate amounts of cytoplasm. The diagnosis of LCC requires immunohistochemical staining to exclude adenocarcinomatous, squamous, and neuroendocrine differentiation. Some genetic alterations commonly associated with adenocarcinoma, including *KRAS*, *EGFR*, and *BRAF* alterations and *ALK* rearrangements, can also be identified in LCC.

Sarcomatoid and SMARCA4-Deficient Tumours

Sarcomatoid Carcinoma

The sarcomatoid carcinoma group comprises three recognized entities: pleomorphic carcinoma, pulmonary blastoma, and pulmonary carcinosarcoma.

Pleomorphic Carcinoma of the Lung

Pleomorphic carcinoma is an aggressive, poorly differentiated, non-small cell lung carcinoma. It includes tumors composed entirely of malignant spindle and/or giant cells, as well as conventional non-small cell lung carcinomas containing at least 10% malignant spindle and/or giant cells. These components may occur in combination with adenocarcinoma, squamous cell carcinoma, or large-cell carcinoma. The characteristic pleomorphic morphology, including malignant giant cells, is illustrated in Figure 7. Immunohistochemistry is used to determine adenocarcinomatous or squamous differentiation, whereas pancytokeratin expression supports the epithelial origin of the tumor, particularly in the spindle cell components. The genetic profile of pleomorphic carcinoma generally reflects the presence of non-small cell lung carcinoma components, with *KRAS* and *EGFR* alterations occurring relatively frequently and *ALK* rearrangements being rare (2).

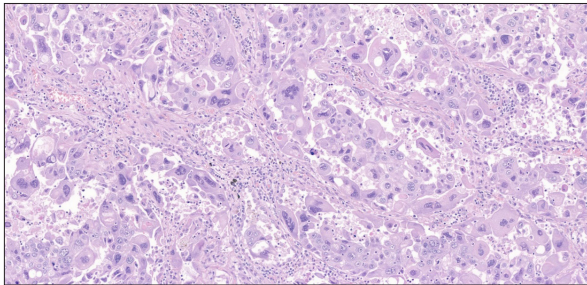


Figure 7. Pleomorphic lung carcinoma with a giant cell component showing diffuse proliferation of atypical, giant, and bizarre cells (H&E, ×40 magnification).

Pulmonary Blastoma

Pulmonary blastoma is an exceptionally rare lung cancer consisting of low-grade fetal adenocarcinoma and primitive mesenchymal stroma, sometimes with specific differentiation into osteosarcoma, chondrosarcoma, or rhabdomyosarcoma. It should be distinguished from pleuropulmonary blastoma, a genetically and morphologically distinct pediatric mesenchymal tumor. Immunohistochemically, tumor cells typically express markers of low-grade fetal adenocarcinoma, such as TTF-1 and CK7,

whereas mesenchymal components may express S100 and desmin, depending on differentiation. A characteristic genetic alteration in pulmonary blastoma is a missense alteration in exon 3 of the *catenin beta 1* (*CTNNB1*) gene, resulting in aberrant nuclear localization of β -catenin, which may serve as a useful diagnostic marker.

Carcinosarcoma of the Lung

Carcinosarcoma is another biphasic malignant lung tumor consisting of non-small cell lung carcinoma components, most commonly squamous carcinoma or adenocarcinoma, and at least one sarcomatous heterologous element, such as rhabdomyosarcoma, chondrosarcoma, or osteosarcoma. Immunohistochemistry is useful for distinguishing carcinosarcoma from pulmonary blastoma, which characteristically demonstrates nuclear β -catenin expression in both the epithelial and mesenchymal components. No specific molecular alterations are characteristic of pulmonary carcinosarcoma. However, tumors with an adenocarcinomatous component may harbor targetable driver mutations, including those typically observed in lung adenocarcinoma. The WHO Classification of Thoracic Tumors recognizes two entities in this group of epithelial tumors: *NUT* carcinoma of the lung and thoracic SMARCA4-deficient undifferentiated tumors.

NUT Carcinoma of the Lung

NUT carcinoma is a poorly differentiated carcinoma that usually shows squamoid differentiation and characteristic rearrangement of the nuclear protein in the testis (*NUTM1*) gene, most commonly resulting in a *BRD4::NUTM1* fusion (2). It is a very aggressive tumor, often found in young individuals and light or never smokers. *NUT* carcinomas typically present as undifferentiated or poorly differentiated carcinomas and may morphologically overlap with squamous cell carcinoma, small cell carcinoma, or other poorly differentiated NSCLC subtypes. The tumor is composed of small to intermediate-sized cells with

brisk mitotic activity, arranged in sheets and nests. Necrosis is frequently present, and characteristic abrupt foci of squamous differentiation with keratinization are observed in approximately one-third of cases (2). Immunohistochemical detection of nuclear NUT expression, confirmed by molecular identification of a *NUTM1* rearrangement, is essential for establishing the diagnosis.

Thoracic SMARCA4- Deficient Undifferentiated Tumor

In the latest WHO Classification of Thoracic Tumors, thoracic *SMARCA4*-deficient undifferentiated tumors are included in epithelial tumors, even though they were previously known as *SMARCA4*-deficient thoracic sarcomas (25). SWI/SNF related BAF chromatin remodeling complex subunit ATPase 4 (*SMARCA4/BRG1*) is the catalytic ATPase subunit of the SWI/SNF complex that regulates chromatin accessibility through ATP-dependent nucleosome remodeling, thereby controlling gene transcription. Loss-of-function alterations in *SMARCA4* disrupt chromatin organization and transcriptional regulation and have been implicated in the pathogenesis of several malignancies, including *SMARCA4*-deficient thoracic tumors (26). *SMARCA4*-deficient undifferentiated tumors are aggressive thoracic malignancies that typically occur in adult male smokers and often present as advanced thoracic masses with mediastinal involvement. Histologically, the tumor is composed of undifferentiated epithelioid cells dispersed in diffuse sheets with large, round nuclei, vesicular chromatin, prominent nucleoli, and occasionally rhabdoid morphology. It is a highly mitotically active tumor, with necrosis commonly present.

The principal immunohistochemical feature is the complete loss of *SMARCA4 (BRG1)* expression in tumor cells. In addition, cluster of differentiation 34 (CD34), SRY-box transcription factor 10 (SOX2), and spalt-like transcription factor 4 (SALL4) may be expressed. In contrast, epithelial differentiation is often limited or absent, and tumors may show variable expression of

cytokeratins or other epithelial markers. Rare cases may show focal positivity for TTF-1, p63, or p40 (2). Tumor cells may express stem cell-associated markers, including SALL4, SOX2, and CD34, while conventional lineage-specific markers are often negative or only focally expressed.

The differential diagnoses include *SMARCB1*-deficient tumors, poorly differentiated or undifferentiated carcinomas, thoracic sarcomas, germ cell tumors, malignant melanoma, and other rhabdoid neoplasms. The genetic profile is characterized by biallelic inactivation of *SMARCA4*, frequently accompanied by *TP53* inactivation. In addition, many cases harbor concurrent alterations in *KRAS*, serine/threonine kinase 11 (*STK11*), and/or *KEAP1*.

Salivary Gland-Type Tumors

Primary pulmonary salivary gland-type tumors are a rare and distinct group of neoplasms defined by characteristic morphologic, immunohistochemical, and molecular features. Compared with other non-small cell lung carcinomas, most of these tumors are associated with a relatively favorable prognosis (27). Because these tumors share many morphological, immunohistochemical, and molecular characteristics with their counterparts in the salivary glands of the head and neck, careful clinicopathological correlation is essential to confirm their origin as primary pulmonary tumors. The WHO Classification of Thoracic Tumors recognizes the following entities within the group of primary pulmonary salivary gland-type tumors: pleomorphic adenoma of the lung, adenoid cystic carcinoma of the lung, epithelial-myoepithelial carcinoma of the lung, mucoepidermoid carcinoma of the lung, hyalinizing clear cell carcinoma of the lung, myoepithelioma, and myoepithelial carcinoma of the lung. Several of these entities are briefly reviewed below.

Adenoid Cystic Carcinoma

Pulmonary adenoid cystic carcinomas (ACCs) are malignant tumors that morphologically resemble

their counterparts in the salivary glands, and generally exhibit indolent clinical behavior. They originate from the bronchial glands and are most commonly located in the trachea and main bronchi, making complete surgical resection challenging. Consequently, local recurrences may occur, often after a prolonged interval following resection. Histologically, ACCs exhibit cribriform, tubular, and solid growth patterns, with characteristic myxoid and hyaline stromal material, as illustrated in Figure 8. The neoplastic population comprises both epithelial and myoepithelial cells, reflecting biphasic differentiation that can be readily confirmed by immunohistochemical analysis. The luminal tumor cells typically express CK7 and CD117 (KIT), whereas the myoepithelial cells express p63, p40, SMA, S100, and/or SOX10. A characteristic pattern of p63 expression is observed, with nuclear staining of peripheral or luminal cells within cribriform structures (28).

Pulmonary ACCs exhibit genomic alterations similar to those observed in adenoid cystic carcinomas of the head and neck. The most common genetic alteration is the fusion of the *MYB* oncogene and *NFIB* transcription factor $t(6;9)(q22-q23;p23-p24)$, and less commonly, $t(8;9)/MYBL1-NFIB$ gene fusion (2). The detection of *MYB-NFIB* and *MYBL1-NFIB* gene fusions can be helpful in establishing a diagnosis. However, the frequency of these alterations in pulmonary ACCs appears to be lower than in their head and neck counterparts, with *MYB-NFIB* and *MYBL1-NFIB* gene fusions identified in approximately 41%–58% of cases (28).

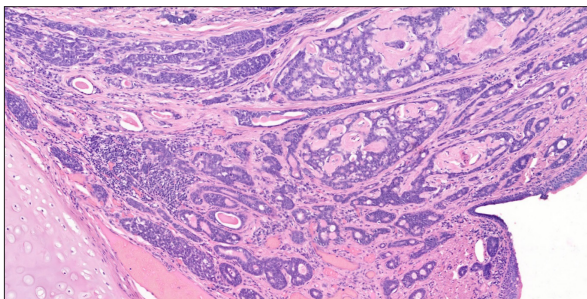


Figure 8. Microscopic features of pulmonary adenoid cystic carcinoma showing a characteristic cribriform architecture composed of epithelial and myoepithelial cells surrounding hyaline stromal material.

Mucoepidermoid Carcinoma

Mucoepidermoid carcinoma (MEC) is the most common salivary gland-type tumor of the lung and, according to the latest WHO Classification of Thoracic Tumors, is divided into two subtypes: low-grade, with a generally favorable prognosis, and high-grade, with a prognosis similar to that of other non-small cell lung carcinomas (2, 28). Representative macroscopic and histopathological features of MEC are shown in Figures 9A and 9B, respectively. The tumor consists of mucin-secreting, squamoid, and intermediate cells arranged in solid and cystic growth patterns (Figure 9). High-grade mucoepidermoid carcinomas are characterized by increased mitotic activity and tumor necrosis. Immunohistochemically, MECs are typically positive for CK7, whereas squamoid cells express p63 and p40. They are negative for pneumocytic markers, including TTF-1 and Napsin A, as well as for S100 and smooth muscle actin (SMA). The characteristic genetic alteration in pulmonary MEC, detected in approximately 76–89% of cases, is a rearrangement of the mastermind-like 2 (*MAML2*) gene. The most common mechanism is the $t(11;19)(q21;p13)$ translocation, resulting in *CRTC1-MAML2* fusion, while a less frequent variant involves *CRTC3-MAML2* fusion (2, 28).

Morphology of low-grade MEC is generally characteristic, whereas the diagnosis of high-grade MEC may be challenging because these tumors can show marked cytological atypia, increased mitotic activity, and reduced morphological features of conventional low-grade MEC. The differential diagnosis includes conventional squamous cell carcinoma and adenosquamous carcinoma. In such cases, detection of a *MAML2* rearrangement may support the diagnosis. However, its absence does not exclude MEC, particularly in high-grade tumors, and molecular findings should always be interpreted in conjunction with morphological and immunohistochemical features.



Figure 9A. Gross specimen of low-grade pulmonary mucoepidermoid carcinoma (black arrow) with characteristic intrabronchial growth, causing obstruction of the bronchial lumen.

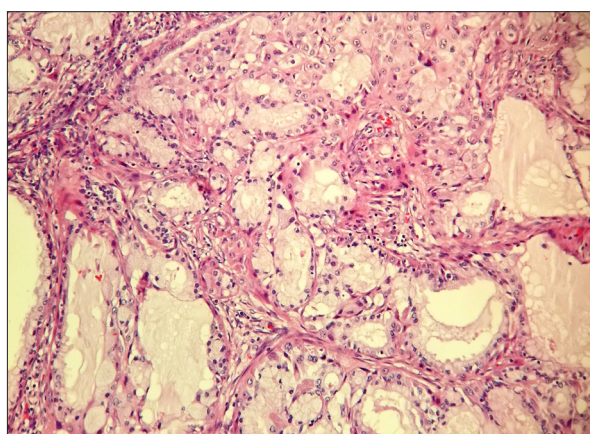


Figure 9B. Microscopic features of low-grade mucoepidermoid carcinoma of the lung, showing the characteristic admixture of mucinous, intermediate, and squamoid cells (H&E, $\times 20$ magnification).

Hyalinizing Clear Cell Carcinoma of the Lung

Hyalinizing clear cell carcinoma (HCC) is an extremely rare, low-grade malignant epithelial tumor composed of cords, trabeculae, and infiltrating nests of clear and eosinophilic cells embedded within a myxohyaline, cellular, and fibrous stroma (2). Representative histopathological features of HCC are shown in Figure 10. This low-grade tumor usually shows minimal mitotic activity and the absence of necrosis. Tumor cells are positive for pancytokeratin, CK7, p40, and

p63, and negative for pneumocytic markers TTF-1 and Napsin A.

The presence of the t(12;22)(q13;q12) chromosomal translocation, resulting in *EWSR1-ATF1* (*Ewing sarcoma breakpoint region 1-activating transcription factor 1*) gene fusion, is an important molecular feature. This alteration is characteristic of hyalinizing clear cell carcinoma, and its detection can provide valuable diagnostic support (29).

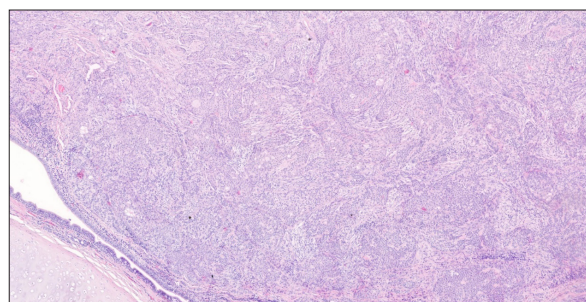


Figure 10. Hyalinizing clear cell carcinoma of the lung. Microscopy demonstrates characteristic nests of clear and eosinophilic cells within a myxohyaline stroma (H&E, $\times 10$ magnification).

Neuroendocrine Neoplasms

Neuroendocrine neoplasms (NEN) of the lung are grouped into a single category in the latest WHO Classification of Thoracic Tumors. They are classified into well-differentiated neuroendocrine neoplasms (NENs), comprising typical carcinoid (TC) and atypical carcinoid (AC), and poorly differentiated neuroendocrine carcinomas, comprising small cell lung carcinoma (SCLC) and large cell neuroendocrine carcinoma (LCNEC). TC and AC are distinguished primarily by their morphological features, mitotic count, and the presence or absence of necrosis, with TC showing fewer than 2 mitoses per 2 mm^2 and no necrosis, whereas AC shows 2–10 mitoses per 2 mm^2 and/or necrosis.

The Ki-67 proliferative index is not included in the diagnostic criteria or classification scheme for pulmonary neuroendocrine neoplasms (NENs). Its primary role is to help distinguish carcinoid tumors (NETs) from large cell neuroendocrine carcinoma (LCNEC) and small cell lung carcinoma (SCLC), particularly in small biopsy specimens affected by crush artifacts (2).

Despite substantial similarities between pulmonary and pancreatic neuroendocrine neoplasms, their classification and terminology retain certain distinctions. One contributing factor is the relatively high frequency of combined pulmonary neuroendocrine carcinomas occurring alongside other histological subtypes of non-small cell lung carcinoma (NSCLC), a phenomenon reported in approximately 25% of cases (2). Additionally, a histologic transformation of NSCLC, most commonly from lung adenocarcinoma, into small cell lung carcinoma (SCLC) is a well-recognized mechanism of acquired therapeutic resistance, primarily described in *EGFR*-mutated lung adenocarcinoma treated with tyrosine kinase inhibitors (TKIs), but also reported in NSCLC following immune checkpoint inhibitor (ICI) therapy (30, 31). This transformation involves a complex interplay of genomic instability, tumor suppressor loss, and epigenetic reprogramming, promoting lineage plasticity and the acquisition of a neuroendocrine phenotype (32).

Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) is considered a precursor lesion of pulmonary neuroendocrine tumors, whereas neuroendocrine carcinomas do not have recognized precursor lesions. Histologically, DIPNECH is characterized by multifocal neuroendocrine cell proliferation within the bronchiolar epithelium and is often accompanied by tumorlets, which are nodular aggregates of neuroendocrine cells measuring less than 5 mm that infiltrate beyond the bronchial wall into the adjacent lung parenchyma. DIPNECH can be associated with clinical symptoms and may present with bronchiolar fibrosis with constrictive features (2).

Carcinoid/Neuroendocrine Tumor of the Lung

Carcinoid tumors are neuroendocrine malignancies with well-differentiated organoid architecture and are classified by the WHO Classification of

Thoracic Tumors into two main subtypes: typical and atypical carcinoid tumors.

The differentiation between the two groups is based on mitotic activity and the presence or absence of necrosis. Typical carcinoids (TC) are well-differentiated neuroendocrine tumors with characteristic neuroendocrine morphology, including organoid patterns with trabecular, rosette, insular, palisading, ribbon, or follicular formation. The tumor cells are uniform, with finely granular nuclear chromatin and moderate to abundant eosinophilic cytoplasm. TCs show low mitotic activity (<2 mitoses/ 2 mm^2) and complete absence of necrosis. Representative histopathological features of typical carcinoids are shown in Figure 11. In contrast, atypical carcinoids with the same architectural patterns are characterized by slightly higher mitotic activity (2–10 mitoses/ 2 mm^2) and/or small foci of necrosis (2).

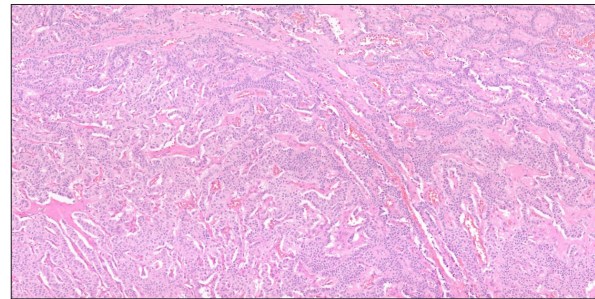


Figure 11. Typical carcinoid showing a characteristic trabecular growth pattern composed of uniform tumor cells arranged in organized trabeculae (H&E, $\times 10$ magnification).

The latest WHO Classification of Thoracic Tumors recognizes a rare subset of pulmonary neuroendocrine neoplasms that have the morphology of an atypical carcinoid (AC) but exhibit elevated proliferative activity, defined by >10 mitoses per 2 mm^2 and/or a Ki-67 index $>30\%$. Although these tumors are analogous to Grade 3 neuroendocrine tumors (NET G3) of the pancreas, the WHO Classification of Thoracic Tumors currently recommends classifying them as LCNEC, with an explanatory note describing the carcinoid morphology and reporting the mitotic count and Ki-67 index (2).

Immunohistochemically, carcinoid tumors are characterized by positivity for low-molecular-weight cytokeratins and diffuse expression of neuroendocrine markers, including chromogranin A, synaptophysin, CD56, and INSM1. Additionally, TTF-1 is positive in peripheral tumors but mostly negative in central tumors. The molecular profiles of carcinoid tumors differ significantly from those of neuroendocrine carcinomas, supporting the view that these entities constitute two distinct groups of neuroendocrine neoplasms. The most commonly mutated genes in carcinoid tumors include *multiple endocrine neoplasia type 1 (MEN1)*, *eukaryotic translation initiation factor 1A, X-linked (EIF1AX)*, and *AT-rich interaction domain 1A (ARID1A)* (2).

Small Cell Lung Carcinoma

Small cell lung carcinoma (SCLC) is the most common and aggressive neuroendocrine neoplasm of the lung. It is usually located in the central airways and is characterized by early involvement of the mediastinal lymph nodes and frequent metastatic spread to distant organs.

The morphological features of this malignant epithelial tumor include small, highly proliferative cells, no larger than three small resting lymphocytes, with scant cytoplasm, finely granular nuclear chromatin, and inconspicuous nucleoli. The tumor cells are typically densely packed in a

sheet-like pattern, as illustrated in Figure 12A. The mitotic count is high, with an average of 60 mitoses/2 mm², and extensive necrosis is frequently associated (2). Crush artifacts and the Azzopardi effect, characterized by the deposition of basophilic nuclear DNA on blood vessel walls, are frequently observed.

SCLC characteristically exhibits cytokeratin expression in a paranuclear dot-like (rim-and-dot) pattern, most commonly detected with AE1/AE3 and CAM5.2. Neuroendocrine markers, including synaptophysin, chromogranin A, and CD56, are positive in most cases. Among these, CD56 demonstrates the highest sensitivity (90–100%), followed by synaptophysin (50–95%) and chromogranin A (83%) (33). Representative immunohistochemical expression of synaptophysin and chromogranin A is shown in Figures 12B and C, respectively. However, approximately 5–10% of SCLCs are negative for all three markers; therefore, the addition of INSM1, a highly sensitive neuroendocrine marker with sensitivity comparable to that of synaptophysin (98%), may be beneficial in diagnostically challenging cases (34). Representative INSM1 immunoreactivity is shown in Figure 12D. TTF-1 is expressed in most SCLCs, although its expression is not specific to the pulmonary origin (2).

The molecular profile of SCLC is characterized by the presence of inactivating mutations in the tumor suppressor genes *TP53* and *RB1* in almost

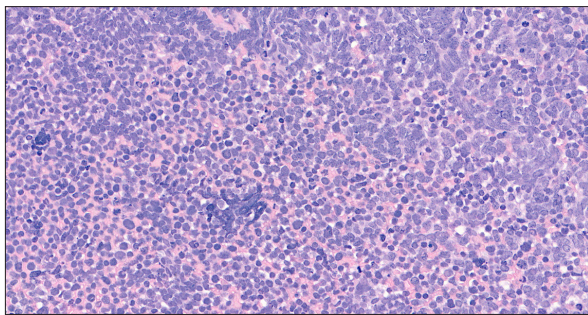


Figure 12A. Small cell lung carcinoma. Hematoxylin and eosin (H&E) staining demonstrates dense sheets of tumor cells with finely granular (“salt and pepper”) chromatin, inconspicuous nucleoli, and scant cytoplasm. Numerous mitotic figures are present, along with scattered apoptotic tumor cells (×20 magnification).

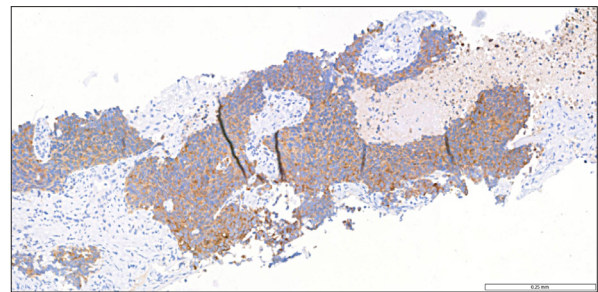


Figure 12B. Synaptophysin immunohistochemical staining showing diffuse positivity in tumor cells, supporting neuroendocrine differentiation.

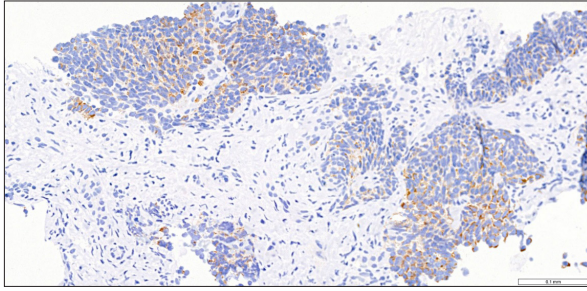


Figure 12C. Chromogranin immunohistochemical staining showing focal cytoplasmic positivity in tumor cells, supporting neuroendocrine differentiation.

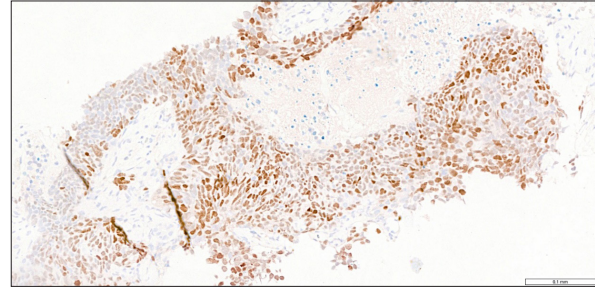


Figure 12D. Immunohistochemical staining for INSM-1 showing strong nuclear positivity in tumor cells.

all cases, and by downregulated Notch signaling in the majority. SCLC is not characterized by frequent activating driver mutations, and molecular subtyping is based on the predominant expression of lineage-defining transcription factors, including *Achaete-scute homolog 1* (*ASCL1*)-(SCLC-A), *neurogenic differentiation factor 1* (*NEUROD1*) - (SCLC-N), *POU class 2 homeobox 3* (*POU2F3*)-(SCLC-P), and *yes-associated protein 1* (*YAP1*) - (SCLC-Y) / Inflamed (SCLC-I) (35). Among these, SCLC-A (*ASCL1*-driven) is the most common subtype, accounting for approximately 50–70% of cases. These molecular subtypes are biologically relevant but are not currently required for the routine diagnosis or classification of SCLC. To date, studies have not demonstrated significant differences in overall survival (OS) or progression-free survival (PFS) among these molecular subtypes. However, the inflammatory subtype (SCLC-I) has been associated with potential benefits from platinum-based chemotherapy combined with immune checkpoint inhibitors (36).

Large Cell Neuroendocrine Carcinoma of the Lung

Large cell neuroendocrine carcinoma (LCNEC) is another high-grade neuroendocrine neoplasm of the lung, distinguished by its distinctive morphological and molecular features. Histologically, LCNEC exhibits organoid, nesting, trabecular, or palisading growth patterns characteristic of neuroendocrine differentiation, as illustrated in Figure 13. The tumor cells are large, with moderate

to abundant cytoplasm, distinct cell borders, and prominent nucleoli. Nuclear chromatin is typically coarsely granular ('salt-and-pepper'), although a vesicular appearance may also be observed. LCNEC is almost invariably associated with extensive necrosis and demonstrates a high proliferative rate, with more than 10 mitoses per 2 mm².

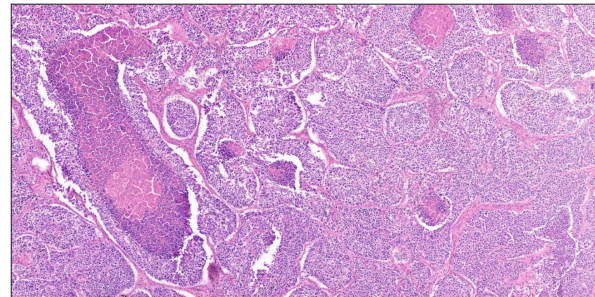


Figure 13. Large cell neuroendocrine carcinoma showing a nested pattern with peripheral palisading and focal central necrosis (H&E staining at ×20 magnification).

Most LCNEC express standard neuroendocrine markers, including synaptophysin, chromogranin, and CD56, and diffuse expression of at least one of these markers is required for diagnosis. Exceptions can be made in cases with unequivocal neuroendocrine morphology and only focal expression of a single neuroendocrine marker (2). TTF-1 expression can be detected in more than half of LCNEC cases, whereas Napsin A and squamous markers (p63, p40, CK5/6) can be positive only in scattered cells. In small diagnostic specimens, a definitive diagnosis of LCNEC generally cannot be established. Such tumors should be designated as NSCLC with neuroendocrine morphology and

positive neuroendocrine markers, with a comment that the findings may suggest LCNEC. A definitive diagnosis of LCNEC should be made only in adequately sampled resection specimens, after careful evaluation of the characteristic morphological features and exclusion of adenocarcinoma, squamous cell carcinoma, and other poorly differentiated carcinomas by morphology and immunohistochemistry (2, 37).

LCNECs exhibit two major molecular subtypes. The first is an SCLC-like subtype characterized by concurrent *TP53* and *RB1* inactivation, whereas the second is an NSCLC-like subtype associated with alterations in the *STK11*, *KEAP1*, and/or *KRAS* genes. Additionally, a small subset of LCNECs harbors molecular alterations typically associated with lung adenocarcinoma, including *EGFR* and *KRAS* alterations as well as *ALK* rearrangements (38).

Selected Mesenchymal Tumors

The latest WHO Classification of Thoracic Tumors recognizes a diverse group of pulmonary mesenchymal neoplasms. In addition to established entities, such as pulmonary hamartoma, pulmonary chondroma, diffuse pulmonary lymphangiomatosis, pleuropulmonary blastoma, and pulmonary artery intimal sarcoma, several newer entities have been incorporated, including congenital peribronchial myofibroblastic tumors, primary pulmonary myxoid sarcoma with an *EWSR1-CREB1* fusion, and perivascular epithelioid cell tumor (PEComatous tumors) (39). Some of these entities are exceptionally rare and have been described only in isolated case reports.

Primary Pulmonary Myxoid Sarcoma with *EWSR1-CREB1* Fusion

Primary pulmonary myxoid sarcoma (PPMS) is a low-grade malignant lung tumor characterized by distinctive histomorphologic features and a recurrent *EWSR1-CREB1* gene fusion. These tumors typically arise in an endobronchial location and may infiltrate the surrounding lung parenchyma

(40). Morphologically, the tumor is composed of spindle-shaped, polygonal, or stellate cells embedded within a myxoid stroma and arranged in a lobulated architecture, often accompanied by a dense chronic inflammatory infiltrate, as shown in Figures 14A and B. Tumor cells typically exhibit mild to moderate cytological atypia and low mitotic activity, generally not exceeding five mitoses per 2 mm². Immunohistochemically, neoplastic cells may show focal expression of epithelial membrane antigen (EMA), while consistently lacking expression of cytokeratins, S100, desmin, CD34, and neuroendocrine markers (2). The principal molecular hallmark of this tumor is the presence of the *EWSR1-CREB1* gene fusion. Given that myxoid neoplasms with overlapping histological features and *EWSR1-CREB1* family gene fusions have been described at various anatomical sites, including the central nervous system, soft tissues, and pulmonary artery, careful exclusion of a metastatic origin is essential for establishing the diagnosis.

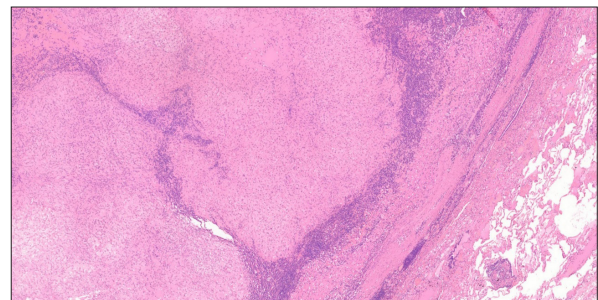


Figure 14A. Primary pulmonary myxoid sarcoma with confirmed *EWSR1::CREB1* fusion shows a lobulated architecture with a lace-like pattern of anastomosing cords and strands of tumor cells within abundant myxoid stroma, accompanied by a surrounding lymphoplasmacytic infiltrate at low magnifications (H&E at ×10 magnification).

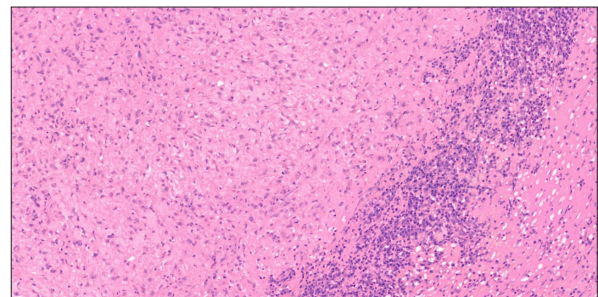


Figure 14B. Primary pulmonary myxoid sarcoma with confirmed *EWSR1::CREB1* fusion (H&E at ×20 magnification).

Molecular Pathology and Clinical Use

The increasing understanding of the molecular landscape of lung cancer has led to the identification of numerous clinically relevant genetic alterations and has substantially expanded the role of molecular testing in diagnosis and treatment selection (41).

Molecular testing in lung tumors should be guided by the clinical setting, tumor morphology, available tissue, and the intended diagnostic or therapeutic application (42). DNA-based next-generation sequencing (NGS) is useful for detecting mutations and small sequence alterations, whereas RNA-based NGS is particularly important for the detection of gene fusions and rearrangements, including ALK, ROS1, RET, and NTRK1–3. Comprehensive molecular testing of advanced non-small cell lung cancer (NSCLC) should include clinically relevant alterations in EGFR, ALK, ROS1, BRAF, KRAS, MET exon 14, RET, NTRK1–3, and ERBB2, as these may represent predictive biomarkers for targeted therapy (43, 44).

PD-L1 immunohistochemistry provides an important biomarker for immunotherapy selection and should be performed using an appropriate validated assay (45). When tissue is insufficient or unavailable, liquid biopsy using circulating tumor DNA may provide a complementary approach, particularly for the detection of actionable genomic alterations, although a negative result does not reliably exclude a tumor alteration and may require tissue-based testing when clinically appropriate (46). Adequate tumor cellularity and appropriate preanalytical handling, including fixation, tissue preservation, and avoidance of unnecessary tissue consumption during immunohistochemical work-up, are essential for reliable molecular analysis. Molecular findings should be interpreted in conjunction with morphology, immunohistochemistry, and clinical information and appropriately incorporated into the integrated pathological report.

Conclusion

The classification of lung tumors has evolved considerably with advances in morphology, immunohistochemistry, and molecular pathology. Morphological assessment remains the foundation of tumor classification, while immunohistochemistry should be applied in a targeted and tissue-sparing manner, particularly in small biopsy and cytological specimens. Certain diagnoses requiring complete evaluation of the tumor, such as AIS, MIA, and adenosquamous carcinoma, should be established only on adequately sampled resection specimens. Preservation of tissue for molecular testing is essential, particularly in advanced disease, where molecular alterations and predictive biomarkers directly guide treatment selection. Molecular findings complement morphological and immunophenotypic assessment and should be integrated into the pathological report to support accurate classification and personalized treatment. Continued advances in molecular characterization are expected to further refine lung cancer classification and expand opportunities for personalized treatment (2, 41).

What Is Already Known on This Topic:

The classification of primary lung tumors has evolved from a predominantly morphology-based approach to an integrated framework incorporating histopathology, immunohistochemistry, and molecular alterations. The 5th WHO Classification of Thoracic Tumors recognizes a broad spectrum of epithelial, neuroendocrine, salivary gland-type, mesenchymal, and other rare pulmonary neoplasms. Accurate histological classification remains fundamental, while immunohistochemistry and molecular testing are increasingly important for resolving diagnostic challenges and identifying clinically relevant alterations that may guide targeted and immune-based therapies. Small biopsies and cytological specimens present particular challenges because the limited tissue must be allocated carefully for diagnosis and predictive molecular testing.

What This Study Adds:

This review provides a practical overview of contemporary pulmonary tumor classifications, emphasizing the integration of morphology, immunohistochemistry, and molecular pathology. It highlights tissue-sparing diagnostic strategies for small specimens, key immunophenotypic and molecular features of major and selected rare lung tumors, and the importance of preserving tissues for predictive biomarker testing. The review also emphasizes that molecular findings should be interpreted in conjunction with morphology, immunohistochemistry, and clinical context to achieve accurate classification and support personalized treatment.

Conflict of Interest: The author declares that she has no conflict of interest.

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