Classification and Pathology of Lung Cancer

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KEYWORDS

• Lung cancer • Classification • Pathology • Immunohistochemistry
• Molecular testing

KEY POINTS

- Lung cancer classification strives to correlate tumor cell morphology with tumor biological characteristics, thus facilitating therapeutic decision-making and effective prognostic outcome prediction in the era of personalized medicine.
- In small biopsy specimens or cytology specimens, major types of lung cancers are established by morphologic evaluation, that is, adenocarcinoma and squamous cell carcinoma.
- When poorly differentiated carcinomas are encountered, judicious application of immunohistochemical stains facilitates such distinction in most cases.
- In resection specimens, lung adenocarcinomas are further divided into low-grade (lepidic adenocarcinoma), intermediate-grade (acinar and papillary adenocarcinomas), and high-grade (solid and micropapillary adenocarcinomas) types of prognostic significance.
- Analysis of neuroendocrine tumors is initiated by the recognition of neuroendocrine morphology, verified by neuroendocrine marker expression when necessary.

INTRODUCTION

Significant progress has been made in the understanding of lung cancer biology, due in large part to advancement in the understanding of tumor biology and pathogenesis. Acquisition of key somatic mutations acts as a sentinel event in lung carcinogenesis, essential for tumor cell growth and division.[1] Molecular detection of driver mutations in specific histologic types of lung cancer can predict favorable response to targeted therapy. The essence of personalized medicine is to tailor individual lung cancer treatment based on accurate histologic classification and biomarker information. Therefore, characterization of histologic type of lung cancer plays an increasingly pivotal role in the multidisciplinary approach in the diagnosis and management of lung cancer. Recognizing the biological diversity of lung cancer, a comprehensive and accurate tumor classification has been developed, which is important for treatment and prognosis. Pathology of lung cancer has expanded to cover both tissue diagnosis

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and selection of specific subtypes of lung cancers for further molecular testing. Confirmatory histologic diagnosis directs surgical resection of early-stage disease, whereas pathologic classification and molecular testing enable selection of tumor type–tailored adjvuant therapy and genotype-based treatment regimen to improve the survivals of advanced-stage patients.

Lung cancers are traditionally divided into non–small cell carcinoma (NSCC) and small cell carcinoma (small cell lung carcinoma, SCLC), with the former accounting for 80% of the cases and the latter accounting for the remaining 20%. SCLCs behave aggressively and are treated nonsurgically in most cases, whereas NSCCs are managed by a combination of surgery and adjuvant therapy. Recognition of the diversity of NSCC has led to its subclassification, culminating in the 2004 and 2015 World Health Organization (WHO) classifications.[2,3] Major types of NSCC include adenocarcinoma, squamous cell carcinoma (SSC), and large cell carcinoma (LCC). Thus, subtype of NSCC is specified, whereas the designation "NSCC" is only preserved in certain small biopsies and cytology specimens. SCLC is grouped with other tumors exhibiting neuroendocrine differentiation. Since the publication of the last volume, significant update in lung cancer classification has occurred for lung adenocarcinomas based on better understanding of tumor biology. This update is manifested by streamlined classification for small biopsies and cytology specimens, with special emphasis on separating adenocarcinomas from the rest of the lung cancers in order to effectively screen cases responsive to current mutation-driven therapeutic paradigms. More detailed histologic subtyping is used in resection specimens to delineate tissue types of prognostic significance. This article discusses current pathologic classification of lung cancer, with an emphasis on updating readers to the new WHO lung adenocarcinoma classification (**Box 1**).[3] This article thus serves as a springboard for effective surgical and medical treatment modalities discussed in other articles in this series.

ADENOCARCINOMA

Adenocarcinoma is the most common type of lung cancer, accounting for more than 40% of lung cancers, 60% of the NSCC, and more than 70% of surgically resected cases.[3,4] The incidence of adenocarcinoma has risen steadily over the past few decades. Lung adenocarcinoma commonly forms a peripherally located mass with central fibrosis and pleural puckering. It can also have a variety of other gross appearances, including centrally located mass, diffuse lobar consolidation, bilateral multinodular distribution, and pleural thickening. By definition, lung adenocarcinoma is a malignant epithelial neoplasm with glandular differentiation or mucin production. When such morphologic features are recognized, the tumor can be designated as adenocarcinoma, even in small biopsy specimens. Lung adenocarcinoma cells usually express pneumocytic markers. Thyroid transcription factor (TTF-1) and NapsinA are expressed in more than 85% of the lung adenocarcinoma cases and thus can serve as markers of adenocarcinoma or adenocarcinoma differentiation in poorly differentiated tumor and in limited biopsy sampling material (**Fig. 1**).[5–7] Tumor classification based on ancillary tests such as immunohistochemistry (IHC) is designated as "NSCC, favor adenocarcinoma" in a small biopsy specimen. Resection specimens allow a more detailed subclassification. There has been significant refinement in adenocarcinoma classification in recent years based on close pathologic and clinical correlation.[3,8] The major histologic types have been validated to bear prognostic significance delineated by the tumor grade.[9–14] Multiple gene alterations can occur in adenocarcinomas, with approved molecular targeted therapy available to improve patient survival (see later disccusion).

Box 1
World Health Organization classification of lung cancer

Adenocarcinoma
- Lepidic adenocarcinoma
- Acinar adenocarcinoma
- Papillary adenocarcinoma
- Micropapillary adenocarcinoma
- Solid adenocarcinoma
- Invasive mucinous adenocarcinoma
- Colloid adenocarcinoma
- Fetal adenocarcinoma
- Enteric adenocarcinoma
- Minimally invasive adenocarcinoma

Squamous cell carcinoma

Neuroendocrine tumors
- Carcinoid tumors
  - Typical carcinoid
  - Atypical carcinoid
- Small cell carcinoma
- Large cell neuroendocrine carcinoma

Large cell carcinoma

Adenosquamous carcinoma

Pleomorphic carcinoma

Spindle cell carcinoma

Giant cell carcinoma

Carcinosarcoma

Pulmonary blastoma

Other and unclassified carcinomas
- Lymphoepithelioma-like carcinoma
- NUT carcinoma

Salivary gland–type carcinomas
- Mucoepidermoid carcinoma
- Adenoid cystic carcinoma
- Epithelial-myoepithelial carcinoma

Mesenchymal tumors, lymphohistiocytic tumors, tumors of ectopic origin, and metastatic tumors

Adapted from Travis WD, Brambila E, Burke AP, et al. WHO classification of tumours of the lung, pleura, thymus and heart. 4th edition. Lyon (France): IARC Press; 2015.

Preinvasive or Minimally Invasive Adenocarcinoma

Adenocarcinoma in situ (AIS) represents relatively small sized tumors ($\leq$3 cm) with neoplastic cells growing along preexisting alveolar structures (lepidic growth pattern) without evidence of stromal, vascular, or pleural invasion. Lepidic is a descriptive term for rind or membranous growth pattern and is now specifically used to describe tumor cells proliferating along the surface of intact alveolar walls.[15] Lepidic growth usually correlates with ground glass opacity on radiograms. Most AISs are the nonmucinous type, with mild to moderate pleomorphic cuboidal to columnar tumor cells linearly lining alveolar walls. There is no secondary papillary or micropapillary growth pattern. A

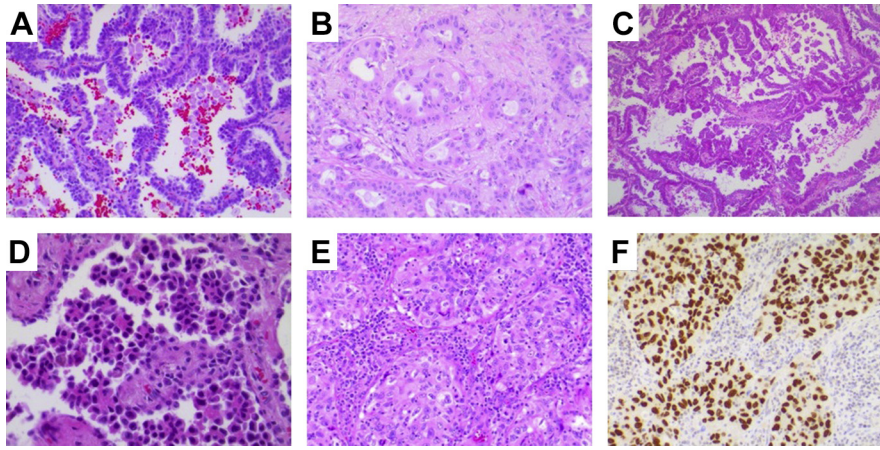

Fig. 1. Adenocarcinomas. (*A*) Lepidic adenocarcinoma (H&E, original magnification ×200). (*B*) Acinar adenocarcinoma (H&E, original magnification ×200). (*C*) Papillary adenocarcinoma (H&E, original magnification ×100). (*D*) Micropapillary adenocarcinoma (H&E, original magnification ×200). (*E*) Solid adenocarcinoma (H&E, original magnification ×100). (*F*) Solid adenocarcinoma (TTF-1 stain, original magnification ×100).

minority of such tumors are of mucinous or mixed type. If the tumor contains a small focus (<5 mm) of invasive growth, the tumor is classified microinvasive adenocarcinoma (MIA). Invasion usually induces formation of a desmoplastic stroma. Invasion can also manifest as nonlepidic growth, such as acinar, papillary, micropapillary, or solid patterns. MIA is defined not only by limited size invasive growth but also by a lack of more advanced invasive pattern, such as tumor necrosis, lymphovascular invasion, and pleural invasion. Both types of tumor are low grade and have a nearly 100% 5-year survival rate.[3]

Invasive Adenocarcinoma

Most invasive adenocarcinomas are composed of mixed morphological subtypes; these are classified according to the predominant architectural structures rather than lumped together as mixed subtype. Each tumor is classified according to one predominant growth pattern, including lepidic, acinar, papillary, micropapillary, and solid patterns (see **Fig. 1**). Each additional subpattern is recorded semiquantitatively as estimated percentage in 5% increments.[3,8] This architectural-driven classification has prognostic significance, with most favorable prognosis for lepidic-predominant adenocarcinomas, intermediate survival rate for acinar and papillary predominant adenocarcinomas, and poor prognosis for solid and micropapillary predominant adenocarcinomas.[11,12,16]

Lepidic Adenocarcinoma

Lepidic growth is commonly seen in lung adenocarcinoma. The lepidic growth pattern denotes tumor cells spreading along preexisting alveolar structures, although there may be sclerotic thickening of alveolar septa. When it is the predominant growth pattern with additional findings that set it apart from previously described AIS and MIA, it is designated as lepidic adenocarcinoma. These additional findings include any one or more of the following: more than 5 mm of invasion (presence of desmoplastic or myofibroblastic stroma); spread through air spaces; lymphatic or vascular

invasion; pleural invasion; tumor necrosis. Although such tumors were previously classified as bronchioloalveolar carcinoma, this term is no longer used because it encompasses a heterogeneous group of adenocarcinomas. This category convenes a significantly better prognosis than other subtypes.[3]

Acinar Adenocarcinoma

Acinar adenocarcinoma is a common type of adenocarcinoma with tumor cells arranged in classic glandular structure on a fibroelastic stroma. It is important to separate demosplastic stroma in this pattern from preexisting alveolar structures with thickened fibroelastic alveolar septa sometimes seen in a lepidic pattern. It is of note that the tumor cells displaying more complex growth patterns, such as a cribriform pattern, likely represent a poor prognostic subtype convening a significant risk of recurrence.[17]

Papillary Adenocarcinoma

The tumor cells form papillary architecture with tumor cells lining the surface of branching fibrovascular cores. The presence of fibrovascular cores separates this tumor type from micropapillary adenocarcinoma.

Micropapillary Adenocarcinoma

The tumor cells form individual cellular tufts without fibrovascular core. The tumor cells appear as detached small and solid individual cell groups. Psammoma bodies may be seen. This subtype of adenocarcinoma has distinctly poor prognosis in the early stage compared with other subtypes of adenocarcinoma.[18–20]

Solid Adenocarcinoma

The tumor cells form patternless sheets and lack any other recognizable patterns, including poorly differentiated/undifferentiated carcinomas expressing pneumocytic markers (such as TTF-1 and NapsinA), which were formerly grouped in the LCC category.[3] It is of note that certain markers commonly associated with squamous cell differentiation, such as p63, and less commonly, p40, can show focal expression in solid adenocarcinoma.[3,21]

Rare Variants of Invasive Adenocarcinoma

Rare variants of invasive adenocarcinoma include invasive mucinous adenocarcinoma, colloid and fetal adenocarcinoma, and enteric adenocarcinoma. Invasive mucinous adenocarcinoma is frequently multicentric and the tumor cells lack expression of TTF-1 commonly seen for lung adenocarcinoma.[22] Enteric adenocarcinoma should be distinguished from metastatic colorectal adenocarcinoma.[23]

SQUAMOUS CELL CARCINOMA

SCCs make up about 20% of lung cancers.[4] Their incidence has declined in recent decades, likely because of changes in smoking behavior. SCC usually occurs in the central portion of the lung, along major airways, and can form cavities when it achieves a large size. On microscopic examination, SCC characteristically shows keratinization and intercellular bridges and exhibits a solid nested growth pattern (**Fig. 2**). The tumor cells usually have hyperchromatic nuclei, visible to inconspicuous nucleoli, and moderate to abundant cytoplasm with delineated intercellular bridges. There can be individual tumor cell keratinization or groups of keratinizing squamous cells forming keratin pearls centrally placed within solid tumor nests. The tumor cells lack glandular

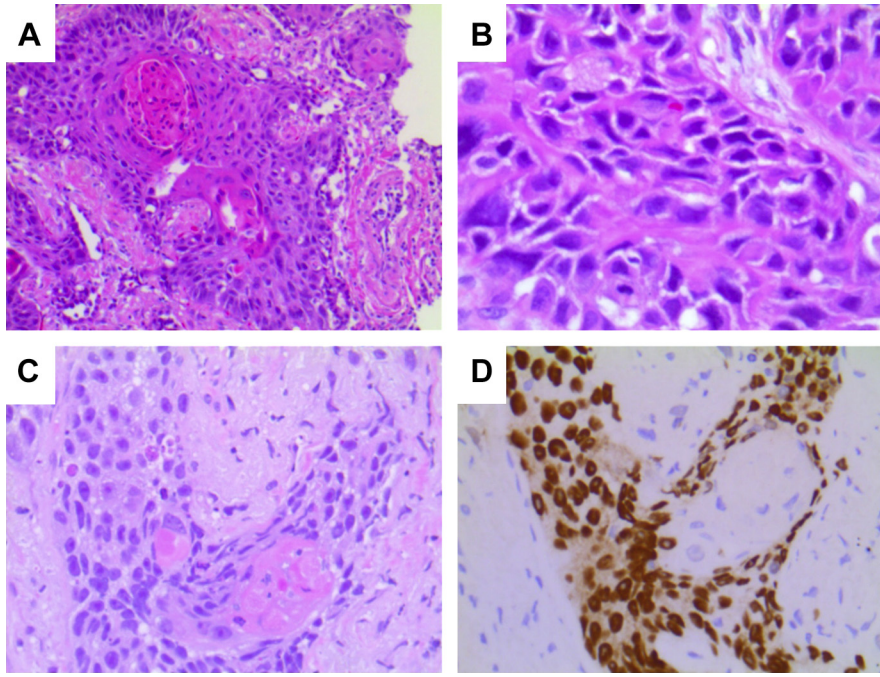

Fig. 2. SCC. (*A*) Keratinizing SCC with keratin pearl formation (H&E, original magnification ×200). (*B*) Intercellular bridge formation (H&E, original magnification ×400). (*C*) Nonkeratinizing SCC without apparent keratinization or discernible intercellular bridges (H&E, original magnification ×400). (*D*) Tumor cells stain positive for p40 (nuclear stain) (p40 stain, original magnification ×400).

structure or mucin production. SCCs are further divided into keratinizing, nonkeratinizing, and basaloid subtypes. Contrary to adenocarcinoma subtypes, such subclassification shows no apparent prognostic utility except for basaloid SCCs, which reportedly display distinct molecular profile conferring intrinsic resistance to cytotoxic chemotherapy.[24] Recognizing morphologic features of squamous cell differentiation, including keratinization, keratin pearl formation, and intercellular bridges, establishes SCC diagnosis, even in small biopsy specimens. When the tumor is poorly differentiated and does not allow confident morphologic classification, selective squamous cell markers, such as p40, CK5/6, CK5, and p63, are used to demonstrate squamous differentiation (see **Fig. 2**).[25] TTF-1 stain is usually negative, although focal weak positive stain for this marker has been reported.[26] Poorly differentiated tumor defined by expression of squamous cell markers in a biopsy material is diagnosed as "NSCC, favor SCC." Although tumors in this category are usually excluded from current molecular testing, identification of driver mutations in SCC, such as discoidin domain receptor 2 (DDR2), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PI3KCA), fibroblastic growth factor receptor 1 (FGFR1), and v-akt murine thymoma viral oncogene homolog 1 (AKT1), may enable future personalized therapy.[27] Establishment of squamous cell differentiation has important implications in chemotherapeutic agent choices so as to avoid certain complications. For instance, treatment with the vascular endothelial growth factor inhibitor bevacizumab in patients with SCC can potentially precipitate life-threatening pulmonary hemorrhage, and therefore, should be avoided.[28,29] Survival rate for SCC is significantly better than adenocarcinoma.

LARGE CELL CARCINOMA

LCCs represent a minority of NSCC cases that are devoid of lineage-specific differentiation, and lack morphologic and immunohistochemical evidence of adenocarcinoma, SCC, or neuroendocrine carcinoma (null immunophenotype). LCC is usually peripherally located, bulky, and necrotic in appearance. The tumor cells are large and polygonal in shape with pleomorphic and vesicular nuclei. The tumor cells form patternless solid sheet or nests. Large cell neuroendocrine carcinoma (LCNEC) is classified in the lung neuroendocrine tumor (NET) category (see later discussion). LCCs represent less than 3% of the lung cancers.[4,23] Tumors with morphologic characteristic of LCC are designated as "NSCC, not otherwise specified (NOS)" in a biopsy or cytology material, not LCC, because the latter can only be ascertained in a resection specimen with the thoroughly analyzed tumor devoid of lineage-specific differentiation.[3,5] It is important to apply ancillary tests such as IHC to avoid inclusion of poorly differentiated NSCC, such as solid adenocarcinoma and nonkeratinizing SCC, in this category. In fact, most LCCs defined by morphologic criteria can be reclassified as adenocarcinoma and SCC using a panel of lineage-specific markers.[30–32] Effective application of such ancillary tests is the likely explanation for the decline of lung cancers categorized as LCC in recent years.[3] Recent evidence suggests that tumors currently classified as LCC with null immunophenotype share molecular characteristics similar to solid adenocarcinoma.[33] Tumors in this category are not necessarily excluded from molecular testing. Metastatic tumors should be excluded in appropriate clinical context. Most LCC cases have adverse outcome, especially for those with null immunophenotype.[34]

OTHER NON–SMALL CELL CARCINOMA TYPES

Adenosquamous carcinoma is a rare type of NSCC, accounting for less than 5% of all lung cancers.[35] It represents a hybrid carcinoma containing both adenocarcinoma and SCC components, with each comprising at least 10% of the tumor.[2,3] From a clinical prospective, adenosquamous carcinoma is usually included in the discussion of adenocarcinomas because therapeutically important mutations occur in adenosquamous carcinoma in similar frequency as in conventional adenocarcinoma[36]; this is especially significant in light of its worse prognosis than that of adenocarcinoma and SCC.[37–39]

Pleomorphic, spindle cell, and giant cell carcinomas are rare types of NSCC, accounting for less than 3% of lung cancers.[3] These tumors demonstrate spindle and/or giant cell differentiation (sarcoma-like differentiation). Such tumors can be ascertained only in resection specimens. In biopsy specimens, tumors with such features are reported as "NSCC with spindle and/or giant cell carcinoma."[3,8] Expression of cytokeratin is important in this setting to exclude a primary pulmonary sarcoma. Because some tumors can show weak or even absent expression of common epithelial markers, multiple anticytokeratin antibodies may be necessary. Epithelial markers commonly used include pancytokeratin, cytokeratin AE1/AE3, CK7, and EMA. Such tumors have a worse prognosis than conventional NSCC.[40]

Carcinosarcoma is composed of NSCC and sarcomatous elements, such as rhabdomyosarcoma, chondrosarcoma, and osteosarcoma.[3] Pulmonary blastoma must be distinguished from pleuropulmonary blastoma occurring in the pediatric population.[41] Carcinomas can rarely arise from bronchial glands, analogous to salivary gland tissue. Major histologic types include mucoepidermoid carcinoma, adenoid cystic carcinoma, and epithelial-myoepithelial carcinoma.[3]

NEUROENDOCRINE TUMORS

NETs as a group are relatively common lung tumors, accounting for about 20% to 25% of lung cancers.[42,43] Their common morphologic, immunohistochemical, and ultrastructural features set them apart from other lung tumors. These features include organoid growth pattern, finely granular or "salt-and-pepper" chromatin pattern, and the expression of several hallmark neuroendocrine markers. Common neuroendocrine markers include chromogranin A, synaptophysin, and CD56. Within this group of tumors, there is a heterogeneous degree of differentiation, with well-differentiated tumors retaining most or all of the above characteristics and poorly differentiated tumors losing some or most of the discernible neuroendocrine differentiation features. This histologic differentiation is epitomized by tumor proliferation rate, which in turn correlates with tumor aggressiveness and prognosis. The 2015 WHO classification separates this group of tumors into 4 major categories, including typical carcinoid, atypical carcinoid, small cell carcinoma (or SCLC), and LCNEC (**Table 1**).[3] Because tumor cell proliferation rate has been shown to provide accurate overall prognostic information, this has been used along with the presence or absence of necrosis to divide NETs into 3 grades of prognostic significance.[44–46] The low-grade NET corresponds to typical carcinoid; intermediate-grade NET corresponds to atypical carcinoid, and high-grade NET corresponds to SCLC and LCNEC. The proliferation rate is expressed as the number of mitoses per microscopic unit area of tumor (usually defined as mitoses per 2 mm^2 or 10 high-magnification microscopic fields). In recent years, utilization of Ki67 labeling index as an adjunct for grading has gained popularity.[47–49] This Ki67 labeling index is especially useful in evaluating small biopsy specimens, whereby it is often impossible to count adequate microscopic fields to give an accurate reflection of proliferation rate.

Typical Carcinoid

Carcinoid tumors are rare, accounting for 1% to 2% of all lung tumors.[48] In the pediatric population, however, carcinoid tumors represent a common tumor type.[50,51]

Table 1
Diagnostic findings in neuroendocrine tumors

	Typical Carcinoid	Atypical Carcinoid	SCLC	LCNEC
Neuroendocrine morphology	Monotonous cells arranged in organoid nesting, palisading, rosettes, trabeculae	Monotonous cells arranged in organoid nesting, palisading, rosettes, trabeculae	Small cell size, finely granular chromatin, inconspicuous nucleoli, scanty cytoplasm	Large cell size, frequent presence of nucleoli, abundant cytoplasm
Mitoses per 2 mm^2	<2	2–10	>10, usually >60	>10, usually >30
Ki67 proliferation index	≤4–5%	≤20–25%	>50%	Usually >40%
Necrosis	No	Focal or punctuate	Often extensively present	Often extensively present
Neuroendocrine marker expression	Yes	Yes	Yes, rarely negative	Yes
Grade	Low grade	Intermediate grade	High grade	High grade

Typical carcinoids are different from other types of lung cancers in their presentation at a relatively younger age (mean age range at presentation 45–55 years) and more frequent presentation at an earlier stage (more than 70% of the cases present as stage I disease), as well as good prognosis (more than 90% 5-year survival rate).[52] There is no direct association with smoking because the prevalence of smoking in patients diagnosed for typical carcinoid is similar to the general population.[53] Carcinoid syndrome is rarely present, unless there is liver metastasis.[54] Carcinoid tumors occur in about 5% of patients with multiple endocrine neoplasia type 1, although most cases occur as nonfamilial (sporadic) isolated tumors.[55]

Typical carcinoids display characteristic morphologic features attributable to neuroendocrine differentiation. The tumor cells are generally small in size and uniform in shape. The chromatin pattern is usually fine or coarse granular without apparent nucleoli. There is a moderate amount of eosinophilic staining cytoplasm, which keeps individual tumor cell nuclei a uniform distance from one another. The tumor cells are typically arranged in organoid nests, with variation of trabeculae, insular, ribbon, and rosettelike arrangements (**Fig. 3**). Some typical carcinoids have spindle cell features with fusiform cells arranged in a fascicular pattern. By definition, typical carcinoids have a low proliferation rate, less than 2 mitoses per 10 high power fields. Ki67 (or MIB-1) labeling index is usually less than 4% to 5%.[49] There is no tumor necrosis. Typical carcinoid expresses common neuroendocrine markers such as synaptophysin, chromogranin, and CD56.

Atypical Carcinoid

Similar to typical carcinoids, atypical carcinoids are relatively common in the younger age group compared with other types of lung cancers and are frequently presented as early-staged disease.[48] The prevalence of smoking in patients diagnosed for atypical carcinoids is twice as high as the general population. The prognosis of atypical

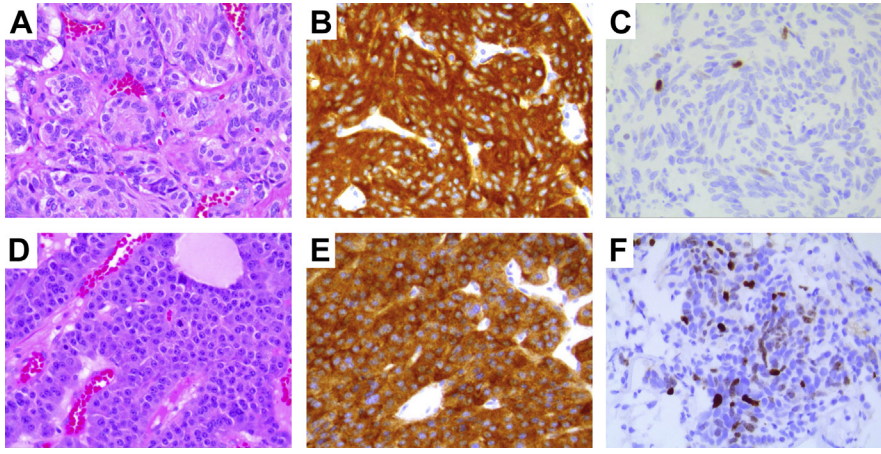

Fig. 3. NETs. (*A*) Typical carcinoid with organoid cell nests (H&E, original magnification ×400). (*B*) Typical carcinoid with positive staining for synaptophysin (synaptophysin stain, original magnification ×400). (*C*) Typical carcinoid with less than 4% Ki67 labeling index (Ki67 stain, original magnification ×400). (*D*) Atypical carcinoid with organoid cell nests (H&E, original magnification ×400). (*E*) Atypical carcinoid with positive staining for synaptophysin (synaptophysin stain, original magnification ×400). (*F*) Atypical carcinoid with greater than 4% Ki67 labeling index (Ki67 stain, original magnification ×400).

carcinoid is significantly lower than typical carcinoid, with 5-year overall survival rate less than 80%.[56]

Atypical carcinoids have cytomorphological features similar to typical carcinoids, although tumor cells in atypical carcinoids tend to display more cytologic atypia (see **Fig. 3**). The defining features of the atypical carcinoids are an intermediate proliferation rate and/or the presence of tumor necrosis. Atypical carcinoids have an intermediate rate of mitosis, between 2 and 10 mitoses per 2 mm^2.[3] Ki67 or MIB-1 labeling index is usually less than 20% to 25%.[46,49] Evaluation of Ki67 labeling index can be important in a small biopsy material to avoid overdiagnosing atypical carcinoids as high-grade neuroendocrine carcinomas.[47] Tumor necrosis is focally present, usually punctuate, and less than 10% of the tumor volume.[49]

Small Cell Carcinoma

Small cell carcinomas or SCLCs comprise slightly more than 10% of all lung cancers.[4,57] Smoking history is present in virtually all cases of SCLC.[4] SCLC is a highly aggressive malignancy. Patients usually have metastatic disease at the time of presentation. Most patients relapse within the first 2 years after treatment and the 2-year survival rate is less than 10% in metastatic patients.[58] SCLC is commonly centrally located in the major airway. SCLC has distinct morphologic features and careful evaluation on the routine hematoxylin and eosin (H&E)-stained section affords a high accuracy of diagnosis. The tumor cells are small in size compared with other types of lung cancers, usually less than the diameter of 3 mature lymphocytes. The chromatin is finely granular without prominent nucleoli. The cytoplasm is scanty, and the cellular borders are inconspicuous (**Fig. 4**). There is a high mitotic rate, usually greater than 10 mitoses per 2 mm^2. There is also a high apoptotic rate and frequent presence of

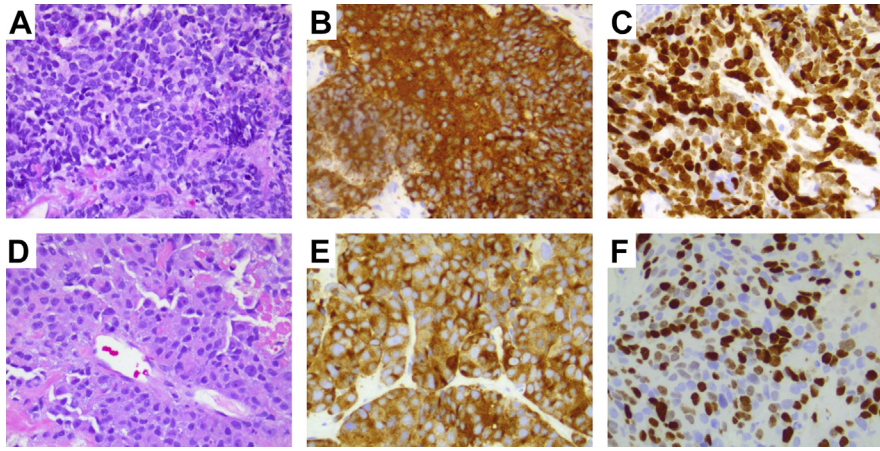

Fig. 4. Neuroendocrine carcinomas. (*A*) Small cell carcinoma (SCLC) with small-sized nuclei and scanty cytoplasm (H&E, original magnification ×400). (*B*) SCLC with positive staining for synaptophysin (synaptophysin stain, original magnification ×400). (*C*) SCLC with greater than 80% Ki67 labeling index (Ki67 stain, original magnification ×400). (*D*) LCNEC with large-sized cells and tumor necrosis (H&E, original magnification ×400). (*E*) LCNEC with positive staining for synaptophysin (synaptophysin stain, original magnification ×400). (*F*) LCNEC with greater than 40% Ki67 labeling index (Ki67 stain, original magnification ×400).

extensive tumor necrosis. In bronchial biopsy material, there is frequent crush artifact. In core needle biopsy or surgical biopsy specimen, there is usually greater nuclear size variation and less crush artifact. The tumor cells form organoid nests or diffuse sheets. The diagnosis is based on light microscopy using routine H&E-stained slides. In small biopsy material with significant crush artifact, IHC can be very helpful in establishing the diagnosis and in excluding other morphologic mimics. Commonly used stains include pankeratin and neuroendocrine markers (CD56, chromogranin, synaptophysin), although their expression levels are usually lower than low- to intermediate-grade NETs. An exception is TTF-1, which is expressed in close to 90% of SCLC.[59,60] High Ki67 (MIB-1) labeling index (>50%, usually 70%–100%) is a hallmark of SCLC, which helps to distinguish it from low- and intermediate-grade NETs to avoid overdiagnosing the latter in small crushed biopsy specimens.[47,49] Cytology is extremely useful and can offer a higher yield of diagnosis than small biopsies with scant intact, viable tumor cells. WHO classification divides SCLC into 2 subtypes: pure SCLC and combined SCLC containing a component of NSCC.

Large Cell Neuroendocrine Carcinoma

LCNEC, like SCLC, is associated with heavy smoking history.[4] It is usually peripherally located in the lung. LCNEC is a highly aggressive neuroendocrine carcinoma. As its name implies, the tumor cells are larger than SCLCs and they have abundant cytoplasm (see **Fig. 4**). Other common features include polygonal cell shape, frequent presence of nucleoli, and low nuclear-cytoplasmic (N:C) ratio.[61] Importantly, there is discernible neuroendocrine architecture, including organoid arrangement, trabecular growth, and rosettelike structures. There is a noticeable absence of cellular architectures commonly associated with adenocarcinoma differentiation. Like SCLC, these tumors show high mitotic rate (>10 mitoses per 2 mm^2) and conspicuous tumor necrosis. Immunohistochemical stains play an important ancillary role. There should be expression of at least one neuroendocrine marker. It is of note that the diagnosis of LCNEC is based on a combination of the above features. Specifically, the diagnosis is not relied solely on immunohistochemical expression of neuroendocrine markers because up to 20% of NSCCs (adenocarcinoma, SCC, and LCC) show demonstrable positive immunohistochemical staining for neuroendocrine markers.[2,22] Such tumors are classified as their NSCC type with neuroendocrine differentiation. Combined LCNEC has components of other types of NSCC or SCLC. LCNEC is a highly aggressive tumor, and 5-year survival rate is reported close to 30%, significantly worse than other types of NSCC.[62]

OTHER PRIMARY TUMORS OF THE LUNG

Besides lung carcinomas as described above, other types of tumors can arise from lung, including mesenchymal tumors, lymphohistiocytic tumors, melanoma, germ cell tumors, and others.[3]

BIOPSY DIAGNOSIS OF LUNG CANCER

Lung cancer diagnosis and classification provide pivotal information for prognosis and guide selection of therapeutic regimens. Most lung cancers are presented in advanced stage, requiring tissue biopsy or cytology diagnosis. The purpose of tissue biopsy includes establishment of malignancy diagnosis based on histomorphological findings, classification of tumor type and grade aided by IHC staining, and obtaining cellular material for targeted therapy-driven molecular testing. To minimize the

occurrence of nondiagnostic or repeat biopsies, a multidisciplinary approach with input from pathology, radiology, pulmonology, surgery, and oncology teams is warranted, with effective tissue sampling strategy preferably established in prospective tumor board case discussion.[25] To maximize the probability of productive biopsy workup, biopsy samples should be prioritized for the following three key steps of analysis: The first step is morphologic evaluation using H&E-stained slides. Diagnosis of better differentiated NSCC can be established based on the presence of glandular structure and/or mucin production (adenocarcinoma) or apparent formation of keratinization and intercellular bridges (SCC). Less well-differentiated NSCC and NET (including SCLC) usually require a second step of IHC confirmation. Concise IHC panel should be selected, guided by morphologic analysis. An effective way to accomplish both steps while preserving tissue material is to precut blank slides for potential IHC stains at the time of initial tissue sectioning for H&E stain. Such strategy minimizes the need to put the tissue block back to the microtome, shortens the diagnosis turn-around time, and maximizes the amount of tissue preserved in the tissue block for the third step of molecular biomarker testing.[25,63] Efficient triage and utilization of small biopsy specimens for molecular testing require effective communication and coordinated effort of a multidisciplinary team to complete an appropriate testing menu.

IMMUNOHISTOCHEMISTRY

The most important ancillary method in lung cancer diagnosis and classification is IHC. IHC stains are performed on tissue slides and can be readily integrated into laboratory diagnostic practice. The significance of applying IHC includes aiding effective and accurate classification of tumors (especially for poorly differentiated tumors in small biopsy specimens), minimizing potential diagnostic errors, improving delineation of tumor types suitable for molecular testing, and utilizing lineage-specific markers for the exclusion of metastatic origin of the tumors. IHC markers are most effective if appropriate markers are judiciously selected and used as a panel. There is significant recent progress in cataloging of the biomarkers, and application of IHC has been emphasized in the current WHO classification.[3] One of the most important applications of IHC is to separate adenocarcinomas from SCC. When these tumors are poorly differentiated, an accurate distinction between the two usually requires IHC analysis, even for resected specimens.[64] In certain instances, IHC provides a more accurate tumor classification because in rare occasions adenocarcinomas can have a solid or pseudosquamous morphology.[65,66] Commonly used markers to ascertain adenocarcinoma differentiation include TTF-1 and NapsinA, expressed in more than 85% of the adenocarcinomas.[6,7,63] Expression of either of these two markers is considered de facto evidence of adenocarcinoma or adenocarcinoma differentiation in NSCC if other tumors that also commonly express these markers (including SCLC and LCNEC) can be excluded. IHC markers commonly expressed in SCC include p40, CK5/6, CK5, and p63 (**Table 2**). A tumor can be considered SCC if any of these markers are expressed in the absence of adenocarcinoma-specific marker expression. One exception is p63, which is less specific than the other markers because it can also be expressed in close to 20% of adenocarcinomas.[21] Using these stains, about 90% of the poorly differentiated NSCC can be classified as either adenocarcinoma or SCC.[65,67] A poorly differentiated carcinoma that is negative for all the above markers (null immunophenotype) can be classified as “NSCC, NOS” in a biopsy specimen.[5] An effective way to achieve diagnostic accuracy while conserving biopsy material is to use antibody cocktails or double labeling.[6,7,68]

Table 2
Differential immunohistochemical characteristics of adenocarcinoma and squamous cell carcinoma

Antibody	Adenocarcinoma	SCC
TTF-1	+	–
NapsinA	+	–
P40	–	+
CK5/6	–	+
CK5	–	+
P63	–	+
Desmoglein-3	–	+

Abbreviation: CK, cytokeratin.

A second effective utilization of IHC is to reclassify or identify adenocarcinoma and SCC differentiation in LCC. Using a panel of IHC, most morphologically diagnosed LCC can be accurately reclassified as adenocarcinoma or SCC.[30,31] Cytokeratin markers are important in diagnosing pleomorphic, spindle cell, and giant cell carcinomas. Multiple epithelial markers, such as pancytokeratin, cytokeratin AE1/3, CK7, and EMA, may be necessary to confirm epithelial differentiation and to exclude pulmonary sarcomas.

IHC is also useful in demonstrating neuroendocrine differentiation in tumors exhibiting neuroendocrine morphology. Commonly used neuroendocrine markers include CD56, synaptophysin, and chromogranin. NETs also express other markers commonly used in a panel IHC workup, including cytokeratins, CK7, TTF-1, NapsinA, and p63. An IHC panel for the confirmation of SCLC diagnosis includes pankeratin (usually dotlike perinuclear staining), neuroendocrine markers (CD56, chromogranin, and synaptophysin), TTF-1 (70%–90% positivity), and Ki67 (70%–100% labeling index).

As a surrogate test for molecular profiling of NSCC (see later discussion), IHC has been evaluated as a rapid and cost-effective testing platform deployable in the routine pathology practice setting in the determination of predictive biomarkers. Several mutation-specific antibodies have been developed. For instance, epidermal growth factor (EGFR) mutation-specific antibodies demonstrate a good specificity, albeit with limited sensitivity.[69,70] In comparison, the sensitivity and specificity of antibodies against anaplastic lymphoma kinase (ALK) and C-ros oncogene 1 (ROS1) are more consistent. Several monoclonal antibodies recognizing ALK protein have shown promising results to screen for ALK gene rearrangement comparable to fluorescence in situ hybridization (FISH) method.[71–73] IHC using an antibody specific for ROS1 protein has achieved high specificity and sensitivity in detecting ROS1-translocated tumors and can act as an effective screening tool.[74]

Lung is a frequent target of metastasis, and metastatic malignancy to lung is more often encountered in clinical practice than primary lung cancer. Although lung metastasis is often multifocal, it can be solitary. Certain lung cancers can also present as multifocal disease. In this regard, IHC plays a pivotal role in separating primary lung cancer from metastasis. IHC can help confirm the presence of metastasis and identify primary tumor tissue of origin in most cases. A 3-tiered approach helps to separate metastasis from lung cancer. Metastatic tumors usually show distinct CK7 and CK20 expression pattern, absence of markers commonly expressed in primary lung

cancers (such as TTF-1 and NapsinA), and expression of organ-associated markers.[75,76]

MOLECULAR TESTING OF LUNG CANCER

Molecular analysis of lung cancer genetic alterations has both advanced the understanding of carcinogenesis and provided a paradigm change in therapeutic targeting and monitoring. Detection of specific genetic alterations has been proved to be effective in predicting treatment response and stratifying prognosis and is potentially applicable in early detection of lung cancer.[77,78] Targeted therapy using drugs specifically designed to inhibit mutation-driven genetic alterations affords more effectiveness and less toxicity than generic chemotherapeutic agents and therefore substantial improvement of outcomes compared with standard chemotherapy in the treatment of advanced NSCC. One of the common mechanisms of carcinogenesis is constitutive activation of receptor tyrosine kinases, such that inhibition of their activity creates an effective modality for anticancer therapy. With the advent of tyrosine kinase inhibitor (TKI) treatments, it is important to screen patients with lung cancer for actionable gene mutations. EGFR mutation and ALK translocation are the most effectively targeted oncogenes in NSCC and are now considered standard of care.[79] Recent advancement in testing methodologies such as next-generation sequencing (NGS) affords multiplex systems to detect multiple gene alterations on one single platform.[80] Noninvasive plasma and serum-based DNA detection and monitoring are emerging molecular tools.[81]

EGFR is a transmembrane glycoprotein receptor. Upon ligand binding, activation of its cytoplasmic tyrosine kinase domain by dimerization and phosphorylation elicits downstream signaling pathways that lead to DNA synthesis and cell proliferation. EGFR gain of function mutations lead to constitutive activation of downstream signaling pathways, which is critical for tumor growth.[82,83] EGFR mutations can be detected in 10% to 30% of NSCC patients. Tyrosine phosphorylation inhibitors or TKIs inhibit EGFR phosphorylation and thus are able to mute the effect of EGFR mutations. Hence, detection of EGFR mutations predicts response to targeted therapy using TKIs. As first-line treatment, TKIs have been shown to produce overall response rates of close to 75% in tumors carrying EGFR mutations.[84] Because EGFR mutations are detected mostly in adenocarcinomas, the current recommendation is to test for EGFR mutations in adenocarcinomas, mixed lung cancers with an adenocarcinoma component (such as adenosquamous carcinoma), and small samples whereby an adenocarcinoma component cannot be excluded.[79] Molecular biological techniques for EGFR mutation detection include screening methods that detect all mutations and targeted methods that detect specific, known types of mutations. Commonly used screening methods include direct DNA sequencing and high-resolution melting analysis. Targeted methods include polymerase chain reaction (PCR) -based targeted methods, such as ARMS (Amplification Refractory Mutation SystemARMS) and SmartAMP (Smart Amplification Process).[85,86] Gefitinib, erlotinib, and afatinib are currently recommended TKIs for first-line treatment of those with sensitizing EGFR mutations in lung cancer.[87–89] Development of an additional mutation T790M is a common mechanism for acquired resistance to TKI treatment. Possible strategies to overcome such acquired drug resistance include platinum-based doublet chemotherapy and utilization of next-generation PKIs.[90]

ALK is another receptor tyrosine kinase. A common form of ALK alteration is the formation of an oncogenic fusion gene with echinoderm microtubule-associated protein-like 4 (EML4-ALK), seen in 4% to 7% of NSCCs, particularly adenocarcinomas.[91]

Thus, ALK testing is similarly recommended for adenocarcinomas and mixed lung cancers with an adenocarcinoma component.[79] ALK gene rearrangements are found to be mutually exclusive with EGFR and KRAS mutations.[92] Commonly used molecular methods for detecting EML4-ALK fusion gene include real-time PCR, FISH, and direct DNA sequencing.[86,93] The ALK competitive binding inhibitor crizotinib is approved for those with ALK gene rearrangement.[90] In contrast to acquired resistance in EGFR TKI therapy, multiple acquired mutations may develop, resulting in drug resistance. Next-generation ALK TKIs may be a promising treatment approach in crizotinib-resistant cases.[90]

Besides EGFR and ALK, a multitude of other biomarkers are being actively evaluated or used as therapeutic targets.[94] ROS1 is a receptor tyrosine kinase that can be rearranged in 1% to 2% of lung adenocarcinomas. ROS1 rearrangement is rarely found coexisting with EGFR and ALK alterations.[92] Crizotinib has been shown to be effective in patients with ROS1 rearrangement and is approved for such treatment.[95] The mesenchymal epithelial transition factor (MET) gene is also a transmembrane tyrosine kinase receptor that can be altered by either overexpression or amplification. Such genetic alterations have been shown to be an adverse prognostic marker.[96] MET amplification is also a common mechanism for acquired resistance to EGFR TKI therapy.[97] Therapeutic coinhibition of both receptors may represent a potential treatment option to overcome such acquired drug resistance. Kirsten rat sarcoma viral oncogene homolog (KRAS) belongs to the RAS family of oncogenes together with HRAS and NRAS. The KRAS gene encodes a GTP-binding protein. Mutations in KRAS are usually mutually exclusive with other oncogenic driver mutations.[98] Development of KRAS inhibitor is still ongoing, and a phase III trial of the downstream RAS pathway inhibitor, selumetinib, has been initiated.[99] Another RAS pathway protein kinase, BRAF, is also a potential target for inhibitor therapy. BRAF mutation occurs in 2% to 3% of NSCC, and V600E is the most common mutation.[94] Other biomarkers under active evaluation include human epidermal growth factor receptor 2, rearranged during transfection, fibroblast growth factor receptor 1 (FGFR1), discoidin domain receptor tyrosine kinase 2 (DDR2), phosphatase and tensin homolog, MAP2K (mitogen-activated protein kinase kinase), and phosphatidylinositol 3 kinase.[94]

Molecular techniques for detecting genomic alterations in lung cancer include screening (or scanning) genotyping methods and targeted genotyping methods. Commonly used screening technologies include Sanger sequencing, pyrosequencing, and high-resolution melt analysis.[100] Targeted assays are designed to detect specific known mutations or “hot-spot” mutations, which afford greater sensitivity. Commonly used targeted assays include Agena MassARRAY Oncocarta panel and SNaPShot multiplex kit.[100] With the necessity to detect an increasing number of gene mutations using often limited biopsy material obtained by minimally invasive procedures, high-efficiency screening and targeted assays have been developed. An ideal detection system should provide adequate sensitivity and specificity in evaluating all clinically relevant genetic alterations in a cost-effective way using a limited sample. This detection system necessitates the implementation of simultaneous evaluation of multiple genes, moving from detecting individual mutations to multiple gene evaluation, or single-tube multiplexed mutation detection. NGS offers a cost-effective approach for detecting multiple genetic alterations with a minimum amount of DNA. NGS provides high-throughput simultaneous sequencing of thousands to millions of short nucleic acid chains in a massively parallel way.[101,102] In targeted NGS, an extended panel of mutations can be screened, covering important mutational hot spots in clinical laboratories.[103,104] NGS can also be used to analyze circulating tumor cells (CTCs) that are shed into the bloodstream from the primary tumor site or

metastatic deposit. Tumor cells also release genetic materials in circulation as cell-free circulating tumor DNA (ctDNA) that can be the target for NGS analysis.[105] Such noninvasive "liquid biopsies" conveniently use peripheral blood to provide a surrogate for direct analysis of solid tumors.[106,107] Genetic profiling of CTCs and ctDNA can be used as a proxy parameter for the detection of mutations, evaluation of tumor burden, monitoring of treatment response, and detection of mutation-based treatment resistance, and as a potential tool for screening and early detection of malignancies.

SUMMARY

Classification and histologic typing of lung cancers, together with tumor molecular profiling, lay the foundation on which the oncologic treatment plan is formulated. Since the publication of the last volume, significant update in lung cancer classification has occurred, especially for the subtyping of adenocarcinomas, based on current understanding of tumor biology. This updating is reflected in streamlined classification for small biopsies and cytology specimens, with an emphasis on separating adenocarcinomas from the rest of the lung cancers in order to select tumors for biomarker testing. More detailed histologic typing is used in resection specimens to delineate tissue types of prognostic significance. Immunohistochemistry is an important ancillary method in lung cancer diagnosis and classification. This article seeks to update readers to the new 2015 WHO classification, which delineates major types of lung cancers as adenocarcinoma, SCC, and NETs. NETs are in turn subdivided into typical carcinoid, atypical carcinoid, small cell carcinoma, and LCNEC based on tumor cell morphology and proliferation rate. Effective application of the recently refined lung cancer classification provides pathologic information fundamental for tumor risk assessment and management decision-making. Molecular profiling has growing importance in identifying subsets of lung cancer with unique sensitivity to targeted therapy.

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