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Update on small cell carcinoma and its differentiation from squamous cell carcinoma and other non-small cell carcinomas

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Small cell lung cancer (SCLC) comprises 14% of all lung cancers, and >30 000 new cases are diagnosed per year in the United States. SCLC is one of the most distinctive malignancies in the entire field of oncology with characteristic clinical properties, responsiveness to specific chemotherapy, genetic features and a highly reliable pathological diagnosis. SCLC is defined by light microscopy, and the most important stain is a good-quality hematoxylin and eosin (H&E)-stained section. The vast majority of cases can be diagnosed on H&E alone; however, in problem cases, immunohistochemistry can be very helpful in making the distinction from other tumors. Cytology is also a powerful tool, often being more definitive than small biopsies with scant tumor cells, crush artifact and/or necrosis. As virtually all SCLCs present in advanced stages, most patients are diagnosed based on small biopsy and cytology specimens. Historically, there has been significant evolution in the histological subclassification of SCLC dating from 1962 when Kreyberg proposed the oat cell and polygonal cell types. The current subclassification recognizes only two subtypes: pure SCLC and combined SCLC. Pathologists need to do their best to make a diagnosis of SCLC or other histological types of lung cancer and this can be achieved in most cases. This review will address some of the diagnostic problems that occur in the minority of cases and outline practical ways to address them. Brief reference will be made to other neuroendocrine lung tumors with an overview of the molecular pathogenesis of this spectrum of tumors.
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Small cell lung cancer (SCLC) comprises 14% of all lung cancers, and >30 000 new cases are diagnosed per year in the United States.[1–4] SCLC is one of the most distinctive malignancies in the entire field of oncology with characteristic clinical properties, responsiveness to specific chemotherapy, genetic features and highly reliable pathological diagnosis.[3] There has even been a specific limited *vs* extensive staging system proposed for SCLC, although the recent IASLC (International Association for the Study of Lung Cancer) staging project and AJCC/UICC 7th Edition proposed to use TNM for staging of SCLC.[5–7] As virtually all SCLCs present in advanced stages, with only 5% presenting as a solitary coin lesion, most patients are diagnosed based on small biopsy and cytology specimens.[3,8,9] Virtually all patients with SCLC are cigarette smokers, usually heavy smokers.[3]

Historically, there has been significant evolution in the histological subclassification of SCLC dating from 1962 when Kreyberg proposed the oat cell and polygonal cell types (Table 1). The major lesson learned from this history is that the creation of an 'intermediate' subtype in the 1981 WHO classification was a failure because (1) clinicians were never sure whether these were SCLCs or non-small cell lung carcinomas (NSCLCs); (2) reproducibility was poor among expert pathologists; and (3) it was not clear there was any relevant clinical implication to this category. Hence, despite the continued existence of a small percentage of problem SCLC cases that are difficult to classify, proposals to recreate an 'intermediate' or 'gray-zone' category overlook this important piece of history. The current subclassification recognizes only two subtypes: pure SCLC and combined SCLC. Pathologists need to do their best to make a diagnosis of SCLC or NSCLC. If

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Table 1 History of small cell lung carcinoma (SCLC) subclassification

Kreyberg 1962[71]	*WHO 1967*[72]	*1973 WP-L WHO 1981*[34]	*IASLC 1998*[73]	*WHO/IASLC 1999*[74]	*WHO 2004*[10]
Oat cell	Lymphocyte-like	Oat cell	Pure SCLC	SCLC	SCLC
Polygonal	Polygonal	Intermediate			
	Fusiform		Mixed (with large cells)		
	Other (containing squamous and glandular foci)	Combined	Combined	Combined SCLC (containing any other NSCLC component)	Combined SCLC (containing any other NSCLC component)

IASLC, International Association for the Study of Lung Cancer; WHO, World Health Organization; WP-L, Working Party for Therapy of Lung Cancer.

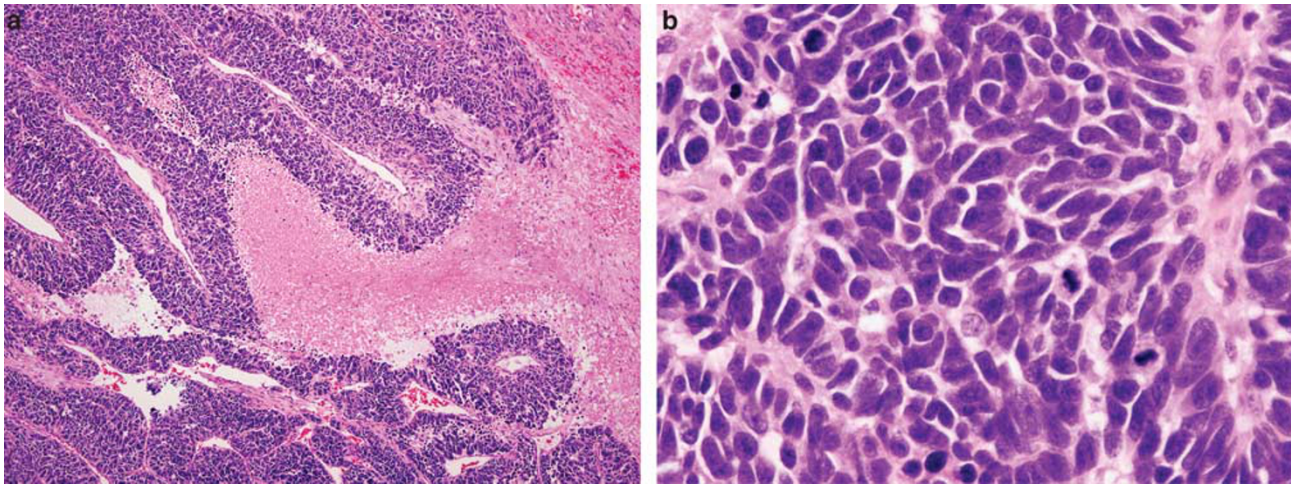

Figure 1 Small cell carcinoma. (**a**) This tumor consists of diffuse sheets of small malignant cells. Extensive necrosis is present. (**b**) The tumor consists of dense sheets of small cells with scant cytoplasm, finely granular nuclear chromatin, frequent mitoses and nucleoli are inconspicuous or absent.

there are uncertainties in a difficult case, the challenging issues should be reflected in a comment added to the diagnosis. Some of the causes of these difficulties and practical ways to address them are discussed in this review. Brief reference will be made to other neuroendocrine lung tumors, with an overview of molecular pathogenesis of these tumors.

Histological features

SCLC is defined by light microscopy as a tumor with cells that have a small size, a round-to-fusiform shape, scant cytoplasm, finely granular nuclear chromatin and absent or inconspicuous nucleoli (Figures 1a and b).[10] Nuclear molding is frequent. Crush artifact can cause smearing or streaming of nuclear chromatin (Figure 2). Necrosis is frequent and often extensive (Figure 1a). Mitotic rates are high, with an average of 80 mitoses per 2-mm^2 area (Figure 1b).[10–12] In small biopsies, mitoses may be difficult to identify and necrosis may be absent because of limited sampling. The tumor usually grows in diffuse sheets, but rosettes, peripheral palisading, organoid nesting, streams, ribbons and rarely, tubules or ductules that fall short of glandular differentiation may be present.[11,13] Basophilic encrustation of vessel walls by DNA from necrotic tumor cells (nuclear debris also known as the Azzopardi effect) is often seen in necrotic areas.[13] A discohesive pattern of growth may resemble malignant lymphoma, especially when infiltrating mediastinal fat.[11] Rarely, a pseudopapillary pattern may occur; this is an artifact when perivascular tumor cells remain viable and the intervening tumor is necrotic.[11] The presence of a very prominent neuroendocrine morphology does not exclude the diagnosis of SCLC. If SCLC has a pure histology, it is classified simply as small cell carcinoma.[10] SCLC is reliably diagnosed in small biopsies and cytology specimens. In fact, cytology may be more reliable than biopsy in some cases (Figure 3). In biopsies, the most important stain is a good-quality hematoxylin and eosin (H&E) stain.

Combined Small Cell Carcinoma

Combined small and large cell carcinoma is defined histologically as a tumor with a mixture of SCLC and at least 10% larger cells that morphologically qualify as a non-small cell carcinoma (Figure 4). In addition to combined small and large cell carcinoma, one can have combined SCLC with squamous cell, adenocarcinoma, spindle cell[14] or giant cell

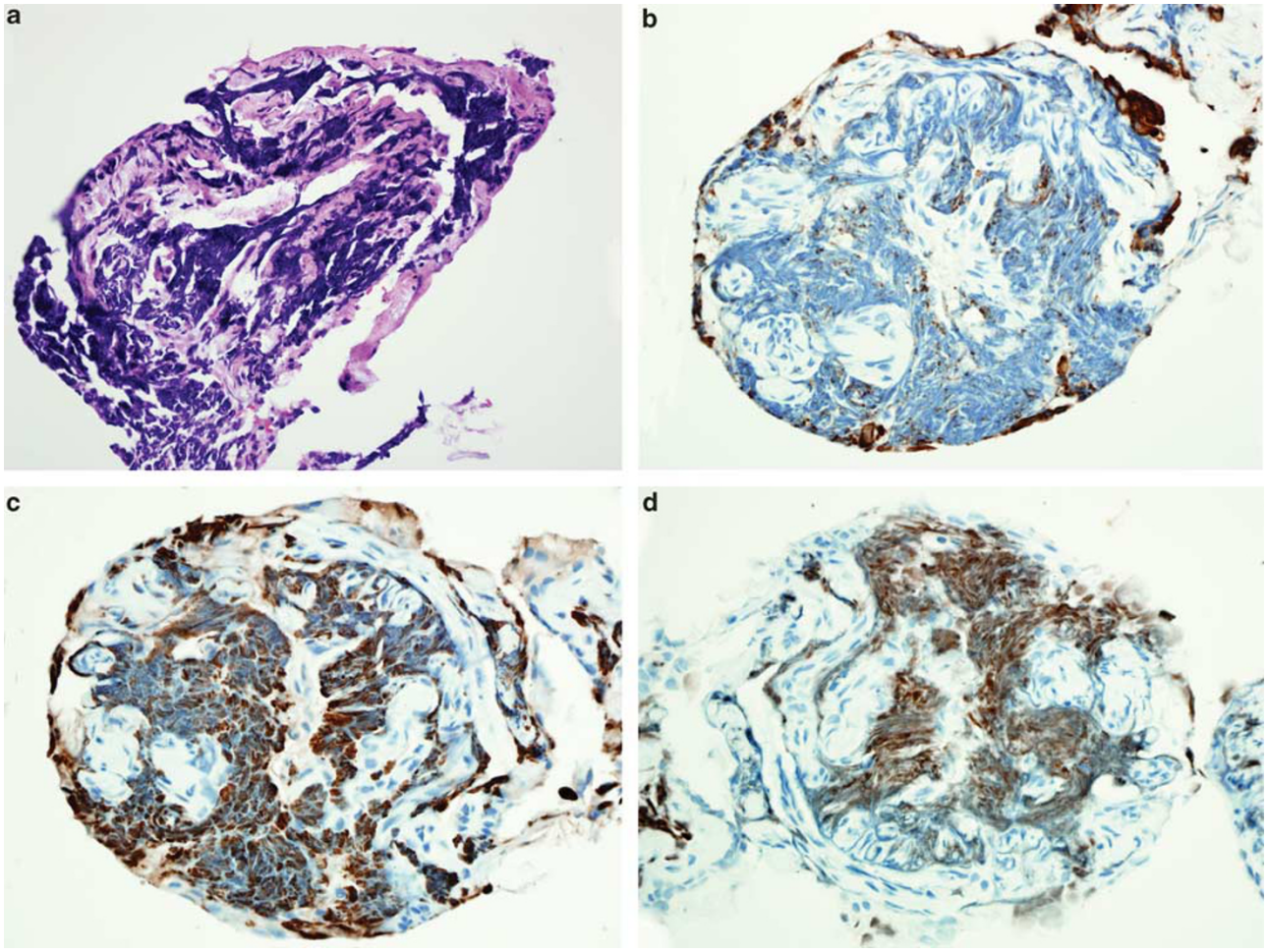

Figure 2 Small cell carcinoma, crush artifact. (**a**) This tumor shows marked crush artifact. A small area of preserved tumor cells elsewhere and cytology correlation enabled a definitive diagnosis. The following positive immunostains were also helpful in conjunction with a negative CD45: (**b**) AE1/AE3, (**c**) Ki-67 and (**d**) TTF-1.

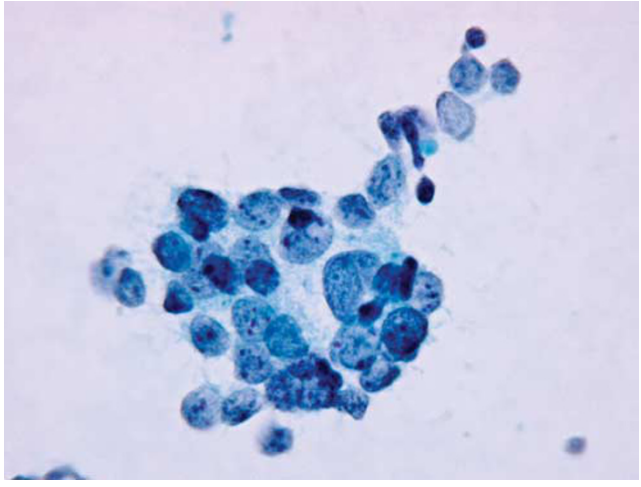

Figure 3 Small cell carcinoma, cytology. This cluster of tumor cells is tightly packed with scant cytoplasm, finely granular nuclear chromatin and nucleoli are absent.

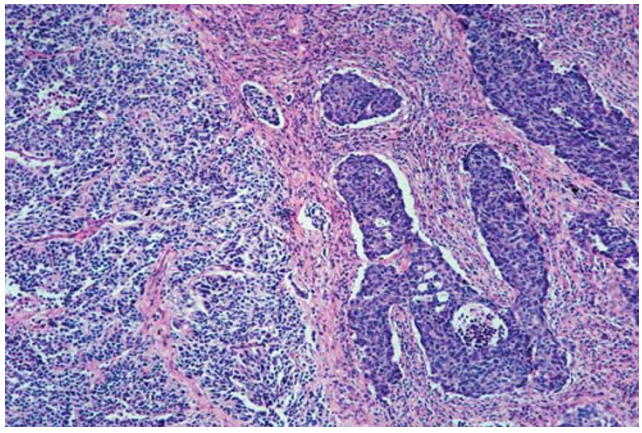

Figure 4 Combined small cell carcinoma and large cell carcinoma. This tumor consists of a mixture of small cell carcinoma (left) and large cell carcinoma (right). The latter has more abundant cytoplasm.

carcinoma. The frequency of combined SCLC varies depending on the tumor sample size, number of histological sections studied, type of specimen (autopsy *vs* surgical *vs* small biopsy) and variation in interpretation.[15,16] In a series of surgically resected cases, Nicholson *et al*[11] found combined

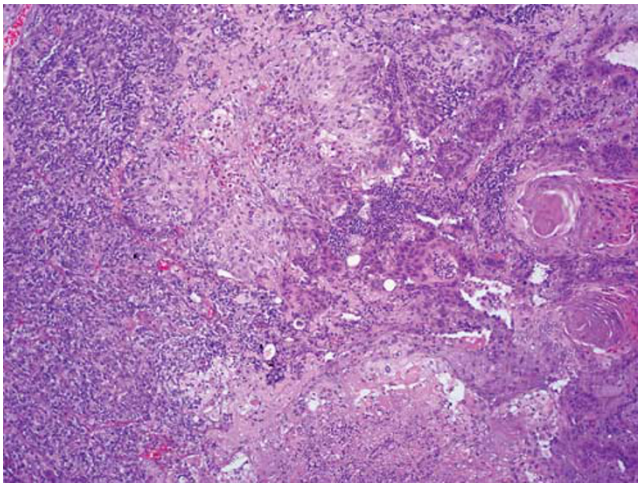

Figure 5 Combined small cell carcinoma and squamous cell carcinoma. This tumor consists of a mixture of small cell carcinoma (left) and squamous cell carcinoma (right).

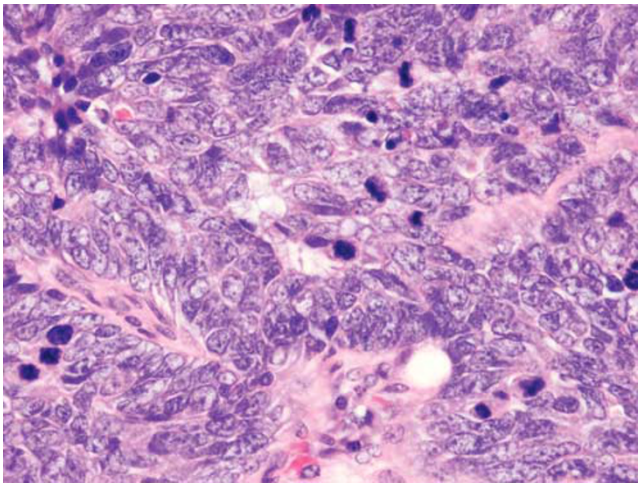

Figure 6 Small cell carcinoma, resected specimen. Tumor cells appear larger than typically seen in small biopsy specimens. However, the cells maintain the morphological features of small cell carcinoma with scant-to-moderate cytoplasm, finely nuclear chromatin and inconspicuous or absent nucleoli.

SCLC in 28% of cases with 16% combined SCLC with large cell carcinoma, 9% with adenocarcinoma and 3% with squamous cell carcinoma. Although the frequent presence of a few large cells in resected SCLC required setting a minimum criterion of 10% for combined SCLC and large cell carcinoma, if there is frank adenocarcinoma or squamous cell carcinoma (Figure 5), no such percentage is required. Tumor cells appear larger in surgical specimens of SCLC because they are better fixed than tumor cells in small specimens (Figure 6).[11,17]

Immunohistochemistry

Although immunohistochemistry is useful in the diagnosis of SCLC, the most important stain is a good-quality H&E stain. In fact, the diagnosis can be established based on a review of H&E-stained sections without immunostains in the majority of cases. Hence, immunohistochemistry is required only in problematic cases. Staining for pancytokeratin such as AE1/AE3 helps to demonstrate that the tumor is a carcinoma rather than a lymphoid lesion (Figures 2b and 7a). CK7 and CK20 are not very useful cytokeratins for SCLC diagnosis because only about half stain with CK7 and $<10\%$ with CK20.[18,19] Although a 'dot-like' pattern of staining for keratin can occur in SCLC, in many cases this pattern is not seen.

The most useful NE markers include CD56, chromogranin and synaptophysin, which are best used as a pane (Figures 7b and c).[11,20] Up to two-thirds of SCLC will be negative for chromogranin and synaptophysin.[21] CD56 will stain approximately 90–100% of cases;[18,22,23] however, it is less specific and interpretation for SCLC diagnosis needs to be performed carefully in the appropriate morphological context. SCLC can stain diffusely and strongly with all three neuroendocrine markers, and this finding should not be used to favor a diagnosis of carcinoid if the morphology is diagnostic, in particular if the tumor has a high mitotic rate and/or proliferation index. However, neuroendocrine marker staining may be focal or weak and only one or two markers may be positive. In $<10\%$ of cases, all neuroendocrine markers may be negative and the diagnosis can still be established if the morphology is diagnostic.

In 70–90% of SCLCs, TTF-1 expression is present (Figures 2d and 7d),[10,12,24–28] but it can be positive in 44–80% of extrapulmonary small cell carcinomas as well; hence, it is not useful in determining the primary site of small cell carcinomas.[29] SCLC show a high proliferation rate by Ki-67, averaging 70–90% (Figures 2c and 7e).[30]

In cases in which all neuroendocrine markers and TTF-1 are negative, lymphoma, melanoma and basaloid carcinoma should be excluded with positive keratin expression and negative squamous markers such as p63.

Negative immunostains or control slides can reveal morphological details that are obscured by poor-quality H&E stains. In particular, negative immunostains can be useful for evaluating nuclear detail and counting mitoses.

Differential diagnosis

The differential diagnosis of SCLC encompasses NSCLC (including large cell carcinoma or basaloid squamous cell carcinoma), malignant lymphoma, chronic inflammation, other neuroendocrine lung tumors (including carcinoids and large cell neuroendocrine carcinoma), malignant melanoma and metastatic carcinoma of the breast or prostate and metastatic neuroendocrine carcinomas from other

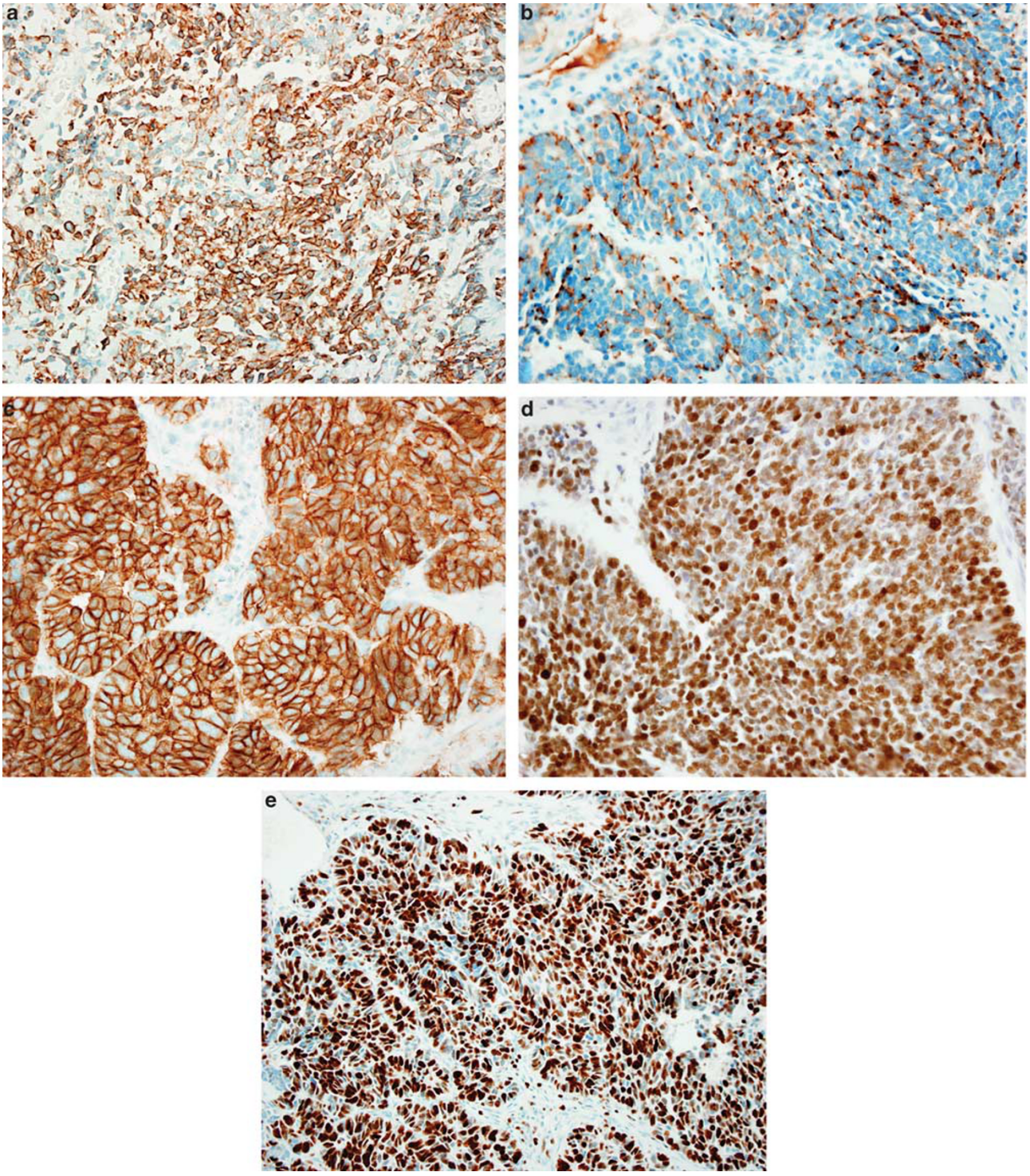

Figure 7 Small cell carcinoma, immunohistochemistry. (**a**) AE1/AE3 shows positive tumor cell staining. (**b**) Chromogranin shows positive cytoplasmic staining. (**c**) CD56 is positive with a membranous pattern. (**d**) TTF-1 shows diffuse positive nuclear staining. (**e**) Small cell carcinoma, Ki-67. (Panel **d**) Ki-67 shows a high proliferation rate with almost 100% tumor cell staining.

sites. In virtually all cases, the diagnosis of SCLC can be made reliably even with small amounts of tumor tissue or cytology samples. In cases of uncertainty, in which the material is suggestive of SCLC, but not unequivocally diagnostic, it can be useful to use the phrase 'consistent with small cell carcinoma' to communicate to clinicians that there is some limitation in the material that precludes an unqualified diagnosis.

As there are major differences in the therapeutic approach to patients with SCLC *vs* NSCLC, a frequent question asked of pathologists in the

Table 2 Light microscopic criteria for distinguishing small cell carcinoma and large cell carcinoma or large cell neuroendocrine carcinoma[a]

Histological feature	*Small cell carcinoma*	*Large cell carcinoma or LCNEC*[a]
Cell size	Smaller (less than diameter of 3 lymphocytes)	Larger
Nuclear/cytoplasmic ratio	Higher	Lower
Nuclear chromatin	Finely granular, uniform	Coarsely granular or vesicular Less uniform
Nucleoli	Absent or Faint	Often (not always) present May be prominent or faint
Nuclear molding	Characteristic	Uncharacteristic
Fusiform shape	Common	Uncommon
Polygonal shape with ample pink cytoplasm	Uncharacteristic	Characteristic
Nuclear smear	Frequent	Uncommon
Basophilic staining of vessels and stroma	Occasional	Rare

LCNEC, large cell neuroendocrine carcinoma.
[a]This is modified from reference.[17]

interpretation of lung biopsy specimens for the diagnosis of lung cancer is whether the tumor is a SCLC or a NSCLC. As the problem of separating SCLC from large cell carcinoma and LCNEC is very similar, the major criteria in separating both of these tumors from SCLC are summarized in Table 2. The distinction of these tumors from SCLC should not rest on a single feature such as cell size or nucleoli, but incorporation of multiple additional features including nuclear-to-cytoplasmic ratio, nuclear chromatin, nucleoli, nuclear molding, cell shape (fusiform *vs* polygonal) and hematoxylin vascular staining.[17,31]

Disagreement among expert lung cancer pathologists over the distinction between SCLC and NSCLC may occur in up to 5–7% of cases.[32,33] Several factors can contribute to interobserver variability including small crushed biopsy specimens, ischemic changes, poor fixation and poor histological sections that are cut too thick or overstained. In some cases, these problems can be overcome by asking the histology laboratory to recut the block and make a good-quality H&E-stained section. When a difficult case is encountered, it is helpful to try to reach a local consensus among colleagues, and if this cannot be achieved, it may be helpful to obtain extramural consultation.

There is a spectrum of cellular morphology in SCLC, which includes larger cells that approach the size of large cell carcinoma (Figure 6). Such cases with tumor cell size at the larger end of the spectrum of SCLC used to be referred to as the intermediate subtype under the 1981 WHO classification.[34] However, this term is no longer accepted. Morphometric data have demonstrated a continuous spectrum of cell size from the smallest SCLC to large cell carcinoma.[17,35] Thus, tumor cells which fall on the border between SCLC and large cell carcinoma may not be able to be distinguished based on cell size alone and require application of multiple morphological criteria (Table 2). A practical rule is that tumor cells of SCLC should measure approximately the diameter of two to three small resting lymphocytes.

The size of the biopsy specimen can also have an effect on tumor cell size. This observation is based on data that indicate that cells of SCLC appear larger in larger specimens, especially open lung biopsies.[11,17] As SCLC is diagnosed by small biopsy in $>90\%$ of cases, most pathologists are not used to seeing this tumor in resected specimens and the threshold for larger cell size needs to be kept in mind when reviewing this tumor in well-fixed open biopsies (Figure 6).

Basaloid variants of large cell or squamous cell carcinoma can be problematic in the differential diagnosis with SCLC. The general morphological criteria for SCLC *vs* large cell carcinoma summarized in Table 2 can also be useful in this differential diagnosis. However, additional histological, cytological and immunohistochemical features that distinguish SCLC and basaloid carcinoma are summarized in Table 3. The most useful differential histological features are that SCLC more often grows in sheets and shows streaming, and basaloid carcinoma is more likely to show a basement membrane-like or a desmoplastic stroma. Distinguishing immunohistochemical features include expression of squamous markers such as p63 by basaloid carcinomas and TTF-1 or neuroendocrine markers by SCLC.[20,26,36]

Pitfalls in the diagnosis of SCLC

There are a number of pitfalls in the diagnosis of SCLC. These include lack of cytology–histology correlation, crush artifact, Merkel cell carcinoma, primitive neuroectodermal tumor (PNET), keratin-negative SCLC, diminished proliferation rate in SCLC after chemotherapy and combined SCLC and large cell carcinoma.

SCLC is readily diagnosed by cytology; hence, it is important to correlate the findings in any biopsy that may be paired with a cytology sample. Often in

Table 3 Differential diagnosis of small cell carcinoma and basaloid carcinoma

Feature	*SCLC*	*Basaloid carcinoma*
Histological patterns		
Nests/organoid	Common	Common
Sheets	Common	Uncommon
Trabecular	Possible	Possible
Streaming	Common	Rare
Rosette-like	Uncommon	Rare
Basement membrane-stroma (hyaline)	Rare	Possible
Desmoplastic	Uncommon	Possible
Cytological features		
Cell size	Usually $\leqslant$ diameter 3 small resting lymphocytes	Usually $>$ diameter 3 small resting lymphocytes
Cell shape	Round to oval or spindled	Round to oval, rarely spindled
N/C ratio	High	Variable—usually low, can be high
Nucleoli	Inconspicuous/absent	Variable—usually present, may be absent
Chromatin	Finely granular	Usually vesicular, can be finely granular
Abrupt keratinization	No (unless combined SCLC and squamous cell carcinoma)	Characteristic
Mitotic rate	High (70–80 per 2 mm^2)	High
Immunohistochemical features		
AE1/AE3	Positive	Positive
P63	Negative	Positive
34βE12 (HMWK)	Negative	Positive
TTF-1	Positive: 70–80%	Negative
Chromogranin/synaptophysin	Positive: 60–70%	Negative
CD56	Positive: 90%	Negative
Ki-67	High: 70–100%	High: 70–100%

very challenging biopsies, the diagnosis may be more readily established based on the cytology sample. If this correlation is not made between biopsy and cytology, it is possible to have a diagnosis of SCLC in one specimen and non-small cell carcinoma in the other specimen.

Crush artifact is a frequent finding in small transbronchial or mediastinal biopsy specimens and can make pathological interpretation very difficult (Figure 2a). Tumor cells of SCLC have a tendency to show a streaming artifact, but this can also occur with NSCLC, carcinoid tumors, lymphoma and chronic inflammation. Lymphoid infiltrates, whether due to small lymphocytic lymphoma or chronic inflammation, can be distinguished from SCLC by their discohesive pattern of growth contrasting with the epithelial clustering and nuclear molding of SCLC. Fortunately, the key immunohistochemical stains useful for SCLC diagnosis including keratin (Figure 2b), chromogranin, CD56, Ki-67, TTF-1 (Figure 2d) and CD45 can work even on small crushed biopsies. These stains can help address most of the problems of differential diagnostic considerations in crushed biopsies. Ki-67 proliferation rates can be particularly helpful in distinguishing carcinoid tumors from SCLC in crushed specimens (Figure 2c).[30] The mitosis-specific marker anti-phosphohistone h3 may also prove to be a useful marker in distinguishing carcinoids from SCLC, but its role in small crushed specimens remains to be determined.[37]

Occasionally, the differential diagnosis with Merkel cell carcinoma or PNET comes into consideration. Particularly, in a non-smoker, a young patient and the presence of skin lesions or chest wall involvement, these entities may rise to the top of the differential diagnosis. Morphologically, tumor cells of PNET are typically more discohesive and mitotic rates may be relatively low compared with SCLC. PNET tends to be keratin negative or only weakly positive and it tends to stain strongly with immunohistochemical staining for CD99, whereas SCLC is usually positive for pancytokeratin and TTF-1 but CD99 staining is negative or weak.[38,39] In a metastatic setting, differential with Merkel cell carcinoma can be problematic, but in contrast to SCLC, Merkel cell carcinomas are often CK20 positive, negative for TTF-1 and may express neurofilament protein.[18,19,29,40–42]

A keratin-negative tumor that looks like SCLC should raise the consideration of malignant lymphoma, malignant melanoma, a crushed carcinoid tumor and PNET. Other stains such as lymphoid markers (CD45 or CD20), melanoma markers (S100, HMB-45) a Ki-67 stain and CD99 may be helpful in this differential diagnosis, respectively. It is extremely unusual to encounter a keratin-negative SCLC. When this occurs and other differential diagnostic considerations seem excluded, several keratin antibodies other than CK7/CK20 may be helpful. When all keratins are negative, if the morphology is characteristic and other tumors have been excluded, the finding of TTF-1 and NE marker expression can help support a SCLC diagnosis.

After chemotherapy, the proliferation rate of SCLC may be markedly diminished to levels more fitting for carcinoid tumors (Figure 8). This can be

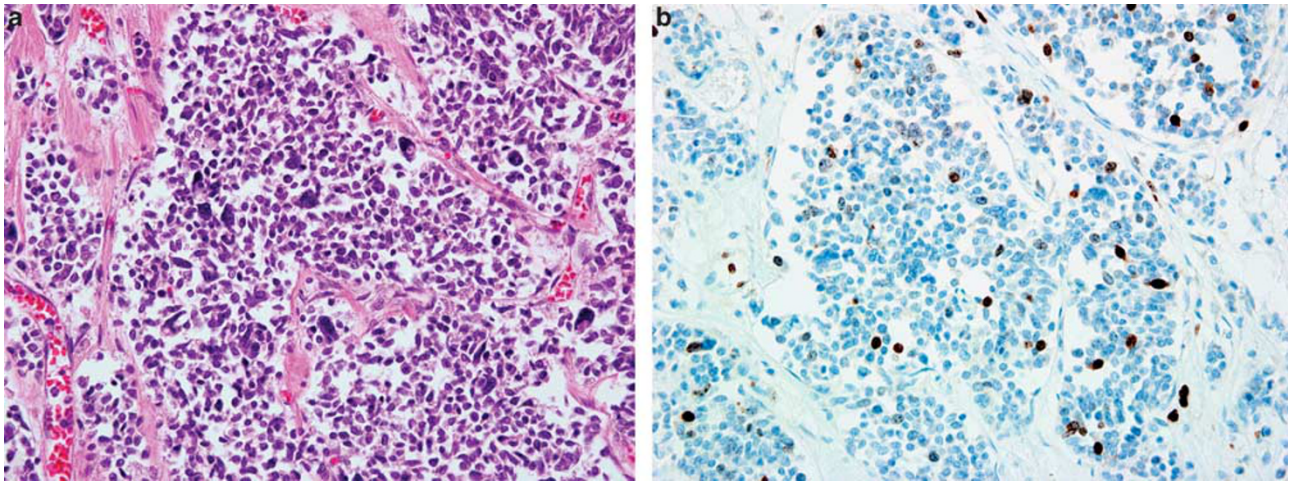

Figure 8 Small cell carcinoma, after chemotherapy. (**a**) Small cell carcinoma at autopsy with a few large pleomorphic cells. (**b**) Ki-67 shows a low proliferation rate of <10%.

confusing in the neoadjuvant setting when tumors are resected after chemotherapy.[43]

Combined SCLC and large cell carcinoma presents a challenge in the differential diagnosis between SCLC *vs* large cell carcinoma or LCNEC. As up to 16% of surgically resected cases are combined SCLC and large cell carcinoma, this is an important issue. Pathologists are not trained very well to identify more than a single population of cells in a given tumor; hence, the tendency is to focus on the first field under the microscope and not to keep searching for a second population. Thus, in these combined tumors, sometimes one pathologist will focus on the small cell component and another on the large cell component. In such cases, the entire set of tumor slides and all cellular components should be reviewed. In most combined tumors, the SCLC component is the predominant component. As the presence of a SCLC component will define the therapy for the patient, the most important decision for a pathologist is the determination of whether a SCLC component is present.

It is extremely unusual to encounter SCLC in a never smoker. When this occurs, the diagnosis should be documented very carefully with a thorough immunohistochemical evaluation to exclude lymphoma, melanoma, carcinoid and PNET. If the diagnosis of SCLC is confirmed, one should consider the very rare possibility of a combined SCLC with an adenocarcinoma component. A growing number of case reports have documented *EGFR* mutations in these cases raising the possibility of treatment with a tyrosine kinase inhibitor.[44]

Other neuroendocrine tumors of the lung

SCLC fits into the spectrum of neurocrine tumors of the lung as a high-grade tumor along with LCNEC and as a low-grade TC and intermediate-grade AC.

LCNEC comprises ~3% of resected lung cancers and carcinoids represent 1–2% of all invasive lung malignancies with ~10% representing AC.[28] The importance of these tumors is much greater than their relative infrequency, because they often come into the differential diagnosis with the much more common SCLC. Although these tumors are frequently discussed together as in this section, carcinoid tumors are only distantly related to high-grade SCLC and LCNEC with major differences in clinical, epidemiological, pathological and molecular features.[3,12,45]

The varied biological behavior of this spectrum of NE tumors is reflected by their differences in survival. TC has a 5-year overall survival of >90% and for AC it ranges between 40 and 60%. Survival data for LCNEC are mainly from series of surgically resected cases, which have shown similar poor survival to that of SCLC.[12] However, a recent analysis of 1211 LCNEC from the SEER data suggests that LCNEC may have a more favorable overall and lung cancer-specific survival compared with SCLC in patients who received definitive surgery without radiation.[46] These data suggest that the survival for LCNEC is more like other large cell carcinomas than SCLC, which may support maintaining this tumor as a variant of large cell carcinoma.[46] Another epidemiological difference between SCLC and LCNEC and carcinoids is the strong association with heavy smoking in the former, which is lacking with TC and AC.[12,45]

Large cell neuroendocrine carcinoma

LCNEC is a high-grade non-small cell neuroendocrine carcinoma that meets the following criteria: (1) neuroendocrine morphology: organoid, palisading, trabecular or rosette-like growth patterns (Figure 9a); (2) non-small cell cytological features: large size, polygonal shape, low N/C ratio, coarse or vesicular

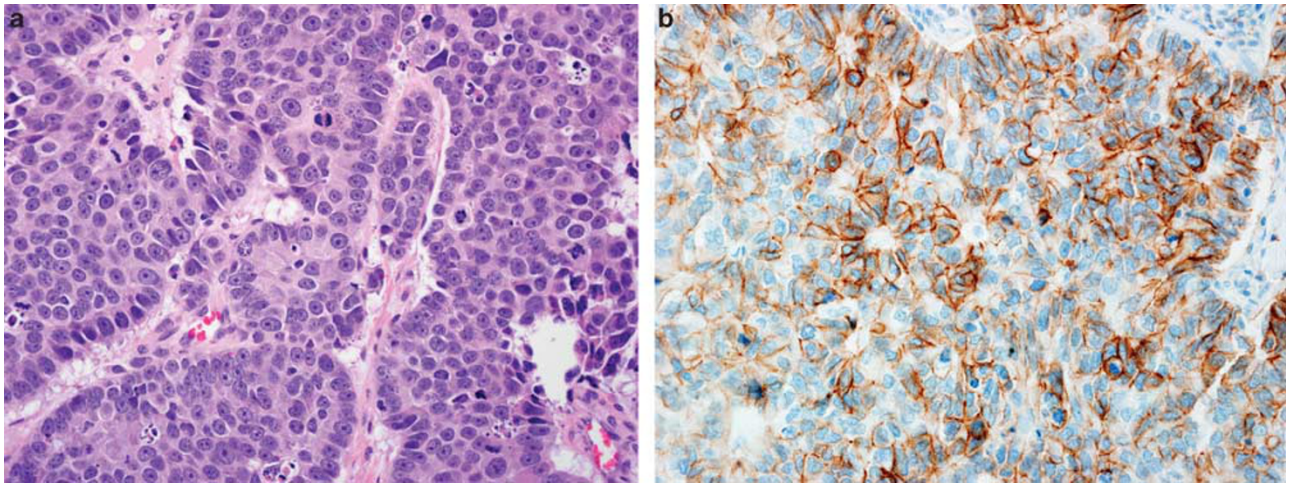

Figure 9 Large cell neuroendocrine carcinoma. (**a**) The tumor grows in organoid nests with peripheral palisading, rosette-like structures and prominent mitoses. Tumor cells have abundant cytoplasm, prominent nucleoli and an atypical mitosis. (**b**) CD56 stains many of the tumor cells.

nuclear chromatin and frequent nucleoli; (3) high mitotic rate ($\geq$11 per 2 mm^2) with a mean of 60 mitoses per 2 mm^2; (4) frequent necrosis; and (5) at least one positive neuroendocrine immunohistochemical marker or neuroendocrine granules by electron microscopy (Figure 9b).[10,31] It is very difficult to diagnose LCNEC based on small biopsy specimens such as needle or bronchoscopic biopsy specimens as it is usually very difficult to be certain of the neuroendocrine morphology without a substantial sampling of the tumor. However, criteria have been proposed to diagnose LCNEC based on cytology.[47] The term 'large cell carcinoma, with neuroendocrine morphology' can be used for tumors resembling LCNEC by light microscopy but lacking proof of neuroendocrine differentiation by electron microscopy or immunohistochemistry.[10] The term 'combined LCNEC' is appropriate for those tumors containing components of other histological types of NSCLC, such as adenocarcinoma or squamous cell carcinoma.[10] The main criteria for distinguishing SCLC from LCNEC are discussed above and summarized in Table 2.

Typical and atypical carcinoid

Both TC and AC are characterized histologically by a uniform population of tumor cells growing in an organoid pattern and having moderate eosinophilic, finely granular cytoplasm with finely granular nuclear chromatin (Figures 10a–c). A spectrum of histological patterns occur in carcinoids including spindle cell, trabecular, palisading, rosette-like, papillary, sclerosing papillary, glandular and follicular patterns.[10] Unusual cytological features can occur such as oncocytic, acinic cell-like, signet-ring, mucin-producing or melanocytic features.[31]

ACs are defined as carcinoid tumors showing mitoses between 2 and 10 per 2 mm^2 area of viable tumor (10 high power fields in certain microscopes) or the presence of necrosis (Figures 10 b and c).[48] The presence of features such as pleomorphism, vascular invasion and increased cellularity is not as helpful in separating TC from AC. In TC, necrosis is absent and mitotic figures are rare ($<$2 per 2 mm^2).[31,48] Necrosis in AC usually is manifest by punctate foci within tumor nests (Figure 10b).

Carcinoid tumors stain for neuroendocrine markers such as chromogranin, synaptophysin and CD56. Reports of TTF-1 expression are variable with one study suggesting peripheral tumors are positive more frequently than central tumors.[49]

Both typical and atypical carcinoid tumors are histologically lower-grade tumors than SCLC and LCNEC as reflected by mitotic rates and proliferation rates. Mitotic rates are high in SCLC and LCNEC, and necrosis tends to be very extensive, but in AC. the rates are low with only up to 10 mitoses per 2 mm^2 and necrosis tends to be focal. In a small crushed specimen, it may be difficult to identify mitoses. In SCLC, tumor cells also tend to have less cytoplasm than those of AC; thus the tumor appears more hyperchromatic. A low proliferation rate ($\leqslant$5%) is seen in TC by Ki-67 staining compared with AC in which it is usually between 5 and 20%.[30,37,50,51] In small crushed biopsies, Ki-67 staining can be helpful in separating TC or AC from the-high-grade LCNEC or SCLC, which have very high proliferation rates (Figure 2c).[30,50]

Ki-67 can also be useful as it will be very high in SCLC (usually $>$50–70%), but low (usually $<$5–15%) in carcinoids.[30,37,51] Precise Ki-67 thresholds for TC *vs* AC are not established.

Genetic alterations in SCLC and other pulmonary neuroendocrine tumors

Our understanding of neuroendocrine lung tumors has been enhanced by genetic studies. Unfortunately,

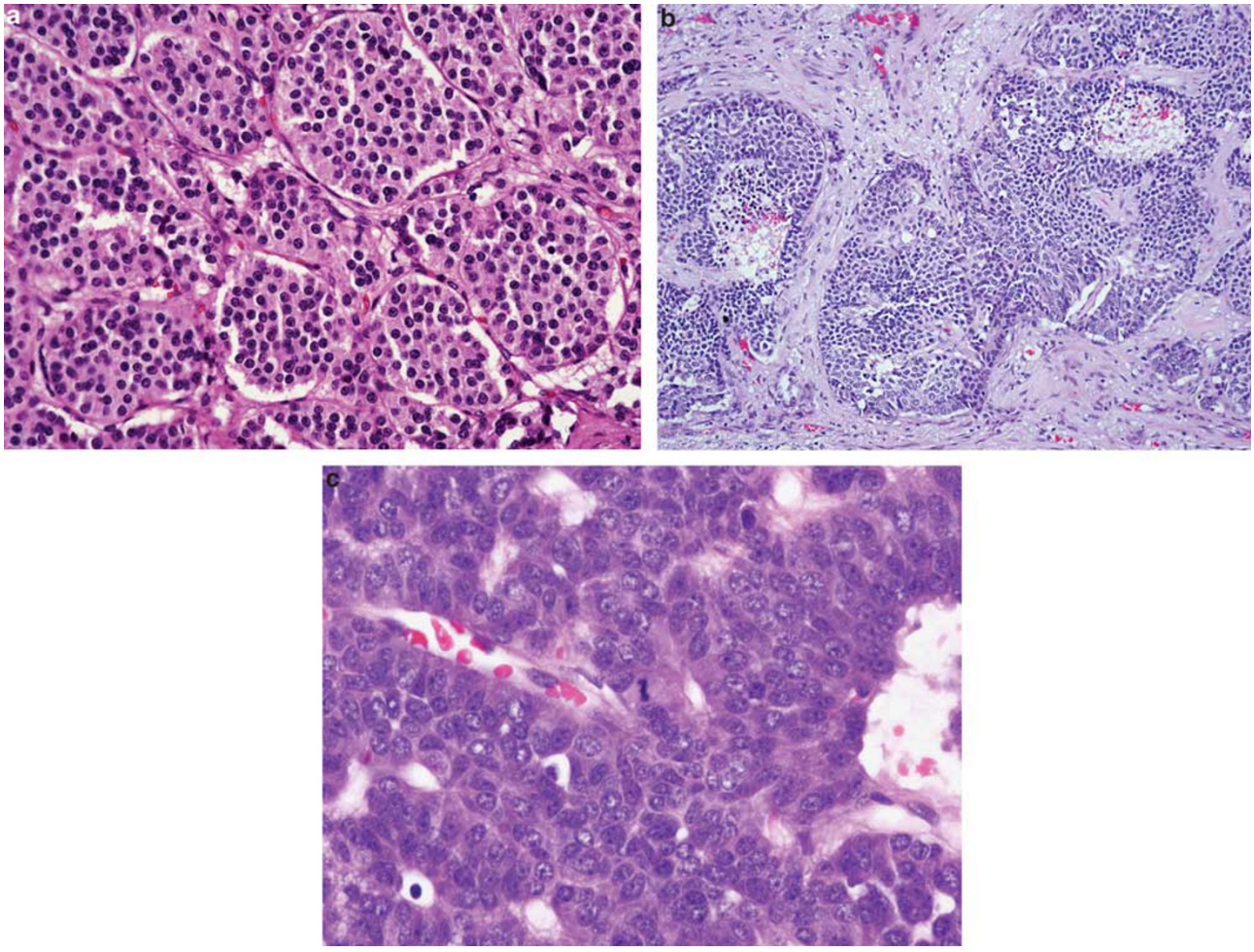

Figure 10 Carcinoid. (**a**) Typical carcinoid. This tumor shows an organoid nesting pattern of uniform cells with a moderate amount of eosinophilic cytoplasm and finely granular nuclear chromatin. (**b**) Atypical carcinoid shows a punctate focus necrosis within sheets and nests of carcinoid tumor cells. Cells have finely granular nuclear chromatin. (**c**) Atypical carcinoid shows single mitoses (center) in one tumor cell.

unlike adenocarcinoma in which molecular targets such as *EGFR* mutations have transformed therapeutic paradigms[45] and recent discoveries have revealed promising targets such as *DDR2* mutation and FRGF1 amplification for squamous cell carcinoma, no breakthroughs exist for SCLC and other pulmonary neuroendocrine tumors that have led to any effective novel therapies.[52] A high percentage of SCLC and LCNEC shows genetic changes with fewer aberrations seen in carcinoids. It is not surprising that many similar genetic abnormalities would be found in SCLC and LCNEC as they are both high-grade neuroendocrine carcinomas. However, some genetic differences have been demonstrated between LCNEC and SCLC,[23,53,54] as well as TC and AC.[54,55] These findings support the concept that these tumors should continue to be classified separately.

Recent data have shown increased insulin-like growth factor 1 receptor protein expression and gene copy number in SCLC with a significant correlation between expression and copy number.[56] Although not yet proven to be effective, IGF1R inhibitors are beginning to be tested in research trials for SCLC.[56]

SCLC and LCNEC show a high frequency of loss of heterozygosity (LOH) for 3p, RB, 5q21, 9p and p53 compared with TC and AC.[54] Significantly more frequent 5q21 LOH was found in SCLC than in LCNEC, as well as in high-grade carcinomas than in carcinoids. In addition, in the spectrum from TC to AC and high-grade SCLC and LCNEC, increasing percentages of P53 abnormalities were demonstrated by immunohistochemistry, LOH and mutation analysis.[54] No P53 mutations were found in TC with 25% in AC, 59% in LCNEC and 71% in SCLC. These data are comparable to other reports in high-grade neuroendocrine carcinomas with p53 expression ranging between 40 and 86% and P53 mutations from 27 to 59%.[50,57–61] In high-grade neuroendocrine tumors, Onuki *et al*[54] found that 58% were G:C to T:A or other transversions and that G:C to T:A transversions are associated with bulky carcinogens found in cigarette smoke. This fits with the frequent heavy cigarette smoking history in LCNEC and SCLC patients.[62] Interestingly in the few AC with mutations, these transversions were not present. This is consistent with the fact that AC patients have

significantly less smoking histories than do LCNEC and SCLC patients.[62] A single *KRAS* mutation was found in one LCNEC, which is not surprising because of the frequency of combined LCNEC and adenocarcinomas.[54]

The $P16^{INK4}$/cyclin D1/Rb pathway that is involved in the regulation of G1 arrest in the cell cycle is frequently affected in NE tumors.[55,63] In SCLC, Rb loss is frequent in SCLC and LCNEC but not in TC and it can be found in 60% of AC. In high-grade tumors, there is an inverse relationship between Rb and P16, and in all neuroendocrine tumors, there is a direct relationship between cyclin D1 and Rb, indicating that p16 and cyclin D1 act exclusively on the Rb pathway for cell-cycle regulation.[55] Igarashi *et al* demonstrated overexpression of cyclin B1 in a high percentage of LCNEC and SCLC. These data demonstrate that the RB pathway of G1 arrest is consistently compromised in SCLC and LCNEC, but is intact in TC with intermediate aberrations in AC.[55,63]

The C-kit protein expression has been found in high-grade pulmonary neuroendocrine tumors. Frequent positive membranous/cytoplasmic expression in high-grade tumors with 77/44% of LCNEC, 70/67% of SCLC was found by Pelosi *et al*[64] but it was found in only 7% of carcinoid tumors. Araki and Casali *et al*.[65,66] found C-kit staining in 55 and 61% of LCNEC, respectively. A significantly worse prognosis ($P = 0.046$) and a higher rate of recurrence (0.037) was found by Casali *et al*[66] for patients with C-kit positive LCNEC. In contrast, neither Pelosi *et al*[64] nor Araki *et al*[65] found any prognostic significance to C-kit expression with LCNEC or SCLC.

LOH at chromosome 11q13, the site of the *MEN1* gene is found in lung carcinoids from familial MEN1 patients.[67] In addition, LOH at this locus and *MEN1* gene mutations can be demonstrated in up to 36% of sporadic carcinoids, particularly AC.[68] *MEN* mutations are very rare in LCNEC and they are not found in SCLC.[68–70] In 1 of 13 LCNEC, Debelenko *et al*[69] found a somatic frameshift in the *MEN1* gene (1226delC), which represented the first mutation observed in a tumor not typically associated with MEN1. On the other allele, neither a deletion or mutation was detected, and wild-type mRNA sequence was expressed. This suggested that the typical two-hit mechanism of MEN1 gene inactivation had not taken place.[69]

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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