



Multiple faces of pulmonary large cell neuroendocrine carcinoma: update with a focus on practical approach to diagnosis

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Abstract: Pulmonary large cell neuroendocrine carcinoma (LCNEC) is a rare and aggressive malignancy that is strongly linked to smoking and notoriously difficult to diagnose and treat. Recent molecular data reveal that it represents a biologically heterogeneous group of tumors, characterized by morphologic and genomic diversity that straddles small cell and non-small cell lung carcinomas (NSCLCs), and in a minority of cases atypical carcinoids. This review provides an update on recent molecular and clinical developments in LCNEC with the main focus on practical approach to pathologic diagnosis using illustrative examples of the main differential diagnostic considerations.

Keywords: Differential diagnosis; large cell neuroendocrine carcinoma (LCNEC); molecular profile; treatment

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Introduction

Pulmonary large cell neuroendocrine carcinoma (LCNEC) represents a minority (~3%) of primary epithelial lung malignancies (1-3) that has been increasing in incidence over the last decade (4). Similar to small cell lung carcinoma (SCLC), the typical patient with LCNEC is an older man (median age of 65 years) with heavy smoking history, although most LCNEC are located in the lung periphery whereas most SCLC are central tumors (2,5). Patients with resectable early-stage disease have significant recurrence rate, generally reported as exceeding 50%; and many patients develop brain metastases (4,6). Patients presenting with distant metastases (stage IV) have very poor survival that is comparable to that of SCLC (2-5,7), although there are significant ranges of patient outcomes reported in different studies. Owing at least in part to its relatively low incidence, difficulty establishing pathologic diagnosis, and biologic heterogeneity, the optimal therapy for LCNEC is

not well-established, and has been a topic of active clinical and scientific investigation over the past decade.

World Health Organization (WHO) definition

LCNEC has been grouped with other neuroendocrine (NE) tumors of the lung in the most recent 2015 WHO classification of thoracic tumors (8). The diagnostic criteria include cytologic features of non-small cell carcinoma (prominent nucleoli and/or abundant cytoplasm, and usually large cell size), NE architecture (organoid nesting, peripheral palisading, trabecular growth pattern, and/or rosette-like structures) with immunohistochemical (IHC) staining for one or more NE markers (synaptophysin, chromogranin, CD56, and more recently INSM1), and high mitotic activity (>10 per 2 mm²) with necrosis that is often geographic (*Figure 1*) (8). Although usually >1 NE marker is expressed in LCNEC and expression of at least one marker is typically diffuse (see below), in the presence of convincing

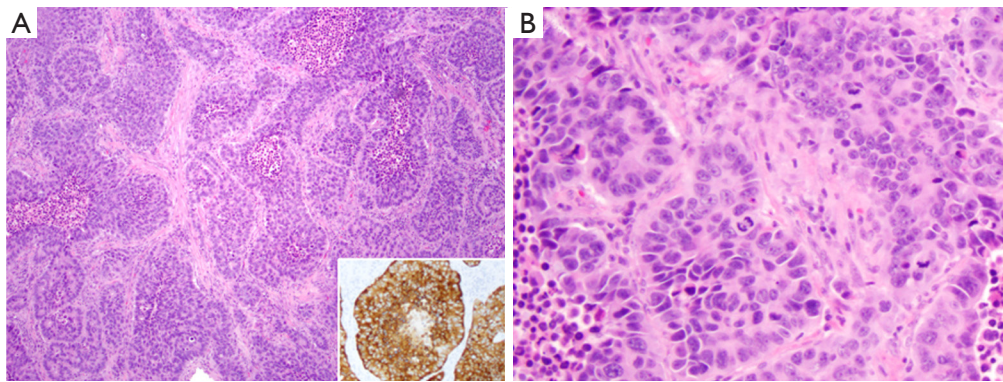


Figure 1 Classic example of LCNEC. (A) Classic morphologic features of LCNEC at scanning magnification include organoid nesting, peripheral palisading, and rosette-like structures; (B) at higher magnification high mitotic count and cytologic features of NSCLC including moderate cytoplasm and prominent nucleoli are present. Typically, strong expression of multiple NE markers is seen, as shown with synaptophysin in the inset of panel (A). Staining method: H&E (A,B), IHC (inset in A). Magnification: $\times 40$ (A); $\times 400$ (B); $\times 100$ (inset in A). LCNEC, large cell neuroendocrine carcinoma; NE, neuroendocrine; NSCLC, non-small cell lung carcinoma; IHC, immunohistochemical.

NE morphology, any extent of NE marker reactivity can be accepted as supportive of LCNEC diagnosis. Although the presence of NE granules by electron microscopy is included in the diagnostic criteria, this approach has been virtually entirely supplanted by IHC in current practice. Any amount of morphologically recognizable adenocarcinoma (ADC), squamous cell carcinoma, giant cell carcinoma or spindle cell carcinoma in combination with LCNEC is sufficient for the diagnosis of combined LCNEC with the corresponding component. Combination with SCLC should be classified as combined SCLC and must contain at least 10% large cells.

Scenarios related to the diagnosis of LCNEC include cases with either NE morphology but lack of NE marker expression or vice versa. According to the WHO criteria, the tumors in the former category are classified as NSCLC or large cell carcinoma (LCC) with NE morphology (8). Given the expanding repertoire of NE markers in recent years with markers like INSM1 (9-11), this uncommon category may become even more rare, and be replaced with a more definitive diagnosis of LCNEC. Conversely, NE marker expression can occur in $\sim 15\%$ of NSCLC that otherwise lack NE morphology (2,11-18). As a group, such tumors are referred to as “NSCLC with NE differentiation” in the thoracic WHO classification. The significance of such expression remains unknown, with some studies showing that this bears no clinical implications (13,14,19). Such tumors should be diagnosed as ADC, squamous cell carcinoma or sarcomatoid/pleomorphic carcinoma (giant

cell and/or spindle cell) based on the combination of defining morphologic features and corresponding non-NE immunomarkers, with a comment describing the positive NE markers. It is widely recommended that NE IHC should be avoided in cases with clear glandular, squamous, giant cell or spindle cell features in the absence of NE morphology (15).

Molecular update

Despite the remarkable advances in understanding of the molecular drivers of lung ADC, until recently, detailed molecular features of LCNEC have remained poorly characterized, largely owing to its relatively low prevalence and difficulty of initial diagnosis. In the last 5 years, a series of studies have been published, which utilized next-generation sequencing (NGS) to characterize the molecular landscape of LCNEC (20-24).

Briefly, the initial NGS study on LCNEC by Rekhtman *et al.* revealed that these tumors are molecularly heterogeneous and can be classified into two major subsets—small cell-like LCNEC (SC-LCNEC) and non-small cell-like LCNEC (NSC-LCNEC)—depending upon the major molecular alterations (Figure 2) (20). SC-LCNEC subset was characterized primarily by *RB1* (retinoblastoma) and *TP53* (tumor protein p53) inactivation, whereas NSC-LCNEC subset was associated with *KRAS*, *STK11* (serine/threonine kinase 11) or *KEAP1* (kelch like ECH associated protein 1) mutations alone or concurrently with *TP53*

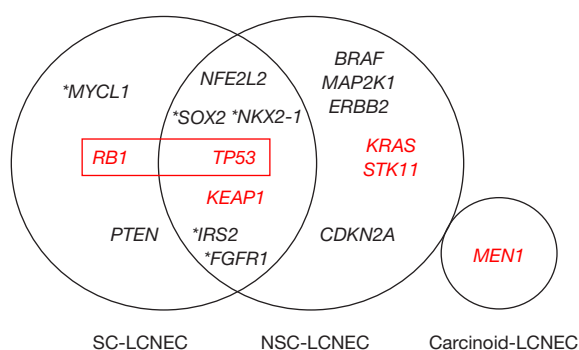


Figure 2 Molecular landscape of LCNEC subsets. Summary diagram showing the molecular heterogeneity of LCNEC with key genetic alterations defining each molecular subset. Most commonly altered genes are highlighted in red. Most alterations are mutations, and some are gene amplifications (denoted with an asterisk). *RB1* and *TP53* are consistently co-altered in SC-like LCNEC (red box). LCNEC, large cell neuroendocrine carcinoma; SC-LCNEC, small cell-like LCNEC; NSC-LCNEC, non-small cell-like LCNEC.

mutations. Additional less common molecular alterations seen almost exclusively in the SC-LCNEC included *MYCL1* amplification and *PTEN* mutations, and those seen exclusively in the NSC-LCNEC involved *BRAF*, *MAP2K1*, *ERBB2*, and *CDKN2A* genes. Furthermore, a small subset of carcinoid-like LCNECs was identified, which was characterized by *MEN1* alterations and lack of *RB1/TP53* alterations (see section on highly-proliferative carcinoids below) (20).

Subsequently, a pivotal study by George *et al.*, which combined whole-exome/genome sequencing and transcriptomic analysis, identified similar SC- and NSC-genomic subsets in LCNEC, which were designated “type II LCNEC” and “type I LCNEC”, respectively. Interestingly, at the transcriptional level, LCNEC with SC-type genomic alterations (type II) were largely distinct from conventional SCLC, whereas LCNEC with NSC-type genomic alterations (type I) exhibited transcriptional similarities to SCLC (23). These findings support that despite genomic overlap with SCLC and NSCLC, the respective subsets of LCNEC do represent distinct entities from their conventional counterparts, and that phenotypes of LCNEC are a result of a complex interplay of genomic and transcriptional programs. Overall, these molecular data provide evidence for biological heterogeneity of LCNEC, and likely reflect distinct cells of origin and pathogenesis of LCNEC subsets merging in the final common phenotypic

appearance of LCNEC.

Regarding the question of whether genomic subsets correspond to specific histopathologic features of LCNEC, the initial study suggested that indeed tumors with SC-like genomic profiles tended to have higher Ki-67 rates and a spectrum of morphologic features closer to SCLC (including greater cell crowding and overall smaller cell size) than LCNEC with NSC-like alterations (20). Conversely, low-level expression of exocrine marker Napsin A was present exclusively in a minority of LCNECs with NSC-like genomic profiles. However, both proliferation rate and morphologic features had substantial overlap between these two molecular subsets. Thus, morphology alone does not fully predict the genomic features. However, the status of *RB1* and *TP53*—the major defining alterations of the SC- versus NSC-LCNEC subsets—can be assessed by IHC, and can thus serve as a surrogate for molecular testing. In recent studies, LCNEC subsets defined by *RB1* and *TP53* alteration using molecular and IHC methods were found to show prognostic and therapeutic differences, as discussed further below (25,26). However, routine use of these markers in clinical practice awaits robust confirmation of clinical utility, including analysis incorporating a combination of genomic profiles, morphologic features, and proliferation rates with clinical outcomes.

Clinical update

Historically, data on systemic therapeutic approaches to stage IV LCNEC has been conflicting, with some studies suggesting benefits of etoposide/platinum regimens used in the treatment of SCLC, and others showing benefits of NSCLC-type therapy (6,7,27–31). More recent data have suggested improved treatment response and survival benefits in patients treated according to the molecular subtype (25,26,32). Most recently, in a study by Zhuo *et al.* (26), despite having a shorter overall survival, more patients with SC-LCNEC responded either completely or partially to the conventional SCLC chemotherapy than those with NSC-LCNEC (47% *vs.* 26%, respectively). However, even in SC-LCNEC, the response rate to platinum/etoposide was lower than historically reported for conventional SCLC (~70%). These findings corroborate prior studies, including that by Naidoo *et al.*, which also found that LCNEC response to chemotherapy was worse than that for SCLC (6). Unfortunately, the major conclusion from the above studies was that patients with advanced LCNEC have extremely poor prognosis irrespective of the type of chemotherapy

or molecular subset. In recent years, immunotherapy has become the mainstay of therapy for NSCLC (33) and was also recently approved for SCLC (34). Efficacy of immunotherapeutic agents in LCNEC awaits assessment in clinical studies, but robust responses to checkpoint inhibitors have been reported in small series (35,36).

Early-stage LCNEC is managed similarly to NSCLC in that localized stage I and II tumors are resected. Overall, the prevalence of early-stage LCNEC significantly exceeds that of SCLC, which is only exceptionally diagnosed at an early-stage. In such instances, unlike LCNEC, resection of SCLC is only recommended for stage I/II N0 disease (37). A recent large retrospective study suggested a survival benefits of surgery in patients with LCNEC up to and including clinical stage IIIA (38). Thus, accurate initial diagnosis of LCNEC *vs.* SCLC may become increasingly important for the decision on surgical management in patients with locally-advanced disease.

Differential diagnostic considerations for LCNEC: the multiple faces

Given the wide morphologic spectrum of LCNEC, a number of entities enter in the differential diagnosis with LCNEC. The main entities include SCLC, atypical carcinoid, basaloid squamous cell carcinoma (BSCC), and solid and/or cribriform ADC or LCC, the latter of which is an extremely rare diagnostic category for fully resected NSCLC lacking morphologic and IHC evidence of glandular or squamous differentiation (39,40). Another important differential diagnostic consideration includes a recently described entity of SMARCA4-deficient undifferentiated thoracic tumors (SD-UTT) with round cell/rhabdoid features. Various morphologic and IHC features of LCNEC overlap with these entities, and in practice, the diagnosis of LCNEC continues to present a challenge, even among expert thoracic pathologists (41-43). Although the most widely recognized and most commonly discussed diagnostic challenge is between LCNEC and SCLC, other aforementioned differentials are also commonly encountered in practice.

This review is aimed at addressing the practical approach to diagnostic challenges with LCNEC using illustrative examples of the main differential diagnoses. Clinical, pathologic and molecular aspects relevant to each case will be discussed.

Diagnostic challenges

LCNEC versus small cell lung carcinoma (SCLC)

Perhaps the most challenging and most widely recognized differential diagnosis of LCNEC is that of SCLC, which is evidenced by variable interobserver reproducibility for distinguishing these entities (41,43,44). The distinction relies upon a combination of morphometric features including cell shape and size, amount of cytoplasm, nuclear-to-cytoplasmic (N:C) ratio, chromatin quality and nucleolar prominence. LCNEC is characterized by larger cell size, moderate-to-large amount of cytoplasm, polygonal cell shape, lower N:C ratio, coarsely granular or vesicular chromatin, and usually prominent nucleoli (*Figure 3*). Conversely, the cells in SCLC are smaller and round to fusiform, with scant cytoplasm, very high N:C ratio, generally smooth nuclear contours, finely granular chromatin, and inconspicuous or absent nucleoli (*Figure 4*). Although objective definition of “prominent nucleoli” and “moderate to abundant cytoplasm” is lacking, we find helpful in our practice to regard nucleoli as prominent if they are visible from medium magnification with a 10× objective, and to consider cytoplasm as abundant when inter-cellular borders are readily visible.

The accuracy of the diagnosis can be further complicated by significant crush artifact seen frequently on endobronchial and transbronchial biopsies (*Figure 3*), which are commonly used for sampling of central lung tumors. The cells can appear small and the artifactual overlap between the cells can mimic nuclear molding (*Figure 3A*). These features, in the presence of NE marker immunoreactivity and a high (50–100%) Ki-67 proliferative index (*Figure 3B,C,D*), can make the distinction between LCNEC and SCLC nearly impossible. Careful examination of all parts of the biopsy on intermediate or high power to look for areas with preserved cytomorphology is critical to make the correct diagnosis. If a crushed biopsy lacks areas with well-preserved morphology, the diagnosis of high-grade NE carcinoma with a comment explaining the possibility of both entities is most appropriate.

Typically, small cell carcinomas are composed of infiltrating sheets of small blue cells with broad areas of necrosis and prominent apoptotic activity. However, nested architecture may also be seen (*Figure 4A*), as can trabecular growth, peripheral palisading and rosette-like structures (45). These features are usually best appreciated on resections—a rare specimen type for SCLC given that

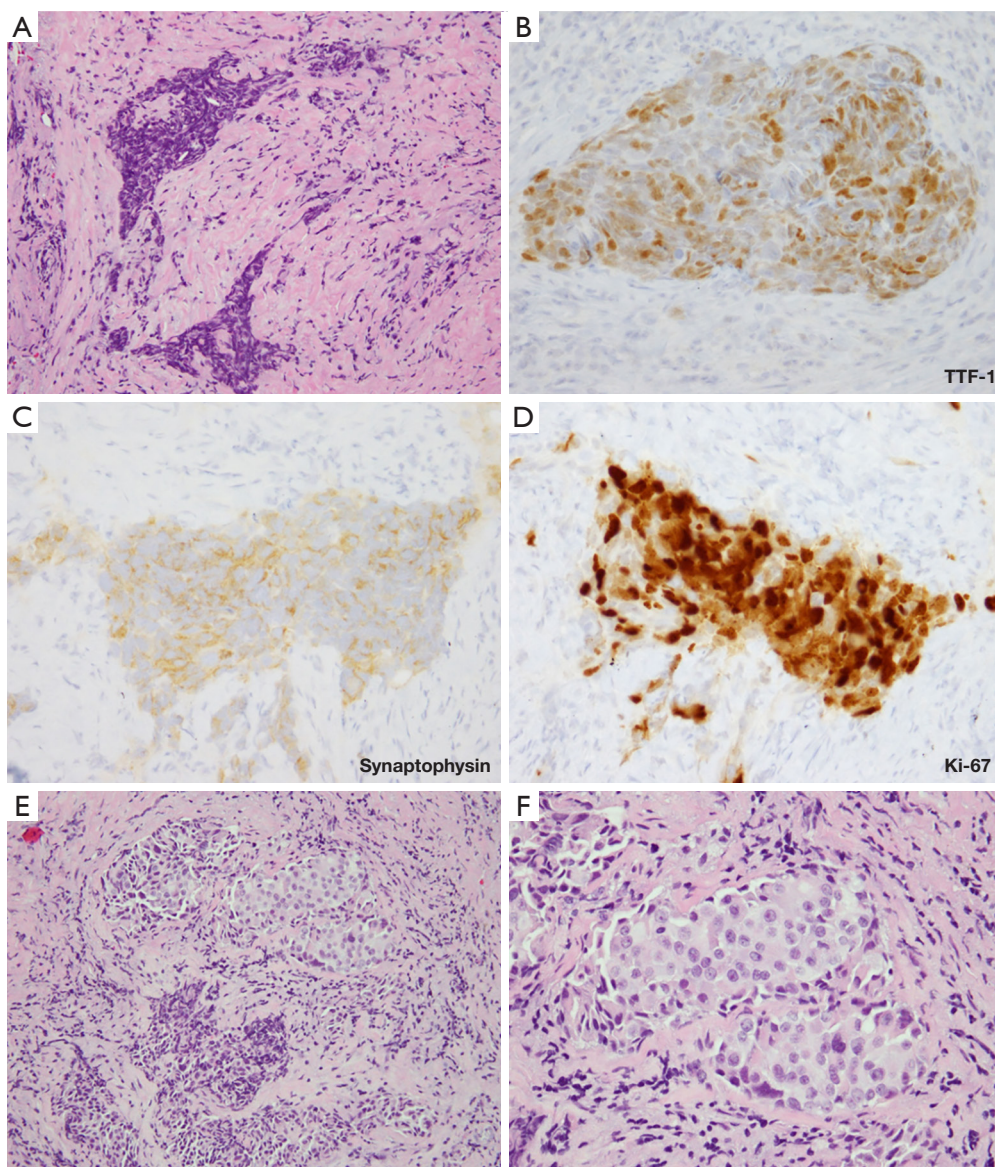


Figure 3 Distinguishing LCNEC from SCLC on a crushed biopsy. On crushed biopsies tumor cells can appear small, presence of nucleoli and abundant cytoplasm may be obscured, and nuclear molding can be exaggerated (A); both LCNEC and SCLC are typically positive for TTF-1 (B) and NE markers (C, synaptophysin) by IHC, and both are characterized by very high Ki-67 proliferative indices (D), which can lead to a major challenge in distinguishing these tumors. Focal area of the tumor with preserved cytology shows the presence of ample cytoplasm at scanning power (E); on higher power, NSCLC cytomorphology with prominent nucleoli, open chromatin and large amount of cytoplasm (F) support the diagnosis of LCNEC. Staining method: H&E (A,E,F); IHC (B,C,D). Magnification: $\times 40$ (A), $\times 400$ (B,C,D,F), $\times 100$ (E). LCNEC, large cell neuroendocrine carcinoma; SCLC, small cell lung carcinoma; NE, neuroendocrine; IHC, immunohistochemical; NSCLC, non-small cell lung carcinoma.

these tumors usually present at advanced, unresectable stage. The presence of at least focal nested architecture has been reported in 94% of resected SCLC (46), yet given the rarity of such specimens pathologists may not

be familiar with the potential of SCLC to exhibit nested/organoid architecture. This can lead to mis-interpretation of SCLC with nested pattern as LCNEC. Furthermore, although typically described as less than the diameter of

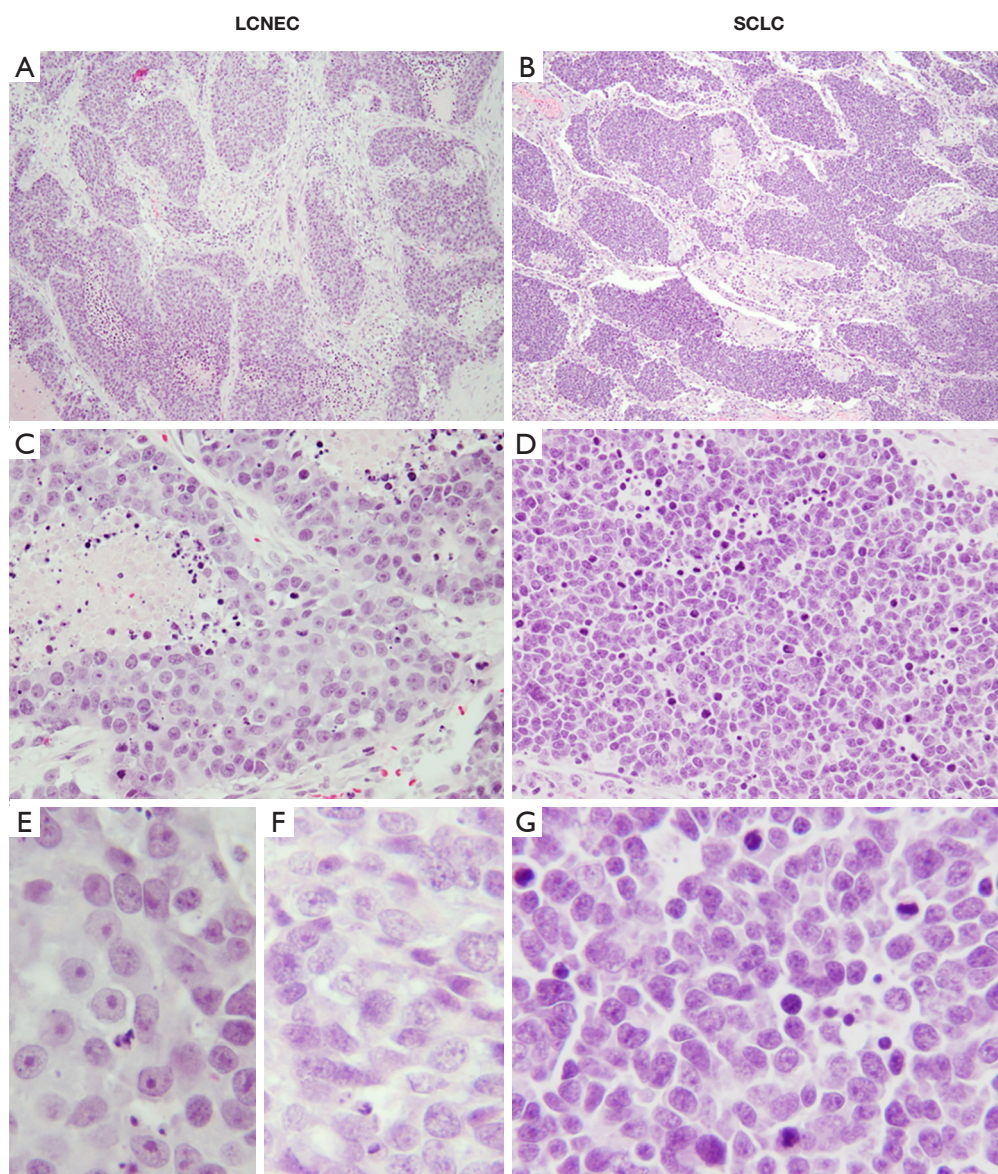


Figure 4 LCNEC versus SCLC in a resection specimen. Similar to LCNEC, SCLC can have nested/organoid architecture (A,B, respectively); cytologic evaluation is the key to making the distinction: while LCNEC has moderate to abundant amount of cytoplasm, and in most cases prominent nucleoli (C,E), SCLC generally has densely packed cells with scant cytoplasm, finely granular and uniform chromatin, and few inconspicuous or absent nucleoli (D,G). However, some cases of LCNEC may show a spectrum of cytomorphic features with the range of nucleolar size and amount of cytoplasm, such that some areas may be difficult to distinguish from SCLC due to more packed cell arrangement, less conspicuous nucleoli and more scant cytoplasm (F). If any areas have convincing morphology of SCLC, such tumors should be classified as combined SCLC/LCNEC. Staining method: H&E (A,B,C,D,E,F,G). Magnification: $\times 40$ (A,B), $\times 400$ (C,D), $\times 1,000$ (E,F,G). LCNEC, large cell neuroendocrine carcinoma; SCLC, small cell lung carcinoma.

three lymphocytes, SCLC cells can display a range of sizes measuring up to the size of LCNEC (45,47-49). Fusiform cell shape and venous incrustation with basophilic nuclear material (Azzopardi phenomenon) are more characteristic

of SCLC, but the diagnosis ultimately rests upon the three main cytologic features: amount of cytoplasm, nucleolar prominence, and chromatin pattern (Figures 3E,F, 4B,C,D,E,F,G). If any amount of tumor has classical small

cell carcinoma cytologic features in the background of LCNEC, the diagnosis of combined SCLC with LCNEC is recommended by the WHO (8,50). It is important to acknowledge that there is a morphologic spectrum in both SCLC and LCNEC, and some cases are difficult to distinguish, though it is suggested that this should apply to approximately 5% of well-preserved specimens (51).

Immunohistochemistry has only a limited role in distinguishing LCNEC and SCLC. One potentially helpful but low-sensitivity feature is focal and weak Napsin A immunoreactivity, which can be seen in up to 15% of LCNEC (52,53), whereas SCLC is consistently negative for this marker. Thus, when present, weak Napsin A labeling in a high-grade NE carcinoma may favor the diagnosis of LCNEC. Additionally, diffuse membranous cytokeratin immunoreactivity has been suggested to favor the diagnosis of LCNEC since SCLC classically has weak dot-like keratin reactivity. However, the dot-like and/or weak staining pattern can also be seen in LCNEC (44,54). Thus, this feature is not widely used in practice.

Genomic overlap between LCNEC and SCLC (*Figure 2*) (20–24) makes the distinction between these entities possible by molecular studies only in cases with NSCLC-like mutational profiles (i.e., NSC-LCNEC subset described above). LCNECs with a SC-like molecular profile (SC-LCNEC) are genomically indistinguishable from SCLC. Overall, potential utility of genomic studies for the distinction between SCLC and LCNEC requires further analysis.

In summary, there is currently no entirely sensitive and specific IHC or molecular marker to separate LCNEC and SCLC, and this remains a distinction that relies entirely on morphologic criteria. Development of objective biomarkers specific for SCLC *vs.* LCNEC would allow greater diagnostic reproducibility. Additionally, development of predictive biomarkers of response to specific systemic therapies—potentially irrespective of LCNEC *vs.* SCLC diagnosis—would also allow objective criteria for guiding treatment decisions. For example, SLFN11 has recently emerged as a promising marker of sensitivity to cytotoxic agents in SCLC (55–57). Its utility in LCNEC and validation of the utility in clinical practice requires further studies.

LCNEC versus adenocarcinoma (ADC) or large cell carcinoma (LCC)

Since the diagnosis of LCNEC by definition requires the

presence of non-small cell carcinoma cytologic features, differentiating solid and/or cribriform ADC or LCC from LCNEC relies on the presence of classical NE architecture and confirmation of this morphologic impression by expression of NE markers. However, architectural features can occasionally be equivocal for LCNEC versus solid/cribriform ADC or LCC with nested organoid-like pattern. Furthermore, as mentioned above, ~15% of ADC/LCC have NE marker immunoreactivity in the absence of NE morphology (13,14,17–19). Thus, distinction of these entities can cause a diagnostic dilemma, particularly in small biopsies or cytology samples.

A helpful consideration for this differential diagnosis is the extent and number of positive NE markers, which is generally significantly different between LCNEC and ADC/LCC with NE marker expression. Most LCNEC show expression of two to three standard NE markers (synaptophysin, chromogranin, CD56), and expression of at least one NE marker is typically diffuse (3,20,53,58). However, ~20% of LCNEC are reported to label for only one NE marker, with some cases showing only focal immunoreactivity for a single marker (53,58). Conversely, NE marker expression in ADC/LCC is usually focal and typically limited to only one marker (11–14, 16,19,53,58,59); however, expression of two NE markers has been reported in 1–4% of ADC (16,19). Thus, while diffuse NE marker immunoreactivity and/or expression of ≥ 2 markers is substantially more common in LCNEC, lower levels of NE marker expression overlap with ADC/LCC. In such cases, the diagnosis hinges on the impression of NE morphology.

A helpful morphologic feature for distinguishing LCNEC with prominent rosette-like structures from cribriform ADC is the shape of the luminal spaces (*Figure 5*). While the rosette-like structures of LCNEC are characterized by punched out lumens (resembling breast DCIS) (*Figure 5A*), the cribriform spaces in ADC are often more collapsed (resembling usual ductal hyperplasia) (*Figure 5B*). Overall, ADCs tend to have more voluminous eosinophilic cytoplasm, whereas the cytoplasm in LCNEC tends to be more scant and commonly has amphophilic quality.

Similar to the differential diagnosis with SCLC, there is no single defining IHC marker for distinguishing LCNEC from ADC/LCC with NE marker expression. However, several IHC features can provide supporting evidence in favor of one versus the other diagnosis, in addition to the extent and number of expressed NE markers. Namely, as mentioned above, while focal and weak Napsin A

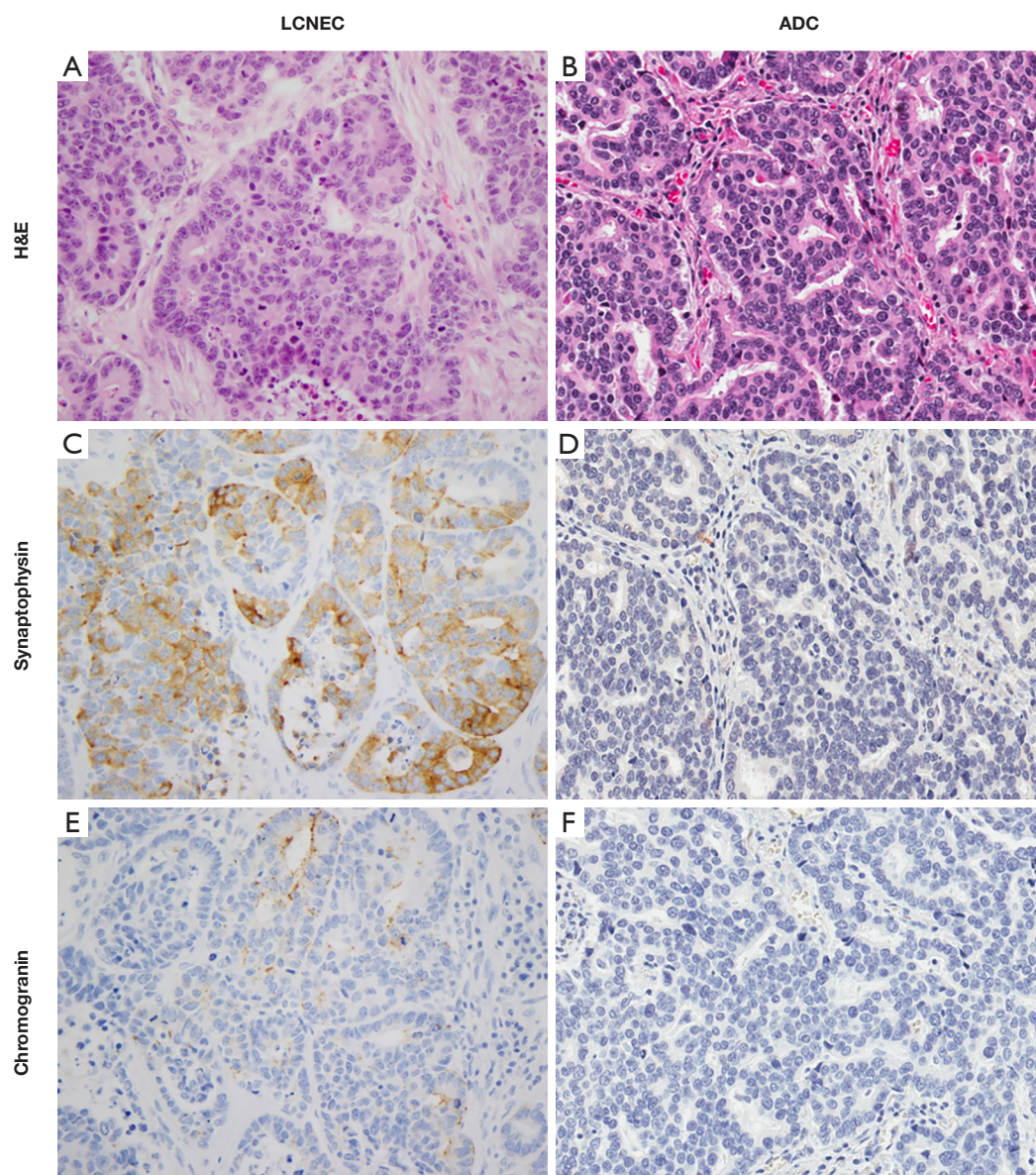


Figure 5 Classical LCNEC versus cribriform ADC. Punched out lumens of rosette-like structures in LCNEC (A) compared with predominantly slit-like spaces of cribriform ADC (B); NE markers are positive in LCNEC (C,E), whereas they are generally negative in cribriform/solid ADC (D,F). However, NE marker expression can be focal in LCNEC, and these markers can be expressed in ADC, causing diagnostic challenges in some cases and emphasizing the importance of interpreting IHC in the context of morphology, especially for cases with focal expression of a single NE marker. See text for details. Staining method: H&E (A,B); IHC (C,D,E,F). Magnification: $\times 200$ (A,B,C,D,E,F). LCNEC, large cell neuroendocrine carcinoma; NE, neuroendocrine; IHC, immunohistochemical; ADC, adenocarcinoma.

immunoreactivity can be seen in up to 15% of LCNEC (52,53), diffuse and strong expression of this marker is unusual for this diagnosis and favors the diagnosis of ADC. Similarly, relative amounts of TTF-1 to Napsin A expression can be used as a supportive feature: strong and diffuse

TTF-1 immunoreactivity in conjunction with negative or very focal/weak staining with Napsin A is a soft feature for LCNEC since most ADCs with strong/diffuse expression of TTF-1 also have strong expression of Napsin A, although there are exceptions (52,53). Another relatively helpful

but neither fully sensitive nor specific feature is the Ki-67 proliferative index. While very high Ki-67 (80–100%) is common in LCNEC, this level of proliferative activity is unusual for ADC/LCC (8,53,60). It is important to note that while these additional IHC features can serve as supporting observations, they are not diagnostic in isolation. Finally, genomic characteristics are insufficient to distinguish LCNEC (specifically NSC-like subset) from ADC/LCC.

It has been suggested that ADC/LCC with NE marker expression may exist in a biologic continuum with NSC-LCNEC, ranging from cases having only weak NE marker expression in the absence of NE morphology to those exhibiting a full set of morphologic and marker expression characteristics of LCNEC in a genomic background of ADC. Diagnostic challenges in a minority of cases may thus reflect this biological continuum (52).

The distinction of LCNEC from solid/cirriiform ADC or LCC with NE marker expression is particularly challenging in small biopsies. It is well recognized that the approach to this differential diagnosis is an integrative one, requiring consideration of all the aforementioned factors. The diagnosis of LCNEC on biopsies has been considered controversial given the difficulty in appreciating NE morphology in high-grade carcinoma in small or fragmented tissue. However, in recent years it has become standard to obtain larger amounts of tissue for thoracic biopsies given increased tissue demands for molecular studies. This allows greater ability to discern NE morphology, and suggest the diagnosis of LCNEC. In a recent study by Baine *et al.*, NE morphologic features were evident in 85% of 1 mm tissue cores from LCNEC (53). In that study, the proposed integrative semiquantitative scoring criteria combining NE morphology (peripheral palisading, rosette-like structures, organoid nesting, and extensive necrosis), NE marker expression and Ki-67 proliferative index was highly sensitive and specific for distinguishing LCNEC from ADC/LCC. Previously, Derks *et al.* had suggested that the presence of ≥ 2 positive NE markers in NSCLC is by itself sufficient to make the diagnosis of LCNEC in a biopsy specimen lacking glandular or squamous differentiation even in the absence of NE morphology (58). However, this method may miss a subset of LCNEC with focal and/or single NE marker immunoreactivity, which can occur in up to 20% of cases (53). Furthermore, expression of ≥ 2 NE markers, particularly at a low level, can be encountered in a minority of ADC/LCC cases (16,19). Therefore, in our practice we

currently limit the use of NE markers in well-preserved specimens to tumors with at least some suggestion of NE morphology. We note that even in the absence of definitive evidence of palisading or rosettes, LCNEC may be suspected in biopsies of high-grade malignant tumors with even subtle nested architecture, cytoplasmic amphophilia and/or stippled chromatin.

In summary, differentiation of LCNEC from ADC/LCC requires a combination of NE morphology and marker expression. In cases where the morphology is equivocal, extent of NE marker reactivity, very high Ki-67 and negative or low Napsin A expression relative to high TTF-1 reactivity can serve as supporting (but not in isolation diagnostic) evidence for LCNEC over ADC/LCC.

LCNEC versus basaloid squamous cell carcinoma (BSCC)

Nested growth pattern with peripheral palisading, high N:C ratios imparting a “blue look” from low power, and large zones of necrosis are the morphologic features shared between LCNEC and BSCC (Figure 6A,B,C,D). Furthermore, some BSCC show prominent rosette-like structures similar to those seen in LCNEC (Figure 6D,E,F). Presence of focal keratinization is diagnostic of BSCC even without confirmatory IHC, but keratinization may be entirely absent in a subset of BSCC. Other distinctive morphologic features are the presence of hyaline stroma and basement membrane-like material in BSCC (Figure 6D,F). Unlike the lack of distinguishing IHC markers for the differential diagnosis of LCNEC with SCLC and the potential overlap between LCNEC and ADC/LCC with NE differentiation, there are reliable and objective markers for the distinction between BSCC and LCNEC. Namely, BSCC consistently express squamous marker p40 (Figure 6G,H), whereas diffuse expression of this marker is not expected in LCNEC. However, focal p40 staining in scattered cells does occur in some LCNEC (20). Although p63 and CK5/6 are highly sensitive squamous markers, they are much less specific than p40, and CK5/6 is also less sensitive; thus, they provide limited to no additional information if p40 is available (61,62). Notably, a potential pitfall in this differential diagnosis is NE marker expression (especially CD56) in BSCC, which has been reported in a subset of these tumors (63–65). However, the diagnosis in this setting can be readily clarified by diffuse expression of p40 and attention to the aforementioned distinctive morphologic characteristics.

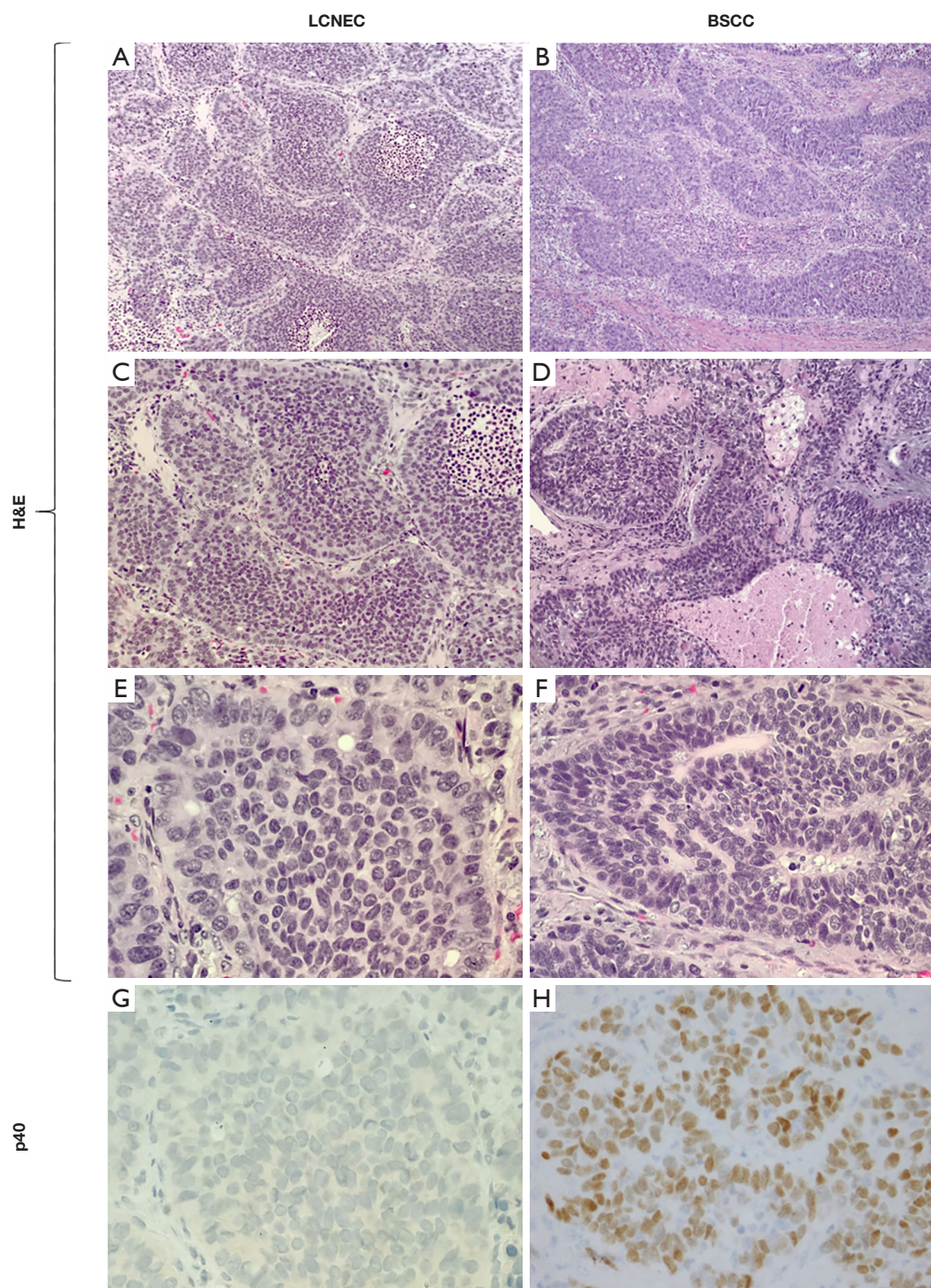


Figure 6 LCNEC versus BSCC. BSCC is a common morphologic mimic of LCNEC, as both tumors have nested architecture and high N:C ratios imparting a “blue” look from low power, peripheral palisading, and extensive necrosis (A,B); notably, some BSCCs have rosette-like structures (D,F) resembling those in LCNEC (C,E); Prominent hyaline stroma and basement membrane material is present in some BSCC (D,F) and can serve as a helpful hint to the correct diagnosis; while LCNEC is negative for p40 by IHC (G), BSCC is strongly and diffusely positive (H), which even in the absence of distinguishing morphologic features resolves this differential diagnosis. Staining method: IHC (A,B,C,D,E,F); H&E (G,H). Magnification: $\times 40$ (A,B), $\times 100$ (C,D), $\times 400$ (E,F,G,H). LCNEC, large cell neuroendocrine carcinoma; BSCC, basaloid squamous cell carcinoma; IHC, immunohistochemical.

LCNEC versus atypical carcinoid

Carcinoid tumors have sharply distinct epidemiology and molecular pathogenesis from LCNEC (and SCLC). Compared to LCNEC/SCLC, they lack strong association with smoking, tend to arise in younger patients, and have significantly more indolent behavior even in stage IV setting. Molecularly, these tumors have low tumor mutation burden and commonly harbor *MEN1* mutations, but lack *RB1* or *TP53* alterations (66). However, a recent study suggested that a small subset of atypical carcinoids has gene expression and methylation profiles resembling LCNEC (so-called “supra-carcinoids”) (67). However, pathological and clinical characteristics of such tumors remain to be defined.

By the 2015 WHO criteria, the key distinguishing feature between LCNEC and atypical carcinoids is the mitotic rate of >10 per 2 mm^2 . However, this distinction also implies characteristic cytomorphologic criteria (Figures 7,8). The prototypical LCNEC shows nuclear pleomorphism, large cell size, nuclear membrane irregularities, coarse or vesicular chromatin, and prominent nucleoli (Figure 7C). In contrast, typical cytologic features of carcinoid tumors include cellular uniformity and monotony, relatively small cell size, smooth nuclear borders, salt-and-pepper chromatin and lack of prominent nucleoli (Figure 7D). It is important to note that similar to other endocrine tumors, marked cytologic atypia may occur in carcinoid tumors, typical and atypical alike, but it is usually focal/random and seen in the background of the more conventional monotonous cytomorphology. While small nucleoli can be present in atypical carcinoids, they should not be a conspicuous feature. Conversely, although some LCNECs may have nuclear monotony, they typically have more marked nuclear membrane irregularities/convolution and more prominent nucleoli.

Necrosis is another distinctive feature, in that it is characteristically punctate/comedo-like in atypical carcinoids (Figure 7D) versus geographic in LCNEC. However, necrosis in some LCNECs may also be limited to punctate areas. Thus, the diagnosis of LCNEC should not be excluded in the absence of broad areas of necrosis.

In contrast, NE architecture is a shared feature between LCNEC and carcinoid tumors. In fact, the concept of NE architecture in LCNEC is adopted from the type of morphologic patterns that can be encountered in carcinoids and well-differentiated neuroendocrine tumors (WD-NETs) of other organs. Both tumors are typically nested

(Figures 7A,B,8B,C) and have prominent rosette-like structures (Figures 5A,8D), trabecular growth pattern, and peripheral palisading.

In most cases of resected primary tumors, the distinction between LCNEC and atypical carcinoids is evident based on the aforementioned cytologic features and the nature of necrosis. Generally, morphologic impression of LCNEC is accompanied by mitotic counts significantly exceeding the threshold of 10 mitoses per 2 mm^2 , whereas the converse is true for atypical carcinoids. Although Ki-67 is not formally included in the 2015 WHO classification of lung NE tumors, it is commonly used in the interpretation of biopsies and cytology specimens (53,69-75), where mitotic counts are more difficult to assess than Ki-67 rate.

Although there is typically a sharp separation of both mitotic and Ki-67 rates in LCNEC versus atypical carcinoids, in recent literature the issue of proliferative overlap among these tumors has started to emerge (76-79). Thus, while most LCNEC have 60–80 mitoses per 2 mm^2 , tumors with intermediate mitotic rates do occur, including those approaching 20 per 2 mm^2 . For Ki-67, LCNEC shows rates that are typically $>50\%$ (Figure 7E), but some cases have intermediate rates in the 20–50% range. Conversely, while the majority of atypical carcinoids have mitotic counts below 10 per 2 mm^2 and Ki-67 of $<20\%$ (usually $<10\%$; Figure 7F), recently data is emerging on the expanded proliferative spectrum in lung carcinoids, particularly in the metastatic setting. More specifically, recent studies have described tumors with morphologic features of carcinoid tumors, but mitotic counts exceeding the atypical carcinoid threshold of 10 per 2 mm^2 and/or surpassing the typical Ki-67 rates of under 20%. However, in most such cases mitotic and Ki-67 rates did not exceed 20 per 2 mm^2 and 50%, respectively. Furthermore, unlike LCNEC (and SCLC), which typically show uniformly high mitotic counts and Ki-67 (Figure 7E), these tumors characteristically have marked intratumor proliferative heterogeneity, featuring proliferative hotspots and other areas of low proliferation rates (Figure 8E). While such tumors are rare among primary carcinoids, a recent study in stage IV lung carcinoids found that proliferative progression with escalation of proliferation rates above the standard thresholds is seen in $\sim 25\%$ of metastatic samples (68).

Although the current WHO classification regards tumors with morphologic features of atypical carcinoids but mitotic counts >10 per 2 mm^2 as LCNEC, recent studies suggest that their clinicopathologic and molecular characteristics

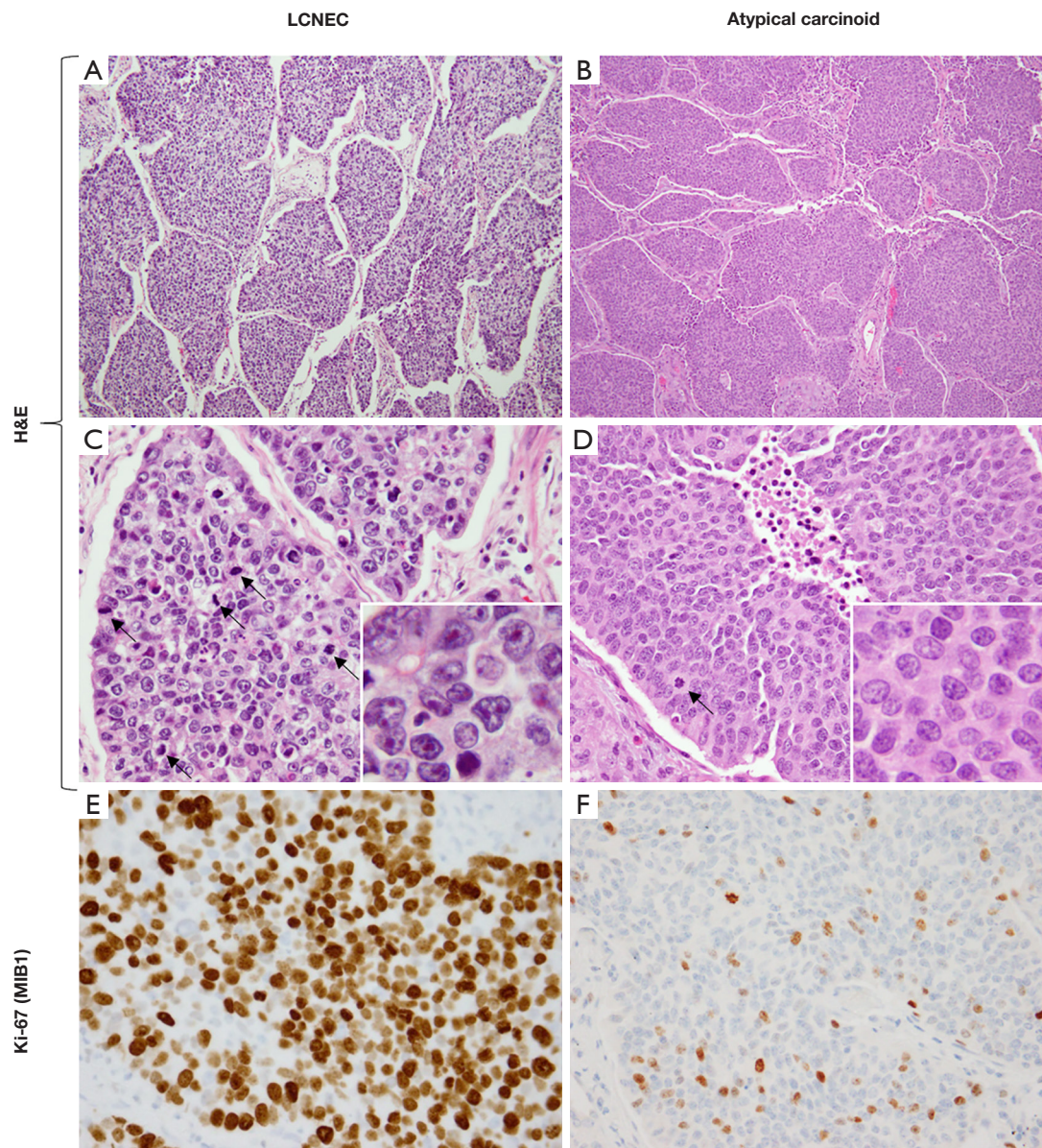


Figure 7 LCNEC versus atypical carcinoid. Organoid nesting is a shared morphologic feature of LCNEC and atypical carcinoids (A,B, respectively); cytologically, LCNEC cells are large with variation in nuclear size, irregular nuclear contours, and prominent nucleoli (C), whereas atypical carcinoid cells are smaller in size and more uniform, with smooth nuclear borders, characteristic salt-and-pepper chromatin, and lack of prominent nucleoli (D); mitotic figures (arrows) in LCNECs are >10 per mm^2 (typically exceeding 20 per mm^2) (C), while those in atypical carcinoids are by definition below this threshold (D) with evidence for rare exceptions recently emerging (see text). When present, necrosis in atypical carcinoids is either single cell or punctate (D); Ki-67 proliferation rate is generally high (>50 – 100%) in LCNEC (E), while that of atypical carcinoids is generally, but not always, $<20\%$; see text (F). Staining method: H&E (A,B,C,D), IHC (E,F). Magnification: $\times 40$ (A,B), $\times 400$ (C,D,E,F), $\times 1,000$ (insets in C and D). LCNEC, large cell neuroendocrine carcinoma.

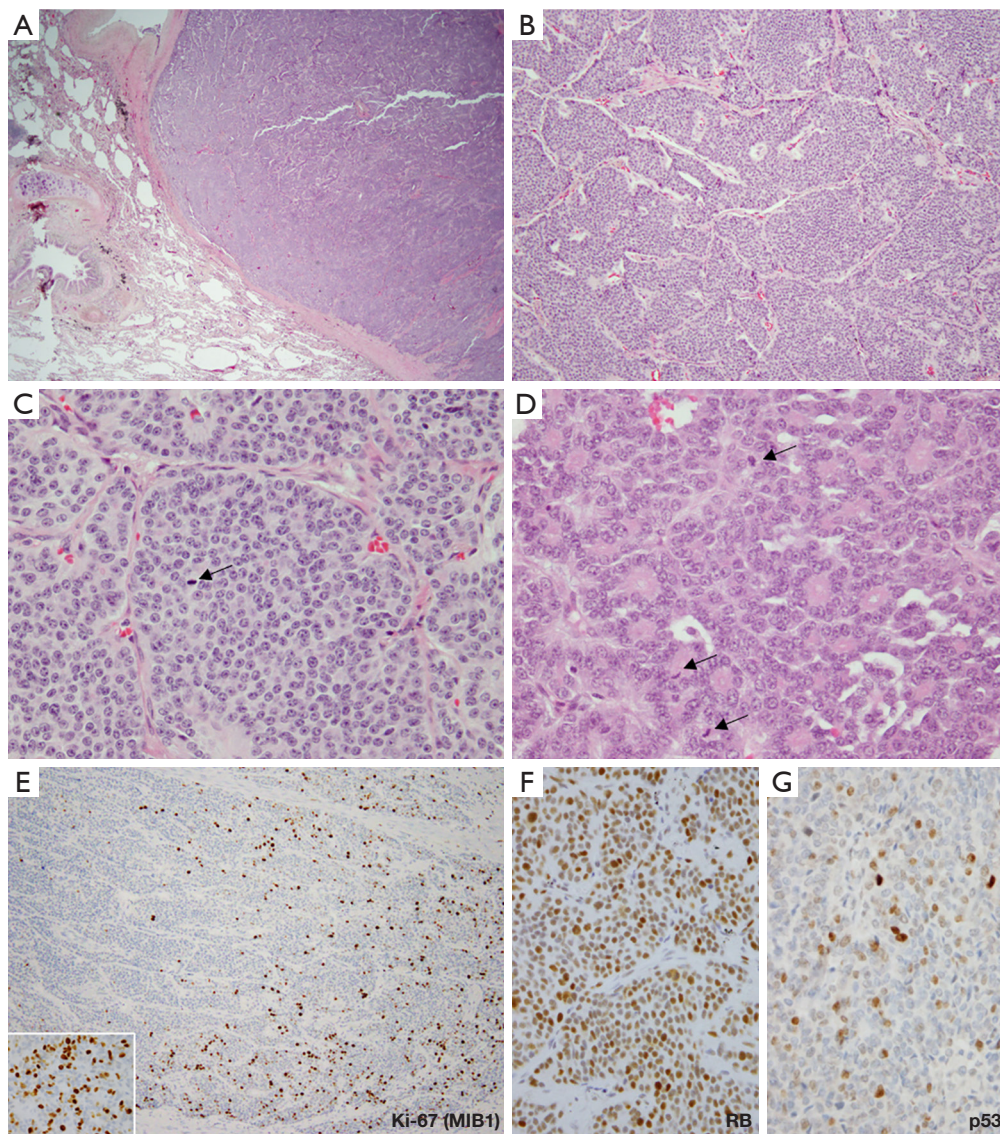


Figure 8 Example of a highly proliferative atypical carcinoid. Illustrated is a rare example of a primary lung tumor exhibiting prototypical morphologic features of carcinoid tumor but proliferation rate exceeding the current WHO ceiling rate of 10 mitoses per 2 mm². Pathologic features linking this tumor to carcinoids rather than LCNEC include lack of geographic necrosis and bland uniform cytomorphology, showing regular nuclear contours, salt and pepper chromatin, and lack of nucleoli (C,D). The tumor is well-circumscribed and peribronchial at low-power (A), and has organoid architecture (B,C) and rosettes (D). Despite the typical morphologic features of carcinoid tumor, mitotic counts (arrows) substantially exceed 10 per 2 mm². Ki-67 shows heterogeneous distribution (E) ranging from <5% up to 40% (inset) in hot-spots. This tumor was included in Rekhtman *et al.* series (68) (case ID LCNEC-36), and was found to have a *MEN1* mutation and lack of *RB1/TP53* alterations, as supported by nuclear retention of RB (F) and wild-type pattern of p53 staining (G) by IHC. Although meeting the WHO 2015 mitotic criteria for LCNEC, approach to such rare tumors awaits a clarification in the upcoming WHO edition. Staining method: H&E (A,B,C,D), IHC (E,F,G). Magnification: ×20 (A,E), ×400 (C,D), ×100 (inset in E), ×200 (F,G). LCNEC, large cell neuroendocrine carcinoma; WHO, World Health Organization; IHC, immunohistochemical.

are akin to carcinoids rather than NE carcinomas (68,76-79). These concepts are analogous to the recent category of WD-NET, grade 3 in the enteropancreatic system, where such tumors were also previously classified as NE carcinomas. The approach to these tumors awaits further studies and the updated thoracic WHO classification.

Presently, for diagnostic purposes, it is important to be aware of these recent data on the expanded proliferation spectrum in carcinoid tumors and potential mitotic and Ki-67 overlap with LCNEC, especially in the metastatic setting. This particularly applies to tumors with intermediate proliferation rates (10–20 mitoses per 2 mm² and/or with Ki-67 of 20–50%). Interpretation of biopsies falling into this “gray-zone” can be challenging. Similar to the pancreatic WD-NET, grade 3, the assessment of RB and p53 by IHC (*Figure 8F,G*) has been suggested to be of potential utility (80,81), since these genes are frequently mutated in LCNEC but they consistently show wild-type pattern in carcinoid tumors (68); however, this requires further validation. Conversely, while IHC for DAXX (death-domain associated protein) and ATRX (alpha thalassemia mental retardation syndrome X-linked) has been used to distinguish pancreatic WD-NET and NEC since ~40% of pancreatic WD-NETs harbor inactivating mutations/loss of expression of these markers (81,82), alterations in these genes are only rarely seen in pulmonary carcinoid tumors (66,67). The utility of comprehensive genomic analysis for distinguishing highly-proliferative carcinoids and LCNEC in biopsies (and rarely resected tumors) falling in the proliferative gray-zone will be of interest to explore in future studies.

LCNEC versus SMARCA4-deficient undifferentiated thoracic tumor (SD-UTT)

A new category of thoracic tumors has been recently described, which was initially designated as SMARCA4 (BRG1)-deficient thoracic sarcoma (83-85). Subsequent studies suggested that these tumors represent undifferentiated/dedifferentiated carcinomas (85,86). Histologically, these tumors are undifferentiated, discohesive and show high-grade round cell to rhabdoid morphology. Notably, these tumors commonly exhibit synaptophysin immunoreactivity (~70% of cases) (86). Morphologically, SD-UTT do not show NE architecture. However, they frequently show geographic necrosis and extremely high mitotic and Ki-67

rates, reflecting their highly aggressive nature. Thus, in a crushed biopsy, these features may closely mimic NE carcinomas. Clinical presentation can also be similar as these tumors present as large typically central masses in heavy smokers, although SD-UTT patients are frequently (but not always) younger than the patients with LCNEC or SCLC. *Figure 9* illustrates one such case, in which initial diagnostic consideration was LCNEC. In addition to the loss of immunoreactivity for SMARCA4 (*Figure 9E*), a set of other IHC markers can be helpful in the diagnosis of SD-UTT and distinguishing these tumors from NE carcinomas. SD-UTT are characterized by the loss of epithelial differentiation, which can be demonstrated by the lack of claudin-4 (epithelial adhesion molecule) and low or absent keratin expression. Furthermore, SMARCA4 deficiency in SD-UTT is typically accompanied with co-deficiency for another SWI/SNF component SMARCA2 (BRM) and frequent expression of stem-cell markers (SALL4, CD34, and SOX2) (85,86).

Other entities in the differential diagnosis of LCNEC

In addition to the major entities discussed above, there is a number of other diagnostic considerations for tumors that enter in the differential diagnosis with LCNEC based on morphologic features and/or overlapping marker expression. In particular, metastases from the breast ductal carcinoma and prostate ADC may enter into consideration, as both of these tumor types can show prominent “endocrine” morphology (relatively monomorphic cytology and nested architecture). In addition, particularly in younger patients and/or never smokers, other rare monomorphic high-grade tumors with high N:C ratio can be encountered in the thoracic cavity and may mimic LCNEC, including NUT carcinoma, EBV-associated carcinoma [which may lack prominent lymphoid infiltrate typical of classic lymphoepithelioma-like carcinoma (87)], and round cell sarcomas (*CIC*-rearranged and even Ewing, which typically exhibits synaptophysin expression) among others. Thus, broader differential diagnosis and IHC workup may be needed in the context of clinicoradiologic findings.

Conclusions

The diagnosis of pulmonary LCNEC can present a major challenge owing to its morphologic and genomic heterogeneity, which overlaps with small cell and non-small cell lung carcinomas, and other entities. Although in recent

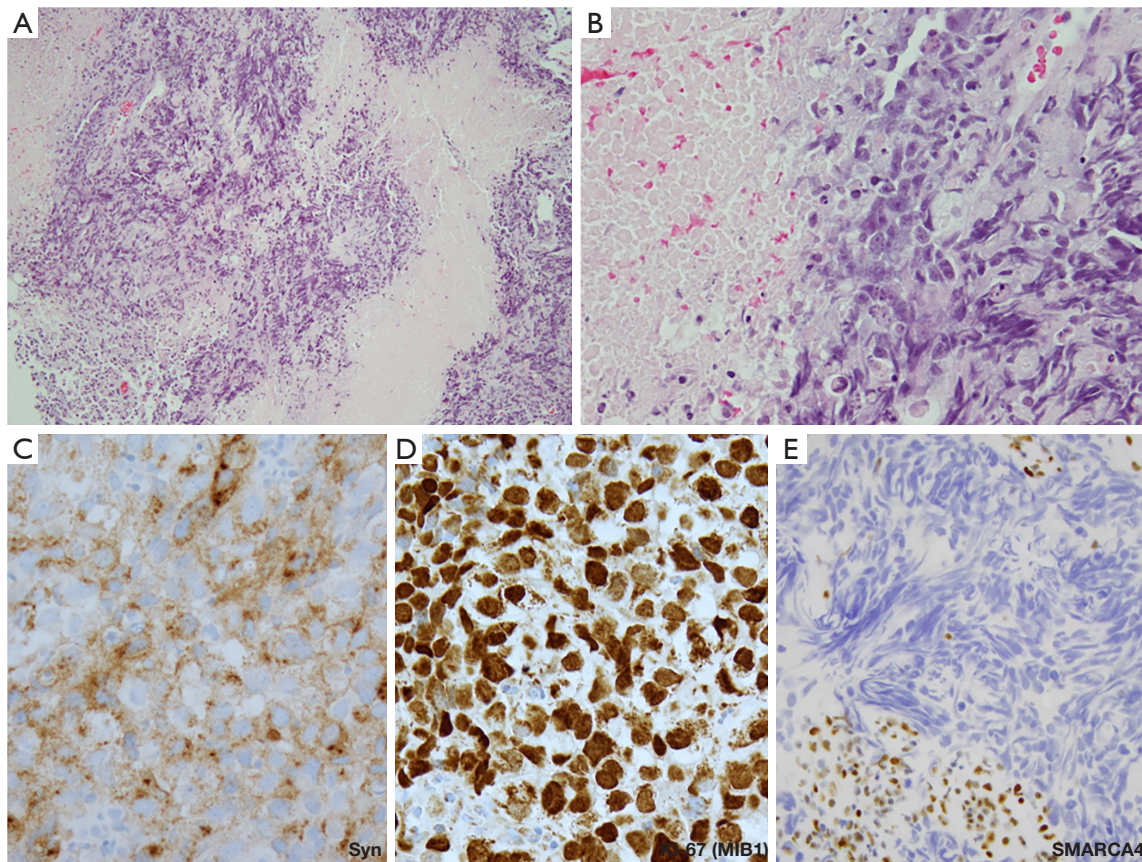


Figure 9 Example of SD-UTT for which the diagnosis of LCNEC was initially considered. Crush artifact and geographic necrosis are commonly seen in both SD-UTT and LCNEC and may limit evaluation of detailed morphology (A); at higher power, focally well-preserved areas demonstrate discohesive round cells with eccentric nuclei and prominent nucleoli (B); frequent expression of synaptophysin (C) and very high Ki-67 index (D) is common in SD-UTT and can further lead to the consideration of LCNEC (or SCLC); however, the loss of SMARCA4 expression (E) together with the loss of SMARCA2 (BRM), negativity for claudin-4, and frequent expression of stem cell markers (SALL4, CD34, or SOX10) support the diagnosis of SD-UTT over LCNEC. Positive labeling for SMARCA4 in left lower corner in E reflects normal expression in benign stromal and inflammatory cells, serving as internal positive controls. Staining method: H&E (A,B), IHC (C,D,E). Magnification: $\times 40$ (A), $\times 400$ (B,C,D,E). LCNEC, large cell neuroendocrine carcinoma; SD-UTT, SMARCA4-deficient undifferentiated thoracic tumor.

years major progress has been made in understanding the molecular landscape of LCNEC, and data linking the molecular subsets to differences in clinical behavior are starting to emerge, translation of these findings to routine clinical practice awaits robust delineation of impact on patient management. Development of objective biomarkers to distinguish LCNEC from entities that enter into its differential diagnosis would be of great value, as would the development of specific biomarkers predictive of responses

to individual systemic therapies.

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