# Controversial issues and new discoveries in lung neuroendocrine tumors

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## Abstract

Lung neuroendocrine (NE) tumors consist of four histologic subtypes, which are usually classified based on a three-tiered prognostic scheme. They are typical carcinoid (TC) as low-grade malignant tumors, atypical carcinoid (AD) as intermediate-grade malignant tumors, and large cell NE carcinoma (LCNEC) and small cell lung carcinoma (SCLC), both of which are high-grade malignant tumors. This three-tiered classification is based solely on histologic grounds and is a source of controversy especially when dealing with borderline or "gray zone" categories (TC vs. AC, AC vs. LCNEC, LCNEC and SCLC). In this review, controversial issues regarding the histologic classification will be discussed, and an innovative grading system that incorporates Ki-67 labeling index will be described. In addition, the recently discovered molecular alterations involved in TC/AC, as well as pathways involved in high-grade NE carcinomas, will be discussed in order to elucidate the differences in pathogenesis and biology between carcinoid tumors and high-grade NE carcinomas.

**Keywords** carcinoid; grading; ki-67 antigen; labeling index; LCNEC; lung; mitoses; molecular; necrosis; neuroendocrine; prognosis; SCLC

## Subtypes and clinical behavior of LUNG neuroendocrine tumors

Neuroendocrine tumors (NETs) of the lung are currently classified into four histological subtypes, namely typical carcinoid (TC), atypical carcinoid (AD), large cell neuroendocrine carcinoma (LCNEC) and small cell carcinoma (SCLC)[1] based on cytomorphological features including the number of mitoses per 2 $mm^2$, the presence (and extent) of necrosis, and the average diameter of tumor cell nuclei (in reference to that of lymphocytes) (Figures 1 and 2). Growing evidence suggests that there are two distinct groups in lung NETs. The one group consists of TC and AC and the other of LCNEC and SCLC, and they are histogenetically unrelated to each other.[2–8] For instance, as discussed later, the differences in genetic alterations between TC and AC and between LCNEC and SCLC are less striking than those between the two groups (TC/AC and LCNEC/SCLC),[2–9] indicating different developmental mechanisms between the two groups. In addition, transition or combined variants are only seen in LCNEC and SCLC.[1] Interestingly, however, the epidemiological (age, gender, smoking habit), clinical (lymph node and distant metastases, association with MEN1 or paraneoplastic syndrome, response to therapy), and survival data suggest that lung NETs could also fit into an operational three-tiered prognostic scheme characterized by progressive malignant potential with significantly different survival curves.[1,10–12] Accordingly, TCs are considered as low-grade malignant tumors with favorable outcomes, ACs are intermediate-grade malignant tumors with more aggressive clinical behavior, and LCNECs and SCLCs are high-grade malignant tumors with unfavorable outcomes.[1,11–14]

The clinical management of patients with lung NET reflects the prognostic scheme. Patients with TC are typically treated by surgery alone irrespective of nodal status while those with SCLC usually present at an advanced stage and receive chemo/radiation therapy. The management of AC and LCNEC appears to be more complex. Some studies have shown that patients with AC harboring regional lymph node metastases have a high likelihood of developing recurrent disease, if treated with surgery alone, and have significantly worse outcomes. Thus, multimodal therapy consisting of surgery and adjuvant chemo and/or radiation therapy is considered in such cases. Similarly, LCNEC, which tends to be located peripherally rather than centrally and presents with early stage (I-II) disease more frequently than SCLC (25% vs. <5%), is often treated with multimodal therapy. The systemic or adjuvant therapy for patients with LCNEC, however, has not been well established. Some advocate SCLC regimens and others recommend NSCLC regimens in this context.[15]

## Issues associated with histologic classification of lung neuroendocrine tumors

Given the prognostic and therapeutic implications, subtyping NE tumors is of paramount importance. Unfortunately, however, it suffers from interobserver variability inherent to its step-wise process in which the aforementioned three categories are primarily separated by the number of mitoses per 2 $mm^2$ and the presence of necrosis, and the distinction of SCLC and LCNEC is essentially based on the mean size of tumor nuclei (and some cytologic features).[16–18] Consequently, it is not infrequent that we encounter borderline or "gray zone" tumors.

The majority of those fall between LCNEC vs. SCLC or AC vs. LCNEC, and to a lesser extent between TC vs. AC. The distinction between the two ends of the spectrum, i.e. TC vs. SCLC, is usually straightforward and reproducible due to the significant differences in the defining criteria; TC is characterized by the virtual absence of mitoses and lack of necrosis along with organoid architecture indicative of well-developed NE differentiation (Figure 1a), while an impressive mitotic count (in average 70 or more mitoses per 2 $mm^2$) and extensive geographic necrosis along with pattern-less pattern or diffuse growth are usually seen in SCLC (Figure 2c).[1,16,17] Of note, the differentiation of SCLC from TC could be problematic,[16] in particular, in small biopsy samples with crush artifact.

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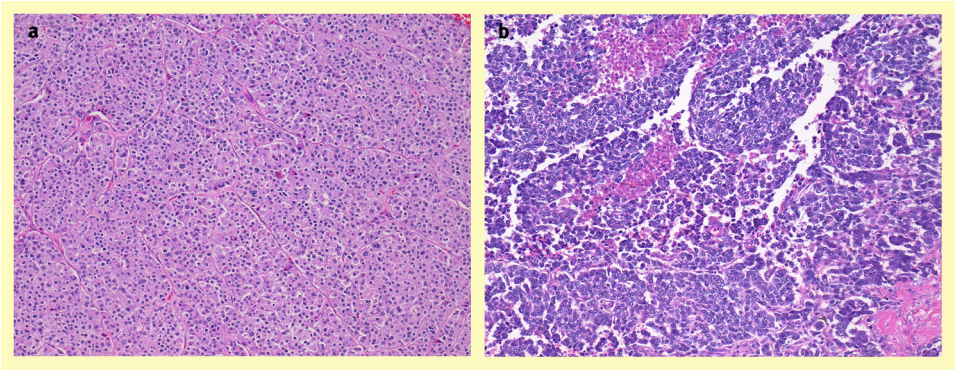


**Figure 1** (**a**) An example of typical carcinoid demonstrating organoid architecture indicative of well-developed neuroendocrine differentiation. No mitosis or necrosis is present. (**b**) An example of atypical carcinoid exhibiting a few small foci of necrosis. A mitotic index was one per high-power field in this case.

### Differentiation between LCNEC and SCLC

The differentiation between LCNEC and SCLC is subject to interobserver variability likely due to overlap in the defining criteria including tumor cell size and cytological features.[16–19] As for the nuclear size, the 3 time rule, which is based on an average tumor cell nuclear/average lymphocyte nuclear size ratio, is usually applied to differentiate LCNEC with "large" nuclei from SCLC with "small" nuclei. However, the study by Marchevsky et al. reported that large nuclei were frequently observed in one third of tumors with the histologic diagnosis of SCC, while one third of those with the histologic diagnosis of LCNEC exhibited small nuclei. Consequently, the 3 time rule was helpful only in one third of the study cases.[19] In the study evaluating interobserver concordance on LCNEC vs. SCLC among 4 pathologists in 129 high-grade lung NETs, Ha et al. has shown that there was a continuum spectrum of tumor nuclear size depicted by morphometric analysis, and tumors with tumor cell nuclear sizes falling in a middle-size range were difficult to classify and lacked the unanimous agreement.[18] Hiroshima et al. also reported that the frequency distribution of tumor nuclear diameter/lymphocyte nuclear diameter (T/L) ratios overlaps between SCLC and LCENC.[20] These studies indicate the presence of borderline high-grade NE carcinomas that fall between LCENC and SCLC. They are characterized by tumor cells with a relatively small amount of cytoplasm and a high N/C ratio (features of SCLC) and a polygonal shape and large nuclei (features of LCNEC) (Figure 2a–c). A morphometric study confirmed that T/L ratios of the borderline category fell between those of SCLC and LCNEC (2.91 ± 0.76, 2.62 ± 0.90 and 3.22 ± 0.86, respectively) (Figure 3).[21] Interestingly, immunohistochemistry and loss of heterozygosity analyses revealed more similarities of the borderline cases to SCLCs than to LCNEC, suggesting that the borderline cases may be phenotypically and genetically close to SCLCs, although there was no difference in patient outcomes between the three categories.[21]

However, the clinical significance of the distinction between LCNEC and SCLC is currently unknown given that there have been conflicting opinions as to whether LCNEC should be treated similarly to SCLC. Recent molecular studies have also reported conflicting results; some provided support for segregation of LCNEC from SCLC as distinct entities[22] while others confirmed molecular resemblance of the two categories.[3,9] Interestingly, some of LCNECs showed unexpected molecular profiles that are commonly seen in conventional adenocarcinoma or squamous cell carcinoma.[23] Thus, several molecular pathways may be involved in the initiation and progression of LCNEC. LCNEC/SCLC may also be clinically heterogeneous, since a small fraction of these have shown longer survival, benefit from adjuvant

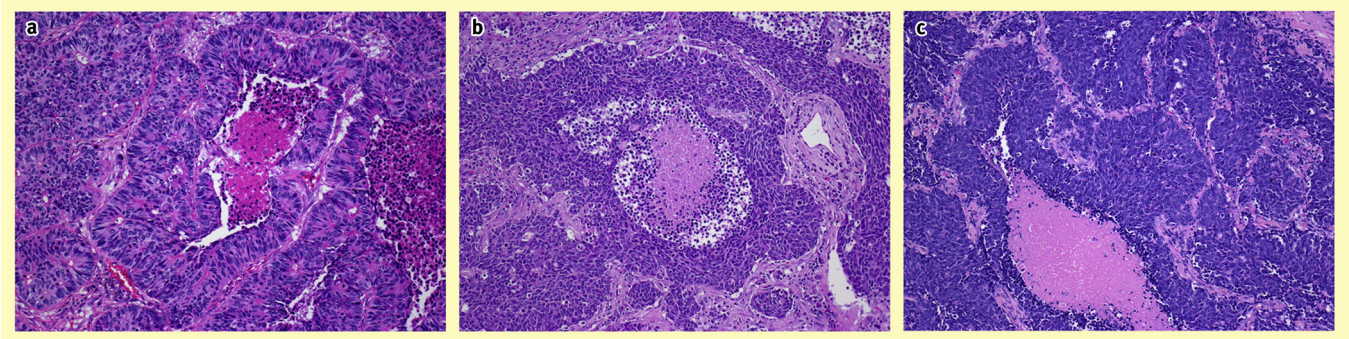


**Figure 2** (**a**) Large cell neuroendocrine carcinoma (LCNEC) with organoid nesting and palisading patterns. Tumor cells harbor abundant eosinophilic cytoplasm, coarsely granular chromatin, and prominent nucleoli. Necrosis is present. (**b**) An example of borderline case. Cell size is larger than that of small cell lung carcinoma (SCLC), but smaller than that of LCNEC. Tumor cells are polygonal and have a relatively small amount of cytoplasm. Rosette-like structures are present. Nuclear chromatin is finely granular, and nucleoli are visible. Necrosis is present. (**c**) SCLC consisting small tumor cells with scant cytoplasm and a high N/C ratio. The nuclei are round or oval with finely granular chromatin and inconspicuous nucleoli. Extensive necrosis is also present.