Thoracic (Lung/Thymus) Neuroendocrine Neoplasms

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Marco Volante and Giuseppe Pelosi

Foreword

Thoracic neuroendocrine neoplasms derive, at least in the majority of cases, from neuroendocrine cells, which are normally present as either single cells scattered in the ciliated epithelium of the airways or clusters (the so-called neuroepithelial bodies) in the lung, but that have not been recognized in the normal thymus, so far. Pulmonary neuroendocrine cells are identified as early as week 7 of gestation in large bronchi and derive from intrapulmonary stem cells through the activation of a specific transcriptional programming, being the human achaete-scute homologue 1 (hASH1) transcriptor factor, encoded by *ASCL1* gene in chromosome 12q, the most studied [1]. Lung neuroendocrine cells share the general morphological, ultrastructural, and immunophenotypic features described in the diffuse neuroendocrine system and possess specific physiological functions acting as sensory chemoreceptors involved in oxygen sensing. Lung neuroendocrine cell functions are mediated by an extraordinary variety of hormonal and receptor interactions. In fact, starting from the gestational phase, lung neuroendocrine cells produce several hormones including serotonin, gastrin-releasing, and bombesin-like peptides, ghrelin, obestatin, calcitonin, calcitonin gene-related peptide, and somatostatin. The role of neuroendocrine cells and neuroepithelial bodies is different in the course of development compared to adult life. In fetal and newborn lung, neuroendocrine cells participate to regulatory mechanisms of air tree branching and cell differentiation and maturation.

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Similar functions are also in place in adult lung during repair processes following injury [2]. In the young and adult population, the oxygen-sensing properties of neuroendocrine cells are tightly interacting with double sensory innervation [3]. A specific oxygen receptor was identified in the cell membrane of neuroendocrine cells of neuroepithelial bodies, belonging to the category of cytochrome b and of NAPDH oxidase [4]. The effects of oxygen sensing cells are automatically evident through the regulation and adaptation of bronchial/bronchiole wall tone, as well as breathing and blood flow control. Such effects are mediated by different neurotransmitters, including serotonin, acetylcholine, and ATP [5]. The complexity of these physiological functions, developmental processes, and hormone productions mirrors the heterogeneous conditions leading to neuroendocrine cell nonneoplastic and neoplastic proliferations and their wide variety of biological and clinical properties.

Classification of Thoracic NENs

The definition of thoracic neuroendocrine neoplasms, according to the most recent World Health Organization (WHO) classification of tumors [6], follows a scheme which encompasses four major categories. The classification scheme should be applied to surgical samples and relies mainly in the combination of morphological parameters which include the evaluation of mitotic index, of the presence of necrosis and of the cell size. Additional cytological and architectural features, such as the pattern of nuclear chromatin, the presence of nucleoli, or the pattern of growth, are descriptive of a given lesion but not definitional per se (Table 9.1). However, in some instances a clear-cut separation between entities encoded by the classification

Table 9.1 Pathological characteristics of thoracic neuroendocrine neoplasms

Parameter	Typical carcinoid	Atypical carcinoid	Large cell neuroendocrine carcinoma	Small cell carcinoma
Mitotic index[a]	<2 × 2 mm^2	2–10 × 2 mm^2	>10 × 2 mm^2	>10 × 2 mm^2
Necrosis[a]	Absent	Absent or present (punctate)	Usually present (extensive)	Usually present (extensive)
Cell size[a]	Variable (variants)	Variable (variants)	Large	Small (<3 small lymphocytes)
Nuclear features	Finely granular chromatin	Finely granular chromatin	Usually vesicular chromatin	Finely granular chromatin
Nucleoli	Occasional, small	Common, small	Present, large	Inconspicuous
Cytoplasm	Variable (variants)	Variable (variants)	Abundant	Scant
Pattern of growth	Organoid/ trabecular	Organoid/ trabecular	Organoid/trabecular/ cribriform	Sheetlike, diffuse
Ki-67[b]	Low (<5%)	Intermediate (<20%)	High (40–80%)	Very high (50–100%)

[a]Definitional parameters for classification
[b]Data on lung NENs

is worrisome since some cases may show borderline pathological features; moreover, the morphological criteria adopted in the classification are somehow subjective or potentially biased by not uniform sampling procedures, and therefore the reproducibility of the classification is not perfect among pathologists [7]. Finally, despite an overlapping classification scheme, neuroendocrine neoplasms in the lung or thymus possess different clinical, biological, immunophenotypic, and molecular characteristics that will be described in detail in this chapter.

Lung Neuroendocrine Neoplasms

Nonneoplastic Conditions and Preinvasive Lesions

The pathological features of neuroendocrine cell alterations in nonneoplastic and preinvasive conditions can be recapitulated by a spectrum of morphological changes ranging from linear hyperplasia to tumorlets whose clinical context of onset and morphological characteristics lack definitive criteria and in several instances coexist in the same tissue sample. The role of these lesions as precursors of lung neuroendocrine neoplasms is postulated for carcinoids [8], mainly those in peripheral location that may be associated with neuroendocrine cell hyperplasia in up to 75% of cases. By contrast, these lesions are probably not associated with the development of high-grade small and large cell carcinomas whose origin seems to be more complex and possibly linked also to other cell types (including type II alveolar cells) [9].

Neuroendocrine Cell Hyperplasia

Clinical Features

Increased number of neuroendocrine cells in the lung is associated with many causative factors.

In the pediatric age, neuroendocrine cell alterations are described in bronchopulmonary dysplasia and dysmaturity, in respiratory distress syndrome, in cystic fibrosis and cystic malformation, in pulmonary hypertension, and in sudden infant death. Bronchopulmonary dysplasia and cystic malformation are complex entities in which developmental errors bring to a disordered growth of different cell types, including epithelial and mesenchymal elements, together with neuroendocrine cells. A form of idiopathic neuroendocrine cell hyperplasia has been also recently described consisting of an obstructive airway disease of unknown etiology and pathogenesis, characterized by tachypnea, crackles, and hypoxia in infants aged less than 2 years [10]. The lung is hyper-expanded with ground-glass opacities [11] and contains hyperplastic bombesin-positive neuroendocrine cells in the alveolar and distal bronchiolar walls in the absence of developmental or inflammatory changes.

In the adult population, some overlapping with pediatric lesions exists, but alterations of the neuroendocrine cell compartment are generally associated with chronic obstructive diseases, smoking-related bronchiolar disease and pneumonia, or more generally to any condition leading to pulmonary injury and repair, as well as in interstitial inflammation and fibrosis [12, 13].

The mechanisms leading to neuroendocrine cell increase are only partly understood. On the one side, experimental mouse models showed that according to the type of induced injury cell regeneration is operated by different cell types, including progenitor cells and neuroendocrine cells [14] as a consequence of the need of expanding the regenerating cell pool in the setting of various basal pulmonary cell plasticities. In some other conditions, neuroendocrine cell hyperplasia is supposed to develop as a consequence of a hypoxic status, a hypothesis supported by experimental evidence in animal models and by the common occurrence of increased neuroendocrine cell number in normal individuals living at high altitudes, as well as patients suffering from hypoventilation syndromes [1].

Pathology

From a pathology viewpoint, neuroendocrine cell hyperplasia is not recognizable at gross examination. Histopathological patterns are recapitulated into two major types:

Linear hyperplasia is defined as an irregular overgrowth of typical triangular- or flask-shaped neuroendocrine cells, located in close contact with the basal membrane of small or large airways, intercalated with mucin and ciliated cells (Fig. 9.1a). Although without numerical cutoffs, in normal conditions neuroendocrine cells do not exceed 0.4% of all bronchial epithelial cells.

Nodular hyperplasia is defined by formation of small clusters of >10–20 neuroendocrine cells in contact with the basal membrane (larger than normal neuroepithelial bodies) (Fig. 9.1b) that may be associated or not with linear hyperplasia.

Tumorlets

Clinical Features

Tumorlets are proliferations of neuroendocrine cells in the bronchial or bronchiolar walls with submucosal extension, with a size of less than 5 mm. Any neuroendocrine cell proliferation of 5 mm or more is by definition a neuroendocrine tumor

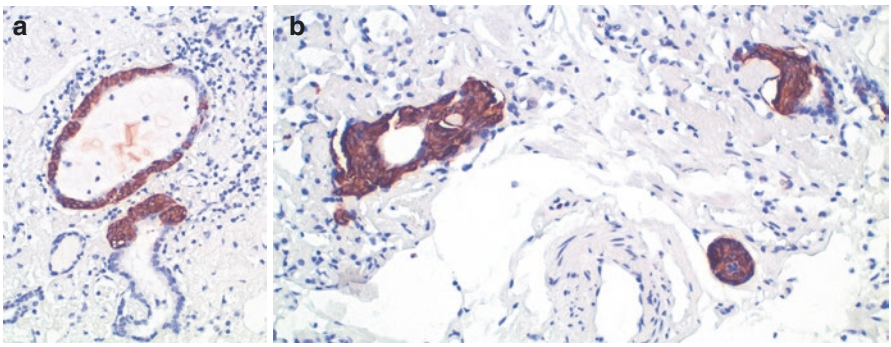

Fig. 9.1 Pulmonary neuroendocrine cell linear (**a**) and nodular (**b**) hyperplasia in a patient with multiple bronchiectasis and chronic inflammation (chromogranin A staining, immunoperoxidase; original magnification 10×)

(carcinoid) [6]. Tumorlets are rare, but their small dimensions probably result in underestimation of their real incidence, if appropriate serial sections are not performed during examination of the surgical specimen. Tumorlets are usually incidental findings at light microscopy, when a variety of pulmonary conditions are examined, including bronchiectasis, chronic inflammation and fibrosis, or tuberculosis, conditions where tumorlets were originally considered as reactive (rather than neoplastic) secondary lesions. However, they may also be occasionally encountered in the lung parenchyma surrounding carcinoid tumors (up to 8% in some series) [15], they can be exceptionally associated with Cushing's syndrome [16], and finally they can even more rarely be responsible for lymph node or distant metastases, thus sharing several features of classical carcinoid tumors of the lung [17]. No clinical relevance has been associated with tumorlets (except for the rare possibility of airway narrowing and/or obliteration), unless they are identified in the context of the rare DIPNECH (see below).

Pathology

Tumorlets can be recognized incidentally at macroscopy as single or multiple nodules. Histologically they are made of oval-, round-, or spindle-shaped cells with minimal atypia and scant, weakly eosinophilic cytoplasm, growing in a more or less dense fibrous stroma in the bronchial or bronchiolar walls, with submucosal extension (Fig. 9.2). Mitotic figures are exceptional and necrosis is invariably absent. Neuroendocrine cell clusters in tumorlets can spread into the surrounding parenchyma although true "spread through air spaces (STAS)" in these lesions is called into question [18]. Neuroendocrine cells in tumorlets do not differ morphologically nor immunohistochemically (production of GRP, serotonin, and calcitonin, as well as of "ectopic" ACTH) from those of lung carcinoids.

Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia (DIPNECH)

Clinical Features

The term diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) may be used to describe a clinical-pathological syndrome, as well as an incidental finding on histological examination. According to the WHO classification [6], the definition of DIPNECH is purely histological. However, DIPNECH encompasses symptomatic patients with airway disease, as well as asymptomatic patients with neuroendocrine cell hyperplasia associated with multiple tumorlets/carcinoid tumors. Due to the lack of uniform classification criteria, its exact incidence and prevalence have not been established. Moreover, DIPNECH is recognized by the WHO classification of lung tumors [6] as a preneoplastic lesion although there are insufficient molecular data to depict pathways of progression from neuroendocrine cell hyperplasia to carcinoids, and this pathological condition represents, if real, a precursor of a small subset of carcinoids.

The term DIPNECH was first introduced in the 2004 WHO classification of lung tumors [19] in light of the observation by Aguayo and coworkers reporting on six

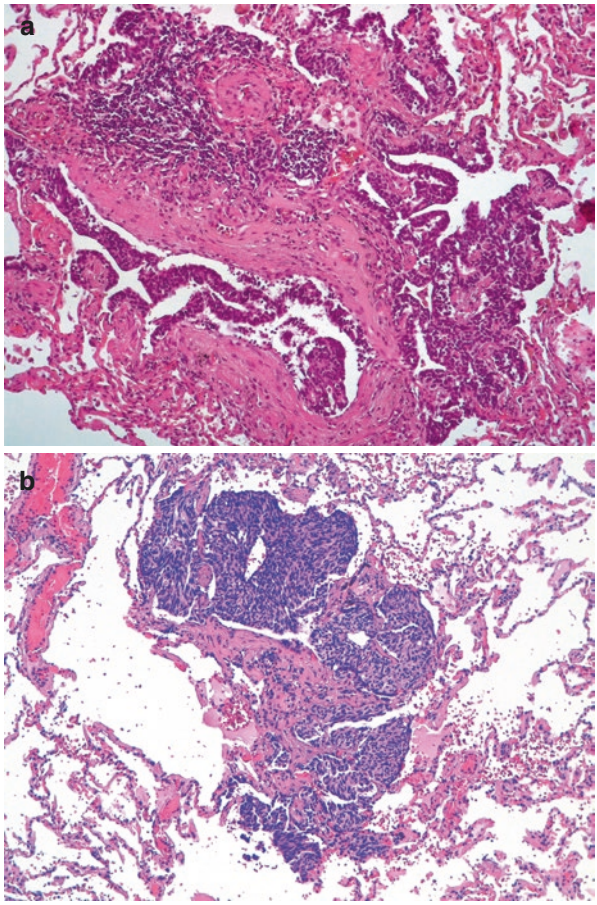

Fig. 9.2 **a**, **b** Examples of lung tumorlets, revealing in both instances (**a**, **b**) an invasive growth that is common to lung tumorlets in contrast to NE cell hyperplasia that is confined to the thickness of the bronchial epithelium (hematoxylin and eosin staining; original magnification 10×)

nonsmoker patients with diffuse hyperplasia of pulmonary neuroendocrine cells, multiple tumorlets and/or carcinoids, and peribronchiolar fibrosis obliterating the small airways [20].

When strict criteria of DIPNECH are applied, patients' characteristics are different from those of reactive neuroendocrine cell hyperplasia and of tumorlets/carcinoid tumors. DIPNECH occurs ten times more frequently in females than males, with a mean age of 58 years, it is not associated with smoking, and it is always symptomatic [21, 22]. DIPNECH has been also diagnosed in the setting of type 1 multiple neuroendocrine neoplasia [23].

DIPNECH presents with chronic symptoms including cough, dyspnea, and wheezing and is often misdiagnosed as asthma, gastroesophageal reflux disease, or chronic obstructive pulmonary disease [24]. Rare DIPNECH cases have been associated with ectopic secretion of adrenocorticotropic and growth hormone [25]. More than half of patients have an obstructive or mixed pulmonary function testing. At imaging, lung nodules are identified at CT scan in about 60% of cases.

DIPNECH-associated specific features are mosaic attenuation with air trapping, which is due to constrictive bronchiolitis, bronchial wall thickening, bronchiectasis, and mucoid impactions [26]. Patients with DIPNECH may remain stable for several years or rapidly deteriorate in few years.

Pathology

DIPNECH is defined by the WHO as "generalized proliferation of scattered single cells, small nodules (neuroendocrine bodies) or linear proliferation of pulmonary neuroendocrine cells" [6]. Although usually confined to the bronchial and bronchiolar epithelium, these proliferations can extend beyond the basement membrane to form tumorlets or carcinoid tumors (when 5 mm or more in diameter). Obviously from this definition, immunohistochemical detection of neuroendocrine cells using pan-neuroendocrine markers, such as chromogranin A and/or synaptophysin, is mandatory for DIPNECH diagnosis. As compared to carcinoids, DIPNECH expresses at a higher extent thyroid transcription factor-1, CD10, and gastrin-releasing peptide/bombesin-like peptide [22].

By some authors, the presence of at least five neuroendocrine cells, isolated or in clusters, located within the basement membrane of the bronchiolar epithelium of at least three bronchioles in combination with at least three carcinoid tumorlets (and in the absence of conditions that could result in secondary neuroendocrine cell hyperplasia), can be used to diagnose DIPNECH in surgical lung biopsy specimens [27]. In addition, in symptomatic cases bronchioles may show a fibrogenic constrictive process leading to constrictive bronchiolitis with mural scarring, luminal narrowing, and/or complete obliteration (Fig. 9.3). Bronchiectasis with mucostasis, emphysematous changes, and mild inflammation may be also present with a peculiar patchy involvement that suggests a thorough examination of the surgical biopsy for a correct pathological diagnosis. DIPNECH may be misdiagnosed with minute meningothelial-like nodules (either isolated or multiple in the setting of so-called meningotheliomatosis) [28], which are tiny aggregates of spindle-shaped cells characterized by nuclear grooving and occasional pseudoinclusions that express EMA, progesterone receptors, and CD56, thus for this latter marker potentially mimicking neuroendocrine cell proliferations.

Neuroendorine Tumors (Carcinoids)

Lung carcinoids are malignant epithelial neoplasms with well-differentiated neuroendocrine morphology and differentiation. They are subdivided into typical and atypical based on mitotic index and presence of necrosis, and histological typing represents the most important prognostic factor.

Epidemiology

Pulmonary carcinoid tumors comprise approximately 27% of all neuroendocrine tumors and account for 1–2% of all lung malignancies with an estimated age-adjusted incidence from 0.1 to 1.5 per 100.000, with a significant increase from 1973 to 2003

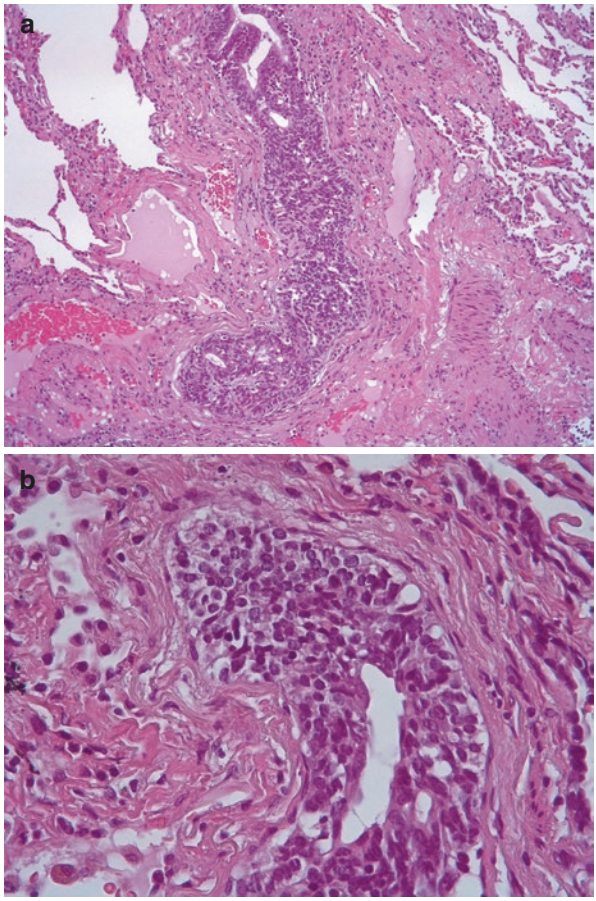

Fig. 9.3 Neuroendocrine cell hyperplasia in a patient with DIPNECH, female, aged 51 years old, with a long clinical history of asthma (hematoxylin and eosin staining; original magnifications: **a** 10×, **b** 20×). Multiple tumorlets and typical carcinoids, from 5.5 to 17 mm in size, were also present

[29, 30]. Typical carcinoids are 70–90% of lung carcinoids. Lung carcinoids more frequently develop in females patients, aged <60 years, and white. Smoking history is usually negative in typical carcinoids, but atypical carcinoids are associated with tobacco smoking in about half of patients. Lung carcinoids may develop in about 5% of patients with MEN1 syndrome [31] (see also section "Inheritance").

Gross, Clinical Presentation and Imaging

Both typical and atypical carcinoids may be central or peripheral and show peculiar pathological and immunohistochemical features depending on their location. In fact, peripheral lesions are associated with presence of spindle cell component, sustentacular cells, a female predominance, and strong association with neuroendocrine hyperplasia, whereas centrally located tumors have more polygonal cell morphology, acinar growth pattern, and only rare association with neuroendocrine hyperplasia [32]. At macroscopy lung carcinoids are relatively well-demarcated nodules, varying in color from yellow-whitish to tan-yellow or brown and ranging from 5 to 95 mm, with a mean size larger in atypical as compared to typical histotype.

About half of patients with carcinoid are asymptomatic, but even when symptoms occur, the tumor may require years before a definitive diagnosis is achieved. Symptoms are site-dependent with peripheral carcinoids usually being asymptomatic and incidentally discovered at imaging studies. Centrally located carcinoids are often symptomatic as a result of partial or complete bronchial obstruction or secondary to its high vascular supply. Cough, hemoptysis, and recurrent pulmonary infections in the same pulmonary segment or lobe are the most frequently reported symptoms. Unilateral wheezing, bronchial asthma refractory to medical therapy, chest pain, and pleural effusion have been occasionally reported [33]. A long-lasting bronchial obstruction can lead to focal bronchiectasis, resulting in partial or complete destruction of the distal lung tissue. Bronchial carcinoid can be associated with paraneoplastic syndromes due to the production and secretion into systemic circulation of several amino peptides and hormonal substances. Carcinoid syndrome occurs in about 8% of bronchial carcinoid, mainly in patients with bronchial carcinoid metastatic to the liver, and is caused by the systemic release of vasoactive substances, in particular serotonin [34]. In actively secreting carcinoids, bronchoscopic management or tumor manipulation during surgical procedures can precipitate the so-called carcinoid crisis: a life-threatening clinical situation characterized by a sudden systemic vasodilatation that leads to a severe cardiovascular collapse. Although bronchial carcinoids are the most frequent cause of ectopic ACTH secretion, Cushing's syndrome is found in 4% of ACTH-secreting carcinoids only. ACTH-functioning tumors are associated with younger age of onset and more advanced tumor stage, although they did not show an independent different survival [16]. GH secretion with acromegaly has been described rarely in pulmonary carcinoids [35].

At imaging, the chest radiograph is abnormal in most cases of bronchial carcinoid, but in approximately 10% it is negative. Centrally located tumors usually present with complete or partial atelectasis, and more rarely a hilar mass can be revealed at chest radiograph. These lesions appear at fibro-bronchoscopy as an esophytic, vascularized mass with smooth bloody surface. CT scan gives an excellent morphological characterization of peripheral and especially centrally located carcinoids that can be purely intraluminal (polypoid configuration), exclusively extraluminal, or more frequently a mixture of intraluminal and extraluminal components ("iceberg" lesion), although pathology still remains mandatory for their correct classification [36]. Bronchocele can be seen in small tumors involving the orifice of a bronchus, and calcifications are present in up to 30% of centrally located carcinoids. In light of the overexpression of somatostatin receptors in carcinoid tumors, somatostatin analog scintigraphy had a role in the past showing a high sensitivity for neuroendocrine cells (over 90% for both primary tumor and metastases), but low specificity because inflammatory conditions and other tumors can also be positive [37]. However, the development of functional imaging evaluation using nuclear medicine techniques during last the two decades provided novel tools for the detection and characterization of lung carcinoids. ^{68}Ga-DOTA-peptide has been shown to be superior to ^{18}F-FDG in terms of the detection rate of pulmonary carcinoids. Moreover, SUVmax ratio of ^{68}Ga-DOTA-peptide and ^{18}F-FDG was an accurate predictor of the carcinoid histotype compared with the SUVmax on ^{18}F-FDG-PET/CT alone [38].

Rate of lymph node metastasis is very different in typical versus atypical carcinoids. In a recent surgical series, positive nodal status was identified in 17.5 typical carcinoids and 45.9 atypical carcinoid cases [39]. Oncological surgical resections (lobectomy or limited sublobar resections in peripheral lesions) associated with regional lymph node sampling are the mainstay of therapy for localized or locally advanced lung carcinoids, either typical or atypical. Typical carcinoids have an excellent prognosis, but in the small proportion of cases with disease progression, both tumor recurrences and metastasis may occur even after 10 or more years from the diagnosis, due to the indolent biologic course of the disease. In atypical carcinoids, the local recurrence rate is also low in the case of limited resection, but the overall prognosis is affected by the high rate of lymph node involvement at diagnosis and by the extent of surgery [40]. Adjuvant treatment, chemotherapy or radiotherapy, has been considered in completely resected atypical carcinoid with mediastinal lymph node involvement, but their real efficacy has been recently called into question [41].

Treatment of metastatic disease is more problematic and no standard strategies have been developed. Various treatment options including somatostatin analogs, peptide receptor radioligand therapy, and biologic systemic therapy, specifically with the mTOR (mechanistic target of rapamycin) inhibitor everolimus, are now available, but the most appropriate treatment algorithms are still not completely designed [42].

In typical carcinoids, the overall 5-year and 10-year survivals range from 90% to 100% and 80% to 90%, respectively, whereas for atypical carcinoids the 5-year and 10-year overall survivals range from 61% to 88% and 35% to 67%, respectively. TNM stage, which has been applied for non-small cell lung cancer, is the key prognostic parameter for both typical and atypical carcinoids [43]. Of note, typical carcinoid with regional lymph node metastasis still have an excellent outcome, especially if with a diameter less than 2 cm [39].

Histopathology

Typical Carcinoid Typical carcinoids have fewer than two mitoses per 2 square mm (usually per 10 high-power fields) and lack necrosis.

Central tumors usually appear as a highly vascularized proliferation of polygonal/round cells with abundant granular and eosinophilic cytoplasm and a central to eccentric round-shaped nucleus with finely granular chromatin with a single, small, inconspicuous nucleolus. These elements are arranged in a mixture of growth patterns, including nesting, solid sheets, trabeculae, ribboning, insular configurations, and rosettes structures (Fig. 9.4). The tumor cell nests are generally dissected by a delicate fibrovascular stroma with dense collagen-rich hyaline stroma that may also contain calcifications, amyloid deposits, and more rarely metaplastic bone and/or cartilage. Cellular pleomorphism may be seen in typical carcinoid, but this feature does not seem to have a prognostic value and does not modify the diagnosis [44]. At intraoperative frozen section examination, a diagnosis of carcinoid can be made in most cases when the tumor has the usual morphological features. However, when there is significant cytological atypia and/or prominent spindle-shaped cell

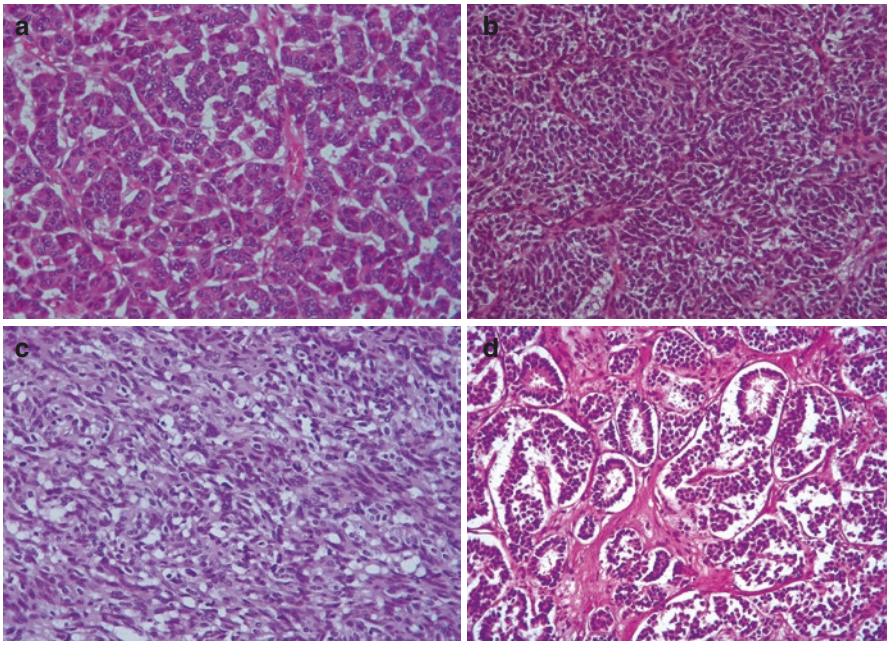

Fig. 9.4 Architectural patterns in typical carcinoid: trabecular (**a**), spindle (**b**), spindle with clear cells (**c**), and pseudo-glandular (**d**) (hematoxylin and eosin staining; all original magnifications 20×)

morphology, distinction from other tumors can be problematic, and the diagnosis may be deferred to prevent a misdiagnosis. The presence of an organoid pattern, stromal hyalinization, spindle-to-ovoid cell proliferation, and finely dispersed nuclear chromatin seems to support a diagnosis of carcinoid tumor [45]. By contrast, distinction between typical and atypical carcinoid may be very problematic at intraoperative consultation, and a diagnosis of pulmonary carcinoid tumor, not otherwise specified, would be preferable and sufficient for therapeutic purposes.

In cytology specimens, typical carcinoids are characterized by hemorrhagic smears containing uniform rounded-to-oval tumor cells, isolated or aggregated in cohesive sheets with the typical finely dispersed nuclear chromatin with inconspicuous nucleoli. The cellular background often shows fragments of delicately vascularized connective tissue with loosely attached tumor cells. However, some cytological features such as nuclear molding and crowding are not discernible features because they may be found on smears with increased cellularity; moreover crush artifact can occur in both carcinoids and high-grade neuroendocrine neoplasms and may cause a misinterpretation of small cell carcinoma. Other artifacts resulting from delayed fixation or poor processing and sampling error are potential causes of incorrect interpretations, leading to up to 49% of discordant diagnoses at definitive histology [46].

Atypical Carcinoid Atypical carcinoid has more than two and up to ten mitoses per 2 square mm (or per 10 high-power fields). Necrosis may be present with punctate foci, but never with large and/or geographic areas (Fig. 9.5).

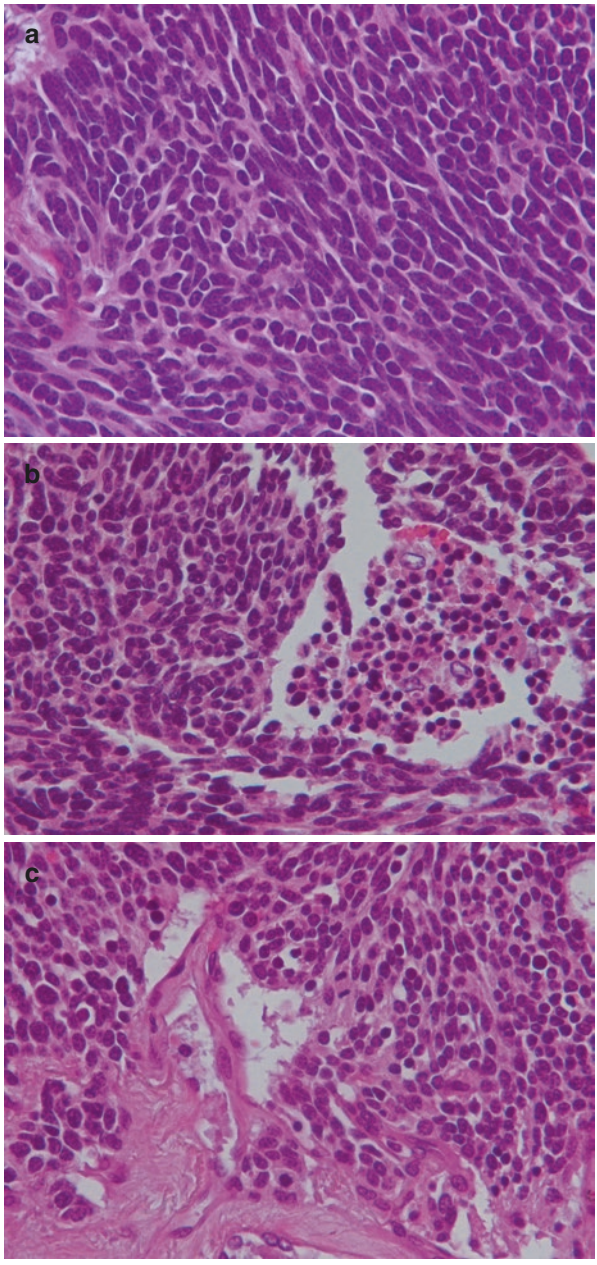

Fig. 9.5 Spindle cell morphology (**a**), punctate necrosis (**b**) and mitotic figure (**c**) in atypical lung carcinoid (hematoxylin and eosin staining; all original magnifications 20×)

Atypical carcinoid was first described as a carcinoid tumor with five to ten mitoses/10 HPF, necrosis, cellular pleomorphism, and increased cellularity [47]. With the recognition of large cell neuroendocrine carcinoma as a distinct high-grade neuroendocrine tumor entity (see section "Large Cell Neuroendocrine Carcinoma"), the criteria to define atypical carcinoid were modified, setting the mitotic index range as it is in the current WHO classification Scheme [6]. As in typical carcinoid, even atypical carcinoids may show several growth patterns including spindle cell, trabecular, palisading, solid/organoid, papillary, and follicular with rosette-like structures. Dense collagen, amyloid, bone, or melanin deposition may be seen. Although cellular pleomorphism, vascular or lymphatic invasion, and hypercellularity are not used in taking typical apart from atypical carcinoids, these features more frequently occur in atypical tumors. Recently, the spread through air spaces (STAS) pattern has been described in lung carcinoids with a higher frequency in the atypical histotype and was significantly correlated with unfavorable parameters, such as high tumor stage, positive nodal status, high Ki-67 index, presence of angioinvasion, and with adverse disease outcome, shorter overall survival, and time to progression [48, 49]. At cytology, atypical carcinoid cells have greater pleomorphism, more coarse chromatin, and more prominent nucleoli than those of typical ones, but these features are not consistent enough to clearly separate these two entities, and mitotic figures and a necrotic background are seen.

Carcinoid Variants

Several histologic variants of typical carcinoid have been described and depict the wide heterogeneity of cytological and architectural patterns in these lesions. Among the most common, the oncocytic variant is characterized by tumor cells with an ample amount of granular oncocytic cytoplasm (as a consequence of mitochondrial accumulation) that has a round-to-oval nucleus with coarse chromatin. Oncocytic areas may be pure or admixed with non-oncocytic ones (Fig. 9.6). Bone formation, the presence of giant cells, and tumor cells with a conspicuous nucleolus are more frequently observed than in conventional cases [50]. Other variants are on record and are mainly to be mentioned as potential pitfalls in diagnostic histopathology. Among those, mucin-producing, clear cell, large spindle, and melanocytic type have been described [51–54].

Immunohistochemical Profile

The use of immunohistochemistry in the diagnostic approach to lung carcinoids partly depends on the type of material available (Fig. 9.7). The definition of the presence of neuroendocrine differentiation in lung carcinoids is mandatory. It may be confirmed by means of several techniques, such as histochemistry (positive reaction with Grimelius or Fontana-Masson stains) and electron microscopy (presence of 30–300 nm electron-dense intracytoplasmic neurosecretory granules, with higher density in typical carcinoids), but immunohistochemistry (IHC) is nowadays the gold standard. Neuroendocrine markers such as chromogranin A (Fig. 9.8), synaptophysin, and CD56 are the most specific and sensitive neuroendocrine markers

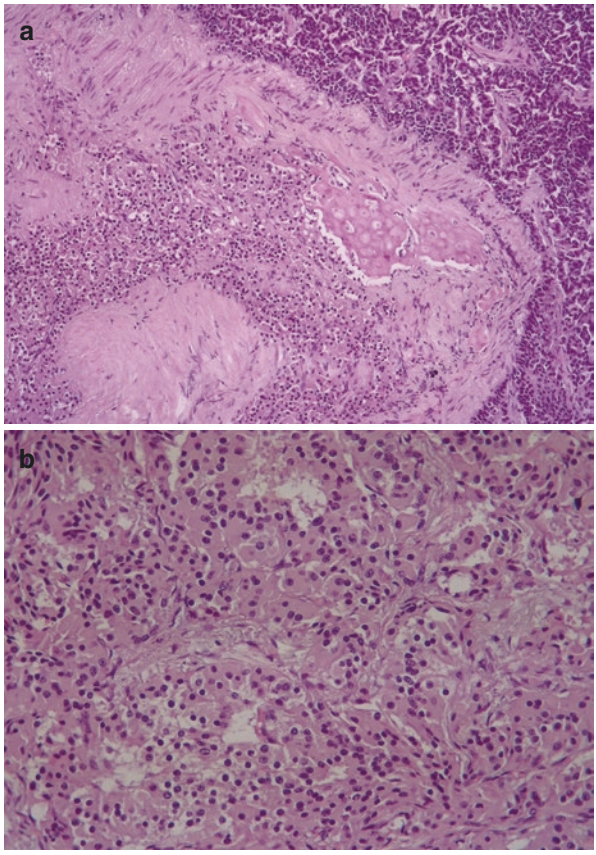

Fig. 9.6 Oncocytic atypical lung carcinoid, admixed with a non-oncocytic component and showing bone formation (**a**); oncocytic cells show the characteristic abundant granular eosinophilic cytoplasm (**b**) (hematoxylin and eosin staining; all original magnifications: a 10×, b 20×)

[55]. Lung carcinoids are also usually reactive for wide-spectrum cytokeratins and CK7, but not for high molecular weight cytokeratins (such as cytokeratin 34betaE12 and/or CK903) nor for napsin A, p40, or p63, features that are helpful to distinguish lung carcinoids from other non-neuroendocrine lung neoplasms. S100 protein may detect the presence of sustentacular cells, which are mainly observed in peripheral lesions. At variance with high-grade neuroendocrine carcinomas, lung carcinoids are almost always negative for PAX-5 [56]. As many other well-differentiated neuroendocrine neoplasms, lung carcinoids express at a high extent the different subtypes of somatostatin receptors, with loss of subtype 2A being associated with more aggressive disease outcome [57].

Different carcinoid histotypes in surgical samples are recognized by means of pure morphological parameters, only, and the use of immunohistochemistry once the neuroendocrine nature is proven is of scarce value. By contrast, in small biopsies or cytological samples, morphological parameters cannot be sufficient alone,

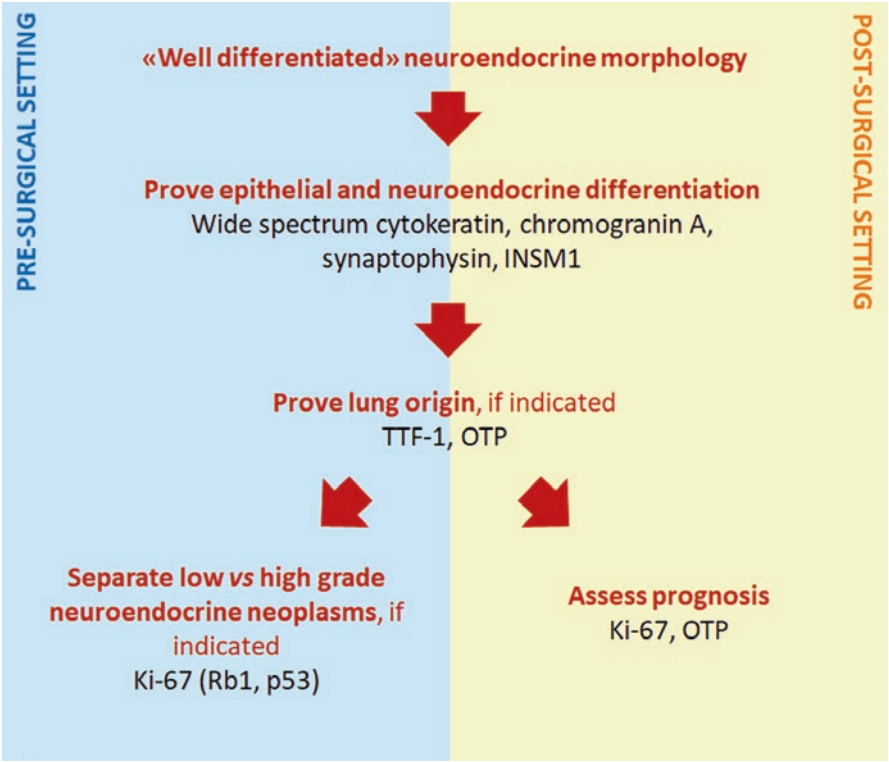

Fig. 9.7 Simplified algorithm of IHC use in lung carcinoids

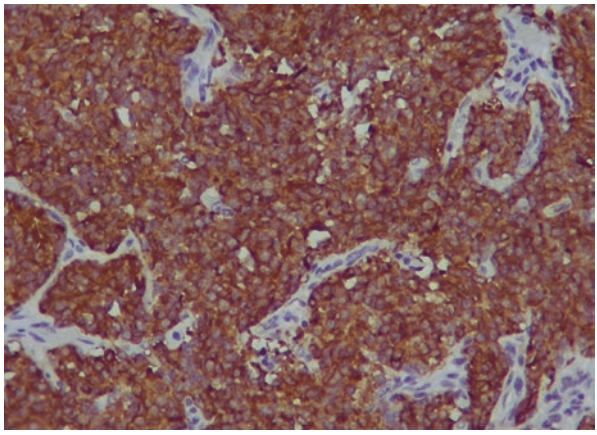

Fig. 9.8 Strong and diffuse chromogranin A staining in typical lung carcinoid (immunoperoxidase; original magnification 20×)

and the pattern of distribution and extent of neuroendocrine markers might be indicative although not supportive of a specific histotype. A diffuse and intense chromogranin A positivity favors a diagnosis of carcinoid, whereas a focal dot-like pattern is more indicative of a high-grade neuroendocrine carcinoma, mainly of the small cell type. The same holds true for hASH-1, a transcription factor whose prevalence of expression increases with the increase of aggressiveness being usually positive at a low prevalence in typical carcinoids and diffusely positive at the other side of the spectrum in small cell carcinoma [58, 59]. By contrast, synaptophysin is usually diffusely positive in both carcinoids and high-grade forms, as well as the novel neuroendocrine marker INSM1 [60]. An important clue in lung carcinoid diagnosis is the identification of the primary lung origin in advanced cases of well-differentiated neoplasms with multiple locations where the clinical definition of the primary site might be not straightforward, despite a strong impact on the management of the patient. Immunohistochemical panels should be specifically designed according to the clinical and radiological pictures and the morphological differentiation of the lesion. Lung carcinoids express TTF1 [61] although mostly in the peripheral location [62]. In this context, metastatic medullary carcinoma of the thyroid may represent a formidable diagnostic challenge, since this latter has morphological as well as immunophenotypic properties of a carcinoid tumor and calcitonin production has been rarely reported in lung carcinoids also [63]. In recent years, the novel marker orthopedia homeobox protein (OTP) has shown to be selectively expressed by lung carcinoids as compared to neuroendocrine tumors of other locations, with a sensitivity of 100% for the typical carcinoid histotype [64] that may be supportive of a lung origin also in cytological samples [65]. The positive expression of other location-specific markers, such as CDX-2, PAX-8, and PDX-1, is indeed supportive for extrapulmonary location and indicative of gastrointestinal or pancreatic origin, according to the phenotypical picture observed. Other differential diagnosis in lung carcinoids includes pulmonary paraganglioma [66] (which expresses neuroendocrine markers and S100 in sustentacular cells but is cytokeratin-negative), glomus tumor (which is positive for smooth muscle actin only), spindle cell neoplasms (especially mesenchymal tumors such as leiomyoma/leiomyosarcoma, schwannoma, and metastatic sarcoma or sarcomatoid spindle cell carcinoma), metastatic melanoma, primary or metastatic meningioma, and various metastatic tumors having a solid growth pattern. In all the above contexts, cytological and architectural features, as well as appropriate immunohistochemical panels, should be integrated to confirm or disprove the diagnosis of carcinoid tumor.

In terms of prediction of clinical behavior, several phenotypical markers have been proposed to be significantly associated with survival, but most of them are directly associated with carcinoid histotype and therefore although of biological interest are of not independent value and limited clinical value. Among the most recent are epithelial-to-mesenchymal transition markers [67], chemokine receptors [68], and IMP3 [69].

Data from gene expression profiling identified several markers potentially applicable in immunohistochemistry in lung carcinoids. However, as for those already mentioned above, most of them are differentially expressed in typical and atypical ones and lose their prognostic value when assessed in comparison to histotyping. Among those, the only biomarker strongly and independently associated to adverse outcome in lung carcinoids is OTP protein loss, either alone or associated with CD44 expression. Since the original publication [70], subsequently validated by the same authors in another independent series [7] and by other groups [71], OTP nuclear expression has been described as a strong independent prognostic factor for recurrence-free survival in carcinoids, including typical ones with locally advanced pathology stage. Among the few others, the lack of central cell cycle proteins KLF4 and p21 expression has been associated with an accumulation of aggressive features in typical carcinoids [72].

Evidence-Based Grading Proposals

Proliferation marker Ki-67, apart from histological type and TNM stage, is the most relevant prognostic indicator in lung carcinoids and has been widely studied and validated since several years [73], and although not coded in the WHO classification system as a prognostic determinant to be mandatory mentioned in the diagnostic report, its assessment is strongly recommended in the clinical practice [36]. Despite even recently called into question as an independent prognostic factor [74], a grading proposal was specifically designed in lung neuroendocrine neoplasms embedding Ki-67 with mitotic index and necrosis [75], and the reliability of this marker in the preoperative setting was recently proved by the high concordance – when carefully assessed – between corresponding presurgical and surgical lung samples [76]. However, no agreement has been reached at the current present on the definition of a grading system for carcinoids, with variable combinations of Ki-67 cutoff levels and morphological criteria [77].

Indeed, Ki-67 relative high expression (using a cutoff of 10% or 20%) further segregates a subgroup of lung carcinoid cases with distinct pathological features and significantly worse outcome independently from the typical or atypical histotype, which, at least in part, resemble the pancreatic “NET G3” group of neoplasms [78, 79] (Fig. 9.9). The presence of aggressive well-differentiated lung neuroendocrine neoplasms that do not have the morphological features of high-grade neuroendocrine carcinomas but exceed canonical proliferative and mitotic indexes of carcinoids has been also strongly suggested in a recent report on stage IV lung carcinoids. In the reported series, up to 27% of cases, mainly in metastatic sites, had mitoses and/or Ki-67 superior than the standard criteria for carcinoids; however, these cases retained well-differentiated morphology and conventional proliferation rates in other samples from same patient, lacked RB1/TP53 alterations (at variance with high-grade neuroendocrine carcinomas), and had a median overall survival of 2.7 years, as compared to <1-year survival of stage IV high-grade neuroendocrine carcinomas [80].

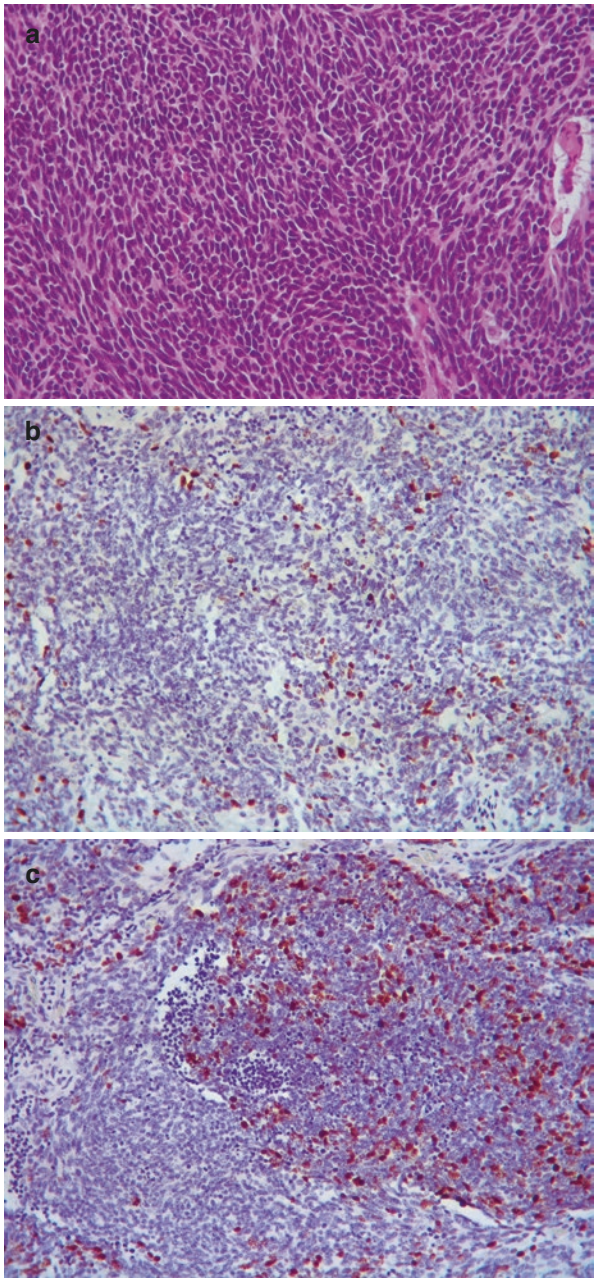

Fig. 9.9 Atypical lung carcinoid with spindle cell morphology (**a**) showing a heterogeneous pattern of staining for Ki-67, with intermediate (**b**) to high proliferation indexes (**c**, in close association with necrotic debris) (**a**, hematoxylin and eosin staining; **b** and **c**, immunoperoxidase; all original magnifications 20×)

Large Cell Neuroendocrine Carcinoma

Epidemiology

Large cell neuroendocrine carcinoma (LCNEC), in the past clustered together large cell carcinomas (LCC) as tumors presenting with neuroendocrine differentiation [81], accounts for 3% of less of all lung cancers, but its prevalence is destined to increase due to heightened diagnostic awareness and increased use of immunohistochemistry for refining poorly differentiated tumors. A recent study dealing with a large Surveillance, Epidemiology, and End Results dataset has reported on a 1–2% prevalence for LCNEC, with female gender, black race, surgery, radiation, and chemotherapy being protective factors for survival in these patients [82]. Early-stage LCNEC patients showed a higher risk of lung cancer-specific death and specific patterns of metastasis with a larger incidence of brain metastases than patients with early-stage non-small cell lung carcinomas (NSCLC) [83]. In particular, patients with isolated liver or brain metastasis or combined invasion patterns to other organs showed poorer survival rates, identifying LCNEC as an aggressive tumor subtype when investigated epidemiologically. Smoke and male gender are considered risk factors for the development of LCNEC, which usually affect elderly patients (with a median age of 65 years) [84]. However, fewer cases of LCNEC arising in non-smokers and/or younger people upon ALK [85] or ROS-1 [86] rearrangement or EGFR mutations [87] are increasingly on record especially in peripherally located lesions. These considerations witness the inherent biological heterogeneity of LCNEC, which may have important patho-biological and clinical implications [88]. According to a recently released common classification framework, LCNEC as defined by current criteria [6] are NENs belonging to the family of neuroendocrine carcinomas, typed as featuring large cells.

Gross, Clinical Presentation and Imaging

There are no specific macroscopic or clinical features of LCNEC compared to conventional NSCLC. At variance with small cell lung carcinoma (SCLC), paraneoplastic syndromes are uncommon, but single case reports of ectopic adrenocorticotropic hormone syndrome [89], Lambert-Eaton syndrome [90], or cancer-associated retinopathy [91] have been well documented. LCNEC present high rate of lymph node (60–80%) and distant metastasis (40%) at the time of diagnostic recognition, similarly to SCLC [84, 92] even if metastatic sites are less frequently reported than in the latter. These findings underline a potentially different natural history of LCNEC as compared to SCLC, as also documented by survival analysis [92, 93]. Tumors may feature central or, more frequently, peripheral location in the form of large, circumscribed, and abundantly necrotic masses infiltrating the pleura, the chest wall, or the adjacent structures (even with Pancoast tumors and Horner syndrome), while cavitation is uncommon. It has recently been observed

that peripheral LCNEC patients had better life expectation compared with central lesions and that the location inside the lung was an independent prognostic factors for overall survival [86]. Even this finding supports once again inherent differences in the origin cells and pathogenesis of LCNEC, also outside the lung, when they are considered as a unitary tumor category. CT scan evaluation usually shows a well-defined and lobulated tumor with no air bronchograms or calcification, where necrosis may cause an inhomogeneous enhancement of the contrast medium to appear especially when dealing with large-sized LCNEC, while this is less apparent in small-diameter (<33 mm) lesions even if they entail some amount of necrosis [94]. The maximum standardized uptake value (SUVmax) on positron emission tomography with 2-deoxy-2-[fluorine-18]fluoro-D-glucose (^{18}F-FDG PET) is commonly high, consistent with highly malignant tumors and correlated with shorter disease-free survival. LCNEC present also with somatostatin receptors, even if at lower levels in comparison with carcinoids [95], but scintigraphic imaging with OctreoScan (indium 111-tagged diethylenetriaminepentaacetic acid pentetreotide scintigraphy), ^{111}In-DOTA-TOC (111In-DOTA-Dphe1-Tyr3-octreotide), or ^{111}In-DOTA-LAN (111In-DOTA-lanreotide), albeit proposed in preoperative staging and in postoperative follow-up of LCNEC patients, did not enter the routine clinical practice.

Histopathology

The diagnosis of LCNEC is usually straightforward on resection specimens by applying the defining criteria settled in the 2015 WHO classification [6], but can be also supported on biopsy specimens by relying on immunohistochemistry findings [96]. Current guidelines stated that non-small cell carcinoma on biopsy samples with neuroendocrine morphology and neuroendocrine marker positivity supports a possible diagnosis of LCNEC; thus, such a diagnosis can be rendered on biopsy samples only if morphology actually suggests neuroendocrine differentiation. As a matter of fact, it has also been observed that neuroendocrine marker staining should not be performed and is not recommended its use for tumors with no obvious neuroendocrine morphological features [97], because some neuroendocrine markers can be even shared by tumors lacking overt neuroendocrine differentiation. Since neuroendocrine morphology may be yet frequently missed in biopsy and cytology samples, there is a potential for LCNEC diagnosis to be missed on small specimens. This is the reason why this tumor type is usually recognized on resection specimens only, even if this is the second most prevalent neuroendocrine tumor after SCLC. At variance with SCLC, there are no reliable criteria for this tumor to be diagnosed on cytological samples due to their large overlap with those of other neuroendocrine tumors or conventional NSCLC, although criteria such as tumor cell size, naked nuclei, thin nuclear membranes, nuclear streaking, neuroendocrine marker positivity, and a necrotic background have been proposed for LCNEC on cytological samples [98].

LCNEC as a tumor entity was proposed by Travis et al. in 1991 [99] by refining the previous Gould and Warren's definition of intermediate cell neuroendocrine carcinoma (intermediate in cell size between well-differentiated neuroendocrine carcinoma, i.e., atypical carcinoid, and SCLC) [100, 101]. LCNEC was described to exhibit neuroendocrine architecture (e.g., organoid and often palisading tumor

islands) and neuroendocrine marker expression, in pure or combined form with other NSCLC and with an intermediate prognosis between atypical carcinoid and SCLC but closer to the latter. These criteria have been largely maintained unchanged over the subsequent three WHO classifications until the last of 2015, with the only change regarding survival that now is considered to largely overlap with SCLC.

In its most classical description, LCNEC is a tumor showing neuroendocrine morphology featuring organoid aggregates or solid to trabecular pattern of growth (Fig. 9.10). Tumor cells are large as opposed to those of SCLC (typically more than three resting lymphocyte diameter), with abundant granular to variably clearer cytoplasm and well-defined cell borders realizing a prominent peripheral palisading or mosaic pattern. Nuclear molding is typically lacking likely due to the cytoplasm abundance that prevents tumor cells to closely juxtapose to each other causing nucleus shape deformation to arise. The chromatin pattern is typically coarse with abundance of heterochromatin and basophilic to amphophile prominent nucleoli (Fig. 9.11), and this is considered the single most important criterion to separate LCNEC from SCLC. Mitoses are plentiful (more than 10 per 2 mm^2, with no upper limits, but a median value of 70 mitotic figures) and may be atypical. The necrosis

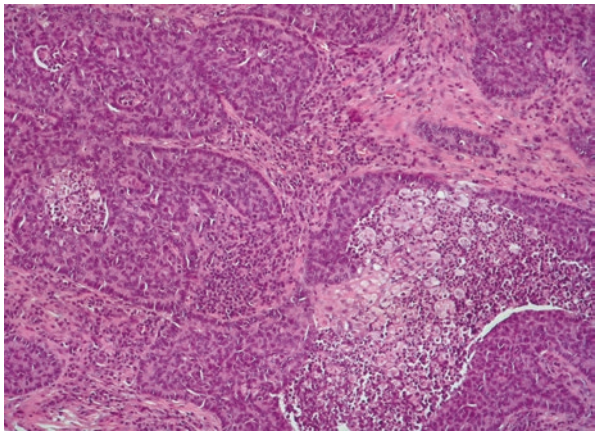

Fig. 9.10 Organoid growth pattern in large cell neuroendocrine carcinoma (hematoxylin and eosin staining; all original magnifications 10×)

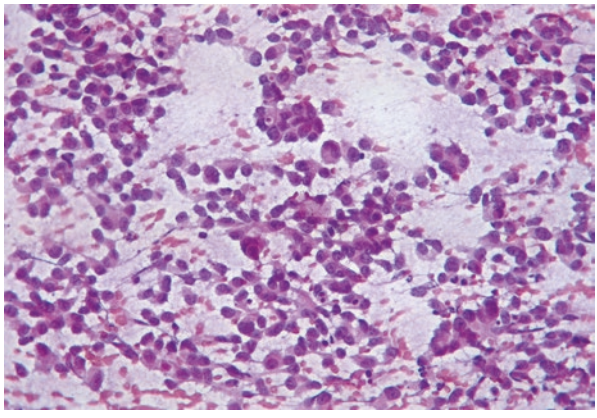

Fig. 9.11 Cytological features in a cytological smear of large cell neuroendocrine carcinoma, showing pleomorphic nuclei with vesicular chromatin and prominent nucleoli (hematoxylin and eosin staining; original magnification 40×)

is variably extensive, sometimes geographic, and peritheliomatous in appearance, with sheets of viable tumor cells being concentrically arranged to survive around vascular channels indicative of complex mechanisms of tumor necrosis [102]. A small subset of LCNEC features histological details that overlap atypical carcinoid, except for showing more mitoses exceeding the allowed number of 10 per 2 mm^2 and more necrosis, this indicating a wide spectrum of morphologic appearance in turn indicative of heterogeneity in cell composition and derivation. Combination of LCNEC with SCLC, for which a 10% percentage of either tumor type is required, is considered a combined variant of SCLC with LCNEC rather than a combined variant of LCNEC with SCLC likely because of the morphologic continuum existing in neuroendocrine carcinomas of small and large cells, which is in turn responsible for the disappointing diagnostic reproducibility between them [103].

The diagnosis of LCNEC is a stepwise process, in which at first neuroendocrine morphology must be recognized through identification of organoid nesting, trabeculae, rosettes, and peripheral palisading, and then LCNEC is identified according to mitotic count and necrosis extent to rule out atypical carcinoid and a combination of morphology and IHC to exclude NSCLC subtypes. Separation from SCLC may be challenging for either the continuous dimensional overlap of small and large cells around three resting lymphocyte/endothelial cell diameter in tumors sharing common neuroendocrine properties or the subjective application of defining criteria [104]. Although a constellation of features regarding cell size, chromatin patterning, and cytoplasmic amount has been advocated to distinguish LCNEC from SCLC, this separation continues to remain challenging and, to some extent, arguable on biologic bases. Difficulties in assessing cell size and cytological features including chromatin pattern may account for disappointingly low inter-observer reproducibility of LCNEC diagnosis even among experts that remain around 50% (just as little as a chance).

It may be useful to briefly comment here the possibility of facing with conventional NSCLC, where IHC and electron microscopy demonstrate neuroendocrine markers but neuroendocrine morphology is lacking by light microscopy and they feature conventional adenocarcinoma, squamous cell carcinoma, or large cell carcinoma realizing the so-called NSCLC with neuroendocrine differentiation. These tumors, which have not been included in the last 2015 WHO classification as independent tumor entities, should be rather classified as adenocarcinoma, squamous cell carcinoma, or large cell carcinoma but commenting on the presence of positive neuroendocrine markers [6]. As a matter of fact, the clinical implications on survival and chemotherapy response have been variably interpreted in the past [105–107], but more recent molecular data favor the biological relationship of NSCLC with neuroendocrine differentiation with the development of LCNEC upon evolution from these precursor lesions [108].

Immunohistochemical Profile

Although recent recommendations for diagnostic IHC on lung cancer have stated that LCNEC diagnosis should be made only when morphology and neuroendocrine markers can be simultaneously demonstrated in the same tumor [109], once obvious

squamous or adenocarcinoma has been reasonably ruled out, a positive decoration for two of three neuroendocrine IHC stains (chromogranin A, synaptophysin, CD56) is supportive on the diagnosis of LCNEC even in small samples. It could be also commented that the greater the expression of neuroendocrine markers, the greater the probability that also the neuroendocrine morphology is as patent as to allow the diagnosis of LCNEC to per rendered according to WHO criteria. For the ultimate diagnosis of LCNEC, the IHC confirmation of neuroendocrine differentiation is compulsory for distinguishing these tumors from mimickers such as conventional NSCLC, with a clear-cut identification of at least one out of two or the three classical and most used neuroendocrine markers (synaptophysin, chromogranin A, and CD56) [96]. There is no proposed clear cutoff value for the extent of tumor cells being positive for neuroendocrine markers to make a diagnosis of LCNEC, but any amount of positive staining of any of these markers should be considered meaningful, if neuroendocrine morphology is clearly patent. Dependency of LCNEC diagnosis on combined evaluation of morphology and IHC is also instrumental to reduce the inter-observer variability and increase the pathologists' diagnostic confidence [110]. Most recently, insulinoma-associated protein 1 (INSM1), an early inducer of NE/neuroectodermal differentiation during ontogenesis and in lung cancer, has been proposed as reliable and sensitive marker of neuroendocrine differentiation in thoracic neuroendocrine tumors, including LCNEC [111]. As the experience on such a marker is still limited, it should not be preferred yet to the other well-consolidated markers of neuroendocrine differentiation. Other IHC markers positive in LCNEC include TTF1 in about one half of instances, different cytokeratin pooling with either dot-like or diffuse cytoplasmic decoration, and rarely and focally p40 or napsin-A expression likely indicating an underlying inapparent keratinizing or glandular differentiation [112, 113].

Small Cell Carcinoma

Epidemiology

Small cell lung carcinoma (SCLC) accounts for about 15% of all lung carcinomas worldwide and for most neuroendocrine neoplasms arising in the lung. Its incidence rate has been decreasing for about the last two decades in both genders after peaking between the mid-1980s and the early 1990s in Western countries, reflecting major changes in smoking habit rather than substantial therapy or diagnosis improvements [110, 114]. Conversely, SCLC incidence is destined to further increase in countries where smoking habit is still largely prevalent in the population of both genders such as Eastern Europe [115]. Epidemiological evidence suggests that the proportion of elderly patients among all cases of SCLC has increased over the past 40 years, with a trend toward a shorter cancer-specific survival while increasing age in the subgroups from 70–74 to 85 or more years [116]. People younger than 40 years with SCLC are uncommon but show similar prognostic factors such as disease stage at clinical presentation, timely diagnosis, and performance status [117]. Female gender and hormone replacement therapy are protective factors for SCLC development

[118], with a prolonged overall and brain metastasis-free survival even in patients bearing limited disease [119]. As a matter of fact, SCLC has been staged for many years as either limited disease (primary tumor and regional lymph nodes within a tolerable radiation field) accounting for 25% of cases or extensive disease (anything beyond limited stage) accounting for 7% of instances. Currently, TNM classification (8th edition) is by far the most preferred and recommended tool for survival and clinical inferences in SCLC patients, because the M descriptors identifying stage IV-A, IV-B, and IV-C are of sure prognostic meaning in either presentation [120]. Most small cell carcinomas are associated with heavy smoking history, either current or former, with significant dose-response relationships for all quantitative smoking variables likely involving mechanistic pathways related to chronic obstructive pulmonary disease [121] and TP53 mutations [122]. Of note, reduction of the pretreatment FEV1/FVC ratio that in turn is diagnostic of obstructive pulmonary disease was independently associated with shorter overall and progression-free survival in limited disease patients, thus confirming once again such a close association with tobacco consumption. However, as many as 2–5% of SCLC patients are never smokers, who show a significantly longer progression-free and overall survival as compared with current or former smokers [123]. Since resected SCLC, whether elective or incidental, exhibit a more favorable clinical course than patients not undergoing surgery [124], it is tempting to speculate that even SCLC may encompass a case mix of diversely behaving tumors not predicted by morphology. According to a recently released common classification framework, SCLC as defined by current criteria are NENs belonging to the family of neuroendocrine carcinomas, typed as featuring small cells [125].

Gross, Clinical Presentation and Imaging

Most SCLC affect major bronchi presenting as hilar/para-hilar mass and huge involvement of regional lymph nodes and vascular channels, whereas 5% or less of them arise in the pulmonary parenchyma most often in the form of low-stage peripheral nodule. In major bronchi, rarely SCLC grow as an endoluminal polypoid tumor, but rather spread in a subepithelial and radial pattern causing diffuse increase of the bronchial wall thickness for concentric stenosis (an airway stenting may be also beneficial) and massive involvement of adjacent structures (nerves, vessels, lymph nodes, lung parenchyma). Clinical symptoms may be local, systemic, or related to paraneoplastic syndromes. Suffice it to say that SCLC make up the most frequent lung cancer histology associated with paraneoplastic syndromes [126], which can be caused by either ectopic hormone production (hyponatremia, Cushing's syndrome) or autoimmune-mediated destruction upon onconeural neoantigen expression by cancer cells (paraneoplastic encephalomyelitis, Lambert-Eaton myasthenic syndrome) [127], the former being associated with poorer outcome, the latter with more prolonged clinical prognosis [128]. Most SCLC are extended diseases at clinical presentation with widespread metastases (liver, bone, brain, adrenal grand, lymph nodes), along with pleural and pericardial effusions. Staging assessment is at the best performed by using TNM classification, as the prognosis of oligometastatic

patients (≤5 metastases in a single organ that tended to locally recur) was significantly superior to patients with polymetastases, thus paving the way to local and systemic combination therapies. No consensus exists on standard imaging modalities for pretreatment staging of SCLC, and there is only low-strength evidence suggesting that FDG-PET/CT is more sensitive than CT alone and bone scintigraphy for detecting osseous metastases [129]. Active magnetic resonance imaging surveillance of brain metastases in SCLC patients has recently been proposed in opposition to the simple prophylactic cranial irradiation to prevent declines in cognitive function [130]. CT scan of SCLC shows characteristically a large solid and lobulated mass in hilar/para-hilar region with bulky mediastinal lymph nodes and invasion of great vessels and mediastinal fat, whereas cavitation is rare. SCLC can also be variably found (6–13% but 34% of all interval cancers) [131] in screening programs with low-dose computed tomography, but prognosis of these patients remains disappointing with no survivors at 3 years after diagnosis. These findings support the widely held belief that low-dose computed tomography screening is ineffective in reducing SCLC-related mortality in an age- or smoking status-independent manner, whereas there was evidence of a differential benefit by female sex. Somatostatin receptor scintigraphy with 111In-pentetreotide (OctreoScan) scintigraphy showed optimal specificity but lower sensitivity for primary SCLC, mediastinal lymph nodes, and distant metastatic disease likely due to variable and inconsistent expression of somatostatin transmembrane receptors by poorly differentiated tumor cells [132].

Histopathology

SCLC diagnosis con be usually rendered on small samples (cytology and biopsy) and surgical resection specimens. As most SCLC are widespread metastatic at clinical presentation, cytology and biopsy samples are most often the only material investigated for clinical purposes of treatment. Small-sized cells, round to spindle shape, irregular nuclear outlines, naked or small clustered nuclei with evenly distributed fine chromatin, no prominent nucleoli, scant to stripped out cytoplasm, chromatin streaking, and apoptotic debris are the typical traits that can be observed in cytological preparations [133]. It has been observed that treatment facilities rather than patients' demography or clinic traits may affect the prevalence ratios of cytology as a confident diagnostic tool in SCLC patients [134], even if a judicious use of IHC improved the inter-observer agreement to good in most cases of small biopsy samples [110]. Cytology of SCLC was not specifically tested with IHC for inter-observer reproducibility, but it correlated well with histopathology, and it is well known the essential role played by IHC in the cytological subtyping of lung cancer [135]. Crush artifacts in both cytology and biopsy samples may hamper diagnostic recognition of SCLC, exposing to the risk of misdiagnosing carcinoid as SCLC (with major diagnostic pitfalls for the clinical handling of patients). Such a situation, however, can be easily overtaken by addressing IHC staining for Ki-67: carcinoids, either typical or atypical, present with a Ki-67 labeling index ranging up to 20–25%, while SCLC exceed to a large extent 50% easily arriving at 90–100%

[136]. Necrosis is variably seen in both cytological and biopsy samples, but mitotic figures are not easily recognizable as one would expect in such proliferating tumors, especially when crush artifacts concur.

Histopathology of SCLC is generally highlighted by small-sized cells not exceeding three resting lymphocytes or endothelial cells, with scant cytoplasm, finely granular to evenly dispersed nuclear chromatin, small or inconspicuous nucleoli, frequent and abundant necrosis up to featuring geographic distribution, and plentiful mitoses (more than ten mitotic figures per 2 mm^2, with a median value of 80). Round-, oval-, and/or spindle-shaped tumor cells are variably admixed with each other in a solid growth pattern with ill-defined borders and prominent nuclear molding sometimes resembling hematologic malignancies, undifferentiated NSCLC, or sarcoma. Giant tumor nuclei may also be seen. Peripherally located tumors show instead more developed neuroendocrine morphology featuring prominent trabecular sheets, organoid solid growth, rosette formation, and more abundant cytoplasm, even if these tumors do not differ in terms of nuclear features and mitotic count [137]. Azzopardi phenomenon [138], featuring basophilic DNA stratification around vascular channels or extracellular matrix collagen fibers, may be noted, albeit it is unspecific, concurrently with geographic necrosis or severe tissue crushing due to fragility of tumor cells. In general, these SCLC characters are sufficiently maintained over tissue samples to ensure inter-observer reproducibility of diagnosis, even in challenging settings such as frozen section examination during surgery. Rarely, in about 5% of instances, SCLC may be observed as asymptomatic peripheral tumor (solitary pulmonary nodule) on routine chest radiography, usually as low-stage tumor with no regional lymph node metastases upon surgery (Fig. 9.12). The survival of these stage I SCLC, after multi-organ scanning and lymph node sampling prior to thoracotomy, is similar to survival of surgically treated stage I NSCLC patients [139]. Interestingly, while these tumors fulfil diagnostic criteria for SCLC, instead they show organoid neuroendocrine patterns of growth with nesting, palisading, trabecular features and rosette formation at variance with centrally located and early aggressive SCLC, mostly presenting as extended disease, which show diffuse, solid, and/or sheetlike patterns simulating hematologic malignancies, thus suggesting a different underlying pathogenesis.

Immunohistochemical Profile

Even if IHC is not strictly required for the diagnosis of SCLC, it is warmly recommended due to the large number of histologic mimickers of this tumor (mainly poorly differentiated NSCLC of squamous lineage, NUT carcinoma, hematologic malignancies, melanoma, sarcomas) [110]. Of minor clinical relevance could seem distinguishing SCLC from LCNEC, because they share similar life expectation and many molecular alterations, but emerging data on the different susceptibilities of LCNEC to diverse chemotherapy regimens [9] and their widely recognized molecular heterogeneity [140, 141] strongly advice performing this separation. Thus, our discussion on IHC will imply two aspects, which are also strictly interconnected with the issue of differential diagnosis: diagnosis of SCLC from other tumor types and separation of SCLC from LCNEC.

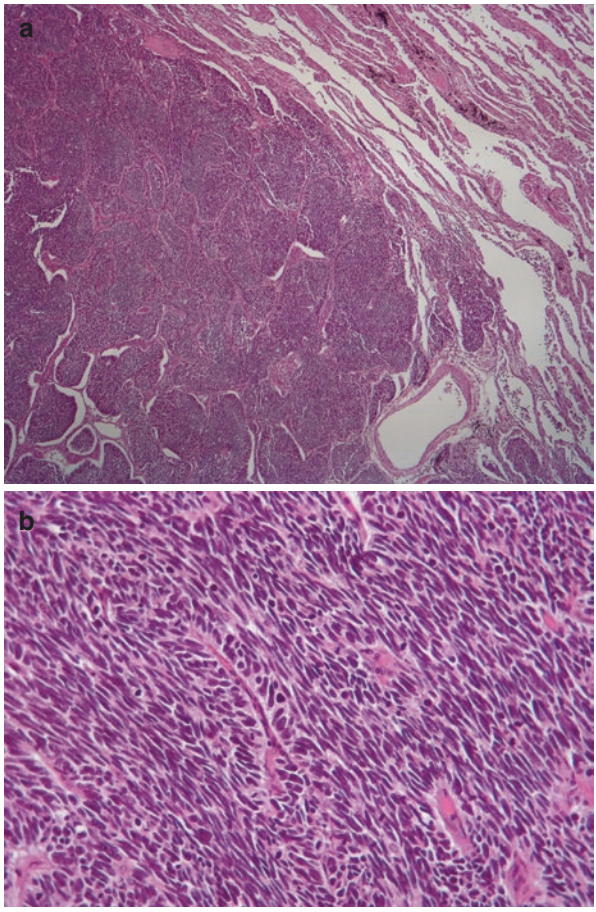

Fig. 9.12 A peripheral small cell lung cancer case, with well-defined borders (**a**) and small cell cytology with numerous mitotic figures (**b**) (hematoxylin and eosin staining; original magnification: **a** 4×, **b** 20×)

A reasonable antibody panel reacting to low and high molecular weight cytokeratins, TTF1, p40, chromogranin A, synaptophysin, retinoblastoma, CD56, NUT protein, and Ki-67 is useful to confirm SCLC diagnosis the morphological impression of facing with SCLC [110]. Low molecular weight cytokeratins highlight epithelial differentiation of tumor cells, with either paranuclear dot-like or cytoplasmic diffuse staining pattern, while high molecular weight cytokeratins or p40 but not p63 are always negative if not in the event of combined variant with squamous cell carcinoma [113, 142]. Pan-NE markers are consistently positive in 85–90% of SCLC, especially synaptophysin and CD56, whereas chromogranin A may be so faint and scattered to require close observation at high power magnification. CD56 is very sensitive in recognizing SCLC [143], but its lack of specificity toward unrelated neoplasms (e.g., small cell sarcomas, melanoma, or NUT carcinoma) obliges a cautious interpretation on the basis of the proper clinical and morphological context. About 10–15% of SCLC may lack overt NE differentiation likely due to different cell lineage derivations as assessed on the basis of differential gene expression:

yes-associated protein 1-SCLC (SCLC-Y) and POU class 2 homeobox 3-SCLC (SCLC-P), both lacking insulinoma-associated protein 1 (INSM1), an early embryonic inducer of NE differentiation, with SCLC-P recapitulating an expression profile closely resembling the rare pulmonary chemosensory tuft cells [144]. These SCLC missing NE differentiation have been called variant subtypes (not to confound with combined variant of SCLC), which are characterized by epithelial-to-mesenchymal transition leading to vimentin accumulation and lack of cytokeratin filaments [145]. At least the SCLC-Y phenotype was found to be associated with shorter patient survival and increased chemoresistance, while the clinical outcomes for SCLC-P patients have not been well defined [146]. INSM1 is accumulated in the nuclei of most SCLC apart from SCLC-P and SCLC-Y and seems the most specific marker, but its sensitivity is not superior to composite marker CD56 plus TTF1 and p16 [147]. It has been proposed, in the appropriate clinical and morphological context, a diagnostic algorithm comprising at first INSM1, then CD56, and lastly p16 and TTF1, in that order, if all previously applied markers were negative. In any case, the lack of NE markers or even cytokeratin filaments should not prevent performing diagnosis of SCLC, provided other alternatives have been reasonably ruled out according to the proper clinical and morphological context. TTF1 reactivity is found in about 90% of SCLC, but its expression is not related to the pulmonary lineage establishment, inasmuch as most extrapulmonary small cell carcinomas are also consistently positive for this marker [148]. TTF1 expression in SCLC is related to the activation of the achaete-scute family bHLH transcription factor 1 (hASH1, product of *ASCL1* gene)/TTF1/ nuclear factor IB (NFIB) axis that potentially contributes to the tumorigenesis and metastatic potential of most SCLC [149] (Fig. 9.13). TTF1 closely correlates with NE differentiation the inhibitory Notch ligand Delta-like protein 3 (DLL3) expression especially in the ASCL1-positive SCLC subset (SCLC-A), which account for at least 70–80% of all SCLC [144]. The truncated form p40 (DNp63) of *p63* gene is consistently negative in SCLC and in general neuroendocrine tumors as a whole, thus making this marker a useful tool in the differential diagnosis with basaloid and nonkeratinizing squamous cell

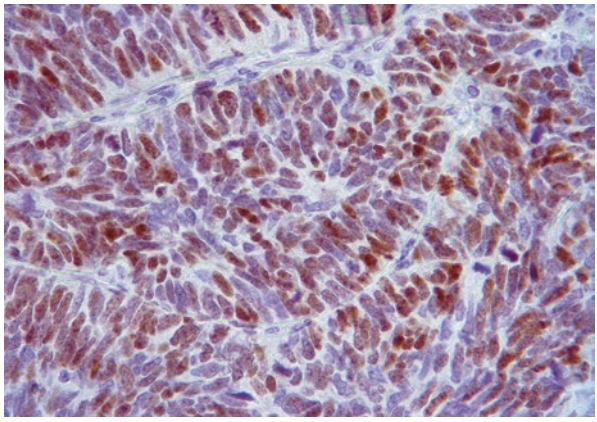

Fig. 9.13 Nuclear staining for hASH-1 in small cell lung carcinoma (immunoperoxidase; original magnification 40×)

carcinomas, which turn out strongly positive for p40 even in biopsy/cytology samples [150]. Retinoblastoma is frequently lost in classical SCLC while is strongly expressed in most NSCLC, but is retained in the variant subtype of SCLC with intermediate to large cell morphology (e.g., SCLC-P) where it is linked to decreased patient survival and increased chemo-refractory tumor response. The antigen Ki-67 is diffusely expressed in SCLC as one would expect from a highly proliferating tumor, with positivity rates approaching 100% [6], even though may sometimes present with some heterogeneity in intratumor distribution that is likely to play some role in histogenesis and pathogenesis of SCLC. Although Ki-67 is not per se diagnostic of SCLC outside its appropriate morphological context, the consistent huge positivity in either classical or variant subtype of SCLC makes Ki-67 a practical marker in the differential diagnosis from low- to intermediate-grade NE tumors (i.e., carcinoids) especially in the setting of limited and/or crushed diagnostic material as seen in biopsy or cytology samples to avoid major pitfalls in the management of patients [136]. In this type of material, beyond carcinoids, SCLC should be differentiated from reactive or neoplastic lymphocytic proliferations, Merkel carcinoma, Ewing sarcoma family tumors (ESFT), and even small cell melanoma. An integration of clinical data with an antibody panel approach including cytokeratins (including cytokeratin 20 for Merkel cell carcinoma), polyomavirus, neuroendocrine markers, CD99 (for ESFT), leukocyte common antigen (for lymphomas), S100 protein/HMB45 (for melanoma) and, if needed, fluorescence in situ hybridization for the relevant gene translocations are fruitful tools in this scenario. An IHC tool that never should miss in the antibody panel approach to SCLC is the nuclear-in-testis (NUT) protein, whose expression in the totality of tumor cells is diagnostic of NUT carcinoma, a rare but deadly form of lung cancer [151]. This tumor, which shows different histologic features and challenging expression profiles, including neuroendocrine differentiation and small blue round cell tumor appearance [152], should always be comprised among diagnostic options while examining small round cell tumors. Differentiating SCLC from LCNEC may be difficult and to some extent a subjective exercise, but is largely based on cytological criteria, such as larger nucleoli, smaller cell size, and lower nuclear-to-cytoplasmic ratio in LCNEC. A panel of three antibodies (BAI3, CDX-2, and VIL1) has been proposed as a useful adjunct to distinguish SCLC (more positive for BAI) from LCNEC (more positive for CDX-2 and VIL1) [153]. Retinoblastoma protein is preserved in about 50% of LCNEC along with cyclin D1 overexpression and p16 loss as opposed to SCLC displaying loss of retinoblastoma and cyclin D1 and hyperproduction of p16 [154] at least in its classical and more frequent form displaying neuroendocrine differentiation (SCLC-A and SCLC-N).

Combined Neuroendocrine-Non-neuroendocrine Carcinoma

Combined variants of LCNEC and SCLC refer to the presence of any other non-neuroendocrine tumor component, such as adenocarcinoma, squamous cell carcinoma, or giant/spindle cell carcinoma [6, 155], for which no cutoff is required for

the non-neuroendocrine components because they are easily recognizable as such, even if IHC characterization may help in diagnosis [156] (Fig. 9.14). However, for SCLC a 10% cutoff is required for LCNEC (see above) or large cell carcinoma to subclassify SCLC as combined variant according to combined (separate/juxtaposed) or composite (intermingled) manners due to the continuity in cell size and nuclear chromatin changes. Combined variant is rare in LCNEC but accounts up to one third of SCLC. In contrast, carcinoid tumors combined with non-small cell lung carcinomas are very rare and supposed to be collision tumors, rather than sharing a common clonal origin, although this remains to be proven by molecular studies since anecdotal cases have been reported sharing a common genetical profile [157]. The neuroendocrine and non-neuroendocrine cell population of combined carcinomas has the same immune-profile as their pure counterparts with regard to the expression of neuroendocrine and lineage-specific markers.

Combined variants of LCNEC and SCLC share the same epidemiology, clinical presentation, prognosis, and neuroendocrine properties as their pure counterparts

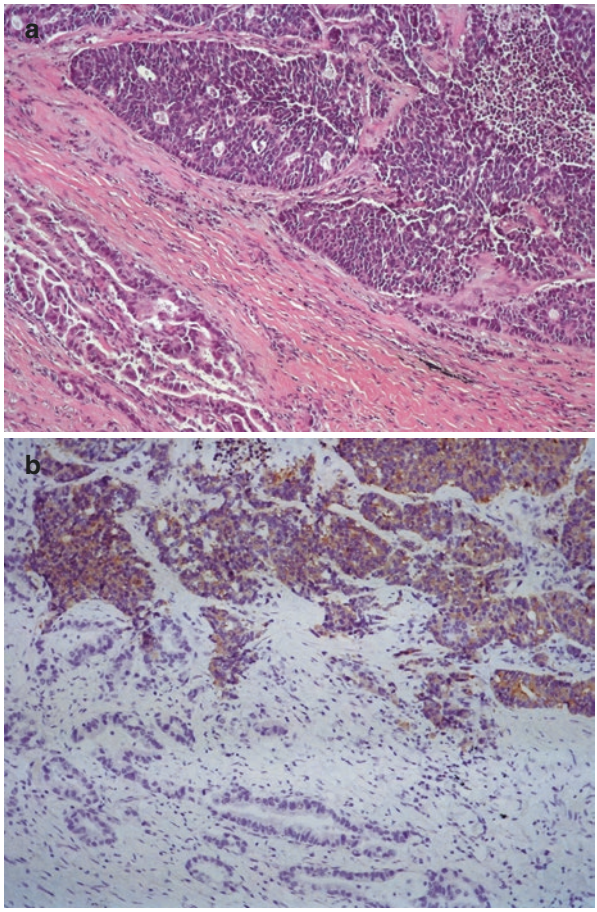

Fig. 9.14 A case of combined lung carcinoma, with acinar adenocarcinoma component and large cell neuroendocrine carcinoma component with necrosis (**a**) and synaptophysin staining (**b**). (**a**, hematoxylin and eosin staining; **b**, immunoperoxidase; all original magnifications 20×)

even if it has been suggested that combined SCLC could have a worse prognosis than pure SCLC, possibly because of a relative chemoresistance of non-SCLC components, which could emerge after therapy on recurrent or metastatic tumors.

Combined variants of LCNEC and SCLC may arise de novo or being the consequence – in the cases of an associated adenocarcinoma component – of histologic transformation as a mechanism of acquired resistance after epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) treatment [158], although data are still scarce and limited to very small series. In both these situations, the genomic alterations in neuroendocrine and non-neuroendocrine components of combined carcinomas are mostly homogeneous, with a high prevalence of *TP53* and *RB1* mutations in the non-neuroendocrine population [159]. Interestingly, mixed high-grade neuroendocrine carcinomas with non-neuroendocrine components (including those of the lung), when studied for regulators of DNA synthesis, repair, or recombination and chromosome disentanglement (such as ribonucleotide reductase, DNA excision repair protein ERCC-1, topoisomerase II-A, and thymidylate synthase), did not show differences for all genes but Topo-IIA between both components, with the thymidylate synthase content, predominant non-NE component, and chemotherapy acting as independent predictors for better prognosis [160].

Thymic Neuroendocrine Neoplasms (T-NENs)

Epidemiology

Neuroendocrine neoplasms of the thymus (T-NENs) make up a heterogeneous family of uncommon middle-aged mediastinal neoplasms accounting for 2–5% of all thymus tumors [161, 162]. T-NENs are classified according the same terminology as the homonymous neoplasms of the lung, i.e., typical carcinoid (TC), atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC), and small cell carcinoma (SCC), and the same unbiased diagnostic criteria, i.e., number of mitoses per 2 mm^2, presence and extent of necrosis, and a constellation of morphologic and immunohistochemical features [163]. However, their biological behavior is quite different, at least for well-differentiated tumors, including TC and AC, which behave on average more aggressively than their pulmonary counterpart (Table 9.2). Pediatric

Table 9.2 Comparative features of carcinoids of lung and thymus

		Lung	Thymus
Age (mean)		40–60 years	45 years
M/F		1:2	3:1
Clinical syndrome	*Cushing*	2%	30–40%
	MEN1	10%	25%
Tumor size (mean)		3 cm	8–10 cm
Histotype	*Typical carcinoid*	90%	10%
	Atypical carcinoid	10%	90%
Lymph node metastases		5–10%	30–45%

and young people instances, mostly but not always belonging to the category of carcinoids, have also been recorded, but most of T-NENs affect adults or elderly patients. T-NENs are not strictly analogous tumors to the pulmonary counterpart, because they present a larger prevalence of AC and LCNEC over TC and SCC [164], a higher association rate with ectopic adrenocorticotropic hormone Cushing's syndrome [165], a more variable dependency on smoking also in female and MEN1 patients [166], and a lower MEN1 genotype-phenotype correlation suggesting the involvement of other genetic factors [167]. As a matter of fact, about one fourth of patients with T-NENs is MEN1-related as opposed to 1–8% of patients bearing such a syndrome who develop T-NENs during life [168]. Most MEN1-related T-NENs correspond histologically to carcinoids, but even poorly differentiated NE carcinomas or purported carcinoids with gross areas of necrosis have been recorded. LCNEC and SCC account for about 15–35% of all T-NENs, with a relative prevalence rate of LCNEC over SCC. Risk factors are largely unknown, inasmuch as high-grade NENs of the thymus are not associated with MEN1 syndrome. It has been estimated that LCNEC and SCC have an incidence of 1 case/20 million individuals and 1 case/50 million individuals, respectively, testifying their substantial rarity as compared with the corresponding neuroendocrine carcinomas of the lung.

Gross, Clinical Presentation and Imaging

Carcinoids usually present with space-occupying mass causing local symptoms (pain, cough, superior vena cava syndrome) to arise according to mediastinal tissue infiltration. Systemic symptoms due to paraneoplastic syndromes are most often due to ectopic hormone secretion, such as Cushing's syndrome (ectopic adrenocorticotropic hormone) [169], hypercalcemia/hypophosphatemia (parathyroid hormone-related protein) [170], acromegaly (antidiuretic hormone or atrial natriuretic peptide) [171], or, exceptionally, carcinoid syndrome (serotonin and other peptides) [161], but paraneoplastic limbic encephalitis [172] and late-onset myasthenia gravis [173] are also on record in thymus carcinoids. TC are unencapsulated and calcified lesions, either circumscribed or locally invasive, while AC are locally infiltrating and metastasizing tumors in most cases. When compared with their pulmonary counterpart, thymus carcinoids present with no significant differences between them in major risk factors, a male preponderance, difficult (delayed) preoperative diagnosis, a higher rate of lymph node and distant metastasis, a larger tumor size on average (delayed detection), low postoperative survival, and a lower rate of carcinoid syndrome as opposed to a higher rate of association of Cushing's syndrome. LCNEC and SCC are detected owing to local symptoms due to infiltration (lung, pericardium, major vessels) or occurrence of distant metastases (bone, liver, lung, brain, adrenal glands, lymph nodes) at the time of clinical presentation. Computed tomography, magnetic resonance, and ^{18}F-fluorodeoxyglucose positron emission tomography imaging play a major role in the identification, staging, preoperative biopsy planning, and follow-up monitoring of thymic epithelial neoplasms, including T-NENs: Furthermore, scintigraphy techniques based on the

bioavailability of somatostatin receptors have been developed in T-NENs by using (68)Ga-DOTA-TOC PET/CT, 111In-OctreoScan, or 99mTc-EDDA/HYNIC-octreotate. LCNEC and SCC have no particular gross presentation, which is the same as in other T-NENs in the form of variably sized (up to 10 cm or more) tumors, usually without the characteristic lobulated growth pattern of thymomas. Of note, cases associated with Cushing's syndrome tend to be smaller likely due to their earlier detection. Cytological criteria do not distinguish TC and AC, which in both instances present as round to oval cells, either single or in small clusters, with scanty cytoplasm, interspersed with some larger cells with moderate to abundant, granular cytoplasm [174]. On cytological grounds, it is not possible to separate TC from AC, while defining criteria for SCC are the same as the pulmonary counterpart with common crush artifacts, nuclear breakdown, and apoptotic bodies. There are no established cytological criteria for thymus LCNEC due to either their rarity or similarities of findings with other T-NENs or more common thymic epithelial cell tumors. From a clinical perspective, TC and AC are low- to intermediate-grade and well-differentiated tumors, while LCNEC and SCC high-grade tumors with similar dismal prognosis. The 10-year actuarial survival rates are 77.92% (median survival 126 months) for TC, 54.55% (median survival 52 months) for AC, and nihil for LCNEC or SCC [161]. As compared with thymic carcinomas, thymic carcinoids show no substantial prognostic differences [175], with younger patients, completeness of resection, adjuvant radiotherapy, no adjuvant chemotherapy, and TNM stage being independent predictors of better overall and/or disease-free survival.

Histopathology

Defining diagnostic criteria for T-NENs settled by 2015 WHO classification are the same as the pulmonary counterparts. The descriptive terms of well-differentiated neuroendocrine carcinoma to indicate carcinoids (Fig. 9.15), either TC or AC, and poorly differentiated neuroendocrine carcinoma to refer to LCNEC and SCC, as stated in the 2004 WHO classifications [19], have been abandoned in the new 4th edition of 2015, inasmuch as LCNEC and even SCC may be highly differentiated in terms of neuroendocrine features. In a perspective of clinical behavior in the decision-making process, TC are considered low-grade tumors, AC intermediate-grade tumors, and the group of LCNEC and SCC high-grade tumors or neuroendocrine carcinomas [163]. Histologically, TC are characterized by less than 2 mitoses per 2 mm^2 and no necrosis, with different growth patterns (trabecular, resetting, lobulated, solid, pseudoglandular, gyriform, festooned) and histologic variants (spindle cell, pigmented, oncocytic, amyloid stroma, angiomatoid), which do not impact on tumor behavior and can be disregarded in a clinical perspective provided that defining criteria are strictly respected but should be accounted for in the differential diagnosis. AC share the same architectural features as TC, with the differences consisting in higher mitotic count (2–10 mitoses per 2 mm^2) and occurrence of even small punctate foci of necrosis. Nuclear pleomorphism may be observed, along with calcifications, diffuse growth pattern, or extensive desmoplastic stroma

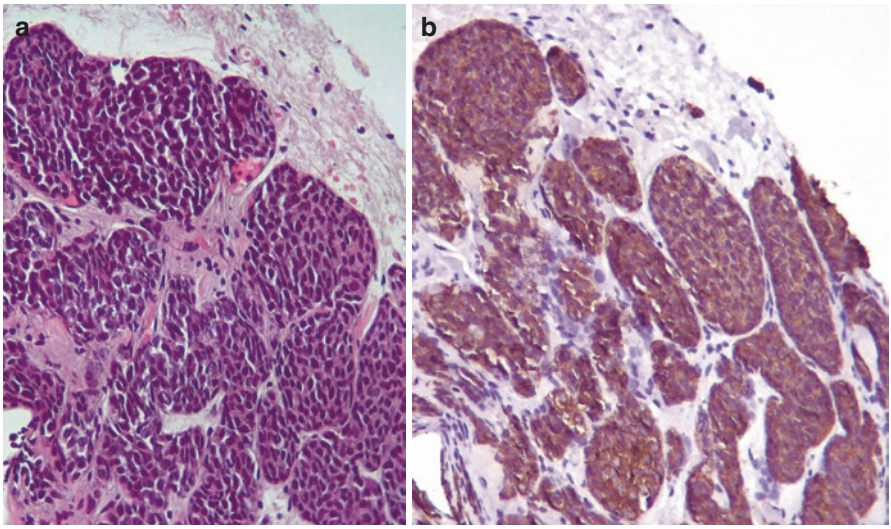

Fig. 9.15 Mediastinoscopic biopsy of a thymic carcinoid with insular arrangement (**a**) and diffuse chromogranin A immune-labeling (**b**). (**a**, hematoxylin and eosin staining; **b**, immunoperoxidase; all original magnifications 20×)

with Indian-file arrangement of tumor cells, which can be relevant to differential diagnosis [164]. The main differential diagnoses of carcinoids include spindle cell type A thymoma (missing diffuse neuroendocrine marker decoration), parasympathetic paraganglioma (missing cytokeratins) [176], extrathyroidal medullary carcinoma in amyloid-rich carcinoid (strong reactivity for calcitonin and carcinoembryonic antigen), metastatic mucinous carcinoma in mucinous carcinoid (missing neuroendocrine markers), and hemangioma in the angiomatoid variant of thymus carcinoid with pseudovascular spaces lined by tumor cells [177].

LCNEC exhibit non-small cell morphology with large tumor cell size, a mitotic rate by far exceeding 10 mitoses per 2 mm^2 (on average 45 mitoses) and extensive necrosis. Some tumors look like AC in terms of general architecture and cell morphology, but differ from them for having too many mitoses and more necrosis [178]. LCNEC co-express epithelial (cytokeratins, often with dot-like staining pattern) and neuroendocrine markers (usually in more than 50% tumor cells and with clear-cut decoration) alongside CD117, TTF1 and, rarely, CD5. The main differential diagnosis of LCNEC is toward thymic carcinomas, which can share reactivity for neuroendocrine markers, usually fainter and focal, more consistent CD5 and CD117 immunoreactivity and extensive positivity for p40, which is always missing in LCNEC. SCC appearance in the thymus is identical to that of the homologous tumors arising anywhere, especially in the pulmonary counterpart. In this regard, TTF1 is not helpful in the differential diagnosis, since it is frequently positive even in extrapulmonary neuroendocrine carcinomas [148]. Therefore, SCC remains basically a histological diagnosis, where expression of neuroendocrine markers is often detectable but not strictly required for the ultimate diagnosis to do. At variance, in

LCNEC the demonstration of neuroendocrine markers is tautologically required for diagnosis, once other histologic mimickers of small blue round cell tumors, either primary or secondary, have been convincingly ruled out. In SCC, mitoses exceed by far the number of 10 per 2 mm^2 (on average, there are 110 mitoses per 2 mm^2) along with small cell morphology (typically less than three times the size of a small resting lymphocyte) and extensive or geographic necrosis. Tumor cells are round to oval or spindle, with evenly distributed chromatin, inconspicuous nucleoli, nuclear molding, and plentiful apoptotic bodies. Most SCCs in the thymus stain for cytokeratins, but negative cases make its separation from other small blue round cell tumors particularly challenging. SCCs are consistently negative for p40, as usually happens for T-NENs. The main differential diagnosis is to distinguish thymus primaries from pulmonary small cell carcinoma, for which an accurate clinicpathologic and imaging correlation is required.

Immunohistochemical Profile

On immunohistochemistry grounds, carcinoids of the thymus exhibit reactivity for epithelial markers (cytokeratins), often with dot-like, paranuclear labeling pattern. Neuroendocrine markers are strongly expressed in TC, with more focal or dispersed distribution in AC [179]. Hormones, such as ACTH, human chorionic gonadotropin, or calcitonin) may be detected in carcinoids of the thymus, usually in a limited amount of tumor cells with no relationship with clinical symptoms of paraneoplastic syndromes. The differentiation of lung and thymus carcinoids proves to be particularly challenging in the setting of low- to intermediate-grade tumors displaying large unresectable or metastatic lesions at the time of diagnosis. TTF1 is a useful marker of pulmonary lineage only when positive in the group of well-differentiated NETs. In this regard, some T-NETs may be reactive for TTF1 even when using the most specific clone 8G7G3/1; thus, TTF1 may not be a reliable maker to exclude the thymic origin in thoracic well-differentiated NETs [180]. Reactivity for PAX-8 in thymus carcinoids helps to differ them from the pulmonary counterpart.

Origin of T-NENs and Combined Tumors

The origin of T-NET is unclear, but evolutionarily conserved neuroendocrine-committed thymus epithelial cells have been detected in the subcapsular region, cortex, and medulla of the thymus gland of reptiles, birds, mice, and humans [181]. Interestingly, subsets of thymus epithelial cells express a variety of neuroendocrine self-proteins belonging to neurohypophysis (oxytocin), tachykinin (neurokinin A), and insulin (IGF1, IGF2, insulin) family peptides, which are likely to be engaged in the self-recognition for immune-tolerance of T lymphocytes toward endocrine organs [182]. Furthermore, ACTH-immunoreactive thymus epithelial cells have been unveiled in the subcapsular region, cortex, and medulla of the human thymus gland [183]. Beyond T-NENs, neuroendocrine differentiation has also been

documented in tumors with no clear-cut neuroendocrine morphology, such as thymic squamous cell carcinoma [184] and, more rarely, thymoma [185], although this finding does not bear direct clinical implications on tumor behavior. These findings, however, account for the great plasticity of thymus epithelial cell ancestors of endoderm derivation, which are also likely to be involved in the development of combined tumors in keeping with similar phenomena occurring in lung NENs. The current 2015 WHO classification identified combined thymic carcinoma as any thymic carcinoma associated with any thymoma or carcinoid, thus excluding SCC and LCNEC. The most frequent combination is thymus squamous cell carcinoma and type B3 thymoma, but also papillary adenocarcinoma or sarcomatoid carcinoma in addition to type A thymoma has been recorded, while combination of different subtypes of thymic carcinomas with each other is quite rare. At variance with lung NENs, where carcinoids are exceptionally found along with non-small cell carcinomas, in the thymus it is possible to face with such a combination of carcinoids with thymoma, thymic carcinoma, or sarcoma-like elements of whatever size or percentage [186]. These combined thymic carcinomas should be listed in their components in 10% increments, starting from the predominant one. Moreover, in the setting of combined thymic carcinomas, associations of LCNEC or SCC with any other thymoma and/or thymic carcinoma are also on record, which yet are considered combined variants of either tumor type, featuring gradual transition or sharp separation from each other. These cases should be listed in their components, but their behavior is expected to be as aggressive as the homologous pulmonary tumors. T-NENs comprising transition forms between TC/AC and LCNEC/SCC within individual tumors have been documented in the past, but have remained an orphan category with only descriptive terminologies being reported on. Diversely graded T-NETs have been interpreted as high-grade NE carcinoma evolving from preexisting carcinoids rather than chance or collision tumors [187].

Molecular Pathology

Inheritance

Most NENs are sporadic in their distribution, in either the lung or the thymus, but about 10% of them are familial or inherited. The most common inherited genetic syndrome underlying NENs development is MEN1 [188], but familial carcinoid tumor syndromes due to rare germline mutation other than MEN1 have been reported in the lung [189]. Likewise, in T-NENs there is a lower MEN1 genotype-phenotype correlation suggesting the involvement of other genetic factors [190]. As a matter of fact, about one fourth of patients with T-NENs is MEN1-related [166] as opposed to 1–8% of patients bearing such a syndrome who develop T-NENs during life. Approximately 50% of patients from MEN1 families will develop the syndrome and the distribution between genders is equal, suggesting an autosomal dominant trait. MEN1 syndrome is due to inactivating mutations (over 1300 different mutations are known) of the tumor suppressor gene *MEN1* mapping to 11q13.1,

whose scaffold protein menin functions in chromatin remodeling through histone modification and epigenetic gene regulation via binding to and inhibition of JunD's (an AP-1 transcription factor) activation of transcription (https://www.ncbi.nlm.nih.gov/gene/4221). About 30–60% of patients bearing MEN1 germline mutations is destined to develop endocrine-neuroendocrine tumors (17% of whom before aging 21 years) [191], which affect the pancreas, parathyroid glands, hypophysis, lung, thymus, thyroid, adrenal glands, and ovaries, beyond meningioma, facial angiofibroma, collagenoma, and lipoma. Less common than the MEN1 syndrome is the von Hippel-Lindau disease (VHL), a dominantly inherited familial cancer syndrome whose germline mutations predispose to a variety of malignant and benign lesions, including hemangioblastomas of the central nervous system, renal clear cell carcinoma, pheochromocytoma, endolymphatic sac tumors, and pancreatic, renal, epididymal, and broad ligament cysts. The VHL gene product encodes protein VHL, which binds to elongin C, elongin B, cullin-2, and Rbx1 to form a complex catalyzing the polyubiquitinylation of specific proteins and targeting them for degradation by proteasomes (https://www.genecards.org/cgi-bin/carddisp.pl?gene=VHL). Neuroendocrine tumors usually affect the pancreas, while pulmonary carcinoids are quite uncommon in VHL [192] and thus far undescribed in the thymus. Neurofibromatosis type 1, inherited as autosomal dominant trait with biallelic inactivation of NF1 gene mapping to 17q11.2 that functions as negative regulator of the RAS signal transduction pathway (https://www.ncbi.nlm.nih.gov/gene/4763), is rarely associated with the development of carcinoids in the thymus while missing in the lung [193]. Tuberous sclerosis complex (TSC) is due to mutations in either the TSC1 or TSC2 gene, which map to 9q34.13 and 16p13.3, respectively, and regulate mammalian target of rapamycin complex 1 (mTORC1) signaling via stimulation of specific GTPases (https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=7248). Although TSC has not been linked to the development of hereditary carcinoids in either the lung or the thymus, somatic mutations of TSC1 or TSC2 genes with upregulation of p-mTOR and ribosomal p70S6-kinase (S6K) indicating PI3K/AKT/mTOR pathway activation have been yet observed in pulmonary carcinoids [194]. Interestingly, high-grade neuroendocrine carcinomas are not components of MEN1, VHL, or NF1 syndromes, but somatic MEN1 mutations have been identified in carcinoid-looking LCNEC and even SCC along with upregulation of eukaryotic initiation factor 4E-binding protein 1 (4EBP1), a downstream activator of mTOR pathway, indicating that this pathway can be engaged even in this subset of patients [195].

Molecular Classification

The current interpretation of lung NEN pathogenesis supports the view that there are major differences in gene alterations between TC/AC on the one hand and SCLC/LCNEC on the other hand, with minor or no differences inside each tumor group. In other words, there should be a close relationship between morphology and underlying molecular alterations making the spectrum of lung NENs a

clinical-pathological but not a pathogenetic one. As a matter of fact, when comparison is performed by means of morphology-based supervised analysis, a statistically significant separation is obtained among the diverse categories of lung NENs in terms of gene mutation and copy number variation (CNV) distribution [196].

In the lung, our current knowledge says that TC and AC show very low mutation rates and recurrent alterations in mechanisms of epigenetic regulation (chromatin remodeling, SWI/SNF complex-dependent DNA packaging, histone methylation and acetylation), with no relevant histology-dependent differences to support a causal relationship of at least some TC with the development of AC. Recurrently altered in carcinoids are chromatin remodeling genes, such as *MEN1*, *PSIP1,* and *ARID1A, with MEN1* mutations also bearing poor prognosis in the setting of AC [197]. Intra- or intertumor heterogeneity of carcinoids is a poorly explored issue due to their relative rarity and reduced metastatic potential at presentation, but incremental proliferation rates have been documented at metastatic sites in the lung with retention [80] of RB1 expression and carcinoid morphology. Conversely, SCLC exhibit high mutation rates and recurrent mutations/deletions in cell cycle regulators (especially *TP53* and *RB1*), chromatin remodeling (*CREBBP*, *EP300, MLL*), copy number variations (MYC family, *FHIT*, *SOX2*, *FGFR1*), somatic genomic rearrangement (*TP73*), and alterations in mechanisms of neuroendocrine differentiation (NOTCH family), with *KMT2D* gene (a histone modifier) mutations correlating with longer survival [196, 198]. In turn, LCNEC share with SCLC the highest mutation rates ever seen in pulmonary NENs, but make up the most heterogeneous tumors on molecular grounds, with some of them resembling carcinoids, some overlapping with SCLC, and some linking to NSCLC (especially adenocarcinoma but also squamous cell carcinoma) on the basis of their patterns of gene alterations. A recent study by George et al. on 75 cases of LCNEC found three main molecular subgroups: one resembling SCLC different from LCNEC type I and LCNEC type II groups. LCNEC type I presented with high neuroendocrine expression (ASCL1^{high}/DLL3^{high}/NOTCH^{low}) and *TP53* mutation similar to SCLC group but with additional *STK11/KEAP1* mutations and lack of *RB1* inactivation; and LCNEC type II, with low neuroendocrine expression (ASCL1^{low}/DLL3^{low}/NOTCH^{high}), combined *TP53* and *RB1* mutations and an upregulation of immune-related pathways [141]. Similarly, Simbolo et al. performed a comparative analysis of AC and LCNEC by means of next-generation sequencing alongside immunohistochemistry for menin and RB1 protein [199]. Transcriptomic and genomic investigation distinguished three separate clusters: (a) cluster 1 showed a large prevalence of LCNEC along with *TP53* and *RB1* gene inactivation while missing MEN1 mutations and Rb1 protein; (b) cluster 3 included especially AC with *RB1*, *MEN1,* and *TP53* mutations while missing menin and RB1; and (c) cluster 2 comprised slightly more AC than LCNEC with intermediate molecular findings. Expectedly, cluster 1 patients run a worse clinical course than the other two ones. These two studies not only support molecular classifications, which are quite independent of morphology but clinically relevant to targeted therapy, but also suggest models of malignancy progression from carcinoids to LCNEC, which are likely to depend on common risk factors.

About 10–15% of human SCLC, SCLC cell lines, and genetically engineered mouse models lack, or express at low levels, neuroendocrine markers: they have been called the "variant subtype of SCLC" with downregulation of neuroendocrine differentiation. A recent reappraisal of SCLC has identified four different subsets of patients according to their molecular profiles: SCLC-A (expressing achaete-scute homologue 1, ASCL1) and SCLC-N (expressing neurogenic differentiation factor 1, NeuroD1) are the neuroendocrine-differentiated forms of SCLC, while SCLC-Y (expressing yes-associated protein 1, YAP1) and SCLC-P (expressing POU class 2 homeobox 3, POU2F3) are the non-neuroendocrine-differentiated ones corresponding to the variant subtype [144]. The first two categories of SCLC make up about 80–85% of all SCLC, with SCLC-Y as the least frequent one with about a 2% prevalence, but virtually all SCLC would be composed of multiple subtypes revealing a still unexplored intratumor heterogeneity. Variant subtypes are characterized by intermediate cells, sometimes resembling NSCLC or LCNEC; downregulation of *TTF1 and DLL3;* upregulation of *REST*, NOTCH, and Hippo/TGFβ pathway; and MYC amplification, with vimentin-expressing epithelial-mesenchymal transition. These phenotype patients have a poorer response to chemoradiotherapy with shorter patient survival and increased chemoresistance (especially SCLC-Y) but vulnerability to Aurora kinase inhibitors as compared to the high-neuroendocrine classical SCLC counterparts [200]. Transformation of high-neuroendocrine classic subtype to low-neuroendocrine variants has been described upon MYC amplification leading to NOTCH pathway and REST activation in tumor cell subsets, which act as transcriptional repressors of neuroendocrine gene expression. These pathways provide a trophic/feeding microenvironment to classical SCLC cells and reveal a high plasticity of cancer stem cells, with a pro-tumorigenic role in the development of SCLC and, to some extent, a linking to NSCLC precursors [201]. Of note, while intra-/intertumor NSCLC genomic heterogeneity resulting from branching evolution is a well-known phenomenon responsible for acquired resistance to targeted treatments, SCLC usually maintain most of mutations in both primary and metastatic foci suggesting a different and linear model of evolution [202]. Many genetic alterations affecting lung NENs involve mechanisms of chromatin opening or gene transcription regulation, such as DNA methylation, histone deacetylation and deubiquitination, and miRNA up-/down-expression. These events include promoter hypermethylation of *RASSF1A* (paralleling tumor grade) [203] and *P15INK4b* [204]; histone modifications by downregulation of H4KM20 and H4KA16 [205]; upregulation of microRNA-129, microRNA-323-3p, microRNA-487b, microRNA-410, microRNA-369-3p, and microRNA-376a; and downregulation of miR-203, miR-224, miR-155, miR-302, miR-34b, miR-181b, miR-193a, miR-5p, and miR-34b [206].

The molecular landscape of T-NENs is largely unknown, but several chromosomal imbalances and aneuploidy status were found in 51–81% and 12% of instances, respectively [207]. Chromosomal losses and gains are differentially distributed among the diverse subtypes of T-NENs, with imbalances per tumor averaging 0.8 in TC (31% aberrant cases), 1.1 in AC (44% aberrant cases), and 4.7 in the LCNEC/SCLC group (75% aberrant cases) [208]. The most frequent overlapping

alteration across histologic variants maps to *MYC* locus-containing 8q24, a downstream target of ß-catenin involved in the development of some thymic and pulmonary neuroendocrine carcinomas. These findings support the current view that TC/AC are different and separate tumor entities with their own specific molecular drivers as opposed to LCNEC/SCC, when tumor separation of T-NENs is accomplished by using the 2015 WHO defining criteria. However, a recent low-coverage whole-genome sequencing study dealing with 63 T-NENs belonging to all different histologic subtypes has found that molecular classification by means of copy number instability (CNI) scores was prognostically effective to identify three tumor categories, somewhat independent of morphology [209]. Moreover, there was a subgroup of tumors fulfilling criteria for LCNEC, which featured carcinoid morphology, strong expression of neuroendocrine markers, Ki-67 averaged 29.5%, and negativity for p53 and enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), a promoter of cell cycle and apoptosis inhibition in SCLC models [210]. This subgroup was defined NET G3, in keeping with homologous lesions recently described in pulmonary and gastroenteropancreatic NENs, where progression of malignancy from low-grade to high-grade histology was noted between primary tumors and paired metastases. Accordingly, a morpho-molecular grading system was devised for better patient stratification and prognostication by identifying T-NET G1 to G3 based on an integrated evaluation of scored CNI and immunohistochemistry findings (Ki-67, chromogranin A, and EZH2 staining).

Novel Insights on the Molecular Pathways of Progression

Data are emerging that at least a certain number of NENs in both the lung and the thymus can take rise from progression of low-grade tumors to neuroendocrine carcinomas/high-grade tumors. This phenomenon holds particularly true in tumor patients undergoing surgical resection, probably because lesions amenable of surgery at presentation are inherently less aggressive. Accordingly, it is expected that resection specimens will capture in large majority TC and most of AC, presumably many LCNEC but only a minority of SCLC. The occurrence of common genetic traits shared by carcinoids and neuroendocrine carcinomas in the lung, thymus, and even gastroenteropancreatic tract supports secondary evolution of NETs to NECs in tumor resection specimens. On the basis of literature data reappraisal on NENs arising in the lung and the thymus, we have recently proposed an alternative and innovative interpretation by identifying a tripartite separation into early aggressive/primary high-grade neuroendocrine tumors (HGNETs), differentiating or secondary HGNETs, and indolent NETs [211].

- P-HGNETs (70–75% of lung NENs; 13% of lung tumors) are the most aggressive ones with widespread metastases at presentation, feature classical SCLC or variant subtype, are usually diagnosed on biopsies of male heavy smokers, present with minimal intertumor heterogeneity indicative of linear mechanisms of

evolution, and are characterized by biallelic inactivation of *TP53* and *RB1*. These tumors would originate through de novo or basal-like mechanisms of carcinogenesis with no intermediate/dysplastic lesions, deriving from cancer stem cells out of a neuroendocrine niche undergoing very early differentiation block. Ki-67 is uniformly high, even approaching 100%. These tumors exhibit high mutation burden.

- S-HGNETs (20–25% of lung NENs; 6% of lung tumors) are less aggressive tumors with longer survival; feature AC, LCNEC, or even SCLC; are usually diagnosed on resection specimens of male smokers; present with marked intertumor heterogeneity indicative of branching mechanisms of evolution; and comprise a variety of different molecular alterations even in common with conventional NSCLC (*TP53* and *RB1* mono-/biallelic inactivation, NOTCH inactivation, *KRAS/LKB1/MEN1* mutation, MYC family gene, *TERT*, *SDHA*, *RICTOR* amplification, and epithelial-mesenchymal transition). These tumors would originate from preexisting lesions (neuroendocrine cell hyperplasia/DIPNECH, neuroepithelial bodies, carcinoids, NSCLC) according to luminal-like mechanisms of sequential acquisition of gene alterations over time, with possibility of intermediate/dysplastic lesions. These tumors would originate from cancer stem cells within a neuroendocrine niche as carcinoids or their precursors or non-neuroendocrine cancer stem cells acquiring neuroendocrine differentiation as NSCLC. Ki-67 is typically heterogeneous within the tumor mass, ranging from 20–25% to 90% or more, with high staining areas intermingled with low staining areas. These tumors exhibit high mutation burden.
- Lastly, I-NETs (5% of lung NENs; 1% of lung tumors) are indolent behaving lesions with long-term survival, feature TC or low-mitotic count AC, are always diagnosed on resection specimens of female nonsmokers, occur in MEN1 or other inherited/familial syndromes, and comprise chromatin remodeling gene/epigenetic alteration mechanisms. These tumors are likely to derive from a neuroendocrine stem cell niche of preinvasive lesions (DIPNECH) through chromatin remodeling gene/epigenetic alteration mechanisms. Ki-67 is uniformly low, typically 10% or less, and these tumors exhibit low mutation burden.

The issue of malignancy progression or transition from low-grade to high-grade histology can be applied even to T-NENs, as previously suggested or more recently demonstrated by Dinter et al. who have identified three different T-NET clusters, independent of histology, for better patient stratification and prognostication, according to an integrated evaluation of scored CNI and immunohistochemistry findings (Ki-67, chromogranin A, and EZH2 staining) [209].

As morphology still remains the backbone of NEN classification but molecular profiling is getting increasingly relevant to clinics and Ki-67 plays an indubitable prognostic role, a morpho-molecular approach is useful in clinical practice by attributing relevance to Ki-67 in the decision-making process by increasing cutoff thresholds. We have recently investigated 16 primary carcinoids and 19 corresponding metastases, either synchronous or metachronous, for Ki-67 expression and

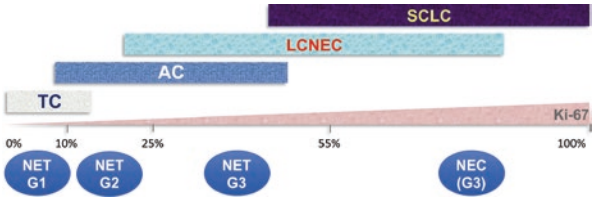

Fig. 9.16 The four different categories of thoracic neuroendocrine neoplasms itemized as NET G1, NET G2, NET G3, and NEC by including Ki-67 proliferation evaluation

morphological definition (TC vs. AC) according to different treatments [somatostatin analogue (SSA), mTOR inhibitor (mTOR-I, everolimus), and platinum and non-platinum chemotherapy)] [212]. Interestingly, survival curves of patients by different treatments paralleled the prediction of survival upon Ki-67 expression (cutoff thresholds 10% and 20%), while histology failed to a large extent, indicating that Ki-67 may have predictive value in lung NENs. By merging Ki-67 and histologic definition of lung NENs according to 2015 WHO classification, we proposed four different categories of tumors with different presentation and treatment options, itemized as NET G1, NET G2, NET G3, and NEC (tautologically G3). This proposal is outlined in Fig. 9.16.

- *Lung NET G1* include indolent behaving NENs with homogeneously low Ki-67 ≤10%, organoid pattern of growth featuring TC or low-mitotic count AC, and with treatment option in the metastatic setting of somatostatin analogues.
- *Lung NET G2* include low to moderate malignant NENs with slightly heterogeneous Ki-67 up to 25%, organoid pattern of growth featuring AC, some TC with "higher" Ki-67 or some carcinoid-like LCNEC, and with treatment options of SSA and/or mTOR-I and/or peptide receptor radionuclide therapy (PRRT) and/or non-platinum CT.
- *Lung NET G3* include moderate to higher malignant NENs with Ki-67 up to 55%; still organoid pattern of growth featuring some AC, carcinoid-like LCNEC, NSCLC-like LCNEC, and some SCLC; and with treatment options of non-platinum CT: alkylating agents, CAPTEM, and gemcitabine.
- *Lung NEC (G3)* include high malignant NENs with quite homogeneously distributed Ki-67 up to 100%; solid to diffuse pattern featuring SCLC, SCLC-like LCNEC, and some NSCLC-LCNEC; and with treatment options of platinum-based CT.

The 55% cutoff to separate, in the lung, NETs characterized by better prognosis but worse response rates to platinum from NECs characterized by worse prognosis but better response rates to platinum is the same as that applied in gastroenteropancreatic NENs to split the previous category of NEC into NET G3 (Ki-67 ranging from 20% to 55%) and NEC (Ki-67 > 55%) [213].

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