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## Divergent differentiation in neuroendocrine lung tumors

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The classification of neuroendocrine lung tumors has been revisited in the latest edition of *World Health Organization Histological Typing of Lung and Pleural Tumors* (1). The concept of neuroendocrine lung tumors has been refined by the recognition of large cell neuroendocrine carcinoma (LCNEO) and by the modification of the criteria for atypical carcinoids.

### Classification of neuroendocrine lung tumors Spectrum of neuroendocrine proliferations

Neuroendocrine lung tumors encompass one spectrum of a distinct subset of tumors, sharing certain morphological, ultrastructural, immunohistochemical and molecular characteristics, which sustain their neuroendocrine phenotype. Tumors with neuroendocrine morphology on light microscopy display organoid nesting, palisading, trabecular and rosette-like structures and include a three-grade spectrum of low-grade typical carcinoids, intermediate grade atypical carcinoid, high-grade LONEC and small cell lung carcinoma (SOLO). They all display the presence of neurosecretory granules at electron microscopy as well as immunohistochemical neuroendocrine markers, the most useful for pathological diagnosis being chromogranin, synaptophysin and neural cell adhesion molecules. The most important criteria for distinguishing between typical and atypical carcinoids and LONEC are mitotic activity and necrosis. A mitotic count of 2-10 mitosis per 2 mm<sup>2</sup>/10.HPF distinguishes atypical

from typical carcinoids, whereas a mitotic count of 11 or more mitoses per 2 mm<sup>2</sup> separates LONEC and SOLO from atypical carcinoids. The second important criteria is necrosis, which is absent in typical carcinoid focal or punctate in atypical carcinoid (eliminating typical carcinoid) and abundant and infarct-like in LONEC and SCLC.

In addition to these classical types of neuroendocrine lung tumors, the spectrum of neuroendocrine proliferations and neoplasms in the lung include a hyperplastic state: neuroendocrine cell hyperplasia and tumorlets occur in the context of associated fibrosis or chronic inflammation (bronchiectasia) or as a preinvasive state (provisional), occurring adjacent to carcinoids, or even isolated from them and called diffuse idiopathic neuroendocrine hyperplasia.

### Divergent differentiation in neuroendocrine lung tumors

Large cell neuroendocrine carcinoma is a category of tumors differing from carcinoids and small cell lung carcinoma but still showing neuroendocrine morphology and markers. These large-cell neuroendocrine carcinomas are the lung neuroendocrine tumors, which display the most frequent divergent differentiation (more than 50%). The most frequent type of divergent differentiation in large cell neuroendocrine carcinoma consists of glandular features with mucin secretion.

Apart from tumors with neuroendocrine morphology, which have been described and properly classified in the World Health Organization (WHO) classification, a subgroup of non-small-cell lung carcinoma (NSLCL) with neuroendocrine differentiation is recognized, although it is not considered as a separate category for histologic classification, essentially because of lack of clinical significance. This obviously defines a category of lung tumors (5-10%) which lacks neuroendocrine morphology but in which immunohistochemical (or ultrastructural) evidence of neuroendocrine differentiation can be demonstrated. A continuous spectrum of malignant tumors from pure LONEC and combined LONEC (with another non-component), to NSOLO with neuroendocrine differentiation emerges, which complicates tumor classification and diagnosis (2).

Obviously, neuroendocrine lung tumor does not escape from the paradigm of multidirectional differentiation, which was stressed long ago in lung tumors (3-9). Evidence of both epithelial (non-neuroendocrine) differentiation in classical neuroendocrine tumors and of neuroendocrine differentiation in non-neuroendocrine tumors has been provided. Mucins are identified in about half of all carcinoids and LONEC. Divergent non-neuroendocrine differentiation toward adenocarcinomatous or squamous differentiation occurs in SOLO spontaneously at the morphological level in a subset of 5% of SOLO (the so-called combined SOLO) and at the level of ultrastructure and immunohistochemistry (mucins and neurosecretory granules) in 20% of them. The increasing frequency of these divergent specializations in SOLO has been demonstrated after therapy (or by induction or selection mechanisms), which have made the post-therapy diagnosis difficult (10). Ultrastructural studies have demonstrated the presence of neuroendocrine granules in association with other specialized differentiation signs (mucus, microvilli, tonofilaments) in a large number of NSOLO and especially in large-cell (undifferentiated) carcinoma (11-13).

### A common endocrine stem cell for lung tumors

Finally, neuroendocrine lung tumors appear to be epithelial tumors characterized by their preferential neuroendocrine differentiation but susceptible to following multidirectional differentiation pathways, as indicated by the occurrence in the same tumor cell cytoplasm (although rare) of neuroendocrine granules, mucin granules,

microvilli and tonofilaments. The derivation of all lung cancer from a common endodermic stem cell is responsible for the frequent multidirectional differentiation in lung tumors, including neuroendocrine phenotype in NSCLC and squamous or glandular signs of differentiation in authenticated neuroendocrine tumors. Only three cell types are candidates for cell division and subsequent proliferation following injury: supra basal intermediate bronchial cells with small mucus granules, neuroendocrine cells and a type-II Clara cell. All three candidates themselves derive from a common endodermic totipotent stem cell which may select one or other differentiation pathway according to the set of genetic alterations which have led to its malignant transformation. The discovery that neuroendocrine cells in normal bronchial mucosa had acquired their APUD phenotype from endodermic stem cells, instead of being derived from the neural crest, helps to explain why several differentiation pathways are potentially available during clonal expansion and are simultaneously displayed in a subgroup of lung tumors.

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## Divergent differentiation in thyroid tumors

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Whenever one thinks of divergent differentiation in thyroid tumors, it is the so-called mixed medullary and follicular (or papillary) carcinoma that comes to mind. The reason for this stems from the well-known dichotomy between follicular and parafollicular cells or, in other words, from the existence in the thyroid of endocrine, thyroglobulin-positive tumors and neuroendocrine, calcitonin-positive tumors. The latter part of this short review will therefore be devoted to the discussion of the diagnosis and histogenesis of mixed endocrine-neuroendocrine carcinomas of the thyroid. In the first part we will 'swim against the stream' and discuss the divergent differentiation of tumors belonging to the group of thyroglobulin-positive thyroid carcinomas. Somewhat provocatively we will focus, respectively, on the follicular variant of papillary carcinoma, considered an example of a peculiar morphological differentiation with limited clinical usefulness, and on the mucoepidermoid carcinoma, taken as a paradigm of a histotype derived from a preexisting papillary carcinoma through a putative genetic mechanism. It is tempting to suggest that the foci of mucoepidermoid carcinoma that can be found in cases of papillary carcinoma represent a sort of balanced divergent differentiation of the latter. Moreover, we think we have obtained enough evidence to suggest that E-cadherin mutation(s) constitute the genetic basis of such divergent differentiation.

The most-important features of the so-called mixed medullary and follicular (or papillary) carcinoma have been extensively reviewed from a pragmatic standpoint. It is now widely accepted that such mixed carcinomas do, although rarely, exist. However, the controversy surrounding their putative histogenesis(es) is far from settled. Such mixed tumors raise diagnostic problems because, in routine diagnostic work, it is not easy to distinguish a *bona fide* mixed carcinoma from a medullary carcinoma that has absorbed or phagocytosed thyroglobulin. It has also become fashionable to try to subclassify the follicular component of the mixed carcinomas as "true follicular, papillary or poorly differentiated. We have previously stressed that the attempt to characterize the follicle cell derived component of mixed carcinomas contrasts with the usual approach to carcinomas exhibiting divergent differentiation elsewhere in the body. Such an attempt implicitly indicates, on the other hand, that we are not at present sure about the clonal nature of these mixed carcinomas. In other words, the possibility that at least some of them may indeed be collision tumors has to be entertained.

In order to progress in the understanding of the histogenesis and possible molecular mechanisms leading to mixed carcinomas, Volante *et al.* have very recently completed a study of 12 *bona fide* mixed carcinomas of the thyroid, *i.e.*, tumors exhibiting the morphological and immunohistochemical features of both medullary and follicle-cell derived neoplasms. These authors isolated the two histological components of the 12 carcinomas by laser-based microdissection and analyzed them for mutations in the *ret* protooncogene and for allelic losses of nine loci on six chromosomes. In female patients, Volante *et al.* also studied the clonal composition of the carcinomas. The results obtained strongly indicate that the follicular and medullary components in mixed carcinomas are not derived from a single progenitor cell since they consistently exhibited a different pattern of mutations and allelic losses. Furthermore, the seven tumors that were suitable for clonal analysis did not yield any signs of monoclonality. These authors also demonstrated that the follicular structures in mixed carcinomas are often oligo- and polyclonal and more frequently exhibit hyperplastic histological features than neoplastic ones, thus indicating that at least a subset of mixed carcinomas appears to include medullary thyroid carcinomas containing hyperplastic follicles.