24 Neuroendocrine Neoplasms

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General Definitions

The subject of neuroendocrine neoplasms, starting with the definition of what *neuroendocrine* means, is thoroughly confusing to the beginner. This chapter reviews the basic concepts and definitions pertaining to this subject.

Let us start with a definition of *neuroendocrine*. As the term implies, there are two components: "neuro" and "endocrine." The "endocrine" quality refers to the secretory nature of neuroendocrine cells: they produce and secrete peptides and amines. The "neuro" quality refers to their ultrastructural similarity to neurons: neuroendocrine cells store their secretory products in granules (i.e., dense-core granules), which bear resemblance to synaptic vesicles. Neuroendocrine cells are different from neurons structurally (no processes) and by the fact that the secretory mode is paracrine rather than synaptic. Also note that not all that secretes is neuroendocrine: for example, thyroid and adrenal cortex are not neuroendocrine because their cells do not possess neurosecretory granules (they are simply endocrine). Thus, at the most basic level, neuroendocrine cells are defined as the presence of neurosecretory granules in nonneurons. Tumors derived from these cells have a characteristic "neuroendocrine morphology" and share expression of "neuroendocrine markers."

Neuroendocrine Markers

In the past, neurosecretory granules were identified by electron microscopy and special stains. Currently these methods have been completely supplanted by immunohistochemical markers. These are called *neuroendocrine markers* and they include synaptophysin (SYN), chromogranin (CHR), neural-specific enolase (NSE), and CD56 (SYN and CHR specifically recognize dense-core granules). Note that these markers also recognize true neurons and neuroblastic cells (primitive neurons).

Neuroendocrine Morphology

Morphologically, unlike adenocarcinoma or squamous cell carcinoma, there is no single feature that defines neuroendocrine neoplasms as a group. Instead, *neuroendocrine morphology* is defined by a constellation of several cytologic and architectural features:

- Neuroendocrine cytology
  - Overall nuclear uniformity/monotony with smooth nuclear contours (unlike typical adenocarcinomas or squamous carcinomas)
  - Evenly dispersed, finely speckled "salt and pepper" nuclear chromatin without prominent nucleoli (Figure 24.1)

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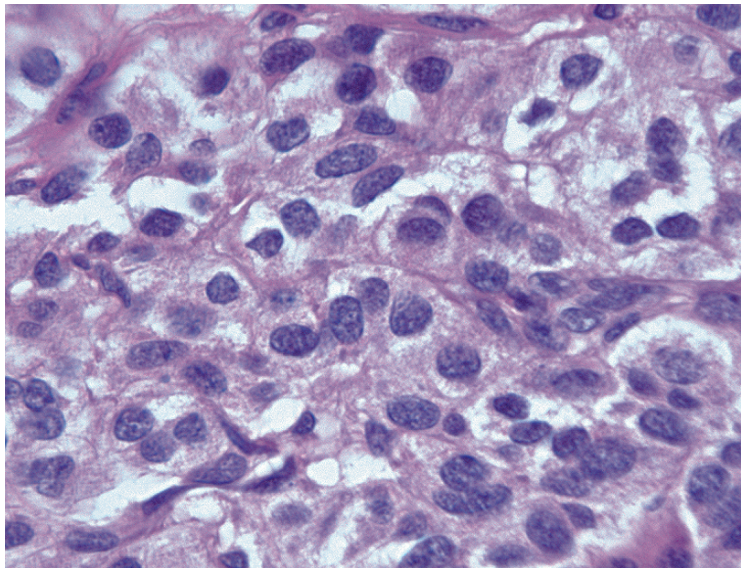

Figure 24.1. Classic neuroendocrine nuclei, with smooth oval nuclear borders, chromatin that is finely speckled throughout ("salt and pepper"), and no nucleoli.

  - Cytoplasmic granularity (corresponding to "neurosecretory granules"; variably evident)
- Neuroendocrine architecture
  - Formation of nests, rosettes, and ribbons/trabeculae
  - Prominent vascularity (in keeping with their secretory nature)

The appearance may be thought of vaguely recapitulating the normal neuroendocrine structures. Recall that nesting is a feature of normal adrenal medulla, and subtle trabecular/ribbon-like structures are present in the islets of Langerhans. The presence of rosettes is not easily explained by resemblance to normal neuroendocrine structures but may relate to their neural lineage, as many neuroglial tumors tend to form rosettes.

The morphology features listed apply to both low-grade (carcinoid, pancreatic neuroendocrine neoplasm, pheochromocytoma) and high-grade (small cell neuroendocrine carcinoma, Merkel cell carcinoma) neuroendocrine neoplasms. Whereas the defining neuroendocrine features are usually very obvious in low-grade neoplasms, they may be quite subtle to barely detectable in high-grade neuroendocrine neoplasms. Nevertheless, even when high grade, the overall uniformity and dispersed finely granular chromatin should be preserved, and at least a hint at neuroendocrine architecture (in the form of nesting, rosettes, or ribbons) is usually present.

What makes recognition of neuroendocrine neoplasms as a group so challenging is the fact that neuroendocrine morphology may be extremely subtle. For example, some neuroendocrine neoplasms rarely display typical neuroendocrine architecture (e.g., Merkel cell carcinoma typically has no nests, rosettes, or trabeculae). In addition, some neuroendocrine neoplasms frankly violate the basic rules of neuroendocrine morphology (e.g., higher grade neuroendocrine neoplasms, most notoriously large cell neuroendocrine carcinoma of the lung, do have prominent nucleoli). Therefore, diagnosis frequently hinges on recognition of subtle morphologic clues and confirmation with immunostains.

Neuroendocrine Cells and Neoplasms

What are the tissues that qualify as neuroendocrine? In addition to neuroendocrine organs (adrenal medulla and paraganglia), the neuroendocrine system includes the so-called diffuse neuroendocrine system. The term *diffuse neuroendocrine system* refers to neuroendocrine

Table 24.1. Major neuroendocrine cell types and corresponding neoplasms.

Location	Neuroendocrine cell type (secreted product)	Corresponding neoplasm	Cytokeratin expression
Intestine and appendix	EC cell (serotonin); D, L cells; other	Carcinoid	Positive
Gastric fundus	ECL cell (histamine)	Carcinoid	Positive
Gastric antrum, duodenum	G cell (gastrin)	Carcinoid	Positive
Lung	Kulchitsky (K) cell	Carcinoid	Positive
Pancreatic Islets of Langerhans	α cell (insulin)	Pancreatic endocrine neoplasm (islet cell tumor)	Positive
	β cell (glucagon)		
	δ cell (somatostatin)		
Thyroid	C cell (calcitonin)	Medullary carcinoma	Positive
Skin	Merkel cell	Merkel cell carcinoma	Positive
Anterior pituitary	Acidophil (PRL, GH)	Pituitary neoplasms	Positive
	Basophil (ACTH, TSH, FSH/LH)		
Parathyroid	(PTH)	Parathyroid neoplasms	Positive
Adrenal medulla and paraganglia	(Epinephrine, norepinephrine)	Pheochromocytoma	Negative
		Paraganglioma (i.e., extraadrenal pheochromocytoma)	
Adrenal medulla and other sites	Neuroblast (catecholamines, variable)	Neuroblastoma, PNET	Negative

ACTH, adrenocorticotropic hormone; EC, enterochromaffin; ECL, enterochromaffin-like; FSH, follicle-stimulating hormone; GH, growth hormone; LH, luteinizing hormone; PNET, primitive neuroectodermal tumors; PRL, prolactin; PTH, parathyroid hormone.

cells dispersed singly or in clusters throughout the body, including pancreas (islets of Langerhans), thyroid (C cells), lungs (Kulchitsky cells), skin (Merkel cells), gastrointestinal tract (many types), and so forth. In fact, neuroendocrine neoplasms may arise in any organ (e.g., prostate, breast, other). The broad definition of neuroendocrine cells also includes parathyroid and anterior pituitary glands. The only endocrine (peptide-secreting) organs excluded as clearly nonneuroendocrine are thyroid gland, adrenal cortex, and the steroid-producing cells of testes and ovaries. Table 24.1 summarizes the major neuroendocrine cell types and their corresponding neoplasms.

A Potentially Confusing Issue: Neuroectodermal Tumors

What about primitive neuroectodermal neoplasms (primitive neuroectodermal tumors and neuroblastoma)? Do these belong to the family of neuroendocrine neoplasms? These tumors do have neurosecretory-type granules (which are SYN/CHR$^+$), and the neuroblastoma secretes catecholamines, so technically they should qualify as neuroendocrine. However, these neoplasms also appear to possess the ultrastructural features of true primitive neurons (such as neurites). Therefore, together with medulloblastoma, they are classified under a separate heading of *primitive neural (neuroblastic/neuroectodermal) tumors*. One could think of these as neuroendocrine neoplasms with primitive neuronal phenotype.

Another Potentially Confusing Issue: Cytokeratin Expression in Neuroendocrine Neoplasms

Another potentially confusing issue with neuroendocrine neoplasms is their status as neural versus epithelial tissues. In the recent past, all neuroendocrine cells were erroneously thought to be neural (neural crest) derived; this is probably what you learned in medical school. However, it appears that neuroendocrine neoplasms actually fall into two groups: the "truly neural" group (pheochromocytoma/paraganglioma) and the "endoderm-derived/epithelial" group

(carcinoid, pancreatic endocrine neoplasm, small cell carcinoma, other). This distinction has practical implications: "neural" neuroendocrine neoplasms are cytokeratin negative, whereas "epithelial" neuroendocrine neoplasms are cytokeratin positive.

Some More (Potentially Confusing) Terminology

To complicate matters further, there are a number of terms that have been applied to neuroendocrine cells over the years. These terms are rarely in routine use today, but they may be encountered in the literature (or on the boards):

- *Amine precursor uptake and decarboxylase* (APUD) cells and *diffuse neuroendocrine system* are the terms for neuroendocrine cells scattered throughout the body (like enterochromaffin-like cells in the stomach). An *APUD-oma* is another term for carcinoid tumor.
- *Chromaffin* is a term applied to adrenal medulla because of its property to stain brown with chromic salts.
- *Enterochromaffin* refers to neuroendocrine cells of the intestine with similar properties, hence "entero" (gut).
- *Enterochromaffin-like* refers to histamine-secreting neuroendocrine cells of the gastric fundus.
- *Argentaffin* and *argyrophil* refer to the property of neuroendocrine cells to take up silver stains without or with a pretreatment step, respectively. Pretreatment gets more cells to stain. Fontana-Masson is a type of argentaffin stain (it also stains melanin), and Grimelius is a type of argyrophil stain. These stains are of historic interest only, because they have been supplanted by immunostains in practice.

Select Neuroendocrine Neoplasms

Neuroendocrine neoplasms encompass such a heterogeneous group of lesions that it may be difficult to see the common thread among them. This section attempts to highlight the common neuroendocrine qualities as well as organ-specific features of neuroendocrine neoplasms.

Note that neuroendocrine neoplasms (particularly low grade) frequently display random nuclear atypia, that is, *neuroendocrine-type atypia* or pleomorphism. Nuclei are smudgy and have bizarre shapes. Neuroendocrine atypia is degenerative in nature, probably owing to the slow growth rate of these neoplasms, and has no correlation with malignant potential. Neuroendocrine atypia is particularly prominent in pheochromocytoma and paraganglioma; do not mistake this for a feature of high-grade malignancy.

Expression of neuroendocrine markers is another defining feature of neuroendocrine neoplasms; expression is usually strong in low-grade lesions and may be weak/focal in high-grade lesions. Note that in the case of small cell carcinoma, diagnosis is based predominantly on morphology: if morphology is classic, expression of neuroendocrine markers is not required for diagnosis. The following discussion of specific neuroendocrine neoplasms emphasizes what constitutes their neuroendocrine qualities.

Well-Differentiated Neuroendocrine Neoplasm (Carcinoid)

This prototypical neuroendocrine tumor has all of the features listed earlier as the hallmarks of neuroendocrine differentiation, including finely speckled chromatin with *no* prominent nucleoli, uniform (monotonous) round nuclei with a smooth nuclear membrane, and frequently a plasmacytoid appearance (eccentrically placed nucleus). The architecture may be nests, rosettes, ribbons, or trabeculae. Delicate fibrovascular septae are characteristic. Neuroendocrine markers are usually strongly expressed.

Poorly Differentiated Neuroendocrine Carcinoma (Small Cell Carcinoma)

Despite the name, diagnosis is not based purely on size, but nuclear size is generally less than three lymphocytes in diameter. The chromatin is finely speckled but is also very dark/hyperchromatic, which may obscure the "salt and pepper" quality in a surgical specimen.

As in other neuroendocrine neoplasms, there are no prominent nucleoli. The unique features include nuclear molding, high nuclear to cytoplasmic ratio with very scant cytoplasm, numerous mitoses and apoptotic bodies, and frequent crush artifact with DNA streaming (known as the *Azzopardi phenomenon*). Nests, trabeculae, and rosettes are uncommon. The most reliable immunostain is CD56, but all may be positive. If morphology is classic, expression of neuroendocrine markers is not required for diagnosis.

Merkel Cell Carcinoma of Skin

Merkel cell carcinoma is a small round blue cell tumor. Cytology and architecture overlap with small cell carcinoma. As in small cell carcinoma, nuclear molding, crush artifact, and necrosis are usually present. Cytology is extremely high grade with numerous mitoses and apoptotic bodies (although Merkel cell carcinoma shows less molding than small cell carcinoma). Stains are usually required to distinguish the two.

Neuroendocrine morphology may be subtle, although neuroendocrine markers are reliably expressed. Hints at the neuroendocrine nature are overall nuclear monotony, despite the high nuclear grade. Although the "salt and pepper" quality of chromatin may be subtle at best, it does show dispersed granularity (so-called dusty look). Also, trabeculae and rosettes may be present (hence the former designation as trabecular carcinoma), although more commonly the pattern is diffuse.

In addition to neuroendocrine markers, Merkel cell carcinoma stains for neurofilament, which is normally a neuronal marker, and CK20 (in classic punctate perinuclear dots). In contrast, small cell carcinoma is negative for neurofilament and CK20 but is positive for TTF-1.

Medullary Carcinoma of Thyroid

Neuroendocrine cytology is as described for carcinoid; speckled chromatin may not be evident in surgical slides due to hyperchromasia but should be more apparent in cytology preparations. Plasmacytoid cytology is common. Neuroendocrine architectural features may be present. The unique features are the presence of amyloid and tendency to form large cellular islands.

Large Cell Neuroendocrine Carcinoma

Large cell neuroendocrine carcinoma is one of the neuroendocrine neoplasms that you would not guess had a neuroendocrine nature based on cytology (nuclei are vesicular, not salt and pepper, and have a single prominent nucleolus). Neuroendocrine classification is based on a subtle hint of neuroendocrine architecture (rosettes and nuclear palisading), expression of neuroendocrine markers, and ultrastructural demonstration of dense-core granules.

Pheochromocytoma (Adrenal)/Paraganglioma (Extraadrenal)

Classic neuroendocrine cytology may be barely discernible in a pheochromocytoma. Some nuclei are carcinoid-like in that they are uniform, round, and finely speckled, but many nuclei are more neuron-like by virtue of large size and single prominent nucleolus. The cytoplasm is abundant, granular, and "amphophilic" (lavender). Nuclear pseudoinclusions (cytoplasmic invaginations) are present in 30% of cases. Hyaline globules are common and, if present, distinguish pheochromocytomas from other adrenal neoplasms. Random nuclear atypia is common.

Although paragangliomas are equated with extraadrenal pheochromocytomas, the morphology is not identical. In paraganglioma, the nuclei are much more carcinoid-like. The architecture is nested and occasionally trabecular, but there are no rosettes. The nest pattern is referred to as *zellballen*, which in German literally means *cell balls*. The zellballen pattern is highlighted by S100 stain, which reacts with sustentacular/supportive cells outlining the nests (sustentacular cells are not visible on H&E stain).

Marker expression is identical in both tumors. Neuroendocrine markers are positive in the nests (chromaffin cells), and S100 highlights sustentacular cells. Unlike all other neuroendocrine neoplasms (e.g., carcinoid), these tumors are cytokeratin negative.

Neuroendocrine Differentiation in Other Types of Carcinoma

As discussed earlier, neuroendocrine neoplasms are diagnosed based on (1) morphology and (2) expression of neuroendocrine markers (electron microscopy and special stains are basically obsolete). If morphology and markers are concordant (which is usually the case), one can comfortably diagnose a neuroendocrine neoplasm. However, there are cases in which morphology and neuroendocrine marker expression are discordant. These distressing situations come in two varieties.

First, expression of neuroendocrine markers may be detected incidentally in an otherwise entirely nonneuroendocrine neoplasm (morphologically), such as a classic adenocarcinoma. In most organs, this is thought to represent a type of occult differentiation with no clear clinical significance. These lesions may be signed out as *Carcinoma with neuroendocrine differentiation by immunohistochemistry*.

Conversely, some high-grade neuroendocrine neoplasms, particularly small cell carcinoma, may express neuroendocrine markers only focally or not at all. Nevertheless, classic morphology trumps the lack of marker expression, once other small cell neoplasms have been excluded.

Note that the first scenario is different from finding small cell carcinoma as a component of another type of carcinoma, such as adenocarcinoma or squamous cell carcinoma. The latter situation is considered to be a form of dedifferentiation to a more primitive phenotype, which does carry a worse prognosis and a need for specific therapy. Such cases are signed out as *Mixed adenocarcinoma/small cell carcinoma* or *Adenocarcinoma with small cell component*.

Sign-Out Terminology for Neuroendocrine Neoplasms in Various Organs

A practical issue of note is that clinical behavior of low-grade neuroendocrine neoplasms (carcinoid, pancreatic endocrine neoplasm, pheochromocytoma) is notoriously difficult to predict based on histologic parameters. Generally, the only definitive sign of malignancy is the presence of metastases. This is why neuroendocrine neoplasms are not staged. However, there are a number of site-specific histologic features that are loosely predictive of a more aggressive phenotype. Given the uncertainty of clinical behavior, there is a range of sign-out terminology applied to neuroendocrine neoplasms (which can vary among institutions):

Primary lung neuroendocrine neoplasms may be signed out as the following:

Carcinoid tumor (low-grade neuroendocrine carcinoma): Typical neuroendocrine morphology is apparent, with <2 mitoses/10 high-power fields [hpf] and no necrosis.

Atypical carcinoid tumor (intermediate-grade neuroendocrine carcinoma): From 2 to 10 mitoses/10 hpf and/or focal necrosis are present.

Small cell (high-grade neuroendocrine) carcinoma: More than 10 mitoses/10 hpf, extensive necrosis, and specific small cell features are present.

Large cell (high-grade neuroendocrine) carcinoma: More than 10 mitoses/10 hpf, extensive necrosis, and specific features are present.

Primary gastrointestinal neuroendocrine neoplasms may be signed out as the following:

Carcinoid tumor: This indicates typical neuroendocrine morphology with no features of concern (e.g., size >1–2 cm, vascular invasion, necrosis, etc.).

Carcinoid tumor, *Malignant carcinoid tumor*, or *Well-differentiated neuroendocrine carcinoma*: Morphology is of typical carcinoid, but the tumor is behaving badly, such as already metastatic. The sign-out terminology is a contentious issue here (a matter of style). Some sign out a lesion as *carcinoid* irrespective of how malignant it behaves. Others advocate that if metastases are present, the lesion should be signed out as *malignant carcinoid* or *well-differentiated neuroendocrine carcinoma*. The third opinion is that the latter two categories should be used if *any* features of concern are present.

Poorly differentiated neuroendocrine carcinoma: The carcinoma morphologically is frankly high grade. Small cell and large cell neuroendocrine carcinomas are included here.

Primary pancreatic neuroendocrine neoplasms may be signed out as the following:

Well-differentiated pancreatic endocrine neoplasm (islet cell tumor): Typical neuroendocrine morphology is apparent, with no features of concern.

Well-differentiated malignant pancreatic endocrine neoplasm (malignant islet cell tumor): This designation is used if any one of the three definitively malignant features are present: (1) metastases, (2) large vessel vascular invasion, and/or (3) invasion of adjacent organs. Lymphatic invasion, large size, microscopically invasive border, and so forth are suspicions but not diagnostic of malignancy.

Poorly differentiated malignant pancreatic endocrine neoplasm (malignant islet cell tumor): morphologically a frankly high-grade carcinoma is present. Small cell and large cell neuroendocrine carcinoma are included here.

Note that pancreas and intestine do not have an "atypical" category corresponding to the "atypical carcinoid" category in the lung. Nevertheless, some pathologists do flag intestinal and pancreatic lesions as "atypical" based on the lung criteria (discussed earlier).

The issue of *metastatic neuroendocrine neoplasm of unknown origin* usually arises for hepatic neuroendocrine neoplasms. The differential diagnosis includes intestinal versus pancreatic metastases (primary hepatic neuroendocrine neoplasms are vanishingly rare). Intestinal carcinoids and pancreatic neuroendocrine neoplasms are histologically identical, and hormone expression is generally not reliable. Therefore clinical correlation is required. These lesions are signed out as "metastatic (well or poorly) differentiated neuroendocrine neoplasm (carcinoid vs. pancreatic endocrine carcinoma)".

One contentious issue is the use of the term *neoplasm* versus *carcinoma* as it pertains to neuroendocrine tumors that are displaying overtly malignant behavior. In principle, these tumors should be called *carcinomas* (i.e., malignant epithelial neoplasms). This terminology is indeed advocated by some authorities. Some authorities even advocate use of the term *low-grade neuroendocrine carcinoma* in place of *carcinoid* tumors to reflect the potential of any of these tumors to metastasize. In contrast, other authorities stress that the term *neoplasm* is preferable (regardless of clinical behavior or morphology) in order to draw a clear distinction between these tumors and typical carcinomas, such as pancreatic adenocarcinoma, which behave in a much more aggressive fashion than any neuroendocrine tumor.