short review

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From morphology to molecular targets—the pathologist's view in diagnosing gastroenteropancreatic neuroendocrine neoplasms

Hanna Henzinger · Iva Brcic

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Summary In the last decade, a number of genetic alterations in gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) have been identified. In addition, differences in tumor morphology as well as proliferation index (Ki-67) or number of mitoses have led to changes in the classification of these neoplasms. According to the new World Health Organization (WHO) classification, GEP-NENs are now divided into two genetically and prognostically different categories: (i) well-differentiated neuroendocrine tumors (NET) subdivided into low (G1), intermediate (2) and high (G3) grade tumors, and (ii) poorly differentiated neuroendocrine carcinomas (NEC). In addition, a group of mixed neuroendocrine–non-neuroendocrine neoplasms (MiNEN) has been defined. This review focuses on the clinical, morphological, immunohistochemical and molecular findings of the GEP-NENs and their key diagnostic features that can help the pathologist to differentiate between tumors in this heterogeneous group. In challenging cases, additional immunohistochemical and/or molecular analysis can be helpful to determine the correct diagnosis and proper treatment for the patient.

Keywords Neuroendocrine neoplasms · Gastroenteropancreatic · Neuroendocrine tumors · Neuroendocrine carcinomas · GEP-NEN

H. Henzinger · I. Brcic (✉)
Diagnostic and Research Institute of Pathology, Medical University of Graz, Neue Stiftingtalstraße 6, 8010 Graz, Austria
iva.brcic@medunigraz.at

H. Henzinger
hanna.henzinger@medunigraz.at

Introduction

Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) are a rare group of tumors with heterogenous morphological and molecular findings. In recent years, the spectrum of genetic alterations in GEP-NENs has been characterized in more detail, leading to changes in the new World Health Organization (WHO) classification [1]: Based on clinical, histological and molecular findings, these tumors are now divided into two genetically distinct categories with different malignant potential and prognosis, namely well-differentiated neuroendocrine tumors (NET) and poorly differentiated neuroendocrine carcinomas (NEC) [2, 3].

The former group can further be subdivided into low (G1), intermediate (G2), or high (G3) grade NET composed of low to moderately atypical cells in an organoid arrangement with expression of immunohistochemical markers of neuroendocrine differentiation (synaptophysin and chromogranin A; Fig. 1). The grade is determined using the proliferation rate (measured with Ki-67) and/or mitotic number (Table 1). G3 NETs are a new subgroup of well-differentiated NETs defined by a proliferation index of >20% or a mitotic rate of >20 per 2 mm^2 [1]. In the 2010 WHO classification, these tumors were considered to be NECs (Table 2); however, it has been shown that they have a better prognosis and different molecular alterations than NECs [4, 5]. The prognosis and potential to metastasize depend on the location of the primary tumor, histological type and differentiation (grade).

Hormone production of NETs may be clinically silent (non-functional (NF)-NET) in >60% of all pancreatic NENs [6] or associated with hormonal hyperfunction syndrome. In contrast to NF-NETs, functional NETs are referred to as insulinomas, VIPomas,

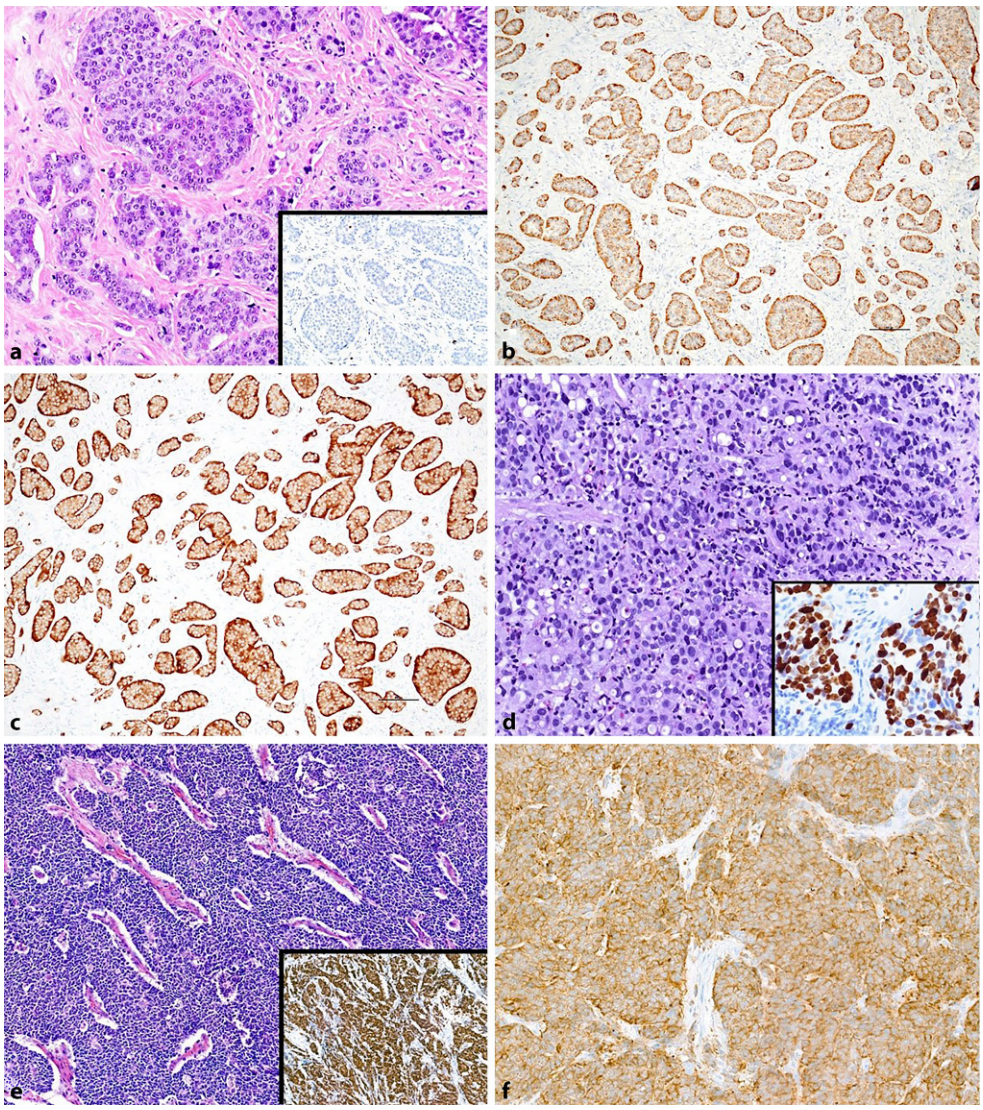

Fig. 1 Histological and immunohistochemical findings. **a** Well-differentiated neuroendocrine tumor, G1 composed of nest-like and glandular formation of uniform bland tumor cells with fine granular chromatin, abundant cytoplasm and round to oval, monomorphic nuclei with a Ki-67 < 3% (*Inset*). **b** Immunohistochemically, tumor expresses chromogranin A and **c** synaptophysin. **d** Large cell neuroendocrine carcinoma consisting of intermediate to large, highly atypical cells with abundant cytoplasm and prominent nucleoli with diffuse growth pattern and Ki-67 of 80% (*Inset*). **e** Small cell neuroendocrine carcinoma comprised of tumor cells with scarce cytoplasm arranged in solid structures with a Ki-67 of 90% (*Inset*) and **f** expression of synaptophysin

glucagonomas, somatostatinomas, etc., according to the hormone that causes hyperfunction [1].

The second group of the new classification comprises NECs, poorly differentiated NENs composed of highly atypical, small or medium-to-large cells, which can be further divided into two different subtypes with distinct cell size and architecture, namely large-cell (LCNEC) and small-cell (SCNEC) NECs. Proliferation rate (measured by Ki-67) is always high (usually above 55%) with an increased number of mitoses (>20/2 mm^2) [1]. NECs make up only 6–8% of GEP-NENs, while 84% are G1 or G2 NETs and up to 8% are G3 NETs [2, 7].

Tumors consisting of at least one neuroendocrine and one non-neuroendocrine component (typically an adenocarcinoma) with each component making up >30% of the tumor are classified as "mixed neuroendocrine–non-neuroendocrine neoplasms" (MiNEN) in the new classification [1].

Table 1 Grading of gastroenteropancreatic NENs according to the 2017 WHO classification

	Proliferation index (Ki-67) (in %)	Mitoses (in mitoses/2 mm^2)
Well-differentiated NENs		
NET G1	<3	<2
NET G2	3–20	2–20
NET G3	>20	>20
Poorly differentiated NENs		
NEC G3 (small- or large-cell)	>20	>20

HPF high power field, *NET* neuroendocrine tumor, *NEC* neuroendocrine carcinoma, *NENs* neuroendocrine neoplasms

Table 2 Comparison of the 2010 and 2017 WHO classification of gastroenteropancreatic neoplasms

WHO 2010	WHO 2017
NET G1/G2	NET G1/G2/G3
NEC G3 (small-cell or large-cell)	NEC G3 (small-cell or large-cell)
Mixed adenoneuroendocrine carcinoma (MANEC)	Mixed neuroendocrine–non-neuroendocrine neoplasm (MiNEN)

NET neuroendocrine tumor, *NEC* neuroendocrine carcinoma

	Histology	Proliferation index and mitoses	IHC	Molecular pathology	5-year-OS
PanNET G1	**Well differentiated**: low to moderate cellular atypia; monotonous nuclei with salt and pepper chromatin; organoid growth pattern (i.e. glandular formation, solid nests); necrosis is rare	Ki-67 <3% or mitoses <2/2 mm^2	Positive: Syn & Chr A, INSM1 SSTR2A (common) Rb1 preserved p53 normal pattern ATRX & DAXX loss	*ATRX* or *DAXX* mutations (40%) Somatic inactivation of MEN1 (40%) PTEN, TSC2 and other mTOR pathway genes mutations (15%) HIF1A and VHL mutations	92%
PanNET G2		Ki-67 3-20% or mitoses 2-20/2 mm^2			62%
PanNET G3		Ki-67 >20% or mitoses >20/2 mm^2			29%
PanNEC	**Poorly differentiated:** marked cellular atypia; LCNEC: large tumor cells with nucleoli, trabecular/nest-like growth SCNEC: small tumor cells, solid/diffuse growth. necrosis is common	Ki-67 >20% or mitoses >20/2 mm^2	Positive: Syn & Chr A (weak), INSM1 SSTR2A (uncommon) Rb1 loss p53 abnormal staining ATRX & DAXX preserved expression	PT53 mutations, RB1 mutations or loss of p16 expression	16%

Fig. 2 Histological, immunohistochemical, genetic and prognostic characteristics of PanNETs and PanNECs (*IHC* immunohistochemistry, *OS* overall survival, *PanNET* pancreatic neuroendocrine tumor, *PanNEC* pancreatic neuroendocrine carcinoma, *Syn* Synaptophysin, *Chr A* Chromogranin A, *INSM1* Insulinoma-associated protein 1, *SSTR2A* Somatostatin receptor 2A)

This review aims to provide a comprehensive overview on histology, immunohistochemistry and molecular attributes of NEC, NET and MiNEN of the gastrointestinal (GI) tract and the pancreas. Clinical, histological, immunohistochemical and genetic findings of pancreatic NETs (PanNETs) and NECs (PanNECs) are also summarized in Fig. 2.

The TNM classification of well-differentiated NETs is described in the eighth edition (2017) of the Union for International Cancer Control (UICC) TNM Classification and the American Joint Committee on Cancer (AJCC), which conform to the European Neuroendocrine Tumor Society (ENETS) classification. The TNM classification of pancreatic and gastrointestinal NECs follows the scheme of the UICC TNM classification for carcinomas of the exocrine pancreas/the digestive system [8].

Neuroendocrine tumors (NET) G1–G3

Histology

Gastroenteropancreatic NETs consist of uniform bland tumor cells with fine granular chromatin (also called salt and pepper chromatin), abundant cytoplasm and round to oval, monomorphic nuclei. The tumors show an organoid architecture, with nest-like, glandular, trabecular, acinar and other growth patterns. Necroses are absent and mitoses are scarce in most NETs, with exception of G3 NETs where per definition, more than 20 mitoses per 2 mm^2 are found. In these high-grade tumors, which are very rare compared to G1 and G2 NETs in the GI tract, Ki-67 is usually below 55% [1, 9]. The determination of

the tumor grade by the number of mitoses or Ki-67 proliferation index is elucidated further in Table 1.

Concerning differential diagnosis of G3 NETs, a multicenter study assessing approximately 200 cases of high-grade NENs found that the most useful morphological criteria for setting apart G3 NETs from NECs comprise organoid growth pattern, close association of vessels to tumor cells and absence of desmoplasia [10].

Immunohistochemistry

NETs stain positive for neuroendocrine markers such as synaptophysin and chromogranin A. In L-cell NETs (e.g. in the appendix), chromogranin B is expressed more commonly than chromogranin A. Functional NETs can also be stained with antibodies for the hormones they produce. In addition, NETs often show a strong expression of somatostatin receptor 2A (SSTR2A), which may be used to indicate eligibility for treatment with somatostatin-analogue therapy [1]. Furthermore, SSTR2A staining can help to discriminate NETs with a high proliferation rate (G3) from NECs, which are only SSTR2A positive in some cases [11]. In addition, loss of ATRX and DAXX nuclear staining is found in a subset of NETs, and this finding can be helpful in challenging cases. p53 and Rb1 stains show normal expression [11, 12].

Insulinoma-associated protein 1 (INSM1) has also been proposed as a very sensitive and specific immunohistochemistry marker for GEP-NENs and has been recommended for diagnostic use by several authors [13–17].

Molecular pathology

In gastrointestinal NETs, epigenetic dysregulation is very common with most NETs showing CpG island methylator phenotype, while somatic mutations appear to be secondary; to date, there is no specific gene mutation that has been described in a substantial amount of cases [1]. The most common alteration is *CDNK1B* mutation, which is present in 8% of small intestine NETs [18]. Copy number variations in chromosomes are frequent. For example, in the small intestine, most NETs display chromosome 18 deletion even at early stages [19, 20], while chromosome 14 gain can be found in advanced stages and may therefore pose a negative prognostic factor [21].

In the pancreas, 40% of NETs display somatic inactivation of *MEN1*. Another 40% have inactivating mutations in *DAXX* or *ATRX*, which have been described as negative prognostic factors [22–24]. Activating mutations of genes involved in the mTOR pathway, including *PTEN* and *TSC2*, are present in about 15% of tumors [25]. Associations of PanNETs with hereditary cancer syndromes (i.e. multiple endocrine neoplasia type 1, neurofibromatosis type 1) and in some cases germline mutations in DNA repair genes (*MUTYH*, *CHEK2* and *BRCA2*) have also been reported [1, 26, 27].

From an evolutionary perspective, it is believed that NETs and NECs are distinct entities; yet a cluster analysis of GEP-NENs has shown that some LCNECs might potentially develop from pre-existing NETs, in particular those where the *CTNNB1* gene is dominantly affected by mutation. The same authors have also previously hypothesized two pathways of NET to NEC evolution in the lung. These concepts remain to be investigated further [28, 29]. Within the NET category however, progression of low-grade tumors to high-grade NETs is possible and G3 NETs can often be found as liver metastases of pulmonary or pancreatic NETs [30, 31].

Neuroendocrine carcinomas (NEC)

Histology

Neuroendocrine carcinomas can be divided into two different subtypes based on morphology. Large cell NEC (LCNEC) consists of highly atypical cells of intermediate to large size with abundant cytoplasm and often prominent nucleoli arranged in an organoid pattern (mostly large nests or trabeculae) or grow diffusely, while the tumor cells in small cell NEC (SCNEC) have scarce cytoplasm and typically form solid structures. In both subtypes, necrosis and a high number of mitoses, including atypical mitoses, are present. The mitotic rate must exceed 20 per 2 mm^2 and/or the Ki-67 proliferation index must be above 20% [1]. In the pancreas, different clinicopathological attributes of LCNEC and SCNEC have not been observed [32].

In diagnostic routine, it is important to differentiate PanNEC, as well as PanNET G3, from acinar cell carcinoma of the pancreas, which often has a similar histological appearance and focally stains positive for neuroendocrine markers [32]. These entities can be distinguished using neuroendocrine and acinar stains, including trypsin and BCL-10 [7, 31, 33].

Immunohistochemistry

In general, NECs show diffuse to weak expression of neuroendocrine markers such as synaptophysin and chromogranin A. Neuron-specific enolase (NSE), CD56, CDX2 and TTF1 may also be expressed by these tumors; however, these markers are not specific for NECs. Hormone production is rarely found [1].

In addition, SSTR2A may be positive in some NECs, but not as frequent as in NETs. Abnormal p53 immunostaining, which is a strong indicator for TP53 mutation, and lack of Rb1 expression are commonly observed in NECs (in contrast to NETs) which makes p53 and Rb1 stains useful for the differential diagnosis of NET G3 and NEC [11, 12]. ATRX and DAXX stains, which are positive as these genes are not affected by mutations in NECs, may also serve this purpose, al-

though they are not as discriminative as p53 and Rb1, since ATRX and DAXX expression is also preserved in the majority of NETs [11].

According to the 2016 ENETS Consensus Guideline, pathology reports on NEC should always include the morphological subtype (LCNEC or SCNEC), Ki-67 index or mitotic rate and staining for synaptophysin and chromogranin A; staining for SSTR2A is optional, but may be useful [34].

Molecular pathology

LCNEC and SCNEC are not genetically distinct. Typical genetic alterations are mutations in *TP53*, which leads to the loss of p53 immunohistochemical expression, mutations in *RB1* or loss of p16 expression [12].

Several studies have also described genetic similarities between gastrointestinal neuroendocrine carcinomas and colorectal adenocarcinomas [35–38], since mutations in *APC, KRAS* and *BRAF* have been found in NECs. *BRAF* mutations appear even more frequently in NECs than in conventional adenocarcinomas and have been associated with an unfavorable prognosis [39].

In PanNECs, *KRAS* mutations as well as *SMAD4* mutations (which are typical for ductal adenocarcinoma of the pancreas) have been reported but do not occur regularly [40]. BCL-2 overexpression is frequent and associated with a high proliferation rate [12]. Inactivation of *ATRX* and *DAXX*, which may be present in PanNETs, does not occur in PanNECs [11, 12].

Mixed neuroendocrine–non-neuroendocrine neoplasms (MiNEN)

Tumors consisting of at least two components, including a neuroendocrine and a non-neuroendocrine component, were previously referred to as "mixed adenoneuroendocrine carcinoma" (MANEC; Table 2). Since the non-neuroendocrine component can also present as a different cancer type (not only adenocarcinoma), "MANEC" was replaced by the term "mixed neuroendocrine–non-neuroendocrine neoplasm" (MiNEN) in the 2017 WHO classification of pancreatic neuroendocrine tumors and the 2019 WHO classification of gastrointestinal tumors. In GI pathology, the term "MANEC" was also formerly used to refer to goblet cell adenocarcinomas (also known as goblet cell carcinoids) of the appendix, which in the recent WHO classification are no longer considered to be neuroendocrine neoplasms [1].

MiNEN components are mostly poorly differentiated, with the non-neuroendocrine tumor component typically being an adenocarcinoma in the GI tract and either a ductal adenocarcinoma and/or an acinar cell carcinoma in the pancreas. Each component should comprise at least 30% of the tumor and grade should be reported separately for both tumor components. The staging of MiNEN is based on the current scheme of the UICC TNM classification of carcinomas of the exocrine pancreas [1]. In the differential diagnosis of MiNEN, acinar cell carcinoma of the pancreas must be distinguished, using a panel of acinar and neuroendocrine immunohistochemical markers (as mentioned in the differential diagnosis of NEC and acinar cell carcinoma) [7, 33].

The molecular features of MiNEN (at least of those with adenocarcinoma components) appear to be more closely related to those of non-neuroendocrine adenocarcinomas than to those of NECs, as *BRAF, KRAS* and *APC* mutations are common while *RB1* mutations are sparse and *TP53* mutations occur in lower frequency compared to NECs [35]. Recent publications have also described microsatellite instability and *ATRX* mutations in MiNEN [41–43]. However, the genetic landscape of MiNEN is heterogenous and complex and remains to be elucidated. Biopsies of these heterogeneous tumors might not include both components, posing an additional difficulty to both diagnostics and analysis of genetic alterations for research [44].

Prognosis

The prognosis of neuroendocrine neoplasms depends on various factors, including primary tumor location, histological type, tumor grade, tumor stage at initial diagnosis (tumor size and metastases), and the presence of vascular invasion. NET G1 and G2 show slow growth with good prognosis. Patients with NET G3 have a better prognosis compared to those with NEC but overall survival is worse than for NET G1 and G2 [2, 7, 45, 46].

Within NEC, two prognostically distinct groups can be defined by using a Ki-67 cut-off of 55%. NECs >55% are associated with an increased rate of mutations in *TP53, KRAS* and *BRAF* and poor overall survival [47–49]. There is no significant difference in the survival of patients with LCNEC compared to SCNEC [32].

MiNEN typically behave in an aggressive way and have an unfavorable prognosis compared to well-differentiated NETs. It is unclear whether the prognosis is better or worse than that of NECs [44]. The crucial prognostic factors for GEP-MiNEN are TNM stage and tumor composition, especially the proliferation rate of the neuroendocrine component [49, 50].

Conclusion

GEP-NENs are a rare, heterogeneous group of tumors that, in difficult cases, represent a diagnostic challenge for pathologists. The subdivision of NENs into distinct groups is crucial for the treatment of these tumors. However, intratumoral heterogeneity limits the accuracy of the grading system in the biopsy material. The multidisciplinary care of patients with GEP-NENs by an experienced team and presentation in a multi-

disciplinary tumor board is crucial for optimized individual and successful therapy.

Take-home message

The subdivision of gastroenteropancreatic neuroendocrine neoplasms (NENs) into distinct groups, namely well-differentiated neuroendocrine tumors (NET) G1–G3 and poorly differentiated neuroendocrine carcinomas (NEC), is crucial for the treatment of these tumors. They can be differentiated by morphology, immunohistochemical expression and molecular alterations.

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