Neuroendocrine Tumors: Classification and Pathological Categorization

M. Anlauf , G. Klöppel and P. Grabowski

Institut für Pathologie, Universitätsklinikum Schleswig-Holstein, Campus Kiel
Michaelisstr. 11, 24105 Kiel and
Klinik für Innere Medizin, Gastroenterologie, Onkologie, Endokrinologie
Zentrum für Neuroendokrine Tumoren Bad Berka Zentralklinik Bad Berka GmbH

Origin and Characteristics

Neuroendocrine tumors of the gastrointestinal tract (GEP NETs) originate in the diffuse endocrine system and were historically designated as clear cells or APUD cells. The diffuse neuroendocrine system regulates important functions of the entire digestive tract and lungs through autocrine, paracrine, or endocrine secretion. This led to a sub-classification of GEP NETs into tumors of the foregut, midgut, and hindgut. GEP NETs express a series of markers that also occur in neural cells and therefore have contributed to the designation of neuroendocrine tumors; these include neuron-specific enolase (NSE), chromogranin A (CgA), and synaptophysin which can be used for morphological characterization of the GEP NETs. Both vesicular monoamine transporters (VMAT) 1 and 2 can be utilized as specific markers for ECL cell tumors of the stomach (VMAT 2-positive) and serotonin-forming tumors (VMAT 1-positive). Only some of the more than 15 different types of neuroendocrine cells dedifferentiate into GEP NETs and express hormones of the diffuse neuroendocrine system, like insulin or gastrin. Immunohistological detection of hormones or messenger substances in GEP NETs, however, is not equivalent with the functional activity of the GEP NETs through an unregulated secretion since this is only the case with one category of GEP NETs. Most GEP NETs express somatostatin receptors (SSTRA), primarily SSTRA2A and SSTRA5 which can be immunohistologically determined.

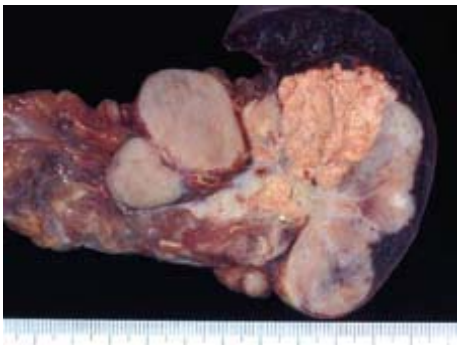

Fig. 1. Well-differentiated neuroendocrine carcinoma with infiltration of the spleen and peripancreatic metastases.

Classification and Division into Stages

The designation by Oberndorfer, who already in 1907 distinguished GEP NETs from the carcinomas of the gastrointestinal tract as carcinoids due to their structure and more benign behavior, is still in use today. However in clinical usage, carcinoid is usually only understood as the serotonin-producing GEP NET of the ileum or appendix leading to carcinoid syndrome. The WHO classification system from 2000 is more just in terms of the morphological and clinical heterogeneity of GEP NETs and sub-classifies them according to highly-differentiated neuroendocrine tumors displaying benign behavior, highly-differentiated neuroendocrine carcinoma with low-grade malignant behavior, and high-grade malignant, mostly small cell poorly-differentiated carcinoma (Table 1). The adjustment of this sub-categorization to the localization of the tumor, its biology, and the clinical symptoms allows for prognostic evaluation of the GET NETs. The categorization based on the WHO

classification system is available for all GEP NETs and is presented in Table 2 as an example for GEP NETs of the pancreas (Table 2).

Table 1. Risk stratification of the GEP NET

1a	Highly-differentiated neuroendocrine tumor (NET)
1b	Highly-differentiated neuroendocrine carcinoma (NEC)
2	Poorly-differentiated neuroendocrine carcinoma (PDEC)

The sub-classification of tumor biology using the WHO overview has established itself in daily clinical practice because it correlates well with the clinical course. In order to also achieve a standardization of the tumor stages of GEP NETs, a consensus proposal for a Tumor-Node-Metastasis (TNM) system for tumors of the foregut (stomach, duodenum, and pancreas), including a grading system and a tumor stage grouping for GEP NETs, was published following a consensus conference of the European Neuroendocrine Tumor Society (ENET) in Frascati, 2006 and is currently undergoing clinical evaluation. The TNM proposal is oriented on the one hand toward the TNM system for solid tumors in the organs of the foregut and on the other toward the WHO classification as far as the grading is concerned. A TNM system for tumors of the midgut and hindgut was published accordingly in October of the past year. In Tables 3 and 4, the TNM system for tumors of the pancreas and ileum are presented as examples. With the TNM system, a tool for standardizing the tumor stages in various clinical studies has finally been created, but which must be validated by the same studies regarding prognostic importance. A comparative description of the TNM system and the WHO classification is located on the homepage of the Center for Neuroendocrine Tumors in Bad Berka (http://www.rhoen-klinikum-ag.com/rka/cms/zbb/deu/3310.html).

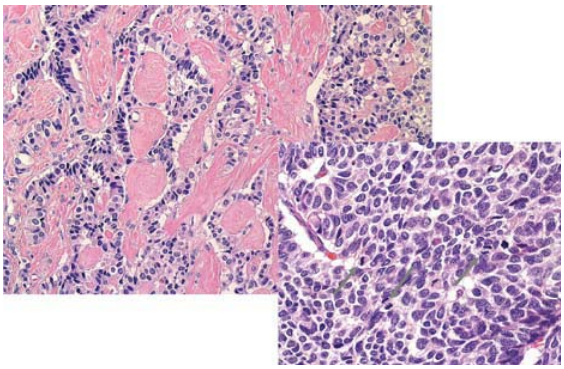

Fig. 2. Differences in architecture and cytology between well differentiated neuroendocrine tumor (A) and poorly differentiated neuroendocrine carcinoma of the pancreas (B).

Precursor Lesions of GEP NETs

Precise understanding of the oncogenesis of GEP NETs is essential for early detection or prevention. Hyperplasia-neoplasia sequence could already be demonstrated for endocrine tumors within the context of predisposition for germline mutation, for example, for medullary thyroid carcinomas or pheochromocytomas concerning endocrine neoplasia (MEN) type II. Also in terms of MEN type I, associated with the neuroendocrine pancreatic tumors, a microadenomatosis can be detected in the pancreas of affected germline carriers (see below). In contrast to the sporadic gastronomas which can cause Zollinger Ellison syndrome and are not associated with MEN type I, duodenal gastronomas emerge multi-centrically in the case of MEN type I and are connected with hyperplastic

Table 2. Classification of Neuroendocrine Tumors of the Pancreas

1. **Well-differentiated neuroendocrine tumor**
   - Benign behavior: confined to pancreas, size <2 cm, non-angioinvasive, ≤2 mitoses/HPF and ≤2 % Ki-67-positive cells
     - functionally active: insulinoma
     - functionally inactive
   - Benign or low-grade malignant behavior (questionable dignity): confined to pancreas, size ≥2 cm, >2 mitoses/HPF, >2 % Ki-67-positive cells or angioinvasive
     - functionally active: gastrinoma, insulinoma, VIPoma, glucagonoma, or ectopic hormonally-induced syndrome
     - functionally inactive

2. **Well-differentiated neuroendocrine carcinoma**
   - Low-grade malignant behavior: invasion of neighboring organs and/or metastases
     - functionally active: gastrinoma, insulinoma, glucagonoma, VIPoma, or ectopic hormonally-induced syndrome
     - functionally inactive

3. **Poorly-differentiated neuroendocrine carcinoma**
   - High-grade malignant behavior

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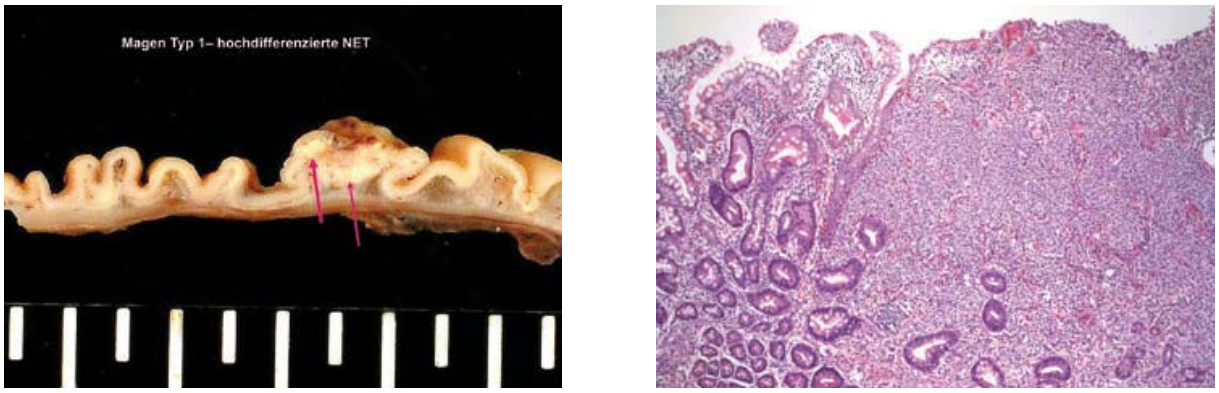

Fig. 3. Well-differentiated neuroendocrine tumor of the stomach. A. Macroscopic aspect. B. Microscopic aspect.

Table 3. TNM Classification for NET of the Pancreas

TNM				
T --- Primary tumor				
TX	Primary tumor cannot be assessed			
T0	No evidence of primary tumor			
T1	Tumor confined to pancreas and <2 cm			
T2	Tumor confined to pancreas and 2-4 cm			
T3	Tumor confined to pancreas and >4 cm			
	or infiltration of duodenum or bile duct			
T4	Tumor infiltrates adjacent organs (stomach, spleen, colon, adrenal glands)			
	or walls of large vessels (truncus coeliacus/A.mesenterica superior)			
	For every T: add (m) in case of multiple tumors			
N --- Regional lymph node metastases				
Nx	Regional lymph nodes cannot be assessed			
N0	No regional lymph node metastases			
N1	Regional lymph node metastases			
M --- Distant metastases				
Mx	Distant metastases cannot be assessed			
M0	No distant metastases			
M1	Distant metastases			
Disease stages				
Stage	I	T1	N0	M0
Stage	IIa	T2	N0	M0
	IIb	T3	N0	M0
Stage	IIIa	T4	N0	M0
	IIIb	every T	N1	M0
Stage	IV	every T	every N	M1

Table 4. TNM Classification of the Neuroendocrine Tumors of the Duodenum and the Proximal Jejunum

TNM	
T --- Primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor infiltrates lamina propria or submucosa and is <1 cm
T2	Tumor infiltrates muscularis propria or is >1 cm
T3	Tumor infiltrates pancreas or retroperitoneum
T4	Tumor infiltrates peritoneum or other organs
	For every T: add (m) in case of multiple tumors
N --- Regional lymph node metastases	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Regional lymph node metastases
M --- Distant metastases	
Mx	Distant metastases cannot be assessed
M0	No distant metastases
M1	Distant metastases

Disease stages				
Stage	I	T1	N0	M0
Stage	IIa	T2	N0	M0
	IIb	T3	N0	M0
Stage	IIIa	T4	N0	M0
	IIIb	every T	N1	M0
Stage	IV	every T	every N	M1

gastrin cells and microtumors that are considered to be precursor lesions. These minigastrinomas and the microadenomas in the pancreas frequently show allelical deletion of the MEN I gene and are therefore viewed as initial neoplasias. Multiple pancreatic microadenomas can also form independently of germline mutations. These microadenomatoses are then mono-hormonal and can as insulin-expressing tumors cause hyperinsulinemic

hypoglycemia, while the glucagon-expressing microadenomatosis of the pancreas is not associated with any functional syndrome. These results illustrate that primarily for the GEP NETs originating within the context of hereditary syndromes, precursor lesions exist whose exact monitoring can be significant for the course of disease.

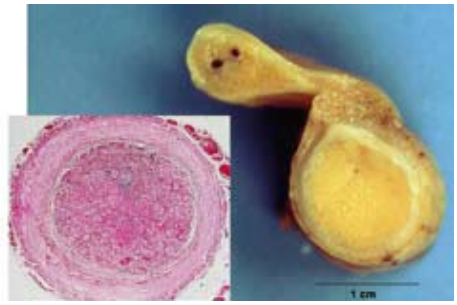

Fig. 4. Well-differentiated neuroendocrine tumor of the appendix (carcinoid of the appendix). Insert. Microscopic aspect.

Specific Tumor Entities of GEP-NETs

Four different NET types occur in the stomach. Type 1 NET of the stomach is characterized by a proliferation of ECL cells which happens within the scope of chronic atrophic gastritis and, as a result, an accompanying hypergastrinemia occurs and leads to multi-focal, highly-differentiated neuroendocrine tumors. These can generally be removed and monitored endoscopically. Type 2 is, likewise, an ECL tumor connected with MEN 1 and hypergastrinemia caused by Zollinger Ellison syndrome with duodenal gastrinoma. These NETs can form metastases. Type 3 of the gastral NETs is a sporadic, solitary tumor that can also form metastases of a size larger than 2 cm. Type 4 is a poorly-differentiated neuroendocrine carcinoma of the stomach which is usually advanced at the time of diagnosis. If clinically indicated, types 2-4 of the gastral NETs should be surgically treated.

In the duodenum there are five different types of GEP-NET which vary regarding their biology and prognosis. Gastrin-producing GEP-NETs are designated as gastrinoma if they cause Zollinger Ellison syndrome which is distinguished through hypergastrinemia, hyperchlorhydria, multiple, in part therapy-resistant ulcers, reflux esophagitis, and diarrhea. Gastrin-producing NETs mainly occur in the proximal duodenum, can appear multi-focally, form metastases in the regional lymph nodes which can exceed the primary tumors in terms of size, and are often incorrectly regarded as pancreatic gastrimomas. They occur sporadically or in association with MEN 1 (see below).

The somatostatin-producing NETs of the duodenum are most frequently localized on the papilla vateri and can appear in connection with double-sided pheochromocytomas within the context of neurofibromatosis type 1. Non-functional NETs of the duodenum can occur as prognostically favorable, benign, highly-differentiated neuroendocrine tumors or as prognostically unfavorable, malignant, poorly-differentiated neuroendocrine carcinomas. Duodenal gangliocytic paragangliomas are a rarity, usually localized adjacent to the papilla vateri, and show mostly benign behavior.

NETs of the distal jejunum and ileum are for the most part localized in the terminal ileum and show regional lymph node metastases, and in 20% liver metastases. In these patients, a carcinoid syndrome can also develop which is characterized by paroxysmal facial redness (flush), diarrhea, and right-ventricular endocardial fibrosis (Hedinger syndrome). NETs of the appendix are also common and can, like the NETs of the jejunum and ileum, produce serotonin. However, NETs of the appendix are associated with a significantly better prognosis since they generally first metastasize at a size larger than 2 cm or upon infiltration of the mesoappendix. In the colon, NETs are seldom and for the most part poorly-differentiated, while the prognosis is more favorable for the more common NETs of the rectum because these are usually highly-differentiated and noticeable as mucosal tumors during endoscopy.

The majority of NETs of the pancreas are functionally active and appear as highly-differentiated NETs or highly-differentiated NECs. Unregulated secretion of insulin (hypoglycemia syndrome, Whipple-Trias),

gastrin (Zollinger Ellison syndrome), vaso-active intestinal polypeptide (Verner Morrison syndrome), glucagon (glucagonoma syndrome), or ACTH (Cushing syndrome) lead to the characteristic syndromes given in parentheses and account for the designation of the tumors as insulinoma, gastrinoma, VIPoma or glucagonoma in terms of the main functional activity. Independent of degree of differentiation, most NETs of the pancreas behave malignantly. The TNM classification of the NETs of the pancreas is given in Table 3. NETs of the pancreas can occur within the context of MEN1 and, as a consequence, a family history and diagnostic screening for pituitary adenomas and hyperparathyroidism should always take place and, if applicable, genetic counseling and testing.

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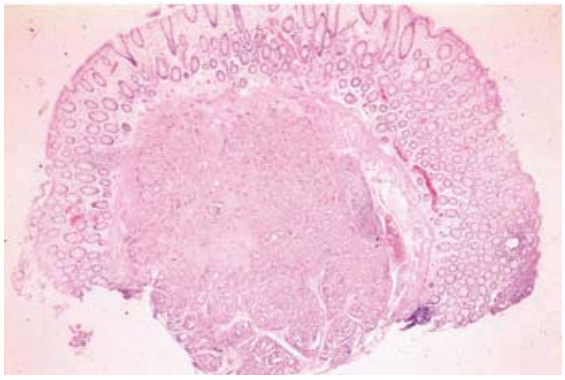

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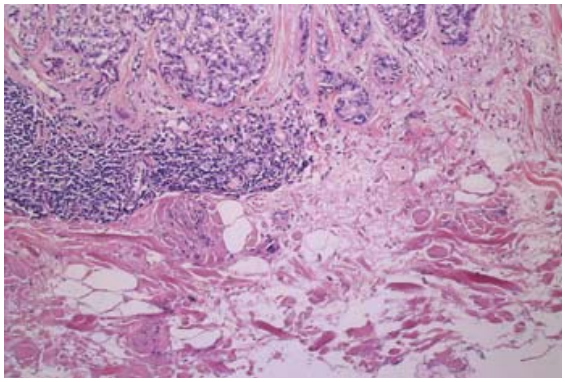

Fig. 5. Well differentiated neuroendocrine tumor of the rectum. A. microscopic overview. Submucosal growth of a well demarcated tumor. B. Microspopic view at the resection border. The tumor margin is separated by fibrous tissue from the resection border.

Hereditary GEP-NETs

Approximately 5-10% of NETs of the intestinal tract and pancreas have a hereditary background. According to frequency, hereditary tumor syndromes with development of GEP NETs are (1) multiple endocrine neoplasia syndrome, (2) neurofibromatosis type 1, (3) von Hippel Lindau syndrome, (4) tuberous sclerosis complex. All of these diseases are based on autosomal-dominant inheritance. This involves tumor-suppressor genes. Loss of heterocygocity (generally chromosomal loss of the second, non-mutated allel) is the basis for tumor occurrence.

The causative genes, epidemiological data, and various clinical appearances are summarized in Table 5. The pathology and clinical picture of MEN 1 disease as the most frequent syndrome is to be briefly described here in more detail. Along with the typical manifestations of hyperparathyroidism and tumors of the adenohypophysis, pancreatic NETs appear in patients with MEN 1 with almost 100% penetration with increasing age. Often this involves so-called microadenomas (size <5 mm). MEN 1-associated microadenomas and macrotumors can express the entire spectrum of pancreatic peptide hormones. They usually behave in an endocrinologically silent manner and are mostly benign. In approximately 10% of cases, the tumors can appear endocrinologically; usually this involves insulin-producing NETs with hypoglycemia syndrome. In approximately 10-25% of cases, patients with MEN 1 additionally develop Zollinger Ellison syndrome (see above). The reason for this are gastrinomas. These are almost exclusively localized in the duodenum and usually occur in multiple. They can already metastasize at a size of less than 2 mm. Detection of the primary in the presence of large lymph node metastases poses a particular interdisciplinary challenge considering the tiny size of these tumors. In parallel, patients with MEN 1 and duodenal gastrinomas develop tumors of the ECL cells of the stomach. Hypergastrinemia as trophic factor, along with the MEN 1

germline mutation, present in all somatic cells, is the cause for the development of these ECL cell tumors.

It is to be presumed that a far larger number of GEP NETs as previously assumed have a hereditary background. It is known from individual population-based studies that some of the GEP NETs appear clustered in families without being able to identify specific factors for this. In addition, it is known that some of the GEP NETs appear in multiple. Approximately 20-40% of the serotonin-producing NETs of the ileum are multiple in appearance. Recently, two disease patterns which accompany multiple glucagon-producing NETs or multiple insulin-producing NETs of the pancreas were described morphologically. The causative factors for the multi-focal tumor development here are unknown.

Table 5. Neuroendocrine Tumor Syndromes: clinical and genetic characteristics. The most frequently occurring tumors (main disease) are underlined for each syndrome. MTC: medullary thyroid carcinoma.

Syndrome	Gene	Neuroendocrine tumors	Other tumors	Specific prevalence*
Multiple endocrine neoplasia type 1 (MEN 1)	MEN 1	Pancreas, parathyroid, hypophysis, adrenal glands, others	Skin (angiofibroma), among others	up to 30%
Multiple endocrine neoplasia type 2 (MEN 2)	RET	Thyroid (MTC), adrenal glands, (pheochromocytoma)	With MEN2b: neuroma	up to 30%
Carney complex	(17q2)	Adrenal glands, testicles, hypophysis	Skin, breast, heart (myxoma)	< 1%
Multiple endocrine neoplasia type 4 (MEN 4)	p27	same as MEN-1		Two families described
Cowden syndrome	PTEN	Thyroid (non-MTC)	Mamma, Kidneys	< 1%
Neurofibromatosis type 1	NF1	Adrenal glands (pheochromocytoma)	Skin, CNS	< 1%

Summary

An exact pathological diagnosis of the heterogeneous group of the neuroendocrine tumors is absolutely essential for patient prognosis and determination of therapy. New knowledge regarding pathogenesis has direct effect not only on the monitoring strategies concerning MEN-1, but also pancreatic NETs without known germline mutation. Introduction of the WHO classification system and the TNM system for NETS of the fore- and midgut allow for a standardized grouping of stages and thus for direct comparison of the studies presently being conducted so that improved pathological diagnostics will have an immediate impact on improving diagnosis and treatment of these tumors.

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Contact:
Klinik für Innere Medizin, Gastroenterologie, Onkologie, Endokrinologie
Zentrum für Neuroendokrine Tumoren Bad Berka
Zentralklinik Bad Berka GmbH
Robert-Koch-Allee 9
99437 Bad Berka
Telephone: 036458-52601
Fax: 036458-53535
Email: d.hoersch.gast@zentralklinik-bad-berka.de

Institut für Pathologie
Universitätsklinikum Schleswig-Holstein, Campus Kiel
Michaelisstr. 11
24105 Kiel
Telephone: (+49) 0431 597 1138
FAX (+49) 0431 597 3462
Email: manlauf@path.uni-kiel.de

Martin Anlauf MD

Patricia Grabowski MD

Günter Klöppel MD