Overlapping but also distinct conditions in the gastroenteropancreatic neuroendocrine system: from hyperplasia to tumor and carcinoma

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Introduction

The presence of hyperplastic tissue changes next to overt neoplasms has always attracted a lot of attention, because the identification of tumor precursor lesions provides insights into tumor pathogenesis and also plays an important role in the diagnosis and treatment of the associated neoplasms. However, it can be very difficult to distinguish hyperplastic tissue changes from developing neoplasms. This is also true for the distinction between a tumor that has not yet metastasized from a tumor that has already done so. Finally, tumors that seemingly have a common origin, may profoundly differ in their nature to an extent that they actually represent two very different entities. In endocrine pathology both issues play an important role. They will here be discussed for the hyperplastic and neoplastic lesions of the gastroenteropancreatic neuroendocrine system.

The hyperplasia-neoplasia sequence

Among the neuroendocrine neoplasms that evolve from hyperplastic lesions are medullary thyroid carcinoma and pheochromocytoma in the setting of multiple endocrine neoplasia type 2 (MEN2) and the hyperplastic changes in the parathyroid glands that may give rise to adenomas in MEN1. In the case of the well differentiated gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs), the NETs according to the terminology of the WHO classification 2010, precursor lesions have been identified in five conditions involving the stomach, the duodenum, the colorectum and the pancreas:

1. ECL (enterochromaffin-like) cell hyperplasia of the corpus-fundic region of the stomach occurring either in autoimmune chronic atrophic gastritis (CAG) or in the setting of MEN1.
2. Gastrin cell hyperplasia in the duodenum associated with MEN1.
(3) neuroendocrine cell hyperplasia associated with chronic inflammatory bowel diseases.

(4) glucagon cell hyperplasia in the pancreas associated with MEN1.

(5) glucagon cell hyperplasia in association with a recently described pancreatic disease called glucagon cell adenomatosis.

**Stomach**

There are three types of well differentiated neuroendocrine neoplasms (NETs or carcinoids) in the stomach, two of them, i.e. type 1 and type 2, are preceded by hyperplastic precursor changes. These two NET types are composed of histamine-producing ECL cells, which because of their neuroendocrine nature express synaptophysin and chromogranin A and can be selectively stained in the stomach with the marker VMAT2 (vesicular monoamine transporter 2). Type 3 of the gastric NETs, which occurs sporadically and is not preceded by any hyperplastic changes, usually also consists of ECL cells, but on rare occasions may be composed of gastrin or other neuroendocrine cells.

The type 1 gastric NETs are always preceded by linear and later nodular ECL cell hyperplasia confined to the fundic glands of the stomach. As the first crucial step towards tumor development ECL-cell nodules develop outside the glands. These microtumors grow slowly (with a Ki67 proliferation index below 2%) in the mucosa and submucosa. They become endoscopically detectable when they measure 0.5-1 cm in diameter and then appear as broad-based, round, polypoid mucosal tumors. Clinically they do not produce any specific symptoms, particularly no hormonal syndrome, but are preferentially found in middle-aged women.

The ECL-cell hyperplasia in CAG is a result of autoimmune destruction of the specific glands (parietal cells) of the corpus-fundic mucosa. The loss of parietal cells has two consequences: (1) insufficient production of intrinsic
factor triggering pernicious anemia via the decreased resorption of vitamin B12 and (2) achlorhydria of the stomach. Achlorhydria stimulates the antral and duodenal G cells to produce gastrin, causing persistent hypergastrinemia, which in turn promotes, via specific receptors, the proliferation of the gastric ECL cells. As a result diffuse linear and micronodular adenomatoid ECL cell hyperplasia develops, out of which multiple ECL NETs arise after a latent period of approximately 10 years. The observation that the tumors can occur in only partial CAG, as for instance in multifocal Helicobacter pylori dependent CAG, and the detection of growth factors such as TGF-alpha and beta-FGF and the anti-apoptotic protein BCL-2 are indications that hypergastrinemia alone probably is not sufficient for these tumors to develop.

The prognosis of type 1 gastric NETs is good, because they are generally so small that they can be totally removed endoscopically. Regional lymph node metastases occur only in very rare cases, in which the tumors are usually larger than 2 cm in size and often infiltrate the muscularis propria.

The type 2 gastric NETs occur in association with MEN1, a hereditary autosomal dominant disorder, in the course of which a Zollinger-Ellison syndrome (ZES), caused by duodenal gastrinoma, has developed. In this condition the associated ECL cell hyperplasia is found not in an atrophic corpus mucosa, but a mucosa that shows diffuse hyperplasia of the acidopeptic glands. When detected, the multiple NETs are mostly smaller than 1.5 cm and limited to the mucosa and submucosa. However, if one of them is larger than 2 cm, shows angioinvasion and/or has invaded the muscularis propria, the tumor has metastasized in approx. 30% of the cases. Clinically, they occur with approximately equal frequency in men and women and do not develop any specific hormonal syndrome.

The ECL cell hyperplasia in type 2 gastric NETs is pathogenetically linked to a heterozygous germline mutation of the MEN1 gene, together with gastrinoma-related hypergastrinemia. Two reports suggested that type 2 NETs also occur in sporadic ZES. Since their publication in 1994/1995, however, these reports have not been confirmed.
Duodenum

There are several types of NET in the duodenum. Two of them, i.e. the gastrin- or somatostatin-producing tumors, may be associated with the MEN1 syndrome and are then preceded by precursor lesions. The other duodenal NETs, sporadic gastrinomas, somatostatin-producing tumors with and without an associated neurofibromatosis type 1 syndrome, serotonin-producing tumors, poorly differentiated neuroendocrine neoplasms and finally gangliocytic paragangliomas, arise from the duodenal mucosa without any preceding changes.

Duodenal gastrinomas arising in the setting of MEN1 are multiple, in contrast to sporadic gastrinomas. In addition to duodenal gastrinomas, somatostatin-producing tumors can also arise in the duodenum of patients with MEN1, although in much lower numbers than gastrinomas. These two NET types are found to be associated with linear and nodular hyperplastic changes of the gastrin and somatostatin cells within the crypts and Brunner’s glands of the duodenal mucosa. These focally accentuated hyperplastic lesions that exhibit enhanced proliferative activity are found in all patients with MEN1, but are absent in patients with sporadic (non-MEN1-associated) duodenal gastrinomas. The fact that the hyperplastic gastrin cell changes occur throughout the duodenal mucosa explains the multifocality of gastrinomas in MEN1 and the failure to cure patients with MEN1-associated ZES by simple excision of a visible tumor.

Like the ECL cell hyperplasia in type 2 gastric NETs, duodenal gastrin cell hyperplasia is also linked pathogenetically to a heterozygous germline mutation of the MEN1 gene. In MEN1 patients all somatic cells harbor a germline mutation of the MEN1 tumor suppressor gene. A loss of heterozygosity (LOH) at the MEN1 gene locus, often combined with LOH of centromere 11, was demonstrated in MEN1-associated duodenal NETs, some of them not larger than 300 µm in diameter. In contrast to tumors, the hyperplastic gastrin cells consistently lack LOH at the MEN1 gene locus. These findings suggest that, though the hyperplastic cells were hyperproliferative and carried the MEN1 germline mutation on one allele, they
had not yet assumed the neoplastic genotype characterized by the allelic loss of 11q13. We do not know what mechanisms enhance the proliferation of gastrin cells and produce hyperplasia, but they could be related to an increased responsiveness of the gastrin cell bearing the germline \textit{MEN1} mutation to certain growth factors or some other means of \textit{MEN1} haploinsufficiency.

Recently, gastrin cell hyperplasia was described in the vicinity of gastrin producing NETs of the duodenum that had been endoscopically removed. All of these tumors were discovered incidentally and none were associated with a Zollinger-Ellison syndrome or the MEN1 syndrome. Since these duodenal gastrin cell tumors were commonly found in patients with \textit{Helicobacter pylori} gastritis and long-term proton pump inhibitor treatment, a pathogenetic relationship with these factors was discussed.

\textbf{Colorectum}

Ulcerative colitis of long duration may be associated with single or multiple small (< 5mm) colorectal NETs, also called microcarcinoids. Some of them are accompanied by hyperplasia of the neuroendocrine cells of the colorectal mucosa.

The ulcerative colitis associated NETs were found by chance in colorectal specimens that were removed because of complications, i.e. bleeding or colorectal adenocarcinoma, related to longstanding ulcerative colitis. Lymph node metastases were not reported. Pathogenetically, it is thought that they are reactive in nature, implying that they develop in response to injury and/or inflammation. The neuroendocrine cell types that are involved in the hyperplastic process have yet to be identified.

\textbf{Pancreas}

In the pancreases of patients with MEN1 there are typically multiple small (< 5 mm) neuroendocrine tumors, a finding that has been referred to as microadenomatosis. The pancreatic microadenomas are often accompanied by one or more macrotumors (diameter >5 mm), some of which may become
insulinomas. Microadenomas are characterized and distinguished from islets by their monohormonal cell composition.

By combining fluorescence *in situ* hybridization of the *MEN1* locus at 11q13 and the centromeric region of chromosome 11q with hormone immunostaining, it was shown that microadenomas are usually composed of glucagon cells and lack one *MEN1* allele. Moreover it was found that a few islets exhibited an increased number of glucagon cells and that these glucagon cells were negative for LOH at 11q13. These results suggest that glucagon cell microadenomas may develop from islets with glucagon cell hyperplasia by acquisition of LOH at 11q13.

The recently described glucagon cell adenomatosis of the pancreas is a neoplastic disease characterized by multiple small glucagon cell microadenomas (smaller than 0.5 cm) and a few macrotumors that are also composed of glucagon cells. In this condition, which is unrelated to MEN1, the islets develop glucagon cell hyperplasia that shows an imperceptible transition to glucagon cell neoplasia.

**NETs and NECs: their distinction and malignant potential**

The neuroendocrine neoplasms are heterogeneous. The WHO classification 2010 of GEP-NENs therefore divides these neoplasms into two groups: (1) the well differentiated neuroendocrine tumors, called NETs, and (2) the poorly differentiated neuroendocrine carcinomas, called NECs.

**NETs**

Typical features of NETs are organoid patterns (trabecular, glandular etc.), little cellular atypia, often hormone expression and occasionally the association with a hormonal syndrome. They are divided according to their proliferative activity into either G1 (Ki67 index < 2%; equivalent to carcinoids) or G2 NETs (Ki67 index 2-20%). Some NET entities evolve from precursor
lesions (see above). During the course of the disease NETs may show an increase in their proliferative activity.

**NECs**

They show a solid/noncohesive growth pattern, extensive necrosis, severe cellular atypia and usually no hormone expression and no hormonal syndrome. NECs are subtyped into small cell and large cell carcinomas. According to their proliferative activity they are G3 tumors (Ki67 index > 20%). NECs show no forerunners such as neuroendocrine cell hyperplasia as do NETs. It also seems that they are genetically distinct from NETs and cannot be considered to be the most dedifferentiated version of a NET.

**General diagnostic features:**

In the context of the identification of potentially metastasizing neoplasms the WHO stratification of the NETs, i.e. the well differentiated NENs, is of importance because this tumor category causes the greatest problems in separating the neoplasms with low metastasizing potential from those with high potential. NECs, in contrast to NETs, are always very aggressive neoplasms that usually have already metastasized at the time of diagnosis.

On the basis of the information and data obtained from the clinical records (tumor localization, symptoms, evidence of systemic disease), macroscopic description (size, gross invasion of adjacent anatomic structures, lymph node involvement) and the microscopic evaluation (histologic differentiation, angioinvasion, invasion of adjacent anatomic structures, grade, Ki67 index, and hormone expression), GEP-NETs are classified according to grade and TNM stage, which has to take the localization of the tumor into consideration. This allows them to be divided into statistically different survival groups, in which survival is largely related to the development of metastases.

**Special diagnostic features:**
Many NETs are well characterized by the WHO classification and the TNM system mentioned above. However, some NETs show very specific behavior with which you must be familiar if you are to assess them appropriately.

_Eosophagus:_ Most of the NENs are NECs.

_Stomach:_ There are three types of NETs in the stomach. Type 1 and 2 are associated with chronic atrophic corpus gastritis and MEN1, respectively. Type 3 NETs are sporadic neoplasms. Type 1 gastric NETs can usually be treated by endoscopic removal, because most of them are discovered while they are still smaller than 1 cm - a stage that has an extremely low risk of metastases.

_Duodenum:_ There are two types of NETs that show specific behavior. The first is duodenal gastrinoma (by definition a gastrin-producing NET with a Zollinger-Ellison syndrome). This neoplasm may be even smaller then 0.5 cm and nevertheless show lymph node metastases. The second is duodenal paraganglioma, which despite its often large size carries a very low risk of metastases.

_Ileum:_ The NETs in the ileum are usually G1, but even when small (< 1 cm) have a great risk of metastases in the regional lymph nodes and the liver.

_Appendix:_ In contrast to the ileum, the appendix NETs below the size of 2 cm only very rarely metastasize to the regional lymph nodes.

_Colon:_ Most of the NENs are NECs.

_Pancreas:_ The NETs of the pancreas can be divided into three biologically different groups: insulinomas, functioning non-insulinomatous NETs, and non-functioning NETs. While insulinomas usually behave benignly, the other two groups follow largely the prognostic criteria of the WHO classification.

_Rectum:_ The behavior of the rectal NETs is similar to that of type 1 gastric NETs.

**Conclusion**

In the GEP-NE cell system there are three well characterized conditions, neuroendocrine cell hyperplasia (NCH), neuroendocrine tumor (NET) and
neuroendocrine carcinoma (NEC). While NCH and NET are pathogenetically linked to each other, NEC belongs to a group of neoplasms of its own.
References


Klöppel G: Classification and pathology of gastroenteropancreatic neuroendocrine neoplasms Endocrine Related Cancer 18, S1 – S16, 2011


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Overlapping and distinct conditions:

Gastroenteropancreatic neuroendocrine

Hyperplasia

Tumor (NET)*

Carcinoma (NEC)*

- Definition according to WHO 2010
- NET – well differentiated NEN
- NEC - poorly differentiated NEN
Gastroenteropancreatic Neuroendocrine Hyperplasia: organ involvement

NE-Hyperplasia:  1 Stomach
                2 Duodenum
                3 Colorectum
                4 Pancreas
Gastroenteropancreatic Neuroendocrine Hyperplasia: stomach: ECL (enterochromaffin-like) cell hyperplasia (I)

Pathogenesis:
- atrophic (autoimmune) gastritis of the corpus-fundic region
- loss of parietal cells with achlorhydria
- stimulation of gastrin secretion from antral/duodenal G-cells
- gastrin-receptor triggered proliferation of ECL-cells
- diffuse linear and nodular adenomatoid ECL-cell hyperplasia
Gastroenteropancreatic Neuroendocrine Hyperplasia: stomach: ECL (enterochromaffin-like) cell hyperplasia (II)

Pathogenesis:
- gastrin hypersecretion by duodenal MEN-1 associated gastrinoma
- gastrin and MEN-1 triggered proliferation of ECL-cells
- diffuse linear and nodular adenomatoid ECL-cell hyperplasia
Gastroenteropancreatic Neuroendocrine Hyperplasia: duodenum: gastrin cell hyperplasia

Pathogenesis (I):
- MEN-1 triggered proliferation of gastrin (and somatostatin) cells
- focal linear and nodular gastrin (and somatostatin) cell hyperplasia
Gastroenteropancreatic Neuroendocrine Hyperplasia:
duodenum: gastrin cell hyperplasia

Pathogenesis (II):

- heterozygous germ line mutation of the tumor suppressor MEN-1 gene triggers proliferation of gastrin (and somatostatin) cells (by their special responsivness to growth factors)

- gastrin (and somatostatin) cell hyperplasia
Gastroenteropancreatic Neuroendocrine Hyperplasia: duodenum: gastrin cell hyperplasia

- Is there a PPI triggered proliferation of gastrin cells in the duodenum causing gastrin cell hyperplasia?

- Merchant et al AJSP 2006
Gastroenteropancreatic Neuroendocrine Hyperplasia:
Colo-rectum: gastrin cell hyperplasia

Pathogenesis:

- Ulcerative colitis of long duration
- Reactive (in response to injury/inflammation) neuroendocrine cell hyperplasia

Gastroenteropancreatic Neuroendocrine Hyperplasia:
Pancreas: glucagon cell hyperplasia

Pathogenesis (I):
- MEN-1 triggered proliferation of glucagon (and non-alpha) cells
- focal glucagon (and non-alpha) cell hyperplasia in individual islets
Gastroenteropancreatic Neuroendocrine Hyperplasia: Pancreas: glucagon cell hyperplasia

Pathogenesis (II):
- heterozygous germ line mutation of the tumor suppressor MEN-1 gene triggers proliferation of glucagon (and non-alpha) cells (by special responsiviness to growth factors)
- glucagon (and non-alpha) cell hyperplasia in individual islets
Gastroenteropancreatic Neuroendocrine Hyperplasia: Pancreas: glucagon cell hyperplasia (II)

Glucagon cell adenomatosis

Pathogenesis:

- glucagon receptor mutation triggered proliferation of glucagon cells
- glucagon cell hyperplasia in islets
Gastroenteropancreatic Neuroendocrine Tumor (NET): The hyperplasia - neoplasia sequence

What are NETs?

Their definition according to the WHO classification 2010
# Neuroendocrine Neoplasms – NENs of the gastroenteropancreatic system

<table>
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<td>1. Well-differentiated endocrine tumour (WDET)*</td>
<td>1. NET G1 (carcinoid)</td>
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<td>2. Well-differentiated endocrine carcinoma (WDEC)*</td>
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**NET, neuroendocrine tumor – well diff.; NEC, neuroendocrine carcinoma – poorly diff.; G, Grade**

*In case that the Ki67 index exceeds 20 %, this NET may be labelled G3*
Gastroenteropancreatic Neuroendocrine Tumor (NET): The hyperplasia - neoplasia sequence

1 Stomach: ECL-cell hyperplasia – ECL cell NET
2 Duodenum: G-cell hyperplasia – G-cell NET
3 Colorectum: NE-cell hyperplasia – NET
4 Pancreas: A-cell hyperplasia – A-cell NET
Gastroenteropancreatic Neuroendocrine Tumor:
The hyperplasia - neoplasia sequence
stomach: ECL – cell NET

Types:
- Type 1: Associated with chronic atrophic (autoimmune) gastritis
- Type 2: MEN-1 associated
Gastroenteropancreatic Neuroendocrine Tumor: The hyperplasia - neoplasia sequence
duodenum: Gastrin (somatostatin) – cell NET

Types:
- Type 1: MEN-1 associated
- Type 2: Associated with PPI administration?
Gastroenteropancreatic Neuroendocrine Tumor:  
The hyperplasia - neoplasia sequence  
colorectum: ? – cell NET

- Ulcerative colitis associated
Gastroenteropancreatic Neuroendocrine Tumor:
The hyperplasia - neoplasia sequence
Pancreas: Glucagon (non-alpha) – cell NET

Types:
- MEN-1 associated glucagon cell NETs
- Glucagon cell adenomatosis
Gastroenteropancreatic Neuroendocrine Tumor: The hyperplasia - neoplasia sequence

Pathogenesis

- Omnipotent stem cell
- Multipotent stem cell
  - Pre-programmed (stem) cell
    - Intrinsic (MEN-1, GR mutation)
    - Extrinsic (gastrin receptor)
    - Stimulation of proliferation
      - NE cell hyperplasia
      - NE Tumor
Gastroenteropancreatic Neuroendocrine Tumor: The hyperplasia - neoplasia sequence

Pathogenesis

Why show the pre-programmed (stem) cell such a type specificity for

- ECL-cells in the stomach
- Gastrin-cells /Somatostatin cells in the duodenum
- Glucagon-cells in the pancreas
Is there a NET – NEC sequence?

Evolve the poorly differentiated NENs, i.e the NECs, from well differentiated NENs, the NETs?

There is usually no NET – NEC sequence!
Gastroenteropancreatic Neuroendocrine Neoplasms

General Features of NETs and NECs

**NET**

Variable histopathology and biology depending on localization - heterogeneity

**NEC - small or large cell type**

Similar histopathology and biology in all parts of the body - homogeneity
Gastroenteropancreatic Neuroendocrine Neoplasms

General Features of NETs and NECs

91 - 97% of GEP-NENs are NETs
- slow growth
- prognosis is often favorable (i.e. stomach, appendix, rectum)
- immunohisto. hormone expression, hormonal syndromes may occur
- surgery, biological response modifiers, chemo, embolization, PRRT

3 - 9% of GEP-NENs are NECs
- explosive growth,
- bad prognosis
- usually no hormone expression, no hormonal syndromes
- preferential locations: esophagus, stomach, ampulla, colon
- chemothrap
Gastroenteropancreatic Neuroendocrine Carcinoma: Pathogenesis

-Omnipotent stem cell

-NE Carcinoma

No hyperplasia – neoplasia sequence!
USCAP 2012:
Overlapping but also distinct conditions in the gastroenteropancreatic neuroendocrine system: from hyperplasia to tumor and carcinoma

Conclusions

In the GEP-NE cell system there are three well characterized conditions: neuroendocrine cell hyperplasia (NCH), neuroendocrine tumor (NET) and neuroendocrine carcinoma (NEC).

While NCH and NET are pathogenetically linked to each other, NEC belongs to a group of neoplasms of its own.