

## **Precursor lesions of neuroendocrine tumors of the GI tract and the pancreas**

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Tumors that develop on the basis of precursor changes such as hyperplastic cellular lesions have always received great attention. The presence of precursor lesions provides insights into the early development of tumors and is also significant for the diagnosis and therapy of the associated neoplasms. Among the endocrine tumors that evolve from hyperplastic lesions are medullary thyroid carcinoma and pheochromocytoma in the setting of multiple endocrine neoplasia type 2 (MEN2) [1;2] and the hyperplastic changes of the parathyroid glands that may give rise to adenomas in MEN1 [3].

In the case of gastroenteropancreatic neuroendocrine tumors (NETs), precursor lesions have been identified in three conditions so far. Best known is the ECL cell (enterochromaffin-like cell) hyperplasia in autoimmune chronic atrophic gastritis of the corpus-fundic region of the stomach [4]. The second condition is the gastrin and somatostatin cell hyperplasia in the duodenum and a glucagons cell hyperplasia in the pancreas associated with MEN type 1 (MEN1) [5-7]. The third condition is chronic inflammatory bowel diseases that are associated with endocrine cell hyperplasia [8]. This review focuses on these three conditions in which endocrine precursor lesions have been described.

### **Stomach**

There are four types of NET in the stomach [9;10], two of them are preceded by precursor lesions, i.e. type 1 and type 2. Both types are composed of histamine-producing enterochromaffin-like cells (ECL cells), which can be selectively stained in the stomach with the marker VMAT2 (vesicular monoamine transporter 2). Type 3 of the gastric NETs, which occurs sporadically, usually also consists of ECL cells and only rarely of gastrin or other endocrine cells [11]. The type 4 gastric NET is a poorly differentiated carcinoma whose hormone production usually remains unrevealed.

The precursor lesion that is observed in type 1 gastric NETs is hyperplasia of the ECL cells in the corpus fundic region of the stomach [12]. There is a linear and

later a nodular pattern of ECL cell hyperplasia in the fundic glands. As the next step towards tumor development the ECL cells outgrow the confinement of the glands and form extraglandular nodules that become the starting point for multiple NETs [4]. These slowly growing tumors become endoscopically visible when measuring 0.5-1 cm in diameter and present as broad-based, round, polypoid mucosal tumors. Clinically these tumors are not associated with any specific symptoms, particularly not with any hormonal syndrome, but occur predominantly in women (70%-80% of the cases). Histologically, the tumors are well differentiated, located in the mucosa and submucosa and composed of intensely chromogranin A and VMAT2 (vesicular monoamine transporter 2) positive ECL cells.

By definition, type 1 gastric NETs develop on the background of chronic atrophic gastritis (CAG) resulting from autoimmune destruction of the specific glands (parietal cells) of the corpus-fundic mucosa [13]. On the one hand, the loss of parietal cells leads to insufficient production of intrinsic factor and can thus trigger pernicious anemia via the decreased resorption of vitamin B12. On the other hand, the loss of hydrochloric acid producing parietal cells causes achlorhydria of the stomach, which for its part stimulates the antral and duodenal G cells to produce gastrin, causing persistent hypergastrinemia. It is assumed that gastrin promotes the proliferation of the ECL cells of the corpus mucosa by binding to specific receptors. As a result diffuse linear and micronodular adenomatoid ECL cell hyperplasia develops [4], out of which the above described multiple ECL tumors arise after a latent period of many 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 [14].

The prognosis of these tumors is good, because they are generally so small that they can be removed endoscopically [15]. Regional lymph node metastases seem to occur only in very rare cases, in which the tumors are larger than 2 cm in size and infiltrate the muscularis propria [9].

ECL cell hyperplasia of the fundic-corporis mucosa without CAG is found in type 2 gastric NETs that present in association with MEN1, a hereditary, autosomal dominant disorder, in the course of which a Zollinger-Ellison syndrome (ZES) has developed [16]. They occur with approximately equal frequency in men and women. The tumors are usually smaller than 1.5 cm and limited to the mucosa and

submucosa. The mucosa shows diffuse hyperplasia of the acidopeptic glands. If angioinvasion is found or the tumor is larger than 2 cm and/or has invaded the muscularis propria, the tumor has metastasized in approx. 30% of the cases [9]. The heterozygous mutation of the *MEN1* gene together with gastrinoma-related hypergastrinemia is probably the basis on which the tumor develops [14]. Two reports suggested that type 2 NETs also occur in sporadic ZES [17;18]. 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 that are associated with the MEN1 syndrome, are preceded by precursor lesions. The other duodenal NETs, sporadic gastrinomas and somatostatin-producing tumors with and without an associated neurofibromatosis type 1 syndrome, serotonin-producing tumors, poorly differentiated neuroendocrine carcinomas and finally gangliocytic paragangliomas, arise from the duodenal mucosa without any preceding changes.

In 1990 it was noticed that many duodenal gastrinomas arising in the setting of MEN1 are multiple, in contrast to sporadic gastrinomas [19]. Recently, it was shown that in addition to gastrinomas, somatostatin-producing tumors can also arise in the duodenum of patients with MEN1 [6]. These multicentric gastrin- and somatostatin-producing NETs were found to be associated with gastrin and somatostatin cell hyperplasia within the nontumorous duodenal mucosa [6;20], with features similar to those described for ECL cells in CAG [4] [21]. Therefore, an analogous classification has been proposed that distinguishes between diffuse, linear and micronodular hyperplasia of gastrin cells associated either with the crypts or with Brunner's glands. The hyperplasia was determined by measuring the gastrin cell density in the nontumorous duodenal mucosa of MEN1 patients using a morphometric approach [20]. A comparison of the density of duodenal gastrin cells in MEN1 patients with that of non-MEN1 patients with and without gastrinomas revealed that gastrin cells were twice as frequent in MEN1 patients. Nodular lesions outside the mucosal glands more than 300  $\mu\text{m}$  in size were classified as microtumors. The proliferative nature of the hyperplastic lesions was confirmed by enhanced Ki-67 expression, contrasting with the lack of Ki-67 expression in single gastrin cells of the mucosal glands outside the hyperplastic foci. The gastrin cell and

somatostatin cell hyperplasia was found in all patients with MEN1 but was absent in patients with sporadic (non-MEN1-associated) duodenal gastrinomas and ZES. Because of the smooth transition from hyperplastic to early neoplastic gastrin cell lesions these alterations were considered to be precursor lesions of the MEN1-associated duodenal gastrinomas. The fact that such gastrin cell hyperplasia occurred at various sites in the duodenal mucosa explains the multifocality of MEN1 gastrinomas and somatostatin-producing tumors and the failure to cure patients with MEN1-associated ZES by simple tumor excision.

Tumor precursor lesions are assumed to show a sequence of genetic changes that lead to overt neoplasia. In MEN1 patients all somatic cells harbor a germline mutation of the MEN1 tumor suppressor gene [3;22;23]. Recently, a loss of heterozygosity (LOH) at the *MEN1* gene locus, often combined with LOH of centromere 11, was demonstrated in approximately 50% of MEN1-associated duodenal NETs [6] (Fig. 4). Allelic loss was detected in tumors as small as 300  $\mu\text{m}$  (gastrin) and 400  $\mu\text{m}$  (somatostatin) in diameter. In contrast to tumors, the hyperplastic gastrin and somatostatin cells consistently lacked LOH at the *MEN1* gene locus (Fig. 4). This finding suggested that though the hyperplastic cells were hyperproliferative and carried the *MEN1* germline mutation, 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 or somatostatin cell bearing the germline *MEN1* mutation to certain growth factors or some other means of *MEN1* haploinsufficiency.

In conclusion, allelic deletion of the second *MEN1* allele seems to be a pivotal event in the development of multifocal gastrin and somatostatin cell neoplasms in the duodenum of MEN1 patients. The observation of distinct deletion patterns in the synchronous MEN1 tumors supports the concept that each gastrin-producing tumor in an individual MEN1 patient arises from an independent cell clone.

Recently, gastrin cell hyperplasia was described in the vicinity of gastrin producing NETs of the duodenum which were endoscopically removed [24]. All these tumors were discovered incidentally and were not associated with a Zollinger-Ellison syndrome or the MEN1 syndrome. Since these duodenal gastrin cell tumors were commonly found in patients with *Helicobacter pylori* gastritis and long-term proton pump inhibitors, a pathogenetic relationship with these factors was discussed.

## Colon and rectum

Small NETs, also called microcarcinoids, have been described in association with ulcerative colitis of long duration [8;25-29]. The described NETs were either solitary or multiple. Some colorectal NETs were accompanied by hyperplasia of the endocrine cells of the mucosa, while in others no endocrine cell hyperplasia was noted [8;26].

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. The NETs were rarely grossly visible. They were composed of trabecular formations of well differentiated neuroendocrine cells staining for synaptophysin, chromogranin and CD56 [8]. 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 endocrine cell types that are involved in the hyperplastic process have yet to be identified.

## Pancreas

The pancreases of patients with MEN1 are characterized by the presence of multiple small endocrine tumors (i.e. up to 5 mm in diameter), a finding that has been referred to as microadenomatosis [30]. The pancreatic microadenomas are often accompanied by one or more macrotumors (diameter >5 mm), some of which may be functionally active [3;30;31].

Opinions vary as to which type of endocrine cell lesion precedes the development of microadenomas in patients with MEN1. Vortmeyer et al. [32] found 11q13 LOH in duct-associated lesions but not in islets and their conclusion was that there is a “non-islet cell origin of pancreatic islet cell tumors.” In our study using a technique combining fluorescence *in situ* hybridization of the *MEN1* locus and the centromeric region of chromosome 11q with hormone immunostaining, we found loss of one *MEN1* allele in all microadenomas and in 19 of 20 so called monohormonal endocrine cell clusters (MECCs) examined. These results therefore indicated that MECCs which were usually composed of glucagon cells represent a minute form of microadenomas. Moreover, since MECCs were often found to be

incorporated in islets and only rarely seen in association with a duct, it was suggested that microadenomas may originate from islets more frequently than from ducts. Islets that were enlarged because of an increased number of glucagon cells but lacked the inclusion of a MECC were always found to be negative for LOH at 11q13. This implies that these islets contain hyperplastic and not neoplastic cells. Interestingly, MECCs were identified more frequently in islets without glucagon cell hyperplasia than in ones with hyperplastic glucagon cells. Thus it seems that glucagon cell hyperplasia of islets is not an obligatory precursor lesion for pancreatic MEN1-associated NETs arising in islets. No precursor lesions have so far been observed in sporadic NETs of the pancreas.

### **Summary and conclusions**

(1) ECL cell hyperplasia in the corpus-fundic mucosa of the stomach is a precursor lesion of multiple NETs that develop either in association with CAG or MEN1 patients with ZES. (2) MEN1-associated duodenal gastrinomas and somatostatinomas are preceded by hyperplastic gastrin and somatostatin cell lesions. Allelic deletions of the *MEN1* gene in microadenomas but not in hyperplastic lesions reflect a pivotal genetic event in the development of multifocal gastrin and somatostatin cell neoplasms in the duodenum of MEN1 patients. (3) Endocrine microadenomatosis of the pancreas is a MEN1-associated feature. The microadenomas in MEN1 show loss of the *MEN1* wild-type allele and therefore represent true “initial” neoplasms. They may originate from islets. (4) Longstanding ulcerative colitis seems to promote the development of endocrine cell hyperplasia and/or small NETs.

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