Precursor lesions of gastroenteropancreatic neuroendocrine tumors

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Introduction

For diagnosis and therapy as well as for the understanding of the tumorigenesis of malignant tumors the identification of lesions that precede neoplastic growth and may represent a sequence of cellular changes from hyperplasia to neoplasia is of great interest.

There are some endocrine tumors that develop on the basis of hyperplastic changes, i.e. medullary thyroid carcinoma or pheochromocytoma. Furthermore, it is likely that the adenomatous changes of the parathyroid in multiple endocrine neoplasia type 1 (MEN1) patients originate from hyperplastic changes. All such conditions, except for the ECL-cell hyperplasia in chronic atrophic gastritis, are associated with an inherited endocrine disorder [1-3].

This lecture focuses on endocrine precursor lesions and microadenomatosis in the duodenum and pancreas of patients with MEN1. Molecular concepts and the potential clinical significance are discussed. In addition, two new disease entities are presented. Both are characterized by non-MEN1-associated microadenomatosis of the pancreas, but one shows glucagon-producing tumors, while the other displays multiple insulinomas.

Duodenum

Gastrin-producing tumors are the most common type of neuroendocrine tumor (NET) in the duodenum [4-6]. Most of them are associated with a Zollinger–
Ellison syndrome (ZES) characterized by elevated fasting gastrin serum levels, a positive gastrin secretin stimulation test and clinical symptoms such as recurrent peptic ulcer disease, gastroesophageal reflux disease and, occasionally, diarrhea [7]. Though duodenal gastrinomas are often small in size (diameter less than 1 cm), they tend to metastasize early to regional lymph nodes and the liver [8,9]. Surgical treatment of sporadic gastrinomas by local excision and lymphadenectomy is in many cases curative, while the same procedure usually does not cure the patient with MEN1-associated duodenal gastrinomas [8].

In 1990 it was noticed that many duodenal gastrinomas arising in the setting of MEN1 are multiple, in contrast to sporadic gastrinomas [10]. Recently, it has been shown that in addition to gastrinomas, somatostatin-producing tumors can also arise in the duodenum of patients with MEN1. These multicentric gastrin- and somatostatin-producing NETs were found to be associated with gastrin and somatostatin cell precursor lesions within the nontumorous duodenal mucosa [9], whose spectrum of proliferative changes was similar to that described for ECL cells in chronic atrophic fundus gastritis [11]. Therefore, an analogous classification has been proposed (Table 1) that distinguishes between diffuse, linear and micronodular hyperplasia of gastrin cells associated either with the crypts or with Brunner’s glands. Lesions more than 300 µm in size, which were encountered less frequently than the hyperplastic changes, were classified as microtumors. The proliferative nature of these lesions was confirmed by enhanced Ki-67 expression, contrasting with the lack of Ki-67 expression in nonhyperplastic gastrin cells. The
hyperproliferative and hyperplastic lesions were found in all patients with MEN1 but were 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 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 gene, which is considered to be a tumor suppressor gene [3,12,13]. Recently, a loss of heterozygosity (LOH) of the \textit{MEN1} gene and/or the centromere 11 was demonstrated in approximately 50\% of MEN1-associated duodenal NETs [14]. Allelic loss was detected in tumors as small as 300\,\mu m (gastrin) and 400\,\mu m (somatostatin) in diameter. In contrast to tumors, the hyperplastic gastrin and somatostatin cells consistently lacked LOH on chromosome 11q13. This finding suggested that though the hyperplastic cells were hyperproliferative and carried the \textit{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 cell bearing the germ cell MEN1 mutation to certain growth factors.
In conclusion, allelic deletion of the second MEN1 gene 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.

**Pancreas**

Most pancreatic NETs are sporadic tumors. Approximately 10% of pancreatic NETs arise in patients with a hereditary background of MEN1 [3,13]. The presence of multiple small endocrine tumors in the pancreas (i.e. up to 5 mm in diameter) has been referred to as microadenomatosis and has been described in association with the MEN1 syndrome [15]. MEN1-associated pancreatic microadenomatosis is usually accompanied by one or more macrotumors (diameter >5 mm), some of which may be functionally active [13,15,16].

Although the identification of the MEN1 gene on 11q13 has led to extensive studies of the genetic and clinical features of the MEN1 syndrome, our knowledge of the development and pathology of pancreatic endocrine neoplasms in MEN1 is mainly based on reports of individual cases. Only few studies on a series of MEN1 patients have been published [15,16]. In a recent study by our group we analyzed pancreatic specimens from 28 MEN1 patients [17]. Previous studies showed that microadenomatosis in MEN1 patients is
highly variable as far as the number of tumors and their hormonal profiles are concerned [15, 16, 18]. We confirmed these observations and established a high penetrance for pancreatic microadenomatosis (> 80%) in MEN1 patients. The hormone-positive microadenomas were usually multihormonal, with glucagon as the predominant hormone. Although many patients had numerous microadenomas they did not appear to be associated with a hormonal syndrome. If symptoms of inappropriate hormone secretion, such as persistent hyperinsulinemic hypoglycemia (PHH), were present, they could be related to one or more insulin-producing macrotumors.

As tiny as the pancreatic microadenomas in MEN1 were (i.e. less than 200 µm), they showed the signs of neoplastic tumors: (1) monohormonality, (2) trabecular growth pattern and (3) a distinct stromal component [15-17]. Furthermore, Vortmeyer et al. [19] and we [20] demonstrated LOH of the MEN1 gene locus in MEN1-associated pancreatic microadenomas.

Opinions vary as to which type of endocrine cell lesion precedes the development of microadenomas in patients with MEN1. Recently, Vortmeyer et al. [19] 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 monohormonal endocrine cell clusters examined. By contrast, LOH on 11q was absent in islets and ductal or acinar structures. Our results indicate that monohormonal endocrine cell clusters
represent a minute form of microadenomas. The frequent presence of single nonneoplastic insulin cells in microadenomas and the occurrence of monohormonal endocrine cell clusters intermingled with islets suggest an islet origin of microadenomas rather than a non-islet origin.

Non-MEN1-related microadenomatosis of the pancreas

Microadenomatosis of the endocrine pancreas has been described in a single case report of a patient with VHL syndrome [21]. In contrast, in a study by Lubensky et al. [22], the smallest tumor encountered in a series of 30 Von-Hippel-Lindau (VHL)-associated PETs was 0.4 cm in diameter, but microadenomatosis was not described in any of their cases. We screened the clinico-pathological records of 425 patients suffering from pancreatic NETs [17] and found only two who fulfilled the criteria of a VHL syndrome. The two patients exhibited solitary functionally inactive clear cell NETs without associated microadenomatosis. These observations do not exclude the possibility that microadenomatosis is a feature of VHL-associated pancreatic NETs, but might suggest that the frequency of this feature is lower than in MEN1.

A special finding was the identification of pancreatic microadenomatosis in 9 patients who had multiple microadenomas but neither clinical nor genetic features of the MEN1 or VHL syndrome. Five of these 9 patients suffered from hyperinsulinemic hypoglycemia and had, in addition insulin-positive microadenomas, insulin-positive macrotumors. Two of the 5 patients with
hyperinsulinemic hypoglycemia had only insulin-positive microadenomas, most of which were less than 1 mm in diameter. Four other patients had multiple small glucagon-expressing neoplasms, three of them with additional macrotumors. None of the four patients with multiple glucagon-producing NETs showed clinical signs of a glucagonoma syndrome. This observation suggests that what we encountered is two new disease entities characterized by pancreatic endocrine microadenomatosis; one causing hyperinsulinemic hyperglycemia, the other without any clinical syndrome but numerous glucagon-producing tumors. The mechanisms that may cause the microadenomatosis in these patients are as yet unknown.

**Brief bullet points**

1. MEN1-associated duodenal and pancreatic endocrine tumors are preceded by proliferative endocrine cell changes and microtumors
2. Allelic deletion of the *MEN1* gene in early neoplastic lesions but not in hyperplastic lesions may reflect a pivotal genetic event in the development of multifocal MEN1-associated endocrine neoplasms.
3. The observation of distinct deletion patterns in very small synchronous tumors supports the concept that each MEN1-associated tumor in an individual MEN1 patient arises from an independent cell clone.
(5) Two types of pancreatic microadenomatosis without any hereditary background were identified that were characterized either by multiple hormonally silent glucagon-producing tumors or multiple syndromatic insulin-producing tumors.
References


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Table 1. Classification of proliferative gastrin cell lesions in the duodenum of patients with MEN1

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Definition</th>
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<tr>
<td>Diffuse hyperplasia</td>
<td>Increase in single gastrin-positive cells (confirmed by morphometric analysis)</td>
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<td>Linear hyperplasia</td>
<td>Formation of chains of five or more gastrin-positive cells and ≥ 2 chains per mm</td>
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<tr>
<td>Micronodular hyperplasia</td>
<td>Nodules of gastrin-positive cells with more than 5 gastrin-positive cells within glands or crypt (30 µm to 90 µm)</td>
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<tr>
<td>Enlarged nodule</td>
<td>Nodules of gastrin-positive cells with solid architecture (≥90 µm to &lt;210 µm)</td>
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<td>Microinvasive lesion</td>
<td>Small clusters of gastrin-positive cells localized in the lamina propria between the glands and surrounded by thickened collagen</td>
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<td>Microtumor</td>
<td>Tumorous lesion with trabecular growth pattern and extensive fibrosis (diameter &gt;250 µm)</td>
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</tbody>
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