Oncogenes, growth factors, receptor expression and proliferation markers in digestive neuroendocrine tumours. A critical reappraisal

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Summary

Background: The main characteristic of the digestive neuroendocrine tumours (dNETs) is the low proliferating activity, even in the presence of malignant, metastatic behavior.

Patients and methods: Considering that dNETs are rare diseases, relatively numerous studies, often including a conspicuous number of patients, have recently investigated the molecular mechanisms of neuroendocrine tumour genesis.

Results: In contrast to non-endocrine tumours of the digestive system such as carcinoma of the pancreas, colon and stomach, dNETs do not show alterations in oncogenes (ras, Myc, fos, jun, Src) or in common tumor suppressor genes [p53, retinoblastoma susceptibility gene (Rb)]. MEN-1 gene alterations will likely be important in a proportion of sporadic dNETs. The role of various growth factors, novel oncogenes and tumour suppressor genes have also been investigated. However, results from these studies are non-conclusive and to date the molecular pathogenesis of these tumours has not been clarified. Studies on somatostatin receptor expression and synthetic analogues, as growth inhibitors in dNETs, although promising, have not reproduced in vivo all the antiproliferative effects showed in in vitro models.

Conclusion: Although various functional genes and molecular mechanisms have been investigated in dNETs, to date the molecular pathogenesis of these tumours remains to be elucidated.

Key words: digestive neuroendocrine tumour, growth factor, oncogene, tumour suppressor gene

Introduction

Digestive neuroendocrine tumours of the gastrointestinal tract (dNETs) are relatively rare tumours and due to the heterogeneity of their clinical and biological aspects their study, although fascinating, is complex.

The pancreas and the mucosa of the gastrointestinal tract contain as many as 16 cell types that belong to the so-called diffuse endocrine system (DES), characterized by the production of peptides or amines from which in theory such tumours can develop. However, in practice, tumour transformation has been demonstrated only for some of the DES cells. For diagnostic purposes most of these tumours are sufficiently characterized by histological features showing solid, trabecular or glandular arrangement of well-differentiated cells. For these characteristics specific immunohistochemical stains for general neuroendocrine markers (chromogranins, synaptophysin, or neuron-specific enolase) and hormonal products, are usually able to identify the neuroendocrine origin of the tumours. They also provide the basis of the so-called 'morphofunctional' classification that has been recently revised [1]. In this classification tumours are divided by site of origin (stomach, pancreas, etc.) and classified by their biological behaviour; furthermore the term 'carcinoid' has been abandoned in favour of tumour or carcinoma, and anatomic, clinical and functional data are utilized in combination.

Although the vast majority of dNET tumours are represented by well-differentiated cells, with the low rate of proliferating cells they often present metastases at the time of the diagnosis. This is one of the most intriguing characteristics of dNET tumours and has triggered the scientific interest of many groups who aim to demonstrate specific molecular features that can explain which mechanisms can account for the ability of these tumour cells to detach from primary malignancy and gain access to the surrounding structures and liver. However, except for tumours developing in patients with specific genomic gene alterations, such as MEN-1 syndrome and von Hippel Lindau disease (VHL), the molecular events determining the uncontrolled growth of neuroendocrine cells are still unclear.

Tumour suppressor genes and oncogenes

The majority of neuroendocrine tumours are sporadic and to date no clear hypothesis on tumour genesis and/or tumour progression has been made.

As in most common carcinomas, a number of tumour suppression genes and oncogenes have been investigated in dNETs. However, due to the great variety of these tumours, their relative rarity and the differences in tissues sampled analysed, no definitive conclusion can be made.
Mutations within the p53 gene are known to be due to common genetic alteration, occurring in about half of all types of cancers arising from a wide spectrum of tissues. In dNETs the p53 somatic mutation and/or over-expression have been reported mostly in the late stage, furthermore, when present, its over-expression has no relationship with the clinical-pathological features [2]. At the present we can assume that p53 mutation/over-expression may be unimportant in the genesis of dNETs [2, 3]. Although the function of the K-ras pathway remains incompletely understood, over 90% of pancreatic non-endocrine cancers show mutations of this oncogene. In dNETs K-ras mutation has been sporadically reported [4, 5] and does not seem to be involved in these tumours. Other common oncogenes such as myc, fos, jun, bel-2 and src, in contrast to their involvement in the tumour genesis of non-endocrine tumours, are generally not important in the molecular pathogenesis of dNETs. Hypothetically, their deregulation may represent one of the pathogenic events in the malignant transformation and/or progression of only few atypical forms of these tumours [6]. The cyclin-dependent kinases are enzymes that regulate various faces of the cell division cycle, and the role of their inhibitors such as p15, p16, p27 and p21 as tumour suppressor genes have recently also been evaluated in dNETs. A high frequency of gene deletion and/or mutation of gene coding for these inhibitors has important implications in human cancer genesis, and in this regard they can be functionally considered as tumour suppressor genes. In particular, high p27 and low p21 expression has been reported in differentiated dNETs, suggesting a potential role of p27 as inhibitor of cell proliferation in these type of tumours [7]. During the last few years, the putative tumour suppressor gene, DPC4, successively designed as Smad4 and involved in signal transduction from the TGF-beta family of cytokines, has been demonstrated to be deleted and/or inactivated in up 50% of pancreatic exocrine carcinomas. Due to the anatomical relationship existing between exocrine and endocrine pancreas, Smad4 gene has been investigated also in pancreatic endocrine tumours. One study reported Smad4 alterations (deletions and/or mutations) in five out of nine non-functioning pancreatic tumours and none in eleven insulinomas, three gastrinomas and two VIPomas [8].

A more recent study tested, among other pancreatic exocrine tumours, 41 cases of non-functioning endocrine tumours and found no Smad4 gene alteration in these tumours whatsoever [9]. Even if it is difficult to explain these different results, it is likely that different methods could account for such discrepancy.

Causal genes that are altered in familial cancer syndromes are usually altered in sporadic disease counterparts. On this basis, knowing that patients with familial germ-line alteration in MEN-1 and VHL genes often develop gastrointestinal and/or pancreatic endocrine tumours (Table 1), it may be expected that these genes could be modified in the respective sporadic form of endocrine neoplasms. Both MEN-1 and VHL genes are considered to act as tumour suppressor genes, located on chromosomes 11q13 and 3p25, respectively. After its cloning (1997) MEN-1 gene has been investigated in different types of sporadic neuroendocrine tumours and its mutation has been recently reported in 16%-42% of patients [10]. In general, when studies involve a small number of patients it is likely that the proportion of gene alterations has been over emphasised, whereas when studies are performed on a relative large number of patients, the percentage of alteration of MEN-1 gene is better defined and conclusions on its significance in the pathogenesis of sporadic neuroendocrine tumours can be made. Specifically, MEN-1 gene was mutated in 31% of 51 sporadic gastrinomas recently investigated, in the same study the presence of a MEN-1 gene mutation correlated only with primary tumour location and not with clinical characteristics and/or tumour growth pattern [11]. Results from studies on von Hippel–Lindau syndrome are interesting – not for the frequency of pancreatic endocrine tumours in VHL patients, nor for VHL gene alterations found in a sporadic form of dNETs, in fact both are very low – but because from these studies the presence of a different new tumour suppressor gene, other than VHL on chromosome 3p, has been proposed. Data supporting these conclusions come from studies on non-functioning pancreatic tumours [12] in which the contemporary presence of rare or absent mutations on VHL gene were accompanied by a 30% allelic loss on chromosome 3p. Then considering that deletion in one allele is accompanied by mutation in the other, the existence of a different mutated gene has been suggested in these cases.

p16, a tumor suppressor gene located on chromosome 9p21, encodes for an inhibitor of cyclin-dependent kinase4 that, when activated, inhibits cell progression at the G1 S junction by phosphorylating protein expression of the retinoblastoma gene (Rb gene). Recent studies in non-endocrine tumours reported that in some tumours p16 gene alteration and/or loss of p16 protein can affect prognosis, recurrence, tumour aggressiveness and patient's survival [13]. p16 inactivation has also been studied in a number of neuroendocrine tumours. Specifically, homozygous deletions and/or hypermethylation have been reported in 92% of a small number of gastrinomas and nonfunctional tumours [14]. In a successive study, performed on a group of 44 gastrinomas, only hypermethylation of a p16 gene promoter island was found in 52% of the tumours [15]. However, in another study on 41 pancreatic endocrine tumours (30 non-functioning, 8 insulinomas, 1 gastrinoma and 1 Vipoma) p16 mutation was found only in 1 insulinoma and no gene alteration nor hypermethylation or homozygous deletion was shown in any of the other tumours [9]. Then, although to date p16 gene hypermethylation is the most common gene alteration reported in gastrinomas, it seems that this alteration is, for unknown reasons, limited mostly to functional neuroendocrine tumours.
Table 1. Gastrointestinal endocrine cell types, their localization and relation to hormonal syndromes.

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>Cell type</th>
<th>Main product</th>
<th>Main localization</th>
<th>Possible syndrome</th>
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<td>Pancreas</td>
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Modified after Rindi et al. (1999) [6].

Growth factors

Growth factors have been variously investigated in different types of dNETs, specifically, production of such factors as alpha and beta fibroblast growth factor (FGF) and transforming growth factor (TGF), platelet-derived growth factor (PDGF) and insulin-like growth factor-I (IGF-I) have been demonstrated by most of dNETs investigated [16, 17]. Although involved in the autocrine stimulation of tumour cells, a specific function for these factors, either in the genesis and/or in the progression of dNETs are not yet elucidated. Interestingly, a high expression of TGF alpha in a group of gastro-intestinal carcinoids has been reported. However, this expression was not accompanied by an intact specific receptor molecule i.e. epidermal growth factor receptor (EGFR), making ineffective the TGF growth factor function and suggesting that this may be related to their indolent behaviour. Among the various growth factors promoting angiogenesis, vascular endothelial growth factor (VEGF), a specific endothelial cell mitogen agent strictly correlated to the tumour progression, has been studied in a group of 48 neuroendocrine tumours. Its expression (from 25% to >50% of positive cells) was found in 78% of 28 gastrointestinal carcinoids; specifically, all the midgut carcinoids were intensely positive, whereas it was found in only 25% of either functioning and non-functioning pancreatic neuroendocrine tumours [18]. These results suggest that VEGF in association with the multiple growth factors synthesized by these tumours may play an important, although not yet understood, role in tumour angiogenesis and indirectly in tumour growth, especially in midgut carcinoids. Furthermore, in nude mice, xenotransplanted with human intestinal endocrine tumour, a protective effect of specific VEGF-antibody on tumour progression has been reported. In fact only in 2 out of 19 VEGF-antibody treated mice developed liver metastasis compared to almost all untreated mice [19].
Peptide receptors

Increasing evidence exists that growth and proliferations, as well as the functional properties of various tumour cells, are modulated by gastrointestinal peptides. The low availability of stable cultured neuroendocrine tumour cell lines still represent a limiting factor in the study of the specific function of these receptors expressed on neuroendocrine tumours. However, for vasoactive intestinal peptide receptors (VIP-1 and VIP-2) and most of all for somatostatin receptors (sst1), numerous studies attempting to elucidate basic mechanisms of receptor functions and clinical applications of specific peptide agonists have been produced during the last few years. VIP receptors have been demonstrated, mostly by [123I]-VIP receptor scintigraphy, to be over-expressed on tumour cells of many intestinal adenocarcinomas as colorectal, pancreatic and gastric cancers, and in most of primary and metastatic digestive neuroendocrine tumours [20]. Although some studies suggest that VIP can regulate the growth and function of tumour cells [21], to date, no specific synthetic agonists for VIP-1 and/or VIP-2 receptor subtypes are available for clinical use. Therefore, radio-iodinated VIP for scintigraphic tumour visualization is the only clinical application available until now. On the contrary, during these years, studies on somatostatin and its receptor family are literally exploding. Many are the reasons accounting for the great interest that somatostatin has stimulated among basic and clinical scientists. Firstly: somatostatin mostly acts as an inhibitory factor that regulates a large number of physiological functions, such as inhibition of endocrine and exocrine secretions, modulation of neurotransmission, motor and cognitive function, inhibition of intestinal motility and intestinal smooth muscle cells contractility [22, 23], absorption of nutrients and ions, vascular contractility and cell proliferations [24, 25]. Secondly: during the last few years five different somatostatin receptor subtypes have been cloned and these are usually expressed in a cell tissue specific manner. Thirdly: synthetic somatostatin analogues, with relative subtype receptor specificity are available either for diagnostic (scintigraphy) and/or for therapeutic purposes in many diseases. Neuroendocrine tumours have been demonstrated to possess a high number of somatostatin receptors i.e., 100 times more than normal tissues. This property makes them a target of somatostatin agonists for the treatment of various disease conditions in somatostatin receptor 

growth arrest by stimulation of tyrosine phosphatase SHP-2, activation of MAP kinase pathway and induction of the cyclin-dependent kinase inhibitor p21 [28]; sst3 acts by a mechanism involving a dephosphorylation cascade, inhibition of cGMP-dependent protein kinase G and MAP kinase [29]. The antiproliferative effect of somatostatin can also result from apoptosis induced by sst3 subtype [30] or by indirect effects of the peptide resulting from the inhibition of secretion of growth promoting hormones and growth factors which specifically regulate tumour growth. These effects, although very impressive, have been mostly evaluated in experimental 'in vitro' conditions in somatostatin receptor subtypes transfected cells. However, more recent data on living patients with dNETs analysed prospectively indicate that the most beneficial antiproliferative effect of somatostatin analogues treatment account for 36% to 70% of tumour growth stabilization lasting from months to years [31, 32].

Conclusions

The molecular pathogenesis of neuroendocrine tumours of the gastrointestinal tract, to date remains largely unknown. Molecular mechanisms involved in the tumour genesis of neuroendocrine compared to non-endocrine tumours such as colo-rectal and pancreatic cancer are different. However, the significant recent advances, involving localization methods, natural history and new treatments, have prompted the attention of many studies on the peculiar characteristics of these tumours that could improve the over-all knowledge on dNETs.

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