Endocrine tumors of the pancreas and gastrointestinal tract
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CHAPTER 1

INTRODUCTION AND OUTLINE OF THE THESIS

HISTORICAL, CURRENT AND FUTURE PERSPECTIVES ON GASTROINTESTINAL AND PANCREATIC ENDOCRINE TUMORS:
A TRIBUTE TO MASSON

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Introduction and outline of the thesis

Abstract

Gastrointestinal and pancreatic endocrine tumors are neoplasms of which the pathogenesis is not completely understood and of which the clinical behavior is difficult to predict. In this review current knowledge on these tumors is discussed and it is suggested that the increasing understanding of the normal development of gastrointestinal and pancreatic endocrine cells, the accumulating data on genetic alterations in endocrine tumors and reappraisal of the hypotheses on their pathogenesis formulated in the past, may help in resolving these issues.

History

When the French-born Canadian pathologist Pierre Masson retired exactly 50 years ago, he left an impressive oeuvre containing interesting concepts on the origin of gastrointestinal endocrine cells and the pathogenesis of gastrointestinal and especially appendiceal carcinoids. He was convinced that gastrointestinal endocrine cells arise from the gastrointestinal epithelium itself and do not, as proposed by others, migrate from a neural plexus towards the mucosa. Moreover, he thought that appendiceal carcinoids originate from undifferentiated cells at the base of the crypts, which bud off the epithelium, form complexes with the subepithelial neural plexus and subsequently acquire their endocrine granules, which in a way might be regarded as a sort of stem cell concept. Concerning carcinoids elsewhere in the gastrointestinal tract, he also described a subepithelial intraneural argentaffin cell as the cell of origin.\(^1\)\(^-\)\(^3\)

In the same period Feyrter recognized that the endocrine cells scattered in the epithelium of various organs, including the gastrointestinal tract, might be regarded as part of a diffuse endocrine/paracrine system\(^4\) and Masson's ideas were forgotten when Pearse showed that these cells share important ultrastructural and biochemical characteristics, especially the Amine-Precursor-Uptake-and-Decarboxylation capacity. These observations were consistent with a common origin of the APUD cells, for which the neural crest appeared the only possible candidate.\(^5\)

However, experiments by Le Douarin and colleagues in quail-chick chimeras provided strong evidence against a neural crest origin of most endocrine cells, except thyroid C-cells, ganglia and paraganglia.\(^6\) Although their results could not completely rule out an ectodermal origin of gastrointestinal endocrine cells, they pointed more towards an endodermal origin, thereby supporting Masson's view and forming a base for the stem cell concept used today.\(^7\)

Developmental biology

Illustrative of this concept is the current knowledge on the development of the endocrine cells in the intestine and pancreas at both the cellular and molecular level, to which data from animal models have contributed significantly. The intestinal epithelium, which is of endodermal origin, contains four main
cell types: enterocytes, goblet cells, Paneth cells and endocrine cells. These are thought to arise from omnipotent stem cells at the base of the crypts of Lieberkühn. Experimental mouse models suggest that the omnipotent stem cells give rise to enterocyte precursors and to (common) precursors of goblet cells, Paneth cells and endocrine cells, sometimes designated as secretory stem cells. This happens under the influence of transcription factors. A transcription factor encoded by a caudal-related homeobox gene and involved in early intestinal development and differentiation is Cdx2. Cdx2 expression is maintained in the adult intestinal epithelium, although expression in Paneth cells is weak. Differentiation into a secretory stem cell and its descendants is thought to be directed by transcription factors encoded by genes of the basic helix-loop-helix (bHLH) family, of which Math1 is (one of) the first in the pathway. Subsequent differentiation towards an endocrine cell is regulated by the transcription factor neurogenin 3 (ngn3). Its downstream target NeuroD/BETA2 is especially required for the development of secretin and cholecystokinin producing intestinal endocrine cells. Endocrine differentiation is repressed by Notch signaling. Notch is a transmembrane receptor which exerts its function via lateral inhibition. Hes1 is an important transcriptional repressor of this pathway. Although an obvious role for ngn3 in the differentiation of goblet cells and Paneth cells has not been established, recently published data suggest its transient expression in a subset of their precursors. The development of the pancreatic endocrine cells, organized in the islets of Langerhans, shows differences from, but also striking similarities to intestinal endocrine development. The pancreas arises from the duodenal region of the embryonic foregut, where expression of the Pancreas Duodenum Homeodomain protein 1 (Pdx1) is required for the formation of the ventral and dorsal primordium, which in a later stage fuse to form the adult organ. At the cellular level, the pancreatic primordia contain Pdx1 positive epithelium, which forms primitive ducts from which the endocrine cells arise. As in the intestine, animal models indicate that ngn3 and neuroD/BETA2 play a role in this process, while Notch inhibits endocrine differentiation. For Notch a role has been suggested in modulating the development of the exocrine pancreas and more recently in keeping the undifferentiated state of pancreas precursor cells. Math1, required in the intestine for differentiation towards a secretory stem cell phenotype, does not seem to play a role in pancreatic development. Other transcription factors downstream of ngn3 and NeuroD/BETA2, involved in endocrine differentiation of the pancreas, are Pax4, Nkx2.2, Nkx6.1, Pax6 and Is11. In the adult pancreas Pdx1 is almost exclusively expressed in the insulin producing β-cells, in which it plays a role in transcriptional activation of the insulin gene, together with NeuroD/BETA2. Although most endocrine cells are organized in the islets of Langerhans, scattered endocrine cells are found in the exocrine ducts.
Developmental biology related to tumorigenesis

The increasing knowledge on embryonic development influences the ideas on tumor pathogenesis and may also be important in endocrine tumor pathology. A tumor is regarded as a clonal expansion that in theory originates from a genetically unstable and deregulated, terminally differentiated cell, from an unstable, deregulated precursor cell already committed to a certain line of differentiation, or from a deregulated, undifferentiated, omnipotent stem cell. Although an origin from a terminally differentiated cell can not be ruled out, it has been suggested that pancreatic endocrine tumors have a precursor cell origin. In part this idea is based on the occurrence of mixed pancreatic tumors, consisting of an endocrine part combined with ductal or acinar features. The concept of derivation from a precursor cell or omnipotent stem cell is especially attractive for these mixed tumors, in which more than one cell type is present, because it provides an overt explanation for their histological features. Another example of a mixed tumor is a goblet cell carcinoid, in which the malignant counterparts of goblet cells, endocrine cells, and Paneth cells can be found, which is suggestive of an origin from an intestinal secretory stem cell, which is thought to be the precursor of precisely these cell types.

In light of the presumed precursor cell origin of certain tumors, including tumors consisting of one cell type, an important consideration is that the transcription factors playing a role in embryonic development may drive tumorigenesis as well. The expression of Math1 in Merkel cell carcinoma (an endocrine tumor of the skin) and its recently reported repression in colonic adenocarcinoma is in support of this. Other examples of transcription factors expressed in tumors, are CDX2 in intestinal tumors, including carcinoids, and Thyroid Transcription Factor 1 (TTF1, involved in thyroidal and pulmonary development) in tumors of the thyroid and lung. Knowledge on the role of transcription factors in (endocrine) tumors may help elucidating their pathogenesis and may even in the far future provide a target for therapeutic options, such as specific intervention of the pathways governed by these molecules and thereby inhibit or stimulate cellular activities of for example proliferation or differentiation. However, at this moment data on the role of the transcription factors mentioned above in gastrointestinal and pancreatic endocrine tumors are limited to reports on the expression of CDX2.

Genetic alterations

More is known about generalized and also some specific molecular genetic abnormalities underlying gastrointestinal and pancreatic endocrine tumors. One of the genetic changes reported repeatedly in gastrointestinal endocrine tumors, especially in midgut carcinoids (carcinoids of jejunum, ileum, ascending colon and appendix) and less frequently in pancreatic endocrine tumors, is loss on chromosomal arm 18q. Because in most cases no apparent alterations have been
found in the tumor suppressor genes DPC4 and DCC, which are located in the affected region, it has been suggested that a currently unknown gene on chromosome 18q is important in the pathogenesis of (especially midgut) endocrine tumors.\textsuperscript{34-40} Because in some midgut carcinoids loss of chromosome 16q21 and gain of chromosome 4p14 have been found in metastases but not in the primary tumor, genes in this region may play a role in tumor progression.\textsuperscript{35} Only in a minority of midgut carcinoids allelic loss on chromosomal arm 11q has been described.\textsuperscript{34,35,41} Chromosome 11q13 harbors the tumor suppressor gene responsible for the hereditary syndrome Multiple Endocrine Neoplasia type 1 (MEN1), which is associated with pancreatic endocrine tumors. Pancreatic endocrine tumors of MEN1 patients and a subset of sporadic cases show loss of heterozygosity of the MEN1 gene. Because allelic losses distal to this gene have been found, it has been suggested that chromosome 11q contains other tumor suppressor genes that may play a role in pathogenesis as well.\textsuperscript{40,43} Another hereditary syndrome associated with pancreatic endocrine tumors is Von Hippel-Lindau (VHL) disease, linked to a tumor suppressor gene on chromosome 3p25-26.\textsuperscript{44} Although pancreatic endocrine tumors frequently show loss in this region, mutations in the VHL gene have not been found in sporadic cases. It is likely that another tumor suppressor gene near the VHL gene plays a role in the pathogenesis of sporadic pancreatic endocrine tumors and there are indications that allelic losses of chromosome 3p are involved in tumor progression.\textsuperscript{45,46} A role in tumor progression has also been suggested for putative tumor suppressor loci at chromosome 6q.\textsuperscript{47} Moreover, allelic losses on the X-chromosome were found in pancreatic endocrine tumors and correlated with aggressive tumor behavior.\textsuperscript{48,49} C-myc is an oncogene that may be involved in the pathogenesis of pancreatic endocrine tumors\textsuperscript{26,50} and genes with a tumor suppressor function on chromosome 9p, e.g. Ink4A/Arf encoding p16, may be of importance in both gastrointestinal and pancreatic endocrine tumors.\textsuperscript{26,51,52} Alterations in tumor suppressor genes and oncogenes commonly involved in human cancer, like TP53, Rb and KRAS, have been described in gastrointestinal and pancreatic endocrine tumors, especially in poorly differentiated endocrine carcinomas, but they do not seem to play a major role in pathogenesis.\textsuperscript{50,53-56}

**Prognostic features**

Although some of the genes mentioned above may be involved in tumor progression and could be associated with an unfavorable prognosis, there are no consistent genetic changes in endocrine tumors that can be used to predict tumor behavior in an individual patient. Also based on histological criteria it is difficult to predict prognosis. Generally, it is not a problem to recognize poorly differentiated endocrine carcinomas, which have histological features of small cell carcinoma. But in the group of better differentiated tumors, metastasis is the only reliable indicator of malignancy. Despite the lack of unequivocal histologi-
eal criteria for malignancy of a primary tumor, the WHO-classification of endocrine tumors published in 2000 distinguishes benign tumors, tumors of uncertain behavior, low-grade malignant and high grade malignant carcinomas of the gastrointestinal tract and pancreas, based on parameters for which prognostic value has been described (for gastrointestinal endocrine tumors site, size, depth of invasion, vasoinvasive growth and for pancreatic endocrine tumors also proliferative activity and functionality (i.e. causing a clinical syndrome by hormone secretion)). In the WHO-classification of tumors of the digestive system also published in 2000, the same prognostic parameters are mentioned for gastrointestinal endocrine tumors, for some sites extended with proliferative activity, although a similar grading system is not explicitly presented. Also the new WHO-classification for pancreatic endocrine tumors (2004) does not contain significant differences compared to the old version. A recent observation in pancreatic endocrine tumors is the correlation between immunohistochemical expression of cytokeratin 19 (Ck19) and aggressive behavior. During embryonic development Ck19 is transiently expressed in all pancreatic cells, including endocrine cells, but after 16 weeks of gestation Ck19 gradually disappears from most cells in the islets of Langerhans, while its expression remains strong in the ducts. In view of the presumed precursor cell origin of pancreatic endocrine tumors, it is tempting to speculate that more aggressive pancreatic endocrine tumors arise from an early precursor cell, that has not yet lost its Ck19 expression or that they arise from a precursor cell, that tends to differentiate towards a ductal phenotype not yet visible histologically, but already evident at the immunohistochemical level. The clinical value of Ck19 as a prognostic marker in pancreatic endocrine tumors remains to be evaluated.

Conclusion
The difficulties described above in accurately predicting prognosis of gastrointestinal and pancreatic endocrine tumors brings us back to the observations of Masson. He already described the unpredictable clinical behavior of gastrointestinal endocrine tumors, and noticed the higher metastatic capability of ileal carcinoids compared to those of the appendix. Although we probably would not agree with his explanation that this is due to the difference in intestinal motility between the ileum and appendix, it might be worthwhile to reconsider his ideas, in particular on the origin of appendiceal carcinoids, and compare and test them with the knowledge and possibilities of today. Especially evaluation of the role of transcription factors involved in differentiation and of genomic alterations in endocrine tumors may help in elucidating their pathogenesis and in establishing reliable prognostic parameters. It is predicted that especially Masson's concepts on origin and pathogenesis will appear much more valid than these were considered over the past 50 years, once the molecular developmental biology of the endocrine cell population in the digestive tract has clarified their
tumorigenesis 50 years from now. Would there then be better proof of Masson's vision than the century it took us to validate his concept?

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OUTLINE OF THE THESIS

In this thesis different pathogenetic and clinicopathological aspects of gastrointestinal and pancreatic endocrine tumors are described.

Chapter 2 is a case report on a gangliocytic paraganglioma of the appendix, a very rare endocrine tumor of uncertain histogenesis.

In chapter 3 the histological, immunohistochemical and molecular characteristics of appendiceal goblet cell carcinoids are compared to these characteristics in conventional appendiceal carcinoids, appendiceal mucinous cystadeno(carci­no)mas and colonic adenocarcinomas to clarify their pathogenesis.

Chapter 4 provides a molecular analysis of an endocrine tumor and a serous papillary adenocarcinoma, which were intimately admixed in a patient with extensively metastasized disease, to find out whether these tumors represent a collision of two different neoplasms or a tumor composed of two components with the same precursor.

In chapter 5 the value of serological tests for detecting gastric body atrophy, a condition in which endocrine cells are affected secundarily, is evaluated.

Chapter 6 addresses general features of pancreatic endocrine tumors and in chapter 7 the origin of the ductules that can be found in pancreatic endocrine neoplasms is investigated.

In chapter 8 the prognostic value of the WHO-classification of endocrine tumors of the lung is evaluated in a group of metastasized endocrine tumors of midgut or unknown origin and compared to the prognostic value of the Capella classification. Also the prognostic value of several clinical and of several immunohistochemical parameters is assessed.

The results are summarized in chapter 9.