Molecular pathology of gastrointestinal neuroendocrine tumours — selected topics

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Abstract

This review focuses on gastrointestinal neuroendocrine tumours, excluding pancreatic endocrine tumours. Neuroendocrine tumours are rare ubiquitous neoplasms. Nevertheless, epidemiological studies have detected a significant increased in prevalence during the last 35 years. Most tumours have an indolent course and many patients have developed metastatic disease at the time of diagnosis, a stage for which there is no available curative treatment. Novel diagnostic, prognostic and predictive markers are needed for the early detection and follow-up of disease progression; and identification of critical signalling pathways is needed to identify targets for effective treatment of these tumours. The complex nature of neuroendocrine cell function and the limited access to biopsy material has restricted elucidation of the tumour biology. The molecular pathology responsible for early tumourigenesis and metastatic processes are still largely unknown. However, it is currently the subject of major investigations and the field has progressed during recent years. This recent progress will be discussed in this review.

Keywords endocrine cells; gastrointestinal neuroendocrine tumours; molecular genetics; tumour biology; neuroendocrine cells; stem cells; tumourigenesis; tumour and novel markers

Overview

Gastrointestinal (GI) neuroendocrine tumours (NETs) are thought to arise from, or differentiate towards, enterochromaffin (EC) cells, which are sparse neuroendocrine cells disseminated throughout the GI tract.[1] GI-NETs as a whole are characterized by the production of several bioactive amines, hormones and peptides, which present to some extent specific variations according to histomorphology, endocrine symptoms and organ of origin. The majority of GI-NET patients have developed metastatic disease in the liver at the time of diagnosis. Surgical debulking and hepatic embolization are employed, but the treatments and surgery are seldom curative. The results of conventional chemotherapy and radiotherapy are disappointing and the current treatments of metastasized ileocaecal neuroendocrine carcinomas aim at controlling tumour growth and hormonal secretion by using somatostatin analogues and interferon alfa (IFN-α) to control hormonal hypersecretion and/or tumour growth.[2] There are a variety of studies in the literature using somatostatin analogues and interferon. However, many of them lack of randomized and perspective planned program excluding documented tumour progression of the patients.[3,4] Furthermore, the molecular mechanisms responsible for the efficacy and ultimately resistance to somatostatin analogues are still largely unknown. Emerging therapies started exploring new signalling pathways such as vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR). Neuroendocrine tumours express VEGF and its receptor VEGFR, which have a pivotal impact on angiogenesis; while mTOR pathway regulates the cell cycle and metabolism. Targeting the VEGF pathway has recently shown therapeutic effect in patients with advanced disease.[5,6] Furthermore, a new combined therapy using somatostatin analogues and a novel oral inhibitor of mTOR showed for the first time promising antitumour activity.[7] It is, therefore, highly important to improve the understanding of neuroendocrine tumour biology, early tumourigenesis, tumour progression and the molecular mechanisms behind somatostatin analogue resistance to overtake the slow progress of curative methods.[8,9]

History

GI-NETs have been divided into foregut (stomach, first and second part of the duodenum), midgut (third and forth part of the duodenum, jejunum, ileum, caecum, appendix; ascending, transverse colon and two-thirds of proximal colon) and hindgut (the distal third of the transverse, descending, sigmoid colon and rectum) carcinoids for many years.[10] The classification of GI-NETs continued to be a critical reason of debate for many years in the pathological and medical world, while the term carcinoid started being considered inadequate. The second edition of the WHO classification in 2000, recognized carcinoid tumours as entities of the endocrine tumours and their name changed to the term neuroendocrine tumours. The recognition of novel histopathological, molecular and clinical features divided these tumours into three major different categories; well differentiated neuroendocrine tumours (benign or uncertain behaviours at the time of diagnosis), well differentiated neuroendocrine carcinomas (low-grade malignant behaviours) and poorly differentiated neuroendocrine carcinomas (high-grade malignant behaviours). The aggressive behaviour of the third group is highly different from the first two categories.[11] The different features for the classification count tumour size, angioinvasion, proliferative activity, histological differentiation, metastatic capacity and invasion of surrounding organs. Hormonal activity and association with specific clinical syndromes and diseases are included as specific parameters.[12] The pathological nomenclature of the gastrointestinal neuroendocrine tumours changed from foregut, midgut and hindgut carcinoids to neuroendocrine tumours of the oesophagus, stomach, duodenum and upper jejunum, distal jejunum and ileum, appendix, colon and rectum.[13,14] The updated WHO edition gave the opportunity to specifically describe the different neuroendocrine tumours of the gastrointestinal tract.[15–17] The European Neuroendocrine Tumor Society (ENETS) recently, to further optimize the last WHO classification, proposed a tumour-node-metastasis (TNM) classification to increase the quality of the management of patients with GI endocrine tumours. The TNM classification is offering the main criteria for the staging and

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grading neuroendocrine of foregut, midgut and hindgut origin.[18,19] To develop an effective grading system has been delayed because severe cytological abnormalities are not correlated to the clinical behaviour and malignancies of several different tumours. However, after critical discussion several clinicians and pathologists approved a new grading system, in agreement with the current WHO histological criteria, that we can briefly. The grading system was based on mitotic count and Ki-67 index and divided in three grades G1, G2 and G3. G1 has <2 mitoses per 2 mm^2 (10 high-power fields, HPF, 40× magnification) and/or Ki-67 index $\leq 2\%$; G2, 2–20 mitoses per 2 mm^2 and/or Ki-67 index between 3% (>2%) and 20% and G3 with >20 or more mitoses per 2 mm^2 and Ki-67 index >20%. The new TNM classification proposal therefore might help clinicians to create a significant stratification to finalize the required therapeutic options and to continue with a proper follow-up after treatment for the patients.[18,19]

Cell biology of gastrointestinal endocrine cells

One of the major reasons why the GI-NETs classification has been delayed is that to classify the cellular origin of different tumours requires exploring the normal GI endocrine cellular biology. The history of the enteroendocrine cells starts with the name of cells of the diffuse endocrine system of the gut.[20,21] In the first part on the nineteen century the major scientific hypothesis was that enterochromaffin (EC) cells shared a common neuroectodermal embryological origin with many different epithelial cells that are able to produce and store several amines and hormones. Pictet et al. firmly corrected this first hypothesis showing that gastrointestinal hormone-producing cells arise from the gastrointestinal endoderm.[22] Nerve cells also produce the majority of the hormones secreted by enteroendocrine cells and this created further problems in understanding the ontological origin of these cells. The enteroendocrine cells are dispersed cells of the GI mucosa. They are a minute fraction, circa 1%, of the entire GI epithelial cellular population. At least 13 highly specialized epithelial cells of endodermal origin have been characterized to be part of the GI tract, excluding pancreas.[3,20,23] They are characterized by the presence of two different secretory vesicles, the large dense core vesicles (LDCVs) and the synaptic-like microvesicles (SLMVs). LDCVs appear like electron-dense granules at the microscope while SLMVs are smaller and resemble the synaptic vesicles at the end of nerves. The secretive function of these vesicles is highly regulated and creates the possibility of a precise release and absorption of different substances, mucosal cell proliferation, and immune control. These cells share a variety of antigens with neural cells, usually named as neuroendocrine markers.[24,25] The characteristics of enteroendocrine cells are very complex and elaborated. There is still a controversial debate ongoing to find a precise method to understand the connections and borders between the scattered endocrine cells, the discrete endocrine organs and the cells of the immune system.[20,21,23]

The role of gastrointestinal stem cells during development

The neuroendocrine cells of the gut arise from a multipotent gastrointestinal stem cell but the differentiation of the diffuse endocrine cell system is poorly understood. The majority of the investigations to understand intestinal development rely on the studies on small intestine of mice, whereas the ones related to carcinogenesis are mainly derived from human colorectal adenomas and carcinomas. These studies will hopefully come up with results that can be implemented and increase our understanding of the human gastrointestinal neuroendocrine tumours.

The mammalian gastrointestinal epithelium rises from endoderm during a network of developmental steps during embryogenesis. Gastrointestinal development probably relies on stem cells and several transcription factors that are working in a complex network. We can separate the intestine cell lineages from the gastric epithelium. The four principal ones are enterocytes, goblet, Paneth and enteroendocrine cells in the intestine and pit, parietal, zymogenic and enteroendocrine cells in the gastric epithelium.[26–28] The transcription factors are temporally and spatially restricted in their action that function to sharply define the different phenotypes of the fully differentiated epithelium and underline the constant renewal during life time.[29,30]

The small intestinal structure is important for the understanding of the location of the intestinal stem cells stem responsible for development and renewal of the GI. The GI mucosa contains epithelial invaginations composing villi and crypts. Villi consist of a single layer of columnar epithelium and contain only fully differentiated cells while crypts are the regions where the cells with proliferative potential reside. Villi contain mature epithelial cells: enterocytes that absorb nutrients, goblet cells that secrete mucus and enteroendocrine cells that function to release GI hormones. The crypts mainly contain undifferentiated cells with the exceptions of differentiated Paneth cells, which reside at the base of the crypts. A variety of studies have proved that the intestinal development is dependent on a highly regulated cross talk mediated by crucial pathways as Wnt, Hedgehog, Notch, P13K and BMP pathways.[30–32] Several evidences supported that the intestinal crypts are monoclonal and this led to the hypothesis that each crypt may derive from its own stem cells.[33,34] Potten et al. considered that stem cells should be able to self renew and hypothesized, in the absence of specific marker, by using a long-term labelling technique, that the stem cells were located in a ring of cell up from the crypt.[35,36] Later Barker et al. elaborated a new theory, based on the importance of Wnt signalling, and its target gene *Lgr/GPR49*, which is expressed specifically at the base of the crypt. *Lgr/GPR49* has become the potential novel marker of intestinal stem cells.[37,38] Recently they managed to single sort $Lgr5^+$ cells and established long-term culture protocols to let singly crypts undergo multiple crypts proliferation while at the same time they generate villus-like domain where all the differentiated cells are present. They proved for the first time that subepithelial myofibroblasts are not essential for the full developments of a crypt; this protocol will help scientists to better understand the relationship between villi and crypt biology.[39]

The role of gastrointestinal stem cells during tumourigenesis

Wnt and Notch are two of the numerous crucial pathways for normal GI development and homeostasis. Despite of this it is well known that these signalling pathways are often dysregulated during pathological conditions.[32] Wnt signalling follows two different pathways, but only the canonical one is very important in the intestine. It includes the function of other partners such as β-catenin, adenomatous polyposis coli (APC), an important tumour