Molecular genetics of gastroenteropancreatic neuroendocrine tumors

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Purpose of review

Gastroenteropancreatic neuroendocrine tumors (GEP NETs) are relatively rare neoplasias arising from the embryonic neural crest, neuroectoderm and endoderm. GEP NETs occur either sporadically or as part of endocrine tumor susceptibility syndromes such as multiple endocrine neoplasia type 1 (MEN1), von Hippel Lindau (VHL) syndrome, neurofibromatosis (NF-1), and possibly tuberous sclerosis (TSC). The overall incidence of GEP NETs shows a significant increase over the past three decades. Improved understanding of the molecular genetics associated with these lesions will likely enhance the diagnosis and treatment of patients with GEP NET.

Recent findings

The molecular and clinical genetics of familial GEP NETs have been further elucidated by the characterization of the tumor suppressor genes, *MEN1*, *VHL*, *NF-1*, *TSC1*, and *TSC2*. The vastly improved technology in the field of cancer genetics with higher resolution of the study of genetic alterations, and the ability of unbiased mutational analyses of entire tumor genomes is likely to further the understanding of the genetic mechanisms of sporadic GEP NET as well.

Summary

Recent advances in the molecular genetics of sporadic and familial GEP NET are reviewed.

Keywords

carcinoid, endocrine, genetic, molecular, pancreas, tumor

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Introduction

Tumors that are derived from the diffuse neuroendocrine system of the gastrointestinal tract and pancreas are fairly rare. They occur in tissues that contain cells derived from embryonic neural crest, neuroectoderm, and endoderm. They, therefore, occur throughout the entirety of the body, albeit with a predilection for the lung and bronchus and the gastro–enter–pancreatic axis. Siegfrid Oberndorfer first coined the term karzinoide (carcinoid; cancer-like) in 1907. Gastroenteropancreatic neuroendocrine tumors (GEP NETs) include gastrointestinal carcinoids (also known as gastrointestinal NETs; GI NETs), as well as the neuroendocrine tumors of the pancreas (PNETs).

GEP NETs are relatively rare in contrast to adenocarcinomas, with an annual incidence of approximately 2.5 to five new cases per 100 000 [1]. However, a substantial increase in the incidence of these tumors has occurred over the last 30 years, with 460–720% increased prevalence [1]. Although the exact mechanism for this finding is unclear, advancements in technologies such as endoscopy and imaging techniques are likely to have contributed to the increased diagnosis of GEP NET. Although GEP NETs are increasingly being diagnosed, a concomitant improvement in outcomes has not been noted. The 5-year overall survival for all carcinoids and intestinal carcinoids in the United States between 1973 and 2002 has remained at 60% [1]. It should be pointed out, however, that there exists significant heterogeneity in outcomes between various groups of GEP NET. This is exemplified by 5-year survival rates of 97 and 30% for benign pancreatic insulinomas vs. nonfunctioning endocrine pancreatic tumors, respectively [2].

Familial gastroenteropancreatic neuroendocrine tumors

Although a majority of GEP NETs are sporadic, the molecular and clinical genetics of tumor susceptibility syndromes, in which GEP NETs may occur, have contributed to the understanding of tumorigenesis in these patients.

Table 1 Gastroenteropancreatic neuroendocrine tumors associated with MEN1 and their approximate penetrance

Tumor type	Penetrance (%)
Enteropancreatic tumors	
Gastrinoma	40
Insulinoma	10
Nonfunctioning	20
Other (glucagonoma, VIPoma, somatostinoma)	2
Foregut carcinoids	
Gastric carcinoids	10
Thymic carcinoid	2
Bronchial carcinoid	2

VIP, vasoactive intestinal peptide.

Multiple endocrine neoplasia type 1

Multiple endocrine neoplasia type 1 (MEN1; OMIM 131100) is an autosomal dominant endocrine tumor susceptibility syndrome causing tumors of the parathyroid, enteropancreatic endocrine tissue, and anterior pituitary [3]. MEN1 is relatively rare (approximately one in 30 000), and a consensus definition of MEN1 is used widely [4]. An MEN1 case has tumor in two of the three principal organs (parathyroid, enteropancreatic endocrine tissue, and anterior pituitary). Similarly, familial MEN1 is defined as one MEN1 case plus one first-degree relative with one of the three principal tumors. GEP NETs are the second most common manifestation of MEN1 after primary hyperparathyroidism and include a spectrum of tumors with variable penetrance in the disease (Table 1).

The *MEN1* gene, localized at chromosome 11q13, was identified by positional cloning [5,6]. Germline *MEN1* mutation is identifiable in 70–90% of typical MEN1 families, and some without an identified mutation may have large deletions or intron mutations not recognizable by polymerase chain reaction. The importance of *MEN1* gene inactivation in tumorigenesis is further substantiated by the fact that sporadic tumors, including GEP NETs, parathyroid adenomas, and bronchial carcinoid frequently harbor *MEN1* gene mutations [5–12]. The patterns of germline and somatic mutations in *MEN1* are similar, and approximately 80% predict truncation or absence of the encoded menin. There is no strong pattern of genotype/phenotype relations among germline and somatic *MEN1* mutations.

Menin is a 67-kDa protein, widely expressed, located in the nucleus, but also detected in cytoplasm and about telomeres [13]. Sequence analysis shows no homologous proteins. Through various protein interaction studies, menin has been suggested to partner with a number of other proteins such as transcription factors, DNA repair factors, cytoskeleton-associated proteins, among others [14–22]. Although the potential protein partners vary in function, most of them are nuclear proteins, generally involved in transcriptional regulation. The role of menin in transcriptional regulation was further substantiated as a large menin complex was demonstrated to exhibit lysine-4 histone methyltransferase activity, and that menin can directly bind to DNA [23,24]. Additionally, menin acts as a molecular adaptor linking the mixed-lineage leukemia (MLL) histone methyltransferase with lens epithelium-derived growth factor (LEDGF) [25•]. A recent study demonstrated that *MEN1*-causing missense mutations lead to a loss of function of menin due to enhanced proteolytic degradation, which may be a mechanism for inactivating menin as a tumor suppressor [26]. Heterozygous knockout of *Men1* in mice provides an excellent model of MEN1 [27–29]. Mice develop normally, but by 16 months frequently develop parathyroid tumors, insulinomas, and prolactinomas. Interestingly, insulinomas in these mice can develop in the absence of chromosome instability or microsatellite instability [30]. In a recent study on MEN1 patients, however, Perren *et al.* [31•] found that loss of heterozygosity (LOH) of the MEN1 locus in all 27 endocrine pancreatic microadenomas and 19 of 20 (95%) monohormonal endocrine cell clusters.

von Hippel Lindau syndrome

The von Hippel Lindau (VHL; OMIM 193300) disease is an autosomal dominant neoplasia syndrome that results from germline mutations in the *VHL* gene [32]. These mutations lead to the development of several benign or malignant tumors and cysts in many organs. VHL is characterized by predisposition to develop hemangioblastomas of the retina and central nervous system (CNS), renal cell carcinomas and renal cysts, pheochromocytomas, endolymphatic sac tumors, as well as pancreatic lesions with marked phenotypic variability. Although cysts are the most common lesion (33–70%) in the pancreas in patients with VHL, endocrine pancreatic tumors occur in 11–17%, and have malignant potential [33].

The *VHL* gene is a tumor suppressor gene on the short arm of chromosome 3 (3p25–26), with three exons encoding the VHL protein [34]. The VHL protein shuttles between the nucleus and cytoplasm, binding to elongin C, elongin B, Cul2, and Rbx1, and degrades alpha subunits of hypoxia-inducible factor in an oxygen-dependent manner [35]. Lack of degradation of this factor due to absence of the VHL protein results in uncontrolled production of factors promoting formation of blood vessels such as vascular endothelial growth factor implicated in tumor development [35]. Germline mutations in the VHL gene are extremely heterogeneous and are distributed widely throughout the coding sequence [32]. They are now identifiable in virtually all families with VHL [32]. The exact molecular mechanism of GEP NET development in VHL is unknown. Analyses of allelic loss identify genetic loci distinct from and mapping proximal to *VHL* within human chromosome 3p VHL kindred under study. Furthermore, chromosome 3p LOH occurs subsequent to VHL mutation and cyst formation

and correlates with malignant progression in VHL-associated GEP NET. These findings suggest that additional genetic alterations, possibly in a stepwise fashion, cause GEP NET in the VHL syndrome [36].

Neurofibromatosis type 1 and tuberous sclerosis

Neurofibromatosis type 1 (NF-1; OMIM 162200) and tuberous sclerosis (TS; OMIM 191100) are both rare autosomal dominant tumor susceptibility syndromes. Ampullary carcinoids, duodenal and pancreatic somatostatinomas, and nonfunctioning GEP NETs have occasionally been reported in such kindreds [37,38].

NF-1 and TS are caused by inactivating mutations in the tumor suppressor genes *NF1* (17q11.2), *TSC1* (9q34), and *TSC2* (16p13.3), respectively. *NF1* encodes the protein neurofibromin, which also regulates *TSC1* and *TSC2* through the mammalian target of rapamycin (mTOR). Loss of function of the *NF1* gene causes mTOR activation and tumor development [39]. Interestingly, disruption of TSC2 in pancreatic beta cells induces beta cell mass expansion in an mTOR-dependent manner [40].

Sporadic gastroenteropancreatic neuroendocrine tumors

Through the clinical and molecular genetic studies of kindreds with GEP NETs, the underlying alterations in these families have been characterized. In contrast, less is known about the genetic mechanism of sporadic GEP NETs, although genes involved in their familial counterparts also play a role in the molecular pathology of sporadic tumors. There exist both similarities and differences in the tumorigenesis of various types of GEP NETs, and is here discussed in two broad groups, those that arise in the pancreas (PNETs) and the gastrointestinal tract (GI NETs) [41].

Sporadic endocrine pancreatic tumors (neuroendocrine tumors of the pancreas)

The occurrence of chromosomal gains, or losses, or both have been extensively studied in PNETs, by LOH analysis, comparative genomic hybridization (CGH), and array CGH analyses. Allelic loss is most commonly seen at chromosome loci 1p (23–75%), 1q (20–88%), 3p (25–62%), 11p (29–52%), 11q (28–66%), and 22q (38–93%), although many other loci throughout the genome show LOH in more than 20% of the tumors [10–12,42–44]. As noted, LOH at 11q13 is common, and *MEN1* gene mutations have been identified in sporadic gastrinomas, insulinomas, glucagonomas, VIPomas, and nonfunctioning tumors. The overall incidence of *MEN1* gene mutations in sporadic PNETs varies between 13 and 38% [11,12,43,44]. In contrast, the *VHL* gene seems not to be mutated in sporadic PNETs [45]. *DPC4/SMAD4* mutations are seen in about 20% of sporadic PNETs [46]. Deletions on chromosome 1, 3p, 6, 11q, 17p, 21q, and 22q as well as gains on chromosomes 4, 7, 14q, and Xq have been associated with malignancy in sporadic PETs [47]. Additional attempts to distinguish benign from malignant PNETs used genomewide expression microarray studies revealing more than three-fold overexpression of 66 transcripts including IGFBP3, which is deregulated in many tumor types. Underexpression (>three-fold) was seen for 119 transcripts, including p21, O6-MGMT, and JunD [48]. When comparing benign and malignant PETs, O6-MGMT was downregulated in malignant tumors and the protooncogene MET was upregulated together with IGFBP1 and IGFBP3 [49]. More recently, Duerr *et al.* [41] using a DNA microarray analysis and hierarchical clustering of 19 PNETs revealed a 'benign' and 'malignant' cluster. FEV, adenylate cyclase 2 (ADCY2), nuclear receptor subfamily 4, group A, member 2 (NR4A2), and growth arrest and DNA-damage-inducible, beta (GADD45b) were the most highly upregulated genes in the malignant group of PNETs.

Sporadic gastrointestinal carcinoids (gastroenteropancreatic neuroendocrine tumors)

Similar to PNETs, chromosomal gains, or losses, or both have been studied in GI NETs using LOH analysis, CGH, and array CGH analyses. Loss of chromosome 18 and, to a lesser extent, loss of chromosome loci 9p and 16q are the most common genetic alterations of GI NETs. Amplification of chromosomal loci is less common in GI NETs than in PNETs and other endocrine tumors, but occurs most frequently on chromosomes 4, 5, 7, 14, 17, and 20 [50•,51–56]. Interestingly, recent findings of genetic alterations in GI NETs using high-resolution array-based CGH analysis are similar to earlier studies using conventional CGH analysis [50•].

Conclusion

Despite the improved understanding of the molecular genetics of familial endocrine neoplasia syndromes, the exact mechanism of GEP NET development is still unclear. With improved technology in the field of cancer genetics, such as large-scale sequencing of entire tumor genomes [57,58], it is likely that significant advancements will occur in the understanding of GEP NET, as well. Further studies of gene function may lead to development of novel therapeutic modalities.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 88–90).

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