Genetics and molecular pathology of neuroendocrine gastrointestinal and pancreatic tumors (gastroenteropancreatic neuroendocrine tumors)

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Purpose of review

Gastrointestinal and pancreatic neuroendocrine tumors (GEP-NETs) originate from cells of the diffuse endocrine system. Most GEP-NETs are sporadic, however, some of them, especially pancreatic endocrine tumors, may occur as part of familial syndromes. The genetic and molecular pathology of neuroendocrine tumor development is incomplete and remains largely unknown. However, the WHO classification introduced in clinical practice will give more insight into genetic and molecular changes related to tumor subtypes.

Recent findings

In sporadic endocrine pancreatic tumors, losses of chromosome 1 and 11q as well as gain on 9q appear to be early invents in development of pancreatic tumors because they are already present in small tumors. Multiple genetic defects may accumulate with time and result in pancreatic neuroendocrine tumor progression and malignancy. Gastrointestinal endocrine tumors (carcinoids) show predominantly genetic alterations concentrated on chromosome 18. There are losses of the entire chromosome as well as smaller deletions. The most frequently reported mutated gene in gastrointestinal neuroendocrine tumors is β-catenin. Overexpression of cyclin D1 and cMyc has also been reported. Recently, a set of genes *NAP1L1*, *MAGE-2D* and *MTA1* has been correlated with malignant behavior of small intestinal carcinoids.

Summary

Molecular profiling of GEP-NETs demonstrates that pancreatic endocrine tumors and gastrointestinal neuroendocrine tumors (carcinoids) display different genetic changes and should, therefore, be considered to be different tumor entities; thereby, also differently managed clinically. Although the number of genetic changes is higher in malignant tumors, we are still far away from defining a malignant profile in GEP-NETs.

Keywords

carcinoid familial GI-NETs, gastrointestinal neuroendocrine tumors, molecular genetics, pancreatic endocrine tumors

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Introduction

Gastrointestinal and pancreatic neuroendocrine tumors (GEP-NETs) originate from cells of the diffuse endocrine system. They comprise approximately *2%* of all malignant gastrointestinal tumors. Most GEP-NETs are sporadic, however, some of them, especially pancreatic endocrine tumors, may occur as part of familial tumors (inherited syndromes) such as multiple endocrine neoplasia type 1 (MEN1 syndrome), von Hippel-Lindau disease (VHL), neurofibromatosis type 1 (NF-1) and tuberous sclerosis (TSC) [1]. The tumors are rather rare and heterogeneous, and it is difficult to predict their behavior and prognosis. Several different tumor classification systems have been used. The tumors are commonly divided into functional (a hormone-related clinical syndrome) or nonfunctional (no hormone-related symptoms). Classification of the gastrointestinal neuroendocrine tumors (GI-NETs) has been based on the embryology and are divided into foregut (stomach and first part of the duodenum), midgut (small intestine: second portion of duodenum, jejunum, ileum, appendix and ascending colon) and hindgut (transverse and descending colon and rectum) [2]. This old type of classification is abandoned today, and the WHO classification of endocrine tumors from 2000 attempts to divide these into well differentiated endocrine tumors (benign or uncertain behavior), well differentiated endocrine carcinomas (low-grade malignant behavior) and poorly differentiated endocrine carcinomas (high-grade malignant behavior) [3]. This classification has further been refined by the European Neuroendocrine Tumor Society

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Table 1 Inherited genetic neuroendocrine syndromes

Syndrome	Gene location (product)	NET frequency (tumor type)
MEN1	11q13 (610-amino acid protein, Menin)	80–100% pancreas + duodenum (NF > gastrinoma > insulinoma) gastric carcinoids
von Hippel–Lindau disease	3p25.5 (213-amino acid protein, *VHL*)	12–17% pancreas (all nonfunctioning)
von Recklinghausen's disease (NF-1)	17q11.2(2485-amino acid protein, neurofibromin)	6% pancreatic (somatostatinoma)
TSC	9q34, (*TSC1*) 16p 13.3 (*TSC2*) (hamartin, tuberin)	<5% pancreas

MEN1, multiple endocrine neoplasia type 1; NET, neuroendocrine tumor; NF-1, neurofibromatosis type 1; TSC, tuberous sclerosis; VHL, von Hippel-Lindau.

(ENETS) with a TNM classification and grading system [4,5•]. The molecular genetic mechanism of neuroendocrine tumor development is complex and remains largely unknown. However, the new classification will give new insight into specific genetic and molecular changes related to tumor subtypes.

Genetics in endocrine pancreatic tumors

Most pancreatic neuroendocrine tumors occur sporadically (90%) Table 1. However, they may be part of four hereditary syndromes: MEN1, VHL, von Recklinghausen's disease (NF-1) and TSC [1]. The MEN1 syndrome includes the following clinical components: primary hyperparathyroidism (>95%) due to parathyroid hyperplasia or adenoma, pancreatic endocrine tumors (25–80%), pituitary tumors (20–40%) and adrenocortical adenomas (10–15%). Apart from these manifestations, neuroendocrine tumors of thymus, lung, stomach and duodenum occur as well as lipomas and ependymomas [6,7]. The MEN1 syndrome is a result of an inactivating mutation of the *MEN1* gene, localized on chromosome 11q13 (Menin gene), which is a tumor suppressor gene [8]. MEN1 pancreatic neuroendocrine tumors (NETs) are located in both pancreas and duodenum with an incidence of 80–90%. Most common are nonfunctioning pancreatic NETs followed by gastrinomas and insulinomas [6]. Multiple tumors in the target organs are caused by a germline *MEN1* gene mutation, followed by a loss of the wild-type allele. *MEN1* gene alteration is an important initiating event in about one-third of sporadic nonfunctioning pancreatic NETs, insulinomas and gastrinomas and is present in the tumor regardless of the size and presence or absence of metastases [9,10]. Somatic *MEN1* gene mutations accompanied by a loss of the wild-type allele are demonstrated in 10–27% of insulinomas and 39–45% of gastrinomas [11,12]. The mutation rate in nonfunctioning NETs is reported to be 15–26%. The rate of 11q13 loss of heterozygosity (LOH) in sporadic pancreatic NETs is about 46%, and LOH is not always accompanied by somatic mutation, therefore, other mechanisms of *MEN1* gene inactivation or other genes may play a role in sporadic tumor development [13–15]. Studies are indicating that additional onco/suppressor genes may reside at 11q distal to the *MEN1* gene and may play a role in the pathogenesis of pancreatic NETs [15].

VHL disease patients develop central nervous system (CNS) and retinal hemangiomas, renal cysts and carcinomas, pancreatic and epididymal cystadenomas and pheochromocytomas [16]. Multiple tumors in the target organs are caused by a germline *VHL* gene mutation followed by a loss of the wild-type allele [17]. Pancreatic nonfunctioning NETs are seen in 12–17% of VHL patients [18,19•]. Loss of heterozygosity at 3p25.5 gene locus is documented in only 30% of sporadic pancreatic NET and is usually not accompanied by somatic *VHL* gene mutation [20]. These data indicate that the *VHL* gene does not play a role in sporadic pancreatic NET development and that another gene telomeric to the *VHL* 3p locus may be involved [21•]. Neurofibromatosis, von Recklinghausen's disease (NF-1), belongs to a group of diseases called phakomatoses and is an autosomal dominant disorder that is clinically characterized by the presence of café au lait spots on the skin, cutaneous or subcutaneous neurofibromas, optic gliomas, benign iris hamartomas and specific dysplastic bone lesions [22]. Patients with NF-1 may also develop ampullary carcinoids, duodenal and pancreatic somatostatinomas as well as pheocromocytomas and paragangliomas. The *NF-1* gene is a tumor suppressor gene that is located on 17q11.2 and encodes a protein called neurofibromin. The latter is also linked with genes responsible for TSC regulating especially *TSC2* through the mammalian target of rapamycin (mTOR) pathway [23]. It has been shown that *NF-1* acts as a negative regulator of mTOR, and therefore, LOH of the *NF-1* gene results in loss of neurofibromin expression, resulting in mTOR activation and possibly tumor development.

TSC is an autosomal dominant disease that also belongs to the phakomatoses. The patients develop hamartomas and astrocytomas together with well differentiated tumors in the brain, heart, skin, kidney, lung and pancreas [24]. The genes that are associated with TSC are *TSC1*, located on 9q34 and *TSC2*, located on 16p13.3 and they encode the proteins hamartin and tuberin, respectively. Various studies have shown LOH of these genes'

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expression in TSC-associated tumors indicating a tumor suppressor role [25,26].

Sporadic endocrine pancreatic tumors: molecular genetics and pathobiology

Genome-wide analyses by comparative genomic hybridization (CGH) indicate that the chromosomal losses occur slightly more frequently than gains, whereas amplifications are uncommon [27,28]. Genetic alterations seem to accumulate during tumor progression. A total number of genomic changes per tumor appear to be associated with both the tumor volume and the stage of the disease [29]. Thus, large or malignant tumors or both, and especially metastasizes harbor a larger number of genetic alterations than small and clinical benign neoplasms [29,30]. Losses of chromosome 1 and 11q as well as gains of 9q appear to be early events in the development of pancreatic tumors because they are already present in small tumors (<2 cm) [29]. These findings point towards a tumor suppressor pathway and chromosomal instability as important mechanisms associated with malignancy in pancreatic endocrine tumors. Variation of genetic changes in functioning versus nonfunctioning pancreatic NETs has been demonstrated in small tumors (<2 cm in diameter) by CGH [30]. Gains of chromosome 4 and losses of 6q were observed in about 50% of functioning tumors, the majority being insulinomas with a size less than 2 cm [30].

A recent study using genome-wide single nucleotide polymorphism (SNP) analysis showed that about 60% of pancreatic endocrine tumors had high genetic imbalances defined by more than four chromosomal aberrations. These tumors were larger than those with lower aberrations [31•].

Homozygous deletion or hypermethylation of the 5′ region of p16/MTS1 or a deletion of the $p16^{INK4a}$ tumor suppressor gene on chromosome 9p21 was demonstrated in sporadic gastrinomas [32,33]. $p16^{INK4a}$ gene alterations were not observed in insulinomas [34]. These findings suggest that other potential tumor suppressor genes on chromosome 9p are involved in pancreatic neuroendocrine development and imply that p16/MTS1 or $p16^{INK4a}$ defect is restricted to gastrin-producing tumors.

Retinoblastoma gene defects on chromosome 13q were not observed in any type of pancreatic NETs [35]. Both benign and malignant insulinomas demonstrated high LOH rates for markers on chromosome 22q (93%) [36]. Cyclin D1 overexpression was observed by both immunohistochemistry and northern blot analysis in 43% of pancreatic NETs [37]. Promoter region CpG island methylation of the estrogen receptor (*ER*) gene has been documented in nine out of 11 pancreatic NETs [36]. Methylation for the Ras-associated domain gene family 1A (RASSF1A) has been reported in 75% of well differentiated endocrine pancreatic tumors and methylation of O^6-MGMT was noticed in 40% of the cases [38,39]. As *RASSF1A* gene mutation is very rarely observed in human cancer, these findings strongly support the methylation mechanism for multiple gene inactivations in pancreatic NET and suggest that the ras pathway is involved via *RASSF1A* methylation [40]. Activation in the ras family of the proto-oncogenes, K-ras, H-ras and N-ras is absent or exceedingly rare in large series of pancreatic NETs. Thereby, the ras oncogene does not play a direct role in the development of most pancreatic NETs with possible exception for some malignant insulinomas [41,42].

Well differentiated NETs only rarely contain p53 mutations [43,44]. Poorly differentiated neuroendocrine carcinoma of any site shows high chromosomal instability and frequent p53 changes [45]. It is likely that p53 alteration is not involved in pancreatic NET initiation, but represents a late progression event in poorly differentiated neuroendocrine carcinoma of the pancreas. Allelic losses for chromosome X markers are frequently observed in malignant, but not benign pancreatic NETs [46].

Published data suggest that multiple genetic defects may accumulate and result in pancreatic NET progression and malignancy. LOH for markers of several different oncosuppressor genes was significantly more common in malignant (40%) than in benign (17%) tumors [47]. More frequent genomic aberrations in metastases than in corresponding primary tumors has been reported in a study [30]. In nonfunctioning pancreatic NETs, a high frequency of loss of chromosomal markers correlates with aneuploidy and a poor clinical outcome [48]. In a recent study [49•] of pancreatic endocrine tumors, analyzing the expression of 112 genes could clearly separate a benign from a malignant gene cluster, further supporting the clinical value of the WHO classification.

Gastric and duodenal neuroendocrine tumors

Both familial and sporadic, benign and malignant NETs of the stomach and duodenum display frequent LOH for the *MEN1* locus at 11q13 [50,51]. LOH of the *MEN1* locus was demonstrated in 75% of gastric enterochromaffin-like (ECL) carcinoids in 23 familial cases and in 41% of 46 sporadic cases [52]. Four out of five poorly differentiated tumors of the stomach showed allelic losses of the *MEN1* gene [53]. 11q13 LOH was accompanied by a somatic mutation in the *MEN1* gene in 33% of sporadic gastrinomas, regardless of metastases [54]. These data support the initiating role of the *MEN1* gene in the development of many foregut gastric carcinoids and duodenal gastrinomas.

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Frequent and diffuse allelic imbalances of multiple chromosomal markers have been reported in aggressive, poorly differentiated tumors of the stomach. Extensive losses of X chromosomal markers were shown in malignant tumors, but absent in benign foregut NET [55].

Gastrointestinal endocrine tumors (carcinoids)

Knowledge about the genetic background of sporadic gastrointestinal NETs is even sparser than that of pancreatic endocrine tumors. Only three studies using CGH or 131 microsatellite LOH markers examined genome-wide allelic imbalances in gastrointestinal NETs [56–58]. The average number (2.9) of genomic changes was lower in gastrointestinal NETs than in pancreatic tumors. There was no clear correlation between the number of aberrations and tumor stage [58]. Furthermore, the number of different chromosomes involved was low, genetic alterations apparently being concentrated on chromosome 18 [56]. The loss of the entire chromosome 18 or of its long arm in 38% of gastrointestinal NETs has been reported. Losses at 18q22 q-ter have been reported in 67% of midgut carcinoids and losses in 50–88% of tumors in other studies [56,59]. The high percentage reported by Lollgen *et al.* [56] is based on microsatellite LOH analysis in which small deletions are detected with a higher sensitivity than obtained by CGH. The loss of chromosome 18 in gastrointestinal NETs is a strong evidence that important candidate tumor suppressor genes are located on this chromosome. Losses of 9p, which are detectable in 50% of gastrointestinal NETs, are rare in pancreatic tumors. Due to the frequent allelic losses on chromosome 18q, the candidate genes *DPC4*, *DCC* and *Smad2* have been analyzed. In most studies, no mutations have been detected in *DPC4*, *DCC* and *Smad 2* [56]. Therefore, other possible tumor suppressor genes located on chromosome 18q remain to be investigated. Patients suffering from MEN1 often develop NETs, most of which are localized in the duodenum and the stomach. Allelic loss of the corresponding chromosomal arm 11q has been detected in these types of endocrine tumors associated with the MEN1 syndrome. Somatic MEN1 mutations have been detected in a small subset of NETs of the ileum and colon indicating that these mutations are not restricted to foregut NETs, but they also occur rarely in midgut and hindgut tumors [12].

The tumor suppressor genes $p16^{INK4a}$ and *TP53* shows no mutations. However, methylation of $p16^{INK4a}$ was significantly more frequent in gastrointestinal NETs than in pancreatic tumors and, thus, represents an additional molecular difference between the two tumor groups [38]. The higher rates of promoter methylation of the *APC*, *MEN1*, *HIC1* and *RASSF1a* genes in gastrointestinal NETs than in pancreatic tumors are also reported in a recent study [60•]. The high rate of *RASSF1a* promoter methylation might explain the frequent expression of extracellular signaling-related kinase (ERK) 1/2, an important downstream point of convergence in the ras-RAF-mitogen-activated protein ERK pathway [61]. About 25% goblet cell carcinoids present *TP53* mutations [62]. The most frequently reported mutated gene in GI-NETs is β-catenin. Mutations in exon 3 of this gene, protecting the corresponding protein from phosphorylation and degradation, have been reported in 38% of GI-NETs [63]. However, nuclear translocation of β-catenin has been reported in only 30% of GI-NETs with absence of exon 3 mutations [64]. Overexpression of cyclin D1 and cMyc may be a downstream effect of the alterations of the Wnt signaling pathway [15]. Recently, a novel molecular pathway has been identified that links the homeobox gene *Hoxc6* with oncogenic signaling through the activator protein-1 pathway through interaction with *JunD* [65•]. In a recent study, candidate marker gene expressions were analyzed using Affymetrix transcriptional profile [66]. Small intestinal carcinoid overexpress the neoplasia-related genes *NAP1L1* (mitotic regulation), *MAGE-D2* (regulate adhesion) and *MTA1* (estrogen antagonism). These marker genes seem to be correlated to malignant behavior of small intestinal carcinoids.

IL-6 is a pleiotropic cytokine with a still controversial role in tumorigenesis in different cancer types. A recent study reports increased serum IL-6 levels in 37% of GEP-NETs. High serum IL-6 levels correlated with GG IL-6-174 genotype and were significantly higher in nonfunctioning GEP-NETs compared with healthy controls [67•].

Conclusion

Molecular profiling of gastroenteropancreatic endocrine tumors demonstrates that pancreatic endocrine tumors and gastrointestinal neuroendocrine tumors display different genetic changes and should, therefore, be considered to be different tumor entities, thereby, also differently managed clinically. The data on gene expression in the different subtypes of neuroendocrine tumors are still sparse, and it is important to provide studies in larger tumor materials to delineate subtypes of neuroendocrine tumors on genetic bases. A recent study in pancreatic tumors has been able to demonstrate differences in gene profiling between malignant and benign tumors. Although the number of genetic changes is higher in malignant tumors, we are still far away from defining a malignant profile in GEP-NETs [68•].

This will hopefully lead to improved treatment in the future. A summary of different genetic changes frequently found in GEP-NETs is found in Table 2 [69•].

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Table 2 Genetic changes frequently found in gastrointestinal and pancreatic neuroendocrine tumors

	Gene(s)	Involvement		
		Frequent	Rare	Absent
Genes characterizing endocrine tumor syndromes	*MEN1*	PET, D	GI, I,	
	VHL		PET	
	NF-1			
	TSC-1/2			
	HRPT-2			PET
	SDHx			PET, gasNET
Wnt signaling pathway	*β-catenin*	gasNET		PET
	APC		gasNET	
TGF-β signaling pathway	*TGFbR2*			PET, gasNET
	Smad4		PET	
	Smad3			PET
Common tumor suppressor genes/oncogenes	*DCC*			PET, gasNET
	p53		PET, gasNET	
	PTEN		PET	
	K-Ras		PET, gasNET	
Mechanisms of tumorigenesis	CIMP pathway	gasNET	PET	
	Chromosomal instability	PET	gasNET	
	MSI		PET, gasNET	

CIMP, CpG island methylator phenotype; D, duodenal endocrine tumor; GEP-NET, gastroenteropancreatic neuroendocrine tumor; gasNET gastrointestinal neuroendocrine tumor; GI, gastrointestinal neuroendocrine tumors; I, ileal endocrine tumor; MSI, microsatellite instability; PET, pancreatic endocrine tumor. Reproduced with permission [69•].

References and recommended reading

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

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- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 92).

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