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Genetics of pancreatic neuroendocrine tumors: implications for the clinic

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Abstract

Pancreatic neuroendocrine tumors (PanNETs) are a common and deadly neoplasm of the pancreas. Although the importance of genetic alterations in PanNETs has been known for many years, recent comprehensive sequencing studies have greatly expanded our knowledge of neuroendocrine tumorigenesis in the pancreas. These studies have identified specific cellular processes that are altered in PanNETs, highlighted alterations with prognostic implications, and pointed to pathways for targeted therapies. In this review, we will discuss the genetic alterations that play a key role in PanNET tumorigenesis, with a specific focus on those alterations with the potential to change the way patients with these neoplasms are diagnosed and treated.

Introduction

Pancreatic neuroendocrine tumors (PanNETs) are the second most common malignancy of the pancreas. These distinctive neoplasms differentiate along lines similar to non-neoplastic pancreatic neuroendocrine cells in the islets of Langerhans. Despite comprising 1- 3% of new pancreatic malignancies, the number of new diagnoses has recently increased, mainly because of the advances in imaging and diagnostic endoscopy, as well as the increased awareness of the disease in both the medical and general population^{1,3}. As a result, non-functioning tumors are more frequently detected incidentally and at a smaller size⁴. Despite this, many patients with PanNETs still present with metastatic disease, highlighting the malignant nature of these tumors^{1,2}. Based on incidence and follow-up data obtained from the SEER registries, PanNETs (excluding poorly differentiated tumors) comprise 10% of all pancreatic cancers⁵. However this analysis underestimates the real prevalence of PanNETs as it considers only overtly malignant tumors (as identified based on medical coding in the SEER database) and not small benign-appearing tumors, such as small non-functional tumors. Indeed, autopsy studies have shown that PanNETs occur in 0.8% to 3% of

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asymptomatic individuals, and up to 10% in one study in which the authors conducted an extensive pathological evaluation of the entire pancreatic gland^{3, 6, 7}.

In recent years, much progress has been made in characterizing the genetic alterations underlying neuroendocrine tumorigenesis in the pancreas. In this review we will discuss the genetic landscape of PanNETs and the clinical implications of this landscape, with a focus on future directions in novel prognostic biomarkers and new treatment targets.

Classification and Pathology

Before discussing the genetics of PanNETs, we first need to define terminology. Some PanNETs do not secrete clinically significant hormones and are designated as non-functional, while other PanNETs secrete hormones that cause clinical symptoms. This latter group, comprising almost half of PanNETs, is classified as functional. Functional PanNETs can be further subclassified based on the clinical syndrome they produce (not based on immunohistochemical hormone expression). The most common functional PanNETs are insulinomas, while gastrinomas, glucagonomas, somatostatinomas, and VIPomas are rarer.

The second set of terminology relates to underlying genetic alterations that predispose to the disease. As will be discussed in detail later, those PanNETs that arise in patients with a genetic disorder that predisposes to the development of PanNETs are designated syndromic or familial, while those that do not are designated sporadic.

The third critical set of terminology is grade. The current 2010-WHO classification system divides the pancreatic neuroendocrine tumors into three grades. Well-differentiated PanNETs are grade 1 (G1) or grade 2 (G2), and the terminology changes to poorly differentiated neuroendocrine carcinoma for grade 3 lesions⁸. This three tier grading system is based solely on the proliferation rate of the neoplastic cells, as determined by the mitotic count and/or the Ki-67 labeling index. This grading is not only essential in the classification of these neoplasms but is also the major risk prognosticator^{9, 10}. Low-grade (G1) PanNETs have a mitotic count of 0–1 per 10 high power fields (HPFs) or a nuclear Ki-67 labeling index of 0–2%. Intermediate-grade (G2) PanNETs are those with 2–20 mitoses per 10 HPFs or a nuclear Ki-67 labeling index of 3–20%⁸. The highest grade (G3) neuroendocrine neoplasms (mitotic counts >20 per 10 HPFs or >20% nuclear Ki-67 labeling index) are classified as pancreatic neuroendocrine carcinomas (PanNECs). As discussed in detail below, recent studies have shown that the G3 category actually includes two different tumor types with different morphological, genetic, and clinical features: 1) otherwise histologically uniform NETs with an elevated proliferative rate and 2) poorly-differentiated NEC with small cell or large cell morphology^{11, 12}.

Genetic Landscape

Familial Syndromes

Although the majority of PanNETs are sporadic, PanNETs may also arise in the context of familial syndromes (less than 10% of all the cases) (Table 1). In addition to providing insights into the management of syndromic patients, the genetic basis for syndromic

PanNETs also provides a foundation for understanding the genetics of sporadic cases, as several of the same genes are altered in both tumor types. Cancer predisposition syndromes are frequently characterized by an inherited deleterious germline mutation in a tumor suppressor gene that leads to increased tumor susceptibility in the pancreas and in other neuroendocrine organs, leading to the development multiple tumors. These syndromes include multiple endocrine neoplasia type 1 (MEN1), von Hippel–Lindau disease (VHL), neurofibromatosis type 1 (NF1), and tuberous sclerosis complex (TSC), which are characterized by germline mutations in the tumor suppressor genes *MEN1*, *VHL*, *NF1*, and *TSC1* or *TSC2*, respectively (Table 1)^{13,15}.

The MEN1 syndrome is an autosomal dominant clinical syndrome with a prevalence of 2–3 per 100,000 – it is one of the most common familial cancer syndromes¹⁶. Pancreatic tumors develop in 30–80% of MEN1 patients, and tumors of the pancreas are the second most frequent manifestation of the syndrome, after tumors in the parathyroid glands. Other organs that can be affected less frequently are the pituitary and the duodenum^{13,16,17}. Multiple microadenomas (<0.5cm) are the most common pancreatic manifestation of MEN1 syndrome, although most patients ultimately develop larger PanNETs¹⁸. Unlike neuroendocrine tumors arising in other organs of MEN1 patients, PanNETs are often non-functional, thus not associated with any distinct clinical syndromes, and only 10% are insulin-secreting insulinomas^{13,19}. Although non-functional PanNETs are usually indolent neoplasms characterized by slow growth²⁰, they can spread to distant organs, and the risk increases with tumor size. Among all neoplastic manifestations that can occur in MEN1 syndrome, non-functioning PanNETs are indeed the ones more frequently responsible for patient mortality with an estimated 10-year disease-specific survival reported to be between 23% and 62%^{21,23}. Patients with MEN1 can also develop small primary duodenal gastrinomas that metastasize to peripancreatic lymph nodes, which can lead to an incorrect impression of a primary pancreatic gastrinoma^{24,25}. *MEN1*, the gene on chromosome 11q whose germline mutation results in MEN1 syndrome, is also the most frequently mutated gene in sporadic PanNETs²⁶. In both sporadic and syndromic PanNETs, *MEN1* functions as a tumor suppressor gene with loss of function of both alleles (either by germline mutations, somatic intragenic mutation, or loss of heterozygosity)^{26,21}.

Patients with VHL syndrome often present with pancreatic involvement. These patients develop pancreatic serous cystadenomas, PanNETs, and mixed serous cystadenoma-PanNETs²⁷. Mixed serous cystadenomas-PanNETs are uncommon outside of VHL, and as such the finding of this unusual neoplasm should raise clinical suspicion for VHL. PanNETs develop in 10% to 17% of VHL patients; they are almost exclusively non-functional and are frequently detected incidentally during follow up for other extra-pancreatic tumors associated with the syndrome^{27,29}. PanNETs in VHL patients are usually well-differentiated neoplasms; however, they can be locally aggressive and some even metastasize^{30,31}. In addition to pancreatic neoplasms, patients with VHL often develop a variety of benign and malignant neoplasms, including clear cell renal cell carcinomas, pheochromocytomas, paragangliomas (mediastinal, abdominal, pelvic), hemangioblastomas, retinal angiomas, endolymphatic sac tumors of the middle ear, and papillary cystadenomas of the epididymis and broad ligament^{15,32}. *VHL*, the gene whose germline alteration results in VHL syndrome, is a tumor-suppressor gene on the chromosome 3 (3p25–26)³³. Although the

precise mechanism that leads to the development of PanNETs is unknown, the mutated VHL protein results in a lack of degradation of the hypoxia-inducible factors (HIF) and ultimately in an uncontrolled production of factors promoting angiogenesis and tumor growth³². In normal tissue, HIF-1 α undergoes ubiquitination by the VHL protein, leading to HIF-1 α degradation³⁴. Under conditions of hypoxia (or in tumors lacking VHL protein), HIF-1 α is not degraded and accumulates in the nucleus, leading to an increased transcription of numerous hypoxia-response genes (such as VEGF and carbonic anhydrase IX (Ca-IX))^{35, 36}.

The involvement of the pancreas in neurofibromatosis (NF1) and tuberous sclerosis complex (TSC) is less common. NF1 is an autosomal dominant clinical syndrome characterized by a wide range of manifestations but most prominently by nervous syndrome abnormalities like neurofibromas^{37,39}. It is caused by germline mutations in the tumor suppressor gene *NF1* on chromosome 17q^{40, 41}. The protein encoded, neurofibromin, is mainly expressed in the nervous system and has a role in the downregulation of the proto-oncogenic mitogen-activated protein kinase (MAPK) and in the consequent inhibition of the phosphatidylinositol 3-kinase (PI3K) pathway (Figure 2)^{40, 42}. “Pancreatic” tumors are reported in 10% of patients with NF1. However, these tumors are frequently somatostatinomas that actually arise in the duodenum^{43,45}. Rare cases of gastrinomas, insulinomas and non-functioning PanNETs have been reported in patients with NF1^{46,50}.

TSC is an autosomal-dominant syndrome characterized by the development of hamartomas in almost every organ, disabling neurologic disorders, and dermatologic features⁵¹. The pancreas is involved in only 1% of the cases, and both functional and nonfunctional PanNETs have been reported. Both the proteins encoded by the affected genes *NF1* and *TSC* play key roles in the mTOR signaling pathway, a promising target for therapy in PanNETs (see discussion below)^{15, 42, 50, 52}.

Sporadic PanNETs

In 2011 all of the protein coding genes (the entire exome) were sequenced in a series of well-characterized sporadic PanNETs, greatly expanding our understanding of the genetic alterations underlying neuroendocrine tumorigenesis in the pancreas. Jiao et al. performed whole exome sequencing of a clinically homogeneous discovery set of 10 metastatic PanNETs, and subsequently the most commonly mutated genes were analyzed in an additional validation set of 58 PanNETs (Table 2)²⁶. Importantly, these studies were performed on primary tumor samples, as recent studies have shown that cell lines derived from neuroendocrine tumors are genetically distinct from primary tumors⁵³. The most commonly somatically mutated genes encoded for proteins implicated in chromatin remodeling: 44% of the tumors had somatic inactivating mutations in *MEN1* and 43% had mutations in genes encoding either one of the two subunits of a transcription/chromatin remodeling complex composed of *DAXX* (death-domain– associated protein) and *ATRX* (a thalassemia/mental retardation syndrome X-linked)²⁶. Mutations in genes encoding proteins in the mTOR pathway were found in 14% of the cases²⁶. Let us consider each of these genes individually.

MEN1—*MEN1*, on chromosome 11q13, is the most frequently mutated gene in sporadic PanNETs, with somatic mutations reported in 25-44% of tumors^{26, 54, 55}. In addition to small intragenic somatic mutations, the *MEN1* gene is also altered by chromosomal alterations (such as loss of heterozygosity)^{56, 58}. The protein encoded by *MEN1* (menin) interacts with numerous positive or negative regulators of transcription to control expression of genes that are involved in a variety of cell functions^{59, 61}. Among others, menin interacts with JunD to inhibit its growth promoting activity^{62, 63}, with Smad3 to inhibit the transforming growth factor- β (TGF- β) signaling pathway⁶⁴, and with other transcription factors to regulate DNA replication (p53/nuclear factor- κ B⁶⁵, NF- κ B, and the metastasis suppressor NM23⁶⁶). Additionally, menin takes part in the nuclear protein complex that promotes site-specific histone methylation, which is a major epigenetic mechanism for maintaining stable gene transcription in terminally differentiated cells^{67, 68}. In pancreatic islet cells, menin regulates histone methylation in promoters of specific target genes that have a role in growth and differentiation of neuroendocrine cells⁶⁷. Menin activates the transcription through histone H3 lysine 4 methylation of the genes encoding cyclin-dependent kinase (CDK) inhibitors: CDKN2C (p18) and CDKN1B (p27). CDK2 plays an important role in G1 to S transition; thus, loss of menin function leads to elevated CDK2 activity and concomitant downregulation of the CDK inhibitors p18 and p27^{67, 69}. The upregulation of CDK2 results in the tissue specific acceleration of G0/G1 to S phase entry and eventually in the increased islet cell proliferation^{67, 69}.

Knockout of the *MEN1* gene in mice leads to the development of β -cell hyperplasia and insulinomas in some animals by 9 months. These data, along with the high rate of *MEN1* mutations in sporadic PanNETs and the large number of microadenomas in patients carrying germline *MEN1* mutations, suggest an early role of *MEN1* inactivation in PanNETs tumorigenesis¹³. Furthermore, it has been suggested that an additional PanNET driver gene could be present at 11q distal to *MEN1*^{28, 70, 71}.

In addition to its role in familial forms of PanNETs, the targeting of *MEN1* in sporadic PanNETs may have clinical implications. *MEN1* mutations may be a prognosticator, although the data on this are inconsistent. It has been suggested that in patients with metastatic PanNETs, the presence of *MEN1* mutation alone and, with stronger evidence, in combination with loss of function of the complex DAXX/ATRAX, might identify a subgroup of patients with prolonged survival²⁶. However some series that specifically examined *MEN1* gene inactivation in primary non-metastatic PanNETs have failed to demonstrate an association between *MEN1* status and prognosis^{54, 58}. There are currently no clinically proven ways to therapeutically target the *MEN1* mutations in PanNETs.

DAXX/ATRAX—Exome sequencing of PanNETs has shown that close to half of sporadic PanNETs (45%) have somatic mutations in either the *DAXX* (25%) or *ATRAX* (18%) genes²⁶. Mutations in these genes were found to be mutually exclusive, which is consistent with their role within the same pathway. Furthermore, the presence of homozygous mutations as well as the high rate of inactivating nonsense mutations in both genes define them as tumor suppressor genes (Figure 1).

Although little is still known about the precise role of loss of the DAXX/ATR complex in the tumor progression, the wild-type proteins play multiple cellular roles. Similar to menin, the DAXX/ATR complex regulates chromatin remodeling. However its function is unique: the DAXX/ATR complex incorporates the histone variant H3.3 at the telomeric ends of chromosomes. Strikingly, loss of ATR/DAXX function is associated with the alternative lengthening of telomeres (ALT) phenotype, a telomerase-independent mechanism of telomere maintenance^{72,74}. ALT is a recombination-based mechanism which is important in the survival of telomerase-negative cancer cells. In PanNETs, ALT phenotype correlates almost perfectly with DAXX/ATR status^{75, 76}. Tumors with intact *DAXX/ATR* do not have ALT, while PanNETs with a *DAXX* or *ATR* mutation have ALT.^{75,76, 77}

About 10% of all human cancers show the ALT phenotype⁷⁸. In some tumors, like in glioblastoma multiforme, a correlation between ALT-positivity and an improved prognosis has been reported⁷⁹. In other tumor types (for example osteosarcoma), no differences in the clinical outcome were observed between tumors with the ALT or telomerase phenotype^{78, 80}. Most likely, among tumors presenting ALT phenotype, a specific correlation between ALT activation and tumor aggressiveness depends on the cancer type. The relationship between *DAXX/ATR* mutation and prognosis for patients with PanNETs remains to be clarified. Jiao et al. reported that patients with mutations in *DAXX* or *ATR* genes had significantly longer survival than patients with wild-type PanNETs, especially patients with metastatic disease. In this study, mutations in both *MEN1* and *DAXX/ATR* were associated with a better prognosis²⁶. In contrast, Marinoni et al. found that nuclear loss of DAXX/ATR correlated significantly with a high tumor stage and a poor prognosis. However, a sub-analysis considering only metastatic patients showed a trend toward longer survival for patients with DAXX/ATR nuclear loss, compared to patients with tumors that retain DAXX/ATR expression⁷⁶. These findings seem consistent with the hypothesis that loss of DAXX /ATR and ALT phenotype may identify a subgroup of metastatic PanNETs characterized by a better prognosis. Further studies are also required to understand the correlation between ALT phenotype and chromosome instability, which has been associated with a poor prognosis^{81, 82}.

There are currently no clinically proven ways to therapeutically target *DAXX/ATR* mutations in PanNETs, but the molecular distinctiveness of the associated ALT phenotype makes this an active area of research interest.

MTOR Pathway—Several observations support a role for aberrant activation of the mammalian target of rapamycin (mTOR) pathway in multiple types of human cancers, including PanNETs, and a number of therapeutic agents targeting different elements of this pathway (such as everolimus) have been developed^{83,84, 84}.

Exome sequencing has shown that almost 16% of well-differentiated PanNETs have somatic mutations in genes encoding proteins involved in the mTOR pathway²⁶. These include *PTEN* (7%), *TSC2* (9%), and *PIK3CA* (1%)²⁶. In a gene expression profile study on a large cohort of patients, proteins encoded by *TSC2* and *PTEN*, two key inhibitors of the mTOR pathway, were under-expressed in the majority of primary PanNETs⁸⁵. The relatively low prevalence of somatic mutations in mTOR pathway genes does not match the high

prevalence of cases with low gene expression. This suggests that in addition to somatic mutations, the up-regulation of the mTOR signaling axis might be achieved through other distinct mechanisms, such as epigenetic changes or post-translational modifications.

One of these mechanisms could involve another mTOR regulator: *PHLDA3*⁵⁴. *PHLDA3* is a novel tumor suppressor gene that represses Akt activity in islet cells. Its locus on chromosome 1q31 has been recently found to undergo loss of heterozygosity (LOH) at a remarkably high frequency in PanNETs (approximately 70% of the cases)⁵⁴. In one study, LOH at the *PHLDA3* locus was associated with advanced stage, whereas absence of LOH was associated with lower tumor grade, suggesting that the LOH of *PHLDA3* may be associated with a more aggressive phenotype. Patients with LOH had also a trend toward poorer survival, but this did not reach statistical significance⁵⁸.

The mTOR pathway is one of the most promising pathways that can be targeted in PanNETs^{26, 84}. For example, Yao et al have reported that everolimus therapy significantly prolonged progression-free survival among patients with advanced PanNETs, and that this therapy is associated with a low rate of severe adverse events.

Using Genetics to Classify Neuroendocrine Tumors of the Pancreas

The WHO currently classifies all neuroendocrine neoplasms of the pancreas with high proliferation rates (>20%) together as neuroendocrine carcinomas. This is done regardless of the other morphologic features of the tumors. In fact, genetic analyses have shown that there are two distinct tumor types included in the umbrella term Grade 3 neuroendocrine carcinomas of the pancreas. Grade 3 neuroendocrine carcinomas of the pancreas that histologically have round nuclei with salt and pepper chromatin (which except for their high mitotic rate look like well-differentiated PanNETs under the microscope) have the same genetic alterations as do well-differentiated PanNETs (*DAXX* and *ATRX* are targeted)¹². By contrast, Grade 3 neuroendocrine carcinomas of the pancreas that histologically look more like carcinomas (small cell carcinoma or large cell carcinoma) do not have *DAXX* and *ATRX* mutations. Instead, the *RB* and *TP53* genes are targeted. These latter tumors also often have extremely high mitotic rates (>50%). These findings have two implications. First, they suggest that Grade 3 neuroendocrine carcinomas do not occur as result of a progressive loss of differentiation of well-differentiated tumors, but rather represent a separate tumor altogether^{11, 12, 86, 87}. Second, they have therapeutic implications, as Grade 3 neuroendocrine carcinomas with extremely high proliferation rates (>50%, small cell carcinoma or large cell carcinomas), may best be treated with different chemotherapies⁸⁸.

Comparison to Other Pancreatic Tumors

The genes with frequent somatic mutations in PanNETs are quite distinct from those altered in pancreatic ductal adenocarcinoma (PDAC), confirming that the genetic differences mirror the clinical and biological differences between these two malignant neoplasms of the same organ. PanNETs had an average of 16 nonsynonymous somatic mutations per tumor, 60% fewer genes mutated than in PDACs. The commonly mutated driver genes in PDAC (*KRAS*, *SMAD4*, *CDKN2A*, *TP53*) are only very rarely altered in PanNETs (*TP53* in 3% of the cases)²⁶.

Chromosomal Alterations

Several chromosome aberrations have been described in PanNETs using comparative genomic hybridization (CGH), and several efforts were made to identify genetic alterations that may discriminate indolent tumors from those likely to progress^{89,92}. An increased number of chromosomal gains and losses (so-called chromosomal instability or CIN), has been found to correlate with more aggressive behavior⁸¹. Indeed, in non-functioning PanNETs, larger tumors have more chromosomal aberrations, and metastatic lesions contain more alterations than do their matched primary tumor^{76, 89}. In functional tumors, particularly insulinomas, the number of chromosomal alterations was significantly increased in metastatic tumors when compared with non-metastatic, and the presence of CIN has been associated with poor outcome^{81, 90}.

Alterations on specific chromosomal regions have been proposed as genetic markers of malignancy and aggressiveness. However, several series have presented a broad spectrum of chromosomal gains and losses, indicating significant heterogeneity, especially among malignant PanNETs^{89, 91, 94}. For example, losses of 6q^{89, 92}, 3p⁹², 11p⁹² and 22q^{91, 94} and gains of 17q⁹², 4p and 4q⁸⁹ have been found to be associated with metastatic disease and in particular 6q loss with malignant insulinoma⁸¹.

Advances in Therapy

Conventional Approaches

Currently, surgery is the only established curative option for PanNETs, and unlike many other pancreatic tumors, surgery also plays a role in the setting of metastatic disease. For example, localized liver metastases are often resected when technically feasible, while it would be unusual for metastatic adenocarcinoma of the pancreas to be resected. Resection of all grossly visible liver metastases can be associated with long-term survival and (in the case of symptomatic functional tumors) symptomatic relief^{95, 96}.

Cytotoxic chemotherapy is also important in specific clinical situations. For patients with neuroendocrine carcinomas (PanNECs), chemotherapy still represents the first line therapy – in these tumors systemic chemotherapy with a regimen of cisplatin and etoposide is recommended^{97, 98}. There are no specific recommendations for second line therapies in PanNECs; however, the successful use of combinations with drugs such as streptozocin, doxorubicin, 5-FU/capecitabine, temozolomide, cisplatin and etoposide have been reported^{99, 100}. By contrast, the use of cytotoxic chemotherapy in well-differentiated PanNETs continues to be a matter of debate. Regimens of streptazocin have been evaluated in combination with numerous other agents, such as 5-fluorouracil (5-FU)/doxorubicin¹⁰¹⁻¹⁰³, and temezolomide has shown promising results either alone or in combination with capecitabine with response rates that vary between 33% and 74%^{104, 105}.

Targeting the Somatostatin Receptor

Most PanNETs express somatostatin receptor (specifically SSTR-2) on the cell surface.^{106, 107}

Currently, somatostatin synthetic analog therapy (e.g., lanreotide, octreotide) is frequently used to treat hormone-related symptoms in functioning PanNETs¹⁰⁸. However, the role of somatostatin analogues in treating patients with non-functioning PanNETs remains uncertain. Multiple studies have shown prolonged progression-free survival in nonfunctional midgut and gastro-entero-pancreatic NETs, though a subgroup analysis on PanNETs did not show a significant improvement in survival^{109, 110}. The expression of SSTRs has been also exploited to develop radiolabeled somatostatin analogs for use in imaging and as a treatment. Nuclear imaging techniques utilizing intravenous injection of radiolabeled somatostatin analogues, such as somatostatin receptor scintigraphy (SRS) and positron-emission tomography (PET), have been shown to localize the primary tumor with more sensitivity than standard imaging techniques^{111, 112}. This concept has been taken further by labeling somatostatin analogues with radionuclides such as indium (111 I), yttrium (90 Y) or lutetium (177 Lu), which can then be utilized for so-called peptide receptor radionuclide therapy (PRRT)^{113, 114}. PRRT is a relatively new therapeutic modality that has been shown very encouraging results in patients with advanced PanNETs, however randomized comparisons between PRRT and the current standard-of-care treatments are still lacking.

Targeting the Vasculature

PanNETs have prominent intratumoral vessels and highly express also a wide variety of pro-angiogenic molecules such as hypoxia inducible factor-1 α (HIF-1 α) and vascular endothelial growth factor (VEGF)^{115, 116}. As in VHL patients, HIF-1 α appears to have a crucial role in tumor progression in sporadic PanNETs, and its overexpression has been proposed as predictor of poor clinical outcome¹¹⁷. As discussed above, lack of degradation of HIF-1 α may be one of the mechanisms leading to enhanced transcription of pro-angiogenic factors such as VEGF^{35, 117}. Considering these characteristics, anti-angiogenic strategies, including the VEGF inhibitor bevacizumab and the VEGF receptor (VEGFR)-targeted tyrosine kinase inhibitor sunitinib, are currently used in clinical practice. A trial evaluating the efficacy of sunitinib in PanNETs showed that the progression free survival in the treatment group was more than double that of the control group, demonstrating the promise of this therapeutic approach.^{118, 119} Other drugs targeting angiogenesis such as bevacizumab and pazopanib are currently under study in PanNETs. A synergistic effect has been proposed for anti-angiogenic agents with other target therapies and conventional chemotherapy by increasing antitumor activity and limiting the drug toxicity^{120, 122}.

MTOR Inhibitors

MTOR inhibitors represent an exciting new potential for “personalized therapy” of PanNETs. Everolimus is an orally available mTORC1 inhibitor derived from rapamycin, a macrolide antibiotic originally isolated from *Streptomyces hygroscopicus*. This class of agents binds the mTORC1 complex inhibiting the downstream signal and therefore the proliferation of tumor cells through the inhibition of the G1/S transition (Figure 2)¹²³. Preclinical studies have confirmed the effective antitumor activity of everolimus in patients with a variety of tumor types^{124, 125}. Everolimus is currently indicated as second line treatment of several solid tumors such as hormone receptor-positive HER2-negative advanced breast cancer and advanced renal cell carcinoma after failure with sunitinib or sorafenib^{126, 127}.

A series of elegant trials on patients with PanNETs has led to the approval of everolimus by the United States Food and Drug Administration (FDA) in 2011 for the treatment of progressive unresectable/metastatic well-differentiated PanNETs. The first of these trials, RADIANT I, was an open-label phase II study of patients with metastatic PanNETs progressing on or after chemotherapy. In this study patients were stratified by prior octreotide therapy, and patients received everolimus alone or in combination with octreotide. Patients receiving the combination of octreotide and everolimus have shown a better survival than those receiving everolimus alone (17 vs 9.7 months)¹²⁸. This may be due to upregulation of the insulin-like growth factor 1 (IGF-1) pathway in patients not receiving octreotide, eventually leading to resistance to everolimus.^{129,131} Because somatostatin analogues act by inhibiting the IGFR/PI3K/Akt axis, the combination of mTOR inhibitors with somatostatin analogues or PI3K inhibitors may overcome resistance mechanisms and increase the response to the drug (Figure 2)^{132, 133}.

In a phase III trial, RADIANT II, patients with advanced NETs with carcinoid syndrome were randomized to treatment with octreotide combined with placebo or with everolimus. The median progression free survival was greater for patients receiving everolimus and octreotide compared with patients in the placebo arm, but the results didn't reach the prespecified level of significance¹³⁴. However imbalances between the study groups (primary tumor site, WHO performance status, and previous use of chemotherapy) favoring the placebo plus octreotide group were noted. When adjusted for these imbalances, a significantly increased progression free survival in favor for treatment with everolimus was observed¹²⁷.

RADIANTIII is the trial that led to the approval in 2011 by the FDA of the use of everolimus in patients with advanced PanNETs. In the placebo controlled phase III trial RADIANT III, single therapy with everolimus was compared with the best supportive care for advanced PanNETs progressive within the previous 12 months. The majority of the patients had received prior treatment with chemotherapy, radiotherapy, somatostatin analogue therapy, or some combination of those. Everolimus, as compared with placebo, was associated with a significant prolongation of the median PFS (11.0 versus 4.6 months) and sub-analyses on the same cohort of patients confirmed its effectiveness across all the subgroups considered (previous chemotherapy, previous therapy with somatostatin analogs, performance status, age, sex, origin, well vs intermediate differentiated tumors)⁸⁴.

Although one could hypothesize that PanNETs with mTOR mutations would respond better to mTOR inhibitors than PanNETs with intact mTOR signaling, this has yet to be proven in clinical trials¹³⁵.

Expert Commentary

Recent genetic analyses of PanNETs have revealed the key genes and pathways driving their tumorigenesis. Specifically, the tumor suppressor gene *MEN1*, the chromatin remodelers *ATRX* and *DAXX*, and the members of the mTOR signaling pathway make up the unique genomic landscape of PanNETs. Although studies to date have provided great insights into the importance of these genetic alterations in PanNET tumorigenesis, much still remains to

be determined, such as the timing and cooperation of these alterations as well as the specific mechanisms by which they enhance tumor growth. These alterations have significant clinical implications, including utility as biomarkers for prognosis as well as targets for novel therapeutic approaches. As these new approaches are developed, we should remember that PanNETs are not a homogeneous group of tumors, but instead represent several subgroups, each characterized by different genetic alterations and clinical features. Further investigations will focus on refining these subgroups based on genetic or other biomarkers to enable personalized therapy of PanNETs. The efficacy of everolimus in PanNETs highlights the great potential of targeted therapies in this tumor type, a potential that can be further realized by further studies of the molecular and clinical features of PanNETs.

Five-Year View

Over the next five years, our understanding of the clinical implications of the inherited and acquired genetic alterations in PanNETs will likely expand further. Larger studies will more clearly define the prognostic implications of driver gene mutations such as *MEN1*, *ATRX*, and *DAXX*. With expanded use of everolimus and other targeted agents, studies will also delineate which subgroups of PanNET patients are more likely to respond to specific targeted agents – studies investigating the impact of mTOR pathway mutations on everolimus response will be particularly critical for effective use of this drug. In addition, new targeted agents will likely be developed to target specific subsets of PanNET patients – approaches targeting the alternative lengthening of telomeres (ALT) phenotype in patients with *ATRX* and *DAXX* mutations show particular promise. Finally, the category of “pancreatic neuroendocrine carcinoma” will likely be further subdivided based on cytologic features of malignancy as well as molecular features, highlighting a subgroup of patients more likely to respond to aggressive cytotoxic chemotherapy. Overall, the future of PanNET therapy lies in integrating molecular findings with clinical and pathological findings to define specific patient subgroups that are likely to respond to particular therapies, making personalized medicine a reality for patients with these neoplasms.

Key Issues

- Pancreatic neuroendocrine tumors (PanNETs) are the second most common pancreatic malignancy and are clinically and biologically distinct from pancreatic ductal adenocarcinoma.
- PanNETs are a feature of many familial cancer syndromes. They occur commonly in patients with multiple endocrine neoplasia type 1 (MEN1) and von Hippel–Lindau disease (VHL), while they are uncommon in patients with neurofibromatosis type 1 (NF1) and tuberous sclerosis complex (TSC).
- Recent high-throughput sequencing studies of sporadic (non-familial) PanNETs have identified many of the key genetic drivers in this tumor type.
- The *MEN1* tumor suppressor gene is somatically mutated in almost half of sporadic PanNETs. This gene likely plays a role in the early steps of PanNET tumorigenesis.

Some studies have suggested that patients with somatic mutations in *MEN1* have an improved prognosis, but data on this are inconsistent.

- Mutations in the genes *ATRX* and *DAXX* occur in almost half of sporadic PanNETs. Loss of *ATRX* or *DAXX* expression (a surrogate for mutation) is associated with the alternative lengthening of telomeres phenotype, a telomerase independent mechanism of telomere maintenance. Like *MEN1*, the data on the relationship between *ATRX* and *DAXX* mutations and prognosis are inconsistent.
- Approximately 15% of PanNETs have somatic mutations in genes that encode components of the mTOR pathway, including *TSC2*, *PIK3CA* and *PTEN*. These mutations highlight the importance of the mTOR pathway in PanNET tumorigenesis, and this pathway can be therapeutically targeted by drugs such as everolimus.
- PanNETs are graded based on proliferation rate, with the highest grade designated as neuroendocrine carcinomas. However, this group is heterogeneous, containing both PanNETs with an elevated proliferation rate and cytologically malignant neuroendocrine carcinomas. These two groups are genetically distinct, with *TP53* and *RB* mutations in the latter group.
- Surgery is a mainstay of treatment of PanNETs, though some studies have examined the use of cytotoxic chemotherapy for advanced cases. Other potential therapeutic targets include the somatostatin receptor and the vasculature.
- Therapies targeting mTOR have recently been approved for use of patients with PanNETs. Patients treated with the mTOR inhibitor everolimus showed improved prognosis compared to placebo in a phase 3 clinical trial. Clinical trials have not yet examined the correlation of mTOR pathway mutations with response to everolimus.

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Figure 1a

Figure 1b

Figure 1.

Loss of ATRX or DAXX function in pancreatic neuroendocrine tumors (PanNETs) with mutated *ATRX* or *DAXX* genes. The wild-type proteins are localized to the nucleus, whereas mutations in these genes correlate with loss of nuclear protein expression. **A)** Immunolabeling for ATRX demonstrating loss of nuclear expression in neoplastic cells, while expression is retained in stromal cell nuclei in well-differentiated PanNET with mutated *ATRX*. **B)** Immunolabeling for DAXX of the same PanNET demonstrating nuclear expression.

Figure 2.

Schematic representation of the mammalian target of rapamycin (mTOR) signaling pathway and crosstalk with the mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase (PI3K) pathway. The mTOR protein is present in two different complexes (mTORC1 and mTORC2) that are activated through two different signaling cascades. MTORC1 is the principal target of rapamycin analogs (rapalogs), including everolimus, while mTORC2 seems not to be sensitive to these agents. Inhibition of mTORC1 by everolimus might cause a lack of inactivation of IRS-1, resulting in an

upregulation by feedback loop mechanisms of the PI3K and MAPK pathways. This seems to be one of the main mechanisms that leads to the development of resistance against mTOR inhibitors. (#) Genes with germline mutations characterizing inherited syndromes with predisposition to PanNETs. (*) Genes with somatic mutations found in sporadic PanNETs. Other abbreviations: 4E-BP1, eIF4E-binding protein 1; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; IGFR, insulin-like growth factor receptor; IRS1, insulin receptor substrate 1; MEK, MAP-ERK kinase; PIP2, phosphatidylinositol (4,5)-biphosphate; PIP3, phosphatidylinositol (3,4,5)-triphosphate; PTEN, phosphatase and tensin homolog deleted at chromosome; S6K1, p70 S6 kinase 1; TSC, tuberous sclerosis complex.

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Table 1
Familial syndromes associated with PanNETs

Familial syndrome	Affected gene	Prevalence of PanNETs
Multiple endocrine neoplasia type 1	<i>MEN 1</i>	30-80 %
Von Hippel–Lindau disease	<i>VHL</i>	10-17%
Neurofibromatosis type 1	<i>NF1</i>	10%
Tuberous sclerosis	<i>TSC1 or TSC2</i>	1%

Table 2
Somatic mutations in PanNETs, percentages taken from Jiao et al

Gene mutated	Mutation prevalence	Pathway involved
<i>MEN 1</i>	44%	Chromatin remodeling (histone methylation)
<i>DAXX</i>	25%	Chromatin remodeling (ALT)
<i>ATRX</i>	18%	Chromatin remodeling (ALT)
<i>TSC 2</i>	9%	MTOR
<i>PTEN</i>	7%	MTOR
<i>PI3KCA</i>	1%	MTOR
<i>TP53</i>	3%	Cellular apoptosis