Introduction

Pancreatic endocrine neoplasms (PENs) are foregut endocrine tumors that arise in the pancreas and appear morphologically similar to other neuroendocrine (carcinoid) tumors throughout the body. However, the biology of endocrine/neuroendocrine tumors tends to depend upon the site of origin. PENs can produce various hormones, though most are nonfunctional. They are generally low grade, well-differentiated tumors. PENs can be classified according to grade, size, or functional status. The nomenclature of these neoplasms can be confusing, partly because of the variable terminology, including different names for the same neoplasm (including “islet cell tumors,” “pancreatic neuroendocrine tumor,” and “pancreatic endocrine tumors”) and partly because well-differentiated malignant tumors can either be called “malignant pancreatic endocrine neoplasms” or “pancreatic endocrine carcinomas.” The authors of the AFIP fascicle prefer to consider all well-differentiated PENs as potentially malignant (with the exception of microadenomas less than 0.5 cm in size). The term “carcinoma” is reserved for poorly differentiated lesions. Similarly, the term PEN will be used in this chapter to refer to well-differentiated tumors.

Sporadic Pancreatic Endocrine Neoplasms

PENs are uncommon neoplasms; the incidence in the United States is about 2.2 per 1,000,000. There is a slight male predominance and the mean age is 58.5 years. The vast majority of PENs are sporadic and up to 90.8% are nonfunctional. A PEN not associated with a syndrome of hormone overproduction is, by default, a nonfunctional tumor. Nonfunctional tumors are discovered incidentally or may be associated with abdominal pain. PENs associated with a syndrome of hormone overproduction are classified as functional PENs and include insulinomas, glucagonomas, somatostatinomas, gastrinomas, VIPomas, serotonin secreting tumors, as well as other rare and mixed hormone-producing entities. The clinical presentation of functional tumors depends on the type of hormone that is secreted.

About 60% of PENs arise in the pancreatic tail, but they can occur throughout the pancreas. Grossly, PENs are usually solid, well-circumscribed, sometimes encapsulated lesions. The cut surfaces can be uniform or heterogenous with solid or spongy, yellow to red parenchyma. PENs have many various histological patterns, including the traditional organoid or trabecular growth pattern seen in well-differentiated neuroendocrine tumors at other sites. The cytologic features can also be typical of other neuroendocrine tumors. The neoplastic cells tend to be small and polygonal with abundant amphophilic cytoplasm and uniform round to oval nuclei with the coarsely stippled “salt and pepper chromatin.” However, variable cellular size, nuclear size, nuclear shape, and chromatin patterns are common. The variation in growth patterns and cytologic features makes PENs a unique and interesting group of endocrine tumors.

Useful immunohistochemical markers include antibodies to most neuroendocrine cells, such as synaptophysin, protein gene product (PGP) 9.5, CD 57 (Leu7) and CD 56 (neural cell adhesion molecule); chromogranin is inconsistently expressed in PENs and its staining can be patchy and dependant on the antibody that is used. When confronted with a metastatic (neuro)endocrine tumor, antibodies to CDX-2 and TTF-1 can be very helpful. PENs tend to lack staining for both of these markers, while TTF-1 is expressed in lung primaries and CDX-2 in gastrointestinal primaries. Immunohistochemical stains can also be used to detect specific peptides such as insulin, glucagon, somatostatin, and pancreatic polypeptide, but the protein expression does not always correlate with the functional status of the PEN.

In an attempt to better predict the prognosis of PENs, several immunohistochemical markers have been studied, but none have replaced classic clinical and morphologic parameters. Ki-67 (MIB-1) proliferation index has been
shown to have prognostic value in PENs and is used by the WHO to distinguish PENs with “benign” behavior from those with “uncertaint” behavior based on a cutoff of 2%. Another proposed grading scheme uses mitoses and ki-67 labeling index to separate low and intermediate grade PENs from high grade pancreatic endocrine carcinomas. However, one group has suggested simplifying the WHO classification system by using clinical parameters of tumor size and metastases with grading information based on necrosis and mitotic rate. Another promising prognostic marker, CK-19, was found to be expressed in more aggressive PENs and its expression was correlated with a shorter disease-free survival and was independent of the WHO criteria.

Genome Wide Study of Sporadic Pancreatic Endocrine Neoplasms

Comparative Genomic Hybridization

Using comparative genomic hybridization, numerous studies have identified many chromosomal alterations in PENs with chromosomal losses being slightly more common than gains. Commonly described alterations include losses at 1p, 3p, 6q, 9p, 10p, 11p, 18q, 22q, Y and X and gains at 4p, 5q, 7p, 7q, 9q, 12q, 14q, 17p, 17q and 20q. One of the most consistent chromosomal alterations is loss of 11q, which harbors the \textit{MEN1} gene. Some chromosomal alterations have been associated with tumor grade, stage, and prognosis. The overall number of genetic changes per tumor, especially gains, has been associated with both tumor size and disease stage. Losses of 3, 6, and 21q and gains of 4, 7, 14q, 17, 20q, and Xq were found to be associated with malignant behavior and/or metastatic disease. Prognostic significance was also linked to changes in the sex chromosome. In males, Speel et al noted that all of the four male patients with malignant insulinomas studied had loss of chromosome Y in addition to gain of Xp. This finding was further supported by Missiaglia et al who showed that PENs from female patients show frequent loss of chromosome X and PENs from male patients show relatively frequent loss of chromosome Y, and that loss of a sex chromosome was associated with the presence of metastases, local invasion, and with poor survival.

Loss of Heterozygosity (LOH)

A number of studies have used LOH to identify chromosomal loci that may harbor potential tumor suppressor genes involved in the pathogenesis of PEN. LOH can detect smaller deletions than CGH. In addition to those losses noted by CGH, additional deletions of interest have been detected by LOH at 3p14.2-3p21, 3p23, 6q22, 7q31–32, 9p, 11q13, 13q14, 17q21, 18q21, and 22q12. LOH at loci 3p25.3-p23, 6q, 17p13, and 22q have been associated with aggressive behavior and metastatic disease. Importantly, these assays have been reported in fine needle aspirations, as well as in histologic material. In addition to their importance as useful markers for clinical behavior, these chromosomal changes direct attention to particular chromosomal regions as candidates for a more detailed analysis with respect to genes involved in PEN development.

Gene Expression Profiling of Sporadic PENs

Several DNA microarray studies using different approaches have reported novel genes that may be important in the pathogenesis and prognosis of PENs. Up-regulated genes potentially involved in pathogenesis of PENs include oncogenes (\textit{MLLT10/AF10}), growth-factor-related genes (\textit{IGFBP3}), cell adhesion and migration molecules (fibronectin), endothelial elements (MCAM/MUC18, PECAM1/CD31, ANGPT2/Ang2), potential biomarkers (SERPINA10, BIN1) and therapeutic targets (LCK/SRC-like kinase, BST2); down-regulated genes include cell cycle check point genes (CDKN1A/p21), cell surface glycoproteins (CD99/MIC2), putative metastasis suppressor genes (NM23, transcription factors (\textit{JUND}) and apoptosis-related genes (\textit{IER3}, \textit{PHLDA2}, \textit{IAPP}, and \textit{SST}). One DNA microarray study compared nonmetastatic with metastatic PENs and found differential expression of genes related to growth-factor-related molecules (\textit{IGFBP1}, \textit{IGFBP3}), cytoskeleton-related molecules (b1-tubulin), cell cycle regulation (\textit{CHEK1}), developmental regulation (\textit{TBX3}), intracellular signaling (\textit{UPK1B}), and DNA damage repair (\textit{MGMT}). While another study found no significant differentially expressed genes between primary tumors and their metastases. Due to poor concordance between these published studies, further analysis of these genes may reveal important insight into the pathogenesis and prognosis of PENs.

Individual Genes in Sporadic Pancreatic Endocrine Neoplasms

Most of the molecular studies of PEN have failed to demonstrate a strong role of the common oncogenes and tumor suppressor genes in the molecular pathogenesis of most PENs. Because of its involvement in \textit{MEN1} patients, the \textit{MEN1} gene has been examined for mutations in sporadic PENs. Although loss of \textit{MEN1} locus at 11p13 occurs in 43–68% of sporadic PENs, somatic mutations have been found in only 15–26%, suggesting involvement of another tumor suppressor gene at this location. Similarly, in patients with sporadic PENs, LOH on chromosome 3p was identified in 33% of cases, but no mutations in \textit{VHL} were detected. As for other tumor suppressor genes
and oncogenes, there appears to be no significant role of TP53, CDKN2A (p16), PTEN, SMAD4/DPC4, ERBB2/HER2, BCL2, KRAS, or BRAF in the pathogenesis of sporadic PENs.18,22,28,39–43

Epigenetic Changes in Sporadic Pancreatic Endocrine Neoplasms

In addition to genetic mutation and chromosomal loss, aberrant epigenetic changes including abnormalities in methylation have been demonstrated in PEN. Chan et al demonstrated that the methylation profile of gastrointestinal neuroendocrine (carcinoid) tumors differs from PENs, reflecting different molecular pathogenesis.54 Among the most commonly methylated genes in PEN are RASSF/RASSF1A, CDKN2A/p16, MGMT, and MLH1.45–47 The hypermethylation of RASSF1 promoter is frequent in many human cancers, and there is an inverse correlation between RASSF1 silencing by methylation and KRAS activation.38,49 As mentioned earlier, it has been shown that well-differentiated PENs lack KRAS or BRAF mutations.32,50 It is therefore interesting that the RASSF1 gene is the most frequently methylated gene in PEN.45–47 This finding suggests that the RAS pathway may still be involved in well-differentiated neuroendocrine tumors mostly by gene silencing of RASSF1 gene through methylation. The frequency of CDKN2A/p16 methylation ranges from 19% to >50% in the literature.46,47,51 The MGMT gene is an important tumor suppressor gene responsible for removing alkylation of DNA at the O6 position of guanine and playing a major role in DNA repair. Methylation of CpG islands in the promoter region of MGMT can cause gene silencing.35 Another tumor suppressor gene important in DNA repair is MLH1. The association between loss of MLH1 function and microsatellite instability in colorectal cancer is well documented. Like microsatellite unstable colorectal cancer, MLH1 methylation leads to MSI in PENs and this was shown to be associated with favorable prognosis.46,53 Similarly, a few studies have suggested that the methylation status of specific tumor suppressor genes is predictive of PEN behavior. In a study of gastrinomas, Serrano et al reported that CDKN2A/p16 gene methylation correlated with malignant tumors associated with lymph node but not liver metastases.28 In a larger study of all types of PEN, House et al found that methylation at the CDKN2A/p16 gene locus was associated with decreased 5-year survival and tumor recurrence within 24 months; two molecular markers that can predict patient outcome after surgical resection. Methylation at 3 or more genes or at the CDKN2A/p16 gene locus is associated with decreased 5-year survival and tumor recurrence within 24 months; 37% of CDKN2A/p16 methylated PEN recurred, compared with 26% of tumors without CDKN2A/p16 methylation.46 They also showed that the methylation of multiple (≥3) tumor suppressor genes may be associated with more aggressive tumors.46

Other Techniques

Telomerase in Sporadic PENs

Chromosomal telomeres (terminal chromosome regions) are made up of several thousand copies of repeating nucleotide sequences. Their main functions are believed to be the stabilization and protection of chromosomal ends.54 In a normal somatic cell, there is approximately 50–100 basepair loss of telomeric DNA from the end of every chromosome during each cell cycle. With progressive erosion of telomeres, these unprotected ends may participate in end-to-end chromosomal fusions, which are lethal to cells.54 Telomerase is an enzyme believed to be involved in the de novo synthesis of telomeric DNA onto chromosomal ends.55,56 Telomerase is not detected in most somatic cells. Conversely, it is activated in most human cancers suggesting an important role in cancer cell survival.57 Telomerase is a ribonucleoprotein complex including a telomerase catalytic subunit (telomerase reverse transcriptase protein subunit, TERT; an internal RNA strand (TR), and an RNA-binding protein (TEP1)). TERT gene expression seems to be the rate-limiting determinant of telomerase activity. A few published studies have shown that telomerase activity is closely related to the malignant potential of human pancreatic endocrine tumors.58,59 In the study by Lam et al, three of ten pancreatic endocrine tumors had telomerase activity. Two of these cases were frankly malignant tumors with liver metastases and the third was pancreatic endocrine tumor occurring in the setting of MEN type 1 and histologically showed an infiltrative border, vascular and perineural tumor infiltration.58 This finding suggested that telomerase activity might be useful in distinguishing between benign and malignant pancreatic endocrine tumors. A more recent study employed real-time quantitative RT-PCR in the quantification of TERT mRNA 23 cases of PEN. Telomerase was negative in 12 out of 12 benign pancreatic tumors (100%) and positive in 6 out of 8 malignant (metastatic or not) pancreatic tumors (75%), resulting in a positive predictive value of 100% and a negative predictive value of 85.7%. In addition, telomerase activity was helpful in identifying metastatic tumors. Sixteen of eighteen nonmetastatic pancreatic tumors were telomerase negative (88.99%), while 5 out of 5 metastatic pancreatic tumors were telomerase positive (80% and 100% positive and negative predictive values, respectively).40

MicroRNA

MicroRNAs are small (about 22 nucleotides in length), single-stranded forms of noncoding RNA that are involved in the normal functioning of our cells. The dysregulation of microRNA has been linked to human disease, including cancer. One study has examined the role of microRNAs in PEN. Comparing nontumor pancreas to pancreatic tumors (insulinomas, nonfunctioning PEN and acinar cell carcinomas), the expression of miR-103 and MIRN107/miR-107 in conjunction
with a lack of expression of MIRN155/miR-155 distinguished tumors from normal. A set of 10 microRNAs were upregulated in PEN and MIRN204/miR-204 was found to be primarily expressed in insulinomas. Interestingly, the overexpression of MIRN21/miR-21 was strongly associated with both a high Ki67 proliferation index and presence of liver metastasis.61

**Hereditary Pancreatic Endocrine Tumors**

In a small subset of patients, PENs are inherited as part of a genetic syndrome. Recognition of these patients is important for both treatment and evaluation of family members. The study of these inherited neoplasms also plays an important role in our understanding of the pathogenesis of PEN. The most common hereditary syndromes associated with the development of PEN are multiple endocrine neoplasia type 1 (MEN1) and von Hippel–Lindau syndrome (VHL).

MEN1 is a rare autosomal dominant disorder with a prevalence that ranges from 1 in 20,000 to 1 in 40,000.62 It is characterized by the combination of parathyroid tumors, pancreatic endocrine cell neoplasms, and pituitary hyperplasia/tumors. Tumors can also arise in the upper gastrointestinal tract, lung, thyroid, thymus, and adrenal gland. Parathyroid tumors occur in nearly all patients by age 50, and pancreatic tumors arise in approximately 80% of patients.63,64 The pancreatic tumors are often multiple, including microadenomas and PENs, and the PENs may undergo malignant transformation. While the majority of PENs are nonfunctional, most patients will have at least one functional tumor. The most common functional tumors are gastrinomas, followed by insulinomas, glucagonomas, and others.

Using genetic linkage analysis, a region on the long arm of chromosome 11 (11q13) was implicated as the potential site that harbored the key gene that is dysfunctional in MEN1 syndromes (Table 23.1).65 The candidate gene was identified and designated the MEN1 gene by Chandrasekharappa et al in 1997.66 Multiple subsequent studies confirmed that MEN1 gene mutations are detected in most MEN1 patients.67,68 This tumor suppressor gene contains 10-exon that encodes for a 610-amino-acid nuclear protein, Menin, that interacts with a variety of transcription factors, DNA repair proteins, DNA repair proteins, and cytoskeletal proteins.69,70 At least 400 different MEN1 mutations have been described.71 It is important to note that mutations in the MEN1 gene locus are also reported in 27–39% of sporadic PENs.23,30,72 The mutations in the MEN1 gene in sporadic PENs appear to be distributed throughout the nine coding exons.23 Mutations are present in the MEN1 gene in both benign and malignant sporadic PENs suggesting that they are an early event in the molecular pathogenesis.23,72

The second syndrome that is associated with the tendency to develop PEN is VHL syndrome. The prevalence ranges from 1 in 30,000 to 1 in 50,000.73 The disease penetrance is over 90% by age 65.74 This autosomal dominant disorder is associated with many tumors, most notably hemangioblastomas, clear cell renal cell carcinomas and pheochromocytomas.74–76

### Table 23.1. Hereditary syndromes associated with pancreatic endocrine neoplasms.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene</th>
<th>Protein product</th>
<th>Protein function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple endocrine neoplasia type-1</td>
<td>MEN1</td>
<td>Menin</td>
<td>Interacts with transcription factors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– JunD, NF-kB, Smad3, Pem</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DNA repair proteins</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– RPA2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DNA processing factors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– nm23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cytoskeletal proteins</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– GFAP, vimentin</td>
</tr>
<tr>
<td>von Hippel Lindau disease</td>
<td>VHL</td>
<td>VHL</td>
<td>Targets several proteins that bind to</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ubiquitin to be degraded by proteosomes:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– Hypoxia-inducible-factor-1 (HIF-1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– Vascular endothelial growth factor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(VEGF)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>– Platelet derived growth factor (PDGF)</td>
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<td></td>
<td></td>
<td></td>
<td>– Tumor growth factor alpha (TGFa)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– Matrix metalloproteinases (MMP)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– MMP-inhibitors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– Atypical protein kinase C (APK-C)</td>
</tr>
</tbody>
</table>

PENs are reported to be present in 17% of patients with VHL and they behave in a malignant fashion in up to 8.3%.77 The majority of PEN are nonfunctional75 and many (60%) have a foamy clear cell appearance.78

Using genetic linkage analysis, a region on the short arm of chromosome 3 (3p25-p26) was found to harbor the VHL gene and the gene was subsequently cloned (Table 23.1).75,79 More than 300 germline mutations have been identified in familial VHL.79 Functioning as a tumor suppressor gene, its product, VHL protein, targets several proteins with which it forms stable complexes that binds to ubiquitin that then get degraded by proteasomes.80,81 Hypoxia-inducible factor-1 (HIF-1) is one of the major proteins regulated by VHL. This protein is involved in erythropoiesis through its ability to induce transcription of mRNA coding for erythropoietin. In patients with VHL disease, loss of functioning VHL results in HIF-1-alpha not binding to ubiquitin and thus not degraded by proteasomes.80,81 In addition to erythropoietin, other factors known to be regulated through the HIF-1-alpha system include vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF)-beta, and transforming growth factor (TGF)-alpha.80,81 VHL also affects several other factors potentially involved in tumorigenesis that are not regulated through the HIF-1-alpha system. These targets include matrix metalloproteinases (MMP) such as MMP1, MMP inhibitors, and atypical protein kinase C.80,81 Although the mechanism of tumorigenesis remains unproven, the combined effect of various angiogenic factors and other growth factors may be to create an autocrine loop that provides an uncontrolled growth stimulus.82 Although loss of 3p is well documented in sporadic PEN,24 mutation of this gene is usually not targeted in sporadic PEN.28 Interestingly, 78% of patients with metastatic...
disease had exon 3 mutations in the *VHL* gene, compared with 46% of patients without metastases, suggesting that detection of exon 3 mutations will be helpful in assessing aggressiveness of PEN and guiding surgical management in these patients.77

**Summary**

Even though two well-known hereditary conditions are associated with PENs, MEN1 and VHL, the molecular pathogenesis of these two diseases has provided only little insight into the much more common sporadic form of the pancreatic neoplasms. Sporadic PENs harbor *MEN1* gene mutations in only a minority of cases and they tend not to have *VHL* mutations, but rather show loss of the locus where the *VHL* gene resides. Abundant research has been performed on sporadic PENs that has provided insight into the molecular pathogenesis of these tumors. Rather than resulting from dysfunction of a single gene, several pathways appear to be involved with the formation of PENs, including losses and gains of genomic material, methylation of some key regulatory genes and expression of particular microRNAs.

While PENs are typically easy to diagnose, their behavior is difficult, if not impossible, to predict. Immunohistochemistry has limited value in assessing prognosis, but new markers are always being developed. Molecular studies have provided some targets that may be helpful in assessing the aggressiveness and metastatic potential of these tumors, and some have even been reported to predict survival (Table 23.2). Some prior studies need to be validated, but a panel of markers utilizing various techniques may be needed to accurately predict the behavior of these tumors.

**Table 23.2. Potential indicators of behavior in pancreatic endocrine neoplasms.**

<table>
<thead>
<tr>
<th>Method</th>
<th>Gene/marker</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC</td>
<td>Ki-67 (MBI-1)</td>
<td>&gt;2% proliferation index = malignant potential</td>
</tr>
<tr>
<td></td>
<td>CK-19</td>
<td>Positive expression = worse prognosis</td>
</tr>
<tr>
<td>CGH</td>
<td>Overall # of genetic changes</td>
<td>Increased # = increased tumor size and disease stage</td>
</tr>
<tr>
<td></td>
<td>Losses of chr 3, 6, 21q, X/Y</td>
<td>Malignant behavior/metastatic disease</td>
</tr>
<tr>
<td></td>
<td>Gains of chr 4, 7, 14q, 17, 20q, Xq</td>
<td>Malignant behavior/metastatic disease</td>
</tr>
<tr>
<td></td>
<td>3p25.3-p23, 6q, 17p13, 22q</td>
<td>Malignant behavior/metastatic disease</td>
</tr>
<tr>
<td>LOH</td>
<td>Gene expression profiling</td>
<td>Several genes, including <em>MET</em> and <em>IGFBP3</em></td>
</tr>
<tr>
<td>Gene expression profiling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylation</td>
<td><em>MLH1</em></td>
<td>Favorable prognosis</td>
</tr>
<tr>
<td></td>
<td><em>CDKN2A/p16</em></td>
<td>Malignant behavior, decreased survival, early recurrence</td>
</tr>
<tr>
<td>Telomerase</td>
<td>Multiple genes</td>
<td>Malignant behavior</td>
</tr>
<tr>
<td></td>
<td>Activity</td>
<td>Malignant behavior/metastatic disease</td>
</tr>
<tr>
<td>microRNA</td>
<td><em>MIRN21/miR-21</em></td>
<td>Malignant behavior/metastatic disease</td>
</tr>
</tbody>
</table>

*IHC* immunohistochemistry, *CGH* comparative genomic hybridization, *LOH* loss of heterozygosity.

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