Endocrine tumors of the pancreas and gastrointestinal tract
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CHAPTER 6

COMMENTARY ON CHAPTER 44

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CHAPTER 44

TUMORS OF THE ENDOCRINE SYSTEM

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Commentary on chapter 44

"Unfortunately I have to open my communication with the confession that I am in no way in a position to present the complete results of a successful research, but at the most to contribute some sporadic observations, which give an idea that the construction of the examined object is much more complicated than heretofore suspected."

This citation comes from the thesis by Paul Langerhans, published in 1869, in which he first describes the pancreatic endocrine cell clusters that were later to be named after him, without being aware of their functional significance.\(^1\)

It is now clear that the pancreas is an organ that consists of an exocrine part containing acini and ductal structures and an endocrine component composed of cells organized in the islets described by Langerhans. Since his time, significant progress has been made at both the morphologic and molecular level in understanding embryonic pancreatic development, the physiologic role of the pancreatic islet cells and the related pathological conditions.

As described in the chapter on pancreatic endocrine neoplasms (PENs) by Klimstra the pancreatic endocrine cells arise from progenitor cells in the primitive ducts of the pancreatic primordia. One of the transcription factors essential in the formation of these ducts and also for the functioning of mature beta cells is the Pancreatic duodenal homeobox 1 protein (Pdx1), which belongs to the homeodomain family of proteins. Other transcription factors of the homeodomain family and basic helix-loop-helix family subsequently involved in the endocrine differentiation of ductal progenitor cells are hepatocyte nuclear factor 6, neurogenin 3, NeuroD1/BETA2, Pax4, Nkx2.2, Nkx6.1, Pax6 and Isil.\(^2\) Although most endocrine cells separate from the ducts and migrate into the exocrine pancreas forming the islets of Langerhans, some of them remain at their site of origin and also some cells with stem cell capacities are thought to remain in the mature pancreas.\(^3\)

This understanding of the embryologic development of the endocrine pancreas is important with respect to the pathogenesis of PENs, because it is now believed that PENs originate from a common precursor cell that proliferates and differentiates towards an endocrine phenotype. If this is the case, the transcription factors involved in normal embryonic pancreatic development might prove to be important in tumorigenesis as well, and it is therefore conceivable that these transcription factors will play a role in diagnosis and prognostication in the near future. A recently described transgenic mouse model in which PENs developed after infection with a c-myc oncogene bearing retrovirus under the control of the pancreas-specific elastase promoter supports this notion. The expression of Pdx1 and the endocrine-specific transcription factors Nkx2.2, Pax6 and Isil could be observed in these neoplasms.\(^4\)

Although our understanding of the biology of PENs has grown, it remains surprisingly difficult to predict the clinical behavior of a PEN. Assessment of associated clinical findings, tumor size, gross local invasion, metastasis and vascular
invasion allows PENs to be subdivided into either functioning or non-functioning tumors of benign behavior, uncertain behavior, low-grade malignancy and high-grade malignancy in the *Revised Classification of Neuroendocrine Tumours of the Lung, Pancreas and Gut* by Capella *et al.* Although the prognostic value of these parameters is disputed, validation of the Capella classification in a group of 82 sporadic PENs shows a statistically significant difference in survival between the neoplasms of different grades. Based on the classification by Capella *et al.*, the World Health Organisation (WHO) separates PENs into either functioning or non-functioning neoplasms of benign behavior, tumors of uncertain behavior, well differentiated carcinomas, and poorly differentiated carcinomas/small cell carcinomas, but with different cut off points for tumor size, with the addition of mitotic activity and proliferation rate (measured by Ki67 labelling). This latter system also does not separate insulinomas from the other PENs. The new WHO classification does not contain essential changes compared to the old classification, but the terms *macroadenoma* and *borderline tumor* will be replaced in order to emphasize the ambiguous clinical behavior rather than implying a certain inherent tumor biology.

As Klimstra indicates, PENs can be classified in more than one way, but because the parameters mentioned earlier seem to be of at least some value and in order to obtain consistent classification and comparable research results, it deserves consideration to consequently follow the WHO-guidelines in classifying PENs until more accurate prognostic parameters have been established. Because PENs are well vascularized neoplasms and angiogenesis is an important feature of tumorigenesis, the prognostic value of tumor microvessel density and the expression of the angiogenic factors of the vascular endothelial growth factor family and their receptors have been investigated recently. Although one study shows that low microvessel density is an unfavorable prognostic factor and although, as mentioned by Klimstra, a role for vascular endothelial growth factor-C in tumor progression has been suggested, the results of different studies are not consistent and do not provide parameters that can be used in clinical practice.

One group of PENs of which the aggressive behavior is easily predictable microscopically on the basis of cell type, high proliferative activity and presence of necrosis, is the poorly differentiated/ small cell carcinomas. However, as Klimstra indicates, these neoplasms are rare and before a diagnosis of a poorly differentiated PEN is made, a metastasis from a primary tumor elsewhere, especially a bronchial carcinoma, has to be excluded. In these cases the clinical data and imaging techniques are important. Additionally, a staining for thyroid transcription factor 1 might be of use, but it should be interpreted with caution. This protein is expressed in the nucleus of normal thyroidal and pulmonary epithelial cells and in a large number of the (adenocarcinomas derived from these tissues. Positivity for thyroid transcription factor 1 has been reported in 90% of the small cell carcinomas of the lung, but also in small cell carcinomas from other sites, such as the prostate and urinary bladder. Therefore both pos-
itive and negative labeling results have to be correlated with the other available data before a final diagnosis is made.

Another important differential diagnosis on the other side of the spectrum mentioned by Klimstra is the one of endocrine microadenomas versus enlarged non-neoplastic islets. Enlarged non-neoplastic islets can be seen in islet cell hyperplasia, which is a poorly defined and rare condition that should not be confused with the much more frequently observed aggregation of islets in chronic pancreatitis, caused by atrophy of the exocrine pancreas. Islet cell hyperplasia is defined as an increase in islet mass due to increased islet size and/or islet number. If the islets cells contain enlarged pleomorphic nuclei, if ductuloinsular complexes consisting of endocrine cell nests budding off ducts are observed and if the patient presents with persistent hyperinsulinemic hypoglycemia, indicative of beta-cell dysfunction, the term *nesidioblastosis* is frequently used. Islet cell hyperplasia predominantly occurs in newborns, but has also been described in adults. In newborns it can be associated with maternal diabetes, erythroblastosis fetalis, hereditary tyrosinemia or Beckwith-Wiedemann syndrome, but in these conditions the histological features of nesidioblastosis are usually not very outspoken. Nesidioblastosis can be focal or diffuse. Especially the focal form, also referred to as *focal adenomatous hyperplasia*, has features reminiscent of an endocrine microadenoma. But in contrast to the conventional microadenoma in which only one cell type is found, the focal variant of nesidioblastosis contains a mixture of different cell types. In some sporadic cases of neonatal focal nesidioblastosis somatic loss of the maternal allele of the imprinted chromosome region 11p15 has been described, which encodes a number of candidate genes. In a part of these cases, the paternal allele of the high affinity sulfonyleurea receptor (*SUR1*) gene, involved in insulin secretion by beta cells and also on chromosome 11p15, shows a germ-line mutation. The fact that not only germ-line mutations, but also somatic genetic alterations have been found, suggests that these lesions might be clonal and maybe should be regarded as a functioning variant of a microadenoma. Although generally the age of the patients (newborns vs adults) and the clinical presentation (hypoglycemia vs asymptomatic coincidental finding) differ, it might turn out that both lesions are more similar than previously thought from a pathogenetic point of view.

Extensive discussion of diffuse nesidioblastosis, which is usually not in the differential of a PEN, is beyond the scope of this commentary. However, the combination of islet cell hyperplasia and the presence of multiple pancreatic endocrine microadenomas should be mentioned here as a typical feature of multiple endocrine neoplasia type 1 syndrome, because as described by Klimstra, most of these patients develop clinically relevant PENs in the course of their disease. In his chapter Klimstra emphasizes that the difference between functioning and nonfunctioning PENs is based on the presence or absence of a clinical syndrome with elevated serum peptide levels and that immunohistochemical evidence of hormone production does not automatically mean that a PEN is clinically func-
tioning. In addition, negative stains do not rule out the diagnosis of a functioning PEN; hormones can be released from the neoplastic cells so quickly that immunohistochemical stains are negative. It is also possible that the neoplasm produces a slightly modified hormone that can not be recognized by the antibodies used for immunohistochemistry.

The differential diagnosis of PENs discussed by Klimstra is important not only in histology, but also in interpreting fine needle aspirates. PENs can be confused with acinic cell carcinoma, solid-pseudopapillary (Hamoudi) neoplasm and pancreatoblastoma. Cytologic features suggestive of acinic cell carcinoma are prominent nucleoli and granular cytoplasm. A solid-pseudopapillary neoplasm is characterized by papillary clusters with fibrovascular stalks. The neoplastic cells contain abundant metachromatic material, nuclear grooves, foamy macrophages and necrosis is often present. In a pancreatoblastoma multiple cell types can be observed in the same neoplasm: small cells with dark nuclei and scanty cytoplasm and larger epithelial cells, occasionally in an acinar arrangement, with abundant granular-to-vacuolated cytoplasm and more prominent nucleoli. In addition to the cytologic features, the patient characteristics (pancreatoblastoma in children, solid-pseudopapillary neoplasm in young females) and imaging techniques can be helpful in making the correct diagnosis.

All in all, it is obvious that our knowledge on the endocrine pancreas and its diseases has increased immensely since the days of Paul Langerhans. However, the words cited from his thesis are still true concerning PENs: we are not yet in a position to present the complete results of a successful research, but we can contribute some observations. Perhaps in the future the pathogenesis of PENs will be unraveled, their biological behavior will be fully understood and the construction of the examined object will seem not as complicated as heretofore suspected.

References


11 http://immunoquery.com


CHAPTER 44

Introduction
The endocrine neoplasms of the pancreas represent an important subset of pancreatic neoplasms both because of the distinctive endocrine paraneoplastic syndromes that may be present and because these neoplasms are often less aggressive and more amenable to curative surgical therapy than carcinomas of the exocrine pancreas. From a pathologic standpoint pancreatic endocrine neoplasms (PENs) represent a diagnostic challenge due to the wide range of histological patterns that occur. Variability in clinical behavior has frustrated attempts to sharply define benign and malignant categories of pancreatic endocrine neoplasms, and attention has recently been directed towards defining prognostic factors and understanding the genetic alterations underlying these neoplasms.

The Normal Endocrine Pancreas
In the normal pancreas, the endocrine cells are largely found within the islets of Langerhans, although approximately 10% of pancreatic endocrine cells are extrainsular, usually distributed within the ducts.1 During development, all of the pancreatic endocrine cells originate from the embryonic ducts and are therefore of endodermal origin.2 The histologic appearance and cellular composition of the islets differ between the regions of the pancreas derived from the dorsal and ventral embryologic pancreatic primordial (Fig. 6.1).

The majority (90%) of the islets consist of roughly spherical collections of cells derived from the embryonic dorsal pancreas.

Figure 6.1 Normal pancreatic islets. Compact islets (A) are roughly spherical, consisting of closely packed lobules of endocrine cells separated by capillaries. Diffuse islets (B) are less well circumscribed and have a trabecular arrangement, with interposed acini between the endocrine cells.
CHAPTER 6

Endocrine microadenoma
Well differentiated pancreatic endocrine neoplasm

Functional pancreatic endocrine neoplasm

- Insulinoma
- Glucagonoma
- Somatostatinoma
- VIPoma
- Gastrinoma
- Carcinoid tumor
- Other ectopic and mixed hormone producing neoplasms

Non-functional pancreatic endocrine neoplasm

(not otherwise specified)
PPoma

Poorly differentiated endocrine carcinoma

- Small cell carcinoma
- Large cell endocrine carcinoma

Mixed endocrine carcinomas

- Mixed ductal-endocrine carcinoma
- Mixed acinar-endocrine carcinoma
- Mixed acinar-endocrine-ductal carcinoma

Table 6.1 Classification of pancreatic endocrine neoplasms.

These compact islets are abundant in the body and tail but are also found in the head of the gland and contain predominantly alpha and beta cells, with minor populations of PP and delta cells. In the portion of the head of the pancreas derived from the embryonic ventral pancreas (essentially, the uncinate process), the islets have a trabecular configuration and are rather ill-defined, with interposition between cords of acinar cells. These diffuse islets also contain many beta cells but are predominantly (70%) composed of PP cells, with few delta and alpha cells.

General Features and Classification

PENs account for roughly 2-4% of clinically detected pancreatic neoplasms. Since endocrine microadenomas are relatively common incidental findings, the prevalence of PENs at autopsy (between 1-10% of autopsies) is significantly higher than the clinical prevalence. Males and females are equally affected. PENs may
Tumors of the endocrine system

arise at any age, but most occur between the ages of 30 and 60 yrs; those arising in patients with multiple endocrine neoplasia 1 (MEN1) syndrome occur at a younger age. Several different classification systems can be applied to endocrine neoplasms of the pancreas (Table 6.1).

The majority are in the well differentiated category, in the sense that they retain the organoid architecture typical of endocrine organs and have a relatively low proliferative rate. PENs measuring less than 0.5 cm are designated as endocrine microadenomas. Microadenomas are the only PENs that can be regarded as completely benign. Most of the remainder of well differentiated PENs are low to intermediate grade malignancies, and despite numerous studies on prognostic factors, no specific classification system has achieved widespread acceptance. Finally, a small group of primary pancreatic neoplasms qualify as high grade endocrine carcinomas due to their diffuse architecture, high proliferative rate, and abundant necrosis.

The well differentiated PENs are also subclassified based on the presence and type of associated clinical syndrome due to inappropriate hormone secretion by the tumor. PENs are designated as insulinoma, glucagonoma, gastrinoma, etc. if the patient exhibits characteristic clinical findings in the presence of elevated serum levels of the responsible peptide or bioamine. These “functional” PENs are listed in Table 6.2.

PENs without a clinical syndrome are termed “nonfunctional”, although most of

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Syndrome</th>
<th>Clinical Findings</th>
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<tr>
<td>Insulinoma</td>
<td>( \beta ) cell</td>
<td>Insulinoma syndrome</td>
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<tr>
<td>Glucagonoma</td>
<td>( \alpha ) cell</td>
<td>Glucagonoma syndrome</td>
</tr>
<tr>
<td>Somatostatinoma</td>
<td>( \delta ) cell</td>
<td>Somatostatinoma syndrome</td>
</tr>
<tr>
<td>VIPoma</td>
<td>Unknown</td>
<td>Verner-Morrison syndrome</td>
</tr>
<tr>
<td>Gastrinoma</td>
<td>G cell</td>
<td>Zollinger-Ellison Syndrome</td>
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Table 6.2 Functional pancreatic endocrine neoplasms.
these PENs also exhibit some evidence of hormone production if serum peptide levels are measured or immunohistochemistry is employed to detect them. A non-functional PEN that is documented to produce a specific hormone may be designated based on the corresponding cell type (e.g., "alpha cell neoplasm", "beta cell neoplasm", etc.), but they should not be labeled as functional tumors in the absence of the appropriate clinical syndrome. The presence or absence of an associated clinical syndrome has prognostic significance; therefore, the relevant clinical distinction is between "syndromic" and "non-syndromic" PENs. However, the "functional" and "nonfunctional" terminology is entrenched in the lexicon. It should be noted that rare PENs have been described that exhibit expression of pancreatic polypeptide (PP) by immunohistochemistry, associated with elevations in serum PP levels. Although these neoplasms have been designated "PPomas", there is no known clinical syndrome associated with PP hypersecretion, so PPomas are categorized as nonfunctional PENs. Many PENs producing somatostatin, calcitonin, and neurotensin also fall in the nonfunctional group. Based on surgical studies, approximately one third of all PENs are nonfunctional; in MEN1 patients with multiple PENs, a greater proportion of the individual tumors are nonfunctional, but most MEN1 patients have at least one PEN that is functional. Insulinomas are the most common functional tumors (45%), followed by gastrinomas (20%), glucagonomas (13%), VIPomas (10%), and somatostatinomas (5%). PENs producing other unusual syndromes (Cushing’s syndrome, carcinoid syndrome, acromegaly, etc.) occur but are rare. PENs may be associated with a number of genetic syndromes, most importantly MEN1 and von Hippel-Lindau (VHL) syndromes. In these cases, the genetic abnormality underlying the syndrome plays a role in the development of the PENs, which often are multiple. The pathologic features of these PENs are generally similar to those occurring sporadically, although the PENs in VHL syndrome patients may have clear cell features and may be associated with a serous cystic neoplasm.

Pathologic Features

The gross appearance of PENs is generally that of a circumscribed, solid mass composed of tan, uniform, fleshy parenchyma (Fig. 6.2). Larger PENs are multinodular, with fibrous septa dividing the neoplasm. Some examples have more abundant fibrous stroma, imparting a firm consistency. Cystic change is a less common phenomenon, usually in the form of a single central locule lined by a thin rim of neoplastic cells (Fig. 6.3). Most PENs have an expansile growth pattern, and small neoplasms may be completely surrounded by a fibrous capsule. It is common to find invasive growth, however, including extension into the adjacent tissues such as the residual pancreatic parenchyma, the peripancreatic soft tissues, vessels, or adjacent organs (e.g., the spleen).
Figure 6.2 Gross appearance of pancreatic endocrine neoplasms. Some examples are relatively small and circumscribed (A), consisting of a uniform tan neoplasm sharply demarcated from the surrounding pancreas. Larger examples (B) exhibit a more multinodular pattern. In this case, there is extensive hemorrhage and cystic degeneration, with gross invasion of the spleen.

Figure 6.3 Cystic pancreatic endocrine neoplasm. There's a central unilocular cyst separated from a fibrous capsule by a thin rim of tan tumor parenchyma.

Figure 6.4 Architectural patterns of pancreatic endocrine neoplasms. Many examples demonstrate a nesting pattern (A), with thin fibrovascular septa separating relatively uniform neoplastic cells. In the gyriform pattern (B), thin interanastomosing trabecula of endocrine cells are found.
CHAPTER 6

Figure 6.5 Cytologic features of pancreatic endocrine neoplasms. The nuclei are round to oval and relatively uniform, and the chromatin is coarsely granular. The cytoplasm is moderate in amount and amphophilic.

Figure 6.6 Some pancreatic endocrine neoplasms demonstrate abundant hyalinized stroma between the nests of neoplastic endocrine cells.

PENs are histologically characteristic at both the architectural and cytologic levels. Many different architectural arrangements have been recognized, all collectively referred to as "organoid" patterns. Cells are arranged in regular nests, ribbons, or trabecula, and it is common for more than one pattern to be found in different regions of a single neoplasm (Fig. 6.4). The trabecular pattern is particularly characteristic, and when thin, complex trabecula are interwoven, the term gyriform is applied to the architecture. The cytology of PENs is often similar to that of other well differentiated endocrine neoplasms such as carcinoid tumors. The nuclei are usually round to oval and uniform (Fig. 6.5), although scattered enlarged nuclei are not uncommon.

Figure 6.7 Less common patterns of pancreatic endocrine neoplasms. Oncocytic PENs (A) have abundant, granular eosinophilic cytoplasm due to numerous mitochondria. Clear cell PENs (B) have foamy to clear cytoplasm due to accumulation of numerous small vesicles.
Tumors of the endocrine system

Figure 6.8 Pleomorphic pancreatic endocrine neoplasms. The nuclei are enlarged and atypical, but there is also abundant cytoplasm and the mitotic rate is not increased.

The chromatin is coarsely granular and clumped, imparting the classic "salt and pepper" appearance characteristic of well differentiated endocrine neoplasms in general. Nucleoli may be inconspicuous, although many PENs have easily identifiable or even prominent nucleoli.

The architectural and cytologic features described above are key to recognizing PENs, but there are many variations in histology that may cause diagnostic dilemmas. The range of patterns one may encounter in PENs exceeds that of nearly all other endocrine neoplasms. The stroma varies considerably in amount. Some PENs have nearly no collagen within the neoplasm (Fig. 6.4A); there is only a thin (but abundant) fibrovascular stroma separating the cellular nests. Other examples contain abundant, hyalinized or amyloid-like stroma that results in the appearance of sparse, thin epithelial cords compressed by broad bands of collagen (Fig. 6.6). Calcifications may be found, including psammoma bodies. The quantity and appearance of the cytoplasm also varies. A moderate amount of amphophilic to basophilic cytoplasm is typical, but PENs with oncocytic cytoplasm or clear cell change have been described (Fig. 6.7). The latter type is reportedly more common in patients with VHL syndrome.

Some PENs have scant cytoplasm, and the resulting high nucleus-to-cytoplasm ratio that may cause confusion with small cell carcinomas or primitive neuroectodermal tumors. The nuclear morphology may also vary; PENs with marked nuclear pleomorphism have been reported, and these cases are commonly confused with ductal carcinomas or other high grade neoplasms (Fig. 6.8).

In these instances, the nuclear atypia is not generally accompanied by an increased proliferative rate and does not appear to have adverse prognostic significance. Mitotic rate is an important measure of aggressiveness in PENs. Well differentiated PENs are defined to have less than 10 mitotic figures per 10 high power microscopic fields (hpf); neoplasms with 10 or more mitoses per 10 hpf are considered high grade endocrine carcinomas (see below). In many PENs, mitotic figures are nearly undetectable, a search of 50 to 50 hpf (or more) being required to find even a single mitotic figure. Some PENs have a higher proliferative rate, and the finding of 2 or more mitoses per 50 hpf places a PEN in a worse prognostic category. Necrosis is also variably present; most commonly it is accompanied
Figure 6.9 Glands in pancreatic endocrine neoplasms. In some cases, the neoplastic endocrine cells form lumina (A), where each gland is formed of cells similar to those in the solid regions of the neoplasm. In other PENs (B), non-neoplastic ductules are entrapped within the tumor. There is very close juxtaposition between the neoplastic endocrine cells and the non-neoplastic glandular epithelium.

by an increase in proliferative rate and signifies a more aggressive PEN. Glands may also be found in PENs and may take several forms. In some cases, the neoplastic endocrine cells form lumina (Fig. 6.9A). Although these gland-forming PENs may be mistaken for adenocarcinomas, the cells lining the lumina retain endocrine differentiation and are cytologically similar to the more abundant solid areas that typically are present. In other cases, non-neoplastic ductules are entrapped within PENs. The neoplastic endocrine cells are often closely juxtaposed to the ductules, but they are cytologically distinct (Fig. 6.9B). Finally, true mixed ductal-endocrine carcinomas occur; these are discussed below (see “Mixed” Endocrine Neoplasms).

Immunohistochemistry and Electron Microscopy

Once the diagnosis of PENs is suspected based on the histologic features, there are several methods to confirm the diagnosis. Classical silver staining techniques to demonstrate neurosecretory granules have largely been replaced by immunohistochemistry. Expression of general endocrine markers including chromogranin, synaptophysin, and neural cell adhesion molecular (CD56) is detectable in essentially all PENs. Synaptophysin expression is commonly more diffuse, and some PENs may demonstrate only focal staining for chromogranin (Fig. 6.10). Expression of peptides such as insulin, glucagon, PP, somatostatin, gastrin, or vasoactive intestinal polypeptide is common, and most functional PENs can be shown to produce the appropriate peptide by immunohistochemistry. In addition, minor cell populations producing a variety of other peptides are commonly detectable. Nonfunctional PENs also contain a variety of peptide cell types, usually (but not inevitably) comprising less than 25% of the total cell
Tumors of the endocrine system

Figure 6.10 Immunohistochemical staining of pancreatic endocrine neoplasms. Most label with both chromogranin (A) and synaptophysin (B). In some PENs, the synaptophysin staining is more intense and diffuse.

population. Other “ectopic” peptides often are produced as well, and exhaustive immunohistochemical labeling for species such as ACTH, bombesin, calcitonin, neurotensin, etc., identifies additional secretory products. Many PENs also immunolabel for glycoproteins such as carcinoembryonic antigen (CEA) and CA19.9\textsuperscript{29,36,37}, especially those with gland formation. Focal acinar differentiation may also be present in scattered cells that label for trypsin or chymotrypsin.\textsuperscript{37,38} A subset of PENs expresses CD99, as do normal islet cells. Labeling of PENs with the proliferation marker MIB1 demonstrates a relatively low proliferative rate in keeping with their low mitotic rate. Generally 1-5\% of the nuclei are labeled, but some examples demonstrate labeling of up to 10\% of cells.\textsuperscript{1}

Although many elegant studies documented the utility of electron microscopy for the diagnosis of PENs, this technique has largely been supplanted by immunohistochemistry. If ultrastructural examination is performed, PENs contain relatively abundant 100-350 nm dense core neurosecretory granules.\textsuperscript{39,40} Most have a nonspecific morphology, with dense cores content separated by a halo from the limiting membrane. Morphologically characteristic secretory granules of alpha or beta cells are sometimes found in the corresponding functional PENs.\textsuperscript{35,41}

**Functional Pancreatic Endocrine Neoplasms**

From a purely morphologic standpoint, there are no specific microscopic findings that distinguish the different types of functional PENs.\textsuperscript{42,43,44} Insulinomas are usually small; many cases measure less than 2 cm, presumably because insulinomas are exquisitely symptomatic and are detected at an early stage. Certain histologic features were classically described to be characteristic of specific functional PENs, such as stromal amyloid in insulinomas or gland formation in gastrinomas, but none are sufficiently specific to allow distinction from other types of PENs. Somatostatinomas occurring in the periampullary duodenum
(glandular duodenal carcinoids) do have a distinctive appearance, including well-formed glands, eosinophilic cytoplasm, and numerous psammoma bodies, but pancreatic somatostatinomas do not share these features.

**Cytologic Findings**

Fine needle aspiration (FNA) is a sensitive technique for the preoperative diagnosis of PENs. Aspirates of PENs produce relatively cellular smears with a clean background. The cells are arranged in clusters and individually. Nuclei are round to oval and uniform, and the characteristic endocrine chromatin pattern is often present. The nuclei are eccentrically located, producing a plasmacytoid configuration to the cells (Fig 6.11). Endocrine differentiation may be documented by immunohistochemical labeling for chromogranin or synaptophysin to confirm the diagnosis.

**Molecular Genetic Features**

Recent cytogenetic and molecular studies have identified many chromosomal alterations in PENs. Interestingly, activation of oncogenes does not appear to play a major role in the development of these tumors. Comparative genomic hybridization studies demonstrate that chromosomal losses are more common than gains. In PENs arising in patients with MEN1 or VHL syndromes, the genetic defect responsible for the syndrome is involved in the pathogenesis of the pancreatic neoplasms. PENs arising in MEN1 patients show a germ line mutation in the *menin* gene on chromosome 11q13 coupled with a somatic (acquired) loss of the normal copy of this gene. Studies on sporadic PENs have also detected relatively common losses at 11q13 or elsewhere on the short arm of chromosome 11 (70%), but specific *menin* gene mutations are only present in approximately 20% of the neoplasms, suggesting involvement of another tumor suppressor gene. Interestingly, insulinomas have a much lower frequency of mutations in the *menin* gene than most other functional PENs and nonfunctional PENs. The VHL gene is usually normal in sporadic PENs.
The chromosomal alterations that have been described in sporadic PENs are relatively consistent and include gains at 4p, 4q, 5q, 7p, 7q, 9q, 12q, 14q, 17p, 17q, and 20q and losses at 1p, 3p, 6q, 9p, 10p, 10q, 11q, 18q, 22q, Y, and X. Some of these losses are associated with more aggressive clinical behavior (see below). The specific genes involved at most of these loci have yet to be determined. Many of the genes targeted in the development of ductal adenocarcinomas of the pancreas have been examined in PENs, and most of these are not targeted in PENs. In particular, K-ras, TP53, p16, and SMAD4 are not mutated in most PENs, although the p16 gene is inactivated via hypermethylation of the promotor in 40% of cases. Her-2/neu amplification is also not commonly detected. It appears that promotor methylation, rather than mutation, may be a relatively common mechanism of tumor suppressor gene inactivation in PENs. Other recent studies have examined gene expression in PENs to determine which genes are significantly over- or underexpressed relative to normal islet cells. Overexpressed genes include putative oncogenes, growth factors, and cell adhesion and migration molecules, whereas the cell cycle regulator p21, the cell surface glycoprotein MIC2 (CD99), and putative metastasis suppressor genes are among those under-expressed. Data from these studies may shed light on pathways important in tumorigenesis in PENs, and some of these species may prove to be prognostically relevant. Some of the genetic alterations in PENs are more commonly detected in larger or more advanced stage neoplasms, suggesting that there is continuing genetic progression in PENs that parallels clinical progression. Fewer gains and losses of genetic material are seen in smaller PENs (less than 2 cm), although losses at 1p and 11q and gains at 9q are already present. In fact, some data suggest that smaller PENs may represent poly- or oligoclonal proliferations from which more aggressive monoclonal neoplasms may arise.

Natural History and Prognostic Considerations

The natural history of PENs is highly variable. Small neoplasms without adverse prognostic features (see below) are readily curable by surgical resection. Many insulinomas fall into this category, since they generally measure less than 2 cm when detected. Most other functional and all nonfunctional PENs are usually larger at diagnosis, and the outcome is much less favorable. Approximately 50-80% of these neoplasms will recur or metastasize, and up to 30% of patients already have metastatic disease at first presentation. Functional PENs with mixed or "ectopic" syndromes are reportedly more aggressive. The five year survival after surgical resection for nonfunctioning PENs is 65%, but the ten year survival is only 45%. Metastases usually occur first in regional lymph nodes and liver, with more distant metastases developing late in the course of the disease. Despite the high rate of metastasis, relatively long survival is typical. Because the disease pro-
gresses slowly, many patients live for several years or even over a decade following the appearance of metastases. Unfortunately, metastatic PENs are relatively resistant to chemotherapy, and cure is unlikely after metastases develop. One of the most controversial aspects of PENs is the predicting of their clinical behavior. For many years, attempts were made to separate PENs into benign and malignant categories; recently, a borderline malignant potential category was proposed as well.¹ Because some PENs that demonstrate malignant behavior have deceptively bland histologic features, it was felt that few pathologic parameters accurately stratify PENs, and only the finding of locally invasive growth, large vessel invasion, or distant metastases could be considered absolute criteria of malignancy. Even with these criteria, however, some “malignant” PENs do not recur after resection and some “benign” PENs lacking these features ultimately prove lethal. More recently, it has been recognized that PENs treated by surgical resection have had the natural history of the neoplasm interrupted, and that a completely resected PEN that does not recur may not have been biologically benign, since malignant neoplasms can be cured by early surgical intervention. Current studies have focused on defining prognostic parameters to predict which resected PENs are most likely to recur or metastasize, essentially treating all clinically relevant PENs as malignant neoplasms.² The exception is the endocrine microadenoma, which can be accepted as benign. Of course, most microadenomas are incidental findings without clinical symptoms. Features of prognostic significance in PENs include tumor size, mitotic rate, presence of necrosis, extrapancreatic invasion, and vascular invasion, in addition to the presence of nodal or distant metastases.¹,²⁹,⁸¹ Peptide production detected in the serum or by immunohistochemistry is not a prognostic factor for nonfunctional PENs.²⁹ Nuclear pleomorphism is also not a useful predictor;²⁸ however, some studies have demonstrated a correlation between overall nuclear grade and prognosis.²⁹ Other factors reportedly predictive of more aggressive behavior include loss of progesterone receptor expression⁸²,⁸³, aneuploidy⁸⁴,⁸⁵, increased Ki67 or PCNA labeling index⁸⁶, loss of heterozygosity (LOH) of chromosome 17p13¹², LOH of chromosome 22q⁶⁵, increased fractional allelic loss⁶⁴, upregulated CD44 isoform expression⁷⁷,⁸⁸, and immunohistochemical expression of cytokeratin 19.⁸⁹ Other allelic losses associated with malignant behavior include loss of chromosomes 1p⁹⁰, 3p⁵¹,⁶⁶,⁹¹, 6q⁵¹,⁹², and X.⁹³ Aberrant methylation of tumor suppressor gene promotores is also more commonly detected in advanced stage PENs⁶⁹, as is upregulation of vascular endothelial growth factor C.⁹⁴ Loss of p27 expression⁹⁵ and methylation of the promoter of the DNA mismatch repair gene hMLH⁹⁶ appear to be markers of indolent behavior. Although no uniform grading system has been employed for PENs (other than the distinction from high grade endocrine carcinomas), a proposal has been made to use the mitotic rate and presence of necrosis to separate low grade PENs from an intermediate grade category.²⁹ Under that proposal, cases demonstrating two or more mitoses per 50 hpf or the presence of necrosis are considered intermediate.
grade, whereas those lacking these features are considered low grade. Alternatively, PENs have been separated into benign, borderline, and low grade malignant categories based on a combination of tumor size, mitotic rate, vascular invasion, gross local invasion, and metastases.1,97

**Differential Diagnosis**

The pathologic differential diagnosis for PENs includes acinar cell carcinoma, pancreatoblastoma, solid-pseudopapillary tumor, and ductal adenocarcinoma. Features helpful for separating these entities are presented in Table 6.3.

<table>
<thead>
<tr>
<th>Histologic Findings</th>
<th>Immunohistochemical Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pancreatic Endocrine Neoplasm</strong></td>
<td>Organoid architecture, hyalinized stroma, coarsely clumped chromatin, few mitosis</td>
</tr>
<tr>
<td><strong>Acinar Cell Carcinoma</strong></td>
<td>Solid and acinar patterns, granular eosinophilic cytoplasm, prominent nucleoli, plentiful mitoses</td>
</tr>
<tr>
<td><strong>Pancreatoblastoma</strong></td>
<td>Solid and acinar patterns, squamoid corpuscles, cellular stroma</td>
</tr>
<tr>
<td><strong>Solid-Pseudopapillary Tumor</strong></td>
<td>Solid and pseudopapillary patterns, nuclear grooves, hyaline cytoplasmic globules, foamy histiocytes</td>
</tr>
<tr>
<td><strong>Ductal Adenocarcinoma</strong></td>
<td>Simple glands, intracellular mucin, desmoplastic stroma, marked nuclear atypia</td>
</tr>
</tbody>
</table>

**Table 6.3** Differential diagnosis of pancreatic endocrine neoplasms. +: positive, -: negative, F: focally positive
CHAPTER 6

The first three share with PENs a solid, hypercellular appearance and a nesting growth pattern. Acinar cell carcinoma and pancreatoblastoma exhibit acinar differentiation and demonstrate well-formed acinar structures and granular, eosinophilic cytoplasm.\textsuperscript{98,99,100} Pancreatoblastomas also have distinctive squamoid corpuscles and a hypercellular stromal component. Both acinar cell carcinoma and pancreatoblastoma consistently produce pancreatic exocrine enzymes and can be distinguished from PENs by immunohistochemical labeling for trypsin and chymotrypsin, which are usually diffusely expressed. However, both acinar cell carcinoma and pancreatoblastoma may also contain a minor component of endocrine cells, so focal labeling for chromogranin and synaptophysin may be found. Solid-pseudopapillary tumors have many histologic similarities with PENs but can be distinguished by the presence of pseudopapillae, nuclear grooves, aggregates of foamy tumor cells and histocytes, and collections of large hyaline globules.\textsuperscript{101} By immunohistochemistry, solid-pseudopapillary tumors do express CD56 and often also synaptophysin, but they are never positive for chromogranin. The hyaline globules of solid-pseudopapillary tumors stain for alpha-1-antitrypsin, and there is consistent positivity for CD10 and nuclear accumulation of beta-catenin. Solid-pseudopapillary tumors express vimentin but are negative or only focally positive for keratin. Pancreatic ductal adenocarcinomas generally are not difficult to distinguish from PENs, with the exception of PENs that exhibit gland formation. Even in such PENs, the glands are found within larger nests of cells, in contrast to the individual infiltrating glands of ductal adenocarcinomas, and intracellular mucin is not present. Ductal adenocarcinomas usually also have a higher mitotic rate and more significant nuclear pleomorphism.

Endocrine microadenomas may be confused with enlarged non-neoplastic islets.\textsuperscript{102} Immunohistochemical staining for islet peptides is helpful. Non-neoplastic islets retain the normal proportions and distribution of peptide cell types, whereas microadenomas generally have a predominance of one cell type (commonly alpha or PP cells).

“Mixed” Endocrine Neoplasms

Minor endocrine elements are relatively common in predominantly exocrine pancreatic neoplasms. Thus, it should not be surprising that rare neoplasms exist in which both endocrine and exocrine components are significantly represented. These “mixed” neoplasms have been arbitrarily defined to contain more than 25% of each component, and endocrine, acinar, and ductal lines of differentiation may all be represented (Fig. 6.12).

Reported mixed neoplasms that contain an endocrine component include mixed ductal-endocrine carcinoma, mixed acinar-endocrine carcinoma, and mixed acinar-endocrine-ductal carcinoma.\textsuperscript{103,104,105,106,107,108,109} In most reported examples, the exocrine elements predominate. The different cell types are usually
Tumors of the endocrine system

Figure 6.12 Mixed endocrine carcinomas. In mixed ductal-endocrine carcinomas (A), there are solid nests of endocrine cells mixed with neoplastic ductal structures that contain mucin and markedly atypical nuclei. A mixed acinar-endocrine carcinoma (B), contains well differentiated endocrine cells with round, centrally located nuclei and pale cytoplasm (bottom) as well as acinar elements that have basally located nuclei with prominent nucleoli and granular, eosinophilic cytoplasm (top).

Tumors of the endocrine system

High Grade Endocrine Carcinoma

High grade endocrine carcinomas are extremely rare in the pancreas. These neoplasms are related to small cell carcinomas, and metastasis from sites such as the lung have to be excluded before an example can be accepted as primary in the pancreas. Most patients are older adults, similar to the distribution of ductal adenocarcinomas. The neoplasms are often large and metastatic at diagnosis, so resected examples are few. Histologically, high grade endocrine carcinomas may be composed of either small or large cells (Fig. 6.13). The neoplastic cells grow in diffuse sheets and have a markedly infiltrative growth pattern. There is often little nesting or other architectural patterns. The principle feature that separates this group from well differentiated PENs is the proliferative rate. More than 10 mitoses per 10 hpf should be found, and often the rate is 50 or more. In addition, there is abundant necrosis. A diagnosis of small cell carcinoma may be rendered for a high grade endocrine carcinoma when there
are predominantly cells with minimal cytoplasm and fusiform nuclei with a granular chromatin pattern and inconspicuous nucleoli. In other high grade endocrine carcinomas the cells are larger, with moderate amounts of cytoplasm, the nuclei are round, and nucleoli are prominent. These large cell endocrine carcinomas must be distinguished from poorly differentiated carcinomas lacking endocrine differentiation, so immunohistochemical labeling for chromogranin or synaptophysin must be performed to confirm the diagnosis. High grade endocrine carcinomas of the pancreas are highly aggressive neoplasms, with a natural history equal to or more virulent than that of ductal adenocarcinomas.

References
CHAPTER 6


Tumors of the endocrine system


Tumors of the endocrine system


CHAPTER 6


