Molecular Genetics of Pancreatic Neoplasms and Their Morphologic Correlates

An Update on Recent Advances and Potential Diagnostic Applications

Michelle D. Reid, MD,1 Burcu Saka, MD,1 Serdar Balci, MD,1 Andrew S. Goldblum,2 and N. Volkan Adsay, MD1

From the 1Department of Pathology, Emory University Hospital, Atlanta GA, and 2Emory University, Atlanta, GA.

Key Words: Pancreas; Pancreatic neoplasms; Molecular genetics

ABSTRACT

Objectives: To summarize the most clinically and biologically relevant advances in molecular/genetic characteristics of various pancreatic neoplasms, with morphologic correlation.

Methods: Whole-exome sequencing of numerous benign and malignant pancreatic tumors, along with the plethora of highly sensitive molecular studies now available for analyzing these tumors, provide mounting evidence to support the long-held belief that cancer is essentially a genetic disease. These genetic discoveries have not only helped to confirm the age-old, morphology-based classifications of pancreatic neoplasia but have shed new light on their mechanisms. Many of these molecular discoveries are currently being used in preoperative diagnosis.

Results: Mutations in KRAS, P16/CDKN2A, TP53, and SMAD4/DPC4 are commonly seen in ductal neoplasia but not in nonductal tumors; ductal adenocarcinomas with SMAD4/DPC4 loss are associated with widespread metastasis and poor prognosis. GNAS and RNF43 mutations have been discovered in most intraductal pancreatic mucinous neoplasms, providing critical molecular fingerprints for their diagnosis. Mutation in DAXX/ATRX is only seen in pancreatic neuroendocrine tumors, making it a useful potential marker in distinguishing these tumors from mimics.

Conclusions: When combined with morphologic observations, molecular studies will increase our understanding of the pathogenesis and morphomolecular signatures associated with specific neoplasms and provide new horizons for precision medicine and targeted therapies.

The pancreas is composed of four distinct compartments (ductal, endocrine, acinar, and mesenchymal), and the tumors arising from these compartments often attempt to recapitulate the cells from which they are derived. Although the morphologic characteristics of these tumors are well described, their molecular genetics remain a constantly evolving milieu, with almost daily new discoveries providing additional information on their pathogenesis as well as potential opportunities for improved diagnostics and therapeutics.

Since the completion of DNA sequencing of 24 pancreatic cancers in 20011 and subsequent intensive studies performed—especially by Johns Hopkins University’s Sol Goldman Pancreatic Cancer Research Center, led by Ralph H. Hruban, MD—our perception of the molecular events involved in the development of pancreatic neoplasia has changed significantly. We herein describe the molecular
pathology of the most common pancreatic neoplasms, with an emphasis on new advances that herald potential impact on the classification, diagnosis, and prognostication of these tumors.

**Genetics of Pancreatic Tumors of Ductal Lineage**

**Ductal Carcinoma (Pancreatobiliary-Type Adenocarcinoma)**

Pancreatic ductal adenocarcinoma (PDA) accounts for more than 85% of all pancreatic neoplasms. These tumors are derived from pancreatic ductal cells and are presumed to contain tumorigenic stem cells. Their morphologic and biologic characteristics are typically described as being of pancreatobiliary type because of their striking resemblance to biliary tract cancers. This histologic appearance is critical for their diagnosis. PDA is one of the most genetically unstable organ cancers. Any given case often shows multiple chromosomal losses and gains. This goes along with the high degree of morphologic heterogeneity observed in these tumors. Most examples show areas of well-formed glandular structures admixed with nonglandular, high-grade patterns. Tumors are firm and solid and have infiltrative borders. They range from well to poorly differentiated and are characterized by well to poorly formed glands, clusters, and single cells, which infiltrate dense fibrotic stroma. Somatic mutations of four key driver genes have been implicated in PDA: KRAS, P16/CDKN2A, TP53, and SMAD4/DPC4.

**Mutations of Oncogenes**

KRAS is the most frequently identified oncogene in ductal adenocarcinoma and is seen in more than 90% of these tumors. Somatic mutations cluster in specific hotspots, most frequently on codon 12. These mutations correlate with increased mucin production in affected epithelial cells. KRAS encodes for small guanosine triphosphatase, which helps to control cell signaling downstream of growth factor receptors, and is involved in the proliferation, differentiation, and survival of tumor cells. Somatic mutations in KRAS have been linked to reduced patient survival in PDA, especially when combined with other genetic mutations (such as in p16/CDKN2A).

Interestingly, KRAS mutation rates highlight the fact that, although the morphology of PDA is similar to that of gallbladder adenocarcinoma and cholangiocarcinoma (hence their universal “pancreatobiliary” lineage), their molecular signatures are distinct. The frequency of KRAS mutations in the pancreatobiliary tree progressively decreases as tumors arise more proximally. The pancreatic duct shows more than 90% of KRAS mutations; the common bile duct, 65%; the common hepatic duct, 45%; and the gallbladder, less than 10%. Interestingly, cases with anomalous union of pancreatic ducts, in which the common bile ducts become exposed to the pancreatic juice, have a high incidence of KRAS mutation. In addition, KRAS mutations are more frequent in ampullary carcinomas than in proximal bile duct cancers.

All of these observations have led to the conclusion that pancreatic juice may be a factor in the induction of KRAS mutation. Also noteworthy is the fact that, as in other sites, this frequency pattern parallels the amount of mucin produced by these tumors. Pancreatic examples tend to be more mucinous than proximal biliary ones. Additional studies, however, are needed to further elucidate these observations.

**Mutations of Tumor Suppressor Genes**

The tumor suppressor genes most frequently implicated in PDA, in order of frequency, are p16/CDKN2A (95%), TP53 (75%), and SMAD4/DPC4 (55%). Somatic mutations of p16/CDKN2A occur in the majority of PDAs and result in loss of p16. p16/CDKN2A is important in cell cycle regulation.

Somatic mutations in TP53 are seen in 75% of PDAs and are the result of intragenic mutation followed by loss of the wild-type allele. TP53 is critical for the cell’s response to stress. Strong nuclear staining of PDA tumor cells with the p53 immunostain suggests mutation of the TP53 gene. Mutant TP53 has even been detected in pancreatic juice from the duodenum of patients with PDA.
Somatic inactivation of the SMAD4/DPC4 gene is seen in approximately half of PDAs. Mutation occurs via homozygous deletion or intragenic mutation coupled with loss of the wild-type allele. SMAD4/DPC4 is important for cell proliferation, motility, and apoptosis, and inactivation results in loss of immunohistochemical expression of Dpc4 protein in tumor cells. Dpc4 antibody staining is therefore a marker of gene inactivation and can assist in the differentiation of PDA from nonneoplastic mimics. PDAs that show SMAD4/DPC4 loss are associated with extensive, widespread metastatic disease and overall poor prognosis. The loss of Dpc4 expression can therefore predict a group of patients who are prone to have widespread metastasis and may help stratify patients for systemic therapeutic regimens. Phase II clinical trials have been conducted using specific therapeutic agents targeting SMAD4/DPC4-inactivated PDA. In a manner similar to Kras, Dpc4 loss is most pronounced in carcinomas of the pancreatic duct and ampullary region, with Dpc4 more commonly preserved in tumors arising proximal to the pancreas (ie, carcinomas in the gallbladder and biliary tree).

Other Genetic Abnormalities

Whole-exome sequencing of PDA revealed approximately 48 nonsynonymous somatic mutations per tumor involving multiple genes. Chromosomal gains, losses, and complex karyotypes have also been identified. Alterations of micro-RNAs, which are small single-stranded noncoding RNAs that regulate gene expression, have also been implicated in PDA tumorigenesis. The altered micro-RNAs in PDA include miR-21 (12.1-fold increase), miR-155 (11.6-fold increase), and miR-221, and these are expressed in PDA but not in benign pancreas. Additional micro-RNAs implicated in PDA include miR-18a, -31, -93, and -224, which are additional potential biomarkers for PDA.

Other Variants of Ductal Carcinoma

Adenosquamous carcinoma of the pancreas is a rare variant of PDA that accounts for 4% of exocrine tumors. The molecular abnormalities in this tumor are similar to those in PDA, and mutations in all four key driver genes are commonplace.

Undifferentiated carcinoma is another rare variant of pancreatic cancer. It is one of the most aggressive pancreatic cancers and is typified by malignant pleomorphic mononuclear cells with or without surrounding nonneoplastic osteoclast-like giant cells. KRAS mutations are often seen in the malignant mononuclear cells but not in the nonneoplastic osteoclast-like giant cells. Tumors with predominant osteoclastic giant cells may in the future prove to have different biology from giant cell–poor tumors. The factors that attract the osteoclastic giant cells to this tumor—and why this tumor is so much more common in the pancreatobiliary tract than elsewhere—are fascinating questions awaiting further studies. Undifferentiated carcinomas also exhibit mutant allelespecific imbalance of the KRAS oncogene (KRAS MASI), which is associated with higher-stage disease and shortened survival. Tumor cells also express p53, while the osteoclastic giant cells do not.

Micropapillary pattern PDA has also been described. This pattern of growth has been attributed to a chain of molecular events that culminate in reversal of cell polarity in which the stroma-facing surface of the tumor cells acquire luminal-surface properties, including the expression of MUC1.

Some PDAs, especially poorly differentiated examples, are characterized by a distinctive neutrophilia (tumor-infiltrating neutrophils), and the reasons for this occurrence remain speculative.

Familial/Syndrome-Associated PDA

A positive family history of pancreatic cancer in one or more first-degree relatives is known to increase one’s risk of PDA. It has now been shown that approximately 10% of pancreatic cancers have a familial basis. Several genetic syndromes have also been implicated as increasing the risk of pancreatic cancer. These include familial breast cancer syndrome, familial atypical multiple mole melanoma syndrome (FAMMM), Peutz-Jeghers syndrome (PJS), Lynch syndrome/hereditary nonpolyposis colorectal cancer (HNPCC), familial adenomatous polyposis syndrome (FAP), and hereditary pancreatitis.

Germline mutations of the BRCA2 gene and other genes in the Fanconi anemia pathway (FANCN/PALB2) have been implicated in familial breast cancer syndrome and are associated with increased risk of familial PDA. Their associated proteins are critical for repairing DNA cross-linkage damage. Germline mutations of BRCA2 cause a 3.5-fold increased risk of pancreatic cancer, whereas germline mutations in FANCN account for a small subset of familial PDAs.

FAMMM syndrome is an inherited autosomal dominant disease that is associated with germline mutations in p16/CDKN2A, a key driver gene in PDA tumorigenesis. Patients

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial breast cancer</td>
<td>BRCA2, FANCN</td>
</tr>
<tr>
<td>Familial atypical multiple mole melanoma</td>
<td>PI6/CDKN2A</td>
</tr>
<tr>
<td>Peutz-Jeghers</td>
<td>STK11/LKB1</td>
</tr>
<tr>
<td>Hereditary nonpolyposis colorectal cancer</td>
<td>hMSH2, hMLH1, hPMS1, hPMS2, hMSH6/GTBP</td>
</tr>
<tr>
<td>Familial adenomatous polyposis</td>
<td>APC</td>
</tr>
<tr>
<td>Hereditary pancreatitis</td>
<td>PRSS1, SPINK1</td>
</tr>
</tbody>
</table>
have an increased risk of cutaneous melanomas and multiple nevi (including atypical ones),\textsuperscript{34,35} and their relatives have a 13- to 22-fold increased risk of PDA.\textsuperscript{36} A detailed family history of melanoma and multiple nevi should be collected in patients with PDA to avoid missing FAMMM-associated tumors.

PJS is associated with germline mutations in the \textit{STK11/\ LKB1} gene, and these patients are at significantly higher risk for PDA (132-fold increased risk).\textsuperscript{37} In patients with PJS, PDAs may also have somatic loss of wild-type \textit{STK11/\ LKB1}. Patients with PJS are also at increased risk for intra- ductal papillary mucinous neoplasms (IPMNs) and their precursors.\textsuperscript{38} This has profound implications for the radiologic screening for, and management of, pancreatic cysts in patients with PJS.\textsuperscript{39}

HNPCC is associated with germline mutations in the genes encoding for proteins associated with DNA mismatch repair (\textit{hMSH2, hMLH1, hPMS1, hPMS2, and \textit{hMSH6/GTBP}}). When mutated, these genes cause defects in the DNA mismatch repair mechanism and increase the risk for colonic adenocarcinoma\textsuperscript{40} and, to a lesser extent, the risk for PDA. These PDAs show microsatellite instability and have characteristic “medullary” morphology, which is recognizable on histologic evaluation.\textsuperscript{41,42} FAP syndrome is associated with germline mutations in the adenomatous polyposis coli (\textit{APC}) tumor suppressor gene, which increases the risk of early colonic polyps and adenocarcinoma as well as PDA (fourfold increased risk).\textsuperscript{43} This “increased” risk for PDA may be partly because of patients’ developing duodenal adenocarcinomas, which invade the pancreas and are misclassified as being pancreatic in origin.

Hereditary pancreatitis leads to chronic pancreatitis in which patients have recurrent episodes of acute pancreatitis starting as early as childhood. Germline mutations in cationic trypsinogen gene (\textit{PRSSI}) and the serine protease inhibitor gene (\textit{SPINK1}) have been identified in these patients\textsuperscript{44} who have a significantly increased risk (up to 53-fold depending on age) of developing PDA.\textsuperscript{45}

In an analysis of prophylactic pancreatectomy specimens from patients with a high risk of developing familial pancreatic cancer, Crnogorac-Jurcevic et al\textsuperscript{46} identified a panel of 76 genes that are commonly dysregulated in the progression from normal to pancreatic intraepithelial neoplasia (PanIN) and PDA. In their series, the \textit{S100P} and \textit{AGR2} genes, as well as \textit{COLLA1}, showed the greatest overexpression in PanIN and PDA. \textit{AGR2} is known to be overexpressed in PanIN, and \textit{S100P} levels are known to increase with increasing PanIN grade.\textsuperscript{47,48}

Mutations of key driver genes are also implicated in familial PDA. The specific genes include \textit{KRAS, TP53}, and \textit{SMAD4/\ DPC4}, and these show similar genetic alterations to those in sporadic PDA.\textsuperscript{5} Interestingly, in a review of PDAs in individuals enrolled in the National Familial Pancreas Tumor Registry (a registry of patients with a family history of pancreatic cancer), no statistically significant differences in tumor morphologies were seen between familial and sporadic PDA.\textsuperscript{49}

The low PDA incidence in the general population makes the use of screening tests for early diagnosis impractical. However, for those with hereditary syndromes and high familial risk, the issue of screening, surveillance, and genetic counseling for mutation carriers is an important one. Studies of pancreatic cancer kindreds have included a detailed clinical history of smoking, alcohol use, and other nonpancreatic malignancies, the presence of which may suggest unrecognized genetic syndromes. Smoking is known to double the risk of pancreatic cancer and is associated with far more nonsynonymous somatic mutations per tumor compared with PDA in those who never smoked.\textsuperscript{50} In one study, the most sensitive screening tests for PDA in pancreatic cancer kindreds were endoscopic ultrasonography (EUS) and pan- creatography, and these were found to be more sensitive than computed tomography (CT), positive emission tomography, and magnetic resonance imaging.\textsuperscript{51} However, others have shown that a screening EUS as well as serial CTs were helpful in diagnosing significant pancreatic neoplasms in asymptomatic high-risk individuals.\textsuperscript{39} Larger studies are needed to determine the most sensitive method(s) for the screening and surveillance of individuals at high risk for developing PDA.

Nonpancreatobiliary-Type Carcinomas of the Pancreas

Medullary carcinoma is a rare, poorly differentiated pancreatic cancer that has been reported in HNPCC syndrome and often shows microsatellite instability similar to its colonic counterpart.\textsuperscript{42} However, medullary carcinoma lacks somatic \textit{KRAS} mutations but may show \textit{BRAF} mutations.\textsuperscript{5} Many of these so-called “pancreatic” medullary carcinomas often prove to be of ampullary origin if examined carefully. True medullary carcinomas of the pancreas appear to be exceedingly uncommon.

Colloid carcinoma is a rare pancreatic cancer that is usually seen in association with intestinal-type IPMN.\textsuperscript{21,52} Compared with PDA, colloid carcinoma shows a significantly lower prevalence of \textit{KRAS} (30\%) and \textit{TP53} (20\%) mutations but often has somatic mutations of the \textit{GNAS} oncogene. This gene is also commonly mutated in IPMNs and IPMN-associated invasive carcinoma.\textsuperscript{55} \textit{GNAS} encodes for a protein that links transmembrane receptors with downstream signaling proteins and plays a critical role in cell signaling. Colloid carcinoma of the pancreas has a significantly better clinical outcome than conventional PDA.\textsuperscript{52}

PanIN

PanIN is the microscopic noninvasive precursor of PDA\textsuperscript{54-56} and shows similar genetic mutations to those seen in its invasive counterpart.\textsuperscript{57-60} Telomere shortening is a very common event in pancreatic cancer development and is seen
in approximately 90% of PanIN-1A lesions. Other events seen in low-grade PanIN include mutations in KRAS, p16/CDKN2A, GNAS, or BRAF; late events, which are seen in high-grade PanIN and invasive carcinoma, include alterations of SMAD4/DPC4 and TP53 genes.

Tumoral Intraepithelial Neoplasms (Cystic and Intraductal Tumors)

It has now become clear that there is a dichotomy in pancreatic ductal carcinogenesis. Although PanINs are the microscopic/incidental forms of dysplasia, mass-forming forms of preinvasive neoplasia are usually present clinically, mostly as cystic neoplasms. These include IPMN, intraductal tubulopapillary neoplasm (ITPN), and mucinous cystic neoplasm (MCN), which are of ductal lineage, are considered neoplastic, and have an established increased malignant risk.

IPMNs

IPMNs are (by definition >1 cm) noninvasive mucin-producing neoplasms arising from the main pancreatic duct and/or its branches. They account for 5% of exocrine pancreatic neoplasms and predominantly arise in the pancreatic head (70%), with fewer cases involving the body (20%) and tail (10%). Cysts can be unifocal or multifocal and are lined by mucin-secreting columnar epithelium of four histologic types (intestinal, gastric foveolar, pancreatobiliary, and oncocytic). Intestinal-type IPMNs typically have papillae lined by tall columnar cells with apical mucin and elongated nuclei. Gastric foveolar-type IPMNs have a more blunted mucin-producing epithelial lining, whereas pancreatobiliary IPMNs form arborizing papillae lined by cuboidal-type epithelium with rounded nuclei and prominent nucleoli. Oncocytic IPMNs have a highly complex papillary meshwork and are lined by cells with abundant granular eosinophilic cytoplasm and rounded nuclei. All IPMNs may show dysplasia (ranging from low to high grade) and some authors believe that they are an additional phenotype of familial pancreatic cancer.

Gastric-type IPMNs express gastric lineage markers MUC5AC and MUC6 and are mostly of small branch–duct type. The typical lesion is an incidentally identified cyst occurring in approximately 8% of elderly patients. Invasion is uncommon (15%), but if it occurs it is most commonly usual-type PDA and is aggressive. In intestinal-type IPMNs, intestinal programming markers are activated. CDX2, the driving transcription factor of intestinal differentiation, is expressed diffusely and strongly almost exclusively in this subtype of IPMN along with its downstream effector, MUC2, which is a tumor suppressor. Intestinal IPMNs are large and complex and approximately 30% are invasive. Invasive carcinoma, however, is of the colloid type and has a good prognosis. Although oncocytic-type IPMN has a highly complex appearance, it is seldom invasive. When invasion occurs, it is usually limited in nature and has a good prognosis. It can be speculated that the causes of striking mitochondria accumulation in this subset of IPMNs may have biologic connotations that lead to higher proliferation rates but less aggressive biology, as with oncocytic tumors in other sites. In addition, MUC6 has been implicated as a marker of the pyloro-pancreatic pathway in oncocytic and pancreatobiliary-type IPMNs. This pathway is distinct from the MUC2/CDX2 intestinal lineage pathway.
The molecular alterations seen in IPMNs are complex. The four key driver genes that are mutated in PDA (KRAS, TP53, p16/CDKN2A, and SMAD4/DPC4) may also be altered in IPMN. Somatic KRAS mutations are seen in 30% to 80% of IPMNs and are more common in cysts with high-grade dysplasia or invasion. P53 is overexpressed in IPMNs with high-grade dysplasia and invasive carcinoma, and somatic TP53 mutations are seen in areas with high-grade dysplasia. DPC4 expression is maintained in most noninvasive IPMNs but lost in one-third of invasive IPMNs, and p16 protein is lost in both noninvasive and invasive IPMN.

Somatic mutations of the GNAS (codon 201) oncogene are seen in more than 60% of IPMNs and involve both in situ and invasive components. Interestingly, GNAS mutations are absent in non–IPMN-related PDAs. The intestinal-type IPMN shows the highest prevalence of GNAS mutations. GNAS mutations have also been detected in duodenal collections of pancreatic juice from patients with IPMN and in patients without visible pancreatic cysts. Wu et al showed that more than 96% of IPMNs have either a GNAS or KRAS mutation, with a little more than 50% showing both. The latter finding has implications for the workup of pancreatic cysts with a differential diagnosis that includes IPMN, MCN, and serous cystadenoma. Wu and coworkers found that most IPMNs had a GNAS and/or KRAS mutation in comparison with none of the serous cystadenomas (sensitivity, 0.96; specificity, 1.0 for distinction between the two cyst types). Because of this finding and also because GNAS mutations were not identified in MCNs in their study, an assay using GNAS and KRAS amplicons could potentially distinguish between these three cyst types (and requires a minimum of 250 µL of cyst fluid for analysis).

Somatic mutations in RNF43, a likely tumor suppressor gene that encodes an E3 ubiquitin ligase, are seen in 75% of IPMNs, and loss of heterozygosity at the RNF43 locus (chromosome 17q) is common. Somatic mutations of the PIK3CA (10% of cases) and STK11/LKB1 genes are rarely seen in IPMNs. Whole-exome sequencing of IPMNs revealed approximately 26 somatic mutations per tumor. There is increased microRNA expression of miR-21, miR-221, and miR-17-3p in IPMN. Micro-RNA biomarkers in cyst fluid are also useful in distinguishing low-grade IPMNs from high-grade IPMNs. They are useful in distinguishing high-grade IPMNs from serous cystadenoma, solid pseudopapillary neoplasm (SPN), and cystic pancreatic neuroendocrine tumors (PanNETs). The complex molecular alterations in IPMN potentially can be exploited in the development of diagnostic assays for evaluating and classifying pancreatic cysts.

ITPNs

ITPNs are extremely rare, relatively indolent, solid tubule–forming epithelial neoplasms that have ductal differentiation but lack mucin production. Tumors consist of back-to-back tubules with variable papillae formation and are lined by cuboidal epithelium showing high-grade dysplasia with easily identifiable mitoses. Invasive carcinoma is seen in up to 40% of cases.

The molecular alterations in ITPNs have only rarely been studied. In a study by Yamaguchi et al, ITPNs showed somatic mutations in the phosphatidylinositol 3-kinase (PIK3CA) gene in approximately 28% of cases. PIK3CA mutations were often associated with immunohistochemical...
overexpression of phosphorylated AKT (indicating activation of the PI3K-AKT pathway) in contrast to IPMNs, which only rarely showed AKT overexpression. Unlike IPMNs and PDAs, ITPNs did not exhibit KRAS mutations. This suggests that phosphatidylinositol 3-kinase activation plays a critical role in ITPNs but not in IPMNs and that KRAS is also not a major actor in ITPNs. The activated phosphatidylinositol 3-kinase pathway may be a potential diagnostic and therapeutic target in these tumors. Immunohistochemical expression of P53 (20% of cases) and p16/CDKN2A (54% of cases) was also seen in ITPNs in the Yamaguchi et al study, and somatic BRAF mutations were rarely seen. P53 was rarely overexpressed, SMAD4 expression was rarely lost, and β-catenin was negative in all ITPNs examined.

**MCNs**

MCNs are mucin-producing cystic tumors that arise outside the pancreatic ductal system. They primarily involve the pancreatic body and tail of perimenopausal women and are characterized by mucin-rich columnar epithelium with underlying ovarian-type stroma (OTS). The nature of this OTS is a fascinating question. Although some authors believe it represents an embryologic remnant of ovary (often noting the close proximity of the left ovary and tail of the pancreas), we believe that OTS is a recapitulation of periductal fetal mesenchyme. In the developing fetus, this mesenchyme is morphologically similar to the OTS that occurs in similar tumors in other organs. It also occurs in the liver, where MCNs can be seen. It is our speculation that periductal fetal mesenchyme remnants are stimulated by the hormonal milieu (in perimenopausal women), and in return this OTS drives the subjacent epithelium into proliferation, thus causing the multicystic cystic tumors.

MCNs show varying degrees of dysplasia (low to high grade), and 15% to 20% of cases are associated with invasive adenocarcinoma, mostly of tubular type. A few invasive MCNs are of undifferentiated sarcomatoid type, but virtually none are colloid-type carcinomas. This distinguishes MCN carcinogenesis from that of IPMN.

MCNs often have similarly mutated genes to those seen in PDA. The frequency of KRAS mutations correlates with increasing grade of dysplasia and/or invasive carcinoma. KRAS mutations are seen less frequently in low-grade dysplasias and commonly in high-grade dysplasias or invasive MCNs. This mutation can also be detected in cyst fluid aspirates, an important part of the diagnostic workup of pancreatic cysts. Somatic mutations of p16/CDKN2A are also more common in high-grade dysplasia. Nuclear overexpression of p53 is seen only in areas of high-grade dysplasia or invasive MCN. Loss of Dpc4 protein is associated with the transition to invasive cancer and is seen in some tumors. These findings suggest that KRAS mutation occurs early in MCN carcinogenesis, whereas SMAD4/DPC4 mutation occurs late. Whole-exome sequencing of MCN identified approximately 16 nonsynonymous somatic mutations per tumor, which is less than those seen in IPMN and PDA. MCNs also contain inactivating somatic mutations of RNF43. However, GNAS mutations have not been reported in MCNs to date—a key finding that makes GNAS a potentially useful distinguishing marker between MCNs and IPMNs on cyst fluid analysis.

**Serous Cystadenoma**

Serous cystadenomas are benign cystic pancreatic tumors that most frequently arise in the pancreatic body and tail of women. Cysts can be small or large and multilocular (microcystic) or unilocular (macrocystic) and are lined by a single layer of low columnar to cuboidal nonmucinous epithelium with clear, glycogen-rich cytoplasm and bland-appearing nuclei.

Somatic mutations of the von Hippel-Lindau (VHL) gene are seen in up to 50% of sporadic cases. In VHL syndrome, in which the VHL gene (chromosome 3p) shows germline mutations, most patients develop serous cystadenomas, and VHL mutations occur in conjunction with somatic inactivation of the second allele. Whole-exome sequencing of serous cystadenomas showed 10 nonsynchronous somatic mutations per tumor. The genetic alterations frequently seen in IPMNs and MCNs (KRAS, GNAS, and RNF43) are not present in serous cystadenomas, which further supports the use of diagnostic molecular assays in distinguishing serous cystadenomas from mucin-producing pancreatic cysts.

An interesting feature of serous cystadenomas is that they seem to be a model of clear-cell–associated tumorigenesis. There is a peculiar vascularization within the epithelium (similar to other VHL-associated clear-cell tumors [capillary hemangioblastomas and clear-cell renal carcinomas]). Molecules implicated in clear-cell tumorigenesis (such as carbonic anhydrase IX and GLUT-1) are also overexpressed in serous cystadenoma.

**Diagnostic Applications of Molecular Genetics in Cystic Pancreatic Lesions**

Because of the increasing sensitivity of new imaging modalities, incidental pancreatic cysts have seen a significant rise. Most of these have a low risk of malignancy and do not require surgical intervention. It is important to accurately distinguish low-risk from high-risk cysts (IPMN, ITPN, and MCN), so as to appropriately stratify patients for either surveillance or surgical intervention, which is associated with significant morbidity and mortality.

The frequency of somatic mutations of KRAS, GNAS, RNF43, PIK3CA, and VHL in specific pancreatic cysts suggests that molecular assays containing a panel of these markers could prove useful in pretreatment diagnosis and...
classification. In addition, finding mutant p16/CDKN2A and TP53 (which is even detectable in pancreatic juice in high-grade IPMNs) would favor high-grade dysplasia or invasive carcinoma in these cysts. Micro-RNA signatures have been identified in pancreatic cysts and show promise as potential biomarkers for diagnosing and predicting high-grade dysplasia. Matthaei et al. recently created a 9-miRNA signature for evaluating cysts and identifying high-grade IPMNs. The genetic alterations seen in key morphologic subtypes of pancreatic cysts may help to further clarify various puzzles in their genesis and progression.

**Genetics of Pancreatic Tumors of Neuroendocrine Lineage**

Neuroendocrine neoplasms of the pancreas are composed of epithelial cells with phenotypic and ultrastructural neuroendocrine differentiation. These tumors arise sporadically in the pancreas as well as in familial syndromes such as VHL syndrome, tuberous sclerosis complex, neurofibromatosis type 1, and multiple endocrine neoplasia 1 (MEN1). In MEN1, neuroendocrine neoplasms are often multiple, but in sporadic cases, tumors are typically solitary masses. Tumors are composed of proliferating epithelial cells with round to oval nuclei containing “salt and pepper” chromatin. The ki-67 index or mitotic count is used to classify neoplasms as well-differentiated (grades 1 and 2) PanNETs or poorly differentiated (grade 3) neuroendocrine carcinomas (PanNECs) of small or large cell type.

Patients with MEN1 syndrome have germline mutations in the MEN1 tumor suppressor gene, and 60% to 70% of them develop PanNETs. Poorly differentiated PanNECs, however, are infrequently associated with MEN1. Up to 17% of patients with VHL syndrome develop PanNECs, most of which are nonfunctional. Among sporadic PanNETs, up to 45% show somatic mutations in MEN1 and a loss of heterozygosity is seen at the MEN1 locus in 30% to 70% of cases, including those without somatic MEN1 mutation. In PanNETs, MEN1 mutations are associated with a better prognosis.

Approximately 45% of sporadic PanNETs have inactivating somatic mutations of the death-domain–associated protein (DAXX) and α-thalassemia/mental retardation syndrome X-linked (ATRX) genes, which are involved in chromatin remodeling. Their mutation results in loss of protein expression on immunohistochemistry. DAXX and ATRX encode for proteins critical to telomere maintenance, and tumors with DAXX/ATRX mutations have the “alternative lengthening of telomeres” phenotype. These mutations are only seen in PanNETs and are not present in microadenomas.

Approximately 15% of PanNETs show somatic mutations of the genes that encode for proteins in the mammalian target of rapamycin (mTOR) cell signaling pathway and include somatic mutations of PIK3CA, PTEN, and TSC2. mTOR pathway alteration can be exploited as a therapeutic target as drugs specifically directed against this pathway have been developed.

Deletion of the VHL gene occurs in up to 25% of sporadic PanNETs. Sequencing of all protein-coding genes in PanNETs yielded an average of 16 nonsynonymous somatic mutations per tumor and are not the somatic mutations frequently seen in PDA. Chromosomal gains and losses have also been identified, and multiple chromosomal abnormalities are associated with a worse prognosis.

PanNECs have recently been shown to have molecular signatures that are distinct from those in PanNETs. TP53 mutations occur frequently in PanNEC and result in aberrant immunohistochemical p53 expression (in 100% of small cell and 90% of large cell carcinomas). Additionally, the retinoblastoma (RB-1) gene is mutated in 74% of cases, which correlates with loss of RB protein in 60% to 90% of PanNECs. These abnormalities are not present in well-differentiated PanNETs. Mutations in ATRX and DAXX have also not been identified in PanNEC.

**Genetics of Pancreatic Tumors of Acinar Lineage**

**Acinar Cell Carcinoma**

Acinar cell carcinoma is a rare malignant tumor of the exocrine pancreas that recapitulates the growth pattern and secretory products of nonneoplastic acini. It is characterized by a stroma-poor proliferation of acinar cells in solid sheets, trabeculae, and acini. Tumor cells have zymogen-rich granular cytoplasm and central nuclei with prominent nucleoli.

Somatic alterations in the APC/β-catenin pathway occur in up to 25% of tumors and include mutations in CTNNB1 and APC. However, in one study, APC gene inactivation was demonstrated in most acinar cell carcinomas. Some tumors may also show microsatellite instability. Numerous large chromosomal losses and gains have been reported in acinar cell carcinoma. Whole-exome sequencing of 23 acinar cell carcinomas revealed a host of genetic alterations including microsatellite instability, large chromosomal gains and losses, and somatic mutations involving the Wnt signaling pathway (including APC and CTNNB1) as well as BRAF. In addition, somatic mutations in genes not previously linked to pancreatic neoplasia are seen in acinar cell carcinoma. This suggests that these tumors are genetically distinct from other pancreatic neoplasms. The genetic mutations commonly seen in PDA (KRAS) and neuroendocrine tumors (DAXX/ATRX) have not been described in acinar cell carcinoma.
Pancreatoblastoma

Pancreatoblastoma is an extremely uncommon, malignant, primary pancreatic tumor that is often seen in children but may also occur in adults. Tumors show multiple lines of histologic differentiation, with acinar, ductal, and mesenchymal components. Squamoid morules are an additional characteristic feature. Primitive embryonal components may be seen.

The squamoid morules in pancreatoblastoma contain biotin-rich, optically clear nuclei (BROCN), which make these tumors members of the family of “BROCN” tumors.102 Tumors with morule formation (the so-called BROCNs) overexpress estrogen receptor (ER)-β and (aberrant) nuclear/cytoplasmic β-catenin, which is postulated to upregulate the Wnt signaling pathway.103 The latter is known to promote keratinization and hair folliculogenesis in embryogenesis, and its activation possibly explains the frequency of morules in this primarily pediatric pancreatic tumor. Expression of ER-β and β-catenin has potential diagnostic potential because both will highlight subtle squamoid morules in histologically challenging cases.

The most common genetic abnormality in pancreatoblastoma is loss of chromosome 11p,104,105 which has also been described in Wilms tumor.106 Somatic alterations in the APC/β-catenin pathway, including mutations in CTNNB1 and APC, are described in pancreatoblastoma.104 The genetic mutations commonly seen in PDA are not seen in pancreatoblastoma.

Genetics of Pancreatic Tumors of Uncertain Lineage

Solid Pseudopapillary Neoplasm

SPNs are distinctive, low-grade, malignant pancreatic tumors whose classification is partly morphologic and partly molecular. Most tumors arise in young women in their 20s to 30s and on imaging are large, circumscribed, solid, and/or cystic. They are composed of poorly cohesive cells surrounding a thin branching vasculature. Tumor cells have scant cytoplasm and nuclei with open chromatin and nuclear grooves. Eosinophilic hyaline globules and clear cells are also seen. Despite their distinctive histomorphology, SPNs fail to show a line of differentiation comparable to any known cell in the normal pancreas.

SPNs show activating somatic mutations in the β-catenin gene (CTNNB1) in 95% of cases. This leads to nuclear accumulation of β-catenin immunohistochemical protein in almost all cases, a feature that supports the diagnosis and distinguishes these tumors from histologic mimics.107,108 In addition, CTNNB1 alterations cause aberrant E-cadherin expression in tumor nuclei,109 which may explain the tumor cells’ notorious proclivity for discohesion. The striking resemblance of SPNs to ependymomas is noteworthy. In fact, although they have striking similarities to ovarian ependymomas, SPNs have not been shown to have glial fibrillary acidic protein or other ependymal markers.

SPNs lack the typical genetic mutations frequently seen in PDA and other cystic pancreatic neoplasms (GNAS, RNF43), a fact that can be used in molecular assays when evaluating cystic pancreatic lesions that include SPN in the differential diagnosis. Whole-exome sequencing of SPNs has revealed only three nonsynonymous mutations per tumor.102

Conclusion

In conclusion, these are exciting times in the field of pancreatic pathology. Although molecular genetic studies have helped to further characterize and verify earlier morphologic observations, numerous others await clarification.

Because PDAs are the most common primary tumors, for a long time they served as the standard by which all other tumors were measured. However, we now know that other pancreatic tumor types have their own specific identifying molecular fingerprints that can be used for diagnostic and therapeutic purposes.

The work established by morphologists who originally typed and classified these neoplasms is now being validated by critical genetic data. With increasing knowledge of the molecular events underlying the development and progression...
of these tumors, their diagnosis and treatment should see vast improvements in the future.

Address correspondence to Dr Adsay: Dept of Pathology, Emory University Hospital, 1364 Clifton Rd, NE, Room H180, Atlanta, GA 30322; nadsay@emory.edu

References


