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Molecular Signatures of Pancreatic Cancer

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Abstract

Context—The introduction of genome- and epigenome-wide screening techniques has dramatically improved our understanding of the molecular mechanisms underlying the development of pancreatic cancer. There are now 3 recognized histologic precursors of pancreatic cancer: pancreatic intraepithelial neoplasia, intraductal papillary mucinous neoplasm, and mucinous cystic neoplasm. Each of these precursor lesions is associated with specific molecular alterations.

Objective—To understand the molecular characteristics of pancreatic ductal adenocarcinoma and its precursor lesions.

Data Sources—PubMed (US National Library of Medicine).

Conclusions—In this review, we briefly summarize recent research findings on the genetics and epigenetics of pancreatic cancer. In addition, we characterize these molecular alterations in the context of the histologic subtypes of pancreatic cancer.

Pancreatic cancer is the fourth leading cause of cancer death in both men and women in the United States. In 2010, it is estimated that 43 140 Americans will be diagnosed and 36 800 patients will die of pancreatic cancer.[1] Most pancreatic cancers are pancreatic ductal adenocarcinomas and the 5-year survival rate for patients with localized disease after surgical resection is 20% and for those with metastatic disease, the survival is only 2%.[1] The poor survival rate is attributed to the late detection of pancreatic cancers; 85% of patients present with advanced disease that is unresectable. Although significant resources have been committed to improving the survival of patients with pancreatic cancer in the past decades, there has been no significant improvement in survival.[1] Research into the molecular mechanisms of pancreatic cancer has revealed that the disease is due to both genetic and epigenetic changes. The introduction of genome- and epigenome-wide screening techniques has expanded the numbers of genes linked to pancreatic cancer.[2–6] In this review, we briefly summarize recent research findings on genetics and epigenetics of pancreatic cancer in the context of histologic variants, precursor lesions, and familial pancreatic cancer.

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GENETICS OF PANCREATIC CANCER

A recent comprehensive study of the pancreatic cancer genome profiled the genetic abnormalities of pancreatic ductal adenocarcinomas. In this study, Jones and colleagues[7] sequenced 20 661 protein-coding genes in 24 ductal adenocarcinomas and demonstrated an average of 48 nonsilent mutations, 6 amplifications, and 8 homozygous deletions per pancreatic cancer. These mutations were associated with 12 core signaling pathways.[7] Based on the frequency of genetically affected genes in pancreatic cancers, a genetic "topographic map" of the pancreatic cancers can be generated in which the most frequent mutations are represented as 4 "mountains" (high-frequency driver genes) involving *KRAS2*, *CDKN2A/p16*, *SMAD4/DPC4*, and *TP53*, with numerous "hills" (low-frequency driver genes) involving *SMARC4A*, *CDH1*, *EPHA3*, *FBXW7*, *EGFR*, *IDH1*, and *NF1*.[7]

1. Oncogenes and Pancreatic Cancer

The most frequently mutated oncogene in pancreatic cancers is *KRAS2* (mutated in >95% of pancreatic cancers), which is activated by point mutations, most often in codon 12.[7,8] The *KRAS2* gene is located on chromosome arm 12p and encodes a membrane-bound guanosine triphosphate (GTP)–binding protein. This GTP-binding protein mediates various cellular functions, such as proliferation, cellular survival, motility, and cytoskeletal remodeling. Activating *KRAS* gene mutations abolish the regulated GTPase activity of the Kras protein, which results in constitutive signaling.[9] Mutations in the *KRAS2* gene are observed in the earliest _ENREF_10pancreatic intraepithelial neoplasia (PanIN) lesions and are considered to be one of the earliest genetic events in pancreatic tumorigenesis.[7,10,11] Several additional signaling pathways downstream from *KRAS2*, including BRAF-MAPK and PI3K-AKT, may also be activated by mutations. The *BRAF* pathway is activated by a point mutation at V600E. *BRAF* gene mutations are observed in 5% of pancreatic cancers that do not possess a *KRAS2* mutation.[12] These cancers are often microsatellite unstable. Similarly, amplifications in the *AKT2* gene are seen in 10% to 20% of pancreatic cancers.[13,14] Amplifications of other oncogenes such as C-*MYC*,[7,15] *KRAS*, and *GATA6*,[16, 15] are less frequent.

2. Tumor Suppressor Genes in Pancreatic Cancer

Three tumor suppressor genes, *CDKN2A/p16*, *TP53*, and *SMAD4/DPC4*, are commonly inactivated in pancreatic cancers.[7,17–20] *CDKN2A/p16* on chromosome arm 9p is inactivated in more than 95% of pancreatic cancers by several different mechanisms, such as homozygous deletion of both alleles of the gene; intragenic mutation in 1 allele, coupled with loss of the other allele; or promoter hypermethylation.[17,21,22] The p16 protein inhibits progression of the cell cycle at the G1-S checkpoint binding of cyclin-dependant kinases (CDKs), including CDK4 and CDK6.[23] The *TP53* gene on chromosome arm 17p is inactivated in 50% to 75% of pancreatic cancers.[7,19,24,25]

p53 proteins play several key roles including maintaining G2-M arrest, regulating G1-S checkpoint, inducing apoptosis, regulating senescence, repairing DNA, and changing cellular metabolism.[26] Inactivation of the *TP53* gene typically occurs through intragenic mutations of 1 allele, accompanied with loss of the other allele.[19] Functional loss of the p53 protein enables cellular survival and division in the presence of DNA damage; this facilitates the accumulation of further genetic abnormalities.[26]

SMAD4/DPC4 on chromosome arm 18q is inactivated in 55% of pancreatic cancers.[27,28] *SMAD4/DPC4* is inactivated by homozygous deletion and by intragenic mutations accompanied by loss of the other allele.[28,29] Smad4 (dpc4) protein has a critical function in the signal transduction cascade that involves transforming growth factor β (TGF-β) and

multiple targets in the TGF-β pathway. Binding of the TGF-β ligand to its receptor triggers a series of reactions including binding of the transcription factor smad2/3 to smad4. Through multiple target genes, the TGF-β pathway normally regulates cellular growth. Loss of smad4 (dpc4) function abolishes the smad4-dependant TGF-β pathway and gives rise to unregulated cellular proliferation.[30] Loss of smad4 nuclear labeling by immunohistochemistry is generally observed late in pancreatic carcinogenesis, such as in PanIN-3 precursor lesions and infiltrating adenocarcinomas.[31] Both *SMAD4/DPC4* mutation and loss of smad4 expression are markers of poor prognosis in pancreatic cancers.[32,33] In contrast to *SMAD4/DPC4* mutation, mutations in *TP53* and *CDKN2A/p16* have not been shown to predict survival.[33] Loss of smad4 protein expression can also be used in the differential diagnosis of carcinomas of unknown primary tumor; *SMAD4/DPC4* mutations with loss of nuclear smad4 labeling frequently occur in pancreatic adenocarcinomas, but not in extrapancreatic malignancies.[34] *SMAD4* mutations have recently been associated with poor prognosis and with the development of widespread metastases in pancreatic cancer.[33,35]

An additional tumor suppressor pathway that can be altered in pancreatic cancers involves *STK11/LKB1* on chromosome arm 19p. Germline mutations of *STK11/LKB1* are responsible for Peutz-Jeghers syndrome and are associated with intraductal papillary mucinous neoplasms (IPMNs) and invasive pancreatic cancer. In addition to germline mutations, somatic mutations of *STK11/LKB1* are observed in 5% of patients with sporadic IPMNs and pancreatic cancers.[36,37] Other tumor suppressor genes, including *TGFBR2*,[38] *MAP2K4*/*MKK4*,[39,40] *FBXW7*,[12] and *ACVR1B*4, are inactivated in a small subset of pancreatic cancers. The genetically altered genes involved in pancreatic cancer are summarized in Table 1.

3. Genetics of Precursor Lesions

There are 3 histologically recognized precursor lesions of pancreatic cancer: PanINs, IPMNs, and mucinous cystic neoplasms (MCNs).[42–45] Pancreatic intraepithelial neoplasia lesions are microscopic papillary or flat noninvasive epithelial neoplasms (<0.5 mm) arising in pancreatic ducts characterized by mucin-containing cuboidal to columnar cells.

Pancreatic intraepithelial neoplasia lesions can be further classified according to the degree of cytologic and architectural atypia as PanIN-1, PanIN-2, and PanIN-3.[43,44] Two distinct genetic events occur in early low-grade PanIN lesions (PanIN-1): telomere shortening and *KRAS2* gene mutations.[8,10,11,46,47] Activating point mutations of *KRAS2* occur in approximately 45% of PanIN-1 lesions.[8,10,11,47] Telomere shortening is found in approximately 90% of PanIN-1 lesions and may contribute to global chromosomal abnormalities in PanINs.[46] Inactivating mutations of *CDKN2A/p16* begin to occur in PanIN-2 lesions, while inactivation of *TP53*, *SMAD4/DPC4*, and *BRCA2* are generally associated with higher-grade PanIN lesions(PanIN-3).[31,48]

Intraductal papillary mucinous neoplasms are mucin-producing epithelial neoplasms, usually with papillary architecture; they arise from the main pancreatic duct or branch ducts.[44] These neoplasms are larger lesions than PanINs (≥1 cm) and therefore can be detected by imaging.[44] Activating point mutations of *KRAS2* occur in approximately 50% of IPMNs with low-grade dysplasia, and the prevalence of *KRAS* mutations increases with the degree of dysplasia.[49–51] Inactivating mutations of *CDKN2A/p16* and *TP53* are found in IPMNs with high-grade dysplasia.[52] Loss of smad4 expression is observed in only a small subset of IPMNs (3%), whereas smad4 loss in PanIN3 occurs in approximately 30% of cases.[53] As described above, somatic mutations of *STK11/LKB1*, with loss of the wild-type allele and corresponding inactivation of stk11 protein, occur in a small proportion of IPMNs.[36,37,53]

Mucinous cystic neoplasms occur predominantly in women.[42] In contrast to IPMNs, MCNs do not have a connection with the pancreatic duct. In addition, MCNs are unique among pancreatic precursor lesions because of an associated ovarian-type stroma.[42] As compared to PanINs and IPMNs, the genetic alterations of MCNs have not been well defined. Studies of MCNs[54–56] have reported a range in the prevalence of *KRAS2* mutations and p53 overexpression, with the prevalence of abnormalities increasing with increasing degrees of dysplasia. One observation is that Smad4 mutation and loss of nuclear expression do not occur in most noninvasive MCNs. As with cancers arising from PanIN-3 lesions, smad4 expression is lost when infiltrating cancers arise from MCNs.[29] This suggests that inactivation of *SMAD4/DPC4* occurs in the late stages of neoplastic progression from MCNs.[29]

4. Genetics of Histologic Variants of Pancreatic Cancer

Several histologic variants of pancreatic cancer have been described, which include adenosquamous carcinoma, colloid carcinoma, medullary carcinoma, signet ring cell carcinoma, undifferentiated carcinoma, and undifferentiated carcinoma with osteoclast-like giant cells.[42] Recognition of these variants is clinically important. Indeed, colloid and medullary carcinomas typically have better prognoses than the typical infiltrating ductal adenocarcinomas, and adenosquamous and undifferentiated carcinomas have worse prognoses than the typical ductal adenocarcinomas.[57–59] Furthermore, medullary carcinomas have distinct mechanisms of pathogenesis. We will briefly describe the genetic characteristics of these histologic variants, but we recommend a more comprehensive review for more in-depth discussion.[60]

Adenosquamous carcinomas contain both glandular and squamous components.[42] The squamous component, by definition, comprises at least 30% of the neoplasm. Adenosquamous carcinomas share similar genetic features with ductal adenocarcinomas, including *KRAS2* mutations and inactivation of *CDKN2A/p16*, *SMAD4/DPC4*, and/or *TP53*.[58] The squamous component expresses p63, which is a helpful finding for identifying squamous components. Recognition of adenosquamous carcinoma is clinically important because it is associated with worse survival than adenocarcinomas.[58]

Medullary carcinomas are characterized by well-defined pushing border, syncytial growth pattern, and poorly differentiated cancer cells.[59,61,62] Similar to medullary carcinomas of the colorectum, medullary carcinomas of the pancreas are often microsatellite unstable; this is caused either by germline or somatic mutation of the mismatch repair genes *MHL1* and *MSH2* or by epigenetic silencing of *MLH1* by promoter methylation.[22,59,61,62] Medullary carcinomas are associated with a better prognosis than ductal adenocarcinomas. Medullary colorectal cancers (with microsatellite instability) respond poorly to 5-fluorouracil–based chemotherapy, but it is not known if this 5-fluorouracil resistance applies to medullary carcinoma of the pancreas.[63]

Colloid carcinomas are characterized by well-differentiated neoplastic cells floating in pools of extracellular mucin; by definition, the mucin pools should comprise at least 80% of the tumor.[57] The neoplastic cells have intestinal differentiation and label with antibodies to MUC2 and/or CDX2.[64,65] Colloid carcinomas are associated with a better prognosis than ductal adenocarcinomas.[57]

Undifferentiated carcinomas lack histologic features of differentiation.[42,59–61] The median survival time for patients with undifferentiated pancreatic adenocarcinoma is only 5 months after surgical resection.[66] Undifferentiated carcinomas are noncohesive cancers characterized by the loss of E-cadherin protein expression.[67] The expression of L1CAM,

COX2, and EGFR proteins in undifferentiated carcinomas have been noted as possible future targets of inhibitor-based treatments.[68]

Undifferentiated carcinomas with osteoclast-like giant cells are composed of cytologically benign, multinucleated, osteoclast-like giant cells admixed with atypical pleomorphic mononuclear cells.[42] Frequently, undifferentiated carcinomas with osteoclast-like giant cells occur in association with noninvasive precursor lesions and share mutations with the associated noninvasive precursor lesions.[69–73]

5. Genetics of Familial Pancreatic Cancer

Up to 10% of pancreatic cancers have a familial basis.[74] Several cohort and case-control studies[75,76] report that individuals with first-degree relatives who have pancreatic cancer are at significantly greater risk for pancreatic cancer, a risk that increases with the number of affected relatives. Thus, the risk for pancreatic cancer in individuals with 1 first-degree relative with pancreatic cancer is 2-fold higher than that for an individual without an affected first-degree relative; persons with 2 affected first-degree relatives have a 6-fold increased risk; and persons with 3 or more affected first-degree relatives have a 14- to 32-fold increased risk for pancreatic cancer.[75,76]

Several genetic syndromes are linked to the development of familial pancreatic cancer. Hereditary breast and ovarian cancer syndrome is an autosomal, dominantly inherited disease characterized by early development of breast and ovarian cancer and germline mutation of *BRCA2* and *BRCA1*.[74] Germline mutation of *BRCA2* increases risk for pancreatic cancer 3.5- to 10-fold.[77–79] *BRCA2* is a member of the Fanconi anemia gene family, and the function of the BRCA2 gene product is to repair DNA interstrand cross-links and double-strand breaks.[80] Pancreatic cancer cells with *BRCA2* mutation are hypersensitive to DNA interstrand cross-linking agents, including mitomycin C, cisplatin, and poly(ADP-ribose) polymerase inhibitors.[81–83] Peutz-Jeghers syndrome is an autosomal, dominantly inherited disease characterized by hamartomatous polyps of the gastrointestinal tract and pigmented macules of the lips and buccal mucosa.[84] Germline mutations of *STK11/LKB1* are responsible for Peutz-Jeghers syndrome, and patients with this syndrome have a very high lifetime risk for pancreatic cancer (up to 132-fold).[84,85] As we described above, pancreatic cancers from patients with Peutz-Jeghers syndrome develop as IPMNs.

Familial atypical multiple mole melanoma (FAMMM) is an autosomal, dominantly inherited disorder characterized by multiple nevi and atypical nevi and an increased risk for malignant melanoma.[86,87] Germline mutations of *CDKN2A/p16* cause FAMMM, and patients with FAMMM and mutated *CDKN2A/p16* have a 47-fold increased risk for pancreatic cancer.[88] Hereditary pancreatitis is characterized by recurrent attacks of pancreatitis at a young age. Germline mutations of *PRSS1* are associated with a markedly increased risk for hereditary pancreatitis and a 53-fold increased risk for pancreatic cancer.[89–92] Variants in SPINK1 are associated with a moderate increased risk for pancreatitis.

Hereditary nonpolyposis colorectal cancer syndrome (HNPCC) is an autosomal, dominantly inherited disease characterized by early onset of right-sided colon cancer as well as an increased risk for endometrial cancer and carcinomas of the small intestine, stomach, endometrium, ovary, bile duct, and kidney.[93] Germline mutations of mismatch repair genes, including *MLH1*, *MSH2*, *PMS1*, *PMS2*, and *MSH6*, are associated with HNPCC. When pancreatic cancers arise in patients with HNPCC, they usually have a characteristic medullary phenotype.

Familial adenomatous polyposis (FAP) syndrome is an autosomal, dominantly inherited disease characterized by the presence of more than hundreds of polyps in the colon at an

early age.[94,95] Germline mutation of *APC* is linked with FAP. Patients with FAP have a 4-fold increased risk for pancreatic cancer.[96]

Genetic syndromes associated with familial pancreatic cancer are summarized in Table 2.

EPIGENETICS OF PANCREATIC CANCER

Epigenetics is defined as heritable changes in gene expression without accompanying changes in DNA sequence.[97] The main epigenetic mechanisms that may affect gene expression include DNA methylation, histone modification, and microRNA expression.

1. DNA Methylation

DNA methylation is the covalent binding of a methyl group (CH_3-) to the 5-carbon of cytosine residues. This methyl-group binding is catalyzed and maintained by a family of enzymes, DNA methyltransferases (DNMTs), including *DNMT1*, *DNMT3A*, and *DNMT3B*. *DNMT1* is involved in preserving parental methylation patterns and transferring these patterns to offspring. *DNMT3A* and *DNMT3B* are involved in de novo methylation.[98–100] Approximately 80% of pancreatic cancers overexpress dnmt1 protein.[101]

A major pattern of DNA methylation occurs in CpG islands. CpG islands are stretches of DNA with a high CG nucleotide content (>50%).[102] The CpG islands are frequently located near the transcriptional start sites of genes. About 60% of human genes have associated CpG islands; for many years CpG islands were thought to be unmethylated except during genomic imprinting and X-chromosome inactivation,[103] but more recent evidence indicates that some CpG islands are methylated in a tissue-specific manner,[104] and CpG island methylation increases with age at many loci.[105,106] Aberrant hypermethylation of promoter CpG islands is tightly associated with gene silencing and may be associated with loss of tumor suppressor function in cancer.[107]

Aberrant Hypermethylation in Pancreatic Cancer—Several classic tumor suppressor genes, as well as increasing numbers of functionally important genes, show aberrant promoter CpG island hypermethylation in a subset of pancreatic cancers. The first tumor suppressor gene that was shown to undergo promoter hypermethylation and silencing in pancreatic cancer was *CDKN2A/p16*.[21] Other genetically inactivated tumor suppressor genes in pancreatic cancers, including *TP53*, *MADH4/DPC4*, and *STK11/LKB1*, have not been shown to undergo epigenetic silencing by DNA methylation.

MLH1 on chromosome arm 3p undergoes DNA methylation in pancreatic cancers and is associated with microsatellite instability in medullary carcinomas.[22,108,109] The *CDH1* gene on chromosome arm 16q, which encodes E-cadherin protein, shows aberrant methylation in a small fraction of pancreatic cancers.[22]

SPARC, located on chromosome arm 5q, encodes a calcium-binding protein that interacts with extracellular matrix.[110] Sparc has effects on cellular migration, proliferation, angiogenesis during wound healing, cell-matrix adhesion, and tissue remodeling.[110] In pancreatic and other cancers, Sparc expression is usually lost through abnormal DNA methylation.[110] Pancreatic cancer–associated peritumoral fibroblasts often express Sparc, and patients with pancreatic cancer and sparc-expressing peritumoral fibroblasts were reported to have a poorer survival in 1 study.[111]

Other cancer-related genes that have been shown to undergo abnormal methylation and induced gene silencing include *RELN*,[112] CCND2,[105] *TFPI2*,[113] *RUNX3*,[114] *SOCS-1*,[115] and *TSLC1*/IGSF4.[116]

Genome-wide screening has made it possible to identify epigenetic alterations in novel genes within the setting of pancreatic cancer. Ueki and colleagues[4] used methylated CpG island amplification with representational difference analysis to identify differentially methylated CpG islands in pancreatic cancer. *PENK* (preproenkephalin) on chromosome arm 8q was one of the genes identified by this method, and more than 90% of pancreatic cancers in this study had aberrantly methylated *PENK*.[4,117] Using oligo-nucleotide microarrays, Sato and colleagues[5] identified a total of 475 candidate genes that were induced by a DNMT inhibitor (5-aza-2′-deoxycytidine) in 4 pancreatic cancer cell lines, but not in HPDE (a nonneoplastic pancreatic ductal epithelial cell line). Of these 475 genes, *UCHL1* on chromosome arm 4p was methylated in all 42 pancreatic cancers studied.[5] *RPRM* on chromosome arm 2q was methylated in 80% of pancreatic cancers studied and was associated with a worse prognosis.[118] More recently, Omura and colleagues[3] applied the methylated CpG island amplification technique to an Agilent 44K promoter microarray (Agilent Technologies, Santa Clara, California) and identified 606 differentially methylated genes in pancreatic cancer cell lines compared to normal pancreas.

A selected list of genes that are aberrantly hypermethylated in pancreatic cancer is summarized in Table 3.

Aberrant Methylation in Precursor Lesions—The discovery of abnormal methylation in pancreatic cancer has been followed by the investigation of methylation in precursor lesions. Many genes that are epigenetically silenced in pancreatic cancers also are silenced or have reduced expression in precursor lesions of pancreatic cancer. For example, global gene expression profiles of IPMN were compared with those of normal pancreatic ductal epithelial samples.[119] *CDKN1C/p57KIP2* on chromosome arm 11p codes for an inhibitor of cyclin/CDK complexes and negative regulator of cellular proliferation.[120,121] Partial methylation of the *CDKN1C/p57KIP2* promoter CpG islands in IPMNs and pancreatic cancer cell lines was correlated with a corresponding decrease in cdkn1c protein expression.[119]

Other genes identified in precursor lesions include *PENK*, *CDKN2A/p16*, *STK11/LKB1*, *SPARC*, *SFRP1*/*SARP2* (chromosome arm 8p), *TSLC1*, *RELN* (chromosome arm 7q), *TFPI2*, *CLDN5* (chromosome arm 22q), and *UCHL1* in IPMNs[37,122,123]; *PENK*, *CDKN2A/p16*, *CLDN5*, *NPTX2*, *RPRM*, *SFRP1*/*SARP2*, and *LHX1* (chromosome arm 11p) in PanINs[117,118,124]; and *CDKN2A/p16* in mucinous cystic neoplasms.[56] A selected list of genes that are aberrantly hypermethylated in pancreatic precursor lesions is summarized in Table 4.

The degree of methylation for these genes positively correlates with the degree of cytologic and architectural atypia. These findings suggest that aberrant CpG island methylation begins in the earliest stages of precursor lesions, such as PanINs, IPMNs, and MCNs, and their prevalence progressively increases during pancreatic carcinogenesis.

Aberrant Hypomethylation in Pancreatic Cancer—In addition to hypermethylation as a mechanism of carcinogenesis, aberrant loss of methylation (hypomethylation of DNA) is also common in pancreatic adenocarcinomas. Hypomethylation can be detected at the genomic scale (global hypomethylation) and at the sequence-specific level (regional hypomethylation). Although global DNA hypomethylation associated with cancer was firstly described in the early 1980s,[125,126] its significance is not known, but it may contribute to genomic instability. Folate and vitamin B12 deficiency can cause global DNA hypomethylation, which is associated with decreased levels of the methyl-group donor S-adenosylmethionine. Decreased DNA methylation results in decreased thymidine synthesis from uracil.[127] Misplacement of uracil into thymidine leads to an imbalance of nucleotide

pools and an increased frequency of DNA strand breaks; this can lead to genomic instability that can promote the development of cancer.[128,129] Pancreatic cancers with defective methylenetetrahydrofolate reductase genotypes have more DNA hypomethylation, which is associated with increased chromosomal loss and genomic instability.[130]

DNA hypomethylation occurs at the 5′ regions of certain genes in pancreatic cancer and is associated with overexpression of the encoded protein. Thus, whereas hypermethylation results in overregulation and silencing of gene and protein expression, hypomethylation can result in loss of regulation and the promotion of gene and protein expression. *S100A4* is linked with hypomethylation at specific CpG sites within the first intron and is associated with protein overexpression.[131,132] Other frequently hypomethylated genes, including *CLDN4* (chromosome arm 7q, encoding claudin-4), *LCN2* (chromosome arm 9q, encoding lipocalin-2), *SFN/14-3-3σ* (chromosome arm 18q), *TFF2* (chromosome arm 21q, encoding trefoil factor 2), *MSLN* (chromosome arm 16p, encoding mesothelin), and *PSCA* (8q, encoding prostate stem cell antigen), are overexpressed in pancreatic cancer cells in comparison with normal pancreatic duct.[132] With oligo-nucleotide microarray technologies, 2 additional genes, *S100P* (chromosome arm 4p) and *SERPINB5* (chromosome arm 18q, encoding maspin), have been identified as being hypomethylated and are overexpressed.[6] A selected list of genes that are aberrantly hypomethylated in pancreatic cancer is summarized in Table 2.

2. MicroRNAs

Aberrant MicroRNA Expression in Pancreatic Cancers and Precursor Lesions —MicroRNAs (miRNAs) are a recently described family of small, nonprotein-coding RNA molecules (18 to 24 nucleotides) that regulate transcription of target messenger RNAs.[133] More than 400 miRNAs in the human genome have been described and many are implicated in the regulation of cellular differentiation, proliferation, and apoptosis.[23] Aberrant miRNA expression has been described in many types of cancers.[134,135] Several mechanisms are involved in aberrant miRNA expression, including genetic (amplification and deletion)[136–138] and epigenetic (chromatin modification, DNA methylation) alterations[139–141] and transcription factor regulation.[142,143]

Pancreatic ductal adenocarcinomas have been shown to aberrantly express numerous miRNAs, including miR-200, miR-34, miR-21, miR-155, miR-221, and miR-222.[144–151] For example, Li and colleagues[152] have demonstrated hypomethylation and overexpression of miR-200a and miR-200b. Aberrant expression of some of these miRNAs is evident in PanINs. For example, miR-155 overexpression is evident in PanIN-2 lesions and aberrant miR-21 expression is evident in PanIN-3 lesions.[153] Similarly, Habbe et al[154] have reported abnormal miR-21 and miR-155 expression in IPMN lesions.

CONCLUSIONS

Pancreatic ductal adenocarinoma continues to be a fatal cancer that is difficult to treat. In the past decade, major advances have been made in the understanding of the earliest histologic and molecular changes that occur in precursor lesions and cancers of the pancreas. Subclassification of pancreatic adenocarcinomas according to its histologic features and molecular alterations could have important therapeutic and prognostic importance. In addition, the identification of molecular signatures that identify the earliest changes of carcinogenesis may lead to the earlier detection of pancreatic cancer. The survival data for pancreatic cancer clearly illustrate that patients do much better with earlier detection and surgical resection regardless of adjuvant chemotherapy or radiotherapy intervention. Understanding the signature of molecular alterations that occur before the development of invasive pancreatic cancer may lead to improved detection and survival in pancreatic cancer.

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Table 1

List of Selected Genes That Are Genetically Altered in Pancreatic Cancer

Gene Symbol	Gene Name	Genetic Alteration	Mechanism of Genetic Alteration	Chromosome Site	Known or Predicted Function	Alteration in Primary Pancreatic Cancer, %	Source, y
CDKN2A/p16	Cyclin-dependent kinase inhibitor 2A	Inactivation	Homozygous deletion (41%), intragenic mutation (38%)	9p21	Cyclin-dependent kinase inhibitor	95	Caldas et al,[17] 1994
KRAS2	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	Activation	Point mutation	12p12.1	Signal transduction, proliferation, cell survival, and motility	>90	Hruban et al,[8] 1993
TP53	Tumor protein p53	Inactivation	Intragenic mutation in 1 allele and loss in the other allele	17p13.1	Cell cycle arrest, apoptosis, senescence, DNA repair, metabolism change	50–70	Redston et al,[19] 1994 Moore et al,[24] 2001 Scarpa et al,[25] 1993
SMAD4/DPC4	Mothers against decapentaplegic, drosophila, homolog of, 4	Inactivation	Homozygous deletion (50%), intragenic mutation in 1 allele and loss in the other allele (50%)	18q21.1	Signal transmission	55	Iacobuzio-Donahue et al,[27] 2004 Hahn et al,[28] 1996
AKT2	v-akt murine thymoma viral oncogene homolog 2	Activation	Amplification	19q13.1-q13.2	AKT pathway, hormone metabolism	10–20	Ruggeri et al,[13] 1998 Cheng et al,[14] 1996
MLH1	mutL homolog 1, colon cancer, nonpolyposis type 2 (*E. coli*)	Inactivation	Heterozygous mutations	3p21.3	DNA mismatch repair	3–15	Goggins et al,[59] 1998 Wilentz et al,[61] 2000
BRCA2	Breast cancer 2, early onset	Inactivation	Homozygous deletion	13q12.3	DNA repair, proliferation, differentiation	7	Goggins et al,[155] 1996
STK11/LKB1	Serine/threonine kinase 11	Inactivation	Homozygous deletion, intragenic mutation in 1 allele and loss in the other allele	19p13.3	Apoptosis regulation	5	Su et al,[36] 1999
BRAF	v-raf murine sarcoma viral oncogene homolog B1	Activation	Point mutation	7q34	Signal transduction, cell growth	5	Calhoun et al,[12] 2003
TGFBR2	Transforming growth factor, β receptor II (70/80 kDa)	Inactivation	Homozygous deletion, homozygous frameshift mutation	3p22	Signal transduction	4	Goggins et al,[38] 1998

Gene Symbol	Gene Name	Genetic Alteration	Mechanism of Genetic Alteration	Chromosome Site	Known or Predicted Function	Alteration in Primary Pancreatic Cancer, %	Source, y
MAP2K4	Mitogen-activated protein kinase kinase 4	Inactivation	Homozygous deletions, missense mutation	17p11.2	MAPK pathway	2	Su et al,[39] 1998 Teng et al,[40] 1997

Abbreviation: MAPK, mitogen-activated protein kinase.

Table 2

Genetic Syndromes Associated With Familial Pancreatic Cancer

Genetic Syndrome	Gene Symbol	Relative Risk of Developing Pancreatic Cancer (Fold)	Histologic Feature of Pancreatic Neoplasm	Extrapancreatic Cancer	Source, y
No familial history	None	1	Ductal adenocarcinoma Intraductal papillary mucinous neoplasm, ductal adenocarcinoma, pancreatoblastoma	Unknown	
Familial adenomatous polyposis	*APC*	4		Colorectum, small intestine, stomach	Giardiello et al,[96] 1993
Familial atypical multiple mole melanoma	*CDKN2A/p16*	13–22	Ductal adenocarcinoma	Melanoma	Gruis et al,[86] 1995 de Snoo et al,[88] 2008
Familial pancreatic cancer	Unknown	2–32	Ductal adenocarcinoma		Amundadottir et al,[75] 2004 Klein et al,[76] 2004
Hereditary breast and ovarian cancer	*BRCA2*, *BRCA1*, *FANCC*, *FANCG*, *PALB2*	3.5–10	Ductal adenocarcinoma	Breast, ovary, prostate	Hruban et al,[77] 1999 Hahn et al,[78] 2003 van Asperen et al,[79] 2005
Hereditary pancreatitis	*PRSS1*, *SPINK1*	53	Ductal adenocarcinoma	None	de las Heras-Castano et al,[89] 2009 Lowenfels et al,[90] 1997 Schneider et al,[91] 2002 Whitcomb et al,[92] 1996
Hereditary nonpolyposis colorectal cancer syndrome	*MLH1*, *MSH2*	Increased	Medullary carcinoma	Colorectum, small intestine, endometrium	Wilentz et al,[61] 2000
Peutz-Jeghers syndrome	*SKT11/LKB1*	132	Intraductal papillary mucinous neoplasm, ductal adenocarcinoma	Small intestine, colorectum, esophagus, stomach, bile duct, lung, breast, ovary, uterus	Zbuk & Eng,[84] 2007 Giardiello et al,[85] 2000

Table 3

List of Selected Genes That Are Aberrantly Methylated in Pancreatic Cancer

Gene Symbol	Gene Name	Epigenetic Alteration	Chromosome Site	Known or Predicted Function	Methylation in Pancreatic Cancer Cell Lines, No. (%)	Methylation in Primary or Xenografted Pancreatic Cancer, No. (%)	Source, y
PENK	Preproenkephalin	Hypermethylation	8q23-q24	Neuropeptide precursor	11/11 (100)	43/47 (91)	Ueki et al,[4] 2001 Fukushima et al,[117] 2002
UCHL1	Ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)	Hypermethylation	4p14	Ubiquitin hydroxylase	22/22 (100)	42/42 (100)	Sato et al,[5] 2003
MDF-1	MAD (yeast Mitosis Arrest DeFicient) related	Hypermethylation	11q13	Glycogen metabolism	45/47 (96)	Not determined	Omura et al,[3] 2008
NPTX2	Neuronal pentraxin II	Hypermethylation	7q21.3-q22.1	Neuronal transport	21/22 (95)	20/20 (100)	Sato et al,[5] 2003
SPARC/ON	Secreted protein, acidic, cysteine-rich (osteonectin)	Hypermethylation	5q31.3-q32	Cell-cycle progression inhibition, cell-matrix interaction	16/17 (94)	21/24 (88)	Sato et al,[110] 2003
RPRM	Reprimo, TP53-dependent G2 arrest mediator candidate	Hypermethylation	2q23.3	P53-induced G2/M cell-cycle arrest	20/22 (91)	16/20 (80)	Sato et al,[118] 2006
BNIP3	BCL2/adenovirus E1B 19 kDa interacting protein 3	Hypermethylation	10q26.3	Hypoxia-induced cell death	9/10 (90)	8/10 (80)	Okami et al,[156] 2004
miR9-1	MicroRNA 9-1	Hypermethylation	1q22	miRNA translation control	42/47 (89)	Not determined	Omura et al,[3] 2008
SERPINB5	Serpin peptidase inhibitor, clade B, member 5 (maspin)	Hypomethylation	18q21.3	Regulation of cell motility and cell death	20/23 (87)	32/34 (94)	Sato et al,[132] 2003 Fitzgerald et al,[157] 2003 Ohike et al,[158] 2003
CCND2	Cyclin D2	Hypermethylation	12p13	Cell-cycle control	19/22 (86)	71/109 (65)	Matsubayashi et al,[105] 2003
ZNF415	Zinc finger protein 415	Hypermethylation	19q13.42		40/47 (86)	Not determined	Omura et al,[3] 2008
CLDN4	Claudin-4	Hypomethylation	7q11.23	Cell adhesion/invasion	17/20 (85)	33/37 (89)	Sato et al,[132] 2003

Gene Symbol	Gene Name	Epigenetic Alteration	Chromosome Site	Known or Predicted Function	Methylation in Pancreatic Cancer Cell Lines, No. (%)	Methylation in Primary or Xenografted Pancreatic Cancer, No. (%)	Source, y
SFN	Stratifin (14-3-3 σ)	Hypomethylation	1p35	P53-induced G2/M cell-cycle arrest	17/20 (85)	36/37 (97)	Sato et al,[132] 2003 Iacobuzio-Donahue et al,[159] 2003
LCN2	Lipocalin-2	Hypomethylation	9q34	Epithelial differentiation	17/20 (85)	34/37 (92)	Sato et al,[132] 2003
TFPI2	Tissue factor pathway inhibitor 2	Hypermethylation	7q22	Serine protease inhibitor	14/17 (82)	102/140 (73)	Sato et al,[113] 2005
CNTNAP2	Contactin-associated protein-like 2	Hypermethylation	7q35-q36	Higher cortical function	39/47 (82)	Not determined	Omura et al,[3] 2008
CDKN1C/p57	Cyclin-dependent kinase inhibitor 1C	Hypermethylation	11p15.5	Cyclin-dependent kinase inhibitor	7/9 (78)	Not determined	Sato et al,[119] 2005
SIP1	Survival of motor neuron protein-interacting protein 1	Hypermethylation	14q13-q21	Assembly of spliceosomal snRNP	11/15 (73)	34/35 (97)	Li et al,[152] 2010
ELOVL4	Elongation of very-long-chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)–like 4	Hypermethylation	6q14	Fatty acid synthesis	32/47 (68)	Not determined	Omura et al,[3] 2008
TFF2	Trefoil factor 2	Hypomethylation	21q22.3	Secretory polypeptide/epithelial repair	13/20 (65)	31/37 (84)	Sato et al,[132] 2003
FOXE1	Forkhead box E1 (thyroid transcription factor 2)	Hypermethylation	9q22	Thyroid transcription factor	14/22 (64)	15/20 (75)	Sato et al,[5] 2003
S100P	S100 calcium-binding protein P	Hypomethylation	4p16	Cell-cycle progression and differentiation	13/23 (57)	30/34 (88)	Sato et al,[132] 2003
RARB	Retinoic acid receptor, β	Hypermethylation	3p24	Cell-growth control	5/9 (56)	4/36 (11)	Ueki et al,[22] 2000
S100A4	S100 calcium-binding protein A4	Hypomethylation	1q21	Motility, invasion, tubulin polymerization	10/20 (50)	28/37 (76)	Rosty et al,[131] 2002 Sato et al,[132] 2003
CDKN2A/p16	Cyclin-dependent kinase inhibitor 2A	Hypermethylation	9P21	Cyclin-dependent kinase inhibitor	3/9 (33)	5/36 (14)	Schutte et al,[21] 1997 Ueki et al,[22] 2000

Gene Symbol	Gene Name	Epigenetic Alteration	Chromosome Site	Known or Predicted Function	Methylation in Pancreatic Cancer Cell Lines, No. (%)	Methylation in Primary or Xenografted Pancreatic Cancer, No. (%)	Source, y
MSLN	Mesothelin	Hypomethylation	16p13.3	Cell surface antigen/cell adhesion	8/20 (40)	34/37 (29)	Sato et al,[132] 2003
SOCS1	Suppressor of cytokine signaling 1	Hypermethylation	16p13.13	Inhibitor of JAK/STAT pathway	6/19 (32)	13/60 (22)	Fukushima et al,[115] 2003
PSCA	Prostate stem cell antigen	Hypomethylation	8q24.2	Cell surface antigen/cell differentiation	6/20 (30)	20/37 (54)	Sato et al,[132] 2003
CADM1/TSLC1	Cell adhesion molecule 1	Hypermethylation	11q23.2	Cell-cell, cell-matrix interaction	4/17 (24)	25/91 (27)	Jansen et al, [116] 2002

Abbreviations: JAK/STAT, Janus kinase/signal transducer and activator of transcription; miRNA, microRNA; snRNP, small nuclear ribonucleoprotein.

Table 4

List of Selected Genes That Are Aberrantly Hypermethylated in Pancreatic Precursor Lesions

Gene Symbol	Gene Name	Precursor Lesions	Methylation in Low-Grade Dysplasia (PanIN-1 or Low-Grade Dysplasia of IPMN or MCN), No. (%)	Methylation in Moderate-Grade Dysplasia (PanIN-2 or Moderate-Grade Dysplasia of IPMN or MCN), No. (%)	Methylation in High-Grade Dysplasia (PanIN-3 or High-Grade Dysplasia of IPMN or MCN), No. (%)	Methylation in Precursor in Total, No. (%)	Source, y
PENK	Preproenkephalin	IPMN	1/6 (17)	4/12 (33)	27/32 (84)	32/50 (64)	Sato et al,[122] 2002
		PanIN	5/67 (7)	5/22 (23)	6/13 (46)	16/108 (15)	Fukushima et al,[117] 2002
		PanIN	1/38 (3)	1/14 (7)	7/12 (58)	9/64 (14)	Sato et al,[124] 2008
CDKN2A/p16	Cyclin-dependent kinase inhibitor 2A	IPMN	0/6 (0)	0/12 (0)	7/32 (22)	7/50 (14)	Sato et al,[122] 2002
		PanIN	4/63 (6)	1/22 (5)	3/14 (21)	8/99 (8)	Fukushima et al,[117] 2002
		PanIN	3/38 (8)	1/15 (7)	3/11 (27)	7/64 (11)	Sato et al,[124] 2008
		MCN	1/10 (10)	1/4 (25)	NA	2/14 (14)	Kim et al,[56] 2003
SPARC/ON	Secreted protein, acidic, cysteine-rich (osteonectin)	IPMN	7/12 (58)	7/12 (58)	16/22 (73)	30/48 (63)	Hong et al,[123] 2008
		PanIN	10/36 (21)	3/14 (21)	3/10 (30)	16/60 (27)	Sato et al,[124] 2008
SFRP1/*SARP2*	Secreted frizzled– related protein 1	IPMN	6/12 (50)	8/12 (67)	21/23 (91)	35/57 (61)	Hong et al,[123] 2008
		PanIN	2/37 (5)	3/15 (20)	10/12 (83)	15/64 (23)	Sato et al,[124] 2008
NPTX2	Neuronal pentraxin 2	PanIN	2/35 (8)	6/13 (46)	4/12 (33)	12/60 (20)	Sato et al,[124] 2008
CADM1/TSLC1	Cell adhesion molecule 1	IPMN	6/12 (50)	8/12 (67)	21/23 (91)	35/57 (61)	Hong et al,[123] 2008
RELN	Reelin	IPMN	3/12 (25)	4/12 (33)	11/23 (48)	18/57 (32)	Hong et al,[123] 2008
TFPI2	Tissue factor pathway inhibitor 2	IPMN	3/12 (25)	5/12 (42)	20/23 (87)	28/57 (49)	Hong et al,[123] 2008
CLDN5	Claudin-5	IPMN	4/12 (33)	5/12 (42)	15/23 (65)	24/57 (42)	Hong et al,[123] 2008
		PanIN	3/35 (9)	1/15 (7)	4/11 (36)	8/61 (13)	Sato et al,[124] 2008
UCHL1	Ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)	IPMN	7/12 (58)	10/12 (83)	21/23 (19)	38/57 (67)	Hong et al,[123] 2008
RPRM	Reprimo, TP53-dependent G2 arrest mediator candidate	PanIN	8/36 (22)	3/15 (20)	8/12 (67)	19/63 (30)	Sato et al,[118] 2006
LHX1	LIM homeobox 1	PanIN	3/37 (8)	1/15 (7)	5/12 (42)	9/64 (14)	Sato et al,[124] 2008

Abbreviations: IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; NA; PanIN, pancreatic intraepithelial neoplasia.