Pathology, genetic alterations, and targets of differentially expressed microRNAs in pancreatic cancer

Abstract: Since their discovery in mammals in 2001, the field of microRNA (miRNA) research has grown exponentially. miRNAs regulate protein translation following binding to conserved sequences within the 3′ untranslated region of messenger RNAs. miRNAs are found to regulate nearly all biological processes, and their expression has been shown to differentially regulate a large number of diseases including cancer. Pancreatic ductal adenocarcinoma (PDAC) was one of the initial groups of cancers to demonstrate differential miRNA expression. Since then, there have been numerous studies linking differential miRNA expression to PDAC. Translational extrapolation of these studies has been done linking diagnostic, prognostic, and therapeutic applications, and multiple review articles and book chapters have been written on these subjects. The intent here is to provide an overview of pancreatic cancer and review the current state of the validated and published findings on the messenger RNA targets of differentially expressed miRNAs in PDAC. We then attempt to summarize these findings to extrapolate them in the hopes of better understanding how altered miRNA expression in PDAC may alter the phenotype of this disease.

Keywords: microRNA, pancreatic cancer, pancreatic ductal adenocarcinoma, target

Endocrine pancreatic cancers

Although pancreatic cancer is the tenth most common type of cancer in terms of incidence, it is the fourth leading cause of cancer-related death in the USA. The annual death rate is approximately 35,000, with a 5-year survival rate of less than 6%. While multiple types of cancers of the pancreas exist, the term “pancreatic cancer” most often refers to pancreatic ductal adenocarcinoma (PDAC) since it comprises 75%–90% of pancreatic tumors. Cancers of the pancreas are often classified based on their origin.

The pancreas is subdivided into the endocrine pancreas, the exocrine pancreas, and the surrounding stroma. The endocrine pancreas is responsible for regulation of carbohydrate metabolism. Cells of the endocrine pancreas form the islets of Langerhans, which are spherical centers containing alpha, beta, and delta cells surrounded by reticular fibers and blood capillaries. These cells compose roughly 1% of the pancreas and are mostly localized in the tail end of the organ. Islet cells as a group secrete insulin, glucagon, somatostatin, and pancreatic polypeptide. Tumors of the endocrine pancreas comprise less than 2% of all pancreatic cancers. They are known as pancreatic neuroendocrine tumors and are classified based on the hormone-secreting properties of the cells. Types of pancreatic neuroendocrine tumors include insulinomas, gastrinomas, glucagonomas, VIPomas, and somatostatinomas.

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Pancreatic neuroendocrine tumors are associated with loss of the multiple endocrine neoplasia type 1 gene (MEN1) and can range from less aggressive to highly malignant disease.\textsuperscript{10,11}

**Exocrine pancreatic cancers**

Acinar cell carcinoma, pancreatoblastoma, and PDAC all arise from the exocrine part of the pancreas, which is composed of acinar cells, centroacinar cells, and ductal cells. Acinar cells are pyramidal-shaped cells that comprise the acini lobules. Acini make up approximately 84% of the pancreas and are responsible for secreting digestive proenzymes that are later activated in the duodenum.\textsuperscript{2,12} These cells have prominent Golgi apparatus to accommodate the secretion of over 20 digestive enzymes packaged in the zymogen granules. The enzymes secreted include proteases (ie, trypsin, chymotrypsins, carboxypeptidases, aminopeptidase, and elastase), amylase, lipases, and nuclease.\textsuperscript{12} Centroacinar cells are present within the acini and are located at the origin of the draining ducts. These cells are much smaller and lack the enzyme containing zymogen granules present in acinar cells. Centroacinar cells precede ductal cells in the formation of the ductal system, which gradually increases in size from intercalated and intralobular ducts to major ducts.\textsuperscript{13} Cells forming the ductal system (cubic, goblet, and cylinder epithelial cells) contribute to 10% of the total pancreas. The ductal system guarantees the delivery of the acini enzymes to the intestine, and serves a secretory function by contributing to the pancreatic juice via secretion of bicarbonate.\textsuperscript{4,12}

The exocrine pancreas gives rise to multiple types of malignancies. Serous cystadenocarcinomas appear to have a centroacinar origin and lack mucinous differentiation. Although serous cystadenocarcinomas tend to form multiple fluid-filled cysts that are fairly benign, a subset shows recurrence and metastasis.\textsuperscript{14} Acinar cells can give rise to acinar cell carcinoma.\textsuperscript{15} These carcinomas are very rare and sometimes secrete digestive enzymes that lead to hypersecretion syndrome. Acinar cell carcinomas have early metastasis to the liver, are highly cellular, and lack the KRAS mutation present in other types of pancreatic cancer.\textsuperscript{16–18} Although very rare, tumors of mixed lineages also occur in the pancreas. Pancreatoblastoma is a prime example of such tumors. Pancreatoblastomas are well demarcated, consist of acinar, ductal, and endocrine cell types, and are more commonly found in early childhood.\textsuperscript{19} Other subtypes of mixed and/or unknown origin also exist.\textsuperscript{7}

Exocrine tumors of the pancreas can also be of ductal cell origin. Adenosquamous, squamous carcinoma, colloid carcinoma, medullary carcinoma, and signet ring cell carcinoma are all examples of exocrine tumors of ductal origin. These carcinomas, however, are very rare in the pancreas.\textsuperscript{20–23} As previously mentioned, PDAC has the highest incidence among the exocrine tumors of the pancreas. As the name suggests, PDAC shows ductal-like differentiation with expression of cytokeratins 7, 8, 13, 18, and 19\textsuperscript{24–26} and mucin-positive cells that are typically cuboidal-shaped.\textsuperscript{27} For these reasons, PDAC has been historically classified as a ductal tumor of the exocrine pancreas. Another feature of this carcinoma is the desmoplastic stroma that the host tissue produces around the tumor. This desmoplastic reaction is composed of pancreatic stellate cells, fibroblasts, and extracellular matrix proteins like fibronectin I and collagens I and III.\textsuperscript{28} This stroma is very similar to the fibrosis caused by chronic pancreatitis (CP), which makes proper pathological diagnosis which can confound proper pathological diagnosis.\textsuperscript{29–31} PDAC is also very aggressive and highly metastatic to the liver and lymph nodes, with only 10%–20% of cases qualifying for resection at the time of diagnosis.\textsuperscript{32–34} These features, along with high stromal content, impose a barrier to effective drug delivery\textsuperscript{35} and contribute to the high mortality of the disease.

**Precursor lesions to PDAC**

Before the full-blown development of PDAC, a number of precursor lesions can be observed in human patients and mouse models of the disease. Pancreatic intraepithelial lesions (PanINs) are the most closely recapitulated lesions in mouse models of PDAC. Subclassification of these lesions depends on the amount of cellular atypia that is found. PanIN-1 lesions are the lowest grade lesions, and are marked by small nuclei and tall ductal “columnar” cells.\textsuperscript{36} As the lesions and atypia progress, the amount of Ki67 immunostaining (or cellular proliferation) increases.\textsuperscript{37,38} PanIN-2 lesions have more papillary architecture to the lesion and also contain nuclear abnormalities, such as expanded nuclei and loss of polarity. PanIN-3 lesions are strictly papillary or micropapillary, and are characterized by luminal necrosis, mitosis, macronucleoli, and loss of polarity.\textsuperscript{39} In terms of mutations, cyclin-dependent kinase inhibitor 2A (p16/CDKN2A) p16 loss is widely found in PanIN-2 lesions (16%–30%);\textsuperscript{39,40} p53 and SMAD family member 4 (SMAD4) loss are late mutations that appear to be exclusive to carcinoma in situ (PanIN-3).\textsuperscript{35,41} The oncogenic Kras is already present in 36% of PanIN-1a lesions, 44% of PanIN-1b lesions, and 87% of cases with PanIN-2 grade or higher.\textsuperscript{42} It is important to note that the early prevalence of this mutation demonstrates the necessity of this oncogenic change for cancer progression.
Risk factors for PDAC

Risk factors for PDAC include family history of certain cancer syndromes, cigarette smoking, obesity, and pancreatitis. Familial atypical multiple mole melanoma and Peutz-Jeghers syndrome have been linked with a risk for PDAC. Cigarette smoking is the only preventable risk factor identified so far and accounts for up to 25% of PDAC cases, although alcohol consumption has also been independently linked. Studies have reported up to an 18-fold increase in the incidence of pancreatic cancer in patients suffering from CP, but whether CP is a precursor or a result of PDAC is controversial. Not only is there an increased incidence of PDAC in patients with CP, but also the desmoplastic reaction mentioned above is histologically similar if not identical to pancreatitis. This implies that PDAC will always happen in the presence of something that looks like CP. However, the confounding factor of PDAC arising in the ductal cells and CP arising in acinar cells has inspired debate. Logsdon and Ji argue that CP is in fact a precursor of PDAC for various reasons. First, the precursor PanIN lesions of PDAC are found in almost all patients with CP. Second, the early Kras oncogenic mutation is found in approximately 30% of CP cases (although reports vary widely). Third, increased Kras activity can lead to PDAC as well as the inflammatory responses that lead to CP. Finally, a new mouse model that expresses the oncogenic Kras exclusively in the acinar cells can reiterate the PanIN progression to PDAC when combined with experimentally induced CP. This recent evidence questions the cell of origin of PDAC and has strengthened the paradigm that CP could be a precursor to PDAC.

Genetic alterations in PDAC

Like all human cancers, PDAC is a disease consisting of multiple genetic changes that are either inherited or accumulate throughout the patient’s lifetime. PDAC is marked by a series of mutations in tumor suppressor genes and oncogenes. Tumor suppressor genes promote tumor growth upon their inactivation and oncogenes upon their activation. The most prevalent altered genes in PDAC are KRAS, receptor tyrosine-protein kinase erbB-2 (HER-2/neu, ERBB2), CDKN2A, TP53, SMAD4, and breast cancer 2, early onset (BRCA2). In a landmark paper, Jones et al used next-generation DNA sequencing on PDAC from 24 patients to demonstrate that PDAC stems from mutations of primarily four different genes, ie, KRAS, SMAD4, TP53, and CDKN2A. It has also been estimated that up to 70% of PDAC overexpresses HER-2/neu. HER-2/neu is part of the epidermal growth factor receptor family, is not expressed in normal ducts, but has an 80%–90% rate of expression in low-grade and high-grade PanINs. CDKN2A protein regulates the cell cycle by controlling the G1/S transition, a major check-point for cell division. CDKN2A protein is encoded in the CDKN2A gene in humans and is lost in 95% of pancreatic cancers by deletion, mutation, or hypermethylation. The TP53 gene encodes for the p53 protein, which is responsible for regulation of various points of the cell cycle and induction of apoptosis. Unregulated cell proliferation due to loss of p53 occurs in 55%–75% of pancreatic cancers. Inactivation of SMAD4 is present in about 50% of PDAC cases. SMAD4 is a downstream target of the transforming growth factor-beta (TGF-β) pathway, and upon activation of the signaling cascade, it travels to the nucleus to transcriptionally regulate cellular growth genes. Lastly, BRCA2 mutations have also been linked to PDAC. BRCA2 mutations lead to a defective BRCA2 protein that is unable to repair double strand breaks in DNA. The mutated gene is present in around 10% of PDAC cases and may be acquired or inherited, and individuals with this germline mutation can have up to a 10-fold increased chance of developing PDAC. The risk is even greater for other types of cancers.

In terms of oncogenic mutations, KRAS is central to PDAC. KRAS is part of the gene family that also contains neuroblastoma RAS viral (v-ras) oncogene homologue (NRAS) and Harvey rat sarcoma viral oncogene homologue (HRAS). The proteins encoded by these genes are G-like regulatory proteins that are involved in the control of cell growth. The Ras family of proteins comprises GTPases that convert guanosine-5'-triphosphate (GTP) into guanosine-5'-diphosphate (GDP). While bound to GTP, they are active and can trigger downstream pathways, but are turned off with the conversion to GDP. As a family, these proteins differ in their C-terminus and in the downstream pathways that they activate. KRAS is mutated in more than 90% of PDAC cases. Mutations in KRAS almost exclusively occur in codons 12, 13, and 61. KRAS and other Ras proteins are GTP-bound. Hydrolysis of GTP to GDP by GTPase-activating proteins turns the protein to an “off” state. Mutations in these codons cause KRAS to be resistant to GTPase-activating protein hydrolysis and are therefore in the constitutively active state. Constitutive KRAS activity leads to continuous induction of the mitogen-activated protein kinase and phosphatidylinositol-3 kinase-mammalian target of rapamycin (mTOR) pathways, which in turn leads to increased cell proliferation, angiogenesis, migration, and increased survival. In addition to the high prevalence and strong oncogenic activity of the KRAS mutations, this onco-
MicroRNAs (miRNAs) are small noncoding RNAs that are processed from larger precursor RNAs. The active, mature miRNA is roughly 21 nucleotides in length and functions by binding through partial complementarity to conserved sequences, typically within the 3’ untranslated region of protein coding messenger RNA (mRNA). Nucleotides 2–8 of the mature miRNA (ie, the so-called 5’ seed sequence) must interact with complete complementarity for the miRNA to be functional. Interaction of the miRNA with the mRNA most often results in reduced translation; sometimes with degradation of the mRNA but other times without mRNA degradation. Discovery and validation of miRNA target genes vary from study to study. One approach involves mining gene expression profiling or RNA sequencing data to look for inverse expression levels of miRNA and mRNA. Published target prediction programs are used to identify putative targets of the miRNA. Studies are validated using luciferase reporter assays to examine reduced luciferase expression following binding of the synthetic pre-miRNA to the target sequence of miRNA cloned downstream of the luciferase reporter gene. Also, Western blotting is a convenient tool to look for reduced expression of the protein in cell lines transfected with pre-miRNA oligo.

PDAC was among the first groups of cancers to be linked to altered miRNA expression. In addition to pancreas tissue, differentially expressed miRNAs have been identified in PanINs, plasma, stool, and cystic fluid of patients with pancreatic disease. Differentially expressed miRNA is defined as those that are either increased or decreased in the tumor compared with normal pancreas or pancreas tissue that is benign and adjacent to the tumor. The definition of the fold change varies, but is commonly a 1.5–2-fold difference in expression. Differential miRNA expression in PDAC and in cystic pancreatic lesions has been reported in a number of excellent, recent review articles and that information will not be repeated here. Instead, the remainder of this review focuses on published, validated target genes of differentially expressed miRNAs in PDAC. The data are summarized in Table 1. The table lists the differentially expressed miRNA, if it is increased or decreased in PDAC, the validated mRNA target gene from the study, the function of the target gene, and finally the citation. We will review what we believe to be the most important targets of differentially expressed miRNAs in PDAC. Due to space limitations, we will not report on each of the studies listed in Table 1.

miR-21

miR-21 is perhaps the most studied, differentially expressed miRNA in PDAC. The expression of miR-21 is increased in nearly all solid tumors, especially those of epithelial origin, as reviewed in Selcuklu et al. The MIR21 gene is found on human chromosome 17q23.1 and is located within exon 1 of the vacuole membrane protein 1 (VMP1) gene. Validated target miRNAs of miR-21 in PDAC include the tumor suppressors programmed cell death 4 (PDCD4), B-cell translocation gene 2 (BTG2), tissue inhibitor metallopeptidase 3, reversion-inducing-cysteine-rich protein with kazal motifs (RECK), and B-cell chronic lymphocytic leukemia/lymphoma 2 (BCL2). miR-21, along with miR-23a and miR-27a, acted synergistically to suppress a group of five tumor suppressors in PDAC. Inhibition of these miRNA with anti-miRNAs resulted in reduced cell proliferation in vitro and reduction in tumor burden in vivo. miR-21-mediated repression of PDCD4 resulted in increased cell proliferation and reduced cell death in vitro. Anti-miR-21 reduced cell proliferation in PDAC cell lines and there was a direct correlation between exposure to anti-miR-21 and increased expression of the tumor suppressors phosphatase and tensin homologue and RECK. A curcumin analog reduced miR-21 expression in a gemcitabine-resistant PDAC cell line; however, no miR-21 target gene associated with the resistance was noted.

miR-200 family

The miR-200 family consists of five miRNAs, ie, miR-200a, miR-200b, miR-200c, miR-141, and miR-429. miR-200c and miR-141 are located on human chromosome 12p13.31, and miR-200a, miR-200b, and miR-429 are present on human chromosome 1p36.33. The miR-200 family has been widely implicated in modulating the epithelial mesenchymal transition in pancreatic and other types of cancer. Epithelial mesenchymal transition is the process by which cells lose their epithelial morphology and become more mesenchymal-like, with increased invasiveness and metastatic potential.
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(Continued)
Epithelial cells express high levels of E-cadherin (CDH1) while mesenchymal cells have high expression in zinc finger E-box binding homeobox 1 (ZEB1), vimentin, and N-cadherin. ZEB1 suppresses CDH1 and the miR-200 family also suppresses ZEB1. Therefore, a feedback loop exists in which low levels of the miR-200c family in mesenchymal cells result in increased levels of ZEB1 and therefore reduced CDH1.\(^{113,114}\)

Reduced levels of the miR-200 family and increased expression of miR-21 was observed in gemcitabine-resistant cells compared with gemcitabine-sensitive cells.\(^{112}\) In addition to the epithelial mesenchymal transition markers, the miR-200 family has also been shown to target the coding sequences of mucin 4 (MUC4) and MUC16.\(^{116}\) MUC4 and MUC16 are highly overexpressed in PDAC and are involved in the metastatic phenotype of pancreatic and ovarian cancer cells.

**miRNAs associated with invasion and metastasis of PDAC**

In addition to the miR-200 family, a number of differentially expressed miRNAs have been shown to regulate invasion and metastasis in PDAC. miR-143 targets several metastasis-regulating genes, including Rho guanine nucleotide exchange factors 1 and 2 and KRAS.\(^{117}\) Transfection of miR-143 mimic into pancreatic cancer cell lines reduced migration and invasion by direct targeting of these genes as well as indirect effects on CDH1, matrix metallopeptidase 2 and matrix metallopeptidase 9.\(^{117}\) miR-141 reduced in vitro invasion and metastasis by targeting TM4SF1, but had no effect on cell proliferation, cell cycle, or apoptosis.\(^{118}\) miR-218 was reported to regulate three prometastatic genes, ie, roundabout axon guidance receptor 1, vesicular overexpressed in cancer prosurvival protein 1, and UDP glycosyltransferase 8.\(^{119,120}\) Enhancer of zeste homologue 2 suppression of miR-218 in PDAC cells reduced in vitro and in vivo cell proliferation as well as metastasis in nude mice through miR-218 targeting of UDP glycosyltransferase 8.\(^{120}\) miR-10a targeting of the homeobox A1 gene increased in vitro invasion, suggesting that increased levels of miR-10a in PDAC patients could increase metastasis. Reduced expression of miR-126 in PDAC cell lines resulted in increased migration, invasion, and reduction of CDH1 levels by targeting ADAM metallopeptidase domain 9.\(^{121}\)

**Apoptosis in PDAC**

A variety of miRNAs that are deregulated in PDAC have been shown to regulate apoptosis. BCL2 is a well-known suppressor of apoptosis. The miRNAs miR-15b, miR-16, and miR-21, each of which is increased in PDAC, target BCL2.\(^{110,122}\) miR-21 resulted in increased levels of BCL2 via direct targeting of the BCL2 3’ untranslated region.\(^{110}\) Apoptosis in MIA PaCa-2 cells was increased or reduced by miR-21 inhibitor or miR-21 mimic, respectively.\(^{110}\) miR-15b and miR-16 are reduced in PDAC tissues.\(^{121}\) Activation of pancreatic stellate cells resulted in reduced miR-15b and miR-16 expression and reduced apoptosis by the miRNAs regulating BCL2.\(^{122}\) Bcl2-Like 11 (BCL2L11 or BIM) is a proapoptotic factor that interacts with other members of the BCL2 family. Apoptosis was reduced in PDAC through direct targeting of BIM by increased levels of miR-17-5p and miR-301a.\(^{124,125}\) When the PDAC cell line CFPA-1 (cystic fibrosis pancreatic adenocarcinoma cell line) was treated with survivin siRNA or miR-203, in vitro cell proliferation and in vivo tumor growth was reduced and apoptosis was increased in vitro.\(^{126}\)

**miRNAs regulating oncogenes in PDAC**

A number of miRNAs have been discovered to regulate critical oncogenes in PDAC. Among these oncogenes, KRAS has been reported to be the target of the most differentially expressed miRNAs in PDAC. Let-7a was shown to regulate KRAS in AsPC-1 pancreatic cancer cells.\(^{127}\) Let-7 has already been

### Table 1 (Continued)

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Differentially expressed PDAC</th>
<th>Validated target gene</th>
<th>Function, target gene</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-424-5p</td>
<td>Increased</td>
<td>SOCS6</td>
<td>ERK1/2 signaling</td>
<td>145</td>
</tr>
<tr>
<td>miR-483-3p</td>
<td>Increased</td>
<td>SMAD4</td>
<td>Tumor suppressor</td>
<td>137</td>
</tr>
<tr>
<td>miR-486</td>
<td>Increased</td>
<td>CD40</td>
<td>Antimetastatic</td>
<td>168</td>
</tr>
<tr>
<td>miR-491-5p</td>
<td>Decreased</td>
<td>TP53, BAD</td>
<td>Tumor suppressor,</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>antiapoptosis</td>
<td></td>
</tr>
<tr>
<td>miR-520h</td>
<td>Increased</td>
<td>ABCG2</td>
<td>Transporter</td>
<td>171</td>
</tr>
<tr>
<td>miR-630</td>
<td>Reduced</td>
<td>IGFR1</td>
<td>Insulin signaling</td>
<td>141</td>
</tr>
</tbody>
</table>

Note: Expression is increased in some studies while other studies report it as reduced.

Abbreviations: EMT, epithelial-mesenchymal transition; miRNA, microRNA; NFκB, nuclear factor kappa-B; PDAC, pancreatic ductal adenocarcinoma; RTK, receptor tyrosine kinase; mRNA, messenger RNA.
implicated in the regulation of KRAS in a number of other solid tumors, including lung cancer.\textsuperscript{128} Let-7 is a family of isoforms that contain the identical 5′ seed sequence and thus would be expected to regulate many of the same genes. Examination of miRNA expression profiling data on the Gene Expression Omnibus show that some members of the let-7 family are reduced in PDAC specimens while others are increased in PDAC. Therefore, it is difficult to draw a conclusion on a let-7-KRAS axis in PDAC. Let-7a was reduced in a variety of PDACs that were resistant to gemcitabine.\textsuperscript{129}

The miRNAs miR-143 and miR-145 were also shown to target KRAS along with Ras responsive element binding protein 1. Low levels of miR-143 and miR-145 in PDAC result in increased levels of Ras responsive element binding protein 1 which then suppress the miR-143 and miR-145 promoter, maintaining a feed forward mechanism that potentiates KRAS signaling.\textsuperscript{130} miR-126 targets KRAS, resulting in increased KRAS protein levels but no change in mRNA.\textsuperscript{123} Interestingly, the interaction between miR-126 and the KRAS 3′ untranslated region did not occur through the traditional 5′ seed interaction but instead by G-U wobbles within this region.\textsuperscript{123}

miR-217 is another miRNA that has been reported to target KRAS. miR-217 along with miR-216a/-216b forms a cluster of three miRNAs with enriched expression in the pancreas.\textsuperscript{131} Of interest is that while the 5′ seed sequence of miR-217 differs from miR-216a/-216b, both miRNAs target KRAS; miR-217 in PDAC\textsuperscript{132} and 216b in nasopharyngeal carcinoma.\textsuperscript{133} Finally, miR-96 is another miRNA that is reduced in PDAC and has been linked to KRAS regulation through direct targeting of KRAS.\textsuperscript{134} Reduced expression of miRNAs, such as miR-217, miR-216a, miR-216b, miR-143, and miR-145, in PDAC will result in increased KRAS expression. As KRAS is mutated in over 90% of human PDAC, it is difficult to separate the contributions, if any, between the activated mutated form of KRAS and any additional bystander effects from miRNA-related increase in KRAS expression.

miRNAs targeting IGF1R

miRNAs have also been implicated in targeting of insulin-like growth factor-1 receptor (IGF1R). IGF1R is mainly involved in differentiation, mitogenesis, and antiapoptotic activities. Activation of the receptor by insulin-like growth factor-1 (IGF1) or IGF2 leads to autophosphorylation of the tyrosine kinase domain and downstream activation of phosphatidylinositol 3-kinase/AKT and mitogen-activated protein kinase pathways. Many cancer types present with elevated expression of IGF1, IGF2, or IGF1R,\textsuperscript{135} including PDAC,\textsuperscript{139} and inhibition of IGF1/IGF1R have been demonstrated to enhance gemcitabine sensitivity in xenografts in mice.\textsuperscript{140} By investigating the effects of adamantyl retinoid-related molecules in PDAC, Farhana et al demonstrated the regulation of IGF1R by miR-150* and miR-630.\textsuperscript{141} Their study showed that adamantyl retinoid-related molecules induce apoptosis in PDAC cell lines by upregulation of miR-150* and miR-630. Pre-miR-150* mimics reduced both v-myb avian myeloblastosis viral oncogene homologue and IGF1R protein levels, while miR-630 mimic reduced IGF1R protein alone. Treatment with the precursors also

Other tumor-suppressive targets of miRNAs in PDAC include the miR-132/-212 cluster that targets retinoblastoma.\textsuperscript{93} These miRNAs were increased in PDAC specimens and were shown to suppress RB1 mRNA and protein. Sel-1 suppressor of lin-12-like (SEL1L) is a putative tumor suppressor that is downregulated in PDAC. SEL1L functions by displacing misfolded proteins from the endoplasmic reticulum to the cytoplasm and is believed to play a role in Notch signaling. Liu et al identified miR-155, an miRNA often upregulated in PDAC, to directly target SEL1L mRNA.\textsuperscript{135}

SMAD4, also known as DPC4 (ie, deleted in pancreatic cancer), is an important tumor suppressor that functions as a coactivator and mediator of TGF-β signaling. The trimeric complex formed between SMADs 2, 3, and 4 stimulates transcription of a number of critical downstream genes in the TGF-β pathway. As previously mentioned, mutations in SMAD4 lead to its inactivation. Another possibility that contributes to reduced SMAD4 activation in PDAC is direct targeting by miRNA. miR-421, an miRNA with increased expression in PDAC, was identified to target SMAD4.\textsuperscript{136} Ectopic expression of miR-421 reduced SMAD4 protein levels in PDAC cell lines, stimulating cell proliferation and colony formation.\textsuperscript{136} Another miRNA with increased expression in PDAC, miR-483-3p, was also found to target SMAD4.\textsuperscript{137} Like miR-421, forced expression of miR-483-3p increased cell proliferation in PDAC cell lines.\textsuperscript{137}
leads to increased apoptosis and a decrease in stem cell-like sphere formation in the PDAC cell lines. IGF1R has also been implicated as a target of miR-100 in the pancreas. Modulation of IGF1R protein levels was reported with miR-100 inhibitor by immunohistochemistry. These studies suggest a tumor-suppressive role for miRNAs targeting IGF1R in PDAC.

Other miRNA/targets of interest in PDAC

Other miRNAs have oncogenic potential by targeting important signaling pathways in PDAC. In addition to direct targeting of tumor suppressors or oncogenes, miRNAs may exert their malignant phenotype by targeting activators or repressors of certain signaling pathways. For example, an miRNA that is overexpressed in cancer can exert its oncogenic effect by direct targeting of a repressor of an active signaling pathway in cancer. Such is the case for the nuclear factor kappa-B (NFκB) signaling pathway and miR-301a, an miRNA with increased expression in PDAC. The NFκB pathway is constitutively activated in most PDACs. Increased levels of miR-301a in PDAC suppress NFκB repressing factor, a repressor of NFκB. Since NFκB promotes transcription of miR-301a, these interactions create a feed forward loop in which high levels of miR-301a repress NFκB repressing factor, which in turn activates NFκB signaling in PDAC.

The expression of miR-424-5p was increased in PDAC and the adjacent benign pancreas when compared with normal pancreatic tissue. Suppressor of cytokine-induced signaling 6 (SOCS6) is negatively regulated by miR-424-5p through direct binding of the miRNA to the SOCS6 3′ untranslated region. SOCS6 is involved in regulating ERK1/2 signaling, and treatment of pancreatic cancer cell lines with an miR-424-5p inhibitor resulted in increased mRNA levels of two ERK1/2 signaling pathway target genes, ie, BCL2 and myeloid cell leukemia sequence 1.

miR-10b has increased expression in both PDAC tissues and in the plasma of PDAC patients. Gene profiling identified Tat-interacting protein 30 (TIP30) as a putative target of miR-10b; this was validated using reporter assays and immunoblotting. TIP30 is an oxidoreductase with tumor-suppressive activity. Knockdown of TIP30 in PDAC cell lines using either pre-miR-10b oligo or siRNA to TIP30 resulted in enhanced epidermal growth factor-dependent invasion. Epidermal growth factor receptor kinase inhibitors attenuated the miR-10b-induced invasiveness in vitro.

Contribution of differentially expressed miRNAs to development and progression of PDAC

PDAC is a disease of genetic alterations that occur throughout the patient’s lifetime. These include genetic (ie, mutations, deletions) and epigenetic (miRNA, chromatin remodeling, DNA methylation) insults. As mentioned previously, the work of Jones et al identified four genes that are primarily mutated in human PDAC. It should be noted that this study focused on protein coding genes and not noncoding RNAs. Many studies have demonstrated altered expression of miRNAs in PDAC and have linked these differentially expressed non-coding RNAs to various target genes (Table 1). However, it has never been demonstrated that altered expression of miRNAs is causative of PDAC or contributes to its progression.

The present article overviews validated mRNAs that are targeted by differentially expressed miRNAs in PDAC. These include tumor suppressors, oncogenes, and mRNAs coding for proteins that are involved in epithelial mesenchymal transition, invasion, metastasis, various signaling pathways, and apoptosis. The critical question is whether these epigenetic modifications of cellular functions are the driver or passenger events in the formation or progression of PDAC. This is a difficult question to answer because association of a gene with a pathway causing PDAC is generally proven by overexpressing or knocking down the gene in a transgenic mouse model of PDAC. For example, Medina et al demonstrated the oncogenic potential of miR-21 by overexpressing the miRNA in mice, leading to a pre-B malignant lymphoid-like phenotype. To our knowledge, no one has published on a mouse model of PDAC that overexpresses or knocks down an miRNA. Given that over 90% of PDACs have KRAS mutations, it is difficult to envisage other genes that are causative of PDAC in the absence of mutated KRAS. Key experiments then are to overexpress oncogenic miRNAs or knockdown tumor-suppressive miRNAs in the pancreas using conditional transgenic approaches. The ability of these changes to induce PDAC in the mice may be studied independently or through enhanced means, such as combining with experimentally induced CP or by crossing with mice harboring a PDAC-causing mutation, as in KRAS G12D mice.

Translational application of differentially expressed miRNAs in PDAC includes diagnostic markers and perhaps therapeutic targets. Additional experiments must be completed to determine if the large number of mRNA target genes that are presently known and those that will be
discovered in the future contribute to the development of PDAC in humans.

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Disclosure
The authors report no conflicts of interest in this work.

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