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EDITORIAL

MicroRNAs in pancreatic ductal adenocarcinoma

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Abstract

Ductal adenocarcinoma of the pancreas is a lethal cancer for which the only chance of long-term survival belongs to the patient with localized disease in whom a potentially curative resection can be done. Therefore, biomarkers for early detection and new therapeutic strategies are urgently needed. miRNAs are a recently discovered class of small endogenous non-coding RNAs of about 22 nucleotides that have gained attention for their role in downregulation of mRNA expression at the post-transcriptional level. miRNAs regulate proteins involved in critical cellular processes such as differentiation, proliferation, and apoptosis. Evidence suggests that deregulated miRNA expression is involved in carcinogenesis at many sites, including the pancreas. Aberrant expression of miRNAs may upregulate the expression of oncogenes or downregulate the expression of tumor suppressor genes, as well as play a role in other mechanisms of carcinogenesis. The purpose of this review is to summarize our knowledge of deregulated miRNA expression in pancreatic cancer and discuss the implication for potential translation of this knowledge into clinical practice.

Key words: MicroRNAs; Pancreatic cancer

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INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer-related mortality in the United States, with 36 800 estimated deaths in 2010, with the great majority being due to ductal adenocarcinomas[1]. Due to the asymptomatic onset of pancreatic cancer, most patients are in advanced or metastatic condition at the time of diagnosis, resulting in poor prognosis. Most patients found to have pancreatic cancer die within 12 mo, and few survive 5 years after diagnosis. The poor prognosis of these patients is due to its late clinical presentation with symptoms, early and aggressive local invasion, and high metastatic potential[2]. Advances in chemo-radiation therapy have been slow over the last few decades, and the overall prognosis in pancreatic cancer has remained essentially unchanged. The only chance of long-term survival with pancreatic adenocarcinoma belongs to

patients with localized disease in whom a potentially curative resection can be performed. Earlier diagnosis and better treatments are urgently needed to improve the survival rate of pancreatic cancer.

Histologically, the pancreas is divided largely into the exocrine and endocrine pancreas, the former consists of ducts and acini, and the latter constitutes the islets that have a hormone secretory function. The most common type of pancreatic cancer, representing about 85% of all pancreatic cancer types[3], arises from the epithelial lining of the exocrine pancreatic duct. Therefore, in this review, we mainly focus on miRNA expression in pancreatic ductal adenocarcinoma (PDAC).

Pancreatic cancer originates from the sequential accumulation of multiple genetic alterations[4]. In the past several decades, significant progress in the identification and characterization of cancer-related gene abnormalities has been made. However, this progress has not yet been effectively translated into new reliable biomarkers that lead to the earlier diagnosis or more effective treatment of this deadly disease. Specific miRNAs affecting tumor suppressor genes or oncogenes may be critical biomarkers that lead to early detection, or potential drug targets for pancreatic cancer.

Although regulation of oncogenes and tumor suppressor genes, by genetic and epigenetic changes has been regarded as being important in the development of pancreatic cancer[5-8], the exact molecular mechanisms of carcinogenesis and of pancreatic cancer progression remain unknown. Gene silencing is frequently caused by epigenetic changes, such as DNA methylation or altered miRNA expression rather than by genetic events such as mutation or deletion. miRNA binding at the 3' untranslated region (UTR) in tumor suppressor genes is an epigenetic change that may contribute to carcinogenesis and cancer progression. Although relatively few genetic mutations have been identified in PDAC, aberrant miRNA expression has been found in both pancreatic tumor tissues and cell lines.

BIOGENESIS, FUNCTION AND TARGETS OF miRNAs

miRNAs are about 22-nucleotide non-protein-coding RNA molecules that regulate gene function in various gene silencing pathways. These molecules are phylogenetically conserved and play important roles in cell survival, proliferation, differentiation, apoptosis and angiogenesis[9,10]. miRNA expression patterns differ, depending upon cell, tissue, and disease types, and changes in these expression patterns have been implicated as an important player in carcinogenesis.

The miRNA, lin-4, was first discovered in 1993 as a small non-coding RNA that regulates *Caenorhabditis elegans* development by negative regulation of lin-14 protein expression[11]. In 2000, the second miRNA, let-7, was identified from *C. elegans* and confirmed as a 21-nucleotide small RNA[12]. Since the discovery of lin-4 and let-7, many more miRNAs have been identified using various experimental and computational methods[13]. In the most recent database (miRBase 15 release), over 15000 mature miRNAs are identified in 133 species[14]. Although they do not encode proteins, miRNAs are transcribed by RNA polymerase Ⅱ as independent units in the nucleus (Figure 1). The primary transcript (pri-miRNA) is processed by the nuclear RNase Ⅲ Drosha and its cofactor DGCR8/Pasha to generate precursor miRNA (pre-miRNA), a 60-70-nucleotide RNA that has a stem loop structure[15-17]. Pre-miRNA is rapidly exported to the cytoplasm by exportin 5 in a Ran-GTP-dependent manner, where it is further processed by a second RNase Ⅲ, dicer, which cuts off the terminal loop and generates a mature about 22-nucleotide miRNA. Mature miRNA is initially part of an imperfect double-stranded RNA duplex called miRNA/miRNA*. This double-stranded RNA duplex binds to a protein (Argonaute 2) as a part of the RNA induced silencing complex (RISC), while the strand of the duplex that is complementary miRNA* is released. The RISC, containing its miRNA, binds to the target mRNA and triggers either mRNA degradation or inhibition of translation, depending on the degree of complementarity between miRNA and its target[18-21].

Each miRNA regulates multiple target genes. In fact, bioinformatics predict that miRNAs may regulate about 50% of all human genes[22]. Therefore, precise identification of miRNA targets is critical to advance our understanding of the role of miRNA regulation in carcinogenesis. Accurate identification of physiologically active miRNA targets is now a considerable impediment to the functional characterization of individual miRNAs.

miRNAs negatively regulate their target mRNAs primarily through base-pairing interactions, which leads to either mRNA degradation or translational inhibition depending upon the degree of match between the "seed sequence" (positions 2-7 at the 5' side) of miRNA and 3'UTR of mRNA (Figure 1). When the seed sequence perfectly or partially matches with target 3' UTR of mRNA, then it may lead to degradation of the mRNA or inhibit translation[18-21]. Based upon publicly available algorithms, each miRNA has several hundred potential target mRNAs. Recent reports have further indicated that secondary structures of mRNA contribute to target recognition sites, due to the fact that there is energetic cost to free base-pairing interactions for accessible targets[23-25]. Kertesz *et al*[26] have shown that target site accessibility is as important as sequence match in the seed sequence region, and that effective miRNA binding requires unpairing of local regions that flank the target, as well as that the target region is unpaired in thermodynamic equilibrium. Thus, simultaneous profiling of miRNA and mRNA, as well as protein expression, has recently been shown to be a timely strategy to achieve the required precision in the identification of functional miRNA targets[27-30].

In summary, miRNAs regulate their targets by direct mRNA cleavage or translational inhibition. miRNAs are coded by genes and are transcribed by RNA polymerase Ⅱ. They have their own regulatory elements and appear as transcriptional units containing either unique or multiple

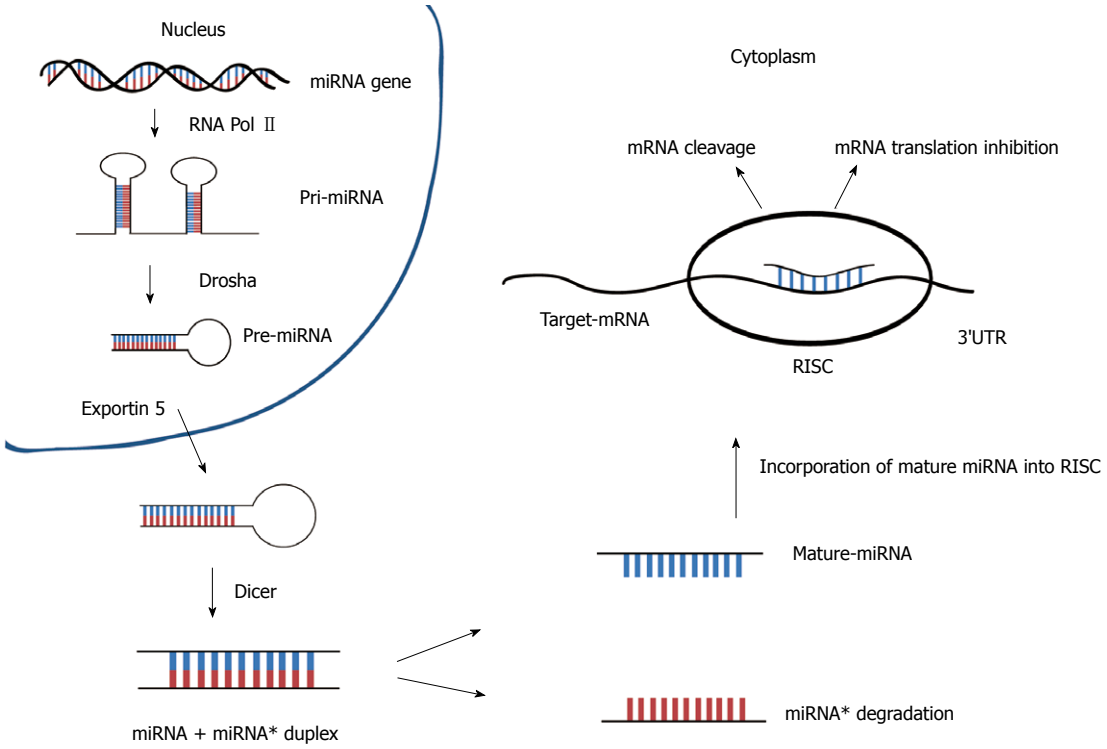

Figure 1 Schematic representation of miRNA biogenesis. miRNA genes are transcribed by RNA polymerase Ⅱ (RNA pol Ⅱ) into long transcripts called primary miRNAs (pri-miRNA) that contain multiple stem-loop/hairpin structures as independent units in the nucleus. pri-miRNA is processed by the nuclear RNase Ⅲ Drosha and its cofactor DGCR8/Pasha to generate precursor miRNA (pre-miRNA). The pre-miRNA is rapidly exported to the cytoplasm by exportin 5, where it is further processed by a second RNase Ⅲ, Dicer, that cuts off the terminal loop and generates a mature about 22-nucleotide miRNA. The mature miRNA is an imperfect double-stranded RNA duplex called miRNA/miRNA*. The double-stranded RNA duplex binds to a protein (Argonaute 2) as a part of the RNA induced silencing complex (RISC), while one of the strands of the duplex, which is complementary miRNA*, is released. The RISC, which contains its miRNA, binds to the target mRNA and triggers either mRNA degradation or inhibition of translation, depending on the degree of complementary between miRNA and its target.

miRNAs (polycistronic). Circumstantial evidence linking miRNAs and carcinogenesis has been observed in over 50% of miRNA genes, which are located within regions of loss of heterozygosity, amplification, fragile sites, viral integration sites, and other cancer-associated genomic regions. Recent high-throughput methodologies have shown deregulated miRNA expression in an increasing number of human cancers, including pancreatic cancer. Differences in miRNA expression patterns have been found to distinguish tumors of different developmental origin, even better than traditional mRNA expression profiling[31].

miRNA AND HUMAN CANCER

The first evidence of miRNA involvement in human cancer came from a study that characterized chromosome 13q14 in chronic lymphocytic leukemia (CLL)[32]. Calin *et al*[32] have shown that miR-15 and miR-16 are deleted or downregulated in about 70% of CLL cases. The tumor suppressive role of miR-15a and miR-16-1 has been supported further by the discovery that expression of both miRNAs inversely correlates with expression of the anti-apoptotic BCL2 protein[33]. BCL2 expression is inhibited by miR-15a and miR-16-1 and these repressions induce apoptosis in leukemic cells. These data suggest a model whereby somatic deletions of miR-15a and miR-16-1 aid leukemogenesis by allowing tumors to escape apoptosis.

Since this first report of aberrant miRNA expression in CLL, deregulation of a number of miRNAs has been found in other human cancers. While some miRNAs, including miR-125b and miR-145 in breast cancer, and let-7 in lung cancer, are reduced, others such as miR-21 and miR-155 in breast cancer, miR-155 in lung cancer, the precursor of miR-155 in Burkitt lymphoma, miR-17-92 cluster and miR-155 in B-cell lymphoma, are overexpressed[31,34-39]. These studies also have shown that miRNA expression signatures correlate well with specific clinical cancer characteristics, and could be used to differentiate normal and cancerous tissues, as well as subtypes of malignancy[40-43].

Deregulation of miRNA in cancer could be caused by: (1) chromosomal regional gain, loss or translocation; (2) aberrant expression and activation of transcriptional factors; (3) epigenetic alterations; and (4) changes in miRNA processing[44]. As described above, the association between chromosomal abnormality and miRNA expression in CLL is due to downregulation of the miR16-1/15a cluster in chromosome 13q14.3[32]. In contrast, upregulation of miR-155 in tumor appears to be due to transcriptional regulation and aberrant miRNA processing[36,45]. miR-155 is encoded in non-coding DNA known as BIC (B-cell integration cluster), located at chromosome 21q21.3, where neither amplification nor loss of heterozygosity is observed. Several studies have shown that miR-155 is in-

Table 1 miRNA deregulation in human pancreatic cancer

miRNA	Lee *et al*[49]	Szafranska *et al*[50]	Bloomston *et al*[51]	Zhang *et al*[52]	Other	Outcome
let-7					↓[53]	
let-7d	↑[1]					
let-7f-1	↑					
miR-10a			↑		↑[54]	
miR-10b			↑			
miR-15b	↑			↑		
miR-16-1	↑					
miR-18a		↑				
miR-21	↑		↑		↑[55, 56]	Poor[55]
miR-23a			↑			
miR-23b			↑			
miR-24-1,2	↑					
miR-29c		↓				
miR-31		↑				
miR-92-1	↑					
miR-93		↑				
miR-95				↑		
miR-96		↓				
miR-99			↑			
miR-100	↑		↑			
miR-100-1/2			↑			
miR-103-2			↑			
miR-107	↑		↑			
miR-125a			↑			
miR-125b-1	↑		↑			
miR-130b		↓				
miR-139	↓					
miR-141		↓				
miR-142-P	↓					
miR-143		↑	↑			
miR-145		↑				
miR-146			↑			
miR-146a		↑				
miR-148a		↓	↓			
miR-148b		↓	↓			
miR-150		↑				
miR-155	↑	↑	↑			Poor[57]
miR-181a	↑		↑			
miR-181b			↑			
miR-181b-1			↑			
miR-181b-2			↑			
miR-181c	↑		↑			
miR-181d			↑			
miR-186				↑		
miR-190				↑		
miR-196a		↑		↑		miR-196a-2; Poor[51]
miR-196b		↑				
miR-199a-1			↑			
miR-199a-2			↑			
miR-200b				↑		
miR-203		↑				Poor[57]
miR-205		↑	↑			
miR-210		↑	↑			Poor[57]
miR-212	↑					
miR-213			↑			
miR-216		↓				
miR-217		↓				
miR-220			↑			
miR-221	↑	↑	↑	↑		
miR-222		↑	↑	↑		Poor[57]
miR-223		↑	↑			
miR-224		↑				
miR-301	↑					
miR-345	↓					
miR-375			↓	↓		
miR-376a	↑					
miR-424	↑					

[1]Arrows indicate increased (↑) or decreased (↓) expression of the specified miRNA.

duced at the transcriptional level by transforming growth factor β/Smad, nuclear factor-κB and activator protein-1 family transcription factors through direct interaction with the miR-155/BIC promoter[46-48]. Further studies have shown that miR-155 processing also regulates mature miR-155 expression levels[36,45], suggesting that overexpression of miR-155 in cancer is due to transcriptional activation and miRNA processing.

miRNA EXPRESSION PROFILE IN NORMAL PANCREATIC TISSUE AND PANCREATIC TUMOR

miRNA expression profiles in pancreatic tumor tissues are different from those identified in normal pancreas or in chronic pancreatitis. Most miRNA expression profile analyses show that miRNAs are deregulated in tumor tissues as compared to normal pancreas, and that the expression pattern is tissue specific. Several studies focusing on miRNA expression profiles in pancreatic tissues have identified a number of differentially expressed miRNAs. Table 1 summarizes the aberrantly expressed miRNAs in human pancreatic cancer and their association with patient survival.

Szafranska *et al*[50] have performed the first comprehensive miRNA expression profile study in tissues from normal pancreas (n = 7), chronic pancreatitis (n = 7), PDAC (n = 10) and 33 human tissues of different non-pancreatic origin, to identify miRNA candidates with a potential for future clinical application from a pool of 377 known and novel miRNAs. The authors have found that two miRNAs, miR-216 and miR-217, are pancreas-specific. These results were in agreement with those of two previous studies[58,59]. Furthermore, both miR-216 and miR-217 are absent or only minimally expressed in pancreatic carcinoma tissues and cell lines. Therefore, miR-216 and miR-217 are potential biomarkers. Based upon clustering analysis, the three pancreatic tissues types can be classified according to their respective miRNA expression profiles. Among 26 miRNAs that have been identified as most prominently deregulated in PDAC, only miR-217 and miR-196a have been found to discriminate between normal pancreas, chronic pancreatitis and tumor tissues. These miRNAs are also potential biomarkers.

Recently, expression of 201 miRNA precursors (representing 222 miRNAs) was profiled in pancreatic adenocarcinoma, paired with benign tissue, normal pancreas, chronic pancreatitis and pancreatic cancer cell lines with the real-time PCR miRNA array[49]. These three cell types could be classified by the clustering algorithm. One hundred miRNA precursors have been identified as aberrantly

expressed miRNAs including known ones in other cancers and novel ones in pancreatic tumor. A list of the top 20 aberrantly expressed miRNA precursors has been proposed as a signature for pancreatic adenocarcinoma.

Bloomston *et al*[51] have identified a large global expression pattern of miRNAs that can differentiate PDAC from chronic pancreatitis with 93% accuracy. Among several deregulated miRNAs in the pancreatic cancers, most notably, miR-21 and miR-155 are uniquely overexpressed in pancreatic tumor, as compared to tissues from normal pancreas and chronic pancreatitis. Both miR-21 and miR-155 have been suggested to play an important role in functioning as a proto-oncogene and have been shown to be overexpressed in several cancers. These authors have performed an miRNA microarray profiling with about 1100 miRNA probes, which included 326 human miRNAs, using microdissected pancreatic tumor tissues.

Zhang *et al*[52] have evaluated 95 miRNAs, selected from pancreatic cancer profiling, and correlated them to their potential biological functions related to cancer biology, cell development, and apoptosis. Among them, eight miRNAs (miR-196a, miR-190, miR-186, miR-221, miR-222, miR-200b, miR-15b, and miR-95) are differentially expressed in most pancreatic cancer tissues and cell lines. All of these eight genes are significantly unregulated, from 3- to 2018-fold, in pancreatic tumors as compared with normal control samples.

In summary, these profiling data may provide novel insights into the miRNA-driven mechanisms involved in pancreatic carcinogenesis, and offer new potential targets for early detection and therapeutic strategies in pancreatic cancer.

miRNAS AS BIOMARKERS FOR PANCREATIC CANCER DIAGNOSIS

Development of biomarkers for pancreatic cancer is especially critical because most patients with this disease remain asymptomatic until the disease progresses to become locally advanced or develops distant metastases. Therefore, most of these patients are surgically inoperable at the time of diagnosis. Sensitive and specific biomarkers for pancreatic cancer are urgently needed to offer better therapeutic options and survival outcome.

Over the years, a number of protein- and DNA-based biomarkers have been proposed as markers of early detection for pancreatic cancer. However, most of these markers fail to have clinical potential, and they have not influenced patients' survival. Since the first discovery of miRNAs by Lee *et al*[11] in 1993, many researchers have investigated expression profiles, biological functions and targets of miRNAs in carcinogenesis and tumor progression, with the purpose of translating the results to clinical settings.

Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) of the pancreas is not likely to be used routinely for screening for PDAC because of its invasive nature. However, this procedure has recently emerged as a specific and minimally invasive modality for preoperative diagnosis and staging of pancreatic cancer. Furthermore, EUS-FNA may also be useful for screening high-risk individuals, as well as for the prognosis and predicting the response to treatment in cases in which the tumor is inoperable[60-62]. Szafranska *et al*[63] have identified potential miRNA markers in EUS-FNA biopsies of pancreatic tissue. The combination of expression pattern of miR-196a and miR-217 can differentiate PDAC cases from healthy controls and chronic pancreatitis in the FNA samples. Furthermore, miR-196a expression is likely specific to PDAC cells and is positively associated with the progression of PDAC.

Carcinogenesis in PDAC develops with a multistep progression from morphologically distinct non-invasive precursor lesions within exocrine pancreatic ducts[64]. These precursors include the intraductal papillary mucinous neoplasms (IPMNs), the mucinous cystic neoplasms, and pancreatic intraepithelial neoplasia (PanIN). Two studies have been carried out to detect expression patterns of miRNA in IPMNs and PanIN. IPMNs are grossly visible, non-invasive, mucin-producing precursors of pancreatic cancer within the main pancreatic duct or one of its branches[65,66]. In contrast, PanINs are non-invasive, microscopic epithelial neoplasms, arising within smaller pancreatic ducts, < 5 mm in diameter, and characterized by cytological and architectural atypia[65,67]. Habbe *et al*[68] have reported significant overexpression of 10 miRNAs in IPMNs (n = 15). miR-155 and miR-21 show the highest relative fold-changes in the precursor lesions. These results have been validated by *in situ* hybridization analysis. miR-155 and miR-21 are upregulated in most IPMNs [83% (53/64) and 81% (52/64)] as compared to normal ducts [7% (4/54) and 2% (1/54)]. With these promising data, the potential use of these miRNAs as biomarkers has been evaluated in pancreatic juices. A total of 15 pancreatic juice samples from 10 patients with IPMNs, and five with other pancreatobiliary disorders obtained at the time of surgical resection were measured for relative levels of miR-155 and miR-21 by quantitative real-time RT-PCR. Upregulation of both miR-155 and miR-21 in the subset of IPMN-associated pancreatic juices was observed, as compared with control samples. These results indicate that aberrant miRNA expression occurs early in the precursor lesion during the multiple stages of pancreatic cancer development, and miRNA profiles may be assessed with more accessible clinical samples, such as pancreatic juice, and could be used as a diagnostic tool.

du Rieu *et al*[69] have investigated miRNAs in PanIN tissues from a conditional Kras (G12D) mouse model (n = 29) and from human origin (n = 38). Expression of miR-21, miR-205 and miR-200 has been found to be positively associated with PanIN progression in the Kras (G12D) mouse model. In the human tissues, expression of miR-21, miR-221, miR-222 and let-7a increases with PanIN grade. The authors, using *in situ* hybridization analysis, have observed that miR-21 expression is concen-

Table 2 miRNAs and their targets involved in human pancreatic cancer

miRNA	Function	Targets	Related cellular events	Ref.
let-7	Suppress	RAS[71]	Inhibit cell proliferation, KRAS expression, and mitogen-activated protein kinase activation	[53]
let-7, miR-200	Suppress		Reverse EMT	[72]
Let-7a	Suppress	RAS	Attenuate KRAS expression and radiosensitize tumor cell	[73]
miR-10a	Oncogenic	HOXB1, 3	Promote metastatic behavior	[54]
miR-21	Oncogenic		Induce cell proliferation, invasion, chemoresistance	[56]
miR-21	Oncogenic		Potentially associated with cell proliferation	[74]
miR-200c	Suppress		Potentially associated with G0/G1 arrest and increased apoptotic rate	
miR-21, miR-221	Oncogenic	PTEN, RECK, CDKN1B	Arrest cell cycle, induce apoptosis, and sensitize the effects of gemcitabine with inhibition of miR-21 or -221	[75]
miR-22	Suppress	SP1, ESR1	Potentially inhibit tumorigenesis	[76]
miR-34	Suppress	BCL2, NOTCH1/2	Inhibit clonogenic cell growth and invasion, induce apoptosis and G1 and G2/M arrest in cell cycle, sensitize to chemotherapy and radiation, and potentially inhibit pancreatic cancer stem cells	[77]
miR-107	Suppress	CDK6	Induce in vitro cell growth downregulation	[78]
miR-155	Oncogenic	TP53INP1	Inhibit apoptosis	[79]
miR-194, miR-200b, miR-200c, miR-429	Oncogenic	EP300	Potentially promote metastatic behavior	[80]
miR-224, miR-486	Oncogenic	CD40	Potentially associated with invasion and metastasis	[81]

BCL2: B-cell CLL/lymphoma 2; CD40: CD40 molecule; CDK6: Cyclin-dependent kinase 6; CDKN1B: Cyclin-dependent kinase inhibitor 1B; EP300: E1A binding protein p300; ESR1: Estrogen receptor 1; HOXB1, 3: Homeobox B1, 3; KRAS: v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; NOTCH1/2: Notch 1/2; PTEN: Phosphatase and tensin homolog; RECK: Reversion-inducing-cysteine-rich protein with kazal motifs; SP1: Sp1 transcription factor; TP53INP1: Tumor protein p53 inducible nuclear protein 1; EMT: Epithelial-to-mesenchymal transition.

trated in the dysplastic ductal epithelial cells. Using PDAC-derived cell lines, they also have noted that miR-21 expression is regulated by Kras (G12D) and epidermal growth factor receptor (EGFR).

Wang *et al*[70] have studied plasma samples from patients with PDAC and have found that four miRNAs (miR-21, miR-210, miR-155 and miR-196a) are able to differentiate pancreatic cancer patients from healthy controls, with moderate accuracy (sensitivity: 64%, and specificity: 89%). In summary, these studies suggest a potential value of miRNAs in the clinical setting as a potential diagnostic tool for PDAC.

miRNAS AS ONCOGENES AND TUMOR SUPPRESSORS

miRNAs are functionally classified into oncogenes or tumor suppressors based upon their targets, thus binding to oncogenes or tumor suppressor genes. Therefore, oncogenic miRNAs are upregulated in tumors, whereas tumor suppressor miRNAs are downregulated. The functions and targets of a handful of miRNAs have been investigated in pancreatic cancer (Table 2).

Torrisani *et al*[53] have reported that tumor suppressor let-7 miRNA is expressed in normal acinar pancreatic cells, but is extensively downregulated in PDAC samples, as compared with adjacent non-involved tissues. Transfection of pancreatic cancer cell lines with let-7 miRNA inhibits cell proliferation, Kras expression, and mitogen-activated protein kinase activation. This study has demonstrated that intracellular restoration of let-7 miRNA reverts neoplastic characteristics of PDAC, suggesting that let-7 miRNA functions as a tumor suppressor in pancreatic cancer. In addition, the results of this study suggest let-7 miRNA as a replacement therapy for pancreatic cancer.

miRNAS AS THERAPEUTIC TARGETS IN PANCREATIC CANCER

Most epithelial tumors, including pancreatic cancer, are believed to progress toward loss of epithelial differentiation and acquisition of a mesenchymal phenotype that leads to enhanced cancer cell invasion and migration[82,83]. The aggressiveness of pancreatic cancer is, in part, due to its drug resistance characteristics, which are also associated with the epithelial-to-mesenchymal transition (EMT). Several studies have shown that the events leading to EMT are regulated by miRNAs[84-89]. Li *et al*[72] have investigated the effects of let-7 and miR-200 on the morphological changes of EMT in gemcitabine-resistant pancreatic cancer cells (GRPCCs). They have found that: (1) the expression of miR-200 and let-7 is significantly downregulated in GRPCCs, which have EMT characteristics; and (2) transfection of GRPCCs with miR-200 rescues the epithelial phenotype by upregulating the epithelial marker E-cadherin and downregulating the mesenchymal markers ZEB1 and vimentin. These authors also have demonstrated that tumor cell sensitivity to gemcitabine is increased after re-expression of miR-200b. These results suggest that EMT could be regulated by miRNAs, and provide a potential strategy for treatment.

RAS mutations are frequent in human tumors and are known to be one of the responsible factors for radiation-induced cell death[90,91]. Using transfection of Lin28 siRNA into pancreatic cancer cells harboring Kras mutation,

Oh *et al*[73] have shown that upregulation with let-7a results in attenuated expression of Kras and increased radiosensitization of pancreatic cancer cells. This suggests that miRNA could be used as a valuable therapeutic option in radioresistant tumors that have Kras mutations.

The main reason for poor survival in pancreatic cancer is the presence of metastasis at the time of diagnosis. Weiss *et al*[54] have shown that miR-10a expression promoted metastasis, and repression of miR-10a inhibited invasion and metastasis in xenotransplantation experiments using zebrafish embryos. They have further identified tumor suppressors HOXB1 and HOXB3 as targets of miR-10a, and have reported that retinoic acid receptor antagonists inhibit miR-10a expression and suppress metastasis. These data suggest new therapeutic applications for miRNA in patients with metastatic pancreatic cancer.

Several studies have reported significant overexpression of miR-21 in pancreatic tumors[49,51], suggesting the potential role of miR-21 in pancreatic cancer. Moriyama *et al*[56] have confirmed that miR-21 is overexpressed in pancreatic cancer cells. They also have observed that miR-21 contributes to cell proliferation, invasion, and chemoresistance. They also have found that mRNA expression of invasion-related genes, matrix metalloproteinase (MMP)-2 and MMP-9, and vascular endothelial growth factor is positively correlated with miR-21 expression. The above studies show that miR-21 functions as an oncogene, and that it is involved in pancreatic cancer chemoresistance. Therefore, miR-21 could be a target for a therapeutic strategy for patients with chemoresistant pancreatic cancer.

Zhang *et al*[74] have found that pancreatic cancer cells treated with trichostatin A (TSA), one of the common histone deacetylase inhibitors[92,93], are arrested in G0/G1 phase, and exhibit an increased in apoptotic rate. The treatment also induces downregulation of miR-21 and upregulation of miR-200c. The data support the oncogenic function of miR-21, and the tumor suppressor function of miR-200, suggesting that epigenetic regulation of miRNAs with histone deacetylase inhibitor could be used as a therapeutic option in pancreatic cancer.

It has been shown that antisense oligonucleotides (ASOs) can inhibit upregulated miRNAs in tumors[94]. Park *et al*[75] have investigated miR-21 and miR-221 biological function using ASOs in pancreatic cancer. ASOs for miR-21 and miR-221 both reduce proliferation of pancreatic cancer cell lines, increase apoptosis by 3-6-fold, and induced G1 arrest. ASOs also increase the levels of the miR-21 targets PTEN and RECK, and the miR-221 target, CDKN1B, at the protein level. The authors have found that ASO targeting of miR-21 and miR-221 sensitizes tumor cells to the effects of gemcitabine, and that ASO-gemcitabine combination treatments generate synergistic antiproliferative effects in pancreatic cancer cells. These results imply that targeting miRNAs with ASOs could be a potential new therapeutic strategy for pancreatic cancer.

In vitro and *in vivo* studies have reported the anticancer activity, with low toxicity, of curcumin (diferuloylmethane)[95,96], a naturally occurring flavonoid from the rhizome of *Curcuma longa*[97,98]. Sun *et al*[76] have investigated whether curcumin affects the expression profiles of miRNAs in pancreatic cancer, and have reported overexpression of miR-22 and downregulation of miR-199a* in pancreatic cancer cells treated with curcumin. The predicted target genes of miRNA-22 are Sp1 transcription factor (SP1) and estrogen receptor 1 (ESR1). The expression of these genes (SP1 and ESR1), which are involved in cell growth, metastasis and apoptosis, is suppressed by upregulation of miR-22. Thus, Sun *et al* have suggested that one of the important anticancer mechanisms of curcumin is modulation of miRNA expression, such as miR-22.

Some cancer stem cells are involved in tumor initiation, self-renewal and survival[99], and miRNAs have been shown to have critical roles in cancer stem cell differentiation. Ji *et al*[77], using cell sorting of $CD44^+/CD133^+$, have examined the roles of miR-34 in p53-mutant human pancreatic cancer cell lines, to find a potential link between stem cells and pancreatic cancer. These authors have observed that miR-34 upregulation results in significant inhibition of clonogenic growth and cell invasion, induction of apoptosis, G1 and G2/M cell cycle arrest, and sensitization of the cells to chemotherapy and radiation. They also have detected an 87% reduction in tumor initiating cells (or cancer stem cells), which was mediated by downregulation of its downstream targets BCL2 and NOTCH. This study has shown that restoration of miR-34 could have significant promise as a novel molecular therapy for human pancreatic cancer *via* inhibiting pancreatic cancer stem cell differentiation.

Aberrations in epigenetic regulation are common in human cancers, and tumor suppressor genes are frequently silenced by this mechanism in nearly all malignancies[100,101]. Recent studies have shown that subsets of miRNAs are also silenced by the same mechanism[102,103]. For example, Lee *et al*[78] have shown that miR-107 is silenced by promoter DNA methylation in pancreatic tumors. These authors treated human pancreatic cancer cell lines with the demethylating agent, 5-aza-2'-deoxycytidine or the histone deacetylase inhibitor, TSA, or with a combination of the two, and identified the upregulation of 14 miRNAs, including miR-107. Retroviral expression of miR-107 in pancreatic cancer cells downregulates *in vitro* cell growth by repressing cyclin-dependent kinase 6, a putative miR-107 target. This study shows that epigenetic mechanisms of miRNA may be involved in pancreatic carcinogenesis.

Tumor protein p53 inducible nuclear protein 1 (TP53INP1) is a pro-apoptotic stress-induced gene. TP53 is able to activate TP53INP1 transcription as a target[104,105]. However, overexpression of TP53INP1 induces cell cycle arrest and apoptosis *in vitro*, independently from TP53. Gironella *et al*[79] have reported that TP53INP1 is expressed in normal tissues but is markedly downregulated or lost in early stages of pancreatic cancer development. TP53INP1 repression by transfection of miR-155 causes loss or significant decrease in expression of TP53INP1. These data suggest that TP53INP1 is an additional potential target of miR-155.

Several studies have suggested that EP300 may func-

tion as a tumor suppressor. This gene is located on chromosome 22q; a region known for its frequent loss of heterozygosity in different cancers, including pancreatic cancer[106-109]. Mees *et al*[80] have classified 16 human PDAC cell lines into three hierarchical groups according to their metastatic potential, and have profiled their mRNA and miRNA expression. The highly metastatic PDAC cell lines, when compared to the non-metastatic cell lines, have shown decreased mRNA and protein expression of EP300, which is related to significant upregulation of EP300-targeting miRNAs (miR-194, miR-200b, miR-200c and miR-429). Using the same 16 human PDAC cell lines, these authors have found markedly reduced expression of CD40 protein, which is involved in the host antitumor immune response[110,111]. CD40-targeting miR-224 and miR-486 are upregulated in the highly invasive and metastatic PDAC[81]. These results show that miRNAs are involved in regulating the metastatic behavior of PDAC, and in modulating metastasis-specific tumor suppressor genes. Targeting of these miRNAs may have potential therapeutic value in PDAC.

miRNAS AS CLINICAL ASPECTS IN PANCREATIC CANCER

Most tumors show deregulation of miRNAs for the initiation and progression of human cancer, therefore, many researchers have been trying to exploit these miRNAs for therapeutic applications, and to develop novel therapies for human cancer[112-115]. Thus, oncogenic miRNAs can be suppressed with ASOs to their precursor or mature forms[94,116], and tumor suppressor miRNAs can be upregulated[53,72].

Numerous miRNA studies have demonstrated that miRNA-directed targeting therapy has therapeutic potential in human cancer. Recent studies have further demonstrated synergistic effects when miRNA-directed therapy is used in combination with conventional chemotherapy or radiotherapy for pancreatic cancer[73,75]. However, currently, there is no miRNA that is used in the clinical setting for treatment of cancer patients. Significant work needs to be done before miRNA-directed therapeutic strategies can be applied. However, current data have shown encouraging preliminary results to support their clinical applications in human cancer.

Several investigators have attempted to utilize miRNA expression profiles as a diagnostic tool to differentiate tumors from normal tissues[43,117,118], and as predictors of clinical outcome. However, there have not been sufficient studies that have investigated the correlation between alterations in miRNA expression and patient outcome in PDAC.

A few miRNA expression patterns have been investigated to predict prognostic outcome from specimens of patients with pancreatic cancer[51,55,57]. Bloomston *et al*[51] have analyzed the association between survival of patients and miRNA expression patterns. In the subgroup analysis of patients with lymph-node positive disease, a panel of six miRNAs (miR-452, miR-105, miR-127, miR-518a-2, miR-187 and miR-30a-3p) was able to differentiate between long-term survivors and short-term survivors who died within 2 years. Furthermore, high expression of miR-196a-2 is associated with poor outcome; patients with high miR-196a-2 expression have a shorter median survival of 14.3 mo when compared with patients with low miR-196a-2 expression, who have a median survival of 26.5 mo.

Dillhoff *et al*[55] have performed *in situ* hybridization after microdissection and tissue microarray analysis of 80 resected pancreatic cancer specimens, and found 79% of the pancreatic cancer samples, 27% of the chronic pancreatitis samples, and 8% of the normal pancreatic samples had positive miR-21 expression. Among the subset of patients with node-negative disease, high miR-21 expression resulted in poorer survival than in patients with low miR-21 expression (median: 27.7 mo *vs* 15.2 mo, $P = 0.037$), although miR-21 expression did not correlate with tumor size, differentiation, nodal status, or T stage.

Greither *et al*[57] have measured the levels of miR-155, miR-203, miR-210, miR-216, miR-217 and miR-222, which are known to be differentially expressed in pancreatic tumors. From 56 microdissected PDACs, they found that elevated levels of miR-155, miR-203, miR-210 and miR-222 were associated with poorer overall survival rates. They further noted that higher expression of all four miRNAs had a 6.2-fold increased risk of tumor-related death as compared to cases in which the expression of these miRNAs was low.

CONCLUSION

Since the discovery of miRNAs, growing evidence has confirmed a link between miRNAs and malignant diseases, and has identified their functions and targets that affect the complex process of carcinogenesis. Like other malignant tumors, PDAC has its unique miRNA expression patterns, which are different from those of other human tumors, and are able to differentiate normal pancreas from benign inflammatory pancreatic tissues and pancreatic cancer. At present, several important oncogenic and tumor suppressor miRNAs, and their molecular targets, have been identified in PDAC. More importantly, this information will lead to new development of prognostic, diagnostic, and treatment strategies. However, additional studies are required to find ways to utilize miRNAs as a therapeutic target in the clinical setting.

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