World Journal of Gastrointestinal Oncology

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DOI: 10.4251/wjgo.v8.i1.18

World J Gastrointest Oncol 2016 January 15; 8(1): 18-29
ISSN 1948-5204 (online)

TOPIC HIGHLIGHT

2016 Pancreatic Cancer: Global view

MicroRNA in pancreatic ductal adenocarcinoma and its precursor lesions

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Author contributions: Hernandez YG and Lucas AL wrote the paper.

Conflict-of-interest statement: Authors declare no conflict of interest for this article.

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Received: April 29, 2015
Peer-review started: May 7, 2015
First decision: September 8, 2015
Revised: November 4, 2015
Accepted: December 1, 2015
Article in press: December 2, 2015
Published online: January 15, 2016

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is the 4th deadliest cancer in the United States, due to its aggressive nature, late detection, and resistance to chemotherapy. The majority of PDAC develops from 3 precursor lesions, pancreatic intraepithelial lesions (PanIN), intraductual papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm. Early detection and surgical resection can increase PDAC 5-year survival rate from 6% for Stage Ⅳ to 50% for Stage Ⅰ. To date, there are no reliable biomarkers that can detect PDAC. MicroRNAs (miRNA) are small noncoding RNAs (18-25 nucleotides) that regulate gene expression by affecting translation of messenger RNA (mRNA). A large body of evidence suggests that miRNAs are dysregulated in various types of cancers. MiRNA has been profiled as a potential biomarker in pancreatic tumor tissue, blood, cyst fluid, stool, and saliva. Four miRNA biomarkers (miR-21, miR-155, miR-196, and miR-210) have been consistently dysregulated in PDAC. MiR-21, miR-155, and miR-196 have also been dysregulated in IPMN and PanIN lesions suggesting their use as early biomarkers of this disease. In this review, we explore current knowledge of miRNA sampling, miRNA dysregulation in PDAC and its precursor lesions, and advances that have been made in using miRNA as a biomarker for PDAC and its precursor lesions.

Key words: Pancreatic cancer; MicroRNA; Biomarkers; Pancreatic intraepithelial lesions; Intraductal papillary mucinous neoplasm

Core tip: Reliable biomarkers are needed to detect pancreatic ductal adenocarcinoma (PDAC) early in order to decrease mortality. In this review, we discuss what the current knowledge is on microRNA (miRNA) in PDAC and its precursor lesions. MiR-21, miR-155, miR-196, miR-210 are dysregulated in tissue, serum, cyst fluid, and stool of PDAC patients. MiR-21, miR-155, and miR-196 are dysregulated in intraductual papillary

mucinous neoplasm and pancreatic intraepithelial lesions demonstrating that these miRNAs may serve as potential biomarkers for early stage lesions and cancer.

Hernandez YG, Lucas AL. MicroRNA in pancreatic ductal adenocarcinoma and its precursor lesions. *World J Gastrointest Oncol* 2016; 8(1): 18-29 Available from: URL: http://www.wjgnet.com/1948-5204/full/v8/i1/18.htm DOI: http://dx.doi.org/10.4251/wjgo.v8.i1.18

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the 4th deadliest cancer in the United States, due to its aggressive nature, late detection, and resistance to chemotherapy[1,2]. The majority of PDAC develops from 3 precursor lesions, pancreatic intraepithelial lesions (PanIN), intraductual papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm (MCN)[3]. The cystic precursor lesions of the pancreas are detectable by certain imaging modalities such as Endoscopic ultrasound (EUS)[4-6], Magnetic Resonance Imaging of the Abdomen with Cholangiopancreatography[7], and computerized tomography scan[8,9]. To date, there is no modality that clearly detects PanIN lesions, although studies have suggested a correlation between multifocal PanIN and lobular atrophy of the pancreas on EUS[10].

Early detection and surgical resection can increase PDAC 5-year survival rate from 6% for Stage Ⅳ to 50% for Stage Ⅰ [11,12]. Detection and surgical removal of precursor lesions has the potential to be curative. Because of this, there has been much research focused on identification of individuals at high-risk of PDAC, detection of early stage lesions, and on the discovery of reliable biomarkers of this deadly disease. Carbohydrate antigen (CA) 19-9 is a poor biomarker of PDAC, as it is elevated in 30%-40% of benign diseases of the pancreas[13,14] with a sensitivity of 79% (70%-90%) and specificity of 82% (68%-91%)[15] for PDAC.

MicroRNA (miRNA) expression has been studied in tumor detection, cancer development and progression, and prognosis[16]. MiRNAs are small noncoding RNAs (18-25 nucleotides) that regulate gene expression by affecting translation of messenger RNA (mRNA)[16-18]. MiRNA function to stabilize mRNA transcripts *via* post-transcriptional gene silencing *via* inhibition of the translation process or cleavage of their target mRNAs[16,19]. Over the last decade, the role of miRNA in cancer development and detection has evolved. MiRNA is very stable in tissue, plasma, stool, and other fluids and can be quantified in very small sample sizes, making it an excellent potential biomarker for the detection of PDAC. Current priorities include: (1) identification of miRNAs that are reliably dysregulated in PDAC; (2) determining which sample source(s) are easily accessible and have the highest yield for detecting these biomarkers; and (3) development of novel ways in which to use this information to detect early onset PDAC and precursor lesions. Array-based analysis is used to evaluate the expression levels of thousands of miRNAs in various tissue types. Subsequent trials have validated these findings by performing quantitative real time PCR (QRT-PCR) and performed receiver operating characteristics (ROC) analysis to determine the sensitivity and specificity of these miRNAs as potential biomarkers for early or advanced disease. Some miRNAs, such as miR-21, miR-155, miR-196a, and miR-210 have stood out as potential biomarkers of this highly fatal disease[20-24]. Our review is aimed at exploring current knowledge of miRNA sampling, miRNA dysregulation in PDAC and its precursor lesions, and advances that have been made in using miRNA as a biomarker for PDAC and its precursor lesions.

ROLE OF MIRNA IN PDAC DETECTION: SAMPLES FROM TISSUE, SERUM, PANCREATIC JUICE, STOOL AND SALIVA

Attention has been paid to circulating serological signatures, autoantibodies, epigenetic markers, circulating tumor cells (TCs), and miRNAs in order to detect PDAC at an earlier stage of disease[25,26]. The use of miRNA for diagnosis and screening is still an evolving field; in the right patient population, an ideal miRNA test would be highly sensitive and specific, minimally-invasive and cost-effective. MiRNA expression in PDAC was first examined in PDAC tissue cells[27]. Now miRNA has been found in serum, blood, whole plasma, stool, saliva, and cyst fluid (Table 1). Current knowledge is described below.

MiRNA in whole pancreas tissue or PDAC biopsies

Szafranska *et al*[27] performed the first analysis comparing miRNA expression in normal pancreas tissue, chronic pancreatitis (CP) tissue and PDAC tissue. On imaging, it can be challenging to distinguish CP from PDAC given the thick stroma and inflammation that may be found in both of these conditions. Furthermore, it is unclear if the aberrant expression of particular miRNAs is secondary to the desmoplastic reaction in CP and PDAC, and not related to tumorigenesis itself. They and others have found that miRNA-216 and miRNA-217 are significantly down-regulated in PDAC and miRNA-143, miR-145, miR-146a, miR-148a, miR-150, miR-155, miR-196a, miR-196b, miR-210, miR-222, miR-223, miR-31 are up-regulated in PDAC[24,27-29]. However, this study also demonstrated that dysregulation of miRNA-196a, miR-196b, miR-203, miR-210, miR-222, miR-217, and miR-375 were found only in PDAC, whereas miRNA-29c, miR-96, miR-143, miR-145, miR-148b, and miR-150 were abnormally expressed in both CP and PDAC. This may suggest that the latter are responsible for causing the desmoplastic reaction as opposed to tumorigenesis.

Table 1 MiRNA in pancreatic ductal adenocarcinoma

MiRNA	Whole pancreas	Serum and plasma	Saliva	Stool	Pancreatic juice
miR-10b		↑[54]			
miR-16		↑[52]			
miR-18a		↑[29,56,57]			
miR-20a		↑[55,134]			↓[62]
miR-21	↑[22,24,27,32,34,38,55,71]	↑[55,71]		↑[63]↔[71]	↑[61,62,71]
miR-24		↑[55]			
miR-27a-3p		↑[134]			↑[62]
miR-29c	↓[27]				
miR-30a-3p	↓[27]				
miR-30c		↓[37]			
miR-31	↑[27]				
miR-34a		↑[37]			
miR-96	↓[27,135]				↓[62]
miR-99a		↑[52]			
miR-101	↑[32]				
miR-106b		↑[54]			
miR-130b	↓[27]				
miR-135b	↑[31]				
miR-139-3p		↓[37,58]			
miR-141	↓[27]				
miR-143	↑[27,71]			↓[71]	
miR-145	↑[27]				
miR-146a	↑[27]				↑[62]
miR-148a	↓[27,29]				
miR-148b	↓[27-29]				
miR-150	↑[27]				
miR-155	↑[22-24,27,32,66,71]	↑[22,54]		↑[63]↓[71]	↑[61,71]
miR-181a,b,d	↑[24]			↑[72]	
miR-185		↑[52,55,134]			
miR-191		↑[55]			
miR-192		↑[37,58]			
miR-194		↑[37]			
miR-196a	↑[22,23,27,49,52,71]	↑[21,22,52,71]		↑[72]↓[71]	↑[62,71]
miR-196b	↑[27]				↑[60]
miR-200a		↑[136]			↑[62]
miR-200b		↑[136]			
miR-205					
miR-210	↑[27,71,137,138]	↑[137]		↑[72], ↔ [71]	↑[60,71]
miR-212	↑[38]	↑[37]			
miR-216	↓[27,38]			↓[63]↓[71]	
miR-217	↓[27]				↓[62]
miR-222	↑[24,27,38]				
miR-223	↑[27]				
miR-375	↓[27,71]			↔[71]	
miR-492		↓[59]			↑[60]
miR-494	↓[27,74]				
miR-508-5p		↓[37]			
miR-513a-5p		↓[37]			
miR-602		↑[37]			
miR-630		↓[37]			
miR-663a		↓[59]			
miR-801		↑[37]			
miR-887		↓[37]			
miR-923		↓[37]			
miR-940			↑[74]	↑[74]	
miR-1290	↑[30]				
miR-1427					↑[60]
miR-3679-5p			↓[74]	↓[72,74]	

miR: MicroRNA; ↑: Up-regulated; ↓: Down-regulated; ↔: Unchanged.

MiR-1290 is elevated in early stage PDAC compared to normal controls[30]. Additionally, miR-135b has been shown to be an effective biomarker for distinguishing PDAC from CP with high sensitivity and specificity[31].

MiR-21, MiR-155, and miR-196 have been demonstrated by multiple groups to differentiate PDAC from non-cancerous lesions of the pancreas[20-24,27,32]. Special attention has been placed on the role of miR-21 in

PDAC, as it has been implicated in tumorigenesis, TC invasion, the desmoplastic reaction, and metastasis of TC[33-36]. Further studies did not demonstrate that miR-21 expression in stromal cells correlated with tumor stage.

MiR-192 has also been found to be present in pancreatic TC, but is seldom seen in stromal cells and not found in adjacent normal pancreas tissue[37]. In this same study, miR-194 expression was detected in PDAC tissue, but not found in the surrounding normal pancreatic tissue. Unfortunately, despite these findings, no significant difference was found between the serum levels of miR-194 in patients with PDAC and healthy controls.

One proposed mechanism for PDAC development includes signaling between the molecular markers of the desmoplastic reaction and TCs[38-41]. Liffers *et al*[29] demonstrated that miR-148a is down-regulated in microdissected PDAC tissue and when over-expressed prevents tumor growth. This suggests that miR-148a may have a crucial role in the molecular signaling by which tumorigenesis occurs. While it is important to find biomarkers that are deregulated in PDAC, it is also important to understand which miRNAs are involved in these aberrant signaling pathways.

MiRNA in serum and plasma of PDAC patients

MiRNAs are known to have organ-specific expression in many human cancers[42,43]. Less than a decade ago, studies found that miRNA could reliably be detected in the serum in both animal models and humans[44,45], and since that time, there has been much research dedicated to identifying which miRNAs have differential expression and the implications of these findings in the detection, staging, treatment, and prognosis of cancers[46-50].

Attempts to use miRNA biomarkers in conjunction with CA19-9 have yielded mixed results. A study examining 847 different miRNAs in patients with PDAC found increased expression of miR-375 in PDAC as opposed to controls. MiR-375 did not improve detection nor predict prognosis in patients with PDAC when compared to CA19-9 alone[51]. Liu *et al*[52] found that using serum miR-16 and miR-196a in combination with CA19-9 increased detection of PDAC and Stage Ⅰ lesions when compared to either modality alone, which suggests that miR-16 and miR-196a may be deregulated early in PDAC. These biomarkers were also up-regulated in studies performed on pancreas tissue, demonstrating that miR-16 and miR-196a can be used as peripheral biomarkers of PDAC. Gao, *et al*[53] also demonstrated that miR-16, when combined with CA19-9, served as a potential biomarker for detection of PDAC when compared to patients with CP.

One limitation of CA19-9 as a biomarker is that it is elevated in a large portion of patients with benign pancreatic diseases. Because of this limitation, studies have evaluated the miRNA expression of patients with PDAC compared to those with benign diseases such as CP or choledocolithiasis. They found that miR-10b, miR-155, and miR-106b were consistently elevated in the serum of patients with PDAC but not in those with benign pancreatic disease[54]. Liu *et al*[55] have demonstrated that up-regulation of miR-20a, miR-21, miR-24, miR-25, miR-99a, miR-185, and miR-191 can be used to distinguish PDAC from healthy controls and CP. Additionally, miR-135b has been shown to be an effective biomarker for distinguishing PDAC from CP with high sensitivity and specificity[31]. MiR-18a levels have also been shown to have increased expression in patients with PDAC and interestingly decrease after surgical resection suggesting that miR-18a levels may be a good marker to not only detect disease but also to monitor disease recurrence[29,56,57]. Zhang *et al*[37] also demonstrated that miR-194, miR-192, miR-602, miR-801, miR-212, miR-34a are up-regulated in PDAC, while miR-923, miR-139-3p, miR-513a-5p, miR-630, miR-30c-1, miR-887, miR-508-5p, and miR-139a-5p were down-regulated in PDAC specimens[37,58]. From these data, they demonstrated that miR-192 is neither present in the stromal cells of the pancreas nor the serum, but it is up-regulated in PDAC TCs and is involved in cell proliferation of PANC-1 TC lines *in vitro*[58]. Lin *et al*[59] performed microarray on 1711 serum miRNAs and found that 23 were down-regulated and 22 were up-regulated in the serum of PDAC patients when compared to normal controls. Of these, miR-492 and miR-663a were found to have decreased expression that was statistically significant in PDAC; however, only miR-663a was found to have a positive correlation with stage of disease[59]. Further studies are needed to determine which miRNAs will be clinically relevant.

Pancreatic juice miRNA

Pancreatic juice sampling requires an invasive endoscopic procedure, but studying the miRNA concentrations of patients with PDAC, benign pancreatic lesions, and healthy controls can shed light on potential biomarkers for detecting disease as they are found in high concentration in cyst fluid. As EUS and endoscopic retrograde cholangiopancreatogram (ERCP) are two methods by which pancreatic masses are frequently detected and sampled, these specimens could be sent for miRNA analysis in order to determine the malignant potential of these lesions. Wang *et al*[60] performed microarray of 49 miRNAs on secretin-stimulated pancreatic juice of a group of patients with PDAC, CP, and normal controls. They demonstrated that miR-205, miR-210, miR-492, and miR-1427 are all significantly elevated in PDAC when compared to controls; however, this statistical significance does not exist when compared to patients with CP[60]. Additionally, by using ROC curves, they determined that combining these 4 miRNAs with serum CA19-9 the sensitivity and specificity of PDAC detection is 91% and 100% respectively, though this analysis was limited by a sample size of 6. Other groups have evaluated the pancreatic juice of patients with PDAC pre-operatively *via* ERCP and from post-operative specimens[61,62]. Sadakari *et al*[61] analyzed the expression

of miR-155 and miR-21 in pancreatic juice sampled *via* ERCP and found that these miRNAs were significantly elevated when compared to patients with CP and healthy controls, though the levels did not correlate with pancreatic juice cytology[61]. Again these findings are consistent with those from pancreatic tissue and serum. Hong *et al*[62] evaluated 158 miRNAs in post-operative fine needle aspiration specimens and found by qRT-PCR that miR-21, miR-27a, miR-146a, and miR-186a were significantly over-expressed in PDAC tissue and miR-217, miR-20a, and miR-96 were significantly down-regulated in PDAC tissue when compared to normal controls[62]. These two studies have demonstrated the feasibility of detecting miRNA from pancreatic juice, thus indicating the potential for using pancreatic juice biomarkers to detect early lesions given the higher concentration of miRNA in this fluid sample.

MiRNA in stool specimens

Frozen stool specimens may serve as potential non-invasive biomarker samples for PDAC. Over-expressed miRNAs from gastrointestinal cancers are shed from the exfoliative cells of the gastrointestinal tract. Intraluminal release of pancreatic juice also allows for detection of miRNAs in the stool[63-69]. Previous studies have largely focused on genetic markers of tumorigenesis and not miRNA[70]. Yang *et al*[63] performed a feasibility study of using stool miRNAs as a potential screening tool for detection of PDAC. They evaluated expression of 5 miRNAs that had been previously shown to be over-expressed in PDAC and found that miRNA-21 and miR-155 were over-expressed and miR-216 was under-expressed in all PDAC stool specimens when compared to normal controls and CP patients. These findings are consistent with what has been found in whole pancreas, pancreatic cyst fluid, and serum specimens. Additionally, with ROC analysis they demonstrated that combining miR-21 and miR-155 in stool samples there was a sensitivity of 93.33% and specificity of 66.67%. When they combined all 3 miRNAs (miR-21, miR-155, and miR-216), the sensitivity and specificity were 83.33% each. Link *et al*[71] selected 7 miRNAs (miR-21, miR-210, miR-143, miR-155, miR-196a, miR-216a, miR-375) and determined that like Yang's group miR-216a was found in lower concentrations in the stool of patients with PDAC. However, unlike Yang's group, they found that miR-155 was down-regulated in this population and miR-21 was unchanged in the stool of controls compared to CP or PDAC[71].

Ren *et al*[72] also evaluated the expression of miR-21, miR-155, miR-181a, miR-196a, and miR-210 and found that miR-181b, miR-196a, and miR-210 were significantly over-expressed in PDAC patients when compared to controls, but only miR-181b and miR-210 were elevated in CP patients, though these elevations were not significant. Ren *et al*[72] established a positive correlation between miR-196a levels and tumor size, which had not been previously described in studies of the serum or stool. Overall, while studies of fecal miRNA have demonstrated feasibility, conflicting data have emerged on which miRNAs are differentially expressed in the stool of PDAC, benign pancreatic disease, and normal controls.

Salivary miRNA

The field of salivanomics has been developing since blood molecules have been found in saliva[73]. As with stool miRNA, salivary miRNA may serve as a non-invasive biomarker for PDAC. Xie *et al*[74] is the only group to have evaluated salivary miRNA in PDAC. They conducted a microarray of 2006 miRNAs and noted that 10, including miR-4433-5p, miR-4665-3p, miR-940, miR-1273g-3p, miR-3676-5p, miR-3679-5p, miR-3940-5p, miR-4327, miR-4442, and miR-5100, were up-regulated or down-regulated in salivary samples. Of these, only miR-940 was significantly up-regulated and miR-3679-5p was significantly down-regulated in the PDAC specimens during the validation phase of the study. Until now, neither has been implicated in PDAC in the serum, stool, whole pancreas, or pancreatic juice. More studies are needed in this area.

ROLE OF MIRNA IN DETECTION OF PRECURSOR LESIONS

The absence of symptoms in early disease makes PDAC a cancer that is detected at very late stages when mortality approaches 100%. Much research has been dedicated to detecting miRNA in patients with PDAC as a novel biomarker for the presence of disease. Given the aggressive nature of PDAC, detection of precursor lesions with malignant potential would be critical to increasing the survival of these patients. PanIN lesions are microscopic PDAC precursor lesions that are graded 1-3 and are categorized based on the level of architectural and cytological atypia that is present[75,76]. Grade 1a is early intraepithelial proliferative lesions that have flat architecture, while grade 1b lesions have papillary architecture. PanIN-2 lesions have moderate abnormalities and PanIN-3 lesions have severe abnormalities, though none of these lesions invade the basement membrane[75,76]. IPMN lesions are mucin-producing cystic tumors, which arise from the epithelium of the pancreatic ducts and have the potential for malignant transformation[77,78]. They are categorized by main duct type (MD) or branch duct type (BD) and histologically are classified as having low-, intermediate-, and high-grade dysplasia[3]. Their malignant potential differs based on their location within the pancreatic ducts, and MD-IPMN carry a 44%-48% risk compared to BD-IPMNs, which only carry a 11%-17% risk of malignant transformation[79-82]. MCN are also mucin-producing epithelial neoplasms with ovarian-type stroma occur primarily in middle-aged females and are located in the body and tail of the pancreas and carry a 12% chance of tumor progression[83-86]. Cystic fluid is analyzed

Table 2 MiRNA in precursor lesions

MiRNA	IPMN	PanIN-1	PanIN-2	PanIN-3
miR-10b			↑[101]	↑[101]
miR-21	↑[23,32,87,88]	↑[100,101]	↑[98,99]	↑[98,99]
miR-92a	↑[93]			
miR-99a	↓[91,93]			
miR-99b	↓[91]			
miR-100	↓[91,93]			
miR-101	↓[32]			
miR-125b	↑[93]			
miR-126	↓ [91]			
miR-130a	↓ [91]			
miR-145	↑[93]			
miR-148			↓[101]	↓[101]
miR-155	↑[23,32]	↑[101]	↑[98]	↑[98]
miR-182		↑[101]		
miR-196a	↑[90]		↑[97]	↑[97]
miR-196b			↑[97]	↑[97]
miR-200a		↑[101]		
miR-200b		↑[101]		
miR-212	↓[93]			
miR-217			↓[101]	↓[101]
miR-221	↑[87]			
miR-296-5p		↑[101]		
miR-342-3p	↓[91]			
miR-483-3p	↑[88,93]			

miR: MicroRNA; ↑: Up-regulated; ↓: Down-regulated; PanIN: Pancreatic intraepithelial lesions; IPMN: Intraductal papillary mucinous neoplasm.

for CEA and amylase as other tumor markers have not demonstrated reliability in detecting malignant lesions.

As cystic neoplasms of the pancreas carry the risk of malignant transformation, determining a way to accurately predict which will progress to invasive carcinomas may guide surgical management and treatment decisions. MiRNA has been examined in PanIN lesions and IPMN as a potential candidate for early detection and the likelihood of progression to cancer. Understanding which miRNAs become deregulated early in the disease process may lead to advances for treatment decisions (Table 2).

MiRNA in IPMN lesions

Given the increased use of abdominal imaging, more pancreatic cystic lesions are being detected. There are guidelines in place to help guide management based on cystic characteristics that are consistent with malignancy[77]. The first study looking at miRNA expression levels in precursor lesions of the pancreas was performed by Habbe *et al*[23] who determined that miR-155 and miR-21 were over-expressed in the IPMN neoplastic epithelium, specifically those with carcinoma-in-situ[23,87]. MiR-155 was also significantly elevated in the pancreatic juice of these patients. While the levels of up-regulation of miRNA-21 and miR-155 correlated with the degree of cellular atypia found in the IPMN lesions, the study lacked long-term outcome data, highlighting the need for large and more longitudinal studies. Caponi *et al*[32] established a relationship between expression of miR-21 in invasive IPMNs and clinical outcome and observed that higher levels of miR-21 were correlated with worse overall and disease-free survival. Furthermore, they demonstrated that miR-155 and miR-21 had higher expression levels in invasive IPMN lesions when compared to non-invasive lesions, suggesting that these miRNAs may serve as early markers of malignant transformation[32,88]. MiR-101 has been shown to be down-regulated in invasive IPMNs when compared to non-invasive IPMNs and normal tissue. This deregulation of MiR-101 has not been described in PDAC samples, which may indicate that miR-101 plays a role in tumor invasion[32,89]. As with studies of miRNA in the serum and tissue of PDAC patients, miR-196a was found to be up-regulated in the pancreatic juice of intestinal-type IPMN[90]. In a recent study miR-100, miR-99b, miR-99a, miR-342-3p, miR-126, miR-130a were found to be down-regulated were up-regulated in high-risk *vs* low-risk IPMN lesions[91]. Furthermore, low miR-99b in IPMN fluid was associated with MD involvement, which is associated with a greater risk for transformation into a malignant neoplasm. Abue *et al*[88] found that miR-483-3p was up-regulated in PDAC cells and plasma when compared to IPMN lesions and may also serve as a useful biomarker in differentiating IPMN lesions with malignant potential from normal tissue and PDAC. The down-regulated miRNAs correlated with high-risk IPMNs and may be involved in cyst invasion and progression. Lee *et al*[92] found that miRNA expression varied amongst pancreatic cystic neoplasm. Specifically, miR-31-5p, miR-4830-5p, miR-99a-5p, and miR-375 were characteristic of serous cyst adenomas (SCA), whereas miR-10-5p, miR-202-3p, miR-210, and miR-375 differentiated MCN from SCA, IPMN, and PDAC[92]. It is unclear why this overlap in miR-375 occurs[92]. Henry *et al*[93] found that miR-92a, miR-99a, miR-100, miR-125b, miR-145, miR-212 and miR-483 were differentially expressed between benign and pre-malignant and malignant lesions of the pancreas and they suggested that a high amount of RNA present in the cystic fluid may suggest the presence of malignant transformation[93]. As previously described miR-21, miR-155, miR-196a have been implicated in both PDAC and IPMN and given these widely replicated results, studies aimed at detecting these biomarkers in serum, saliva, and stool could help to determine, in a non-invasive way, if they are increased in pre-malignant lesions.

MiRNA in panin lesions

Currently, PanIN lesions are found in the neighboring pancreatic tissue of patients with PDAC; however, there is no consistent way to detect the presence of these lesions[10,94-96]. Identifying a biomarker to detect PanIN lesions may be critical in early detection of PDAC. Slater *et al*[97] demonstrated that miRNA-196a and miR-196b were elevated in PDAC and PanIN-2/3 lesions in both animal models of PDAC and humans with PDAC. Ryu *et al*[98] demonstrated that miR-155

is up-regulated in PanIN-2 and PanIN-3 lesions when compared to neighboring healthy pancreatic tissue, but not in PanIN-1 lesions. Furthermore, miR-21 has been shown to be over-expressed in PanIN-2[98,99] and PanIN-3[98,99], but not PanIN-1 lesions suggesting that this is a marker for later disease. These are significant findings as miR-155 and miR-21 have been shown to be up-regulated in IPMN lesions and PDAC suggesting that they are early markers for cells with malignant potential. Yu *et al*[100] found that miR-196b was up-regulated in PanIN-3 lesions, which correlates with previous studies that have found that miR-196b is up-regulated in PDAC lesions[100,101]. Importantly, miR-21, miR-155, miR200a, miR-200b, miR-182, and miR-296-5p were deregulated as early as PanIN-1 lesions and remained deregulated until progression to PDAC, with the exception of miR-200c that normalized in PanIN-3 lesions. A recent publication describing miRNA expression in PanIN lesions found that miRNA-148a and miR-217 were down-regulated while miR-10b was up-regulated in PanIN-2 and PanIN-3 lesions[101]. While miR-21 has been shown repeatedly to be over-expressed in PDAC, there are conflicting studies on its deregulation in early PanIN lesions suggesting that it may represent a later and more aggressive dysregulation in the progression to PDAC. A non-invasive method to detect advanced PanIN lesions would represent a significant advance in the field.

DISCUSSION

While PDAC is the fourth most common cause of cancer-related deaths, there is still no reliable way to detect early disease and patients present with late-stage disease with a nearly 100% mortality. Research in the field of biomarkers shows a great deal of promise as current research aims to understand the molecular mechanisms and stromal microenvironment of this deadly tumor. MiRNA are small nucleotides that control the genetic expression in all cells and importantly in an organ-specific manner. Abberant miRNA expression has been identified in various cancers[102-106], and factors such as transcriptional deregulation, epigenetic alterations, mutations, DNA copy number abnormalities, and defects in the miRNA biogenesis pathway may account for these differences in expression[107,108]. C-myc and p53 are two transcriptional factors that have been associated transcriptional deregulation of miRNA[109-111], Epigenetic regulation of miRNAs by DNA methylation and histone tail modification play a role in miRNA expression through chromatin remodeling[105,112-114]. Both germ-line and somatic mutations are responsible for miRNA expression levels in various types of cancers[115-117]. It has been described by Calin *et al*[118], that miRNAs are located a fragile sites on the chromosome, minimal regions of loss of heterozygosity, minimal regions of amplifications, and common breakpoints, thus increasing the risk for DNA copy abnormalities. DNA copy abnormalities have been found in melanoma, breast cancer, ovarian cancer, leukemia, colorectal cancer[119-122]. Lastly, defects in miRNA biogenesis pathway may contribute to varying expression levels and cancer phenotype as miRNA undergoes complex processing intracellulary prior to reaching its mature form[123-127]. In addition to the aforementioned mechanisms, dietary components, such as folate, retinoids, curcumin, and Vitamin D have been implicated in the modulation of miRNA expression[128-130]. Some miRNAs have been shown to increase muscle loss in cancer cachexia and specifically, increased miR-21 levels have been shown to increase muscle breakdown in pancreatic cancer[131,132]. Deeper understanding of the regulatory mechanisms of miRNA expression will hopefully give new insight to the factors responsible for miRNA deregulation and lead to miRNA-based diagnostic testing and miRNA-directed therapy for PDAC.

Some limitations that exist with the current miRNA research at this time include standardization of extraction, reproducibility of testing, diagnostic yield in the various sample methods, and small sample sizes. Additionally, despite finding biomarkers for this disease, there is limited evidence that miRNA will impact PDAC-related mortality. Dysregulation of miRNA affects the cell cycle, proliferation, apoptosis, epigenetics, oncogenesis, tumor differentiation, tumor invasion, tumor metastasis and migration, prognosis, and chemoresistance in numerous cancers[133]. Increased efforts to understand the biological function of miRNA expression and its effects on cancer development are needed.

Despite these limitations, great advances have been made in this field and now miRNA expression is being analyzed not just in pancreatic tissue and cystic fluid, but also in stool, saliva, and serum; which would lead to non-invasive ways by which to analyze the expression levels of miRNA in patients at high risk. There have been great efforts to identify which of the greater than 2000 miRNAs are deregulated in PDAC and its precursor lesions and miRNA-21, miR-155, and miR-196b seem to be dysregulated in both early lesions and advanced cancer and show promise as potential screening tools in the future.

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P- Reviewer: Slotwinski R
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