

# Can miRNA Biomarkers Be Utilized to Improve the Evaluation and Management of Pancreatic Cystic Lesions?

## Abstract

This article reviews the current strategies and challenges of diagnosing pancreatic cystic lesions, and presents an overview of molecular tools that are available to enhance diagnostic accuracy. Specifically, we highlight the emergence of microRNAs (miRNAs) as diagnostic markers. miRNA signatures have been reported for both solid tissue and biofluid specimens, including cyst fluid, collected from patients with solid and cystic pancreatic lesions. These miRNA signatures offer the opportunity to improve molecular characterization of pancreatic lesions, to help guide clinical management through early diagnosis and informed prognosis, and to provide novel therapeutic targets for pancreatic cancer.

## Keywords

microRNA • miRNAs • pancreatic cystic neoplasms • cystic fluid • Laboratory Developed Test (LDT) • Clinical Laboratory Improvement Amendments (CLIA) • College of American Pathologists (CAP) • mucinous cystic neoplasms (MCN) • intraductal papillary mucinous neoplasms (IPMN) • cystadenomas (SCA) • solid pseudopapillary neoplasm (SPEN) • endoscopic ultrasound (EUS)

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## 1. Background

The management of pancreatic cystic lesions presents a clinical challenge. With technological advances and widespread utilization of radiologic imaging, these lesions are often identified incidentally. Recent radiology studies suggest pancreatic cysts are identified in up to 20% of adults without history of pancreatic disease who are undergoing magnetic resonance imaging (MRI) studies for non-pancreatic indications [1,2]. Unlike most hepatic and renal cysts, many pancreatic cystic lesions have malignant potential. Therefore, accurate and rapid identification of those that are malignant and pre-malignant has important clinical implications.

Pancreatic cysts may be broadly classified into non-neoplastic cysts, cystic neoplasms, and necrotic degeneration of solid tumors. Non-neoplastic cysts have no malignant potential and include pseudocysts, retention cysts, and benign epithelial cysts. Cystic neoplasms include both mucinous and non-mucinous cystic varieties. Mucinous cysts are pre-malignant and comprise mucinous cystic neoplasms (MCN) and intraductal papillary mucinous neoplasms (IPMN). Non-mucinous cysts have low or no malignant potential and include serous cystadenomas (SCA) (Table 1). Finally, some pancreatic cancers, such as neuroendocrine or acinar cell carcinoma, may undergo necrotic degeneration with formation of a cystic cavity resembling the aforementioned pancreatic cysts.

Definitive characterization of these different types of pancreatic cystic lesions relies mainly on the combination of diagnostic imaging and analysis of cyst fluid obtained during endoscopic ultrasound-guided fine needle aspiration (EUS-FNA). Unfortunately, the imaging findings of pancreatic cysts can be similar, making differentiation between benign and pre-malignant lesions difficult [3-6]. In addition, current cyst fluid analyses fail to clearly distinguish among the different types of pancreatic cysts, thus preventing the prediction of their behavior [6,7]. The accurate classification of pancreatic cysts is important since pre-malignant lesions may require surgical resection, while benign, non-malignant cysts can be monitored without the need for surgery. In this review we present an overview of pancreatic cystic neoplasms and discuss recent data supporting the potential clinical utility of miRNA profiling as an ancillary tool in diagnosis.

Table 1. Classification of pancreatic cysts.

Benign, Not Pre-malignant	Pre-malignant/ Malignant
Serous cystadenoma	Intraductal papillary mucinous neoplasm
Pseudocyst	Mucinous cystic neoplasm
Lymphoepithelial cyst	Solid pseudopapillary neoplasm
Lymphangioma	Cystic neuroendocrine tumor
Retention cyst	Metastatic cyst (e.g., ovarian cystadenocarcinoma)

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## 2. Differential Diagnosis

Sixty percent (60%) of pancreatic cystic lesions are cystic neoplasms (mostly composed of SCA, MCN, IPMN), 30% are pseudocysts and approximately 10% are the result of degeneration of solid neoplasms [8]. Pseudocysts are sequelae of acute pancreatitis and require at least 4 – 6 weeks to arise [9]. A thin capsule of non-epithelialized granulation or fibrotic tissue forms a wall around amylase-rich fluid. Symptoms, when present, typically consist of abdominal pain, but gastric outlet and/or biliary obstruction may occur as well.

Serous cystadenomas are benign pancreatic cystic neoplasms, which very rarely become malignant. SCAs account for over 30% of pancreatic cystic neoplasms and typically occur in women over the age of 60 [10]. SCAs are multicystic, and 30% have a lobular 'honeycomb' appearance due to dense septations producing multiple small cysts (Figure 1A). As with the majority of pancreatic cystic lesions, SCAs are often discovered

incidentally. SCAs may present with nonspecific symptoms due to compression of adjacent organs by the cyst. The natural history of SCAs is not well described; however, they appear to grow over time. Malignant transformation is extremely rare with only a few case reports of serous cystadenocarcinoma [11]. On pathology, SCAs are lined by glycogen-containing cuboidal epithelial cells (Figure 1B). SCAs are typically monitored with serial imaging due to their tendency to grow, although the frequency of imaging is debatable with some advocating imaging every 12 months while others suggest biennial surveillance [12-14]. Because these are benign lesions, surgical resection is reserved for patients with symptoms, cystic lesions without a clear diagnosis where the potential for malignancy cannot be ruled out, and large (>4 cm) lesions.

Mucinous cystic neoplasms are pre-malignant parenchymal lesions that almost exclusively occur in women, usually between 40 and 50 years old [10]. They arise in the body-tail of the pancreas in approximately 95% of patients and are defined by the presence

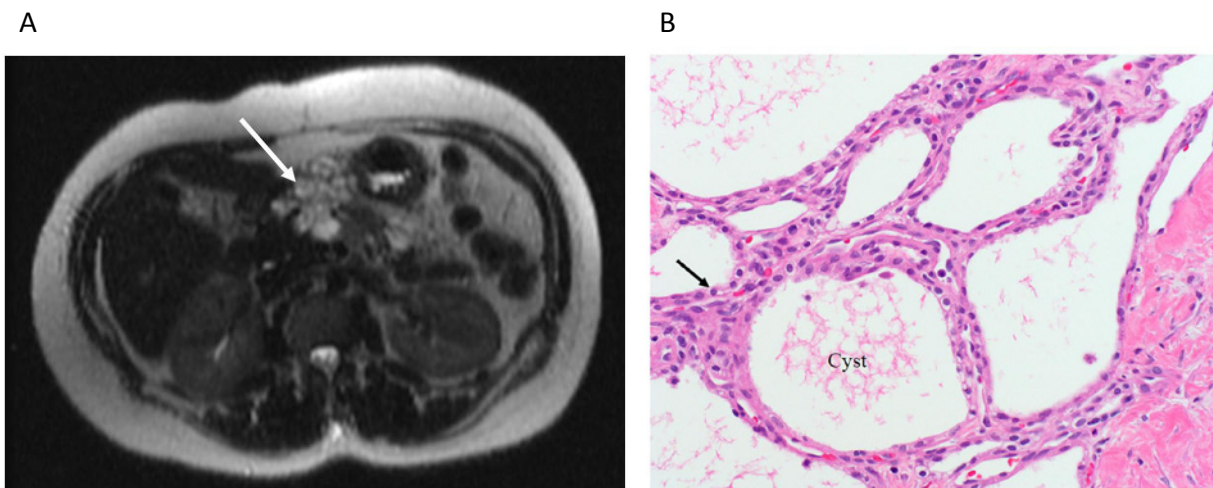


Figure 1. (A) MRI of serous cystadenoma; (B)- Histology of serous cystadenoma with a microcystic pattern. Cysts are lined by bland cuboidal cells (arrow) with clear or palely eosinophilic cytoplasm.

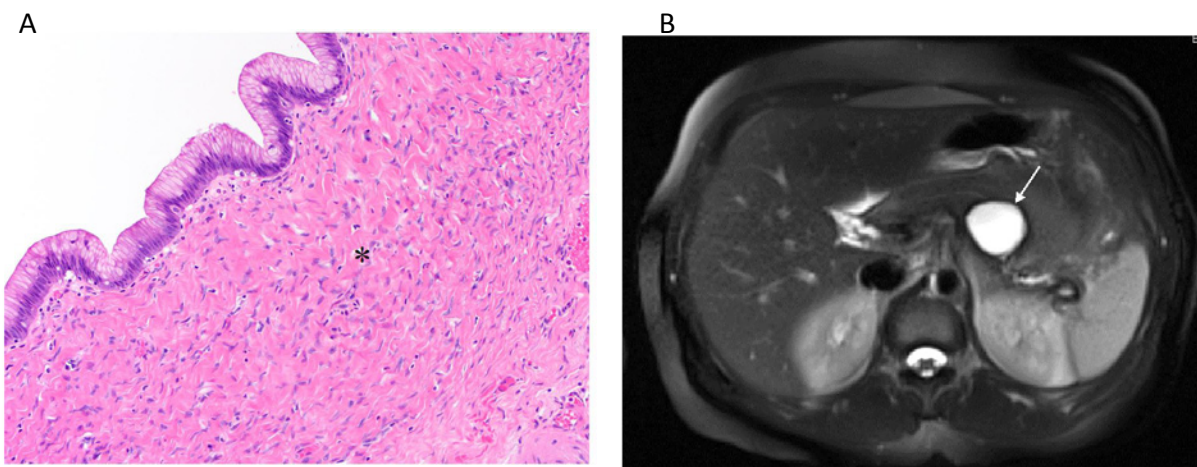
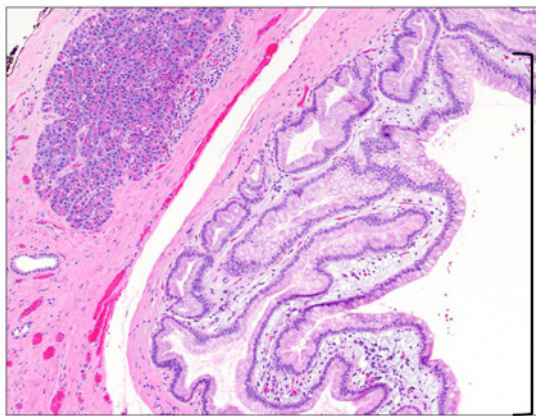


Figure 2. (A) Histology of mucinous cystic neoplasm showing low-grade mucinous epithelium and underlying 'ovarian-type' stroma (\*). (B) MRI of mucinous cystic neoplasm.

of ovarian-like stroma (Figure 2A) [10]. MCNs usually appear smooth, well-defined, and unilocular or with a few septations (Figure 2B). Unlike SCAs, presence of symptoms in mucinous cystic lesions is associated with malignancy. Features that are risk factors for malignancy in MCN include older age (> 55 years), large size (diameter >6 cm), and presence of thick cyst wall, mural nodules, or peripheral eggshell calcification [10]. The true incidence of malignancy in MCNs is unknown. Recent studies suggest incidence rates of invasive cancer can range from 12% to 29% with only 5.5% carcinoma in situ [15]. The pre-malignant nature of MCNs combined with the lack of reliable tools to definitively diagnose malignancy in MCNs preoperatively and the relatively young age (40-50 years) of these patients drives consensus recommendations for surgical resection of these lesions in all cases. Patients who are poor surgical candidates may be followed with serial imaging and monitored for symptoms [16].

Intraductal papillary mucinous neoplasms are mucinous cysts that arise from the pancreatic ductal epithelium of the main duct, side branches, or both (Figure 3). IPMNs occur most commonly in men between the ages of 50 and 60. Symptoms may include steatorrhea and diabetes; 15% to 30% of patients with IPMN also present with acute pancreatitis, most likely due to obstructive pancreatitis from mucus plugging the ducts. It is important to accurately differentiate among the clinical subtypes of IPMN as early as possible, since each subtype varies in malignant potential and management. The three subtypes of IPMN are main duct (MD; diffuse or segmental dilation of the main duct >5 mm), branch duct (BD; dilation of one or more side branches), and mixed type (both main duct and side branch involvement). By pathology, IPMN may also be classified as gastric, intestinal, or pancreaticobiliary type. The gastric type is typically low grade while the intestinal and pancreaticobiliary types are more aggressive [17]. While histological grading may hold some predictive value, this is currently only available following surgical resection.

The various IPMN subtypes are usually differentiated by radiology; however, diagnostic accuracy of radiologic imaging compared to surgical pathology for branch duct BD-IPMN and



**Figure 3.** Histology of MD-IPMN showing mixed gastric and intestinal-type epithelium and mild dysplasia (area within bracket).

mixed type IPMN is 49% and 73%, respectively [18]. The mean risk of malignancy in MD-IPMN is 62% [16], and the malignant potential of mixed type IPMN is believed comparable to this. Therefore, surgical resection is recommended for all patients with MD- or mixed type IPMN who are surgical candidates [16]. However, nearly 20% of BD-IPMNs originally diagnosed by radiology are later found by surgical pathology to be mixed type IPMNs [18]. Because only approximately 15% of BD-IPMNs undergo malignant transformation [19] these patients are often followed conservatively (unless there are other indications suggestive of malignancy). Therefore, misclassification of IPMNs may lead to inappropriate management of patients who have different risks of malignancy.

Revised International Association of Pancreatology (IAP) consensus criteria from 2012 recommend surgical resection of BD-IPMN in patients with obstructive jaundice and a cystic lesion in the head of the pancreas, solid component, main pancreatic duct >10 mm, mural nodule, main duct involvement (thickened wall, mural nodule or intraductal mucin), or cytology suspicious or positive for malignancy [16]. The previous criteria from 2006 also supported surgery for cyst size >3 cm [20], however, the recent criteria deemphasize size as the sole criteria in the decision to operate and indicate to 'strongly consider' surgery in young, otherwise healthy patients with cysts >3 cm.

Patients who do not undergo surgical resection should be followed with surveillance imaging. A repeat CT or MRI with MRCP should occur 3-6 months following the initial imaging. If the lesion appears stable, surveillance recommendations depend on cyst size ranging from every 3-6 months for cysts >3 cm to every year or other year for cysts <2 cm [16].

Other less common pancreatic cystic neoplasms include solid pseudopapillary neoplasms (SPENs), which occur almost exclusively in young women of childbearing age and account for 1-2% of pancreatic cystic neoplasms. About 10-15% of SPENs are malignant, and to date, no predictors of aggressive behavior have been identified [21]. These patients usually present with nonspecific abdominal pain and occasionally with an abdominal mass palpable on examination. SPENs typically appear as a large well-defined encapsulated mass with peripheral solid component and cystic degeneration in the center with areas of hemorrhage (Figure 4) [22]. Pathology reveals characteristic pseudopapillae with cystic spaces containing hemorrhage and cholesterol clefts in myxoid stroma alternating with solid tissue. All patients with SPEN should undergo surgical resection. Local invasion and/or limited metastases occur in less than 20% of patients with SPEN and are not a contraindication to surgical resection. Long-term survival is excellent for these patients with 5-year survival rates of 95% [22].

Less commonly observed lesions such as neuroendocrine or acinar cell tumors occasionally undergo cystic degeneration. Cystic neuroendocrine tumors account for only 8% to 17% of pancreatic neuroendocrine tumors and are usually asymptomatic [23]. Acinar cystadenocarcinoma is extremely rare with fewer than 10 cases reported in the literature. These tumors typically present with abdominal pain and a multilocular cystic lesion [24].

Execution of the appropriate treatments as outlined above requires the definitive diagnosis of each type of pancreatic cyst. Unfortunately, a major problem with managing pancreatic cystic lesions is that often, despite extensive radiologic and endoscopic evaluation, the detailed diagnosis of the lesion remains unclear [25]. The most cost-effective approach to asymptomatic patients with incidentally discovered pancreatic cystic lesions requires the consideration of each patient's age, cyst location, cyst size, and preoperative health score (as defined by the American Society of Anesthesiologists) [26]. Three management options were evaluated in a recent study to determine which was most cost-effective: a conservative approach with radiologic follow-up, an aggressive approach with surgical resection for all surgical candidates, or a EUS-directed approach. The EUS approach was the most cost-effective strategy, with subsequent decision for radiologic follow-up or surgery based on a combination of cytology and CEA from EUS-FNA in addition to the patient's surgical risk.

### 3. Current Diagnostic Tools

#### Radiologic Imaging

Evaluation of pancreatic cysts includes a combination of radiologic imaging, endoscopic ultrasound, and cyst fluid analyses. For radiologic characterization of pancreatic cysts, patients typically undergo a "pancreatic protocol" abdominal CT scan and/or MRI [27]. MRI is preferred over CT scans, with its enhanced ability to detect septa, nodules, and ductal communication [27]. Magnetic resonance cholangiopancreatography (MRCP) is superior to CT in characterizing IPMN by demonstrating ductal communication, main duct involvement, and small branch duct cysts (Table 2) [28]. Furthermore, recent concern over radiation exposure from repeated CT may favor the use of MRI for surveillance of pancreatic cysts [29]. Overall accuracy of radiologic imaging for the histologic diagnosis of pancreatic cysts is about 40% to 60% [30-32], while both CT and MRI predict the presence of malignancy in pancreatic cysts with 73% to 79% accuracy [32,33].

#### Endoscopic Imaging

Both MRI and EUS have modest sensitivity (58% to 67%) for detecting mural nodules and both have historically demonstrated approximately 51% accuracy for diagnosing mucinous lesions [34]. However, a recent study refined the EUS criteria for differentiating mucus from a nodule, and improved diagnostic accuracy in the detection of these EUS criteria to 79% [35]. MRI and EUS may be complementary techniques, and used in combination may potentially increase diagnostic yield for mucinous cysts [36].

Diagnosis of MD-IPMN specifically may be aided by other endoscopic procedures including endoscopic retrograde cholangiopancreatography (ERCP) with pancreatoscopy and intraductal ultrasound (IDUS). Endoscopic visualization of mucin emerging from the major or minor papilla is pathognomonic for MD-IPMN (Figure 7). On ERCP, findings consistent with MD-IPMN include diffuse or segmental pancreatic duct dilation

with filling defects. Pancreatoscopy, which involves direct endoscopic visualization within the pancreatic duct using special instruments, can be helpful in differentiating MD-IPMN from chronic pancreatitis by visualizing intraductal tumor [37-39]. Similarly IDUS with the insertion of a tiny ultrasound probe into the pancreatic duct can aid in diagnosis of MD-IPMN with identification of the frond-like tumor within the duct [40].

#### Cyst Fluid Analysis

Imaging alone whether by radiology or EUS is often inadequate to accurately characterize pancreatic cystic lesions. With advances in endoscopy allowing the safe sampling of pancreatic cyst fluid via EUS- FNA [41], collection of cyst fluid for analysis for various biochemical markers, DNA markers, and cytology has become more routine. This is important because cytology alone is generally non-diagnostic, with most studies demonstrating less than 50% sensitivity for diagnosis of the cyst lesion [7,42-44], and at best allowing differentiation between mucinous and



Figure 4. MRI of solid pseudopapillary neoplasm with thin enhancing rim, internal septations, and hemorrhage.

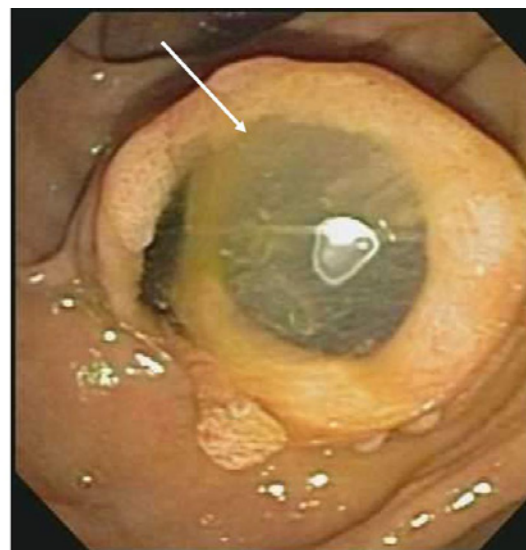


Figure 5. 'Fish mouth' papilla on ERCP with mucin at major papilla.

serous cystic lesions. Diagnostic yield of cytology is higher for SPEN and possibly cystic neuroendocrine tumors. A multicenter study of patients with histologically proven SPEN demonstrated EUS-FNA cytology accuracy of 75% [45].

Few cyst fluid markers have proven valuable and none are definitively diagnostic (Tables 2 and 3). CEA is the most extensively studied and allows the differentiation of mucinous and non-mucinous cystic lesions. Although elevated CEA is consistent with a mucinous cyst, the cutoff level remains debated; studies use cutoff levels from 110 to 800 ng/mL [46]. In addition, CEA cutoffs can vary depending on the laboratory performing the assay. The higher the CEA, the greater the specificity while sensitivity is sacrificed. The commonly used cutoff of 192 ng/mL yields modest sensitivity (73%) and specificity (84%) [34]. CEA less than 5 ng/mL is 95% specific for a SCA or pseudocyst. Cyst fluid amylase lower than 250 units/L can exclude a pseudocyst with 98% specificity and 44% sensitivity [46]. A recent study in surgically resected IPMN concluded that serum biomarkers, such as CA 19-9 and CEA, may be helpful in predicting invasive carcinoma within IPMN [47].

Clinical utility of molecular DNA markers in cyst fluid has been a strong focus in diagnosing malignant pancreatic cystic lesions. A peer-reviewed multicenter study suggested high specificity (96%) for malignancy when high amplitude *k-ras* mutation was followed by allelic loss, but very low sensitivity (37%) [48]. This same mutation pattern showed perfect specificity for diagnosing a mucinous cystic lesion; however its sensitivity was lacking (19%). Presence of *k-ras* mutation alone had 96% specificity and 45% sensitivity for detecting mucinous lesions. The conclusion of this study was that in the presence of these mutations, the likelihood that the cyst is malignant and mucinous is high; however, their absence is not helpful in ruling out the malignancy or the presence of a mucinous cyst. Thus, better biomarkers are necessary to diagnose both malignant and mucinous pancreatic cystic lesions.

#### 4. MicroRNA in Pancreatic Ductal Adenocarcinoma

Better biomarkers are needed to diagnose the various pancreatic cystic lesions and to predict malignant transformation into pancreatic ductal adenocarcinoma (PDAC). Understanding of changes in microRNA (miRNA) expression levels between normal/benign and malignant tissue may offer new diagnostic capabilities and insights. Over the past decade, these molecules have been shown to aid in the diagnosis, prognosis, and prediction of response to chemotherapy for many human cancers, including PDAC [49-52].

##### Tissue candidate biomarkers

Multiple studies have demonstrated that analysis of the miRNA profiles can distinguish normal pancreas tissue from chronic pancreatitis and PDAC in surgically resected tissue specimens. miRNA expression changes associated with PDAC include up-regulation of numerous miRNAs (miR-21, miR-27a, miR-146a, miR-200a, miR-196a, miR-196b, miR-135b, miR-150, miR-155, miR-210, miR-221 and miR-222) and down-regulation of others (miR-217, miR-216, miR-130b, miR-148a, miR-148b, miR-96, miR-20a, miR-34) [51,53-65]. In addition, changes in expression of specific miRNAs, such as miR-196a, miR-196b, and miR-148a, have been associated with different developmental stages of pancreatic intraepithelial neoplasias (PanINs), the precursor lesions involved in the progression to PDAC [56,66,67].

Clinical tests must be robust, reproducible, highly sensitive, very specific, and capable of detecting multiple miRNAs, all in clinical specimens with limited cellularity. Rigorous testing is required for laboratory-developed tests (LDTs) in compliance with CLIA guidelines and College of American Pathologists (CAP) regulations to demonstrate

Table 2. Laboratory markers and pancreatic cysts.

Type of cyst	CEA	Amylase	Cytology	<i>k-ras</i>
Pseudocyst	↓	↑	Cyst content	-
SCA	↓	↓	Hemosiderin-laden macrophages, PAS+ cuboidal epithelial cells	-
MCN	↑	↓	Mucinous cells	+
IPMN	↑	↑	Mucinous cells	+

Table 3. Diagnostic markers for pancreatic cysts.

Marker	Sensitivity	Specificity
Cytology	32%	100%
CEA <5 ng/mL (SCA or pseudocyst vs. mucinous lesions)	54%	94%
CEA >192 ng/mL (mucinous vs. non-mucinous lesions)	73%	84%
Amylase <250 U/L (SCA, MCN vs. pseudocyst)	44%	98%
<i>k-ras</i> mutation + allelic loss (malignant vs. non-malignant)	37%	96%

accuracy, precision, analytical sensitivity, and specificity. The diagnostic potential of miRNAs was first realized in a LDT, the miR*Inform*<sup>®</sup> Pancreas (Asuragen), which differentiates PDAC from normal and chronic pancreatitis tissue using formalin fixed paraffin embedded specimens [50]. This first miRNA-based diagnostic test interrogates the expression levels of two miRNAs, miR-196a and miR-217, to allow differential diagnosis of PDAC with sensitivity and specificity of 95%. It measures the increased proportion of ductal adenocarcinoma cells (indicated by up-regulation of miR-196a) relative to the decline in the number of acinar cells observed in PDAC (resulting in reduced expression levels of miR-217). The score is defined as a simple difference in expression ( $\Delta\text{Ct}$ ) between miR-196a and miR-217, and the specimens are classified according to a cutoff point of 0.5  $\Delta\text{Ct}$ . A score of  $\Delta\text{Ct} \geq 0.5$ , indicates a diagnostic negative (benign), while  $\Delta\text{Ct} < 0.5$  indicates a diagnostic positive (PDAC). Evaluation of the ability of this  $\Delta\text{Ct}$  (miR-196a – miR-217) to distinguish PDAC from normal and chronic pancreatitis tissues was critical to ensure successful development and validation of a preoperative molecular test on pancreatic fine needle aspirations (FNAs), for which final pathology is not always available and/or is more difficult to obtain.

Subsequently, a number of miRNA-based LDTs for other cancer indications have been launched commercially. These include the MiRview<sup>®</sup> LDT series from Rosetta Genomics, comprising tests for cancer of unknown primary (CUP), lung cancer and kidney cancer. The CUP test is based on the detection of 64 miRNAs that can differentiate between 42 types of tumors to identify the 42 different types of primary tumor of origin in primary and metastatic cancer. Another MiRview<sup>®</sup> test can differentiate squamous from non-squamous non-small cell lung cancer (NSCLC), with 96% sensitivity and 90% specificity. There is also a test for lung cancer that identifies the four main subtypes of lung cancer, and another test that classifies common types of kidney tumors.

New strategies are needed to improve the diagnostic accuracy of cytology-based diagnosis of EUS-FNA specimens, as this procedure can be challenging due to small amount of cellular material, presence of overt inflammation, fibrosis and blood, as well as foci of necrosis in the suspicious masses [68-71]. In order to improve the diagnostic accuracy of cytology, a 7-miRNA classifier was developed at Asuragen to use as an adjunct on EUS-FNA specimens with indeterminate and non-diagnostic cytology. This classifier employs an algorithm to calculate a score that dichotomizes the specimens into PDAC (score  $\geq 0.5$ ) and inconclusive (score  $< 0.5$ ) result categories. A multicenter clinical validation study of this classifier test has been recently completed and confirms the improved sensitivity and specificity of the “molecular cytology” for diagnosis of PDAC in EUS-FNA specimens with indeterminate and non-diagnostic cytology [72]. The addition of miRNA-based testing to EUS-FNA cytology may allow more accurate preoperative diagnosis of the approximately 15% of patients with inadequate cytology.

### Biofluid candidate biomarkers

A blood-based miRNA test would be an invaluable screening tool for diagnosis of PDAC, as well as for detection of occult metastatic disease in patients with PDAC. The development of such a diagnostic approach has the potential to reduce the invasiveness of tests, lower costs, enable monitoring of disease status, and minimize the radiation exposure risk associated with cross-sectional imaging. It is believed that circulating nucleic acids are sequestered within two types of lipid vesicles: microvesicles (~100 nm–1  $\mu\text{M}$  in diameter), and exosomes (~30–100 nm), which protect them from degradation [73-78]. Initial reports indicate that circulating miRNAs could participate in cell-to-cell communication [79] although the specific functions and mechanisms of action of circulating miRNAs remain largely elusive.

Circulating miRNAs have emerged in a handful of recent studies as promising candidate biomarkers for pancreatic cancer. A miRNA panel consisting of miR-21, miR-210, miR-155, and miR-196a from plasma was shown to have 64% sensitivity and 89% specificity for diagnosing PDAC [80]. Another panel of 7 miRNAs, including miR-16, miR-21, miR-155, miR-181a, miR-181b, miR-196a and miR-210, was evaluated in combination with CA 19-9 [81]. The top performing candidates, miR-16 and miR-196a independently distinguished between PDAC and chronic pancreatitis, and in combination with CA 19-9 discriminated patients with PDAC from a combination of normal controls and chronic pancreatitis with a sensitivity of 92.0% and specificity of 95.6%, and from chronic pancreatitis only with sensitivity of 88.4% and specificity of 96.3%. In addition, elevated serum miR-196a correlated with patients having unresectable PDAC and poorer survival [82].

Although the results described above are tantalizing, most of the data come from single-institution studies. Several elements must be considered to ensure successful clinical validation of promising miRNAs initially discovered in any biofluid specimen, as evidenced by promising lung cancer miRNAs [83]. Some of the most significant confounding factors may include choice of sample type (e.g. whole blood, serum, and plasma), sample numbers, study design, sample collection method, sample storage and shipment, and others. Hence, all results must be validated in multi-center, appropriately powered prospective studies with protocols emphasizing biomarker normalization, quality control and SOP standardization before they can be applied in the management of patients with pancreatic cancer.

### Therapeutic miRNA candidates

In addition to aiding in diagnosis and prognosis of PDAC, a greater understanding of the aberrant expression of specific miRNAs in various pancreatic lesions may advance therapeutics. Down-regulation of miR-21 in cell cultures led to increased cell death when treated with gemcitabine [84]. Analogously, overexpression of miR-21 resulted in increased cell proliferation and chemoresistance to gemcitabine [85]. Another clinical study found that patients with high expression levels of miR-21 in their tumor tissue had decreased overall survival both in the metastatic

and in the adjuvant setting, along with increased gemcitabine resistance [86]. Similarly, surgically resected patients with low miR-21 expression levels demonstrated increased survival with adjuvant chemotherapy [87]. miR-142-5p, miR-204, miR-221, and miR-10b are among other miRNAs with a potential therapeutic role in PDAC [88]. A study reported that low levels of miR-10b in FNA samples collected from patients who underwent neoadjuvant therapy were associated with improved response to gemcitabine-based neoadjuvant therapy, longer time to metastasis and improved overall survival of 50% after 2 years [52]. It appears that miR-10b expression levels could be used as an indicator of localized pancreatic disease, similarly to miR-143 which was negatively correlated with lymph nodes spreading ( $r = -0.64$ ;  $P = 0.0004$ ) [89]. Two groups reported miRNA signatures that showed utility in predicting survival in patients with nodal disease. The miRNAs in these signatures did not overlap and included: miR-452, miR-105, miR-127, miR-518a-2, miR-187 and miR-30a-3p [55], as well as miR-155, miR-203, miR-210 and miR-222 [66]. Modulating expression levels of various miRNAs may promote apoptosis of cancer cells and provide targeted therapy for PDAC [90].

### 5. miRNA in Pancreatic Cysts

While efforts continue to better diagnose, prognosticate, and treat PDAC, much attention has focused on pancreatic cystic neoplasms, which may be precursors to PDAC. As described earlier, diagnosis and prediction of behavior of pancreatic cystic lesions is currently difficult and limited. The solution to this clinical dilemma may be provided by the application of miRNA analysis on preoperatively collected specimens, such as pancreatic juice and cyst fluid. For example, expression of miR-155 was shown to be elevated in 60% IPMN pancreatic juice specimens as compared to disease-free controls [91]. In pancreatic cystic fluid, elevated expression levels

of miR-21, miR-221, and miR-17-3p were able to differentiate the mucinous cysts from those the non-mucinous specimens with  $p < 0.01$  [92]. The key candidate, miR-21, was able to resolve those diagnostic entities with a median specificity of 76% and a sensitivity of 80%. Recently, Matthaei et al. used cyst fluid specimens collected in the operating room to build a 9-miRNA classifier, which predicted the degree of dysplasia within the epithelial lining of an IPMN and identified cysts that likely needed surgical resection [93]. This classifier consisted of miR-18a, miR-24, miR-30a-3p, miR-92a, miR-342-3p, miR-99b, miR-106b, miR-42-3p, and miR-532-3p, and predicted with 89% sensitivity and 100% specificity which cyst fluid specimen came from a cyst requiring resection vs. a more conservative management. Other studies of surgical pathology specimens found miRNAs that were down-regulated in PDAC compared to serous cystadenoma (e.g. miR-21) and up-regulated in IPMN and PDAC (e.g. miR-196a and miR-183) relative to normal pancreatic tissue [94,95].

Our own exploratory work on surgically resected pancreatic cyst specimens has revealed several miRNA models that can accurately differentiate serous cystadenoma, mucinous cystic neoplasm, and IPMNs [3]. Specifically, we identified four different 4-miRNA models, which distinguished with high degree of accuracy: 1/ MCN from SCA, IPMN, and PDAC; 2/ SCA from MCN, IPMN, and PDAC; 3/ PDAC from IPMN; 4/ MCN from BD-IPMN. Further studies are needed to examine the ability of miRNA to identify the various cystic lesions using cyst fluid specimens. Such a transition will require appropriately powered prospective studies with specimens collected from consecutive patients, stored in a standardized manner, and equivalently monitored for quality control. Additionally, most of those lesions would be required to have undergone surgical resection to achieve the gold standard diagnosis, which will necessitate a multicenter effort. We believe that a combination of the top candidate biomarkers identified in resected tissues with those

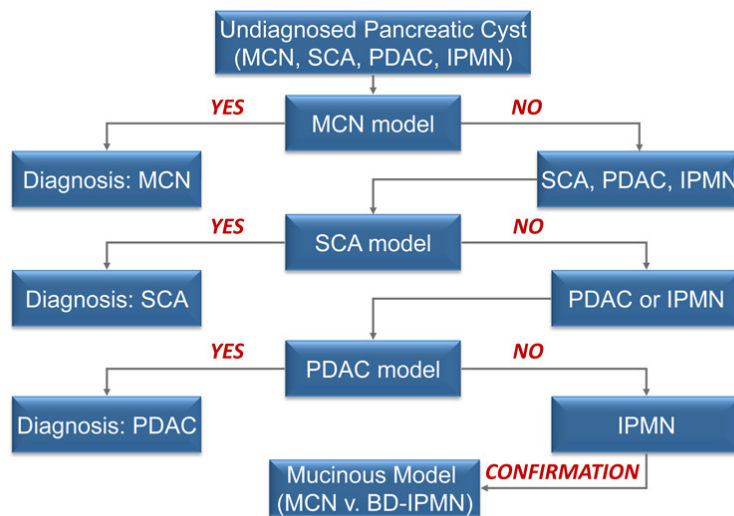


Figure 6. Future proposed clinical algorithm utilizing cyst-type specific miRNA models for improved diagnostic accuracy pancreatic cystic neoplasms.

identified in cyst fluid specimens may provide an opportunity for improved molecular characterization of cystic lesions. Figure 6 shows hypothetically how these models may be used clinically. Larger studies will also be needed to examine the ability of relative expression levels of various miRNAs to differentiate among the different degrees of dysplasia in mucinous lesions.

## 6. Conclusions

Despite improved imaging modalities and increasing clinical awareness of pancreatic cysts, there is currently no preoperative diagnostic assay that has sufficient sensitivity and specificity to accurately predict the biological behavior of a pancreatic cyst.

Studies to date have shown that aspirations of cyst content, both neoplastic and non-neoplastic, can be used to develop biomarkers which can be used in conjunction with FNA cytology to determine which lesions can be monitored and which may require surgical resection. The literature discussed above suggests that knowledge of miRNA expression changes in pancreatic cyst fluid may improve diagnostic yield and provide additional prognostic information that could be utilized in the evaluation and management of pancreatic cysts. Further large clinical validation studies on EUS-FNA specimens collected prospectively from consecutive patients and using standardized protocols are necessary to confirm the utility of miRNAs as diagnostic tools for management of pancreatic cysts.

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