Endoscopically Acquired Pancreatic Cyst Fluid MicroRNA 21 and 221 Are Associated With Invasive Cancer

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OBJECTIVES: **Pancreatic cysts are a group of lesions with heterogeneous malignant potential. Currently, there are no reliable biomarkers to aid in cyst diagnosis and classification. The objective of this study was to identify potential microRNA (miR) biomarkers in endoscopically acquired pancreatic cyst fluid that could be used to distinguish between benign, premalignant, and malignant cysts.**

METHODS: **A list of candidate miRs was developed using a whole-genome expression array analysis of pancreatic cancer (pancreatic ductal adenocarcinoma) and nonmalignant samples overlapped with existing literature and predicted gene targets. Endoscopically acquired pancreatic cyst fluid samples were obtained from a group of 38 patients who underwent cyst fluid aspiration and surgical resection. Selected miR expression levels in cyst fluid samples were assessed by quantitative real-time-PCR. Additionally, *in situ* hybridization (ISH) on corresponding cyst tissue samples was performed to identify the source and validate the expression level of fluid miRs.**

RESULTS: **Of the six miRs that were profiled in the study, two showed differential expression in malignant cysts. miR-221 was expressed at significantly higher levels in malignant cysts compared with benign or premalignant cysts ($P=0.05$). miR-21 was also expressed at significantly higher levels in malignant cysts ($P<0.01$). Additionally, the expression of miR-21 was significantly higher in premalignant cysts than benign cysts ($P=0.03$). The differential expression of miR-21 among cyst categories was confirmed by ISH.**

CONCLUSIONS: **In this small single-center study, miRs are potential pancreatic cyst fluid diagnostic biomarkers. In particular, miR-21 is identified as a candidate biomarker to distinguish between benign, premalignant, and malignant cysts. Additionally miR-221 may be of use in the identification of more advanced malignant disease.**

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INTRODUCTION

Cystic neoplasms of the pancreas (CNP) are an increasingly recognized heterogeneous group of diseases with variable malignant potential. They range from benign pancreatic cysts (e.g., serous cystadenomas and pseudocysts) to premalignant or malignant cysts (intraductal papillary mucinous neoplasm (IPMN) or mucinous cystic neoplasms (MCNs)) (1). They are common, as it has been estimated that IPMNs now constitute up to 25% of resected pancreatic lesions (2,3). The major clinical challenges with CNPs are their accurate diagnosis and cancer risk assessment during a patient's lifetime. Currently, a set of clinical and radiologic parameters are used to diagnose, risk stratify, and manage CNPs (4,5). In addition, pancreatic cyst fluid cytology and tumor markers (e.g., pancreatic cyst fluid carcino embryonic antigen or DNA) have been used with variable success in evaluating these patients (6,7). Even with these techniques, there is currently lack of an accurate

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Table 1. Candidate cyst fluid miRs

miR	Literature	PDAC targets[a]	*In silico* tumor vs. normal (*P* value)[b]
21	• Increased in PDAC (9–12) • Associated with poor prognosis (10) • Increased in IPMN (19) • Involved in gemcitabine resistance (13,14)	PTEN RECK PDCD4 VEGF	0.03
155	• Increased in PDAC (9–12) • Associated with poor prognosis (19) • Increased in IPMN (19)	TP53INP1 FOXP3 PIK3R1	0.06
181c	• Increased in PDAC (9,12) • Associated with poor prognosis in other cancers (23)	KRAS NOTCH4 BCL-2	0.04
196a	• Increased in PDAC (11,15) • Associated with poor prognosis (16)	HOXB8 HMGA2 Annexin A1	0.01
217	• Decreased in PDAC (15)	KRAS	0.06
221	• Increased in PDAC (9–12) • Involved in gemcitabine resistance (14)	PTEN TIMP3 P27	0.1

IPMN, intraductal papillary mucinous neoplasm; PDAC, pancreatic ductal adenocarcinoma.
[a]Pancreatic ductal adenocarcinoma.
[b]Tumor vs. normal samples (25).

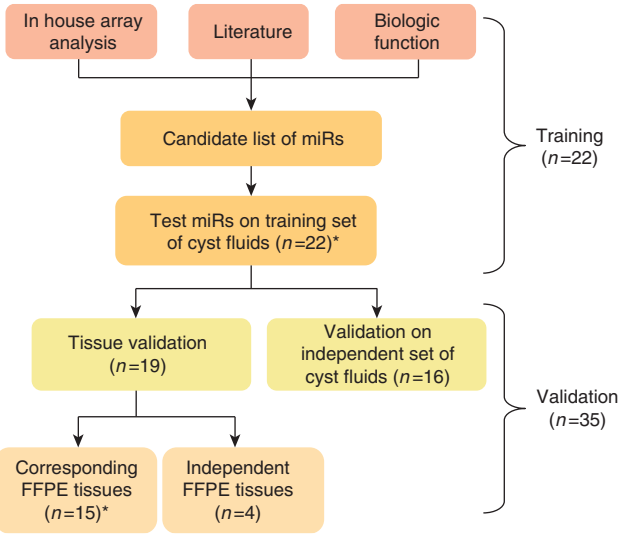

Figure 1. Experimental design. *Cyst fluid and tissue samples from corresponding patients. FFPE, formalin-fixed and paraffin-embedded.

method to diagnose the degree of dysplasia within a cyst without surgical resection. There remains a need for better pancreatic cyst fluid markers to aid in diagnosis and lifetime risk prediction of cancer.

MicroRNAs (miRs) are a family of small noncoding RNA molecules with 21–25 nucleotides, which function primarily as negative regulators of gene expression by binding to multiple target messenger RNAs (8). Growing preclinical and clinical data has implicated them in tumor initiation, progression, and response to treatment in pancreatic ductal adenocarcinoma (PDAC) (9–18). Similar data is emerging on the role of miRs in CNPs with limited and conflicting studies involving pancreatic cyst fluid (19–24). To date, miR profiling was not possible in up to 8% of non-endoscopically acquired pancreatic cyst fluid (24). Moreover, a controversy still exists about which miRs, especially miR-21, are associated with CNP malignant transformation, their site of origin, and which ones may be useful as diagnostic tools.

This study evaluates a panel of preselected miRs (based on current literature (**Table 1**) and an in-house whole-genome array analysis) in a well-characterized cohort of endoscopically acquired pancreatic cyst fluid with confirmed pathologic diagnosis and matching histology (9–17,25). This study aims to assess the diagnostic value of pancreatic cyst fluid miR markers in differentiating benign from malignant and premalignant CNP, and their tissue site of origin.

METHODS

Identification of candidate miRs

In order to generate a set of candidate miRs to evaluate in pancreatic cyst fluid, data from multiple sources were used (**Figure 1**). A whole-genome, survival-based array analysis that we recently reported comparing tumor ($n=25$) vs. nonmalignant samples ($n=7$) was used to begin to generate a list of miRs (25). This candidate list was further refined via existing literature to identify miRs previously implicated in pancreatic tumorigenesis (9–17). Those miRs with regulatory functions in pathways known to be active in PDAC were given particular consideration.

Pancreatic cyst fluid and tissue acquisition

The UCLA pancreatic database was used to identify 38 patients who underwent endoscopic ultrasound-guided pancreatic cyst fluid sampling and surgical resection. At the time of sampling, a portion of pancreatic cyst fluid was snap frozen immediately and stored at −80°C for future analysis, without RNAase protection. Pancreatic cyst tissue from surgical specimens was formalin-fixed and paraffin-embedded. Pancreatic cysts were histopathologically classified as benign, premalignant or malignant. Premalignant cysts included MCNs and IPMNs with varying degrees of dysplasia. The designation of malignant cysts was reserved for those samples that contained an invasive component (**Table 2**).

Initially, the pancreatic cyst fluid from a training set of 22 samples was analyzed for miR expression by quantitative real-time PCR (qRT-PCR). This analysis was then validated in a set of 16 additional pancreatic cyst fluid samples. Based on the results of the pancreatic cyst fluid analyses, a further validation step was performed on tissue specimens. *In situ* hybridization (ISH) for miR-21 was performed on a total of 19 formalin-fixed and paraffin-embedded tissues. Fifteen of these samples correlated to cyst fluid samples from the training set. An additional group of four independent malignant samples was added because there were few

Table 2. Histopathologic classification of cyst fluid and tissue samples

	Cyst fluid		Tissues
Type	Training	Validation	Validation
Benign	*n*=7	*n*=9	*n*=3
Serous cystadenoma	2	3	2
Other	5[a]	6[b]	1[c]
Premalignant	*n*=11	*n*=7	*n*=8
MCN	2	2	2
IPMN—low/int. grade	7	5	4
IPMN—high grade	2	0	0
Malignant	*n*=4	*n*=0	*n*=8
IPMN—invasive	4	0	8

IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasms.
[a]Pseudocyst-1, mesothelial cyst-1, treated cancer with no residual tumor-1, pancreatitis-1, normal pancreas-1.
[b]Pseudocyst-5, lymphoepithelial cyst-1.
[c]Mesothelial cyst-1.

malignant cyst fluid samples with available, corresponding tissue samples (**Figure 1**, **Table 2**).

miR profiling of cyst fluid samples by qRT-PCR

RNA was isolated from 50 µl samples of cyst fluid using QIAzol lysis reagent (Qiagen, Valencia, CA). Total RNA was then purified using the miRNeasy Mini Kit (Qiagen). RNA concentration and purity was assessed via spectrophotometry (Nanodrop 1000, Thermo Fisher Scientific, Wilmington, DE). Complementary DNA was synthesized from isolated RNA using the miScript II RT Kit (Qiagen). qRT-PCR for the miRs of interest was performed using the miScript SYBR Green PCR Kit (Qiagen). All primers were purchased from Qiagen. A standard curve was generated for each miR, as well as the housekeeping RNA, RNU6B. Log starting quantities were calculated for each sample based on the standard curves. Relative expression was calculated by subtracting the log starting quantity of RNU6B in a given sample from that of each target miR in the corresponding sample.

miR-21 ISH

Five micrometer sections of formalin-fixed and paraffin-embedded tissues were incubated at 60 °C for 1 h, deparaffinized in xylene, and rehydrated with graded alcohol washes. Slides were then washed three times with diethyl pyrocarbonate-treated phosphate-buffered saline, digested with 5 µg/ml proteinase K at 37 °C for 30 min, washed again twice with diethyl pyrocarbonate-treated phosphate-buffered saline, submerged in graded alcohol for 1 min each, and air-dried completely. Slides were then hybridized at 55 °C for 2 h with 50 nmol/l locked nucleic acid-modified digoxigenin-labeled probes for miR-21 (Exiqon, Vedbæk, Denmark). After hybridization, stringency washes were performed at 55 °C with 5×, 1× and 0.2× saline-sodium citrate buffer respectively. The slides were then placed in a blocking solution for 1 h at room temperature followed by incubation overnight at 4 °C with alkaline phosphatase-conjugated anti-digoxigenin F_{ab} fragment in blocking solution. After three washes in phosphate-buffered saline/0.1% Tween 20, the slides were incubated for 4–48 h with 4-nitro-blue tetrazolium and 5-bromo-4-chloro-3′-indolylphosphate substrate (Roche, Mannheim, Germany) forming dark-blue 4-nitro-blue tetrazolium-formazan precipitate. Slides were then counterstained with Nucleic Fast Red (Vector, Burlingame, CA) for 1 min. The slides were washed with water, dehydrated in alcohol solutions and mounted with Eukitt mounting medium (Electron Microscopy Sciences, Hatfield, PA). Slides were graded for level of staining on a scale of negative (0), low (1), moderate (2) or high (3). Two independent scorers were used, who were blinded to the clinical and pathologic data. Any discrepancies in scoring were reviewed by both scorers and a consensus score was agreed upon.

Statistical analysis

Relative expression in base 10 logarithm of target miRs was calculated as described above. One-way analysis of variance was first performed to identify miR whose expression might be significantly correlated with malignancy degree: benign, premalignant, and malignancy. The pairwise two-tailed *t*-tests were then carried out to study the differences between specific groups of cysts. The *P* values were finally adjusted for multiple testing by using a Benjamini and Hochberg approach (**Table 3**). An adjusted *P* value of <0.05 was considered significant.

RESULTS

Selection of candidate miRs and pancreatic cyst fluid samples

By means of the filtering process described in the METHODS section, we identified miR-21, miR-155, miR-181c, miR-196a, miR-217, and miR-221 as the focus of our study (**Table 1**). Thirty-eight endoscopically acquired pancreatic cyst fluid samples with histopathologically confirmed diagnoses, used in the training (22 samples) or validation (16 samples) components of the study, included 16 benign samples (5 serous cystadenomas, 11 other), 18 premalignant samples (MCN ($n=4$), IPMN with low-grade dysplasia ($n=12$), IPMN with high-grade dysplasia (HGD, $n=2$)) and 4 invasive IPMN cancer samples.

Cyst fluid miR-21 levels are elevated in premalignant and malignant cysts

Of the six candidate miRs selected as described in the METHODS, 4 (miR-155, miR-181c, miR-196a and miR 217) did not demonstrate any significant differences in pancreatic cyst fluid expression levels among benign, premalignant, and malignant cysts in the training set ($n=22$). In contrast, miR-21 expression progressively increased from its lowest values in benign cyst fluid to its highest values in malignant cyst fluid. Premalignant cyst fluid contained an intermediate amount of miR-21 (**Figure 2a**). The differences in miR-21 expression between all three groups were statistically significant (benign vs. premalignant: $P=0.032$, benign and premalignant vs. malignant: 7.9×10^{-5}, **Table 3**). In addition to the

Table 3. Differential miR expression in benign, premalignant, and malignant cysts[a]

Marker	ANOVA	Benign vs. premalignant	Benign vs. malignant	Benign vs. pre + malignant	Benign + pre vs. malignant
miRNA21	0.001[b]	0.032[c]	0.000[b]	0.004[b]	0.000[b]
miRNA221	0.020[c]	0.329	0.096	0.268	0.007[b]
miRNA155	0.484	0.727	0.900	0.727	0.949
miRNA196a	0.440	0.853	0.493	0.853	0.493
miRNA217	0.293	0.746	0.491	0.683	0.491
miRNA181c	0.055	0.148	0.135	0.135	0.189

ANOVA, analysis of variance; miR, microRNA.

[a]All data from cyst fluid training set, n=22. Except for ANOVA, all P values from two-tailed t-test. All t-test P values are adjusted for multiple testing based on Benjamini and Hochberg approach.

[b]$P<0.01$.

[c]$P<0.05$.

training set of samples, a validation set of 16 cyst fluid samples were profiled for miR-21 expression. Again, miR-21 expression was significantly different between benign cyst fluid and premalignant cyst fluid ($P=0.01$, **Figure 2b**). The validation group did not contain any malignant cysts. Cumulatively, these data provide compelling evidence that cyst fluid miR-21 levels increase during malignant transformation of pancreatic cysts.

Cyst fluid miR-221 levels are elevated in malignant cysts

The second miR in our candidate group to exhibit differential expression was miR-221. In the training set, miR-221 expression was significantly higher in malignant cyst fluid compared with benign or premalignant fluid. However, expression levels were scattered over a wide range, and not significantly different between benign and premalignant fluid samples (**Figure 2c**, **Table 3**). As it included only benign and premalignant samples, the validation set was not profiled for miR-221. Increased levels of miR-221 in malignant but not premalignant cyst fluid suggest that miR-221 elevation is a late step in malignant transformation.

ISH for miR-21 confirms increased expression in premalignant and malignant cysts

In order to confirm that cyst fluid miR-21 originates from cyst epithelium and to validate the PCR fluid measurements, ISH of corresponding surgically resected tissues was performed. A set of 19 formalin-fixed and paraffin-embedded tissues, 15 matched to samples from the cyst fluid training set and 4 additional cyst derived cancers, were selected. ISH for miR-21 was performed on these tissues, and staining was scored as negative, low, or high (**Figure 3**). Malignant cysts demonstrated the most intense staining. In fact, all samples classified as miR-21 high were malignant. Most benign samples were miR-21 negative, and the majority of premalignant samples were miR-21 low (**Figure 3b**). The fourth case in **Figure 3a** illustrates that miR-21 is higher in the invasive component of the malignant cyst as compared with the cyst wall. Beyond confirming that miR-21 is expressed more abundantly in advancing malignancy, these histopathologic studies demonstrate that the source of cyst fluid miR-21 is the ductal epithelium lining the cyst wall or the infiltrative cancer originating from the cyst wall (**Figure 3a**).

DISCUSSION

Our study confirms the potential diagnostic role for endoscopically acquired pancreatic cyst fluid miR markers in evaluating pancreatic cystic neoplasms and presents new information on the source of the miRs within the fluid of these cystic neoplasms. Although pathologically distinct, MCNs and IPMNs are considered to be the preneoplastic type of CNPs. MCNs and IPMNs display various degrees of epithelial dysplasia: low grade, intermediate grade, high grade, and invasive (malignant), with evidence supporting a variable duration and completion of progression from a premalignant to an invasive lesion (1,26,27). Currently, no available pancreatic cyst fluid biomarker can accurately determine the histologic grade. IPMNs also have different underlying genetic and biologic features compared with pancreatic intraepithelial neoplasia-derived PDAC. Whereas invasive IPMNs appear to have a better prognosis than pancreatic intraepithelial neoplasia-derived PDAC when lymph nodes are uninvolved, they have the same dismal clinical course when lymph node spread is present (28).

Our understanding of the potential role of miRs in PDAC and CNP pathogenesis and malignant progression, as well as their role as prognostic and predictive markers in these diseases is currently evolving. Initial studies of miRs in pancreatic neoplasms focused on their differential expression in pancreatic intraepithelial neoplasia-derived PDAC compared with nonmalignant tissues (e.g., chronic pancreatitis and normal pancreas) using either surgical resection or endoscopic biopsy material. This revealed a group of miRs, which were increased or decreased in PDAC compared with nonmalignant controls. The most common PDAC-specific miRs included increased expression of miR-21 and miR-155 (9,12,15). In addition to their role in tumor initiation, miRs have also been implicated in the clinical progression of PDAC, as miR-21 is associated with survival and treatment response in PDAC (12,29,30).

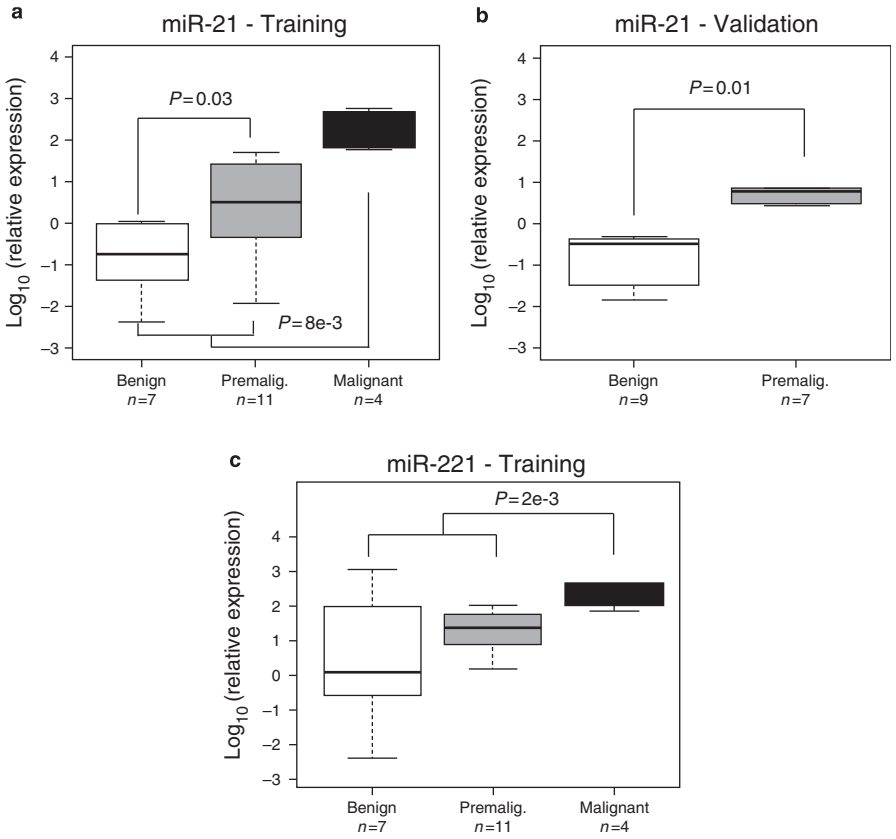

Figure 2. Differential miR expression in benign, premalignant, and malignant cyst fluids. The plots highlight median (horizontal bar), interquartile range (box), and lower and upper adjacent values (vertical bars) for each cyst category. (**a**) miR-21 expression in the training set of cyst fluid samples. (**b**) miR-21 expression in the validation set of cyst fluid samples. (There were no malignant samples in this data set). (**c**) miR-221 expression in the training set of cyst fluid samples. miR, microRNA; premalig., premalignant.

More recent studies have focused on the role of miRs in CNP. Using surgically resected specimens and laser capture microdissection techniques, various studies have proposed different miR patterns associated with IPMN, as well as with malignant transformation in IPMN. Initial small studies have demonstrated elevated levels of miR-21 and miR-155 in noninvasive IPMNs as compared with normal parenchyma remote from the disease site, with both being most frequently seen in high-grade compared with low-grade IPMNs (19). Interestingly, miR-101 has a lower expression in invasive IPMNs correlating with a possible increased expression of its target Enhancer of zeste homolog-2 in IPMN malignant progression (21). These findings have recently been validated in larger cohorts of CNPs showing the correlation between elevations of tissue miR-21, miR-155, and decreased miR-101 level in invasive vs. noninvasive IPMNs, with miR-21 being an independent prognostic biomarker in invasive IPMNs (23).

To date, there have been only two studies looking at miRs in pancreatic cyst fluid, with the fluid being collected *ex-vivo* at the time of surgical resection in both studies. Using five preselected miRs, Ryu *et al.* (20) found statistically elevated miR-21, miR-221, and miR-17-3 p in mucinous (including three invasive IPMNs) compared with non-mucinous cyst fluid, but no difference for miR-155 and miR-191. In a separate study, Matthaei *et al.* (24) performed initial high-throughput miR profiling on 15 *ex-vivo* pancreatic cyst fluid specimens, yielding a panel of 18 miRs, which together with miR-21 (which was not found to be important on the initial screening) were tested in another 49 pancreatic cyst fluids, including 20 serous cystadenoma, 2 low-grade IPMNs, 11 intermediate-grade IPMNs, 6 high-grade IPMNs, 5 PanNETs, and 5 solid pseudopapillary neoplasms, but no invasive cancer. Five of the 65 (8%) samples did not yield results due to insufficient RNA yield to profile all candidates, failure to amplify >10% of miRNAs, or low recovery of miRNA fraction. A logistic regression model using nine miRs (miR-24, miR-30a-3p, miR-18a, miR-92a, miR-342-3p, miR- 99b, miR-106b, miR-142-3p, and miR-532-3p) allowed prediction of cyst pathology requiring resection (high-grade IPMNs, PanNETs, and solid pseudopapillary neoplasms) vs. conservative management (low-grade IPMNs, serous cystadenomas) with a sensitivity of 89%, a specificity of 100%, and area under the curve of 1. Interestingly, this study also showed a lack of correlation between profiled miRs from the microdissected tissue cyst wall and the corresponding cyst fluid. Furthermore, this study did not demonstrate miR-21 to be significantly predictive of malignant transformation (24).

Our study is notable for several reasons. First, despite the potential for RNA degradation, we demonstrate the feasibility of isolating and quantifying miRNA on endoscopically acquired

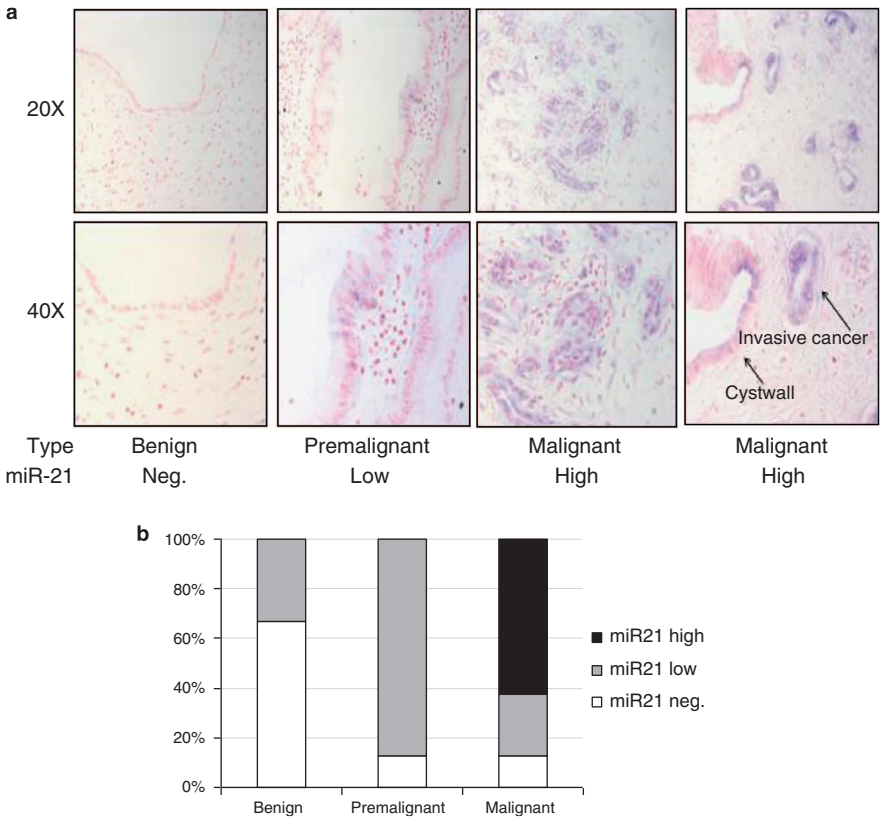

Figure 3. *In situ* hybridization (ISH) for miR-21 in pancreatic cyst tissue samples. (**a**) Representative images of miR-21 ISH. Staining intensity increases from benign to premalignant to malignant cysts. The site of miR-21 staining is the ductal epithelium in the cyst wall. (**b**) Distribution of miR-21 expression between cyst categories as assessed by ISH histoscores. miR, microRNA; neg. negative.

pancreatic cyst fluid. All our pancreatic cyst fluid samples were stored at −80 °C long-term without adding any RNA protectant agent. miR isolation and quantification via qRT-PCR was possible in all the 38 samples chosen for our study. Secondly, we demonstrate for the first time the localization of the fluid derived miR to the cyst epithelial wall and demonstrate a correlation between tissue levels of miR (miR-21) by ISH, degree of cellular atypia, and pancreatic cyst fluid level of miR. Other studies have demonstrated a disconnect between miR profiling levels from the pancreatic cyst wall (based on laser capture microdissection) and the pancreatic cyst fluid.

Finally, we validated the important role of miR-21 and possibly miR-221 as markers of malignant progression within mucinous pancreatic cysts, such as MCN and IPMN. At the same time, we provide data on other miRs felt to be important in PDAC, which we did not find to be important diagnostically in pancreatic cystic neoplasms, such as miR-155, miR-181c, miR-196c, and miR-217. Specifically, we showed a statistically significant difference between benign, premalignant, and malignant pancreatic cyst fluid levels of miR-21. This is important, as there is still a need for improved pancreatic cyst fluid markers to assist with diagnosis and risk stratification. Pancreatic cyst fluid carcino embryonic antigen has an 80% accuracy at distinguishing mucinous from non-mucinous lesions, but does not have a role in diagnosing or predicting the development of malignancy in a cyst (6). Molecular DNA analysis of pancreatic cyst fluid is now also available commercially. However one multi-institutional prospective study (the PANDA study) and several retrospective, single institutional studies, have failed to convincingly show its defined role (7). Whether the presence of a k-ras mutation, the presence of allelic imbalance, or the quantity/quality of DNA is used, the operating characteristics of these molecular analyses remains poor (7). Although there are other newer pancreatic cyst fluid markers currently being evaluated (e.g., plectin-1, guanine nucleotide binding protein, alpha stimulating activity polypeptide), pancreatic cyst fluid miRs, especially miR-21, offer the greatest potential as predictive markers not only of the presence of invasive cancer at the time of evaluation but also of histologic progression and cancer development over time (31–33). Our findings have clinical implications for accurate diagnosis and cancer risk stratification to assist with long-term surveillance strategies. In addition, the development of anti-miR treatments may allow for future intervention to decrease the risk of malignant progression (34).

How miR-21 and miR-221 are involved in malignant progression in IPMNs remains unclear. Studies in PDAC cells show that these miRs are involved in proliferation, invasion, and chemoresistance to gemcitabine through modulation of several direct and indirect targets, such as PTEN, whose expression is related to miR-21 (13,29). Other downstream targets of miR-21 include RECK, PDCD4, and VEGF, as well as 17 genes in the KEGG-database signaling pathways of PDAC (23). Recent studies in miR-21-overexpressing mice established by Cre/Tet-off technologies confirmed

the oncogenic role of miR-21 in a lymphoma model, demonstrating its impact on tumor initiation, maintenance, and invasion (35). Finally, clinical studies in PDAC have shown that miR-21 overexpression was predictive of shorter survival in patients treated in both the adjuvant and the palliative setting (29,30,36). In invasive IPMN, miR-21 is the only miR significantly associated with overall survival and disease-free survival (23).

Limitations of this current study include the preselection of candidate miRs using PDAC data. Although it is recognized that the molecular pathogenesis of pancreatic intraepithelial neoplasia-derived PDAC and CNP, especially IPMN, are different, the paucity of literature and in-house data about miRs and CNPs results in us relying heavily on the PDAC literature to identify suitable markers. The second major limitation of our study is the lack of invasive IPMN cyst fluid in the validation portion of the study. Although we included 12 patients with invasive IPMN in the initial cyst fluid training phase and the tissue portion of our study (unlike the study by Matthaei *et al.* (24), which did not include any patients with invasive IPMN), we were unable to identify any more patients with invasive IPMN for the validation portion of the study. Additionally, the small numbers of pathologically confirmed pancreatic cyst fluids collected over the duration of the study prevented the separate analysis of MCNs and IPMNs: clinically similar but pathologically distinct entities. Finally, the retrospective design of the study and its initial promising results should be considered as hypothesis generating and lead to the development of larger prospective studies focusing on miR expression in endoscopically acquired pancreatic cyst fluid in resected and non-resected patients.

CONFLICT OF INTEREST

Guarantor of the article: James J. Farrell, MD.

Specific author contributions: Conceiving : Farrell and Donahue; Initiating: all authors; Writers: Farrell and Donahue.

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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Pancreatic cysts represent a diverse group of lesions with varying malignant potential.
- ✓ Diagnostic ability is currently limited by the lack of cyst fluid markers that are reliably associated with malignant cysts.
- ✓ MicroRNAs have been identified as potential biomarkers in various diseases including pancreatic cysts.

WHAT IS NEW HERE

- ✓ Expression of miR-21 is significantly higher in cyst fluid of malignant vs. premalignant vs. benign cysts making it a candidate marker to accurately diagnose pancreatic cysts.
- ✓ Surgically resected tissue samples corresponding to cyst fluid samples confirm that the source of cyst fluid miR-21 is the epithelial wall.
- ✓ Expression of miR-221 is significantly elevated in malignant but not premalignant cysts. miR-221 may be a useful marker of more advanced disease.

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