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Cyst Fluid Biomarkers for Intraductal Papillary Mucinous Neoplasms of the Pancreas: A Critical Review from the International Expert Meeting on Pancreatic Branch-Duct-Intraductal Papillary Mucinous Neoplasms

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Introduction

The recognition of pancreatic cysts and intraductal papillary mucinous neoplasms of the pancreas (IPMN) has increased largely secondary to greater utilization of high-quality cross-sectional abdominal imaging.[1, 2] Although the general characteristics of IPMNs, radiographic diagnosis, cyst fluid composition, and their delineation from other pancreatic tumors have been well established, several issues regarding their growth and progression into malignancy remain poorly described. The degree of neoplastic transformation within IPMN is highly variable, from those with an entirely innocuous cell population typically resembling gastric epithelium and lacking any cytologic atypia, to those that have progressively increasing degrees of cytoarchitectural atypia. Though some patients with highly dysplastic and invasive IPMN may present with clinical symptoms or characteristic

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radiographic findings, including jaundice, an associated pancreatic mass, or main pancreatic duct dilation; increasingly IPMN are incidentally discovered. Once IPMN are radiographically diagnosed, there is currently no reliable way to determine the level of epithelial dysplasia or to predict the time frame of progression to high-grade dysplasia or cancer.[3–6] A biologic marker of IPMNs is urgently needed – one that can be easily obtained and tested without significant morbidity for the patient.

An evidence-based expert meeting on pancreatic branch-duct IPMNs (BD-IMPN) was held in Verona, Italy and the authors reviewed the current role of existing technologies and molecular markers for predicting the biological behavior of IPMNs. The status of endoscopic ultrasound (EUS) and EUS-guided fine needle aspiration (FNA) cytology, cyst fluid biochemistry, mucins, cytokines, DNA, and microRNA profiles were critically reviewed by the group in order to identify the most promising clinically relevant biomarkers, and to target analyses for further development.

Endoscopic ultrasound

Endoscopic ultrasound (EUS) is a highly sensitive imaging modality for the evaluation of pancreatic cystic lesions that was developed as a diagnostic modality, but rapidly gained a role in IPMN for morphologic assessment and for its interventional capabilities, namely aspiration of cyst fluid and fine-needle aspiration (FNA). Diagnosis of IPMN based solely on EUS findings requires attention to cyst size, characteristics of the cyst wall, internal characteristics of the cyst, communication with the MPD, and the existence of any background lesions. Using EUS morphologic parameters, the sensitivity, specificity and accuracy to differentiate between benign and malignant, or potentially malignant, cystic lesions has been reported to be between 56%–91%, 45%–60% and 51%–72%, respectively[7, 8] Although evaluation and the differential diagnosis of cystic lesions based solely on EUS morphology is feasible; inter-observer variability, operator dependency, and moderate diagnostic performance limit its accuracy without the addition of cyst fluid aspiration and FNA cytology, especially for determination of high-risk IPMN without overt malignant features.

Cyst Fluid Cytology Analysis

Information gained from EUS morphology is enhanced in the context of additional clinical information, cross-sectional imaging, and cyst fluid analysis. EUS-FNA is a safe and accurate technique to obtain cytological or histological samples from pancreatic masses and it can also be used to aspirate fluid from cystic lesions. Diagnostic accuracy has varied considerably among studies and may reflect differences in sampling methods, cytopathologist skill, and success in clinical pathological correlation.[7] Cyst fluid cytology is an accurate test for the diagnosis of a malignant pancreatic cystic lesion,[9] however, the sensitivity of cytology is often hampered by the low cellular content of the pancreatic cyst fluid. Additionally, distinguishing lesional cells from gastrointestinal contamination is often difficult, though crucial, to making an accurate interpretation.[10, 11] The positive predictive value of EUS-FNA for invasive malignancy is very high, however, its utility to determine the level of dysplasia in mucinous lesions remains to be improved.[5] The main issue is that in

the absence of frank malignancy, determination of high from low or intermediate-grade dysplasia is difficult to determine from FNA cytology.

The neoplastic epithelial cells of serous cystic neoplasms are rarely identified on EUS-FNA specimens[12] though a recent report has identified vascular endothelial growth factor-A (VEGF-A) and VEGF receptor 2 to be overexpressed only in these cysts.[13] The cytological features of IPMNs and MCNs are similar to each other, showing mucinous neoplastic epithelial cells. MCNs may have large secretory epithelial cells with evidence of mucin secretion or atypia.[14] Unfortunately, the cellularity of the cyst fluid is seldom sufficient to distinguish IPMN from MCN.[10] EUS-FNA of pancreatic cystic lesions has a very low false positive rate, but a high false negative rate in the diagnosis of malignancy, mainly due to sampling error, occurring in up to 30% of cystic lesions vs. 12% of solid lesions.[15] The combination of no high-risk stigmata, no worrisome features, and no high-grade atypia on cytology have comprised an EUS+EUS-FNA “triple test,” that may provide a negative predictive value of 99% for conservative management.[16] EUS-FNA cytology is a screening tool that contributes to the evaluation of pancreatic cysts and BD-IPMN. When positive for malignancy, it is critically useful, however, when negative, its utility in risk stratification and surgical decision making for IPMN is limited.[5] In this setting, additional analysis of the cyst fluid for markers of malignancy is far more useful.

Cyst Fluid Tumor Markers

The fluid contents of cystic lesions of the pancreas are often analyzed for cytology,[17] however, the low cellular content of cyst fluid has hampered the use of cytologic analysis. A variety of cyst fluid markers have been studied to help differentiate between the major types of cystic neoplasms (table 1).[18] The presence of extracellular mucin in aspirated cyst fluid is moderately predictive of a mucinous neoplasm.[19] Furthermore, cyst fluid tumor markers such as carcinoembryonic antigen (CEA), CA 72-4, CA 125, CA 19-9, and CA 15-3 have been tested for their use in the diagnosis of pancreatic cystic neoplasms.[20] Of these, cyst fluid CEA concentration is reported to be the most accurate marker to differentiate mucinous from non-mucinous pancreatic cystic lesions with an accuracy of 79% and sensitivity and specificity of 73% and 84%, respectively. The 2012 international consensus guidelines restated that a cutoff value of 192 – 200 ng/mL is approximately 80% accurate for the diagnosis of a mucinous pancreatic cystic lesion.[11] It is important to note that though cyst fluid CEA levels help differentiate serous from mucinous cystic lesions, the value will not differentiate between IPMN and MCN, or correlate with the level of dysplasia or malignancy.

Cyst Fluid Amylase

Theoretically, one may speculate that the cyst fluid amylase concentration might be higher in IPMNs than MCNs, as IPMNs involve the pancreatic duct and MCNs do not. However, cyst fluid amylase concentration has been shown not to distinguish MCNs from IPMNs.[9, 11]

Cyst Fluid Mucin Analysis

Mucins (MUCs) are heavily glycosylated high-molecular-weight glycoproteins whose polypeptide chains have domains rich in threonine and serine.[21–23] Pancreatic MUCs play

an important role in the lubrication, moisturization, and protection of the duct lining. MUCs are also involved in the renewal and differentiation of the epithelium, modulation of cell adhesion, and cell signaling. Alterations in MUC glycosylation have been observed in tumor tissues and it has been hypothesized that they are presumably important in carcinogenesis and tumor invasion.[24–26]

Pancreatic MUCs are classified in different categories: MUC1, MUC3, MUC4 are membrane-associated glycoproteins; MUC2, MUC5A, MUC5B and MUC6 are gel-forming mucins, and MUC7 is a soluble mucin. Normal pancreatic tissue expresses MUC1 mainly on the luminal surface of centro-acinar cells and in intra- and interlobular ducts, but not in the main pancreatic duct, acini, or islets. MUC2, MUC4, MUC5AC and MUC7 are not expressed in the normal pancreas.[27]

MUCs by IPMN histotype—Over 19 human MUC genes exist, and immunohistochemical MUC expression patterns can distinguish the different histopathologic types of IPMN (gastric, intestinal, pancreatobiliary).[28–39] The gastric subtype of IPMN typically express MUC5AC but not MUC1 or MUC2 and are almost uniformly low-grade[3], while the intestinal subtype of IPMN typically express MUC2. The pancreatobiliary subtype of IPMN typically express MUC1 and frequently contain high grade dysplasia.[40, 41] In some cases, studies using different antibodies for immunohistochemical analysis have given conflicting results and, in other cases, the lack of specific antibodies have made it difficult to perform appropriate functional studies.

Cyst fluid MUC levels measured by enzyme-linked immunoassays of aspirates from 40 patients found that gastric-subtype IPMN expressed lower levels of MUC2 (p=0.02), MUC4 (p=0.02), and MUC5AC (p=0.04) compared to non-gastric cysts. There was no difference in MUC1 expression with very low or undetectable levels in the majority of cysts. Intestinal cysts contained higher levels of MUC2 (p=0.03) and tended to have increased levels of MUC4 (p=0.05) compared to non-intestinal cysts. Pancreatobiliary cysts were not significantly associated with a distinct mucin profile.[42]

MUCs by IPMN level of dysplasia—Numerous studies have evaluated the dysregulation of mucins in IPMNs, but the results are still controversial. Some studies revealed that IPMNs usually express MUC2, MUC5AC and mostly MUC4, whereas they don’t express MUC1.[34, 35, 39, 43] This is in contrast to invasive ductal adenocarcinomas, which are characterized by an overexpression of MUC1 and the absence of MUC2. In a study of cyst fluid from patients with IPMN that underwent pancreatectomy, it was determined that MUC1 expression was very low in the cyst fluid across all groups of IPMN and that there was no correlation between MUC1 levels and the degree of cyst dysplasia. MUC5AC expression was much higher than MUC1 across all IPMN groups, but there was no association between the level of dysplasia and MUC5AC expression. On the other hand, MUC2 and MUC4 levels tended to cluster by the degree of dysplasia with higher levels of expression in highly dysplastic and invasive cystic lesions.[42]

MUCs in invasive adenocarcinoma—It has been demonstrated that non-invasive mucinous cystic neoplasms of the pancreas express MUC5AC and MUC2, and are MUC1-

negative; compared to ductal adenocarcinoma where MUC1 is overexpressed in the invasive component and its expression correlated with a poor outcome.[44] Masaki et al. specifically identified that sialynated MUC1 was detected in adenocarcinoma, whereas it was not observed in specimens from normal pancreas, chronic pancreatitis or ductal hyperplasia.[45] Similarly, the *de novo* expression of MUC4 in adenocarcinoma and in pancreatic carcinoma cell lines has been reported.[24, 27, 46, 47] Both the *MUC1* and *MUC4* genes encode numerous alternatively spliced isoforms, some of which are variants devoid of the mucin hallmark -- the tandem repeat array.[48] The role of these isoforms in IPMN histology remains to be determined.

MUC1 and MUC4 are implicated in almost all of the steps associated with the development of metastases as they possess anti-adhesive properties.[23] These mucins are upregulated in pancreatic ductal adenocarcinoma where they also lose their strictly apical localization. The steric hindrance caused by the overexpression of the two extended glycoproteins disturbs the cell–cell and/or cell–matrix interactions with a mechanism that facilitates the release of tumor cells from the tissue into the circulation.[49]

One of the most recent studies on this topic demonstrated a significant change in mucin expression patterns in pancreatic adenocarcinoma throughout disease progression. MUC1 and MUC4 were differentially glycosylated as the disease progressed from early pancreatic intraepithelial neoplasia to metastatic disease.[50] Additionally, a recent study evaluated cyst fluid RNA obtained by EUS–FNA for MUC expression in solid and cystic benign and malignant focal pancreatic lesions.[51] The prevalence of MUC1, MUC2, MUC4, and MUC7 mRNA in ductal adenocarcinoma was 58, 51, 19, and 73.0%, respectively. Sixty-three percent of IPMNs were positive for MUC1 and 47% were positive for MUC2 expression. MUC7 expression was highly significant for adenocarcinoma ($p = 0.007$) and borderline for IPMN ($p = 0.05$). In this study, although there was not a difference in MUC7 expression in adenocarcinoma and chronic pancreatitis, MUC7 could be considered a potential marker of malignancy as it was positive in 73 % of cancers. It could be useful in those cases where the cytological diagnosis is difficult because of a strong desmoplastic reaction or necrosis or, potentially, in cases in which a focal lesion with malignant evolution toward carcinoma grows in the context of an IPMN.

In summary, IPMNs have different histological patterns of growth and each histological subtype has a unique biology with different patterns of mucin expression (table 2). Though evaluation of mucin expression by immunohistochemistry, ELISA, and RNA analysis has proven feasible, and patterns of expression correlating with dysplasia and subtype have been identified; there is currently no established method for the preoperative subtype identification of IPMN. Cyst fluid mucin expression may hold promise for determination of invasive cancer, IPMN histologic subtype, and possibly the level of IPMN dysplasia.

Cyst fluid cytokines and prostaglandins

Inflammation and cancer have a complex interaction, often falling on either side of the spectrum of neoplasia. Pro-inflammatory cytokine-mediated inflammation has been shown to be associated with the pathogenesis of gastrointestinal malignancy[52–55] Similarly, continued inflammation in the setting of chronic pancreatitis may lead to dysplasia and

ultimately invasive pancreatic adenocarcinoma. However, inflammation may not only be a risk factor for adenocarcinoma, but it may also serve as a marker of neoplasia. Specific to IPMNs, it has been demonstrated that cytotoxic T-cells can be identified in the lesions and that there is a decrease in CD8+ cells with a corresponding increase in T-regulatory cells with increasing levels of cyst dysplasia.[56] Cytokine markers of a Th1 and Th2 immune response have been shown to discriminate pancreatic cancer from chronic pancreatitis or normal pancreatic tissue in both serum and pancreatic juice samples.[57, 58] Inflammation in pancreatic IPMN may lead to severe dysplasia and be reflected in inflammatory mediators that can be measured in the cyst fluid. Alternatively, increasing levels of cyst dysplasia may initiate an immune response that can be quantified by evaluation of cyst fluid cytokines. In both instances, quantification of the immune response in IPMN cyst fluid may serve as a biomarker of dysplasia.

It follows, then, that inhibition of cytokine signaling may also be a marker of dysplasia. Aberrant methylation can silence the suppressor of cytokine signalling-1 (*SOCS-1*) gene, and when methylated it was found to be associated with 22% of tested pancreatic ductal adenocarcinomas and 6% of IPMNs, as opposed to no methylation in normal ductal epithelia, pancreatic intraepithelial neoplasia, or in non-invasive IPMNs.[59]

Exploratory analysis of cyst fluid from 5 IPMNs was performed utilizing a multiplex bead-based microarray protein assay that evaluated levels of 89 inflammatory mediator proteins.[60] In this study, granulocyte-macrophage colony-stimulating factor (GM-CSF) and hepatocyte growth factor (HGF) were found to a greater degree in inflammatory cysts compared to IPMNs. The same assay was also used to compare BD-IPMN and mixed IPMN.[61] The studies were limited by their exploratory nature and lack of statistical power or validation, but support the ability to measure multiple inflammatory mediators in the cyst fluid of IPMNs using a small volume of effluent (<100uL) to search for markers of specific cyst histotypes.

Utilizing a multiplex sandwich immunoassay of Th1/Th2 markers, the cyst fluid from 40 patients (19 high risk lesions with high grade dysplasia or invasive disease, and 21 low risk lesions with low or moderate grade dysplasia) were evaluated. In a univariate analysis, IL1β, IL5, and IL8 had higher levels of expression in the presence of high grade dysplasia or invasive carcinoma. Multivariate analysis revealed that IL1β levels alone predicted high from low risk cysts, and remained a significant predictor on logistical regression when corrected for IPMN type (main or side branch) and cyst size. IL1β levels had a high sensitivity and specificity with a likelihood ratio of 17× to distinguish low from high risk cysts, and in a validation set maintained a high positive predictive value for correctly identifying high risk cysts, and a high negative predictive value for correctly identifying low risk cysts.[62] Prostaglandins represent another molecular marker of inflammation that may be well disposed to measurement in IPMN cyst fluid. In a small number of patients with BD and MD-IPMNs, prostaglandinE2 (PGE2) levels in the cyst fluid were measured by ELISA and found to differentiate low from high risk (high grade dysplasia and invasive carcinoma) lesions. Prospective validation is warranted.[63]

In conclusion, there appears to be evidence of an immune cell infiltrate into IPMNs that may correspond with the level of dysplasia. Cyst fluid analysis of mediators of inflammation including IL1β and PGE2, in particular, could provide information that differentiate these groups.

Cyst Fluid DNA Analysis

DNA is a very stable molecule and less prone to degradation than RNA or proteins. Importantly, neoplastic cells that line pancreatic cysts appear to shed their DNA into the cyst fluid. In 2009, the multicenter PANDA (Pancreatic Cyst DNA Analysis) study was conducted to investigate the role of genetic markers found in the pancreatic cyst fluid of 113 patients.[64] The authors concluded that the presence of a *KRAS* mutation in the cyst fluid had 95% specificity and 45% sensitivity for diagnosing mucinous cysts. The combination of *KRAS* mutations and allelic loss yielded high specificity (96%) but a low sensitivity (37%) for distinguishing malignant from benign cysts.[64] Subsequent studies have shown varied results using the same panel of markers[65–69] and a recent prospective, single center study found that the accuracy of this panel was just over 50% for identifying mucinous pancreatic cysts.[70]

Initial studies based on a candidate gene approach identified a number of key genes frequently mutated in IPMNs, including *KRAS, p16/CDKN2A, SMAD4,* and *TP53*.[71] Interestingly, these genes were also mutated in invasive pancreatic ductal adenocarcinoma (PDAC), although the prevalence of mutations in these genes was higher in invasive cancers than in IPMNs.[71–76] Mutations in other genes, such as *PIK3CA, LKB1/STK11* and *BRAF* have been also shown to occur in a small subset of IPMNs.[77–79]

Using parallel DNA sequencing, Wu et al. tested a panel of 169 cancer genes for the presence of somatic mutations in DNA from surgically resected IPMN.[16] This confirmed a high mutational frequency of the *KRAS* gene and unexpectedly found frequent mutations in the *GNAS* gene, all located in the “hotspot” codon 201. More specifically, mutations in *GNAS* and *KRAS* were present in 66% and 81% of specimens, respectively, with a mutation in at least one of the two genes present in 95% of IPMNs.[16] In contrast, no *GNAS* or *KRAS* mutations were identified in a large series of serous cystadenomas (SCA) and no *GNAS* mutations were present in other cyst types or in pancreatic adenocarcinoma. Whole-exome sequencing of mucinous pancreatic cysts found IPMNs characterized by *KRAS, GNAS, RNF43, TP53, p16/CDKN2A* and *SMAD4* gene mutations and MCNs by *KRAS, RNF43, TP53, p16/CDKN2A* and *SMAD4* gene mutations (Table 3).[80]

Only a few studies have validated the role of genetic alterations using next-generation sequencing.[81–83] Amato et al. used next-generation targeted sequencing technology to assess the mutational status of 51 cancer genes in 48 surgically resected IPMNs.[81] The authors showed that 96% of all IPMN analyzed harbored at least one mutated gene, with *GNAS* and *KRAS* being the most commonly mutated genes (79% and 50%, respectively). *RNF43* was the third most frequently altered gene. In addition to these gene mutations, other mutations were found with lower frequency in *TP53* (10%) and *BRAF* (6%). Furthermore, the authors performed deep sequencing on seven cyst fluid samples to assess the presence of the same mutations found in the microdissected neoplastic epithelial cells from corresponding IPMNs.

Ten out of 13 mutations found in the tumors were also found in the cyst fluid samples, substantiating the feasibility of detecting mutant DNA molecules in cyst fluid, even when these mutant molecules were at a low concentration.

Other studies have confirmed the high prevalence of *GNAS* mutations in IPMNs, with mutations found in 57%–64% of cases of IPMNs.[16, 76, 80, 82] Although both *GNAS*[16, 76, 82] and *KRAS*[72, 74–76] mutations occur commonly in patients with IPMNs, both mutations appear to occur early in the neoplastic transformation of IPMNs; specifically, *KRAS* mutations have been detected in over 90% of IPMNs with low- or intermediate-grade dysplasia.[83, 84] *GNAS* mutations, on the other hand, are found in IPMNs with low- through high-grade and invasive tumors.[82, 85]

Although cyst fluid genetic analysis has been predominantly centered on mutations of specific genes, another line of research has focused on epigenetic alterations. Hong et al. performed CpG island amplification followed by microarray analysis to compare the methylation status in IPMNs with normal pancreatic ductal epithelium.[86] They found differential methylation in 2,259 genetic regions, and identified a series of genes including *BNIP3*, *PTCHD2*, *SOX17*, *NXPH1*, and *EBF3*, which were characteristically hypermethylated in IPMNs with high-grade compared with low-grade dysplasia.

There are limitations to DNA-biomarker analysis, and the studies conducted to date have analyzed surgically resected IPMN. Little is known about the genetic features of IPMNs undergoing surveillance, thus, there may be a bias in prevalence and clinico-pathological mutated genes as the lesions selected for operative intervention likely contained high-risk or worrisome features. Secondly, the prevalence of mutated genes varied between studies. Intra-tumor genetic heterogeneity may explain some of the variability observed, since various neoplastic clones are thought to develop within a neoplastic lesion.[80] This highlights one of the limitations of molecular testing, which is that usually only a portion of a cyst is sampled.

In conclusion, DNA-based biomarkers may be used as diagnostic tools. However, further research is needed to identify a combination of genetic markers found only in the cyst fluid that will not only identify mucinous from serous lesions, but IPMN from MCN, and help identify patients with high-grade dysplasia or invasive disease, who may benefit from surgical resection. The authors find promise in further evaluation of KRAS and GNAS in cyst fluid in particular.

Cyst Fluid miRNA Analysis

Study of epigenetic mechanisms regulating the multistep processes involved in transcription or translation may provide insight into biomarkers associated with IPMN progression.[87] MicroRNAs (miRNAs) are small (19 to 25 nucleotides) noncoding RNAs that regulate the stability and translation of mRNA transcripts. Micro-RNA evaluation is feasible due to their small size and stability in formalin-fixed, paraffin-embedded (FFPE) tissue[88] and biofluids. Deregulation has been recognized in numerous human malignancies, including pancreatic ductal adenocarcinoma,[89] and specific miRNA expression patterns can discriminate between PanIN II and PanIN III lesions,[90] and pancreatic adenocarcinoma from chronic

pancreatitis.[91, 92] Elevated levels of miR-21 and miR-155 were identified in surgically resected specimens of non-invasive IPMNs and both were more frequently detected in IPMNs with carcinoma in-situ compared to non-invasive IPMNs. Follow-up assessment of cyst fluid from resected lesions identified significantly elevated concentrations of miR-21, miR-217, and miR-17-3p in mucinous (10 MCN, 16 IPMN) compared to non-mucinous cysts.[93]

Assessment of FFPE sections from 65 invasive IPMNs and 16 non-invasive IPMNs supported a role for these particular microRNAs with miR-21 and miR-155 significantly overexpressed in invasive IPMN compared to non-invasive IPMNs, while the contrary relationship was observed for miR-101.[94]

Global miRNA expression analysis was examined in a study of 10 low grade, 5 moderate grade, 5 high grade, and 10 invasive IPMNs.[95] Hierarchical clustering revealed 15 miRNAs that were different between low and intermediate-grade compared with high-grade and invasive IPMNs. Specifically, three miRNAs (miR-10a, miR-146 and miR-155) were elevated in high-grade and invasive IPMNs compared to low and moderate-grade IPMNs.

In an attempt to utilize miRNAs to discriminate high-grade dysplastic pancreatic cystic neoplasms likely to require surgical resection compared to those suitable for observation, high-throughput miRNA expression profiling was performed in low-grade (n = 10) and high-grade (n = 12) IPMN tissue as well as cyst fluid from 3 low-grade and 4 high-grade IPMNs.[96] Subsequent qRT-PCR validation was performed in 23 IPMN tissues and 19 IPMN cyst fluid samples resulting in a subset of 18 miRNAs that discriminitated cysts requiring surgery (high-grade) from those suitable for observation (low-grade IPMNs). Logistic regression analysis led to the final identification of a 9-miRNA model, which allowed separation of all but one high-grade IPMN from low-grade IPMNs/SCAs with a sensitivity and specificity of 89% and 100%, respectively. Of note miR-21 was upregulated in low-/intermediate-/high-grade IPMNs, however, failed to predict the risk of malignant transformation. While limited by the mixed nature of the cysts that were assessed (IPMNs, SCAs, PNETs) this investigation highlighted the disparity in miRNA expression profiles between cyst fluid and resected tissue specimens, underlining the need for the development of a miRNA cyst fluid profile rather than extrapolation from resected tissue derived miRNA profiles.

Table 4 summarizes those miRNAs found dysregulated in IPMN tissue, cyst fluid, or serum. Certainly, further studies are needed to ascertain whether miRNA expression in cyst fluid and tumor tissue obtained with fine-needle aspiration biopsy can potentially differentiate high-grade from low-grade lesions.

Conclusion

Determination of the biologic behavior of an IPMN is critical to identify patients for whom surgical resection is indicated and potentially preventative or curative. Great strides have recently been made in study of the natural history of the disease and in establishing diagnostic criteria to better define IPMNs from other pancreatic cysts. What remains to be

elucidated, however, is identification of IPMN at high-risk for malignant transformation or underlying malignancy, and a reliable molecular marker within the cyst fluid is needed. The status of EUS, EUS-FNA cytology, cyst fluid biochemistry, mucins, cytokines, DNA, and microRNA profiles in IPMN were critically reviewed. The group concluded that the ideal biomarker which could predict malignant potential should be easily obtained, widely applicable, and inexpensive. We predict that this molecular marker will likely be a conglomerate of the current known biomarkers explained herein, combined with additional proteomic analysis, and concluded that based on the available data, there is particular promise in creation of a biologic signature consisting of mucins 1, 2, 4, 5AC; IL1β, PGE2, KRAS, GNAS, and the 9 miRNA panel. We forsee this signature as having a high positive predictive value and working alongside clinical factors that have already been validated such as mural nodularity, solid component, cyst growth rate, and duct obstruction; but having its greatest utility in the evaluation high risk pre-malignant cysts that are otherwise indistinguishable radiographically from benign cysts and those that will contain low-grade dysplasia indefinitely.

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Abbreviations (in order of appearance)

IPMN	Intraductal papillary mucinous neoplasm of the pancreas
BD	Branch-duct
MPD	Main pancreatic duct
SCN	Serous cystic neoplasm
MCN	Mucinous cystic neoplasm
miRNA	micro-ribonucleic acid
MUCs	mucins
PDAC	pancreatic ductal adenocarcinoma

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Table 1

Cyst Fluid Glycoprotein and Amylase Expression in Intraductal Papillary Mucinous Neoplasms

	Amylase, U/L	CEA, ng/mL	CA 72-4, U/mL	CA 19-9, U/mL	CA 125, U/mL	CA 15-3, U/mL
Cut off	6,800	192	7	2,900	9	57
Sensitivity, %	66	73	80	68	83	19
Specificity, %	81	84	61	62	37	94
Accuracy, %	69	79	72	66	60	57

Table 2

Mucin Expression Profiles in Various Intraductal Papillary Mucinous Neoplasms Histotypes

TYPE	Main involvement	MUC1	MUC2	MUC5AC	Type of invasive carcinoma (%)
Intestinal IPMN	MD	−	+	+	Colloid (30–50)
Pancreatobiliary IPMN	MD	+	−	+	Tubular (>50)
Gastric IPMN	BD	−	−	+	Tubular (10–30)
Oncocytic IPMN	MD	+/−	−	+	Oncocytic (NA)
ITPN	MD	+	−	−	Tubular (NA)

IPMN, intraductal papillary mucinous neoplasm; ITPN, intraductal tubulopapillary neoplasm; MD, main duct; BD, branch duct; NA, not available.

Table 3

DNA Analysis of Pancreatic Cyst Fluid Can Identify Patterns of Genetic Alterations that Define Cyst Type

	KRAS	GNAS	RNF43	CTNNB1	VHL
IPMN	+	+	+		
MCN	+		+		
SPN				+	
SCA					+

IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; SPN, solid-pseudopapillary neoplasm; SCA, serous cystadenoma.

Table 4

MicroRNAs Associated with Various Histologic Classifications of Intraductal Papillary Mucinous Neoplasms

Histologic classification of IPMN	First author	Tissue Source	Up-regulated	Down-regulated
Lesion				
Non-invasive IPMN	Habbel[97], Caponi[94], Nakhara[98]	Tissue	miR-21, miR-155, miR-107, miR-223, miR-181c, miR-181a, miR-221, miR-210, miR-16, miR-100	miR-101
	Li[99]	Serum	miR-1290	--
Invasive IPMN	Lubezky[95], Caponi[94], He[100]	Tissue	miR-21, miR-155, miR-708	miR-101, miR-217, miR-218
PDAC vs non-invasive IPMN	Lee[101]	Tissue	miR-21-5p, miR-708	miR-485-3p, miR-375
	Jiao[102]	Tissue		miR-16, miR-126
	Li[99]	Serum	miR-1290	--
Degree of dysplasia				
HG IPMN	Lubezky[95], Habbe[97]	Tissue	miR-10a, miR-21, miR-146, miR-155	--
IG IPMN	Lubezky[95], Habbe[97]	Tissue	miR-21, miR-155, miR-708	miR-217
LG IPMN	Lubezky[95], Habbe[97]	Tissue	miR-21, miR-155, miR-708	miR-217
HG/Invasive IPMN vs LG/IG IPMN	Lubezky[95]	Tissue	miR-21, miR-146a, miR-150, miR-214, miR-503, miR-424, miR-708, miR-155	miR-217, miR-216a, miR-216b, miR-148, miR-375, miR-130b
	Matthei[96]	Tissue, cyst fluid	miR-18a, miR-24, miR-30a-3p, miR-92a, miR-99b miR-106b, miR-142-3p, miR-342-3p, miR-532-3p	DiffPairs analysis
LG IPMN vs HG IPMN with evidence of invasion	Lubezky[96]	Tissue	miR-10a, miR-21, miR-24-2, miR-132, miR-146a, miR-146b, miR-155, miR-196, miR-424, miR-503, miR-708	miR-217, miR-216a, miR-216b, miR-148, miR-375, miR-130b
IPMN morphological subtype				
Intestinal	Aso[103], Habbe[97]	Tissue, serum	miR-196a, miR-21, miR-155	--
Gastric	Habbe[97]	Tissue	--	miR-155

IPMN, intraductal papillary mucinous neoplasm; HG, high grade; IG, intermediate grade; LG, low grade; PDAC, pancreatic ductal adenocarcinoma.