Cyst Fluid Biomarkers – Diagnosis and Prediction of Malignancy for Cystic Lesions of the Pancreas

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Pancreatic cysts · Biomarkers · Intraductal papillary mucinous neoplasm · Mucinous cystic neoplasm · Serous cystadenomas

Summary

Pancreatic cysts are common, and are identified in 2–13% of individuals undergoing cross-sectional imaging. Intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs) are pancreatic cysts which are precursors to pancreatic adenocarcinoma. Currently available tools are imperfect at differentiating IPMNs and MCNs from other, benign types of pancreatic cysts. The role of molecular markers in the evaluation of pancreatic cysts and the identification of cysts with high-grade dysplasia or invasive adenocarcinoma is reviewed.

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Introduction

Pancreatic cysts are common, and are identified in 2–13% of individuals undergoing cross-sectional imaging [1–3]. Two of the three precursors to pancreatic adenocarcinoma, i.e. intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs), are pancreatic cysts, which are easily identified on computed tomography and magnetic resonance imaging. Identification of these two pancreatic cyst precursors offers the potential of identifying, and potentially screening, for pancreatic adenocarcinoma [4]. In clinical practice, however, this approach is complicated by two factors. First, there are many different types of pancreatic cysts, including cysts with no risk of malignant transformation like serous cystadenomas (SCAs) or pseudocysts. Second, most IPMNs and MCNs will not progress to invasive pancreatic

adenocarcinoma. Thus, two key clinical questions are: i) how to differentiate IPMNs and MCNs from other benign types of pancreatic cysts which require no follow-up; ii) how to identify IPMNs and MCNs which harbor high-grade dysplasia or an associated invasive pancreatic adenocarcinoma, and require surgical resection, from IPMNs and MCNs which can be safely followed. The currently available tools are imperfect at answering these two questions. This is evidenced by the fact that in large surgical series over 20% of patients are found to have low-risk cysts, while up to 78% of resected IPMNs do not harbor high-grade dysplasia or invasive adenocarcinoma [5, 6]. Thus, better tools are required to answer these questions. One potential avenue which has been explored is whether molecular markers, i.e. analysis of DNA, RNA, protein or metabolomic changes within the cyst, could aid the diagnosis of cyst type as well as management.

Recent genetic analyses have examined the somatic mutations that occur in each type of pancreatic cyst, and all types of neoplastic pancreatic cysts have been analyzed by whole exome sequencing, providing great insights into the genetic alterations underlying the development of each cyst type [7]. These studies revealed that histologically defined cyst types possess unique combinations of driver gene alterations so that the genetic changes mirror the cyst morphology (table 1). This highlights the importance of somatic mutations in defining the morphological and clinical features of pancreatic cysts and underscores their potential as biomarkers to preoperatively diagnose pancreatic cysts.

Intraductal Papillary Mucinous Neoplasms and Mucinous Cystic Neoplasms

IPMNs, with MCNs, are precursors to pancreatic adenocarcinoma. IPMNs are associated with a number of mutations, several of which are also found in pancreatic adenocarcinoma. The commonest of these is a somatic mutation at the oncogenic hotspots in *KRAS* which is identified in approximately 80% of IPMNs if sensi-

Table 1. Genetic profile of pancreatic cysts

Cyst type	SCA	MCN	IPMN	PDAC	SPN
<i>KRAS</i>		+	+	+	
<i>GNAS</i>			+	(+)	
<i>RNF43</i>		+	+	+	
<i>CTNNB1</i>		+	+	+	+
<i>VHL</i>	+				

SCA = Serous cystadenoma; MCN = mucinous cystic neoplasm; IPMN = intraductal papillary mucinous neoplasm; PDAC = pancreatic ductal adenocarcinoma; SPN = solid pseudopapillary neoplasm.

tive techniques are used [8]. These *KRAS* mutations occur in all grades of dysplasia as well as histologic subtypes, and are not a predictor of the presence of high-grade dysplasia [9]. In contrast, loss of tumor suppressor genes occurs later in IPMN development. Although loss of *CDKN2A* (p16) (either due to mutation, deletion, or methylation) occurs in both non-invasive IPMNs and IPMNs with associated invasive carcinomas, it is more frequent in IPMNs with associated invasive carcinomas [10]. Similarly, *TP53* mutations and aberrant expression of the encoded protein are most prevalent in areas of high-grade dysplasia and invasive carcinoma associated with IPMNs [9, 11, 12]. Loss of expression of the protein encoded by the tumor suppressor gene *SMAD4* is also much more prevalent in IPMN-associated invasive carcinomas compared to non-invasive IPMNs. In short, the alterations of these four major driver genes in IPMNs mirror those in pancreatic ductal adenocarcinoma (PDAC); *KRAS* mutations are early alterations, loss of *CDKN2A* is intermediate, and loss of *TP53* and *SMAD4* are late alterations. In addition to these well-known PDAC driver genes, IPMNs also harbor somatic mutations in genes that are not commonly mutated in PDAC. Approximately 60% of IPMNs have somatic mutations in *GNAS*, which encodes a protein that couples transmembrane receptors to their downstream signaling proteins [8, 13]. *GNAS* is a known oncogene with previously described mutations in pituitary adenomas and other uncommon neoplasms. Mutations in IPMNs occur at the previously described oncogenic hotspot (codon 201), resulting in the substitution of an arginine with histidine (R201H), cysteine (R201C), or serine (R201S). *GNAS* mutations occur most frequently in IPMNs with intestinal subtype (82%), and are found at a lower prevalence in gastric- (61%), pancreaticobiliary- (38%), and rarely in oncocytic-type IPMNs [8]. Loss-of-function mutations in the ubiquitin ligase-encoding gene *RNF43* occur in 40–75% of IPMNs [7, 8]. Loss of heterozygosity at the *RNF43* locus on chromosome 17q is also reported in 11% of IPMNs [8]. Alterations in the phosphatidylinositol-3-kinase (PI3K) pathway, including somatic mutations in the oncogenes *PIK3CA* and *AKT1* as well as in the tumor suppressor gene *PTEN*, have been reported but are rare in IPMNs [8, 14]. Additional markers which have shown promise for identifying high-grade dysplasia in IPMNs include hypermethylation of *SOX17*, which in one study was associated with an accuracy of 84% for identifying IPMNs with high-grade dysplasia [15]. Elevated cyst fluid telomerase levels have also been associated with an increased

risk of high-grade dysplasia [16]. These studies are promising but require validation in prospective studies and by other groups.

Similar to IPMNs, MCNs also have the potential to give rise to invasive pancreatic adenocarcinoma [4], and contain mutations in many of the same genes. The commonest mutation present in MCNs is in *KRAS*, which is present in 50% of MCNs [8]. Mutations in *CDKN2A*, *TP53*, and *SMAD4* occur less frequently, and later in tumorigenesis in MCNs. Inactivating mutations in the tumor suppressor gene *RNF43* also occur in MCNs, though at a lower prevalence than in IPMNs [7, 8]. To date, *GNAS* mutations have not been identified in MCNs, suggesting that these mutations are specific to IPMNs and not shared among other mucin-producing precancerous pancreatic cysts [7, 8].

Several studies have found that mRNA markers have a high sensitivity and specificity (>80%) for identifying cyst type and IPMNs with high-grade dysplasia or invasive pancreatic adenocarcinoma [17, 18]. However, all the studies are single-center, retrospective studies with very few markers evaluated in more than one report. Additional larger studies are therefore required.

The best known protein marker associated with IPMNs is cyst fluid carcinoembryonic antigen (CEA). The role of CEA was established in a multicenter, prospective co-operative study in 2004 which found that the accuracy of cyst fluid CEA was superior to endoscopic ultrasound, cytology, or other tumor markers including CA 72-4, CA 125, CA 19-9, and CA 15-3 for identifying IPMNs or MCNs [19]. A recent meta-analysis of 18 studies with 1,438 patients found that cyst fluid CEA had a sensitivity of 63% and a specificity of 88% for identifying IPMNs and MCNs [20]. In addition to only moderate sensitivity, obtaining sufficient cyst fluid to assess CEA levels is often not possible, particularly in very small cysts, or if the fluid is very viscous. This issue was highlighted by a prospective European study in which CEA levels were obtained in only half of the cysts tested [21]. Some studies have suggested that a high cyst fluid CEA is associated with high-grade dysplasia or invasive cancer in IPMNs. However, these studies were small and retrospective, and there was a significant overlap between the CEA level in IPMNs with low- or intermediate-grade dysplasia and those with high-grade dysplasia or invasive adenocarcinoma. In contrast, much larger studies, including a prospective study and a meta-analysis, have found no association between CEA level and the presence of high-grade dysplasia or invasive carcinoma [19, 22–24]. Thus, cyst fluid CEA level is not helpful in IPMNs with high-grade dysplasia or invasive carcinoma. In addition, cyst fluid CEA levels are elevated in both IPMNs and MCNs and are therefore not useful in differentiating between them.

Cyst fluid amylase is another protein marker which is often evaluated. Low amylase levels can be useful in excluding a pseudocyst, as shown in a large meta-analysis which found that a cyst fluid amylase level of <250 IU/l had a very high specificity (98%) for excluding a pseudocyst [25]. In contrast, a high cyst fluid amylase level is not helpful, as it is found in multiple types of pancreatic cysts including SCAs, IPMNs, and MCNs [26]. Some authors have hypothesized that IPMNs, which have a communication between the main pancreatic duct and the cyst, would have a high cyst fluid amylase

level, whereas MCNs, which have no connection with the main pancreatic duct, should have a low amylase level. However, studies have shown that cyst fluid amylase levels are similar in IPMNs and MCNs, and thus cannot be used to differentiate these two types of cysts [27]. There has been an interest in using cyst fluid glucose levels to differentiate IPMNs and MCNs from other types of cysts [28, 29]. A recent study evaluated this in 153 patients all of whom had undergone surgical resection [30]. Using a cutoff of ≤ 50 mg/dl, cyst fluid glucose had 92% sensitivity and 87% specificity for identifying IPMNs and MCNs from other cyst types, and had a higher accuracy of 90 versus 69% compared with cyst fluid CEA [30].

A number of other protein markers have been evaluated, and results indicate that using multiplex assays detecting many markers or oligomarkers is superior to single markers. Markers identified using 'omics' platforms or when combining markers include IL-1 β , AFM, REG1A, LCN, PIGR, and MUC5AC. One of the most promising markers is MUC(mucin)5AC-WGA/endorepellin. MUC are heavily glycosylated high-molecular-weight glycoproteins, with different MUC protein expressions reported in different histological types of IPMNs, as well as grades of dysplasia [31]. MUC1 is found in IPMNs with the pancreaticobiliary subtype, MUC2 in those with the intestinal subtype, and MUC5AC in those with gastric subtype. A number of studies have examined the potential of MUC proteins in identifying IPMNs with high-grade dysplasia. A recent study in over 140 patients found that MUC5AC had a sensitivity and specificity of over 90% for identifying IPMNs from other types of cysts, and was associated with high-grade dysplasia [32]. Additional studies are needed to validate these results. One other promising marker for identifying IPMNs which require surgical resection is mAb Das-1, a monoclonal antibody which is found in premalignant conditions of the gastrointestinal tract. In preliminary studies, it had 85% sensitivity and 95% specificity for identifying IPMNs with high-grade dysplasia or invasive cancer and is now being evaluated in a multicenter study [33].

Serous Cystadenomas

SCAs have a risk of malignant transformation of 0.1%, and are considered benign cysts, which do not require surveillance [34–36]. Therefore, differentiating SCAs from IPMNs and MCNs is important if unnecessary follow-up or surgical resection is to be prevented. Genetic analyses of SCAs have revealed that these cysts are genetically distinct from other types of pancreatic cysts. Somatic mutation in the *VHL* gene occurs in over 40% of SCAs. Loss of heterozygosity at the *VHL* locus on chromosome 3p is found in over 60%, with aneuploidy of chromosome 3p also reported, indicating a critical role for loss of this tumor suppressor gene in the formation of SCAs [7, 8]. Almost 70% of SCAs have a mutation in *VHL*, loss of heterozygosity of chromosome 3, or aneuploidy in chromosome 3p [8]. Mutations in other genes, including *KRAS*, *CDKN2A*, *TP53*, *SMAD4*, and *RNF43*, are rare, while loss of other chromosomes including 10p have been reported [8, 37]. One pro-

tein marker which has shown promise in identifying SCAs is vascular endothelial growth factor(VEGF)-A, which is a critical regulator of vascular growth and function. This protein has been found to be elevated in patients with SCAs [38], with a recent study in 149 patients finding that a VEGF-A level of greater than 5,000 pg/ml had 100% sensitivity and 82% specificity for differentiating SCAs from IPMNs and MCNs [39]. Combining VEGF-A with CEA was associated with 96% sensitivity and 100% specificity [39]. These results are promising and now require prospective validation.

Solid Pseudopapillary Neoplasms

Solid pseudopapillary neoplasms (SPNs) are rare cysts which classically present in young women. These cysts are aggressive, with metastatic spread occurring in 8% [40]. They have a distinct genetic profile which is quite different to other pancreatic cysts, with almost all SPNs containing a somatic mutation in the *CTNNB1* gene, which is a key component of the Wnt signaling pathway [7, 8]. They have an average of only three somatic mutations per tumor, one of the lowest mutation rates of any tumor sequenced to date [7]. In contrast to IPMNs, MCNs, and SCAs, mutations in *KRAS*, *CDKN2A*, *TP53*, *SMAD4*, and *VHL* are extremely rare.

Conclusion

Molecular markers show promise for the diagnosis and management of pancreatic cysts. There are several areas which are important to consider in future studies. The first is how to incorporate multiple different markers together, and how to combine them with clinical features. The second issue is the lack of high-quality studies, with much of the current data coming from small, retrospective, single-center studies in which the findings are not reproducible in other centers. In initial studies, the performance of future markers should be compared with the gold standard, i.e. surgical pathology, with promising markers then evaluated in prospective, multicenter studies. To be incorporated into clinical practice, a marker panel needs to show analytical reproducibility, clinical validation, and clinical utility, to improve quality of life, and to be cost-effective.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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