Molecular markers contribute to the clinical diagnosis for pancreatic cystic neoplasms

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Abstract

A pancreatic cystic neoplasm (PCN) is a rare pancreatic disease. Malignant PCNs are usually identified incidentally while evaluating other lesions. However, PCNs are being identified more frequently owing to the increased use of abdominal imaging. Malignant PCNs have complicated and diverse biological behaviors, including various malignant risk factors, diverse molecular features, natural history, and complex pathological classifications. Although many diagnostic methods, such as cross-sectional imaging and endoscopic evaluation, have been developed, malignant PCNs are still difficult to differentiate from benign tumors. On searching for related articles in the recent decade, we found that some molecular biomarkers such as carcinoembryonic antigen could be useful for discriminating between malignant tumors and benign tumors. However, cytopathologic evaluation is the most useful method for differentiating between benign and malignant lesions. Although cytopathologic evaluation has a specificity of 100% for identifying malignancies, its accuracy is often hampered by the low cellularity of PCN cells in the cystic fluid. Herein, we review the progress in the use of cellular and molecular markers for the accurate identification of PCNs.

Keywords: Pancreatic cystic neoplasm; Biomarker; Diagnosis

Introduction

Pancreatic cystic neoplasms (PCNs) can be classified into epithelial and non-epithelial types on the basis of the composition of epithelial and mesenchymal tissue. PCNs account for approximately 10% to 15% of all pancreatic cystic lesions.[1] PCNs mainly include the following four types: intraductal papillary mucinous neoplasms (IPMNs), mucinous cystic neoplasms (MCNs), serous cystic neoplasms (SCNs), and solid pseudopapillary neoplasms (SPNs). Among the four types of PCNs, SCNs are benign, while the other types tend to have malignant potential. According to a research for observing the results of a selective surgical approach to patients with PCNs for 15 years, including 1424 operative and non-operative patients, among them, the incidence of main duct IPMNs (MD-IPMNs), branch duct IPMNs (BD-IPMNs), MCNs, serous cystadenomas (SCAs, a type of SCNs), and SPNs was about 25%, 26%, 11%–18%, 13%–23%, and 2%, respectively.[2]

PCNs are often identified on abdominal computed tomography (CT) or magnetic resonance imaging (MRI). The reported prevalence of PCNs on CT is up to 2.6% in 78 patients who were unsuspected for PCNs when underwent abdominal CT at an outpatient center over a 1-year period, whereas MRI has increased the ability to identify PCNs, with a reported prevalence of 13.5% to 45% in 152 patients with unknown pancreatic disease over an observation of 26-months.[3] However, CT or MRI cannot always clearly distinguish between non-cystic and cystic tumors. Although endoscopic ultrasound (EUS) can reveal the septa, solid nodules, and main duct dilatation, it is relatively limited for the identification of micro-cystic lesions, which may appear solid. Owing to the small tumor size or inappropriate tissue samples, endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNA) does not always result in a definitive diagnosis.

The pathological classification of PCNs is complex [Table 1], and the prognosis varies for each type. The prognosis of IPMNs is the worst. However, MCNs, SCNs, and SPNs have better prognosis. Therefore, accurate classification and early diagnosis of PCNs are particularly important. Currently, many markers have been studied,

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Table 1: Classification of pancreatic cystic neoplasms (PCN).

Classification	Benign	Malignant
Epithelial neoplastic	Intraductal papillary mucinous adenoma	Intraductal papillary mucinous carcinoma
	Mucinous cystic adenoma	Mucinous cystadenocarcinoma
	Serous microcystic adenoma	Serous cystadenocarcinoma
	Serous macrocystic adenoma	Solid pseudopapillary neoplasm
	Serous oligocystic adenoma	Cystic ductal adenocarcinoma
	Accessory splenic epidermoid cyst	Cystic pancreatoblastoma
	Cystic neuroendocrine tumors	Cystic acinar cell carcinoma
	Acinar cell cystadenoma	Cystic neuroendocrine carcinoma
	Cystic hamartoma	Cystic metastatic epithelial neoplasm
	Dermoid cyst	
	Von Hippel-Lindau associated serous cystic adenoma	
Non-epithelial neoplastic	Lymphangioma	Sarcomas

including clinical features, imaging features, cellular markers, and molecular markers.

To date, only a few studies have evaluated the association between molecular markers and malignant PCNs. However, recently, there has been a growing interest in the identification of the subtypes of PCNs or malignant PCNs by using new molecular biomarkers [Table 2], which can be obtained from cystic fluid via EUS-FNA. Herein, we review the progress in the use of molecular markers for the clinical diagnosis of PCNs.

IPMNs

IPMNs are common in elderly men (average age, 65–75 years); they arise from the pancreatic ducts and are located in the head and uncinate part of the pancreas. On histological examination, columnar epithelial cells of IPMNs demonstrate a spectrum of dysplasia, ranging from low- to high-grade tumors that may show progression to invasive cancer. The cystic fluid of IPMNs is viscous and mucin-rich, with elevated amylase levels.[4] IPMNs can be divided into three types on the basis of the origin site or location: MD-IPMNs, BD-IPMNs, and mixed type IPMNs (MT-IPMNs). Furthermore, IPMNs can be pathologically divided into gastric, intestinal, pancreatobiliary, and oncocytic subtypes.[5] The pancreatobiliary subtype can progress to tubular carcinomas, while the intestinal subtype might progress to colloid carcinomas. The 5-year survival of patients with the intestinal, oncocytic, gastric, and pancreatobiliary subtypes was 95%, 75%, 70%, and 35.6%, respectively.[6,7]

MD-IPMNs present as tubular cysts, characterized by segmental or diffuse dilatation of the main pancreatic duct, with a diameter of >5 cm on CT/MRI scans. BD-IPMNs are observed as oval macro-cystic masses, sometimes lobulated, with or without internal septa; they usually involve the main pancreatic duct, but do not show any concomitant dilatation. MT-IPMNs are cysts that communicate with both the main duct and the branch ducts. The enhanced images of irregular septa and nodules or solid components indicate adenocarcinomas among the IPMNs.[8,9]

MRI or magnetic resonance cholangiopancreatography (MRCP) has a higher sensitivity for identifying IPMNs than multi detector computed tomography (MDCT) does, with sensitivities of 96.8% and 86%, respectively.[4] However, MRI and CT have similar accuracy for differentiating between malignant and benign IPMNs, with accuracies of 74%–75% and 74%–78%, respectively.[10] In general, MRI is better than CT, as it has higher contrast resolution for profiling the involvement of the main duct and for detecting mural nodules and septa. Moreover, MRI is beneficial for patients who require long-term follow-up, as it avoids repeated radiation exposure.[11] Moreover, although EUS has a higher sensitivity for identifying mural nodules, it is more invasive and lacks specificity. In fact, EUS has a lower accuracy rate of 68% compared to CT and MRI for differentiating between malignant and benign IPMNs.[10] However, pancreatoscopy has a higher diagnostic accuracy for MD-IPMNs than for BD-IPMNs, and it can be used to differentiate between MD-IPMNs and chronic pancreatitis.[3]

Molecular markers

DNA biomarkers

We found that more than half of all cases of IPMNs have *KRAS* and *GNAS* mutations. The use of the combination of *KRAS* and *GNAS* mutations for identifying IPMNs has a sensitivity of 84% and specificity of 98%.[12] Moreover, the incidence of *KRAS* mutations is not different among the various grades of dysplasia, whereas the incidence of *GNAS* mutations is higher in more advanced lesions and these mutations are detected only in IPMNs. Loss of function mutations in the ring finger protein 43 (*RNF43*) gene can be found in 40% to 75% of IPMNs.[13]*PIK3CA* mutations are often found in advanced IPMNs. In contrast, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mutations are rarely detected in MCNs and pancreatic ductal adenocarcinomas (PDACs), showing that they are exclusively associated with IPMNs.[14]

Some genetic mutations are associated with histological subtype of IPMNs. *KRAS* mutations are observed in 100% of cases of the pancreatobiliary subtype and in 83% of

Table 2: Biomarkers of pancreatic cystic neoplasms.

Biomarkers	IPMN	MCN	SCN	SPN
KRAS	√	√		
GNAS	√			
RNF43	√	√		
PIK3CA	√	√		
SHH	√			
CDKN2A (p16)	√	√		
BRG1	√			
TP53	√	√		
PTEN		√		
hTERT	√			
hMLH1	√			
SOX11				√
SOX17	√			
VHL			√	
VEGF-A			√	
CTNNB1				√
TFE3				√
Tcf-3				√
miR-21	√			
S100	√			
MUC	√			
mAb Das-1	√			
Plectin-1	√			
Interleukin-1β	√			
CD138				√
CD10				√
Ki-67				√
Her-2	√			
α-inhibin			√	
β-catenin				√

√: Molecular that can be used to identify the IPMN/MCN/SCN/SPN. IPMN: Intraductal papillary mucinous neoplasm; MCN: Mucinous cystic neoplasm; SCN: Serous cystic neoplasm; SPN: Solid pseudopapillary neoplasm. *RNF43:* Ring finger protein 43; *PIK3CA*: Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; SHH: Sonic hedgehog signaling molecule; *CDKN2A* (*p16*): Cyclin dependent kinase inhibitor 2A; *TP53*: Tumor protein p53; *PTEN:* Phosphatase and tensin homolog; *hTERT*: Human telomerase reverse transcriptase; hMLH1: Human MutL homolog 1; *SOX11*: SRY-box transcription factor 11; *VHL*: Von Hippel-Lindau; VEGF-A: Vascular endothelial growth factor A; *CTNNB1:* Catenin beta 1; *TFE3*: Transcription factor binding to IGHM enhancer 3; *Tcf-3*: Transcription factor 3; S100A: S100 calcium binding protein; MUC: Mucin protein; mAb Das-1: Monoclonal antibody Das-1.

cases of the gastric subtype, but only in 39% of cases of the intestinal subtype. *GNAS* mutations are observed in 100% of cases of the intestinal subtype, in 71% of cases of the pancreatobiliary subtype, in 51% of cases of the gastric subtype, and rarely in cases of the oncocytic subtype.[15,16] The sonic hedgehog signaling molecule (*SHH*) gene was detected in 68.8% of cases of the intestinal subtype and in 92.8% of cases of the pancreatobiliary subtype.[16] SHH can also be used to differentiate between IPMNs and pancreatitis, as it can be detected in the pancreatic juice of patients with IPMNs but not in patients with pancreatitis.

Some genes have been used for identifying advanced IPMNs, including inactivation of cyclin dependent kinase inhibitor 2A (*CDKN2A* [p16]) and *BRG1* and mutation in tumor protein p53 (*TP53*) and human telomerase reverse transcriptase (*hTERT*).[13] Inactivation of *BRG1* was reported in 53.3% of IPMNs and its loss of expression was correlated with an increasing degree of dysplasia.[17] The promoter hypermethylation at CpG islands is observed in higher-grade IPMNs, such as the mismatch repair genes MutL homolog 1 (*hMLH1*) and O6-alkylguanine DNA alkyltransferase.[16] Hypermethylation of SRY-box transcription factor 17 (*SOX17*) can be used to identify advanced IPMNs, with an accuracy of 84%.[18]

RNA markers

Over 80% of IPMNs show high expression of microRNAs (miRNAs) miR-155 and miR-21, especially IPMNs with PDACs. The over-expression of miR-21 in PDACs is associated with reduced overall survival and disease-free survival.[12] Moreover, a set of miRNAs—including miR-24, miR-30a-3p, miR-18a, miR-92a, miR-342-3p, miR-99b, miR-106b, miR-142-3p, and miR-532-3p—can be used to differentiate between high-grade dysplasia and low-grade dysplasia in IPMNs. The sensitivity and specificity of this miRNA panel were 89% and 100%, respectively.[19]

Protein markers

S100 calcium binding proteins such as S100A6 and S100A11 have higher expression in IPMN cells than in normal or pancreatitis-associated epithelial cells. S100A4 and S100P are more commonly expressed in IPMN-derived carcinomas.[16] Mucin proteins such as MUC1, MUC2, and MUC6 are specific for invasive IPMNs and can be used to identify the subtypes of IPMNs. MUC1 can be used for identifying the pancreatobiliary subtype, with 60% of tubular adenocarcinomas expressing MUC1 and only 1% expressing MUC2. In contrast, MUC2 can be used for identifying the intestinal subtype.[20] MUC6 shows high expression in the pancreatobiliary and oncocytic subtypes. MUC5AC is produced by all types of IPMNs. MUC4 is expressed more frequently in advanced IPMNs and is not detected in normal pancreatic tissues.[21] Monoclonal antibody Das-1 (mAb Das-1) can be detected in the cystic fluid and in histological specimens, with high specificity (100% *vs*. 95%) and sensitivity (89% *vs*. 85%).[22] Plectin-1 has a sensitivity of 84% and specificity of 83% for the identification of advanced IPMNs. Interleukin-1β can be utilized to identify advanced IPMNs, with sensitivity, specificity, and accuracy of 79%, 95%, and 92%, respectively.[12] Human epidermal growth factor receptor (HER-2) protein expression was detected in 29% of intraductal papillary mucinous carcinomas.[23]

MCNs

MCNs are often found in middle-aged women (average age, 48–55 years). Typically, MCNs do not communicate with the pancreatic ducts, and are frequently located in the pancreatic body and tail. The presence of ovarian-type stroma in MCNs is a unique identifier for diagnosis, and the MCNs contain dense cells and estrogen and progesterone hormone receptors.[5,24] CT and MRI revealed that MCNs have an oval, macrocapsule-like appearance, are unilocular or multilocular, and have fewer than six cysts, each measuring >2 cm in size. The presence of thick and

enhancing septa, mural nodules or solid components, peripheral eggshell-like calcification, and irregularly thick cystic walls are possible markers of invasive carcinomas.[4,25] The septa are typically more peripherally located in the tumor. Peripheral calcification is observed in up to 15% of patients.[8] Septa and mural nodules can be best visualized with EUS and contrast-enhanced EUS (CE-EUS), and EUS has an accuracy of 84% to 96% for identifying MCNs.[26] CT is better than MRI for detecting peripheral/septal calcification, and MDCT has a specificity of 83% for differentiating between malignant and benign MCNs.[4] In contrast, MRI is especially useful for identifying small MCNs with other cystic lesions, and MRCP is useful for observing the smallest communication between the cyst and the ductal system.[27]

In MCNs, *KRAS* mutations appear early and increase along with the dysplasia grade; *KRAS* mutations are found in 3% to 26% of low-grade MCNs and in 50% to 100% of high-grade MCNs. Inactivation of *TP53* is observed in 25% to 56% of high-grade MCNs.[28] The inactivation of *SMAD4* occurs late during the neoplastic progression of MCNs.[29]*RNF43* alterations occur in 12% of low-grade MCNs and in 25% of high-grade MCNs.[30,31]*CDKN2A* genes are only found in 14% of low-grade MCNs, but are observed in 50% to 59% of high-grade MCNs.[28,32] However, *GNAS* mutations are not detected in MCNs. In addition, malignant MCNs contain *PIK3CA* and phosphatase and tensin homolog (*PTEN*) gene mutations and show disruptions in the hedgehog and Wnt signaling pathways.[24]

SCNs

SCNs are frequent in elderly women (average age, 61–65 years), and they can be located in any part of the pancreas. The cysts are histologically lined with thin cuboidal and flat epithelial cells with clear cytoplasm, which are PAS-positive owing to the high levels of glycogen.[4,25] SCNs are usually divided into SCAs, serous cystadenocarcinomas, and solid serous adenomas. SCAs, including micro-cystic, oligo-cystic, macro-cystic, and VHL-related types, are more common than the other types.[33] The cystic fluid of oligo-cystic SCAs is thin and clear, whereas the fluid of micro-cystic SCAs is bloody. Oligo-cystic or macro-cystic SCAs account for 10% to 15% of all SCAs, and they are more often found in a younger population; however, it is difficult to distinguish between SCAs and MCNs or IPMNs.[5] Micro-cystic SCAs are typically a well-circumscribed, lobulated, micro-cystic mass, with a honeycomb appearance; they are composed of many, small, separated cysts (0.2–2 cm, >6 cysts), with fibrous septa originating from a central calcified scar. The central calcified scar is highly specific and best observed on CT.[4,34] The absence of wall enhancement and a wall thickness of <2 mm can be helpful for distinguishing between macro-cystic SCAs and other macro-cystic tumors of the pancreas.[35] MDCT can be used to differentiate SCNs from IPMNs and MCNs, with a specificity of 90% and 100%, respectively. In contrast, EUS has an accuracy of 76% for identifying SCNs.[4] In addition, researchers of the CONTACT study who used needle-based confocal laser endomicroscopy (nCLE) observed a dense capillary network on the surface of SCAs; nCLE had an accuracy of 87%, sensitivity of 69%, and specificity of 100% for the diagnosis of SCAs.[36]

SCAs can be identified on the basis of a biochemical profile of carcinoembryonic antigen (CEA) levels <5 ng/mL (sensitivity of 50% and specificity of 95% for SCAs/pseudocyst) and amylase levels <250 U/L (sensitivity of 44% and specificity of 98% for SCA, mucinous cystadenoma, and mucinous cystadenocarcinoma).[37] Most SCAs (89%–100%) have tumor suppressor gene *VHL* mutations, including loss of heterozygosity and chromosome 3p aneuploidy.[38] When the cut-off is >5000 pg/mL, elevated levels of vascular endothelial growth factor A (VEGF-A) have a sensitivity of 100% and specificity of 83.7% for differentiating between SCNs and other cystic lesions.[39] Furthermore, expression of α-inhibin on immunohistochemistry has a sensitivity of 80% for identifying SCAs.[37]

SPNs

SPNs are rare and are often observed in relatively young women (average age <35 years), usually in the pancreatic body and tail. Resected SPNs show a large single tumor (5–10 cm) with cystic and solid components.[4,9] SPNs have bloody cystic fluid; cytology revealed monomorphic cells with round nuclei and eosinophilic, foamy cytoplasm and fibrovascular stroma, and the cells are usually positive for vimentin and α-1-antitrypsin.[40] In addition, SPNs may cause local invasion, metastasis, or recurrence in 8% to 20% of cases.[41]

MDCT revealed that SPNs present as oval or lobulated macro-cysts, which are usually well circumscribed and have mixed solid, cystic, and hemorrhagic components, with peripheral calcification.[25] Irregular peripheral calcification can be observed in more than 65% of patients.[42] However, calcification is less common in smaller lesions.

SPNs are characterized by *CTNNB1* gene mutations, but lack mutations in *KRAS*, *GNAS*, *RNF43*, and *VHL*.[12,25] Immunohistochemistry of SPNs revealed strongly positive nuclear staining for β-catenin, with a sensitivity of 100% and a specificity of 92.3%.[43] Among 20 cases, all the cases were positive for vimentin and CD10. A total of 95% of cases were positive for progesterone receptor.[44] Other markers included CD138, Ki-67, SRY-box transcription factor 11 (*SOX11*), transcription factor binding to IGHM enhancer 3 (*TFE3*), and transcription factor 3 (*Tcf-3*). Furthermore, patients with a Ki-67 index of ≥4% were significantly associated with poorer recurrence-free survival and disease-specific survival.[45]

Conclusions

MRI is the preferred imaging for non-invasive diagnosis, especially IPMNs. MRI and MRCP are as effective as EUS for detecting mural nodules, internal septa, and communication with pancreatic ducts. CT can help in better observation of calcification in MCNs, SCAs, and SPNs. Some novel techniques, such as contrast-enhanced har-

monic EUS and nCLE, have been developed and may improve the diagnosis of PCNs. Although imaging can be used to identify PCNs with certain accuracy, imaging alone is not sufficient for differentiating between the subtypes of PCNs.

The serum CEA level has a sensitivity of 18% for identifying malignant IPMNs, but the level is too low in serum to be used for diagnosis.[16] The CEA level in cystic fluid is more advantageous than the level in serum, which can be used to distinguish between mucinous and non-mucinous PCNs, and it is not correlated with the level of dysplasia or malignancy. A CEA level of ≥192 ng/mL in the cystic fluid has a sensitivity of 52% to 78% and specificity of 63% to 91% for identifying mucinous PCNs.[25] However, high levels of CEA cannot be used to further differentiate between IPMNs and MCNs. A CEA level of <5 ng/mL in the cystic fluid has a sensitivity of 100% and a specificity of 86% for non-mucinous PCNs, such as SCAs and pseudocysts. Cytopathologic evaluation is the most useful method for differentiating between benign and malignant lesions. Although the specificity of cytopathologic evaluation for identifying malignant tumors is 100%, its accuracy is often hampered by the low cellularity of PCN cells in the cystic fluid.[46]

Molecular gene markers can be used to differentiate between the subtypes of PCNs, increase diagnostic accuracy, and identify advanced PCNs. *GNAS* and *PIK3CA* mutations are specific for IPMNs. Pancreatobiliary IPMNs may be diagnosed by identifying the incidence of mutations in the *KRAS* gene (100%) and *SHH* gene (92.8%) and the expression of the MUC1 protein (60% possibility). Intestinal IPMNs may be diagnosed by identifying the incidence of mutations in the *GNAS* gene (100%) and the expression of the MUC2 protein (100% expression in colloid carcinomas). *SMAD4* mutations are unique to MCNs. *VHL* gene mutations and elevated VEGF-A levels are specific for SCAs. SPNs are characterized by activating mutations in *CTNNB1*.

In the future, many studies are still needed to determine and validate these biomarkers in order to incorporate these biomarkers during the clinical diagnosis and treatment of PCNs. Multicenter, prospective, systematic studies should also include patients with PCNs to determine more effective criteria that comprise a combination of several molecular markers or clinical and molecular markers. We believe that the treatment methods for PCNs will be further improved with the continuous development in imaging, endoscopy, and molecular testing and the increasing safety of surgical techniques.[47]

Conflicts of interest

None.

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