Biomarkers of Pancreatic Cancer

Shin Hamada  Tooru Shimosegawa
Division of Gastroenterology, Tohoku University Graduate School of Medicine, Sendai, Japan

Introduction
Pancreas cancer is one of the main causes of cancer-related death, since most of the patients present at advanced clinical stages, and even in operable cases the prognosis is unfavorable [1]. Lack of an adequate screening method for pancreatic cancer hampers early detection of curable disease. Specific markers in the blood samples of pancreatic cancer patients have been identified by antibody-based detection of cell surface antigen, such as CA19-9 [2]. Those classical tumor markers have contributed to the management of pancreatic cancer treatment as clinical indicators of disease progression during chemotherapy or recurrence after surgery. Despite their clinical usefulness, classical tumor markers are not effective for the early detection of small pancreatic cancers, since elevated classical tumor marker levels indicate the presence of a significant number of cancer cells.

Previously, suspicious pancreatic cancer cases were mainly diagnosed based on imaging studies due to difficulties in obtaining cancer tissue. Recent development of the endoscopic pancreatic juice cytology, endoscopic brushing cytology or biopsy from the stenotic duct is widely performed for the histological evidence of pancreatic cancer, but still suffers from low sensitivity. In some cases, novel markers are tested for the diagnosis of cystic neoplasms. In addition, advances in endoscopic ultrasonography-guided fine needle aspiration biopsy enabled sampling of the cancer tissue before surgery or treatment, which delineates the individualized therapeutic strategy against pancreatic cancer, via the assessment of prognosis- or therapy resistance-related factors. Furthermore, novel transcriptomic or metabolomic biomarkers in the clinical samples collected by non-invasive methods, e.g. blood or saliva samples, are now applied for the diagnosis of pancreatic cancer. These methods will be beneficial for the screening and early detection of pancreatic cancer.

Key Words
CA19-9 · CEA · DUPAN-2 · S100P · hENT1 · MicroRNA

Abstract
Pancreatic cancer has a high mortality rate since early diagnosis is difficult and radical operation is challenging. Classical tumor markers are reliable parameters to determine disease progression during chemotherapy or recurrence after surgery, but they are not adequate to identify suspected disease or for screening. Endoscopic brushing cytology or biopsy from the stenotic duct is widely performed for the histological evidence of pancreatic cancer, but still suffers from low sensitivity. Recently, several molecules were found to be specifically expressed in pancreatic cancer, and these novel molecular markers are reported to improve the sensitivity of cytology or biopsy. In some cases, novel markers are tested for the diagnosis of cystic neoplasms. In addition, advances in endoscopic ultrasonography-guided fine needle aspiration biopsy enabled sampling of the cancer tissue before surgery or treatment, which delineates the individualized therapeutic strategy against pancreatic cancer, via the assessment of prognosis- or therapy resistance-related factors. Furthermore, novel transcriptomic or metabolomic biomarkers in the clinical samples collected by non-invasive methods, e.g. blood or saliva samples, are now applied for the diagnosis of pancreatic cancer. These methods will be beneficial for the screening and early detection of pancreatic cancer.
cules specifically expressed in pancreatic cancer, such as the calcium-binding protein S100P [4], and some of them indeed improved the diagnostic sensitivity combined with cytology or biopsy. Also, in cystic lesions of the pancreas, novel markers are assessed to determine the nature of pancreatic cysts.

The advent of endoscopic ultrasonography-guided fine needle aspiration biopsy (EUS-FNA) dramatically changed the diagnosis of pancreatic cancer, with EUS-FNA providing the ideal sensitivity and feasibility for cancer diagnosis [5]. This method enabled not only accurate diagnosis, but also the collection of cancer tissue before surgery or chemotherapy even in inoperable cases. Recently, expression levels of multiple molecules were found to correlate with a poor prognosis or resistance to chemotherapy, such as ATP-binding cassette (ABC) transporters [6]. Recent studies have tried to examine the expression status of multiple molecules within the FNA specimen [7], and further evaluation will lead to the establishment of individualized therapeutic strategies based on the prediction of prognosis or response to chemotherapy.

For early detection and effective screening of pancreatic cancer, non-invasive methods to obtain clinical samples have gained renewed interest in order to target novel biomarkers, such as transcriptomic or metabolomic biomarkers [8, 9]. Their clinical accuracy and effectiveness should be examined carefully, but these non-invasive approaches seem promising to establish a screening system identifying patients who are possibly at high risk for pancreatic cancer or have asymptomatic curable disease.

Biomarkers of pancreatic cancer and their application in the clinical setting are summarized in figure 1. In the following section, potential utility of biomarkers in pancreatic cancer treatment and in future applications as novel diagnostic or therapeutic tools are discussed.

**Utility of Classical Tumor Markers in the Clinical Setting**

In pancreatic cancer treatment, several tumor markers are employed, such as CA19-9 or carcinoembryonic antigen (CEA). CA19-9 is a carbohydrate antigen which belongs to sialyl-Lewis (a) [10]. CEA is a glycoprophosphatidylinositol-anchored protein [11], which is widely used as a tumor marker of gastrointestinal tract cancers. These markers indicate false-positive results in a small subset of benign diseases by chance, but continuous elevation in these markers strongly suggests progressive disease during chemotherapy or recurrence after operation. Of note, patients with a Lewis-negative (a–/b–) status (approximately 10% of the population) are not able to synthesize CA19-9 from the precursor carbohydrate antigen DUPAN-2. In such cases, DUPAN-2 is a better parameter in monitoring than CA19-9 [12].

Prediction of the patient’s prognosis using these tumor markers has been studied, and several reports have sug-
gested a positive correlation between these tumor markers and clinical outcome. In patients with locally advanced or metastatic unresectable pancreatic cancer, elevated serum CA19-9 and CEA levels correlate with poor prognosis by multivariate analysis [13]. Another study has indicated the usefulness of tumor markers to determine the efficacy of a chemotherapeutic regimen, which may affect the choice of chemotherapeutic agents [14]. In this study, serum CA19-9 levels were routinely measured and the results were used as one of the parameters assessing the efficacy of chemotherapy.

Tumor markers not only assist in the treatment of advanced pancreatic cancer, they also play a beneficial role during the follow-up of intraductal papillary mucinous neoplasms (IPMN) of the pancreas. Usually, the branch-type IPMN without invasive growth has a favorable prognosis, but the detection of invasive cancer cells within cystic lesions is difficult. Once the IPMN-derived invasive cancer has developed, the prognosis turns unfavorable. Furthermore, IPMN patients are predisposed to the occurrence of concomitant ductal adenocarcinoma [15]. To identify the clinical features of invasive carcinoma arising from the branch duct in IPMN patients, we assessed 159 patients with branch-duct IPMN [16]. In summary, 12 patients had invasive IPMN and 7 patients had concomitant ductal adenocarcinoma. The analyses of these patients' clinical characteristics elucidated the contribution of tumor markers for the prediction of invasive IPMN or concomitant ductal adenocarcinoma. Interestingly, serum CEA levels were independently associated with invasive IPMN. Similarly, elevated CA19-9 levels were associated with concomitant ductal adenocarcinoma.

These results indicate the indispensable role of the classical tumor markers as useful clinical parameters to determine the efficacy of therapeutic methods and in decision making during long-term follow-up of high-risk patients, such as patients with branch-type IPMN.

**Novel Biomarkers of Pancreatic Cancer and Cystic Neoplasms**

Endoscopic retrograde cholangiopancreatography (ERCP) has been improved regarding mechanical and technical aspects. For the diagnosis of pancreatic cancer, endoscopic sampling of pancreatic juice, brushing cytology from the stenotic pancreatic or bile duct and direct forceps biopsy is widely performed during ERCP. Although these interventions are sometimes complicated by post-ERCP pancreatitis, these diagnostic methods provided histological evidence of pancreatic cancer. However, these strategies suffered from a low sensitivity, and novel markers which complement these strategies were explored.

Recently, the calcium-binding protein S100P was reported to be expressed exclusively in pancreatic cancer cells [4]. The expression of S100P was also confirmed in PanIN-2 or -3 lesions, indicating the possibility of earlier detection in clinical applications. Expression levels of S100P mRNA were higher in pancreatic juice obtained from pancreatic cancer and IPMN patients than from pancreatitis cases by quantitative real-time reverse transcription PCR [17]. In this report, microdissected epithelium from pancreatic cancer, IPMN and PanIN lesions had higher expression levels of S100P mRNA than normal pancreatic duct epithelium, which might help to differentiate benign pancreatic duct stenosis from the malignant one. The clinical efficacy of S100P mRNA measurement in brushing cytology samples from patients with pancreatic duct stenosis is currently assessed in our department.

In addition, several approaches to improve the sensitivity of brushing cytology have also been made using different markers. Immunocytochemical assessment of the p53 protein in brushing cytology samples displayed higher sensitivity than conventional Papanicolau staining [18]. Another group reported elevated expression of hTERT (human telomerase reverse transcriptase) in pancreatic juice samples from patients with ductal adenocarcinoma or malignant IPMN, even in cytology-negative cases [19]. These molecular markers themselves have the possibility of false-positive results, and a combined diagnostic strategy including conventional cytology or biopsy will be beneficial for an accurate diagnosis.

However, cystic lesions of the pancreas are now frequently found at an asymptomatic state due to the improvement in imaging techniques. Accurate diagnosis of these lesions is not easy due to the lack of adequate markers of lesions necessitating treatment. A recent report suggested the usefulness of the assessment of glycan variants in the differentiation of cystic neoplasms (mucinous cystic neoplasms and IPMN) from benign cystic lesions (serous cystadenomas and pseudocysts) [20]. Although the risk of tumor dissemination following cyst fluid sampling from cystic lesions of the pancreas remains to be determined, improvements in the sampling method will help to improve the diagnosis of cystic lesions in the pancreas.
Biomarkers in EUS-FNA Specimens

In this decade, the arrival of EUS-FNA changed the diagnostic strategy of pancreatic cancer. The advantage of EUS-FNA is the sampling of sufficient cancer tissue even in inoperable cases. Until now, numerous reports have suggested the predictive value of multiple molecules regarding prognosis or sensitivity to chemotherapeutic agents, but most studies had a retrospective design. Analysis of these molecules in EUS-FNA samples will enable to prospectively study pancreatic cancer with respect to prognosis and therapeutic efficacy. In 53 EUS-FNA specimens, Salek et al. [7] assessed genetic alterations of well-known genes which are frequently mutated in pancreatic cancer, such as K-ras, p53, p16 and Smad4. Unfortunately, this study failed to find any positive predictive factor for pancreatic cancer patients, but confirmed the possibility of genetic studies using EUS-FNA specimens.

Immunohistochemical assessment of pancreatic cancer tissue provided several candidate molecules for the prediction of prognosis in pancreatic cancer patients. For example, higher expression of CDCP1 (CUB domain-containing protein 1), which is reported to facilitate anchorage-independent growth and migration of cancer cells, is correlated with the overall survival of pancreatic cancer patients [21]. Similarly, L1-CAM (L1-cell adhesion molecule) expression is reported to correlate with perineural invasion of pancreatic cancer cells and poor survival [22]. On the other hand, higher expression of B7-H3, a co-stimulatory molecule for immune responses, correlates with a better postoperative prognosis [23]. By assessing the expression status of these molecules in EUS-FNA specimens in a prospective manner, accurate classification of pancreatic cancer patients will be achieved.

The fact that most of the pancreatic cancer patients have inoperable disease due to distant metastases or locally advanced tumor emphasizes the importance of disease-controlling therapy, such as systemic chemotherapy or chemoradiation. In patients with unresectable pancreatic cancer, gemcitabine is widely used as a first-line chemotherapeutic agent. Factors determining gemcitabine sensitivity have been studied extensively, and a correlation between hENT1 (human equilibrative nucleoside transporter 1) and gemcitabine sensitivity was established [24]. Pancreatic cancer cells highly express hENT1, which contributes to gemcitabine uptake in cancer cells. Another group reported that in addition to hENT1 higher expression of dCK (deoxycytidine kinase), which metabolizes gemcitabine to its active form, correlates with gemcitabine efficacy [25]. Determination of these markers in EUS-FNA specimens will help to predict response to chemotherapy, which may affect the choice of regimens.

Novel Biomarkers for the Non-Invasive Diagnosis of Pancreatic Cancer

The above-mentioned diagnostic methods include invasive procedures, e.g. tissue sampling by endoscopy, which may cause complications. In addition, the demand for less invasive diagnostic methods is increasing in these decades. These situations encouraged the development of non-invasive biomarkers in pancreatic cancer. Saliva, for example, can be easily obtained in most patients, without using a special technique. Recent reports suggested the possible utility of saliva for the differentiation between pancreatic cancer patients and patients with normal or chronic pancreatitis. Zhang et al. [8] could differentiate pancreatic cancer patients from those without cancer using transcriptome profiles in saliva supernatants with a sensitivity of 90.0% and a specificity of 95.0%. In this report, 4 mRNA biomarkers were combined to discriminate pancreatic cancer cases; these biomarkers were selected from 12 mRNA biomarkers discovered in saliva samples from pancreatic cancer patients. A similar approach has been made by another group, which assessed metabolites in saliva by comprehensive analysis. Using mass spectrometry, pancreatic cancer cases were successfully detected based on the pancreatic cancer-specific signature [9]. These methods will affect pancreatic cancer diagnosis in the future.

In addition, small non-coding RNA is now attracting increased attention as a regulator of various biological processes, including cancer progression. Among them, microRNAs play a central role in the regulation of cellular functions, such as migration, invasion and stem cell functions. Altered microRNA expression has been reported for numerous cancers, including pancreatic cancer [26]. Interestingly, recent research has suggested that these microRNAs can be detected in peripheral blood samples, and therefore assist in cancer detection [27]. The expression levels of microRNAs are affected by the cellular function of cancer cells, and several microRNAs are highly expressed in pancreatic cancer cells. Among those microRNAs, miR-200a and miR-200b are highly expressed in pancreatic cancer cell lines, and their expression levels were significantly elevated in the sera from pancreatic cancer patients [28]. This suggests that microRNA itself could be a biomarker for pancreatic cancer.
Although these new diagnostic methods should be prospectively examined regarding reliability and concordance with existing modalities, treatment outcome and screening may be improved using biomarkers obtained using less invasive diagnostic procedures.

**Closing Remarks**

Asymptomatic pancreatic cancer is hard to detect, but possibly curable. Recent research identified novel biomarkers of pancreatic cancer, but screening for early pancreatic cancer is still challenging. Future work should be addressed to the development of diagnostic techniques with a higher sensitivity to detect even asymptomatic cases.

**Disclosure Statement**

There are no conflicts of interest.

**References**


