Int J Clin Exp Pathol (2009) 2, 1-10
www.ijcep.com/IJCEP803009

Review Article

Diagnostic and Prognostic Biomarkers in Pancreatic Carcinoma

John J. Liang[1], Eric T. Kimchi[2], Kevin F. Staveley-O'Carroll[2] and Dongfeng Tan[3]

Departments of Pathology[1] and Surgery[2], Pennsylvania State University Hershey Medical Center, Hershey, PA, USA and [3]Department of Pathology, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA

Received 12 March 2008; Accepted and available online 12 April 2008

Abstract: Pancreatic ductal carcinoma, one of the leading causes of cancer mortality, is typically diagnosed at an advanced stage, which significantly contributes to its high mortality rates. Studies have demonstrated that resection of small pancreatic tumors and tumors at lower stages correlates with improved survival. Detection of pancreatic carcinoma at an early, surgically resectable stage is the key to decreasing mortality and improving survival. Identification of sensitive diagnostic biomarkers as screening tools is crucial in detecting preinvasive pancreatic neoplasms. Numerous new DNA-, RNA- and protein-based biomarkers have been extensively investigated. This review aims to provide an update on these molecular markers, including biomarkers from blood, tissue as well as pancreatic juice and cystic fluid. These biomarkers hold potential utility in early diagnosis and prognostification of pancreatic ductal carcinoma, though many of which need to be validated in large-scale prospective studies before they can be used in clinical settings.
Key Words: Pancreatic ductal carcinoma, molecular biomarkers

Introduction

Invasive ductal adenocarcinoma of the pancreas accounts for only about 2% of all cancers in the United States. However, it is the fourth leading cause of cancer deaths. The high mortality is largely attributed to the lack of early detection and screening methods, advanced stage at diagnosis, and ineffective medical treatment. Complete surgical resection is the only curative option for this disease. Unfortunately, most patients present with advanced disease and are not amenable for curative surgery. Only about 15% of patients are able to undergo curative resection at the time of diagnosis [1]. Therefore, early diagnosis of pancreatic cancer is critical for improvement of survival. While early diagnosis may increase survival, this is often difficult due to non-specific symptoms or a complete lack of symptoms at presentation. Imaging analysis, such as conventional computed tomography scanning and endoscopic ultrasound (EUS), is only useful to define the location, the extent of a mass, and determine resectability. However, imaging alone is of very limited use in determining malignant potential or the type of neoplasm [2-4]. Image-guided or EUS-guided fine needle aspiration (FNA) or needle core biopsies have played a very important role in the diagnosis and staging of pancreatic cancers, particularly in unresectable tumors [4, 5]. Interpreting FNA specimens from the pancreas can be very difficult as samples are often scant and bloody [6], and are non-diagnostic in 11% to 30% of specimens evaluated [7-10]. Molecular markers may hold the key and provide an additional means to enable early detection of cancer, to distinguish benign reactive cells from malignant cells, and to differentiate tumor types in these very limited samples.

Fortunately, a large number of potential proteins and DNA-based markers have been identified in pancreatic cancers using gene expression arrays and proteomics. Their clinical utilities as screening, diagnostic and prognostic tools are under active investigation. Though the diagnostic accuracy of any single marker is inadequate, the combination of several biomarkers may provide the necessary

sensitivity and specificity to accurately diagnose pancreatic cancer. Our goal for this review is to provide an update on potentially useful diagnostic and prognostic serum, tissue and pancreatic fluid biomarkers in pancreatic adenocarcinoma.

Serum Biomarkers

Blood biomarker studies are attractive because a large number of biomarkers are present in the serum. In addition, blood samples are safe to collect and readily available, providing a very practical modality to screen large populations.

CA19-9

The CA19-9 antigen is an oligosaccharide (sialyl-Lea) present within the MUC1 mucin-type glycoprotein [11-13]. The sensitivity of immunoassays for CA19-9 was reported in the range of 70% to 95%, and the specificity in the range of 72% to 90% [14, 15]. CA19-9 is the most commonly used serum marker to monitor treatment response, recurrence and prognosis of pancreatic cancer [16, 17 18]. In a study reported by Ferrone *et al* [17], the authors found that the perioperative CA19-9 levels can predict stage and survival in patients with resectable pancreatic adenocarcinoma. Both a postoperative decrease in CA19-9 and a postoperative CA19-9 value of less than 200 U/mL are strong independent predictors of survival. In addition, Kilic *et al* concluded that CA19-9 levels could be useful in determining the resectability of the tumor [18]. Based on their findings, Boeck *et al* postulated that a more than 20% reduction in CA19-9 levels 8 weeks after chemotherapy predicts an improvement in survival [19-21]. However, CA19-9 levels are also elevated in a number of benign and other malignant conditions, and it is not recommended for diagnostic purpose by the American Society of Clinical Oncology (ASCO) [22].

MUC1

The MUC1 antigen is expressed in both benign and malignant tissues from pancreatic and nonpancreatic organs. However, it is usually overexpressed in cancerous tissues and displays an aberrant oligosaccharide profile giving rise to the expression of neomarkers such as sialyl-Lea (CA19-9), sialyl-Lex, and sialyl-Tn (TAG-72) [23-25]. By using an immunoassay with monoclonal antibody PAM4 as the capture reagent, and a polyclonal anti-MUC1 antibody as the probe, Gold *et al* found that MUC-1 could distinguish not only cancer from normal, but also cancer from chronic pancreatitis [26]. The sensitivity and specificity of the immunoassay for pancreatic cancer were 77% and 95%, respectively. The PAM4-based immunoassay of circulating MUC1 was superior to CA19-9 for both sensitivity and specificity in the discrimination of pancreatic cancer and pancreatitis ($P<0.003$). These results are very promising and encouraging.

MUC4

MUC4 is a transmembrane apomucin, which was reported to be specifically expressed in pancreatic adenocarcinoma and other cancer cell lines, but not in chronic pancreatitis and normal pancreas. In one study, MUC4 mRNA was expressed in 75% pancreatic adenocarcinoma tissue samples (12 of 16) and pancreatic adenocarcinoma tumor cell lines (11 of 15), in 0% chronic pancreatitis (0 of 10) and normal pancreas tissues (0 of 7) [23]. Saitou *et al* found that MUC4 was expressed in 32% (43/135) patients with invasive pancreatic ductal adenocarcinoma and high MUC4 expression was significantly correlated with poor survival. These studies suggest that MUC4 may provide a valuable diagnostic and prognostic marker for pancreatic adenocarcinoma [27]. Singh and colleagues reported that decreased expression of MUC4 via plasmid transfection of anti-MUC4 into a pancreatic cell line resulted in reduced clonogenicity, cell proliferation, and tumor volume [28]. These studies indicate that MUC4 expression may play an important role in the development and progression of pancreatic adenocarcinoma. Its utility as a diagnostic marker needed to be validated in large scale studies.

CEACAM1

CEACAM1 (previously biliary glycoprotein 1) is a member of the human carcinoembryonic antigen (CEA) family, which is expressed in a number of epithelia, including bile ducts as well as in monocytes, granulocytes, activated T cells, B cells, and a subset of natural killer cells [29]. The expression of CEACAM1 has been shown to be lower in cancers of the breast, colon, and prostate than in their corresponding normal tissues [30]. However,

CEACAM1 gene expression was reported to be elevated in pancreatic adenocarcinoma compared with normal pancreas or chronic pancreatitis [31]. By measuring CEACAM1 messenger RNA levels using quantitative RT-PCR and protein using a double determinant enzyme-linked immunosorbent assay and immunohistochemistry, Simeone *et al* found that CEACAM1 not only had higher expression levels in pancreatic adenocarcinomas compared with noncancerous pancreas but was also superior to CA19-9 in distinguishing cancer from normal [30]. CEACAM1 itself is no better than CA19-9 in distinguishing cancer from chronic pancreatitis. However, the combination of CEACAM1 and CA19-9 was superior to either one alone.

MIC-1

Macrophage inhibitory cytokine 1 (MIC-1) is a member of the transforming growth factor-beta superfamily [32]. Oligonucleotide microarray and serial analysis of gene expression data have demonstrated that MIC-1 RNA levels were higher in primary pancreatic adenocarcinomas, intraductal papillary mucinous neoplasms, and pancreatic cancer cell lines than in non-neoplastic pancreatic ductal epithelium [33]. MIC-1 expression was localized to the malignant epithelium in pancreatic adenocarcinomas by in situ hybridization [33]. Overexpression of MIC-1 has been reported in carcinoma of pancreas, colon, prostate, breast, and stomach [34-36]. MIC-1 may contribute to the malignant progression by inducing tumor cell invasion through up-regulation of the urokinase-type plasmaminogen activator (uPA) activation system via extracellular signal-regulated kinase-1/2-dependent pathway [36]. A recent study found that serum MIC-1 is a more sensitive marker of pancreatic cancer than CA19-9 in differentiating patients with pancreatic cancer from healthy controls ($p = 0.003$), but not in distinguishing pancreatic cancer from chronic pancreatitis ($p=0.63$) [37]. These results suggest that serum MIC-1 could be particularly helpful in the early detection of pancreatic cancer in high-risk populations as part of their pancreatic screening protocols [38, 39].

Studies by Tessler *et al* suggested that a CA19-9 level higher than 37 U/mL in combination with weight loss of more than 20 lb (9 kg) and a bilirubin level higher than 3 mg/dL may diagnose 45.4% patients with pancreatic adenocarcinoma [20]. The sensitivity increased to 83.3% when combined with imaging studies [20]. Surprisingly, the combination of all five markers, MIC-1, CA19-9, tissue inhibitor of metalloproteinase 1 (TIMP-1), osteopontin, and hepatocarcinoma-intestine-pancreas / pancreatitis-associated protein, was no more accurate than MIC-1 or CA19-9 alone in a study reported by Koopmann *et al* [37]. Therefore, more studies are needed to identify a better panel of biomarkers.

By using surface-enhanced laser desorption /ionization time-of-flight mass spectrometry (SELDI-TOF-MS), Ehmann *et al* identified three specific protein peaks through comparative serum protein expression profiling of pancreatic cancer with a sensitivity of 100% and a specificity of 98% [52]. Unfortunately, the same results were not achieved in their validation set. In addition, samples from patients with chronic pancreatitis were not included in the initial screening.

Circulating Tumor Cells and DNA/RNA Markers

Circulating tumor cells (CTCs) are known to exist in the peripheral blood of cancer patients. They can be present as low as one per ten million normal blood cells. No good method with sufficient sensitivity is currently available to reliably measure a statistically significant number of CTCs at the early stage of disease. However, the discovery of CTCs has led to increased interest in the analysis of DNA and RNA in peripheral blood samples. By measuring the levels of α-1,4-acetyl-glucosaminyltransferase (alpha4GnT) mRNA extracted from peripheral blood mononuclear cells, pancreatic adenocarcinoma can be diagnosed with a sensitivity of 76% and a specificity of 83% [53]. Although increased levels of alpha4GnT mRNA were detected in 40% of chronic pancreatitis patients and 17.1% of cancer-free volunteers, the expression levels were significantly lower than those seen in pancreatic cancer patients [53]. Recently, the levels of cytokeratin-19 (CK-19) mRNA in blood, bone marrow and peritoneal lavage from patients with pancreatic adenocarcinoma were analyzed by using CK-19-specific nested-PCR and quantitative fluorogenic RT-PCR [54]. CK-19 mRNA expression was increased in 64% blood samples and 30% peritoneal lavage samples

from patients with pancreatic cancer. Although CK-19 is suitable for the distinction between malignant and benign pancreatic disease in combination with other tumor-specific markers, levels of CK19 mRNA expression were neither sensitive nor specific enough for clinical use as a single marker [54]. These studies indicate that development and identification of RNA or DNA biomarkers from peripheral blood are a promising area. The development of sensitive and reliable methods to efficiently isolate circulating tumor cells is also warranted.

K-ras mutations are present in approximately 90% pancreatic adenocarcinoma, and can be easily detected using molecular assays due to a one codon mutation. Mutant *K-ras* has been detected in the blood of patients with pancreatic adenocarcinoma [40, 41]. However, *K-ras* mutations are also detected in patients with chronic pancreatitis, PanIN lesions without invasive pancreatic adenocarcinoma and smokers [42-45]. This significantly limits the usefulness of *K-ras* mutations as a diagnostic marker. Sensitive assays which can accurately quantify levels of DNA mutations and differentiate malignancy from benign conditions may exploit this difference and aid in the diagnosis of pancreatic cancer. Several promising assays have been developed for this purpose. By combining DNA ligation and PCR amplification techniques, LigAmp can detect *K-RAS2* mutant DNA at 0.01% in mixtures of different cell lines and has been used to quantify *K-ras* mutations in pancreatic duct fluid from patients with pancreatic cancer [46]. Dressman *et al* reported a molecular strategy, BEAMing, that can be used to detect single base pair mutations such as *K-ras* [47]. BEAMing can detect mutant adenomatous polyposis coli (APC) DNA molecules in the plasma of patients with colorectal cancer at levels ranging from 0.01% to 1.7% of the total APC molecules [47, 48]. With these newer quantitative assays, quantifying mutant *K-ras* levels could become a useful diagnostic marker for pancreatic adenocarcinoma.

Tissue Biomarkers

Although tissue markers are less useful in terms of early detection and early diagnosis of pancreatic adenocarcinoma, they play an important role in tumor classification and assessment of treatment response. Tissue markers are extremely useful in samples obtained by FNA. The use of these markers could increase the diagnostic sensitivity and the ability to differentiate malignant from benign conditions, such as chronic pancreatitis.

Human Equilibrative Nucleoside Transporter 1

By using frozen sections of pancreatic tumors, Giovannetti *et al* [55] found that patients with high levels of human equilibrative nucleoside transporter-1 (hENT1) mRNA had a significantly longer overall survival (median 25.7 months) than patients with a low level of hENT1 expression (median 8.5 months). This suggests that hENT1 levels may provide a useful prognostic tool in predicting survival.

microRNAs

Recently, microRNAs (miRNAs or miRs) have gained recognition as a family of molecules involved in oncogenesis. miRNAs are functional 22 nt noncoding RNAs that negatively regulate gene expression. A microRNA expression signature has been identified in pancreatic adenocarcinoma [56]. Reverse transcription in situ PCR demonstrated that three of the top differentially expressed miRNAs (miR-221, -376a and -301) in pancreatic adenocarcinoma were specifically localized to tumor cells and not to stroma, normal acini or ducts [56]. Aberrant microRNA expression may offer new clues to pancreatic tumorigenesis and provide new diagnostic biomarkers for pancreatic adenocarcinoma.

Telomerase

Telomeres are non-coding repeated tandem DNA sequences at the ends of chromosomes that normally function to protect the terminal sequences from loss during replication and prevent the ends of chromosomes from joining aberrantly. The major components of telomerase are human telomerase RNA (hTR or TERC), telomerase-associated protein 1 and its catalytic subunit (hTERT). The nucleolar expression of hTERT has been shown to be associated with prognosis in patients with colon, lung, and urothelial cancers [57-62]. By using a semi-quantitative telomeric repeat amplification protocol, Mishra *et al* reported that the sensitivity and specificity of telomerase activity in detecting pancreatic

adenocarcinoma in solid masses was 79% [95% confidence interval (CI), 64-89%] and 100% (95% CI, 55-100%), respectively [63]. hTERT may provide an important diagnostic marker in pancreatic adenocarcinoma.

By using SELDI, some protein fragments in serum appear to perform as well or better than serum CA19-9 as diagnostic markers [64, 65]. In one study, protein chip arrays and SELDI-TOF-MS were used to identify appropriate tissue biomarkers [66]. In this study it was highly reliable in distinguishing between tumors and adjacent normal tissues, as well as between early and more advanced cancers by panels of protein peaks.

S100p and Other Markers

Immunohistochemical analysis has been widely used to evaluate the levels of biomarker expression in tissue samples. Very recently, Lin and colleagues [67] reported that S100P and von Hippel-Lindau gene product (pVHL) are a pair of sensitive and specific markers for identifying primary pancreatic ductal carcinoma and pancreatic intraepithelial neoplasm, and up-regulation of S100P and down-regulation of pVHL may play a role in early tumorigenesis in pancreatic ductal carcinoma. By using a semi automated image analysis system, Tsiambas *et al* reported that HER2/neu protein expression was significantly associated with grade (P=0.019), but not with stage (P=0.466) of pancreatic adeno-carcinoma [68]. The authors also found that evaluation of HER2/neu protein expression based on digital image analysis compared to conventional microscopy, improved the accuracy and reliability of immunohistochemical estimation. The mitogen-activated kinase protein 4 kinase 4 (MAP4K4, also called hepatocyte progenitor kinase-like/germinal center kinase-like kinase), is a serine/threonine kinase and belongs to the mammalian STE20/MAP4K family. Recent studies have shown that MAP4K4 is overexpressed in many types of human cancer cell lines and tumors compared to normal tissue [69]. By using immunohistochemistry, our group has found that a high level of MAP4K4 is associated with a worse prognosis, a higher frequency of recurrence, an increased number of positive lymph nodes, and a larger tumor size in patients with stage II pancreatic adeno-carcinoma. Furthermore, our data indicates that MAP4K4 is an independent prognostic marker for stage II pancreatic adenocarcinoma (Liang *et al*, Manuscript in preparation).

Markers in Pancreatic Fluid and Cyst Aspirate

Pancreatic fluid contains numerous important biomarkers for pancreatic carcinoma, including DNA, RNA and protein markers. Telomerase activity was detectable in 80% of surgically resected pancreatic carcinomas and in 75% to 95 % of pancreatic fluid samples obtained from patients with pancreatic adenocarcinoma [70-72]. The levels of hTERT expression can differentiate ductal adenocarcinoma from adenoma and pancreatitis [70-72]. By using methylation-specific PCR (MSP) assays, a panel of five genes (*Cyclin D2*, *FOXE1*, *NPTX2*, *ppENK*, and *TFPI2*) was identified from pancreatic fluid collected endoscopically or surgically from 155 individuals [73]. With a cut-off of at least 1% methylation in two or more of these genes, the panel proved to have a sensitivity of 82% and a specificity of 100% in identifying pancreatic cancer in endoscopically collected juice.

The expression levels of different proteins in pancreatic juice can be quantified using isotope-code affinity tag and MS/MS method. With this approach, nine proteins with at least a two-fold change in samples from both pancreatic cancer and pancreatitis patients were identified [74, 75]. Twenty-one proteins were specifically altered in the pancreatic-cancer specimens and 18 specifically in the pancreatitis specimens [74, 75]. The potential utility of proteomics or other high-throughput discovery technologies in identifying new markers remains to be further investigated.

The cystic fluid from cystic lesions of the pancreas is another important source for biomarker study [76]. Unfortunately, there is no sensitive test available in distinguishing benign lesions, low-grade and high-grade malignant tumors [77]. Although markers such as amylase, CA19-9, and carcinoembryonic antigen have a relative high specificity in distinguishing benign from neoplastic lesions, their sensitivity is low (< 50%) [78].

By using immunocytochemistry, 116 ascites specimens were analyzed for CDX2 expression [79]. The authors found that CDX2 is a specific and sensitive marker to detect gastrointestinal and pancreatic malignancies in ascites

cytology specimens [79].

P53 gene mutations occur in later stages of pancreatic adenocarcinoma in approximately 70% of cases [49]. By using pancreatic fluid samples and brush cytology specimens, Sturm and colleagues reported p53 gene mutations were present in 40% to 50% of patients with pancreatic cancers [50]. By using a panel of molecular markers (p53 mutations, *K-ras* mutations, and methylated p16), Yan and colleagues found that patients at risk for development of pancreatic cancer could be stratified from a negligible risk to over a greater than 50% risk of developing an early pancreatic cancer. The most commonly associated molecular marker in cases of pancreatic cancer was the presence of p53 mutations [51]. The combination of imaging analysis, cytology and current available biomarkers has great promise in providing future direction in the detection of pancreatic cancers [80].

Conclusions

Detection of pancreatic adenocarcinoma at an early surgically resectable stage is the key to decrease mortality and improve survival. Identification of sensitive and specific diagnostic markers as screening tools is crucial in detecting premalignant neoplasms and early pancreatic cancers. There are numerous new protein, DNA, and RNA-based markers that are currently being investigated. Many of them need validation in large scale studies before they can be used in clinical settings. FNA specimen, pancreatic juice, and cystic fluid are important sources for marker studies. Until more sensitive and specific biomarkers are discovered, we will continue to rely on the combination of imaging analysis, cytology and available biomarkers to diagnose pancreatic adenocarcinoma.

Please address all correspondences to Dongfeng Tan, MD, Department of Pathology and Laboratory Medicine, Unit 85, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA. Tel: 713-745-4977, Fax: 713-745-1105; Email: dtan@mdanderson.org

References

[1] Chua YJ and Cunningham D. Adjuvant treatment for resectable pancreatic cancer. *J Clin Oncol* 2005; 23:4532-4537.

[2] Chen CH, Tseng LJ, Yang CC, Yeh YH and Mo LR. The accuracy of endoscopic ultrasound, endoscopic retrograde cholangiopancreatography, computed tomography, and transabdominal ultrasound in the detection and staging of primary ampullary tumors. *Hepatogastroenterology* 2001;48:1750-1753.

[3] Tascilar M, Skinner HG, Rosty C, Sohn T, Wilentz RE, Offerhaus GJ, Adsay V, Abrams RA, Cameron JL, Kern SE, Yeo CJ, Hruban RH and Goggins M. The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma. *Clin Cancer Res* 2001;7:4115-4121.

[4] Jhala NC, Jhala DN, Chhieng DC, Eloubeidi MA and Eltoum IA. Endoscopic ultrasound-guided fine-needle aspiration. A cytopathologist's perspective. *Am J Clin Pathol* 2003;120:351-367.

[5] Volmar KE, Vollmer RT, Jowell PS, Nelson RC and Xie HB. Pancreatic FNA in 1000 cases: a comparison of imaging modalities. *Gastrointest Endosc* 2005;61:854-861.

[6] Schwartz MR. Endoscopic ultrasound-guided fine-needle aspiration. *Cancer* 2004;102:203-206.

[7] Shin HJ, Lahoti S and Sneige N. Endoscopic ultrasound-guided fine-needle aspiration in 179 cases: the M. D. Anderson Cancer Center experience. *Cancer* 2002;96:174-180.

[8] Eloubeidi MA, Jhala D, Chhieng DC, Chen VK, Eltoum I, Vickers S, Mel Wilcox C and Jhala N. Yield of endoscopic ultrasound-guided fine-needle aspiration biopsy in patients with suspected pancreatic carcinoma. *Cancer* 2003;99:285-292.

[9] Jhala NC, Jhala D, Eltoum I, Vickers SM, Wilcox CM, Chhieng DC and Eloubeidi MA. Endoscopic ultrasound-guided fine-needle aspiration biopsy: a powerful tool to obtain samples from small lesions. *Cancer* 2004;102:239-246.

[10] Klapman JB, Logrono R, Dye CE and Waxman I. Clinical impact of on-site cytopathology interpretation on endoscopic ultrasound-guided fine needle aspiration. *Am J Gastroenterol* 2003;98:1289-1294.

[11] Baeckstrom D, Hansson GC, Nilsson O, Johansson C, Gendler SJ, Lindholm L: Purification and characterization of a membrane-bound and a secreted mucin-type glycoprotein carrying the carcinoma-associated sialyl-Lea epitope on distinct core proteins. *J Biol Chem* 1991;266:21537-21547.

[12] Baeckstrom D, Nilsson O, Price MR, Lindholm L and Hansson GC. Discrimination of MUC1 mucins from other sialyl-Le(a)-carrying glycoproteins produced by colon carcinoma cells using a novel monoclonal antibody. *Cancer Res* 1993;53:755-761.

[13] Ho JJ, Siddiki B and Kim YS. Association of sialyl-Lewis(a) and sialyl-Lewis(x) with MUC-1 apomucin in a pancreatic cancer cell line. *Cancer Res* 1995;55:3659-3663.

[14] Steinberg W. The clinical utility of the CA 19-9 tumor-associated antigen. *Am J Gastroenterol*

1990;85:350-355.
[15] Ritts RE and Pitt HA. CA 19-9 in pancreatic cancer. *Surg Oncol Clin N Am* 1998;7:93-101.
[16] Ziske C, Schlie C, Gorschluter M, Glasmacher A, Mey U, Strehl J, Sauerbruch T and Schmidt-Wolf IG. Prognostic value of CA 19-9 levels in patients with inoperable adenocarcinoma of the pancreas treated with gemcitabine. *Br J Cancer* 2003;89:1413-1417.
[17] Ferrone CR, Finkelstein DM, Thayer SP, Muzikansky A, Fernandez-delCastillo C and Warshaw AL. Perioperative CA19-9 levels can predict stage and survival in patients with resectable pancreatic adenocarcinoma. *J Clin Oncol* 2006;24:2897-2902.
[18] Kilic M, Gocmen E, Tez M, Ertan T, Keskek M and Koc M. Value of preoperative serum CA 19-9 levels in predicting resectability for pancreatic cancer. *Can J Surg* 2006;49:241-244.
[19] Boeck S, Stieber P, Holdenrieder S, Wilkowski R and Heinemann V. Prognostic and therapeutic significance of carbohydrate antigen 19-9 as tumor marker in patients with pancreatic cancer. *Oncology* 2006;70:255-264.
[20] Tessler DA, Catanzaro A, Velanovich V, Havstad S and Goel S. Predictors of cancer in patients with suspected pancreatic malignancy without a tissue diagnosis. *Am J Surg* 2006;191:191-197.
[21] Boeck S, Schulz C, Stieber P, Holdenrieder S, Weckbach S and Heinemann V. Assessing prognosis in metastatic pancreatic cancer by the serum tumor marker CA 19-9: pretreatment levels or kinetics during chemotherapy? *Onkologie* 2007;30:39-42.
[22] Locker GY, Hamilton S, Harris J, Jessup JM, Kemeny N, Macdonald JS, Somerfield MR, Hayes DF and Bast RC Jr. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *J Clin Oncol* 2006;24:5313-5327.
[23] Andrianifahanana M, Moniaux N, Schmied BM, Ringel J, Friess H, Hollingsworth MA, Buchler MW, Aubert JP and Batra SK. Mucin (MUC) gene expression in human pancreatic adenocarcinoma and chronic pancreatitis: a potential role of MUC4 as a tumor marker of diagnostic significance. *Clin Cancer Res* 2001; 7:4033-4040.
[24] Burdick MD, Harris A, Reid CJ, Iwamura T and Hollingsworth MA. Oligosaccharides expressed on MUC1 produced by pancreatic and colon tumor cell lines. *J Biol Chem* 1997;272:24198-24202.
[25] Beum PV, Singh J, Burdick M, Hollingsworth MA and Cheng PW. Expression of core 2 beta-1,6-N-acetylglucosaminyltransferase in a human pancreatic cancer cell line results in altered expression of MUC1 tumor-associated epitopes. *J Biol Chem* 1999;274:24641-24648.
[26] Gold DV, Modrak DE, Ying Z, Cardillo TM, Sharkey RM and Goldenberg DM. New MUC1 serum immunoassay differentiates pancreatic cancer from pancreatitis. *J Clin Oncol* 2006;24: 252-258.
[27] Saitou M, Goto M, Horinouchi M, Tamada S, Nagata K, Hamada T, Osako M, Takao S, Batra SK, Aikou T, Imai K and Yonezawa S. MUC4 expression is a novel prognostic factor in patients with invasive ductal carcinoma of the pancreas. *J Clin Pathol* 2005;58:845-852.
[28] Singh AP, Moniaux N, Chauhan SC, Meza JL and Batra SK. Inhibition of MUC4 expression suppresses pancreatic tumor cell growth and metastasis. *Cancer Res* 2004;64:622-630.
[29] Prall F, Nollau P, Neumaier M, Haubeck HD, Drzeniek Z, Helmchen U, Loning T and Wagener C. CD66a (BGP), an adhesion molecule of the carcinoembryonic antigen family, is expressed in epithelium, endothelium, and myeloid cells in a wide range of normal human tissues. *J Histochem Cytochem* 1996;44:35-41.
[30] Simeone DM, Ji B, Banerjee M, Arumugam T, Li D, Anderson MA, Bamberger AM, Greenson J, Brand RE, Ramachandran V and Logsdon CD. CEACAM1, a novel serum biomarker for pancreatic cancer. *Pancreas* 2007;34:436-443.
[31] Logsdon CD, Simeone DM, Binkley C, Arumugam T, Greenson JK, Giordano TJ, Misek DE, Kuick R and Hanash S. Molecular profiling of pancreatic adenocarcinoma and chronic pancreatitis identifies multiple genes differentially regulated in pancreatic cancer. *Cancer Res* 2003;63:2649-2657.
[32] Bootcov MR, Bauskin AR, Valenzuela SM, Moore AG, Bansal M, He XY, Zhang HP, Donnellan M, Mahler S, Pryor K, Walsh BJ, Nicholson RC, Fairlie WD, Por SB, Robbins JM and Breit SN. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. *Proc Natl Acad Sci USA* 1997;94:11514-11519.
[33] Koopmann J, Buckhaults P, Brown DA, Zahurak ML, Sato N, Fukushima N, Sokoll LJ, Chan DW, Yeo CJ, Hruban RH, Breit SN, Kinzler KW, Vogelstein B and Goggins M. Serum macrophage inhibitory cytokine 1 as a marker of pancreatic and other periampullary cancers. *Clin Cancer Res* 2004;10:2386-2392.
[34] Buckhaults P, Rago C, St Croix B, Romans KE, Saha S, Zhang L, Vogelstein B and Kinzler KW. Secreted and cell surface genes expressed in benign and malignant colorectal tumors. *Cancer Res* 2001;61:6996-7001.
[35] Welsh JB, Sapinoso LM, Su AI, Kern SG, Wang-Rodriguez J, Moskaluk CA, Frierson HF Jr. and Hampton GM. Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. *Cancer Res* 2001;61:5974-5978.
[36] Lee DH, Yang Y, Lee SJ, Kim KY, Koo TH, Shin

SM, Song KS, Lee YH, Kim YJ, Lee JJ, Choi I and Lee JH. Macrophage inhibitory cytokine-1 induces the invasiveness of gastric cancer cells by up-regulating the urokinase-type plasminogen activator system. *Cancer Res* 2003;63:4648-4655.

[37] Koopmann J, Rosenzweig CN, Zhang Z, Canto MI, Brown DA, Hunter M, Yeo C, Chan DW, Breit SN and Goggins M. Serum markers in patients with resectable pancreatic adenocarcinoma: macrophage inhibitory cytokine 1 versus CA19-9. *Clin Cancer Res* 2006;12:442-446.

[38] Brentnall TA, Bronner MP, Byrd DR, Haggitt RC and Kimmey MB. Early diagnosis and treatment of pancreatic dysplasia in patients with a family history of pancreatic cancer. *Ann Intern Med* 1999;131:247-255.

[39] Canto MI, Goggins M, Yeo CJ, Griffin C, Axilbund JE, Brune K, Ali SZ, Jagannath S, Petersen GM, Fishman EK, Piantadosi S, Giardiello FM and Hruban RH. Screening for pancreatic neoplasia in high-risk individuals: an EUS-based approach. *Clin Gastroenterol Hepatol* 2004;2: 606-621.

[40] Mulcahy HE, Lyautey J, Lederrey C, qi Chen X, Anker P, Alstead EM, Ballinger A, Farthing MJ and Stroun M. A prospective study of K-ras mutations in the plasma of pancreatic cancer patients. *Clin Cancer Res* 1998;4:271-275.

[41] Yamada T, Nakamori S, Ohzato H, Oshima S, Aoki T, Higaki N, Sugimoto K, Akagi K, Fujiwara Y, Nishisho I, Sakon M, Gotoh M and Monden M. Detection of K-ras gene mutations in plasma DNA of patients with pancreatic adenocarcinoma: correlation with clinico-pathological features. *Clin Cancer Res* 1998; 4:1527-1532.

[42] Kalthoff H, Schmiegel W, Roeder C, Kasche D, Schmidt A, Lauer G, Thiele HG, Honold G, Pantel K and Riethmuller G et al. p53 and K-RAS alterations in pancreatic epithelial cell lesions. *Oncogene* 1993;8:289-298.

[43] Schutte M, Hruban RH, Geradts J, Maynard R, Hilgers W, Rabindran SK, Moskaluk CA, Hahn SA, Schwarte-Waldhoff I, Schmiegel W, Baylin SB, Kern SE and Herman JG. Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. *Cancer Res* 1997; 57:3126-3130.

[44] Berger DH, Chang H, Wood M, Huang L, Heath CW, Lehman T and Ruggeri BA. Mutational activation of K-ras in nonneoplastic exocrine pancreatic lesions in relation to cigarette smoking status. *Cancer* 1999;85:326-332.

[45] Tada M, Komatsu Y, Kawabe T, Sasahira N, Isayama H, Toda N, Shiratori Y and Omata M. Quantitative analysis of K-ras gene mutation in pancreatic tissue obtained by endoscopic ultrasonography-guided fine needle aspiration: clinical utility for diagnosis of pancreatic tumor. *Am J Gastroenterol* 2002;97:2263-2270.

[46] Shi C, Eshleman SH, Jones D, Fukushima N, Hua L, Parker AR, Yeo CJ, Hruban RH, Goggins MG and Eshleman JR. LigAmp for sensitive detection of single-nucleotide differences. *Nat Methods* 2004;1:141-147.

[47] Dressman D, Yan H, Traverso G, Kinzler KW and Vogelstein B. Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations. *Proc Natl Acad Sci USA* 2003;100: 8817-8822.

[48] Diehl F, Li M, Dressman D, He Y, Shen D, Szabo S, Diaz LA Jr, Goodman SN, David KA, Juhl H, Kinzler KW and Vogelstein B. Detection and quantification of mutations in the plasma of patients with colorectal tumors. *Proc Natl Acad Sci USA* 2005;102:16368-16373.

[49] Caldas C, Hahn SA, da Costa LT, Redston MS, Schutte M, Seymour AB, Weinstein CL, Hruban RH, Yeo CJ and Kern SE. Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adeno-carcinoma. *Nat Genet* 1994;8:27-32.

[50] Sturm PD, Hruban RH, Ramsoekh TB, Noorduyn LA, Tytgat GN, Gouma DJ and Offerhaus GJ. The potential diagnostic use of K-ras codon 12 and p53 alterations in brush cytology from the pancreatic head region. *J Pathol* 1998;186:247-253.

[51] Yan L, McFaul C, Howes N, Leslie J, Lancaster G, Wong T, Threadgold J, Evans J, Gilmore I, Smart H, Lombard M, Neoptolemos J and Greenhalf W. Molecular analysis to detect pancreatic ductal adenocarcinoma in high-risk groups. *Gastroenterology* 2005;128:2124-2130.

[52] Ehmann M, Felix K, Hartmann D, Schnolzer M, Nees M, Vorderwulbecke S, Bogumil R, Buchler MW and Friess H. Identification of potential markers for the detection of pancreatic cancer through comparative serum protein expression profiling. *Pancreas* 2007;34:205-214.

[53] Ishizone S, Yamauchi K, Kawa S, Suzuki T, Shimizu F, Harada O, Sugiyama A, Miyagawa S, Fukuda M and Nakayama J. Clinical utility of quantitative RT-PCR targeted to alpha1,4-N-acetylglucosaminyltransferase mRNA for detection of pancreatic cancer. *Cancer Sci* 2006;97:119-126.

[54] Hoffmann K, Kerner C, Wilfert W, Mueller M, Thiery J, Hauss J and Witzigmann H. Detection of disseminated pancreatic cells by amplification of cytokeratin-19 with quantitative RT-PCR in blood, bone marrow and peritoneal lavage of pancreatic carcinoma patients. *World J Gastroenterol* 2007;13:257-263.

[55] Giovannetti E, Del Tacca M, Mey V, Funel N, Nannizzi S, Ricci S, Orlandini C, Boggi U, Campani D, Del Chiaro M, Iannopollo M, Bevilacqua G, Mosca F and Danesi R. Transcription analysis of human equilibrative nucleoside transporter-1 predicts survival in pancreas cancer patients treated with gemcitabine. *Cancer Res* 2006;66:3928-

3935.
[56] Lee EJ, Gusev Y, Jiang J, Nuovo GJ, Lerner MR, Frankel WL, Morgan DL, Postier RG, Brackett DJ and Schmittgen TD. Expression profiling identifies microRNA signature in pancreatic cancer. *Int J Cancer* 2007;120:1046-1054.
[57] Chadeneau C, Hay K, Hirte HW, Gallinger S and Bacchetti S. Telomerase activity associated with acquisition of malignancy in human colorectal cancer. *Cancer Res* 1995;55:2533-2536.
[58] Kanaya T, Kyo S, Takakura M, Ito H, Namiki M and Inoue M. hTERT is a critical determinant of telomerase activity in renal-cell carcinoma. *Int J Cancer* 1998;78:539-543.
[59] Lantuejoul S, Soria JC, Moro-Sibilot D, Morat L, Veyrenc S, Lorimier P, Brichon PY, Sabatier L, Brambilla C and Brambilla E. Differential expression of telomerase reverse transcriptase (hTERT) in lung tumours. *Br J Cancer* 2004; 90:1222-1229.
[60] Liu YC, Chen CJ, Wu HS, Chan DC, Yu JC, Yang AH, Cheng YL, Lee SC and Harn HJ. Telomerase and c-myc expression in hepatocellular carcinomas. *Eur J Surg Oncol* 2004;30:384-390.
[61] Niiyama H, Mizumoto K, Sato N, Nagai E, Mibu R, Fukui T, Kinoshita M and Tanaka M. Quantitative analysis of hTERT mRNA expression in colorectal cancer. *Am J Gastroenterol* 2001;96:1895-1900.
[62] Yoo J, Park SY, Kang SJ, Kim BK, Shim SI and Kang CS. Expression of telomerase activity, human telomerase RNA, and telomerase reverse transcriptase in gastric adenocarcinomas. *Mod Pathol* 2003;16:700-707.
[63] Mishra G, Zhao Y, Sweeney J, Pineau BC, Case D, Ho C, Blackstock AW, Geisinger K, Howerton R, Levine E, Shen P and Ibdah J. Determination of qualitative telomerase activity as an adjunct to the diagnosis of pancreatic adenocarcinoma by EUS-guided fine-needle aspiration. *Gastrointest Endosc* 2006;63:648-654.
[64] Bhattacharyya S, Siegel ER, Petersen GM, Chari ST, Suva LJ and Haun RS. Diagnosis of pancreatic cancer using serum proteomic profiling. *Neoplasia* 2004;6:674-686.
[65] Koopmann J, Zhang Z, White N, Rosenzweig J, Fedarko N, Jagannath S, Canto MI, Yeo CJ, Chan DW and Goggins M. Serum diagnosis of pancreatic adenocarcinoma using surface-enhanced laser desorption and ionization mass spectrometry. *Clin Cancer Res* 2004;10:860-868.
[66] Scarlett CJ, Smith RC, Saxby A, Nielsen A, Samra JS, Wilson SR and Baxter RC. Proteomic classification of pancreatic adenocarcinoma tissue using protein chip technology. *Gastroenterology* 2006;130:1670-1678.
[67] Lin F, Shi J, Liu H, Hull ME, Dupree W, Prichard JW, Brown RE, Zhang J, Wang HL and Schuerch C. Diagnostic utility of S100P and von Hippel-Lindau gene product (pVHL) in pancreatic adenocarcinoma-with implication of their roles in early tumorigenesis. *Am J Surg Pathol* 2008; 32:78-91.
[68] Tsiambas E, Karameris A, Dervenis C, Lazaris AC, Giannakou N, Gerontopoulos K and Patsouris E. HER2/neu expression and gene alterations in pancreatic ductal adenocarcinoma: a comparative immuno-histochemistry and chromogenic in situ hybridization study based on tissue microarrays and computerized image analysis. *Jop* 2006;7:283-294.
[69] Wright JH, Wang X, Manning G, LaMere BJ, Le P, Zhu S, Khatry D, Flanagan PM, Buckley SD, Whyte DB, Howlett AR, Bischoff JR, Lipson KE and Jallal B. The STE20 kinase HGK is broadly expressed in human tumor cells and can modulate cellular transformation, invasion, and adhesion. *Mol Cell Biol* 2003;23:2068-2082.
[70] Hiyama E, Kodama T, Shinbara K, Iwao T, Itoh M, Hiyama K, Shay JW, Matsuura Y and Yokoyama T. Telomerase activity is detected in pancreatic cancer but not in benign tumors. *Cancer Res* 1997;57:326-331.
[71] Suehara N, Mizumoto K, Tanaka M, Niiyama H, Yokohata K, Tominaga Y, Shimura H, Muta T and Hamasaki N. Telomerase activity in pancreatic juice differentiates ductal carcinoma from adenoma and pancreatitis. *Clin Cancer Res* 1997;3:2479-2483.
[72] Ohuchida K, Mizumoto K, Yamada D, Yamaguchi H, Konomi H, Nagai E, Yamaguchi K, Tsuneyoshi M and Tanaka M. Quantitative analysis of human telomerase reverse transcriptase in pancreatic cancer. *Clin Cancer Res* 2006;12:2066-2069.
[73] Matsubayashi H, Canto M, Sato N, Klein A, Abe T, Yamashita K, Yeo CJ, Kalloo A, Hruban R and Goggins M. DNA methylation alterations in the pancreatic juice of patients with suspected pancreatic disease. *Cancer Res* 2006;66: 1208-1217.
[74] Chen R, Pan S, Yi EC, Donohoe S, Bronner MP, Potter JD, Goodlett DR, Aebersold R and Brentnall TA. Quantitative proteomic profiling of pancreatic cancer juice. *Proteomics* 2006;6: 3871-3879.
[75] Chen R, Pan S, Cooke K, Moyes KW, Bronner MP, Goodlett DR, Aebersold R and Brentnall TA. Comparison of pancreas juice proteins from cancer versus pancreatitis using quantitative proteomic analysis. *Pancreas* 2007;34:70-79.
[76] Kosmahl M, Pauser U, Peters K, Sipos B, Luttges J, Kremer B and Kloppel G. Cystic neoplasms of the pancreas and tumor-like lesions with cystic features: a review of 418 cases and a classification proposal. *Virchows Arch* 2004;445:168-178.
[77] Pelaez-Luna M and Chari ST. Cyst fluid analysis to diagnose pancreatic cystic lesions: an as yet unfulfilled promise. *Gastroenterology* 2006;130:1007-1009; discussion 1009.
[78] van der Waaij LA, van Dullemen HM and Porte

RJ. Cyst fluid analysis in the differential diagnosis of pancreatic cystic lesions: a pooled analysis. *Gastrointest Endosc* 2005;62:383-389.

[79] Kobayashi M, Ueyama Y, Nakanishi H, Ishida H, Takahashi E, Nakamura S, Koshikawa T and Yatabe Y. Immunocytochemical detection using CDX2: An aid for discerning tumor involvement in ascites cytology. *Cancer* 2006;108:114-118.

[80] Moparty B, Logrono R, Nealon WH, Waxman I, Raju GS, Pasricha PJ and Bhutani MS. The role of endoscopic ultrasound and endoscopic ultrasound-guided fine-needle aspiration in distinguishing pancreatic cystic lesions. *Diagn Cytopathol* 2007;35:18-25.