JOP. J Pancreas (Online) 2010 Nov 9; 11(6):536-544.

EDITORIAL

Molecular and Clinical Markers of Pancreas Cancer

James L Buxbaum[1], Mohamad A Eloubeidi[2]

[1]Divisions of Gastroenterology and Hepatology, University of Southern California. Los Angeles, CA, USA. [2]University of Alabama in Birmingham. Birmingham, AL, USA

Summary

Pancreas cancer has the worst prognosis of any solid tumor but is potentially treatable if it is diagnosed at an early stage. Thus there is critical interest in delineating clinical and molecular markers of incipient disease. The currently available biomarker, CA 19-9, has an inadequate sensitivity and specificity to achieve this objective. Diabetes mellitus, tobacco use, and chronic pancreatitis are associated with pancreas cancer. However, screening is currently only recommended in those with hereditary pancreatitis and genetic syndromes which predispose to cancer. Ongoing work to identify early markers of pancreas cancer consists of high throughput discovery methods including gene arrays and proteomics as well as hypothesis driven methods. While several promising candidates have been identified none has yet been convincingly proven to be better than CA 19-9. New methods including endoscopic ultrasound are improving detection of pancreas cancer and are being used to acquire tissue for biomarker discovery.

Pancreas cancer has the darkest prognosis of any gastrointestinal cancer with the mortality approaching the incidence [1]. While the overall five year survival is less than 4%, those recognized early, with tumor involving only the pancreas have a 25-30% five-year survival after surgery [2]. Given the limited treatment options there has been considerable focus on clinical and molecular harbingers of early disease.

Currently Available Tools to Evaluate for Pancreas Cancer

There are no population wide screening tests for pancreas cancer. The best established marker is CA 19-9 which is a sialylated Lewis antigen of the MUC1 protein with an overall sensitivity of 80% and specificity of 90% [3]. Unfortunately, CA 19-9 may be positive in patients with non malignant diseases including cirrhosis, chronic pancreatitis, cholangitis, as well as other gastrointestinal cancers [4]. Patients with certain blood types are incapable of expressing the antigen recognized by CA 19-9 [3]. Furthermore, only 65% of those with resectable pancreas cancer have elevated CA 19-9 levels [5]. Additionally, CA 19-9 is a poor screening test. At the Samsung Medical Center in South Korea 70,940 asymptomatic patients were screened using CA 19-9. However, among 1,063 with elevated levels only 4 had pancreas cancer and only 2 had resectable disease [6]. Nonetheless, CA 19-9 is widely used to evaluate patients with suspected pancreas cancer and those undergoing treatment.

Most cases of pancreas cancer are diagnosed by the discovery of a mass by computed tomography (CT) or magnetic resonance imaging. Endoscopic ultrasound (EUS), in which a high frequency ultrasound transducer is positioned in close proximity to the gland using an endoscope, has improved detection [7]. Additionally, EUS guided fine needle aspiration has improved the accuracy of diagnosing small lesions (less than 3 cm) from 66.7% for percutaneous methods to 86.1% [8]. EUS-FNA of the pancreas is associated with less malignant seeding compared to percutaneous methods and the region which is traversed is also typically removed during surgical resection [9]. However, the ability of EUS to detect malignancy in the setting of chronic pancreatitis is limited due to acoustic artifact and the yield of FNA is diminished by compromised visualization as well as desmoplastic tissue changes [10, 11, 12]. Emerging techniques such as elastography which measures the distensibility of tissue may improve the performance of EUS in this arena [13]. Analysis of molecular markers in FNA tissue shows promise and is likely to be the strategy of the future [14].

Precursor lesions of pancreas cancer include intraductal papillary mucinous neoplasms (IPMN), mucinous

Key words CA-19-9 Antigen; Diabetes Mellitus; Endosonography; Molecular Epidemiology; Pancreatic Neoplasms; Tobacco; Tumor Markers, Biological

Correspondence Mohamad A Eloubeidi
American University of Beirut School of Medicine, P.O. Box 11-0236 Riad El Solh 110, 72020 Beirut, Lebanon
Phone: +961-1.350.000; Fax: +961-1.366.098
E-mail: me75@aub.edu.lb

URL http://www.serena.unina.it/index.php/jop/article/view/3395/3696

cysts, and pancreatic intraepithelial neoplasia (PanIN). Cystic lesions can be detected and sampled by EUS as well as radiographic methods and are the primary target for screening which is performed in high risk families. The evolution of cysts into malignancy is thought to involve the enlargement of the cysts, thickening of the lining, and the invagination of popcorn like dysplastic nodules into the cyst [15]. PanIN is microscopic and thus difficult to detect [16]. Akin to the Vogelgram seen for colonic neoplasia, PanIN progresses through stages 1-3 marked by worsening histologic dysplasia and the accumulation of additional genetic mutations [17, 18].

There are now molecular markers to help differentiate which cysts are mucinous and later to help determine which might be malignant. In the breakthrough cooperative pancreatic cyst study, lesions were sample by needle aspiration and a panel of potential markers in the fluid analysis was evaluated. Brugge *et al*. demonstrated that carcinoembryonic antigen (CEA) at a level greater than 192 ng/mL optimally predicted that a cyst was mucinous [19]. The pancreatic cyst fluid DNA analysis (PANDA) investigators reported that a DNA quality and mutational analysis can improve the differentiation of malignant from benign mucinous cysts [20]. Analysis of EUS-FNA tissue for K-*ras* point mutations can also help differentiate pseudo-tumoral chronic pancreatitis from pancreas cancer thus improving the accuracy of cytopathology from 86% to 90% [14]. Due to increased risk of complications, inability to evaluate the parenchyma, and relatively low yield of cytologic brushings, endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangiography have less of a role in the diagnostic evaluation for pancreas cancer, though they are important in the management of biliary obstruction in patients with locally advanced and metastatic disease who are eligible for chemotherapy. A new panel of markers assessing for the absence of tumor suppressor genes may improve the yield of cytologic brushings [21].

Genetic and Familial Pancreas Cancer

While there is no population screening for pancreas cancer, surveillance programs are widely used to evaluate individuals with genetic syndromes strongly associated with pancreatic malignancy and those with extensive family history. Patients with Peutz-Jeghers syndrome harbor the STK11/LKB1 mutation. In addition to developing buccal hyperpigmentation and hamartomatous polyps throughout the gastrointestinal tract, these patients have an increased risk of multiple types of cancer. They have an extraordinarily high risk of pancreas cancer, 132 times that of the general population [22]. The most common germline mutation associated with pancreas cancer is the familial breast and ovarian cancer mutation (BRCA2) though the relative risk of disease is only 3.5. Others associated with increased risk of pancreas cancer include the familial atypical multiple mole melanoma (FAMMM) syndrome associated with the p16/CDKN2A mutation and the ataxia telangiectasia syndrome [23, 24].

Individuals with hereditary pancreatitis develop recurrent pancreatitis in childhood often resulting in advanced chronic pancreatitis by adolescence. The best understood mutation is in the cationic trypsinogen gene (PRSS1). In these patients trypsin is resistant to autolysis, one of the cardinal mechanisms to down regulate premature pancreatic enzyme activity [22]. Mutations in the serine protease inhibitor, Kazal type 1 (SPINK1) gene, an intra-pancreatic inactivator of trypsin is also associated with familial pancreatitis [25]. Finally, mutations in the cystic fibrosis gene (CFTR) are correlated with pancreatitis. Patients with genetic pancreatitis are at a 50 fold increased risk of pancreas cancer.

Additionally, may patients present with a very strong family history of pancreas cancer without a known genetic abnormality. This risk increases with the number of family members involved; 4.5 fold for one first degree relative, 8.4 for two, and 32 fold for three [26]. The palladin gene which encodes a cytoskeletal protein has been implicated in at least one pancreatic cancer kindred [17].

Screening for pancreas cancer is now performed for patients with the high risk genetic syndromes described above as well as those with multiple family members who have pancreas cancer. Experts recommend that patients with a greater than ten fold increased risk in pancreas cancer undergo screening which includes those with hereditary pancreatitis, Peutz-Jeghers syndrome, and FAMMM [24]. This also includes those who have two family members with pancreas cancer of the same lineage with one of these being a first degree family member or in those with three family members in the same lineage. Additionally, patients who have the BRCA1, BRCA2, p16 mutations and a first or second degree relative with pancreas cancer are recommended to undergo screening. Typically this is performed by performing cross sectional imaging or EUS (often alternating annually) beginning at age 30 for those with Peutz-Jeghers and 40 for those with familial cancer and hereditary pancreatitis [27, 28]. Magnetic resonance imaging is often favored over CT to minimize the risk of long term radiation. The aim of these test are to identify small masses, pancreatic cysts (primarily IPMN have been reported), and PanIN [28]. Patients with abnormalities then undergo EUS-FNA or ERCP and subsequently resection [27, 29]. Canto *et al*. report that amongst a cohort of 76 patients followed for 3 years with annual EUS and CT scan that 8 lesions were detected including 6 IPMN, 1 PanIN3, and 1 cancer associated with a cyst; 2 lesions were missed by CT alone [27].

Proposed and Confirmed Clinical Risk Factors for Pancreas Cancer

With the exception of those with genetic cancer syndromes or concerning family history there are few known risk factors for pancreas cancers. The best

established risk factors include diabetes mellitus, tobacco use, and chronic pancreatitis. For patients who have these clinical risk factors there are no screening recommendations. However it is important to educate these patients, particularly smokers, regarding the risk of pancreas cancer.

More than 50% of patients with pancreas cancer have diabetes mellitus which is a stronger association than that observed for pregnancy or obesity [30]. Meta-analysis demonstrated that patients who have diabetes for greater than five years have a two fold relative risk of pancreas cancer though other studies indicate that long term diabetes may not be a strong risk factor for pancreas cancer [30, 31]. A growing body of data suggests that new onset diabetes mellitus may be a consequence of the development of pancreas cancer and may resolve following removal of malignant tissue [32]. Chari *et al*. demonstrated that patients who present with new onset diabetes within the past 3 years have a 7.94 (95% CI: 1.61-12.74) observed to expected ratio of pancreas cancer [33]. Furthermore, the pancreas cancer risk associated with onset of diabetes mellitus correlates inversely with the duration of disease; i.e. the more recent the onset the stronger the correlation with pancreas cancer [34, 35]. Additionally, in comparison to controls with new onset diabetes mellitus type II without malignancy, patients with new onset diabetes prior to pancreas cancer have higher fasting glucose levels in the 12 months prior to diagnosis [36].

Fuchs *et al*. have demonstrated in 118,339 health care providers in the Nurses Health Study and 118,339 physicians in the Health Professional Study that the relative risk of pancreas cancer for current smokers was 2.5 fold, this decreased by 50% in two years, and returned to baseline 10 years after quitting smoking [37]. The risk of cancer also increases in parallel with the quantity of tobacco products consumed [38]. Overall in this population 25% of pancreas tumors were a consequence of smoking. Chronic pancreatitis has also been strongly associated with pancreas cancer. In a large retrospective cohort of 2,015 subjects with chronic pancreatitis the incidence ratio for cancer was 14.4 in patients with a five year history of chronic pancreatitis [39]. There was no difference between patients with alcoholic *versus* non alcoholic chronic pancreatitis.

Additionally, it has been proposed that obesity is a risk factor for pancreas cancer. Stolzenberg-Solomon *et al*. report that patients with a body mass index (BMI) of 35 kg/m^2 compared to 18.5-25 kg/m^2 have a hazard ratio of 1.45 (95% CI: 1.04-2.02) for the development of pancreas cancer, though this association weakens to a hazard ratio of 1.33 (95% CI: 0.95-1.86) when diabetes is accounted for [40]. However, other data suggest that obesity may not be an independent risk factor thus this remains a topic of ongoing investigation [41]. Alcohol and coffee have been proposed but do not appear to be risk factors for pancreas cancer [38].

While surveillance is performed in those with genetic syndromes, hereditary pancreatitis, and a strong family history there are no clear guidelines for those with clinical risk factors. Given that new onset diabetes may precede the clinical presentation of pancreas tumors, it has been proposed that CT scans in these patients could detect early lesions [42]. A prospective study employing cross sectional imaging in patients over 50 with recent onset diabetes showed that 6 out of 115 had tumors [43]. Unfortunately, five of the tumors were large and none were resectable, thus the authors did not advocate the widespread application of this strategy. In addition to population wide anti-tobacco campaigns, patients with chronic pancreatitis and those with genetic syndromes that predispose to pancreas cancer should be counseled to avoid smoking to reduce their already considerable risk [23]. Patients with hereditary pancreatitis who smoke are at an additional 3 fold risk of pancreas cancer, thus increasing from 50 fold to 150 fold [25].

Ongoing Quest for Molecular Markers

The Holy Grail for pancreas cancer investigators is to identify early markers which predict the development of pancreas cancer, uncover early resectable disease, and guide therapy. As previously described CA 19-9 levels are inadequate to identify early pancreas cancer in the population. Universal cross sectional imaging is impractical and would be associated with high cost and potential radiation related morbidity.

There are two major approaches to molecular marker discovery. In the high throughput "shotgun" strategies thousands of contenders are screened simultaneously. In the traditional hypothesis driven approach, interactions between molecules known to be important to pancreas cancer development are studied to identify novel molecules and pathways.

High Throughput Approach: Strategies and Yield

Several advanced technologies are being employed to evaluate for markers of pancreas cancer. The underlying principle is to analyze tissue from those with pancreas cancer compared to normal tissue. DNA arrays involve the use of microchips to which are appended the "negative" sequences to thousands of portions of genes [44]. Tissue from those with and without pancreas cancer is processed to yield RNA which is used to generate cDNA sequences which are differentially marked using fluorescence tags. The cDNA is then exposed to the microchips which anneals to the corresponding negative sequences. Differences in the fluorescence pattern between the pancreas cancer and control can be used to rapidly identify differential gene expression.

Another critical high throughput technique is proteonomics. The potential advantage of proteonomics is that proteins levels may be more clinically relevant as gene expression does not necessarily correlate with the quantity and nature of the proteins they encode [45, 46, 47]. As is the case in

gene arrays tissue from those with and without pancreas cancer are compared. While there are several methods, most rely on an initial fractionation step whether by two dimensional electrophoresis or more sophisticated methods such as protein chip technology [48]. In most cases the samples are then analyzed by mass spectroscopy in which the components are separated based on molecular weights and the quantities of different mass size can be compared between malignant and control samples [49, 50, 51]. Subsequently, the proteins are identified by iterative techniques and sequencing [51]. In order to quantitatively compare levels between samples from patients with pancreas cancer and healthy controls, isotope-coded affinity tagging may be used [52]. In this process proteins (cysteine residues) from the cancer and control samples are labeled with heavy and light isotopes of a reagent and the samples are then combined and analyzed simultaneously by mass spectrometry [50]. Peaks corresponding to the protein from the two groups appear immediately adjacent (due to the slight difference in weight between the isotopes) and their ratio allows quantification of the differences in that particular protein in patients with cancer as compared to controls.

High throughput analysis can also be performed to look at RNA. MicroRNA are relatively short stable non-coding RNA sequences which bind to target RNA and prevent translation into protein [53]. Aberrant microRNA expression has been found to be important in the development of leukemia due to its role in gene translation [54]. Arrays containing probes for hundred of known microRNA's are being used to study their role in pancreas cancer with encouraging results [54]. Major challenges include correctly identifying the genes with which specific microRNA interact [53]. Messenger RNA has also been evaluated using microarray technology [55].

Subsequently, expression of potentially important genes and protein levels (as well as epigenetic markers) from patients with pancreas cancer, control, and those with benign pancreatic disease, particularly chronic pancreatitis, must be compared among groups of patients. While high throughput techniques have generated several important candidates much work remains [56]. Problems with high throughput methods include reproducibility and in particular identification of markers which can be measured by readily available clinical laboratory methods [46, 48, 52, 57, 58].

Candidate Markers

High throughput methods have identified a number of candidate molecular markers. In subsequent validation studies several have not been found to be more discriminating than CA 19-9 including hepato-carcinoma - intestine - pancreas / pancreatitis - associated protein (HIP-PAP), ostepontin, tissue inhibitor of metalloproteinase 1 (TIMP 1), DUPAN-2, CA 242, CA 72-4, CA 195, MMP-7, cathepsin D, integrin B1, and plasminogen [59, 60, 61, 62, 63, 64].

In a trial comparing 50 pancreas cancer patients to 50 chronic pancreatitis and to 50 healthy controls, macrophage inhibitory cytokine-1 (MIC-1) had a greater accuracy (92%) to distinguish pancreas cancer from normal patients than CA 19-9 (71%) but did not perform better in differentiating patients with pancreas cancer from those with chronic pancreatitis (67%) compared to CA 19-9 (67%) [59]. S100A6 level were measured in the pancreatic juice of 26 patients with pancreas cancer, 37 with IPMN, and 30 with chronic pancreatitis. S100A6 did significantly discriminate between those with chronic pancreatitis and those with cancer and IPMN, but did not discern between cancer and IPMN [64]. CEACAM1 which is part of the CEA family performs better than CA 19-9 in discriminating between those with pancreas cancer and normal controls and its level increases in patients with PanIN-3 versus PanIN-1 or PanIN-2 [65]. Nonetheless, it does not differentiate effectively between those with chronic pancreatitis and cancer.

After initially being identified by microarray of pancreas cancer tissue the presence of microRNA (miR)-155 has been demonstrated in precursor lesions [2, 66]. MiR-155 was present in PanIN-2 and to an even greater extent in PanIN-3 indicating that increased expression of the microRNA correlated with development of pancreas cancer via the PanIN pathway [2]. Its expression was also demonstrated to be 11.5 fold greater in IPMN tissue than control pancreas specimens [66]. Microarray technology has been used to identify messenger RNA transcripts in saliva which may assist in differentiating those with and without pancreas cancer [55].

Other potential candidates identified primarily by high throughput methods which are under investigation include alpha-1,4-N-acetylglucosaminyltransferase, cyclin I, GD12, annexin A2, annexin A8, claudin 18, insulin-like growth factor I (IGF-I), growth factor I binding protein-I (IGFBP-1), insulin-like growth factor-binding protein 2 (IGFBP-2), MBD3L2, DPMI, ACRV1, insulin-like apolipoprotein A-I, REG4, and transthyretin [1, 44, 51, 52, 55, 57, 60, 67, 68, 69, 70, 71]. MicroRNA 16, 143, 155, 196a, 217, and 223 also are potential candidates under active investigation [54].

Hypothesis Driven Research

Hypothesis driven research has concentrated on several pathways important to the development of pancreas cancer including stimulation of proto-oncogenes, inactivation of tumor suppressor gene, dysregulation mechanisms including aberrant methylation and telomerase activity, and the role of proteins known to be important in gastrointestinal cancers including the mucin family. Proto-oncogenes that promote abnormal proliferation are thought to be important in pancreas cancer. K-*ras*, which encodes a signal transduction protein, has been found to have a mutation in codon 12 in greater than 90% of pancreas cancer cases [72]. However, it is frequently detected in the serum and

pancreatic juice only in those with advanced disease [73, 74]. A prospective study showed that only 38.1% of those with pancreas cancer were seen to have mutated K-*ras* samples in pancreatic juice and bile [75]. Additionally, K-*ras* mutations are seen in chronic pancreatitis and in smokers; as high as 39% of heavy smokers have K-*ras* mutations [76]. Recently Shi *et al*. employed a quantitative assay to detect single nucleotide K-*ras* mutations which may enable better discrimination between mutations seen in those with malignancy *versus* benign disease based on quantitative levels in pancreatic juice, bile, and serum [73, 77].

Tumor suppressor genes including SMAD4, APC, and p53 have also been of interest. When used alone p53 is not sensitive enough to function as a tumor marker; in one series only 56% of pancreas carcinoma were found to have the mutation [78]. The leading mechanism for inactivation of tumor suppressor gene is chromosomal loss. One method of detection is to measure the presence of microsatellites, which are well described DNA sequence repeats. The loss of one of the pair of microsatellite repeats disappears, termed loss of heterozygosity correlates with the silencing of the nearby tumor suppressor genes. As part of the PANDA study a group of investigators found that a panel of makers including loss of heterozygosity and K-*ras* could be used to differentiate malignant from nonmalignant mucinous cysts [20].

Another mechanism through which tumor suppressor genes may be silenced is by hypermethylation of key gene promoter regions (CpG islands) [79]. It has been demonstrated that more than 1% methylation of 2 of 5 key tumor suppressor genes (Cyclin D2, FOX E1, NPTX2, ppENK, and TFP12) occurred in 82% of patients with pancreas cancer compared to none of the controls [80]. Progressive methylation abnormalities have been correlated with dysplastic progression in PanIN lesions [79].

Telomerase is an enzyme which stabilizes chromosomes by placement of repeat sequences at their ends. It has a particularly important role in proliferating cells including lymphocytes, germ cells, and malignant cells. Ohuchida *et al*. have demonstrated that the relative telomerase activity from the pancreatic juice of those with cancer is elevated relative to those with chronic pancreatitis and other benign diseases [63].

CA 19-9 is an antigen expressed by the MUC1 protein. Like other members of the mucin family it is a glycosylated extracellular protein implicated in a number of malignancies [4]. MUC1 levels are elevated in pancreas cancer. It also expresses an aberrant antigen profile in this setting [62]. Other MUC1 antigens of interest in those with pancreas cancer include CA 15-3 and CA 27.29. The PAM4 antibody against MUC1 is more specific for pancreas cancer than antibodies to other MUC1 antigens which are seen in other tumors [62]. Gold *et al*. demonstrated in a group of 43 healthy individuals, 87 patients with pancreatitis, and 53 patients with pancreas cancer that PAM4 antibody is more sensitive (71% *versus* 59%) and specific (96% *versus* 63%) than CA 19-9. This improvement was largely a result of less false positive results for patients with chronic pancreatitis when the PAM4 was used, 5%, compared to CA 19-9, 37%.

A combination of hypothesis driven and high throughput methods is resulting in the development of additional potential biomarkers. In a groundbreaking study published in *Science* a combination of approaches including gene sequencing and microarrays were used to delineate twelve core pathways including K-*ras*, hedgehog, and TGF-B signaling among others [81]. The associated genes identified to be involved in these processes have potential as markers of pancreas cancer.

Diabetes and Molecular Markers

The correlation of new onset diabetes and pancreas cancer also represents fertile ground in the search for molecular markers [82]. Surgeons have noted that peripheral insulin sensitivity improves after resection of malignant tissue raising the question of whether there is a substance secreted by the tumor which might cause diabetes. In 1994 it was found in a group of patients with pancreas cancer that those who also had diabetes had significantly elevated levels of the protein amylin relative to those with cancer but no diabetes, those with diabetes but no cancer, as well as those without either disease [83]. Furthermore, those who underwent resection were found to have normalization of amylin levels. Amylin is a protein co-secreted with insulin and which inhibits glucose uptake and glycogen synthesis by skeletal muscles [84]. Thus amylin was proposed as a serum marker of pancreas cancer associated with glucose intolerance.

However, prospective studies by Chari *et al*. demonstrated that amylin had a sensitivity of only 39% and specificity of 93% as a tumor marker, significantly less than CA 19-9 [85]. Amylin is elevated in chronic pancreatitis as well as biliary obstruction and other gastrointestinal cancers [86]. Nonetheless, while enthusiasm for amylin has decreased, *in vitro* data has demonstrated that the media from pancreas cancer cell lines injected into mice can lead to impaired glucose tolerance suggesting the presence of a yet undiscovered humoral factor [87]. Co-culture of the tumor media with hepatocytes and myocytes appears to impact glucose metabolism, implicating that the substance may interact with these tissues [88, 89, 90]. Both proteomic and hypothesis driven approaches are being used in the quest for such a serum factor [91, 92].

Other groups are investigating changes in pancreatic function in the setting of malignancy. Kolb *et al*. demonstrated that in pancreas cancer associated diabetes as opposed to diabetes mellitus type II, islet cells express increased glucagon and decreased insulin [93]. They propose that an insulin to glucagon ratio greater than 7.4 ng/mU can differentiate pancreas cancer associated diabetes mellitus from diabetes

mellitus type II with a sensitivity and specificity of 77% and 69%, respectively.

Markers of Therapy

Additionally, markers used to predict therapeutic response are being evaluated. Gemcitabine is the mainstay of modern chemotherapy for pancreas cancer. It is transported into cells by the human equilibrative nucleoside transporter 1 (hENT-1) protein. High hENT-1 protein expression has been found to be strongly predictive of treatment response to chemotherapy and survival [94, 95]. Evidence suggests that levels of the most established tumor marker, CA 19-9 also predicts therapeutic response. In a cohort of 424 patients with pancreas cancer who underwent resection, those with a preoperative CA 19-9 less than 1,000 had a median survival of 2.3 years versus 1 year for those with a CA 19-9 greater than 1,000 [96]. Necrotic pancreatic cancer cells undergoing apoptosis express the inactivated complement component iC3b which is important for phagocytosis [97]. Marten *et al*. have demonstrated that soluble levels of iC3b predict recurrence 4 months prior to image confirmed recurrence [98]. Potentially, this marker may also have role in those at high risk for cancer including those with familial cancer syndromes.

Approach to the Gland

Potential molecular markers are sought in the pancreatic tissue, juice as well as other body fluids including serum and urine. To minimize invasive procedures ideal markers should optimally be detected in the serum and other body fluids. An important consideration is that pancreatic tumor cells and secreted molecules are found in markedly higher concentrations in the pancreas and pancreatic juice compared to the serum [99, 100]. CA 19-9 and CEA levels in the pancreatic juice are 30-1,000 times higher than in serum [101]. It has been reported that one potential marker, the HIP/PAP protein, is 1,000 times more concentrated in the pancreatic juice compared to serum [102]. Additionally, molecules and protein in the serum are overwhelmed by high concentrations of albumin, transferrin, and immunoglobulins. Thus it is logical to first obtain pancreas tissue to identify differential markers of pancreas cancer and then look for their presence further from the gland. EUS-FNA can be used to readily and safely obtain pancreas tissue to enable this process and has a burgeoning role in biomarker discovery [103].

Conclusions

While the landscape of pancreas cancer is currently bleak, several auspicious developments are ongoing. While there is no screening test for pancreas cancer, those with genetic syndrome, hereditary pancreatitis, and those with strong family history may benefit from surveillance by EUS and cross sectional imaging. New onset diabetes, tobacco use, and chronic pancreatitis have been demonstrated to be risk factors. These findings are important for patient education and represent a fertile territory for biomarker discovery. Both hypothesis driven and high throughput searches for molecular markers to predict disease, early diagnosis, and treatment response are underway. Challenges include differentiation of cancer from chronic inflammatory diseases of the pancreas and achieving reproducible results among diverse patients. Minimally invasive methods including EUS-FNA to acquire tissue may facilitate these important efforts.

Conflict of interest The authors have no potential conflict of interest

References

1. Karanjawala ZE, Illei PB, Ashfaq R, Infante JR, Murphy K, Pandey A, et al. New markers of pancreatic cancer identified through differential gene expression analyses: claudin 18 and annexin A8. Am J Surg Pathol 2008; 32:188-96. [PMID: 18223320]

2. Ryu JK, Hong SM, Karikari CA, Hruban RH, Goggins MG, Maitra A. Aberrant MicroRNA-155 expression is an early event in the multistep progression of pancreatic adenocarcinoma. Pancreatology 2010; 10:66-73. [PMID 20332664]

3. Steinberg W. The clinical utility of the CA 19-9 tumor-associated antigen. Am J Gastroenterol 1990; 85:350-5. [PMID 2183589]

4. Duffy MJ, Sturgeon C, Lamerz R, Haglund C, Holubec VL, Klapdor R, et al. Tumor markers in pancreatic cancer: a European Group on Tumor Markers (EGTM) status report. Ann Oncol 2010; 21:441-7. [PMID: 19690057]

5. Goggins M. Molecular markers of early pancreatic cancer. J Clin Oncol 2005; 23:4524-31. [PMID 16002843]

6. Kim JE, Lee KT, Lee JK, Paik SW, Rhee JC, Choi KW. Clinical usefulness of carbohydrate antigen 19-9 as a screening test for pancreatic cancer in an asymptomatic population. J Gastroenterol Hepatol 2004; 19:182-6. [PMID 14731128]

7. Ardengh JC, de Paulo GA, Ferrari AP. Pancreatic carcinomas smaller than 3.0 cm: endosonography (EUS) in diagnosis, staging and prediction of resectability. HPB (Oxford) 2003; 5:226-30. [PMID 18332991]

8. Harewood GC, Wiersema MJ. Endosonography-guided fine needle aspiration biopsy in the evaluation of pancreatic masses. Am J Gastroenterol 2002; 97:1386-91. [PMID: 12094855]

9. Micames C, Jowell PS, White R, Paulson E, Nelson R, Morse M, et al. Lower frequency of peritoneal carcinomatosis in patients with pancreatic cancer diagnosed by EUS-guided FNA vs. percutaneous FNA. Gastrointest Endosc 2003; 58:690-5. [PMID: 14595302]

10. Ardengh JC, Lopes CV, Campos AD, Pereira de Lima LF, Venco F, Modena JL. Endoscopic ultrasound and fine needle aspiration in chronic pancreatitis: differential diagnosis between pseudotumoral masses and pancreatic cancer. JOP. J Pancreas (Online) 2007; 8:413-21. [PMID: 17625292]

11. Varadarajulu S, Tamhane A, Eloubeidi MA. Yield of EUS-guided FNA of pancreatic masses in the presence or the absence of chronic pancreatitis. Gastrointest Endosc 2005; 62:728-36. [PMID: 16246688]

12. Fritscher-Ravens A, Brand L, Knöfel WT, Bobrowski C, Topalidis T, Thonke F, et al. Comparison of endoscopic ultrasound-guided fine needle aspiration for focal pancreatic lesions in patients with normal parenchyma and chronic pancreatitis. Am J Gastroenterol 2002; 97:2768-75. [PMID: 12425546]

13. Janssen J, Schlorer E, Greiner L. EUS elastography of the pancreas: feasibility and pattern description of the normal pancreas, chronic pancreatitis, and focal pancreatic lesions. Gastrointest Endosc 2007; 65:971-8. [PMID 17531630]

14. Bournet B, Souque A, Senesse P, Assenat E, Barthet M, Lesavre N, et al. Endoscopic ultrasound-guided fine-needle aspiration biopsy coupled with KRAS mutation assay to distinguish pancreatic cancer from pseudotumoral chronic pancreatitis. Endoscopy 2009; 41:552-7. [PMID: 19533561]

15. Adsay NV, Merati K, Basturk O, Iacobuzio-Donahue C, Levi E, Cheng JD, et al. Pathologically and biologically distinct types of epithelium in intraductal papillary mucinous neoplasms: delineation of an "intestinal" pathway of carcinogenesis in the pancreas. Am J Surg Pathol 2004; 28:839-48. [PMID: 15223952]

16. Hruban RH, Takaori K, Klimstra DS, Adsay NV, Albores-Saavedra J, Biankin AV, et al. An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. Am J Surg Pathol 2004; 28:977-87. [PMID: 15252303]

17. Pogue-Geile KL, Chen R, Bronner MP, Crnogorac-Jurcevic T, Moyes KW, Dowen S, et al. Palladin mutation causes familial pancreatic cancer and suggests a new cancer mechanism. PLoS Med 2006;3:e516. [PMID 17194196]

18. Klein WM, Hruban RH, Klein-Szanto AJ, Wilentz RE. Direct correlation between proliferative activity and dysplasia in pancreatic intraepithelial neoplasia (PanIN): additional evidence for a recently proposed model of progression. Mod Pathol 2002; 15:441-7. [PMID 11950919]

19. Brugge WR, Lewandrowski K, Lee-Lewandrowski E, Centeno BA, Szydlo T, Regan S, et al. Diagnosis of pancreatic cystic neoplasms: a report of the cooperative pancreatic cyst study. Gastroenterology 2004; 126:1330-6. [PMID: 15131794]

20. Khalid A, Zahid M, Finkelstein SD, LeBlanc JK, Kaushik N, Ahmad N, et al. Pancreatic cyst fluid DNA analysis in evaluating pancreatic cysts: a report of the PANDA study. Gastrointest Endosc 2009; 69:1095-102. [PMID: 19152896]

21. Khalid A, Pal R, Sasatomi E, Swalsky P, Slivka A, Whitcomb D, Finkelstein S. Use of microsatellite marker loss of heterozygosity in accurate diagnosis of pancreaticobiliary malignancy from brush cytology samples. Gut 2004; 53:1860-5. [PMID 15542529]

22. Canto MI. Strategies for screening for pancreatic adenocarcinoma in high-risk patients. Semin Oncol 2007; 34:295-302. [PMID 17674957]

23. Whitcomb DC, Pogue-Geile K. Pancreatitis as a risk for pancreatic cancer. Gastroenterol Clin North Am 2002; 31:663-78. [PMID: 12134623]

24. Brand RE, Lerch MM, Rubinstein WS, Neoptolemos JP, Whitcomb DC, Hruban RH, et al. Advances in counselling and surveillance of patients at risk for pancreatic cancer. Gut 2007; 56:1460-9. [PMID 17872573]

25. Rosendahl J, Bodeker H, Mossner J, Teich N. Hereditary chronic pancreatitis. Orphanet J Rare Dis 2007; 2:1. [PMID 17204147]

26. Klein AP, Brune KA, Petersen GM, Goggins M, Tersmette AC, Offerhaus GJ, et al. Prospective risk of pancreatic cancer in familial pancreatic cancer kindreds. Cancer Res 2004; 64:2634-8. [PMID 15059921]

27. Canto MI, Goggins M, Hruban RH, Petersen GM, Giardiello FM, Yeo C, et al. Screening for early pancreatic neoplasia in high-risk individuals: a prospective controlled study. Clin Gastroenterol Hepatol 2006; 4:766-81. [PMID 16682259]

28. Ulrich CD. Pancreatic cancer in hereditary pancreatitis: consensus guidelines for prevention, screening and treatment. Pancreatology 2001; 1:416-22. [PMID 12120218]

29. Brentnall TA, Bronner MP, Byrd DR, Haggitt RC, Kimmey MB. Early diagnosis and treatment of pancreatic dysplasia in patients with a family history of pancreatic cancer. Ann Intern Med 1999; 131:247-55. [PMID 10454945]

30. Gullo L, Pezzilli R, Morselli-Labate AM. Diabetes and the risk of pancreatic cancer. N Engl J Med 1994; 331:81-4. [PMID 8208269]

31. Everhart J, Wright D. Diabetes mellitus as a risk factor for pancreatic cancer. A meta-analysis. JAMA 1995; 273:1605-9. [PMID 7745774]

32. Pannala R, Leirness JB, Bamlet WR, Basu A, Petersen GM, Chari ST. Prevalence and clinical profile of pancreatic cancer-associated diabetes mellitus. Gastroenterology 2008; 134:981-7. [PMID: 18395079]

33. Chari ST, Leibson CL, Rabe KG, Ransom J, de Andrade M, Petersen GM. Probability of pancreatic cancer following diabetes: a population-based study. Gastroenterology 2005; 129:504-11. [PMID: 16083707]

34. Chari ST, Leibson CL, Rabe KG, Timmons LJ, Ransom J, de Andrade M, Petersen GM. Pancreatic cancer-associated diabetes mellitus: prevalence and temporal association with diagnosis of cancer. Gastroenterology 2008; 134:95-101. [PMID: 18061176]

35. Gupta S, Vittinghoff E, Bertenthal D, Corley D, Shen H, Walter LC, McQuaid K. New-onset diabetes and pancreatic cancer. Clin Gastroenterol Hepatol 2006; 4:1366-72. [PMID 16945591]

36. Pannala R, Leibson CL, Rabe KG, Timmons LJ, Ransom J, de Andrade M, et al. Temporal association of changes in fasting blood glucose and body mass index with diagnosis of pancreatic cancer. Am J Gastroenterol 2009; 104:2318-25. [PMID: 19513024]

37. Fuchs CS, Colditz GA, Stampfer MJ, Giovannucci EL, Hunter DJ, Rimm EB, et al. A prospective study of cigarette smoking and the risk of pancreatic cancer Arch Intern Med 1996; 156:2255-60. [PMID 8885826]

38. Ghadirian P, Simard A, Baillargeon J. Tobacco, alcohol, and coffee and cancer of the pancreas. A population-based, case-control study in Quebec, Canada. Cancer 1991; 67:2664-70. [PMID 2015568]

39. Lowenfels AB, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, et al. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. N Engl J Med 1993; 328:1433-7. [PMID 8479461]

40. Stolzenberg-Solomon RZ, Adams K, Leitzmann M, Schairer C, Michaud DS, Hollenbeck A, et al. Adiposity, physical activity, and pancreatic cancer in the National Institutes of Health-AARP Diet and Health Cohort. Am J Epidemiol 2008;167:586-97. [PMID 18270373]

41. Berrington de Gonzalez A, Sweetland S, Spencer E. A meta-analysis of obesity and the risk of pancreatic cancer. Br J Cancer 2003; 89:519-23. [PMID: 12888824]

42. Pelaez-Luna M, Takahashi N, Fletcher JG, Chari ST. Resectability of presymptomatic pancreatic cancer and its relationship to onset of diabetes: a retrospective review of CT scans and fasting glucose values prior to diagnosis. Am J Gastroenterol 2007; 102:2157-63. [PMID: 17897335]

43. Damiano J, Bordier L, Le Berre JP, Margery J, Dupuy O, Mayaudon H, Bauduceau B. Should pancreas imaging be recommanded in patients over 50 years when diabetes is discovered because of acute symptoms? Diabetes Metab 2004; 30:203-7. [PMID: 15223996]

44. Iacobuzio-Donahue CA, Maitra A, Olsen M, Lowe AW, van Heek NT, Rosty C, et al. Exploration of global gene expression patterns in pancreatic adenocarcinoma using cDNA microarrays. Am J Pathol 2003;162:1151-62. [PMID 12651607]

45. Petricoin EF, Ardekani AM, Hitt BA, Levine PJ, Fusaro VA, Steinberg SM, et al. Use of proteomic patterns in serum to identify ovarian cancer. Lancet 2002; 359:572-7. [PMID 11867112]

46. Chen R, Pan S, Brentnall TA, Aebersold R. Proteomic profiling of pancreatic cancer for biomarker discovery. Mol Cell Proteomics 2005; 4:523-33. [PMID 15684406]

47. Paradis V, Degos F, Dargère D, Pham N, Belghiti J, Degott C, et al. Identification of a new marker of hepatocellular carcinoma by serum protein profiling of patients with chronic liver diseases. Hepatology 2005; 41:40-7. [PMID 15690480]

48. Scarlett CJ, Smith RC, Saxby A, Nielsen A, Samra JS, Wilson SR, Baxter RC. Proteomic classification of pancreatic adenocarcinoma tissue using protein chip technology. Gastroenterology 2006; 130:1670-8. [PMID: 16697731]

49. Link AJ, Eng J, Schieltz DM, Carmack E, Mize GJ, Morris DR, et al. Direct analysis of protein complexes using mass spectrometry. Nat Biotechnol 1999; 17:676-82. [PMID 10404161]

50. Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. Nat Biotechnol 1999; 17:994-9. [PMID 10504701]

51. Chen R, Pan S, Yi EC, Donohoe S, Bronner MP, Potter JD, et al. Quantitative proteomic profiling of pancreatic cancer juice. Proteomics 2006; 6:3871-9. [PMID 16739137]

52. Chen R, Yi EC, Donohoe S, Pan S, Eng J, Cooke K, et al. Pancreatic cancer proteome: the proteins that underlie invasion, metastasis, and immunologic escape. Gastroenterology 2005; 129:1187-97. [PMID: 16230073]

53. Lee EJ, Gusev Y, Jiang J, Nuovo GJ, Lerner MR, Frankel WL, et al. Expression profiling identifies microRNA signature in pancreatic cancer. Int J Cancer 2007; 120:1046-54. [PMID 17149698]

54. Szafranska AE, Davison TS, John J, Cannon T, Sipos B, Maghnouj A, et al. MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. Oncogene 2007; 26:4442-52. [PMID 17237814]

55. Zhang L, Farrell JJ, Zhou H, Elashoff D, Akin D, Park NH, et al. Salivary transcriptomic biomarkers for detection of resectable pancreatic cancer. Gastroenterology 2010; 138:949-57. [PMID: 19931263]

56. Hanash SM, Pitteri SJ, Faca VM. Mining the plasma proteome for cancer biomarkers. Nature 2008; 452:571-9. [PMID 18385731]

57. Ehmann M, Felix K, Hartmann D, Schnölzer M, Nees M, Vorderwülbecke S, et al. Identification of potential markers for the detection of pancreatic cancer through comparative serum protein expression profiling. Pancreas 2007; 34:205-14. [PMID 17312459]

58. Ransohoff DF. Bias as a threat to the validity of cancer molecular-marker research. Nat Rev Cancer 2005;5:142-9. [PMID 15685197]

59. Koopmann J, Rosenzweig CN, Zhang Z, Canto MI, Brown DA, Hunter M, et al. Serum markers in patients with resectable pancreatic adenocarcinoma: macrophage inhibitory cytokine 1 versus CA19-9. Clin Cancer Res 2006; 12:442-6. [PMID 16428484]

60. Chen R, Brentnall TA, Pan S, Cooke K, Moyes KW, Lane Z, et al. Quantitative proteomics analysis reveals that proteins differentially expressed in chronic pancreatitis are also frequently involved in pancreatic cancer. Mol Cell Proteomics 2007; 6:1331-42. [PMID 17496331]

61. Kuhlmann KF, van Till JW, Boermeester MA, de Reuver PR, Tzvetanova ID, Offerhaus GJ, et al. Evaluation of matrix metalloproteinase 7 in plasma and pancreatic juice as a biomarker for pancreatic cancer. Cancer Epidemiol Biomarkers Prev 2007; 16:886-91. [PMID 17507610]

62. Gold DV, Modrak DE, Ying Z, Cardillo TM, Sharkey RM, Goldenberg DM. New MUC1 serum immunoassay differentiates pancreatic cancer from pancreatitis. J Clin Oncol 2006; 24:252-8. [PMID 16344318]

63. Ohuchida K, Mizumoto K, Ishikawa N, Sato N, Nagai E, Yamaguchi K, et al. A highly sensitive and quantitative telomerase activity assay with pancreatic juice is useful for diagnosis of pancreatic carcinoma without problems due to polymerase chain reaction inhibitors: analysis of 100 samples of pancreatic juice from consecutive patients. Cancer 2004; 101:2309-17. [PMID 15476274]

64. Ohuchida K, Mizumoto K, Yu J, Yamaguchi H, Konomi H, Nagai E, et al. S100A6 is increased in a stepwise manner during pancreatic carcinogenesis: clinical value of expression analysis in 98 pancreatic juice samples. Cancer Epidemiol Biomarkers Prev 2007; 16:649-54. [PMID 17416753]

65. Simeone DM, Ji B, Banerjee M, Arumugam T, Li D, Anderson MA, et al. CEACAM1, a novel serum biomarker for pancreatic cancer. Pancreas 2007; 34:436-43. [PMID 17446843]

66. Habbe N, Koorstra JB, Mendell JT, Offerhaus GJ, Ryu JK, Feldmann G, et al. MicroRNA miR-155 is a biomarker of early pancreatic neoplasia. Cancer Biol Ther 2009; 8:340-6. [PMID 19106647]

67. Sun ZL, Zhu Y, Wang FQ, Chen R, Peng T, Fan ZN, et al. Serum proteomic-based analysis of pancreatic carcinoma for the identification of potential cancer biomarkers. Biochim Biophys Acta 2007; 1774:764-71. [PMID 17507299]

68. Ishizone S, Yamauchi K, Kawa S, Suzuki T, Shimizu F, Harada O, et al. Clinical utility of quantitative RT-PCR targeted to alpha1,4-N-acetylglucosaminyltransferase mRNA for detection of pancreatic cancer. Cancer Sci 2006; 97:119-26. [PMID 16441422]

69. Wolpin BM, Michaud DS, Giovannucci EL, Schernhammer ES, Stampfer MJ, Manson JE, et al. Circulating insulin-like growth factor binding protein-1 and the risk of pancreatic cancer. Cancer Res 2007; 67:7923-8. [PMID 17699799]

70. Wolpin BM, Michaud DS, Giovannucci EL, Schernhammer ES, Stampfer MJ, Manson JE, et al. Circulating insulin-like growth factor axis and the risk of pancreatic cancer in four prospective cohorts. Br J Cancer 2007; 97:98-104. [PMID: 17533398]

71. Takayama R, Nakagawa H, Sawaki A, Mizuno N, Kawai H, Tajika M, et al. Serum tumor antigen REG4 as a diagnostic biomarker in pancreatic ductal adenocarcinoma. J Gastroenterol 2010; 45:52-9. [PMID 19789838]

72. Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perucho M. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell 1988; 53:549-54. [PMID 2453289]

73. Shi C, Fukushima N, Abe T, Bian Y, Hua L, Wendelburg BJ, et al. Sensitive and quantitative detection of KRAS2 gene mutations in pancreatic duct juice differentiates patients with pancreatic cancer from chronic pancreatitis, potential for early detection. Cancer Biol Ther 2008; 7:353-60. [PMID 18075308]

74. Shi C, Eshleman SH, Jones D, Fukushima N, Hua L, Parker AR, et al. LigAmp for sensitive detection of single-nucleotide differences. Nat Methods 2004; 1:141-7. [PMID 15782177]

75. Trümper L, Menges M, Daus H, Köhler D, Reinhard JO, Sackmann M, et al. Low sensitivity of the ki-ras polymerase chain reaction for diagnosing pancreatic cancer from pancreatic juice and bile: a multicenter prospective trial. J Clin Oncol 2002; 20:4331-7. [PMID 12409332]

76. Berger DH, Chang H, Wood M, Huang L, Heath CW, Lehman T, Ruggeri BA. Mutational activation of K-ras in nonneoplastic exocrine pancreatic lesions in relation to cigarette smoking status. Cancer 1999; 85:326-32. [PMID 10023699]

77. Shi C, Chandrasekaran A, Thuluvath PJ, Karikari C, Argani P, Goggins M, et al. Ultrasensitive detection of KRAS2 mutations in bile and serum from patients with biliary tract carcinoma using LigAmp technology. J Mol Diagn 2009; 11:583-9. [PMID: 19815696]

78. Sturm PD, Hruban RH, Ramsoekh TB, Noorduyn LA, Tytgat GN, Gouma DJ, 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; 186:247-53. [PMID: 10211112]

79. Sato N, Fukushima N, Hruban RH, Goggins M. CpG island methylation profile of pancreatic intraepithelial neoplasia. Mod Pathol 2008; 21:238-44. [PMID 18157091]

80. Matsubayashi H, Canto M, Sato N, Klein A, Abe T, Yamashita K, et al. DNA methylation alterations in the pancreatic juice of patients with suspected pancreatic disease. Cancer Res 2006; 66:1208-17. [PMID 16424060]

81. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008; 321:1801-6. [PMID 18772397]

82. Chari ST. Detecting early pancreatic cancer: problems and prospects. Semin Oncol 2007; 34:284-94. [PMID 17674956]

83. Permert J, Larsson J, Westermark GT, Herrington MK, Christmanson L, Pour PM, et al. Islet amyloid polypeptide in patients with pancreatic cancer and diabetes. N Engl J Med 1994; 330:313-8. [PMID 8277951]

84. Cluck MW, Chan CY, Adrian TE. The regulation of amylin and insulin gene expression and secretion. Pancreas 2005; 30:1-14. [PMID 15632693]

85. Chari ST, Klee GG, Miller LJ, Raimondo M, DiMagno EP. Islet amyloid polypeptide is not a satisfactory marker for detecting pancreatic cancer. Gastroenterology 2001; 121:640-5. [PMID: 11522748]

86. Brand RE, Ding XZ, Young CM, Adrian TE. The specificity of amylin for the diagnosis of pancreatic adenocarcinoma. Int J Gastrointest Cancer 2002; 31:123-8. [PMID 12622423]

87. Basso D, Brigato L, Veronesi A, Panozzo MP, Amadori A, Plebani M. The pancreatic cancer cell line MIA PaCa2 produces one or more factors able to induce hyperglycemia in SCID mice. Anticancer Res 1995;15:2585-8. [PMID 8669828]

88. Basso D, Valerio A, Brigato L, Panozzo MP, Miola M, Lucca T, et al. An unidentified pancreatic cancer cell product alters some intracellular pathways of glucose metabolism in isolated rat hepatocytes. Pancreas 1997; 15:132-8. [PMID 9260197]

89. Valerio A, Basso D, Brigato L, Ceolotto G, Baldo G, Tiengo A, Plebani M. Glucose metabolic alterations in isolated and perfused rat hepatocytes induced by pancreatic cancer conditioned medium: a low molecular weight factor possibly involved. Biochem Biophys Res Commun 1999; 257:622-8. [PMID 10198261]

90. Basso D, Greco E, Fogar P, Pucci P, Flagiello A, Baldo G, et al. Pancreatic cancer-associated diabetes mellitus: an open field for proteomic applications. Clin Chim Acta 2005; 357:184-9. [PMID 15946661]

91. Basso D, Valerio A, Seraglia R, Mazza S, Piva MG, Greco E, et al. Putative pancreatic cancer-associated diabetogenic factor: 2030 MW peptide. Pancreas 2002; 24:8-14. [PMID 11741177]

92. Basso D, Greco E, Fogar P, Pucci P, Flagiello A, Baldo G, et al. Pancreatic cancer-derived S-100A8 N-terminal peptide: a diabetes cause? Clin Chim Acta 2006; 372:120-8. [PMID 16678810]

93. Kolb A, Rieder S, Born D, Giese NA, Giese T, Rudofsky G, et al. Glucagon/insulin ratio as a potential biomarker for pancreatic cancer in patients with new-onset diabetes mellitus. Cancer Biol Ther 2009; 8:1527-33. [PMID 19571666]

94. Farrell JJ, Elsaleh H, Garcia M, Lai R, Ammar A, Regine WF, et al. Human equilibrative nucleoside transporter 1 levels predict response to gemcitabine in patients with pancreatic cancer. Gastroenterology 2009; 136:187-95. [PMID: 18992248]

95. Giovannetti E, Del Tacca M, Mey V, Funel N, Nannizzi S, Ricci S, et al. Transcription analysis of human equilibrative nucleoside transporter-1 predicts survival in pancreas cancer patients treated with gemcitabine. Cancer Res 2006; 66:3928-35. [PMID 16585222]

96. Ferrone CR, Finkelstein DM, Thayer SP, Muzikansky A, Fernandez-delCastillo C, Warshaw AL. Perioperative CA19-9 levels can predict stage and survival in patients with resectable pancreatic adenocarcinoma. J Clin Oncol 2006; 24:2897-902. [PMID 16782929]

97. Hoimes CJ, Moyer MT, Saif MW. Biomarkers for early detection and screening in pancreatic cancer. Highlights from the 45th ASCO annual meeting. Orlando, FL, USA. May 29-June 2, 2009. JOP. J Pancreas (Online) 2009;10:352-6. [PMID 19581733]

98. Marten A, Buchler MW, Werft W, Wente MN, Kirschfink M, Schmidt J. Soluble iC3b as an early marker for pancreatic adenocarcinoma is superior to CA19.9 and radiology. J Immunother 2010; 33:219-24. [PMID: 20139773]

99. Jimeno A, Hidalgo M. Molecular biomarkers: their increasing role in the diagnosis, characterization, and therapy guidance in pancreatic cancer Mol Cancer Ther 2006; 5:787-96. [PMID 16648548]

100.Al-Haddad M, Wallace MB. Molecules and markers for endosonographers: what do we need to know and measure? Endoscopy 2006; 38(Suppl 1):S50-3. [PMID: 16802224]

101.Zhou L, Lu Z, Yang A, Deng R, Mai C, Sang X, et al. Comparative proteomic analysis of human pancreatic juice: methodological study. Proteomics 2007; 7:1345-55. [PMID 17443640]

102.Rosty C, Christa L, Kuzdzal S, Baldwin WM, Zahurak ML, Carnot F, et al. Identification of hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein I as a biomarker for pancreatic ductal adenocarcinoma by protein biochip technology. Cancer Res 2002; 62:1868-75. [PMID 11912167]

103.Laurell H, Bouisson M, Berthelemy P, Rochaix P, Dejean S, Besse P, et al. Identification of biomarkers of human pancreatic adenocarcinomas by expression profiling and validation with gene expression analysis in endoscopic ultrasound-guided fine needle aspiration samples. World J Gastroenterol 2006; 12:3344-51. [PMID: 16733850]