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Advances in Biomedical Imaging, Bioengineering, and Related Technologies for the Development of Biomarkers of Pancreatic Disease: Summary of a National Institute of Diabetes and Digestive and Kidney Diseases and National Institute of Biomedical Imaging and Bioengineering Workshop

Kimberly A. Kelly, PhD[*], **Michael A. Hollingsworth, PhD**[†], **Randall E. Brand, MD**[‡], **Christina H. Liu, PhD**[§], **Vikesh K. Singh, MD, MSc**[‖], **Sudhir Srivastava, PhD, MPH**[§], **Ajay D. Wasan, MD, MSc**[‡], **Dhiraj Yadav, MD, MPH**[‡], and **Dana K. Andersen, MD**[¶]

[*]Department of Biomedical Engineering, University of Virginia, Charlottesville, VA

[†]Eppley Cancer Institute, University of Nebraska School of Medicine, Omaha, NE

[‡]Division of Gastroenterology, Hepatology and Nutrition, Department of Medicine and Departments of Anesthesiology and Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA

[§]Office of Cancer Nanotechnology Research and the Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, Bethesda, MD

[‖] Division of Gastroenterology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD

[¶]Division of Digestive Diseases and Nutrition, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

Abstract

A workshop sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases and the National Institute of Biomedical Imaging and Bioengineering focused on research gaps and opportunities in the development of new biomarkers of pancreatic disease. The session was held on July 22, 2015, and structured into six sessions: 1) introduction and overview, 2) keynote address, 3) new approaches to the diagnosis of chronic pancreatitis, 4) biomarkers of pain and inflammation, 5) new approaches to the detection of pancreatic cancer, and 6) shed exosomes, shed cells, and shed proteins. Recent advances in the fields of pancreatic imaging, functional markers of pancreatic disease, proteomics, molecular and cellular imaging, and detection of circulating cancer cells and exosomes were reviewed. Knowledge gaps and research needs were highlighted. The development of new methods for the non-invasive determination of pancreatic

Corresponding Author: Kimberly A. Kelly, PhD, Associate Professor of Biomedical Engineering, Robert M. Berne Cardiovascular Research Center, University of Virginia, 415 Lane Road Bldg MR5 Rm 1214, Charlottesville, VA 22908 Tel: 434-243-9352 kak3x@virginia.edu.

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pathology, the use of cellular markers of pancreatic function, inflammation, pain, and malignancy, and the refinement of methods to identify cells and cellular constituents of pancreatic cancer were discussed. The further refinement of sophisticated technical methods, and the need for clinical studies to validate these new approaches in large-scale studies of patients at risk for the development of pancreatic disease was repeatedly emphasized.

Keywords

Biomarkers; Chronic pancreatitis; Pancreatic cancer; Molecular imaging; Exosomes; Circulating tumor cells

Introduction

Chronic pancreatitis and pancreatic cancer are typically diagnosed at advances stages of disease. The inability to detect early disease when intervention can reverse the disease or improve the outcome of treatment is well known, and is overshadowed by the fact that both benign and malignant diseases of the pancreas are increasing in their incidence, world-wide. For these reasons, the National Institute of Diabetes and Digestive and Kidney Diseases and the National Institute of Biomedical Imaging and Bioengineering convened a workshop entitled “Advances in Biomedical Imaging, Bioengineering, and Related Technologies for the Development of Biomarkers of Pancreatic Disease: Gaps, Needs, and Opportunities” on July 22, 2015 at the University Club in Pittsburgh, PA. The purpose of the workshop was to bring together experts from diverse areas of biotechnology to examine the latest developments in methods to detect and assess both benign and malignant pancreatic disease, and to discuss the research challenges and opportunities for further development and validation of biomarkers of early stage disease.

Seventeen speakers participated in the workshop, which included an overview and introduction to the workshop, a keynote address, and sessions devoted to a) New Approaches to the Diagnosis of Chronic Pancreatitis, b) Biomarkers of Pain and Inflammation, c) New Approaches to the Detection of Pancreatic Cancer, and d) Shed Exosomes, Shed Cells, and Shed Proteins. Recent developments were reviewed, and knowledge gaps and research needs were highlighted. This summary includes the key elements of each session, and identifies priority areas for further research.

Overview of Pancreatic Disease and the Need for Further Biomarker Research

Diseases of the pancreas are common maladies, and diseases of the exocrine pancreas are a major cause of hospitalization in the US. Exocrine pancreatic disorders including pancreatitis and cancer are known to arise from infections, trauma, chemical or drug exposure, genetic etiologies, or combinations of these and other factors. The genesis and progression of pancreatic diseases are complex, in that different etiologies can lead to similar pathology, which in turn may lead to multiple and different outcomes for which it is difficult to accurately predict proper therapeutic intervention.

Pancreatitis presents with different features of duration and incidence, and is characterized as acute (days), recurrent acute (months) and chronic (years). Whereas the causes of these conditions are understood for many cases (genetic, autoimmune, alcohol, obstruction or trauma), the etiology of a significant percentage of cases of pancreatitis is not known. Cancer is believed to proceed from multiple acquired genetic, epigenetic and other environmental insults to premalignant lesions over years, and may include pancreatitis as a predecessor, contributor to or a consequence of disease progression. It is difficult to accurately image the pancreas in the clinical setting to a degree that allows for accurate discrimination of overlapping features of chronic inflammation, premalignant and malignant lesions. In addition, there are no highly specific or sensitive biomarkers for different types of early pancreatic diseases to complement existing imaging technology. As a result, benign and malignant diseases are usually diagnosed only at an advanced stage, when the opportunity for reversal of disease or effective treatments is severely limited. Thus there is a great need for advances in biomedical imaging, biomarkers, and the development of related technologies to accurately diagnose and monitor pancreatic disease at its earliest stage.

New Approaches to the Diagnosis of Chronic Pancreatitis (CP)

Overview

Several studies have defined the natural history of CP [1-3]. The clinical presentation and course of CP can be highly variable, ranging from asymptomatic disease detected incidentally, to presentation with a variety of clinical symptoms including abdominal pain, acute pancreatitis, diabetes, steatorrhea or complications of CP. The time between onset of symptoms, clinical diagnosis and disease course varies based on age, etiology, genetic factors and ongoing exposure to risk factors such as alcohol and tobacco consumption. Moreover, clinical features in CP correlate poorly with morphologic findings on imaging studies [4]. CP-associated morphologic changes are common in patients who are older[5], drink[6], smoke[7], and are obese[8] without any clinical features of CP. The challenge therefore includes both early detection of disease prior to development of structural and functional alterations as well as monitoring its progression.

Traditionally, cross sectional imaging such as abdominal computed tomography (CT) and magnetic resonance imaging have been used to diagnose CP. A limitation of these approaches is poor sensitivity to detect early stage disease. The use of endoscopic retrograde cholangiopancreatography (ERCP) for diagnosing CP has been abandoned due to the risk of post-ERCP pancreatitis and poor correlation of ductal changes on pancreatography to histology [9].

The standard criteria for diagnosing CP by endoscopic ultrasound (EUS) include 5 ductal features (ductal dilation, ductal irregularity, dilated side branches, calcifications, hyperechoic duct wall margins) and 4 parenchymal features (hyperechoic foci and strands, lobularity, cysts). EUS is not required for diagnosing CP in patients with pancreatic calcifications as these are often detected on CT scans. The standard EUS criteria are generally weighted equally but there is evidence that the ductal features more strongly correlate with moderate to severe fibrosis [10]. Using histology as the reference, 3 studies have evaluated EUS criteria for diagnosing noncalcific CP and have reported sensitivities of

75-91% and specificities of 85-100%[11-13]. However, these studies had many limitations including surgical resection for cancer in 95% of patients[11], inclusion of patients with a high pretest probability of CP based on the presence of risk factors[12], and variability in the threshold number of criteria used to define CP on histology and EUS[11-13]. In general, as the threshold number of EUS criteria increases, the specificity for a diagnosis CP increases but the sensitivity decreases. Given the high interobserver variability between endosonographers for standard EUS criteria [14,15], the Rosemont classification was developed to determine the probability of CP based on assigned weights and numerical thresholds for individual EUS criteria [16]. However, the Rosemont classification is not widely utilized in clinical practice due to its complexity, lack of comparison to a histologic standard and poor interobserver variability [17,18]

EUS elastography is an imaging technique based on the finding that different diseases have different tissue density and compressibility [19]. EUS elastography has been largely used to differentiate benign versus malignant solid pancreatic masses but, despite high accuracy [20], it has not replaced fine needle aspiration in clinical practice. In addition, mass-forming CP tends to occur in those patients with more advanced disease. While one study reported differences in strain ratios obtained on elastograms between normal patients and those with established CP [21], there have been no studies to differentiate normal patients from those with suspected CP.

Direct pancreatic function testing by collection of pancreatic juice (PJ) after stimulation with secretin or cholecystokinin to measure bicarbonate or lipase output respectively, can detect secretory dysfunction in patients with at least 30% pancreas damage [22]. Endoscopic collection of PJ by the Dreiling's tube method for the measurement of bicarbonate and/or lipase has been shown to have good correlation, but is cumbersome, available only at a few institutions, and requires an oroduodenal tube placement which needs confirmation by fluoroscopy [23]. Studies of pancreatic function testing have shown a high sensitivity (>90%) in detecting advanced CP but a lower sensitivity (70%–75%) and positive predictive value (45%) in detecting early CP [24]. Furthermore, some abnormal pancreatic function tests in the setting of normal imaging may be the result of smoking or genetic defects in CFTR function rather than CP [25,26]. Therefore, in patients with suspected CP who do not have obvious morphologic evidence of disease, pancreatic function testing can serve as a useful test to exclude the presence of CP. However, its low positive predictive value may lead to over-diagnosis in patients in whom abnormalities in bicarbonate section may be unrelated to CP.

PJ collected from the duodenum (preferably after stimulation with secretin to facilitate fluid secretion) can be used to test for biomarkers other than bicarbonate and lipase. An example of this was a recent proof of concept pilot study which found the PJ concentration of prostaglandin E2 (PGE2) to be elevated and differentially expressed in patients with definite CP (defined by using M-ANNHEIM criteria) and minimal change CP (MMCP - defined by presence of at least one of the following: clinical risk factors, ≥3 EUS features of CP or PJ bicarbonate concentration of <80 mEq/l). Median PJ PGE2 was elevated in CP (307 pg/ml, IQR (249–362)) and MCCP (568 pg/ml, (418–854)) when compared with normal controls (104 pg/ml, (68–206)) ($P \leq 0.001$). Area under receiving operator curve (AUROC) for diagnosis of CP and MMCP was 0.9 and 0.62, respectively, for PJ bicarbonate concentration

alone; AUROC was 1.0 and 0.94 for the combination of PJ bicarbonate and PGE2 concentrations [27]. These findings suggest that PJ PGE2 concentration could be a potential biomarker for the diagnosis of CP even at an early stage. Preliminary data from other studies have reported on the utility of proteomic analyses of PJ for diagnosing early stage CP (see section on Biomarkers of Pain and Inflammation). However, these findings need to be validated in a larger cohort of patients.

Non-invasive imaging techniques such as ultrasound elastography (USE) and magnetic resonance elastography (MRE) represent noninvasive and less costly alternatives to biopsy for assessing inflammation and fibrosis [28]. Other advantages include short acquisition time (minutes), reproducibility, the ability to assess the entire organ, and monitoring of changes over time by sequential studies. The premise is that inflammation and fibrosis increases the stiffness of normal tissue. Shear waves applied to an organ travel faster in the presence of inflammation and fibrosis when compared with normal tissue and result in differential pattern of wavelength images. USE and MRE techniques are increasingly applied to assess liver cirrhosis and differentiating inflammation from fibrosis. MRE is shown to be superior to USE and laboratory test-based fibrosis screening panels for assessing liver fibrosis [29].

Preliminary results of MRE of the pancreas in humans suggest the feasibility of its use to differentiate normal pancreas from disease states such as CP, malignancy and benign tumors. In a recent study of 20 healthy volunteers, imaging at 40 Hz vibration was found to provide better and uniform estimates of sheer stiffness across the different regions of the pancreas (vs. 60 Hz vibration frequency used for the liver) with less inter- and intra- subject coefficients of variation. In contrast to the 2D technique utilized for MRE of the liver, a 3D technique was suggested to be better for MRE of the pancreas [30].

Research Gaps and Opportunities

Developing accurate biomarkers on imaging studies and in biological specimens for early detection of CP and at different stages of disease progression is vital. In addition to diagnosis and monitoring disease progression, availability of biomarkers will allow for use in trials to measure efficacy of interventions designed to delay or interrupt the fibro-inflammatory process and development of structural and functional changes. Due to low disease prevalence, multicenter collaborative efforts will be needed to achieve adequate sample sizes for these approaches.

Specific research priorities include the following:

1. Evaluate the effect of genetic polymorphisms (e.g. CFTR variants) and environmental factors that may confound bicarbonate and enzyme concentrations in pancreatic juice.
2. Collection of biological specimens at different stages of CP to help facilitate development of biomarkers for different stages of CP that correlate with the morphological and clinical alterations of the disease.

3. Optimization of technology for MR and EUS elastography of the pancreas and clinical studies across the spectrum of pancreatic diseases to fully appreciate its utility as a biomarker of pancreatic disease.

4. Development of technology for the combined imaging of pancreatic nerves and parenchymal fibrosis in an effort to understand how pancreatic pain develops and changes through the stages of CP.

Biomarkers of Pain and Inflammation

Overview

Acute pancreatitis (AP) is the most common gastrointestinal disorder leading to hospital admission in the United States. Common causes for AP include alcohol and gallstones. Patients who present with recurrent acute pancreatitis are at a very high risk of developing CP. Patients with CP experience intractable, difficult to treat abdominal pain and the disease is associated with pancreatic acinar cell loss and fibrosis, and can result in exocrine and endocrine insufficiency. CP is a risk factor for developing pancreatic ductal adenocarcinoma (PDAC) [31].

In AP, initial injury signals likely start at the acinar cell level, where injured acini produce pro-inflammatory cytokines and chemokines leading to the recruitment of inflammatory leukocytes into the pancreas. One of the initially recruited leukocytes includes neutrophils, which play pathogenic role locally within the pancreas and in distant organs such as the lung [32]. Soon after, inflammatory monocytes follow into the pancreas and differentiate into macrophages with pro-inflammatory properties (classically activated or M1s) and exacerbate the pancreatic injury [33]. In addition to cellular infiltrates, recent studies showed the importance of IL-6 trans-signaling in promoting the lung injury associated with severe acute pancreatitis [34].

Earlier and recent studies show that T cells and macrophages are the predominant immune cell infiltrates in CP [35]. Macrophages in CP are found in close proximity to key pro-fibrogenic pancreatic stellate cells [36]. Experimental studies suggest an interaction between the stellate cells and macrophages, mediated via IL-4 receptor signaling, which results in fibrosis in CP [37]. In contrast to AP, macrophages in CP display alternate activation (M2) consistent with immunosuppressive environment associated with CP.

Standard patient care recommendations include CT with contrast in AP especially when suspicion for necrosis or other complications exist. In CP, EUS, MRI, and magnetic resonance cholangiopancreatography (MRCP) are used as diagnostic tools. Inflammation is reported with the use of these imaging modalities based on parenchymal enlargement, changes in tissue density, indistinct pancreatic margins and/or changes in surrounding fat. Although helpful, such reports do not provide in depth analysis of the inflammatory process. However, recent experimental non-invasive means of imaging immune responses and in particular macrophages have been developed, including the use of biocompatable quenched-fluoresence probes that can be activated by cleavage, and probes that are taken up by specific cell types (e.g. macrophages) and activated by internal enzymes and proteases.

Investigations into the progression of PDAC from early stages to metastasis identified molecules that contribute to perineural invasion by pancreatic cancer cells [38,39]. As one example, CDK5, a kinase with homology to cyclin dependent kinases, but with distinct functions that include contributions to neural tissue patterning and nociception, is overexpressed and hyperactivated in a high percentage of human pancreatic cancers, especially in areas of perinerual invasion [39]. The concept that pancreatic cancers undertake tissue remodeling including perineural invasion during progression led to investigations of the influence of inflammation and tumor growth on neurolymphatic remodeling [40], nociception, and other pain related processes.

Recent unpublished results show differences in remodeling of nerves and lymphatics during non-malignant inflammation processes as compared to malignant tumor growth. One aspect of these results suggests that tumors influence neurolymphatic remodeling in areas that extend into the tumor microenvironment beyond the zone of tumor cell contact. As such, this field effect presents a potential target for imaging of tumor specific effects, not seen in cases of acute inflammation, that would be of greater dimensions than the area occupied by individual tumor cells, and therefore may be amenable to detection by imaging. Potential targets to detect areas of tumor specific tissue remodeling as compared to inflammation associated remodeling include agents (antibodies, peptides, small molecules) that detect lymphatics, nerves, and molecules that are specifically associated with remodeling these cell types in the tumor microenvironment and in the non-malignant inflammatory microenvironment.

Another molecular imaging candidate, divalent zinc (Zn^{2+}), is essential for the secretory function of several tissues including the pancreas, mammary glands, and prostate [41]. Insulin produced by pancreatic β-cells is stored in granules as a zinc complex which is released in response to high glucose. As a result, the local concentration of Zn^{2+} in the extracellular space of β-cells during insulin secretion increases to ~400 μM. Zn^{2+} is known to modulate several aspects of pancreatic function including glucagon secretion from α-cells. The normal human prostate has been reported to have the highest levels of free Zn^{2+} [42] while malignant prostate cells undergo a metabolic transformation that results in less accumulation of Zn^{2+} [43]. This loss of tissue Zn^{2+} in prostate cancer offers a potential opportunity to distinguish malignancy from benign prostatic hyperplasia, prostatic intraepithelial neoplasia, and inflammation. It has also been shown that Zn^{2+} levels are markedly decreased in PDAC as compared with normal pancreatic ductal/acinar epithelium due to down-regulation of ZIP3 transporters [44]. Hence, an imaging technique for monitoring Zn^{2+} secretion from secretory cells could provide new insights into the development and progression of cancer that other biological signatures cannot provide.

Several gadolinium-based contrast agents capable of detecting free Zn^{2+} ions by MRI at biological concentrations were recently reported. As one model compound, GdDOTA-diBPEN has little affinity for human serum albumin (HSA) in the absence of Zn^{2+} but after binding two Zn^{2+} ions, the 2:1 complex then binds to HSA and this results in a 2.6-fold increase in r_1 relaxivity to 17 $mM^{-1}s^{-1}$ [45]. Although this r_1 relaxivity enhancement is limited by the rate of water exchange, it was sufficient to monitor release of Zn^{2+} and insulin from pancreatic β-cells in control mice, prediabetic (obese) mice, and diabetic

mice [46]. A second generation version of GdDOTA-diBPEN with one acetate side-chain replaced by propionate yielded a complex with identical Zn^{2+} binding properties but with a r_1 of 57 $mM^{-1}s^{-1}$ (8.6-fold increase) when exposed to Zn^{2+} and HSA. This new agent nicely enhances the mouse prostate *in vivo* by MRI but only after co-injection of glucose. This suggests a secretory mechanism involving glucose stimulated Zn^{2+} release from the prostate, similar to the pancreas [46]. These results demonstrate that it is possible to design functional MRI contrast agents that respond to alterations in important biological ions *in vivo* at doses acceptable for translation to human imaging.

Additional molecular imaging targets are being sought by proteomics studies, which comprehensively map the protein content of samples of interest. Information about protein content, i.e. the proteome, is complementary to the DNA and RNA content, i.e. the genome and transcriptome, as there is no clear universal quantitative correlation between the DNA, the RNA and the protein content. The disconnect between RNA and protein abundance is particularly striking for secreted proteins due to the numerous, individually controlled post-transcriptional steps needed for the successful secretion of a protein. Given the role of the exocrine pancreas in secreting enzymes, proteomic analysis is particularly useful for assessing pancreatic disease, especially to gain insights into the pathophysiological state of a chronically inflamed pancreas, to monitor pancreatic function in this particular disease context and to diagnose different forms of pancreatitis.

Characterization of proteomes of pancreatic fluid specimens collected from chronic pancreatitis patients as well as normal, truly healthy controls resulted in the first normative pancreatic fluid proteome map[47]. Proteome maps of CP-related pancreatic fluid specimens clearly reflected known functional insufficiencies. Finding these expected changes for the pancreatic enzymes lends credibility to discovery of other proteins with similar disease-dependent differences in abundance.

Given the dearth of modalities for robustly, quickly and non-invasively diagnosing and staging CP, the diagnostic potential of urine as a non-invasively obtainable source of biomarkers has also been investigated, in order to minimize the need for the highly invasive collection of pancreatic fluid specimens and/or expensive, slow and subjective imaging. These research efforts resulted in several promising biomarker candidates, but also highlighted the need for much higher throughput in order to enable the comprehensive proteome mapping of biostatistically relevant sample numbers. To this end, a unique urine proteomics “pipeline” is being developed to meet the need for higher throughput.

Research Gaps and Opportunities

There is a need for new agents that allow for imaging of specific molecules and cell types in the tumor and inflammatory microenvironments.

Specific research priorities include the following:

1. Novel cellular targets that need to be identified include nerves, lymphatics, and inflammatory cells.

2. Imaging agents that allow for discrimination of the causes of different types of pain (nociception), such as from inflammation, swelling, perineural invasion, and remodeling of the neural microenvironment, need to be developed and validated.

3. Molecular targets need to be identified and validated, including molecules that contribute to remodeling of the tumor microenvironment (proteases, tumor-associated secreted proteins, proinflammatory factors other factors to be discovered by techniques such as proteomics) and which discriminate malignant and non-malignant microenvironments. These targets include important ions and metals (e.g. Zn^2) involved in cellular function.

Keynote Lecture: The Potential of Optical Imaging for the Early Detection of Pancreatic Cancer

Although not currently routinely used clinically, optical imaging approaches and technology are being developed that could change clinical practice. In the area of biomedical and molecular imaging, it will be necessary to develop image-guided approaches for multiplexed molecular characterization of cancer and methods to visualize small numbers of cancer initiating cells. Imaging and sensing will need to move from detection limits of 1 cm masses to 1 mm, or even 100 μm diameter masses, and new technologies with this sensitivity need to be developed. Optical imaging has the sensitivity for this level of detection and there are a number of recent advances that will enable the use of optics in the clinic for cancer detection[48]. Developments in the field of optical imaging will be useful in informing diagnosis, prognosis and therapy, and for guiding biopsies for multiparameter molecular analyses. Dr. Contag's lecture focused on his laboratory's technologies that enable miniaturization of 3-D scanning confocal microscopes to examine tissue in situ for early anatomic and molecular indicators of disease, in real time, and at cellular resolution[49]. These new devices will lead to a shift from the current diagnostic paradigm of biopsy followed by histopathology and recommended therapy, to one of non-invasive point-of-care diagnosis with the possibility of treatment in the same session. On the horizon are insertable, implantable and wearable micro-optical devices for the early detection of cancer.

New Approaches to the Detection of Pancreatic Cancer

Overview

This session focused on recent developments in molecular imaging probes and techniques as well as the identification of at risk populations in addition to those with a hereditary predisposition to PDAC. New approaches for the detection of PDAC that utilize non-invasive medical imaging have played an important role in the diagnosis of disease and clinical decision-making. Medical imaging modalities include MRI, CT, positron emission tomography (PET), single-photon emission computed tomography (SPECT) and US. Based on their image resolutions and sensitivities, these imaging modalities are being used for different clinical purposes. For example, CT and MRI have excellent image resolution, but poor sensitivity, so these modalities are used for anatomical evaluation. US offers poor image resolution but is widely used to detect tissue or blood flow abnormalities and to provide functional information on the region under investigation. Although exposing

patients to ionizing radiation, PET or SPECT have excellent sensitivity to monitor tumor growth and metastasis; 18FDG-PET is the standard image protocol for cancer detection, although its utility is variable for PDAC.

PDAC is often diagnosed at an advanced stage due to the lack of early and clearly-defined symptoms. PDAC diagnosis is therefore associated with a dismal prognosis. Few effective treatment options exist, and there is an overall 5-year survival rate of approximately 7% [50]. Although it does improve to 26% if found at a local stage (node negative), the vast majority of these early-staged patients die from unrecognized metastatic disease. Currently, no cost-effective screening approach for sporadic PDAC exists in the general population limiting candidates for surveillance to high-risk individuals with a hereditary predisposition for developing a PDAC [51]. A major obstacle in developing a cheap surveillance test, such as a serum-based biomarker, is the inability to detect a curable PDAC since tumors detected at a size of greater than 1 cm have typically metastasized. While the association between diabetes mellitus (DM) and PDAC has been known for 150 years, its role in early detection of PDAC has only recently been investigated.

Molecular imaging can afford better understanding in the disease pathway, stratifying patient treatment, monitoring therapeutic response or drug distribution, and monitoring cancer recurrence. Imaging agents that bind and amplify the signal of molecular targets have actively been developed to detect changes of the tissue at the microscopic level. Numerous imaging agents have demonstrated great potential for cancer diagnosis, staging and treatment planning at the preclinical stage; some have led to a number of agents being approved for human trials. A review highlighting molecular imaging agents that illuminate various cell populations in PDAC (i.e., epithelial, endothelial, and stromal tumor cells) can be found in a recent publication by Dimastromatteo et al [52].

Before development of an imaging agent or probe, one or more molecular target(s) need to be identified. Candidate targets should be highly abundant, bioavailable, unique, and have readily-available or easily-designed ligands that can bind to these targets with high affinity. Target selection based on complex metabolic pathways is daunting. Phage display screening and biochemical techniques based on functional proteomics allowed the identification of binding partners of 15 peptides that are specific for pancreatic cancer [53]. Ligands for the molecular targets can be antibodies, aptamers, peptides, nanoparticles or small molecules. These ligands are designed based on the imaging modality and should have high affinity, signal specificity and favorable pharmacokinetics.

US molecular imaging is an emerging imaging modality that is particularly suited for earlier detection of PDAC. Advantages of US molecular imaging include: cost effectiveness, no ionizing radiation, bedside-ready, real-time measurement, and most importantly, can be combined with endoscopy. In particular, EUS using targeted contrast agents can increase diagnostic accuracy and reliability. Currently US-based contrast agents are microbubbles, about 7um in size, with a gas core (filled with perfluorocarbon, sulphur hexafluoride, nitrogen or air), and surrounded by a lipid-based shell. Because of their size, these microbubbles remain in the intravascular space and oscillate in an acoustic field providing signal in deep tissue. Additionally, their lipid shell allows the microbubbles to carry 10-1000

ligands for the purpose of targeted contrast enhancement. Using Vascular Endothelial Growth Factor Receptor Type 2 (VEGFR2), which is highly expressed on early neoplastic lesions in human pancreas, targeted contrast microbubbles under B-mode and nonlinear contrast-enhanced US imaging detected small foci of PDAC in transgenic mice [54]. Novel imaging targets are being discovered and validated to further increase diagnostic accuracy of US molecular imaging. For example, Thymocyte Differentiation Antigen 1 (Thy1) was identified as a specific biomarker of PDAC neovasculature by proteomic analysis from patients with PDAC, chronic pancreatitis and normal pancreas; US imaging with microbubbles targeting Thy1 accurately detected human Thy1-positive PDAC xenografts and PDACs that express endogenous Thy1 in genetic mouse models of PDAC [55].

The use of PET for cancer imaging is a well established and an accepted molecular imaging modality in both clinical and research settings. Although non-specific for cancer, the most acceptable PET imaging for the staging of cancers is 18-fluorodeoxyglucose (18-FDG, an analog of glucose) which delineates areas of elevated glucose intake indicative of tumor activities. Using specially designed radiopharmaceuticals, PET offers quantitative measurements of biological and receptor-based processes. It was noted that many serum biomarkers used for clinical diagnosis have limitations, with the most prominent shortcoming being high false-positive diagnosis of malignant disease. As such, probing the sites of biomarker secretion with imaging tools could be a useful strategy to better understand disease status. PET can be used to broaden the diagnostic utility of serum CA19.9, which is the only currently utilized biomarker in the management of PDAC patients. A study designed to test this hypothesis was conducted utilizing a fully human monoclonal antibody against CA19.9 (5B1) as part of a PET radiotracer which showed improved specificity and tumor localization in patient derived xenograft (PDXs) of PDAC than 18FDG-PET [56].

Dynamic Contrast Enhanced MRI (DCE-MRI) is a widely used imaging methodology with a broad range of preclinical and research applications. These applications include quantification of myocardial, pulmonary, and renal perfusion and solid tumor imaging in breast, prostate, and brain. Based on the distribution and tissue perfusion of gadolinium (a clinically used contrast agent), a combination of DCE-MRI and data modeling methods can generate a range of parameters. These parameters can be used to characterize tissue microvasculature, blood perfusion and tissue physiology of the pancreas and pancreatic tumors, such as transfer constant (K^{trans}) of water, extracellular extravascular volume (v_e), blood volume (v_b), mean vascular transit time (t_c), and vascular transit time heterogeneity (α^{-1}) [57]. A novel tracer-kinetic modeling approach for dual agent (iron and gadolinium) DCE-MRI was developed to differentiate blood flow and microvascular permeability [58]. By quantifying these DCE-MRI parameters (which are non-invasive imaging biomarkers), it is possible to identify changes in microvasculature and tissue characteristics associated with malignant pathology earlier and with greater precision. The diagnostic capability of DCE-MRI in the pancreas was first tested in 2001 [59] followed by reports showing that DCE-MRI parameters could predict response of PDAC to sorafenib and gemcitabine [60], could correlate with fibrosis and microvascular density [61] and could assess tumor microvascularity in normal and pancreatic cancer patients [62]. Although DCE-MRI is successful in detecting

1mm foci of disease in breast and prostate cancer, technical challenges for DCE-MRI of the pancreas are associated with motion artifact, which can degrade the spatial resolution of the image to 3mm. Special pulse sequences are being developed to improve image quality of DCE-MRI in human pancreata. There is an ongoing clinical trial on the use of DCE-MRI in the management of pancreatic cancer (NCT02070705).

Over 90% of pancreatic cancers are sporadic and asymptomatic at early stage. To address the need for advances in research innovation and subsequent translation to clinical practice for the early detection of sporadic pancreatic cancer, a white paper on a strategic map for innovation was generated after the Early Detection of Sporadic Pancreatic Cancer Summit Conference in 2014 [63]. This paper concluded that because pancreatic cancer is relatively uncommon, initial screening should be restricted to subjects at high risk for the disease. A rational, evidence-based strategy can then be used to identify patients who may benefit from screening and early detection procedures. Ideally, initial screening strategies for PDAC would include the use of biomarkers that are detectable in easily collectable biosamples such as blood, saliva, urine or stool and applied to a high-risk population. An abnormal result would allow for the enrichment of the high-risk cohort (increased prevalence of advanced precursor lesions or PDAC); thereby, identifying a subset of the high-risk group that the performance of more expensive and potentially invasive procedures to examine the pancreas is warranted [63].

Up to 80% of PDAC patients are either hyperglycemic or diabetic during the presymptomatic phase and older patients with new onset diabetes mellitus (DM) have about an eight-times higher risk of having PDAC than the general population within the first 3 years of DM diagnosis. As such, recognition of new-onset diabetes as an early indication of PDAC could lead to the identification of a population of non-hereditary high-risk patients that are candidates for PDAC surveillance [64]. Type 2 DM is more prevalent than PDAC-associated DM (PDAC-DM) in the general population; the two forms of DMs are clinically indistinguishable. The development of multiple approaches such as phenotypic, mechanistic, discovery-based and candidate biomarker assessments are underway to differentiate those older patients with new-onset PDAC-DM from type 2 DM. Standard clinical risk factors for DM do not distinguish PDAC-DM from type 2 DM. However, the symptom of weight loss does appear to precede diagnosis of DM in PDAC in many patients and is more common in PDAC-DM than type 2 DM or PDAC patients without DM [65,66]. The following pathophysiologic alterations in PDAC-DM have been observed: 1) insulin levels in PDAC are comparable to that of glycemic controls; 2) despite significant weight loss PDAC have similar insulin resistance to controls; 3) insulin resistance is significantly ameliorated by PDAC resection and 4) adrenomedullin (AM), which inhibits insulin secretion, is up-regulated at both protein and gene expression levels in PDAC [67,68]. In regards to the latter, it was discovered that exosomes were involved in delivering AM to β cells that lead to β-cell dysfunction and death suggesting that PDAC-DM is an “exosomopathy” [69]. The first clinical trial of screening for PDAC in new-onset DM is ongoing (http://www.mayo.edu/research/clinical-trials/cls-20123050).

Research Gaps and Opportunities

A variety of research gaps and opportunities were identified. The early development of metastases when the PDAC tumor is small and asymptomatic is a major factor in the poor survival seen in this cancer, current imaging modalities (CT, MRI, and EUS) are unable to detect advanced precursor lesions (PanIN 3 lesions or intraductal papillary mucinous neoplasm (IPMN) with high-grade dysplasia) or the microscopic spread of disease, which exists in the majority of patients who, despite having undergone an apparently successful resection of their PDAC with complete removal of macroscopic disease (R0 resection), still die from tumor recurrence. Although targeted microbubbles can improve the sensitivity of both transabdominal and endoscopic ultrasound, the specificity of contrast-enhanced ultrasound for differentiating benign from malignant pancreatic lesions is relatively low. Improving the specificity of ultrasound techniques would greatly increase the usefulness of this imaging modality for the early detection and characterization of pancreatic lesions and allow detection of PDAC with very high diagnostic accuracy. Similarly, there is no standardized protocol and data model for DCE-MRI across clinical and research centers. Due to the low incidence of PDAC, it has been recognized that it is not feasible to screen the general population for PDAC. Since only up to 10% of PDAC cases are hereditary, there is a need to identify other patient populations that can be screened for PDAC such as those with new onset diabetes.

Cross-imaging modality research priorities for PDAC are the identification and validation of additional molecular markers and tools that can address the following needs:

1) Development and validation of an imaging platform which allows for single scan identification and evaluation of a tumor with any associated metastases--local and distant

2) Further development of validation of methods to differentiate PDAC from benign diseases and normal tissues, such as DCE-MRI

3) Development and validation of methods to detect malignant transformation of intraductal papillary mucinous neoplasms (IPMNs) to PDAC

4) Development and validation of non-invasive methods to detect advanced precursor lesions (e.g., PanIN-3)

5) Development of methods to accurately identify the extent of viable tumor following neoadjuvant or adjuvant therapy administration

6) Development of a consortium of scientists and clinicians to rapidly translate the promising preclinical methods for detection and assessment of disease to clinical practice: such a consortium might focus on a) technical development and imaging protocols for improving the specificity of ultrasound techniques to differentiate benign from malignant pancreatic lesions, b) standardization in image acquisition and data analysis for DCE-MRI, c) research in contrast enhanced MRI which includes combining DCE-MRI techniques with molecular IV contrast agents to further improve the sensitivity of pancreatic cancer detection, d) elucidating the complex metabolic abnormalities (DM, weight loss,

cachexia) that occur in PDAC and the validation of methods to discriminate PDAC-associated diabetes from new onset type 2 diabetes, and e) creation of a large repository of biospecimens collected and stored with stringent protocols from non- hereditary high-risk groups to address the challenge of the early detection of sporadic PDAC. This could include funding a national consortium to screen subjects aged >50 years with new-onset DM.

Shed exosomes and shed cells

Overview

The development of effective and relevant biomarkers of advanced precancerous pancreatic lesions and early stage, preclinical PDAC has been extremely challenging for numerous reasons. One of the biggest impediments to previous efforts (e.g., blood-based proteomics and imaging approaches) is lack of specificity of signal; this is particularly relevant in the setting of early diagnosis of patients at risk for PDAC, as prophylactic actions in response to a suspected malignancy, namely surgical resection, is associated with significant morbidity. Circulating tumor cells (CTCs) have emerged as a potential way to detect and monitor therapeutic responses as well as potential patient treatment stratification with a minimally invasive blood draw. CTCs are shed early on from the primary tumor into the blood stream and represent clones from the primary tumor. The ability of CTCs to seed metastases as well as their representation of the primary tumor means that analysis of CTCs can be seen as “liquid biopsies” that allow sampling over time and can more accurately inform about patient disease status.

Circulating pancreas cells (CPCs) may represent an ideal source of material that could be used for PDAC risk stratification and even diagnosis of PDAC in patients at elevated risk. As opposed to other assays that seek to identify a surrogate marker of advanced neoplasia, CPCs represent a unique subpopulation of pancreas epithelial cells that have gained additional abilities to gain access to the blood stream and persist in the absence of cell-to-cell contacts and in the face of shear forces in the blood stream—many of the same characteristics acquired by advanced cancer cells during the metastatic cascade. Previously, dogma maintained that epithelial cell seeding of the bloodstream only occurred once large, clinically detectable tumors had formed. However, work utilizing genetically engineered mouse models of PDAC [70] has clearly shown that epithelial cells in circulation can be detected in the absence of large tumors and prior to the clinical diagnosis of cancer. In this study, using genetic lineage labeling, Rhim et al were able to identify significant numbers of pancreas epithelial cells in the circulation only when the pancreas contained PanIN2, PanIN3 and overt PDAC. Further analysis of these CPCs have revealed that the vast majority had altered transcriptional and genetic signatures, suggesting that such cells likely derive from advanced neoplastic lesions, as opposed to normal pancreas tissue. Thus, CPCs may represent a specific and direct sampling of advanced neoplastic pancreas cells that can be obtained via the peripheral venous circulation. This is especially important, as CPCs may actually represent a “liquid biopsy” of advanced lesions in the pancreas, as opposed to a biomarker, obviating the need for invasive procedures to obtain visualization of sub-millimeter lesions.

These exciting results from faithful and robust genetic mouse models of PDAC have been recapitulated in patients at risk for PDAC. In studies focusing on patients with pancreatic cystic lesions (such as IPMNs), Rhim et al were able to utilize a unique microfluidic device to capture significant numbers of CPCs from a portion of these patients [71]. These data provide evidence that epithelial cells can indeed disseminate in the blood stream in the absence of a clinical diagnosis. Based on these data in patients and in genetic mouse models, it has been hypothesized that CPCs represent a highly relevant and unique population of pancreas cells that likely derive from advanced PanIN and PDAC lesions and that the study of these cells will lead to the effective identification of patients poised to develop PDAC or who already harbor this disease, but of a size which is too small to detect on current clinical assays.

Thus, CPCs, as opposed to many other logical biomarker approaches, represent the most specific source material of early PDAC, as these cells likely represent a liquid biopsy of the relevant lesions from the pancreas. Robust clinical studies are already underway to determine the effectiveness of this approach. However, next-generation capture techniques and ultrasensitive genomic-based assays are being developed to build upon previous successful studies in order to potentially achieve an even more precise method to identify the earliest stages of PDAC, long before detection is possible on cross sectional imaging.

Exosomes are vesicles that are secreted from tumor cells and contain protein, DNA, RNA, and miRNAs from the tumor. Like CTCs, the enumeration of exosomes could allow early detection of cancer, monitoring therapeutic responses, and inform patient treatment stratification. One of the major challenges emerging in the field of exosome utilization for clinical use is the lack of robust and reproducible methods for the isolation of a pure vesicular population. There is a lack of clear consensus for an optimal method of isolation of a pure exosomal population that is devoid of contamination with similar sized microvesicles of different origins. This is a major hurdle for the utilization of exosomes and its components for clinical use and early detection and screening of cancer [72]. In addition to population heterogeneity, many proteins and other biomolecules may bind/associate nonspecifically to the exosomal surface and confound the analysis of exosomal composition. In traditional methods, exosomes are purified preferably by a combination of differential centrifugation and ultracentrifugation, filtration, concentration, and immmunocapture using antibody-impregnated beads. The ultrafiltration is performed using a 100 to 220 nm filter followed by a 100,000 × g ultracentrifugation in a sucrose gradient to create an exosome pellet. These methods allow for the removal of cellular debris and larger microvesicles while concentrating exosomes. High-performance liquid chromatography (HPLC)-based protocols can potentially allow one to obtain highly pure exosomes, although the methodology has limitations. Exosome purification kits are commercially available; however, the isolation technology needs further improvement as kits have been reported to isolate non-exosomal contaminants in some cases. A recent study that conducted a comprehensive evaluation of a few methods of exosome isolation including ultracentrifugation, OptiPrep density-based separation, and immunoaffinity capture using anti-EpCAM coated magnetic beads, found immunoaffinity capture to be the most effective method. Although comprehensive, it has been suggested that the serial isolation steps do not allow complete recovery of the secreted exosomes at any given time. In addition, it was presumed that only a fraction of secreted

exosomes may be captured during purification because they are reabsorbed by cells through phagocytosis. At this time, because of their small size, visualization by electron microscopy is the only accepted method for the assessment of the purity of exosomal population. Exosomes appear as spherical or cup-shaped bilayered membrane-bound vesicles in electron micrographs. Some speculate that the cup shape may be formed due to membrane shrinkage during the fixation procedure.

New approaches to purifying and enumerating shed exomes in PDAC are currently in development. A miniaturized platform has been developed for point-of-care exosome analysis in which two core technologies are combined: 1) in-flow filtration devices to enrich exosomes directly from biological fluids and 2) a nano-plasmonic chip to profile exosomal proteins. This device uses size-based sonophoresis to selectively enrich exosomes. The plasmonic chip, termed nano-plasmonic exosome (nPLEX), provides high-throughput exosome protein profiling. The nPLEX detection is based on optical transmission through an array of nanoholes. The strategy provides an ideal sensing scheme for exosomes, as the probing depth (< 200 nm) of the sensor could be matched to exosome size. Preliminary data show a promising potential of using exosome diagnostics for non-invasive identification of PDAC.

Research Gaps and Opportunities

Techniques are needed to efficiently capture and study CTCs, exosomes and their contents. Specific research priorities include:

1. Standardization of methods for exosome isolation, purification, and characterization.
2. Validation studies of isolated CTCs from patients at high risk for developing PDAC to determine when cancer cells first appear in the blood.
3. Development and validation of technical methods to sensitively enumerate CTCs
4. Development of technology to improve the capture of CTCs and exosomes for further analysis

Conclusions

The workshop highlighted some of the exciting new developments in anatomical, tissue, and cellular imaging which are expanding the ability to detect early-stage pancreatic disease. Molecular imaging, with probes combined with magnetic resonance, ultrasound, and other modalities, are providing new opportunities to non-invasively detect malignancy at a cellular level. The ability to capture shed cells and shed exosomes has the potential to dramatically change the way clinicians can screen for PDAC in high-risk subjects. These and other technological advances have the potential to bring the detection and treatment of pancreatic disease to a new level, and may change our ability to alter the course of benign and malignant disease. Much work needs to be done to refine and validate these new methods, and more research is clearly needed to achieve the goal of transforming our ability to effectively treat patients with pancreatic disease. The workshop made clear, however, that

this goal is now more attainable than ever before, and engaged both scientists and clinical investigators in this quest.

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References

1. Ammann RW, Buehler H, Muench R, et al. Differences in the natural history of idiopathic (nonalcoholic) and alcoholic chronic pancreatitis. A comparative long-term study of 287 patients. Pancreas. 1987; 2:368–377. [PubMed: 3628234]
2. Lankisch PG, Lohr-Happe A, Otto J, et al. Natural course in chronic pancreatitis. Pain, exocrine and endocrine pancreatic insufficiency and prognosis of the disease. Digestion. 1993; 54:148–155. [PubMed: 8359556]
3. Layer P, Yamamoto H, Kalthoff L, et al. The different courses of early- and late-onset idiopathic and alcoholic chronic pancreatitis. Gastroenterology. 1994; 107:1481–1487. [PubMed: 7926511]
4. Wilcox CM, Yadav D, Ye T, et al. Chronic pancreatitis pain pattern and severity are independent of abdominal imaging findings. Clin Gastroenterol Hepatol. 2015; 13:552–60. quiz e28-29. [PubMed: 25424572]
5. Bhutani MS, Arantes VN, Verma D, et al. Histopathologic correlation of endoscopic ultrasound findings of chronic pancreatitis in human autopsies. Pancreas. 2009; 38:820–824. [PubMed: 19657310]
6. Yusoff IF, Sahai AV. A prospective, quantitative assessment of the effect of ethanol and other variables on the endosonographic appearance of the pancreas. Clin Gastroenterol Hepatol. 2004; 2:405–409. [PubMed: 15118979]
7. van Geenen EJ, Smits MM, Schreuder TC, et al. Smoking is related to pancreatic fibrosis in humans. Am J Gastroenterol. 2011; 106:1161–1166. quiz 1167. [PubMed: 21577244]
8. Al-Haddad M, Khashab M, Zyromski N, et al. Risk factors for hyperechogenic pancreas on endoscopic ultrasound: a case-control study. Pancreas. 2009; 38:672–675. [PubMed: 19506531]
9. Vitale GC, Davis BR, Zavaleta C, et al. Endoscopic retrograde cholangiopancreatography and histopathology correlation for chronic pancreatitis. Am Surg. 2009; 75:649–653. discussion 653. [PubMed: 19725285]
10. LeBlanc JK, Chen JH, Al-Haddad M, et al. Endoscopic ultrasound and histology in chronic pancreatitis: how are they associated? Pancreas. 2014; 43:440–444. [PubMed: 24622076]
11. Varadarajulu S, Eltoum I, Tamhane A, et al. Histopathologic correlates of noncalcific chronic pancreatitis by EUS: a prospective tissue characterization study. Gastrointest Endosc. 2007; 66:501–509. [PubMed: 17640639]
12. Albashir S, Bronner MP, Parsi MA, et al. Endoscopic ultrasound, secretin endoscopic pancreatic function test, and histology: correlation in chronic pancreatitis. Am J Gastroenterol. 2010; 105:2498–2503. [PubMed: 20606675]

13. Chong AK, Hawes RH, Hoffman BJ, et al. Diagnostic performance of EUS for chronic pancreatitis: a comparison with histopathology. Gastrointest Endosc. 2007; 65:808–814. [PubMed: 17466199]
14. Wallace MB, Hawes RH, Durkalski V, et al. The reliability of EUS for the diagnosis of chronic pancreatitis: interobserver agreement among experienced endosonographers. Gastrointest Endosc. 2001; 53:294–299. [PubMed: 11231386]
15. Gardner TB, Gordon SR. Interobserver agreement for pancreatic endoscopic ultrasonography determined by same day back-to-back examinations. J Clin Gastroenterol. 2011; 45:542–545. [PubMed: 20921903]
16. Catalano MF, Sahai A, Levy M, et al. EUS-based criteria for the diagnosis of chronic pancreatitis: the Rosemont classification. Gastrointest Endosc. 2009; 69:1251–1261. [PubMed: 19243769]
17. Stevens T, Lopez R, Adler DG, et al. Multicenter comparison of the interobserver agreement of standard EUS scoring and Rosemont classification scoring for diagnosis of chronic pancreatitis. Gastrointest Endosc. 2010; 71:519–526. [PubMed: 20189510]
18. Del Pozo D, Poves E, Tabernero S, et al. Conventional versus Rosemont endoscopic ultrasound criteria for chronic pancreatitis: interobserver agreement in same day back-to-back procedures. Pancreatology. 2012; 12:284–287. [PubMed: 22687386]
19. 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; 65:971–978. [PubMed: 17531630]
20. Xu W, Shi J, Li X, et al. Endoscopic ultrasound elastography for differentiation of benign and malignant pancreatic masses: a systemic review and meta-analysis. Eur J Gastroenterol Hepatol. 2013; 25:218–224. [PubMed: 23169307]
21. Janssen J, Papavassiliou I. Effect of aging and diffuse chronic pancreatitis on pancreas elasticity evaluated using semiquantitative EUS elastography. Ultraschall Med. 2014; 35:253–258. [PubMed: 24327468]
22. Wu B, Conwell DL. The endoscopic pancreatic function test. Am J Gastroenterol. 2009; 104:2381–2383. [PubMed: 19806083]
23. Stevens T, Conwell DL, Zuccaro G Jr, et al. A prospective crossover study comparing secretin-stimulated endoscopic and Dreiling tube pancreatic function testing in patients evaluated for chronic pancreatitis. Gastrointest Endosc. 2008; 67:458–466. [PubMed: 18294508]
24. Ketwaroo G, Brown A, Young B, et al. Defining the accuracy of secretin pancreatic function testing in patients with suspected early chronic pancreatitis. Am J Gastroenterol. 2013; 108:1360–1366. [PubMed: 23711627]
25. Kadiyala V, Lee LS, Banks PA, et al. Cigarette smoking impairs pancreatic duct cell bicarbonate secretion. JOP. 2013; 14:31–38. [PubMed: 23306332]
26. LaRusch J, Jung J, General IJ, et al. Mechanisms of CFTR functional variants that impair regulated bicarbonate permeation and increase risk for pancreatitis but not for cystic fibrosis. PLoS Genet. 2014; 10:e1004376. [PubMed: 25033378]
27. Abu Dayyeh BK, Conwell D, Buttar NS, et al. Pancreatic juice prostaglandin e2 concentrations are elevated in chronic pancreatitis and improve detection of early disease. Clin Transl Gastroenterol. 2015; 6:e72. [PubMed: 25630864]
28. Venkatesh SK, Yin M, Ehman RL. Magnetic resonance elastography of liver: technique, analysis, and clinical applications. J Magn Reson Imaging. 2013; 37:544–555. [PubMed: 23423795]
29. Singh S, Venkatesh SK, Wang Z, et al. Diagnostic performance of magnetic resonance elastography in staging liver fibrosis: a systematic review and meta-analysis of individual participant data. Clin Gastroenterol Hepatol. 2015; 13:440–451. e6. [PubMed: 25305349]
30. Shi Y, Glaser KJ, Venkatesh SK, et al. Feasibility of using 3D MR elastography to determine pancreatic stiffness in healthy volunteers. J Magn Reson Imaging. 2015; 41:369–375. [PubMed: 24497052]
31. Yadav D, O'Connell M, Papachristou GI. Natural history following the first attack of acute pancreatitis. Am J Gastroenterol. 2012; 107:1096–1103. [PubMed: 22613906]

32. Oiva J, Mustonen H, Kylanpaa ML, et al. Patients with acute pancreatitis complicated by organ dysfunction show abnormal peripheral blood polymorphonuclear leukocyte signaling. Pancreatology. 2013; 13:118–124. [PubMed: 23561969]
33. Xue J, Sharma V, Hsieh MH, et al. Alternatively activated macrophages promote pancreatic fibrosis in chronic pancreatitis. Nat Commun. 2015; 6:7158. [PubMed: 25981357]
34. Zhang H, Neuhofer P, Song L, et al. IL-6 trans-signaling promotes pancreatitis-associated lung injury and lethality. J Clin Invest. 2013; 123:1019–1031. [PubMed: 23426178]
35. Emmrich J, Weber I, Nausch M, et al. Immunohistochemical characterization of the pancreatic cellular infiltrate in normal pancreas, chronic pancreatitis and pancreatic carcinoma. Digestion. 1998; 59:192–198. [PubMed: 9643678]
36. Apte MV, Pirola RC, Wilson JS. Pancreatic stellate cells: a starring role in normal and diseased pancreas. Front Physiol. 2012; 3:344. [PubMed: 22973234]
37. Schmitz-Winnenthal H, Pietsch DH, Schimmack S, et al. Chronic pancreatitis is associated with disease-specific regulatory T-cell responses. Gastroenterology. 2010; 138:1178–1188. [PubMed: 19931255]
38. Swanson BJ, McDermott KM, Singh PK, et al. MUC1 is a counter-receptor for myelin-associated glycoprotein (Siglec-4a) and their interaction contributes to adhesion in pancreatic cancer perineural invasion. Cancer Res. 2007; 67:10222–10229. [PubMed: 17974963]
39. Eggers JP, Grandgenett PM, Collisson EC, et al. Cyclin-dependent kinase 5 is amplified and overexpressed in pancreatic cancer and activated by mutant K-Ras. Clin Cancer Res. 2011; 17:6140–6150. [PubMed: 21825040]
40. Fink DM, Connor AL, Kelley PM, et al. Nerve growth factor regulates neurolymphatic remodeling during corneal inflammation and resolution. PLoS One. 2014; 9:e112737. [PubMed: 25383879]
41. Kelleher SL, McCormick NH, Velasquez V, et al. Zinc in specialized secretory tissues: roles in the pancreas, prostate, and mammary gland. Adv Nutr. 2011; 2:101–111. [PubMed: 22332039]
42. Costello LC, Franklin RB. Novel role of zinc in the regulation of prostate citrate metabolism and its implications in prostate cancer. Prostate. 1998; 35:285–296. [PubMed: 9609552]
43. Franklin RB, Costello LC. Zinc as an anti-tumor agent in prostate cancer and in other cancers. Arch Biochem Biophys. 2007; 463:211–217. [PubMed: 17400177]
44. Sanyal R, Darroudi F, Parzefall W, et al. Inhibition of the genotoxic effects of heterocyclic amines in human derived hepatoma cells by dietary bioantimutagens. Mutagenesis. 1997; 12:297–303. [PubMed: 9237777]
45. Esqueda AC, Lopez JA, Andreu-de-Riquer G, et al. A new gadolinium-based MRI zinc sensor. J Am Chem Soc. 2009; 131:11387–11391. [PubMed: 19630391]
46. Lubag AJ, De Leon-Rodriguez LM, Burgess SC, et al. Noninvasive MRI of beta-cell function using a Zn2+-responsive contrast agent. Proc Natl Acad Sci U S A. 2011; 108:18400–18405. [PubMed: 22025712]
47. Paulo JA, Kadiyala V, Lee LS, et al. Proteomic analysis (GeLC-MS/MS) of ePFT-collected pancreatic juice in chronic pancreatitis. J Proteome Res. 2012; 11:1897–1912. [PubMed: 22243521]
48. Garai E, Sensarn S, Zavaleta CL, et al. A real-time clinical endoscopic system for intraluminal, multiplexed imaging of surface-enhanced Raman scattering nanoparticles. PLoS One. 2015; 10:eD123185.
49. Rogalla S, Contag CH. Early cancer detection at the epithelial surface. Cancer J. 2015; 21:179–187. [PubMed: 26049697]
50. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015; 65:5–29. [PubMed: 25559415]
51. Canto MI, Harinck F, Hruban RH, et al. International Cancer of the Pancreas Screening (CAPS) Consortium summit on the management of patients with increased risk for familial pancreatic cancer. Gut. 2014; 62:339–347. [PubMed: 23135763]
52. Dimastromatteo J, Houghton JL, Lewis JS, et al. Challenges of Pancreatic Cancer. Cancer J. 2015; 21:188–193. [PubMed: 26049698]
53. Reynolds F, Panneer N, Tutino CM, et al. A functional proteomic method for biomarker discovery. PLoS One. 2011; 6:e22471. [PubMed: 21811618]

54. Pysz MA, Machtaler SB, Seeley ES, et al. Vascular endothelial growth factor receptor type 2-targeted contrast-enhanced US of pancreatic cancer neovasculature in a genetically engineered mouse model: potential for earlier detection. Radiology. 2015; 274:790–799. [PubMed: 25322341]
55. Foygel K, Wang H, Machtaler S, et al. Detection of pancreatic ductal adenocarcinoma in mice by ultrasound imaging of thymocyte differentiation antigen 1. Gastroenterology. 2013; 145:885–894. e3. [PubMed: 23791701]
56. Viola-Villegas NT, Carlin SD, Ackerstaff E, et al. Understanding the pharmacological properties of a metabolic PET tracer in prostate cancer. Proc Natl Acad Sci U S A. 2014; 111:7254–7259. [PubMed: 24785505]
57. Huang W, Li X, Morris EA, et al. The magnetic resonance shutter speed discriminates vascular properties of malignant and benign breast tumors in vivo. Proc Natl Acad Sci U S A. 2008; 105:17943–17948. [PubMed: 19004780]
58. Jacobs I, Strijkers GJ, Keizer HM, et al. A novel approach to tracer-kinetic modeling for (macromolecular) dynamic contrast-enhanced MRI. Magn Reson Med. Apr 4.2015 [Epub ahead of print].
59. Obuz F, Bora S, Sarioglu S. Malignant islet cell tumor of the pancreas associated with portal venous thrombus. Eur Radiol. 2001; 11:1642–1644. [PubMed: 11511884]
60. Akisik MF, Sandrasegaran K, Bu G, et al. Pancreatic cancer: utility of dynamic contrast-enhanced MR imaging in assessment of antiangiogenic therapy. Radiology. 2010; 256:441–449. [PubMed: 20515976]
61. Bali MA, Metens T, Denolin V, et al. Tumoral and nontumoral pancreas: correlation between quantitative dynamic contrast-enhanced MR imaging and histopathologic parameters. Radiology. 2011; 261:456–466. [PubMed: 21852570]
62. Yao X, Zeng M, Wang H, et al. Evaluation of pancreatic cancer by multiple breath-hold dynamic contrast-enhanced magnetic resonance imaging at 3.0T. Eur J Radiol. 2012; 81:e917–922. [PubMed: 22695786]
63. Kenner BJ, Chari ST, Cleeter DF, et al. Early Detection of Sporadic Pancreatic Cancer: Strategic Map for Innovation-A White Paper. Pancreas. 2015; 44:686–692. [PubMed: 25938853]
64. Pannala R, Basu A, Petersen GM, et al. New-onset diabetes: a potential clue to the early diagnosis of pancreatic cancer. Lancet Oncol. 2009; 10:88–95. [PubMed: 19111249]
65. Pannala R, Leirness JB, Bamlet WR, et al. Prevalence and clinical profile of pancreatic cancer-associated diabetes mellitus. Gastroenterology. 2008; 134:981–987. [PubMed: 18395079]
66. Hart PA, Kamada P, Rabe KG, et al. Weight loss precedes cancer-specific symptoms in pancreatic cancer-associated diabetes mellitus. Pancreas. 2011; 40:768–772. [PubMed: 21654538]
67. Gibbons C, Dackor R, Dunworth W, et al. Receptor activity-modifying proteins: RAMPing up adrenomedullin signaling. Mol Endocrinol. 2007; 21:783–796. [PubMed: 17053041]
68. Aggarwal G, Ramachandran V, Javeed N, et al. Adrenomedullin is up-regulated in patients with pancreatic cancer and causes insulin resistance in beta cells and mice. Gastroenterology. 2012; 143:1510–1517. e1. [PubMed: 22960655]
69. Korc M. Pancreatic cancer-associated diabetes is an “exosomopathy”. Clin Cancer Res. 2015; 21:1508–1510. [PubMed: 25645860]
70. Rhim AD, Mirek ET, Aiello NM, et al. EMT and dissemination precede pancreatic tumor formation. Cell. 2012; 148:349–361. [PubMed: 22265420]
71. Rhim AD, Thege FI, Santana SM, et al. Detection of circulating pancreas epithelial cells in patients with pancreatic cystic lesions. Gastroenterology. 2014; 146:647–651. [PubMed: 24333829]
72. Tauro BJ, Greening DW, Mathias RA, et al. Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. Methods. 2012; 56:293–304. [PubMed: 22285593]