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Protein Signatures and Tissue Diagnosis of Pancreatic Cancer

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Abstract

Background—Endoscopic ultrasound-guided fine needle aspiration (EUS-guided FNA) fails to diagnose up to 25% of patients with pancreatic ductal adenocarcinoma (PDAC). Proteomics may help to overcome this clinical dilemma. We hypothesized that soluble protein signatures can differentiate PDAC from benign tissues.

Study Design—Tissues were obtained from resected surgical specimens, lysed, and homogenates collected for analysis with a 41-protein multiplex assay. Analyte concentrations were normalized to total protein. Statistical analysis was performed to evaluate for differences in PDAC vs benign tissue.

Results—Tissues were obtained from 159 patients, 82 patients with PDAC naïve to therapy and 77 with benign pancreatic pathology. Fourteen analytes had a receiver operating characteristic (ROC) curve area of >0.75 for predicting PDAC vs benign tissue. A recursive partitioning model using only two analytes, IL-1RA and TGF α , provided an accuracy, sensitivity, and specificity of 91.2%, 90.2%, and 92.2%, respectively. A penalized logistic regression model found 12 analytes that provide diagnostic value to a protein signature. The average area under the ROC after 50 10-

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Study conception and design: Underwood, Gerber, Nguyen, Delitto, Han, Thomas, Trevino, Gooding, Hughes. Acquisition of data: Underwood, Gerber, Nguyen, Delitto. Analysis and interpretation of data: Underwood, Hughes, Gooding. Drafting of manuscript: Underwood, Hughes, Gooding. Critical Revision: Underwood, Gerber, Nguyen, Delitto, Han, Thomas, Forsmark, Trevino, Gooding, Hughes

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fold cross validations was 0.951. Accuracy, sensitivity, and specificity of this model were 91.2%, 87.8%, and 94.8%, respectively. Applying the scenario of 80% disease prevalence in patients undergoing EUS with FNA for a pancreatic head mass, positive predictive value is 98.5% (95% CI 93.0%–99.7%) and negative predictive value is 66.0% (95% CI 54.9%–75.6%).

Conclusion—Protein signatures from pancreatic specimens can differentiate PDAC from benign tissue. Further work to validate these findings in a unique sample set is warranted.

Précis

Distinguishing pancreatic cancer from benign disease remains a clinical dilemma in the preoperative setting. Protein signatures obtained from the tumor microenvironment can distinguish pancreatic ductal adenocarcinoma from benign tissue.

Graphical Abstract

Keywords

Pancreatic; endoscopic ultrasound; fine needle aspirate; molecular diagnostics; proteomics

Introduction

For a patient presenting with a pancreatic mass, obtaining adequate tissue for diagnosis is challenging. Presently, the gold standard for biopsy of a pancreatic mass is endoscopic ultrasound-guided fine needle aspiration (EUS-guided FNA), but this fails to provide a definitive diagnosis in up to 15–25% of patients with pancreatic ductal adenocarcinoma (PDAC) (1–4). These non-diagnostic EUS-guided FNA result in the need for repeat procedures, delays in treatment, exclusion from neoadjuvant therapy clinical trials, and decision making in the absence of definitive data. Thus, a non-diagnostic EUS-guided FNA also places considerable stress on the patient and the treating physician. Further, as neoadjuvant therapeutic approaches gain favor, the ability to obtain adequate sample to confirm a PDAC diagnosis has become increasingly critical (5).

Highlighting this problem is considerable literature exploring optimum EUS-guided FNA sampling and analysis techniques. Randomized trials have evaluated needle type and gauge, number of needle passes, stylet use, use of suction, and presence of on-site cytopathology to determine best practices (6–10). Elastography, fluorescence in situ hybridization (FISH), K-RAS analysis, microRNAs (miRNA), immunostaining, and RNAseq analysis have all been proposed as supplementary tests to cytopathology but none have gained widespread use (11–14). Here, we explore the inflammatory milieu of the PDAC microenvironment as a potential diagnostic supplement to cytopathology. Desmoplastic stroma represents as much as 80% of the overall PDAC tumor volume (15). It is composed of extracellular matrix, stellate cells, fibroblasts, and immune cells. The interaction between the pancreatic cancer cells and surrounding tumor-associated stroma is presently the subject of intense investigation. Included in this interaction is secretion of cytokines, chemokines, and growth factors. Previous work by our lab demonstrated that the inflammatory milieu within the pancreatic cancer microenvironment correlates with clinico-pathologic parameters, chemoresistance, and survival. It found 23 proteins that were significantly different in concentration between PDAC and chronic pancreatitis or PDAC and benign tissue (16). Thus, we hypothesized that protein signatures derived from these differences can differentiate PDAC from benign tissue.

Methods

Patient Sample Selection

All studies were approved by the Institutional Review Board at the University of Florida (IRB201600873). Informed consent was obtained from all patients. Tissue samples were obtained from a prospectively maintained tissue bank. Consecutive patients with pathologically confirmed PDAC and an adequate tissue sample who did not receive neoadjuvant chemotherapy or radiation were included. The collection period was from October 14, 2011 – April 23, 2018. Tissue from patients with other malignant diagnoses of the pancreas were excluded. Benign and chronic pancreatitis specimens were collected from patients undergoing pancreatic resection for benign disease and chronic pancreatitis, respectively. No benign or chronic pancreatitis specimens were collected from patients with PDAC. Forty-six benign pancreas samples were available in our tissue bank, and 31 additional benign specimens were obtained from the Network for Pancreatic Organ Donors (nPOD). nPOD collects pancreatic tissue for research purposes in organ donors who have been declared brain dead. The clinico-pathologic parameters and circumstances of death for these patients are unavailable. While the tissue grossly represents normal pancreas, this was unable to be confirmed with histology.

Tissue Collection and Preparation

Immediately after removal of the surgical specimen, malignant, chronic pancreatitis, or benign pancreatic tissue was collected by sharp dissection on a back table in the operating room. For benign specimens, normal pancreatic tissue remote from any pathology and characterized by the operative surgeon as visibly uninvolved by inflammation is taken. It is important to note that none of the benign specimens were taken from patients undergoing surgery for malignant disease. In patients undergoing surgery for chronic pancreatitis, tissue involved by chronic inflammation was collected. Tumor tissue was taken from patients with

PDAC. Samples were immediately transported on ice in DMEM media supplemented with 10% fetal bovine serum (Lonza Group, Basel, Switzerland) and antibiotic/antimycotic solution (Corning Inc., Corning, NY, USA). Samples were flash frozen in liquid nitrogen within 20 minutes of collection and stored at -80°C . nPOD specimens were resected at the time of organ donation immediately after the donor organs are procured (17, 18). These tissues have been infused with University of Wisconsin Solution.

At the time of processing, tissues were thawed and weighed. They were sharply divided into small pieces and placed into 2mL lysing matrix D tubes (MP Biomedicals, Santa Ana, CA, USA). For every 30 mg of tissue, 500 μL of cell lysis buffer (Cell Signaling, Danvers, MA, USA) with Protease/Phosphatase Inhibitor (Cell Signaling Technology, Danvers, MA, USA) was added. Samples underwent bead homogenization at 50 Hz for 40 seconds for three cycles (Qiagen TissueLyser, Venlo, Netherlands). Samples were placed on ice for two minutes between each cycle. Lysates were collected and centrifuged at 13,000 RCF for 10 minutes. Supernatants were then collected and analyzed for total protein concentration (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific, Waltham, MA, USA).

Soluble Protein Analysis

Homogenates were probed for 41 unique analytes using a commercially available, multiplex assay per the manufacturer's protocol (Catalog# HCYTMAG-60K-PX41, Millipore Sigma, Burlington, MA, USA) and as previously described (16). The multiplex assay was selected for its large inflammatory panel to provide data for exploratory analysis since the particular proteins that would contribute to a multivariate model were unknown, and the promiscuity of a customized bead could be avoided. Data were acquired with the MAGPIX system (Luminex Corporation, Austin, TX, USA) and analyzed using MILLIPLEX Analyst 5.1 (Millipore Sigma, Burlington, MA, USA). Individual protein concentrations were normalized to total protein concentration to yield individual analyte concentrations in pg/mg of total protein.

Statistical Analysis

Group comparisons in table 1 used Fishers' Exact test for gender, the Wilcoxon test for age and an exact chi-square test for race. Analyte expression concentrations were natural log transformed and then standardized to have mean zero and standard deviation one. Analytes that lacked complete, informative results for all 159 subjects were excluded, reducing the panel of analyzed proteins to 31. Variable importance was measured as the area under a receiver operating characteristic (ROC) curve for each analyte. Two classifiers were developed. The first, recursive partitioning, was presented with three loss functions. One loss function treated both types of misclassification (false positive, false negative) equally. The other two models unequally penalized misclassification by penalizing either false negatives (for improved sensitivity) or false positives (for improved specificity). The second classifier used penalized logistic regression in which the likelihood function is optimized based on cross-validation. The optimized regression equation was itself tested by 50 repetitions of leave-10-out cross-validation. This process replicated the complete penalized regression process including selecting the maximum likelihood value, shrinking the regression coefficients, and predicting outcome based on the remaining non-zero coefficients.

Presentation of the penalized regression is based on average cross-validated estimates. For generalizing model results beyond the observed prevalence of PDAC in our cohort, Bayes Theorem was used to derive the resulting positive and negative predictive values for a range of disease prevalence. All statistical analyses were conducted using R software version 3.5.1 (19). Penalized logistic regression was conducted with the R package penalized (20).

Results

Clinico-pathologic Parameters

Tumor tissue was collected from 82 patients with surgically resectable PDAC who did not receive neoadjuvant therapy and 77 patients with non-malignant conditions. Patient clinicopathologic parameters are displayed in Table 1. Mean age ($\pm$ standard deviation) was significantly higher in patients undergoing surgery for PDAC than benign pancreatic conditions (69.7 ± 9.1 years vs. 54.2 ± 9.1 years, $p < 0.001$). A higher percentage of males underwent surgery for PDAC than benign pancreatic conditions (61% vs 45.7%, $p = 0.10$). The majority of patients were Non-Hispanic, White in both groups. Sixty-nine (84.1%) patients underwent a pancreatoduodenectomy for PDAC. Thirty-one of the benign specimens (40.8%) were obtained from unused pancreata from deceased transplant donors. Tissue was collected from twenty-seven patients (35.1%) undergoing surgery for chronic pancreatitis. Of the patients whose samples were categorized as benign tissue, 18 underwent pancreatoduodenectomy, 15 underwent distal pancreatectomy, 12 underwent Frey's procedure, and 1 underwent a Puestow Procedure. Oncologic factors for patients with PDAC including stage, tumor differentiation, lymph node status, and margin status are displayed in Table 1.

Heatmap Pattern Analysis

Of the 41 analytes assayed, 10 were excluded as the analyte concentrations were under the lower limits of the assay's detection thresholds. Thus, 31 analytes underwent complete data analysis. Analyte concentrations were logged and standardized with a mean of zero and standard deviation of one. A supervised heatmap of analyte concentrations is shown in Figure 1. The samples are organized by tissue type as shown on the left. There are patterns of homogeneity between patients with PDAC and benign tissue. The PDAC samples tend to have analytes with higher z-scores while the benign samples tend to have lower z-scores. Pancreatitis samples appear more heterogeneous. The samples on the left cluster tend to have higher z-scores, similar to PDAC and the right cluster tends to have lower z-scores, similar to benign tissue. The patterns within the heatmap support our hypothesis that tissue-type differences in analyte concentrations might be exploited for diagnostic purposes.

Area Under the Receiver Operating Characteristic Curves

Univariate analysis was performed on all 31 analytes to develop ROC curves. Figure 2 demonstrates the sorted area under the curve (AUC) for each of the thirty-one analytes. A PDAC diagnostic cut-off of an AUC greater than 0.75 for any single analyte identified 14 analytes for additional assessment (Figure 2). Of note, Interleukin-1 Receptor Antagonist (IL-1RA) independently had an AUC of 0.94.

Recursive Partitioning Models

Recursive partitioning was performed using individual protein concentrations which are normalized to total protein and then natural log transformed and standardized with a mean of zero and standard deviation of one. The model was run three times to optimize for either accuracy, sensitivity, or specificity. Figure 3 demonstrates the recursive partitioning model optimized for accuracy when the weight of a false negative and false positive were equal. The accuracy of this classification method was 91.2%. Sensitivity and specificity were 90.2% and 92.2%, respectively. eFigure 1 and eFigure 2 demonstrate the recursive partitioning model optimized for sensitivity and specificity, respectively.

Penalized Logistic Regression Model

A penalized logistic regression model was used for multivariate analysis to reduce the rate of false discovery and to enhance ability to generalize to other data. Prior to building the model, the data is first natural log transformed and standardized with a mean of zero and standard deviation of one. Twelve of the thirty one analytes had non-zero coefficients (Figure 4A) suggesting they hold diagnostic value when applied in this model. The logistic model formula is $\text{Ln} [p / (1-p)] = -.316 * \text{Flt-3L} - .041 * \text{Fractalkine} - 1.308 * \text{GRO} + .049 * \text{IL-10} - .054 * \text{PDGF-AA} - .062 * \text{IL-15} + .0002 * \text{TGF}\alpha + 1.972 * \text{IL-1Ra} + .150 * \text{IL-1a} + .078 * \text{IL-6} + 1.191 * \text{IL-8} + .982 * \text{IP-10}$, where p = the probability of malignant tissue. This method demonstrated a ROC with an AUC of 0.975 (95% CI: 0.948–1) (Figure 4B). The ROC curve after 50 10-fold cross validations is displayed in Figure 4C. The AUC decreased slightly to 0.951 (95% CI: 0.913–0.989).

A risk score for likelihood of PDAC was generated from this model for each sample (Figure 5A). The risk score ranged from 0–1.0. The risk-score cutoff with the highest diagnostic accuracy was 0.492. At this cutoff, there were 4 false positives and 10 false negatives. The accuracy, sensitivity, and specificity were 91.2%, 87.8%, and 94.8%, respectively. Figure 5B demonstrates the logistic model predicted risk score with chronic pancreatitis as a separate group from other benign pancreatic tissues. The majority of the pancreatitis samples fell under the cutoff of 0.492. There was a single false positive. In our cohort the prevalence of PDAC was $81/159 = 52\%$. Positive predictive value (PPV) and negative predictive value (NPV) for the penalized logistic regression model were calculated across a range for disease prevalence (Figure 6). In general, for a patient undergoing EUS-guided FNA for a pancreatic mass, the prevalence of PDAC is approximately 80% (1, 4). Under this assumption of 80% prevalence, our model provides for a PPV of 98.5% (95% CI 93.0%–99.7%) and NPV of 66.0% (95% CI 54.9%–75.6%).

Discussion

The above data demonstrate differences in protein signatures present in PDAC, chronic pancreatitis, and benign tissue. Importantly, in the context of a potential future application in EUS-guided FNA, protein signatures from chronic pancreatitis tissue were classified with benign tissue with the exception of a single false positive. Univariate and multivariate models were able to classify tissue as PDAC or benign with a high degree of accuracy,

sensitivity, and specificity. If validated in EUS-guided FNA specimens, these models could provide objective data that could assist the cytopathologist in diagnosis of PDAC.

Efforts to improve diagnostic yield of EUS-guided FNA have been proposed but have yet to gain clinical acceptance. Early efforts focused on immunostaining of a number of proteins, including IMP3, S100, p53, and MIB-1, and have yielded varied results (21, 22). Imaging adjuncts to EUS have also been investigated. Elastography is an ultrasound modality which measures tissue stiffness. It has been identified as a sensitive modality to assist with the diagnosis of PDAC during EUS-guided FNA, but is presently limited by poor specificity (11, 23). Similarly, RNA sequencing has been shown to distinguish malignant from benign lesions with high sensitivity (87%) but lower specificity (75%). Further, there was inadequate RNA in 9 of the 48 enrolled subjects, limiting this adjunct in a similar way to cytopathology (13). MicroRNA signatures also hold promise to improve the diagnostic accuracy of EUS-guided FNA with specificity of 85–95% at the cost of sensitivity of 81–83% (24, 25). Combination FISH and K-ras analysis hold promise with higher sensitivity (87.9%) and specificity (93.8%), but are not readily available at many centers and need further validation (12).

Protein signatures from the tumor microenvironment have been previously been explored in a number of other cancers for a variety of purposes. Similar to PDAC, about 22% of FNAs from thyroid nodules yield indeterminate pathology (26). Many of these patients undergo unilateral thyroid lobectomy to determine pathology. Adjunctive tests to cytopathology could potentially spare patients from surgery. Galectin-3, Hectortin-1, Mesothelial-1 (HBME-1), and CD44v6 have been proposed as molecular markers separately or in combination to improve the accuracy of FNA with sensitivity and specificity as high as 88% and 98%, respectively (27, 28). Protein signatures in ovarian cancer have been proposed as serum biomarkers as well as to predict prognosis and chemosensitivity (29–31). Similar biomarker and prognostic markers have been proposed in lung cancer and breast cancer (32–34). Additional investigation into protein signatures in PDAC may provide similar utility in prognosis and chemosensitivity and deserves investigation.

Interestingly, in our work, IL-1RA alone is quite accurate for identifying PDAC with an AUC of 0.94. Previous work has shown both IL-1RA and IL-1 β to be increased in the serum of pancreatic cancer patients (35). As its name suggests, IL-1RA is an antagonist of the IL-1 receptor. As such, it blocks the pro-inflammatory response to IL-1 β . The reason for the elevated IL-1RA levels in tumor is unclear. It may be present to attenuate pancreatic inflammation seen in many PDAC patients. An immunosuppressive microenvironment is known to be important for PDAC progression but a relationship between immune tolerance and IL-1RA is speculative (36). There is conflicting data on the role of IL-1RA in pancreatic cancer. It has previously been reported to increase pancreatic intraepithelial neoplasia (PanIN) cell proliferation (37).

Conversely, other work has shown it to inhibit cell proliferation, migration, and invasion in PDAC cell lines (38). Further work to investigate the role of IL-1RA in the tumor microenvironment is warranted. While IL-1RA is accurate for the diagnosis of PDAC, multivariate models improve upon this by explaining more variation than a single variable

alone. It is important to understand how the reported models can be used in a clinical context. Logistic regression models provide a predicted probability of tissue malignancy. Penalized estimation of logistic regression coefficients shrinks coefficients toward zero to reduce model overfitting and false discovery associated with a large panel of predictors. Fifty 10-fold cross validations provide an internal validation to reduce optimism and give more realistic accuracy prior to validation from an external sample set. This model allows for a clinical provider to use the risk score in the context of pre-test probability to make informed decisions. For instance, given that greater than 75% of atypical or suspicious cytology is ultimately malignant, a high risk score could help inform a clinical decision in a patient with atypical or suspicious cytology (1, 3). Alternatively, recursive partitioning provides a rapid classifier that depends on a small number of proteins rather than a complete Luminex panel. It can be used to provide a categorical diagnosis, rather than a continuous risk score. We provide examples of added flexibility in which the recursive partitioning parameters can be changed to optimize the classifier for either sensitivity or specificity approaching 100%. This allows for the model to be used to objectively rule-in or rule-out PDAC based on the clinical scenario. Trials to validate this work in EUS-guided FNA samples would be necessary prior to clinical applicability of any of these models.

Our study has a number of limitations. It is a single-center, retrospective analysis. Tissues were obtained at the time of surgical resection with sharp dissection, not EUS-guided FNA. There is ischemic time prior to tissue processing which may lead to proteolysis, although every attempt was made to minimize this time. It is challenging to acquire benign and chronic pancreatitis samples due to the lower frequency of surgery in this population. Unused pancreata from transplant donors (nPOD samples) were obtained and clinico-pathologic parameters were not available for these patients. As noted in the methods, the collection process differs due to infusion of University of Wisconsin Solution. As these samples are normal pancreatic tissue, they may not be representative of the population undergoing EUS-guided FNA. The risk scores of these samples were significantly lower than the other benign samples and pancreatitis samples. Including the benign, non-inflamed tissues in the analysis may be seen as a limitation to this work. This study is limited to PDAC and benign specimens. Patients presenting with a pancreatic mass could have other forms of malignant pancreatic disease (e.g. neuroendocrine tumors) and the protein signatures from these tumors are unknown. Endoscopists occasionally biopsy regional lymph nodes due to accessibility during EUS-guided FNA. The possible differences in these protein signatures is unknown. Future work will need to address these limitations.

Conclusions

The work presented here needs to be replicated in specimens obtained by EUS-guided FNA, but shows promise in the ability to differentiate between benign and malignant pancreatic tissues. Further efforts to validate this work in a unique sample set is warranted.

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Abbreviations

AUC	area under the curve
EUS-guided FNA	endoscopic ultrasound-guided FNA
FNA	fine needle aspiration
FISH	fluorescence in situ hybridization
HBME-1	Hector Battifora mesothelial-1
IL-15	interleukin 15
IL-1RA	interleukin 1 receptor antagonist
miRNA	microRNA
nPOD	Network for Pancreatic Organ Donors
PDAC	pancreatic ductal adenocarcinoma
ROC	receiver operating characteristic

References

1. Turner BG, Cizginer S, Agarwal D, et al. Diagnosis of pancreatic neoplasia with EUS and FNA: a report of accuracy. *Gastrointest Endosc*. 2010 1;71(1):91–8. [PubMed: 19846087]
2. Chen G, Liu S, Zhao Y, et al. Diagnostic accuracy of endoscopic ultrasound-guided fine-needle aspiration for pancreatic cancer: a meta-analysis. *Pancreatol*. 2013 May-Jun;13(3):298–304. [PubMed: 23719604]
3. Eloubeidi MA, Chen VK, Eltoun IA, et al. Endoscopic ultrasound-guided fine needle aspiration biopsy of patients with suspected pancreatic cancer: diagnostic accuracy and acute and 30-day complications. *Am J Gastroenterol*. 2003 12;98(12):2663–8. [PubMed: 14687813]
4. Shin HJ, Lahoti S, Sneige N. Endoscopic ultrasound-guided fine-needle aspiration in 179 cases: the M. D. Anderson Cancer Center experience. *Cancer*. 2002 6 25;96(3):174–80. [PubMed: 12115306]
5. Youngwirth LM, Nussbaum DP, Thomas S, et al. Nationwide trends and outcomes associated with neoadjuvant therapy in pancreatic cancer: An analysis of 18 243 patients. *J Surg Oncol*. 2017 8;116(2): 127–32. [PubMed: 28407261]
6. Yamabe A, Irisawa A, Bhutani MS, et al. Efforts to improve the diagnostic accuracy of endoscopic ultrasound-guided fine-needle aspiration for pancreatic tumors. *Endosc Ultrasound*. 2016 Jul-Aug; 5(4):225–32. [PubMed: 27503153]
7. Storm AC, Lee LS. Endoscopic ultrasound-guided techniques for diagnosing pancreatic mass lesions: Can we do better? *World J Gastroenterol*. 2016 10 21;22(39):8658–69. [PubMed: 27818584]

8. Cazacu IM, Luzuriaga Chavez AA, Saftoiu A, et al. A quarter century of EUS-FNA: Progress, milestones, and future directions. *Endosc Ultrasound*. 2018 May-Jun;7(3):141–60. [PubMed: 29941723]
9. Collins BT, Murad FM, Wang JF, Bernadt CT. Rapid on-site evaluation for endoscopic ultrasound-guided fine-needle biopsy of the pancreas decreases the incidence of repeat biopsy procedures. *Cancer Cytopathol*. 2013 9;121(9):518–24. [PubMed: 23983161]
10. Schmidt RL, Witt BL, Matynia AP, et al. Rapid on-site evaluation increases endoscopic ultrasound-guided fine-needle aspiration adequacy for pancreatic lesions. *Dig Dis Sci*. 2013 3;58(3):872–82. [PubMed: 23053888]
11. Kongkam P, Lakananurak N, Navicharern P, et al. Combination of EUS-FNA and elastography (strain ratio) to exclude malignant solid pancreatic lesions: A prospective single-blinded study. *J Gastroenterol Hepatol*. 2015 11;30(11):1683–9. [PubMed: 26238152]
12. Reicher S, Boyar FZ, Albitar M, et al. Fluorescence in situ hybridization and K-ras analyses improve diagnostic yield of endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic masses. *Pancreas*. 2011 10;40(7):1057–62. [PubMed: 21705950]
13. Rodriguez SA, Impey SD, Pelz C, et al. RNA sequencing distinguishes benign from malignant pancreatic lesions sampled by EUS-guided FNA. *Gastrointest Endosc*. 2016 8;84(2):252–8. [PubMed: 26808815]
14. Szafranska AE, Doleshal M, Edmunds HS, et al. Analysis of microRNAs in pancreatic fine-needle aspirates can classify benign and malignant tissues. *Clin Chem*. 2008 10;54(10):1716–24. [PubMed: 18719196]
15. Chu GC, Kimmelman AC, Hezel AF, DePinho RA. Stromal biology of pancreatic cancer. *J Cell Biochem*. 2007 7 1;101(4):887–907. [PubMed: 17266048]
16. Delitto D, Black BS, Sorenson HL, et al. The inflammatory milieu within the pancreatic cancer microenvironment correlates with clinicopathologic parameters, chemoresistance and survival. *BMC Cancer*. 2015 10 24;15:783. [PubMed: 26498838]
17. Campbell-Thompson M, Wasserfall C, Kaddis J, et al. Network for Pancreatic Organ Donors with Diabetes (nPOD): developing a tissue biobank for type 1 diabetes. *Diabetes Metab Res Rev*. 2012 10;28(7):608–17. [PubMed: 22585677]
18. Liu CW, Atkinson MA, Zhang Q. Type 1 diabetes cadaveric human pancreata exhibit a unique exocrine tissue proteomic profile. *Proteomics*. 2016 5;16(9):1432–46. [PubMed: 26935967]
19. R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing V, Austria. URL <https://www.R-project.org/>.
20. Goeman Jelle J. (2018). Penalized R package, version 0.9–51. URL <https://CRAN.R-project.org/package=penalized>.
21. Burnett AS, Bailey J, Oliver JB, et al. Sensitivity of alternative testing for pancreaticobiliary cancer: a 10-y review of the literature. *J Surg Res*. 2014 8;190(2):535–47. [PubMed: 24969546]
22. Senoo J, Mikata R, Kishimoto T, et al. Immunohistochemical analysis of IMP3 and p53 expression in endoscopic ultrasound-guided fine needle aspiration and resected specimens of pancreatic diseases. *Pancreatol*. 2018 3;18(2):176–83. [PubMed: 29305088]
23. Mei M, Ni J, Liu D, et al. EUS elastography for diagnosis of solid pancreatic masses: a meta-analysis. *Gastrointest Endosc*. 2013 4;77(4):578–89. [PubMed: 23199646]
24. Brand RE, Adai AT, Centeno BA, et al. A microRNA-based test improves endoscopic ultrasound-guided cytologic diagnosis of pancreatic cancer. *Clin Gastroenterol Hepatol*. 2014 10;12(10):1717–23. [PubMed: 24662333]
25. Frampton AE, Krell J, Prado MM, et al. Prospective validation of microRNA signatures for detecting pancreatic malignant transformation in endoscopic-ultrasound guided fine-needle aspiration biopsies. *Oncotarget*. 2016 5 10;7(19):28556–69. [PubMed: 27086919]
26. Yoon JH, Moon HJ, Kim EK, Kwak JY. Inadequate cytology in thyroid nodules: should we repeat aspiration or follow-up? *Ann Surg Oncol*. 2011 5;18(5):1282–9. [PubMed: 21331807]
27. Bartolazzi A, Gasbarri A, Papotti M, et al. Application of an immunodiagnostic method for improving preoperative diagnosis of nodular thyroid lesions. *Lancet*. 2001 5 26;357(9269):1644–50. [PubMed: 11425367]

28. Cantara S, Marzocchi C, Pilli T, et al. Molecular Signature of Indeterminate Thyroid Lesions: Current Methods to Improve Fine Needle Aspiration Cytology (FNAC) Diagnosis. *Int J Mol Sci*. 2017 4 6;18(4).
29. Jin C, Xue Y, Li Y, et al. A 2-Protein Signature Predicting Clinical Outcome in High-Grade Serous Ovarian Cancer. *Int J Gynecol Cancer*. 2018 1;28(1):51–8. [PubMed: 28976449]
30. Muinao T, Deka Boruah HP, Pal M. Diagnostic and Prognostic Biomarkers in ovarian cancer and the potential roles of cancer stem cells - An updated review. *Exp Cell Res*. 2018 1 1;362(1):1–10. [PubMed: 29079264]
31. Trachana SP, Pilalis E, Gavalas NG, et al. The Development of an Angiogenic Protein “Signature” in Ovarian Cancer Ascites as a Tool for Biologic and Prognostic Profiling. *PLoS One*. 2016;11(6):e0156403. [PubMed: 27258020]
32. Gocheva V, Naba A, Bhutkar A, et al. Quantitative proteomics identify Tenascin-C as a promoter of lung cancer progression and contributor to a signature prognostic of patient survival. *Proc Natl Acad Sci U S A*. 2017 7 11;114(28):E5625–E34. [PubMed: 28652369]
33. Lee HB, Kang UB, Moon HG, et al. Development and Validation of a Novel Plasma Protein Signature for Breast Cancer Diagnosis by Using Multiple Reaction Monitoring-based Mass Spectrometry. *Anticancer Res*. 2015 11;35(11):6271–9. [PubMed: 26504062]
34. Skoog P, Ohlsson M, Ferno M, et al. Tumor tissue protein signatures reflect histological grade of breast cancer. *PLoS One*. 2017;12(6):e0179775. [PubMed: 28650989]
35. Poch B, Lotspeich E, Ramadani M, et al. Systemic immune dysfunction in pancreatic cancer patients. *Langenbecks Arch Surg*. 2007 5;392(3):353–8. [PubMed: 17235586]
36. Wang HC, Hung WC, Chen LT, Pan MR. From Friend to Enemy: Dissecting the Functional Alteration of Immunoregulatory Components during Pancreatic Tumorigenesis. *Int J Mol Sci*. 2018 11 13;19(11).
37. Liou GY, Bastea L, Fleming A, et al. The Presence of Interleukin-13 at Pancreatic ADM/PanIN Lesions Alters Macrophage Populations and Mediates Pancreatic Tumorigenesis. *Cell Rep*. 2017 5 16;19(7):1322–33. [PubMed: 28514653]
38. Zhuang Z, Ju HQ, Aguilar M, et al. IL1 Receptor Antagonist Inhibits Pancreatic Cancer Growth by Abrogating NF-kappaB Activation. *Clin Cancer Res*. 2016 3 15;22(6): 1432–44. [PubMed: 26500238]

Figure 1:

Semi-supervised cluster heatmap analysis organized by tissue type. The PDAC tissue, represented in yellow, has a pattern of homogeneity with samples tending to have higher z-scores (red). The benign tissue, represented by blue, also demonstrates homogeneity with lower z-scores (green). Chronic pancreatitis, represented by orange, is more heterogenous. The left cluster appears similar to PDAC with high z-scores, while the right cluster appears similar to benign tissue with low z-scores.

Figure 2:
Sorted area under the receiver operating characteristic curve (AUC) for predicting PDAC vs. benign tissue for all 31 analytes included in the analysis. Fourteen analytes had an AUC greater than 0.75.

Figure 3:

A recursive partitioning model with equal weight for false positives and false negatives, optimizing for accuracy. An IL-1RA level ≥ 0.095 identifies 70 samples as having PDAC with 67 true positives and 3 false positives. IL-1RA < 0.095 and TGF α < 0.412 identifies 79 samples as benign tissue with 71 true negatives and 8 false negatives. IL-1RA < 0.095 and TGF α ≥ 0.412 identifies the remaining 10 samples as PDAC with 7 true positives and 3 false positives. Reported protein concentrations are normalized to total protein and then natural log transformed and standardized with a mean of zero and standard deviation of one.

Figure 4.

(A) Data was first normalized to total protein and then natural log transformed and standardized with a mean of zero and standard deviation of one. A penalized logistic regression model demonstrates 12 of the 31 one analytes as having non-zero coefficients. (B) The area under the receiver operating characteristic curve (AUC) for this model was 0.975 (95% CI: 0.948–1). (C) After 50 10-fold cross-validations the AUC fell slightly to 0.951.

Figure 5.

(A) Individual samples are given a logistic model-predicted risk score to predict the likelihood of PDAC vs benign. Benign samples are represented by green circles and PDAC samples are represented by red triangles. The most accurate risk score cutoff was 0.492. For samples with a risk score <0.492 , 73/83 samples were benign. For samples with a risk score >0.492 , 72/76 samples were PDAC. The accuracy, sensitivity, and specificity were 91.2%, 87.8%, and 94.8%, respectively. (B) Chronic pancreatitis is separated from benign tissue and displayed by a blue cross. All but one pancreatitis sample have a risk score of <0.492 , consistent with benign tissue.

Figure 6.

NPV, PPV of cross-validated Luminex logistic model. Positive (gray) and negative (black) predictive values using the cross-validated logistic model optimized for accuracy are displayed across disease prevalence. Under the assumption of equivalent diagnostic properties for tissue obtained from FNA and from surgical resection, then with 80% prevalence of PDAC in patients undergoing EUS-guided FNA, the PPV and NPV would be approximately 98.5% and NPV of 66.0%, respectively.

eFigure 1:

A recursive partitioning model with a false negative having five times the weight of a false positive, optimizing for sensitivity. An IL-1RA ≥ -0.5 predicts 96 samples as having PDAC with 77 true positives and 19 false positives. An IL-1RA level < -0.5 , IL-15 ≥ -1.556 , and TGFA < 0.205 predicts all 49 samples as benign tissue with no false negatives. An IL-1RA level < -0.5 and IL-15 ≥ -1.556 predicts 7 patients as having PDAC with 3 true positives and 4 false positives. An IL-1RA level < -0.5 , IL-15 ≥ -1.556 , and TGFA ≥ 0.205 predicts 7 patients as having PDAC with 2 true positives and 5 false positives. The sensitivity of this model was 100%. The accuracy and specificity were 82.4% and 74.6%, respectively. Reported protein concentrations are normalized to total protein and then natural log transformed and standardized with a mean of zero and standard deviation of one.

eFigure 2:

A recursive partitioning model with a false positive having five times the weight of a false negative, optimizing for specificity. This model incorporates IL-1RA and eotaxin. An IL-1RA < 0.095 predicts 89 patients as benign tissue with 74 true negatives and 15 false negatives. An IL-1RA ≥ 0.095 and eotaxin < -0.141 predicts 7 samples as benign with 2 true negatives and 5 false negatives. An IL-1RA ≥ 0.095 and eotaxin ≥ -0.141 predicts 63 samples as PDAC with 62 true positives and 1 false positive. The specificity of this model was 98.4%. The accuracy and sensitivity were 86.8% and 79.2%, respectively. Reported protein concentrations are normalized to total protein and then natural log transformed and standardized with a mean of zero and standard deviation of one.

Table 1.

Patient Clinico-Pathologic Parameters

Parameter	PDAC (n = 82)	Benign* (n = 77)	p Value
Age, y, mean $\pm$ SD	69.7 $\pm$ 9.10	54.2 $\pm$ 14.6	<0.0001
Sex, m, n (%)	50 (61.0)	21 (45.7)	0.1001
Race, n (%)			0.0407
White, non-Hispanic	74 (90.2)	38 (84.4)	
African American	3 (3.7)	6 (13.3)	
Hispanic	4 (4.9)	0 (0.0)	
Asian	1 (12)	0 (0.0)	
American Indian	0 (0.0)	1 (2.2)	
Pathology, n (%)			
PDAC	82 (100.0)	0 (0.0)	
Benign pancreatic tissue	0 (0.0)	19 (24.7)	
Pancreatitis	0 (0.0)	27 (35.1)	
Transplant donor	0 (0.0)	31 (40.2)	
Operation, n (%)			
Pancreatoduodenectomy	69 (84.1)	18 (23.7)	
Distal pancreatectomy	11 (13.4)	15 (19.7)	
Total pancreatectomy	2 (2.44)	0 (0.0)	
Frey's procedure	0 (0.0)	12 (15.8)	
Puestow procedure	0 (0.0)	1 (13)	
Transplant donor	0 (0.0)	31 (40.8)	
T stage, n (%)			
T1	1 (12)	-	
T2	4 (4.9)	-	
T3	76 (92.7)	-	
T4	1 (12)	-	
N stage, n (%)			
N0	15 (18.3)	-	
N1	65 (79.3)	-	
N2	2 (2.4)	-	
Differentiation, n (%)			
Well	6 (7.4)	-	
Moderate	40 (49.4)	-	
Poor	33 (40.7)	-	
Undifferentiated	2 (2.5)	-	
Positive lymph node, mean $\pm$ SD	3.9 $\pm$ 3.89	-	
Total lymph node, mean $\pm$ SD	22.4 $\pm$ 7.51	-	

Parameter	PDAC (n = 82)	Benign* (n = 77)	p Value
Lymph node ratio, mean $\pm$	0.17 $\pm$ 0.165	-	
SD			
Tumor size, cm, mean $\pm$ SD	3.69 $\pm$ 1.862	-	
Lymphovascular invasion, n (%)	68 (82.9)	-	
Perineural invasion, n (%)	81 (98.8)	-	
R0 resection, n (%)	62 (76.0)	-	

* Patient demographics were not available for the 31 specimens obtained from transplant donors

PDAC, pancreatic ductal adenocarcinoma