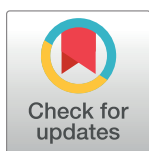


RESEARCH ARTICLE

Renalase is a novel tissue and serological biomarker in pancreatic ductal adenocarcinoma

Yasheen Gao^{1,2}, Melinda Wang^{2,3}, Xiaojia Guo^{2,3}, Joanna Hu⁴, Tian-min Chen^{2,3}, Sade M. B. Finn⁵, Jill Lacy³, John W. Kunstman⁶, Charles H. Cha⁷, Melena D. Bellin^{5,8}, Marie E. Robert^{9‡}, Gary V. Desir^{2,3‡}, Fred S. Gorelick^{2,3,10‡*}

1 Yale University, New Haven, Connecticut, United States of America, **2** Department of Medicine, Veterans Affairs Connecticut Health System, Yale University School of Medicine, West Haven, Connecticut, United States of America, **3** Department of Medicine, Yale University School of Medicine, New Haven, Connecticut, United States of America, **4** Yale Cancer Center, New Haven, Connecticut, United States of America, **5** Department of Surgery, University of Minnesota Medical School, Minneapolis, Minnesota, United States of America, **6** Department of Surgery, Yale University School of Medicine and VA Connecticut, New Haven, Connecticut, United States of America, **7** Department of Surgery, Hartford Healthcare Saint Vincent's Medical Center, Bridgeport, Connecticut, United States of America, **8** Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota, United States of America, **9** Department of Pathology, Yale University School of Medicine, New Haven, Connecticut, United States of America, **10** Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut, United States of America



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☞ These authors contributed equally to this work.

‡ These authors also contributed equally to work.

* fred.gorelick@yale.edu

Abstract

Dysregulated expression of the secretory protein renalase can promote pancreatic ductal adenocarcinoma (PDAC) growth in animal models. We characterized renalase expression in premalignant and malignant PDAC tissue and investigated whether plasma renalase levels corresponded to clinical PDAC characteristics. Renalase immunohistochemistry was used to determine the presence and distribution of renalase in normal pancreas, chronic pancreatitis, PDAC precursor lesions, and PDAC tissues. Associations between pretreatment plasma renalase and PDAC clinical status were assessed in patients with varied clinical stages of PDAC and included tumor characteristics, surgical resection in locally advanced/borderline resectable PDAC, and overall survival. Data were retrospectively obtained and correlated using non-parametric analysis. Little to no renalase was detected by histochemistry in the normal pancreatic head in the absence of abdominal trauma. In chronic pancreatitis, renalase immunoreactivity localized to peri-acinar spindle-shaped cells in some samples. It was also widely present in PDAC precursor lesions and PDAC tissue. Among 240 patients with PDAC, elevated plasma renalase levels were associated with worse tumor characteristics, including greater angiolymphatic invasion (80.0% vs. 58.1%, $p = 0.012$) and greater node positive disease (76.5% vs. 56.5%, $p = 0.024$). Overall survival was worse in patients with high plasma renalase levels with median follow-up of 27.70 months vs. 65.03 months ($p < 0.001$). Renalase levels also predicted whether patients with locally advanced/borderline resectable PDAC underwent resection (AUC 0.674; 95%CI 0.42–0.82, $p = 0.04$). Overall tissue renalase was increased in both premalignant and

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malignant PDAC tissues compared to normal pancreas. Elevated plasma renalase levels were associated with advanced tumor characteristics, decreased overall survival, and reduced resectability in patients with locally advanced/borderline resectable PDAC. These studies show that renalase levels are increased in premalignant pancreatic tissues and that its levels in plasma correspond to the clinical behavior of PDAC.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the seventh leading cause of cancer death worldwide with an estimated 430,000 annual deaths and an overall survival rate of less than 10% [1, 2]. PDAC is often not detected until after it has disseminated systemically, is resistant to drug therapies, and is recognized as an urgent unmet medical need [3, 4]. A better understanding of the biology and requirements for PDAC growth is crucial to improving the diagnostic and therapeutic approaches to this disease.

Renalase (RNLS) is a novel secretory protein [5–7] that can be highly expressed in PDAC tissue [8]. Recombinant RNLS can promote the growth of human PDAC cell lines, and its inhibition leads to experimental tumor cell death in both *in vitro* and *in vivo* [8]. Overall PDAC patient survival correlates inversely with RNLS tissue levels in tumor samples [8]. These studies suggest that RNLS could be an important factor in maintaining PDAC growth and influence patient outcomes. Whether RNLS activity is associated with PDAC development, including in precursor lesions, has not been explored.

The risk of developing PDAC is increased in several premalignant lesions including chronic pancreatitis [13], pancreatic intraepithelial neoplasia (PanIN), and pancreatic cystic neoplasms. PanIN [9], the most common of these, progresses step-wise from non-invasive neoplasms (grades 1–3) to invasive PDAC with KRAS mutations being one of the earliest genetic events [10–12]. Pancreatic cystic neoplasms, comprising intraductal papillary mucinous neoplasms (IPMNs) and less frequently, mucinous cystic neoplasms (MCNs), can also progress to PDAC [13].

Since RNLS is a secretory protein, changes in its tissue levels could be reflected in its plasma levels. As seen with other tumors and biomarkers (e.g., CA19.9, CEA and PSA), it is possible that plasma RNLS levels could parallel tissue levels of RNLS in PDAC and serve as a new biomarker in PDAC.

Here we show that RNLS is present in premalignant (chronic pancreatitis, PanINs, and mucinous pancreatic cystic neoplasms) and malignant PDAC tissue suggesting a potential role for pancreatic RNLS in PDAC development. We also observed that elevations of a distinct form of plasma RNLS appear to correspond to PDAC prognosis in a subset of pancreas cancer patients with locally advanced disease at presentation.

Methods

Tissue specimens

Human pancreatic tissue samples from resection specimens of normal pancreas, chronic pancreatitis, PDAC precursor lesions (PanIN, IPMN, and MCN), and PDAC, were obtained from Yale Surgical Pathology (Yale HIC approval number: 2000021579). Additional chronic pancreatitis tissue samples were obtained from University of Minnesota Medical Center (IRB Approval Number: 0609M91887). Studies were given exceptions by the Veterans

Administration HIC and Research committees under protocols FG0011-2020 and FSG0012-2020 (Oct 28, 2020)

Immunohistochemistry protocol

Immunohistochemistry was performed as described [8]. Sections (5- μ m) from formalin-fixed paraffin-embedded tissues were mounted on slides and de-paraffinized and hydrated. After antigen retrieval in a pressure cooker containing citrate buffer (1g NaOH, 2.1 g citric acid in 1 L H₂O pH 6), sections were blocked with DAKO Dual endogenous enzyme block (Agilent, Santa Clara, CA, USA) for 10 min and 2.5% normal horse serum for 1 hr followed by incubation with primary antibody and isotype control IgG overnight at 4°C. The m28-RNLS monoclonal antibody was diluted at 1 μ g/mL in buffer (TBS/1% Tween with 300 mM NaCl, pH = 7.4). ImmPRESS peroxidase-anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA) was used to detect primary antibodies. The color was developed using a Vector DAB substrate kit and tissue was counterstained with hematoxylin (Vector Laboratories). Hematoxylin and eosin stained and RNLS IHC stains were examined at the light microscope to confirm the histologic diagnosis and document the distribution of RNLS immunopositivity by a pancreaticobiliary pathologist (MER). RNLS IHC staining was categorized as present or absent in all components of the pancreas (benign exocrine, endocrine, pancreatic ducts and stroma; neoplastic tissues, either in-situ or invasive). The specificity of labeling on each tissue was confirmed by either labeling in tissue sections in the absence of the m28-RNLS primary antibody or 2 using m28-RNLS antibody that had been pre-incubated with the peptide antigen (RP-220) [14].

Immunofluorescence protocol

The slides were deparaffinized and processed as above for immunofluorescence microscopy. After antigen retrieval, sections were blocked with TBS/0.3% TritonX-100/10% goat serum) for 1 hr and incubated with a cocktail of m28-RNLS (1:100) plus α -smooth-muscle actin (α SMA) mouse monoclonal (1:400 Sigma-Aldrich, St. Louis, MI, USA) at 4°C overnight. Alexa 488-conjugated goat anti-rabbit (1:2000, Invitrogen Corporation, Carlsbad, CA) and Alexa 594-conjugated goat anti-mouse (1:2000, Invitrogen Corporation, Carlsbad, CA) were used to detect the primary antibodies. Tissue autofluorescence was quenched using a commercial preparation (Vector TrueVIEW Autofluorescence Quenching Kit, Vector Laboratories, Burlingame, CA, USA). The specificity of labeling on each tissue was confirmed by labeling tissue sections in the absence of the m28-RNLS and α SMA primary antibodies.

Measurement of plasma RNLS and CA-19.9 levels

Plasma RNLS levels were determined by assaying the denaturation acid-sensitive pool and non-acid treated pool by ELISA as described [15]. Here we refer to the acid-treated value as RNLS and the untreated value as non-acid treated RNLS. The median value was used to separate low and high RNLS levels Serum CA19-9 levels were obtained from medical records before the first date of PDAC treatment.

Because of potentially spurious CA19-9 values in patients with biliary obstruction, patients with total bilirubin > 2.0 mg/dL were excluded from the CA19-9 analysis. CA19-9 levels of < 10 U/mL were also excluded because artifactually low CA19-9 levels can be seen in patients, especially those who have Lewis a-b-blood group antigen [16]. A threshold CA19-9 value of 300 U/mL was designated elevated based on studies showing that pre-operative CA19-9 values above 300 U/mL suggest advanced disease and unresectable cancer [17].

Clinical data collection

Prospectively collected plasma samples were obtained from consented patients with pathologically confirmed PDAC prior to initiation of treatment as part of the intake procedure for the Yale Gastrointestinal Tumor Biorepository from April 2012 to March 2019 (YGTB; Yale HIC #1203009817). Following definitive treatment, clinical and pathologic information were used to retrospectively annotate the plasma samples. Sociodemographic and clinicopathologic data were extracted from oncology visit notes, operation reports, and surgical pathology reports. Management was determined by the treating oncology team. Patients with locally advanced/borderline resectable (LA/BR) PDAC were determined at the time of diagnosis by cross-sectional imaging using a dedicated pancreatic contrast administration protocol and reviewed by a multidisciplinary PDAC management team that included an experienced pancreatic surgeon. Specifically, definitions of “resectable”, “borderline resectable”, and “locally advanced” were ascribed to patients by treating surgeons in accordance with the published National Comprehensive Cancer Network (NCCN) guidelines germane to the year of diagnosis [18]. For the purposes of this study, patients who were identified as LA/BR at diagnosis who underwent resection with curative intent were considered “resectable” versus those who did not undergo surgery with curative intent. Any patients undergoing surgery for palliative purposes were not considered resectable.

Statistical analyses

Nonparametric statistical analyses were performed using SPSS Version 24 (IBM Statistics; Armonck, NY). Bivariate nonparametric analyses of sociodemographic and treatment-related variables were performed using the calculated cut-off value for RNLS. Multivariate analyses were performed for statistically significant variables within the bivariate analysis. Sensitivity and specificity values were calculated using the calculated cut-off value for RNLS and pre-determined cut-off value for CA19-9. Kaplan-Meier survival analyses were performed using log rank analysis to calculate statistical significance to calculate overall survival and resectability of patients. All quantitative data were reported as median (range) or mean when median was unavailable and a p-value < 0.05 was used to determine statistical significance.

Results

RNLS immunoreactivity in human pancreatic tissue

We examined the presence and distribution of RNLS in normal human pancreas (n = 11), chronic pancreatitis (n = 32), PDAC precursor lesions (PanIN: n = 5, IPMN: n = 6, MCN: n = 4), and PDAC (n = 9) by immunohistochemistry. Sociodemographic and labeling characteristics for the patients examined are summarized in **Tables 1** and **2**.

Among normal human pancreatic tissue from patients who had undergone Whipple procedure for duodenal adenoma (n = 7), there was little to no RNLS labeling (**Fig 1A**). In patients who had undergone distal pancreatectomy associated with trauma-related splenectomy (n = 4) there was granular RNLS labeling in the apical cytoplasm of pancreatic acinar cells as well as diffuse RNLS labeling in pancreatic islets and ducts (**Fig 1B**). One of these tissues showed RNLS labeling in spindle-shaped cells surrounding pancreatic acinar cells, a cellular distribution more often seen in chronic pancreatitis. Normal tissue from the pancreatic body or tail from non-abdominal trauma cases were not available for analysis. Representative images showing the spectrum of RNLS labeling in normal pancreas cases are presented in **S1 Fig**.

Chronic pancreatitis tissues were obtained from patients with the following etiologies: alcoholic chronic pancreatitis (n = 5), genetic chronic pancreatitis (n = 6), idiopathic chronic

Table 1. Sociodemographic characteristics for RNLS immunohistochemistry patients.

Characteristics	Histological variant					
	Normal Pancreas (n = 11) ^a	Chronic Pancreatitis (n = 32) ^b	PanIN (n = 5)	IPMN (n = 6)	MCN (n = 4)	PDAC (n = 9)
Age ^c , years	70 (23–94)	56.8 (27–85)	71 (62–82)	70 (44–78)	52 (26–74)	67 (51–80)
Male ^d	4 (36.4)	13 (40.6)	2 (40.0)	4 (66.7)	0	5 (55.6)
White ^d	7 (63.6)	28 (87.6)	5 (100)	5 (83.3)	4 (80)	8 (88.9)
BMI ^c , kg/m ²	28.0 (25.8–30.1)	24.3 (13.3–37.1)	26.0 (18.9–28.7)	28.4 (14.9–39.6)	22.6 (13.3–34.1)	30.4 (23.3–34.9)
Current or past smoker ^d	4 (36.4)	17 (53.1)	3 (60)	4 (66.7)	2 (40)	5 (55.6)
Alcohol use ^d	0	5 (15.6)	0	0	1 (20)	1 (11.1)
Hypertension ^d	3 (27.3)	13 (40.6)	2 (40)	2 (33.3)	1 (20)	3 (33.3)
Cardiovascular Disease ^d	0	3 (8.4)	1 (20)	0	0	1 (11.1)
Chronic Kidney Disease ^d	0	2 (6.3)	0	0	1 (20)	1 (11.1)
Diabetes ^d	0	9 (28.1)	3 (60)	2 (33.3)	2 (40)	2 (22.2)

^a Seven normal pancreas cases were taken from patients who had undergone Whipple surgery for duodenal adenoma. Four normal pancreas cases were taken from patients who had undergone splenectomy associated with trauma.

^b Five cases were taken from patients who had alcohol induced chronic pancreatitis. Four cases were taken from patients with idiopathic chronic pancreatitis. Six cases were taken from patients who had genetic chronic pancreatitis. One case came from a patient who had chronic pancreatitis due to pancreatic divisum. One case was taken from a patient who had chronic pancreatitis due to Sphincter of Oddi dysfunction. Two cases were taken from patients who had chronic pancreatitis associated with benign inflammatory pseudocyst. Thirteen cases were taken from patients with PDAC precursor lesions and PDAC.

^c Data are given as median (range).

^d Data are given as number (percentage).

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pancreatitis (n = 4), chronic pancreatitis due to pancreatic divisum (n = 1), chronic pancreatitis due to Sphincter of Oddi dysfunction (n = 1), chronic pancreatitis adjacent to a benign inflammatory pseudocyst (n = 2), chronic pancreatitis associated with precursor lesions (n = 9), and chronic pancreatitis associated with PDAC (n = 4).

Overall, RNLS labeling in chronic pancreatitis was present, but variable, as noted below and in [Table 2](#) and [S2 Fig](#). RNLS immunoreactivity was found in spindle-shaped cells surrounding pancreatic acinar cells in six of the nineteen non-tumor associated chronic pancreatitis cases ([Fig 2A](#)) and two of the thirteen tumor-associated chronic pancreatitis cases ([Fig 2B](#)). These included one case each of the following etiologies: 1) Mutations in the cystic fibrosis transmembrane conductance regulator (CTFR), 2) Mutation in chymotrypsin C (CTRC), 3) Idiopathic chronic pancreatitis, 4) Alcoholic chronic pancreatitis, 5) Pancreatic divisum, 6) Associated with a pseudocyst, 7) Associated with MCN and 8) Chronic pancreatitis associated with PDAC. To determine the identity of the spindle-shaped containing RNLS immunoreactivity, we performed double-label (RNLS and α SMA- smooth muscle actin)

Table 2. Labeling characteristics for RNLS immunohistochemistry patients.

Pancreatic tissue type	n	Benign ductal epithelium	Acinar cell	Islets	Neoplastic cells	Stellate cells
Normal pancreas (trauma)	4	4/4 (100)	4/4 (100)	4/4 (100)	N/A	1/4 (25)
Normal pancreas (duodenal adenoma)	7	1/7 (14)	3/7 (43)	1/7 (14)	N/A	0/7 (0)
Chronic pancreatitis (Non-tumor associated)	19	6/19 (32)	8/19 (42)	6/19 (32)	N/A	6/19 (32)
Chronic pancreatitis (adjacent to pancreatic precursor lesion/PDAC)	13	12/13 (92)	13/13 (100)	12/13 (92)	N/A	2/13 (15)
Precursor Lesion (PanIN)	5	5/5 (100)	5/5 (100)	5/5 (100)	5/5 (100)	0/5 (0)
Precursor Lesion (IPMN)	6	6/6 (100)	6/6 (100)	5/6 (83.3)	6/6 (100)	0/6 (0)
Precursor Lesion (MCN)	4	4/4 (100)	4/4 (100)	4/4 (100)	4/4 (100)	0/4 (0)
PDAC	9	4/9 (44)	4/9 (44)	5/9 (55)	9/9 (100)	0/9 (11)

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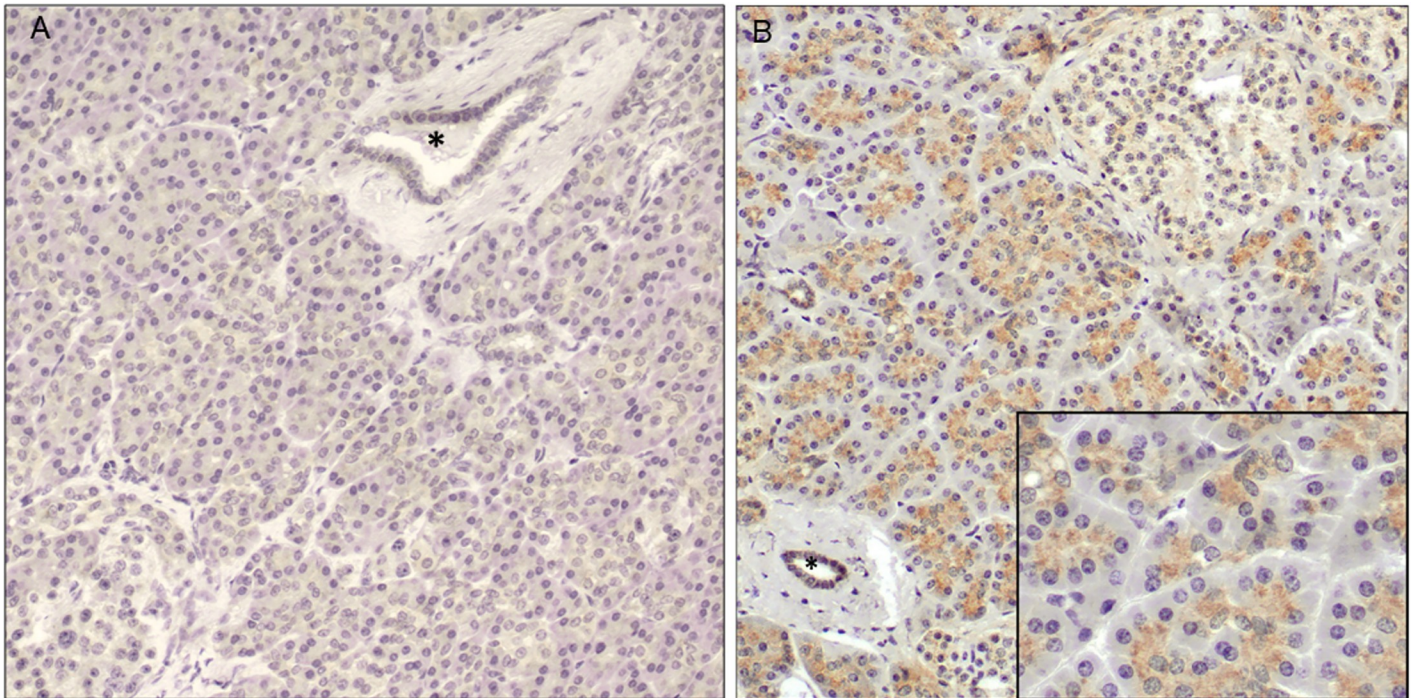


Fig 1. RNLS labeling in normal human pancreas. Representative images of RNLS labeling detected by immunohistochemistry using the m28-RNLS antibody in the normal human pancreas from (A) a patient who had undergone Whipple procedure for duodenal adenoma with essentially no signal and (B) a patient who had undergone splenectomy for trauma, showing granular cytoplasmic staining in acinar cells. The asterisk indicates a normal pancreatic duct. Original magnification 200x; inset 400x.

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immunofluorescence on six non-tumor associated and three tumor associated chronic pancreatitis cases. In a chronic pancreatitis case (associated CTSC mutation) we observed co-localization of RNLS and α SMA (Fig 2C–2E), suggesting that RNLS may be present in stellate cells. In the remainder of the cases the labeling was faint, and it was unclear whether the markers co-distributed. In non-tumor associated chronic pancreatitis samples, RNLS immunoreactivity was also observed in acinar cells (8/19 samples), pancreatic ducts (6/19) and islet cells (6/19). (Table 2). Granular cytoplasmic RNLS labeling of acinar cells, pancreatic ducts and islet cells was also present in regions of chronic pancreatitis associated with neoplastic conditions, including PanIN (n = 2), IPMN (n = 4), MCN (n = 3), and PDAC (n = 5). Finally, RNLS labeling was noted in stromal cells, including mononuclear inflammatory cells and fibroblasts, in regions of chronic pancreatitis associated with neoplastic precursor lesions in four samples.

We labeled for RNLS immunoreactivity in PDAC precursor lesions (n = 15) including PanIN (n = 5), IPMN (n = 6), and MCN (n = 4), and in PDAC tumors (n = 9). In all premalignant and malignant PDAC tissues there was diffuse RNLS labeling in in-situ and invasive neoplastic epithelium, adjacent benign duct cells, acinar cells, and islets (Fig 3A–3D). In six of the fifteen precursor lesion cases and three of the nine PDAC cases, RNLS labeling was noted in stromal cells (comprising fibroblasts, endothelial cells and inflammatory cells) (Fig 3E). Additional representative images of RNLS labeling in PDAC precursor lesions and PDAC are presented in S3 Fig and S4 Fig. In-situ and invasive pancreatic ductal neoplasms and precursor lesions showed increased RNLS staining compared to benign samples (normal pancreas and most cases of pancreatitis). Specifically, in precursor and PDAC samples, RNLS was present in most cell types, including all benign elements. The intensity of labeling often appeared greater in neoplastic than in benign epithelium but was not quantified because of limitations of the labeling technique.

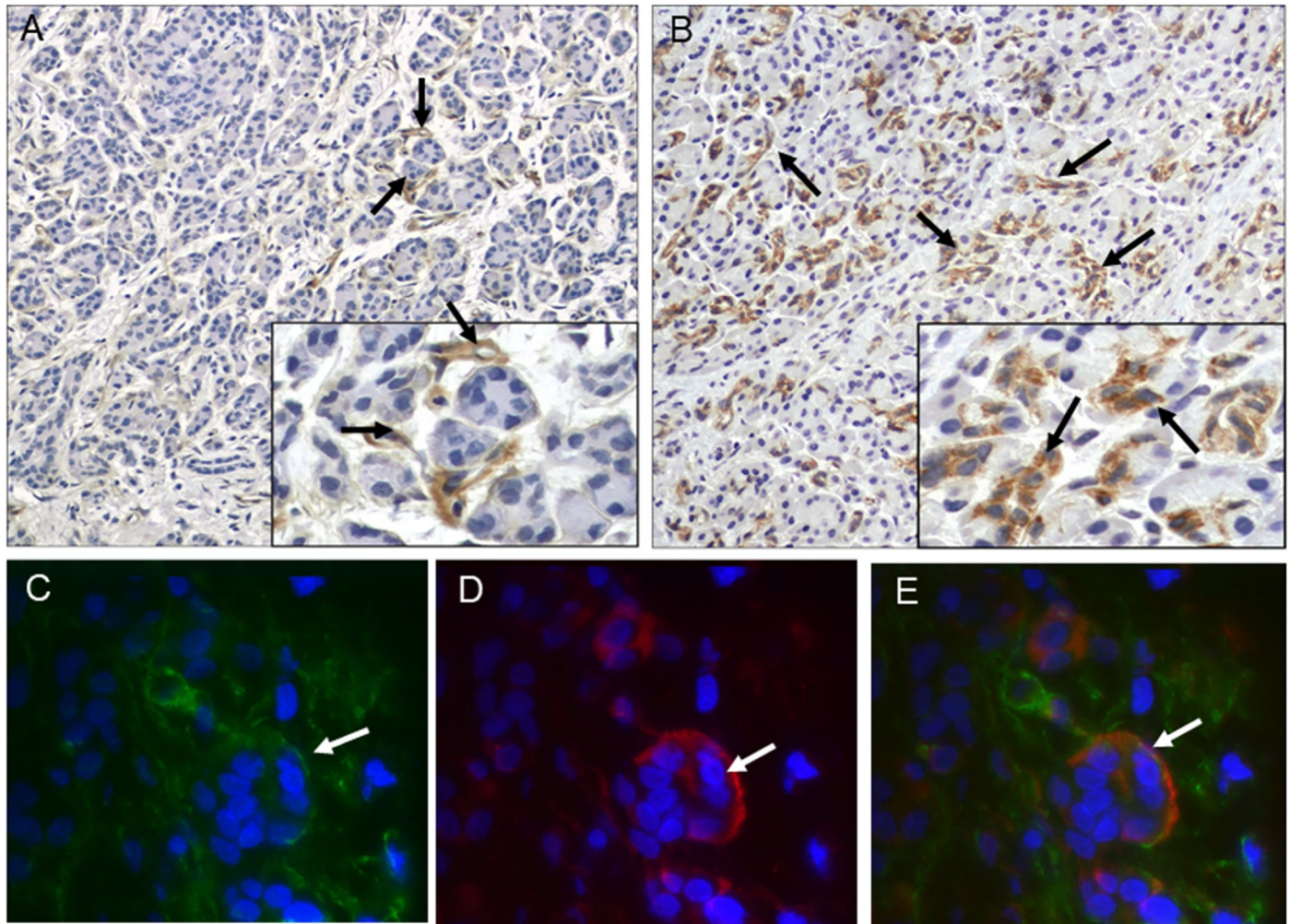


Fig 2. RNLS is present in spindle-shaped cells in human chronic pancreatitis. Representative images of RNLS immunoreactivity using the m28-RNLS antibody in (A) genetic chronic pancreatitis (CTRC mutation) and (B) PDAC-associated chronic pancreatitis. (C-E) Immunofluorescence labeling of RNLS with m28-RNLS antibody (green) and alpha-smooth muscle actin (red) in a patient with genetic chronic pancreatitis (CTRC mutation). The white arrows point to cells positive for both proteins (stellate cells). Original magnification A and B: 200x, insets 400x. Original magnification C-E: 10000x.

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Plasma RNLS in patients with PDAC

Three hundred and forty-seven patients with biopsy confirmed PDAC were identified. Of those, 240 patients had both plasma RNLS levels drawn prior to any PDAC treatment and medical records available for review. Median acid-treated RNLS level was 13291.15 ng/ml for all patients. Among patients with high or low acid-treated plasma RNLS, the median was 17337.52 vs 10135.56 ng/ml, respectively. The difference between these two groups was statistically different ($p < 0.001$). Median non-acid treated RNLS level was 1774.30 ng/ml. Median high non-acid treated RNLS level was 2824.05 ng/ml and median low non-acid treated RNLS level was 647.03 ng/ml ($p < 0.001$). High acid-treated plasma RNLS was associated with younger age at diagnosis but not associated with *race/ethnicity*, gender, or BMI. Patients with higher serum protein levels at diagnosis also had higher plasma RNLS levels at diagnosis (7.30 vs. 7.00, $p = 0.004$). Patients with high acid-treated RNLS levels also exhibited worse PDAC disease attributes (Table 3). This included higher angiolymphatic invasion (80.0% vs. 58.1%, $p = 0.012$), and greater node positive disease (76.5% vs. 56.5%, $p = 0.024$) particularly hepatic

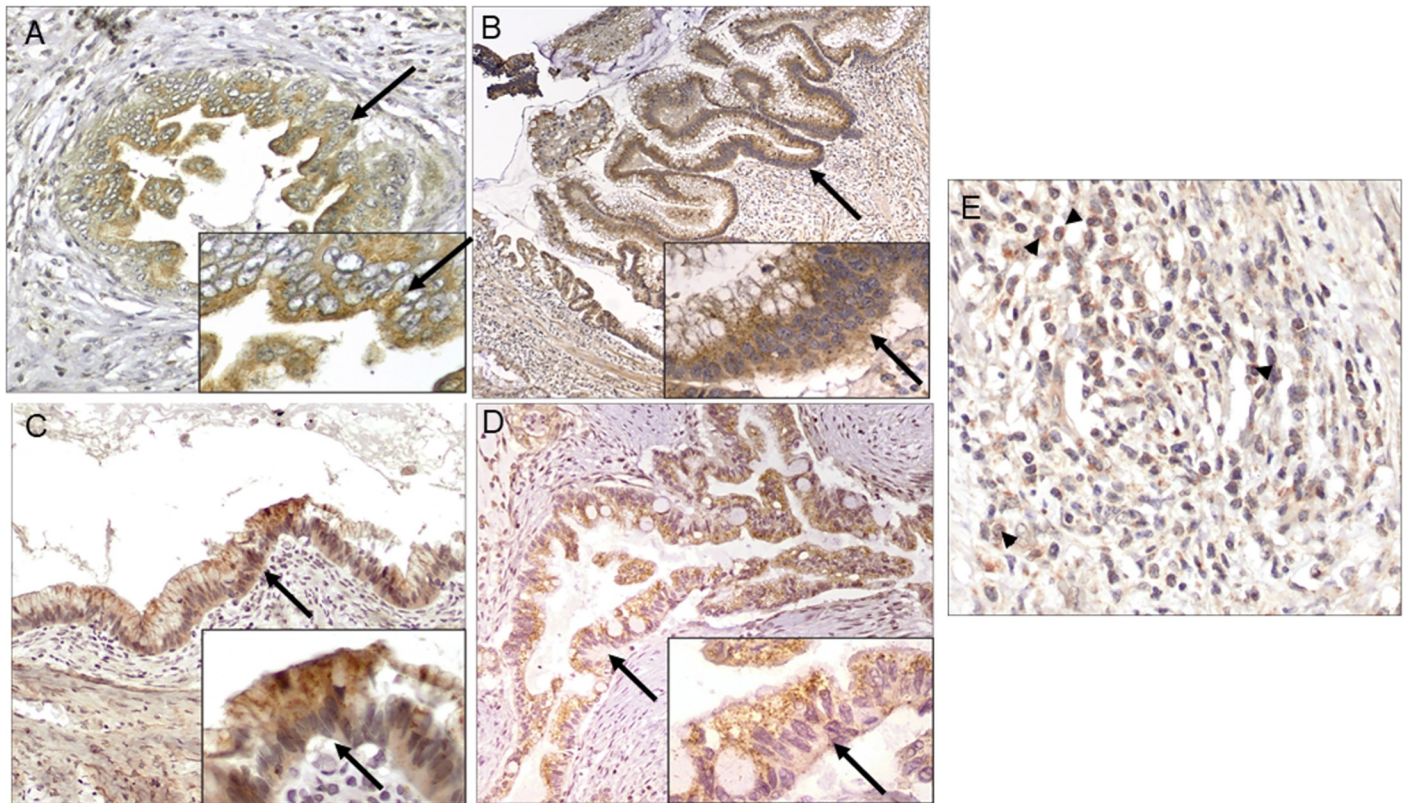


Fig 3. RNLS distribution in human precursor and PDAC tissue. Representative images of RNLS labeling detected by immunohistochemistry using the m28-RNLS antibody in: (A) PanIN, (B) IPMN, (C) MCN, and (D-E) PDAC. Intense granular cytoplasmic staining is present in the majority neoplastic epithelial cells, and in a subset of mononuclear inflammation near tumor. The black arrows point to ductal cells in (A) PanIN, (B) IPMN, (C) MCN, and (D) PDAC. The black arrowheads point to mononuclear inflammatory cells in the PDAC stroma. Original magnification: A, E 400x, inset 600x; B 100x, inset 600x; C, D 200x, insets 400x.

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artery lymph node positivity (26.7% vs. 6.7%, $p = 0.022$). Patients with higher plasma acid-treated RNLS levels did not exhibit larger tumor size, or greater rates of margin positivity (Table 3). In concordance with worse disease features, patients with higher plasma acid-treated RNLS levels were less likely to be deemed clinically resectable at diagnosis (39.2% vs. 58.0%, $p = 0.004$) and were more likely to have disseminated disease (distant metastasis) at diagnosis (25.8% vs. 13.4%, $p = 0.022$). Higher plasma acid-treated RNLS levels at diagnosis were associated with decreased survival with a median of 27.70 months vs. 65.03 months ($p < 0.001$; Fig 4A). The survival difference persisted when acid-treated RNLS levels were divided into tertiles ($n = 80$ per group; Bottom tertile = 19.13 months, Middle tertile = 38.47 months, Top tertile = 65.03 months; $p < 0.001$; Fig 4B) and into quartiles ($n = 60$ per group; Bottom quartile = 19.13 months, Bottom middle quartile = 38.47 months, Top middle quartile = 70.43 months, Top quartile = 65.03 months, $p < 0.001$; Fig 4C). There was no correlation between non-acid treated RNLS levels and either tumor characteristics or survival (S1 Table, S5 Fig).

Plasma RNLS in patients with locally advanced/borderline resectable PDAC

Among patients with PDAC and available plasma RNLS levels, 76 patients presented with LA/BR PDAC according to the treating oncology team at diagnosis. Median acid-treated RNLS

Table 3. Sociodemographic and clinicopathologic features among patients with PDAC with high vs. low plasma RNLS.

Factor	Low RNLS	High RNLS	Total	p-value
<i>Sociodemographic Characteristics</i>				
Age at Diagnosis	68.00 (9.83)	66.00 (9.62)	68.00 (9.80)	0.018
Race/ethnicity				0.236
White	114/120 (95.0%)	107/120 (89.2%)	221/240 (92.1%)	
Black	5/120 (4.2%)	10/120 (8.3%)	15/240 (6.3%)	
Other	1/120 (0.8%)	3/120 (2.5%)	4/240 (1.7%)	
Male	71/120 (59.2%)	75/120 (62.5%)	146/240 (60.8%)	0.692
BMI at Diagnosis	26.37 (5.29)	26.34 (6.92)	26.29 (6.04)	0.719
Albumin	4.00 (0.50)	4.00 (0.50)	4.00 (0.49)	0.237
Total Protein	7.00 (0.71)	7.30 (0.69)	7.10 (0.73)	0.004
<i>Clinicopathologic Characteristics</i>				
Perineural Invasion	61/75 (81.3%)	46/50 (92.0%)	107/125 (85.6%)	0.122
Angiolymphatic Invasion	43/74 (58.1%)	40/50 (80.0%)	83/124 (66.9%)	0.012
Differentiation				0.734
Well	3/75 (4.0%)	1/50 (2.0%)	4/125 (3.2%)	
Moderate	37/75 (49.3%)	23/50 (46.0%)	50/125 (48.0%)	
Poor	35/75 (46.7%)	26/50 (52.0%)	61/125 (48.8%)	
Node Positive	43/76 (56.6%)	39/51 (76.5%)	82/127 (64.6%)	0.024
HALN Positive	3/45 (6.7%)	8/30 (26.7%)	11/75 (14.7%)	0.022
Tumor Size \geq 2cm	58/73 (79.5%)	44/49 (89.8%)	102/122 (83.6%)	0.144
Any Margin	15 (19.7)	15 (29.4)	30 (23.6)	0.237
<i>Whipple Characteristics</i>	Low (n = 53)	High (n = 36)	Total (n = 89)	
Neck Margin	1 (1.9)	2 (5.6)	3 (3.4)	0.563
Bile Duct Margin	0	0	0	–
Anterior Circumferential Margin	0	0	0	–
Posterior Circumferential Margin	11 (20.8)	6 (16.7)	17 (19.1)	0.785
Vascular Groove	2 (3.8)	0 (0.0)	2 (2.2)	0.513
Uncinate Process	10 (18.9)	6 (16.7)	16 (18.0)	1.000
Duodenal	0 (0.0)	1 (2.8)	1 (1.1)	0.404
Invades Duodenum	25 (47.2)	19 (52.8)	44 (49.4)	0.669
Gastric	0	0	0	–
Pancreatic Duct	13 (24.5)	13 (36.1)	26 (29.2)	0.248
CBD	12 (22.6)	9 (25.7)	21 (23.9)	0.801
Peripancreatic soft tissue	43 (81.1)	32 (88.9)	75 (84.3)	0.386
Any margin	12 (22.6)	9 (25.0)	21 (23.6)	0.805
Whipple	53 (68.8)	36 (70.6)	89 (69.5)	1.000
<i>Distal Pancreatectomy Characteristics</i>	Low (n = 24)	High (n = 15)	Total (n = 39)	
Peripancreatic soft tissue	16 (69.6)	13 (86.7)	29 (76.3)	0.273
Pancreatic	0	0	0	–
Anterior	1 (4.3)	1 (6.7)	2 (5.3)	1.000
Posterior	2 (8.7)	4 (26.7)	6 (15.8)	0.188
Spleen	0 (0.0)	1 (6.7)	1 (2.6)	0.395
Any Margin	3 (13.0)	6 (40.0)	9 (23.7)	0.115

Low RNLS is defined as RNLS \leq 13291.15ng/ml (median of data), high RNLS defined as $>$ 13291.15ng/ml (median of data). Total is defined as data for all patients included in the dataset. P-value column includes p-value between low and high RNLS groups.

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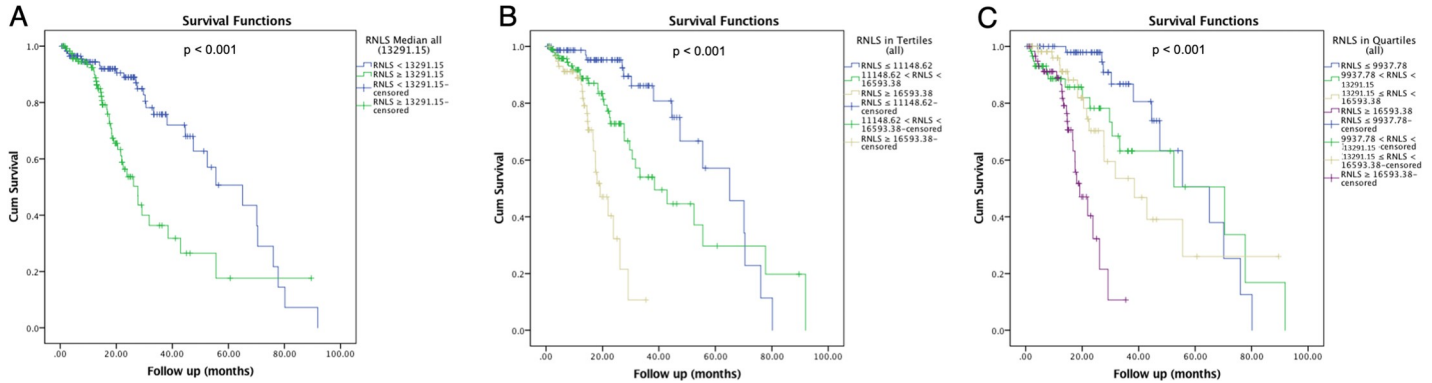


Fig 4. High plasma RNLs levels are associated with worse survival in patients with PDAC. (A) When RNLs is cut by median, (B) by tertiles, and (C) by quartiles.

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level among all LA/BR PDAC patients was 13601.55 ng/ml. Plasma acid-treated RNLs levels did not correlate with age, race/ethnicity, gender, BMI, serum albumin, or total blood protein at diagnosis (Table 4). High acid-treated RNLs level, defined as values above the median, did not correlate with high CA19-9 levels (Table 4). Of note, only 22 of the total 76 patients had CA19-9 values at diagnosis that met the exclusion criteria. Additionally, acid-treated RNLs levels were not associated with the reported tumor size, tumor location, or vessel encasement on imaging at initial presentation (Table 4). Among LA/BR patients, high acid-treated RNLs

Table 4. Patient characteristics for low vs. high plasma RNLs levels in patients with locally advanced/borderline resectable (LA/BR) pancreatic adenocarcinoma (PDAC).

Factor	Low RNLs (n = 38)	High RNLs (n = 38)	p-value
Age at Diagnosis	67.0 (46–90)	63.5 (37–86)	0.651
Race/ethnicity			0.744
White	35 (92.1%)	33 (86.8%)	
Black	2 (5.3%)	3 (7.9%)	
Other	1 (2.6%)	2 (5.3%)	
Gender			0.818
Male	20 (52.6%)	22 (57.9%)	
Female	18 (47.4%)	16 (42.1%)	
Chronic Kidney Disease	1 (2.6%)	2 (5.3%)	1.000
Hypertension	12 (31.6%)	14 (36.8%)	0.809
Heart Failure	1 (2.6%)	0 (0.0%)	1.000
Coronary Artery Disease	4 (10.5%)	2 (5.3%)	0.674
BMI at Diagnosis	25.45 (17.81–40.04)	26.34 (16.46–68.11)	0.875
Albumin at Diagnosis	4.10 (2.50–4.70)	4.00 (3.20–4.70)	0.232
Total Protein at Diagnosis	7.10 (5.60–8.20)	7.15 (5.70–8.30)	0.555
High CA19-9 at Diagnosis ^a	6 (50.0%)	2 (20.0%)	0.204
Large Vessel Encasement	10 (26.3%)	12 (31.6%)	0.801
PDAC Location			0.377
Head	31 (81.6%)	28 (73.7%)	
Body	6 (15.8%)	6 (15.8%)	
Tail	1 (2.6%)	4 (10.5%)	
PDAC Size by imaging	30.50 (15.00–90.00)	31.50 (18.00–92.00)	0.705

^a Only includes patients with total bilirubin < 2.0 and CA19-9 >10 (n = 22).

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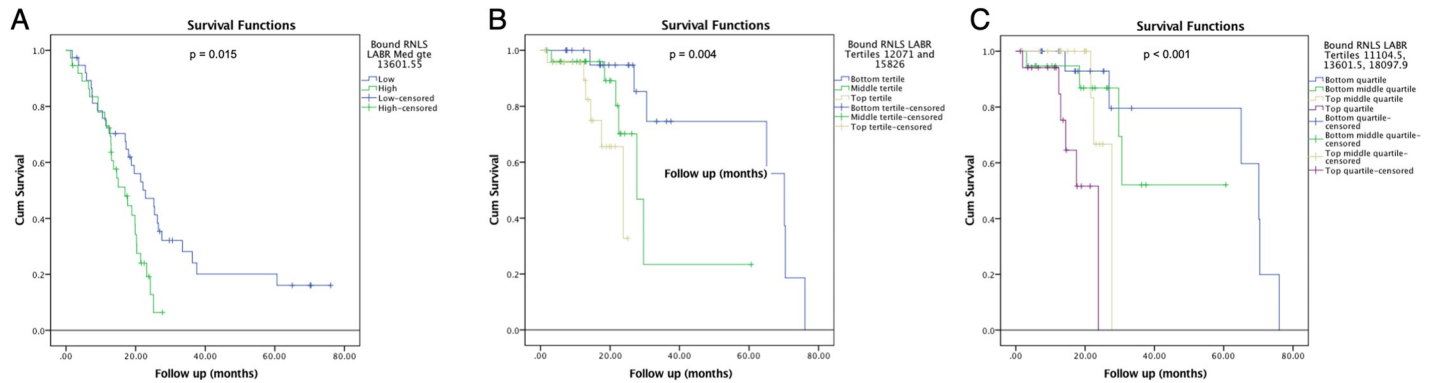


Fig 5. High plasma RNLS levels are associated with worse survival in patients with LA/BR PDAC. (A) When RNLS is cut by median, (B) by tertiles, and (C) by quartiles.

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levels were associated with worse overall survival than were low RNLS levels ($p = 0.015$, Fig 5A). At a median follow-up of 19.60 months, median overall survival was 22.83 months among patients with low RNLS and 16.93 months among those with high RNLS levels. Survival correlations were especially discriminatory when RNLS levels were analyzed using tertiles ($n = 25$ per group; Bottom tertile = 70.13 months, Middle tertile = 27.70 months, Top tertile = 23.83 months; $p = 0.004$, Fig 5B) and quartiles ($n = 19$ per group; Bottom quartile = 60.63 months, Bottom middle quartile = 43.67 months, Top middle quartile = 25.81 months, Top quartile = 18.62 months; $p < 0.001$, Fig 5C). As a continuous variable, RNLS level was also associated with survival ($p = 0.002$). CA19-9 levels were not as predictive of survival. Median survival among those with low vs. high CA19-9 levels was 21.47 vs. 19.60 months ($p = 0.548$). There was no significant difference in survival when compared to values for non-acid treated RNLS (S6 Fig).

Of the 76 patients with LA/BR PDAC, 18 (23.7%) underwent successful resection. Reasons for no eventual surgery in 58 patients were persistent local disease in 37 (48.7%), metastatic disease in 7 (9.2%), poor surgical candidacy in 4 (5.3%), and death in 3 (3.9%). The remaining 7 (9.2%) underwent exploratory laparoscopy and were subsequently found to have either unresectable local (5) or metastatic (2) disease upon surgical examination. Low RNLS levels were associated with greater adjusted odds ratio (aOR) of undergoing resection (aOR = 0.29 (0.09–0.93, $p = 0.036$, Table 5). Of patients with low RNLS levels, 34.2% underwent resection compared to 13.2% patients with high RNLS levels, a > 2-fold difference. On the other hand, serum CA19-9 levels showed no relationship to resection status (aOR = 0.33, $p = 0.272$, Table 5).

Table 5. Treatments for high vs. low RNLS levels in patients with locally advanced/borderline resectable pancreatic adenocarcinoma as compared to CA19-9.

RNLS				
Factor	Low (n = 38)	High (n = 38)	aOR (95%CI)	p-value
Resection	13 (34.2%)	5 (13.2%)	0.29 (0.09–0.93)	0.036
Chemotherapy	36 (94.7%)	36 (97.3%)	2.00 (0.17–23.06)	0.578
Radiotherapy	18 (48.6%)	21 (58.3%)	1.48 (0.59–3.73)	0.408
CA19-9				
Factor	Low (n = 14)	High (n = 8)	aOR (95%CI)	p-value
Resection	3 (42.9%)	3 (20.0%)	0.33 (0.05–2.37)	0.272
Chemotherapy	6 (85.7%)	15 (100.0%)	–	1.000
Radiotherapy	5 (71.4%)	7 (46.7%)	0.35 (0.05–2.41)	0.286

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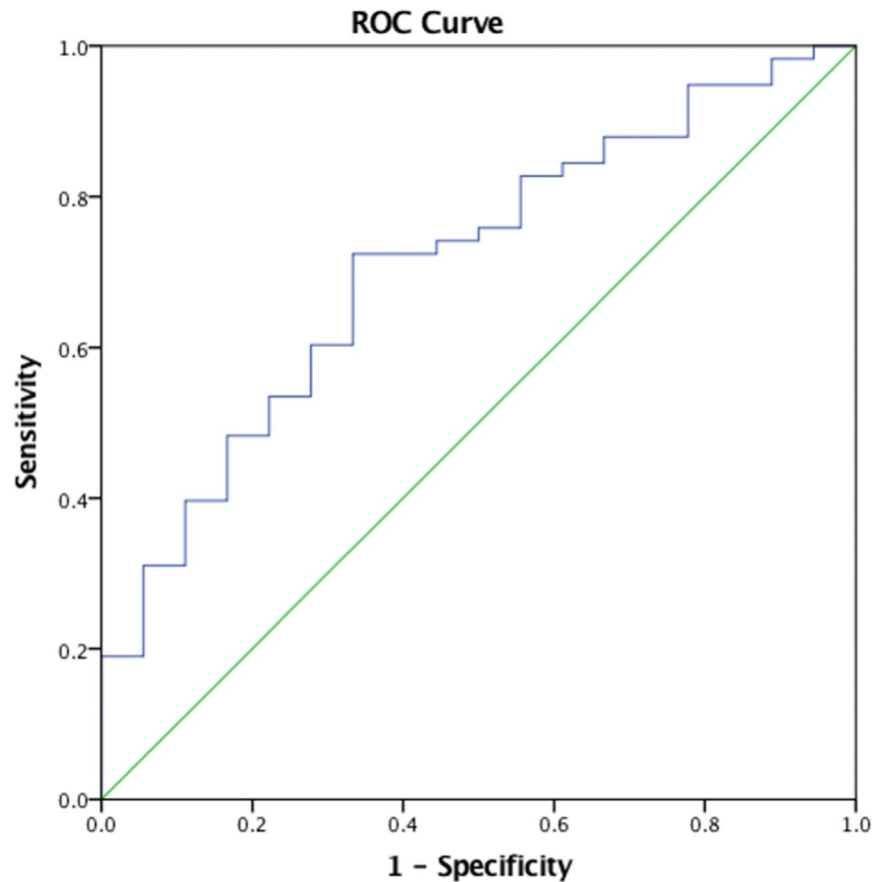


Fig 6. Plasma RNLS levels predict patients which will undergo resection in patients with locally advanced/borderline resectable pancreatic adenocarcinoma in ROC Curve. AUC (area under curve) = 0.674.

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Further, receiver operating characteristic (ROC) analysis shows that lower plasma RNLS levels at diagnosis predicted resection among patients with LA/BR disease (AUC (area under curve) = 0.674; 95% CI 0.53–0.82; $p = 0.037$, Fig 6). The cut-off value with the highest sensitivity and specificity as determined by ROC analysis, 12548.46 ng/mL, was similar to the median of 13601.55 ng/mL and used as the cut-off for the designation of low vs high RNLS status. When using a cut-off value specified by AUC analysis, low RNLS was associated with resection with a sensitivity of 70.69% and a specificity of 66.67% (Table 6). Low CA19-9 levels showed lower sensitivity and lower specificity when compared to RNLS levels at AUC or median cut-off.

Discussion

Our data show that little to no RNLS immunoreactive protein was present in normal pancreatic head tissues but that RNLS was detectable to varying degrees in premalignant PDAC and

Table 6. Sensitivity and specificity tests of CA19-9 and RNLS levels to predict resection in patients with locally advanced/borderline resectable pancreatic adenocarcinoma.

	Sensitivity (95% CI)	Specificity (95% CI)
RNLS (Median)	43.10% (20.16%-56.77%)	27.78% (9.69%-53.48%)
RNLS (AUC)	70.69% (57.27%-81.91%)	66.67% (40.99%-86.66%)
CA19-9	0.00% (0.00%-45.93%)	50.00% (24.65%-75.35%)

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in PDAC tissues. The finding suggests a potential role for RNLS in the development of PDAC. Additionally, we found that high plasma RNLS levels correspond to worse PDAC tumor characteristics, worse overall survival, and less resectability in a subset of patients with LA/BR PDAC.

In a subset of samples primarily represented by chronic pancreatitis, RNLS was also present in stromal cells, including stellate cells with a myofibroblastic phenotype and other unidentified cell types. Stellate cells have important roles in the deposition of the extracellular matrix (stellate cells) and signaling to PDAC cells. The presence of RNLS in stellate cells shown in at least one chronic pancreatitis patient suggests that this protein could have a role in stellate cell function. Previous studies have shown that tumor microenvironment may exert selective pressures, including host immune response, proliferative or survival ability of cancer cells, or physiological restraints, which lead to the predominance of highly malignant PDAC cells [19, 20]. We have previously shown that RNLS is present in tumor-associated macrophages adjacent to melanoma, which can promote tumor growth through a STAT3-mediated mechanism [21]. Therefore, these findings suggest that RNLS could play a role in the tumor microenvironment during PDAC development. Future studies will determine the identity of the RNLS-labeled stromal cells that do not correspond to activated stellate cells and if RNLS levels can distinguish activated versus non-activated stellate cells.

Though essentially absent in other normal pancreatic head tissues taken from patients undergoing a Whipple procedure for duodenal adenomas, RNLS labeling was present in pancreatic tissue of patients who had undergone splenectomy with distal pancreatectomy for abdominal trauma. Normal pancreatic tissues from the pancreatic body or tail obtained in the absence of abdominal trauma were not available for analysis. Whether the RNLS immunoreactivity in the pancreatic tail reflects a local increase as an acute phase reactant [22, 23] in response to trauma or true differences in the RNLS content between distinct regions of the pancreas remains unclear.

A major finding of our study is that plasma levels of acid-treated RNLS correspond to PDAC prognosis. High plasma acid-treated RNLS levels were also associated with worse overall survival, angiolymphatic invasion, node positive disease, including sentinel hepatic artery lymph node positive disease, and metastasis. Plasma acid-treated RNLS levels were not associated with tumor size or surgical margin status among patients treated with Whipple or distal pancreatectomy surgery. It may be relevant that node positivity and metastasis reflect the clinically prognostic finding of tumor spread outside of the pancreas but that the effect of tumor size and margin status on prognosis in PDAC is controversial [24, 25]. Overall, these findings suggest that plasma acid-treated RNLS levels correlate with poor prognostic histopathologic features in PDAC. On the other hand, non-acid treated RNLS levels did not correspond with PDAC prognosis. Previous studies have found that non-acid treated plasma RNLS, considered its free form, is associated with mortality in patients with chronic kidney disease [15]. Our findings suggest that acid treated RNLS, hypothesized to represent a RNLS form in which its antigenic epitopes for RNLS antibody are hidden prior to acid treatment, however, may be associated with PDAC. The functional significance of this difference requires further evaluation.

Previous studies have suggested that tissue expression of RNLS is higher in select cancers than in benign tissues, including PDAC [26–30]. Tissue RNLS has been also described as a survival factor during ischemic or toxic injury and as a cytokine that activates several pathways, including PI3K/AKT, MAPK, p-ERK1/2, protein kinase B, and JAK/STAT pathways [28–30]. In PDAC tissue studies, high RNLS tumor expression was associated with worse overall survival [8]. Conditions that block RNLS can inhibit both in vitro and in vivo PDAC tumor growth [8]. Further studies would benefit from understanding the mechanism of elevated

plasma RNLS levels in patients with PDAC, whether different forms of RNLS have distinct tumor effects, if tissue RNLS contributes the levels of plasma RNLS or whether the changes in plasma levels represents host responses to the tumor. Knowledge of whether plasma RNLS levels change with PDAC resection and can predict recurrence could also be clinically useful.

When considering patients who presented with LA/BC PDAC, our data show that reduced plasma RNLS, but not CA19-9, can predict both whether LA/BR PDAC patients will be subject to resection and their overall survival; CA-19 blood levels were not found to be predictive of these outcomes. Specifically, low plasma RNLS levels at diagnosis were associated with eventual eligibility for PDAC resection and with increased overall survival. There was a more than 2-fold increase in the rate of resection in patients with low plasma RNLS levels compared to high plasma RNLS levels. Previous studies have found survival improvements with resection in appropriately selected patients with locally advanced/borderline resectable PDAC [31]. Though our study includes a limited number of patients, we found a non-significant trend towards improved survival in patients undergoing resection. Future studies should prospectively assess the role of RNLS in resection clinical decision making and survival outcomes. Additionally, whether plasma RNLS levels can predict chemoresistance should be considered in future studies to determine the potential utility of plasma RNLS in tailoring chemotherapeutic strategies. Together with tumor characteristic findings in resected PDAC samples, these findings suggest that plasma RNLS levels may reflect a tumor biology that predict outcome independent of radiologic tumor size, location, or vessel encasement at presentation. Plasma RNLS levels could also reflect characteristics that were not analyzed such as genetic, epigenetic or stromal microenvironment characteristics that portend worse outcomes. Future studies should also consider measuring RNLS levels in patients throughout the development of PDAC from normal controls to chronic pancreatitis to develop a broader understanding of how RNLS may change throughout PDAC development.

Our study has a few limitations. First, the small sample size of each tissue type and immunohistochemical protocol limits quantitative comparisons of the levels of RNLS in pancreatic tissue between each group. Future studies using greater numbers of tissue samples and an automated immunohistochemistry platform are needed to quantitatively compare tissue levels of RNLS across tissues. For plasma RNLS studies, the retrospective cohort study design limited the ability to accurately assess survival over long periods of time to predict resectability without bias from independent treatment team decisions. We were also limited in analyzing factors that may play a role in determining a patient's candidacy for surgery such as comorbidities, fitness, and anatomical limitations that were not uniformly present in chart review. Without serial plasma RNLS measurements or concurrent tissue expression of RNLS on plasma RNLS samples, we cannot correlate plasma RNLS with underlying PDAC tissue expression. The small sample size of this study also limited power. A larger sample size may have yielded a potential relationship between CA19-9 and RNLS that was not seen here among LA/BR patients. Additionally, for example, we found the median survival among patients who underwent resection versus no resection was 29.67 months vs. 30.57 months ($p = 0.060$). This small sample size makes it difficult to evaluate this parameter in this cohort. Finally, there may have been selection bias associated with the type of patients who obtained care at our institution and subsequently were included in the biobank.

In conclusion, a key finding of our study is that RNLS is increased in both premalignant and malignant PDAC tissue compared to normal pancreas, suggesting a potential role for RNLS in the early development of PDAC. In addition, we found that the relationship between plasma RNLS levels and clinical outcomes of patients with PDAC complements published data that correlated tissue RNLS expression and PDAC survival. These findings suggest that plasma RNLS could be used as a predictive biomarker in patients with PDAC and guide therapies

such as resectability in LA/BR PDAC. The RNLS levels in tissue and plasma suggest a potential pathophysiological mechanism of RNLS for the development of PDAC and severe PDAC disease. Further studies should explore the potential mechanism of action of RNLS in pre-malignant pancreatic tissue and its expression in stromal cells including stellate cells. Additionally, further studies should also explore disease progression patterns, plasma RNLS levels and tumor RNLS expression after resection, and the origin of plasma RNLS compared to tumor expression of RNLS. To further assess the ability of plasma RNLS to predict resectability in LA/BR pancreatic disease, larger prospective studies are needed that examine changes in plasma RNLS levels following neoadjuvant treatment. If plasma and tissue RNLS indeed reflects tumor biology and pathophysiology, it holds promise as a guide to surgical interventions and potential therapies that inhibit the pro-survival effects of RNLS in PDAC.

Supporting information

S1 Fig. RNLS labeling in normal pancreas.

(TIFF)

S2 Fig. RNLS labeling in chronic pancreatitis.

(TIFF)

S3 Fig. RNLS labeling in PanIN and MCN lesions.

(TIFF)

S4 Fig. RNLS labeling in IPMN and PDAC lesions.

(TIFF)

S5 Fig. Association between high and low RNLS levels with PDAC survival.

(TIFF)

S6 Fig. Association between high and low RNLS levels with survival in locally advanced/borderline resectable PDAC.

(TIFF)

S7 Fig. Acid-treated vs non-treated plasma RNLS in PDAC.

(TIFF)

S1 Table. Demographics of PDAC patients.

(TIFF)

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Author Contributions

Conceptualization: John W. Kunstman, Charles H. Cha, Marie E. Robert, Gary V. Desir, Fred S. Gorelick.

Data curation: Yasheen Gao, Melinda Wang, Sade' M. B. Finn, Jill Lacy, John W. Kunstman, Charles H. Cha, Marie E. Robert.

Formal analysis: Yasheen Gao, Melinda Wang, Xiaojia Guo, Tian-min Chen, Charles H. Cha, Marie E. Robert, Gary V. Desir.

Funding acquisition: Gary V. Desir, Fred S. Gorelick.

Investigation: Yasheen Gao, Melinda Wang, Xiaojia Guo, Marie E. Robert.

Methodology: Yasheen Gao, Melinda Wang, Tian-min Chen, Gary V. Desir.

Project administration: Fred S. Gorelick.

Resources: Xiaojia Guo, Joanna Hu, Tian-min Chen, Sade' M. B. Finn, Jill Lacy, John W. Kunstman, Charles H. Cha, Melena D. Bellin, Marie E. Robert.

Software: Melinda Wang.

Supervision: Fred S. Gorelick.

Validation: Melena D. Bellin.

Writing – original draft: Yasheen Gao, Melinda Wang.

Writing – review & editing: Joanna Hu, John W. Kunstman, Charles H. Cha, Marie E. Robert, Gary V. Desir, Fred S. Gorelick.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018; 68(6):394–424. <https://doi.org/10.3322/caac.21492> PMID: 30207593
2. Rawla P, Sunkara T, Gaduputi V. Epidemiology of Pancreatic Cancer: Global Trends, Etiology and Risk Factors. *World J Oncol.* 2019; 10(1):10–27. <https://doi.org/10.14740/wjon1166> PMID: 30834048
3. Hidalgo M. New insights into pancreatic cancer biology. *Ann Oncol.* 2012; 23 Suppl 10:x135–8. <https://doi.org/10.1093/annonc/mds313> PMID: 22987949
4. Nolen BM, Brand RE, Prosser D, Velikkhatnaya L, Allen PJ, Zeh HJ, et al. Prediagnostic serum biomarkers as early detection tools for pancreatic cancer in a large prospective cohort study. *PLoS One.* 2014; 9(4):e94928. <https://doi.org/10.1371/journal.pone.0094928> PMID: 24747429
5. Xu J, Li G, Wang P, Velazquez H, Yao X, Li Y, et al. Renalase is a novel, soluble monoamine oxidase that regulates cardiac function and blood pressure. *J Clin Invest.* 2005; 115(5):1275–80. <https://doi.org/10.1172/JCI24066> PMID: 15841207
6. Desir GV, Tang L, Wang P, Li G, Sampaio-Maia B, Quelhas-Santos J, et al. Renalase lowers ambulatory blood pressure by metabolizing circulating adrenaline. *J Am Heart Assoc.* 2012; 1(4):e002634. <https://doi.org/10.1161/JAHA.112.002634> PMID: 23130169
7. Desir GV, Wang L, Peixoto AJ. Human renalase: a review of its biology, function, and implications for hypertension. *J Am Soc Hypertens.* 2012; 6(6):417–26. <https://doi.org/10.1016/j.jash.2012.09.002> PMID: 23107895
8. Guo X, Hollander L, MacPherson D, Wang L, Velazquez H, Chang J, et al. Inhibition of renalase expression and signaling has antitumor activity in pancreatic cancer. *Sci Rep.* 2016; 6:22996. <https://doi.org/10.1038/srep22996> PMID: 26972355
9. Hruban RH, Maitra A, Goggins M. Update on pancreatic intraepithelial neoplasia. *Int J Clin Exp Pathol.* 2008; 1(4):306–16. PMID: 18787611
10. Hingorani SR, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell.* 2003; 4(6):437–50. [https://doi.org/10.1016/s1535-6108\(03\)00309-x](https://doi.org/10.1016/s1535-6108(03)00309-x) PMID: 14706336
11. Peters MLB, Eckel A, Mueller PP, Tramontano AC, Weaver DT, Lietz A, et al. Progression to pancreatic ductal adenocarcinoma from pancreatic intraepithelial neoplasia: Results of a simulation model. *Pancreatology.* 2018; 18(8):928–34. <https://doi.org/10.1016/j.pan.2018.07.009> PMID: 30143405
12. Hezel AF, Kimmelman AC, Stanger BZ, Bardeesy N, Depinho RA. Genetics and biology of pancreatic ductal adenocarcinoma. *Genes Dev.* 2006; 20(10):1218–49. <https://doi.org/10.1101/gad.1415606> PMID: 16702400

13. Lennon AM, Wolfgang CL, Canto MI, Klein AP, Herman JM, Goggins M, et al. The early detection of pancreatic cancer: what will it take to diagnose and treat curable pancreatic neoplasia? *Cancer Res.* 2014; 74(13):3381–9. <https://doi.org/10.1158/0008-5472.CAN-14-0734> PMID: 24924775
14. Wang L, Velazquez H, Moeckel G, Chang J, Ham A, Lee HT, et al. Renalase prevents AKI independent of amine oxidase activity. *J Am Soc Nephrol.* 2014; 25(6):1226–35. <https://doi.org/10.1681/ASN.2013060665> PMID: 24511138
15. Chang J, Guo X, Rao V, Gromisch E, Chung S, Kluger H, et al. Identification of Two Forms of Human Plasma Renalase, and Their Association With All-Cause Mortality. *Kidney international reports.* 2020; 5(3):362–8. <https://doi.org/10.1016/j.ekir.2019.12.002> PMID: 32154458
16. Tempero MA, Uchida E, Takasaki H, Burnett DA, Steplewski Z, Pour PM. Relationship of carbohydrate antigen 19–9 and Lewis antigens in pancreatic cancer. *Cancer research.* 1987; 47(20):5501–3. PMID: 3308077
17. Winter JM, Yeo CJ, Brody JR. Diagnostic, prognostic, and predictive biomarkers in pancreatic cancer. *Journal of surgical oncology.* 2013; 107(1):15–22. <https://doi.org/10.1002/jso.23192> PMID: 22729569
18. [NCCN.org](https://www.nccn.org). NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines). 2019.
19. Takahashi K, Ehata S, Koinuma D, Morishita Y, Soda M, Mano H, et al. Pancreatic tumor microenvironment confers highly malignant properties on pancreatic cancer cells. *Oncogene.* 2018; 37(21):2757–72. <https://doi.org/10.1038/s41388-018-0144-0> PMID: 29511349
20. Ball CR, Oppel F, Ehrenberg KR, Dubash TD, Dieter SM, Hoffmann CM, et al. Succession of transiently active tumor-initiating cell clones in human pancreatic cancer xenografts. *EMBO Mol Med.* 2017; 9(7):918–32. <https://doi.org/10.15252/emmm.201607354> PMID: 28526679
21. Hollander L, Guo X, Velazquez H, Chang J, Safirstein R, Kluger H, et al. Renalase Expression by Melanoma and Tumor-Associated Macrophages Promotes Tumor Growth through a STAT3-Mediated Mechanism. *Cancer Res.* 2016; 76(13):3884–94. <https://doi.org/10.1158/0008-5472.CAN-15-1524> PMID: 27197188
22. Haulik L, Toth B, Issekutz A, Gartner B. [Pancreatic injury in blunt abdominal trauma: early versus late diagnosis and surgical management]. *Magy Seb.* 2001; 54(5):309–13. PMID: 11723735
23. Heuer M, Hussmann B, Lefering R, Taeger G, Kaiser GM, Paul A, et al. Pancreatic injury in 284 patients with severe abdominal trauma: outcome, course, and treatment algorithm. *Langenbecks Arch Surg.* 2011; 396(7):1067–76. <https://doi.org/10.1007/s00423-011-0836-1> PMID: 21847623
24. Li D, Hu B, Zhou Y, Wan T, Si X. Impact of tumor size on survival of patients with resected pancreatic ductal adenocarcinoma: a systematic review and meta-analysis. *BMC cancer.* 2018; 18(1):985. <https://doi.org/10.1186/s12885-018-4901-9> PMID: 30326871
25. Tummers W, Groen J, Sibinga Mulder B, Farina-Sarasqueta A, Morreau J, Putter H, et al. Impact of resection margin status on recurrence and survival in pancreatic cancer surgery. *British Journal of Surgery.* 2019; 106(8):1055–65. <https://doi.org/10.1002/bjs.11115> PMID: 30883699
26. Akkoc R, Aydin S, Goksu M, Ozcan Yildirim S, Eroksuz Y, Ogeturk M, et al. Can renalase be a novel candidate biomarker for distinguishing renal tumors? *Biotechnic & Histochemistry.* 2020:1–6. <https://doi.org/10.1080/10520295.2020.1825805> PMID: 33956551
27. Guo X, Hollander L, MacPherson D, Wang L, Velazquez H, Chang J, et al. Inhibition of renalase expression and signaling has antitumor activity in pancreatic cancer. *Scientific reports.* 2016; 6(1):1–10. <https://doi.org/10.1038/s41598-016-0001-8> PMID: 28442746
28. Hollander L, Guo X, Velazquez H, Chang J, Safirstein R, Kluger H, et al. Renalase expression by melanoma and tumor-associated macrophages promotes tumor growth through a STAT3-mediated mechanism. *Cancer research.* 2016; 76(13):3884–94. <https://doi.org/10.1158/0008-5472.CAN-15-1524> PMID: 27197188
29. Wang Y, Safirstein R, Velazquez H, Guo XJ, Hollander L, Chang J, et al. Extracellular renalase protects cells and organs by outside-in signalling. *Journal of cellular and molecular medicine.* 2017; 21(7):1260–5. <https://doi.org/10.1111/jcmm.13062> PMID: 28238213
30. Yu X, Han P, Wang J, Sun H, Shao M. Renalase overexpression in ER-positive breast cancer. *International Journal of Clinical and Experimental Pathology.* 2018; 11(3):1297. PMID: 31938225
31. Del Chiaro M, Rangelova E, Halimi A, Ateeb Z, Scandavini C, Valente R, et al. Pancreatotomy with arterial resection is superior to palliation in patients with borderline resectable or locally advanced pancreatic cancer. *HPB.* 2019; 21(2):219–25. <https://doi.org/10.1016/j.hpb.2018.07.017> PMID: 30093144