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Osteopontin Splice Variant as a Potential Marker for Metastatic Disease in Pancreatic Adenocarcinoma

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Abstract

Background and Aims—Osteopontin (OPN) is a phosphoprotein that activates pathways that induce cancer cell survival and metastasis. Our aim was to examine the expression pattern of OPN splice variants a, b and c in fine needle aspirates and to determine their correlation with stage-adjusted pancreatic ductal adenocarcinoma (PDA) survival.

Methods—Endoscopic ultrasound-guided fine needle aspiration was performed in patients with solid pancreatic masses. The tissue was collected and analyzed for the expression of OPN isoforms by RT-PCR. Survival curves of stages and overexpression of OPN splice variants (a, b, c) were estimated according to the Kaplan-Meier and the log-rank test.

Results—EUS-FNA was performed in 46 patients with solid pancreatic lesions (40 PDA & 6 chronic pancreatitis). OPNa was highly expressed in 39/40 (98%), OPNb in 24/40 (60%), while OPNc was present in 10/40 (25%) of PDA samples. The median survival was lower in patients whose FNA samples expressed OPNb than those without (406 days vs. 749 days, p=0.049). There was no significant difference in survival in patients with OPNc. Cox proportional hazard model demonstrated that OPNb expression had a trend towards decrease overall survival (p=0.06), with these patients having a hazard of death 3 times higher than those without. OPNc was found to significantly correlate with metastatic disease (p=0.009) in PDA patients.

Conclusions—Our data shows for the first time that in FNA samples there is a strong association between OPNc and presence of metastasis in PDA, and OPNb and poor survival.

Keywords

pancreatic cancer; fine needle aspirates; cytology; osteopontin

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDA) is the 4th leading cause of the cancer death in the United States.(1) The overall 5-year survival rate is less than 5%, with 85% of patients with PDA being diagnosed after the tumor has infiltrated into adjacent organs or when distant metastases are present.

OPN gene is subject to alternative splicing resulting in 3 isoforms: OPNa, the full-length isoform, OPNb lacks exon 5 and OPNc lacks exon 4.(2) Recent studies have shown that OPNc is expressed in invasive, but not in noninvasive breast and liver tumor cell lines.(3;4) It has been suggested that this isoform may accelerate tumor progression by conveying anchorage independence and inducing the expression of oxidoreductases.(3;5) We have recently identified OPNc as being highly expressed in invasive PDA tissues from smokers. (6)

To date, there has been very limited information about the role of OPN and its isoforms in pancreatic malignancies. In this study, our primary aim was to analyze the expression pattern of OPN splice variants a, b and c in endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) of pancreatic lesions and to determine their correlation with the presence of metastatic disease and other clinicopathological parameters at the time of presentation. Our secondary aim was to evaluate whether the presence of specific OPN splice variants in PDA lesions correlated with the stage-adjusted PDA survival.

PATIENTS and METHODS

Patients

We prospectively enrolled 46 consecutive patients for the evaluation of solid pancreatic lesions seen on imaging study with a final diagnosis of pancreatic ductal adenocarcinoma (PDA) in 40 and focal chronic pancreatitis in 6 who underwent an initial EUS-FNA at Thomas Jefferson University Hospital from October 2010 to March 2012. Patients were excluded if (1) they had the inability to provide informed consent, (2) if it was anticipated that they would be unavailable for an office follow-up, or (3) the patient had coagulopathy (international normalized ratio >1.5 or platelets <50,000). The study was granted approval by the institutional review board and informed consent was obtained from all patients prior to the procedure.

Patients in the current study were considered to have a pancreatic malignancy if there was (1) cytologic or histologic evidence of malignancy based upon material obtained by EUS-FNA, ERCP, surgical or percutaneous biopsy or (2) clinical course based upon a 6 month follow-up in which that patient developed radiographic evidence of local or distant metastasis, or death attributed to a malignant pancreatic lesion based upon autopsy results. A lesion was defined as being benign based upon EUS-FNA results, a minimum of a 6 month clinical follow-up and absence of any of the above defined criteria for malignancy.

Patient details were analyzed for demographic characteristics, presenting clinical features, laboratory data, and imaging studies from patient interviews and medical records.

Specimen Acquisition by EUS-FNA and Specimen Preparation

All EUS-FNA procedures were performed by 3 experienced faculty endoscopists with greater than 5 years of experience. Once the pancreatic lesion was visualized, a curvilinear echoendoscope (Olympus GF UCT 140 or UCT 160, Melville, NY) was used to perform the EUS-FNA. A 22-gauge FNA needle (Echotip, Wilson-Cook, Winston-Salem, NC) was utilized to obtain cytological samples. Aspirates were placed onto glass slides and preserved with Diff-Quik stain (American Scientific Products, McGraw Park, Illinois, USA). Any additional material was sprayed into Hanks's solution and sent for cell block processing. One or more needle passes of the pancreatic lesion were performed until the technician established the presence of an adequate number of cells to make a diagnosis. One pass of aspirated material was then collected exclusively to be analyzed for OPN isoforms.

Patients with a confirmed malignant lesion by EUS-FNA had the tumor's clinical stage at diagnosis classified according to the tumor-node-metastasis (TNM) system and International Classification of Diseases for Oncology.(7)

RNA isolation and Semi-quantitative PCR

Total RNA was isolated from aspirated centrifuged samples using Trizol reagent (Life Technologies, Gaitherburg, MD). RNAs were quantified, DNase-digested, and cDNAs were prepared using ImProm-II^{TM} Reverse Transcription System (Promega), then subjected to semi-quantitative PCR using master mix (Promega). The primers used were OPNa forward: 5′-ATCTCCTAGCCCCACAGAAT-3′, reverse: 5′-CATCAGACTGGTGAGAATCATC-3′; OPNb forward: 5′-AAAATCAGTGACCAGTTCATCAG-3′, reverse: 5′-ATCTCCTAGCCCCAGAGAC-3′; OPNc forward: 5′-TCAGGAAAAGCAGAATGCTG-3′, reverse: 5′-GTCAATGGAGTCCTGGCTGT-3′. Upstream and downstream primers that could anneal with the 3′-untranslated region of human GAPDH were included in the PCR reaction as an internal standard, forward: 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′, reverse: 5′-CATGTGGGCCATGAGGTCCACCAC-3′. The linear range of amplification for each set of primers was determined to ensure that we used a number of cycles in the linear range. PCR products were electrophoresed on 2% agarose gels and band intensities were quantified by densitometry using Kodak Electrophoresis Documentation and Analysis System 290 (EDAS 290) in reference to GAPDH. All the samples were adequate for measurement of OPN expression.

Statistical analysis

All experiments were performed 4 to 6 times. Data was analyzed and the expression of OPN splice variants a, b and c were correlated with different clinicopathological parameters including mass location, tumor stage at presentation, and the presence of metastases. Data were analyzed for statistical significance by ANOVA with post-hoc student *t* test analysis. Continuous normally distributed variables were analyzed by Student-t-test. Cross-tabulations of OPN splice variants (a, b, c) and survival status were run along with Fishers Exact Test. Kaplan-Meier plots and log-rank chi square statistics were performed for univariate association of each gene subset with survival, unadjusted for grade. Three Cox Proportional Hazards regression models were run to assess the effect of each splice variant

subset on survival, adjusting for grade. These models were adjusted for 2-category grade. Analyses were performed with the assistance of a computer program (JMP 5 Software SAS Campus Drive, Cary, NC). Differences were considered significant at $P \leq 0.05$.

RESULTS

Patient characteristics

From October 2010 to March 2011, EUS-FNA was performed on 46 patients with solid pancreatic lesions without any complications. The mean age of the patients was 66.7 years (SD ± 14.7); 58% were male (74% White, 12% African American, 7% Hispanic, 7% other). The clinical presentation in these patients was obstructive jaundice (46%), weight loss (33%), poor appetite (57%), malaise (14%) and abdominal pain (67%). Overall 40/46 (87%) of lesions had a final diagnosis of pancreatic ductal adenocarcinoma and 6/46 (13%) were diagnosed with chronic focal pancreatitis. The patient characteristics are summarized in Table 1.

Patients with Pancreatic Ductal Adenocarcinoma (n=40)

Cytology established a definitive diagnosis of malignancy in 40 cases by EUS-FNA. The mean age of patients was 68.1 years (SD ± 11.7) and 57% were male. Of the patients diagnosed with pancreatic adenocarcinoma, 21 (53%) had a history of hypertension, 9 (23%) were diabetic, 21 (53%) were smokers and 9 (23%) had a history of alcohol abuse. Three patients had a history of concomitant chronic pancreatitis. The mean CA 19-9 levels at time of presentation were 1143 U/ml (range 2–16,597). The aspirated pancreatic lesions ranged from 7mm to 53mm, with a mean size of 31mm and a median size of 30mm. A median of 5 passes (range, 1–8 passes) with the FNA needle was needed to obtain adequate samples from lesions for definitive diagnosis (Table 2).

Pancreatic cancer at the time of diagnosis was: a) Stage I in 3/40 (7.5%) patients, b) Stage II in 12/40 (30%) patients, c) Stage III in 8/40 (20%) patients and d) Stage IV metastatic disease in 17/40 (43%) patients.

Expression of OPN isoforms in FNA samples from PDA patients

As a first step to investigate the expression profiling of distinct OPN splice variants in PDA, we analyzed OPNa, OPNb and OPNc mRNA levels in the pancreatic cancer EUS-FNA cytology specimens. Relative to GAPDH, band intensities were quantified by densitometry. As seen in table 3, OPNa was expressed in 39/40 (97.5%) of PDA patients with relative mRNA levels of 186 ± 36 (mean ± SD) transcripts/μl cDNA (range 116–241). OPNb was expressed in 24/40 (60%) of PDA patients with relative mRNA levels of 179.6 ± 41 (mean ± SD) transcripts/μl cDNA (range 94–233). OPNc was expressed in 10/40 (25%) of PDA patients with relative mRNA levels of 192.1 ± 21 (mean ± SD) transcripts/μl cDNA (range 164–222). Representative UV light visualization of ethidium bromide gel electorphoresis for OPN isoforms and GAPDH in PDA patients is seen in Figure 1.

In patients with PDA, there was no statistical difference in the overall demographics or clinical presentation in patients who expressed OPNa, OPNb and OPNc.

There was no significant difference in the expression of OPNa between smokers and non-smokers in patients with PDA (100% versus 94% respectively; p=0.45). Similarly, there was no significant difference in the expression of OPNb between smokers and non-smokers in patients with PDA (72% versus 55% respectively; p=0.33). OPNc was expressed in 9/21 (43%) of smokers compared to only 1/19 (5%) non-smokers (OR=13.5 [95% CI=1.5–120.8]; p=0.009). These data indicate that OPNc in FNA samples has significant association with the smoking status of PDA patients.

There was no statistically significant difference in the expression of OPNa between patients with stage 4 metastatic and non-metastatic PDA (100% versus 96% respectively; p=1). OPNb was expressed in 11/17 (65%) patients with stage 4 metastatic and compared to 14/23 (61%) patients with non-metastatic PDA (p=1). OPNc was expressed in 8/17 (47%) patients with metastatic disease compared to only 2/23 (9%) patients without metastasis (OR=9.3 [95% CI=1.6–52.9]); this was found to be statistically significant with a p=0.009. OPNc was expressed in 80% of patients with metastatic adenocarcinoma compared to only 44% of patients with OPNa and OPNb expression (p=0.01). These data indicate that OPNc in FNA samples has significant association with metastasis in PDA patients. These results are illustrated in Figure 2.

Patients with Chronic Pancreatitis (n=6)

Cytology and clinical follow-up of at least 6 months established a diagnosis of chronic focal pancreatitis in 6 patients. The mean age of patients was 59.3 years (SD ± 13) and 66% were male. Of the patients diagnosed with chronic pancreatitis, 3 (50%) had a history of hypertension, 1 (17%) was diabetic, 4 (67%) were smokers and 5 (83%) had a history of alcohol abuse. The mean CA 19-9 levels at time of presentation were 22U/ml (range 8–43). The aspirated pancreatic lesions ranged from 10mm to 33mm in size, with a mean and median size of 23mm. A median of 6 passes (range, 1–10) with the FNA needle was needed to obtain adequate samples from lesions for definitive diagnosis.

Expression of OPN in patients with chronic pancreatitis

As seen in table 3, OPNa was expressed in 3/6 (50%) chronic pancreatitis patients with relative mRNA levels of 147 ± 32 (mean ± SD) transcripts/μl cDNA (range 120–183). OPNb was expressed in 1/6 (17%) chronic pancreatitis patient with relative mRNA levels of 173 transcripts/μl cDNA. OPNc was not expressed in any patient with chronic pancreatitis. Representative UV light visualization of ethidium bromide gel electrophoresis for OPN isoforms and GAPDH in PDA patients is seen in Fig 3. Focal pancreatic inflammation was not associated with the presence of OPNc.

Comparison of OPN isoform expression in PDA and chronic pancreatitis

OPNa and OPNb were expressed in 97.5% and 60% respectively in patients with PDA in comparison to 50% and 17% of patients with chronic pancreatitis (Tables 3 &4); this difference was found to be statistically significant with a $p<0.05$. Twenty-five percent of patients with PDA expressed OPNc as compared to none with chronic pancreatitis. Thus, OPNc isoform expression in cell cytology itself was found to be an excellent marker for distinguishing pancreatic malignancy from chronic pancreatitis by EUS-FNA.

Correlation of OPN isoform expression in PDA and stage-adjusted patient survival

The median survival of the 40 subjects with PDA was 162 days (range = 73–626). In Kaplan-Meier univariate analysis, the median survival time was significantly longer in patients that expressed OPNa when compared to those without (579 days vs. 113 days, p=0.003); however meaningful statistical interpretation is difficult since only 1 patient in our cohort did not express OPNa. The median survival was lower in patients' FNA samples that expressed OPNb than those without (406 days vs. 749 days, *p*=0.049). There was no significant difference in survival in patients with and without OPNc (530 days vs. 441 days, p=1). The associations of OPN isoform expression with disease-specific survival are illustrated in Figure 4.

A multivariate Cox's regression model (Table 4) also revealed that patients with OPNb expression had a trend towards overall worse prognosis than those without OPNb (*p*=0.06). Patients with OPNb expression were found to have a hazard of death 3 times higher than those without (95% CI, 0.94–11.37). While there was a trend showing an association between improved survival and OPNa, it should be noted that OPNa was expressed in 39/40 patients, making meaningful statistical interpretation difficult (hazard ratio=0.11, 95% CI, 0.01–1.06; p=0.06). No statistically significant association was found between survival for OPNc when adjusted for PDA stage (hazard ratio=1.13, 95% CI, 0.39–3.24; p=0.74). This was also consistent with the unadjusted results.

DISCUSSION

PDA is an aggressive disease in which progression of the cancer is dependent upon accumulation of genetic modifications that support metastasis and physiologic alterations. Physiologic changes seen in PDA are often regulated by cell-signaling molecules, which target signal transduction pathways and, eventually gene expression.(8–10) OPN is a secreted glycoprotein often significantly over-expressed in invasive cancers, including PDA. In this study, we demonstrate for the first time that in FNA samples, there is a strong association between the expression of OPNc isoform and the presence of metastasis in PDA. We also show that OPNb may also be a prognostic marker in patients with PDA as it was associated with a poorer overall survival. Furthermore, the consistent presence of OPNa in FNA samples and the consistent absence of OPNc in chronic pancreatitis suggest a discriminatory role for the individual OPN isoforms at the time of presentation. Various genetic abnormalities have been demonstrated in pancreatic cancer cytology specimens obtained by EUS-FNA. The most frequent is the K-*ras* codon 12-point mutation which is detected in 70–100% of pancreatic ductal cancers and rarely in chronic focal pancreatitis. Detection of the K-*ras* point mutation by EUS-FNA could also be a good method for clarification of the presence of malignancy.(11;12)

Osteopontin (OPN) is a secreted glycoprotein which acts as both an extracellular matrix component and a cytokine.(3;13) OPN serves various functions in cell adhesion and migration,(14;15) inflammatory response,(16) and apoptosis.(17) It is often significantly over-expressed in cancer. OPN over expression correlates with metastatic potential of transformed cell lines,(18) and OPN concentrations are substantially elevated in the blood of patients with metastatic cancer.(19;20) Since OPN has an adhesive function, it is possible

that it plays a role in tumor cell invasion and metastasis, processes in which adhesive interactions between tumor cells and extracellular matrix are critical. In addition, expression of OPN in cancer patients has been associated with a more advanced cancer on presentation(21) and a decrease overall survival(18).

Our analyses reported here have found that patients with PDA who presented with metastasis had higher levels of OPNc expression in comparison to patients without metastatic disease (Fig 2). There was no difference in the levels of OPNa and OPNb expression in patients with or without metastasis. These findings suggest that OPN isoforms exhibit differential expression patterns in tumors according to their stage of presentation. This makes OPNc a marker for the metastatic potential of pancreatic tumors, which could give rise to novel diagnostic approaches. OPNc can also serve as a good marker for pancreatic malignancy because it is absent from patients with chronic pancreatitis (Fig 3, table 3).

OPNc has previously been correlated with increased invasiveness and metastasis in several carcinomas. Mirza *et al* hypothesized that OPNc increased metastasis by supporting anchorage-independent growth of cancer cells. OPNc was shown to induce the expression of oxidoreductases, consistent with protection from anoikis during anchorage-independent growth.(22) It therefore promoted adhesion of tumor cells and supported tumor invasiveness.(5) Tilli *et al* found that OPNc over-expression stimulated cell proliferation, migration, invasion, and tumor colony formation in patients with ovarian cancer.(23).

While the exact effect of OPNc on tumor progression is unknown, there are several plausible explanations. OPNc has been show to stimulate cell proliferation rates independently of growth factors, a feature of a proteins typically involved in tumor progression.(24) OPNc could also cause stimulation of growth signal autonomy by various strategies, such as alteration of extracellular growth signals, of transcellular transducers of these signals or of intracellular circuits that translate those signals into action.(23;25) OPNc could affect the expression of genes involved in multiple aspects of tumor progression and malignant growth, including those involved in self-sufficiency in growth signals.(23;26;27)

Our current study also demonstrated that OPNb mRNA overexpression was associated with a mortality rate three times higher than in PDAs without OPNb overexpression ($P = 0.06$) even when adjusted for the stage of presentation. Previous studies have demonstrated that cancer cells overexpressing OPNb can significantly enhance cell proliferation and survival, independently of growth factors which could account for poorer survival in this sub-set of patients.(23) These results provide further evidence of the potential role of OPNb on favoring poorer survival, which is not associated with stimulated cell death promoted by OPNa. OPNb may therefore be a useful molecular marker for predicting poorer prognosis. The above stated observation correlates with Hahnel et al who showed that elevated mRNA expression levels of OPNb splice variant are a negative prognostic marker for soft tissue sarcoma patients.(28) We hypothesize that the different roles of the OPN splice variants in angiogenesis, cellular invasion, and cancer progression may account for our observations.

The different expressions of OPNa, OPNb and OPNc in PDA specimens and the important observation of OPNc expression in metastatic disease observed herein, could be the basis for future trials to better investigate the potential use of OPNc as a prognostic biomarker. Examining the expression of these isoforms in PDA tissues with different tumor subtypes and clinical behavior could also provide valuable information about the involvement of these isoforms in PDA tumorigenesis and progression.

Previous data from our laboratory have shown that OPNc is highly expressed in metastatic pancreatic lesions from smokers (Sullivan et al). In this study, we show that OPNb, and not OPNc, correlates with poor survival. It not known, however, whether the expression of the OPNb in pancreatic lesions would mirror its expression profile in FNA samples. It is also unknown whether OPN isoforms are differentially expressed in the solid PDA and FNA samples. Studies to address these questions are currently ongoing in our lab.

Our study has several limitations. This is a single center study. It also remains to be determined whether osteopontin isoforms can aid in clinical decision making in the management of individual patients. Certain pancreatic lesions may also have had a significant amount of desmoplasia which may have decreased the cancer cells available for OPN analysis and hence led to a false negative analysis.

In conclusion, our study suggests that the OPN splice variant OPNc is highly expressed in patients with metastatic pancreatic ductal adenocarcinoma. Although the exact role of OPNc in pancreatic ductal adenocarcinoma remains to be defined, the ability of OPNc to mediate carcinogenic effects in PDA is novel and could provide a potential target to control pancreatic cancer aggressiveness. In addition, overall survival from PDA may be reduced in patients who express OPNb, even when adjusted for PDA stage of presentation.

Acknowledgments

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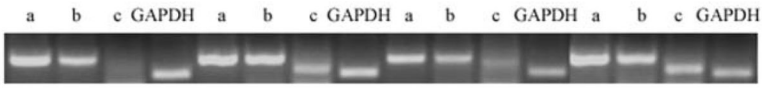

Fig 1.

Representative ethidium bromide agarose gel with the PCR product of FNA samples from 4 cytologically confirmed PDA patients showing expression of OPNa, OPNb, and OPNc (208-bp, 209-bp bands, and 109-bp respectively). OPNa and OPNb were expressed in almost all PDA FNA samples. OPNc was present in 80% of patients with metastatic disease

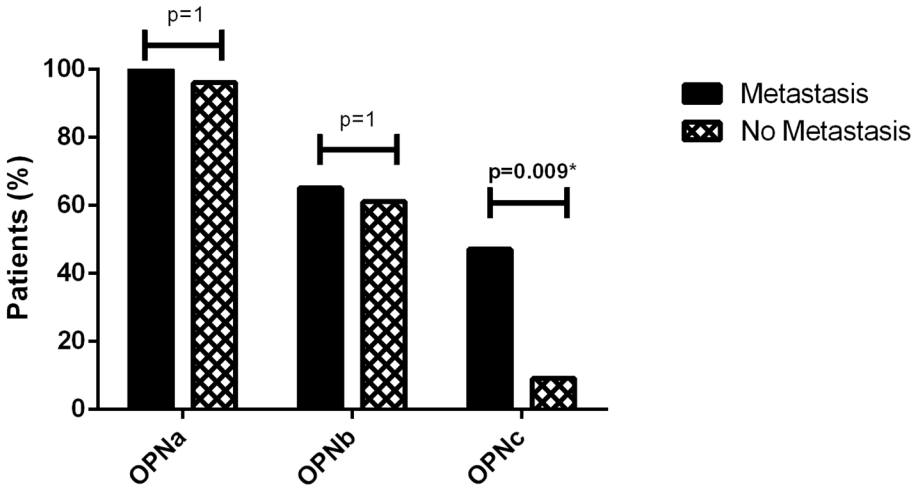

Fig 2.

There was no statistically significant difference in the expression of OPNa and OPNb between patients with metastatic and non-metastatic PDA (100% versus 96%, and 61% versus 47% respectively; p=1). OPNc was expressed in 47% patients with metastatic disease compared to only 9% without metastasis (p=0.009).

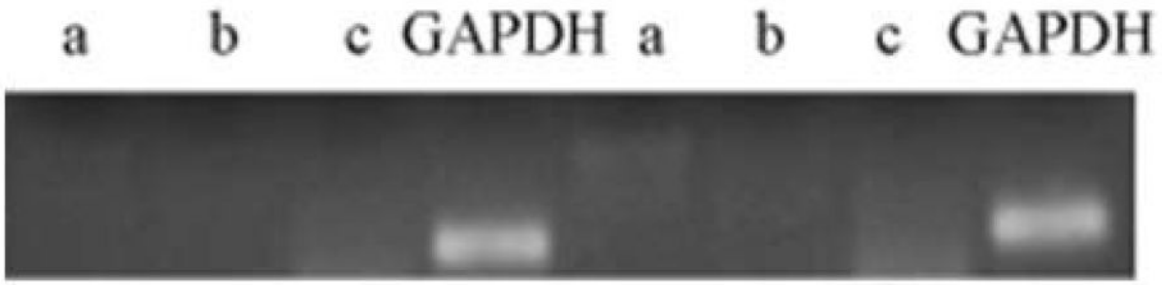

Fig 3.

Representative ethidium bromide agarose gel with the PCR product of FNA samples from cytologically confirmed chronic pancreatitis showing expression of OPNa, OPNb, and OPNc (208-bp, 209-bp bands, and 109-bp respectively). OPNb and OPNc were absent in almost all chronic pancreatitis FNA samples.

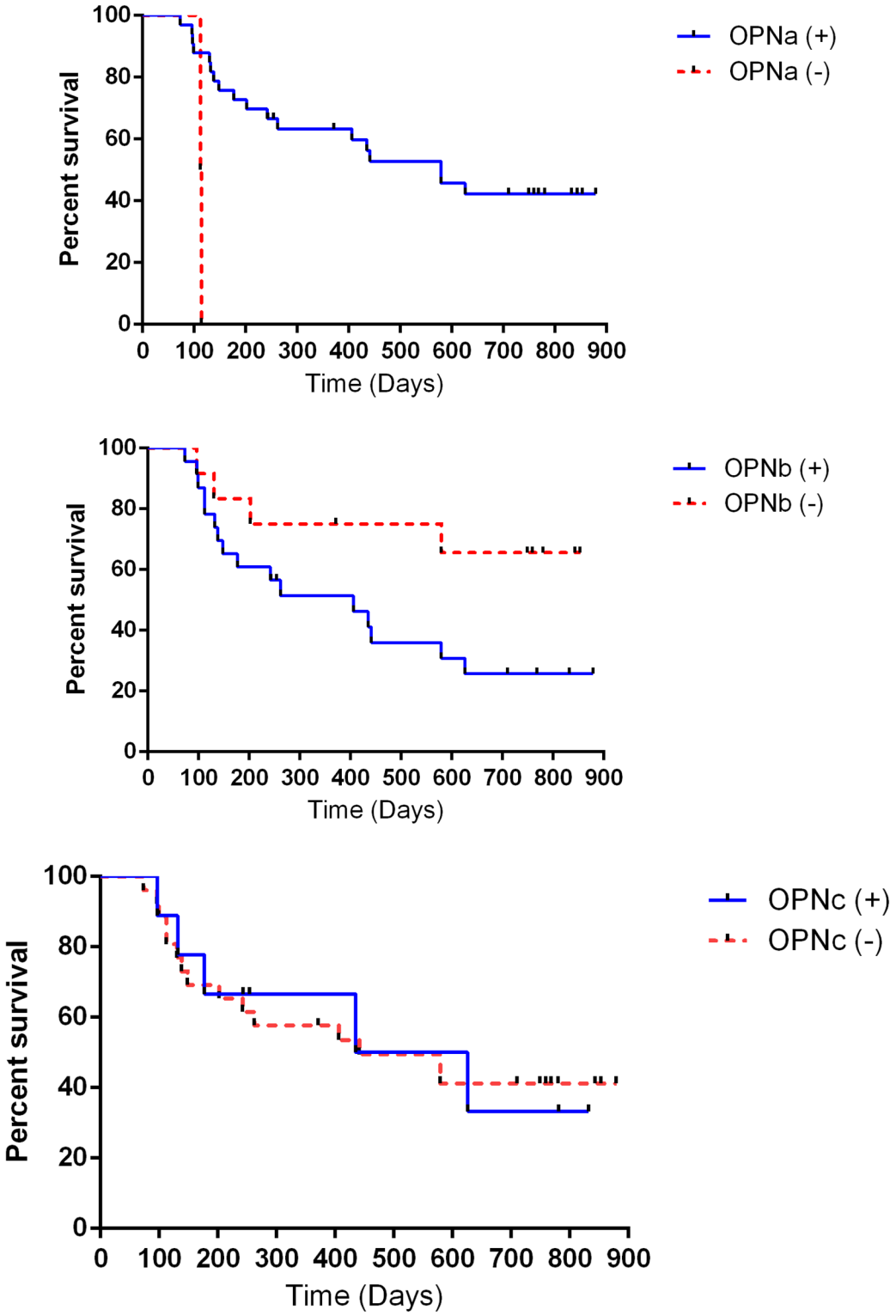

Fig 4.

Fig 4A, B and C. Kaplan-Meier Analysis: Association of OPN splice variants mRNA expression levels with disease-specific survival. OPNb (**B**) expression was associated with

overall worse prognosis (hazard ratio=3, p=0.06). OPNc (**C**) showed no correlation with survival. Meaningful statistical interpretation of survival in patients with OPNa (**A**) was difficult since only 1 patient in our cohort did not express this isoform.

Table 1

Demographics and clinical features of the evaluated PDA and chronic pancreatitis patients

	Pancreatic Adenocarcinoma (n=40)	Chronic Pancreatitis (n=6)
Mean Age yrs (range)	68.1 (45–89)	57.8 (44–71)
Gender N (%)		
Male	23 (57.5)	4 (66)
Female	17 (42.5)	2 (34)
Race N (%)		
White	24 (60)	4 (66.7)
African American	7 (17.5)	1 (16.7)
Hispanic	4 (10)	1 (16.7)
Other	5 (12.5)	0 (0)
History of Smoking N (%)	21 (53)	4 (67)
History of Alcohol Use N (%)	9 (23)	5 (83)
Diabetes N (%)	9 (23)	1 (17)
Mass Location N (%)		
Head	29 (72.5)	5 (83.3)
Neck	1 (2.5)	0 (0)
Body	5 (12.5)	1 (16.7)
Tail	5 (12.5)	0 (0)
Largest Mass Diameter (mm)		
Median (range)	30 (7–53)	23 (10–33)
CA 19-9 (U/ml)		
Mean (range)	1143 (2–16,597)	22 (8–43)
Cancer Stage N (%)		
I	3 (7.5)	-
II	12 (30)	-
III	8 (20)	-
IV (Metastasis)	17 (42.5)	-
Site of Metastasis N (%)		
Liver	10 (25)	-
Peritoneal Cavity	4 (10)	-
Lung	2 (5)	-
Bone	1 (2.5)	-

Table 2

Demographics and clinical features of patients evaluated according to the OPN splice variants in patients with PDA.

	OPNa (n=39)	OPNb (n=24)	OPNc (n=10)	P-value
Demographics				
Age (years)	68.2	67.5	62.5	
Male Sex (%)	45	40	30	0.68
White (%)	57	59	62	0.96
Mean Body Mass Index	26.6	27	29.9	
Clinical Presentation				
Jaundice (%)	45	50	63	0.59
Abdominal Pain (%)	77	75	100	0.26
Anorexia (%)	68	65	88	0.39
Past Medical History				
Diabetes (%)	32	30	13	0.48
Hyperlipidemia (%)	64	65	50	0.68
Hypertension (%)	64	65	50	0.68
Chronic Pancreatitis (%)	14	15	0	**0.005***
Social History				
Smoker (%)	40	40	23	0.59
Alcohol Abuse (%)	37	42	43	0.89
Laboratory Parameters				
Mean Leukocyte Count	7.8	7.9	8.7	0.82
Mean CA 19-9	374	329	175	0.52
Mean Serum Albumin	3.7	3.6	3.7	0.81
Metastasis on Presentation (%)	44	44	80	**0.01***

*Significant association with the absence of OPNc and absence of malignancy, and significant association of metastatic disease with the presence OPNc.

Table 3

Presence of OPN isoforms in PDA and chronic pancreatitis patients.

	Pancreatic Ductal Adenocarcinoma	Chronic Pancreatitis
OPNa Expression (%)	97.5	50
OPNb Expression (%)	60	17
OPNc Expression (%)	25	0

Table 4

Multivariate Cox Proportional Hazards Regression Results for Patient Survival

	Hazard Ratio	95% Hazard Ratio Confidence Limits	p-value
OPNa Expression	0.11	0.01–1.06	0.06
OPNb Expression	3.0	0.94–11.37	0.06
OPNc Expression	1.13	0.39–3.24	0.74