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Osteopontin Expression is Associated with Improved Survival in Patients with Pancreatic Adenocarcinoma

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Abstract

Background—Osteopontin (OPN) is a secreted protein of the extracellular matrix. It has been used as a marker for tumor aggressiveness and correlated with clinical outcomes in several solid tumors, such as liver, lung, and breast. We determined the OPN expression and its influence on survival in patients with resected pancreatic adenocarcinoma.

Methods—Tissue microarrays were constructed from 245 resected pancreatic adenocarcinomas. Immunohistochemical staining for OPN was undertaken and compared to normal pancreas ($n = 12$). OPN expression was then correlated with patient demographics, tumor size, grade, node, and margin status. Survival curves were created by the Kaplan–Meier method and compared by log rank analysis.

Results—In total, 181 (74 %) of pancreatic adenocarcinoma tissues expressed OPN compared to 7 (58 %) of normal controls ($p = 0.004$). Expression was observed predominantly in the cytoplasm of the tumor cells. The median and 2 year overall survival was longer when OPN was expressed (17.1 vs. 11.6 months, and 38 vs. 24 %, respectively, $p = 0.04$). Multivariate analysis showed OPN expression and T stage to be independent predictors of overall survival, while other histopathologic factors such as tumor grade, tumor size, and nodal status were not.

Conclusions—These results suggest that the presence of OPN expression in pancreatic adenocarcinoma may have a protective effect independent of tumor stage. This emphasizes the importance of the interaction between pancreatic cancer cells and their stromal elements.

Pancreatic adenocarcinoma is the fourth most common cause of cancer-related death in the United States with nearly all of the estimated 43,000 patients diagnosed dying within one year.¹ Surgical resection offers patients the only hope for cure. When curative resection is possible and disease is confined to the pancreas without lymphatic or hematogenous spread of disease, 5 year survival is still less than 20 % with a fraction of patients being resectable at the time of diagnosis.² The delayed clinical presentation of symptoms and the unique tumor biology of pancreatic cancer, which tends to have early local invasion and high metastatic potential, contributes to the poor prognosis observed in this disease.³

The hallmark of tumors associated with pancreatic ductal adenocarcinoma is the inflammatory response and dense desmoplastic reaction.⁴ Stromal elements include

inflammatory lymphocytes, macrophages, fibroblasts, and stellate cells. In the past, researchers have primarily focused their studies on the pancreatic cancer cell but that focus has shifted more recently to efforts to delineate the role that the surrounding stroma plays on the pancreatic cancer cell and targeting the stroma for therapeutic development.⁵ Recent evidence suggests that inflammatory cells may play a pro-tumorigenic role while the extracellular matrix has both pro- and anti-tumorigenic effects in the development and progression of pancreatic cancer.⁶

Osteopontin (OPN) is a phosphorylated and glycosylated secretory protein of the extracellular matrix.⁷ One source of OPN is from macrophages in the tumor stroma.⁸ OPN may contribute to tumorigenesis through several different functions including binding and activation of matrix metalloproteinases, stimulation of migration and invasion, enhancement of metastatic ability, and protection from apoptosis.^{9–12} OPN has been used as a marker for tumor aggressiveness and correlated with clinical outcomes in several solid tumors, such as liver, lung, and breast.^{13–15} Its usefulness as a biomarker for prognosis in pancreatic adenocarcinoma remains controversial as results published to date have conflicted.^{7,16} This is the largest study to date assessing OPN's role as a biomarker in pancreatic adenocarcinoma. The purpose of this study was to determine the impact of OPN expression relative to other typical clinicopathologic variables on outcomes in patients undergoing resection for pancreatic adenocarcinoma.

MATERIALS AND METHODS

Between December 2001 and 2009, 265 patients underwent resection for pancreatic adenocarcinoma at the Ohio State University. After approval by our institutional review board, we reviewed the clinical charts of 245 of these patients whose tumors had been included in our ongoing tissue microarray building process. No particular selection process was utilized to select these cases as they were chosen in parallel by a single pathologist (WLF) during creation of our comprehensive periampullary cancer database. Demographic information obtained included age, gender, and major comorbidities (e.g. hypertension, diabetes, chronic obstructive pulmonary disease, coronary artery disease, etc.). Data on tumor location, differentiation, size, T stage, nodal status, and margin status were collected. Overall stage was determined by criteria set forth by the American Joint Committee on Cancer.¹⁷

Tissue Microarray and Immunohistochemistry

Our method for tissue microarray (TMA) creation has been described elsewhere.¹⁸ Briefly, formalin-fixed, paraffin-embedded tissues were obtained from the archival files at the Ohio State University Department of Pathology. Two tissue cores (2 mm diameter each) were punched out of each donor paraffin block and transferred to each of the recipient TMA blocks with a precision instrument (Beecher Instruments, Silver Spring, MD). Paraffin-embedded tissue was cut at 4 μ m and placed on positively charged slides. TMAs were constructed from 245 resected pancreatic adenocarcinomas and from normal pancreas from patients without pancreatic cancer or other pancreas disease ($n = 12$).

Immunohistochemical staining was performed on the arrayed tissue. Our methods for immunohistochemistry have been previously described.¹⁸ Briefly, slides were heated to 60 °C for 60 min, cooled, deparaffinized and rehydrated prior to immunostaining. OPN primary antibody (NCL-O-Pontin; Novocastra Laboratories, Newcastle upon Tyne, UK) was used at a dilution of 1:50. Slides were counterstained in Richard Allen hematoxylin, dehydrated through graded ethanol solutions, and coverslipped. The positive and negative controls stained appropriately.

OPN staining was considered as positive if at least 5 % of cells stained. Expression was scored from 0 to 3+ where 0 = negative, 1+ = weak, 2+ = moderate, and 3+ = strong staining. The intensity of staining (i.e., 1+ to 3+) was considered independent of the proportion of cells that stained. In other words, intense staining in 5 % of cells was still considered 3+ whereas faint staining in 50 % of cells may have been considered 1+. Patients were then divided into two categories based upon the presence (e.g., 1+ to 3+) or absence (e.g., 0) of OPN expression and then correlated with histopathologic characteristics. All stains were read by a single pathologist (J. R.) who was blinded to tumor stage and clinical characteristics. In the event where one core showed no expression or, more often, no cancer cells yet the second of the pair did show OPN expression, the sample was considered positive and the intensity was scored according to the positive staining core.

Statistical Analysis

All analyses were completed utilizing SPSS version 14.0 software (SPSS, Chicago, IL). Parametric scale data were compared by two-tailed Student's *t* test. Nonparametric data were compared by Fisher's exact test or by χ^2 analysis, where appropriate. Overall survival was considered from the time of resection until the time of death from any cause. Dates of death were obtained from the Social Security Death Index Web site reported as of September 28, 2010 (<http://ssdi.genealogy.rootsweb.com>) or patient hospital records. Survival curves were generated by the Kaplan–Meier method and compared by log rank analysis. Multivariate analysis was undertaken by the Cox proportional hazard method. Median data are presented unless stated otherwise.

RESULTS

Patient Characteristics

The demographic and clinicopathologic characteristics of the 245 patients with pancreatic adenocarcinoma were similar between those that did and did not express OPN (Table 1). Patients with pancreatic cancer tended to be in their seventh decade of life and mostly male. Commensurate with their advanced age, most had major comorbidities, including chronic obstructive pulmonary disease, coronary artery disease, diabetes mellitus, and hypertension. Tumors with and without OPN expression were similar based upon differentiation, tumor size, T stage, location, margin-negative resection, and nodal status. Although, on average, tumor size was similar in those with and without OPN expression, OPN positivity was more common in tumors <2 cm in size (78 vs. 46 %, $p = 0.0002$). Tumors <2 cm in size tended to have less nodal metastases (55 vs. 68 %, $p = 0.14$) and less lymphovascular invasion (29 vs. 46 %, $p = 0.07$) compared to those at least 2 cm in size, though this was not significant.

In total, 181 (74 %) of pancreatic adenocarcinoma tissues expressed OPN compared to 7 (58 %) of normal controls ($p = 0.004$; Table 2). Moderate (2+) and strong (3+) stains for OPN were observed only in the pancreatic cancer tissue but not in the normal pancreatic tissue. OPN expression, when present, was observed predominantly in the cytoplasm of the tumor cells (Fig. 1). Normal pancreatic ducts stained only weakly (1+) positive for OPN or were negative (0) (Fig. 2; Table 2).

Survival

Forty-nine patients were still alive after resection of their pancreatic adenocarcinomas at the time of our study with a median follow-up of 39.8 months (range 11.5–237 months). Overall median survival was 14 months with 2- and 5-year survival of 29 and 4 %, respectively. Univariate analysis was utilized to assess the impact of several variables on survival for pancreatic cancer (Table 3). Factors assessed included age, gender, comorbidity, resection margins, tumor size, T stage, tumor differentiation, lymphovascular invasion (LVI), neural

invasion, nodal metastases, and OPN expression. Univariate predictors of overall survival included LVI and OPN expression. Tumors positive for LVI had decreased survival compared to tumors without LVI. The 1 year overall and 2 year overall survival was longer when LVI was absent (64 vs. 52 %, and 44 vs. 23 %, respectively, $p = 0.024$). Tumors positive for OPN had a survival advantage compared to tumors that did not express OPN. The median and 2 year overall survival was longer when OPN was expressed (17.1 vs. 11.6 months, and 38 vs. 24 %, respectively, $p = 0.04$) (Fig. 3). Five year survival was 16 vs. 11 % when OPN was expressed vs. OPN negative, respectively. The intensity of OPN staining, when present, was not predictive of survival.

Variables most likely to impact survival by univariate analysis (i.e., $p < 0.2$) were entered into the multivariate analysis model (Table 3). Significant predictors of survival included age, T stage and OPN expression, while other histopathologic factors such as tumor grade, LVI, margins, and nodal status were not predictive.

DISCUSSION

Pancreatic cancer is the deadliest gastrointestinal cancer with an annual mortality almost equal to its incidence.¹ Given the poor outcomes in spite of our best surgery and chemotherapy, prognostic markers and novel potential therapeutic targets are being sought. In this study, we used tissue microarrays from a single institution experience to determine expression of the glycoprophosphoprotein OPN and its influence on survival in patients with resected pancreatic adenocarcinoma. We found that traditional markers of outcome such as nodal metastasis and margin status were not predictive of survival. However, OPN expression offered a significant survival advantage independent of other clinicopathologic factors.

Here we describe the largest study to date assessing OPN's role as a biomarker in pancreatic adenocarcinoma in which we analyzed OPN expression in 245 resected pancreatic cancers compared to normal pancreas using tissue microarrays. Our findings that nearly three-quarters of the tumor cells express OPN in the cytoplasm of the pancreatic cancer tissue microarrays further validates earlier studies that OPN expression is significantly higher in cancer cells as compared to normal pancreatic tissue.⁷ We did not find any significant differences between groups in the distribution of gender, age, or comorbidities based upon OPN expression. The pancreatic cancers in our study tended to be larger, >3.5 cm and with extension beyond the pancreas, with >80 % being T3. This may represent a selection bias, as larger tumors were more likely to have adequate tissue for inclusion in our tissue microarrays. As well, more than 65 % of the patients had lymph node metastases, which could be attributed to pancreatic cancer's tendency to spread early. However, tumor stage and nodal status were not significant predictors of survival by univariate or multivariate analysis.

OPN has been linked to clinical outcomes in several solid tumors. In liver, lung, and breast cancers, OPN expression has been associated with inferior survival compared to patients whose tumors did not express OPN.^{13–15} Both stroma and tumor-derived OPN is believed to regulate tumor progression and metastasis in breast cancer.¹⁹ OPN was previously identified through global gene expression profiling to be overexpressed in pancreatic cancer.²⁰ However, its usefulness as a biomarker for prognosis in pancreatic adenocarcinoma has remained controversial as results published to date have conflicted.^{7,16} An earlier study by Koopmann et al.¹⁶ that utilized in situ hybridization for OPN on 14 tissue micro-arrays identified OPN expression in tumor-infiltrating macrophages but failed to demonstrate signal in pancreatic tumor cells, the surrounding stroma, or normal pancreas tissue. This is in

contrast to a later study by Kolb et al.⁷ that demonstrated 60 % OPN expression in pancreatic cancer cells by immunohistochemistry.

Interestingly, in our study OPN expression imparted a significant survival advantage of 50 % that persisted in multivariate analysis. This is in contrast to the worse prognosis observed in other solid tumors when OPN is expressed in which OPN is believed to contribute to metastasis.^{13–15} Because our study involved only resected pancreatic adenocarcinomas, any patients who had evidence of gross metastasis at the time of surgery would not have been resected and therefore would have been excluded from consideration in this particular study. Patients with OPN expression also were more likely to have tumors <2 cm in size, which may help explain the correlation with improved survival. The association with tumor size suggests that OPN expression may be lost as tumors grow, thus selecting for a more aggressive phenotype. Alternatively, the trend toward association between tumors <2 cm in size and other favorable characteristics may reflect small sample size. Still, when controlling for typical pathologic characteristics likely to impact outcome, OPN expression had the greatest impact on survival.

In conclusion, OPN appears to be produced by both pancreatic and stromal elements, and is present at increased levels in pancreatic cancer tissue as compared to normal pancreas. Our results suggest that the presence of OPN expression in pancreatic adenocarcinoma may have a protective effect independent of tumor stage in resectable tumors. This emphasizes the importance of the interaction between pancreatic cancer cells and their stromal elements.

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FIG. 1. OPN expression in pancreatic adenocarcinoma. Immunohistochemical staining for OPN ($\times 200$) demonstrates negative (**a**), weak (**b**), moderate (**c**), or strong (**d**) cytoplasmic staining in pancreatic adenocarcinoma cells (*arrows*)

FIG. 2.
OPN expression in benign pancreas. Immunohistochemical staining for OPN ($\times 200$) shows negative (**a**) or weak (**b**) cytoplasmic staining in normal pancreatic ducts cells

Proportion surviving**P = 0.048**

FIG. 3.
Kaplan–Meier overall survival curve for patients with pancreatic adenocarcinoma by OPN expression

TABLE 1

Demographics and clinicopathologic characteristics of patients with resected pancreatic cancer based on osteopontin (OPN) expression

Characteristic	All (<i>n</i> = 245)	OPN negative (<i>n</i> = 64)	OPN positive (<i>n</i> = 181)	<i>p</i>
Age, median (range) (year)	66 (30–87)	66 (30–87)	66 (38–85)	0.42
Gender, M/F	144/101	40/24	104/77	0.56
Comorbidities	146 (60 %)	38 (59 %)	108 (60 %)	0.77
Differentiation				0.36
Well	10 (4 %)	5 (8 %)	5 (3 %)	
Moderate	139 (57 %)	34 (53 %)	105 (58 %)	
Poor	93 (38 %)	24 (38 %)	69 (38 %)	
Unknown	3 (1 %)	1 (1 %)	2 (1 %)	
Median tumor size cm (range)	3.7 (0.8–17.5)	3.5 (0.8–8.0)	3.8 (1.0–17.5)	0.69
T stage				0.21
T1	9 (4 %)	5 (8 %)	4 (2 %)	
T2	28 (11 %)	6 (9 %)	22 (12 %)	
T3	205 (84 %)	52 (81 %)	153 (85 %)	
Unknown	3 (1 %)	1 (2 %)	2 (1 %)	
HOP location (vs. body/tail)	202 (82 %)	57 (89 %)	145 (80 %)	0.13
Negative margins	185 (76 %)	47 (76 %)	138 (77 %)	0.74
Lymph node metastasis	161 (66 %)	43 (67 %)	118 (65 %)	0.88

HOP head of pancreas

TABLE 2

Immunohistochemistry results for osteopontin in pancreatic cancer compared with normal pancreas

Osteopontin	Pancreatic cancer	Normal pancreas
0	64/245 (26 %)	5/12 (42 %)
1+	109/245 (44 %)	7/12 (58 %)
2+	51/245 (21 %)	0
3+	21/245 (9 %)	0

TABLE 3Potential predictors of overall survival in 245 patients with pancreatic cancer who underwent resection^a

Variable	Univariate	Multivariate
Age	0.07	0.008 (HR 1.02, 95 % CI 1.01–1.04)
Margins	0.132	0.511
T stage	0.118	0.012 (HR 1.62, 95 % CI 1.11–2.36)
Nodal status	0.122	0.447
Lymphovascular invasion	0.024	0.152
OPN (negative vs. positive)	0.048	0.001 (HR 1.83, 95 % CI 1.27–2.65)

HR hazard ratio, *CI* confidence interval, *OPN* osteopontin^aData presented as *p* values and HRs with 95 % CIs for those reaching statistical significance