Sp1, a New Biomarker That Identifies a Subset of Aggressive Pancreatic Ductal Adenocarcinoma

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Abstract

Pancreatic adenocarcinoma is one of the leading causes of cancer-related deaths in the United States. Sp1 is a sequence-specific DNA binding protein that is important in the transcription of a number of regulatory genes involved in cancer cell growth, differentiation, and metastasis. In this study, we investigated Sp1 expression in pancreatic ductal adenocarcinoma and its association with clinical outcome. We studied 42 patients with primary pancreatic adenocarcinoma. The expression of Sp1 in pancreatic adenocarcinoma was evaluated by immunohistochemical staining. All 42 patients had clinical follow-up information and were evaluated for survival. Sp1 protein was aberrantly overexpressed in a subset of primary pancreatic adenocarcinoma. These tumors all developed metastasis, whereas none of the primary tumors without lymph node metastasis showed Sp1 overexpression. Statistically, Sp1 overexpression was associated with higher stage, higher grade, and lymph node metastasis ($P < 0.001$, $P = 0.036$, and $P < 0.0001$, respectively). Additionally, patients of this subset had a much shorter overall survival than patients without Sp1 overexpression, as evidenced by Kaplan-Meier plots and the log-rank test ($P = 0.002$). The 5-year overall survival rate was 19% in patients with Sp1 overexpression, compared with 55% in patients without Sp1 overexpression. The median survival was only 13 months for patients with Sp1 overexpression, compared with 65 months for patients without Sp1 overexpression. In conclusion, Sp1 is a new biomarker that identifies a subset of pancreatic ductal adenocarcinoma with aggressive clinical behavior. It can be used at initial diagnosis of pancreatic adenocarcinoma to identify patients with an increased probability of cancer metastasis and much shortened overall survival. (Cancer Epidemiol Biomarkers Prev 2008;17(7):1648−52)

Introduction

Pancreatic ductal adenocarcinoma is the fourth leading cause of cancer-related deaths in the United States (1, 2). Historical data show that once recognized, patients had a mean survival time of 6 months and a 5-year survival rate of only 4% (3). The only form of curative treatment was surgical resection of early-stage pancreatic adenocarcinoma (4). However, 80% of these tumors recurred within 2 years of surgery (5). More recent studies have shown improved survival. Various adjuvant and neo-adjuvant treatments have been developed and are used in conjunction with surgical resection (6). Retroperitoneal resection has been advocated to attempt to eliminate lymph node metastasis (7). Systemic chemotherapy has also emerged as an effective option to treat this cancer (8). These approaches have shown promising efficacy that may substantially prolong patient survival. To improve outcome further, biomarkers have attracted much attention with the hope that these markers can predict tumor behavior and also serve as therapeutic targets, thus providing tools for the individualized treatment of pancreatic adenocarcinoma (9).

Sp1 protein is a well-characterized, sequence-specific, DNA-binding protein that is part of the Sp/XKLF (specificity protein/Krüppel-like factor) family of transcription factors in many cellular and viral genes (10). Although Sp1 protein is heavily expressed in many embryonic cells such as human umbilical vein endothelial cells, Sp1 is down-regulated in many fully differentiated cells (11). Sp1 protein is now believed to be critical in the regulation of cell growth, differentiation, and apoptosis (12). Its role in cancer has also been the focus of study (11). Sp1 overexpression was shown in sarcoma, colonic adenocarcinoma, and gastric adenocarcinoma (13-15), as well as in a cell line derived from pancreatic adenocarcinoma (16). In the present study, we examined the status of Sp1 protein expression in tumor tissues of surgically resected human pancreatic adenocarcinoma and analyzed the relationship between the expression of Sp1 protein and tumor differentiation, clinical tumor staging, tumor metastasis, and patient survival.

Materials and Methods

Patients and Tumor Samples. Forty-two patients underwent Whipple resections for pancreatic adenocarcinoma, ductal type, between 2000 and 2006, at the University of Massachusetts Medical Center. The tumor slides and corresponding tissue blocks were obtained from the archives. The routine formalin-fixed, paraffin-embedded sections were reviewed and two pathologists
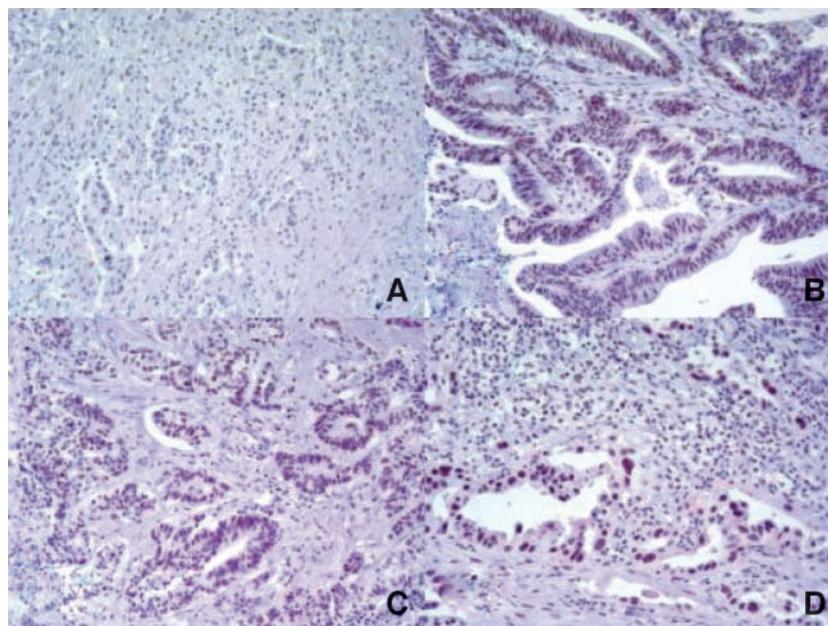
Figure 1. Representative negative (**A**) and positive (**B-D**) immunohistochemical stains of Sp1 protein in the nuclei of well-differentiated (**B**), moderately differentiated (**C**), and poorly differentiated (**A** and **D**) pancreatic ductal adenocarcinomas. Magnification, ×200.

confirmed the diagnoses. Staging was based on pathologic findings according to the American Joint Committee on Cancer. By the use of the tumor-node-metastasis classification system, we identified 2 tumors as stage IA, 4 tumors stage IB, 11 tumors stage IIA, 19 tumors stage IIB, 3 tumors stage III, and 2 tumors stage IV. Lymph node metastasis was found in samples from 22 of 42 (52%) patients. Clinical follow-up was obtained by reviewing the patient's medical record. Overall survival was measured from the date of tumor resection to the date of death or was censored as of the date of the last follow-up visit for survivors. The Institutional Review Board at our institution approved this study.

Immunohistochemical Analysis. Immunohistochemical studies were done on 5-μm sections of formalin-fixed, paraffin-embedded tissue as previously described (17). Every tumor was read blinded by two pathologists. The tissue blocks that were most representative and contained most tumor volume were selected for staining. Antigen retrieval was carried out with 0.01 mol/L citrate buffer (pH 6.0) in an 800-W microwave oven for 15 min before immunostaining. The slides were stained on the DAKO Autostainer (DAKO Corporation) using EnVision (DAKO) staining reagents.

The sections were blocked for endogenous protein binding and peroxidase activity with an application of

Table 1. Clinical and pathologic data of pancreatic adenocarcinoma shown by Sp1 immunohistochemical staining results

	SP1-positive tumors ($n = 17$)	SP1-negative tumors ($n = 25$)	P
Sex, n (%)			
Female	4 (22)	14 (78)	0.057
Male	13 (54)	11 (46)	
Age, y			
Mean (SD)	66.1 (11.9)	65.7 (10.4)	0.853
Tumor differentiation, n (%)			
Well	2 (22)	7 (78)	0.036
Moderate	6 (30)	14 (70)	
Poor	9 (69)	4 (31)	
Tumor stage, n (%)			
IA	0	2 (100)	<0.001
IB	0	4 (100)	
IIA	0	11 (100)	
IIB	14 (74)	5 (26)	
III	2 (67)	1 (33)	
IV	1 (50)	1 (50)	
Tumor size, cm			
Mean (SD)	4.2 (3.0)	3.0 (1.6)	0.071
Tumor metastasis, n (%)			
No	0	20 (100)	<0.001
Yes	17 (77)	5 (23)	
No. lymph nodes			
Median	15	16	0.879
Mean (SD)	15.78 (10.22)	16.60 (11.32)	
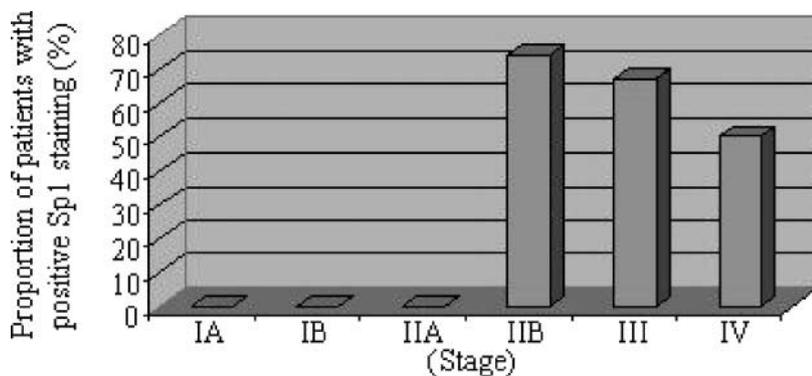
Figure 2. Association of Sp1-positive staining with tumor stage.

Dual Endogenous Block (DAKO) for 10 min, followed by a buffer wash. The sections were then incubated with a mouse monoclonal antibody specific for SP1 (DAKO) at 2 μg/mL for 30 min, followed by a buffer wash. Sections were then incubated with EnVision+ Dual Link reagent (a polymer conjugated with goat anti-mouse immunoglobulin and horseradish peroxidase) for 30 min. The sections were then washed and treated with diaminobenzidine and hydrogen peroxide to produce the visible end product. A toning solution (diaminobenzidine enhancer, DAKO) was used to enrich the final color. The sections were counterstained with hematoxylin, dehydrated, and coverslipped with mounting media.

Negative control sections were stained by replacing the primary antibody with nonimmune mouse IgG (Vector) at a concentration of 2 μg/mL. Positivity of the primary antibody was defined as strong brown nuclear staining. Negative staining was defined as no staining or weak diffuse background granules. For carcinoma with Sp1 overexpression, each tumor had at least 20% of the tumor cells staining positive. The intensity of the staining has mild variation within the same tumor, but the average staining intensity among all Sp1-positive tumors is very similar. In contrast, for carcinoma without Sp1 overexpression, essentially no tumor cells stained positive.

Statistical Analysis. Age, sex, size of the tumor, tumor stage, and tumor differentiation were collected as baseline variables. The distribution of each baseline variable was compared for Sp1-positive and Sp1-negative subgroups with the Wilcoxon rank sum test for continuous variables and the Fisher exact test for categorical variables. Overall survival was estimated by the Kaplan-Meier method and evaluated with the use of log-rank test for univariate analysis. The Cox proportional hazard model was used to assess the simultaneous contribution of the covariates of tumor differentiation, tumor stage, and SP1 status. Two-sided $P < 0.05$ was considered to indicate statistical significance.

Results

Seventeen of the 42 (40%) cases of pancreatic adenocarcinoma showed Sp1 overexpression (Fig. 1) with at least 20% positive cells for each case. The remaining 25 cases were negative. Table 1 provides the Sp1 status and the relevant clinical characteristics of the 42 patients with pancreatic adenocarcinoma. Age, gender, and tumor size were not associated with Sp1-positive status ($P = 0.853$, $P = 0.057$, and $P = 0.071$, respectively). Positive Sp1 staining was associated with higher tumor stage, higher grade, and lymph node metastasis ($P < 0.001$, $P = 0.036$, and $P < 0.0001$, respectively), which are established pathologic predictors of clinical outcome for pancreatic adenocarcinoma. None of the stage IA, IB, and IIA tumors was Sp1 positive whereas 74% of stage IIB, 67% of stage III, and 50% of stage IV were Sp1 positive (Fig. 2). All tumors that showed Sp1 expression had lymph node metastasis, whereas none of the tumors without lymph node metastasis was Sp1 positive (Fig. 3A). Among all patients with metastasis, 77% of the primary tumors were Sp1 positive (Fig. 3B). Therefore, as a marker to detect tumor lymph node metastasis, the positive staining for Sp1 protein in primary pancreatic adenocarcinoma has a sensitivity of 77%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 80%.
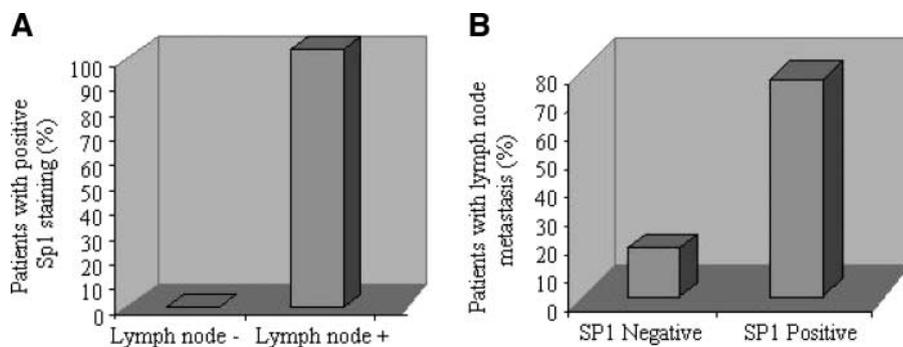
Figure 3. A. All patients with tumors staining positive for Sp1 had lymph node metastasis. **B.** Seventy-seven percent of patients with lymph node metastasis had tumors staining positive for Sp1.
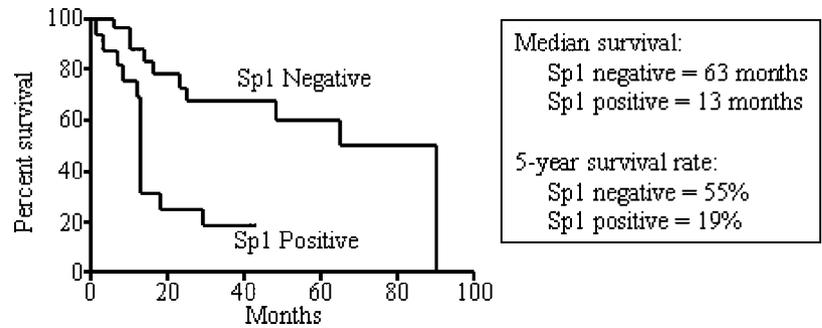
Figure 4. Kaplan-Meier plots and log-rank tests for survival of patients with Sp1-negative and Sp1-positive pancreatic ductal adenocarcinomas.

Kaplan-Meier plots and log-rank tests of survival (Fig. 4) in patients with pancreatic adenocarcinoma showed that patients with negative Sp1 staining in their primary tumor had significantly longer overall survival than patients with positive Sp1 staining (Fig. 4; $P = 0.0012$). The 5-year overall survival rate was 55% in patients with negative Sp1 staining versus 19% in patients with positive Sp1 staining. The median survival was 63 months in patients with negative Sp1 staining, whereas the median survival was only 13 months in patients with positive Sp1 staining in their primary tumors.

The results of multivariate analysis for overall survival are shown in Table 2. The factors listed in the table, positive Sp1 staining, tumor differentiation, and tumor stage, are included as potential copredictors. Multivariate Cox proportional hazards regression analysis showed that for positive Sp1 staining the hazard ratio was 2.995 (95% confidence interval, 8.466-1.060; $P = 0.039$). This result indicates that positive Sp1 staining in the primary adenocarcinoma is a possible predictor of clinical outcome, independent of tumor differentiation and staging.

Discussion

Normal pancreatic ductal epithelial cells express very low levels of Sp1, which are not detected by the immunohistochemical staining method. The positive staining in the nuclei of pancreatic adenocarcinoma cells illustrated in this study represented aberrant overexpression of Sp1 protein. In the present study, we showed that using this method to detect the overexpression of Sp1 protein as a biomarker has identified a subset of pancreatic ductal adenocarcinoma characterized by aggressive clinical behavior. We provide evidence that overexpression of Sp1 is associated with the presence of lymph node metastasis and advanced cancer stages, specifically stages IIB to IV, and the patients with Sp1-positive cancer had a much shortened overall survival. The ability of Sp1 overexpression to predict the clinical behavior was independent of tumor differentiation and tumor stage as evidenced by the results of multivariate Cox proportional hazards regression analysis.

The application of Sp1 as a prognostic marker may improve clinical management. Additionally, the overexpression of Sp1 has 100% specificity for the presence of lymph node metastasis. This is very useful because lymph node removal during surgical resection of pancreatic adenocarcinoma may be challenging, and inadequate lymph node dissection may lead to false-negative lymph node status and understaging of the tumor. Furthermore, surgical retroperitoneal lymph node dissection, in addition to conventional resection of the tumor, has been advocated in recent years, despite the fact that such procedure may lead to severe complications. The ability of Sp1 to identify patients with lymph node metastasis can assist in individualized decision making and treatment option and selectively apply retroperitoneal dissection to those who indeed need it.

The mechanisms that regulate Sp1 expression and the aberrant Sp1 overexpression are not well understood. Enhanced Sp1 action has been shown to be due to both increased gene expression and posttranslational modification of Sp1 protein (18, 19). Studies using cell lines derived from cancers have also shown that enhanced Sp1 actions promote tumor cell growth and metastasis (20, 21). Recently, such enhanced Sp1 protein action was further shown directly in patients with gastric

Table 2. Stepwise selection of Sp1 overexpression, tumor differentiation, and tumor stage as significant variables for patient survival were analyzed using the Cox proportional hazard model

	Hazard ratio (95% confidence interval)	SE	z	$P > \|z\|$
Positive Sp1	2.995168 (1.059714-8.465526)	1.587777	2.07	0.039
Tumor differentiation				
2 vs 1	0.1671525 (0.0400883-0.6969601)	0.1217696	−2.46	0.014
3 vs 1	0.2119732 (0.0463751-0.9688955)	0.1643576	−2.00	0.045
Tumor stage				
II vs I	6.610593 (1.006433-43.42064)	6.348517	1.97	0.049
III vs I	5.287212 (0.6780914-41.22543)	5.540249	1.59	0.112
IV vs I	21.42855 (1.899969-241.6791)	26.48975	2.48	0.013

adenocarcinoma and was associated with advanced stage and poor survival. Aberrant Sp1 overexpression was also found in cell lines derived from pancreatic cancers (22, 23). Our study, for the first time, has described the expression of Sp1 in patients with pancreatic adenocarcinoma and showed the association of Sp1 with advanced stage and poor survival.

Although there are many Sp1 site–dependent target proteins, it may be of interest to note that Sp1 has recently been shown to regulate osteopontin expression, a molecule that mediates cancer metastasis, in cells derived from colon adenocarcinoma (24), and our results in this study clearly showed that every pancreatic adenocarcinoma with Sp1 overexpression had lymph node metastasis. Additionally, Sp1 has also been shown to mediate p53-associated cellular actions (25), and p53 is one the most frequently mutated genes in pancreatic adenocarcinoma (26). It is therefore logical that these two proteins may function synergistically. In an era that focuses on molecular targeting as a therapeutic strategy to treat patients with pancreatic adenocarcinoma, our finding also suggests that Sp1 protein may serve as a therapeutic target for tumors with Sp1 overexpression, especially considering the availability of Sp1 site–specific inhibitors (27, 28). Development of quantitative molecular techniques to measure Sp1-positive staining is also needed to determine if the intensity of staining correlates with the aggressiveness of tumor.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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