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# Role of Sparc as a Prognostic Factor in Pancreatic Tumors

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## Abstract

**Background:** Pancreatic adenocarcinoma is one of the most aggressive solid neoplasms. Evidence exists for interactions between pancreatic cancer cells and stromal fibroblasts that affect the invasive phenotype of pancreatic cancer. Secreted Protein Acidic and Rich Cysteine (SPARC) is a protein involved in cell matrix interactions, and is highly expressed in a wide range of human malignant neoplasms. SPARC could play a role in tumor progression at the site of interface between neoplastic cells and the surrounding host cells.

**Methods:** We collected cases with diagnose of pancreatic infiltrating ductal adenocarcinoma. Samples were staining with anti-SPARC probe and listed in 3 categories depending on the staining intensity: negative or weak intensity (0-1+), moderate (2+) and high (3+).

**Results:** Results indicated that from all the variants, only SPARC high expression could be a prognostic factor. Patients with strong positivity for SPARC had a median survival of 11.9 months compared with 16.7 months for those which SPARC expression was moderate, weak or absent.

**Conclusion:** In this study, we demonstrated that stromal SPARC expression could be a potent marker of poor prognosis, independent of common clinical parameters.

**Keywords: Pancreas; Adenocarcinoma; SPARC**

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## Abbreviations

SPARC: Secreted Protein Acidic and Rich Cysteine; ECM: Extra Cellular Matrix; EMT: Epithelial Mesenchymal Transition; FAK: Focal Adhesion Kinase

## Introduction

Pancreatic infiltrating ductal adenocarcinoma is one of the most aggressive solid neoplasms which incidence is increasing in the last years. Therapy for pancreatic infiltrating ductal adenocarcinoma has poor results without substantial changes in survival time. Besides the potential aggressiveness of neoplastic cells themselves, the host response at the site of primary invasion has been considered an important factor in pancreatic cancer progression. Indeed, evidence exists for interactions between pancreatic cancer cells and stromal fibroblasts that affect the invasive phenotype of pancreatic cancer [1]. Secreted Protein Acidic and Rich in Cysteine (SPARC), also known as osteonectin and BM-40 is a protein involved in cell matrix interactions. Its expression is believed to be regulated spatially and temporally throughout develop. Localization by in situ hybridization and immunohistochemistry has revealed synthesis of high levels of SPARC mRNA and protein respectively by a variety of tissues undergoing remodeling during normal development or in response to injury. High levels of the protein are detected in the "culture shock" which occurs when most attachment dependent mesenchymal cells are placed in culture [2]. SPARC expression is believed to modulate reversible interactions between cells and Extra Cellular Matrix (ECM) through: a) inhibiting cell spreading and disassembly of focal adhesion; b) inhibiting cell cycle progression; c) abrogating growth factor-mediated chemotaxis; and d) regulating the production of ECM. Although there are differences of expression reported using the different commercial antibodies, SPARC is highly expressed in a wide range of human malignant neoplasms, and the deregulated expression of SPARC is often correlated with disease progression and/or poor prognosis [3-11]. Interestingly, in certain tumor types, strong expression of SPARC has been detected predominantly in the stroma adjacent to the neoplastic cells [12-14]. All this backgrounds indicates that SPARC could play a role in tumor progression at the site

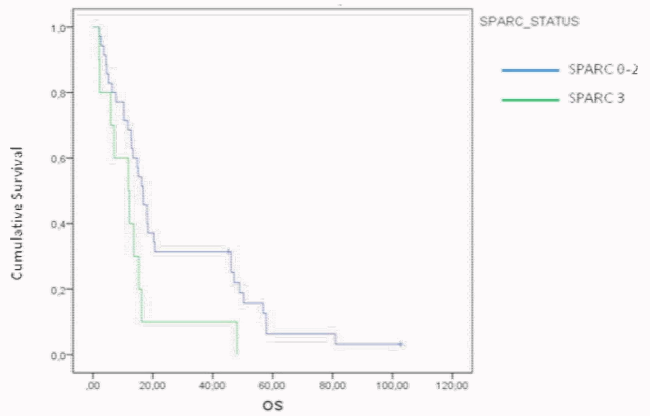


**Figure 1:** Overall Survival (os) depending on SPARC Status (3 vs 0-2) (Times is expressed in months).

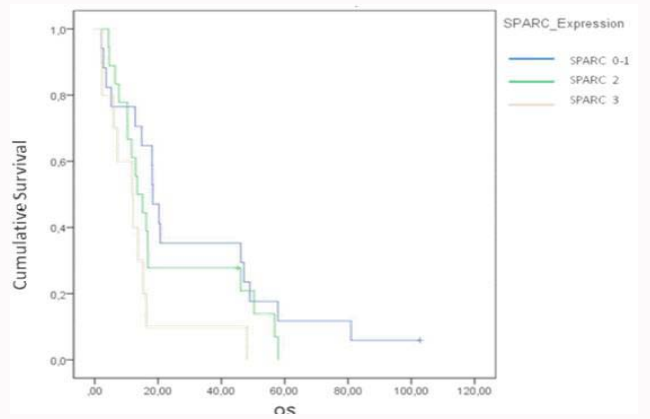


**Figure 2:** Overall Survival (OS) regarding SPARC expression. Light Brown line is SPARC 3+, Green line is SPARC 2+ and Blue line is SPARC 0-1+ (Time is expressed in months).

of interface between neoplastic cells and the surrounding host cells. So, the hypothesis of our study is that SPARC plays an important role in the tumor infiltration in the surrounding tissue. This role could mean SPARC expression level could be consider as a survival prognostic factor in pancreatic tumors.

## Material and Methods

Patients with pancreatic adenocarcinoma diagnosis, after curative intention surgery at University Hospital Santiago were included. Samples were provided by Hospital Bio bank. Patients with other malignancies within the last 5 years, incomplete medical records or insufficient sample were excluded. The medical reports included age, sex, recurrence and survival outcomes. A pathological report will be reviewed for Tumor invasion (T), lymph node metastasis, staging according AJCC 7$^{th}$ edition, lymphatic and venous capillary invasion and resection margins. The study was done in accordance with good clinical practice guidelines and the Declaration of Helsinki. All patients alive provided written informed consent. Approvals for the study protocol were obtained from independent ethic committee. From each tumor sections 3 nm thick were stained. Staining was performed according to the protocol of Cenbimo® Cish Probe (Lugo, Spain); shortly: after treatment with proteinase, sections were incubated for 60 minutes with the anti-SPARC probe. As detection system we used the Envision Plus Detection Kit (DAKO, Carpinteria, CA). Nuclei were counterstained with hematoxylin. We used a specifically developed CISH probe to detect SPARC to avoid differences of expression using different commercial antibodies. SPARC expression was analyzed for both carcinoma and the normal juxta-tumoral pancreatic tissue as positive or negative. The staining of the slides was examined microscopically and interpreted in a blinded fashion by three pathologists. We established 3 categories for SPARC immunolabeling: Grade 0-1: There is not immunostaining or it is very weak; Grade 2: moderate immunostaining; Grade 3: intense immunostaining.

### Statistical analysis

The statistical analysis will be performed using SPSS 19.0 statistical software. Comparisons of categorical variables were tested using the chi-square test or Fisher's exact test as a function of sample size of subgroups. Overall Survival was defined since the date of surgery to death for any cause or the last known alive date. The survival curves will be derived from Kaplan-Meier estimates, and the curves will be compared used log-rank test and Cox proportional hazard regression model. All tests will be two sided, and P values of <0.05 will be considered significant. Multivariate model was generated using a Cox forward stepwise regression.

## Results and Discussion

Patients were included between 1995 and 2011, in total 45 patients. The characteristics are shown in Table 1. Tumors histology was as follow: 91.1% were ductal adenocarcinomas, with 6.7% of mutinous and 2.1% of adenosquamous. In all patients, primary tumours were located at pancreatic head. Regarding tumor size, all of them were similar, averaging 2.79 ± 1.4 cm. 75.6% had T3 tumor (tumor extends beyond the pancreas without the involvement of the superior mesenteric artery), 66% had lymph node infiltration and 1 patient has metastatic disease which was totally excised, finally 71.1% had vascular and/or perineural invasion. Following the scale set for SPARC immunolabeling, 3 tumors were scored as 0; 14 tumors were scored as 1+; 18 of them were 2+ and 10 as 3+. There was a relationship between staining intensity and extension of immunostaining. We have also analyzed the cases following the pathological stage, and thus only five of the patients had stage I, of which the majority (3) had an expression of SPARC 1+. Most patients (39) had stage II, and the expression of SPARC in the same was variable, being mostly SPARC 1+ and 2+ (14 and 16 respectively), and only 9 were 3+; only one patient was stage IV and its expression of SPARC belonged to 3+ score. Referring to relation between infiltrated lymph nodes and SPARC expression 15.6% of the cases were pN0 and SPARC 1+; 15.6% of the cases were pN0 and SPARC 2+; and only 2.2% of the cases were pN0 and SPARC 3+; while cases pN1, 22.2% were SPARC 1+ 24.4% SPARC 2+ and 20.0% SPARC 3+. With a median follow up of 102 months, OS was 15.1 months (CI 95% 11.9 to 18.2). OS taking into account the results of SPARC: OS012 16.7 (CI 95% 12.9-20.5) vs. OS3 11.8 (4.1-19.5) HR 0.452 (CI 95% 0.216-0.949) p 0.031. No other prognostic factors were showed either with univariate or multivariate analysis (Figure1,2). We found that SPARC expression in peri tumoral fibroblasts is a robust marker of poor prognosis in patients with pancreas adenocarcinoma who undergo surgery with curative intent. Patients whose pancreatic cancer stroma labeled strong positive for SPARC had a median survival of 11.8 months compared with 16.7 months for those whose stroma did not express or express weekly to moderate SPARC. The mechanism by which stromal SPARC expression implies a worse prognosis is not known. We believe the most likely explanation for our findings is that peri tumoral fibroblast

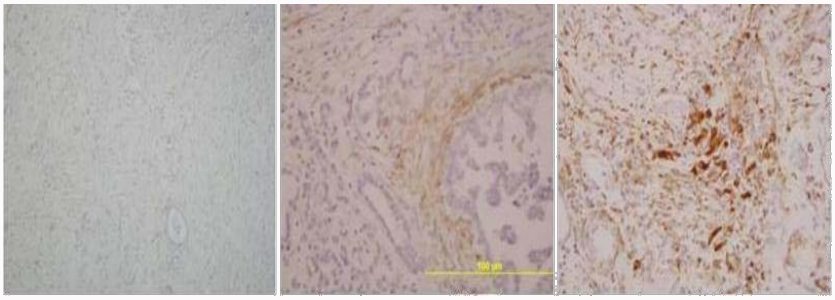

**Figure 3:** The images show stained pancreatic adenocarcinomas using the SPARC Cenbimo® Cish Probe. The left figure shows staining Grade 1 (no staining or very weak), central image shows staining Grade 2 (moderate), and the right one is a Grade 3 (intense staining).

**Table 1:** Patients Characteristics.

| Patients Characteristics | | |
|---|---|---|
| **Age** | Median (Range) | 64 (37-79) |
| **Sex** | Male | 57.80% |
| | Female | 42.20% |
| **Stage** | IA | 6.70% |
| | IB | 4.40% |
| | IIA | 22.20% |
| | IIB | 64.40% |
| | IV | 2.20% |
| **Grade** | Well differentiated | 24.40% |
| | Moderately differentiated | 53.30% |
| | Poorly differentiated | 4.40% |
| | Unknown | 17.80% |
| **Infiltration** | Vascular | 8.90% |
| | Perineural | 26.70% |
| | Perineural | 35.60% |
| | None | 8.90% |
| | Unknown | 28.80% |

SPARC expression is a marker of activated fibroblasts. Underlying mechanisms by which SPARC might favor metastatic dissemination, it was shown that SPARC expression directly correlated with MMP-2 and MMP-9 levels and activity [7]. Matrix cellular proteins have important roles in the exchange of information between tumor cells, stromal cells and their surrounding extracellular matrix, as they were shown to interact with cell surface receptors, the structural matrix, and soluble extracellular factors such as growth factors and proteases. The Epithelial Mesenchymal Transition (EMT) is a relevant process during normal development in which epithelial cells lose their polarity and develop a mesenchymal phenotype [15]. It has been proposed that similar EMT-like changes might occur in order that cancer cells can acquire its invasive and metastatic phenotype [15]. EMT is characterized by loss of inter cellular adhesion (E- to N- cadherin switch), down-regulation of epithelial markers (cytokeratins) and up-regulation of mesenchymal markers (vimentin) and the acquisition of a fibroblast-like motile and invasive phenotype. The transcription factor Snail and other members of its family have been implicated in the promotion of EMT, acting as a repressor of epithelial and activator of mesenchymal genes [16-18]. Interestingly, SPARC expression in melanoma cells has been associated with several features involved in EMT; indeed, SPARC was shown to decrease E-cadherin and increase vimentin expression through phosphorylation of FAK and or induction of the transcriptional factor Snail [19,20]. In addition, it was shown that down regulation of SPARC in melanoma cells significantly decreased membrane bound and extracellular soluble N-cadherin levels [21]. These evidences suggest that SPARC mediates the switch from E-cadherin to N-cadherin expression in melanoma cells, with the subsequent enhancement of cell migration and invasive capacity. In addition, a gene expression profile study demonstrated that most aggressive human metaplastic breast tumors differentially over expressed several molecules associated to EMT, including SPARC and several collagens [22]. In this study, over expression of Snail in MCF7 human breast cancer cells induced a decrease in E-cadherin levels and a concomitant increase in SPARC and vimentin, indicating that SPARC expression is under the modulation of Snail [22]. Some EMT changes are also mediated by extracellular matrix components such as collagens (Figure 3). The binding of several native collagens to certain integrins leads to signal transduction by the transient activation of Focal Adhesion Kinase (FAK) and the phosphorylation of paxillin, which ultimately leads to an increase in cell motility [23]. Moreover, SPARC was shown to modulate cell survival and invasion of glioma cells through the activation of FAK and ILK (Shi et al, 2007). Overall, the above mentioned evidence suggests that SPARC may act at several stages of the EMT, thus contributing to the enhancement of the malignant phenotype. SPARC could be a potential target for anti neoplastic therapy. The prognostic influence of stromal behavior suggests there may be a need for therapeutic efforts that target the supportive micro environment. In this studied the expression of SPARC in stromal fibroblast, tumor epithelia by immunostaining and plasma SPARC by Elisa in a phase III trial patients [24]. This study found no differences in overall survival when studying SPARC in the tumor; however, we must consider several differences from our study. In this sense, to begin, they used a standard monoclonal antibody, and we have used a specific probe to rule out differences of expression obtained using different commercial antibodies. Also the results obtained with the antibody staining in most cases was zero or low, in contrast to our study in which three independent pathologists, blinded, detected tumors with high expression, and it is precisely at this level of expression where the differences are significant, matching with the results of Hidalgo, when the expression is moderate or null.

## Conclusion

In summary, stromal SPARC expression is a potent marker of poor prognosis, independent of common clinical parameters including tumor size, margin status and lymph node metastasis.

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## Ethical Standards

This study has been approved by the appropriate ethics committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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