# Epithelial and Stromal Expression of Syndecan-2 in Pancreatic Carcinoma

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**Abstract.** *The aim of the study was to determine the expression and prognostic role of syndecan-2 in patients with pancreatic adenocarcinoma. Patients and Methods: Syndecan-2 expression and its relationship with established prognostic features were assessed in a series of 53 patients with pancreatic ductal adenocarcinoma. Results: Epithelial expression was observed in 23 (43.4%) and stromal in 30 (56.6%) pancreatic carcinomas, respectively. In normal pancreatic tissue, the epithelial expression was moderate or strong in single or small clusters of acinar cells and negative in ductal cells. Normal pancreatic stroma did not express syndecan-2. Statistical analysis showed that stromal expression had no influence on survival but epithelial expression was positively correlated with survival time, and patients with higher epithelial syndecan-2 expression had a distinctly longer survival (p=0.029). Conclusion: Our results support a potential role for syndecan-2 in pancreatic carcinogenesis and cancer progression. Moreover, expression of syndecan-2 might serve as a prognostic marker.*

Syndecans comprise a family of cell surface transmembrane heparan sulfate proteoglycans that mainly serve as co-receptors and play an important role in a variety of cellular functions, including cell proliferation, migration, and cell cell and cell matrix interactions. They control or influence tissue repair, metabolism, formation of tumors and the development of immune response (1-3). Four syndecans (from 1 to 4) have been recognized, each with distinct structure and pattern of expression. They are composed of a short *C*-terminal cytoplasmic domain, an *N*-terminal signal peptide, an ectodomain containing several consensus sequences for glycosaminoglycan attachment, and a transmembrane domain with a putative protease cleavage site at the proximal side (1, 2). All adhesive cells express at least one syndecan, and most express multiple syndecans (4). The most extensively studied member of syndecan family is syndecan-1 (SDC1) which is mostly expressed in epithelial cells (2, 4). Several studies have shown that SDC1 plays an important role in the regulation of cancer growth and behavior in different carcinomas including pancreatic adenocarcinoma and cystic tumors of the pancreas (5-11). Syndecan-2 (SDC2) was originally isolated from human lung fibroblasts and is found in abundance in fibroblasts, endothelial cells and hepatocytes but, in contrast to SDC1, is deficient in epithelial cells (12, 13). An early recognizable role of SDC2 was cell adhesion and migration, but recently, SDC2 has also been implicated in tyrosine kinase signaling pathway activation, angiogenesis and tumorigenesis (14-18). Syndecan-2 is critical mediator in the tumorigenesis of colon carcinoma cells. Normal colon epithelial cells did not express SDC2, whereas colon carcinoma cells showed SDC2 up regulation, resulting in an increased SDC2 expression that proved crucial for tumorigenicity (16-18). Park *et al*. (16) demonstrated SDC2 to be necessary for cell cycle progression and cell matrix interaction in colon cancer cells. Moreover, an increased level of SDC2 led to a less adhesive phenotype and loss of contact inhibition (19). Several other studies investigated the role of SDC2 in different types of carcinoma (16-22), however, to our knowledge there are no studies focused on SDC2 in pancreatic cancer. The aim of the study was thus to determine the expression and prognostic role of SDC2 in patients with pancreatic adenocarcinoma who were treated by pancreaticoduo-denectomy for cure.

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## Patients and Methods

*Patients*. The study included 53 patients treated with pancreaticoduodenectomy for pancreatic ductal adenocarcinoma at the University Department of Surgery, Sestre Milosrdnice University Hospital, Zagreb, between January 1, 2001 and December 31, 2008. Patients with other histological subtypes of carcinoma were not included in the study. All patients included in the study underwent curative pancreaticoduodenectomy. The operation was considered to be curative if there was no cancer at the resection margins marked with the ink at microscopic evaluation. None of the patients was treated with chemotherapy or radiation before the surgery, and none had secondary cancer. All patient identifiers were removed and replaced by unique study numbers, linked to the original identifiers by a single file kept under high security. The follow-up of patients and the retrieval of archival tissue block were conducted under Institutional Review Board approval.

Twenty-eight of the patients were females and 25 were males, with a median age at the time of diagnosis of 62.5 (range 32-81, interquartile range 56.3-67.8) years. Overall follow-up ranged from 1 to 96 (median 14, interquartile range 10-28) months. Twenty-nine (54.7%) patients died of pancreatic cancer and the median time of death was 12 (range 1-64, interquartile range 5-19) months after the diagnosis.

The TNM stage was assigned by the pathologist according to the criteria of the International Union against Cancer (23). Six (11.3%) patients had stage T1, 9 (16.9%) stage T2, 37 (69.9%) stage T3 and 1 (1.9) had stage T4 disease. Twenty-three patients (43.4%) had metastases in regional lymph nodes, 24 (45.3%) were without metastases, and in 6 (11.3%) patients, regional lymph nodes could not be assessed. Only three (5.7%) patients had distant metastases. The median size of tumors was 3.5 (range 2-10, interquartile range 2.75-5.75) centimetres. Ten (18.9%) tumors were well differentiated, 29 (54.7%) moderately and 14 (26.4%) poorly differentiated.

*Immunohistochemistry*. Specimens were fixed in 10% buffered formalin, embedded in paraffin, cut at 5 μm thickness, and routinely stained with hematoxylin and eosin. The diagnosis of pancreatic ductal adenocarcinoma was histologically confirmed in all cases. All slides submitted to immunohistochemistry analysis also contained areas of normal, non-neoplastic pancretic tissue which served as an internal control. Deparaffinization and immunohistochemical staining were performed following the microwave streptavidin immunoperoxidase (MSIP) protocol on a DAKO Tech-Mate™ Horizon automated immunostainer (DAKO, Copenhagen, Denmark). Primary monoclonal antibody to syndecan-2 ((1F10/B8): sc-73516, dilution 1:100; Santa Cruz Biotechnology, Inc., CA, USA) was used. Colon cancer tissue served as a positive control and removal of the primary antibody was used as a negative control. To evaluate the intensity of SDC2 expression in pancreatic cancer, the percentage of positively stained cells was examined in all the tumor on the representative slide. The expression of SDC2 was evaluated separately in the epithelial and stromal cells. The level of epithelial SDC2 expression was graded on a scale of 0-3 and expressed as 0, no positive carcinoma cells; 1, low, up to 10% of positive carcinoma cells; 2, moderate, more than 10%-30% of positive carcinoma cells; and 3, high, more than 30% of positive carcinoma cells. The stromal tissue surrounding cancer cell was evaluated and scored likewise: 0, no positive stromal cells; 1, low, up to 10% of positive stromal cells; 2, moderate, more than 10%-30% of positive stromal cells; and 3, high, more than 30% of positive stromal cells. The intensity of the stain did not influence the score. All samples were examined independently by three observers (A.G., B. K. and D. T.) and any difference was resolved by a joint review.

Smirnov-Kolmogorov test was used to analyze the data distribution before statistical analysis. Statistical analysis was performed using $\chi^2$-test, Spearman rank correlation coefficients (rho), Kaplan-Meier test and multivariate Cox proportional-hazards regression test. The levels of statistical significance were set at $p<0.05$. Statistical analyses were performed using MedCalc for Windows, version 11.0 (MedCalc Software, Mariakerke, Belgium).

## Results

Epithelial SDC2 expression was observed in 23 (43.4%) pancreatic carcinomas (Figure 1A). Sixteen (30.2%) had low, 4 (7.5%) moderate and 3 (5.7%) a high intensity of epithelial expression. In normal, non-neoplastic pancreatic tissue, the epithelial expression was moderate or strong in single or small clusters of acinar cells and negative in ductal cells (Figure 1B). Immunohistochemical reaction was cytoplasmatic and granular.

Stromal SDC2 expression was found in 30 (56.6%) pancreatic carcinomas (Figure 1C). Twenty-two (41.5%) had low and 8 (15.1%) had moderate intensity of SDC2 stromal expression. None of the carcinomas had a high intensity of stromal expression. The normal, non-neoplastic pancreatic stroma did not express SDC2. In pancreatic cancer, there was no statistically significant association between the level of epithelial SDC2 expression and age ($p=0.546$), gender ($p=0.103$), tumor differentiation ($p=0.080$), tumor size ($p=0.583$), T stage ($p=0.268$), nodal status ($p=0.206$) nor distant metastases ($p=0.969$). Stromal expression did not correlate with gender ($p=0.228$), tumor differentiation ($p=0.284$), tumor size ($p=0.528$), T stage ($p=0.674$), nodal status ($p=0.733$), nor distant metastasis ($p=0.595$). The patients with SDC2 stromal expression were significantly older compared to patients without SDC2 stromal expression, and age was positively correlated with intensity of stromal expression (rho=0.340, $p=0.014$). Expression of SDC2 in epithelial cells did not correlate with expression in stromal cells in pancreatic carcinoma ($p=0.847$).

Differences between epithelial and stromal expression of SDC2 in the patients who died of carcinoma and the patients who were alive at the end of the follow-up period were not significant but showed a tendency to reach significance ($p=0.088$ and $p=0.080$, respectively). On Kaplan-Meier analysis, epithelial ($p=0.381$) and stromal ($p=0.328$) expression of SDC2 were not associated with survival. On multivariate Cox proportional hazards regression analysis which included patients' age, sex, tumor size, TNM stage and histological grade, the intensity of stromal SDC2 expression had no influence on survival (odds ratio, OR=0.699; 95% confidence interval, CI: 0.371-1.318,

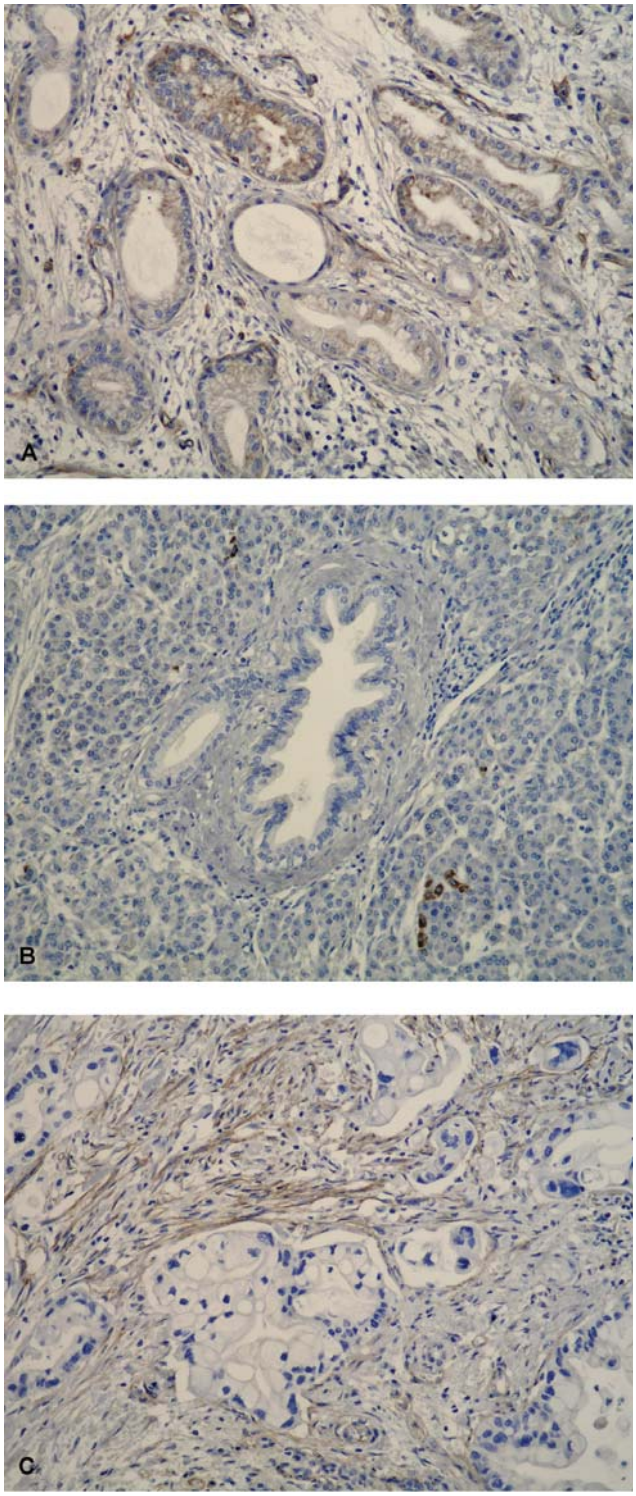


Figure 1. *A: Syndecan-2 (SDC2) was expressed in epithelial cells of pancreatic adenocarcinoma. Immunohistochemical reaction in tumor cells was granular and cytoplasmic (×200). B: In normal pancreatic tissue, the epithelial expression of SDC2 was moderate or strong in single or small clusters of acinar cells and negative in ductal cells (×200). C: The pancreatic carcinoma in general showed weak stromal expression of SDC2 (×200).*

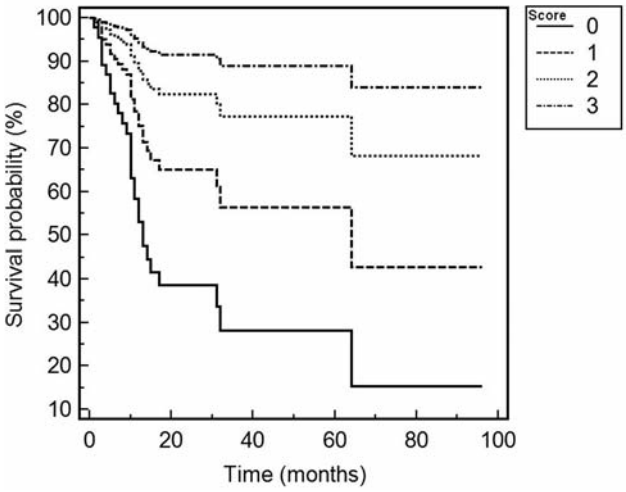


Figure 2. *Impact of epithelial syndecan-2 expression on the survival of patients with pancreatic adenocarcinoma (Cox proportional hazards regression analysis).*

$p$=0.271), but epithelial SDC2 expression positively correlated with survival time (OR=0.452, 95% CI: 0.222-0.918, $p$=0.029) and the patients with higher epithelial SDC2 expression had a distinctly longer survival (Figure 2).

## Discussion

In our study of 53 patients with pancreatic adenocarcinoma, we analyzed epithelial and stromal expression of SDC2 in the tumors and compared with this different clinicopathological factors. The intensity of epithelial expression in the majority of positive cases was weak but did positively correlate with survival in multivariate analysis: patients with higher epithelial SDC2 expression had significantly longer survival. Considering that the ductal cells of normal pancreatic tissue showed no expression of SDC2, it seems that expression of SDC2 during carcinogenesis directly influenced the prognosis of patients with pancreatic carcinoma.

Several studies investigated syndecan in pancreatic cancer, but all were focused on SDC1 (9-11), and to our knowledge there is no available investigation addressing the expression of SDC2 in pancreatic cancer. Conejo *et al*. (9) found that pancreatic cancer cells lines expressed *SDC1* mRNA and protein, and that SDC1 was markedly overexpressed in cancer cells in comparison with normal pancreatic cells. In addition, these cells also released SDC1 into the culture medium. In the normal pancreas, by *in situ* hybridization and immunohistochemistry, the same study showed that SDC1 expression was evident at low levels in the ductal cells and less frequently in acinar cells. In contrast, in pancreatic cancer tissues, SDC1 was present at moderate to high levels in the majority of the cancer cells and also in metastatic lesions of

pancreatic tumors (9). In our study, we observed similar pattern of expression for SDC2, which was expressed in epithelial and stromal cells of pancreatic ductal carcinoma but not in ductal cells of normal pancreatic tissue.

In a more recent study, Juuti *et al*. (10) showed that low epithelial SDC1 expression in cancer cells was associated with a worse overall survival compared to strong epithelial SDC1 expression in patients with pancreatic cancer who underwent surgery for cure, but these results were not confirmed in multivariate analysis. On the contrary, patients with stromal SDC1-positive pancreatic cancer had a worse outcome than did patients with stromal SDC1-negative tumors, and stromal expression of SDC1 was an independent prognostic factor (10). On the basis of these results, they suggested that SDC1 has a different function in cancer depending on the site of expression (10). In pancreatic cancer, epithelial SDC1 is produced by epithelial cancer cells (9), but the origin of stromal SDC1 is still unresolved. One hypothesis is that epithelial cells shed the extracellular portion of the transmembrane SDC1 molecule into the tumor stroma (9). On the other hand, it is also known that mesenychimal cells are capable of producing SDC1 and that the SDC1 produced by stromal cells is different from epithelial SDC1 (24).

Our results showed that expression of epithelial SDC2 was directly associated with better outcome, while stromal expression had no significant influence. Likewise, we found no significant association between epithelial and stromal expression of SDC2. It seems that epithelial and stromal SDC2, as well as epithelial and stromal SDC1, have different functions and that the functions are related to the site of expression. Expression of stromal SDC2 in our study positively correlated with the patients' age, but we are unable to explain this observation at present. Previous studies on different tumors have shown that increased SDC2 expression in cancer cells in general has been correlated to a worse clinical outcome, and to lymph node metastasis (17, 20, 22, 25, 26). Our results showed an exception in pancreatic cancer, where increased epithelial SDC2 expression indicated a better prognosis.

Similar results were reported for epithelial SDC1 in pancreatic carcinoma and in cystic tumors of the pancreas where loss of SDC1 indicated malignancy (10, 11). Moreover, Conejo *et al*. (9) showed that SDC1 mRNA level in gastrointestinal malignancies (esophageal, gastric, colon and liver cancer) was not significantly different from the levels observed in the corresponding normal samples. They also suggested that SDC1 overexpression in pancreatic cancer may be of importance in the pathobiology of this tumor and that its role in pancreatic cancer could be different from that in other gastrointestinal malignancies (9). Our results also suggest that SDC2 expression could play multiple roles in tumorigenic activity and perform various tissue- and or tumor stage-specific functions.

In conclusion, our results support a potential role for SDC2 in pancreatic carcinogenesis and cancer progression, although its role in pancreatic cancer could be somewhat different from that in other malignancies. Moreover, expression of SDC2 might serve as prognostic marker that might help in further stratifying the risk of death for patients with pancreatic cancer who undergo surgery for cure. Additional studies are necessary to identify SDC2 function in pancreatic carcinogenesis and its prognostic role in pancreatic cancer.

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