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Reduced expression of the membrane skeleton protein beta1-spectrin (SPTBN1) is associated with worsened prognosis in pancreatic cancer

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Summary. Spectrins are members of the superfamily of F-actin cross linking proteins that are important as scaffolding proteins for protein sorting, cell adhesion, and migration. In addition, spectrins have been implicated in TGF-beta signaling. The aim of the present study was to analyze the expression and localization of beta1-spectrin (SPTBN1) in pancreatic tissues. mRNA levels of SPTBN1 in cultured pancreatic cancer cell lines, as well as in normal pancreatic tissues (n=18), chronic pancreatitis (n=48) and pancreatic cancer tissues (n=66) were analyzed by real time quantitative RT-PCR. Localization of SPTBN1 in pancreatic tissues was determined by immunohistochemistry. SPTBN1 staining was assessed semi-quantitatively in 55 cancer tissues and survival analysis was carried out using the Kaplan-Meier method. Median SPTBN1 mRNA levels were 6.0-fold higher in pancreatic cancer tissues compared to the normal pancreas ($p<0.0001$) and 2.2–fold higher compared to chronic pancreatitis tissues ($p=0.0002$). In the normal pancreas, SPTBN1 was present in the cytoplasm of normal ductal cells and occasionally in pancreatic acinar and centroacinar cells. In pancreatic cancer tissues, SPTBN1 was present in the cytoplasm of pancreatic cancer cells. Low SPTBN1 protein expression indicated a tendency for worsened prognosis with a median survival of 14.0 months, versus 23.8 months for patients whose tumors expressed moderate/high levels of SPTBN1. In conclusion, reduced SPTBN1 expression correlated with shorter survival of pancreatic cancer patients, suggesting a tumor suppressor function of this gene, as has already been shown for other malignancies of the gastrointestinal tract.

Key words: ELF, Cytoskeleton, TGF-ß, Smad, Pancreatic cancer

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Introduction

Spectrins are members of the superfamily of F-actin cross linking proteins, and are essential components of the membrane skeleton, the membrane associated cytoskeletal network. This membrane skeleton is found on the cytoplasmatic surfaces of most intracellular membrane compartments as well as on the plasma membranes of many cells. The elastic filaments built by spectrins consist of two alpha- and two beta-spectrin-proteins, and are encoded by two alpha spectrin genes (α_1 and α_2) and five beta spectrin genes ($ß_{1-5}$). They form elongated heterodimers of two subdivisions, α and ß, with motifs called "spectrin repeats", and are composed of triple helical coiled coils connected by helical linkers. Due to their modular structure made of these repeats, spectrins can expand and contract. As spectrins are essential components of the cytoskeletal network, they, among other functions, influence differentiation, polarization, cell adhesion, and protein sorting. Spectrins provide essential mechanical support, preventing for example loss of membrane material to environmental shear stresses (Mohandas et al., 1983;

Speicher and Marchesi, 1984; Grum et al., 1999; Bennett and Baines, 2001; Mohler and Bennett, 2005; Tang et al., 2005; Chakrabarti et al., 2006; Baines, 2009). Associated with different binding domains, spectrins also act as adapter molecules mediating several physiological protein-protein interactions subsequently regulating signal transduction. By assembling an extensive intercellular network, spectrins interact with a large number of proteins such as actin, ankyrin, and adducin (Hartwig, 1994; Davis et al., 2009).

Transforming growth factor-ßs (TGF-ßs) belong to a family of cytokines that induce signaling pathways through binding to specific serine-threonine kinase receptors at the cell surface. Specifically, TGF-ß binds to the type II TGF-ß receptor, which then associates with the type I receptor. Downstream signaling occurs via phosphorylation of receptor Smad molecules (Smad2 and Smad3) that associate with co-Smads (Smad4) and then translocate to the nucleus, where they form transcriptional complexes to either repress or activate transcription of target genes. TGF-ßs regulate a variety of functions, including cell growth, cell differentiation, adhesion, motility, apoptosis, angiogenesis, immunosuppression and others (Derynck and Zhang, 2003; Siegel and Massague, 2003).

Beta-spectrins have been identified as important regulatory elements for TGF-ß signaling through Smad mediated pathways (Tang et al., 2003). The mouse homolog of ß1 spectrin (SPTBN1), the adaptor protein ELF (embryonic liver fodrin), was first identified from endodermal stem cells dedicated to foregut filiation (Mishra et al., 1998, 1999).

ELF associates with Smad3 and the TGF-ß receptor complex, and it subsequently interacts with Smad4, leading to translocation to the nucleus, and activation of gene expression e.g. of a number of growth and differentiation factors. Deficiency of ELF results in a mislocalization of Smad3/4 and subsequent disruption of the TGF-ß dependent transcriptional response (Tang et al., 2003). Therefore, ELF and its human homologue SPTBN1 seem to be important in the carcinogenesis of many gastrointestinal tumors (Katuri et al., 2005; Mishra et al., 2005; Baek et al., 2008).

Alterations in normal TGF-ß signalling play an important role during carcinogenesis of various cancers, such as those of the pancreas, colon, stomach, breast, prostate, and many others. TGF-ßs exert paradoxical effects on normal and neoplastic epithelial cells. It is generally thought that TGF-ßs act as tumor suppressors early in the carcinogenic process and as tumor promoters in advanced tumors (Truty and Urrutia, 2007).

Pancreatic cancers frequently over-express TGF-ß isoforms and overexpression is associated with a worsened prognosis (Friess et al., 1993), despite the observation that some cultured pancreatic cancer cell lines are growth inhibited by TGF-ßs (Wagner et al., 1999). It has been shown that TGF-ß signaling in pancreatic cancer cells is blocked by mutations of the tumor suppressor Smad4 (DPC4) in approximately 50% of cases (Hahn et al., 1996). In addition, inactivating mutations of the TGF-ß receptor type II gene have been found in around 5% of pancreatic cancers and it has been recently shown that genetic alterations resulting in disruption of the TGF-ß signalling pathway are present in almost all cases of pancreatic cancer (Jones et al., 2008). Epigenetic changes, such as enhanced expression of inhibitory Smads like Smad7, contribute to the resistance of pancreatic cancer cells towards the growth inhibitory effects of TGF-ßs (Kleeff, Ishiwata et al., 1999). On the other hand, TGF-ßs exert tumor promoting effects by acting strongly immunosuppressive, by enhancing angiogenesis, and by inducing epithelial to mesenchymal transition (EMT) resulting in a more migratory and invasive phenotype (Truty and Urrutia, 2007; Welsch et al., 2007).

In view of the essential role of TGF-ß signaling in the pathogenesis of pancreatic cancer and the importance of ß-spectrin (specifically SPTBN1) in TGF-ß signaling in other gastrointestinal malignancies, in the present study we analyzed the expression and localization of the ß-spectrin SPTBN1 in pancreatic cancer.

Materials and methods

Tissue sampling

Pancreatic tissue specimens were obtained from patients who underwent pancreatic resection or through an organ donor program from previously healthy individuals between January 2003 and September 2005. All pancreatic cancer patients had histological confirmed pancreatic ductal adenocarcinoma according to the WHO classification (Klöppel et al., 2000) and underwent macroscopic complete (R0/R1) resections. Since during the study period, the pathological processing and reporting changed at our institution (Esposito et al., 2008), no analysis regarding R0 and R1 resections has been carried out. None of the patients received neoadjuvant radio-chemotherapy. All patients were advised to receive adjuvant therapy; however, data for adjuvant therapy were heterogeneous and not available for a number of patients. Detailed information regarding other clinical and histopathological information is presented in table 1. The Human Subjects Committee of the University of Heidelberg, and the Technische Universität München, Germany, approved all studies. Written informed consent was obtained from all patients.

Cell culture

Pancreatic cancer cells were routinely grown in RPMI medium supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 μg/ml streptomycin. For the isolation of pancreatic stellate cells, the outgrowth method was used (Erkan et al., 2007). Cell populations between passage 3 and 6 were used. A 1:1 (vol/vol) mixture of low glucose (1000 mg/L) DMEM

with Ham's F12 medium supplemented with 10% FCS, L-glutamine (2 mmol/l), penicillin/streptomycin, and amphotericin was the standard medium (SM-10%). Cells were maintained at 37°C in a humid chamber with 5% CO_2 and 95% air atmosphere.

Real-time quantitative polymerase chain reaction (QRT-PCR)

All reagents and equipment for mRNA/cDNA preparation were supplied by Roche Applied Science (Mannheim, Germany). mRNA was prepared by automated isolation using the MagNA pure LC instrument and isolation kit I (for cells) and kit II (for tissues). cDNA was prepared using the first-strand cDNA synthesis kit for RT-PCR (AMV) according to the manufacturer's instructions. QRT-PCR was carried out using the LightCycler FastStart DNA SYBR Green kit. The number of specific transcripts was normalized to the housekeeping gene peptidylprolyl isomerase B (PPIB) and presented as copies/10,000 copies PPIB. All primers were obtained from Search–LC (Heidelberg, Germany).

Immunoblotting

Protein levels were assessed by immunoblotting using a specific rabbit polyclonal SPTBN1 antibody (sc28272; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Briefly, cells were lysed in a lysis buffer containing 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 2mM EDTA pH 8.0 and 1% SDS. 20 μg proteins were separated on 6% gels and electroblotted onto nitrocellulose membranes. Membranes were then incubated in blocking solution (5% milk in 20 mM Tris HCl, 150 mM NaCl, 0.1% Tween-20), followed by overnight incubation with the rabbit polyclonal SPTBN1 antibody (dilution 1:500 in blocking solution). The membranes were then washed in TBS-T and incubated with anti-rabbit horseradish peroxidase-conjugated secondary antibodies (dilution 1:3000 in blocking solution) (Amersham Bioscience, Buckinghamshire, UK). Antibody detection was performed with an enhanced chemiluminescence reaction (ECL, Amersham Bioscience). Equal loading and transfer was confirmed using GAPDH antibodies (Santa Cruz Biotechnology, Inc.).

Immunohistochemistry

Paraffin-embedded human pancreatic tissue sections (3 μm thick) were subjected to immunostaining as described previously (Erkan et al., 2007, 2009). Sections were deparaffinized in Roticlear (Carl Roth GmbH, Karlsruhe, Germany) and rehydrated in progressively decreasing concentrations of ethanol. Antigen retrieval was performed by boiling with citrate buffer (pH 6.0) twice for 7.5 min. Endogenous peroxidase activity was quenched by incubating the slides in methanol containing 3% hydrogen peroxide in methanol. The sections were incubated at 4°C overnight with the primary rabbit polyclonal SPTBN1 antibody (sc28272; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) in DAKO antibody diluent (S3022; DAKO Corporation, Carpinteria, CA) (dilution 1:500). The slides were rinsed with washing buffer (1x TBS, 0.1% BSA, 0.05% Tween20) and incubated with EnVision+ System Labeled polymer HRP anti-rabbit antibody (K4003; DAKO) for 45 min at room temperature. Tissue sections were then washed in washing buffer and subjected to 100 μl DAB-chromogen substrate mixture (K3468; DAKO), followed by counterstaining with hematoxylin (Carl Roth GmbH). Sections were washed, dehydrated in progressively increasing concentrations of ethanol, and mounted with xylene-based mounting medium. Slides were visualized using the Axioplan 2 imaging microscope (Carl Zeiss Lichtmicroskopie, Göttingen, Germany). Additionally, to confirm the specificity of the primary antibodies, tissue sections were incubated in the absence of the primary antibody and with negative control rabbit IgG. Under these conditions, no specific immunostaining was detected. Semiquantitative analysis was carried out as described before (Reiser-Erkan et al., 2008). Briefly, scores were given separately for the stained area and for the intensity of staining. Quantification was made as follows; <33% of the cancer cells: 1, 33-66% of the cells: 2, >66% of the cancer cells: 3; intensity of staining: absent: 0, weak/moderate: 1, strong: 2. Each section had a final grade that derived from the multiplication of the area and intensity scores. SPTBN1 expression was considered to be absent/low for grade 0-1, and moderate/high for grades of 2-6. Slides were evaluated independently by two researchers (XJ, IE).

Statistical analysis

Graph presentations were made using GraphPad Prism 5 for Windows (GraphPad, San Diego, CA). Differences in mRNA expression levels in normal, chronic pancreatitis and pancreatic cancer tissues were compared using the Mann–Whitney U test. Analysis of overall survival was carried out using the Kaplan-Meier method for estimation of event rates and the Log-rank (Mantel-Cox) or the Gehan-Breslow-Wilcoxon test for survival comparisons between patient groups. In addition, univariate and multivariable analyses were carried out for clinical and histopathological parameters using a Cox proportional hazards model. Statistical analyses were performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL) and GraphPad Prism 5 for Windows (GraphPad, San Diego, CA). The level of statistical significance was set at $p<0.05$.

Results

First, we determined SPTBN1 mRNA expression levels in the normal pancreas, chronic pancreatitis and pancreatic cancer tissues. To this end, bulk tissue

samples were analyzed from 66 pancreatic cancer patients (34 female, 32 male, median age 65 years). According to the 6th edition of the UICC classification, there were 1 stage IB, 11 stage IIA, 53 stage IIB, and 1 stage IV tumor (table 1). All cases were ductal adenocarcinoma (Klöppel et al., 2000). In addition, tissue samples from 48 chronic pancreatitis patients (13 female, 35 male, median age 47 years) and 18 healthy organ donors (6 female, 12 male, median age 49 years) were analyzed (figure 1). This analysis revealed median (95% confidence interval) SPTNB1 mRNA levels in the normal pancreas of 119 (112-331) copies/10,000 PPIB copies and in chronic pancreatitis of 331 (297-682) copies/10,000 PPIB copies. In contrast, SPTNB1 mRNA levels in pancreatic cancer were 717 (709-1216) copies/10,000 PPIB copies. Thus, median SPTBN1 mRNA levels were 6.0-fold higher in pancreatic cancer compared to the normal pancreas ($p<0.0001$) and 2.2–fold higher compared to CP samples ($p=0.0002$). In addition, SPTBN1 expression was also increased in areas next to the tumor mass with median (95% confidence interval (CI)) mRNA levels of 749 (118-1813) copies/10,000 PPIB copies (fig. 1). There was no difference in SPTBN1 mRNA expression depending on age or gender of the patients.

Next, the exact localization of SPTBN1 was determined in human pancreatic tissue specimens. In the normal pancreas, SPTBN1 was occasionally present in the cytoplasm of pancreatic acinar and centroacinar cells (Fig. 2A-D) but not in islet cells (Fig. 2A). In contrast, SPTBN1 was consistently expressed in the cytoplasm of normal pancreatic ductal cells (Fig. 2E).

In chronic pancreatitis, as well as in chronic pancreatitis-like areas next to pancreatic cancer, SPTBN1 was strongly present in small ducts and in tubular complexes (Fig. 3A, B). In pancreatic cancer tissues, SPTBN1 was present in the cytoplasm of pancreatic cancer cells (Fig. 3C-E). SPTBN1 expression was also weakly present in cancer cells of poorly differentiated tumors (Fig. 3F).

SPTBN1 expression was quantified as described in the Methods section in 55 pancreatic cancer specimens and survival of patients with low (n=39) versus high (n=16) SPTBN1 expression was compared using the Kaplan-Meier method and the Log-rank (Mantel-Cox) or the Gehan-Breslow-Wilcoxon test. Low SPTBN1 protein expression indicated a tendency for worsened prognosis ($p=0.059$ Log-rank test; $p=0.045$ Gehan-Breslow-Wilcoxon test). Median survival in patients with low SPTBN1 expression was 14.0 months (95%CI 9.9-18.1 months) in comparison to 23.8 months (95% CI 16.2-31.4 months) in patients whose tumors expressed moderate/high levels of SPTBN1 protein in the cancer cells. 1-/2-year survival rates were 58%/26% and 81%/45% in low and high SPTBN1 expressing tumors, respectively (Fig. 4). Multiple Cox regression analyses were carried out next. Univariate analysis regarding survival demonstrated a tendency for reduced survival for T3/T4 versus T1/T2, G3 versus G1/2, and SPTBN1 low versus moderate/high expressing tumors (Table 2). In multivariable analysis, the association of these factors with survival did not reach statistical significance (table 2). Univariate analysis regarding SPTBN1 staining

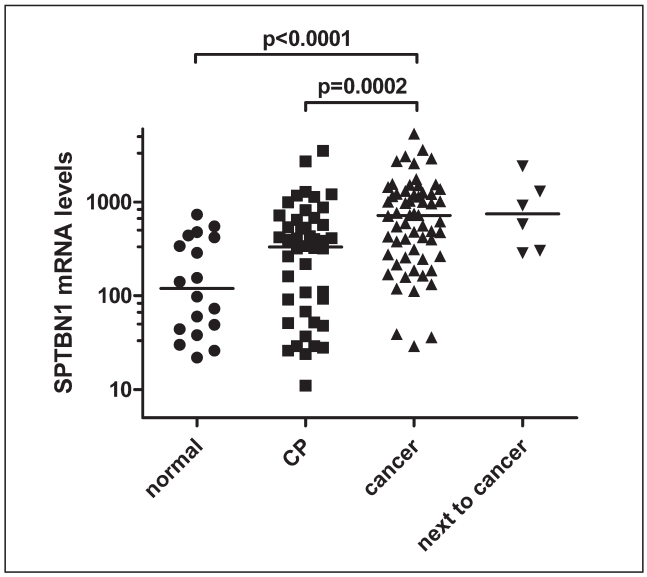

Fig. 1. Expression of SPTBN1 mRNA in normal pancreatic tissues (n=18), as well as chronic pancreatitis (CP) (n=48), pancreatic cancer (n=66), and areas next to cancer (n=6) bulk tissues using QRT-PCR, as described in the Materials and Methods section. Horizontal lines represent the median expression level.

Table 1. Demographic data and characteristics of patients included into the quantitative real-time PCR (QRT-PCR) and immunohistochemical (IHC) analysis

	QRT-PCR analysis	IHC analysis
patients	66	55
median age (range)	65 (39-83) years	65 (41-81) years
female	34 (52%)	25 (45%)
male	32 (48%)	30 (55%)
TNM		
T1	0 (0%)	0 (0%)
T2	4 (6%)	2 (4%)
T3	62 (94%)	52 (94%)
T4	0 (0%)	1 (2%)
N0	13 (20%)	11 (20%)
N1	53 (80%)	44 (80%)
M0	65 (98%)	51 (93%)
M1	1 (2%)	4 (7%)
grading		
G1	4 (6%)	6 (11%)
G2	34 (52%)	26 (47%)
G3	28 (42%)	23 (42%)
survival status		
dead		38 (69%)
alive/lost on follow-up		17 (31%)

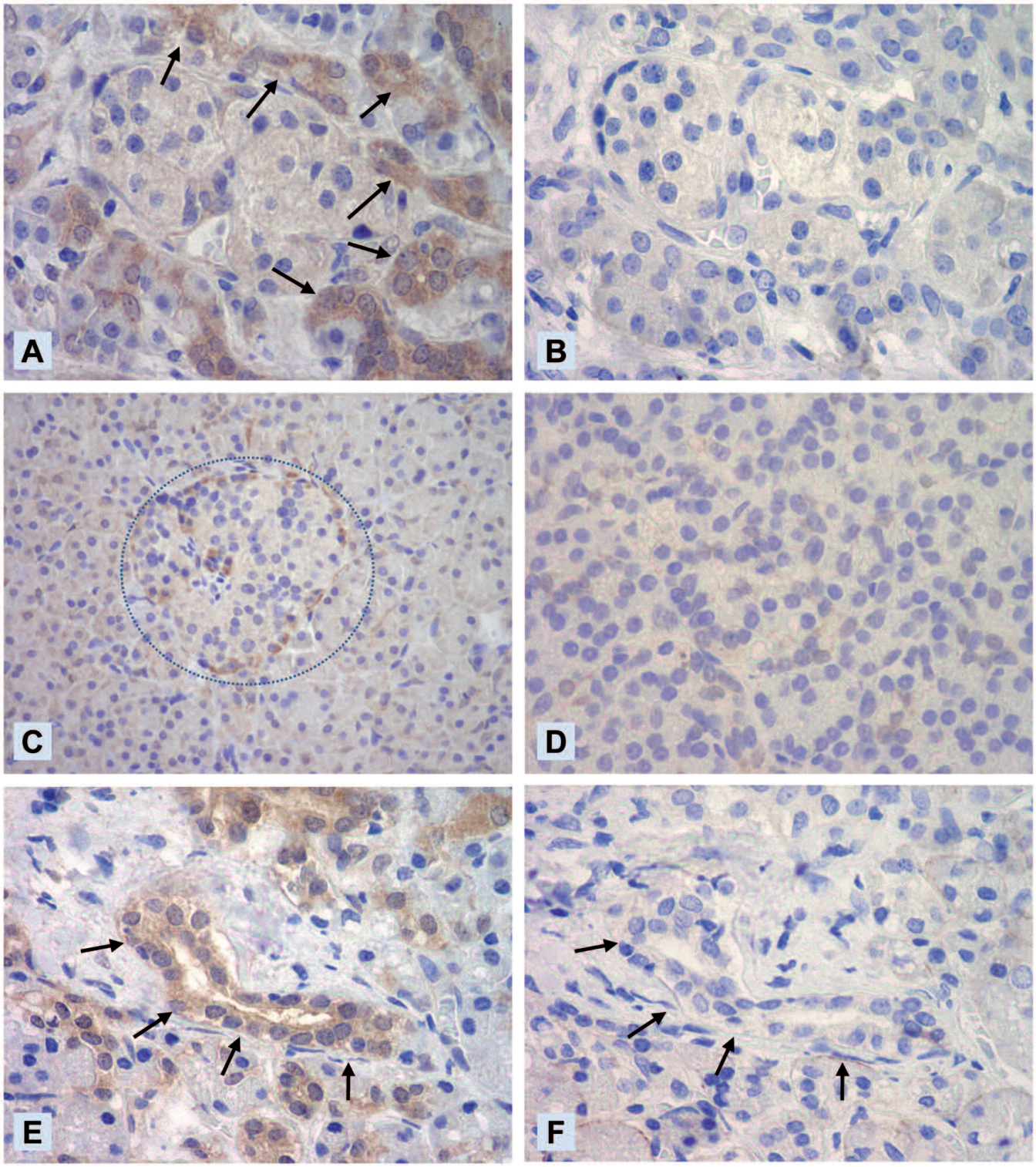

Fig. 2. Immunohistochemistry in normal pancreatic tissues using a specific SPTBN1 antibody as described in the Materials and Methods section. Arrows in **A** depict stained acinar cells surrounding an islet. **B.** Negative control of **A. C, D.** Normal pancreatic tissues with mostly SPTBN1 negative acinar cells (circle in **C** depicts an area with scattered SPTBN1 positive acinar/centroacinar cells). **D** demonstrates an area with SPTBN1 negative acinar cells. **E.** Arrows depict SPTBN1 positive ductal cells. **F.** Negative control of **E**. A, B, D-F, x 400; C, x 200

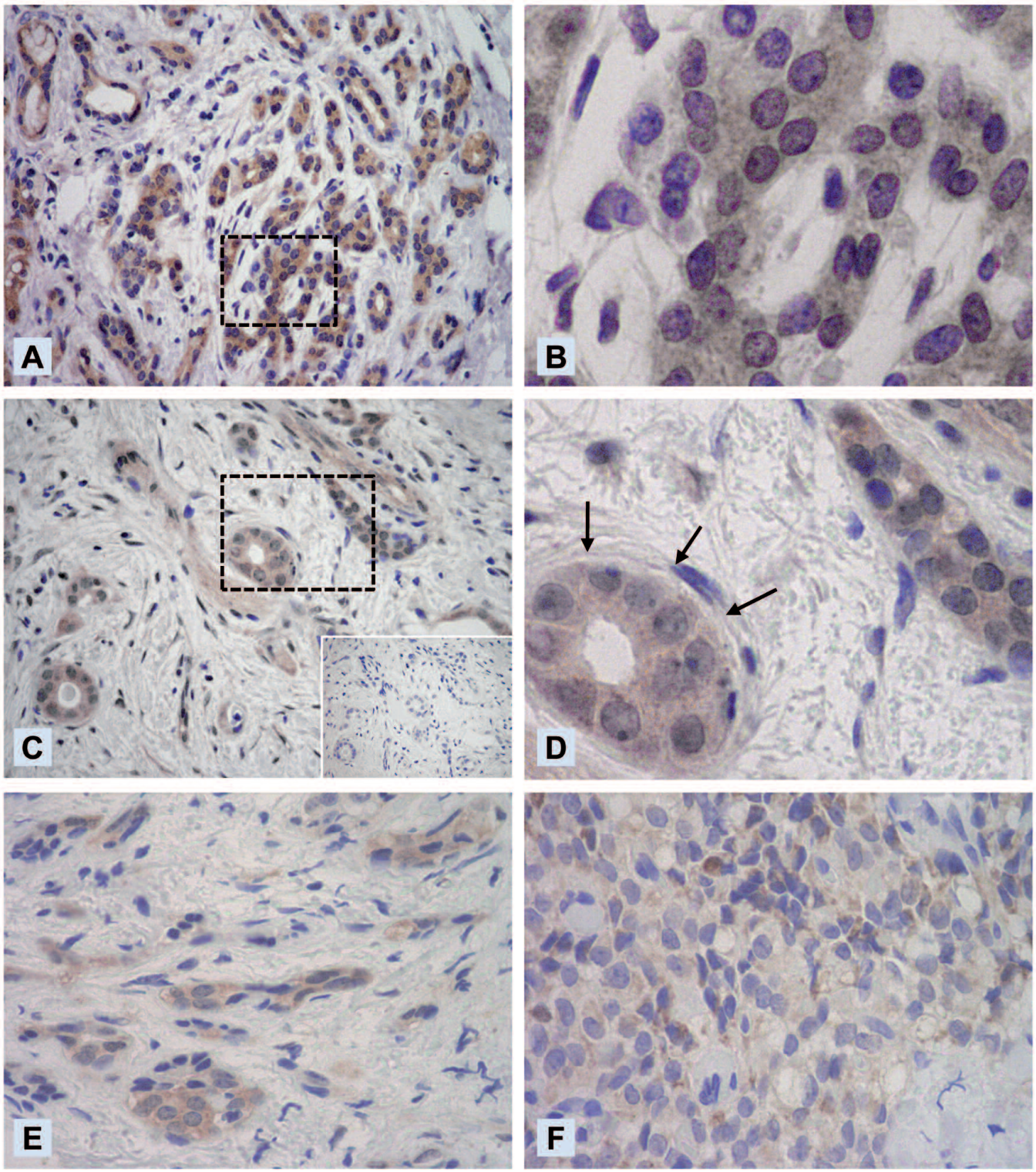

Fig. 3. Immunohistochemistry in pancreatic tissues using a specific SPTBN1 antibody as described in the Materials and Methods section. **A, B.** Chronic-pancreatitis like changes with tubular complexes next to pancreatic cancer. **B.** Higher magnification of **A. C, D.** Pancreatic cancer tissues (ductal adenocarcinoma, grade 2). Inset in **C.** Negative control. **D.** Higher magnification of **C**. Arrows depict SPTBN1 positive cancer cells. **E.** Pancreatic cancer tissues (ductal adenocarcinoma, grade 2). **F.** Poorly differentiated pancreatic cancer tissues (ductal adenocarcinoma, grade 3). A, C, E, F, x 400; B, D, x 1,000

demonstrated a significant association of SPTBN1 staining and survival (p=0.019) and a tendency for a correlation of SPTBN1 staining and grading (p=0.11) (Table 3).

Expression analysis in eight cultured pancreatic cancer cell lines, as well as in pancreatic stellate cells, demonstrated variable expression of SPTBN1 mRNA in all cell lines ranging from 380-1569 copies/10,000 PPIB copies (fig. 5). Immunoblot analysis generally confirmed the mRNA expression data showing variable levels of two immunoreactive, closely spaced bands with a size of approximately 260-270 kDa in all cell lines (some cell lines only displayed one of the two bands). In addition, an immunoreactive band of approximately 230 kDa was present in 4 of the 8 pancreatic cancer cell lines (Fig. 5).

Table 2. Univariate and multivariable analysis of clinical and histomorphological factors associated with survival.

	univariate analysis p value	multivariable analysis p value	odds ratio	95% confidence interval
T3/4 vs. T1/T2	0.072	0.19	13.9	0.79-293
N1 vs. N0	0.94	0.63	1.03	0.45-2.36
M1 vs. M0	0.36	0.98	1.63	0.57-4.7
G3 vs. G1/2	0.083	0.92	3.05	0.86-10.8
SPTBN1 moderate/strong vs. low staining score	0.066	0.11	0.49	0.23-1.05
male vs. female	0.84	0.91	0.97	0.7-1.34

Table 3. Univariate analysis of histomorphological factors associated with SPTBN1 expression.

	univariate analysis p value
T3/4 vs. T1/T2	0.49
N1 vs. N0	0.97
M1 vs. M0	0.22
G3 vs. G1/2	0.11
survival	0.019

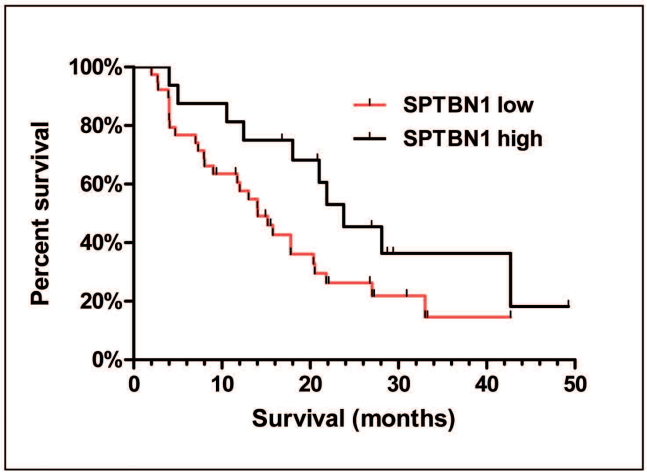

Fig. 4. Survival analysis in 55 pancreatic cancer patients with high (black line) and low (red line) SPTBN1 immunohistochemistry score using the Kaplan-Meier analysis.

Discussion

Pancreatic cancer is associated with an extremely poor prognosis with an overall median survival time of 5-8 months and a 5 year survival rate of less than 5% when all stages are combined (Jemal et al., 2009). The only chance for cure or prolonged survival is macroscopic complete resection (Loos et al., 2008);

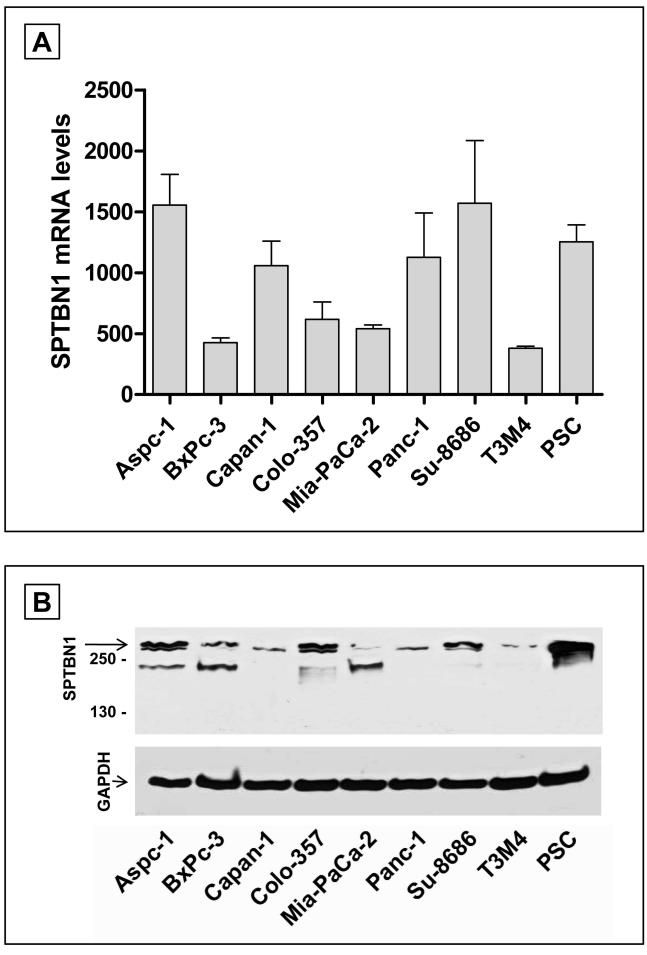

Fig. 5. Expression of SPTBN1 mRNA **(A)** and protein **(B)** in cultured pancreatic cancer cell lines and stellate cells as described in the Materials and Methods section (upper panel). Data are presented as mean (±SD) from three independent experiments **(A)**. GAPDH immunoblotting (**B** lower panel), was used as a loading and transfer control. Size markers in kDa are indicated on the left.

however, for the vast majority of patients only non-curative strategies can be offered, since around 85% of these patients have locally advanced or metastatic tumors at the time of diagnosis. Palliative treatments such as chemotherapy and/or radiotherapy significantly improve the prognosis only in a few pancreatic cancer patients, as most of the cancers do not respond to cytotoxic agents and radiation. A better understanding of the pathophysiological and molecular alterations may thus lead to more effective treatment options.

In the present study, we analyzed the expression and localization of SPTNB1, a scaffolding protein that is important for protein sorting, cell adhesion and the development of differentiated epithelial cells. In addition, SPTNB1 plays a role in TGF-ß signaling via Smad molecules (Tang et al., 2003). We now show that in bulk tissues, SPTBN1 mRNA levels were significantly over-expressed in pancreatic cancer and in the tissue around the tumor mass compared to chronic pancreatitis and the normal pancreas. SPTNB1 was strongly present in ducts and tubular complexes in chronic pancreatitis and in chronic pancreatitis-like areas of pancreatic cancer. Thus, SPTBN1 was present in the early tissue components that have been suggested to belong to the spectrum of pancreatic cancer progression (Esposito et al., 2007). SPTNB1 was also present in the cytoplasm of pancreatic cancer cells but only weakly in cancer cells of poorly differentiated tumors, suggesting reduced expression in later stages and differentiation, which is in part supported by the univariate regression analysis demonstrating reduced SPTNB1 expression in G3 versus G1/2 grade tumors, although this correlation did not reach statistical significance. Nonetheless, this is also in line with the observation that reduced SPTBN1 protein expression correlated with a worsened prognosis.

The association of SPTBN1 and TGF-ß signaling is intriguing. TGF-ßs influence numerous cellular procedures, like cell growth, differentiation and apoptosis, angiogenesis, extracellular matrix deposition and local immune function. TGF-ß signaling is mediated by cell surface receptor activation with subsequent phosphorylation of Smad proteins and transfer to the nucleus, where activated Smad complexes interact with cell- and tissue specific transcriptional regulators, to modulate the expression of a number of target genes (Derynck and Zhang, 2003; Siegel and Massague, 2003). SPTBN1 seems to interfere with TGF-ß signaling by interacting with Smad3/Smad4 and the TGF-ß receptor complex in the cytoplasm and assisting in transporting the Smad complex to the nucleus. Thus, deficiency of ELF, the mouse homologue of SPTBN1, results in mislocalization of Smad3 and Smad4, and loss of the TGF-ß-dependent transcriptional response (Tang et al., 2003).

Disturbances in the signaling cascade of TGF-ßs bear a wide variety of cellular consequences and have been described as causative in the development and progression of various malignancies of e.g. the pancreas, colon, breast, melanoma, prostate, stomach, neuroendocrine, gynecologic, skin, and nervous system (Massague et al., 2000).

In pancreatic cancer, TGF-ßs are over-expressed and correlate with worsened prognosis (Friess et al., 1993). During disease progression, pancreatic cancer cells acquire a number of genetic and epigenetic alterations that render them resistant to the growth (and tumor) suppressive effects of TGF-ßs, such as Smad4 mutations, mutations or reduced expression of TGF-ß receptors, increased expression of inhibitory Smad molecules and others (Hahn et al., 1996; Kleeff et al., 1999a,b; Jones et al., 2008). It has therefore been hypothesized that TGF-ßs act as tumor suppressors in early stage pancreatic cancer and as tumor promoters in advanced stages. TGF-ß exerts tumor promoting effects by acting strongly immunosuppressive, by inducing angiogenesis and epithelial to mesenchymal transition (EMT) that promote a more migratory and invasive phenotype.

The link between TGF-ß and SPTBN1 and the the exact molecular mechanisms of the interaction of these proteins in pancreatic cancer is currently not known but it might be speculated that reduced SPTBN1 expression in advanced tumors contributes to the resistance of the tumor suppressive effects of the overexpressed TGF-ßs. The link between TGF-ß signalling and SPTBN1 has been analyzed in other gastrointestinal tumors using different mouse models, demonstrating an important role of ELF in the carcinogenesis of these tumors (Grady and Markowitz, 2002; Katuri et al., 2005; Mishra et al., 2005; Tang et al., 2005, 2008; Kim et al., 2006; Kitisin et al., 2007; Xu and Pasche, 2007; Baek et al., 2008).

In addition to its effects on TGF-ß signalling, ß-spectrins are important for microtubule bundling, and as dynamic scaffolding proteins and essential components of the membrane associated cytoskeletal network, ß-spectrins like SPTBN1 influence other important functions of the cytoskeleton, and might therefore play a role in tumor cell motility, adhesion, migration, and invasion (Bennett and Baines, 2001; Chakrabarti et al., 2006; Baines, 2009).

In conclusion, SPTBN1 is expressed by pancreatic cancer cells and over-expressed in bulk pancreatic cancer tissues. Reduced expression correlates with worsened prognosis of pancreatic cancer patients, suggesting a tumor suppressor function of this gene, as has been shown for other malignancies of the gastrointestinal tract. Since the exact molecular mechanisms of the effects of SPTBN1 in pancreatic cancer cells still remain unclear, further analysis of the functional consequences of reduced SPTBN1 expression has to be carried out in pancreatic cancer cells and in appropriate animal models.

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