Pathology - Research and Practice 215 (2019) 152685

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

SPARC expression in desmoplastic and non desmoplastic pancreatic carcinoma and cholangiocarcinoma

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ARTICLE INFO

Keywords:
SPARC
Stromal desmoplasia
Pancreatic adenocarcinoma
Cholangiocarcinoma
Pancreatobiliary carcinoma

ABSTRACT

Background: The pancreatobiliary carcinomas are characterized by presence of desmoplastic stroma. Overexpression of secreted protein acid and rich in cysteine (SPARC), a matrix producing agent has been documented in pancreatic ductal adenocarcinomas, with survival benefits. This study was targeted to see if SPARC expression in pancreatobiliary carcinomas is responsible for stromal desmoplasia and its prognostic significance.

Methods: In this retrospective study 48 cases of pancreatic cancer and 27 cases of cholangiocarcinoma were analyzed. The expression pattern of SPARC and vascular endothelial growth factor (VEGF) (angiogenic factors) was evaluated by immunohistochemistry on formalin fixed paraffin embedded tissues. Immunoreactivity was scored semi quantitatively based on stain intensity and stain distribution. SPARC expression was correlated with tumor histology, stromal desmoplasia, VEGF expression, various histological parameters and overall survival in patients. Real time polymerase chain reaction was performed in few cases to validate the immunohistochemistry expression pattern.

Results: SPARC expression was high in peritumoral stroma in pancreatic carcinoma than in pancreatic controls; however, SPARC expression pattern was not grossly different in desmoplastic and non-desmoplastic pancreatobiliary carcinomas and in cholangiocarcinomas. No definite correlation was noted between SPARC expression and histological markers of severity and overall survival data.

Conclusions: The relevance of SPARC expression in pancreato-biliary carcinomas though may still be important for therapeutic decision making, it is not responsible for peritumoral stromal desmoplasia in these tumors and it does not have any significant prognostic implication.

1. Introduction

Secreted protein acidic and rich in cysteine (SPARC), also known as Osteonectin, or basement membrane-40 protein, is a member of the matricellular family of proteins, which interacts with the structural proteins, growth factors, and cell surface receptors of the extracellular matrix (ECM). This interaction results in a broad spectrum of biological effects, including tissue remodeling, wound repair, morphogenesis, cellular differentiation, cell proliferation, cell migration, and angiogenesis [1–6]. SPARC expression has been studied in various tumors, and its' role as a prognostic tumor marker appears to be organ specific. In colorectal, ovarian and prostatic carcinomas while SPARC acts as a tumor suppressor protein via suppression of the vascular endothelial growth factor (VEGF) mediated angiogenesis; in pancreatic ductal adenocarcinoma (PDCA), breast carcinomas, melanomas, and glioblastomas, SPARC protein promotes the epithelial to mesenchymal (EMT) transition and increased matrix metalloprotease secretion facilitating invasion and metastasis [4,7,8].

PDCA is the most common and aggressive pancreatic neoplasm. The histological hallmark of PDCA is stromal desmoplasia [9]. The stromal proliferation in pancreatic neoplasm starts right from the beginning of the development of the PanIN lesions [10]. This proliferating peritumoral stroma is composed of pancreatic stellate cells, inflammatory cells, ECM proteins, growth factors, cytokines and is believed to

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https://doi.org/10.1016/j.prp.2019.152685
Received 18 July 2019; Received in revised form 24 September 2019; Accepted 6 October 2019
0344-0338/

promote the aggressive nature of PDCA, by facilitating tumor growth, metastasis, and resistance to chemo-radiotherapy [11]. This may be the reason why it is difficult to treat and prevent dismal progression in PDCA. The SPARC protein is believed to interact with the components of ECM and facilitate angiogenesis. From the available publications, it was understood that SPARC expression is frequently lost in PDCA cells through aberrant hypermethylation of its CpG island, but is often overexpressed in stromal fibroblasts immediately adjacent to the cancer cells, suggesting its role in the tumor-stroma interactions [12,13]. Its expression in the peritumoral stroma was found to be a marker of poor prognosis in studies performed with immunohistochemical stains (IHC) [14–18]. Some studies also suggested better survival after nab-paclitaxel and gemcitabine therapy in SPARC-high tumors, suggesting its role as a potential biomarker [14,17,19,20].

We hypothesized that SPARC protein possibly stabilizes the ECM in pancreatobiliary tumors resulting in the characteristic peritumor desmoplasia. The basis of this belief was a study by Mantoni TS et al. who suggested that tumor cells and peritumoral stellate cell secrete transforming growth factor type 1 (TGF1) resulting in desmoplasia in PDCA [16]. The characteristics stromal desmoplasia in intrahepatic cholangiocarcinoma and perihilar cholangiocarcinomas may result in a high rate of perineural and vascular tumor invasion, metastasis, and resistance to therapy [21–25]. Taking a cue from the PDCA, we also hypothesized that the SPARC mediated ECM interaction may also be responsible for peritumoral stromal desmoplasia and aggressive behavior in biliary tract carcinomas [26,27]. Hence, the present study aimed to assess the expression patterns of SPARC protein in desmoplastic and non-desmoplastic PDCA, periampullary carcinoma, and cholangiocarcinomas, as well as the prognostic implication of SPARC expression in these tumors.

2. Methods

The study was approved by the Institutional Ethics Committee. It was a retrospective cross-sectional study. Formalin-fixed, paraffin-embedded (FFPE) archival tissue blocks were chosen from patients who underwent surgical resection for pancreatic carcinomas, including PDCA and periampullary carcinomas restricted to the head of the pancreas (n 48), and cholangiocarcinomas (n 27). On histological evaluation, in total, 23 cases of PDCA and 18 cases of cholangiocarcinoma showed desmoplastic reaction (in total = 41). Rest of the 25 cases of PDCA/ periampullary carcinomas and nine cholangiocarcinomas did not show significant peritumoral desmoplasia (in total = 34). As controls, 20 FFPE blocks of histologically confirmed normal pancreatic tissues collected from the trauma center, where partial pancreatectomy was performed in road traffic accident victims. Also, FFPE blocks from the resected common bile duct (CBD) margin from 18 Whipple's pancreaticoduodenectomy specimens were included (in total 38 controls). Desmoplasia, which is a result of the proliferation of alpha-smooth muscle actin-positive fibroblasts (also known as the myofibroblasts, or activated stellate cells) and increased deposition of ECM components, leads to reduced elasticity of tumor tissue with a concomitant increase in tumor interstitial fluid pressure (IFP). Increased IFP results in a decreased rate of perfusion of therapeutic agents and decreased efficacy [28]. We defined desmoplasia as relatively hypocellular dense extratumoral stroma, which appeared hyalinized or sclerotic on histological examination. The usual moderately cellular, fibroblast and myofibroblast rich non-sclerotic stroma was not considered. Sections taken from tumor and peritumoral stromal interface were examined by two pathologists under microscope and FFPE block having at least 50% representation of the tumor and desmoplastic stroma each were chosen for further work up. The topographic and histological detail of cases included in this study was analyzed. Overall survival data was retrieved from available cases and was defined as the interval between the dates of diagnosis to the date of last follow up.

2.1. Immunohistochemical staining

Sections cut from FFPE blocks were dewaxed and rehydrated. Endogenous peroxidase activity was blocked using 4% hydrogen peroxidase followed by antigen retrieval by treating with Tris-EDTA (pH 9) / citrate (pH 6). Primary monoclonal antibodies for SPARC (ab14174, 1:400, pH 9, AbCam, Cambridge, UK) and VEGF (ready to use, pH6, Spring Biosciences) were used to incubate the sections at 4°C, in a humid chamber overnight. Universal polymer based secondary antibody (Skytek Laboratories, USA) was used, and the reaction product was developed with 3, 3″-diaminobenzidine and counterstained with hematoxylin. FFPE section taken from invasive front of a colorectal carcinoma with desmoplastic stromal reaction was used as the positive control, and section from the same control without use of primary antibody was used as the negative control for SPARC staining. For VEGF staining, sections from adult nephrectomy specimen were used as the positive control for VEGF staining where the renal tubules showed strong positivity. Secondary antibody only section was used as the negative control.

Interpretation for SPARC and VEGF stains were made on the tumor and peritumoral stroma separately by two pathologists blinded about any detail. H scores were generated in these different anatomical regions (stain intensity x stain distribution, i.e., positive percentage cells). Six distribution grades were employed: 0 = no staining (< 10% cells positivity); 1+ = 10–20% of tumor cell positivity; 2+ = 21–40% of tumor cell positivity; 3+ = 41–60% of tumor cell positivity; 4+ = 61–80% of tumor cell positivity; 5+ > 80% of tumor cell positivity. Intensity was graded as: 1+ = mild; 2+ = moderate; 3+ = marked. A final H score of < 5 was taken as low expression whereas & > 5 was considered as high expression. Any discrepancy between the two observers was discussed and a consensus was reached on a multiheader microscope. This categorization was done for the purpose of statistical analysis, and the cutoff was decided arbitrarily. H scores of different patient group and controls were compared.

2.2. Image analysis

Sections cut from the chosen blocks were stained with Sirius red stain and the section was digitalized. The digitalized image was then annotated and segmented using the Image Proplus 6® software. Post-segmentation, areas of tumor-desmoplasia were calculated (Fig. 1). For categorization, a total fibrosis score of > 30% (i.e., > 1/3rd of the tumor area) was considered desmoplastic, and tumors with a fibrosis score of < 30% were considered non-desmoplastic. The fibrosis scores were correlated with 'H' scores of IHC stains performed and SPARC mRNA levels in the peritumoral stroma.

2.3. Real-time polymerase chain reaction (PCR) study

Real-time PCR was performed only to assess SPARC mRNA expression to validate the IHC findings. In the total of 40 FFPE samples were selected randomly for RNA extraction. RNA was extracted by using Zymoresearch quick RNA FFPE kit (CA, USA) and the isolated RNA was quantified both qualitatively and quantitatively via spectrophotometric based absorbance studies using NanoDrop™ 8000 Spectrophotometer (Thermo Scientific) and gel electrophoresis. The total RNA (3 mg) was reverse transcribed using the RT2 first strand kit (Qiagen, Hilden, Germany). For the qPCR analyses Sso Fast Evagreen (Syber green) from Bio Rad was used on an ARIAMX real-time PCR machine from Agilent Technologies, USA, under the following conditions: 98 °C for 2 min; then 40 cycles of 98 °C for 10 s, 52 °C (annealing temperature) for 10 s, and 60 °C for 20 s; followed by a melt curve analysis. Primer sequences for *SPARC* mRNA used were: 5′ AAG ATC CAT GAG AAT GAG AAG-3′ (forward) and 5′-AAA AGC GGG TGG TGC AAT G-3′ (reverse).19 To normalize and to check the integrity of mRNA, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene was amplified

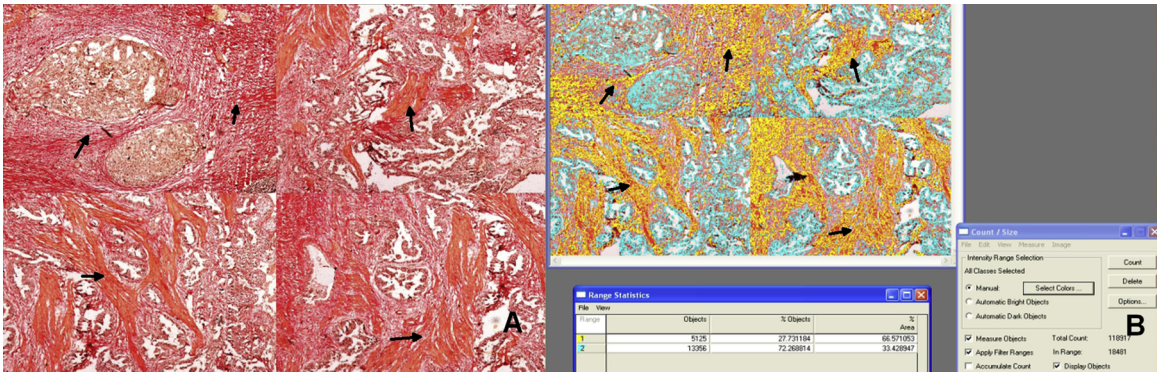

Fig. 1. Shows methods of highlighting the desmoplastic stroma with Sirius red stain (arrows), method of segmentation of digitalized biopsies by using the Image Pro Plus software (arrows showing segmented collagen) and method of estimation of stromal fibrosis/ collagenization.

in the same PCR conditions. The primer sequence for *GAPDH* was 5′-ACCCACTCCTCCACCTTTGAC-3′ (forward), 5′-TGTTGCTGTAGCCA AATTCGTT-3′ (reverse). The qPCR was run in triplicates, and relative gene expression was calculated according to the comparative cycle threshold (Ct) method using GAPDH as an endogenous control.

2.4. Statistical analysis

Data were analyzed using statistical software STATA-14.0. Qualitative/categorical data were expressed as frequency and percentage, and the quantitative data expressed as mean ± standard deviation and median (minimum-maximum). Fischer exact test was used to analyze the association between the categorical variables. Mann Whitney test (Ranksum) was used to compare the continuous variables with skewed distribution. Kaplan Meier (survival analysis) was used to find the overall survival with associated factor. $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Patient characteristics

The median age of patients with pancreatic (PDCA/ periampullary) carcinomas was 55.5 ± 10.7 years and 50.8 ± 12.7 years for cholangiocarcinoma. Out of the 48 pancreatic carcinoma patients, 40 were male patients, and 8 were female patients, and out of the 27 cholangiocarcinoma patients, 19 were males, and 8 were females.

3.2. SPARC protein expression

SPARC stain was expressed in the cell cytoplasm and was assessed in both tumor cells (and epithelial cells in controls) and peritumoral stroma (or, peri-epithelial stroma in controls). SPARC H scores while was low in the tumor cells and benign pancreatic acinar and ductal cells of controls, in peritumoral stroma SPARC H score was high (Table 1). Differential SPARC expression between the tumor cells and control epithelium was not statistically significant (p = 1). However, SPARC expression in the peritumoral stroma was significantly high in comparison to the normal periductal stroma in controls (p= < 0.001). Forty-one out of 48 pancreatic carcinoma cases (85.1%) showed high SPARC expression in the stroma, whereas none of the controls showed high expression of SPARC protein (Table 1; Fig. 2).

In control CBD epithelium while SPARC expression was high, in cholangiocarcinomas, SPARC expression was low (p= < 0.001). Both in the peritumoral stroma in cholangiocarcinoma (19 out of 27) and periductal stroma in CBD controls (13 out of 17) showed high SPARC H scores, and the difference was not statistically significant (Table 1). On histological examination, the control CBD samples collected from Whipple's' pancreaticoduodenectomy specimen also showed both periductal fibrosis and inflammation, and none of them had normal histological findings.

3.3. SPARC stain expression in desmoplastic and non-desmoplastic tumors

SPARC H scores were high in peritumoral stroma of both desmoplastic as well as the non-desmoplastic pancreatic tumors; while, in cholangiocarcinomas, the desmoplastic tumors were mostly SPARC-high in comparison to the non-desmoplastic cholangiocarcinomas, however, this difference was not statistically significant (p = 0.4) (Table 2).

3.4. Correlation of SPARC expression with histological features of tumor aggressiveness

There was no significant correlation of SPARC expression, either in tumor epithelium or stroma with histological features of tumor aggressiveness, such as tumor differentiation, tumor stage, and lymph node metastasis in both pancreatic and cholangiocarcinomas, except the tumor size, which was borderline significant. Stromal SPARC-high pancreatic carcinomas were smaller in size than the SPARC-low tumors.

Table 1
Expression of SPARC immunohistochemical stain in pancreatic and cholangiocarcinoma.

	SPARC in Pancreatic tumor and Normal pancreatic tissue						SPARC In Cholangiocarcinoma and Normal CBD					
	Tumor epithelium (48) [n (%)]	Normal Pancreatic Epithelium (20) [n (%)]	*p* value	Tumor Stroma (48) [n (%)]	Normal Pancreatic Stroma (20) [n (%)]	*p* value	Tumor (27) n (%)]	Normal CBD Epithelium (17) [n (%)]	*p* value	Tumor Stroma (27) [n (%)]	Normal CBD Stroma (17) [n (%)]	*p* value
SPARC low	46 (95.83)	19 (95.00)	1	7 (14.58)	20 (100.00)	< 0.001	27 (100.00)	5 (29.41)	< 0.001	8 (29.63)	4 (23.53)	0.73
SPARC high	2 (4.17)	1 (5.00)		41 (85.42)	0.0		0.00	12 (70.59)		19 (70.37)	13 (76.47)	

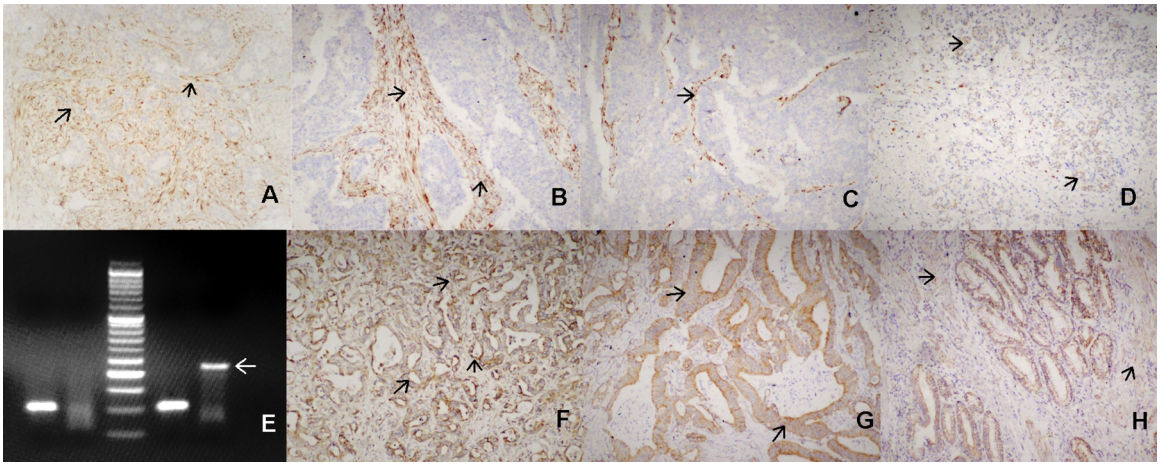

Fig. 2. Figures show high SPARC expression in desmoplastic stroma of a pancreatobiliary carcinoma (arrows, A x100; B x200), in comparison to the low SPARC expression in peritumoral stroma in another pancreatobiliary carcinoma (arrows, C x 200). Focal epithelial and stromal SPARC stain expression was noted in control pancreas (arrows, D x 100). An image of gel electrophoresis shows SPARC mRNA expression band in one of the samples (arrow). VEGF stain shows diffuse positivity in pancreatobiliary carcinoma with variable stromal expression (arrows, F x100; G x200). VEGF stain expression in CBD control shows expression in epithelium and patchy stromal positivity [arrows] (H x 100).

However, a similar correlation was not identified with SPARC expression in tumors and cholangiocarcinomas (Table 3).

3.5. Correlation of % area of fibrosis along with the expression of SPARC and VEGF

The stromal expression of SPARC and VEGF stains was high in both desmoplastic and non-desmoplastic tumors, and the difference was not statistically significant. SPARC expression and VEGF expression patterns in pancreatic and cholangiocarcinomas were almost identical (Table 4) (Fig. 2).

3.6. Validation of immunohistochemical SPARC expression with qPCR data

The qPCR cycle threshold (Ct) values were interpretable in 23 cases out of the 40 cases subjected to RNA quality extracted from FFPE blocks. SPARC and GAPDH amplicon sizes were 217 bp and 100bps, respectively. A correlation was noted between the SPARC immunohistochemistry scores and SPARC mRNA ΔCt values both in pancreatic and cholangiocarcinomas (Fig. 3).

3.7. Impact of SPARC IHC expression in the overall survival

Kaplan Meir survival analysis was performed to find the overall survival proportion of SPARC- high and SPARC- low pancreatic ($p = 0.43$) and cholangiocarcinomas ($p = 0.19$), and its comparison by the Log Rank test. No significant correlation was, however noted in either of the tumors (Fig. 3).

4. Discussion

In this study, we hypothesized that SPARC protein might be responsible for the formation of peritumoral desmoplastic stroma in pancreatobiliary carcinomas and examined if SPARC protein expression has any prognostic significance, as was reported earlier. We found that SPARC expression in the peritumoral stroma in pancreatic carcinomas though was significantly higher than in controls; in cholangiocarcinomas, stromal SPARC expression was not significantly different from the control used (Table 1). When SPARC expression was compared between the desmoplastic and non-desmoplastic pancreatic and cholangiocarcinomas, no significant difference of expression was noted (Table 2). No significant correlation between SPARC expression and histological parameters of tumor aggressiveness and overall survival was noted. Immunohistochemical SPARC expression and SPARC mRNA Ct values were correlated. No correlation was found between SPARC expression and VEGF immunohistochemical expression; a proposed mechanistic relate reported earlier. Our study shows that SPARC protein may not have a particular role in peritumoral stromal desmoplasia in pancreatobiliary carcinomas, or its role may be tumor specific. Favorable prognostic implication of SPARC was not found in this study.

The peritumoral expression of SPARC protein in desmoplastic and non-desmoplastic pancreatic and cholangiocarcinomas is in concordance with the previous studies done by Infante et al., Mantoni et al. and Sato et al., though, none of them had looked for the differential expression of SPARC protein in desmoplastic and non-desmoplastic tumors [12,15,16]. We also noted low SPARC expression in pancreatic acinar cells and in periductal stroma in controls included in this study, which was in contrast to the findings of the Gundewar et al., who did

Table 2
Differential expression pattern of SPARC immunohistochemical stain between desmoplastic and non-desmoplastic pancreatic carcinoma and cholangiocarcinoma.

Parameter	Tumor		Tumor			Stroma		Stroma		
	Desmoplastic		Non Desmoplastic			Desmoplastic		Non Desmoplastic		
	Low SPARC [n (%)]	High SPARC [n (%)]	Low SPARC [n (%)]	High SPARC [n (%)]	*p* value	Low SPARC [n (%)]	High SPARC [n (%)]	Low SPARC [n (%)]	High SPARC [n (%)]	*p* value
Pancreatic cancer	22 (95.65)	1 (4.35)	24 (96.00)	1 (4.00)	1	2 (8.70)	21 (91.30)	5 (20.00)	20 (80.00)	0.4
Cholangiocarcinoma	18 (100.00)	0 (0.00)	9 (100.00)	0 (0.00)	N/A	4 (22.22)	14 (77.78)	4 (44.44)	5 (55.56)	0.4

N/A- Not available/ could not be calculated.

Table 3
Correlation of SPARC expression with histological features of tumor aggressiveness.

Parameters			Tumor Differentiation				Largest dimension of tumor		Tumor stage			Tumor stage			Lymph node		
			Well diff [n(%)]	*Mod Diff* [n(%)]	*Poorly Diff* [n(%)]	*p value*	*Mean(± S.D)* Median(min.-max)	*p value*	*Stage 1 &2* [n(%)]	*Stage 3&4* [n(%)]	*p value*	*Stage 1* [n(%)]	*Stage 2,3 & 4* [n(%)]	*p value*	*Negative* [n(%)]	*Positive* [n(%)]	*p value*
Pancreatic carcinoma	Tumor	Low SPARC	10	32	1	0.1	2.95 (± 1.5)	0.7	15	28	0.5	2	41	1.0	20	23	1.0
			(23.5)	(74.4)	(2.3)		2.5 (1-6.5)		(34.9)	(65.1)		(4.6)	(95.4)		(46.5)	(53.4)	
		High SPARC	2	0	0		2.35 (± 1.2)		0	2		0	2		1	1	
							3 (1.5-3.2)			(100)			(100)		(50)	(50)	
	Stroma	Low SPARC	1	5	1	0.2	3.87 (± 1.72)	0.05	2	5	1.0	1	6	0.3	5	2	0.2
			(14.3)	(71.5)	(14.3)		3 (1-6)		(28.6)	(71.4)		(14.2)	(85.7)		(71.4)	(28.6)	
		High SPARC	11	27	0		2.75 (± 1.41)		13	25		1	37		16	22	
			(28.9)	(71.0)			2.45 (1-6)		(34.2)	(65.7)		(2.6)	(97.3)		(42.1)	(57.87)	
Cholangio carcinoma	Tumor	Low SPARC	3	20	4		3.05 (± 2.12)	0.5	15	12		4	23		15	12	
			(11.1)	(74.0)	(14.8)		2.5 (0.6-12.3)		(55.6)	(44.4)		(14.8)	(85.9)		(55.6)	(44.4)	
		High SPARC	0	0	0		0		0	0		0	0		0	0	
	Stroma	Low SPARC	0	6	2	0.5	3.83 (± 3.6)	0.4	6	2	0.2	2	6	0.5	6	2	0.2
				(75)	(25)		3.2 (0.6-12.3)		(75)	(25)		(25)	(75)		(75)	(25)	
		High SPARC	3	14	2		2.7 (± 1.03)		9	10		2	17		9	10	
			(15.7)	(73.6)	(10.5)		2.5 (1.5-5)		(47.4)	(52.6)		(10.5)	(89.5)		(47.4)	(52.6)	

not identify SPARC expression in normal pancreatic ducts, acini, centroacinar cells, islets, or surrounding non-epithelial components [17]. We could not arrange histologically normal biliary controls. The tissue included as biliary controls was the resected margin of the CBD from Whipple's pancreatoduodenectomy specimens performed for pancreatic head lesions. In all such cases, due to distal obstruction, the CBD had inflammation and periductal fibrosis. Both the epithelium and periductal CBD stroma in these controls showed relatively high SPARC expression, which was one of the limitations of this study.

As discussed previously, we did not find any correlation between expression of SPARC protein and histological markers of tumor aggressiveness, as tumor differentiation, tumor grade and lymph node involvement in both pancreatic and cholangiocarcinomas (Table 3). It was in concordance with the finding of Ormanns et al. who examined the association of clinicopathological variables as sex, age group, disease stage, tissue origin, tumor grading and the number of metastatic sites within the stromal as well as the cytoplasmic SPARC expression subgroup and detected no statistically significant interaction [20]. However, we found that SPARC-high pancreatic tumors were smaller in size. Though, from this observation we cannot draw any conclusion, as no direct correlation between SPARC expression and overall survival was noted in pancreatic carcinoma patients.

In contrast to our findings, Infante et al., found that poorly differentiated tumors (regardless of SPARC) and well and moderately differentiated tumors with the presence of stromal SPARC expression had a significantly worse prognosis (hazard ratios 2.59, 3.25 and 4.56, respectively; $p < 0.0001$) compared to the SPARC-negative stroma tumors [15]. Hence, our findings are in contrast to those who reported that SPARC could be an independent prognostic marker in pancreatic carcinomas. Miyoshi et al., identified that high SPARC expression is a significant independent poor prognostic factor (hazard ratio (HR) 2.92 (95% confidence interval 1.63–5.50), $p = 0.01$) [29]. Similarly, Infante et al., and Gundewar et al. also concluded that peritumoral SPARC expression was a poor prognostic marker in PDCA. Nakashima et al. concluded that SPARC expression in the peritumoral stroma is a poor prognostic marker for patients with biliary tract carcinoma after surgery [15,17,26]. In a study by Ormanns et al., though high SPARC expression in the peritumoral stroma was not correlated with the progression-free survival in PDCA, high SPARC expression in the tumor epithelium was correlated with inferior progression-free survival and overall survivals (PFS, 6.2 vs 8.6 months, $p = 0.004$ and OS 7.8 vs 8.4 months, $p = 0.032$) [20]. However, the importance of SPARC protein expression possibly lies in its therapeutic implication, as Von Hoff DD et al., had demonstrated that gemcitabine plus nab-paclitaxel significantly increases the overall survival of pancreatic carcinoma to 17.8 months from 8.1 months in peritumoral stromal SPARC-high tumors [19]. However, in this study we did not examined this perspective.

It was previously hypothesized that SPARC possibly acts by suppressing the angiogenesis factors, as VEGF and platelet-derived growth factors [30–32]. The role of SPARC in inhibiting the synthesis and secretion of VEGF has also been reported in gliomas [33]. Chlenski et al. interpreted that SPARC is an inhibitor of angiogenesis in Schwann cells [34]. In a study on colon carcinomas, Liang et al. found that increased VEGF expression correlates with decreased SPARC expression. Similarly, Reduction of SPARC up-regulated the expression of VEGF, causing an increase in micro vessel density and poor clinical outcome [35]. However, we did not identify any definite inverse correlation between peritumoral stromal SPARC expression and VEGF expression, and we did not assess the micro vessel density in tumor stroma (Table 4).

In conclusion, we could not find an exclusive evidence to state the SPARC protein expression is responsible for peritumoral stromal desmoplasia in pancreatobiliary carcinomas. Our results also indicate that SPARC might act as a tumor-specific manner, as high SPARC expression was noted around the peritumoral stroma in pancreatic carcinomas, but, not in cholangiocarcinomas. We also did not find a particular prognostic significance of SPARC expression in these tumors. It seems

Table 4
Correlation of % area of fibrosis along with the stromal expression of SPARC and VEGF.

Parameters	% fibrosis	SPARC in Stroma			VEGF in Stroma		
		Low SPARC n(%)	High SPARC n(%)	*p* value	Low VEGF n(%)	High VEGF n(%)	*p* value
Pancreatic carcinoma	Low < 30% (Non-desmoplastic)	2 (28.6)	13 (33.3)	1	6 (40)	9 (30)	0.5
	High > 30% (Desmoplastic)	5 (71.4)	26 (66.7)		9 (60)	21 (70)	
Cholangio carcinoma	Low < 30% (Non-desmoplastic)	3 (37.5)	4 (21.1)	0.6	1 (14.3)	7 (35)	0.6
	High > 30% (Desmoplastic)	5 (67.5)	15 (78.9)		6 (85.7)	13 (65)	

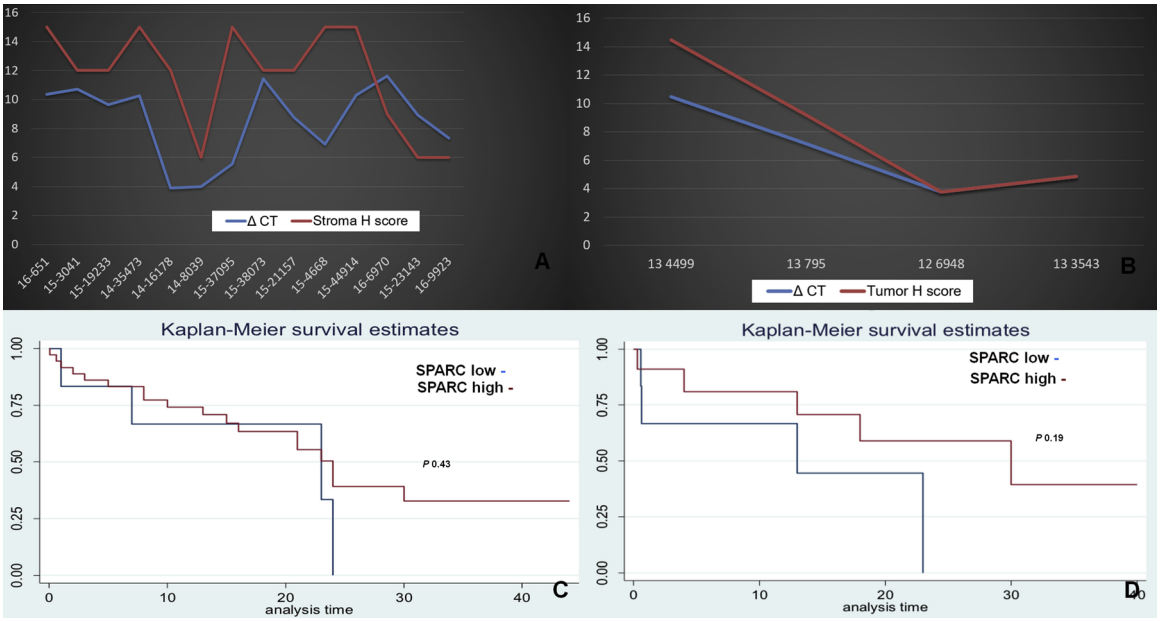

Fig. 3. A & B show correlation of Ct values of SPARC mRNA expression with SPARC IHC stains performed on formalin fixed tissue in pancreatic carcinoma (A) and in cholangiocarcinomas (B). C & D show overall survival analyses in SPARC high and SPARC low pancreatic (C) and cholangiocarcinoma (D).

that SPARC expression should only be explored in pancreatobiliary carcinomas keeping in mind its therapeutic benefits, not as a prognostic marker.

Funding information

We declare no source of funding for the work

Declaration of Competing Interest

None of the author declares any conflict of interest

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