

ORIGINAL ARTICLE

Tenascin C expression is upregulated in pancreatic cancer and correlates with differentiation

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J Clin Pathol 2004;57:1151–1155. doi: 10.1136/jcp.2003.015818

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Accepted for publication 16 March 2004

Background: Tenascin C is a large, hexameric, extracellular matrix protein that is present during embryonic development but essentially absent in adult tissues. It is involved in remodelling processes, such as wound healing and tumour development. Tissue expression of tenascin C correlates with prognosis in colorectal, cervical, and breast cancer and in carcinoma of the papilla of Vater.

Aim: To study the expression of tenascin C in pancreatic cancer and to compare the staining results with the patients' clinicopathological data.

Material and methods: Formalin fixed, paraffin wax embedded specimens from 146 patients with pancreatic adenocarcinoma were stained with an anti-tenascin C monoclonal antibody.

Results: Tenascin C immunoreactivity was seen in most samples of pancreatic carcinoma: staining was weak in 72 (49%), moderate in 52 (36%), strong in 10 (7%), and negative in 12 (8%) samples. Tenascin C expression correlated with age (≤ 66 v > 66 years) and poor differentiation (grades 1–2 v 3). There was no correlation between tenascin C expression and survival, clinical stage, tumour size, nodal status, distant metastasis, tumour location, or sex.

Conclusion: Tenascin C expression was increased in most pancreatic carcinomas, but contrary to the results in other cancers, it is not a prognostic factor in pancreatic cancer.

Tenascin is a large (180–300 kDa), hexameric glycoprotein located mainly in the extracellular matrix (ECM). It is a member of an adhesion modulating family of the ECM. It is expressed transiently during fetal development and is found in restricted locations in normal adult tissue.¹ It is sparsely expressed in the basement membrane of skin,² colon mucosa,³ and ductal salivary glands.⁴ It is seen in the vessel walls of different organs and in visceral smooth muscle.⁵ Tenascin is expressed in healing wounds,⁶ atherosclerosis,⁷ and hyperproliferative skin diseases.²

“Tenascin C concentrations have been associated with tumour recurrence and prognosis, although the findings are contradictory”

In addition to being present in areas around growing or differentiating epithelia, tenascin C is prominent in the stroma of a variety of tumours.⁵ Interactions between tumour cells and the ECM are important for tumour invasion and the development of metastases.^{8–9} The expression of tenascin C in tumour stroma suggests altered cell–matrix interactions, which may facilitate epithelial tumour cell invasion and metastases. Tenascin C concentrations have been associated with tumour recurrence and prognosis, although the findings are contradictory.^{10–15} In laryngeal and hypopharyngeal cancers, the accumulation of tenascin in the blood vessels is an indicator of an unfavourable prognosis; it correlates with metastasis, early tumour recurrence, and a lethal outcome.¹⁴ In breast cancer, the expression of tenascin C in the invasion border of the tumour is a predictor of local and distant recurrence.¹⁰ In colorectal cancer, tenascin has been reported to be a predictor of a worse prognosis in two studies.^{11–16} In contrast, patients with cervical or gastric cancers whose tumours were tenascin positive had a better prognosis.^{12–17} To our knowledge, there are no previous studies on tenascin C and prognosis in pancreatic cancer; therefore, we have studied the prognostic value of tenascin C

expression in a series of patients with pancreatic adenocarcinoma.

MATERIALS AND METHODS

Patients and tissue samples

The series consisted of 216 patients with histologically verified pancreatic adenocarcinoma who underwent surgery at the Helsinki University Central Hospital, Finland, between 1974 and 1998. Paraffin wax embedded specimens obtained from the files of the department of pathology, University of Helsinki, were available in 146 patients. Table 1 shows the clinical data. Sixty five of the patients were men and 81 were women. The median age at the time of diagnosis was 66 years (range, 34–82). The median survival was 10 months (range, 0–21.7 years). There were seven patients who survived more than five years, and six patients were still alive at the end of the follow up (range, 4.7–21.7 years). All patients included in our study underwent surgery, either pancreaticoduodenectomy for cure (n = 94), non-radical pancreaticoduodenectomy (n = 39), palliative bypass (n = 8), or diagnostic laparotomy (n = 5). Staging was done according to the UICC TNM classification.¹⁸ The patients' survival data were obtained from patient records, Statistics Finland, and the Population Registry.

For benign controls we chose 10 chronic and 10 acute pancreatitis samples and four samples of normal pancreas.

Histology

Histological specimens were re-evaluated by one pathologist (SN) from haematoxylin and eosin and Herovici stained slides. The diagnosis of ductal adenocarcinoma of the pancreas was confirmed. Histological grade was re-evaluated and the most representative sample of the primary tumour was chosen for immunohistochemistry.

Abbreviations: CI, confidence interval; ECM, extracellular matrix; PBS, phosphate buffered saline

Table 1 Distribution of tenascin C according to preoperative characteristics in 146 patients with pancreatic cancer

Clinicopathological variable	Patients	Tenascin C >20% n (%)	χ^2	p Value
Age				
≤66	73	37 (51)	4.0	0.04
>66	73	25 (34)		
Sex				
Female	81	31 (38)	1.3	0.3
Male	65	31 (48)		
Grade				
1	15	6 (40)	4.3	0.1
2	92	34 (37)		
3	39	22 (56)		
Grade				
1–2	107	40 (37)	4.2	0.04
3	39	22 (56)		
Stage				
I	27	11 (41)	0.5	0.9
II	48	19 (40)		
III	29	13 (45)		
IV	41	19 (46)		
Not available	1			
T				
1	9	5 (56)	1.3	0.7
2	28	10 (36)		
3	73	31 (42)		
4	35	16 (46)		
Not available	1			
N				
0	90	39 (43)	0.003	1.0
1	42	18 (43)		
Not available	14			
M				
0	135	56 (41)		0.3
1	10	6 (60)		
Not available	1			
Tumour size				
≤2 cm	26	11 (42)	0.5	0.8
>2–4 cm	73	33 (45)		
>4 cm	32	12 (38)		
Not available	15			
Curativity				
Intent to cure	94	37 (39)	1.0	0.3
Non curative	52	25 (48)		
Location				
Head	129	52 (40)	2.1	0.1
Body and tail	15	9 (60)		
Not available	2			

Immunohistochemistry for tenascin

Sections (4 µm) were freshly cut from formalin fixed and paraffin wax embedded blocks. Sections were dewaxed in xylene and rehydrated in a series of graded concentrations of ethanol and distilled water. For antigen retrieval, the sections were pretreated in trypsin/phosphate buffered saline (PBS) solution for 30 minutes, immersed in 0.6% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity, and then immersed in normal horse serum diluted 1/67 in PBS for 15 minutes to block non-specific binding sites. Tenascin specific antihuman monoclonal antibody (clone DB7; Biohit Diagnostics Oy, Helsinki, Finland) was applied overnight at a dilution of 1/2000 in PBS containing 0.1% sodium azide and 0.5% bovine serum albumin at room temperature. Sections were then treated with biotinylated horse antimouse immunoglobulin (1/200 dilution; Vector Laboratories, Burlingame, California, USA). Antibody binding sites were visualised by an avidin–biotin immunoperoxidase technique (Vectastain ABCComplex; Vector Laboratories) and incubated with the second layer antibody and peroxidase labelled avidin–biotin complex for 30 minutes each. The peroxidase staining was visualised with 3-amino-9-ethylcarbazole (Sigma Chemical Co, St Louis, Missouri, USA). Counterstaining was performed with Mayer's haematoxylin.

Interpretation of immunohistochemical staining

The staining intensity of the tumour stroma was evaluated by a pathologist (SN) who was unaware of the clinical data. An extracellular staining reaction in the stroma around the tumour cells was considered positive. Staining was scored as negative (≤ 5%), weak (> 5% to 20%), intermediate (> 20% to 60%), or strong (> 60%) according to the percentage of the positively stained area. The cutoff value for statistical analysis of association was set at 20%, which divided the series into two approximately equally sized groups. For positive controls, we stained tenascin C positive breast cancer tissue samples for each staining batch. The smooth muscle cells in blood vessels stained positively in all specimens and acted as an internal positive control. For negative controls we used sections where PBS or non-immune mouse serum was used instead of primary antibody.

Statistical analysis

The χ^2 test and Fisher's exact test in case of very low expected frequencies were used to test for the association between factors. Survival curves were calculated according to the Kaplan–Meier product limit method. The disease specific overall survival was calculated from the date of diagnosis to death from pancreatic cancer, and patients who died of

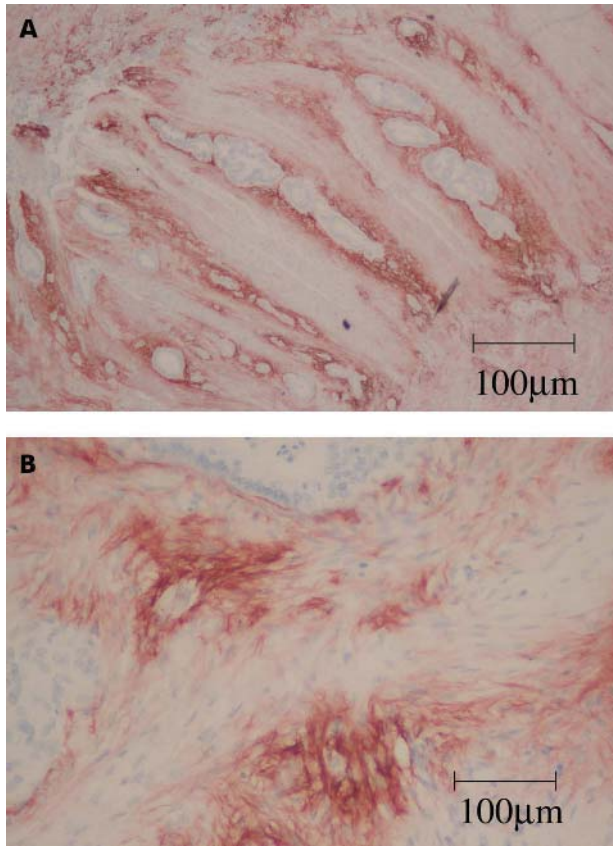


Figure 1 Immunohistochemical staining for tenascin C using monoclonal antibody DB7. (A) Tenascin C expression seen as undulating bands in the stroma surrounding well differentiated ductal cancer cells and (B) tenascin C staining is seen in poorly differentiated ductal cancer cells.

ordered categories. A p value of less than 0.05 was considered significant. The power to detect relative hazards of 2.0, 1.8, and 1.5 was 98%, 93%, and 66%, respectively.

RESULTS

Most of the pancreatic cancer samples expressed tenascin C (table 2). Tenascin C was found mainly in the stroma surrounding malignant ductal cells. When expression was strong, staining was seen as strong undulating bands around tumour cells in the adjacent stroma (fig 1). Twelve samples were negative for tenascin C, 72 samples stained weakly, 52 had intermediate staining, and 10 stained strongly in the stroma.

The staining pattern of tenascin C in chronic pancreatitis samples was similar to that in pancreatic cancer: positivity for tenascin C was seen diffusely in the stroma surrounding ductal cells. In acute pancreatitis, there was a distinct pattern of tenascin C expression: tenascin C was expressed as running bands between lymphatic cells at the edges of remaining pancreatic tissue bordering necrotic areas. The expression of tenascin C was stronger in acute pancreatitis than in chronic pancreatitis. In acute pancreatitis no samples were negative, and four showed weak staining, three intermediate, and three strong. In chronic pancreatitis four samples were negative, and one showed weak staining, four intermediate, and one strong staining. In normal pancreatic samples there was no tenascin expression in the pancreatic tissue. In one section there was some positivity surrounding a large bile duct.

Tenascin C expression correlated with low differentiation (grades 1–2 v 3) and with age (≤ 66 v > 66 years). In grade 1 and 2 tumours, 37% of the samples had intermediate or strong tenascin C expression, whereas in grade 3 tumours 56% of the samples had intermediate or strong expression ($p = 0.038$). The tumours of younger male patients had stronger tenascin C expression compared with those of older male patients and all women. The tumours of 23 of the 35 men under 66 years had intermediate or strong tenascin C expression, whereas in those over 66 years only eight of the 30 tumours had intermediate or strong tenascin C expression. There was no significant correlation between tenascin C

intercurrent causes were censored. The significance of the difference in survival between groups was calculated using the log rank test or log rank for trend in case of three or more

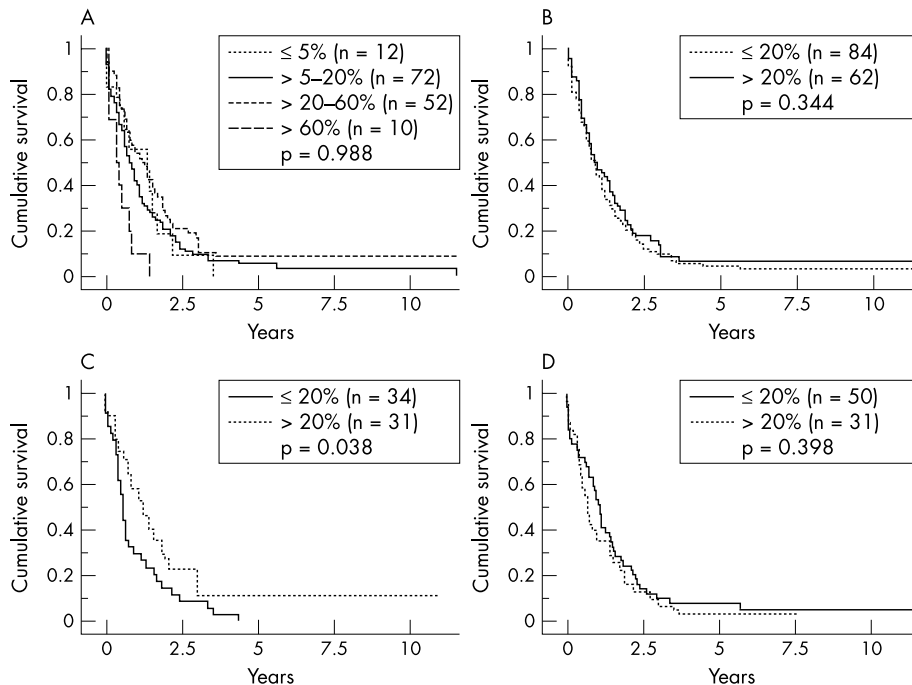


Figure 2 (A, B) Kaplan–Meier survival curves for all patients with pancreatic cancer grouped by tenascin C immunoreactivity. (C) In the subgroup of male patients, survival in those with tenascin C positive tumours (> 20% of the stroma stained) was significantly better than in those with tenascin C negative ($\leq 20\%$ of the stroma stained) tumours. (D) In the female patients there were no significant differences in survival between patients with tenascin C negative or positive tumours.

Table 2 Univariate analysis of the relation between preoperative characteristics and survival of 146 patients with pancreatic cancer

Clinicopathological variable	Patients n (%)	1 year CS (%)	95% CI	2 year CS (%)	95% CI	5 year CS (%)	95% CI	χ^2	p Value
Tenascin									
Negative ($\leq 5\%$)	12 (8)	56	26–85	19	0–2	0	0–0	<0.01	1.0
Faint ($>5\text{--}20\%$)	72 (49)	42	30–53	21	12–30	6	0–11		
Moderate ($>20\text{--}60\%$)	52 (36)	54	40–67	25	13–37	9	0–16		
Strong ($>60\%$)	10 (7)	10	0–29	0	0–0	0	0–0		
Tenascin									
$\leq 20\%$	84 (58)	44	33–54	21	12–29	5	0–10	0.9	0.34
$>20\%$	62 (42)	47	34–60	21	11–31	7	0–14		
Sex									
Female	81 (55)	46	36–57	21	12–30	6	0–12	0.03	0.9
Male	65 (45)	43	31–55	20	10–30	5	0–11		
Age (years)									
≤ 66	73 (50)	52	40–63	26	16–37	9	2–16	5.0	0.03
>66	73 (50)	38	27–50	15	7–23	3	0–7		
Tumour location									
Head	129 (88)	47	38–56	23	15–30	7	2–11	4.3	0.04
Other	15 (10)	33	10–57	7	0–19	0	0–0		
NA	2 (1)								
TNM stage									
I	27 (18)	67	49–84	33	16–51	11	0–23	9.9	0.002
II	48 (33)	46	32–60	27	15–40	6	0–13		
III	29 (20)	39	21–58	18	4–32	7	0–17		
IV	41 (28)	32	18–46	7	0–15	0	0–0		
NA	1 (1)								
Tumour size									
≤ 2 cm	26 (18)	68	50–87	20	4–36	0	0–0	3.3	0.07
$>2\text{--}4$ cm	73 (50)	51	39–62	25	15–35	10	3–17		
>4 cm	32 (22)	19	5–32	13	1–24	3	0–9		
NA	15 (10)								
Tumour stage									
1	9 (6)	78	51–100	22	0–49	0	0–0	3.3	0.07
2	28 (19)	46	28–65	32	15–49	11	0–22		
3	73 (50)	43	32–55	22	13–32	7	1–13		
4	35 (24)	37	21–53	9	0–18	0	0–0		
NA	1 (1)								
Lymph node metastasis									
N0	90 (62)	56	45–66	27	18–36	7	2–12	3.4	0.07
N1	42 (29)	32	18–46	15	4–26	7	0–15		
NA	14 (10)								
Distant metastasis									
M0	135 (92)	48	39–56	22	15–30	6	2–11	14.7	0.0001
M1	10 (7)	10	0–29	0	0–0	0	0–0		
NA	1 (1)								
Curativity									
Intent to cure	94 (64)	56	46–66	29	20–38	9	3–14	18.1	<0.0001
Non-curative	52 (36)	25	13–37	6	0–12	0	0–0		
Grade of differentiation									
1	15 (10)	80	60–100	40	15–65	27	4–49	16.3	<0.0001
2	92 (63)	47	37–58	23	15–32	6	0–10		
3	39 (27)	26	12–39	8	1–16	0	0–0		

CI, confidence interval; CS, cumulative survival; NA, not available.

expression and clinical stage, tumour size, nodal status, distant metastasis, tumour location, or sex (table 1).

In univariate analysis, surgery with intent to cure ($p < 0.0001$), high histological differentiation ($p < 0.0001$), absence of distant metastases ($p = 0.0001$), less advanced stage ($p = 0.002$), tumour location in the head of the pancreas ($p = 0.04$), and young age ($p = 0.03$) correlated with a longer survival. The association between survival and nodal status (N), tumour stage (T), and tumour size approached significance ($p = 0.07$) (table 2). Tenascin C was not associated with survival in the entire patient group (fig 2A, B). In the subgroup of male patients ($n = 65$), tenascin C expression ($> 20\%$) was associated with longer survival ($p = 0.038$; $\chi^2 = 4.3$) (fig 2C). Male patients with $\leq 20\%$ of tenascin C expression had 29% (95% confidence interval (CI), 14% to 45%), 15% (95% CI, 3% to 27%), and 0% (95% CI, 0% to 0%) one, two, and five year survival rates, respectively. Male patients with $> 20\%$ tenascin C expression had 58% (95% CI, 41% to 75%), 26% (95% CI, 10% to 41%), and 11% (95% CI, 0% to 23%) one, two, and five year survival

rates, respectively. In other subgroups, divided by sex, age, tumour location, and stage, tenascin C expression was not associated with survival (data not shown).

DISCUSSION

In our study, the immunohistochemical expression of tenascin C was evaluated in pancreatic adenocarcinoma, in addition to samples of normal pancreas and specimens from patients with pancreatitis. Normal pancreas did not express tenascin C, but in pancreatitis and pancreatic cancer tenascin C expression was upregulated. Tenascin C was expressed in 134 (92%) of the tumour specimens and high tenascin C expression ($> 20\%$) was associated with high grade (grades 1–2 v 3) (table 1).

The stroma surrounding malignant cells differs from normal ECM. A recent study has shown that the ECM protein tenascin C is expressed in pancreatic tumours.¹⁹ In our study, we found that tenascin C was abundant in the stroma around tumour cells but was not expressed in normal pancreatic stroma. Moreover, expression was enhanced in

Take home messages

- Tenascin C is not expressed in normal pancreatic tissue but is upregulated in pancreatic cancer and in acute and chronic pancreatitis
- Although increased tenascin C expression was associated with poor grade of differentiation it was not significantly associated with pancreatic cancer specific survival
- Therefore, tenascin C expression is not of prognostic value in pancreatic cancer

poorly differentiated tumours. This finding is in accordance with the antiadhesive functions of tenascin C, as described by Midwood and co-workers.^{20 21}

Tenascin C seems to play an important role in angiogenesis,^{11 22} cell proliferation,²³ and migration.²⁴ It has been reported to be a predictor of survival in colorectal,¹¹ breast,^{10 25} and cervical cancers.¹² In contrast to these studies, we found no significant association between tenascin C expression and survival. Surprisingly, in male patients, tenascin C expression seemed to predict longer survival ($p = 0.038$). This might suggest that hormones modulate the function of tenascin C. Studies indicate that tenascin C expression in prostatic stroma correlates with androgen concentrations. High tenascin expression is also seen during puberty when androgen concentrations increase.²⁶ In our study, the expression of tenascin C correlated with age. Younger men had stronger tenascin C expression than older ones ($p = 0.002$). The difference between age groups was not seen in women. However, in both younger and older men, tenascin C expression predicted a longer survival (data not shown).

“Surprisingly, in male patients, tenascin C expression seemed to predict longer survival”

In a previous study, 6 kb tenascin C mRNA was found in normal pancreatic tissue,²⁷ suggesting that normal pancreatic tissue has a potential to express tenascin C, if necessary. This is in accordance with our findings that pancreatic tissue normally does not express tenascin C, but in inflammation (acute and chronic pancreatitis) pancreatic tissue expresses tenascin C abundantly. In conclusion, tenascin C expression is upregulated in pancreatic cancer and in acute and chronic pancreatitis. Although increased tenascin C expression was associated with poor grade of differentiation it was not significantly associated with pancreatic cancer specific survival in the present study.

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J Clin Pathol 2004 57: 1151-1155
doi: 10.1136/jcp.2003.015818

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