Pathol. Oncol. Res. (2012) 18:299–307
DOI 10.1007/s12253-011-9444-1

RESEARCH

Expression of Galectin-3 in Pancreatic Ductal Adenocarcinoma

Matthias M. Gaida · Sylvia T. Bach · Frank Günther · Billur Baseras · Darjus F. Tschaharganeh · Thilo Welsch · Klaus Felix · Frank Bergmann · Gertrud M. Hänsch · Moritz N. Wente

Received: 16 April 2011 / Accepted: 1 August 2011 / Published online: 12 September 2011

Abstract Galectin-3 influences neoangiogenesis, tumor cell adhesion, and tumor-immune-escape mechanisms. Hence, the expression of galectin-3 in pancreatic ductal adenocarcinoma (PDAC) was evaluated. Galectin-3 expression in PDAC cell lines was proven by the presence of intracellular protein and by release into the supernatant. Furthermore, galectin-3 was found in the majority of human tissue samples. Serum concentrations of galectin-3 in PDAC patients did not differ significantly from healthy donors and did not correlate with established tumor markers. In conclusion, galectin-3 is expressed in PDAC tissues suggesting a role in tumor development; however, no relationship between expression and clinical findings could be established.

Keywords Pancreatic cancer · Galectins · Galectin-3 · Expression

Abbrevations

CEA	Carcinoembryonic antigen
CPB	Cyclophylin B
H&E	Haematoxylin and eosin
IQR	Interquartile range
PBS	Phosphate-buffered saline
PDAC	Pancreatic ductal adenocarcinoma
TMA	Tissue microarray

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth most common cancer-related death in Western countries [1]. The dismal prognosis with an overall 5-years survival rate of less than 5% is due to aggressive and invasive growth, early metastasis, and resistance to radiation and chemotherapy [2]. Because disease specific symptoms are lacking, diagnosis is often late, and curative therapy options are rather limited [3]. Hence, there is an intensive search for new therapeutic regimens and for markers for an early diagnosis as well.

In that context, galectins have been evaluated, because in numerous malignancies their involvement in tumor neoangiogenesis [4], tumor cell adhesion [5], tumor cell migration [6], and tumor-immune-escape [7] was reported. Moreover, enhanced serum galectin concentrations were found, for example in patients with bladder cancer, leading to the suggestion that galectins might serve as a serum "biomarker"

Electronic supplementary material The online version of this article (doi:10.1007/s12253-011-9444-1) contains supplementary material, which is available to authorized users.

M. M. Gaida · S. T. Bach · D. F. Tschaharganeh · F. Bergmann
Institute of Pathology, University of Heidelberg,
69120 Heidelberg, Germany

S. T. Bach · F. Günther · B. Baseras · G. M. Hänsch
Institute of Immunology, University of Heidelberg,
69120 Heidelberg, Germany

T. Welsch · K. Felix · M. N. Wente
Department of Surgery, University of Heidelberg,
69120 Heidelberg, Germany

Present Address:
F. Günther
Institute of Hygiene and Microbiology, University of Würzburg,
97080 Würzburg, Germany

Present Address:
M. N. Wente (✉)
Department of Medical Scientific Affairs, Aesculap AG,
Am Aesculap-Platz,
78532 Tuttlingen, Germany
e-mail: moritz.wente@aesculap.de

[8, 9]. In general, galectins are a family of animal lectins that are able to bind beta-galactosides; beside tumor development, galectins are also involved in inflammatory processes, artherosclerosis and wound repair and are considered to be widely distributed throughout the body [10].

Of special interest is galectin-3 (LGALS3), a structurally unique member of the galectin family by the presence of tandem repeats in the N-terminal region, which has been detected intracellularly located in the nucleus or cytoplasm, as well as on the cell surface, and in the extracellular area [11, 12]. Galectin-3 has been described to promote fibroblast proliferation, macrophage migration, collagen synthesis and the development of fibrosis [13]. Galectin-3 has been extensively evaluated in cardiac remodelling and heart failure, where it has been described as an independent prognostic marker for patients with acute and chronic heart failure [13–15].

In addition, galectin-3 was also found to be overexpressed in various malignancies including papillary thyroid carcinoma [16], bladder cancer [8] or colon cancer [6, 17]. In laryngeal squamous cell carcinoma galectin-3 expression correlates with an advanced tumor stage [18], in renal cell cancer with enhanced metastasis [19] and with a dismal survival prognosis in patients with malignant melanoma [20].

In the pancreas, galectin-3 expression has been initially described to be upregulated in chronic pancreatitis, also a disease with fibrosis and tissue remodelling [21]. Furthermore, a strong galectin-3 expression has been determined in solid pseudopapillary tumors, potentially associated with and involved in metastatic processes in this rare tumor entity of the pancreas [22].

Galectin-3 is also expressed in PDAC tissue, but according to a study by Berberat et al. it did not correlate with the clinical tumor stage or with its histological differentiation [23]. These data were in contrast to a study of Shimamura et al., who described a correlation between low galectin-3 expression and advanced clinical stage, distant metastases and low histological grade of differentiation and dismal survival prognosis [24]. Therefore, to resolve the given discrepancies, the aim of the present study was to evaluate galectin-3 expression in PDAC cell lines and to analyse a large series of human PDAC tissue samples regarding galectin-3 expression in relationship to clinical and pathological parameters.

Materials and Methods

Patients and Collection of Tissue Samples

PDAC tumor tissue samples were obtained from 130 patients (56 female, 74 male; age range from 39 to 85 years; mean: 65.3 years; median 66 years). The tissue specimens were formalin-fixed and paraffin-embedded. H&E staining was performed, and the tumor stage was evaluated according to the criteria established by the World Health Organization (2000) [25] and the UICC criteria (2009) [26]. Follow-up information was available for 104 patients: 61 patients died of the cancer within 25 to 1187 days after surgery (mean: 427 days, median: 347 days), 36 patients were alive after a follow-up of 10 to 1110 days (mean: 557.9 days, median: 663.5 days), and 7 patients died of non-cancer related disease and were excluded from further analysis (Table 1).

Serum was obtained from 83 patients with primary PDAC and from 16 patients with synchronic or metachronic metastases (37 female, 62 male; age range from 41 to 80 years; mean: 62.2 years; median 63 years). For comparison, serum from age and gender matched healthy volunteers (n=19) was collected (Table 2). From 19 patients, serum could be obtained one day preoperatively and 7 days postoperatively.

The use of tissue and serum samples for scientific purposes was approved by the ethic committee of the University of Heidelberg, and informed consent was obtained from the patients and from the donors as well.

Immunohistochemistry

Paraffin-embedded tissue sections (4 μm) were used for the immunohistochemical analysis. Immunostaining was performed as previously described [27], using the avidin-biotin complex method. As primary antibody a mouse monoclonal

Table 1 Patients included in the histological study

Gender (F : M) (n=130)	56 : 74
Age (years)	39–85 (mean: 65.3) (median: 66)
Location of tumor (n=130)	Head: 98
	Body: 8
	Body and tail: 13
	Tail: 11
Tumor stage	
T	pT3: 126
	pT2: 2
	pT1: 2
N	pN1: 110
	pN0: 20
M	pM1: 14
	pM0 : 116
Grade	G3: 39
	G2: 86
	G1: 5
Survival (n=104 patients; days)	Death: 61 patients; 25–1187 (mean 427; median: 347)
	Alive: 36 patients; 10–1110 (mean: 557.9; median: 633.5)
	Non cancer related death: 7 patients

Table 2 Patients included in the analysis of galectin-3 serum concentrations

	PDAC and Metastasis of PDAC
Gender (F : M) (n=99)	37 : 62
Age (years) (n=99)	31–80 (mean: 62.2) (median: 63)
Location of tumor, PDAC (n=77)	Head: 61
	Body: 7
	Body and tail: 1
	Tail: 8
Tumor stage	
T (n=76)	pT4: 4
	pT3: 68
	pT2: 2
	pT1: 2
N (n=72)	pN1: 56
	pN0: 16
M (n=93)	pM1: 28
	pM0 : 65
Grade (n=72)	G3: 23
	G2: 40
	G1: 9
Diagnosis	PDAC: 83
	Metastasis of PDAC: 16
CEA in μg/l (n=71)	0–1458 (mean: 27.75; median: 2.2)
CA19-9 in kU/l (n=79)	0.6–80458 (mean: 2370.86; median: 266)

antibody to galectin-3 (BD Pharma, Heidelberg, Germany; diluted 1: 100) was used. Galectin-3 staining was performed on tissue microarrays of 112 and on whole tissue sections of 18 PDAC patients. For TMA analysis two representative samples of each patient were analyzed. To validate the immunohistochemical results obtained from the microarrays, 20 of the tissue microarray cases were additionally stained for galectin-3, using whole tissue tumor sections. An isotype-specific negative control to the primary antibody (mouse IgG1, DAKO Cytomation, Hamburg, Germany) was performed to detect the specificity of the antibody.

To evaluate galectin-3 expression, a semiquantitative score was established, where score 0 means no expression; focal expression with 1–75% positive cells was scored 1, and diffuse expression with more than 75% positive cells was scored 2. The intensity of expression was also described as no staining (score 0), weak staining (score 1), moderate staining intensity (score 2) or strong staining intensity (score 3) (Fig. 1).

The severity and activity of the inflammation was evaluated microscopically on whole tumor sections, using a previously established score [28]. The severity was determined as absent (score 0), mild (score 1), moderate (score 2), or severe (score 3), depending on the accumulation and density of inflammatory cells (lymphocytes, plasma cells, macrophages) and formation of lymph follicles. The activity of inflammation was semiquantitatively scored as absent (score 0), mild (score 1) or moderate to severe (score 2), depending on the density of intratumoral neutrophil granulocytes (Table 3).

Cell Culture

Human PDAC cell lines ASPC-1, BxPc-3, Capan-1, MiaPaCa-2, PANC-1 and Su8686 were all purchased from American Type Culture Collection (ATCC, Rockville, MD, USA); COLO-357 and T3M4 were a gift from R. Metzgar (Duke University, Durham, NC, USA). Cell lines were cultured in RPMI1640 medium containing 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 μg/mL) (Invitrogen, Karlsruhe, Germany) at 37°C in humidified air with 5% CO_2. For supernatant collection, cells were seeded at 2×10^5 / well. After 24 h, medium was replaced by serum-free medium and cells were incubated for another 24 h. Then medium was collected and ELISA was performed.

Quantitative RT-PCR Analysis

Eight human pancreatic cancer cell lines (AsPC-1, BxPc-3, Capan-1, Colo-357, MiaPaCa2, PANC-1, Su8686, and T3M4) were tested. mRNA and cDNA preparation kits were purchased from Roche Applied Sciences (Mannheim, Germany). For mRNA preparation, automated MagNA PURE-LC instrument and corresponding isolation kit I (for cells) was used. Primers were obtained from Search-LC (Heidelberg, Germany). cDNA for RT-PCR was prepared by using a 1st strand cDNA Synthesis kit. Subsequently, RT-PCR was performed with the LightCycler-FastStart DNA SYBR Green kit. CPB was used as a housekeeping gene to normalize the expression of specific galectin-3 transcripts and presented as adjusted copies/10 k copies CPB, as previously described [29].

Galectin-3 ELISA

Galectin-3 in cell culture supernatants and in the serum samples were measured using the commercially available galectin-3 ELISA kit QIA112-1 (Calbiochem, Darmstadt, Germany) according to the manufacturer's instructions. All samples were measured in duplicates.

Cytofluorometry

Eight human pancreatic cancer cell lines (AsPC-1, BxPc-3, Capan-1, Colo-357, MiaPaCa2, PANC-1, Su8686, and

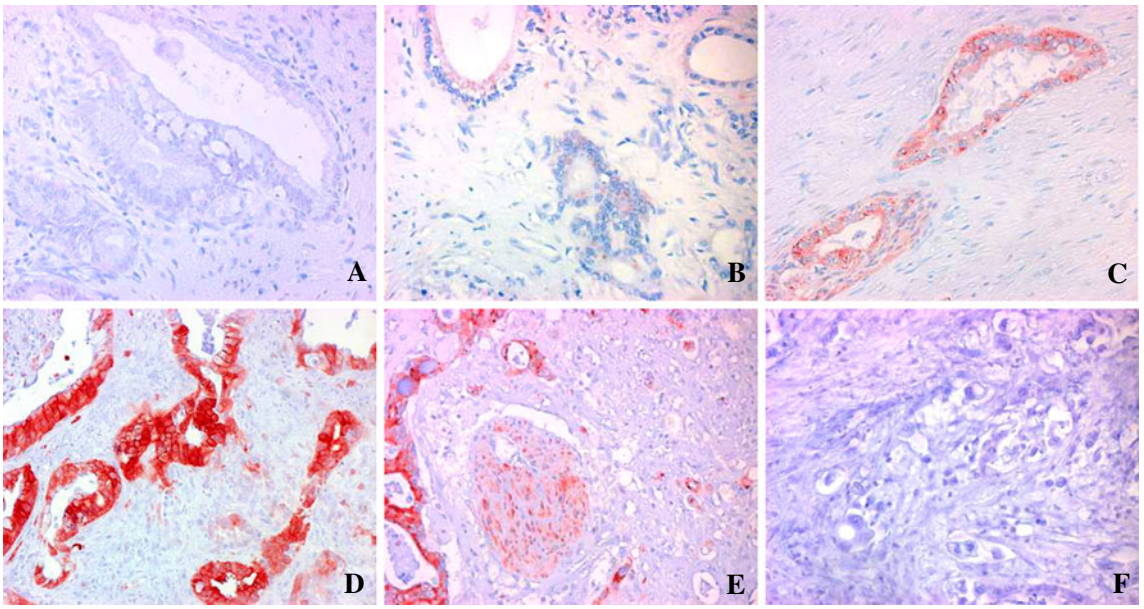

Fig. 1 Immunohistochemical staining of galectin-3. Expression of galectin-3 in tissue (**a**–**d**). Galectin-3 was determined in biopsies of pancreatic tissue of patients (n=130) by use of a specific antibody. An example for a negative result is shown in (**a**); in (**b**) an example for weak, in (**c**) for moderate, and in (**d**) for intense expression of galectin-3 is given, respectively. **e** shows nerve tissue that was used as internal positive control. **f** is the negative control without staining

T3M4) were suspended in PBS containing 1% bovine serum albumin and 0.1% sodium azide ($1x10^5$/100 µl) and incubated with the antibody to galectin-3 (1 µg) or for comparison with mouse IgG (BD Pharmingen, Heidelberg, Germany). After 30 min, a FITC-labeled antibody to mouse IgG (raised in goat; Immunotech, Marseille, France) was added for 30 min. Then, the cells were washed, resuspended in 1% paraformaldehyde in PBS and antibody binding was measured by FACScalibur® using CellQuest® as software (Becton and Dickinson, Heidelberg, Germany). For intracellular galectin-3 determination, the permeabilisation Kit (Becton and Dickinson) was used according to the recommendation of the supplier. Galectin-3 expression was measured as mean fluorescence intensity (MFI).

Table 3 Distribution and intensity of galectin-3 expression in tissue

Galectin-3 distribution score	Cases of PDAC (n=130)
0	25
1	81
2	24
Galectin-3 intensity score	Cases of PDAC (n=130)
0	25
1	41
2	54
3	10

Statistical Analysis and Correlation

For statistical analysis of survival the non-parametric Logrank test was performed. Correlation of galectin-3 expression with clinical or pathological parameters respectively was performed using Spearmans-Rho analysis, non-parametrical Mann-Whitney U- and Chi-Square test with Yates correction. Significance levels were defined at $p<0.05$. The statistical analyses were performed by using SPSS version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Graphs were made using OriginPro7.5 software (Additive Software, Friedrichsdorf, Germany).

Results

Expression of Galectin-3 in PDAC Tissue, and Correlation to Clinical and Pathological Parameters

Tissues of 130 patients with PDAC were screened for the expression of galectin-3 by immunohistochemistry. In 105 patients (80.8%) galectin-3 was expressed, predominantly with a focal pattern (81/105). In the other positive cases, the expression pattern was diffuse (Fig. 1, Table 3). Strong galectin-3 expression in tumor cells was seen in 10 cases (10.5%), whereas 53 cases (50.5%) showed moderate and 41 cases (39.0%) weak expression. The immunostaining was predominantely cytoplasmatic in all investigated cases

Table 4 Correlation of galectin-3 distribution in tissue with activity and severity of intratumoral inflammation

Inflammation		Galectin-3 Distribution (%) 0	1	2	*p*
Activity					
0	*n*=4	0 (0)	2 (50)	2 (50)	0.695
1	*n*=62	16 (25.8)	35 (56.5)	11 (17.7)	
2	*n*=64	9 (14.1)	44 (68.7)	11 (17.2)	
Severity					
0	*n*=0	0	0	0	0.222
1	*n*=12	6 (50)	4 (33.3)	2 (16.7)	
2	*n*=87	14 (16.1)	57 (65.5)	16 (18.4)	
3	*n*=31	5 (16.1)	20 (64.5)	6 (19.4)	
		n=25	*n*=81	*n*=24	

(*n*=112), whereas in some cases additionally a membraneous or nuclear staining was seen. As internal positive control nerves revealed a positive immunostaining. Furthermore, in all cases where tumor infiltrating macrophages were present, they stained positive for galectin-3. In addition, tumor infiltrating lymphocytes revealed galectin-3 positivity. The intensity correlated with the distribution pattern of galectin-3 expression ($p<0.001$). Neither distribution pattern nor the intensity of galectin-3 expression correlated with the intratumoral inflammation (Tables 4 and 5) or with clinical or pathohistological parameters (Supplementary Table 1, Supplementary Table 2). In addition, no relationship between galectin-3 expression and patient survival could be established, as patients with either galectin-3 negative or positive tissues showed a comparable overall survival (galectin-3 negative: mean 669.7 (95% CI [521.5–817.9]) days; galectin-3 positive: mean 611.7 (95% CI [515.5–708]) days; $p=0.602$) (Fig. 2). Furthermore, galectin-3 intensities did not correlate with patient survival (data not shown).

Galectin-3 Serum Concentrations in Patients with PDAC

Galectin-3 serum concentrations were tested in patients (*n*=99) and in healthy individuals (*n*=19). Compared to the mean values obtained from the healthy individuals, only in 49 patients with PDAC elevated concentrations were found; and the mean values of the groups did not differ significantly from each other (Fig. 3a). Comparing the serum levels of PDAC patients without metastasis (lymph node and/or distant organ metastases; T1-4, N0, M0) (*n*=15) with those having tumor manifestations in lymph nodes and/or distant organs (*n*=78) a tendency towards higher galectin-3 concentrations was seen in the latter group ($p=0.076$; Mann-Whitney-*U*-test) (Fig. 3b).

The galectin-3 serum levels neither correlated with serum concentrations of CA19-9 (mean 2370.86 kU/l; $p=0.575$) or CEA (mean 27.75 µg/l; $p=0.715$) (Table 2, Fig. 4), nor with clinical or pathological parameters (Table 6).

Serum samples of PDAC patients (*n*=19) were collected preoperatively and 7 days postoperatively after pancreatic resection. In 16 patients (84.2%), a significant decrease of the serum galectin-3 concentrations was seen from the preoperative to the postoperative status (Fig. 5).

Expression of Galectin-3 in PDAC Cell Lines

The galectin-3 expression was tested in established PDAC cell lines. Galectin-3 mRNA was detected in all cell lines albeit in different copy numbers (mean: 4532 copies/10 k copies CPB; interval: 1652–11760 copies/10 k copies CPB). By cytofluorometry, galectin-3 protein was found intracellularly. Surface expression of galectin-3 could not be detected (Fig. 6, Supplementary Table 3).

Table 5 Correlation of galectin-3 staining intensity in tissue with activity and severity of intratumoral inflammation

Inflammation		Galectin-3 Intensity (%) 0	1	2	3	*p*
Activity						
0	*n*=4	0 (0)	0 (0)	4 (100)	0 (0)	0.892
1	*n*=62	16 (25.8)	17 (27.4)	24 (38.7)	5 (8.1)	
2	*n*=64	9 (14.1)	24 (37.5)	26 (40.6)	5 (7.8)	
Severity						
0	*n*=0	0 (0)	0 (0)	0 (0)	0 (0)	0.127
1	*n*=12	6 (50)	2 (16.7)	3 (25)	1 (8.3)	
2	*n*=87	14 (16.1)	31 (35.6)	35 (40.2)	7 (8.1)	
3	*n*=31	5 (16.1)	8 (25.8)	16 (51.6)	2 (6.5)	
		n=25	*n*=41	*n*=54	*n*=10	

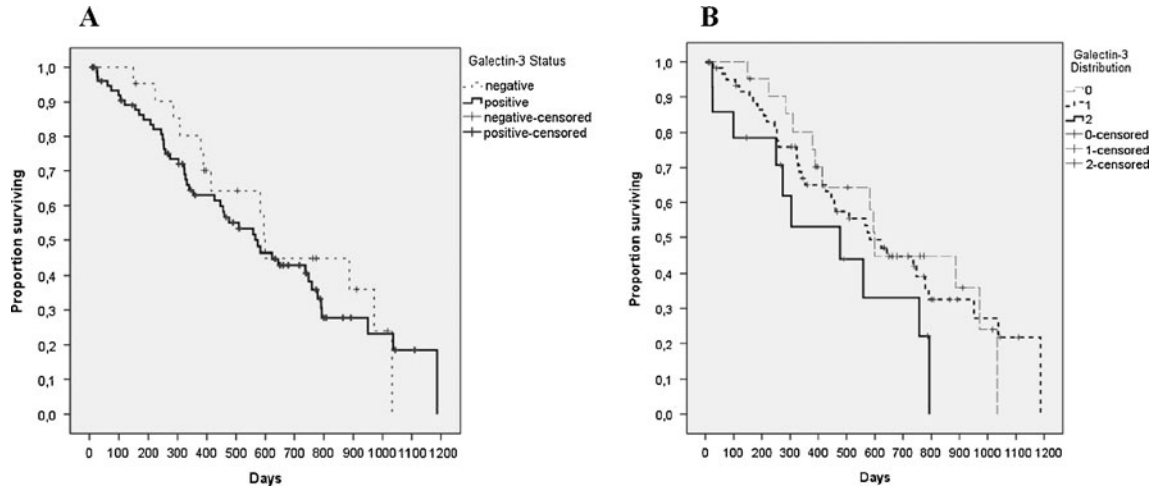

Fig. 2 Correlation of galectin-3 expression and survival. Galectin-3 expression in the pancreas did not correlate with survival, which was analysed according to Kaplan-Meier. **a** 97 patients were analysed. Expression of galectin-3 did not have a significant effect on survival (mean survival in galectin-3 negative patients 669.7 days, in galectin-3 positive patients 611.7 (log-rank test; $p=0.602$). **b** Subdividing the different groups of galectin-3 distribution patterns in pancreatic cancer tissue revealed no significant differences between the three groups (no galectin-3 expression, focal galectin-3 expression, high galectin-3 expression). Patient survival of the group with only focal galectin-3 expression in tissue (1–75% positive pancreatic cancer cells) compared with the group with high galectin-3 expression (>75% of positive pancreatic cancer cells) showed a trend for an unfavourable survival prognosis (log-rank test; $p=0.145$; mean: Distribution 1: 644.2 days; Distribution 2: 444.3 days)

To assess whether the tumor cells release galectin-3, supernatants of three cell lines (Capan-1, COLO-357 and T3M4) were collected and galectin-3 concentrations were measured by ELISA. Three independent tests resulted in an average release of 2594 pg/ml for COLO-357, of 942 pg/ml for Capan-1 and of 589 pg/ml for T3M4 (Supplementary Table 3).

Discussion

The aim of the present study was to determine the expression of galectin-3 in PDAC tissue, to correlate galectin-3 expression with clinical and pathological parameters and to assess whether or not galectin-3 in serum could qualify as a tumor marker. Galectin-3 appeared as an attractive candidate,

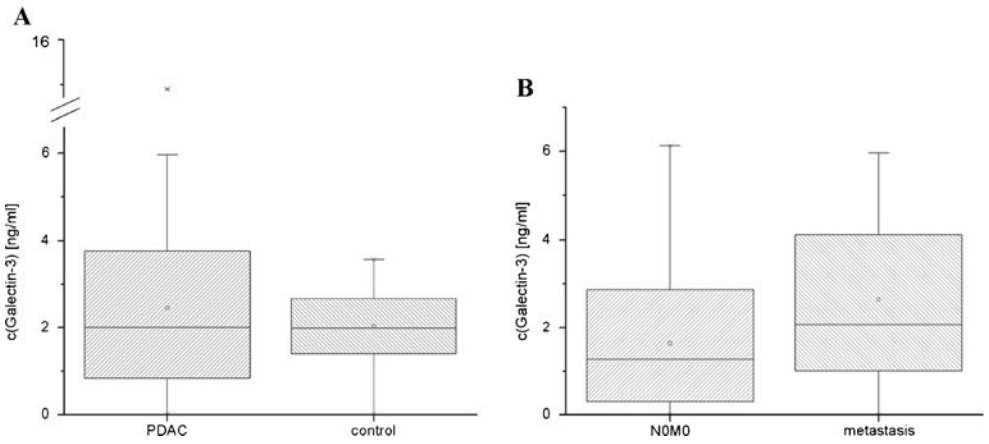

Fig. 3 Galectin-3 concentration in blood sera. Analysis of galectin-3 serum concentrations was determined in serum samples of PDAC patients ($n=99$) and healthy individuals ($n=19$) by ELISA. The data are summarised as box-and-whiskers blot, with the box containing 50% of the values. The median is depicted as horizontal bar, the mean as dot, and the IQR as the box length. In (**a**), the direct comparison of all patients (mean 2.447 ng/ml; median 2.003 ng/ml) and the healthy individuals ("control") (mean 2.031 ng/ml; median 1.988 ng/ml) is shown. There was no significant difference between the two groups as tested by ANOVA and by Mann-Withney-*U*-test ($p=0.869$). In (**b**), patients with lymph node and/or distant organ metastasis ($n=78$) (mean 2.641 ng/ml; median 2.0915 ng/ml) were compared to patients without metastasis (N0M0; $n=15$) (mean 1.640 ng/ml; median 1.262 ng/ml). Again, there was no difference between the groups ($p=0.076$)

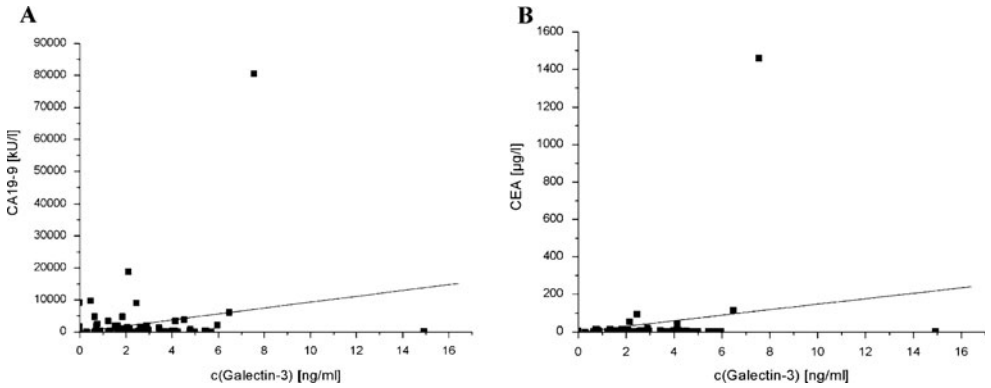

Fig. 4 Relationship between galectin-3 serum concentrations and that of the established tumor markers CEA and CA19-9. The linear regression analysis comprising 71 patients (CEA and galectin-3) (**a**) and 79 patients (CA19-9 and galectin-3) (**b**) revealed no correlation between galectin-3 serum concentrations and concentrations of the tumor markers (r=0.267, p=0.715 and r=0.226, p=0.575, respectively)

Table 6 Correlation of galectin-3 serum levels with clinical and pathological parameters

		c(Galectin-3) in ng/ml (median 2,003 (U-14,929 ng/ml); undetectable n=12)		p
		<2.003	≥2.003	
Sex				
Male	n=62	35 (56.4)	27 (43.6)	0.381
Female	n=37	14 (37.8)	23 (62.2)	
Age				
<63	n=48	28 (58.3)	20 (41.7)	0.116
≥63	n=51	21 (38.9)	30 (61.1)	
T				
1	n=2	0 (0)	2 (100)	0.164
2	n=2	1 (50)	1 (50)	
3	n=68	36 (52.9)	32 (47.1)	
4	n=4	2 (50)	2 (50)	
N				
0	n=16	11 (68.8)	5 (31.2)	0.156
1	n=56	28 (50)	28 (50)	
M				
0	n=65	34 (52.3)	31 (47.7)	0.305
1	n=27	11 (40.7)	16 (59.3)	
Grade				
1	n=9	4 (44.4)	5 (55.6)	0.737
2	n=40	24 (60)	16 (40)	
3	n=23	13 (56.5)	10 (43.5)	
Tumor size				
<3.5 cm	n=19	11 (57.9)	8 (42.1)	0.814
≥3.5 cm	n=25	13 (52)	12 (48)	
CEA				
<2.2 µg/l	n=34	18 (52.9)	16 (47.1)	0.715
≥2.2 µg/l	n=37	15 (40.5)	22 (59.5)	
CA19-9				
<266 kU/l	n=39	22 (56.4)	27 (43.6)	0.575
≥266 kU/l	n=40	18 (45)	22 (55)	

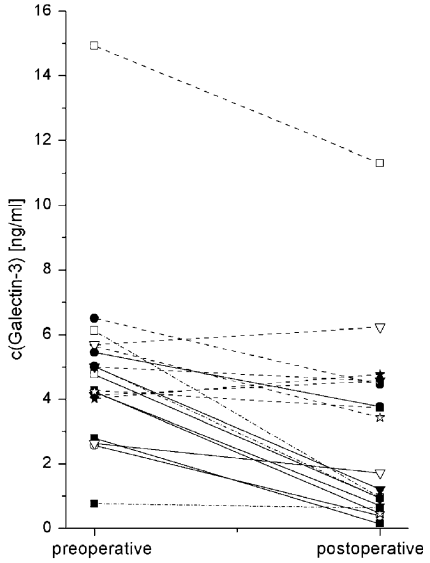

Fig. 5 Galectin-3 serum concentration pre- and postoperatively. In 19 patients, the galectin-3 serum concentration was measured one day before surgery and 7 days postoperatively. In 16 patients a decline of the galectin-3 concentration was seen postoperatively (each symbol represent an individual patient; by using non parametric Wilcoxon test, the decline was significant; $p<0.001$)

because it is found in various human malignancies, including malignant melanoma [30], laryngeal squamous cell carcinoma [18] or renal cell cancer in advanced stage [19]. Moreover, galectin-3 expression serves as diagnostic marker in papillary thyroid cancer [31].

We have now found galectin-3 expression in tissue of PDAC patients. In line with previous data by Berberat et al. comprising a smaller group of patients ($n=33$), expression of galectin-3 did not correlate with clinical or pathological parameters [23]. Moreover, no correlation between severity and activity of intratumoral inflammation and galectin-3 was seen. Different kinetics of galectin-3 expression by the tumor cells and the manifestation of the inflammatory response could account for the discrepancy. Another explanation is that the inflammatory environment does not promote the galectin-3 expression by the tumor cells.

In vitro data indicated that numerous pancreas tumor cells lines synthesize galectin-3 and in all lines tested galectin-3 protein was found intracellularly. Release of galectin-3 into the environment was also seen, although so far the trigger for galectin-3 release has not yet been identified.

Release from the tumor cells could also account for the galectin-3 found in the serum of the patients. That the presence of galectin-3 is linked to the presence of the tumor could be shown by analysing the serum galectin-3 concentrations prior and 7 days after surgery: here in the majority of patients (16 of 19) a drastic decrease in the galectin-3 serum concentration was seen in the post-operative samples. Despite this relationship, galectin-3 is not suitable as a biomarker for pancreatic tumours, because when analysing 99 samples of PDAC patient serum, galectin-3 expression was only found in 87 (88%) of the patients, and accordingly, the mean values between patients and donors did not differ significantly, and there was no correlation of serum galectin-3 concentrations with clinical or pathological parameters. In that, PDAC differed from other malignancies such as bladder cancer or thyroid cancer, where high galectin-3 serum concentration were found [9, 32], but is in line with data from patients with biliary tract carcinoma, where serum galectin-3 concentrations did not differ between the carcinoma group and the control group [33], or with data by Sakaki et al., who could not find a correlation of galectin-3 serum concentrations with the clinical and pathological features such as tumor stage and histological grading in patients suffering bladder cancer [9].

In summary, in this large series of PDAC patients, neither expression of galectin-3 in PDAC tissue samples, nor galectin-3 serum concentrations correlated with clinical and pathological parameters or with the intratumoral inflammation, although in vitro data showed that pancreatic tumor cells lines are able to produce and release galectin-3.

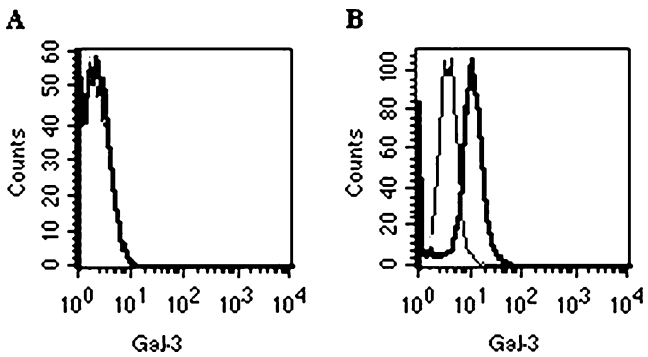

Fig. 6 Galectin-3 expression by pancreatic cancer cells detected by FACS. Expression of galectin-3 in pancreatic cancer cells, analysed by cytofluorometry: (**a**) galectin-3 was not detected on the surface of the cells (here as example the cell line Capan-1 was used); (**b**) reveals the finding that galectin-3 was seen intracellularly. The histograms shown illustrate the isotype FITC anti-mouse IgG1 (narrow line) and the anti-human galectin-3 FITC reactive cells (thick line)

Acknowledgements We thank Birgit Prior, Institute of Immunology, University of Heidelberg and Sarah Messnard, Institute of Pathology, University of Heidelberg for excellent technical support. We also thank Thomas Giese, Institute of Immunology, University of Heidelberg for performing quantitative RT-PCR.

Part of this work has been financially supported by a grant of the "Heidelberg Surgery Foundation" to G.M.H. and M.N.W. All authors declare that they have no conflict of interest to disclose.

References

1. Jemal A, Siegel R, Xu J, Ward E (2010) Cancer statistics, 2010. CA Cancer J Clin 60:277–300
2. Li D, Xie K, Wolff R, Abbruzzese JL (2004) Pancreatic cancer. Lancet 363:1049–1057
3. Warshaw AL, Fernandez del Castillo C (1992) Pancreatic carcinoma. N Engl J Med 326:455–465
4. Thijssen VL, Postel R, Brandwijk RJ, Dings RP, Nesmelova I, Satijn S, Verhofstad N, Nakabeppu Y, Baum LG, Bakkers J, Mayo KH, Poirier F, Griffioen AW (2006) Galectin-1 is essential in tumor angiogenesis and is a target for antiangiogenesis therapy. Proc Natl Acad Sci USA 103:15975–15980
5. Goetz JG, Joshi B, Lajoie P, Strugnell SS, Scudamore T, Kojic LD, Nabi IR (2008) Concerted regulation of focal adhesion dynamics by galectin-3 and tyrosine-phosphorylated caveolin-1. J Cell Biol 180:1261–1275
6. Hittelet A, Legendre H, Nagy N, Bronckart Y, Pector JC, Salmon I, Yeaton P, Gabius HJ, Kiss R, Camby I (2003) Upregulation of galectins-1 and -3 in human colon cancer and their role in regulating cell migration. Int J Cancer 103:370–379
7. Rubinstein N, Alvarez M, Zwirner NW, Toscano MA, Ilarregui JM, Bravo A, Mordoh J, Fainboim L, Podhajcer OL, Rabinovich GA (2004) Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection; A potential mechanism of tumor-immune privilege. Cancer Cell 5:241–251
8. Canesin G, Gonzalez-Peramato P, Palou J, Urrutia M, Cordon-Cardo C, Sanchez-Carbayo M (2010) Galectin-3 expression is associated with bladder cancer progression and clinical outcome. Tumour Biol 31:277–285
9. Sakaki M, Oka N, Nakanishi R, Yamaguchi K, Fukumori T, Kanayama HO (2008) Serum level of galectin-3 in human bladder cancer. J Med Invest 55:127–132
10. Yang RY, Rabinovich GA, Liu FT (2008) Galectins: structure, function and therapeutic potential. Expert Rev Mol Med 10:e17
11. Dumic J, Dabelic S, Flogel M (2006) Galectin-3: an open-ended story. Biochim Biophys Acta 1760:616–635
12. Liu FT, Hsu DK (2007) The role of galectin-3 in promotion of the inflammatory response. Drug News Perspect 20:455–460
13. de Boer RA, Yu L, van Veldhuisen DJ (2010) Galectin-3 in cardiac remodeling and heart failure. Curr Heart Fail Rep 7:1–8
14. de Boer RA, Lok DJ, Jaarsma T, van der MP, Voors AA, Hillege HL, van Veldhuisen DJ (2011) Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction. Ann Med 43:60–68
15. Lok DJ, van der MP, de la Porte PW, Lipsic E, Van WJ, Hillege HL, van Veldhuisen DJ (2010) Prognostic value of galectin-3, a novel marker of fibrosis, in patients with chronic heart failure: data from the DEAL-HF study. Clin Res Cardiol 99:323–328
16. Cvejic D, Savin S, Petrovic I, Selemetjev S, Paunovic I, Tatic S, Havelka M (2005) Galectin-3 and proliferating cell nuclear antigen (PCNA) expression in papillary thyroid carcinoma. Exp Oncol 27:210–214
17. Zaia PL, Oshima CT, de Oliveira LF, ndrade Scherholz PL, Manoukian FN (2010) Immunoexpression of galectin-3 in colorectal cancer and its relationship with survival. J Gastrointest Cancer.
18. Choi JY, Cho SI, Do NY, Kang CY, Lim SC (2010) Clinical significance of the expression of galectin-3 and Pim-1 in laryngeal squamous cell carcinoma. J Otolaryngol Head Neck Surg 39:28–34
19. Sakaki M, Fukumori T, Fukawa T, Elsamman E, Shiirevnyamba A, Nakatsuji H, Kanayama HO (2010) Clinical significance of Galectin-3 in clear cell renal cell carcinoma. J Med Invest 57:152–157
20. Vereecken P, Awada A, Suciu S, Castro G, Morandini R, Litynska A, Lienard D, Ezzedine K, Ghanem G, Heenen M (2009) Evaluation of the prognostic significance of serum galectin-3 in American Joint Committee on Cancer stage III and stage IV melanoma patients. Melanoma Res 19:316–320
21. Wang L, Friess H, Zhu Z, Frigeri L, Zimmermann A, Korc M, Berberat PO, Büchler MW (2000) Galectin-1 and galectin-3 in chronic pancreatitis. Lab Invest 80:1233–1241
22. Geers C, Moulin P, Gigot JF, Weynand B, Deprez P, Rahier J, Sempoux C (2006) Solid and pseudopapillary tumor of the pancreas–review and new insights into pathogenesis. Am J Surg Pathol 30:1243–1249
23. Berberat PO, Friess H, Wang L, Zhu Z, Bley T, Frigeri L, Zimmermann A, Büchler MW (2001) Comparative analysis of galectins in primary tumors and tumor metastasis in human pancreatic cancer. J Histochem Cytochem 49:539–549
24. Shimamura T, Sakamoto M, Ino Y, Shimada K, Kosuge T, Sato Y, Tanaka K, Sekihara H, Hirohashi S (2002) Clinicopathological significance of galectin-3 expression in ductal adenocarcinoma of the pancreas. Clin Cancer Res 8:2570–2575
25. Hamilton SR, Aaltonen LA (2000) World Health Organisation classification of tumours. Pathology and genetics of tumours of the digestive system. IARC, Lyon
26. Sobin LH, Gospodarowicz MK, Wittekind C (2009) TNM classification of malignant tumours. Wiley-Blackwell, Oxford
27. Bergmann F, Breinig M, Hopfner M, Rieker RJ, Fischer L, Kohler C, Esposito I, Kleeff J, Herpel E, Ehemann V, Friess H, Schirmacher P, Kern MA (2009) Expression pattern and functional relevance of epidermal growth factor receptor and cyclooxygenase-2: novel chemotherapeutic targets in pancreatic endocrine tumors? Am J Gastroenterol 104:171–181
28. Ceyhan GO, Bergmann F, Kadihasanoglu M, Erkan M, Park W, Hinz U, Giese T, Müller MW, Büchler MW, Giese NA, Friess H (2007) The neurotrophic factor artemin influences the extent of neural damage and growth in chronic pancreatitis. Gut 56:534–544
29. Erkan M, Kleeff J, Esposito I, Giese T, Ketterer K, Büchler MW, Giese NA, Friess H (2005) Loss of BNIP3 expression is a late event in pancreatic cancer contributing to chemoresistance and worsened prognosis. Oncogene 24:4421–4432
30. Prieto VG, Mourad-Zeidan AA, Melnikova V, Johnson MM, Lopez A, Diwan AH, Lazar AJ, Shen SS, Zhang PS, Reed JA, Gershenwald JE, Raz A, Bar-Eli M (2006) Galectin-3 expression is associated with tumor progression and pattern of sun exposure in melanoma. Clin Cancer Res 12:6709–6715
31. Xu XC, el-Naggar AK, Lotan R (1995) Differential expression of galectin-1 and galectin-3 in thyroid tumors. Potential diagnostic implications. Am J Pathol 147:815–822
32. Saussez S, Glinoer D, Chantrain G, Pattou F, Carnaille B, Andre S, Gabius HJ, Laurent G (2008) Serum galectin-1 and galectin-3 levels in benign and malignant nodular thyroid disease. Thyroid 18:705–712
33. Koopmann J, Thuluvath PJ, Zahurak ML, Kristiansen TZ, Pandey A, Schulick R, Argani P, Hidalgo M, Iacobelli S, Goggins M, Maitra A (2004) Mac-2-binding protein is a diagnostic marker for biliary tract carcinoma. Cancer 101:1609–1615