

## The expressions and clinical significances of tissue and serum galectin-3 in pancreatic carcinoma

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### Abstract

**Purpose** Galectin-3, a member of the beta-galactoside-binding protein family, is involved in many biological processes, including cell proliferation, regulating cell cycle, angiogenesis, tumorigenesis, metastasis, etc. The aim of this study is to elucidate the relationship between galectin-3 and clinicopathological variables and to evaluate the clinical significance of serum galectin-3 in the diagnosis of pancreas carcinoma.

**Methods** Galectin-3 expression in 78 pairs of pancreatic carcinoma tissues and the adjacent nontumorous tissues was tested by immunohistochemistry. The relationship between galectin-3 expression and clinical variables was analyzed. A sensitive method of time-resolved fluorescence immunological assay (TRFIA) for the detection of galectin-3 was established, and serum galectin-3 in cases with different pancreatic diseases was measured by TRFIA and ELISA. Further we compared the sensitivity and specificity of determining galectin-3, carcinoembryonic antigen (CEA) and carbohydrate antigen 199 (CA199) for diagnosis

of pancreatic carcinoma and assessed the complementary diagnostic value of galectin-3, CEA and CA199 for pancreatic carcinoma.

**Results** Immunohistochemistry showed that galectin-3 expression was significantly higher in the human pancreatic carcinoma tissues than in the adjacent nontumorous tissues. The expression levels were correlated with the differentiation degree with the higher expression in poor differentiation tissues. Serum galectin-3 detected by both TRFIA and ELISA was much higher in patients with pancreatic carcinoma than in other groups. Serum galectin-3 was not correlated with CEA and CA199. Combined determination of these three markers has the complementary diagnostic value for human pancreatic carcinoma and may increase the diagnostic sensitivity to 97.5%.

**Conclusions** Galectin-3 is overexpressed in pancreatic carcinoma tissues, and it is correlated with the tumor differentiation. Serum galectin-3 is higher in cases with pancreatic carcinoma than in benign pancreatic diseases and healthy persons. Combined determination of serum galectin-3, CEA and CA199 may improve the diagnostic power for pancreatic carcinoma.

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Carcinoembryonic antigen · Carbohydrate 199 ·  
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### Introduction

Pancreatic adenocarcinoma is currently the fourth leading cause of cancer-related mortality in Western societies with an overall 5-year survival rate less than 5% (Jemal et al. 2008). Most patients present late in the course of the disease. Therefore, only 20% will be able to have potentially

curative therapy (Michl et al. 2006). Operative resection is usually not feasible and, even resection is undertaken, the perioperative mortality is high. Prognosis is greatly improved when patients are diagnosed at an early operable disease stage. The methods of identification of asymptomatic pancreatic neoplasms in patients are BUS, CT, MRI, ERCP and tumor markers in serum. Until now, however, no single clinical method meets the sensitivity and specificity criteria required for the purposes. Recognized serum markers for pancreatic carcinoma, such as carcinoembryonic antigen (CEA) or carbohydrate antigen CA 199 (CA199), lack early diagnosis value. So, more accurate and effective molecular markers of pancreatic tumor are needed to increase the early diagnosis rate of pancreatic tumor and to improve prognosis of the patients.

Galectins are members of the animal lectins, an ancient family of carbohydrate-binding proteins with an affinity for  $\beta$ -galactosides, sharing a conserved carbohydrate recognition domain (CRD) of about 130 amino acids (Barondes et al. 1994). To date, fifteen mammalian galectins have been found (Houzelstein et al. 2004). They are subdivided into three groups. While some of galectins contain a single CRD, called prototype ones, and are biologically active as monomers (galectins-5, -7, -10), as homodimers (galectins-1, -2, -11, 13–14, -15), whereas tandem-repeat galectins are composed of two nonidentical CRDs joined by a not long peptide (galectins-4, -6, -8, -9, -12), and the rest, called the unique chimera type as oligomers (galectin-3), contains a single CRD with a not short N-terminus (Jemal et al. 2008). It is evident that the galectins have a fine specificity in binding tissue- or developmentally specific ligands (Ahmad et al. 2004). Galectins play an essential role in the function and development of multicellular organisms, including development, differentiation, cell–cell adhesion, cell–matrix interaction, growth regulation, apoptosis, RNA splicing and tumor metastasis (Danguy et al. 2002).

Galectin-3 is one of the most extensively studied members of the beta-galactoside-binding protein family. It is a 31-kDa gene product, localized mainly in the cytoplasm and expressed on the cell surface (Kawachi et al. 2002). This protein is involved in different biological processes, such as cell-to-cell and cell-to-matrix interactions (Matarrese et al. 2000), induction of pre-mRNA splicing (Dagher et al. 1995), cell proliferation (Inohara et al. 1998), regulating cell cycle (Lin et al. 2000), angiogenesis (Nangia-Makker et al. 2000) and, what is more important, tumorigenesis and metastasis (Takenaka et al. 2004).

Galectin-3 was reported to be overexpressed in pancreatic carcinoma tissues in a proteomics study (Chen et al. 2009). However, there has been no commercial kit for detection of serum galectin-3, and whether serum galectin-3 could become a serum marker for pancreatic carcinoma has not been known. The goal of this study was to elucidate

the relationship between galectin-3 and clinicopathological variables, to establish a sensitive method of time-resolved fluorescence immunological assay (TRFIA) for detecting serum galectin-3 and to evaluate the clinical significance of serum galectin-3 in the diagnosis of pancreas carcinoma.

## Patients and methods

### Patients and specimens

Seventy-eight pairs of pancreatic carcinoma tissues and their adjacent nontumorous tissues were obtained from the patients who had undergone pancreaticoduodenectomy at Affiliated Hospital of Nantong University, Nantong Tumor Hospital and Shanghai Renji Hospital, respectively, between 2002 and 2008. Our study was approved by the research ethics committee of the institute, and written informed consent was obtained from each subject. All patients were histologically documented cases of adenocarcinoma based on WHO histological classification of tumors of the pancreas, with 60 moderately or poorly differentiated and 18 well-differentiated adenocarcinomas. The TNM classification system appointed by the American Joint Committee on Cancer (AJCC) was used for the clinical staging. Of the 78 samples analyzed, the mean age of the patient group was 50 years (range, 36–80 years).

A total of 137 serum samples were analyzed. Preoperative serum samples were obtained from patients at Affiliated Hospital of Nantong University, including 49 patients with pancreatic adenocarcinoma, 16 with benign pancreatic cystic neoplasms, 36 with acute pancreatitis and 36 healthy volunteers.

Clinical variables and pathologic findings, including gender, age at diagnosis, tumor location, tumor diameter, lymphatic and distant metastasis, histological differentiation, TNM staging and serum CA199 concentration, were gathered.

### Immunohistochemistry

The paraffin-embedded tissues from 78 matched pairs of pancreatic adenocarcinoma and nonmalignant pancreatic tissues from the same patients were sectioned into 4- $\mu$ m thickness and placed on the glass slides. The sections were deparaffinized using a graded ethanol series, and endogenous peroxidase activity was blocked by soaking in 0.3% hydrogen peroxide. Antigen retrieval was performed by microwave heating at high power (750 W) in 10 mmol/l sodium citrate buffer (pH 6.0) for three cycles of 5 min each. After rinse in PBS (pH 7.2), 10% goat serum was applied for 1 h at room temperature to block nonspecific reactions. The sections were then incubated overnight at 4°C with

mouse monoclonal antibodies against galectin-3 (diluted 1:200; Santa Cruz Biotechnology, Santa Cruz, CA, Argentina). Negative control slides were also processed in parallel using a nonspecific immunoglobulin IgG (Sigma, St. Louis, MO, USA) at the same concentration as the primary antibody. All slides were processed using the peroxidase-antiperoxidase method (Dako, Hamburg, Germany). After rinse in PBS, the peroxidase reaction was visualized by incubating the sections with diaminobenzidine tetrahydrochloride in 0.05 mol/l Tris buffer (pH 7.6) containing 0.03% H<sub>2</sub>O<sub>2</sub>. After rinse in water, the sections were counterstained with hematoxylin, dehydrated and coverslipped. Referring to previous publication (Masunaga et al. 2000), galectin-3 staining intensity was scored as 0 (negative), 1 (weak), 2 (medium) and 3 (strong). Extent of staining was scored as 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%) and 4 (76–100%) according to the percentages of the positive staining areas in relation to the whole carcinoma area. The sum of the intensity and extent score was used as the final staining score (0–7) for galectin-3. According to the final staining score, the immunohistochemical evaluation results were scored as – (0–2), + (3–4), ++ (5–6) and +++ (7).

#### TRFIA of serum galectin-3

Optimal concentrations of reagents for TRFIA assay were determined on the basis of experimental data in a trial-and-error process. To attain the highest possible sensitivity, 4 mg/l mouse monoclonal antibodies (Santa Cruz), 80 µg/l goat polyclonal antibodies (Biotin) (Santa Cruz) and 500 µg/l europium labeled streptavidin (PE, USA) were, respectively, selected as the concentrations of an optimal system for galectin-3 detection. Microplates were coated with 100 µl/well monoclonal antibodies at a concentration of 4 mg/l in carbonate buffer (pH 9.6). The coated plates were stored at 4°C overnight and then washed four times with PBS and 0.05% Tween 20, followed by incubation in blocking solution (PBS containing 10 g/l FCS, 250 µl/well) at 4°C for about 48 h. After the blocking solution was poured off, serum samples, negative control (PBS containing 10 g/l BSA) and a series of galectin-3 standard dilutions (0.78–100 ng/ml) were loaded (50 µl) on to the plate, together with the goat polyclonal antibodies (Biotin) (100 µl/well). The plates were then incubated at room temperature for 2 h on an orbital shaker (Stuart Scientific, Stone, UK) set at 60 rpm, washed 4 times and then 100 µl of europium labeled streptavidin, diluted in assay buffer, was added to all wells. After an additional 1 h of shaker incubation at room temperature, the plates were washed 4 times and 200 µl of enhancement solution (PE, USA) was added to all wells. The plates were then shaker incubated for 5 min at room temperature and read using a Victor™ X5 automatic time-resolved fluorescence detector (PE, USA).

#### ELISA detection of serum galectin-3

Serum samples were also analyzed with a commercially available ELISA kit, Galectin-3-detect ELISA, according to the manufacturer's recommendations. Briefly, serum samples were incubated in a 96-well capture plate on a plate shaker for 90 min at room temperature. The plate was then washed 5 times with wash buffer. Galectin-3 enzyme conjugate was added, and the plate was incubated for 60 min, and the wash was repeated. Serum galectin-3 levels were determined by color change upon addition of tetramethylbenzidine substrate followed by addition of substrate stop solution. Absorbance values were read at 450 nm using a Victor™ X5 automatic time-resolved fluorescence detector. Galectin-3 concentrations were determined by interpolation from the standard curve.

#### Testing serum levels of CEA and CA199

The serum samples were also tested for CEA and CA199 by chemiluminescence immunoassay and the reagents were from Abbott, USA.

#### Statistical analysis

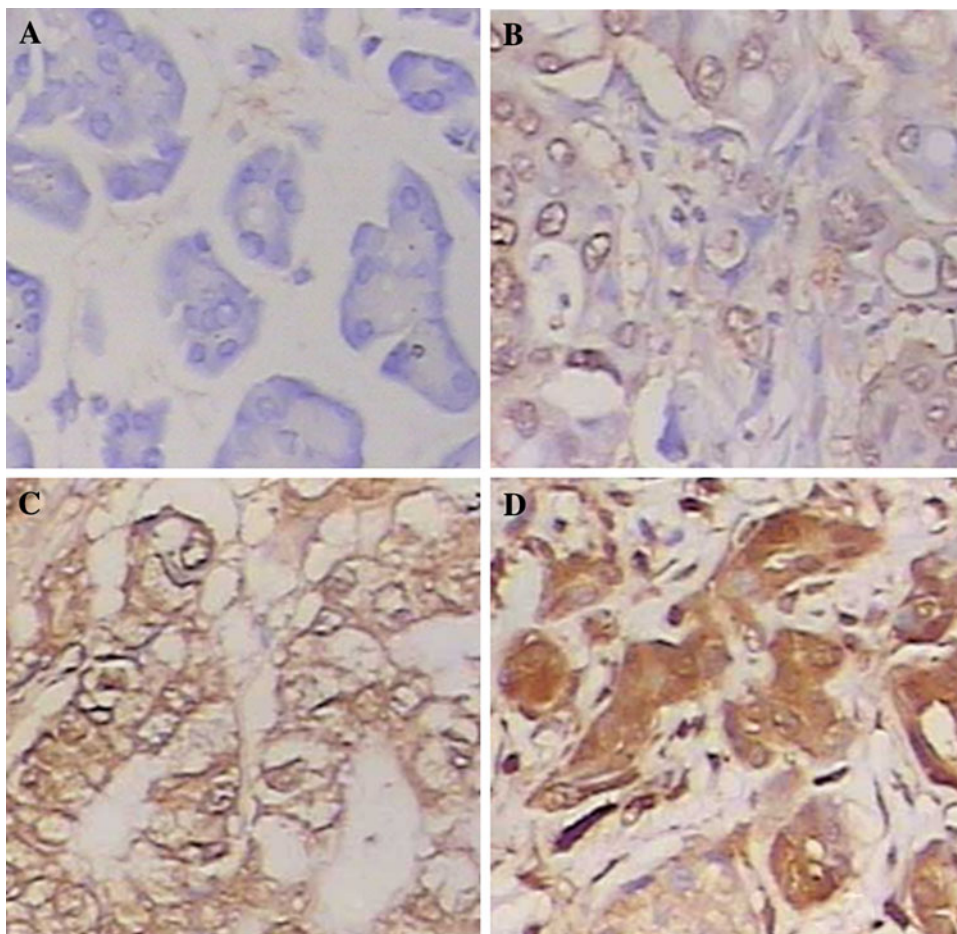
The Stat View 5.0 software package was used for statistical analysis. Statistical analyses were performed with chi-square test or Fisher's exact test for any 2 × 2 tables. A rank sum test was used to analyze the relationship between clinical pathological variables and the level of galectin-3. The correlations between serum galectin-3, CEA and CA199 were tested by Spearman rank correlation. Receiver operating characteristic (ROC) curves were used to assess the diagnostic value of serum markers. Probability values of less than 0.05 were considered statistically significant in all analyses.

## Results

#### The expression of galectin-3 protein in pancreatic carcinoma and adjacent nontumorous tissues

After the galectin-3 expression in paraffin-embedded, formalin-fixed, surgically resected pancreatic adenocarcinoma tissue sections was analyzed by immunohistochemistry, a strong galectin-3 staining was predominantly observed in the cytoplasm and weak staining in the nucleus of tumor cells (Fig. 1b, c, d). In contrast, galectin-3 expression was very low or negative in adjacent nontumorous tissue sections (Fig. 1a). When the scores of the nuclear and cytoplasmic staining were pooled together, we found that galectin-3 was positively expressed in 82.1% (64/78) of

**Fig. 1** Immunohistochemical staining of galectin-3 in adjacent nontumorous and pancreatic adenocarcinoma tissues. Tissue sections were stained with antibodies against galectin-3 and counterstained with hematoxylin. **a** Galectin-3 negative staining (–) was shown in adjacent nontumorous tissues. **b–d** Galectin-3 immunoreactivity was detected in well, moderately and poorly differentiated pancreatic adenocarcinoma with staining (+, ++ and +++) predominant in the cytoplasm ( $\times 400$ )



pancreatic carcinoma tissues and in 26.9% (21/78) of adjacent nontumorous tissues ( $\chi^2 = 47.7952$ ;  $P = 0.000$ ).

#### Association of galectin-3 expression with clinicopathological variables in pancreatic carcinoma

The association of galectin-3 expression with the clinicopathological variables is shown in Table 1. Galectin-3 expression was not associated with gender, age, tumor location, tumor diameter, lymphatic or distant metastasis, TNM stage and CA199 level ( $P > 0.05$ ), but was significantly correlated with histological differentiation ( $P = 0.0152$ ). Galectin-3 was higher expressed in poorly differentiated tissues than in well-differentiated ones.

#### Serum galectin-3 determined by TRIFA or ELISA

Serum galectin-3 levels were determined by TRIFA or ELISA in cases with pancreatic adenocarcinoma, benign pancreatic cystic neoplasms, and acute pancreatitis, as well as healthy subjects. By TRIFA, the median and range of galectin-3 level in patients with pancreatic adenocarcinoma were 4.93 and 0.85–23.80 ng/ml, which were higher than

those in patients with benign pancreatic cystic neoplasms and acute pancreatitis and healthy subjects (Table 2; Fig. 2), respectively. The sensitivity of ELISA for determination of galectin-3 was very low, with the median of 0 ng/ml in all groups. However, galectin-3 detected by ELISA was also higher in cases with pancreatic adenocarcinoma than in other groups (Table 2; Fig. 2). On the basis of ROC curves, the area under the curve (AUC) was used to diagnose pancreatic carcinoma. By TRIFA, the sensitivity and specificity of galectin-3 for the diagnosis of pancreatic carcinoma were 75.5 and 90.9%, respectively, if the cutoff value was selected as 3.77 ng/ml (Fig. 3). By ELISA, the sensitivity and specificity were 32.2 and 97.7%, respectively, with the cutoff value of 0.26 ng/ml (Table 3; Fig. 3).

#### Serum levels of CEA and CA199 in pancreatic carcinoma

Serum CEA and serum CA199 were higher in pancreatic carcinoma groups than in other groups (Table 4,  $P < 0.05$ ). The median of CEA level in pancreatic adenocarcinoma was 6.10 ng/ml, which was higher than that in benign pancreatic cystic neoplasms (1.80 ng/ml), acute pancreatitis (2.24 ng/ml) and healthy subjects (0.86 ng/ml) (Table 4;

**Table 1** Associations of galectin-3 expression with clinicopathological variables

Variables	<i>n</i>	Positive (%)	–	+	++	+++	<i>Z</i> *	<i>P</i> *
<b>Gender</b>								
Male	44	34 (77.3)	10	14	18	2	–0.279	0.7801
Female	34	30 (88.2)	4	16	12	2		
<b>Age</b>								
<65	42	33 (78.6)	9	16	15	2	–0.790	0.4293
≥65	36	31(86.1)	5	14	15	2		
<b>Tumor location</b>								
Head and neck	50	42 (84.0)	8	20	22	0	–0.178	0.8590
Body and tail	28	22 (78.6)	6	10	8	4		
<b>Tumor diameter (cm)</b>								
≤3	26	26 (100)	0	12	14	0	1.807	0.0707
>3	52	38 (73.1)	14	18	16	4		
<b>Lymph node metastasis</b>								
Absent	32	26 (81.3)	6	10	16	0	0.303	0.7618
Present	46	38 (82.6)	8	20	14	4		
<b>Distant metastasis</b>								
Absent	62	50 (80.6)	12	22	28	0	–0.580	0.5617
Present	16	14 (87.5)	2	8	2	4		
<b>Histological differentiation</b>								
Well	18	12 (66.7)	6	8	4	0	9.797	0.0075
Moderately	26	26 (92.3)	2	6	16	2		
Poorly	34	28 (82.4)	6	16	10	2		
<b>TNM stage</b>								
I, II	32	28 (87.5)	4	16	12	0	–0.520	0.6033
III, IV	46	36 (78.3)	10	14	18	4		
<b>CA199 (U/ml)</b>								
<1,000	59	48 (81.4)	11	20	26	2	0.521	0.6023
≥1,000	19	16 (84.2)	3	10	4	2		

\*The *Z* and *P* value were calculated by rank sum test

Fig. 4). The median of CA199 level in pancreatic adenocarcinoma was 111.6 U/ml, which was higher than that in benign pancreatic cystic neoplasms (15.80 U/ml), acute pancreatitis (24.48 U/ml) and healthy subjects (21.60 U/ml) (Table 4; Fig. 4). The sensitivities of CEA and CA199 for the diagnosis of pancreatic carcinoma was 69.4 and 67.3%, respectively, when the cutoff values were selected as

3.82 ng/ml and 41.61 U/ml, respectively, according to ROC curves (Table 5; Fig. 5).

Serum galectin-3 level is complementary to serum levels of CEA and CA199

Spearman rank correlation analysis was performed on serum levels of galectin-3, CEA and CA199. The data showed that galectin-3 was not correlated with either CEA ( $r = 0.1321$ ,  $P = 0.373$ ) or CA199 ( $r = 0.0920$ ,  $P = 0.5384$ ), suggesting that these 3 markers are complementary in the diagnosis of pancreatic carcinoma. The sensitivity of each marker alone for diagnosis of pancreatic adenocarcinoma was not so high, but the combination of 3 markers increased the sensitivity to 97.5% (Table 6).

**Discussion**

The diagnosis for patients with pancreatic adenocarcinoma, one of the most common cancer types, remains challenging despite intensive efforts (Jemal et al. 2010). With the increasing incidence of pancreatic carcinoma, the markers for early detection are urgently needed to improve the prognosis in patients with this highly aggressive disease. A non-invasive diagnostic test is a key to help screening from high-risk individuals. Clinical proteomics and peptidomics have rapidly developed over the past years, resulting in the discovery of potential biomarkers for cancer diagnosis. Therefore, identification of serum protein markers of pancreatic carcinoma could help providing a noninvasive diagnostic screening tool.

In this study, the overexpression of tissue galectin-3 protein was detected in pancreatic carcinoma patients by immunohistochemistry, with highly elevated galectin-3 in the majority of tumors (64/78). Different from this, there was less galectin-3 expression in adjacent nontumorous tissues. The overexpression of tissue galectin-3 protein was positively correlated with tumor histological grade. These findings agree with previous reports (Berberat et al. 2001).

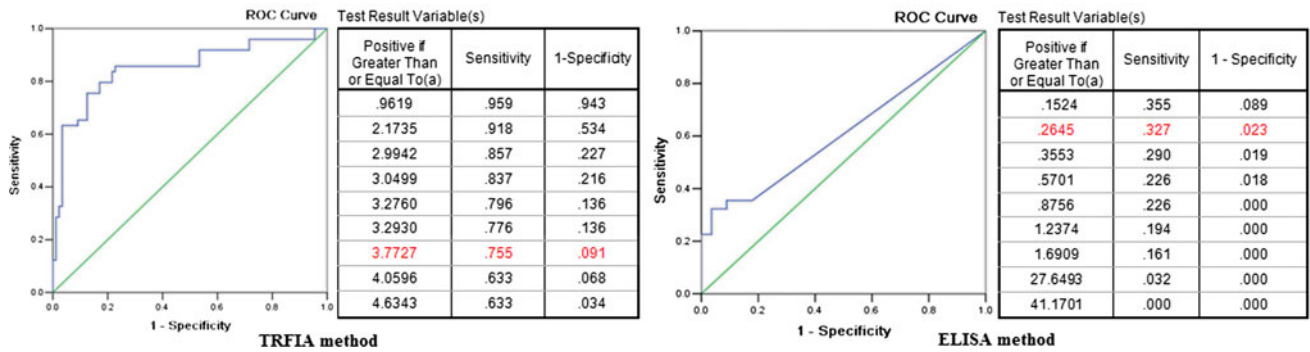
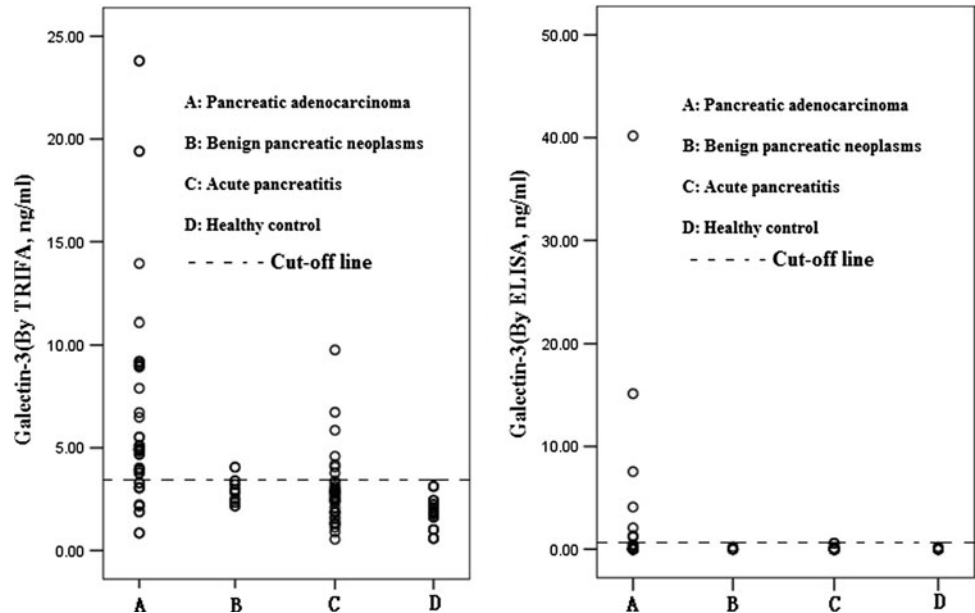
A body of clinical and experimental evidence has shown the significance of galectin-3 expressions in other primary

**Table 2** Comparison of serum galectin-3 level detected by TRIFA and ELISA

Subgroups	<i>n</i>	Galectin-3 (ng/ml), TRFIA			Galectin-3 (ng/ml), ELISA		
		(Median, range)	<i>Z</i> *	<i>P</i> *	(Median, range)	<i>Z</i> *	<i>P</i> *
Pancreatic adenocarcinoma	49	4.93 (0.85–23.80)			0 (0–40.17)		
Benign pancreatic neoplasms	16	2.83 (2.17–4.06)	3.778	0.0002	0 (0–0.22)	2.106	0.0352
Acute pancreatitis	36	2.62 (0.55–9.76)	4.874	0.0000	0 (0–0.59)	2.831	0.0046
Healthy control	36	1.88 (0.59–3.94)	6.379	0.0000	0 (0–0.20)	2.506	0.0122

\*The *Z* and *P* value were calculated by rank sum test, while the pancreatic adenocarcinoma group compared with the other groups, respectively

**Fig. 2** Scatterplot of serum galectin-3 level detected by TRIFA and ELISA



**Fig. 3** ROC curves of serum galectin-3 as detected by TRIFA and ELISA for the diagnosis of pancreatic adenocarcinoma

**Table 3** Comparison of diagnostic sensitivity of galectin-3 detected by TRIFA and ELISA

Subgroups	n	Galectin-3 (≥3.77 ng/ml) TRIFA		Galectin-3 (≥0.26 ng/ml) ELISA	
		n	%	n	%
Pancreatic adenocarcinoma	49	37	75.5*	16	32.7**
Benign pancreatic neoplasms	16	2	12.5	0	0
Acute pancreatitis	36	6	16.7	2	5.6
Healthy control	36	0	0	0	0

\* and \*\*  $P < 0.05$ , pancreatic adenocarcinoma group compared with the other groups, respectively

$P < 0.05$ , the sensitivity of galectin-3 detected by TRIFA compared with that detected by ELISA

Statistical analyses were performed by chi-square test or Fisher’s exact test

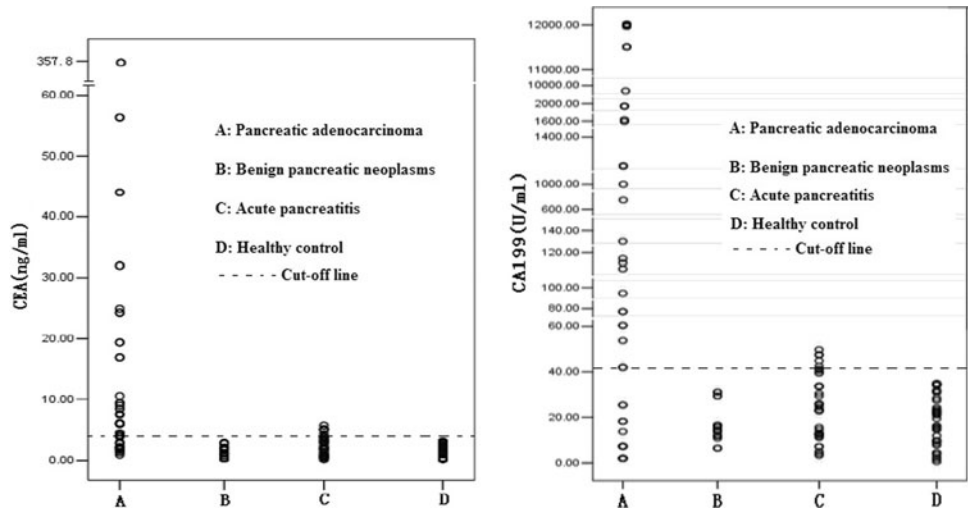
and secondary malignant tumors (Raz et al. 1990). In digestive system, higher levels of galectin-3 were reported in colon (Arfaoui-Toumi et al. 2010), stomach (Okada et al. 2006) and liver cancer (Matsuda et al. 2008). Galectin-3 has also been detected overexpressed in other tumors (Chiu et al. 2010; Szöke et al. 2007; Righi et al. 2010; Sakaki et al. 2010; Abdou et al. 2010; Koo and Jung 2011; Choi et al. 2010; Saffar et al. 2011; Brustmann 2008; Canesin et al. 2010). The mechanisms by which galectin-3 brings into its effects largely remain unknown. Some scientists assumed that pancreatic carcinoma cell-associated MUC4, overexpressed in pancreatic carcinoma, helps in the docking of tumor cells on the endothelial surface. During cancer progression, MUC4-galectin-3 may expose the surface adhesion molecules, which in turn promotes a stronger attachment (locking) of tumor cells to the endothelial surface (Senapati et al. 2011). Galectin-3-dependent mechanism modulated the regulation of MUC1/EGFR (epidermal

**Table 4** Serum levels of CEA and CA199 in different pancreas diseases and healthy subjects

Subgroups	n	CEA (ng/ml)			CA199 (U/ml)		
		(median, range)	Z*	P*	(median, range)	Z*	P*
Pancreatic adenocarcinoma	49	6.10 (0.91–357.80)			115.60 (2.00–12,000.00)		
Benign pancreatic neoplasms	16	1.80 (0.19–2.91)	4.723	0.0000	15.80 (6.50–31.10)	3.007	0.0026
Acute pancreatitis	36	2.24 (0.07–5.72)	4.839	0.0000	24.48 (3.36–49.72)	3.508	0.0005
Healthy control	36	0.86 (0.07–3.11)	5.898	0.0000	21.60 (0.62–34.94)	3.899	0.0001

\* The Z and P value were calculated by rank sum test, while the pancreatic adenocarcinoma group compared with the other groups, respectively

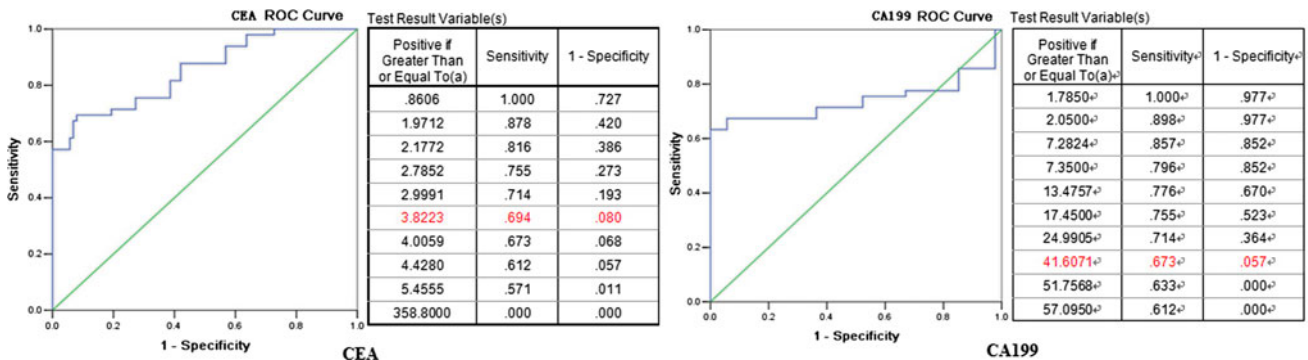
**Fig. 4** Scatterplot of serum levels of CEA and CA199 in different pancreas diseases and healthy subjects



**Table 5** Serum levels of CEA and CA199 for the diagnosis of pancreatic carcinoma

Subgroups	n	CEA ( $\geq 3.82$ ng/ml)		CA199 ( $\geq 41.61$ U/ml)	
		n	%	n	%
Pancreatic adenocarcinoma	49	34	69.4*	33	67.3*
Benign pancreatic neoplasms	16	0	0	0	0
Acute pancreatitis	36	7	19.4	5	13.9
Healthy control	36	0	0	0	0

\*  $P < 0.05$ , pancreatic adenocarcinoma group compared with the other groups, respectively  
 Statistical analyses were performed by chi-square test or Fisher’s exact test



**Fig. 5** ROC curve of serum levels of CEA and CA199 for the diagnosis of pancreatic adenocarcinoma

**Table 6** Complementary value of galectin-3, CEA and CA199 to diagnose pancreatic carcinoma

	Sensitivity	Specificity	Accuracy
Galectin-3 positive (a)	75.5*	90.9	85.4
CEA positive (b)	69.4 <sup>#</sup>	92.0	83.9
CA199 positive (c)	67.3 <sup>#</sup>	94.3 <sup>Δ</sup>	84.7
a+b	92.5	83.6	84.7
a+c	92.0	85.7	85.1
b+c	90.0	86.8	84.3
a+b+c	97.5	78.8	84.7

\*  $P < 0.05$ , a versus (a+b+c); <sup>#</sup>  $P < 0.05$ , b and c versus (a+b), (a+c), (b+c) and (a+b+c); <sup>Δ</sup>  $P < 0.05$ , c versus (a+b+c); Statistical analyses were performed by chi-square test or Fisher's exact test

growth factor receptor) functions to the EGFR-stimulated cell growth of pancreatic carcinoma cells (Merlin et al. 2011). Free circulating galectin-3 and cancer-associated MUC1 promotes embolus formation and thus spreading tumor cells in the circulation (Zhao et al. 2010). With galectin-3, cancer-associated MUC1 led to exposure of smaller cell surface adhesion molecules/ligands including CD44 and ligand(s) for E-Selectin by causing MUC1 polarization (Zhao et al. 2009). Galectin-3 also plays a role as a mediator of vascular endothelial growth factor (VEGF)- and basic fibroblast growth factor (bFGF)-mediated angiogenic response (Markowska et al. 2010). These findings suggest that galectin-3 binding protein may be a promising therapeutic strategy with high expression of galectin-3 binding protein (Kim et al. 2011).

In this study, we identified serum galectin-3 as a potential tumor marker for pancreatic carcinoma by both TRFIA and ELISA methods. Elevated galectin-3 protein levels could be detected in the serum of patients with pancreatic carcinoma. The application of galectin-3 as a new marker strongly improves the diagnostic power of conventional serum tumor marker panels consisting of CA 199 and CEA.

TRFIA method was used to detect galectin-3 in this study. The results showed that with internal controls, TRFIA data are reproducible. Diagnostic agreement between ELISA and TRFIA was evaluated using different cutoffs on the bases of ROC curve. Though ELISA kits for galectin-3 detection are widely used, it is evident that commercial ELISA kits revealed much lower sensitivity than TRFIA. This study provided an opportunity to compare the performance of the two methods to detect galectin-3. The detection results with ELISA kits were disappointing. Obviously, TRFIA for the detection of galectin-3 is superior to ELISA because of its higher sensitivity. Our finding is in agreement with many previous studies on the detection of other biomolecules (Aceti et al. 1988; Maple et al. 2001). The greater sensitivity of TRFIA benefits from the use of a

highly detectable “tracer” and the sensitivity of labeled reagent techniques may be greatly increased by improving the signal-to-noise ratio. On the other hand, the greater specificity of TRFIA is related to the lack of background which, in the ELISA, produces false-positive interpretations. Also, TRFIA is simple to perform, accurate, reproducible and amenable to automation with a large dynamic range. Because of its better analytical performance, TRIFA is more suitable to the determination of serum galectin-3 than conventional ELISA.

In this study, 3 tumor markers displayed their diagnostic value for pancreas carcinoma. The best performance of these 3 markers in sensitivity was galectin-3 (75.5%) and the best in specificity was CA199 (94.3%). The diagnostic power of galectin-3 in combination with the conventional tumor markers CA 199 and CEA, by performing ROC analysis, has been greatly improved. Combined determination of these 3 markers would provide a higher sensitivity level for the diagnosis of pancreas carcinoma.

It is a limitation of this study that we did not validate the discriminating ability of markers in another cohort of pancreatic cancer patients by estimating the sensitivity and specificity independently. However, the bootstrap estimate of bias in calculating the AUC was quite small, resulting in a decrease in the AUC only by a very small value. In consequence, our presented data about the sensitivity and specificity may serve as a measure of the diagnostic power.

## Conclusion

Galectin-3 is overexpressed in pancreatic carcinoma tissues. TRFIA is capable of sensitive and reproducible detection of serum galectin-3, which may serve as a novel marker of pancreatic carcinoma. Combined determination of serum levels of galectin-3, CEA and CA199 may improve the diagnostic power for pancreatic carcinoma.

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**Conflict of interest** We declare that we have no conflict of interest.

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