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Clinical significance of serum survivin in patients with pancreatic ductal adenocarcinoma

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Abstract. – OBJECTIVE: We investigate the clinical significance of survivin levels in pancreatic ductal adenocarcinoma (PDAC) patients.

PATIENTS AND METHODS: The serum level of survivin from patients with PDAC (n = 80) and age-matched healthy volunteers (n = 80) were analyzed by enzyme-linked immunosorbent assays (ELISA) prior to surgical resection. Expression levels were correlated with clinicopathological parameters.

RESULTS: Serum survivin concentrations were significantly elevated in PDAC patients when compared to healthy sera (p = 0.001). High serum survivin levels were significantly associated with perineural invasion, venous invasion, lymph node status (N stage), histologic grade and T stage, but not with the tumor size, age, gender of the patients or tumor location. The median overall survival of the normal serum survivin group was longer than that of the elevated serum survivin group. The independent factors associated with overall survival were advanced pancreatic cancer and elevated serum survivin level.

CONCLUSIONS: Patients with elevated serum survivin level at diagnosis demonstrated poor overall survival. Pretreatment survivin level may predict the prognosis of patients with PDAC.

Key Words:
Pancreatic ductal adenocarcinoma, Prognosis, Survivin.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths in Western countries and has the poorest survival rate (< 5%) among the common malignancies[1,2]. Although surgical resection shows promise as an effective treatment for PDAC, a lack of effective tools for diagnosis in the earliest stages results in low 5-year survival rates, which drop rapidly from > 50% in patients with stage I to < 5% in patients with more advanced stages[3-5]. Both imaging techniques and serological markers, such as carbohydrate antigen19-9 (CA19-9) and carcinoembryonic antigen (CEA), have relatively poor diagnostic sensitivity and specificity[6,7]. In addition, it is still difficult to distinguish chronic pancreatitis (CP) patients, a high-risk population of PDAC, from PDAC patients[8-11]. Therefore, to improve the prognosis of PDAC, it is urgent to develop specific and noninvasive biomarkers for PDAC diagnosis, especially for early-stage tumors.

Survivin is a member of the inhibitor of apoptosis protein (IAP) family originally discovered in the baculovirus[12]. Structurally, survivin is a unique IAP protein[13], organized as a stable dimmer[14], containing only one baculoviral IAP repeat domain and a –COOH terminus coiled-coiled domain[15]. Survivin is unique for its expression in a wide range of embryonic and foetal tissues but is undetectable in terminally differentiated normal adult tissues. However, it is re-expressed in transformed cell lines and several human cancer cells at a frequency of 34-100%[15-16]. As a prognostic factor, survivin expression is significantly associated with poor clinical outcome in cancers, such as neuroblastoma, colorectal cancer, breast cancer, lung cancer,esophageal cancer, medulloblastoma and pediatric acute lymphoblastic leukemia[17-24].

Survivin expression in pancreatic cancer has been widely studied. Satoh et al[25] has reported that expression of survivin may be upregulated during an early step of tumorigenesis and during the development of cancer by reducing the cancer cell apoptosis. Kami et al[26] and Ekeblad et al[27] has reported that survivin expression in pancreatic cancer tissues could be a useful prognostic marker in

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pancreatic cancer patients. Lee et al[28] has found survivin expression seems to have a potential as a predictive marker for chemotherapy.

Although elevated expression of survivin was found in many cancer tissues including PDAC tissues and its association with tumour behaviour and patient prognosis, there are few studies analyzing the serum levels of survivin in patients with PDAC. In the present work, we have determined the serum levels of survivin in patients with PDAC with a special interest to determine the levels and prognostic value of their serum levels.

Patients and Methods

Patients and Clinical Data

For this study, blood sera (n = 80) of patients with PDAC and the sera of 80 controls was collected from Jan 1, 2007-Dec 30, 2012. All blood samples were collected before any therapeutic procedures, including surgery chemotherapy, and radiotherapy. Blood samples were taken directly before surgery. After centrifugation of the peripheral blood, serum samples were stored at −20°C until assayed. PDAC diagnosis was confirmed by histological examination or fine-needle aspiration cytology.Histological typing of the tumors was performed according to WHO criteria. All the enrolled PDAC patients showed the tumor histotype of pancreatic ductal adenocarcinoma. Tumors were staged according to the sixth edition of the American Joint Committee on Cancer tumor-node-metastasis (TNM) system. Overall survival was calculated from the date of operation to the date of death or last follow-up. Patients who did not survive the first 30 days after surgery were excluded from the survival analysis. Patients' clinicopathological characteristics are summarized in Table I. Median age was 46 years (range, 32-71 years). 54 patients had stage I and II disease, and 18 patients had stage III. Disease and 6 had stage IV Disease. 18 patients had only palliative resection and treated combination chemotherapy with epirubicin, cisplatin and 5-FU. Of 37 patients who had curative surgery, 25 patients underwent adjuvant treatment with radiotherapy. Recurrent cancer developed in 28 patients, and the median time to recurrence was 163 days (range, 51-1370 days).

The 80 healthy controls at the Healthy Physical Examination Center of the affiliated hospital of medical college, Qingdao university. The health condition checkup included a detailed history; physical, radiological, and endoscopic examinations; blood tests; tumor marker tests (CA19-9, CEA); and abdominal sonography. Subjects with no evidence of pancreatic disease or other abnormalities were enrolled as cancer-free controls.

The study was approved by the Ethics Committee of the Affiliated Hospital of Medical College, Qingdao University. Written consent for using the samples for research purposes was obtained from all patients prior to surgery or blood drawing.

The Measurement of Serum Survivin Levels

Survivin (Quantikine Human survivin Immunoassay, R&D System, Minneapolis, MN, USA) concentrations was determined by means of an enzymelinked immunosorbent assay (ELISA) method according to the manufacturer's instructions. All specimens were assayed twice. The minimum detectable dose (MDD) of survivin ranged from 1.58 pg/ml to 9.96 pg/ml.

Statistical Analysis

All statistical analysis was performed using SPSS statistical software (SPSS Inc., Chicago, IL, USA). Data are presented as mean (SE). We used Student *t*-tests to compare the differences in serum survivin concentrations between the cancer group and control group. The relationship between serum survivin level and each of the clinicopathological parameters was analyzed by ^{2} analysis. Survival curves were plotted with method of Kaplan-Meier. The statistical difference in survival between different groups was compared by the log-rank test. Survival correlation with the prognostic factors was further investigated by multivariate analysis using Cox proportional hazards model with backward stepwise likelihood ratio. $p < 0.01$ was considered statistically significant.

Results

Serum Levels of Survivin

The serum survivin levels were significantly higher in PDAC patients than in healthy controls (70.38 ± 12.2 pg/ml vs 91.4 ± 14.6 pg/ml, $p < 0.01$). The cut-off value was set at 82.58 pg/ml, based on the data of the 80 healthy control sera. Increased serum survivin levels were found in 63.75% (51/80) of patients with PDAC and 36.25% (29/80) was found less 82.58 pg/ml.

Serum Survivin Level and Clinicopathological Features in PDAC Patients

Using 82.58 pg/ml as the cutoff value, these PDAC patients were then divided into group A (n = 51) as those with the higher level (≥ 82.58 pg/ml) and group B (n = 29) as those with lower level (< 82.58 pg/ml). χ^2 analysis showed that the preoperative serum survivin levels correlated well with perineural invasion, venous invasion, lymph node status (N stage), histologic grade and tumor stage, but not with the tumor size, age, gender and lymph node status (N stage) of the patients or tumor location (Table I). Spearman analysis revealed a correlation between serum survivin level and T stage, perineural invasion, venous invasion, recurrence and histological differentiation (Table II). These observations support the hypothesis that the progression of PDAC is associated with increased serum survivin levels.

Association between Serum Levels of Survivin and Prognosis, and Survival Rates for PDAC Patients

A log-rank test and Kaplan-Meier analysis were used to calculate the effect of serum levels of survivin on survival. The log-rank test showed that the serum levels of survivin attested remarkably to patients' survival time ($p < 0.0001$; Figure 1). More specifically,the median survival time of patients with high serum levels of survivin was only 9 months, whereas the median survival time of those with low levels of survivin was 26

Table I. Relationship between serum levels of survivin and clinicopathological factors.

Clinicopathological factors	Groups[a]		
	A (n)	B (n)	P[b]
Age (yr)			NS
< 50 (n=32)	23 (71.8%)	9 (28.2%)	
> 50 (n=48)	28 (58.3%)	20 (41.7%)	
Gender			NS
M (n=62)	40 (64.5%)	22 (35.5%)	
F (n=18)	11 (61.1%)	7 (38.9%)	
Location			NS
Head (n=55)	34 (61.8%)	21 (38.2%)	
Body (n=3)	3 (0%)	0 (100%)	
Tail (n=7)	4 (57%)	3 (43%)	
Body & Tail (n=15)	10 (66.7%)	5 (33.3%)	
Stage (TNM)			< 0.01
I (n=17)	7 (41.2%)	10 (58.8%)	
II (n=37)	23 (62.1%)	17 (37.9%)	
III (n=18)	15 (83.3%)	3 (16.7%)	
IV (n=6)	6 (100%)	0 (0%)	
Lymphatic invasion			< 0.01
Positive (n=47)	30 (63.8%)	17 (36.2%)	
Negative (n=33)	12 (63.6%)	12 (36.4%)	
Perineural invasion			< 0.01
Yes (n=55)	44 (80%)	11 (20%)	
No (n=25)	7 (28%)	18 (72%)	
Vein invasion			< 0.01
Yes (n=50)	40 (80%)	10 (20%)	
No (n=30)	11 (36.6%)	19 (63.4%)	
Cell differentiation			< 0.01
Well (n=10)	2 (20%)	8 (80%)	
Moderate (n=57)	37 (64.9%)	20 (35.1%)	
Poor (n=8)	8 (100%)	0 (0%)	
Others (n=5)	4 (80%)	1 (20%)	
Recurrence			< 0.01
Yes (n=56)	45 (80.3%)	11 (19.7%)	
No (n=24)	6 (25%)	18 (75%)	

[a]Patients were grouped by preoperative serum levels of survivin. In group A (n = 51), serum survivin levels were > 82.58 pg/ml, and in group B (n = 29), survivin levels were 82.58 pg/ml. p were determined by ξ^2 test. NS: No significance.

Table II. Spearman correlation analysis between serum survivin levels and clinical pathologic factors.

Variables	Spearman correlation	*p* value
Age	-0.09	NS
Gender	-0.12	NS
Location	0.07	NS
Stage (TNM)	0.783	< 0.0001
Lymphatic invasion	0.656	< 0.01
Perineural invasion	0.430	< 0.001
Vein invasion	0.586	< 0.0001
Cell differentiation	0.52	< 0.0001
Recurrence	0.12	NS

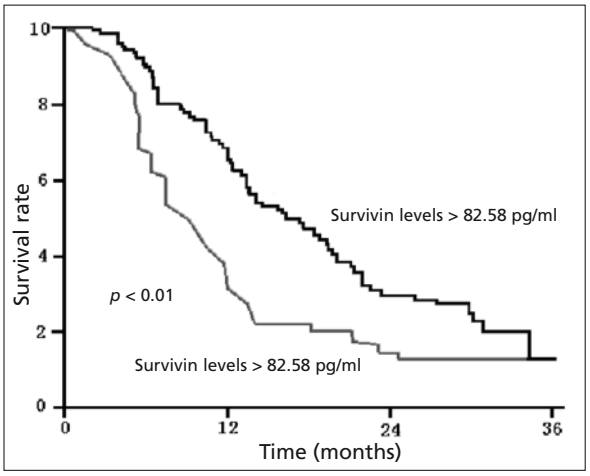

Figure 1. The serum Survivin level corresponded with the progression of PDAC.

months. The cumulative 5-year survival rate was 38.4% in the low serum levels of survivin group (n= 29), whereas in the high serum levels of survivin group (n = 51; Figure 1), the survival rate was only 9.28%. Clinical stage, serum levels of survivin, T classification, histological differentiation, perineural invasion and vein invasion were analyzed using univariable and multivariable Cox regression analyses. Univariable analysis revealed that clinical stage, serum levels of survivin and histological differentiation were significant predictors of ESCC (Table III). Multivariable analysis showed that clinical stage, serum levels of survivin and histological differentiation were independent predictors for PDAC on the basis of changes in likelihood interactions between the parameters listed in univariable regression analysis (Table III).

Discussion

Identification of targets for early detection of PDAC is important to improve the prognosis of the patients with this pernicious disease. Currently, carcinoembryonic antigen and cytokeratin-19 fragments are routinely used as serum markers for detection of PDAC. Due to the low sensitivity and specificity of detection of these markers[6,7], additional serum markers must be established for early detection and diagnosis of PDAC.

Survivin, an IAP, has been studied as a prognostic marker in various cancers. Adida et al[17] reported that surviving expression in neuroblastoma correlated with unfavorable histology, aggressive and disseminated disease. In colorectal cancer and breast cancer, patients with surviving positive tumors had a decreased apoptotic index and worse survival rates than those with survivin-negative tumors[19,29]. In addition, survivin expression correlated with poor prognosis in esophageal cancer and non-small cell lung cancer[21,22]. Survivin expression in pancreatic cancer tissues could also be a useful prognostic marker in pancreatic cancer patients[26,27].

In the present study, we detected survivin levels in the serum of patients are significantly higher than in a control population of healthy blood

Table III. Univariate and Multivariate analysis various prognosic parameters in PDAC patients.

Characteristic	Univariate analysis		Multivariate analysis	
	Regression coefficient (SE)	*p* value	95%CI	*p* value
Survivin levels	0.317 (0.042)	< 0.001	2.89 (1.98-5.83)	0.003
Stage (TNM)	0.156 (0.06)	0.027	2.74 (1.56-5.14)	0.014
Lymphatic invasion	0.178 (0.013)	0.042	0.54 (1.12-4.34)	0.015
Perineural invasion	0.65 (0.18)	0.012	0.84 (0.53-1.27)	0.002
Vein invasion	0.54 (0.12)	0.034	2.34 (1.48-5.13)	0.021
Cell differentiation	0.74 (0.28)	< 0.01	2.46 (1.75-5.54)	0.004
Recurrence	0.33 (0.18)	0.093	1.64 (1.15-3.38)	0.084

donors. Notably, serum survivin levels were higher in patients with perineural invasion, lymph node status (N stage) and venous invasion compared to those without. Thus, survivin concentrations may be as the suitability as a routine serum marker for the detection of metastatic disease in PDAC.

In addition, serum survivin levels was much higher in PDAC patients with advanced stages and poor differentiation, however, no significant differences were found in the serum levels of survivin in PDAC patients with, ages germ and tumor size. Serum levels of survivin is useful for predicting the prognosis of patients with PDAC.

Evaluation of the survival data showed that the survival was significantly shorter in patients with survivin concentrations > 82.58 pg/ml, and high survivin concentrations predict poor prognosis for patients with PDAC, especially in those presenting with metastasis. In addition, the uni- and multivariate analyses in the present study showed the prognostic significance of clinicopathologic factors such as TNM and differiation. These findings indicate that the results obtained from the present series of cases are applicable to PDAC in other counties. In addition, Cox regression analysis showed that survivin was an independent variable.

In the present study, we have assessed the prognostic value of serum survivin in PDAC patients and identified survivin as an independent prognostic factor. Due to the relatively limited number of controls and patients in this study, further studies are required. In addition, studies with serum samples obtained at different phases of PDAC progression could further elucidate the role of survivin in growth and metastasis of PDAC.

Conclusions

Our findings provide evidence that survivin has important role at different phases of metastatic spread and that measurement of serum survivin, in particular, could be of clinical value when identifying patients at high risk for progression.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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