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ORIGINAL RESEARCH PAPER

Changes in the expression of serum markers CA242, CA199, CA125, CEA, TNF-α and TSGF after cryosurgery in pancreatic cancer patients

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Abstract The presence of serum tumor markers, carbohydrate antigen 242 (CA242), carbohydrate antigen 199 (CA199), carbohydrate antigen 125 (CA125), carcinoembryonic antigen (CEA), tumor-supplied group of factors (TSGF) and tumor necrosis factor-α (TNF-α), is closely associated with invasion and metastasis of many malignancies. The expression of these markers were measured in serum taken from 37 pancreatic cancer patients prior to treatment. Levels of CA242, CA199, CA125, CEA and TNF-α expression correlated with tumor size, clinical stage, tumor differentiation, lymph node and liver metastasis ($P < 0.05$). One month after cryosurgery, serum levels of these markers were significantly reduced compared with levels prior to cryosurgery ($P < 0.05$), whereas there was no significant difference was found between serum levels before and after chemotherapy ($P > 0.05$). Thus, cryosurgery is more effective than chemotherapy for decreasing CA242, CA199, CA125, CEA, TSGF and TNF-α serum levels in these patients.

Keywords Chemotherapy · Cryosurgery · Pancreatic cancer · Tumor markers

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Introduction

Pancreatic cancer (PC) is one of the most lethal malignancies (Michalski et al. 2007), and the 5 year survival rate for PC patients is less than 5 % (Hirata et al. 2007). Due to a lack of easily observable symptoms, patients cannot be diagnosed at an early stage and therefore less than 20 % of PC patients are candidates for surgical resection. Moreover, the post-operative 5-year survival rate is only 15–25 % (Carpelan et al. 2005; Wagner et al. 2008). Chemotherapy and radiotherapy are common methods for treating advanced-stage PC, but the median survival time after such treatments is less than 10 months (Eickhoff et al. 2006; Wilkowski et al. 2006). It is therefore essential to identify new and effective therapies for PC.

Cyosurgical techniques have been used since the end of the 20th century with the availability of high-resolution imaging techniques and new freezing equipment, and have been used to treat many types of tumors (Gage and Baust 2007; Mouraviev and

Polascik 2006; Xu and Niu 2003; Xu and Niu 2008). PC patients respond well to cryosurgery, with few surgical complications or deaths associated with this treatment (Kovach et al. 2002). A previous study confirmed that eight PC patients treated with cryosurgery survived for 24 months or more (Xu and Niu 2008). However, the prognosis of PC patients after cryosurgery is still undefined and needs further evaluation.

It has been difficult to identify non-invasive and reliable methods for monitoring the prognosis of PC patients. This has been complicated by the discovery that different mechanisms for tumor development and metastasis exist between animal models of PC and humans. Tissue-based assays for monitoring cancer progression require invasive pancreatic biopsies and are therefore unsuitable for routine use in the clinic. However, the serum tumor markers, CA242, CA199, CA125, CEA, TSGF and TNF-α, have been closely linked to PC metastasis and progression and are widely used for monitoring the prognosis of PC patients (Banfi et al. 2000; Giraudo et al. 1998; Jiang et al. 2004; Schlieman and Bold 2003) although complications arising from tumor invasion and metastasis have their general acceptance as prognostic indicators.

In this study, the serum expression levels of potential PC biomarkers were investigated before and after treatment. The present study was designed to investigate changes in CA242, CA199, CA125, CEA, TSGF and TNF-α expression in PC patients after cryosurgery and to determine whether these tumor biomarkers are suitable for monitoring PC progression following cryosurgery.

Materials and methods

Patients and samples

Thirty-seven PC patients, 22 males and 15 females, were enrolled in a study at Fuda Hospital in Guangzhou, China, with an average age of 58 ± 15.3 years. A total of 22 tumors were located in the head of the gland, five in the body and ten in the tail. According to the tumor node metastasis (TNM) standard, only one tumor was stage I, five were stage II, seven were stage III and 24 were IV stage. Of the 37 patients, 18 underwent cryosurgery and 19 underwent chemotherapy. Twelve healthy individuals with no tumor history were selected as normal controls, including seven males and five females, with an average age of 47 ± 14.2 years. Informed consent was obtained from each patient and healthy individual. Protocols conformed to the ethical guidelines of the Declaration of Helsinki in 1975 were and approved by the Research Ethics Committee of Fuda Hospital of the Chinese Academy of Sciences.

Peripheral blood samples (5 ml) were collected from patients 1 day prior to cryosurgery or chemotherapy and from healthy individuals (controls) using a sterile needle. The cryosurgery and chemotherapy were performed according to the previous procedure (Xu et al. 2008). After treatment, blood samples were collected from patients after 10 d and after 1 month. All blood samples were centrifuged at $\sim 4{,}000 \times g$ for 5 min and serum supernatants were stored at −20 °C.

Measuring CA242, CA199, CA125, CEA, TSGF and TNF-α serum levels

CA242, CA199 and CA125 expression levels were determined using a chemiluminescence assay kit, according to the manufacturer's instructions (Roche, Shanghai, China). CEA expression levels were determined using an immunoradiometric assay (IRMA) kit, according to the manufacturer's protocol (Chemclin Biotechnology, Beijing, China). TNF-α expression levels were determined using ELISA according to the manufacturer's instructions (Sun Biomedical Technology, Beijing, China). TSGF expression levels were determined using a biochemical assay kit, according to the manufacturer's protocol (Newland Life Technology, Fujian, China).

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences Predictive Analytics Software (SPSS PASW Statistics ver. 18.0; SPSS Inc., Chicago, IL, USA). Correlations between tumor markers and PC tumor pathology were determined using Spearman's correlation. Groups were compared using t tests and results are presented as the mean ± SD. A probability value of <0.05 was considered to be statistically significant.

Results and discussion

Serum levels of CA242, CA199, CA125, CEA, TSGF, TNF-α correlate with PC pathology

Thirty-seven PC patients were clinically diagnosed by imaging and histopathology. CA242, CA199 and CA125 serum levels showed a significant correlation with sex and tumor location ($P < 0.05$). As previously reported, CA199 and CEA expression levels showed a significant correlation with age ($P < 0.05$), (Kang et al. 2007). These results indicate that age, sex and tumor location may be factors influencing PC progression. Serum levels of CA199, CA242, CA125, CEA and TNF-α showed a significant correlation with tumor size, tumor differentiation, clinical stage and metastasis to lymph nodes and liver ($P < 0.05$), implying that CA199, CA242, CA125, CEA and TNF-α play important roles in tumor progression and are significant predictors of PC invasion and metastasis (Tables 1, 2). This may be related to the function of CA199, CA242, CA125 and CEA in cellular adhesion, an important factor in proliferation, migration and differentiation of tumor cells (Giannini et al. 2001; Jiang et al. 2004). Therefore, these proteins may facilitate PC tumor progression and metastasis. TNF-α is a multifunctional cytokine and reported to promote the growth and metastasis of tumors (Giraudo et al. 1998). However, TSGF expression levels were not associated with the clinical stage, tumor size, differentiation and metastasis of PC ($P < 0.05$), indicating that TSGF expression related to blood vessel hyperplasia in malignant tumors is not an index for assessing PC prognosis.

Serum levels of CA242, CA199, CA125, CEA, TSGF and TNF-α change after treatment

Tumor markers are widely used as indicators to monitor the effectiveness of therapy and cancer

Table 1 Relations between CA242, CA199 and CA125 expression levels and PC pathology

Pathological features	No	CA242 (U/ml)	*P*	CA199 (U/ml)	*P*	CA125 (U/ml)	*P*
Sex			0.112		0.121		0.078
Male	22	125.7 ± 18.2		21333.7 ± 9865.4		609.2 ± 201.8	
Female	15	98.1 ± 19.5		34312.9 ± 15412.2		241.4 ± 19.9	
Age			0.433		0.014		0.078
<60	14	124.3 ± 20.1		6123.8 ± 3433.2		187.8 ± 29.4	
>60	23	100.2 ± 16.3		$^{\Delta}$35476.4 ± 12876.3		$^{\Delta}$533.2 ± 101.3	
Tumor position			0.511		0.213		0.234
Head	22	125.8 ± 23.2		26732.4 ± 8876.9		411.1 ± 119.2	
Body and tail	15	111.4 ± 19.6		14765.8 ± 5653.1		278.4 ± 67.8	
Tumor size			0.028		0.044		0.041
<4 cm	15	67.2 ± 19.4		13777.4 ± 7652.9		168.4 ± 32.0	
>4 cm	22	$^{\Delta}$145.3 ± 18.2		$^{\Delta}$38731.6 ± 12111.7		$^{\Delta}$782.2 ± 229.3	
Clinical stage			0.021		0.005		0.013
I–III	13	50.4 ± 17.4		929.8 ± 381.2		42.9 ± 24.7	
IV	24	$^{\Delta}$129.9 ± 13.5		$^{\Delta}$32887.3 ± 11098.3		$^{\Delta}$512.3 ± 234.1	
Differentiation			0.031		0.045		0.01
Low	9	74.2 ± 22.6		13061.8 ± 5671.2		56.3 ± 16.9	
Moderate and well	28	129.4 ± 18.8		$^{\Delta}$35642.8 ± 10912.1		$^{\Delta}$578.9 ± 121.4	
Lymph nodes metastasis			0.019		0.015		0.016
–	12	66.6 ± 18.4		5566.4 ± 1234.1		57.8 ± 19.2	
+	25	131.2 ± 26.4		$^{\Delta}$38992.4 ± 12135.8		$^{\Delta}$634.9 ± 157.1	
Liver metastasis			0.037		0.047		0.04
–	13	77.6 ± 21.5		12312.4 ± 4308.2		87.4 ± 30.6	
+	24	128.919.4		$^{\Delta}$36123.9 ± 12522.1		$^{\Delta}$644.8 ± 218.4	

Table 2 Relations between CEA, TSGF and TNF-α expression levels and PC pathological features

Pathological features	No.	CEA (ng/ml)	*P*	TSGF (U/ml)	*P*	TNF (pg/ml)	*P*
Sex			0.014		0.333		0.212
Male	22	23.7 ± 4.9		16.2 ± 3.8		11.2 ± 3.7	
Female	15	$^{\Delta}$4.8 ± 1.6		16.8 ± 2.4		14.4 ± 2.8	
Age			0.023		0.431		0.278
<60	14	4.5 ± 1.7		14.1 ± 3.9		11.5 ± 3.6	
>60	23	$^{\Delta}$18.5 ± 5.5		16.3 ± 3.8		15.6 ± 4.8	
Tumor position			0.213		0.213		0.444
Head	22	11.6 ± 3.8		15.4 ± 3.5		13.4 ± 3.7	
Body and tail	15	13.3 ± 3.2		15.9 ± 2.8		14.6 ± 2.8	
Tumor size			0.037		0.544		0.027
<4 cm	15	10.3 ± 4.6		16.5 ± 2.1		9.7 ± 2.6	
>4 cm	22	$^{\Delta}$18.9 ± 5.7		16.9 ± 2.2		16.5 ± 3.9	
Clinical stage			0.012		0.621		0.031
I–III	13	3.6 ± 0.6		14.9 ± 2.7		10.4 ± 2.4	
IV	24	$^{\Delta}$18.2 ± 5.7		15.5 ± 2.4		16.1 ± 3.2	
Differentiation			0.01		0.377		0.026
Low	9	4.5 ± 1.2		15.9 ± 2.1		8.4 ± 1.6	
Moderate and well	28	$^{\Delta}$19.9 ± 6.3		17.6 ± 1.9		15.4 ± 3.1	
Lymph nodes metastasis			0.013		0.389		0.038
–	12	4.9 ± 1.2		15.8 ± 2.4		11.8 ± 3.2	
+	25	$^{\Delta}$22.4 ± 5.3		17.1 ± 2.6		16.4 ± 3.3	
Liver metastasis			0.047		0.478		0.018
–	13	11.1 ± 3.9		15.2 ± 1.9		8.6 ± 2.7	
+	24	$^{\Delta}$17.2 ± 3.8		16.2 ± 1.8		17.8 ± 3.3	

recurrence. We confirmed that CA242, CA199, CA125, CEA, TSGF and TNF-α expression in PC patients before treatment was significantly higher than in healthy individuals ($P < 0.05$; Figs. 1, 2). However, serum levels of these factors gradually decreased in PC patients after cryosurgery. Moreover, levels of CEA, TSGF and TNF-α expression in patients 1 month after cryosurgery were not significantly different from the levels present in healthy individuals ($P > 0.05$; Figs. 1, 2). However, serum levels of CA242, CA199, CA125, CEA, TSGF and TNF-α in patients 10 days and 1 month after chemotherapy remained significantly higher than levels in healthy individuals ($P < 0.05$; Figs. 3, 4). Expression levels of CA199, CEA and TSGF 10 days after cryosurgery were significantly lower than levels in patients before treatment ($P < 0.05$). Furthermore, CA199, CA242, CA125, CEA, TSGF and TNF-α expression in patients 1 month after cryosurgery was significantly lower than in patients prior to cryosurgery ($P < 0.05$; Figs. 1, 2). However, at early (10 days) or later (1 month) times after chemotherapy the expression levels of all markers were not significantly different from levels prior to chemotherapy ($P > 0.05$; Figs. 3, 4).

These results indicate that cryosurgery is a more effective treatment for decreasing the expression of tumor markers than chemotherapy. Previous studies found that the median survival time of patients with locally advanced PC was only 6–10 months following chemotherapy and that CA199 levels were not obviously reduced (Okusaka et al. 2006; Park and Park 2007). However, Xu et al. (2008) reported that CA199 levels in PC patients were reduced from 210 to 48 U/mL after cryosurgery, consistent with our study. It is established that tumor markers are released from tumor cells during the cell cycle. Cryosurgery can

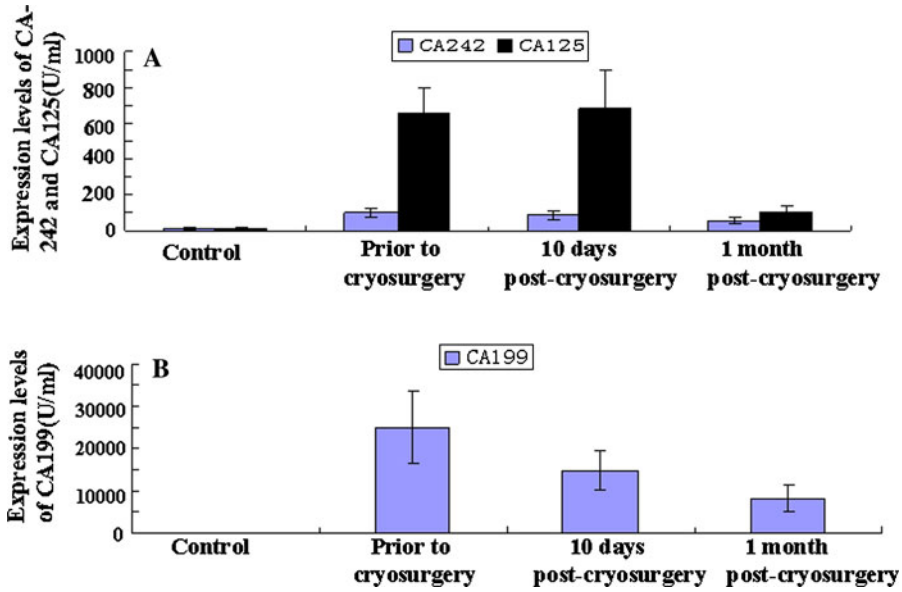

Fig. 1 Serum levels of CA242, CA199 and CA125 in PC patients treated with cryosurgery and in healthy individuals. PC patients were treated with cryosurgery alone. CA242, CA125 (**a**) and CA199 (b) expression levels were measured in serum samples taken from healthy individuals and from patients 1 day before treatment, and 10 days or 1 month following cryosurgery. *Number of patients* 18; *bars* represent SD

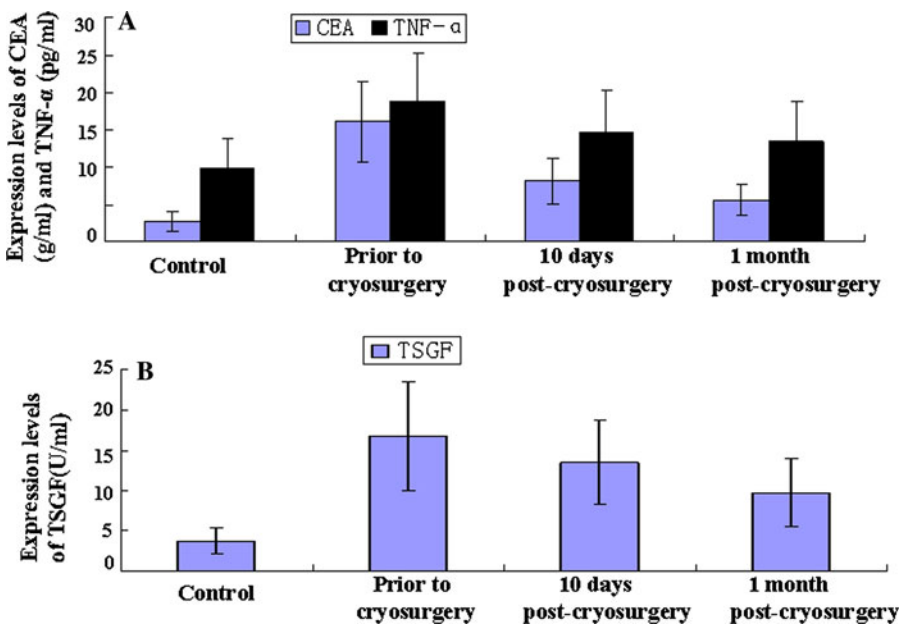

Fig. 2 Serum levels of CEA, TSGF and TNF-α in patients treated with cryosurgery and in healthy individuals. PC patients were treated with cryosurgery alone. CEA, TNF-α (**a**) and TSGF (**b**) and expression levels were measured in serum samples taken from healthy individuals and from patients 1 day before treatment, and 10 days or 1 month following cryosurgery. *Number of patients* 18; *bars* represent SD

induce the formation of extracellular and intracellular ice crystals and cause high levels of necrosis in tumor cells via freezing and thawing, leading to a reduction in CA242, CA199, CA125, CEA, TSGF and TNF-α levels after cryosurgery (Xu et al. 2003). Since high levels of serum tumor markers are closely associated with poor differentiation, rapid progression, and metastasis in PC, monitoring post-operative changes of CA242, CA199, CA125, CEA, TSGF and TNF-α expression may be an effective index for evaluating the therapeutic efficacy of cryosurgery.

Taken together, the dynamic changes observed in CA242, CA199, CA125, CEA, TSGF and TNF-α expression suggests that these biomarkers may serve as indicators for predicting disease progression in PC patients after cryosurgery. The use of these serum

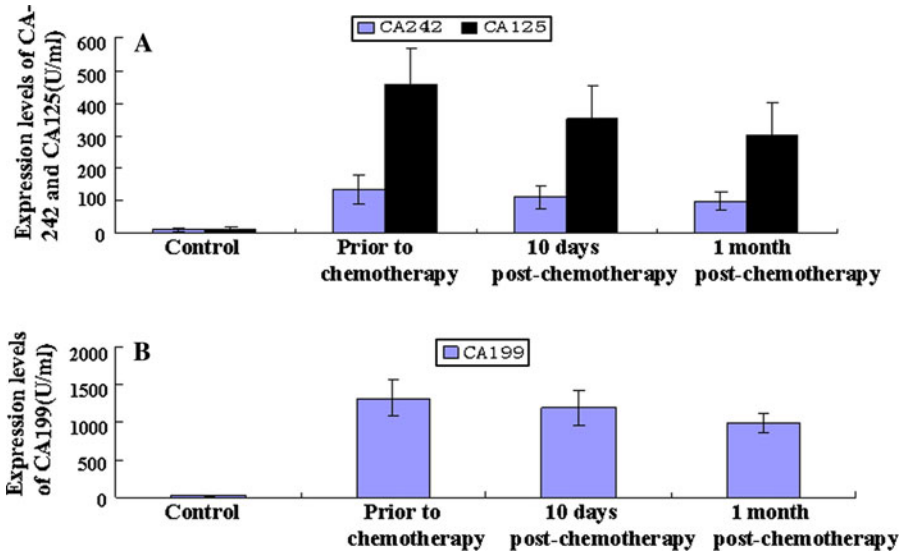

Fig. 3 Serum levels of CA242, CA199 and CA125 in patients following chemotherapy and in healthy individuals. PC patients were treated with chemotherapy alone. CA242, CA125 (**a**) and CA199 (**b**) expression levels were measured in serum samples taken from healthy individuals and from patients 1 day before treatment, and 10 days or 1 month following chemotherapy. *Number of patients* 19; *bars* represent SD

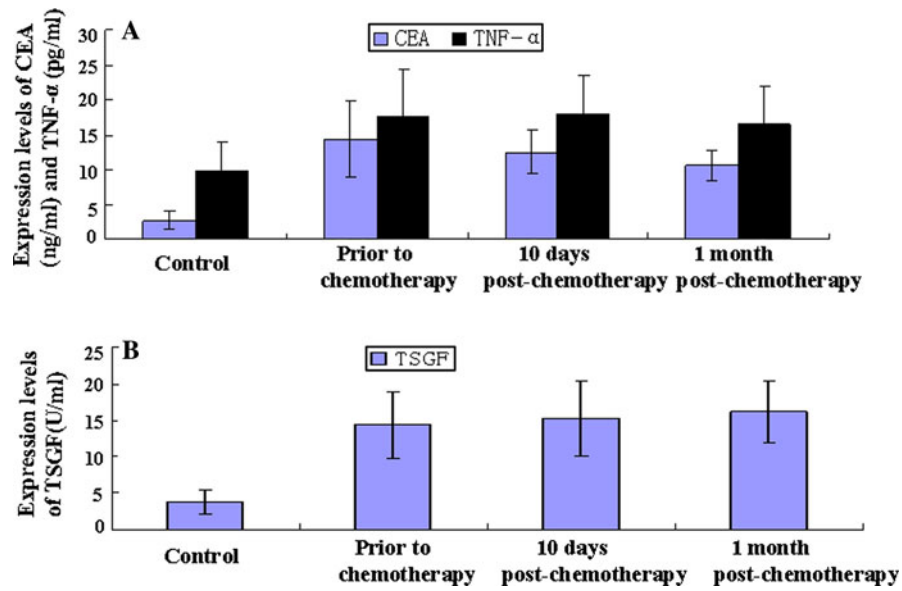

Fig. 4 Expression levels of CEA, TSGF and TNF-α in patients who following chemotherapy and healthy individuals. PC patients were treated with chemotherapy alone. CEA, TNF-α (**a**) and TSGF (**b**) expression levels were measured in serum samples taken from healthy individuals and from patients 1 day before treatment, and 10 days or 1 month following chemotherapy. *Number of patients* 19; *bars* represent SD

biomarkers may help to improve clinical management and to develop new therapeutic options for PC patients.

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