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RESEARCH

The Efficacy Evaluation of Cryosurgery in Pancreatic Cancer Patients with the Expression of CD44v6, Integrin-β1, CA199, and CEA

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Abstract Increased expression of cell adhesion molecule CD44v6, integrin-β1, carbohydrate antigen 199 (CA199), and carcinoembryonic antigen (CEA) are closely associated with the progression and metastasis of numerous cancers. In this study, peripheral blood mononuclear cell (PBMC) and serum samples were collected from 37 pancreatic cancer patients and 12 healthy people. A novel triplex TaqMan real-time reverse transcription polymerase chain reaction assay was used to measure the expression levels of CD44v6 and integrin-β1 gene in PBMCs, while chemiluminescence and enzyme-linked immunosorbent assay were used to measure the levels of CA199 and CEA expression in serum. The results showed that both the levels of CD44v6 and integrin-β1 expression had significant correlation with clinical stage, lymph node, and liver metastasis of pancreatic cancer ($P < 0.05$). Age, tumor size, tumor differentiation, clinical stage, lymph nodes, and liver metastasis were significantly associated with the levels of CA199 and CEA expression ($P < 0.05$). The levels of CD44v6, integrin-β1, CA199, and CEA expression in the patients prior cryosurgery and chemotherapy were significantly higher than those in the control group ($P < 0.05$), whereas no significant difference was found between the patients 1 month post cryosurgery and control group ($P > 0.05$). The expression levels of CD44v6, integrin-β1, CA199, and CEA in the patients 1 month post cryosurgery were significantly lower than those in the patients prior cryosurgery ($P < 0.05$). Interestingly, no significant difference was found for the CD44v6, integrin-β1, CA199, and CEA levels between the patients prior and post-chemotherapy ($P > 0.05$). The higher expression of CD44v6, integrin-β1, CA199, and CEA are closely related to the progression and metastasis of pancreatic cancer and may play a important role in the curative evaluation of cryosurgery of pancreatic cancer.

Keywords CD44v6 · Integrin-β1 · CA199 · CEA · Pancreatic cancer

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Introduction

Pancreatic cancer is one of the solid malignancies because of its rapid growth and propensity to invade adjacent organs and metastasize [1]. Most patients with pancreatic cancer have difficulties with early diagnosis because of the absence of early disease-specific signs and symptoms. Therefore, less

than 20% of the patients are candidates for surgical resection and even if surgery is performed, the postoperative 5-year survival rate is only 15–25% because of the high-recurrence rate [2–4]. This poor outcome is mainly due to difficulties in early detection, lack of effective treatment, and limited understanding of the biological characteristics of this disease. Nearly 25% of the patients have showed a 5-year survival period post surgery if the cancer were diagnosed at an early stage [5]. Thus, the identification of biological markers that could provide prognostic information about the invasive or metastatic potential of pancreatic cancer patients becomes essential for this disease.

Very complex processes are involved in tumor cell invasion and metastasis. The propensity for the cancer cells to degrade and adhere to the basement membrane and then metastasize to distant organs is one of the most critical aspects of cancer development [6]. The cell adhesion molecules, including integrins, cadherins, the superfamily of immunoglobulins and the CD44, not only mediate cell–cell recognition and cell–matrix interactions, but also associate with tumor cellular proliferation, migration, and penetration of basement membrane [7, 8]. CD44v6 (v6) expression, which is closely related to the progression, metastasis and prognosis of tumor, has been studied in several malignant neoplasms [9–11]. Integrins are membrane-spanning complexes and each integrin is composed of one α and one β subunit. Recently, several studies have confirmed that the alteration of the expression of the integrin-β1 gene was associated with the invasion and metastasis of many malignant neoplasms [12−14]. Among several molecular markers, CA199 and CEA are those more frequently used for serum detection in pancreatic cancer. CA199 and CEA are a kind of the ganglioside lipoprotein and their serum levels have been widely used in prognosis monitoring of pancreatic cancer patients.

Pancreatic cancer has a low-resection rate, which means, in the case of successful resection, the median survival and overall survival period of patients are only 6.9 and 20.2 months, respectively [15]. Chemotherapy and radiotherapy were two main methods of treating pancreatic cancer in advanced stage, but the treatment effects are very limited. Most studies showed that median survival time of patients conducting chemotherapy and radiotherapy is less than 10 months [16, 17]. Therefore, it was very essential to explore a new treating method for pancreatic cancer. Recently, Argon-Helium cryosurgery has provided a novel therapeutic approach and has been used in the treatment of tumors, especially unrespectable tumors [18–20]. Xu [18] in Fuda hospital showed that pancreatic cancer patients' survival time extended obviously after cryosurgery. However, the application of cryosurgery in the treatment of pancreatic cancer still stays in a primary stage, its efficacy and safety required to be proved by new experimental data.

The aim of this study was to detect the CD44v6, integrin-β1, CA199, and CEA expression levels in pancreatic cancer patients and analyze its possible clinical use in assessing the curative effect of cryosurgery of pancreatic cancer.

Materials and Methods

Patients and PBMCs

Thirty-seven pancreatic cancer patients were enrolled in Fuda Hospital in Guangzhou, China, including 22 males and 15 females, at an average age of 58 ± 15.3 years. Totally, 22 heads, 5 bodies and 10 tails of pancreatic cancer were diagnosed by pathology. According to the standard of TNM, only 1 patient belonged to I stage and 5, 7, and 24 patients belonged to II, III, and IV stage, respectively. Among 37 patients, 18 patients only performed cryosurgery and 12 patients only conducted chemotherapy. 12 healthy people in Fuda hospital were selected randomly as normal control and no tumor history was found among them, including seven males and five females, at an average age of 47 ± 14.2 years.

The peripheral blood samples of the healthy people and the patients prior surgery, 10 days and 1 month post surgery were collected. PBMCs were isolated by the Ficoll-paque plus (Invitrogen, Shanghai, China) according to the manufacturer's protocol. Serum samples were extracted and stored in −20 °C until use.

Total RNA Extraction and cDNA Synthesis

Total RNA of PBMCs was extracted with an RNA isolation plus (TaKaRa, Dalian, China) and treated with Dnase I (TaKaRa, Dalian, China) to avoid contamination of genome DNA in keeping with the manufacturer's process. Next, cDNA was synthesized with an M-MLV reverse transcriptase kit (TaKaRa, Dalian, China) according to manufacturer instructions.

Primers and Probes for the Triplex qRT-PCR

The human CD44v6 (GenBank ID: XM002821884, AB468969, BC004372, and L05415), integrin-β1 (GenBank ID: NM003032, NM173217, NM002211, NM133376, and NM033668), and *β-actin* gene sequences were retrieved from the GenBank database (GenBank ID: NG022872, XM096887, XM096458, XM096372, XM096268, XM09 2509, and M10277) and aligned using DNAStar program (DNASTAR, Inc., Madison). Only the highly conserved regions were used to design the primers and TaqMan probes for these genes by using Beacon Designer software version

7.0 (Palo Alto, CA, USA). We also conducted BLAST search to confirm the specificity of the nucleotide sequences chosen for the design of the primers and probes. The optimal primers and probes (Table 1) were synthesized by Invitrogen (Invitrogen, Shanghai, China).

Preparation of Standard Plasmids for the Triplex qRT-PCR

Three conventional RT-PCR amplifications for CD44v6, integrin-β1, and *β-actin* gene from PBMC cDNA were performed, respectively, with the primers in Table 1. The PCR fragments were purified and cloned into pMD18-T vector (TaKaRa, Dalian, China) according to manufacturer's instructions. Plasmid DNA from recombinant clones was extracted using a plasmid isolation kit (Biowatson, Shanghai, China), following manufacturer protocol and sequenced by Invitrogen (Invitrogen, Shanghai, China). The concentration of CD44v6, integrin-β1, and *β-actin* plasmids DNA was measured with an ultraviolet spectrophotometer (Eppendorf, Jerman) and the copy number was calculated by formula:

$$\text{Copy number (copies/}\mu\text{l)} = \frac{NA(\text{copies/mol}) \times \text{concentration (g/}\mu\text{l)}}{\text{MW(g/mol)}}$$

where, NA is Avogadro's number and MW is base number × 340.

The Triplex qRT-PCR Assay

The triplex qRT-PCR reaction system contained 2.5 μl of 10 × buffer, 2 μl of 2.5 mM dNTPs, 0.4 μl of 10 μM C–F primer, 0.4 μl of 10 μM C–R primer, 0.2 μl of FAM-labeled CD44v6 probe, 0.5 μl of 10 μM I–F primer, 0.5 μl of 10 μM I–R primer, 0.25 μl of ROX-labeled integrin-β1 probe, 0.4 μl of 10 μM *β*-R primer, 0.4 μl of 10 μM *β*–F primer, 0.2 μl of HEX-labeled *β-actin* probe, 0.5 μl of 5 U Hotstart ExTaq polymerase (HS-ExTaq) (TaKaRa, Dalian, China), 1 μl template DNA and 15.75 μl distilled water. The reaction process was as follows: 94 °C for 3 min followed by 40 cycles of 94 °C for 15 s and 61 °C for 45 s.

The recombinant plasmids (10^{10} copies/μl) of CD44v6, integrin-β1 and *β-actin* gene were diluted serially tenfold using TE buffer. To construct the standard curves of the triplex qRT-PCR assay, serial dilutions from 10^3 to 10^9 copies/μl of the CD44v6, integrin-β1, and *β-actin* plasmids DNA were amplified using this assay (Note: each dilution of these plasmids was amplified in the same tube). The standard curves were obtained by plotting the Ct values against the copy number of the serial diluted plasmids.

Investigation of Expression Levels of CD44v6, Integrin-β1 in PBMC

The serially diluted recombinant plasmids for CD44v6, integrin-β1, and *β-actin* gene, along with the cDNAs of PBMCs collected from 37 patients and control group were tested simultaneously. The copy number of CD44v6, integrin-β1, and *β-actin* gene in PBMCs was extrapolated from the standard curves of CD44v6, integrin-β1, and *β-actin* gene in each run. Negative control (sterile water) was also performed for each run. For each sample, the copy number of CD44v6 and integrin-β1 gene in a million PBMCs was expressed as the number of CD44v6 or integrin-β1 copies per 10^6 *β-actin* copies, then, the log value of copy number in a million PBMCs was calculated by Excel software.

Investigation of Expression Levels of CA199 and CEA in Serum

The expression levels of CA199 in serum samples were determined by chemiluminescence assay according to the

Table 1 Primers and TaqMan fluorogenic probes used in this research

Gene	Name	Sequence(5′–3′)	GenBank	Amplicon size(bp)
CD44v6	C–F	CCAGGCAACTCCTAGTAGTACAAC	L05415	107 bp
	C–R	GGGAGTCTTCTCTGGGTGTTTG		
	C[a]	TGCCATCTGTTGCCAAACCACTGTTCCT		
Integrin-β1	I–F	TTCGATGCCATCATGCAAGTTG	NM002211	110 bp
	I–R	CCATCTCCAGCAAAGTGAAACC		
	I[b]	AGCAGCCGTGTAACATTCCTCCAGCC		
β-actin	*β*–F	CGGGACCTGACTGACTACCTC	M10277	136 bp
	β–R	CCATCTCTTGCTCGAAGTCCAG		
	β[c]	TCCTTAATGTCACGCACGATTTCCCGCT		

[a] CD44v6 probe was labelled with 5′-6-carboxy-fluorescein (*FAM*) and 3′-6-carboxytetramethyl-rhodamine (*TAMRA*)

[b] Integrin-β1 probe was labelled with 5′-6-carboxy-X-rhodamine (*ROX*) and 3′-Black Hole Quencher 1 (*BHQ1*)

[c] *β-actin* probe was labelled with 5′-6-carboxy-X-rhodamine (HEX) and 3′-Black Hole Quencher 2 (*BHQ2*)

kit instructions of manufacturer (Roche, Shanghai, China). According to the ELISA kit instructions of CEA (Tianjing, Shanghai, China), the levels of CEA expression in serum samples were measured.

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). The potential correlation between the molecular marker expression and the clinical and pathological characteristics of the patients was tested by Spearman's correlation. A P value <0.05 was considered to be statistically significant.

Results

Standard Curves of the Triplex qRT-PCR

Detectable fluorescent signals above threshold were observed at 16.78, 16.76, and 17.20 cycles for the amplifications of the CD44v6, integrin-β1, and *β-actin* plasmids, respectively (data not shown). Linear standard curves of the CD44v6, integrin-β1, and *β-actin* gene were obtained from 10^3 to 10^9 copies per reaction with Ct values ranging from 16.78 to 36.72 cycles (R^2: 0.999; reaction efficiencies: 109.1%), 16.76–36.62 cycles (R^2: 0.999; reaction efficiencies: 107%), and 17.20–36.85 cycles (R^2: 0.998; reaction efficiencies: 109.5%), respectively (Fig. 1a–c).

The Relationship between the Levels of CD44v6, Integrin-β1, CA199, CEA Expression, and Pathological Characteristic of Pancreatic Cancer

As shown in Table 2, sex, tumor size, and tumor differentiation were significantly associated with the expression levels of CD44v6 ($P < 0.05$) but were not significantly correlated with those of integrin-β1 ($P > 0.05$). Age was not significantly associated with both the CD44v6 and integrin-β1 expression levels ($P > 0.05$). The CD44v6 levels in body and tail of pancreatic cancer were higher than those in head of pancreatic cancer, however, no statistically significant difference was found between them ($P > 0.05$). Clinical stage, lymph node metastasis and liver metastasis were significantly associated with the levels of CD44v6 and integrin-β1 expression ($P < 0.05$). Age, tumor size, tumor differentiation, clinical stage, lymph nodes, and liver metastasis were significantly associated with both the levels of CA199 and CEA expression ($P < 0.05$). Sex had significant correlation with the levels of CEA expression ($P < 0.05$).

The Levels of CD44v6, Integrin-β1, CA199, and CEA Expression in the Control Group and Pancreatic Cancer Patients Prior and Post Cryosurgery

As shown in Table 3, the CD44v6, integrin-β1, CA199, and CEA expression levels in patients prior and 10 days post cryosurgery were significantly higher than those in the control group ($P < 0.05$). For CD44v6, integrin-β1, and CEA expression levels, no significant difference was found between the patients 1 month post cryosurgery and the control group ($P > 0.05$), however, CA199 expression levels in the patients 1 month post cryosurgery were significantly higher than those in the control group ($P < 0.05$). Compared with the expression levels in the patients prior cryosurgery, CD44v6, integrin-β1, and CEA levels in patients 10 days post cryosurgery showed no significant change ($P > 0.05$), while the CA199 levels were significantly decreased ($P < 0.05$). CD44v6, integrin-β1, CA199, and CEA expression levels in the patients 1 month post cryosurgery were significantly decreased compared with those in the patients prior cryosurgery ($P < 0.05$).

The Levels of CD44v6, Integrin-β1, CA199, and CEA Expression in the Control Group and Pancreatic Cancer Patients Prior and Post Chemotherapy

As shown in Table 4, CD44v6, integrin-β1, CA199, and CEA expression levels in the patients prior, 10 days and 1 month post chemotherapy were significantly higher than those in the control group ($P < 0.05$). Compared with the patients prior chemotherapy, the CD44v6, integrin-β1, CA199, and CEA expression levels in the patients 10 days and 1 month post chemotherapy had no significant change ($P > 0.05$).

Discussion

The modified expression of CD44v6 and integrin-β1 in malignant tumors could facilitate tumor progression and metastasis [21, 22]. Many studies have been done to clarify the role of CD44v6 and integrin-β1 in different human neoplasms [23–27]. However, most of them used animal models and cell lines as experimental objects, whose mechanism of tumor development differs greatly from that of human. Besides, some conventional assays, such as immunohistochemistry, RT-PCR, ELISA, and molecular hybridization assays were used for the detection of CD44v6 and integrin-β1 in the previous studies [28–33]. As only tissue samples can be tested by immunohistochemistry assay, surgery becomes essential for the collection of clinical specimens. Conventional RT-PCR assay could not

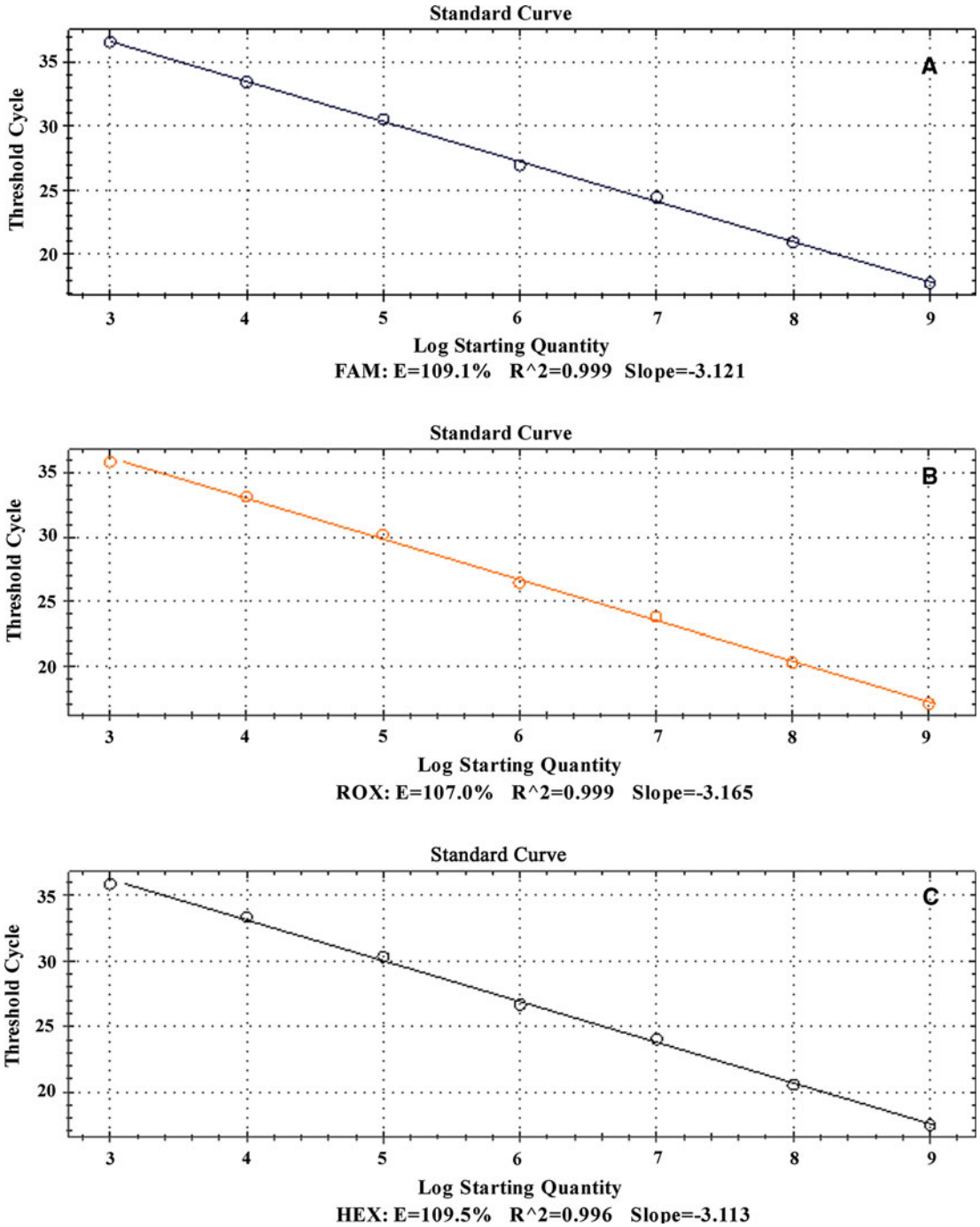

Fig. 1 The triplex qRT-PCR standard curves generated from CD44v6, integrin-β1, and *β-actin* plasmids amplication plots. Standard curves were plotted between copy number of recombinant plasmids and CT. **a** CD44v6 plasmid ranged from 10^3 to 10^9 copies/μl; **b** Integrin-β1 plasmid ranged from 10^3 to 10^9 copies/μl; **c** *β-actin* plasmid ranged from 10^3 to 10^9 copies/μl

quantitatively detect the gene expression level in the tissues or cells. ELISA and molecular hybridization assays lack sensitivity, resulting in many samples with lower expression levels cannot be tested positive. Thus, the CD44v6 and integrin-β1 expression levels cannot be precisely quantified in former studies, leading to the arise of many conflicting conclusions. In this study, the TaqMan triplex qRT-PCR assay was used to measure the expression levels of CD44v6 and integrin-β1 gene in the 37 pancreatic cancer patients. It not only realized the quantitative analysis of the gene expression, but also was more sensitive and specific than the conventional methods. Besides, this assay could amplify the CD44v6, integrin-β1, and human *β-actin* gene in a single reaction and constructed the standard curves

Table 2 Relation between levels of CD44v6, integrin-β1, CA199, and CEA expression and pathological feature of pancreatic cancer

Pathological features	Case	CD44v6 (log copies)	*P*	Integrin-β1 (log copies)	*P*	CA199 (U/ml)	*P*	CEA (ng/ml)	*P*
Sex			0.037		0.941		0.121		0.014
Male	22	4.65 ± 0.64		4.21 ± 0.50		21333.71 ± 9865.42		23.71 ± 4.93	
Female	15	4.17 ± 0.69		4.20 ± 0.58		34312.92 ± 15412.23		4.87 ± 1.66	
Age			0.301		0.802		0.014		0.023
<60	14	4.58 ± 0.64		4.17 ± 0.47		6123.84 ± 3433.22		4.56 ± 1.72	
>60	23	4.37 ± 0.72		4.23 ± 0.53		35476.42 ± 12876.31		18.57 ± 5.53	
Tumor position			0.068		0.666		0.213		0.213
Head	22	4.28 ± 0.69		4.18 ± 0.48		26732.44 ± 8876.96		11.65 ± 3.82	
Body and tail	15	4.70 ± 0.64		4.25 ± 0.55		14765.84 ± 5653.16		13.37 ± 3.22	
Tumor size			0.038		0.056		0.044		0.037
<4 cm	15	4.17 ± 0.61		4.02 ± 0.42		13777.45 ± 7652.97		10.38 ± 4.62	
>4 cm	22	4.64 ± 0.69		4.33 ± 0.53		38731.63 ± 12111.71		18.96 ± 5.73	
Tumor differentiation			0.047		0.614		0.005		0.012
Low	9	4.10 ± 0.63		4.08 ± 0.45		929.83 ± 381.21		3.66 ± 0.63	
Moderate and well	28	4.57 ± 0.68		4.25 ± 0.52		32887.33 ± 11098.34		18.25 ± 5.73	
Clinical stage			0.039		0.041		0.045		0.010
I–III	13	4.19 ± 0.75		4.01 ± 0.47		13061.84 ± 5671.21		4.57 ± 1.26	
IV	24	4.60 ± 0.63		4.35 ± 0.52		35642.85 ± 10912.11		19.94 ± 6.33	
Lymph nodes metastasis			0.035		0.047		0.015		0.013
−	12	4.13 ± 0.55		4.02 ± 0.45		5566.46 ± 1234.05		4.96 ± 1.24	
+	25	4.61 ± 0.71		4.30 ± 0.51		38992.47 ± 12135.82		22.41 ± 5.30	
Liver metastasis			0.004		0.047		0.047		0.047
−	14	4.04 ± 0.67		4.02 ± 0.49		12312.48 ± 4308.24		11.18 ± 3.92	
+	23	4.70 ± 0.59		4.32 ± 0.49		36123.95 ± 12522.15		17.27 ± 3.84	

Table 3 The levels of CD44v6, integrin-β1, CA199, and CEA expression in patients prior and post-cryosurgery and control group

Groups	*N*	CD44v6 (log copies)	*P*	Integrin-β1 (log copies)	*P*	CA199(U/ml)	*P*	CEA(ng/ml)	*P*
Control	12	3.85 ± 0.23		3.80 ± 0.32		16.62 ± 5.47		2.70 ± 5.72	
Prior cryosurgery	18	4.44 ± 0.63	0.013a	4.28 ± 0.32	0.028a	25029.71 ± 5433.72	0.001a	11.61 ± 3.78	0.017a
10 days post cryosurgery	18	4.06 ± 0.42	0.037a	4.11 ± 0.38	0.017a	14821.93 ± 3451.32	0.004a	8.21 ± 2.44	0.032a
			0.058b		0.286b		0.037b		0.142b
1 month post cryosurgery	10	3.41 ± 0.49	0.109a	3.74 ± 0.41	0.109a	8211.43 ± 813.73	0.008a	5.60 ± 1.73	0.079a
			0.043b		0.043b		0.014b		0.041b
			0.043c		0.345c		0.032c		0.221c

[a] Compared with control

[b] Compared with the patients prior cryotherapy

[c] Compared with the patients 10 days post cryotherapy

successfully after optimizing reaction conditions, saving the time and money greatly. Lastly, this assay use *β-actin* gene as an internal control. It can determine the copy number of CD44v6 and integrin-β1 gene in a single cell due to the steady expression of *β-actin* gene in the different tissues or cells. Thus, the error in sample processing and RNA extraction of the different samples can be eliminated.

In this study, a systematic analysis of the relationship between the CD44v6 and integrin-β1 mRNA expression levels and pancreatic cancer was conducted for the first time. The results found that in the patients with tumors larger than 4 cm, with moderate or well-differentiated degree, at the IV clinical stage, with lymph node or liver metastasis, the levels of CD44v6 expression were

Table 4 The levels of CD44v6, integrin-β1, CA199, and CEA expression in patients prior and post-chemotherapy and control group

Groups	*N*	CD44v6 (log copies)	*P*	Integrin-β1 (log copies)	*P*	CA199 (U/ml)	*P*	CEA (ng/ml)	*P*
Control	12	3.85 ± 0.23		3.80 ± 0.32		16.62 ± 5.47		2.70 ± 5.72	
Prior chemotherapy	12	4.57 ± 0.68	0.022a	4.33 ± 0.29	0.017a	1311.65 ± 241.66	0.000a	14.33 ± 4.38	0.011a
10 days post chemotherapy	12	4.33 ± 0.33	0.027a	4.24 ± 0.33	0.024a	1183.54 ± 226.87	0.000a	12.55 ± 3.87	0.017a
			0.153b		0.277b		0.112b		0.156b
1 month post chemotherapy	8	4.21 ± 0.51	0.039a	4.18 ± 0.43	0.043a	984.84 ± 124.65	0.000a	10.45 ± 3.23	0.024a
			0.144b		0.245b		0.076b		0.056b
			0.321c		0.376c		0.136c		0.273c

[a] Compared with control

[b] Compared with the patients prior chemotherapy

[c] Compared with the patients 10 days post chemotherapy

significantly higher than those in the patients with tumors smaller than 4 cm, with low differentiated degree, at the I–III clinical stage, without lymph node or without liver metastasis, respectively ($P < 0.05$). The results proved that the rising CD44v6 expression levels can be regarded as an important biological index for the diagnosis, prediction of metastasis, and prognosis of pancreatic cancer, which is consistent with the views of previous studies [34, 35]. The abnormal expression of integrin-β1 was correlated with the clinical stage, lymph node, and liver metastasis ($P < 0.05$), which suggested that integrin-β1 was one of the possible indicators that affect the metastasis of pancreatic cancer. The possible reason is that CD44v6 and integrin-β1 can enhance the adhesion of tumor cells to mesothelial cells and extracellular matrix, promoting the release and activation of proteolytic enzyme in tumor cells [36]. Besides, tumor cells with high CD44v6 and integrin-β1 expression levels may possibly be assisted by the "camouflage" from lymphocytes and evade the identification and elimination from the human immune system, thus gaining easier access to lymph nodes and liver and forming the metastasis [37].

Although many studies have demonstrated that CA199 and CEA are important biological markers for predicting metastatic potential, the consistent view has not been reached so far because of the complicate process of tumor invasion and metastasis. The results showed that the expression levels of CA199 and CEA were significantly correlated with the age ($P < 0.05$), which was consistent with the previous finding [38]. This indicates that age is one of the possible factors that affect the development of pancreatic cancer. Furtherly, the levels of CA199 and CEA expression had significant correlation with tumor size, tumor differentiation, clinical stage, lymph nodes, and liver metastasis ($P < 0.05$), implying that expression levels of CA199 and CEA play an important role in tumor progression and are significant predictors for the metastasis of pancreatic cancer. The reason is that CA199 and CEA are a kind of serum mucoprotein correlated with cellular adhesion effect which is involved in the proliferation, migration, and differentiation of tumor cell. Actually, many previous studies have strongly supported the results in this research [39, 40].

Lately, argon-helium cryosurgery, as a new tumor ablation technology, has been widely used in the treatment of various solid tumors. Some studies have demonstrated that cryosurgery is more effective than conventional pancreatic resection with a significantly low rate of adverse effect [41, 42]. The change of biological functions that occur during and after surgery have been studied in many malignant tumors. A number of studies have proved that the modified expression of biological markers can be used as a important indicator for evaluating the efficacy and safety of surgery. This study confirmed that the levels of CD44v6, integrin-β1, CA199, and CEA expression in the patients prior cryosurgery and chemotherapy were significantly higher than those in the control group ($P < 0.05$), proving the close association between CD44v6, integrin-β1, CA199, and CEA expression and the development of pancreatic cancer. After the treatment of cryosurgery and chemotherapy, CD44v6, integrin-β1, CA199, and CEA expression levels were gradually decreased. However, CA199 levels in the patients 10 days post cryosurgery were significantly decreased compared with those prior cryosurgery ($P < 0.05$). For the patients 1 month post cryosurgery, CD44v6, integrin-β1, CA199, and CEA levels were significantly lower than those prior cryosurgery ($P < 0.05$). Moreover, no significant difference was found for the CD44v6, integrin-β1 and CEA levels between the patients 1 month post cryosurgery and control ($P > 0.05$). Interestingly, both the CD44v6, integrin-β1, CA199, and CEA levels in the patients 10 days and 1 month chemotherapy were still significantly higher than the control ($P < 0.05$) and were not significantly decreased compared with those prior chemotherapy ($P > 0.05$). This implies

that cryosurgery possibly plays an important role in inhibiting the development and metastasis of pancreatic cancer. The timely and effective evaluation of cryosurgery efficacy in the treatment of pancreatic cancer may be realized through observing the changes of the levels of CD44v6, integrin-β1, CA199, and CEA expression.

In conclusion, expression of CD44v6, integrin-β1, CA199, and CEA are closely related to the invasion and metastasis of pancreatic cancer. It may play a vital role in predicting the prognosis and therapeutic effect and thus establish an individual therapy scheme for the patients with pancreatic cancer.

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