ORIGINAL RESEARCH

Prognostic value of CRM1 in pancreas cancer

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Abstract

Purpose: Pancreatic cancer is a highly aggressive malignant tumour with poor prognosis. The median survival is only 6 months. This study investigated the prognostic value of nuclear export protein chromosomal region maintenance/exportin 1/Xpo1 (CRM1) expression in pancreas cancer.

Methods: CRM1 expression was detected, by Western blot, in pancreatic tissue from 69 cancer patients and 10 normal subjects.

Results: CRM1 showed increased expression in pancreatic cancer tissue ($P = 0.007$). The high expression of CRM1 was associated with increased serum levels of CEA ($P = 0.002$) and CA19-9 ($P = 0.005$). There was an association between CRM1 expression and tumour size ($P = 0.01$), lymphadenopathy ($P = 0.004$) and liver metastasis ($P = 0.003$). High CRM1 expression was not correlated with the other clinicopathological parameters. High CRM1 expression was a prognostic indicator for progression-free survival (PFS) ($P = 0.006$) as well as overall survival (OS) ($P = 0.001$). Expression of CRM1 was an independent prognostic parameter for poorer PFS and OS (95% CI, 1.27–5.39).

Conclusions: CRM1 expression demonstrated prognostic value in pancreatic cancer. Prospective studies are required

to determine the prognostic role of high expression of CRM1 in pancreatic cancer.

Pancreatic cancer is a highly aggressive malignant tumour and is one of the leading causes of cancer-related mortality in the western world.¹ At the time of diagnosis, 20% of patients present with a resectable tumour, 50% with metastatic disease, and 30% with locally advanced tumour.² More than 30,000 people develop pancreatic cancer each year in the United States, and almost all are expected to die from the disease. Although the incidence of pancreatic cancer is only about 10 in 10⁵, it is the fourth leading cause of cancer-related death. Almost half the patients with pancreatic cancer will die within 6 months from diagnosis.³ One reason for the poor prognosis is the insensitivity to most therapies such as chemotherapy, radiotherapy and immunotherapy. Surgical resection offers the only potential chance for cure. The 5-year survival rate is < 5%, and the median survival time after diagnosis is 6 months. Furthermore, only about

20% of patients survive longer than 5 years after curative resection.⁴

Understanding the clinical, pathological and molecular markers that predict outcome is particularly useful when evaluating new treatment for patients with resectable pancreatic cancer. While clinicopathological markers provide some information about prognosis after surgery, these markers alone do not accurately predict survival. Tumour diameter >2.5–3.0 cm, positive lymph nodes, positive margins, and poor differentiation are all features that have consistently been associated with a worse prognosis.^{5,6} Numerous genetic (*K-ras*, *p16* and *SMAD4* [7-9] and epigenetic alterations occur during the development of pancreatic adenocarcinoma.¹⁰⁻¹⁵ Although our understanding of the molecular and genetic changes that lead to pancreatic cancer has improved, few molecular markers predict prognosis, or response to therapy reliably, to justify their use in clinical decision making.

The human nuclear export protein chromosomal region maintenance/exportin 1/Xpo1 (CRM1) mediates the nuclear export of proteins and messenger RNAs and, thus, is an important regulator of subcellular distribution of key molecules.¹⁶⁻¹⁸ In addition to cell-biology studies that suggested a fundamental role for CRM1 in the regulation of mitosis, Noske et al. reported that the expression of this protein is a prognostic factor in human ovarian cancer.¹⁹ However, more data is required from the other cancers to further evaluate the prognostic value of CRM1 expression.

In this study we have investigated the expression of CRM1 and evaluated its prognostic value in pancreatic cancer. The correlation between CRM1 expression and the clinicopathological parameters of pancreas cancer, including CA19-9 and CEA, was also analyzed.

Materials and methods

The study was approved by the Ethics Review Board and all subjects gave informed consent. Samples of pancreatic tissue were taken, at surgery, from 69

patients with I and II stage pancreatic cancer and from 10 of normal subjects. Patients with pancreatic cancer were admitted from January 2002 to December 2006 to the Cancer Center, Hospital of Qingdao University. All patients were followed up after diagnosis. Pathological grades: G1, well-differentiated; G2, moderately differentiated; G3, poorly differentiated and undifferentiated. Physical examination was performed every 3 months in all patients for the first 2 years and then twice/year. Radiographic studies, and liver ultrasound were performed every 12 months; computed tomography scans were performed whenever clinically indicated. Blood tests, including electrolyte and liver function profiles and complete blood cell counts, were repeated at every follow-up visit. Progression-free survival (PFS) was calculated as the period from surgery until the date of the first recurrence.

Treatment

The 69 cancer patients received surgery followed by gemcitabine chemotherapy for 6 months.

Western blot

Samples were homogenized in a solution of 10 mM HEPES, 10 mM KCl, 1.5 mM MgCl₂, 0.5mM DTT, 0.5 mM PMSF, 10 µg/ml aprotinin, and 10 µg/ml leupeptin at pH 7.9. Protein extracts were then subjected to SDS-PAGE and transferred to a polyvinylidene difluoride membrane. Monoclonal antibody against CRM1 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The membrane was incubated with primary and secondary antibodies, respectively, for 1 hour at room temperature. Signals were developed by ECL Kit (Amersham Pharmacia Biotech Inc., Buckinghamshire, UK). The expression of CRM1 protein in human ovarian carcinoma cell line ES-2 is used as positive control.²⁰ Blot quantitation was done with a Molecular Dynamics Laser Densitometer (Model PSD) and the Image Quant Version 1 software.^[21] The level of CRM1 expression of sample higher than that of ES-2 was defined as high expression of CRM1.

TABLE 1. Patient demographics

		No. of Patients
Age (yr)		
Median	64.7	
Range	35–79	
<60		21
≥ 60		48
History of symptoms		
Median (days)	72	
<70 days		31
≥ 70 days		38
Sex		
Male		36
Female		33
Tumour size		
< 2 cm		40
≥ 2 cm		29
Metastasis		
Absent		63
Detected		6
Lymph nodes		
Positive		31
Negative		38
Grade		
G1		25
G2		16
G3		28
Duration follow-up (months)		
Median	42.5	
Range	6–59	
Relapse		25
Metastasis		31
Deaths		51

Statistical analysis

Statistical significance of the correlation between expression of CRM1 and several clinicopathological parameters was assessed by Fisher's exact test, χ^2 test, or χ^2 test for trends as indicated. The probability of overall survival as a function of time was determined by the Kaplan-Meier method and the log-rank test. Multivariate survival analysis was performed using the Cox regression model. $P < 0.05$ was considered as

TABLE 2. CRM1 expression in pancreas cancer and normal tissues.

	Pancreatic cancer	Normal tissue	<i>P</i>
CRM1 expression			0.0013
Low	30	10	
High	39	0

FIGURE 1. Expression of CRM1 in Pancreatic Cancer and Normal Pancreatic tissues. Lane 1: lysis of ES-2 cells as positive control, Lanes 2-12: lysis of pancreatic cancer tissues, Lanes 13-14: lysis of normal pancreatic tissues.

significant. For the statistical evaluation, the SPSS software version 12.0 was used (SPSS, Inc., Chicago, IL).

Results

Clinical and Pathological Characteristics.

The demographics and the pathological characteristics of the pancreatic cancer patients are shown in Table 1. The median age was 64.7 yr (range 35-79). Most patients ranged from 55-70 yr. During follow-up. 25 patients relapsed, 31 metastasised and 51 died

CRM1 Protein Expression

Compared with the low CRM1 expression in normal tissue, CRM1 expression in pancreatic cancer tissue showed increased levels ($P = 0.0013$) (Figure 1). 39 of 69 patients had high CRM1 expression, none of the 10 samples from normal subjects expressed increased CRM1 (Table 2). This suggests that CRM1 might be a prognostic factor in pancreatic cancer.

TABLE 3. CRM1 expression with serum CA19-9, CEA , clinicopathological factors

Characteristic	All cases	CRM1 low (%)	CRM1 high (%)	<i>P</i>
CA19-9 (<i>n</i> = 69)				0.005
Normal	13	11 (84.6)	2 (15.4)	
Increased	56	19 (51.8)	37 (48.2)	
CEA (<i>n</i> = 69)				0.002
Normal	37	25 (67.6)	12 (32.4)	
Increased	32	5 (15.6)	27 (84.4)	
Sex				0.26
Male	36	14 (38.9)	22 (61.1)	
Female	33	16 (48.5)	17 (51.5)	
Tumour size				0.011
< 2 cm	40	24 (67.5)	16 (32.5)	
≥ 2 cm	29	6 (50.0)	23 (50.0)	
Lymph nodes				0.004
Negative	31	22 (71.0)	9 (29.0)	
Positive	38	8 (21.1)	30 (78.9)	
Metastasis				0.041
M ₀	63	29 (46.0)	34 (54.0)	
M ₁	6	1 (16.7)	5 (83.3)	
Histological grade				0.083
G1	25	14 (56.0)	11 (44.0)	
G2	16	6 (37.5)	10 (62.5)	
G3	28	10 (35.7)	18 (64.3)	
Age (yr)				0.27
<60	21	10 (47.6)	11 (52.4)	
≥ 60	48	20 (41.7)	28 (58.3)

FIGURE 2. Overall Survival (OS) Curves of CRM1.

High CRM1 expression and clinicopathological factors.

Increased expression of CRM1 was associated with increased serum level of CEA ($P = 0.002$) and CA19-9 ($P = 0.005$). There was also an association between CRM1 expression and tumour size ($P = 0.011$) lymphadenopathy ($P = 0.004$) and metastasis ($P = 0.041$). High expression of CRM1 was not correlated with sex, histological grade or age (Table 3).

FIGURE 3. Progression Free Survival (PFS) Curves of CRM1.

High CRM1 expression and overall survival and progression-free

High expression of CRM1 was a prognostic indicator for overall survival ($P = 0.004$) and PFS ($P = 0.011$). Presence of metastases and histological grade were also prognostic indicators for both OS and PFS (Fig. 2).

Necrosis rate post chemotherapy, history of symptoms and tumour size were prognostic indicators for overall survival but not for progression-free survival (Fig. 3).

Multivariate survival analysis demonstrated that high CRM1 expression, lymphadenopathy, tumour size, presence of metastasis, and worse histological grade were related to shorter survival (Table 4).

Discussion

In the present study, 39 of 69 patients with resectable pancreatic cancer demonstrated high expression of CRM1 in tumour tissue. Patients with high CRM1 expression showed less survival time than those with low CRM1 expression. Several clinicopathological parameters, including serum level of CEA and CA19-9, tumor size, lymph node and metastasis were also observed to be associated with high CRM1 expression.

TABLE 4. Multivariate survival analysis for overall survival of patients with pancreatic cancer

Characteristic	RR(95%CI)	P
CRM1 expression		
High	1.00	
Low	2.83 (1.27-5.39)	0.006
Serum CA19-9 (n = 69)		
Normal	1.00	
Increased	2.64 (0.78-6.91)	0.06
Serum CEA (n = 69)		
Normal	1.00	
Increased	2.43 (1.26-5.49)	0.07
Sex		
Male	1.00	
Female	2.62 (0.85-6.72)	0.098
History of Symptoms		
<70	1.00	
≥70	2.83 (0.45-4.36)	0.13
Tumour size		
< 2 cm	1.00	
≥ 2 cm	2.45 (1.22-3.19)	0.036
Lymph nodes		
Negative	1.00	
Positive	2.71 (1.74-3.49)	0.004
Metastasis		
M ₀	1.00	
M ₁	2.31 (1.55-3.81)	0.006
Histological grade		
G1-G2	1.00	
G3	1.41 (1.56-4.75)	0.023
Age (yr)		
<60	1.00	
≥60	1.51 (0.72-4.33)	0.22

RR, relative risk; CI, confidence interval.

Pancreatic cancer is a high aggressive malignant tumour.²² The prognosis of patients with advanced stage pancreas cancer is much worse than for those with early stage disease. Unfortunately, more than 80% of pancreatic cancer patients were in an advanced stage when diagnosed. Prognostic factors include tumour size, lymph node status, elevation of serum CEA and CA19-9 levels. These clinical characteristics reflect the frequency of advanced disease at diagnosis among our patient population. A

biomarker or prognostic factor specific for early stage pancreatic cancer would be more valuable than CEA and CA19-9. Here, we document high CRM1 expression in early stage pancreatic cancer. This biomarker could be applied to early diagnosis of pancreas cancer.

Primary tumour spread is associated with shorter prediagnostic symptom duration and this could be related to more aggressive biological behavior.²³ In the present study, the correlation of the presence of metastases with the time from onset of symptoms to diagnosis, tumour size, or pathological grade was demonstrated. The history of symptoms, the presence of metastases, pathological grade and tumour size were identified as independent factors influencing outcome. By detecting specific early stage biomarkers in the potential patients, combined with CT scan or PET-CT, the disease may be diagnosed with tumours smaller than 1-2cm and may, therefore, increase the rate of early diagnosis. Reducing the time from onset of symptoms to diagnosis may benefit prognosis.

Several studies have demonstrated that CRM1 is important in nuclear-cytoplasmic transport and in the control of mitosis. Regarding transport function, CRM1 is the main nuclear export receptor in humans.²⁴⁻²⁷ Mechanistic studies have demonstrated that some important molecules related to tumour biology depend on the CRM1 nuclear export pathway.²⁸⁻³⁰ Also, it has been demonstrated that CRM1 functions in complex with ras-related nuclear protein guanosine triphosphatase (RAN-GTPase) to control several processes during cellular mitosis. Therefore, CRM1 may be associated with carcinogenesis.

The cellular mechanisms responsible for the worse prognosis of tumors with an increased expression of CRM1 are unknown. Our results suggest a link between over-expression of CRM1 and increased serum levels of CEA and CA19-9. This is also supported by the association between high CRM1 expression and worse pathological grade. No evidence is found to support that CEA and CA19-9 expression are correlated with differentiation. It is possible that another

CRM1 target is involved, which is important for cell differentiation. Additional studies will be required to analyze the molecules related to cell differentiation that are regulated by CRM1 in pancreas cancer.

It should be emphasized that this is a retrospective study with limitations. In our study group, data on therapy as well as intraoperative residual tumour were not available for all patients and could not be included in the multivariate analysis. Additional large-scale prospective and retrospective studies are needed to investigate whether CRM1 expression is indeed of practical utility as a prognostic predictor.

In general, when the determination of the immunoreactive pattern of CRM1 expression is combined with other clinicopathological factors, this may improve the prognostic evaluation of pancreatic cancer patients. CRM1 is a prognostic factor in human pancreatic cancer and may benefit identification of individuals who are at high risk for poor survival. The high expression of CRM1 in 39 of 69 patients (56.5%) and in none of 10 normal subjects suggests it may be a potential biomarker for early diagnosis of pancreas cancer. Additional prospective studies are required to confirm the prognostic value of CRM1.

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