World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Gastroenterol 2017 March 21; 23(11): 2012-2022

DOI: 10.3748/wjg.v23.i11.2012

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Expression of CRM1 and CDK5 shows high prognostic accuracy for gastric cancer

Yu-Qin Sun, Jian-Wei Xie, Hong-Teng Xie, Peng-Chen Chen, Xiu-Li Zhang, Chao-Hui Zheng, Ping Li, Jia-Bin Wang, Jian-Xian Lin, Long-Long Cao, Chang-Ming Huang, Yao Lin

Yu-Qin Sun, Hong-Teng Xie, Peng-Chen Chen, Xiu-Li Zhang, Yao Lin, College of Life Sciences, Fujian Normal University, Fuzhou 350108, Fujian Province, China

Yu-Qin Sun, Jian-Wei Xie, Hong-Teng Xie, Chao-Hui Zheng, Ping Li, Jia-Bin Wang, Jian-Xian Lin, Long-Long Cao, Chang-Ming Huang, Department of Gastric Surgery, Fujian Medical University Union Hospital, Fuzhou 350001, Fujian Province, China

Author contributions: Sun YQ and Xie JW contributed equally to this article; Huang CM and Lin Y conceived and designed the study; Sun YQ, Xie JW, Xie HT, Chen PC and Zhang XL performed the experiments; Zheng CH, Li P, Wang JB, Lin JX and Cao LL analyzed and interpreted the data; Sun YQ and Xie JW drafted the manuscript.

Supported by National Natural Science Foundation of China, No. 81441123 (to Huang CM), No. 31640053 (to Lin Y); National Key Clinical Specialty Discipline Construction Program of China, No. [2012]649; Key Scientific and Technological Project of Fujian Province, China, No. 2014Y0025 (to Huang CM); and Natural Science Foundation of Fujian Province, China, No. 2014J01322 (to Xie JW), No. 2016Y0029 (to Lin Y).

Conflict-of-interest statement: The authors declare that no conflict of interest exists.

Data sharing statement: All available data can be obtained by contacting the corresponding author.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Manuscript source: Unsolicited manuscript

Correspondence to: Dr. Yao Lin, Professor, College of Life Sciences, Fujian Normal University, Qishan Campus, Fuzhou 350108, Fujian Province, China. yaolin@fjnu.edu.cn
Telephone: +86-591-22868688

Received: November 3, 2016
Peer-review started: November 4, 2016
First decision: December 28, 2016
Revised: December 30, 2016
Accepted: January 4, 2017
Article in press: January 4, 2017
Published online: March 21, 2017

Abstract

AIM

To evaluate the predictive value of the expression of chromosomal maintenance (CRM)1 and cyclin-dependent kinase (CDK)5 in gastric cancer (GC) patients after gastrectomy.

METHODS

A total of 240 GC patients who received standard gastrectomy were enrolled in the study. The expression level of CRM1 and CDK5 was detected by immunohistochemistry. The correlations between CRM1 and CDK5 expression and clinicopathological factors were explored. Univariate and multivariate survival analyses were used to identify prognostic factors for GC. Receiver operating characteristic analysis was used to compare the accuracy of the prediction of clinical outcome by the parameters.

RESULTS

The expression of CRM1 was significantly related to size of primary tumor (P = 0.005), Borrmann type (P = 0.006), degree of differentiation (P = 0.004), depth of invasion (P = 0.008), lymph node metastasis (P = 0.013), TNM stage (P = 0.002) and distant metastasis

(P = 0.015). The expression of CDK5 was significantly related to sex (P = 0.048) and Lauren's classification (P = 0.011). Multivariate Cox regression analysis identified that CRM1 and CDK5 co-expression status was an independent prognostic factor for overall survival (OS) of patients with GC. Integration of CRM1 and CDK5 expression could provide additional prognostic value for OS compared with CRM1 or CDK5 expression alone (P = 0.001).

CONCLUSION

CRM1 and CDK5 co-expression was an independent prognostic factors for GC. Combined CRM1 and CDK5 expression could provide a prognostic model for OS of GC.

Key words: Gastric cancer; CRM1; CDK5; Prognosis

© The Author(s) 2017. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Our study shows that low expression of chromosomal maintenance (CRM)1 and cyclin-dependent kinase (CDK)5 was associated with poor prognosis of gastric cancer patients. The expression of CRM1 or CDK5 influenced the prognostic value of each other. Combined CRM1 and CDK5 expression had better prognostic power than their individual expression had.

Sun YQ, Xie JW, Xie HT, Chen PC, Zhang XL, Zheng CH, Li P, Wang JB, Lin JX, Cao LL, Huang CM, Lin Y. Expression of CRM1 and CDK5 shows high prognostic accuracy for gastric cancer. *World J Gastroenterol* 2017; 23(11): 2012-2022 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i11/2012.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i11.2012

INTRODUCTION

Gastric cancer (GC) is the third leading cause of cancer-related death worldwide, although its incidence and mortality have decreased dramatically over the last 50 years[1]. In 2011 there were about 420000 new cases diagnosed (70% men and 30% women) and 300 000 deaths due to this disease in China[2-4]. Clinically, the prognostic classification model for outcomes of GC patients is mainly the TNM staging system based on the histopathological score[5], whereas the underlying molecular and cellular processes during carcinogenesis of GC are ignored. Patients with the same TNM stage may have wide variations in survival owing to different genetic mutation status[6]. Therefore, a better understanding of the molecular pathology might provide better prognostic biomarkers and guidance for more precise treatment for GC patients.

The human nuclear export protein chromosomal maintenance (CRM)1 (also known as exportin 1) has been reported to control multiple processes during cellular mitosis and is important in mediating nuclear export of cargo proteins that contain specific leucine-rich nuclear export signal (NES) consensus sequences[7,8]. Previous studies have demonstrated that CRM1 is important for the functions of proteins such as epidermal growth factor receptor, p53, p27, cyclin-dependent kinase (CDK)5 and Akt1[9-13]. The prognostic value of CRM1 expression has been reported in many types of cancer including ovarian cancer[14], osteosarcoma[15], glioma[16], pancreatic cancer[17] and esophageal squamous cell carcinoma[18]. However, whether CRM1 expression contributes to the development or progression of GC is not known.

CDK5 is a proline-directed serine/threonine kinase and participates in a variety of pathological and physiological functions[19,20]. Increasing evidence suggests a role for CDK5 in cancer tumorigenesis and progression[21,22]. Our previous work has demonstrated that in GC, CDK5 downregulation is an independent prognostic factor and the nuclear localization of CDK5 is critical for its tumor-suppressor function[23]. Given that CRM1 regulates CDK5 cytoplasm localization in neurons[12], we hypothesized that the functional correlation between CRM1 and CDK5 may affect the prognostic power of each molecule. In the present study, we examined the expression of CRM1 and CDK5 in 240 gastric tumor tissues and analyzed their correlation with patient clinicopathological features.

MATERIALS AND METHODS

Patients and specimens

The study cohort was composed of samples from 240 patients (178 men and 62 women, mean age: 59.5 years) with gastric adenocarcinoma, who had undergone gastrectomy at the Department of Gastric Surgery, Fujian Medical University Union Hospital, between January 2009 and December 2009. Following surgery, routine chemotherapy was given to patients with advanced disease and no radiation treatment was administered to any of the patients. Eligibility criteria for patients included in this study were: (1) histologically proven adenocarcinoma; (2) no other gastric tumors such as gastric stromal tumor; (3) no history of gastrectomy or other malignancy; (4) no prior neoadjuvant chemotherapy; and (5) availability of complete clinicopathological and survival data (Figure 1). The study was performed with the approval of the Ethics Committee of Fujian Medical Union Hospital. Written consent was given by the patients for their information and specimens to be stored in the hospital database and used for research.

Clinicopathological and survival data

The clinical and pathological data were recorded prospectively for the retrospective analysis. The clinicopathological data for the 240 GC patients included age, sex, size of primary tumor, location of primary tumor, degree of differentiation, histological

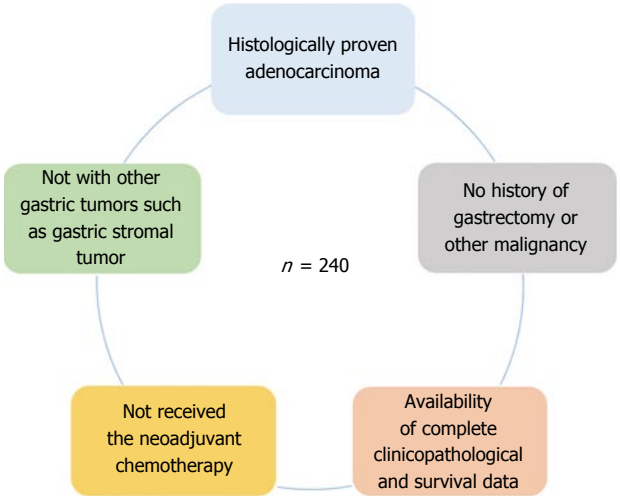


Figure 1 Eligibility criteria for patient inclusion.

type, Lauren's classification, Borrmann type, depth of invasion, lymph node metastasis, TNM stage, vessel invasion and distant metastasis. The pathological stage of the tumor was reassessed according to the 2010 International Union Against Cancer on GC TNM Classification (seventh edition)[5]. Overall survival (OS) was defined as the time from curative surgery to death or the last clinical follow-up. After surgery, all patients were followed by outpatient visits, telephone calls and letters every 3 mo in the first 2 years, every 6 mo in the next 3 years, and every year afterwards or until death. The deadline for follow-up was October 2015. All patients had follow-up records for > 5 years.

Immunohistochemistry

Paraffin blocks that contained sufficient formalin-fixed tumor specimens were serial sectioned at 4 μm and mounted on silane-coated slides for immunohistochemistry analysis. The sections were deparaffinized with dimethylbenzene and rehydrated through 100, 100, 95, 85, and 75% ethanol. Antigen retrieval treatment was done in 0.01 mol/L sodium citrate buffer (autoclaved at 121 ℃ for 2 min, pH 6.0) and endogenous peroxidase was blocked by incubation in 3% H_2O_2 for 10 min at room temperature. The sections were then washed in phosphate-buffered saline (PBS) and blocked with 10% goat serum (ZhongShan Biotechnology, China) for 30 min and incubated with rabbit anti-human CRM1 (ab24189, 1:200 dilution; Abcam, Cambridge, MA, United States) or CDK5 (sc-173, 1:150 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, United States) antibody in a humidified chamber at 4 ℃ overnight. Following three additional washes in PBS, the sections were incubated with horseradish-peroxidase-conjugated secondary antibody for 30 min at room temperature. The visualization signal was developed with diaminobenzidine solution and all slides were counterstained with 20% hematoxylin. Finally, all slides were dehydrated and mounted on coverslips. For negative controls, the primary antibody diluent was used to replace primary antibody.

Evaluation of immunostaining intensity

The stained tissue sections were reviewed under a microscope by two pathologists who were blinded to the clinical parameters, and scored independently according to the intensity of cellular staining and the proportion of stained tumor cells[6]. The CRM1 and CDK5 proteins were immunohistochemically stained yellowish to brown in the cytoplasm and/or nuclei of cancer cells. The expression pattern of CRM1 and CDK5 was all or none in tumor tissues, suggesting the score for the proportion of stained tumor cells was unavailable. The staining intensity was scored as 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining, yellow brown), and 3 (strong staining, brown) (Figure 2). The CRM1 and CDK5 protein expression was considered low if the score was $\leqslant 1$ and high if it was $\geqslant 2$.

Statistical analysis

IBM SPSS version 19.0 (SPSS, Chicago, IL, United States) was used for all statistical analyses. χ^2 and Fisher's exact tests were used to analyze categorical data. Univariate survival analysis was performed using the Kaplan-Meier method, and the significance of difference between groups was analyzed using the log-rank test. The stepwise Cox proportional hazards regression model was used for multivariate survival analysis, with adjustments for variables that may have been significant prognostic factors according to the univariate analysis. Receiver operating characteristic (ROC) analysis was used to compare the accuracy of the prediction of clinical outcome by the parameters. All *P* values were two-sided and statistical significance was determined at $P < 0.05$.

RESULTS

Expression status of CRM1 and CDK5 in GC

We examined CRM1 and CDK5 protein expression in tumor tissues from 240 GC patients using immunohistochemistry. The expression of CRM1 and CDK5 proteins were scored as low in 149 (62.08%) and 91 (37.92%) samples, and high in 91 (37.92%) and 149 (62.08%) samples, respectively. Based on the combined expression of CRM1 and CDK5, we classified the patients into three subtypes: CRM1 and CDK5 high ($n = 63$), CRM1 or CDK5 low ($n = 114$) and CRM1 and CDK5 low ($n = 63$).

Correlation between CRM1 and CDK5 expression and clinicopathological parameters in GC patients

The correlation between expression of CRM1 and CDK5 and the clinicopathological features were analyzed (Table 1). CRM1 expression was significantly related to size of primary tumor ($P = 0.005$), Borrmann type (*P*

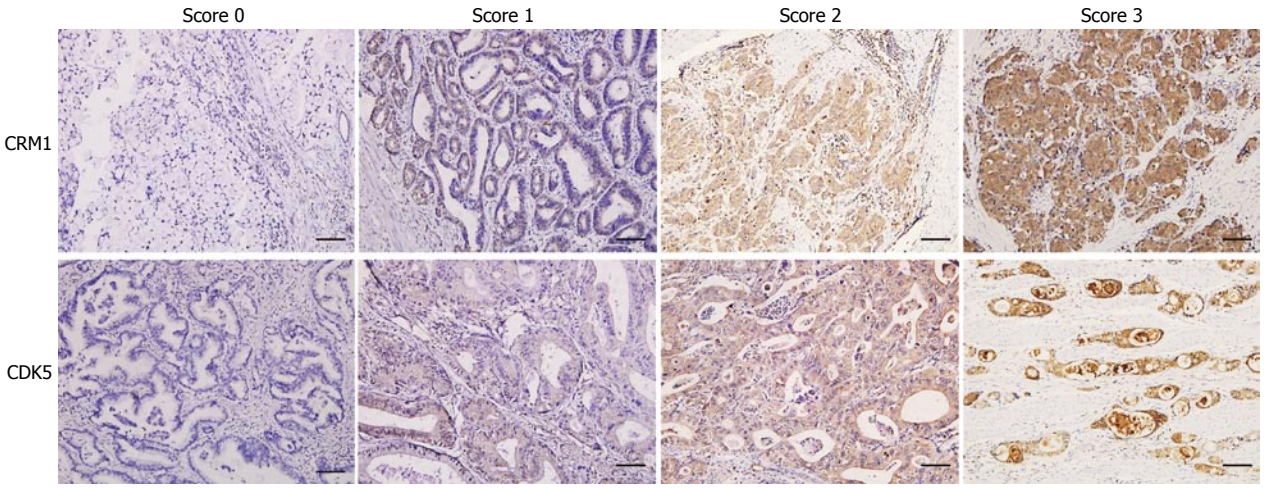


Figure 2 Immunohistochemical staining of CRM1 and CDK5 expression in gastric cancer tissue and the criteria for immunohistochemistry scoring. Score 0: no staining, Score 1: weak staining, Score 2: moderate staining, Score 3: strong staining. The protein expression was considered low if the score was ≤ 1 and high if it was ≥ 2. Scale bar = 100 μm.

= 0.006), degree of differentiation (P = 0.004), depth of invasion (P = 0.008), lymph node metastasis (P = 0.013), TNM stage (P = 0.002) and distant metastasis (P = 0.015). The expression of CDK5 was significantly related to sex (P = 0.048) and Lauren's classification (P = 0.011). The correlation between combined CRM1 and CDK5 expression and the clinicopathological features was also analyzed. The combined CRM1 and CDK5 expression was significantly related to size of primary tumor (P = 0.026), degree of differentiation (P = 0.007), Lauren's classification (P = 0.019), lymph node metastasis (P = 0.015), TNM stage (P = 0.035) and vessel invasion (P = 0.021) (Table 2).

Prognostic value of CRM1 and CDK5 expression

To elucidate the prognostic value of CRM1 and CDK5 expression, univariate Kaplan-Meier and multivariate Cox regression analyses were used. Univariate analysis revealed that OS was significantly associated with size and location of primary tumor, Borrmann type, degree of differentiation, depth of invasion, lymph node metastasis, TNM stage, vessel invasion, distant metastasis, CRM1 and CDK5 expression, but not with sex, age at surgery, histological type, and Lauren's classification (Table 3). The hazard ratio and 95%CI for OS were compared among the subgroups. OS was shorter in patients with low expression of CRM1 or CDK5 in comparison to the corresponding patients with high CRM1 or CDK5 expression (Figure 3).

The 3- and 5-year cumulative survival rates were 54.1% and 39.7% for patients with low CRM1 expression, and 67.0% and 61.5% for those with high CRM1 expression. The mean survival time for patients with low and high expression of CRM1 was 44.6 and 56.5 mo, respectively. Clearly, GC patients with low expression of CRM1 had a poorer prognosis than those with high CRM1 expression (P < 0.05) (Figure 4A). The 3- and 5-year cumulative survival rates were 49.5% and 39.3% for GC patients with low expression of CDK5, and 63.6% and 53.4% for those with high CDK5 expression. The mean survival time for GC patients with low and high expression of CDK5 was 43.4 and 53.1 mo, respectively, suggesting a shorter OS for GC patients with low expression of CDK5 (P < 0.05) (Figure 4B).

We evaluated the prognostic value of the combined CRM1 and CDK5 expression. The patients with simultaneous high expression of CRM1 and CDK5 displayed better survival in comparison with the rest of the patients in Kaplan-Meier analysis (Figure 4C). The 3- and 5-year cumulative survival rates were 47.6% and 34.3% for the simultaneous low CRM1 and CDK5 expression patient group, 55.9% and 45.2% for the CRM1 or CDK5 low expression patient group, and 73.0% and 66.7% for the simultaneous high CRM1 and CDK5 expression patient group, respectively. The mean survival time was 41.5 mo for patients with CRM1 and CDK5 low expression; 46.9 mo for those with CRM1 or CDK5 low expression; and 61.1 mo for those with CRM1 and CDK5 high expression (Table 3).

The clinicopathological parameters that were correlated with patient survival in univariate analysis were included in multivariate analysis. CRM1 and CDK5 coexpression status, tumor size, tumor location, and TNM stage were independent prognostic factors for patients with GC, whereas vessel invasion and Borrmann type were not (Table 4).

Improvement of CDK5 prognostic model with CRM1 expression

In our previous work, we demonstrated that down-regulation of CDK5 in GC was an independent prognostic factor. To improve the prognostic accuracy of OS in GC patients, we combined CRM1 and CDK5 expression to generate a predictive model. ROC analysis was applied to compare the prognostic accuracy between

Table 1 Relationships between CRM1 and CDK5 protein expression (immunohistochemical staining) in gastric cancer tissues and various clinicopathological variables

Variables	Total	CRM1 expression				CDK5 expression			
		Low (n = 149)	High (n = 91)	χ^2	P value	Low (n = 91)	High (n = 149)	χ^2	P value
Gender									
Male	178	110	68	0.024	0.877	61	117	3.893	0.048[1]
Female	62	39	23			30	32		
Age at surgery (yr)									
≤ 60	120	78	42	0.867	0.352	46	74	0.018	0.894
> 60	120	71	49			45	75		
Size of primary tumor (cm)									
≤ 5	99	51	48	7.995	0.005[1]	35	64	0.470	0.493
> 5	141	98	43			56	85		
Location of primary tumor									
Upper 1/3	56	33	23	5.290	0.152	22	34	1.718	0.633
Middle 1/3	59	39	20			21	38		
Lower 1/3	103	59	44			37	66		
More than 1/3	22	18	4			11	11		
Borrmann type									
Early stage	10	4	6	10.118	0.006[1]	5	5	0.774	0.679
Ⅰ + Ⅱ type	89	46	43			32	57		
Ⅲ + Ⅳ type	141	99	42			54	87		
Degree of differentiation									
Well/moderate	96	49	47	8.287	0.004[1]	30	66	3.021	0.082
Poor and not	144	100	44			61	83		
Lauren's classification									
Intestinal type	46	33	13	2.254	0.176	25	21	6.527	0.011[1]
Diffuse type	294	116	78			66	128		
Histological type									
Papillary	7	4	3	2.958	0.398	3	4	7.052	0.070
Tubular	187	112	75			63	124		
Mucinous	20	13	7			10	10		
Signet-ring cell	26	20	6			15	11		
Depth of invasion									
T1	40	18	22	11.908	0.008[1]	15	25	2.145	0.543
T2	27	13	14			8	19		
T3	62	38	24			21	41		
T4	111	80	31			47	64		
Lymph node metastasis									
N0	63	29	34	10.781	0.013[1]	23	40	4.868	0.182
N1	40	29	11			11	29		
N2	43	26	17			14	29		
N3	94	65	29			43	51		
TNM stage									
Ⅰ	44	18	26	15.074	0.002[1]	15	29	1.058	0.787
Ⅱ	55	33	22			19	36		
Ⅲ	123	82	41			49	74		
Ⅳ	18	16	2			8	10		
Vessel invasion									
Negative	230	141	89	1.423	0.233	88	142	0.278	0.598
Positive	10	8	2			3	7		
Distant metastasis									
Negative	222	133	89	5.940	0.015[1]	83	139	0.352	0.553
Positive	18	16	2			8	10		

[1]P < 0.05, statistical significance. CRM: Chromosomal maintenance; CDK: Cyclin-dependent kinase.

combined CRM1 and CDK5 expression and CRM1 or CDK5 expression alone. Combination of CRM1 and CDK5 expression showed significantly higher prognostic accuracy [area under the curve (AUC): 0.622, 95%CI: 0.551-0.694, P = 0.001] than CRM1 expression alone (AUC: 0.585, 95%CI: 0.512-0.657, P = 0.024) or CDK5 expression alone (AUC: 0.575, 95%CI: 0.503-0.648, P = 0.045) (Figure 5). All these results indicated that the combined CRM1 and CDK5 expression provided better prognostic power for GC patient OS.

DISCUSSION

Increasing evidence has demonstrated that the karyoplasm localization of CDK5 is important for its multiple pathological and physiological functions, including neuronal migration during brain development, neuronal cell survival and tumor development and

Table 2 Relationships between different CRM1 and CDK5 protein expression status in gastric cancer tissues and various clinicopathological variables

Variables	Total	CRM1 and CDK5 High expression	CRM1 or CDK5 Low expression	CRM1 and CDK5 Low expression	χ^2	*P* value
Gender						
Male	178	42	87	49	2.553	0.279
Female	62	21	27	14		
Age at surgery(yr)						
≤ 60	120	35	54	31	1.109	0.574
> 60	120	28	60	32		
Size of primary tumor (cm)						
≤ 5	99	22	42	35	7.275	0.026[1]
> 5	141	41	72	28		
Location of primary tumor						
Lower 1/3	56	18	19	19	10.848	0.093
Middle 1/3	59	14	32	13		
Upper 1/3	103	22	52	29		
More than 1/3	22	9	11	2		
Borrmann type						
Early stage	10	2	5	3	6.035	0.197
Ⅰ + Ⅱ type	89	20	38	31		
Ⅲ + Ⅳ type	141	41	71	29		
Degree of differentiation						
Well/moderate	96	18	43	35	10.027	0.007[1]
Poor and not	144	45	71	28		
Lauren's classification						
Intestinal type	46	17	24	5	7.875	0.019[1]
Diffuse type	194	46	90	58		
Histological type						
Papillary	7	2	3	2	11.127	0.850
Tubular	187	44	87	56		
Mucinous	20	5	13	2		
Signet-ring cell	26	12	11	3		
Depth of invasion						
T1	40	8	17	15	10.996	0.088
T2	27	4	13	10		
T3	62	16	27	19		
T4	111	35	57	19		
Lymph node metastasis						
N0	63	15	22	26	15.845	0.015[1]
N1	40	9	22	9		
N2	43	7	26	10		
N3	94	32	44	18		
TNM stage						
Ⅰ	44	8	17	19	13.543	0.035[1]
Ⅱ	55	14	24	17		
Ⅲ	123	33	65	25		
Ⅳ	18	8	8	2		
Vessel invasion						
Negative	230	62	105	63	7.757	0.021[1]
Positive	10	1	9	0		
Distant metastasis						
Negative	222	55	106	61	4.191	0.123
Positive	18	8	8	2		

[1]$P < 0.05$, statistical significance. CRM: Chromosomal maintenance; CDK: Cyclin-dependent kinase.

progression[23-27]. CDK5 has no intrinsic nuclear localization signal and its nuclear localization relies on p27[12]. In the absence of p27, two weak NESs on CDK5 bind to CRM1, leading to the cytoplasmic shuttle of CDK5[12]. In this study, low CDK5 expression was associated with poorer prognosis (Figure 4B), which was consistent with our previous discovery that CDK5 acted as a tumor suppressor in GC[23]. However, CRM1 is usually considered as an oncogene and involved in the nuclear export of a number of proteins including p53, p21, c-ABL and FOXOs[28-30]. Forgues *et al*[31] found that cytoplasmic sequestration of CRM1 is frequently associated with hepatocellular carcinoma. In this work, high CRM1 expression was associated with longer GC patient survival (Figure 4A), suggesting that CRM1 exerts a tumor suppressive role in GC. Considering the oncogenic role of CDK5 in many other types of cancer such as hepatocellular carcinoma[24], breast cancer[32]

Table 3 Univariate analysis of the correlation between clinicopathological parameters and survival of patients with gastric cancer

Clinicopathological parameters	Cumulative survival rates (%)		Mean survival time (mo)	Log-rank test	*P* value
	3 yr	5 yr			
Gender					
Male	66.1	48.3	49.022	0.092	0.762
Female	56.6	48.0	49.324		
Age at surgery (yr)					
≤ 60	60.8	48.1	49.510	0.022	0.882
> 60	57.2	47.9	49.285		
Size of primary tumor (cm)					
≤ 5	84.8	73.4	66.451	44.251	0.000[1]
> 5	41.1	30.4	37.516		
Location of primary tumor					
Upper 1/3	51.8	38.7	44.354	28.888	0.000[1]
Middle 1/3	42.4	33.9	39.508		
Lower 1/3	76.5	66.7	61.597		
More than 1/3	31.8	22.7	30.500		
Borrmann type					
Early stage	90.0	90.0	72.186	41.770	0.000[1]
Ⅰ + Ⅱ type	81.9	71.5	64.835		
Ⅲ + Ⅳ type	42.6	30.4	38.102		
Degree of differentiation					
Well/moderate	70.8	60.3	57.397	8.644	0.003[1]
Poor and not	49.8	39.9	44.056		
Lauren's classification					
Intestinal type	66.8	50.7	53.287	0.649	0.420
Diffuse type	56.2	47.4	48.471		
Histological type					
Papillary	57.1	57.1	50.857	1.026	0.752
Tubular	57.2	47.0	48.339		
Mucinous	75.0	53.6	53.850		
Signet-ring cell	60.2	48.2	51.110		
Depth of invasion					
T1	97.5	94.9	78.311	64.970	0.000[1]
T2	88.9	74.1	67.889		
T3	59.2	46.0	48.764		
T4	37.8	25.2	34.461		
Lymph node metastasis					
N0	88.9	80.8	70.120	59.862	0.000[1]
N1	69.5	69.5	61.079		
N2	58.1	34.9	43.674		
N3	33.0	23.3	32.911		
TNM stage					
Ⅰ	97.7	95.4	78.211	71.616	0.000[1]
Ⅱ	76.1	61.3	60.241		
Ⅲ	40.7	29.2	38.186		
Ⅳ	27.8	16.7	22.518		
Vessel invasion					
Negative	60.8	49.3	50.492	8.264	0.004[1]
Positive	20.0	20.0	23.400		
Distant metastasis					
Negative	60.7	50.6	51.544	20.223	0.000[1]
Positive	16.7	16.7	22.518		
CRM1 expression					
Low	54.1	39.7	44.590	7.707	0.005[1]
High	67.0	61.5	56.540		
CDK5 expression					
Low	49.5	39.3	53.058	6.234	0.013[1]
High	63.6	53.4	43.438		
CRM1/CDK5 expression					
CRM1 and CDK5 Low	47.6	34.3	41.487	13.683	0.001[1]
CRM1 or CDK5 Low	55.9	45.2	46.873		
CRM1 and CDK5 High	73.0	66.7	61.069		

[1]$P < 0.05$, statistical significance. CRM: Chromosomal maintenance; CDK: Cyclin-dependent kinase.

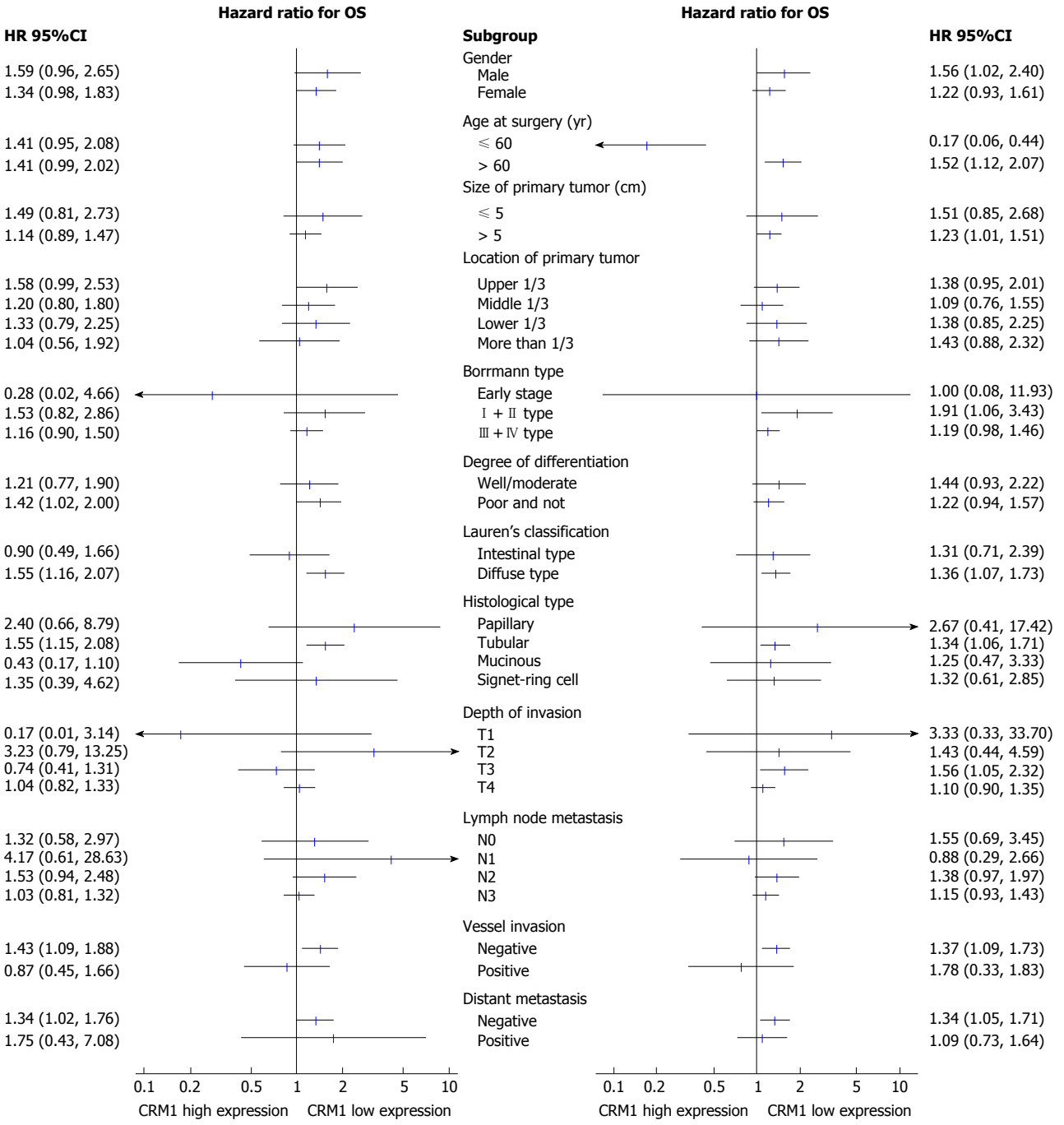


Figure 3 Forest plot showing hazard ratios (oblongs) and 95%CI (bars) for overall survival of subgroups from the 240 gastric cancer patients with different CRM1 (left) and CDK5 (right) expression status. HR: Hazard ratio; OS: Overall survival; CRM: Chromosomal maintenance; CDK: Cyclin-dependent kinase.

Table 4 Multivariate analysis of the correlation between clinicopathological parameters and survival time of patients with gastric cancer

Covariates	Coefficient	Standard error	HR	95% CI for HR	*P* value
Tumor location (cardia *vs* others)	0.451	0.202	1.570	1.057-2.333	0.026[1]
Tumor size (≥ 5 *vs* < 5 cm)	0.723	0.232	2.060	1.309-3.243	0.002[1]
Vessel invasion (positive *vs* negative)	NA	NA	NA	NA	NA
TNM stage (stage Ⅲ and Ⅳ *vs* Ⅰ and Ⅱ)	1.086	0.243	1.961	1.839-4.768	0.000[1]
CDK5 and CRM1 expression					
(low/high *vs* high/high)	0.568	0.254	1.765	1.074-2.903	0.025[1]
(low/low *vs* high/high)	0.769	0.269	2.158	1.274-3.657	0.004[1]
Borrmann type (type early, Ⅰ, Ⅱ *vs* Ⅲ, Ⅳ)	NA	NA	NA	NA	NA

[1]$P < 0.05$, statistical significance. NA: Not available.

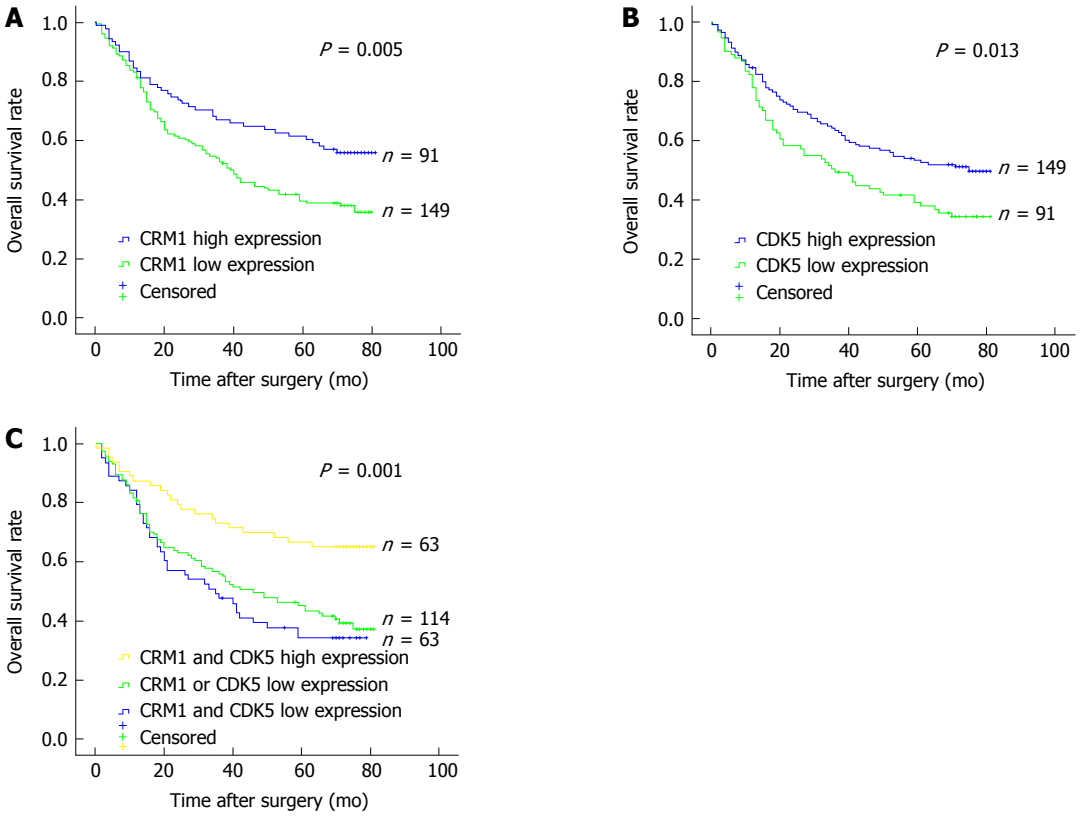
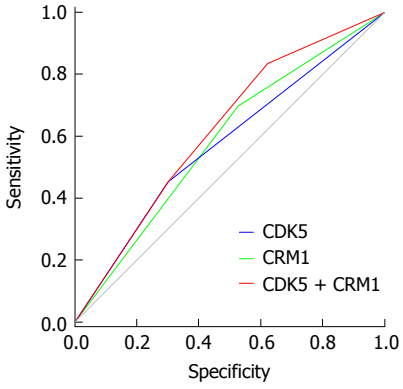


Figure 4 Kaplan-Meier analysis of the correlation between expression of CRM1 (A), CDK5 (B) and combined CRM1 and CDK5 expression (C) and the overall survival of gastric cancer patients. CRM: Chromosomal maintenance; CDK: Cyclin-dependent kinase.



	AUC	95%CI	*P*
CDK5	0.575	0.503-0.648	0.045
CRM1	0.585	0.512-0.657	0.024
CDK5 + CRM1	0.622	0.551-0.694	0.001

Figure 5 Receiver operating characteristic analysis of the sensitivity and specificity of the predictive value of the combined CRM1 and CDK5 expression model, CRM1 expression model and CDK5 expression model. CRM: Chromosomal maintenance; CDK: Cyclin-dependent kinase.

and neuroendocrine thyroid cancer[25], it is possible that the shift of CDK5 function in GC affects the function of CRM1. In addition, we recently found that CDK5RAP3, a binding protein of the CDK5 activator p35, negatively regulates the β-catenin signaling pathway by repressing glycogen synthase kinase-3β phosphorylation and acts as a tumor suppressor in GC[33]. The differential expression or activities of other CDK5-binding partners such as CDK5RAP3 may also affect the functions of CDK5 and CRM1 among different cancer types.

The fact that either CDK5 or CRM1 expression could influence the prognostic power of the other (Figure 4C) seemed to support this hypothesis. Further analysis with ROC revealed that combination of CRM1 and CDK5 expression showed significantly higher prognostic accuracy than CRM1 or CDK5 expression alone (P = 0.001) (Figure 5), indicating that combined CRM1 and CDK5 expression show more prognostic power for OS of patients with GC. Taken together, our present study suggested that CRM1 and CDK5 should receive considerable attention as effective markers for predicting therapeutic outcomes, but the profound molecular roles of CRM1 and CDK5 in GC remain far from being fully elucidated and need further research.

In addition, we found that low CRM1 expression was associated with lymph node metastasis in GC (Table 1). This suggested that the identification of CRM1 expression in preoperative mucosal biopsies from GC patients may indicate the necessity for a more aggressive lymphadenectomy, although further studies in a larger cohort of patients are needed.

In conclusion, our results suggested that combined CRM1 and CDK5 expression was an independent prognostic factor for OS and showed more prognostic power in GC patients. Considering the inferior prognosis of the CRM1 and/or CDK5 low patients, more frequent

follow-up is probably needed for these patients after surgery.

COMMENTS

Background

To evaluate the prognostic value of the expression of chromosomal maintenance (CRM)1 and cyclin-dependent kinase (CDK)5 for gastric cancer (GC) patients after gastrectomy.

Research frontiers

CDK5 downregulation was an independent prognostic factor and the nuclear localization of CDK5 was critical for its tumor suppressor function in GC. Given that CRM1 regulates CDK5 karyoplasm localization in neurons, we hypothesized that the functional correlation between CRM1 and CDK5 may affect the prognostic power of each molecule. In the present study, we examined the expression of CRM1 and CDK5 in 240 gastric tumor tissues and analyzed their correlation with patient clinicopathological features.

Innovations and breakthroughs

CRM1 and CDK5 coexpression was an independent prognostic factor for patients with GC. The present results suggested that combined CRM1 and CDK5 expression could provide a better prognostic model for overall survival (OS) of GC patients.

Applications

The presented results suggested that combined CRM1 and CDK5 expression was an independent prognostic factor for OS of GC patients and showed more prognostic power than individual factors alone. Considering the inferior prognosis of the CRM1 and/or CDK5 low patients, more frequent follow-up is probably needed for these patients after surgery.

Peer-review

The authors investigate whether combined expression of CDK5 and CRM1 correlates with clinic-pathological parameters in GC. The manuscript is sound and the experiments/correlations are well-performed.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Yang L**. Incidence and mortality of gastric cancer in China. *World J Gastroenterol* 2006; **12**: 17-20 [PMID: 16440411 DOI: 10.3748/wjg.v12.i1.17]

3 **Chen W**, Zheng R, Zeng H, Zhang S. The updated incidences and mortalities of major cancers in China, 2011. *Chin J Cancer* 2015; **34**: 502-507 [PMID: 26370301 DOI: 10.1186/s40880-015-0042-6]

4 **Chen W**, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; **66**: 115-132 [PMID: 26808342 DOI: 10.3322/caac.21338]

5 **Washington K**. 7th edition of the AJCC cancer staging manual: stomach. *Ann Surg Oncol* 2010; **17**: 3077-3079 [PMID: 20882416 DOI: 10.1245/s10434-010-1362-z]

6 **Shou ZX**, Jin X, Zhao ZS. Upregulated expression of ADAM17 is a prognostic marker for patients with gastric cancer. *Ann Surg* 2012; **256**: 1014-1022 [PMID: 22668812 DOI: 10.1097/SLA.0b013e3182592f56]

7 **Fukuda M**, Asano S, Nakamura T, Adachi M, Yoshida M, Yanagida M, Nishida E. CRM1 is responsible for intracellular transport mediated by the nuclear export signal. *Nature* 1997; **390**: 308-311 [PMID: 9384386 DOI: 10.1038/36894]

8 **Ossareh-Nazari B**, Bachelerie F, Dargemont C. Evidence for a role of CRM1 in signal-mediated nuclear protein export. *Science* 1997; **278**: 141-144 [PMID: 9311922 DOI: 10.1126/science.278.5335.141]

9 **Stommel JM**, Marchenko ND, Jimenez GS, Moll UM, Hope TJ, Wahl GM. A leucine-rich nuclear export signal in the p53 tetramerization domain: regulation of subcellular localization and p53 activity by NES masking. *EMBO J* 1999; **18**: 1660-1672 [PMID: 10075936 DOI: 10.1093/emboj/18.6.1660]

10 **Saji M**, Vasko V, Kada F, Allbritton EH, Burman KD, Ringel MD. Akt1 contains a functional leucine-rich nuclear export sequence. *Biochem Biophys Res Commun* 2005; **332**: 167-173 [PMID: 15896313 DOI: 10.1016/j.bbrc.2005.04.109]

11 **He W**, Wang X, Chen L, Guan X. A crosstalk imbalance between p27(Kip1) and its interacting molecules enhances breast carcinogenesis. *Cancer Biother Radiopharm* 2012; **27**: 399-402 [PMID: 22690887 DOI: 10.1089/cbr.2010.0802]

12 **Zhang J**, Li H, Herrup K. Cdk5 nuclear localization is p27-dependent in nerve cells: implications for cell cycle suppression and caspase-3 activation. *J Biol Chem* 2010; **285**: 14052-14061 [PMID: 20189989 DOI: 10.1074/jbc.M109.068262]

13 **Lo HW**, Ali-Seyed M, Wu Y, Bartholomeusz G, Hsu SC, Hung MC. Nuclear-cytoplasmic transport of EGFR involves receptor endocytosis, importin beta1 and CRM1. *J Cell Biochem* 2006; **98**: 1570-1583 [PMID: 16552725 DOI: 10.1002/jcb.20876]

14 **Noske A**, Weichert W, Niesporek S, Röske A, Buckendahl AC, Koch I, Sehouli J, Dietel M, Denkert C. Expression of the nuclear export protein chromosomal region maintenance/exportin 1/Xpo1 is a prognostic factor in human ovarian cancer. *Cancer* 2008; **112**: 1733-1743 [PMID: 18306389 DOI: 10.1002/cncr.23354]

15 **Yao Y**, Dong Y, Lin F, Zhao H, Shen Z, Chen P, Sun YJ, Tang LN, Zheng SE. The expression of CRM1 is associated with prognosis in human osteosarcoma. *Oncol Rep* 2009; **21**: 229-235 [PMID: 19082467]

16 **Shen A**, Wang Y, Zhao Y, Zou L, Sun L, Cheng C. Expression of CRM1 in human gliomas and its significance in p27 expression and clinical prognosis. *Neurosurgery* 2009; **65**: 153-159; discussion 159-160 [PMID: 19574837 DOI: 10.1227/01.NEU.0000348550.47441.4B]

17 **Huang WY**, Yue L, Qiu WS, Wang LW, Zhou XH, Sun YJ. Prognostic value of CRM1 in pancreas cancer. *Clin Invest Med* 2009; **32**: E315 [PMID: 20003838]

18 **van der Watt PJ**, Zemanay W, Govender D, Hendricks DT, Parker MI, Leaner VD. Elevated expression of the nuclear export protein, Crm1 (exportin 1), associates with human oesophageal squamous cell carcinoma. *Oncol Rep* 2014; **32**: 730-738 [PMID: 24898882]

19 **Choi JH**, Banks AS, Estall JL, Kajimura S, Boström P, Laznik D, Ruas JL, Chalmers MJ, Kamenecka TM, Blüher M, Griffin PR, Spiegelman BM. Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARgamma by Cdk5. *Nature* 2010; **466**: 451-456 [PMID: 20651683 DOI: 10.1038/nature09291]

20 **Hisanaga S**, Endo R. Regulation and role of cyclin-dependent kinase activity in neuronal survival and death. *J Neurochem* 2010; **115**: 1309-1321 [PMID: 21044075 DOI: 10.1111/j.1471-4159.2010.07050.x]

21 **Lindqvist J**, Imanishi SY, Torvaldson E, Malinen M, Remes M, Örn F, Palvimo JJ, Eriksson JE. Cyclin-dependent kinase 5 acts as a critical determinant of AKT-dependent proliferation and regulates differential gene expression by the androgen receptor in prostate cancer cells. *Mol Biol Cell* 2015; **26**: 1971-1984 [PMID: 25851605 DOI: 10.1091/mbc.E14-12-1634]

22 **Tripathi BK**, Qian X, Mertins P, Wang D, Papageorge A, Carr S, Lowy DR. CDK5 negatively regulates Rho by phosphorylating and activating the Rho-GAP and tumor suppressor functions of DLC1. *Cancer Res* 2014; **74**: 1574-1574 [DOI: 10.1158/1538-7445.AM2014-1574]

23 **Cao L**, Zhou J, Zhang J, Wu S, Yang X, Zhao X, Li H, Luo M, Yu Q, Lin G, Lin H, Xie J, Li P, Hu X, Zheng C, Bu G, Zhang YW, Xu H, Yang Y, Huang C, Zhang J. Cyclin-dependent kinase 5 decreases in gastric cancer and its nuclear accumulation suppresses gastric tumorigenesis. *Clin Cancer Res* 2015; **21**: 1419-1428 [PMID: 25609066 DOI: 10.1158/1078-0432.CCR-14-1950]

24 **Ehrlich SM**, Liebl J, Ardelt MA, Lehr T, De Toni EN, Mayr D,

Brandl L, Kirchner T, Zahler S, Gerbes AL, Vollmar AM. Targeting cyclin dependent kinase 5 in hepatocellular carcinoma--A novel therapeutic approach. *J Hepatol* 2015; **63**: 102-113 [PMID: 25660209 DOI: 10.1016/j.jhep.2015.01.031]

25 **Pozo K**, Castro-Rivera E, Tan C, Plattner F, Schwach G, Siegl V, Meyer D, Guo A, Gundara J, Mettlach G, Richer E, Guevara JA, Ning L, Gupta A, Hao G, Tsai LH, Sun X, Antich P, Sidhu S, Robinson BG, Chen H, Nwariaku FE, Pfragner R, Richardson JA, Bibb JA. The role of Cdk5 in neuroendocrine thyroid cancer. *Cancer Cell* 2013; **24**: 499-511 [PMID: 24135281 DOI: 10.1016/j.ccr.2013.08.027]

26 **Merk H**, Zhang S, Lehr T, Müller C, Ulrich M, Bibb JA, Adams RH, Bracher F, Zahler S, Vollmar AM, Liebl J. Inhibition of endothelial Cdk5 reduces tumor growth by promoting non-productive angiogenesis. *Oncotarget* 2016; **7**: 6088-6104 [PMID: 26755662]

27 **Zhang J**, Li H, Yabut O, Fitzpatrick H, D'Arcangelo G, Herrup K. Cdk5 suppresses the neuronal cell cycle by disrupting the E2F1-DP1 complex. *J Neurosci* 2010; **30**: 5219-5228 [PMID: 20392944 DOI: 10.1523/JNEUROSCI.5628-09.2010]

28 **Connor MK**, Kotchetkov R, Cariou S, Resch A, Lupetti R, Beniston RG, Melchior F, Hengst L, Slingerland JM. CRM1/Ran-mediated nuclear export of p27(Kip1) involves a nuclear export signal and links p27 export and proteolysis. *Mol Biol Cell* 2003; **14**: 201-213 [PMID: 12529437 DOI: 10.1091/mbc.E02-06-0319]

29 **Vigneri P**, Wang JY. Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR-ABL tyrosine kinase. *Nat Med* 2001; **7**: 228-234 [PMID: 11175855 DOI: 10.1038/84683]

30 **Vogt PK**, Jiang H, Aoki M. Triple layer control: phosphorylation, acetylation and ubiquitination of FOXO proteins. *Cell Cycle* 2005; **4**: 908-913 [PMID: 15917664 DOI: 10.4161/cc.4.7.1796]

31 **Forgues M**, Difilippantonio MJ, Linke SP, Ried T, Nagashima K, Feden J, Valerie K, Fukasawa K, Wang XW. Involvement of Crm1 in hepatitis B virus X protein-induced aberrant centriole replication and abnormal mitotic spindles. *Mol Cell Biol* 2003; **23**: 5282-5292 [PMID: 12861014 DOI: 10.1128/MCB.23.15.5282-5292.2003]

32 **Chiker S**, Pennaneach V, Loew D, Dingli F, Biard D, Cordelières FP, Gemble S, Vacher S, Bieche I, Hall J, Fernet M. Cdk5 promotes DNA replication stress checkpoint activation through RPA-32 phosphorylation, and impacts on metastasis free survival in breast cancer patients. *Cell Cycle* 2015; **14**: 3066-3078 [PMID: 26237679 DOI: 10.1080/15384101.2015.1078020]

33 **Wang JB**, Wang ZW, Li Y, Huang CQ, Zheng CH, Li P, Xie JW, Lin JX, Lu J, Chen QY, Cao LL, Lin M, Tu RH, Lin Y, Huang CM. CDK5RAP3 acts as a tumor suppressor in gastric cancer through inhibition of β-catenin signaling. *Cancer Lett* 2017; **385**: 188-197 [PMID: 27793695 DOI: 10.1016/j.canlet.2016.10.024]

P- Reviewer: Liebl J **S- Editor**: Yu J **L- Editor**: Kerr C
E- Editor: Zhang FF

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327

© 2017 Baishideng Publishing Group Inc. All rights reserved.