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Basic Study

Low expression of CDK5RAP3 and DDRGK1 indicates a poor prognosis in patients with gastric cancer

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Abstract

AIM

To investigate the effects of different levels of expression of CDK5RAP3 and DDRGK1 on long-term survival of patients undergoing radical gastrectomy.

METHODS

The expression of CDK5RAP3 and DDRGK1 was detected by immunohistochemistry in 135 patients who received standard gastrectomy were enrolled in the study. Western Blot was used to detect the expression of CDK5RAP3 and DDRGK1 in gastric cancer and its adjacent tissues and cell lines. The correlations between the expression of CDK5RAP3 and DDRGK1 and clinicopathological factors were analyzed, and the value of each parameter to the prognosis of the patients was compared. Receiver operating characteristic analysis was used to compare the accuracy of the prediction of clinical outcome by the parameters.

RESULTS

CDK5RAP3 and DDRGK1 expression was down-regulated in the gastric cancer compared to its respective adjacent non-tumor tissues. The expression of CDK5RAP3 was closely related to the age of the patients ($P = 0.035$) and the T stage of the tumor ($P = 0.017$). The expression of DDRGK1 was correlated with the sex of the patients ($P = 0.080$), the degree of tumor differentiation ($P = 0.036$), the histological type ($P = 0.036$) and the N stage of the tumor ($P = 0.014$). Low expression CDK5RAP3 or DDRGK1 is a poor prognostic factor for gastric cancer patients. Prognostic analysis showed that the co-expression of CDK5RAP3 and DDRGK1 was an independent prognostic factor correlating with the overall survival of gastric cancer patients. Combined expression analysis of CDK5RAP3 and DDRGK1 may provide a more accurate prognostic value for overall survival.

CONCLUSION

The co-expression of CDK5RAP3 and DDRGK1 is an independent prognostic factor for gastric cancer, which can provide a more accurate model for the long-term prognosis.

Key words: Gastric cancer; CDK5RAP3; DDRGK1; Prognosis

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Core tip: The expression of CDK5RAP3 and DDRGK1 was down-regulated in gastric cancer tissues. Low expression CDK5RAP3 or DDRGK1 is a poor prognostic factor for gastric cancer patients. The co-expression of CDK5RAP3 and DDRGK1 is an independent prognostic factor for the overall survival of patients with gastric cancer. Moreover, we also found that co-expression of CDK5RAP3 and DDRGK1 can provide a more accurate model for the long-term prognosis of gastric cancer.

Lin JX, Xie XS, Weng XF, Zheng CH, Xie JW, Wang JB, Lu J, Chen QY, Cao LL, Lin M, Tu RH, Li P, Huang CM. Low expression of CDK5RAP3 and DDRGK1 indicates a poor prognosis in patients with gastric cancer. *World J Gastroenterol* 2018; 24(34): 3898-3907 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v24/i34/3898.htm> DOI: <http://dx.doi.org/10.3748/wjg.v24.i34.3898>

INTRODUCTION

Although the morbidity and mortality of primary gastric cancer has declined in recent decades, it is still the third most common cause of cancer-related deaths worldwide^[1-3]. At present, the etiology and pathogenesis of gastric cancer has not yet been fully clarified. There is also a lack of specific and highly effective therapeutic drugs available for use in clinical practice. The symptom specificity of early gastric cancer is not obvious, so most patients are already in advanced stages before receiving medical treatment, which seriously affects the prognosis of patients. Therefore, searching for molecular markers that can be used as an independent prognostic factor for gastric cancer is of great significance for the early diagnosis and targeted treatment of gastric cancer.

The cyclin-dependent kinase 5 activating binding protein (CDK5RAP3, also called C53) was first identified as a binding protein of the cyclin-dependent kinase 5 (CDK5) activators P35 and P39^[4]. In recent years, an increasing number of studies have been conducted on the role of CDK5RAP3 in tumors, but its expression and role in different tumors has been found to be different. An *et al*^[5] reported that CDK5RAP3 inhibited the phosphorylation and activation of p38 by promoting the binding of p38 and p53-induced protein phosphatase 1 to inhibit tumor proliferation. However, Stav *et al*^[6] found that the expression of CDK5RAP3 in most cancer tissues was increased, which is of great significance in the diagnosis of lung cancer. The expression and function of CDK5RAP3 are also controversial in the same types of tumors. Mak *et al*^[7] found that CDK5RAP3 was highly expressed in hepatocellular cancer and that it could promote the metastasis of hepatoma cancer cell by activating p21-activated protease 4 and down-regulating the expression of tumor suppressor gene p14. However, Zhao *et al*^[8] showed that the expression of CDK5RAP3 protein was down-regulated in hepatocellular cancer and that down-regulation of CDK5RAP3 expression was associated with a poor prognosis.

Recent studies have shown that DDRGK1 interacts with CDK5RAP3^[9]. DDRGK1 was cloned from human liver in 2010 by Lemaire *et al*^[10]. DDRGK1 is located on the short arm of chromosome 20 (20p13), also known as UFBP1, C20orf116, or dJ1187M17. The DDRGK1 sequence is highly conserved and exists in many tissues and organs. Its N-terminal 1-28 amino acid residue region is highly hydrophobic and is an endoplasmic reticulum anchor sequence; 65-69 amino acid residues

are nuclear localization signals. The 229-273 amino acid residues near the C-terminus are the protein PCI domain^[11]. Studies have shown that proteins containing a PCI domain are primarily responsible for the construction and assembly of protein complexes^[12]. Xi *et al.*^[13] found that DDRGK1 interacts with IκBα and regulates its stability, thereby regulating the transcriptional activity of NF-κB. At present, there are few studies on the co-expression of CDK5RAP3 and DDRGK1 in gastric cancer and its impact on prognosis. In this study, we examined the expression of CDK5RAP3 and DDRGK1 in 135 cases of gastric cancer, and analyzed their correlation with clinicopathological features and long-term prognosis of the patients.

MATERIALS AND METHODS

Human gastric tumor tissues

The gastric cancer specimens were obtained from 135 patients with gastric adenocarcinoma, who had undergone D2 lymph node dissection and gastrectomy for gastric cancer at the Department of Gastric Surgery, Fujian Medical University Union Hospital (Fujian, China) with available detailed clinic pathologic parameters, between January 2013 and June 2015. All patients received their first diagnosis of gastric cancer and received no other treatment, such as chemotherapy, before surgery. All diagnoses were confirmed by pathology after surgery. Gastric cancer was confirmed by hematoxylin and eosin (H&E) staining in all cases. The clinicopathological data of the 135 GC patients included age, sex, size of the primary tumor, location of the primary tumor, degree of differentiation, histological type, Borrmann type, depth of invasion, lymph node metastasis, distant metastasis and TNM stage. The pathologic stage of the tumor was re-assessed according to the TNM classification of gastric cancer (eighth edition) of the International Union against Cancer (2016). The clinical and pathological data were recorded prospectively for the retrospective analysis. This study was approved by the ethics committee of Fujian Medical University Union Hospital and written consent was obtained from all patients involved.

Immunohistochemistry

Paraffin sections containing sufficient formalin fixed tumor tissue were sectioned continuously at a thickness of 4 μm and were mounted on silage coated slides for immunohistochemical analysis. The slices were deparaffinized with xylene and rehydrated in 95%, 85% and 75% ethanol. Antigen retrieval was performed by subjecting the slides to high-pressure sterilization at 121 °C for 2 min in 0.01 mol/L sodium citrate buffer solutions (pH 6.0). Endogenous peroxidase activity was blocked by incubating the slides with 3% H₂O₂ at room temperature for 10 min. The slices were then washed in phosphate buffered saline (PBS) solution and blocked in 10% goat serum (Zhongshan Biotechnology

Co. Ltd.) for 30 min. Next, the sections were incubated with diluted rabbit anti-human CDK5RAP3 (ab157203, 1:200 dilution; Abcam) or DDRGK1 (21445-1-AP, 1:50 dilution; Proteintech) overnight in a humidified chamber at 4 °C. After three washes in PBS, the sections were incubated with the secondary antibody conjugated to horseradish peroxidase at room temperature for 30 min. The signal was developed with diaminobenzidine solution, which was followed by counterstaining in 20% hematoxylin. Finally, all slides were dehydrated and mounted on cover glass. For negative controls, non-specific antibody diluent was substituted for the primary antibody.

Evaluation of immunostaining intensity

The immunohistochemistry (IHC) of the tissue sections were examined by two experienced pathologists, who scored the slides according to the intensity of cell staining and the proportion of positively stained tumor cells. The definition for the evaluation of CDK5RAP3 and DDRGK1 staining intensity was as follows: no staining (score of 0), weak staining (light yellow, score of 1), moderate staining (yellow brown, score of 2) and strong staining (brown, score of 3). The positive proportion of stained tumor cells was scored as follows: ≤ 5% positive cells (score of 0), 6% to 25% positive cells (score of 1), 26% to 50% positive cells (score of 2), ≥ 51% positive cells (score of 3). If the total score (intensity × percentage score) was less than 3, the protein expression was considered low, however, if the score was 4 or higher, the protein expression was considered high (Figure 1A).

Western blot

After the cells grew to 90%-100% confluence, the cells were flushed twice with pre-cooled PBS and then extracted with RIPA lysis solution (Thermo Fisher Scientific, Waltham, MA, United States) containing a 10% cocktail (Roche, South San Francisco, CA, United States). Protein samples (40 μg per lane) were separated on 10% polyacrylamide gels by the SDS-PAGE method and transferred to PVDF membranes. Then, at room temperature, 5% skim milk was used to block the PVDF membrane for 1 h. The membrane was then incubated at 4 °C with the primary anti-CDK5RAP3 (ab157203, 1:1000 dilution; Abcam), anti-DDRGK1 (21445-1-AP, 1:1000 dilution; Proteintech), or anti-GAPDH (ab8245, 1:5000 dilution; Abcam) and washed with TBS-T 3 times, 5 min each time, then incubated at room temperature with the HRP secondary antibody (Cell Signaling Technology) for 1 h. GAPDH was used as an internal control. Finally, the membrane was washed with TBS-T for 30 min and the protein bands were detected by an enhanced chemiluminescence method (Amersham Corporation, Arlington Heights, IL, United States).

Follow-up

All patients were followed up once every three months

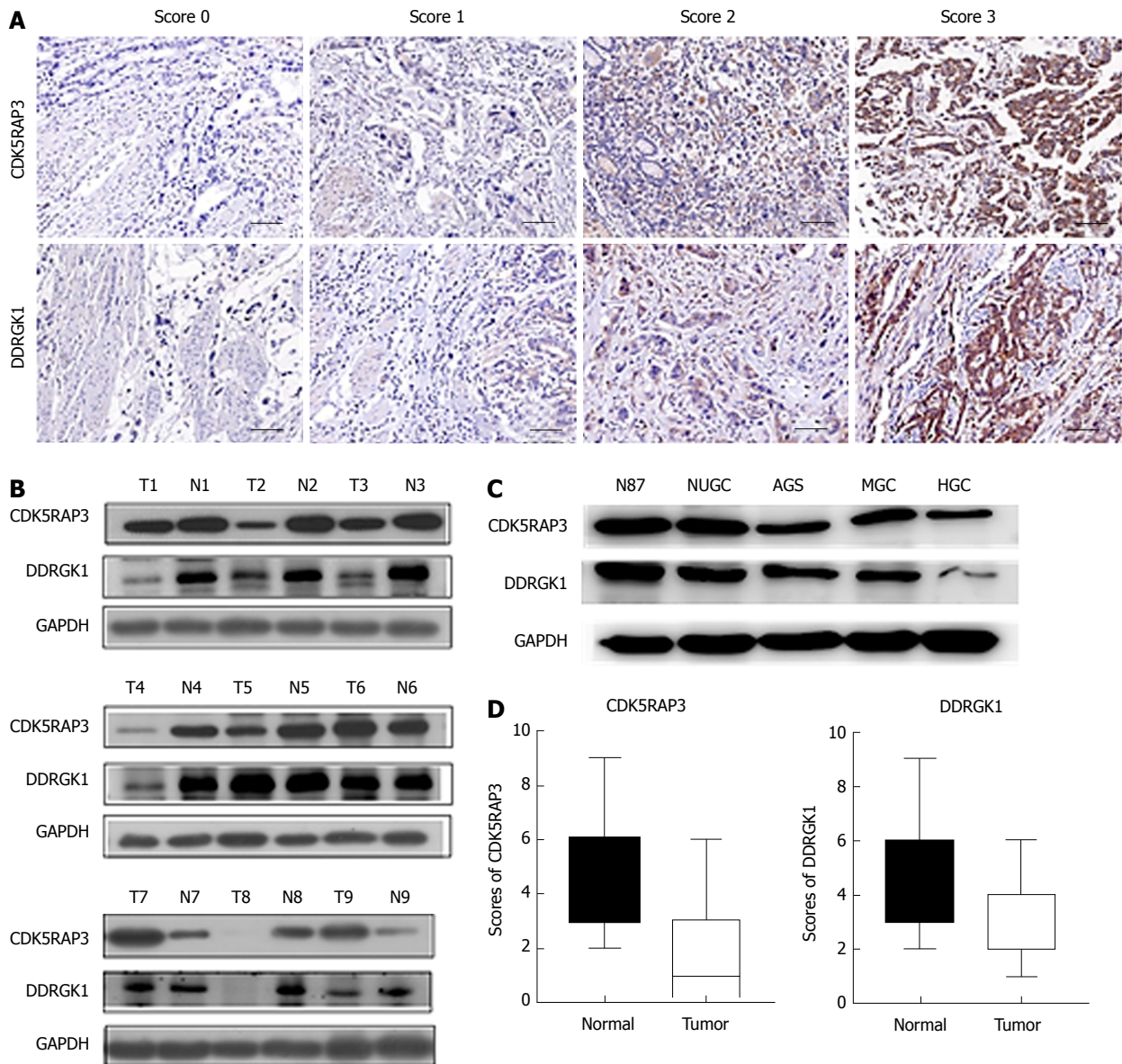


Figure 1 Expression levels of CDK5RAP3 and DDRGK1 in gastric cancer and adjacent non-tumor tissues. A: Immunohistochemical staining of CDK5RAP3 and DDRGK1 expression in gastric cancer tissue and the criteria for immunohistochemistry scores following the intensity of positive signals. Scale bar = 100 μ m. B: Western blot of CDK5RAP3 and DDRGK1 in gastric cancer and adjacent non-tumor tissues in nine patients. C: Western blot of CDK5RAP3 and DDRGK1 in five gastric cancer cells. D: CDK5RAP3 and DDRGK1 expression scores are shown as box plots, with the horizontal lines representing the median; the bottom and top of the boxes representing the 25th and 75th percentiles, respectively, and the vertical bars representing the range of data. The expression of CDK5RAP3 and DDRGK1 in gastric tumor tissues and respective adjacent non-tumor tissues was compared using the *t*-test. $n = 135$ ($P < 0.001$).

for the first two years and were then followed up every six months for the next three to five years. The last follow-up time point was January 2018. Follow-up routine examinations, including a physical examination, laboratory tests (CA19-9, CEA and CA72-4), chest X-ray, abdominal CT, B ultrasound, and gastroscopy were performed each year. The total survival time was defined as the time from surgery to the last follow-up, or the time of death, or the expiration of the follow-up database (e.g., lost to follow-up, death from other diseases, etc.)

Statistical analysis

All of the data were processed by the SPSS23.0 statistical software package. Appropriate test methods, such as the χ^2 test or Fisher's exact test, were selected

according to the type of variables and the purpose of comparison. The survival rate was calculated by the Kaplan-Meier method, and the subsequent survival curve was plotted. The log-rank test was used to compare the survival rates. Cox regression was used to analyze the independent factors that affected the prognosis. The area under the ROC curve was used to compare the prognostic ability of different indexes. The difference was statistically significant when $P < 0.05$.

RESULTS

Expression status of CDK5RAP3 and DDRGK1 in gastric cancer

Of 135 patients with primary gastric cancer, 109 patients (80.7%) had low expression of CDK5RAP3 and 26

Table 1 Relationship between CDK5RAP3 and DDRGK1 protein expression in gastric cancer tissues and various clinicopathological variables

Variables	Total	CDK5RAP3 expression				DDRGK1 expression			
		Low	High	χ^2	<i>P</i> value	Low	High	χ^2	<i>P</i> value
Gender				1.659	0.198			3.057	0.080
Male	107	84	23			74	33		
Female	28	25	3			24	4		
Age (yr)				4.441	0.035			0.719	0.397
> 60	91	78	13			64	27		
≤ 60	44	31	13			34	10		
Tumor size(cm)				0.125	0.723			0.043	0.835
> 5	53	42	11			39	14		
≤ 5	72	67	15			59	23		
Location of tumor				3.860	0.277			1.537	0.674
Lower 1/3	55	48	7			37	18		
Middle 1/3	20	15	5			16	4		
Upper 1/3	45	36	9			34	11		
More than 1/3	15	10	5			11	4		
Borrmann type				0.285	0.593			1.312	0.252
I + II Type	31	24	7			25	6		
III + IV Type	104	85	19			73	31		
Degree of differentiation				0.187	0.666			4.414	0.036
Well/moderate	57	47	10			36	21		
Poor and not	78	62	16			62	16		
Histological type				1.271	0.736			8.547	0.036
Papillary	58	48	10			39	19		
Tubular	42	32	10			27	15		
Mucinous	10	9	1			9	1		
Signet-ring cell	25	20	5			23	2		
Depth of invasion				5.674	0.017			1.428	0.232
T1 + T2	21	13	8			13	8		
T3 + T4	114	96	18			85	29		
Lymph node metastasis				0.008	0.927			6.023	0.014
Negative	20	16	4			10	10		
Positive	115	93	22			88	27		
TNM stage				1.383	0.240			2.632	0.105
I + II	44	33	11			28	16		
III + IV	91	76	15			70	21		
Distant metastasis				1.761	0.184			0.639	0.424
Negative	128	102	26			92	36		
Positive	7	7	0			6	1		

patients (19.3%) had high expression. The DDRGK1 immunohistochemical score showed low expression in 98 cases (72.6%) and high expression in 37 cases (27.4%) (Table 1). Western blotting was used to detect the expression of CDK5RAP3 and DDRGK1 in the tumor and adjacent tissues of nine patients with gastric cancer. It was found that the expression of CDK5RAP3 and DDRGK1 in six patients was higher in respective adjacent non-tumor tissues than that in gastric cancer tissues (Figure 1B). In addition, the expression of CDK5RAP3 and DDRGK1 in gastric cancer cell lines decreased with the decrease of differentiation degree of the gastric cancer cell lines (Figure 1C). We also found that the histological scores of CDK5RAP3 and DDRGK1 in adjacent tissues were higher than those in cancer tissues, with a statistically significant difference (Figure 1D).

Relationships between CDK5RAP3 and DDRGK1 protein expression in gastric cancer tissues and clinicopathological parameters

We analyzed the relationship between CDK5RAP3 and

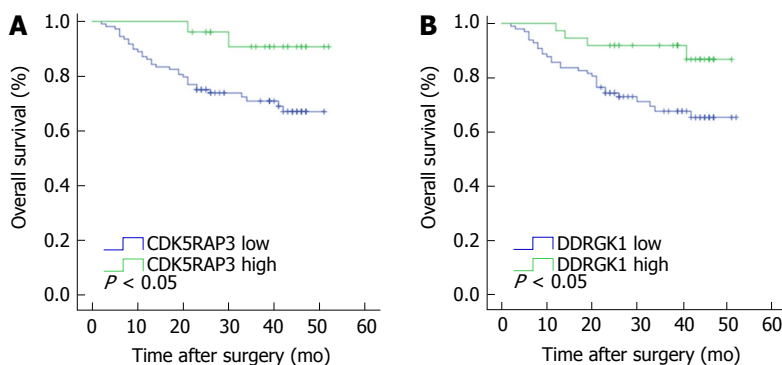
DDRGK1 protein expression in gastric cancer tissues and various clinicopathological data. The expression of CDK5RAP3 in gastric cancer was correlated with the age ($P = 0.035$) and T stage of the tumor ($P = 0.017$). However, the expression of DDRGK1 in gastric cancer was closely correlated with the differentiation degree ($P = 0.036$), the histological type ($P = 0.036$) and N stage of tumor ($P = 0.014$), as shown in Table 1. The co-expression level of CDK5RAP3 and DDRGK1 was related to sex ($P = 0.024$), T stage ($P = 0.026$), N stage ($P = 0.048$) and TNM stage ($P = 0.016$), as shown in Table 2.

Expression levels of CDK5RAP3 and DDRGK1 are correlated with the prognosis of patients with gastric cancer

The median follow-up time was 30.0 mo, and the three-year survival rate was 70.7%. Survival analysis showed that the three-year survival rate of gastric cancer patients with low expression of CDK5RAP3 was 71.1%, which was lower than that of patients with high expression of CDK5RAP3 (90.8%, Figure 2A).

Table 2 Relationship between different CDK5RAP3 and DDRGK1 protein expression status in gastric cancer tissues and various clinicopathological variables

Variables	Total	C53 and DDRGK1 low expression	C53 and/or DDRGK1 high expression	χ^2	P value
Gender					
Male	107	59	48	5.077	0.024
Female	28	22	6		
Age (yr)					
> 60	91	55	36	0.022	0.881
≤ 60	44	26	18		
Tumor size (cm)					
> 5	53	32	21	0.005	0.943
≤ 5	82	49	33		
Location of tumor					
Lower 1/3	55	33	22	1.481	0.687
Middle 1/3	20	12	8		
Upper 1/3	45	29	16		
More than 1/3	15	7	8		
Borrmann type					
I + II Type	31	20	11	0.342	0.559
III + IV Type	104	61	43		
Degree of differentiation					
Well/moderate	57	31	26	1.296	0.255
Poor and not	78	50	28		
Histological type					
Papillary	57	33	25	4.415	0.220
Tubular	42	22	20		
Mucinous	10	8	2		
Signet-ring cell	25	18	7		
Depth of invasion					
T1 + T2	21	8	13	4.972	0.026
T3 + T4	114	73	41		
Lymph node metastasis					
Negative	20	8	12	3.913	0.048
Positive	115	73	42		
TNM stage					
I + II	44	20	24	5.754	0.016
III + IV	91	61	30		
Distant metastasis					
Negative	126	75	53	2.034	0.154
Positive	7	6	1		

**Figure 2** Kaplan-Meier analysis of the correlation between the expression of CDK5RAP3 and DDRGK1 and the overall survival of gastric cancer patients.

The survival time of gastric cancer patients with low expression of DDRGK1 was significantly lower than that of patients with high expression of DDRGK1 (67.7% vs 91.9%, Figure 2B). When combining analysis of CDK5RAP3 and DDRGK1, the three-year survival rate of gastric cancer patients with low expression of CDK5RAP3 and DDRGK1 was 64.2%, which was

significantly lower than that of the patients with high expression of CDK5RAP3 and DDRGK1 (Figure 3A). We compared the prognostic value of high expression of CDK5RAP3 and low expression of DDRGK1 to low expression of CDK5RAP3 and high expression of DDRGK1, and there were no significant differences between these survival curves (Supplementary Figure

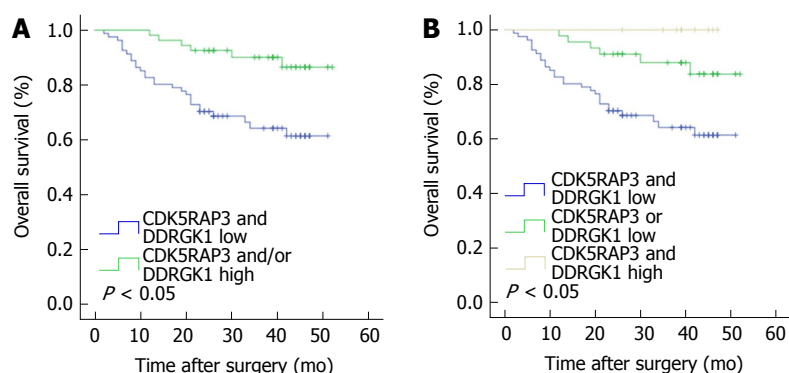


Figure 3 Kaplan-Meier analysis of the correlation between the combined expression of CDK5RAP3 and DDRGK1 with the overall survival of gastric cancer patients.

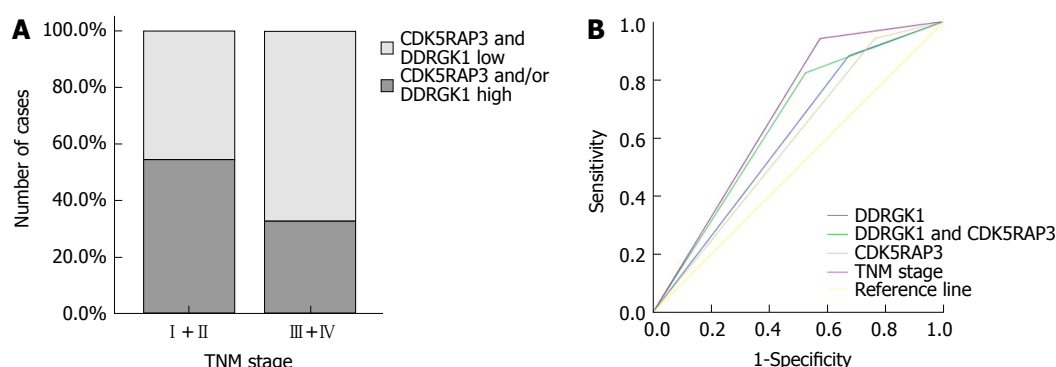


Figure 4 Receiver operating characteristic analysis of the sensitivity and specificity of the predictive value of DDRGK1 expression model, CDK5RAP3 expression model, the combined CDK5RAP3 and DDRGK1 model and the TNM model. A: Co-expression of CDK5RAP3 and DDRGK1 were significantly correlated with TNM stage. B: The area under the ROC curve was 0.649 (0.548-0.751) for the co-expression of CDK5RAP3 and DDRGK1 model, 0.598 (0.486-0.693) for the CDK5RAP3 expression model, 0.605 (0.501-0.708) for the DDRGK1 expression model, and 0.659 (0.562-0.756) for the TNM model.

1). So we combined the two groups into a group for further analysis. Further stratification analysis showed that the prognosis was the best when CDK5RAP3 and DDRGK1 were both highly expressed, and the prognosis was the worse when either CDK5RAP3 or DDRGK1 was highly expressed, while the worst prognosis was correlated with low expression of both CDK5RAP3 and DDRGK1 (Figure 3B). In addition, we used immunoprecipitation combined with mass spectrometry (in an HGC cell line) to find CDK5RAP3 binding protein and potential downstream targets. The results of string analysis show that CDK5RAP3 can bind DDRGK1 (Supplementary Figure 2).

Univariate and multivariate analyses of the prognosis in the entire group

Univariate analysis showed that the overall survival was correlated with the T status ($P = 0.026$), N status ($P = 0.031$), M status ($P = 0.005$), TNM stage ($P = 0.001$), and the expression level of CDK5RAP3 ($P = 0.023$) and DDRGK1 ($P = 0.015$) in gastric cancer tissues and the co-expression level of CDK5RAP3 and DDRGK1 in gastric cancer tissues ($P = 0.001$) (Table 3). Multivariate Cox prognostic analysis showed that the co-expression levels of CDK5RAP3 and DDRGK1 ($P = 0.009$) and the TNM stage ($P = 0.007$) were both independent

prognostic factors in gastric cancer patients (Table 4).

The relationship between the expression of CDK5RAP3 and DDRGK1 and TNM stage

As shown in Figure 4, we established a ROC curve to compare the expression of CDK5RAP3 or DDRGK1 alone and the expression of CDK5RAP3 and DDRGK1 together with TNM stage in gastric cancer prognostication. The results showed that the area under the curve of the combination of CDK5RAP3 and DDRGK1 (AUC: 0.649, 95%CI: 0.548-0.751, $P = 0.009$) was larger than that of CDK5RAP3 or DDRGK1 expression alone (CDK5RAP3: AUC: 0.589, 95%CI: 0.486-0.693, $P = 0.120$, DDRGK1: AUC: 0.605, 95%CI: 0.501-0.708, $P = 0.069$). In addition, the prognostic value of the combined expression of CDK5RAP3 and DDRGK1 was closer to that of the TNM stage (AUC: 0.683, 95%CI: 0.591-0.776, $P = 0.001$).

DISCUSSION

In recent years, although some progress has been made in the treatment of gastric cancer, the prognosis of gastric cancer patients is still not optimistic because the majority of patients are only diagnosed in moderate or advanced stages, and the effect of adjuvant therapy is limited.

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

Clinicopathological parameters	Three-year cumulative survival rate	Log-rank test	P value
Gender			
Male	75.4	0.395	0.53
Female	71.4		
Age (yr)			
> 60	75.4	0.163	0.686
≤ 60	73.8		
Tumor size (cm)			
> 5	68.3	0.988	0.32
≤ 5	79.3		
Location of tumor			
Lower 1/3	78.2	3.438	0.329
Middle 1/3	83.6		
Upper 1/3	63.3		
More than 1/3	86.7		
Borrmann type			
I + II	76.9	0.373	0.541
III + IV	74		
Degree of differentiation			
Well/moderate	75.5	0.111	0.739
Poor and not	74.2		
Histological type			
Papillary	70	4.386	0.223
Tubular	85.6		
Mucinous	70		
Signet-ring cell	69.1		
Depth of invasion			
T1 + T2	91.7	4.95	0.026
T3 + T4	71.7		
Lymph node metastasis			
Negative	95	4.629	0.031
Positive	71.2		
TNM stage			
I + II	92.3	11.424	0.001
III + IV	66.3		
Distant metastasis			
Negative	76.7	8.015	0.005
Positive	42.9		
CDK5RAP3 expression			
Low	71.1	5.168	0.023
High	90.8		
DDRGK1 expression			
Low	67.7	5.971	0.015
High	91.9		
CDK5RAP3/DDRGK1 expression			
CDK5RAP3 and/or DDRGK1 high	90.1	10.415	0.001
CDK5RAP3 and DDRGK1 low	64.2		

Therefore, finding new biomarkers will help to improve earlier diagnosis and treatment of gastric cancer. DDRGK1 is not only a target protein of ufmylation, but is also an integral component of the ufmylation modification system. Ufmylation mediated by DDRGK1 plays an important role in carcinogenesis^[14,15]. Shiwaku *et al.*^[16] found that the amino acid sequence of CDK5RAP3 contained an ubiquitin protein ligase binding region. Wu's study^[9] found that CDK5RAP3 can interacted with DDRGK1 and UFL1 (called RCAD in their study) and regulate the stability of CDK5RAP3 and DDRGK1. Therefore, based on these previous reports and our finding, we speculate

that the function of CDK5RAP3 and DDRGK1 is related. However, the expression of CDK5RAP3 and DDRGK1 in gastric cancer and their influence on clinicopathological characteristics and prognosis have not previously been reported.

CDK5RAP3 is widely expressed in various tissues and cells of the whole body, including the heart, brain, skeletal muscle, placenta, lung, liver, kidney and pancreas^[17]. In early embryonic development, CDK5RAP3 regulates cell cycle progression, epidermal cell adhesion and migration^[18]. Recent studies have suggested that CDK5RAP3 plays an important role in various cancers such as lung cancer, liver cancer, and head and neck cancer^[6,19,20]. Our study found that the three year survival rate of patients with low expression of CDK5RAP3 was lower than that of patients with high expression of CDK5RAP3 ($P < 0.05$), and the expression of CDK5 RAP3 was correlated with tumor T stage, suggesting that CDK5RAP3 is involved in gastric cancer. It may play a role in suppressing cancer, which is also consistent with our previous research results^[21]. In the case of DDRGK1, some studies have shown that DDRGK1 is a tumor suppressor. The ufmylation of DDRGK1 itself is essential for its combination with UFL1 and activation of the UFL1 ubiquitin ligase. If DDRGK1 is unable to undergo ufmylation, it cannot bind and activate UFL1 activity, thereby blocking the ufmylation of the nuclear receptor co-activator ASC1 and, inhibiting the binding of ASC1 and the transcription factors p300 and SRC1 to the downstream target genes of the estrogen receptor ERα^[11,14].

In this study, the survival of gastric cancer patients with low expression of DDRGK1 was significantly shorter than that of patients with high expression of DDRGK1 ($P < 0.05$), and the expression of DDRGK1 was related to tumor differentiation, histological type and N stage. It has also been suggested that DDRGK1 may play a role in suppressing the progression of gastric cancer.

In addition, we found that patients with low expression of CDK5RAP3 and DDRGK1 had the worst prognosis while patients with high expression of both proteins had the best prognosis, and the other patients were between them. Further analysis showed that the accuracy of prognostication with a combination of CDK5RAP3 and DDRGK1 was higher than that of CDK5 RAP3 or DDRGK1 alone. We showed that the combined expression of CDK5RAP3 and DDRGK1 had a better ability to predict the overall survival rate of gastric cancer patients. Xi *et al.*^[13] found that DDRGK1 interacted with IkBa and regulated its stability, thereby regulating the transcriptional activity of NF-κB and its target gene expression. However, Wang's study^[9] of CDK5RAP3 found that down-regulation of CDK5RAP3 increased cell invasiveness and increased the transcriptional activity of NF-κB. CDK5RAP3 binds to RelA to inhibit its phosphorylation and increase the binding of HDAC to RERA, thereby inhibiting the transcriptional activity of NF-κB. CDK5RAP3 and DDRGK1 can interact with

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
CDK5RAP3 expression (high <i>vs</i> low)	-1.226	0.735	0.294	0.070-1.239	0.095
DDRGK1 expression (high <i>vs.</i> low)	-0.979	0.536	0.376	0.131-1.074	0.068
CDK5RAP3 and DDRGK1 expression (low/low <i>vs.</i> high and/or high)	1.178	0.453	3.247	1.336-7.891	0.009
Depth of invasion (T3,T4 <i>vs</i> T1,T2)	1.071	1.045	2.920	0.376-22.635	0.305
Lymph node metastasis (positive <i>vs</i> negative)	1.538	1.020	1.974	0.631-34.367	0.132
Distant metastasis (positive <i>vs</i> negative)	0.861	0.544	2.365	0.815-6.8631	0.113
TNM stage (stage III and IV <i>vs</i> I and II)	-1.630	0.608	7.195	0.060-0.645	0.007

each other, and their roles in the NF- κ B pathway are similar. Therefore, we hypothesized that their impact on prognosis may be related to the overlapping of the two tumor suppressing effects. However, the interaction between CDK5RAP3 and DDRGK1 in gastric cancer has not been fully elucidated. Further manipulation of gene expression in different gastric cancer cell lines and investigation of the characteristics and mechanism of these genes effects on gastric cancer are needed in additional studies.

In summary, low expression of CDK5RAP3 and DDRGK1 are closely related to the prognosis of gastric cancer, and the co-expression of CDK5RAP3 and DDRGK1 is an independent prognostic factor correlated with the overall survival of gastric cancer patients.

ARTICLE HIGHLIGHTS

Research background

Although the morbidity and mortality of the primary gastric cancer has declined in recent decades, it is still the third most common cause of cancer-related deaths worldwide. The symptoms of early gastric cancer are not highly specific. Therefore, misdiagnosis and missed diagnosis may occur. Therefore, finding new biomarkers will help to improve earlier diagnosis and treatment of gastric cancer. In recent years, an increasing number of studies on the prognostic indicators of gastric cancer have been published. However, the expression of CDK5RAP3 and DDRGK1 in gastric cancer and its influence on prognosis have not yet been reported.

Research motivation

At present, the etiology and pathogenesis of gastric cancer has not yet been fully clarified. There is also a lack of specific and highly effective therapeutic drugs available for use in clinical practice. The symptom specificity of early gastric cancer is not obvious, so most patients are already in advanced stages before receiving medical treatment, which seriously affects the prognosis of patients. Therefore, searching for molecular markers that can be used as an independent prognostic factor for gastric cancer is of great significance for the early diagnosis and targeted treatment of gastric cancer. A series of studies on tumor prognostic factors is expected to provide a new target for the treatment of gastric cancer while providing new targets for the treatment of gastric cancer. The expression of CDK5RAP3 and DDRGK1 in gastric cancer and their influence on clinicopathological characteristics and prognosis have not previously been discussed.

Research objectives

The aim of this study is to identify novel effective biomarkers to classify patients with low or high survival. This would provide a guide to clinicians to select therapeutic strategies for patients and provide personalized therapy according to the predicted survival rate. In this study, we investigated two interacting proteins, CDK5RAP3 and DDRGK1, which may help determine patient

management strategies.

Research methods

We used immunohistochemistry to detect the expression of CDK5RAP3 and DDRGK1 in gastric cancer and adjacent tissues. Western Blot was used to detect the expression of CDK5RAP3 and DDRGK1 in gastric cancer and its adjacent tissues and cell lines. According to immunohistochemistry scores, the patients were divided into CDK5RAP3 high expression group and CDK5RAP3 low expression group, DDRGK1 high expression group and DDRGK1 low expression group, and the relationship between the expression level and clinicopathological data was analyzed. Furthermore, based on the combined expression of CDK5RAP3 and DDRGK1, we classified the patients into three subtypes: CDK5RAP3 and DDRGK1 high ($n = 9$), CDK5RAP3 or DDRGK1 low ($n = 45$) and CDK5RAP3 and DDRGK1 low ($n = 81$). Then, we used the Kaplan-Meier method to analyze the effect of different expression patterns on prognosis.

Research results

Our research found that the expression of CDK5RAP3 and DDRGK1 was down-regulated in gastric cancer. Low expression of CDK5RAP3 or DDRGK1 is a poor prognostic factor for gastric cancer patients. Moreover, prognostic analysis showed that the co-expression of CDK5RAP3 and DDRGK1 was an independent prognostic factor correlating with the overall survival of gastric cancer patients. Combined expression analysis of CDK5RAP3 and DDRGK1 may provide a more accurate prognostic value for overall survival. This study presents two interacting proteins, which may be useful to determine patient management strategies. These makers may predict the prognosis of gastric cancer patients through an analysis of CDK5RAP3 and DDRGK1 protein expression in preoperative biopsy and tumor specimens.

Research conclusions

This study found that low expression of CDK5RAP3 and DDRGK1 are closely related to the poor prognosis of gastric cancer patients, and the co-expression of CDK5RAP3 and DDRGK1 is an independent prognostic factor correlated with the overall survival of gastric cancer patients. These two interacting proteins, CDK5RAP3 and DDRGK1, may be helpful in determining patient management strategies, and to predict the prognosis of gastric cancer patients. We hypothesized that CDK5RAP3 and DDRGK1 were key genes which may participate in the biological regulation of gastric cancer. The mechanism of their role in gastric cancer has not been fully elucidated and further studies are needed. With advances in technology, humans may find more effective and new indicators in the future to guide treatment, improve prognosis, and reduce the recurrence rate and mortality of patients with gastric cancer.

Research perspectives

This study found the prognostic value of two interacting proteins, CDK5RAP3 and DDRGK1, by detecting the expression of both in clinical specimens, combined with detailed clinicopathological data analysis. This study provided ideas for finding new tumor prognosis related molecules. Manipulation of both CDK5RAP3 and DDRGK1 expression in different gastric cancer cell lines, such as overexpression or knockdown, will be needed for future research. Further study is necessary to investigate the characteristics of cancer cells and explore the mechanism of CDK5RAP3 and DDRGK1 affecting the development of

gastric cancer by an *in vitro* cell model and *in vivo* xenograft model.

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