European Review for Medical and Pharmacological Sciences 2017; 21: 2372-2376

Higher RABEX-5 mRNA predicts unfavourable survival in patients with colorectal cancer

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Abstract. – OBJECTIVE: The aim of the present study was to clarify the expression pattern and prognostic role of RABEX-5 mRNA in colorectal cancer (CRC) patients.

PATIENTS AND METHODS: RABEX-5 mRNA levels in 187 CRC were examined by Real-time polymerase chain reaction. Then, the association of RABEX-5 mRNA levels with clinicopathological features was analyzed. Survival curves were plotted using the Kaplan-Meier method and differences in survival rates were analyzed using the log-rank test. The influence of each variable on survival was examined by the Cox multivariate regression analysis.

RESULTS: RABEX-5 mRNA expression was significantly upregulated in CRC tissues compared with the adjacent noncancerous tissues ($p < 0.01$). By statistical analyses, high RABEX-5 mRNA expression was observed to be closely correlated with histology/differentiation ($p = 0.010$), N classification ($p = 0.004$), and TNM stage ($p = 0.004$). Kaplan-Meier curves showed that patients with high RABEX-5 mRNA expression showed unfavorable overall survival (OS) than the low RABEX-5 mRNA expression group ($p < 0.001$). Finally, univariate and multivariate analyses showed that RABEX-5 mRNA expression was an independent predictor of overall survival ($p < 0.05$).

CONCLUSIONS: RABEX-5 mRNA may be a promising biomarker for the detection and prognosis evaluation of CRC.

Key Words:
RABEX-5 mRNA, Colorectal cancer, Prognosis.

Introduction

Colorectal cancer (CRC) is one of the most common gastrointestinal tumors with millions, with an estimated over 1 million new cases and more than half million deaths in 2012[1]. In China, the incidence of colorectal cancer is increasing, and it is the leading 6 cause of cancer-related death[2]. Despite recent advances in chemotherapies and molecular-targeted therapies, the long-term prognosis for patients with advanced disease and metastasis remains poor[3,4]. Therefore, it is crucial to identify and characterize novel molecular markers for accurate prognosis prediction, and the development of new therapeutics.

Ras-associated binding (Rab)-GTPases are members of the Ras family of small GTPases which has been reported to regulate many cell functions and biological processes, such as proliferation migration, invasion, and apoptosis[5,6]. The small GTPase RAB-5 is a master regulator of early endocytic trafficking[7]. The study showed that RAB-5 is activated by an exchange of bound GDP with GTP[8]. RABEX-5 was identified as an interactor of Rabaptin-5 and was found to possess GEF activity toward RAB-5 and related GTPases[9]. Recently, aberrant expression of RABEX-5 was found in several tumors, including CRC[10]. However, The correlation of RABEX-5 mRNA expression with the clinical behavior and prognosis of CRC was also evaluated. In the present study, we firstly reported that RABEX-5 mRNA may help predict the prognosis of patients with CRC.

Patients and Methods

Patients and Tissue Samples

One hundred eighty-seven patients with CRC were collected at Qingdao Municipal Hospital from July 2008 to July 2010. All tissues were histologically examined, and pathologists confirmed the diagno-

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sis. All tissues were obtained at the time of surgery and immediately stored in liquid nitrogen. The histology was confirmed and staged according to the tumor-node-metastasis (TNM) staging system of the International Union Against Cancer. Before surgery, none of the patients received anti-cancer treatment involving chemotherapy, Written informed consent was obtained from all patients, and the study was approved by the Ethics Committee of Qingdao Municipal Hospital.

RNA Extraction, Reverse Transcription and Quantitative Real-time PCR (qPCR)

Total RNAs in colorectal cancer and adjacent non-cancerous tissues were extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) based on the instructions to the users. First-strand cDNA was generated using the PrimeScript TM RT reagent Kit (Takara, Dalian, Niaoning, China). The cDNA was amplified with SYBR Premix Dimer Eraser TM (TaKaRa, Dalian, Niaoning, China). Real-time polymerase chain reaction (RT-PCR) was performed using an ABI 7900 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The amplification profile was denatured at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. U6 snRNA was used as internal standard. The qRT-PCR primers for RABEX-5 mRNA and U6 were purchased from Beijing Tianshi gene company. Expression levels of RABEX-5 mRNA were calculated by the $-\Delta Ct$ method.

Statistical Analysis

SPSS version 19 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The chi-square test was used to determine the correlations between RABEX-5 mRNA expression and clinicopathological characteristics. The associations of overall survival with RABEX-5 mRNA expression levels were determined by log-rank tests and presented as Kaplan-Meier curves. Univariate Cox regression analysis was used to examine the prognostic significance of RABEX-5 mRNA expression and all clinical covariates. Then, a multivariate model was used to identify independent prognostic factors overall survival. A two-tailed *p*-value less than 0.05 was considered to have statistical significance.

Results

RABEX-5 mRNA was Upregulated in CRC

The relative expression level of RABEX-5 mRNA was determined using real-time qPCR in a total of 187 pairs of CRC and adjacent non-cancerous tissues. As shown in Figure 1, the results showed that RABEX-5 mRNA was significantly upregulated in CRC tissues compared with that in paired adjacent non-tumor tissues ($p < 0.05$).

Upregulation of RABEX-5 mRNA is Associated with Advanced Clinicopathological Features of CRC

We next investigated the correlation between RABEX-5 mRNA expression and CRC clinicopathological features. 187 patients were divided into low-RABEX-5 mRNA group (n=93) and high-RABEX-5 mRNA group (n=94) by using the median level of RABEX-5 mRNA as the cutoff. As shown in Table I, the data revealed that RABEX-5 mRNA expression in CRC was significantly correlated with histology/differentiation ($p = 0.010$), N classification ($p = 0.004$) and TNM stage ($p = 0.004$). However, there were no significant correlations between RABEX-5 mRNA expression and other clinicopathological factors of patients, such as age ($p = 0.332$), gender ($p = 0.318$), tumor size ($p = 0.083$) and depth of invasion ($p = 0.493$).

The Association between RABEX-5 mRNA Expression and Overall Survival of Patients with CRC

Then, we used Kaplan-Meier analysis to assess the impact of RABEX-5 mRNA on patients' overall survival. As shown in Figure 2, upregulated expression of RABEX-5 mRNA was correlated with poor overall survival, while low expression

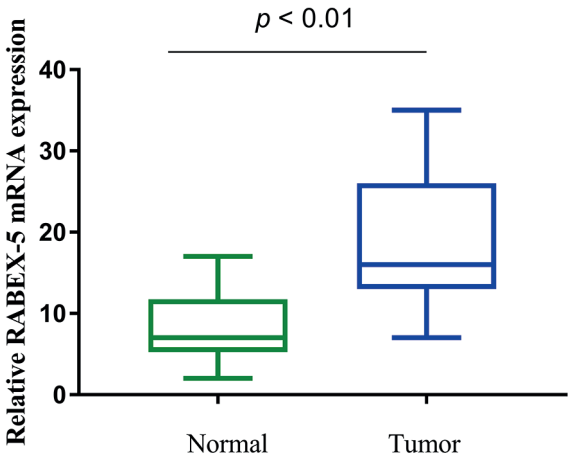

Figure 1. RABEX-5 mRNA expression was determined by qPCR in CRC tissues and matched normal tissues.

Table I. Correlation between RABEX-5 mRNA levels and the 187 CRC patients' clinicapathological parameters.

Clinicopathological features	No. of cases	RABEX-5 mRNA expression		
		Low (n = 93)	High (n = 94)	*p*-value
Age				0.332
<60	81	37	44	
≥60	106	56	50	
Gender				0.318
Male	116	61	55	
Female	71	32	39	
Tumor size (cm)				0.083
<5	98	52	36	
≥5	99	41	58	
Histology/differentiation				0.010
Well + moderate	83	50	33	
Poor	104	43	61	
Depth of invasion				0.493
T1 + T2	75	35	40	
T3 + T4	112	58	54	
N classification				0.004
Negative	95	57	38	
Positive	92	36	56	
TNM stage				0.004
I-II	101	60	41	
III	86	33	53	

of RACK1 was correlated with better overall survival ($p = 0.001$).

The univariate analysis showed that histology/differentiation, N classification, TNM stage, and RABEX-5 mRNA levels were significantly related to overall survival ($p < 0.012$, $p = 0.003$, $p = 0.006$, and $p = 0.001$, Table II). Meanwhile, multivariate analysis confirmed the adverse prognostic value of RABEX-5 mRNA expression in patients with CRC ($p = 0.003$).

Discussion

Colorectal carcinoma is one of the major causes of cancer death throughout the world[11]. Prognostic at an early stage is a useful way that decreases and avoids mortality[12]. It is known to us that effective screening methods for early detection and biomarkers for prognostic prediction could reduce the mortality rate of cancer[13,14]. Recently, some functional protein and mRNA were identified as a potential biomarker for early detection and poor prognosis of CRC. For instance, Wu et al[15] showed that measuring the serum level of activin A might be used as a reliable diagnostic and screening tool for CRC. Alexopoulou et al[16] found that kallikrein-related peptidase mRNA was significantly higher in CRC tissues, and its high expression was significantly associated with the poorer prognosis of patients with CRC. In the present study, our attention focused on RABEX-5 mRNA.

The potential effect of RABEX-5 has been reported in several studies. Usually, RABEX-5 functions

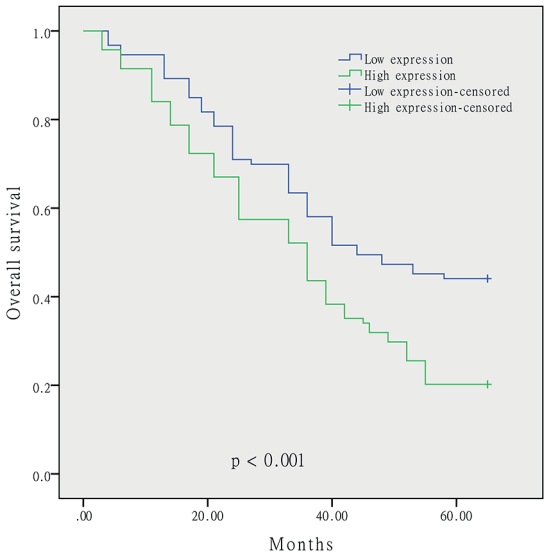

Figure 2. Kaplan-Meier analysis of overall survival related to the expression of RABEX-5 mRNA. Patients with high expression of RABEX-5 mRNA had a poorer prognosis than patients with low expression of RABEX-5 mRNA ($p < 0.001$).

Table II. Prognostic factors in Cox proportional hazards model.

	Univariate analysis			Multivariate analysis		
Variable	**Risk ratio**	**95% CI**	***p***	**Risk ratio**	**95% CI**	***p***
Age						
≥ 60 vs. < 60	1.214	0.663-1.894	0.377	-	-	-
Gender						
Male vs. Female	1.784	0.784-2.213	0.229	-	-	-
Tumor size (cm)						
<5 vs. ≥5	1.662	0.834-2.344	0.121	-	-	-
Histology/differentiation						
Well + moderate vs. Poor	2.424	1.231-5.773	0.012	2.127	1.044-4.889	0.014
Depth of invasion						
T1 + T2 vs. T3 + T4	1.672	0.822-1.989	0.214	-	-	-
N classification						
Negative vs. Positive	2.822	1.349-6.231	0.003	2.763	1.192-5.332	0.006
TNM stage						
I-II vs. III	3.345	1.763-6.112	0.006	2.774	1.458-5.563	0.009
RABEX-5 mRNA						
Low vs. High	2.788	1.664-5.568	0.001	2.544	1.384-5.112	0.003

as a tumor promoter. For instance, Zhang et al[17] showed that knockdown of RABEX-5 suppressed cancer cell proliferation, colony formation and migration ability *in vitro* and inhibited tumor growth *in vivo*. Kang et al[18] reported that high RABEX-5 levels in gastric cancer were associated with advanced tumor invasion, metastasis, and poor prognosis. The similar findings were also found in non-small-cell lung cancer[19]. Another study by Wang et al[20] proved the mechanism that RABEX-5 promoted wound healing, migration and the invasive abilities of gastric cancer cells by activating the VEGF signaling pathway. Although the expression levels of RABEX-5 has been reported in the previous study, the function of RABEX-5 in the progression of CRC was largely unknown.

In the present study, we found that the expression levels of RABEX-5 mRNA were increased significantly in CRC tissues in comparison with matched normal tissues. Then, we proved that RABEX-5 mRNA was correlated with histology/differentiation, N classification, and TNM stage, suggesting that up-regulation of RABEX-5 mRNA may play a positive regulator in CRC. Moreover, we performed Kaplan-Meier analysis, and the results showed that overall survival rate of patients with high RABEX-5 mRNA expression was significantly poorer than that of the remaining cases. Finally, Cox regression analysis demonstrated that RABEX-5 mRNA expression was an independent prognostic factor for the survival time of patients with CRC. Therefore, our data supported the idea that the expression of RABEX-5 mRNA would have a real prognostic value in CRC patients.

Conclusions

Our findings showed that increased expression of RABEX-5 mRNA was seen in CRC, and high expression of RABEX-5 mRNA may be a significant prognostic factor for poor survival in patients with CRC.

Conflict of interest

The authors declare no conflicts of interest.

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