Decreased expression of *RASSF10* correlates with poor prognosis in patients with colorectal cancer

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Abstract

Ras association domain protein 10 (*RASSF10*) was reported to act as a prognostic indicator in various types of cancer and it was proved to be tumor suppressor gene in colorectal cancer (CRC). The purpose of this study was to evaluate the prognostic significance of *RASSF10* in CRC.

Quantitative real-time polymerase chain reaction was used to detect the messenger RNA (mRNA) expression while enzyme-linked immunosorbent assay was taken to measure the protein expression of *RASSF10* in tumor tissues and adjacent normal tissues from 102 patients with CRC. The relationship between *RASSF10* expression level and clinical characteristics of CRC patients was analyzed by chi-squared test. In addition, the association between overall survival of CRC patients and *RASSF10* expression was estimated by Kaplan–Meier analysis. Cox regression analysis was used to evaluate the prognostic value of *RASSF10*.

The expression level of *RASSF10* in tumor tissues was significantly lower than that in the normal tissues both at mRNA and protein levels. Moreover, the expression level was correlated with lymph-node-metastasis and tumor-node-metastasis stage. Kaplan–Meier analysis suggested that patients with high expression level of *RASSF10* had a longer overall survival than those with low level (log-rank test, $P < .001$). Besides, *RASSF10* might be a potential biomarker in the prognosis of CRC according to cox regression analysis.

The down regulated of *RASSF10* is found in CRC and it may be an ideal prognostic marker.

Abbreviations: 95% CI = 95% confidence interval, CRC = colorectal cancer, ELISA = enzyme-linked immunosorbent assay, GAPDH = glyceraldehyde-3-phosphate dehydrogenase, HR = hazard ratio, mRNA = messenger RNA, RASSF = Ras association domain protein, RT-PCR = real-time polymerase chain reaction, TNM = tumor node metastasis.

Keywords: colorectal cancer, prognosis, *RASSF10*

1. Introduction

Colorectal cancer (CRC) was the third most commonly diagnosed cancer in male and the second in female.[1,2] What's more, the morbidity of CRC was increasing in Asian, especially in China, due to the progressive "Westernization" of lifestyles.[3] CRC was the consequences of the accumulation of genetic and epigenetic alterations, so it was difficult to determine the risk factors for CRC.[4] At present time, the commonly used prognostic marker for CRC in clinical practice was tumor-node-metastasis (TNM) system.[5] However, TNM system could cause substantial under-treatment and over-treatment for CRC patients.[6] Therefore, it was urgently need to exploit novel and reliable biomarkers for the prognosis of CRC.

Ras association domain protein 10 (*RASSF10*) was a novel member of RASSF family and characterized by the inclusion of an N-terminus, which was described from a predicted sequence with homology to RASSF9/P-CIP1.[7] It located at chromosome 11p15.2 and contained a CpG island which was easy to be methylated leading to tumorigenesis.[8] In the previous studies, *RASSF10* was proved to act as a tumor suppressor in several cancers such as lung cancer, thyroid cancer, hepatocellular carcinoma, esophageal squamous cell carcinoma, and gastric cancer.[9–13] *RASSF10* was considered to be a tumor suppressor and could inhibit tumor growth by activating P53 signaling in CRC according to the study of Guo et al.[14] However, its clinical significance in CRC was never reported.

In this study, we aimed to detect the expression and its prognostic significance of *RASSF10* in patients with CRC. The expression level of *RASSF10* in CRC tissues both at messenger RNA (mRNA) and protein levels was detected. Meanwhile, the association between clinical characteristics and *RASSF10* expression was evaluated by chi-squared test. Besides, the overall survival of CRC patients according to the level of *RASSF10* was estimated and cox regression analysis was used to analyze the prognostic value of the gene in CRC, in order to find a novel indicator for CRC prognosis.

2. Materials and methods

2.1. Patients and tissue samples

One hundred two patients with CRC were enrolled in this study at Chinese PLA General Hospital from December 2008 to March 2010. The study was permitted by the Ethnic
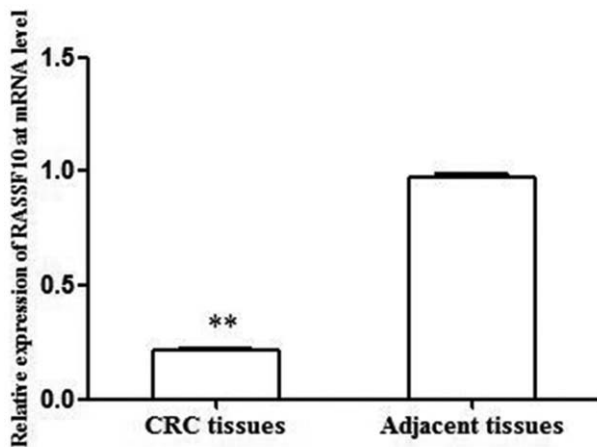
Figure 1. Relative mRNA expression of *RASSF10* in CRC tissues and corresponding normal tissues. *RASSF10* mRNA expression was decreased in CRC tissues compared to that in corresponding normal tissues ($P < .001$). CRC = colorectal cancer. RASSF10 = Ras association domain protein 10.
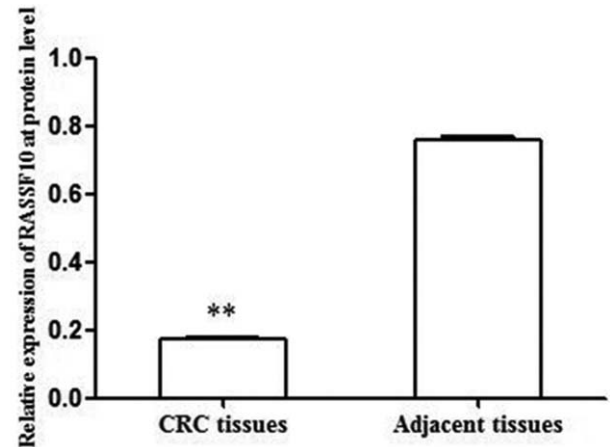
Figure 2. Relative protein expression of *RASSF10* in CRC tissues and corresponding normal tissues. *RASSF10* protein expression was lower in CRC tissues than that in corresponding normal tissues ($P < .001$). CRC = colorectal cancer, RASSF10 = Ras association domain protein 10.

Committee of Chinese PLA General Hospital and all patients had signed written informed consents in advance. None of the patients had received any physical therapy and chemotherapy before sampling.

Pathological specimens and adjacent normal tissues were collected from CRC patients and frozen in liquid nitrogen immediately. Then all samples were stored at −80°C until use, respectively. The detailed clinicopathologic characteristics of patients including age, gender, histological type, depth of invasion, location, lymph node metastasis, and TNM stage were recorded in database. A 5-year follow-up was conducted and patients who were died from unexpected events or other diseases were excluded from our study.

2.2. RNA extraction and quantitative RT-PCR analysis

Total RNA was extracted from the collected specimens using Trizol agent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. DNaes I was used to treat the residual DNA in RNA samples. The concentration and quality of the RNA samples were detected by UV absorbance (A260/A280) and 1% agarose gel electrophoresis, respectively. The first chain of cDNA was compounded through a Prime Scrip RT reagent kit (Takara Biotechnology Co, Ltd). Real-time polymerase chain reaction (RT-PCR) was performed with SYBR Green I assay (Takara) in the Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) acted as internal control. The relative expression of *RASSF10* was calculated with $2^{-\Delta\Delta Ct}$ method. The sequences of primers used in this study were as followed. *RASSF10*: forward 5′-CCATGACCCAGGAGAAAC-AG-3′; reverse 5′-GCTGGCGAATTGTGTGGTC-3′. *GAPDH*: forward 5′-CATGAGAAGTATGACAACAGCCT-3′; reverse 5′-AGTCCTTCCACGATACCAAAGT-3′.

2.3. ELISA analysis

Total protein was extracted from all samples. The expression of RASSF10 protein was measured by enzyme-linked immunosorbent assay (ELISA) kits (DSA00-R&D systems) according to the manufacturer's protocol. Each experimental was in triplicate.

2.4. Statistical analysis

SPSS 18.0 software was used for all statistical analysis and GraphPad Prism 5 was used for designing the figures in this study. All quantitative variables were shown as mean±standard deviation. According to the average expression level of *RASSF10*, the patients were divided into high expression group and low expression group. The difference of the *RASSF10* expression in collected specimens was analyzed by Student *t* test. Chi-squared test was used to analyze the relationship between the gene expression level and clinical characteristics of CRC patients. The association between *RASSF10* expression and overall survival was analyzed with Kaplan–Meier analysis. Hazard ratios (HRs) with the corresponding 95% confidence intervals (95% CIs). Besides, the prognostic significance of *RASSF10* was evaluated by cox regression analysis. $P < .05$ was considered as statistical significance.

3. Results

3.1. Relative mRNA expression of RASSF10 in collected specimens

Quantitative RT-PCR was used to detect the relative mRNA expression of *RASSF10* in CRC tissues and adjacent normal tissues. As shown in Fig. 1, the expression level of *RASSF10* in tumor tissues was significantly lower than that in the adjacent normal tissues (0.215 ± 0.093 vs. 0.974 ± 0.126, $P < .001$).

3.2. Relative protein expression of RASSF10 in collected specimens

The protein expression of RASSF10 in collected specimens was measured by ELISA analysis. The result demonstrated that RASSF10 protein expression was decreased in tumor tissues compared to that in adjacent normal tissues (0.172 ± 0.075 vs. 0.759 ± 0.098, $P < .001$, Fig. 2).

3.3. Clinical characteristics of CRC patients and their correlation with RASSF10 expression level

In order to analyze the correlation between *RASSF10* expression and clinical characteristics, the patients were divided into high

Table 1

Relationship between *RASSF10* expression and clinical characteristics of patients with CRC.

Characteristics	Cases, n	High, n	Low, n	χ^2	P
Gender				1.522	.217
Male	53	16	37		
Female	49	30	19		
Age				0.103	.748
>65	47	25	22		
≤65	55	21	34		
Histological type				0.813	.367
Well, moderate	56	23	33		
Poor, mucinous	46	23	23		
Depth of invasion				0.002	.969
T1 + T2	49	27	22		
T3 + T4	53	19	34		
Location				0.010	.919
Colon	56	21	35		
Rectum	46	25	21		
Lymph node metastasis				6.796	.009
Yes	50	34	16		
No	52	12	40		
TNM stage				5.511	.019
I + II	58	16	42		
III + IV	44	30	14		

CRC = colorectal cancer, RASSF10 = Ras association domain protein 10, TNM = tumor node metastasis.

Figure 3. Kaplan–Meier analysis for estimating the overall survival of patients with different expression of *RASSF10* in colorectal cancer. Patients with high *RASSF10* expression had a longer overall survival than those with low expression (log-rank test, $P < .001$). RASSF10 = Ras association domain protein 10.

expression group and low expression group according to *RASSF10* average expression level (0.172 ± 0.075). Chi-squared test suggested that *RASSF10* level was significantly correlated with lymph node metastasis ($P = .009$) and TNM stage ($P = .019$) (Table 1). However, there were no significant relationship between *RASSF10* expression level and age, gender, histological differentiation, the depth of invasion, and tumor location.

3.4. Prognostic value of RASSF10 in CRC

The overall survival of CRC patients with different expression of *RASSF10* was analyzed by Kaplan–Meier analysis with log-rank test. The results indicated that patients with high expression level of *RASSF10* had a longer overall survival than those with low expression level (48.9 vs. 35.9 months, log-rank test, $P < .001$, Fig. 3). Cox regression analysis was used to analyze the prognostic significance of *RASSF10* in CRC and the results were listed in Table 2. The results of univariate analysis indicated that *RASSF10* expression level was significantly correlated with CRC prognosis (HR = 3.060, 95% CI = 1.608–5.821, $P = .001$). Then the multivariate cox regression analysis demonstrated that *RASSF10* could act as an independent biomarker in the prognosis of CRC (HR = 3.333, 95% CI = 1.823–6.095, $P < .0001$).

4. Discussion

CRC is one of the most common malignancies and the third-leading cause of cancer-related death, causing over 600,000 deaths every year all over the world.[15,16] Although great advance has been got in the treatment of CRC, the prognosis of CRC is still poor due to its frequently metastasis.[2] Therefore, it is necessary to explore effective molecular marker for the prognosis of CRC.

RASSF10 belongs to the RASSF family which play important roles in various pathological pathways such as microtubule
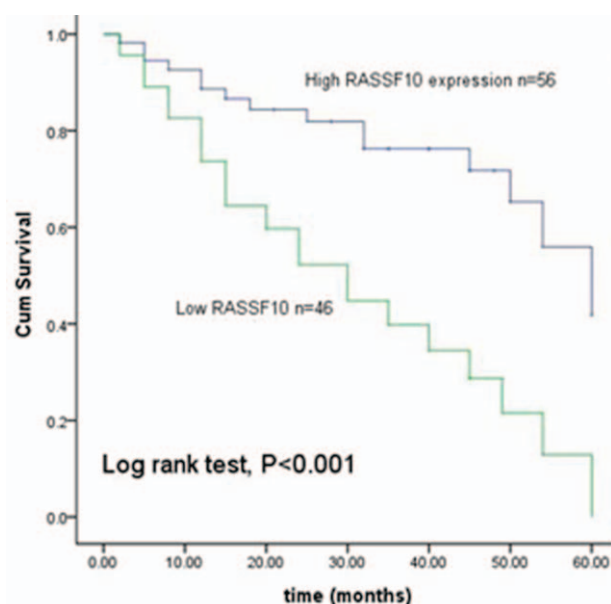
stability, cell division, migration, apoptosis and adhesion, and modulating NFjB activity and the duration of inflammation.[17] According to the previous studies, we found that many of the family members were tumor suppressor genes, which were easily methylated then leading to the silencing of the according transcript in neoplasia.[18] In the study of Zhang et al,[19] the promote methylation and silencing of *RASSF2* was detected in cervical cancer tissues which indicated that abnormal methylation of *RASSF2* might involved in cervical carcinogenesis. Calvisi et al[20] had indicated that *RASSF1A*, *RASSF2*, and *RASSF5* were significantly correlated with human hepatocellular carcinoma and inactivating these genes would inhibit the treatment of the cancer. Other RASSF family associated with cancers including neuroendocrine tumors of the lung, bladder cancer, gastric cancer, melanoma, CRC, nonsmall cell lung cancer, and so on were also covered.[21–24] As respect to the role of RASSF family in CRC, there were also some studies. For instance, in the study of Akino et al,[25] *RASSF2* was proved to be a tumor inhibitor in CRC which played a pivotal role in the early stage of CRC via regulating Ras signaling. The aberrant promoter hypermethylation of *RASSF5* was detected in colorectal tumorigenesis and the results suggested that the gene was correlated with colorectal tumor.[26] Fernandes et al[27] revealed that *RASSF1A*, *RASSF2*, and *RASSF5* took part in CRC development, although the mechanisms of action remained poorly understood. Guo et al[14] indicated that reduced expression of *RASSF10* was associated with *RASSF10* promoter region methylation significantly in CRC leading to loss of expression. Despite *RASSF10* was detected in CRC, its prognostic value was still unclear.

In this study, we detected the expression level of *RASSF10* in CRC tissues and correspondingly normal tissues both at mRNA and protein levels. The present data indicated that *RASSF10* expressed lower in CRC tissues compared with normal tissues. What's more, the expression level was significantly correlated

Table 2

Univariate and multivariate analyses with cox regression analysis adjusted for the clinical factors for the prognostic value of *RASSF10* in CRC patients.

	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
RASSF10 (low vs. high)	3.060	1.608–5.821	.001	3.333	1.823–6.095	<.0001
Age (>65 vs. ≤65)	1.109	0.611–2.014	.733	—	—	—
Gender (male vs. female)	1.131	0.595–2.150	.707	—	—	—
Histological differentiation (poor vs. well)	1.267	0.667–2.406	.470	—	—	—
The depth of invasion						
(T3 + T4 vs. T1 + T2)	1.115	0.597–2.083	.733	—	—	—
Location (colon vs. rectum)	0.783	0.420–1.459	.441	—	—	—
Lymph node metastasis (yes vs. no)	0.607	0.315–1.170	.136	—	—	—
Tumor stage (III + IV vs. I + II)	1.213	1.035–3.682	.045	—	—	—

CI = confidence interval, CRC = colorectal cancer, HR = hazard ratio, RASSF10 = Ras association domain protein 10.

with lymph node metastasis and TNM stage. These results suggested that *RASSF10* might involved in the development of CRC.

In the study of Hill et al,[28] *RASSF10* was proved to act as a prognostic marker for gliomagenesis. While according to the research of Deng et al,[29] we found that methylated *RASSF10* promoter was an independent predictor for the survival of patients with gastric cancer. Therefore, we investigated the prognostic value of *RASSF10* in CRC. First, we analyzed the overall survival of patients with CRC according to the expression level of *RASSF10* through Kaplan–Meier analysis with log-rank test. The results showed that patients with low expression level of *RASSF10* had a shorter overall survival than those with high expression level which revealed *RASSF10* might be related to the prognosis of CRC. Then cox regression analysis was conducted to estimate the prognostic value of *RASSF10* in CRC and the outcome showed it cloud be an independent prognostic indicator.

In conclusion, the expression level of *RASSF10* is decreased in CRC tissues, compared with correspondingly normal tissues. In addition, the expression level is significantly associated with lymph node metastasis and TNM stage. Besides, we prove that *RASSF10* may be a potential prognostic marker for patients with CRC.

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