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

Junxun Ma, PhD, Suijie Zhang, PhD, Yi Hu, PhD*, Xiaoyan Li, PhD, Fang Yuan, PhD, Danyang Sun, PhD, Lijie Wang, PhD, Fan Zhang, PhD, Guangying Chen, PhD, Pengfei Cui, PhD

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.5,6 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.

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Department of Oncology, Chinese PLA General Hospital, Beijing, China.
*Correspondence: Yi Hu, Department of Oncology, Chinese PLA General Hospital, Beijing 100853, China (e-mail: ny546@sina.com).
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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
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. DNAse 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) 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 $2^{-\Delta\Delta C_{t}}$ method. The sequences of primers used in this study as followed: RASSF10: forward 5'-CCATGACCACCGAGAACAG-3'; reverse 5'-GCTGCTGCTTGTGTC-3'. GAPDH: forward 5'-CATGAGAAGTCAGACAGCCT-3'; reverse 5'-AGTCCCTTCACGATAACAGT-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
expression group and low expression group according to RASSF10 average expression level \((0.172 \pm 0.075)\). 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 \((48.9 \text{ vs. } 35.9 \text{ months, } P < .001, \text{ Fig. 3})\). 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. Although great advance has been got in the treatment of CRC, the prognosis of CRC is still poor due to its frequently metastasis. 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 NF-κB activity and the duration of inflammation. 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. In the study of Zhang et al., 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. 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.

As respect to the role of RASSF family in CRC, there were also some studies. For instance, in the study of Akino et al., 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. Fernandes et al. revealed that RASSF1A, RASSF2, and RASSF5 took part in CRC development, although the mechanisms of action remained poorly understood. Guo et al. 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...
with lymph node metastasis and TNM stage. These results suggested that RASSF10 might be 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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