Int J Clin Exp Pathol 2018;11(9):4276-4282
www.ijcep.com /ISSN:1936-2625/IJCEP0079969

Review Article

High RASEF expression is associated with a significantly better prognosis in colorectal cancer

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Received May 20, 2018; Accepted July 31, 2018; Epub September 1, 2018; Published September 15, 2018

Abstract: This study mainly studied the correlation of RASEF expression and the clinical index of colorectal cancer by tissue microarray (TMAs, HCol-Adel180sur-06) containing tissue samples of 90 colorectal cancers. The results showed that RASEF was significantly highly expressed both in nuclei (3.07±1.95 vs 1.83±1.74, P=0.000) and cytoplasm (7.74±2.08 vs 5.83±1.97, P=0.000) compared to their para-carcinoma tissues, which was in line with the data of the Oncomine database. The correlation between RASEF expression and microsatellite instability, analyzed by Spearman's correlation analysis showed that RASEF expression in colorectal cancer cytoplasm was correlated significantly with the mismatch repair genes MLH1 (P=0.037; r=0.227) and MSH6 (P=0.038; r=0.224). Additionally, high RASEF expression was associated with a significantly better prognosis (45.3% vs 8%, P=0.041), which was consistent with the data of the Human Protein Atlas. Subsequently, Cox analysis of multi-factor survival showed that RASEF expression was an independent predictive factor for colorectal cancer (P=0.001). Thus, we speculated that RASEF may be a suppressor gene, and may inhibit the development of colorectal cancer through participating in DNA repair processes.

Keywords: RASEF, high expression, colorectal cancer, prognosis

Introduction

Colorectal cancer is one of the most common gastrointestinal tumors with a high death rate and an apparent increase in incidence. Colorectal cancer incidence is mainly concentrated in North America and other developed countries [1]. Although the incidence and death rates of colorectal cancer declined about 3% per year inrecent years, death rate remains high. Therefore, the prevention, treatment, and prognosis of colorectal cancer remains a significant problem in the global public health field [2]. The characterization of the molecular mechanisms and independent predictive factors of colorectal cancer should improve diagnosis and treatment.

The RAS (Ras-related protein) superfamily of GTPases consists of five different subfamilies including RAS, RHO, RAB, and ARF families, and the closely related Gα family [3]. The Rabs formed the largest branch of the RAS superfamily, which act as indispensable regulators of vesicular transport pathways [4-6]. Traditionally, Rab1, 2, 3, and other more than 70 members have been numbered in order of discovery over the last decade [8]. Since the Rabs perform the necessary function of regulation in cellular vesicular transport, studies have demonstrated that Rabs played a key role in tumorigenesis and some cancer progression [6]. For example, in a cancer type-dependent manner, Rab25 could functions as an oncogene or a tumor suppressor as it regulates apical transport as well as recycling of vesicles to the plasma membrane [3]. Studies have revealed that several members' abnormal expression might perform an important role in the occurrence, development, or metastasis of colorectal cancer [3, 9, 10]. Few studies have revealed the relationship of RASEF expression and human cancers, especially colorectal cancer.

The RASEF (RAS and EF-hand domain-containing protein), a member of Rab GTPase protein

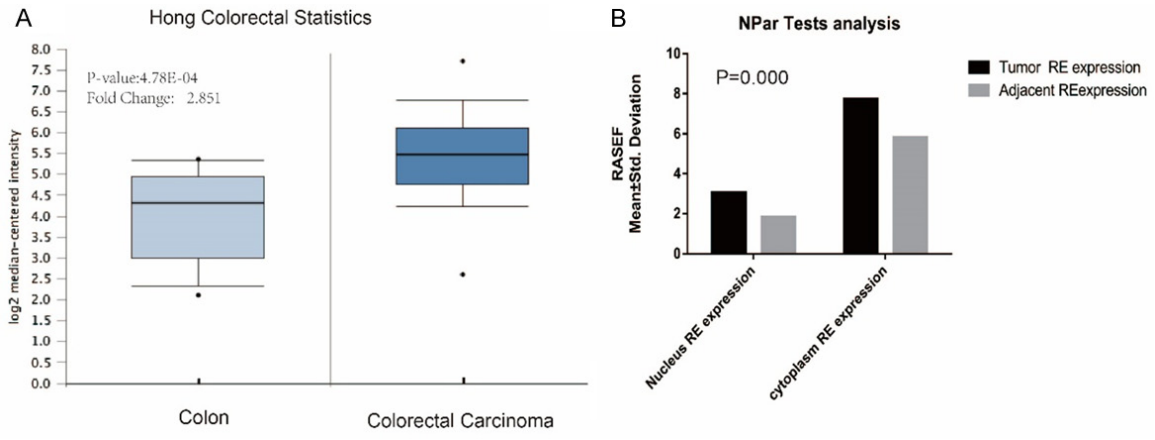

Figure 1. RASEF expression is significantly elevated in colorectal cancer. A. RASEF mRNA expression analysis using Oncomine. RASEF was significantly overexpressed in colorectal carcinoma compared with normal colon samples from the Hong Colorectal Statistics (P=4.78E-4). B. Similar results were observed in the colorectal microarray data (P=0.000). P<0.05 was considered significant.

family, contains a Rab GTPase domain in the C-terminal region, a coiled coil motif in an internal region as well as 2 EF hand domains which were of importance for binding to calcium ions in the N-terminus [11, 12]. However, little is known about RASEF and human cancers especially colorectal cancer, so unraveling the mechanism of tumor progression and further discovery of novel prognostic markers for prediction and treatment evaluation is urgently required.

Materials and methods

Clinical data

The colorectal cancer tissue microarray (HColAdel180sur-06) contained 90 colorectal cancer patients' tissue samples and 90 paired para-carcinoma tissues were made by Shanghai Outdo Biotech Co., Ltd to research the expression of RASEF. The colorectal cancer tissue microarray contained well-documented clinicopathological information: among these patients, 47 were men, other 42 were women as well as 1 was lost to follow-up, while the age of these patients' were range from 24 to 90 with a median age of 70, and the tumor size varied from 1.5 cm to 15 cm, with the median size of 5.9 cm. 8 of these colorectal cancer patients' clinical grade were stage I, 48 were stage II, 30 were stage III, and 2 were stage IV. 4 patients' clinical stage was not obtained.

All the colorectal cancer patients were clinicopathologically diagnosed as colorectal cancer and received no extra treatment before surgery. The operation time was from July 2006 to May 2007 and the eventual follow-up time was in August 2014, containing 87-97 months. The results of statistical analysis showed that during the follow-up time, 56 of the 90 patients died of colorectal cancer, though the other 34 patients were still alive, while the median follow-up time was about 92 months.

Immunohistochemistry

Two-step immunohistochemistry: first, tissue sections were treated with EDTA buffer to retrieval antigen; and then incubated with primary antibody which anti-RE (1:800, Proteintech) at 4°C overnight; the very next sections were incubated with secondary antibody (HRP-labeled anti-mouse antibody, DAKO). It was visualized using diaminobenzidine (DAB) system and hematoxylin re-dying after washed with PBS. The RE expression was observed and analyzed by pathologist under microscope, scored and grouped with positive staining rate and intensity, and the positive staining rate was defined according to the proportion of positively stained cancer cells: "Negative" is 0, "1%-25%" for 1, "26%-50%" for 2, "51-75%" for 3, "76-100%" for 4. The score of staining intensity was defined as: "Negative" is 0, "1+" for 1, "2+" for 2, "3+" for 3. Thus, patients were divided into two groups according to the scores of "positive staining rate score" multiplied by "staining intensity score". It was divided into a high expression group when the score is

Table 1. Correlation between RASEF expression and clinical indicators of colorectal cancer patients: Spearman's correlation analysis

				Gender	Age	Tumor size	Pathological grading	T	N	M	Clinical grading
Spearman's rho	Cytoplasm RE expression	Tumor	Correlation Coefficient	-.035	-.017	.064	.010	-.081	.005	.125	.054
			Sig. (2-tailed)	.752	.878	.562	.927	.480	.960	.251	.628
			N	85	81	85	86	79	86	86	84
		Adjacent	Correlation Coefficient	-.032	-.017	-.103	-.107	-.142	.066	-.156	-.010
			Sig. (2-tailed)	.779	.885	.356	.336	.220	.552	.160	.930
			N	82	78	82	83	76	83	83	81
	Nucleus RE expression	Tumor	Correlation Coefficient	.024	.088	.154	.162	.156	.007	.010	.055
			Sig. (2-tailed)	.824	.433	.156	.133	.167	.950	.927	.616
			N	86	82	86	87	80	87	87	85
		Adjacent	Correlation Coefficient	-.067	-.101	-.001	.242*	.100	.027	.146	.068
			Sig. 2-tailed)	.549	.378	.992	.027	.389	.808	.188	.547
			N	82	78	82	83	76	83	83	81

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

Table 2. The ccorrelation between RASEF expression and microsatellite instability: spearman's correlation analysis

				MLH1	MSH6	MSH2	PMS2
Spearman's rho	Cytoplasm RE expression	Tumor	Correlation Coefficient	.227*	.224*	.201	.209
			Sig. (2-tailed)	.037	.038	.068	.056
			N	85	86	83	84
		Adjacent	Correlation Coefficient	-.190	-.113	-.185	-.074
			Sig. (2-tailed)	.090	.310	.102	.516
			N	81	83	79	80
	Nucleus RE expression	Tumor	Correlation Coefficient	-.116	.069	.013	.047
			Sig. (2-tailed)	.286	.526	.904	.672
			N	86	87	84	85
		Adjacent	Correlation Coefficient	-.274*	-.035	-.144	.033
			Sig. (2-tailed)	.013	.750	.204	.769
			N	81	83	79	80

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

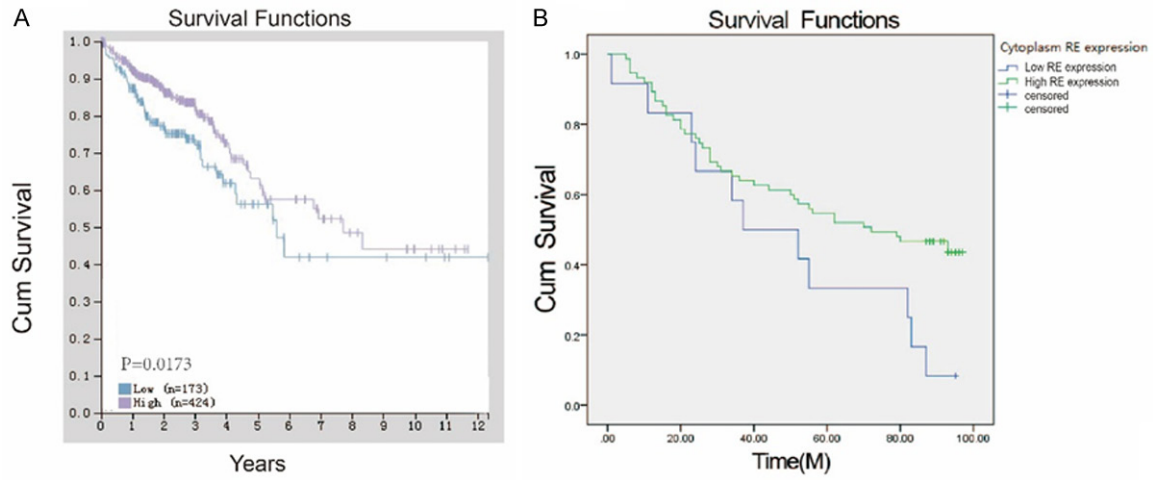

Figure 2. High RASEF was associated with a significantly better prognosis in colorectal cancer. A. High RASEF expression associated with better prognosis in colorectal carcinoma compared with low RASEF expression (P=1.73e-2) from the Human Protein Atlas. B. Similar results were observed in the correlation of RASEF expression in cancer cytoplasm and the prognosis of colorectal cancer from colorectal microarray data (P=0.041). P<0.05 was considered significant.

equal or more than 6, while less than 6 wasconsidered low expression group.

Statistical analysis

We statistically analyzed the differential expressions of RASEF in colorectal cancer tissues and their adjacent tissues useing NPar Test. The RASEF mRNA expression in colorectal specimens was obtained from the public available cancer microarray database ONCOMINE (https://www.oncomine.org/). The correlations between RASEF expression and colorectal cancer patients' clinical indexes were analyzed by Pearson's correlation. The relationships between the overall survival time of colorectal cancer patients and RASEF expression were analyzed by the Kaplan-Meier method and the log-rank test. Finally, statistically significant variables in univariate analysis were included in Cox multivariate regression survival analysis. P<0.05 was considered significant.

Results

RASEF expression was significantly elevated in colorectal cancer

To characterize the role of RASEF in colorectal cancer, we analyzed RASEF mRNA expression

Table 3. Cox multivariate regression analysis of the independent predictors of RASEF in colorectal cancer patients

	B	SE	Wald	Df	Sig.	Exp(B)	95.0% Exp(B) confidence interval	
							Lower limit	Upper limit
Cytoplasm RE expression in cancer	-1.191	.361	10.897	1	.001	.304	.150	.616
Nucleus RE expression in cancer	1.743	.548	10.113	1	.001	5.713	1.952	16.723
N	.624	.369	2.865	1	.091	1.866	.906	3.844
M	1.636	.860	3.621	1	.057	5.137	.952	27.709
Clinical stage	.241	.406	.352	1	.553	1.273	.574	2.821

A. The degree of freedom was reduced because of the constant or linear variation. B. Constant or linear covariance. Clinical stages = T + N + M. **Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

in colorectal specimens from the public available cancer microarray database ONCOMINE (https://www.oncomine.org/). We found that RASEF expression was significantly increased in colorectal carcinoma (**Figure 1A**) compared to colon.

Beyond that, we also evaluated the levels of RASEF protein in colorectal cancer tissue microarray and did statistical analysis of results of immunohistochemistry showing that the RASEF could expressed in both cytoplasm and nucleus, while the expression of RASEF between colorectal tissues and para-carcinoma tissues were significantly elevated both in cytoplasm of colorectal cancer (7.74±2.08 VS 5.83±1.97, P=0.000) and in the nucleus (3.07±1.95 VS 1.83±1.74, P=0.000). The results are shown in **Figure 1**.

The correlation between RASEF expression and colorectal cancer clinical parameters

The correlation between RASEF expression and colorectal cancer clinical parameters were analyzed by the Spearman's correlation analysis, and the results showed that there was no significant correlation between the expression of RASEF and the clinical indicators of colon cancer. The results are shown in **Table 1**.

The correlation between RASEF expression and Microsatellite Instability

Spearman's correlation analysis showed that RASEF expression in colorectal cancer cytoplasm was correlated significantly with the mismatch repair gene MLH1 (P=0.037; r=0.227) and MSH6 (P=0.038; r=0.224). The results are shown in **Table 2**.

The relationships between the overall survival time of colorectal cancer patients and RASEF expression

Kaplan-Meier method and the log-rank test analysis results showed that patients with RASEF high expression had a significantly better prognosis (45.3% vs 8%, P=0.041), which was consistent with the data of the Human Protein Atlas (https://www.proteinatlas.org/). The analysis results were shown in **Figure 2**.

Subsequently, multivariate analyses of clinical factors associated with survival and RASEF expression in colorectal cancer demonstrated that RASEF expression was an independent predictive factor (P=0.001). Detailed results are shown (**Table 3**).

Discussion

Colorectal cancer is one of the most common gastrointestinal tumors with a high death rate and an apparent increase in incidence. The prevention, treatment, and prognosis of colorectal cancer remain unresolved issues in the global public health field. Therefore, finding independent predictive factors of colorectal cancer should have a significant sense on diagnosing and treating colorectal cancer.

The RAB protein is a small GTP binding protein that forms the largest branch of the RAS superfamily which plays a vital role in multiple types of tumorigenesis, as well as regulates the proliferation, migration, and invasion of tumor cells. It has been reported that Rab proteins' expression have a closely connection with tumorigenesis and development progression like breast

cancer [13, 14], renal cancer [15], gastric cancer [16], liver cancer [17, 18], ovarian cancer [19], glioblastoma multiforme [20], head and neck cancer [21], and esophageal squamous cell carcinoma [22]. An increasing number of studies report that some Rabs proteins' expression have close correlation with colorectal cancer [10, 23, 24]. However, as a member of Rabs protein family, there was little research on the correlation between RASEF and human cancers. Studies reported that high RASEF expression could promote cell growth via enhanced ERK signaling and was associated with poor prognosis of NSCLC, so that RASEF could serve as a novel diagnostic biomarker and a therapeutic target for lung cancer.

Our experiment mainly studied the correlation between the RASEF expression and the clinical index of colorectal cancer. The results showed that RASEF was significantly highly expressed both in colorectal cancer nuclei (3.07±1.95 vs 1.83±1.74, P=0.000) and cytoplasm compared to their para-carcinoma tissues (7.74±2.08 vs 5.83±1.97, P=0.000), which was in line with data of Oncomine database and also previous studies reporting that RAB has important roles in ccRCC [1, 3, 11]. Spearman's analysis on the correlation between RASEF expression and Microsatellite Instability showed that RASEF expression in colorectal cancer cytoplasm was significantly correlated with MLH1 and MSH6. Mismatch repair gene abnormality and microsatellite instability could serve as preliminary evidence of the tumorigenesis as most colorectal cancers have proficient MMR [25], which demonstrated that the high expression of RASEF might correlate with inhibiting tumorigenesis or the development of colorectal cancer through interacting with MLH1 and MSH6.

Moreover, patients with high RASEF expression had a significantly better prognosis (45.3% vs 8%, P=0.041), which was consistent with the data of the Human Protein Atlas (https://www.proteinatlas.org/). The Cox multi-factor survival analysis showed that RASEF expression was an independent predict factor for colorectal cancer (P=0.001). Thus, we speculate that RASEF may be serve as a suppressor gene, and may inhibit the process of colon carcinogenesis through participating in DNA repair processes. Further work focusing on the RASEF expression affection on colorectal cancer cellular function and metastasis ability should be done to support this.

In conclusion, we unveiled the prognostic significance of RASEF in colorectal cancer, and suggested RASEF expression to be a potential prognostic and therapeutic marker for this disease.

Acknowledgements

The study was supported by National Nature Science Foundation of China (to M.L.) [Grant number: 81372612] National Nature Science Foundation of China (to G.L.) [Grant number: 81302059] and Nature Science Foundation of Heilongjiang Province of China (to G.L.) [Grant number: LC2013C35].

Disclosure of conflict of interest

None.

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