Expression Patterns of Ras-Association Domain Family Genes and Their Prognostic Values in Gastric Cancer

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Research article

Keywords: RASSF family members, Gastric cancer, Expression Patterns, Prognosis

Posted Date: February 21st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-737041/v1

Abstract

Background

Ras-association domain family (RASSF) members are a set of proteins involved in cell cycle control and apoptosis and they are generally considered as tumor suppressors.

Objective

The present study aimed to elucidate the expression patterns of RASSFs and their prognostic values in gastric cancer.

Methods

The Oncomine and UALAN online databases were used for analyzing the expression patterns of RASSFs in gastric cancer patients and Kaplan-Meier online database was used for analyzing the prognostic values of RASSFs. The STRING database was used to establish the protein-protein interaction network. Gene ontology (GO) analysis and the Kyoto Encyclopedia of Genes and Genomics (KEGG) enrichment analysis were performed by using clusterProfiler package. Finally, MEXPRESS was used for analyzing DNA methylation.

Results

It was found that RASSF 1 was upregulated in gastric cancer and associated with a better prognosis, whereas RASSF7 was also upregulated in gastric cancer, but associated with a worse prognosis. Enrichment analysis further explained RASSFs association with Ras by regulation of GTP/GDP binding. RASSF genes showed different methylation levels in gastric cancer.

Conclusions

In conclusion, RASSF members are differentially expressed in gastric cancer, and could be potential prognostic biomarkers in gastric cancer.

Introduction

Gastric cancer is a major malignancy of the gastrointestinal tract, accounting for a large proportion of cancer-related deaths globally[1]. Diagnosis of early-stage gastric cancer and prediction of the prognosis of gastric cancer patients significantly reduce mortality. Currently, extensive investigation has been carried out to identify biomarkers for diagnosis and prognosis, with some achievements[2]. However, there

are still no biomarkers that can be reliably used for early diagnosis and prognosis of gastric cancer. Therefore, there is still a need for further studies to find robust diagnostic and prognostic biomarkers.

The Ras family of small GTPases (Ras GTPases), which include K-Ras, H-Ras, and N-Ras, act as a molecular switch and play a role in the regulation of cell proliferation (*i.e.*, G1 to S cell cycle restriction checkpoints), metabolism, and transformation[3, 4]. Ras GTPase-activating proteins (Ras GAPs), such as neurofibromin (NF1) or p120GAP, regulate the transition between inactive GDP-bound Ras and active GTP-bound Ras[5]. *Ras* gene mutations, which are found in more than 19% of cancers[6], lead to the accumulation of GTP-bound Ras and Ras signaling cascades[4]. Besides, deregulation of wild-type Ras signals in the absence of *Ras* mutations exists in many tumors[3]. Two main mechanisms account for the deregulation of wild-type Ras signals[3]: over-activation of Ras positive regulators or loss of function of negative regulators. Ras-association domain family (RASSF) belongs to the latter one[3].

RASSF consists of 10 members[7]. All these 10 members contain the Ras-related domain, which enables them to combine with Ras, and form scaffolds to reduce the promoting effects of Ras on cell growth and survival[3, 8], and link Ras with pathways regulating apoptosis, aging, autophagy, inflammation, and DNA repair[9]. RASSF1-6 each owns their Ras-related domain at the C-terminal[10] and RASSF7-10 at the N-terminal[11]. RASSF1-6 each also possesses the SARAH protein-protein interaction motif, which is a conserved mediator in the Hippo pathway [12–14].

It has been reported that RASSF expression is often downregulated in cancers, mainly due to promoter hypermethylation[7]. Hypermethylation of RASSF family genes is widespread in different tumors, including lung cancer [15], thyroid neoplasm [16], multiple myeloma [17], breast cancer [18], bladder cancer[19], and RASSF1 promoter hypermethylation is one of the most common methylation events in human cancers [20]. Unlike other members of the RASSF family, RASSF8 promoter remains unmethylated in most cancers; however, previous studies have revealed that the transcript factor E4BP4, as a key transcriptional modulator, inhibits RASSF8 expression through histone methyltransferases, G9a and SUV39H1 [21], leading to RASSF8 down-regulation in some cancers.

Previous studies have reported the expression patterns of several individual RASSF family proteins and their diagnostic values in different cancers[15–18, 20], but there has been no study on RASSF expression in gastric cancer. Therefore, the present study aimed to explore the expression patterns of RASSF members and their prognostic values in gastric cancer, based on online data mining tools and an R package.

Methods

Search for information on RASSFs through the GeneCards database

The GeneCards database (https://www.genecards.org), developed and maintained by the Crown Human Genome Center at the Weizmann Institute of Science, Rehovot, Israel, provides gene-centric information that is automatically mined and integrated from data sources, producing a web-based card for thousands of human genetic entries.[22] RASSFs were searched on GeneCards for their chromosomal locations and other information.

Oncomine database analysis of RASSF family expression patterns in gastric cancer

The Oncomine Platform (https://www.oncomine.org/) collects microarray datasets from Gene Expression Omnibus (GEO) and Array Express, measuring either mRNA expression or DNA copy number variations on primary tumors or cell lines, and provides a set of online data-mining-tools. The gene expression patterns of individual members of the RASSF family in gastric cancer were obtained from the Oncomine database. The Student's T-test was used to calculate the *P* values of differences in RASSF family gene expression between gastric cancer and normal gastric samples. The threshold parameters of the *P* value and fold change were delimited to 0.01 and 1.5, respectively. The significant difference in the expression was defined when the *P* value was < 0.01 and the fold change was ≥ 1.5.

UALCAN database analysis for validation

UALCAN [23], like the Oncomine database, is an open portal site (http://ualcan.path.uab.edu) that provides an online analysis of data from The Cancer Genome Atlas (TCGA). In this study, we used it to further analyze and compare the expression patterns of RASSF genes between gastric cancer and normal gastric samples. A *P* value of less than 0.01 was considered statistically significant.

Kaplan-Meier plotter survival analysis

The prognostic value of the gene expression of RASSF members was analyzed by using the Kaplan-Meier online database (http://www.kmplot.com) [12]. A total of 18,674 human malignant tumor samples, including 1,065 from gastric cancer patients, were evaluated. The detection probe IDs were: 204346_s_at (RASSF1), 203185_at (RASSF2), 227167_s_at (RASSF3), 226436_at (RASSF4), 223322_at (RASSF5), 229147_at (RASSF6), 204927_at (RASSF7), 225946_at (RASSF8), 210335_at (RASSF9), 238755_at (RASSF10). Each probe ID was entered into the database, without specific restrictions, such as cancer subtypes. The samples were divided into the high-expression and low-expression groups according to Auto select best cutoff generated from the online tool. The associations of the gene expression with the overall survival (OS), first progression (FP), and post-progression survival (PPS) were verified by the K-M survival curve and log-rank test. A *P* value of less than 0.05 was considered statistically significant.

Construction of a protein-protein interaction network

The STRING database (https://string-db.org/) is an online tool to construct a functional association network by integrating a large amount of protein-protein interaction (PPI) data that has been

experimentally proven or bioinformatically predicted. A functional PPI network was established among interactors with a confidence score ≥ 0.4 as identified by the STRING database [24].

Gene function annotation and pathway enrichment analysis

Gene ontology (GO) analysis and the Kyoto Encyclopedia of Genes and Genomics (KEGG) enrichment analysis were conducted for 50 interactors selected by STRING analysis by using the clusterProfiler package, an R package for comparing biological themes among gene clusters [25–27]. The top 20 enriched GO and KEGG categories obtained from the analysis results were selected in the present study.

MEXPRESS for methylation analysis

MEXPRESS (https://mexpress.be/) is an online tool for easy visualization and integration of gene expression, DNA methylation, and clinical data from TCGA [28, 29]. As mentioned above, RASSF members are mostly hypermethylated in cancers; so, MEXPRESS was used to determine the associations between gene expression and DNA methylation of RASSF members in gastric cancer.

Results

Expression pattern of RASSF genes in gastric cancer

Chromosomal locations and other information of all RASSF members, as concluded from the GeneCards, are shown in Table 1.

Table 1
Basic information on RASSF genes as concluded from the GeneCards

Approved symbol	Gene ID	HGNC ID	Synonym(s)	Exon	Chromosomal location
RASSF1	11186	HGNC :9882	123F2	8	3p21.31
			RDA32		
			NORE2A		
			RASSF1A		
			REH3P21		
RASSF2	9770	HGNC :9883	CENP-34	15	20p13
			RASFADIN		
RASSF3	283349	HGNC :14271	RASSF5	8	12q14.2
RASSF4	83937	HGNC :20793	AD037	11	10q11.21
RASSF5	83593	HGNC :17609	RAPL; Maxp1; NORE1; NORE1A; NORE1B; RASSF3	7	1q32.1
RASSF6	166824	HGNC :20796	-	17	4q13.3
RASSF7	8045	HGNC :1166	HRC1; FAP88; HRAS1; CFAP88; C11orf13	6	11p15.5
RASSF8	11228	HGNC :13232	HOJ1; C12orf2	11	12p12.1
RASSF9	9182	HGNC :15739	P-CIP1, PAMCI, PCIP1	3	12q21.31
RASSF10	644943	HGNC :33984	-	1	11p15.3

The Oncomine database analysis showed that RASSF1, RASSF2, RASSF4, RASSF7, and RASSF9 were over-expressed while RASSF5 and RASSF6 were down-regulated in gastric cancer (Fig. 1).

The UALCAN database analysis was then used to verify the expression of RASSFs in TCGA datasets. There was a clear trend that the expression levels of RASSF1, RASSF4, RASSF6, and RASSF7 were significantly higher in gastric cancer than in normal gastric tissues (Fig. 2). Thus, The Oncomine and UALCAN analyses both indicated that the expression levels of RASSF1, RASSF4, and RASSF7 were upregulated in gastric cancer; however, the expression level of RASSF6 were divergent in two analyses.

The prognostic values of RASSF genes in gastric cancer

Kaplan-Meier survival curves for the prognostic values of RASSF members in gastric cancer are shown in Table 2 and Fig. 3. It was revealed that upregulation of RASSF1 (HR = 0.74 95% CI 0.62 − 0.89, and *P* = 0.001) and RASSF6 (HR = 0.69, 95% CI 0.55 − 0.88, and *P* < 0.001) were associated with better prognosis-longer period of time for OS; however, upregulation of RASSF2 (HR = 1.33, 95% CI 1.09 − 1.62, and *P* = 0.005), RASSF3 (HR = 1.35, 95% CI 1.05 − 1.73, and *P* = 0.020), RASSF7 (HR = 1.42, 95% CI 1.18 − 1.7, and *P* = 0.001), RASSF8 (HR = 1.77, 95% CI 1.43 − 2.2, and P < 0.001) and RASSF9 (HR = 1.41, 95% CI 1.17 − 1.69, and *P* < 0.001) was associated with poor prognosis shorter period of time for OS. The same trend was observed for FP and PPS (Fig. 3).

Table 2
Median overall survival of gastric adenocarcinoma patients with different expression levels of RASSFs.

RASSF	Low expression cohort (months)	High expression cohort(months)	HR (95%CI)	*P* value
RASSF1	23.9	30.7	0.74(0.62 − 0.89)	0.001
RASSF2	36.4	27.9	1.33(1.09 − 1.62)	0.005
RASSF3	85.6	40.7	1.35(1.05 − 1.73)	0.020
RASSF4	56.9	44.7	1.15(0.92 − 1.44)	0.230
RASSF5	63.7	45.1	1.13(0.87 − 1.45)	0.360
RASSF6	28.7	63.7	0.69(0.55 − 0.88)	0.002
RASSF7	34.37	21.6	1.42 (1.18 − 1.7)	< 0.001
RASSF8	97	28.27	1.77 (1.43 − 2.2)	< 0.001
RASSF9	45	25.9	1.41(1.17 − 1.69)	< 0.001
RASSF10	40.2	75.5	0.8 (0.64 − 1)	0.051
HR: Hazard ratio; CI, confidence interval;				

Functions and interactions of RASSF genes

The STRING analysis on the PPI network revealed that interrelationships among RASSF members and other proteins were intricate (Fig. 4). The top 20 enriched GO and KEGG categories obtained with clusterProfiler package are shown in Fig. 5. GO analysis showed accumulation in the binding of GTP and GDP, indicating that RASSFs might regulate the activation and inactivation of Ras by participating in Ras

GTPases-RasGAPs interactions as previously described. KEGG analysis revealed that RASSF proteins were strongly related to Ras protein signal transduction and carcinogenesis.

RASSF methylation in gastric cancer

The DNA methylation levels of RASSF1, RAFFS4, and RASSF7 were significantly increased in gastric cancer, compared with normal gastric tissues (Fig. 6).

Discussion

The present study investigated for the first time the expression patterns of RASSF genes in gastric cancer and their prognostic values in gastric cancer.

The Oncomine and UALCAN online databases were used to explore the expression pattern of RASSF genes in gastric cancer and the Kaplan-Meier plotter was used to analyze their prognostic values. It was found that RASSF1, RASSF4 and RASSF7 were upregulated in gastric cancer both in the Oncomine and UALCAN database analyses, while RASSF6 was found down-regulated in the Oncomine analysis but up-regulated in the UALCAN analysis. The reason for the discrepancy for RSAAF6 gene expression between the two database analyses may be due to the fact that the Oncomine and UALCAN databases contain different gastric cancer pathological types; UALCAN mostly contains gastric adenocarcinoma whereas Oncomine holds different histological types.

In the present study, we found that RASSF1 was associated with a better prognosis of gastric cancer, which is consistent with previous observations on tumor suppressors[30]. However, RASSF1 was highly expressed in gastric cancer as shown in both the Oncomine and UALCAN online databases, whereas a high methylation level was shown with MEXPRESS. The reason for the inconsistence between the gene expression and DNA methylation may be due to the fact that, in addition to DNA methylation, there are other regulatory mechanisms responsible for RASSF1 expression at the mRNA and protein levels. A previous study showed that RASSF1 expression was upregulated by reactive oxygen species (ROS)-mediated pathway in lung cancer[31]. Under the hypoxic condition within lung cancer, RASSF1A, an isoform of RASSF1, is phosphorylated by ROS-activated protein kinase C α, binding of hypoxia-inducible factor 1 (HIF1), and increasing the nuclear translocation and subsequent transcriptional activity of HIF1, which, in turn, leads to increased expression of RASSF1A[31]. However, it is unknown whether or not a hypoxic condition is responsible for the high level of RASSF1 gene expression in gastric cancer is unknown, and further investigation is required to reveal the regulatory mechanisms for RASSF1 gene expression in gastric cancer.

Although RASSF4 was upregulated in gastric cancer as determined by both in the Oncomine and UALCAN database analyses, it did not have any prognostic value. RASSF4 is proven as a tumor suppressor and previous studies have shown that is down-regulated in non-small lung cancer [32], osteosarcoma [33], multiple myeloma [17], and downregulated in non-small cell lung cancer, which is opposite to our

observation in the present study in gastric cancer. Further studies with clinical samples obtained from gastric cancer patients are needed to verify our observation using online databases.

In the present study, RASSF7 was up-regulated in gastric cancer and associated with a worse prognosis. Previous studies have demonstrated that RASSF7 is up-regulated in hepatocellular carcinoma (HCC) [34] and non-small cell lung cancer [35]. Moreover, RASSF7 overexpression is significantly associated with increased serum alpha-fetoprotein levels, poor tumor histology, and T staging in HCC patients; overexpression of RASSF7 promotes the proliferation of HCC cells and cell cycle transformation in the G1-S phase, and inhibit cell apoptosis, whereas down-regulation of RASSF7 gene inhibits cell growth and induces cell cycle arrest and apoptosis in G1-S phase[34]. Although the present study indicates that RASSF7 might be used as a prognostic biomarker for gastric cancer, further clinical studies are needed to confirm its prognostic value. Moreover, extensive investigation is also required to reveal its pathogenic role in gastric cancer as an oncogene.

There are a few limitations in the present study. First, the present study was carried out solely based on online bioinformatic analysis tools, and thus further validation using clinical samples is required. Second, the present study mainly focused on RASSFs at the DNA and RNA levels, and thus further investigation on RASSFs at the protein level is required.

Conclusions

The gene expression levels of some RASSF members are differentially expressed in gastric cancer and associated with the prognosis of patients with gastric cancer. Further investigation is needed to verify the prognostic values of RASSFs in the clinical setting.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

Funding

Qingdao People's Livelihood Science and Technology plan(20-3-4-14-nsh)

Author contribution

Fei Wen: Conceptualization; Data curation; Methodology; Writing-original draft.

Haixia Qu: Conceptualization.

Xiangjun Jiang: Conceptualization (lead); Funding acquisition (lead).

Acknowledgements

Not applicable.

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Figures

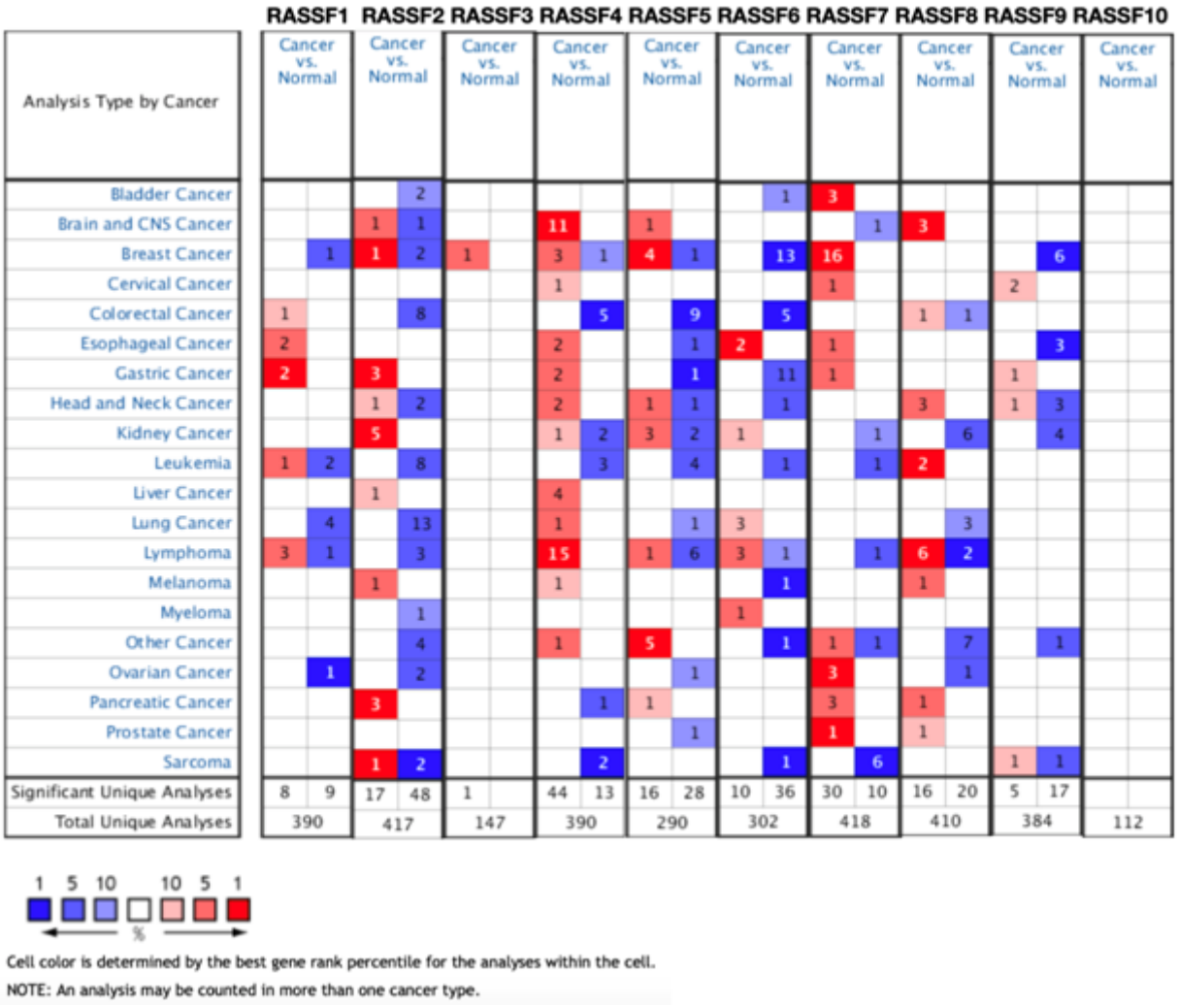

Figure 1

The expression levels of RASSF genes in different types of cancers, as revealed by the Oncomine database analysis. Red and blue stand for increased and decreased levels of RASSF gene datasets, respectively, defined by $P < 0.01$ and the fold change ≥ 1.5.

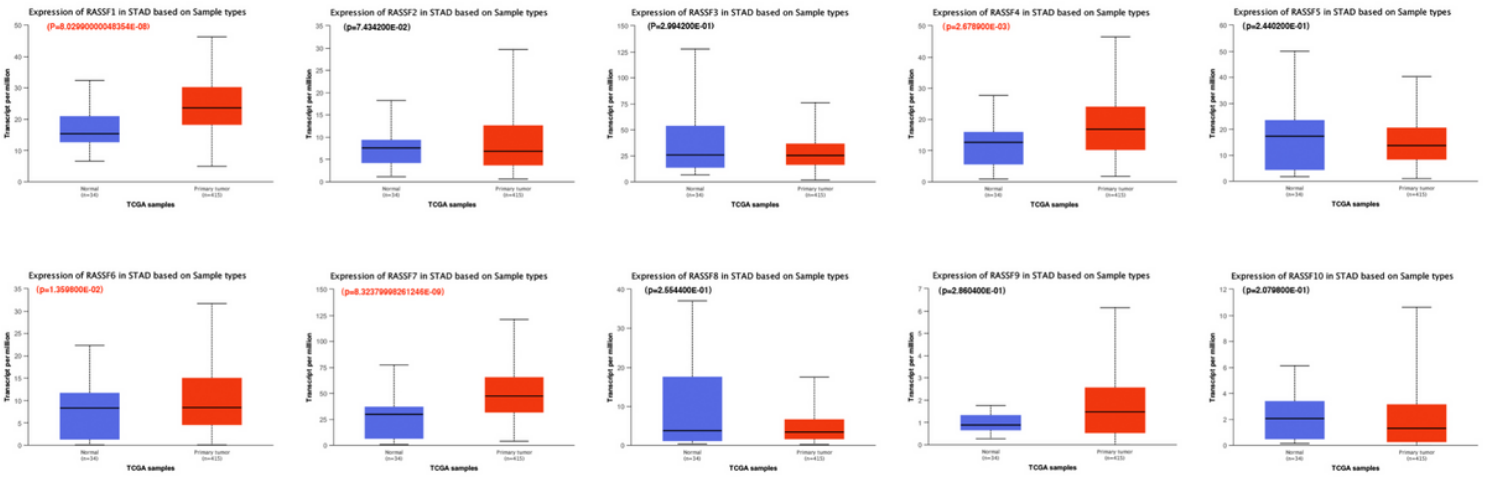

Figure 2

The expression levels of RASSF genes in the normal gastric tissues and gastric adenocarcinoma tissues, as revealed by the UALCAN database analysis. STAD, stomach adenocarcinoma.

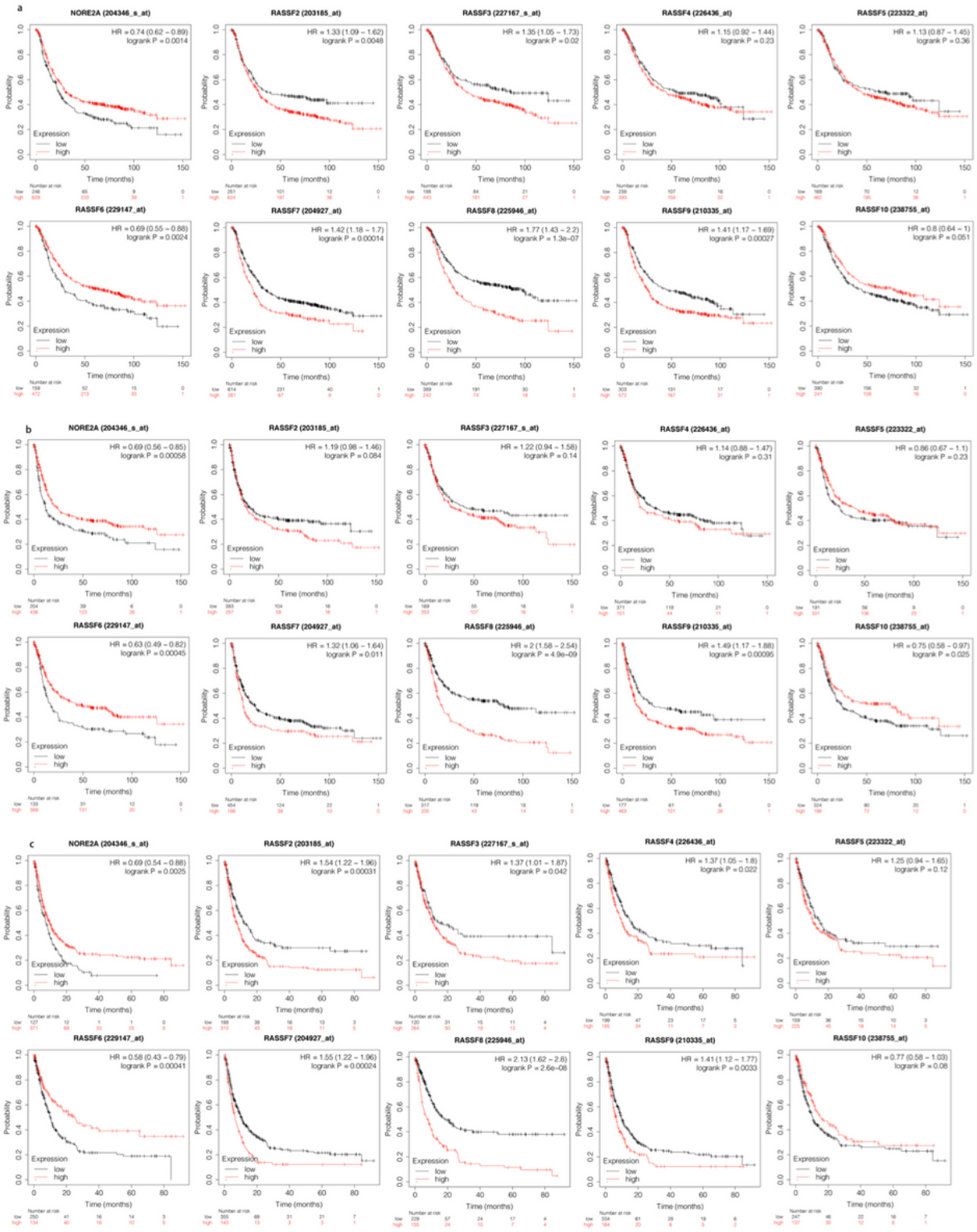

Figure 3

Prognostic values of RASSF genes in gastric adenocarcinoma, as analyzed by Kaplan-Meier plotter survival analysis for the overall survival (OS, a), first progression(FP, b) and post progression survival(PPS, c) .

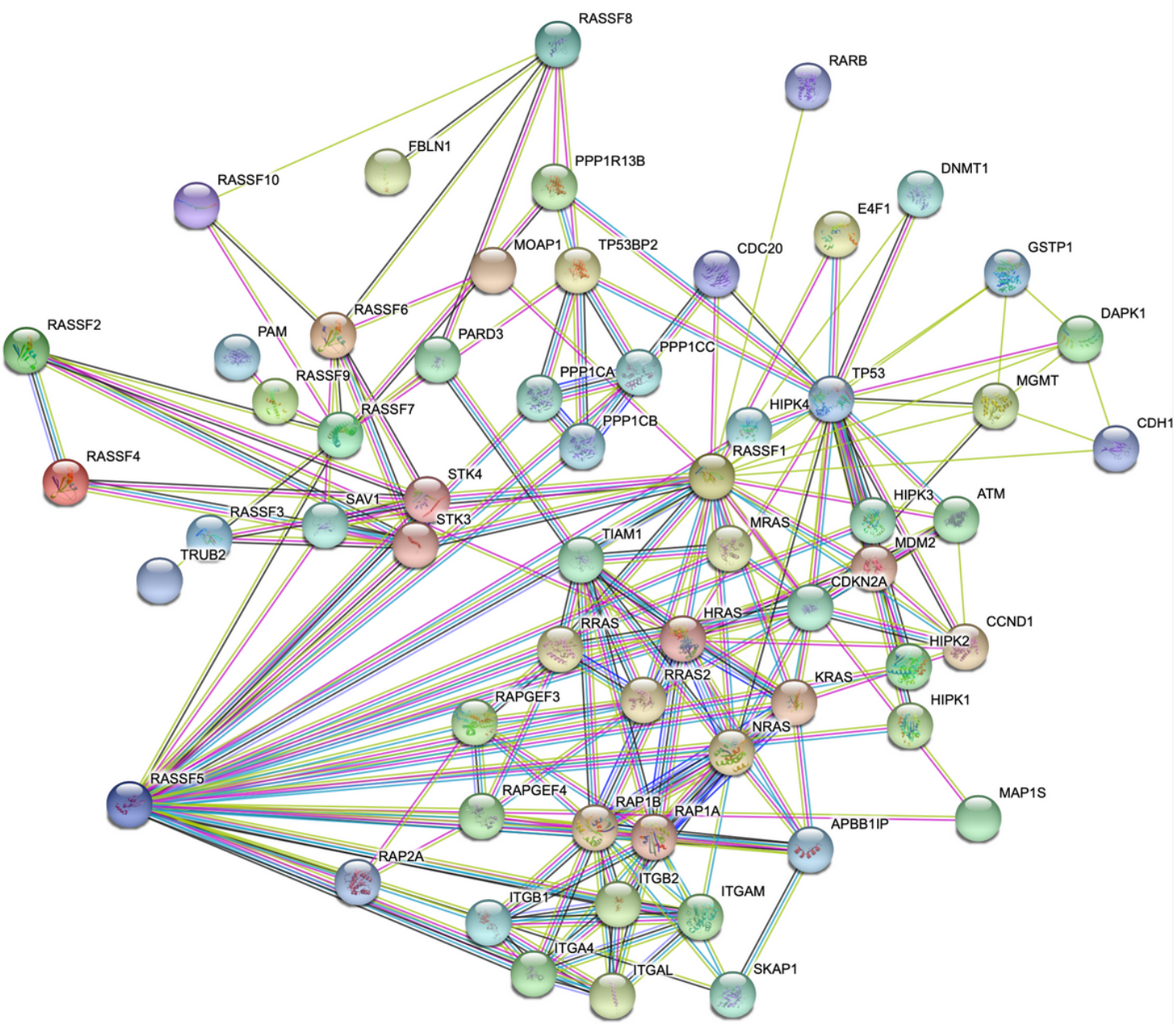

Figure 4

Potential protein-protein interactions between RASSFs and connected proteins, as constructed by the STRING analysis.

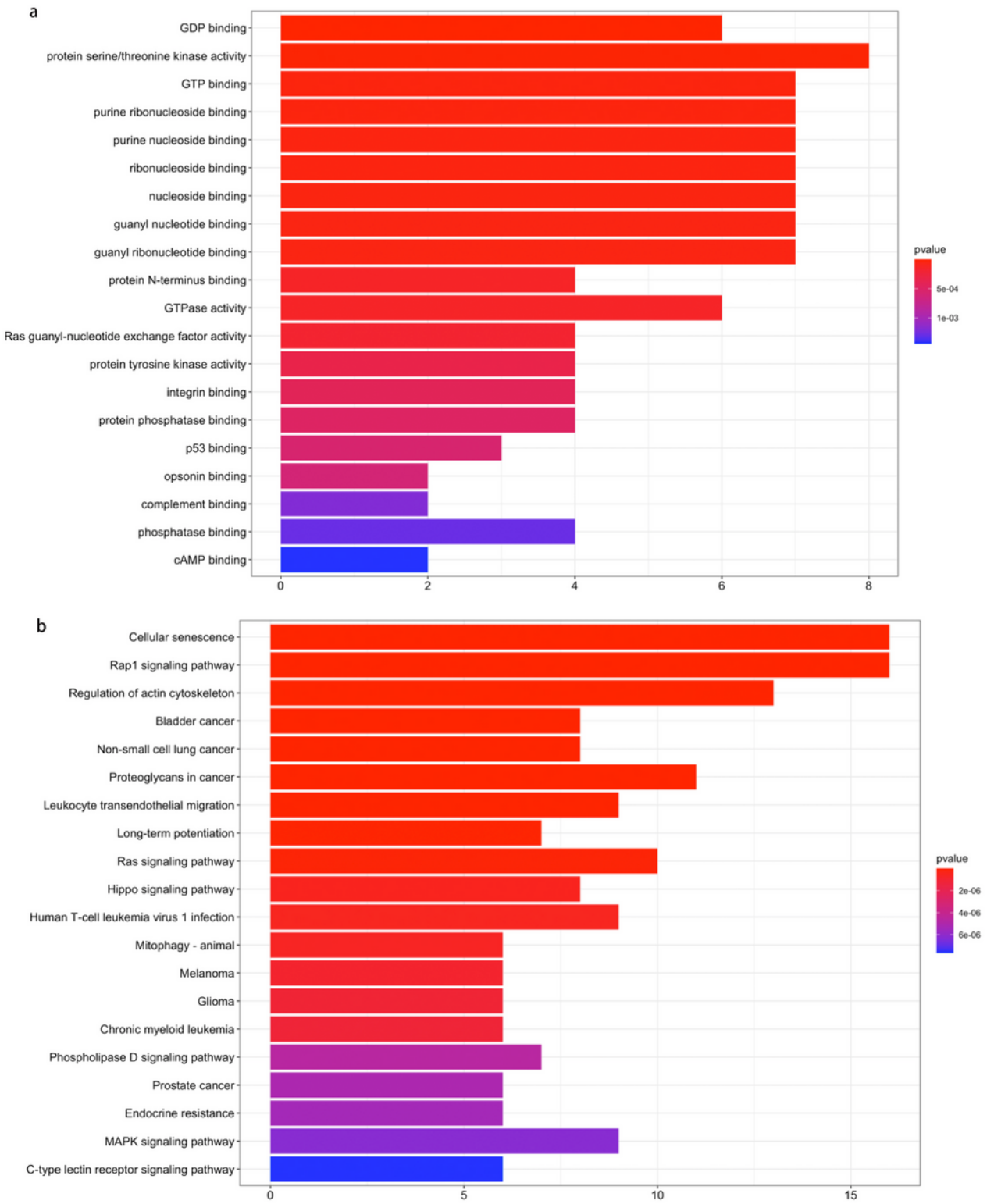

Figure 5

The top 20 enriched categories from the GO (a) and KEGG (b) analyses of 50 interactive proteins identified from the STRING analysis.

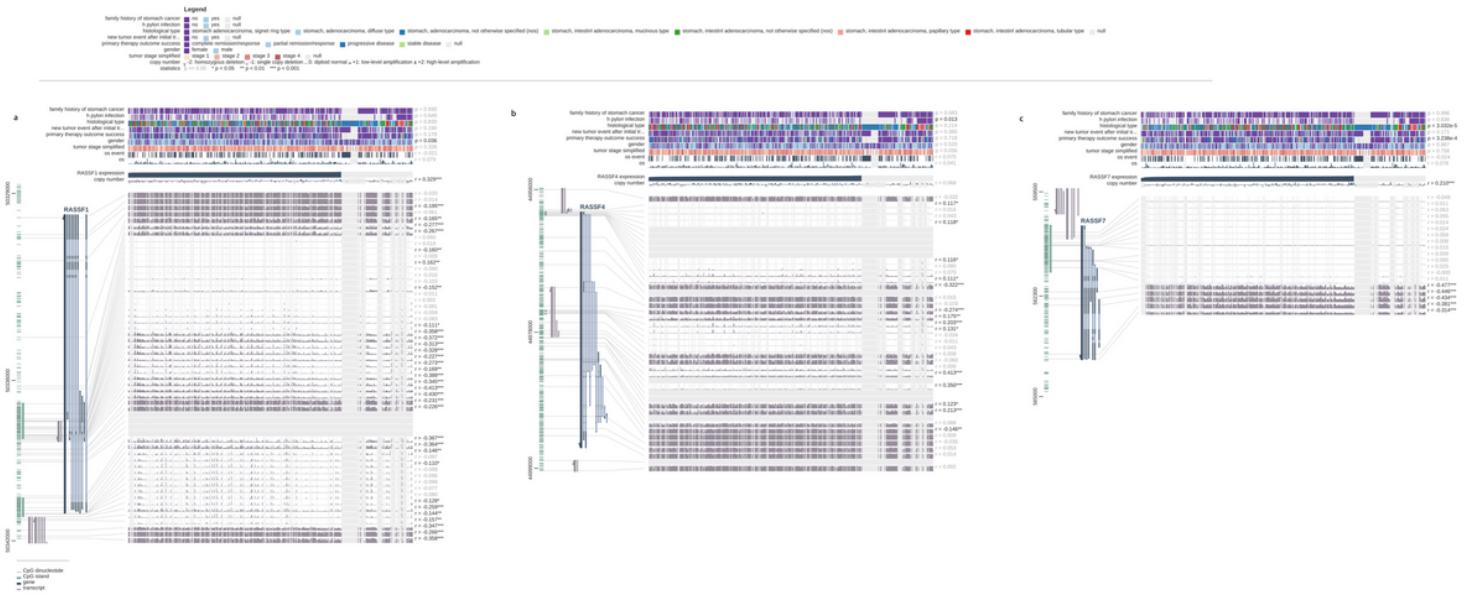

Figure 6

DNA methylation of RASSF1 (A), RASSF4 (B), and RASSF7 (C) in gastric cancer.