

Downregulation of regulator of G protein signaling 2 expression in breast invasive carcinoma of no special type: Clinicopathological associations and prognostic relevance

CHENGLONG WANG¹, QIAN YE², YIJIA CAO³, JUAN TAN², FEI WANG², JIN JIANG² and YOUDE CAO²

¹Department of Pathology, Chongqing Traditional Chinese Medicine Hospital, Chongqing 400021; ²Department of Pathology, College of Basic Medicine, Chongqing Medical University; ³Department of Endocrine and Breast Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, P.R. China

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Abstract. Changes in the expression of regulator of G protein signaling 2 (RGS2) are involved in the genesis and development of a number of malignancies. However, the association between changes in the expression of RGS2 and breast invasive carcinoma of no special type (BIC-NST) remains unknown. The present study found that, in comparison to normal breast tissue, BIC-NST exhibited low expression of RGS2 mRNA and protein, as detected using data mining and immunohistochemical analysis. The low expression of RGS2 was associated with the positive status of hormone receptor expression in BIC-NST. Kaplan-Meier analysis revealed that patients with low RGS2 expression had a significantly poorer overall survival rate. Furthermore, multivariate Cox regression analysis demonstrated that the RGS2 low expression was an independent high-risk factor. Gene set enrichment analysis using data from The Cancer Genome Atlas supported these results. In summary, the results of the current study indicate that RGS2 acts as a suppressor gene in the progression of BIC-NST. To the best of our knowledge, the present study is the first concerning the association between RGS2 and hormone receptors in BIC-NST, as well as that between RGS2 expression and the prognosis of patients with BIC-NST. However, the effect of RGS2 in breast cancer requires further investigation.

Introduction

Disease-free survival in patients with breast cancer has significantly improved since the 1990s, owing to the combination of early breast cancer screening and a greater number of treatment approaches, including adjuvant chemotherapy, hormonal treatment and targeted therapy (1-3). However, there are certain patients who are only treated with maintenance therapy who receive an initial diagnosis of advanced-stage breast cancer or multidrug resistance (4). Breast cancer is the most commonly diagnosed cancer in women all over the world, and is the first and the second cause of cancer-associated mortality in women in developing and developed countries, respectively (5). Therefore, it is necessary to identify candidate biomarkers that are associated with the development of breast cancer and investigate therapeutic targets to provide patients with breast cancer with treatment options.

G-protein regulating factors negatively regulate the recycling of G-protein-coupled receptors; these factors accelerate GTP hydrolysis by binding to the α -subunit of a heterotrimeric G protein to deactivate G-protein signaling (6,7). With regard to malignant tumors, regulator of G-protein signaling 2 (RGS2) is one of the most well-characterized RGS genes. RGS2 can also inhibit the translation of mRNA into protein by binding to the ϵ -subunit of eukaryotic initiation factor 2B (8). Alteration of RGS2 expression is associated with a number of types of cancer, including prostate cancer (9), ovarian cancer (10), mantle cell lymphoma (11), acute myeloid leukemia (12) and fibrolamellar hepatocellular carcinoma (13). However, the association between RGS2 and breast cancer remains obscure. On the one hand, a large-sample study revealed that RGS2 is upregulated in breast cancer (14); on the other hand, another more recent study demonstrated that low expression of RGS2 is associated with breast cancer using MCF7 and MCF10A cells (15). The aim of the present study was to assess the association of RGS2 with breast cancer. Furthermore, according to the World Health Organization Classification of Tumors of the Breast (16), breast invasive carcinoma of no special type (BIC-NST) is the largest group among the different classifications of breast cancer. Thus, given the considerable heterogeneity of breast cancer, the present study focused on BIC-NST.

Correspondence to: Professor Youde Cao, Department of Pathology, College of Basic Medicine, Chongqing Medical University, 1 Yixueyuan Road, Yuzhong, Chongqing 400016, P.R. China
E-mail: 420306100@qq.com

Abbreviations: RGS2, regulator of G protein signaling 2; BIC-NST, breast invasive carcinoma of no special type; IHC, immunohistochemistry; GSEA, gene set enrichment analysis

Key words: breast invasive carcinoma of no special type, regulator of G protein signaling 2, prognosis, immunohistochemistry, data mining

Materials and methods

Patients and samples. Paraffin-embedded breast cancer samples from patients who had undergone radical mastectomy were obtained from the archives of the Department of Pathology at the First Affiliated Hospital of Chongqing Medical University (CQMU) (Chongqing, China) between January and December 2011. Two pathologists screened 196 BIC-NST samples according to the criterion of 2012 WHO Classification of Tumors of the Breast and reached a consensus on the diagnosis. The patients enrolled in the present study were followed up every other year, and the last follow-up was in June 2016. All protocols in this study were approved by the Ethics Committee of the First Affiliated Hospital of CQMU, and each patient enrolled provided written informed consent.

RGS2 gene expression data in breast cancer and relevant clinical information were downloaded from The Cancer Genome Atlas (TCGA) (<https://cancergenome.nih.gov>). Data from 513 patients with breast cancer were analyzed, following the exclusion of all male patients and those female patients without BIC-NST, or those lacking complete information. Of the 513 patients, there were 93 paired datasets that included cancer tissue and adjacent tissue.

Immunohistochemistry (IHC) experimental procedures. RGS2 protein expression in 4- μ m sections of 196 BIC-NST samples was detected by IHC. The polyclonal RGS2 antibody (cat. no. ab36561; dilution, 1:200) was purchased from Abcam (Cambridge, UK). Briefly, slides were deparaffinized using xylene, and rehydrating using a gradient ethanol series. Microwave antigen retrieval was performed using sodium citrate solution (10 mmol/l, pH 6.0) for 4 min in high heat (~100°C) and 10 min in low heat (~35°C), and the slides were cooled for 30 min. Slides were incubated with 3% hydrogen peroxide for 10 min at room temperature to block endogenous peroxidase activity. Samples were incubated with RGS2 antibody for 1 h at room temperature. Following incubation with Polymer Helper reagent and a poly-horseradish peroxidase-conjugated anti-mouse/rabbit IgG secondary antibody (cat. no. PV-9000; dilution ready-to-use; OriGene Technologies, Inc., Beijing, China) twice, for 20 min at room temperature each time, the sections were visualized using 3,3'-diaminobenzidine solution (Dako; Agilent Technologies, Inc., Santa Clara, CA, USA) and were counterstained with hematoxylin for 20 sec at room temperature. Image acquisition was performed using a light microscope (Olympus BX45; Olympus Corporation, Tokyo, Japan), and images were captured at magnification, x200 and x400.

IHC evaluation. The slides were independently reviewed by two pathologists who were blinded to any information on the clinical characteristics and follow-up data. RGS2 expression level scores were calculated according to the sum of IHC intensity (0, negative; 1, weak staining; 2, moderate staining; and 3, strong staining) and the rate of positive staining (0, 0%; 1, 1-50%; and 2, 51-100%). Differences in scoring were resolved by reaching a consensus. The total cumulative scores of 0-3 and 4-5 were defined as the low- and high-expression groups, respectively. Representative IHC staining images are shown in Fig. 1.

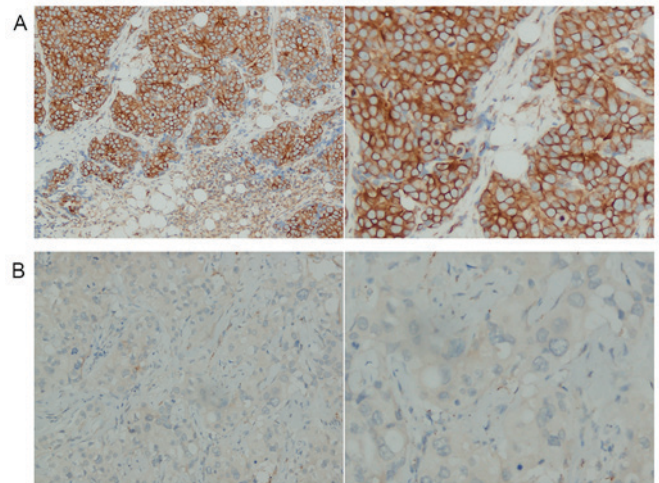


Figure 1. IHC staining of RGS2 protein in BIC-NST samples. (A) Representative IHC staining image showing high RGS2 expression in BIC-NST. (B) Representative IHC staining image showing low RGS2 expression in BIC-NST. Magnification: Left, x200; right, x400; IHC, immunohistochemistry; RGS2, regulator of G protein signaling 2; BIC-NST, breast invasive carcinoma of no special type.

Gene set enrichment analysis (GSEA). GSEA was used to assess different enriched gene sets corresponding to each gene from microarray data. Every enriched gene set is sorted according to a common biological function. Thus, the association between the targeted gene and biological function could be indirectly shown by GSEA. GSEA software version 2.2.2 was downloaded from the Molecular Signatures Database, Broad Institute (<http://software.broadinstitute.org/gsea/>) (17). The enrichment of the C2 curated gene sets and the C5 Gene Ontology (GO) gene sets were analyzed by comparing low-expression and high-expression RGS2 subtypes. The number of permutations was set at 1,000.

Statistical analysis. Differences in expression between cancer tissue and adjacent tissue from the 93 paired TCGA datasets was analyzed by paired Student's t-test. The association between RGS2 expression and clinical pathological parameters was evaluated by the χ^2 test. The association between RGS2 expression and overall survival was assessed using Kaplan-Meier method with log-rank test, and univariate and multivariate Cox regression analyses. $P < 0.05$ was considered to indicate a statistically significant difference. Statistical analysis was conducted using SPSS version 19.0.0 (IBM Corp., Armonk, NY, USA) and statistical drawing was performed using GraphPad Prism version 6.01 (GraphPad Software Inc., La Jolla, CA, USA).

Results

mRNA and protein RGS2 expression is downregulated in BIC-NST. The expression of RGS2 mRNA and protein in tumor and adjacent normal breast tissue was detected by analysis of the TCGA datasets and IHC analysis of tissue samples. As shown in Fig. 2, the expression level of RGS2 mRNA in breast cancer tissue was significantly lower than that in adjacent normal breast tissue ($P < 0.001$), as was the protein expression level of RGS2 ($P < 0.001$).

Table I. Association between patient characteristics and expression status of RGS2 in the Chongqing Medical University cohort.

Characteristic	Low RGS2, n (%)	High RGS2, n (%)	χ^2	P-value
Age, years			3.300	0.069
<60	112 (86.2)	50 (75.8)		
≥60	18 (13.8)	16 (24.2)		
Histological grade ^a			10.332	0.006
Grade 1	30 (23.1)	30 (45.5)		
Grade 2	60 (46.2)	22 (33.3)		
Grade 3	40 (30.7)	14 (21.2)		
ER status			8.790	0.003
Negative	32 (24.6)	30 (45.5)		
Positive	98 (75.4)	36 (54.5)		
PR status			5.085	0.024
Negative	38 (29.2)	30 (45.5)		
Positive	92 (70.8)	36 (54.5)		
HER-2 status			2.454	0.293
Negative	80 (61.5)	48 (72.7)		
Positive	40 (30.8)	14 (21.2)		
Uncertain ^b	10 (7.7)	4 (6.1)		
Ki-67, %			1.807	0.179
<14	50 (38.5)	32 (48.5)		
≥14	80 (61.5)	34 (51.5)		
Distant metastasis			3.142	0.076
No	124 (95.4)	66 (100.0)		
Yes	6 (4.6)	0 (0.0)		
Tumor size, cm			9.771	0.002
≤2	60 (46.2)	46 (69.7)		
>2	70 (53.8)	20 (30.3)		
Lymph node metastasis			2.274	0.132
No	64 (49.2)	40 (60.6)		
Yes	66 (50.8)	26 (39.4)		
Clinical stage			1.143	0.285
I-II	102 (78.5)	56 (84.8)		
III-IV	28 (21.5)	10 (15.2)		

^aHistological grading was performed according to the World Health Organization Classification of Tumors of the Breast, 2012 (4th edition). ^bThese samples were not examined using fluorescence in situ hybridization. RGS2, regulator of G protein signaling 2; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2.

Association between RGS2 expression and clinical characteristics. Following IHC analysis, the relevant clinicopathological analysis of the 196 BIC-NST samples was performed (Table I). HER2 status is primarily determined by immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) on formalin-fixed and paraffin-embedded samples. Definition of HER status is performed as follows:

Table II. Association between patient characteristics and expression status of RGS2 in The Cancer Genome Atlas cohort.

Characteristic	Low RGS2, n (%)	High RGS2, n (%)	χ^2	P-value
Age, years			9.307	0.002
<60	99 (48.5)	81 (65.9)		
≥60	105 (51.5)	42 (34.1)		
ER status			27.172	<0.001
Negative	33 (16.2)	52 (42.3)		
Positive	171 (83.8)	71 (57.7)		
PR status			22.132	<0.001
Negative	55 (27.0)	65 (52.8)		
Positive	149 (73.0)	58 (47.2)		
HER-2 status			0.163	0.687
Negative	169 (82.8)	104 (84.6)		
Positive	35 (17.2)	19 (15.4)		
Distant metastasis			0.556	0.456
No	198 (97.1)	121 (98.4)		
Yes	6 (2.9)	2 (1.6)		
Tumor size, cm			0.029	0.866
≤2	53 (26.0)	33 (26.8)		
>2	151 (74.0)	90 (73.2)		
Lymph node metastasis			9.469	0.002
No	82 (40.2)	71 (57.7)		
Yes	122 (59.8)	52 (42.3)		
Clinical stage			5.381	0.020
I-II	154 (75.5)	106 (86.2)		
III-IV	50 (24.5)	17 (13.8)		

RGS2, regulator of G protein signaling 2; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2.

When the tumor is classified as 3+ by IHC staining, HER2 status considered positive. When the tumor is classified as 0 or 1+ by IHC staining, HER2 status is considered negative. If the tumor is classified as 2+ by IHC staining, HER2 status requires additional confirmation using FISH (18). In the present study, there were 14 cases of breast cancer which were classified as 2+ by IHC staining, but these samples were not examined using FISH as this protocol was not performed. Consequently, these cases were defined as 'uncertain' (Table I).

RGS2 protein expression was significantly negatively associated with the following clinical pathological characteristics: Histological grade (P=0.006), estrogen receptor (ER) status (P=0.003), progesterone receptor (PR) status (P=0.024) and tumor size (P=0.002). The TCGA BIC-NST sample was divided into two groups according to the expression of RGS2: The low-expression group (n=204, the lowest 35%), and the over-expression group (n=123, the highest 35%).

Table III. Univariate analysis for RGS2 and clinical characteristics in The Cancer Genome Atlas cohort.

Characteristic	HR	95% CI	P-value
RGS2 expression (low vs. high)	0.324	0.154-0.683	0.003
Age (≥ 60 vs. <60 years)	2.014	1.213-3.342	0.007
ER status (positive vs. negative)	1.017	0.567-1.825	0.954
PR status (positive vs. negative)	0.799	0.477-1.338	0.393
HER-2 status (positive vs. negative vs. uncertain)	0.517	0.207-1.295	0.159
Distant metastasis (presence vs. absence)	3.642	1.770-7.495	<0.001
Tumor size (>2 vs. ≤ 2 cm)	1.736	0.876-3.439	0.114
Lymph node metastasis (presence vs. absence)	1.676	0.970-2.894	0.064
Stage (III-IV vs. I-II)	2.277	1.363-3.803	0.002

HR, hazard ratio; 95% CI, 95% confidence interval; RGS2, regulator of G protein signaling 2; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2.

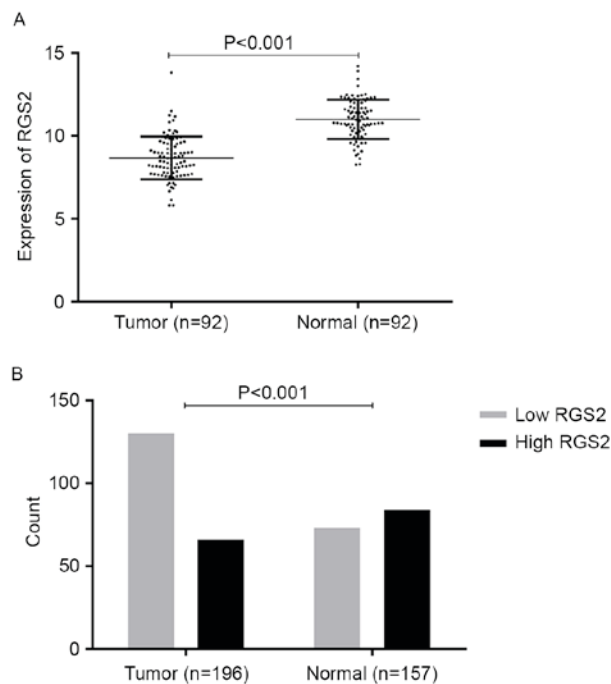


Figure 2. RGS2 expression in BIC-NST tissues and normal breast tissues. (A) In The Cancer Genome Atlas cohort, 92 paired mRNA expression datasets in patients with BIC-NST were analyzed using paired Student's t-test. (B) In the Chongqing Medical University cohort, protein expression levels in 196 tumor tissues and 157 normal tissues were analyzed using the χ^2 test. RGS2, regulator of G protein signaling 2; BIC-NST, breast invasive carcinoma of no special type.

As shown in Table II, age ($P=0.002$), ER status ($P<0.001$), PR status ($P<0.001$) and lymph node metastasis ($P=0.002$) were significantly negatively associated with RGS2 mRNA expression.

Low expression levels of RGS2 are associated with poor survival rate in BIC-NST patients. Follow-up data from the TCGA cohort and the CQMU cohort were assessed, and the association between the RGS2 expression level and overall survival rate were analyzed using the Kaplan-Meier method and log-rank test. The median follow-up time in

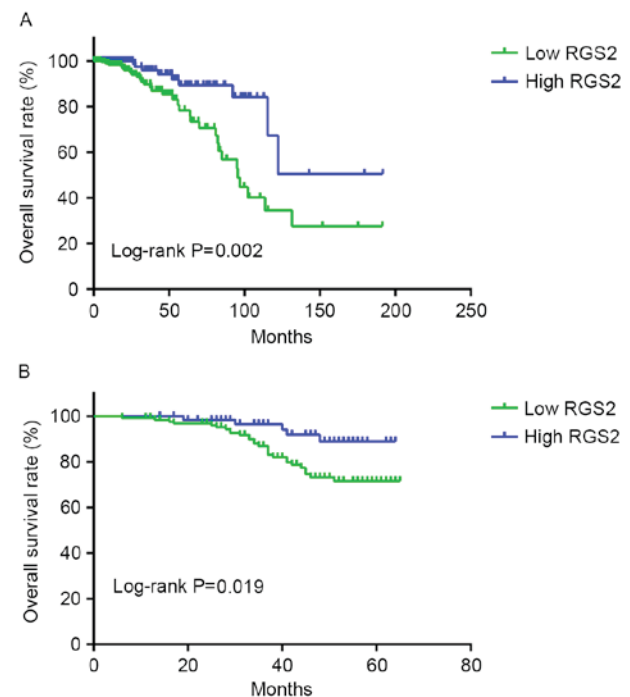


Figure 3. Kaplan-Meier analysis for different mRNA expression levels of RGS2 and overall survival in patients with breast invasive carcinoma of no special type. Analysis of (A) The Cancer Genome Atlas and (B) Chongqing Medical University cohorts showed that patients with low RGS2 expression exhibited a poor overall survival rate compared with those expressing high levels of RGS2.

the TCGA and CQMU cohorts was 27 months (range, 0-238 months) and 44 months (range, 6-65 months), respectively. As shown in Fig. 3, the group with low expression of RGS2 mRNA had a significantly lower overall survival rate ($P=0.002$), as did the group with low expression of RGS2 protein ($P=0.019$). The results of univariate analysis in the TCGA and CQMU cohorts are presented in Tables III and IV, respectively. To evaluate the prognostic value of RGS2, the two groups of data were analyzed independently using multivariate Cox regression analysis. In the TCGA cohort, as demonstrated in Table V, the low expression of

Table IV. Univariate analysis for RGS2 and clinical characteristics in the Chongqing Medical University cohort.

Characteristic	HR	95% CI	P-value
RGS2 expression (low vs. high)	0.337	0.130-0.874	0.025
Age (≥ 60 vs. < 60 years)	4.036	1.777-9.169	0.001
Histological grade ^a (grade 3 vs. 2 vs. 1)	0.816	0.521-1.278	0.375
ER status (positive vs. negative)	0.989	0.470-2.080	0.977
PR status (positive vs. negative)	0.944	0.457-1.949	0.877
HER-2 status (positive vs. negative vs. uncertain)	0.835	0.464-1.503	0.547
Ki-67 (≥ 14 vs. < 14 %)	0.544	0.274-1.080	0.082
Distant metastasis (presence vs. absence)	56.107	8.760-359.364	< 0.001
Tumor size (> 2 vs. ≤ 2 cm)	2.276	1.119-4.629	0.023
Lymph node metastasis (presence vs. absence)	5.898	2.427-14.332	< 0.001
Stage (III-IV vs. I-II)	8.547	4.123-17.718	< 0.001

^aHistological grading was performed according to the World Health Organization Classification of Tumors of the Breast 2012 (4th edition). HR, hazard ratio; CI, confidence interval; RGS2, regulator of G protein signaling 2; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2.

Table V. Multivariate Cox regression analysis for RGS2 and clinical characteristics in The Cancer Genome Atlas cohort.

Characteristic	HR	95% CI	P-value
Age (≥ 60 vs. < 60 years)	2.073	1.013-4.243	0.046
RGS2 expression (low vs. high)	0.435	0.196-0.965	0.041
Stage (III-IV vs. I-II)	2.938	1.390-6.208	0.005
Distant metastasis (presence vs. absence)	0.831	0.266-2.597	0.750

HR, hazard ratio; CI, confidence interval; RGS2, regulator of G protein signaling 2.

Table VI. Multivariable Cox regression analysis for RGS2 and clinical characteristics in the Chongqing Medical University cohort.

Characteristic	HR	95% CI	P-value
Age (≥ 60 vs. < 60 years)	5.832	2.149-15.830	0.001
RGS2 expression (low vs. high)	4.602	1.467-14.432	0.009
Lymph node metastasis (presence vs. absence)	3.993	1.406-11.340	0.009
Stage (III-IV vs. I-II)	5.021	2.201-11.454	< 0.001
Distant metastasis (presence vs. absence)	7.517	0.773-73.114	0.082
Tumor size (> 2 vs. ≤ 2 cm)	1.495	0.686-3.261	0.312

HR, hazard ratio; CI, confidence interval; RGS2, regulator of G protein signaling 2.

RGS2 [hazard ratio (HR), 0.435; 95% confidence interval (CI), 0.196-0.965; $P=0.041$], age (> 60 years) (HR, 2.073; 95% CI, 1.013-4.243; $P=0.046$) and advanced disease stage (III-IV) (HR, 2.938; 95% CI, 1.390-6.208; $P=0.005$) were significantly associated with unfavorable survival in BIC-NST independently. In the CQMU cohort, as shown in Table VI, the low expression of RGS2 (HR, 4.602; 95% CI, 1.467-14.432; $P=0.009$), age (> 60 years) (HR, 5.832; 95% CI, 2.149-15.830; $P=0.001$), advanced stage (III-IV) (HR, 5.021; 95% CI, 2.201-11.454; $P<0.001$) and lymph node metastasis

(HR, 3.993; 95% CI, 1.406-11.340; $P=0.009$) were significantly independently associated with poor patient outcome in BIC-NST.

GSEA of RGS2 mRNA expression in TCGA microarray. To investigate the biological function of RGS2, TCGA microarray data was analyzed using GSEA software. The C2 curated gene set results indicated that the significant gene set enrichment in the RGS2 low expression group contained 'VANTVEER BREAST CANCER ESR1 UP' (enrichment score, 1.8211;

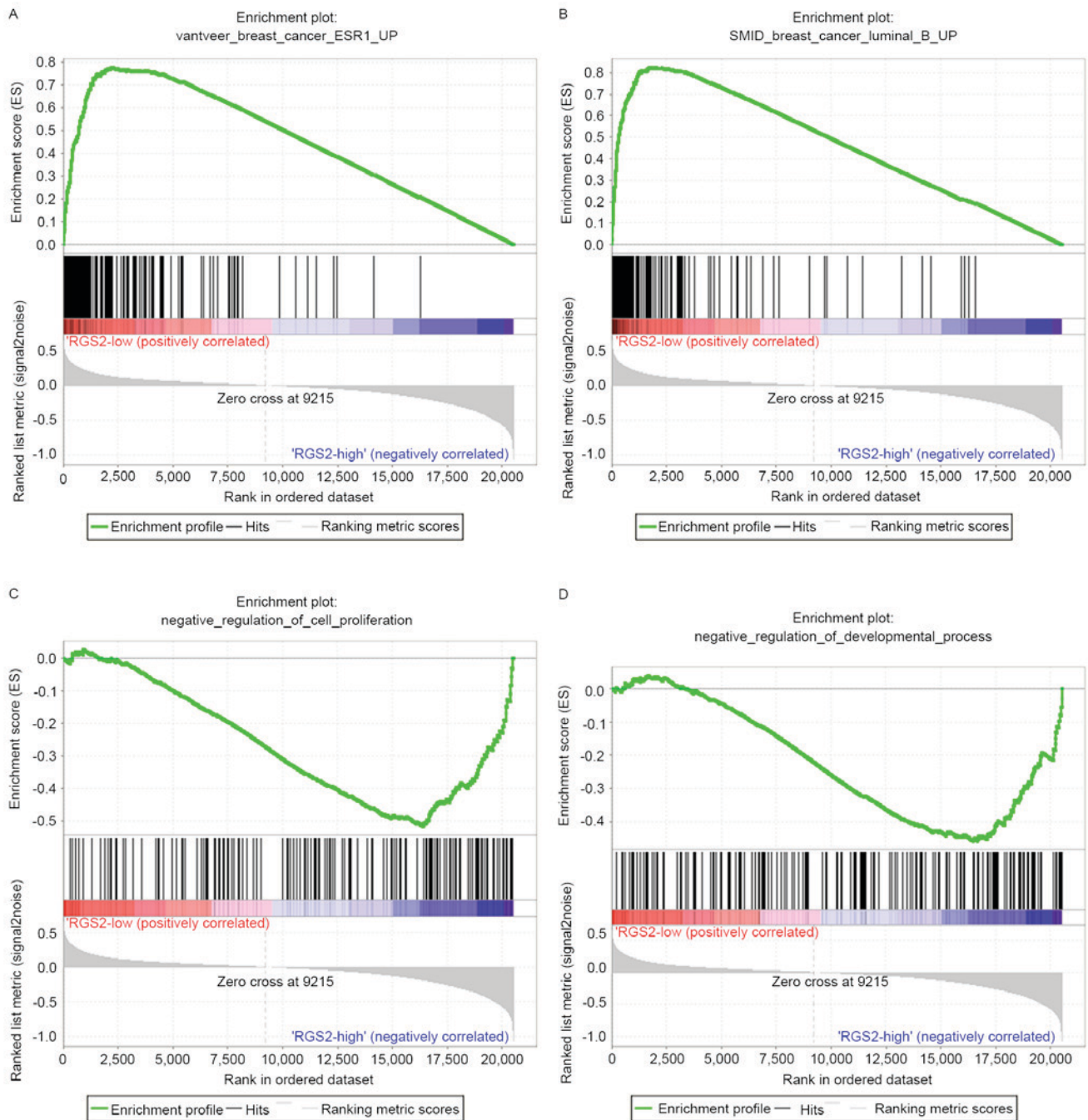


Figure 4. Gene set enrichment analysis of RGS2 expression in The Cancer Genome Atlas cohort. (A) Genes associated with upregulation of estrogen receptor 1 expression in breast cancer were significantly associated with low RGS2 expression. (B) Genes associated with upregulation in luminal B breast cancer were significantly associated with low RGS2 expression. (C) Genes associated with the negative regulation of cell proliferation were significantly associated with high RGS2 expression. (D) Genes associated with negative regulation of developmental process were significantly associated with high RGS2 expression. RGS2, regulator of G protein signaling 2.

nominal $P=0.002$; false discovery rate, 0.1435; Fig. 4A) and 'SMID BREAST CANCER LUMINAL B UP' (enrichment score, 1.8208; nominal $P<0.001$; false discovery rate, 0.1295; Fig. 4B). C5 GO gene set results demonstrated that the significant gene set enrichment in the RGS2 overexpression group included 'NEGATIVE REGULATION OF CELL PROLIFERATION' (enrichment score, -1.8292; nominal $P<0.001$; false discovery rate, 0.2224; Fig. 4C) and 'NEGATIVE REGULATION OF DEVELOPMENTAL PROCESS' (enrichment score, -1.8096; nominal $P<0.001$; false discovery rate, 0.2326; Fig. 4D).

Discussion

The present study was a large-sample comprehensive investigation into protein and mRNA levels, laboratory experiments and data mining; its workflow may become a trend of large-sample retrospective studies. The present study demonstrated that the RGS2 expression level was significantly downregulated in tumor tissues of BIC-NST compared with adjacent normal tissues. Although Smalley *et al* (14) revealed that RGS2 is overexpressed in the majority of breast cancer cases, no

comparison was made with normal tissues. Additionally, the aforementioned study also demonstrated that RGS2 expression was lower in the breast cancer cell line compared with that in the normal cells. The results of the *in vitro* aspect of this study validated the results of the present study. Lyu *et al* (15) demonstrated that RGS2 expression was downregulated in breast cancer MCF-7 cells compared with normal mammary epithelium MCF-10A cells. The results of the present study were consistent with the results of Lyu *et al* (15) on the one hand; on the other hand, the present study expands these results from the cellular level to the level of human tissue, promoting the translation of the study from the laboratory bench to the clinic.

At the mRNA and protein levels, different results were obtained regarding the association between the clinicopathological features and RGS2 expression. There are a number of processes involved in the translation of mRNA to protein, including splicing, cleavage and modification. Thus, the expression of the gene is not always entirely consistent with that of the protein. However, RGS2 mRNA and protein expression was significantly downregulated in the ER-positive and PR-positive groups. GSEA revealed that the gene set enrichment in the RGS2 low-expression group was associated with estrogen receptor 1 and the luminal B subtype of breast cancer (19). According to the PAM50 Gene Expression Assay (20), the luminal B subtype of breast cancer is an ER-positive breast cancer. Therefore, the results of GSEA supported the results of the present study with regards to biological function. ER acts as a nuclear transcription factor when activated by estrogenic hormones and promotes the growth of the normal mammary epithelium (21). ER-positive cells in invasive breast cancer are frequently over-proliferative, resulting in harm to patients (22). The expression of PR is regulated by ER, so PR expression status is used to assess whether the estrogen-ER pathway is intact and functional (21). We hypothesize that the low expression of RGS2 may be a potential factor in the development of BIC-NST. To the best of our knowledge, the present study is the first to reveal that the low expression of RGS2 is associated with the positive status of hormone receptors in BIC-NST.

The Kaplan-Meier survival curve and Cox regression analysis demonstrated that low expression of RGS2 was significantly associated with the poor prognosis of patients with BIC-NST, and that it represents an independent risk factor. Through GSEA, the overexpression of RGS2 was found to be associated with the negative regulation of a number of biological processes. In the present study, the expression of RGS2 was negatively associated with pathological grade. Previous research revealed that there is an association between high pathological grade and poor survival outcome in patients with BIC-NST (23,24); this indirectly indicates that the low expression of RGS2 was associated with unfavorable prognosis in patients with BIC-NST. However, as TCGA data does not contain information regarding pathological grade, there was no way to validate the association between the expression of RGS2 mRNA and pathological grade in patients with BIC-NST. Taken together, these results indicate that RGS2 serves a role in the suppression of oncogene expression. To the best of our knowledge, the present study is also the first to report that low RGS2 expression was associated with poor outcome in patients with BIC-NST.

There are several limitations to the present study. The mechanism that underlies the association between the expression of RGS2 and the hormone receptors remains unknown. The subject of the present study focused solely on BIC-NST, among the various types of invasive breast cancer. This is the largest group and is thus the most likely to be experienced by patients; however, other types of invasive breast cancer exhibit different pathological features and gene profiles (25-29). Thus, the effect of RGS2 in invasive breast cancer requires further investigation.

In summary, the expression of RGS2 is under-regulated in patients with BIC-NST, and its low expression is closely associated with positive hormone receptor status. The low expression of RGS2 is predictive of poor prognosis in patients with BIC-NST, and is an independent risk factor. The results of the present study indicate that RGS2 may be a tumor suppressor gene in BIC-NST. Further study is required to investigate the precise mechanistic pathway underlying the effect of RGS2 in BIC-NST and to expand the study of RGS2 to other subtypes of invasive breast cancer.

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