Evaluation of the TRIP13 level in breast cancer and insights into potential molecular pathways

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Research

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Abstract

Background: TRIP13 is a member belonging to a large AAA+ ATPases protein super family. Emerging evidences had shown that TRIP13 may serve as an oncogene However, the function of TRIP13 in BC has not yet been elucidated.

Methods: By utilizing the multiple database across BC, we presented the expression of TRIP13 in BC tissue and normal control. We then verified the expression of TRIP13 in patients with BC by immunohistochemical (IHC) staining. Kaplan-Meier plots were used to perform the survival analysis. Further, gene ontology (GO) analysis, gene set enrichment analysis (GSEA), and PPI (protein-protein interaction) network were performed to explore the biological function and potential regular pathway of TRIP13 in BC.

Results: The multiple database and immunohistochemical (IHC) showed that higher TRIP13 expression in BC tissue compared to normal tissue. TRIP13 was highly exprssed in lung metastasis lesion compared with primary tumor in our BALB/C mice 4T1 BC models. Kaplan-Meier plots also revealed that high TRIP13 expression correlated to poor survival in patients with BC. Moreover, GSEA analysis revealed that TRIP13 was primarily enriched in the processes of cell division and proliferation. Finally 10 hub genes with a high score of connectivity were filtered from the PPI network, including MAD2L1, CDC20, CDC5L, CDK1, CCNA2, BUB1B, RAD51, SPO11, KIF11 and AURKB.

Conclusion: High TRIP13 expression predicted poor prognosis and contributed tumor growth and metastasis in the BC. Thus, ARL3 may be a prognostic marker and therapeutic target for glioma. TRIP13 may be a favorable biomarker and effective therapeutic target for BC. Keywords: TRIP13; breast cancer; metastasis; bioinformatic analysis, prognosis

Background

Breast cancer (BC) is the most common malignancy among women worldwide and its prevalence is increasing[1, 2]. In China, breast cancer had become the leading cause of mortality in females, and the population is getting younger [3]. It was estimated that 20–30% BC patients will experience distant metastasis[4]. Although recent advances therapy in cancer have been remarkable, but the underlying molecular mechanisms of BC progression have not been fully elucidated. Therefore, searching for new therapeutic targets is urgent task for the treatment of BC.

Thyroid hormone receptor interactor 13 (TRIP13, also called pachytene checkpoint 2 and 16E1BP), was first ascertained that interacts with human papilloma virus E1 proteins, had been found to play a key role in meiotic recombination, chromosome synapsis and spindle assembly checkpoint[5, 6]. Accumulating evidence has shown that TRIP13 protein levels are operational in several human cancers, including ovarian cancer, colorectal cancer, prostate cancer, and Wilms' tumor, etc[7–10]. Aberrant expression of TRIP13 might be related to the occurrence and development of tumor, and the up-regulation of TRIP13

can promote the cell proliferation, migration and increase the resistance to chemotherapeutic drugs[9–11]. However, the function of TRIP13 in BC has not yet been elucidated.

In this study, we used integrated bioinformatics analysis and experiments to identify that TRIP13 may be a novel oncogene in BC. Thus, multiple methods were performed to unravel the role of TRIP13 plays in the developments of BC. Our study purpose was to obtain a better understanding and novel threpy target of BC development and thus to prevent the development of BC.

Materials And Methods

Breast carcinoma datasets

The breast cancer gene expression profiles GSE29431 and GSE42568 were obtained from the publicly available Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). The GSE29431 dataset contained 68 samples, including 12 normal tissues and 54 tumor tissues obtained from BC patients. GSE42568 dataset contained 17 normal tissues and 109 tumor tissues. Furthermore, the gene expression profiling of 1218 BC patients and clinical information were selected from TCGA (http://tcga-data.nci.nih.gov) . Then sort out the matched clinical and expression information for further research.

ONCOMINE database analysis

The transcriptional levels of TRIP13 in BC was examined in the Oncomine database (https://www.oncomine.org) [12], a publicly accessible cancer microarray database, was used to investigate the transcriptional levels of TRIP13 in BC. Fold changes and a *p*-value of 0.05 was considered significant for the comparison of cancer and normal.

UALCAN analysis

UALCAN (http://ualcan.path.uab.edu) includes TCGA RNA-seq datasets and clinical data from 31 cancer types, which allow us to analyze the relationship between the gene expression and clinical characteristics[13]. Here, we evaluated the relationship between TRIP13 and clinical characteristics including cancer stage, pathological type and other criteria through UALCAN database.

Prognostic survival analysis

The Kaplan-Meier plotter (http://kmplot.com/analysis), an public online tool that can be used to assess the impact of 54675 genes on survival in several cancer types including breast cancer, ovarian cancer, liver and gastric cancer[14]. Prognostic value analysis including the overall survival (OS), relapse free survival (RFS), post progression survival (PPS) and distant metastasis free survival (DMFS) were performed using the Kaplan-Meier method. p-value < 0.05 was considered statistically significant.

LinkedOmics analysis (functional enrichment analysis)

The LinkedOmics database (http://www.linkedomics.org) is a web-based data-mining platform for analyzing TCGA cancer datasets[15]. The LinkedOmics database was used to probe genes differentially expressed in correlation with TRIP13 in the TCGA BC cohort. presenting in volcano plots, heat maps. Correlation data results were signed , ranked, and used to GSEA perform analysis of Gene Ontology (GO). GO term can be divided into three parts: biological process (BP), cellular component (CC), and molecular function (MF). The rank criterion was the *p*-value < 0.01, FDR < 0.25, and 1000 simulations were performed.

Gene set enrichment analysis(GSEA)

Gene set enrichment analysis (GSEA) can be used to identify the biological mechanisms pathways according to the expression matrix in order. In order to probe the downstream signaling pathway correlate to TRIP13. The GSE2034 databset was divided into two groups based on the median expression level of TRIP13 expression. Then the GSEA software (v2.1.0, Broad Institute) would calculatedthe gene sets with a high enrichment score with TRIP13. Enrichment results were considered significant at with *p* value < 0.05 and FDR < 0.25.

PPI network construction analysis

String Database (https://string-db.org) is an online public database to gain insights into the functional associations between proteins[16, 17]. The protein-protein interaction (PPI) network of TRIP13 was constructed by the String. Setting the a combined score > 0.7 were considered high confidence. Then, the PPI network was visualized by the Cytoscape software (version 3.5.1).

Patients tissue specimens

20 paraffin-embedded BC tissues and paired normal control were obtained from the Department of Pathology, Third Affiliated Hospital of Southern Medical University. All paraffin-embedded tissues were cut into 2.0 μm slices and transferred to glass slides for furder use. Informed consent of specimens was obtained from each patient before surgery. The study was approved by the ethics committee of The Third Affiliated Hospital of Southern Medical University.

Cell lines and animal models

Breast cancer cells 4T1 were obtained from the American Type Culture Collection (ATCC) and preserved in the Key Laboratory of Molecular Tumor Pathology, Southern Medical University. 4T1 was cultured in RPMI 1640 (GIBCO, USA) supplemented with 10% fetal bovine serum (HyClone, USA) at 37 $^{\circ}$C in a humidified atmosphere of 5% CO_2.

6-week-old female BALB/c mice were housed in a specific pathogen-free environment. All mice were purchased from the Animal Center of Southern Medical University , Guangzhou, China. 10×10^4 4T1 cells were injected into the mammary fat pad of each mouse. After 30 days, all mice were sacrificed and their primary tumor and lung were removed. Collected organs were fixed in formalin solution and then

embedded in paraffin for further research. All study protocol for mice were approved by the Institutional Animal Care and Use Committee of Southern Medical University.

Immunohistochemical analysis

Specimens were embedded in paraffin, and then cut into 2.0 μm slices for immunohistochemical analysis. IHC staining was performed in our tissue sections by the by following protocol. First, the sections were stepwise dewaxed and ethanol to water. To block endogenous peroxidase activity, sections were immersed in the hydrogen peroxide solution. Subsequently, the sections were incubated with anti-TRIP13 antibodies (Proteintech, 19602-1-AP, 1:100 dilution) overnight at 4 °C. Next day, incubating secondary antibody 1 hour and then DAB solution 1 min at room temperature, sections were counterstained with hematoxylin for 5 min and washed by PBS three times for 5 min. Stained tissue sections were evaluated under a light microscope. The results were recorded as a sum of the staining intensity and percentage of positive tumor cells.

Statistical analysis

All statistical analyses were performed by default of the web resources. A two-tailed Student's t-test and χ^2 test were conducted to compare differences between the conditions using SPSS 22.0. Survival curves of TRIP13 expression in BC patients were analyzed using the Kaplan–Meier method. *p*-value < 0.05 was considered statistically significant. (*, *p*-value < 0.05; **, *p*-value < 0.01; ***, *p*-value < 0.001).

Results

TRIP13 is overexpressed in breast cancer

We initially evaluated the TRIP13 transcriptional levels in tumor from the Oncomine database analysis. Data in the Oncomine database revealed that TRIP13 was highly expressed in many tumors including BC,compared to the normal samples (Fig. 1A). Details of 6 sub-datasets analysis showed high transcriptional level of TRIP13 in BC samples (Fig. 1B-G and Table 1). Further, UALCAN analysis indicated that the mRNA expression of TRIP13 was significantly higher in BC patients than the healthy controls in sub-group analysis based on clinical stages, pathological features, menopause status, lymph node stages and ethnicity analysis(Fig. 2). And TRIP13 was higherly correlated with the clinical feature of tumor depth (P < 0.001) in BC patients (Table 1).

Table 1
The situation of each sub-database

Database	Sample size	Sample size of normal	Sample size of BC	Fold change	P value
TCGA breast	450	61	389	4.424	2.05E-48
	137	61	76	3.713	1.44E-22
	97	61	36	2.565	1.49E-13
Curtis breast	176	144	32	4.147	1.12E-14
	1700	144	1556	2.379	9.28E-97
Richardson breast	47	7	40	9.008	2.61E-8

Table 2
Clinical features of BC patients with differential expression of TRIP13 in TCGA.

	TRIP13 expression (%)		
	Low (n = 389)	High (n = 389)	P value
Age			P = 0.01
< 60	192 (45.7)	228 (54.3)	
> 60	197 (55.0)	161 (45.0)	
Tumor depth			P < 0.001
T1 T2 T3 T4 Lymph node - + Distant metastasis	136 (66.0) 201 (43.4) 36 (45.6) 16 (53.3) 189 (50.1) 200 (49.9)	70 (34.0) 262 (56.6) 43 (54.4) 14 (46.7) 188 (49.9) 201 (50.1)	P = 0.943 P = 0.281
- +	380 (49.7) 9 (64.3)	384 (50.3) 5 (35.7)	

To verify the expression of TRIP13 in BC, 20 BC tumor samples and paired normal tissue were obtained and assessed by IHC staining. Results showed that TRIP13 is expressed to a higher proportion and

expression levels in BC tumor tissues(88.9%) compared to the normal tissues (53.3%) (Fig. 3A, D), and this was confirmed by quantification of IHC staining ($p < 0.001$) (Fig. 3E).

In order to access the potential ability of TRIP13 used for diagnosis in breast cancer, ROC curve analysis were performed and the AUC value were calculated. As is shown, the AUC of TRIP13 for diagnosing BC in GSE29431 was 0.9028, and in GSE42568 was 0.9047 (Fig. 4A, B). For BC diagnosis, the sensitivity and specificity of TRIP13 were applicable. Thus, TRIP13 may serve as a potential diagnostic indicator in BC.

TRIP13 expression is higher in lung metastasis lesion in BC than that in primary lesions

In order to determine the role of TRIP13 in BC, we established an animal model of breast cancer. Interestingly, in our six 4T1 BC BALB/C mice models, we found that TRIP13 was expressed to higher levels of lung metastasis tissue samples compared to primary lesions (Fig. 3C, E). It may be suggest that TRIP13 plays an important role in promoting BC metastasis.
High Trip13 Expression Is Poor Survival-associated

To further evaluate the prognostic value of TRIP13 in BC patients, Kaplan-Meier plotter survival analysis was performed. Survival analysis demonstrated that higher TRIP13 expression is significantly associated with shorter OS and RFS in BC (Fig. 4C, D). Similarly, high TRIP13 mRNA expression was also associated with decreased PPS and DMFS (Fig. 4E, F). This result indicated that TRIP13 may serve as a novel prognostic marker in BC.

Go Analysis Of Trip13 Functional Annotations In Bc

The Function enrichment tool of LinkedOmics was used to analyze mRNA microarray data from BC patients in TCGA. As shown in the volcano plot (Fig. 5A), 6179 genes showed significant positive correlations with TRIP13, and 7543 genes presented significant negative correlations. The heat map presented the top 50 significant genes positively and negatively correlated with TRIP13 (Fig. 5B). Significant GO analysis showed that gene sets expressed correlated with TRIP13 in BC were located primarily in the chromosome segregation, DNA replication and spindle organization in terms of biological process (Fig. 5C). In terms of cellular component, chromosomal region was the most significantly enriched term(Fig. 5D). What's more, some molecular function terms, such as catalytic activity, helicase activity and DNA binding were significantly enriched(Fig. 5E). These elaborated the basic and specific functions of TRIP13 in BC.

Functional And Pathway Enrichment Of Trip13 In Bc

To further explore the potential mechanisms of TRIP13 in BC, gene set enrichment analysis (GSEA) was performed to compare the gene microarray profiles of TRIP13 in BC samples. The GSE2034 databset contains 286 BC tissues divided into two groups based on the median expression level of TRIP13. $TRIP13^{low}$ (n = 143) and $TRIP13^{high}$ (n = 143) groups. The top 20 positively enriched gene sets related to TRIP13 expression in BC presented in blue column histogram (Fig. 6A). GSEA analysis showed the

significant functional pathway associate with TRIP13 were mitotic nuclear division, cell cycle and chromosome segregation, which participate primarily in DNA replication and cell proliferation process (Fig. 6B). Additionally, mTORC1, P53 and PI3K-AKT-mTOR signaling pathway may also suggest the metastatic activity of TRIP13 (Fig. 6C–6E).

Ppi (protein-protein Interaction) Network And Module Analysis

Network construction identified 10 hub genes significantly interacted with TRIP13, namely MAD2L1 (Mitotic Arrest Deficient 2 Like 1), CDC20 (Cell Division Cycle 20), CDC5L (Cell Division Cycle 5 Like), CDK1 (Cyclin Dependent Kinase 1e), CCNA2 (Cyclin A2), BUB1B (BUB1 Mitotic Checkpoint Serine/Threonine Kinase B), RAD51 (DNA Repair Protein RAD51 Homolog 1), SPO11 (Meiotic recombination protein SPO11), KIF11 (Kinesin Family Member 11) and AURKB (Aurora Kinase B) (Fig. 6A). Then, Pearson correlation coefficients were calculated by correlation analysis between TRIP13 and hub genes (Fig. 6B – 6I), ranging from 0. 72 (TRIP13 vs. CDC20) to 0.84 (TRIP13 vs. CCNA2). Along with TRIP13, the transcription level of hub genes were also elevated in BC (Figure S1). And these hub genes also often predict a poor prognosis (Figure S2).

Disscussion

TRIP13 is a member belonging to a large AAA + ATPases protein super family. The ATPases are involved in various cellular activities, including protein degradation, DNA replication and chromosome synapsis[6, 18, 19]. Previous studies had shown that TRIP13 plays critical roles in meiotic recombination, DNA repair and spindle assembly checkpoint[20, 21]. TRIP13 was first reported as an oncogene in head and neck cancer that can promote tumor growth and enhance repair of DNA damage[9]. And accumulating researches have demonstrated that TRIP13 plays an oncogenic role in multiple human cancers and usually associated with poor survival[9, 10, 22, 23]. Here, we determined to explore the role of TRIP13 in BC.

According to the analysis of BC datasets from Oncomine and UALCAN, TRIP13 was found to be highly expressed in BC tumor compared with normal control (FIGURE1-2), and this result was validated by the IHC analysis of our BC patient samples (FIGURE 3A). Further, the AUC of TRIP13 in two gene expression profiles were more than 0.7, which were very applicable. Additionally, higher expression level of TRIP13 was significantly associated to poor prognosis (FIGURE 4C-4F). Thus, these results suggest that TRIP13 overexpression occurs in BC and deserves identification as a potential diagnostic and prognostic marker.

Surgery and radiation therapy today can effectively control many cancers at the primary lesion, but the development of metastatic tumor always result a unfavourable prognosis[24]. Britta Weigelt and his colleagues pointed out that the metastatic capacity of tumor is an inherent character, and not a necessarily late, acquired phenotype[25]. Distant metastasis of tumor cells occurs early in many cancer patients. To explore the role of TRIP13 in BC metastasis, we established an animal model of BC. The murine mammary carcinoma cell 4T1 share highly carcinogenic and reliably metastasize to multiple distant organs. The 4T1 mouse model, which to some extent resembles the formation of human breast cancers, allow us to simulate the progress of breast cancer metastasis in patients[26, 27]. Interestingly, s,

compared to primary BC lesions, TRIP13 expression is higher in lung metastatic lesions in our BALB/C mice 4T1 BC model (FIGURE 3C). This result may be suggest that TRIP13 plays an critical role in metastasis during BC progression.

Since TRIP13 plays several critical physiological functions, its dysregulation may bring variation in various signaling pathways. To get more insights into the potential molecular functions of TRIP13 and its interactive network in BC, we performed multiple bioinformatics analysis of BC datasets. Here, we present the homologous changes in gene transcriptome caused by TRIP13 expression through volcano plot and heat maps (FIGURE 5A,B). The variation results a widespread impact of TRIP13 on the BC transcriptome. Results of GO annotation analysis showed that were mostly enriched in chromosome segregation, DNA replication and spindle organization, etc(FIGURE 5 C-E). And these annotations were mainly related to the processes of cell proliferation and differentiation. Previous studies have demonstrated that TRIP13 promoted cell proliferation and invasion via interacting with YWHAQ and YWHAZ in CRC[10]. And TRIP13 mutations predispose to chromosome missegregation and tumorigenesis[8]. These results suggest that elevated TRIP13 in BC cells may contributed to unnatural activation of these processes, leading to the development of BC.

Nest, GSEA was performed to explore the potential molecular mechanisms of TRIP13 driven BC progression and metastasis. The results showed that TRIP13 expression was significantly associated with mitotic nuclear division, cell cycle progression, DNA replication and chromosome segregation. The cell cycle process has four successive phases and each phase is accurately regulated by the checkpoints[28]. During the evolution of cancer cell, this proofreading mechanism is abolished due to dysfunctional checkpoints. Thus, mismatched DNA can then duplicated, leading to a uncontrolled proliferation and malignant phenotype[29]. So we supposed that TRIP13 mediates BC progression through regulating tumor cell cycle signaling. In addition, MTORC1, P53 and PI3K-AKT-mTOR signaling pathway that often activated in diverse cancers were also involved in TRIP13-related BC progression. Evidences showed that mTOR-related mechanisms contribute to the malignant phenotype of multiple cancers. mTORC1 promotes carcinogenesis by driving transcription of oncogenes and inducing angiogenesis, etc. mTORC2 plays a role in activating Akt and other AGC family proteins which promote cell proliferation[30, 31]. Loss of P53 and PI3K/AKT/mTOR activation can promote tumor development and metastasis[32, 33]. These results are consistent with the molecular pathways involved in BC tumorigenesis and metastasis. These fingdings were critical to understand how TRIP13 aberration can result in physiological dysfunction and even cancer such as BC.

For mining regulators potentially responsible for TRIP13 dysregulation, 10 hub genes were filtered from the PPI network, MAD2L1, CDC20, CDC5L, CDK1, CCNA2, BUB1B, RAD51, SPO11, KIF11 and AURKB (Fig. 7A). These hub genes might also similarly interact with each other via various signaling pathways in BC. And their physiological functions are primarily involved in cell cycle control and may act as transcription activatior[34, 35]. CDK1, CCNA2 and AURKB were the members of the of serine/threonine protein kinase family. Progression through the cell cycle is driven by the cyclin-dependent kinase (CDK) family and the cyclins[36–38]. Further, most of the hub genes are associated with poor overall survial

(Supplementary Fig. 1). Now, drugs targeting the cell cycle-regulatory CDK 4 and 6 have been approved for the treatment of breast cancer, and inhibitors targeting other CDKs are in clinical trials recently[39]. These targets can be incorporated into the comprehensive treatment of BC. To gain more accurate correlation results, lots of further experiments are required to verified the current results and elaborate the molecular mechanisms.

In summary, Our studies showed multi-level evidences for the value of TRIP13 and its potential as a novel therapeutic target in BC. Further studies are needed to testify these notions. Finally, these findings would contribute to a better understanding of the role of TRIP13 in BC.

Conclusion

The expression patterns, diagnostic and prognostic values, functional enrichment, and PPI networks of TRIP13 in patients with BC were investigated. Our study demonstrated that TRIP13 is elevated in breast cancer tissues, especially in lung metastasis lesion. And our study provides noval and promising insights for regular network and pathway of TRIP13 in BC. Taken together, these results suggested that TRIP13 could promotes tumor development and may serve as a novel and potential diagnostic or therapeutic target in BC.

Declarations

Ethics committee approval and consent to participate

This study was approved by the ethics committee of The Third Affiliated Hospital of Southern Medical University.

Data availability statement

The datasets analyzed in this study can be accessed in the Oncomine, UALCAN, LinkedOmics and STRING web database. Further access to data are available from the corresponding author upon reasonable request.

Author contributions

LJ, HJ and LX design the study. Study supervision: LL, LX and SJ. Data collection: LJ, HJ, GY and ZL. Data analysis: LJ, SJ, GY and HJ. Results interpretation: LX,, LJ, HJ, GY, and SJ. Drafting manuscript: LJ, HJ, GY, SJ and LX. All authors have read and approved the final submission of this manuscript.

Conflivt of interest

All authors declare no conflict of interest.

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Consent for publication

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Supplementary Material

Supplementary Figure 1 The transcription of hub genes in BC (UALCAN). (A) MAD2L1, (B) CDC20, (C) CDK1, (D) CCNA2, (E) BUB1B, (F) RAD51, (G) KIF11, (H) AURKB and (I) CDC5L in BC tissues compared with normal tissues. The *p* value was set at 0.05.

Supplementary Figure 2 Overall survival analysis of hub genes that coregulated with TRIP13 in patients with BC (Kaplan–Meier plotter). (A) MAD2L1, (B) CDC20, (C) CDK1, (D) CCNA2, (E) BUB1B, (F) RAD51, (G) KIF11, (H) AURKB and (I) SPO11.

Figures

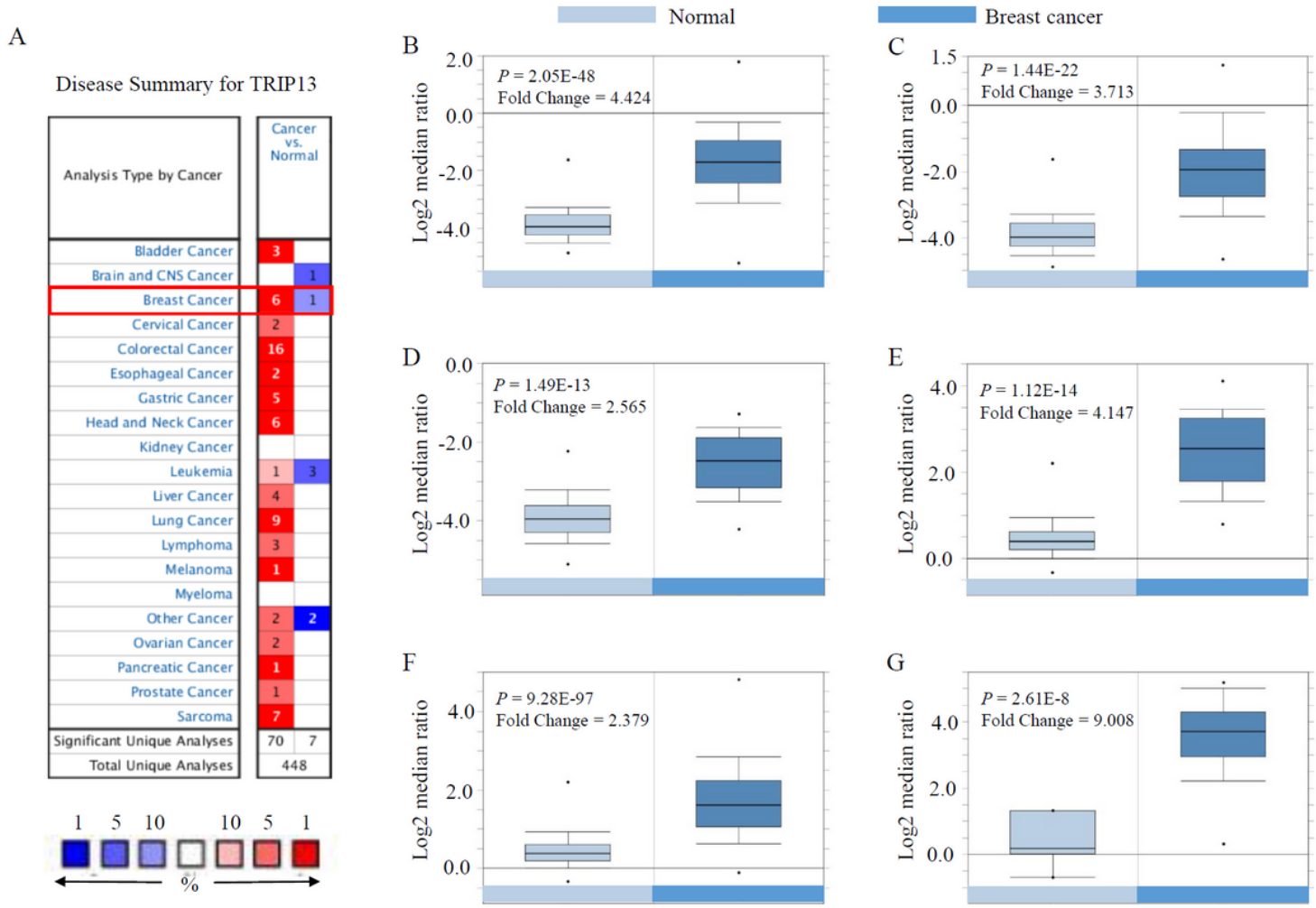

Figure 1

TRIP13 transcription in breast carcinoma (Oncomine). (A). mRNA expression of TRIP13 in different tumors. Graphs show the number of datasets with statistically significant mRNA overexpression (red) or down-regulation (blue) of the target gene (cancer vs. normal tissue and cancer vs. cancer). p value

thresholds were 0.01. (B-G). Box plots showing TRIP13 mRNA levels in sub-database derived from Oncomine.

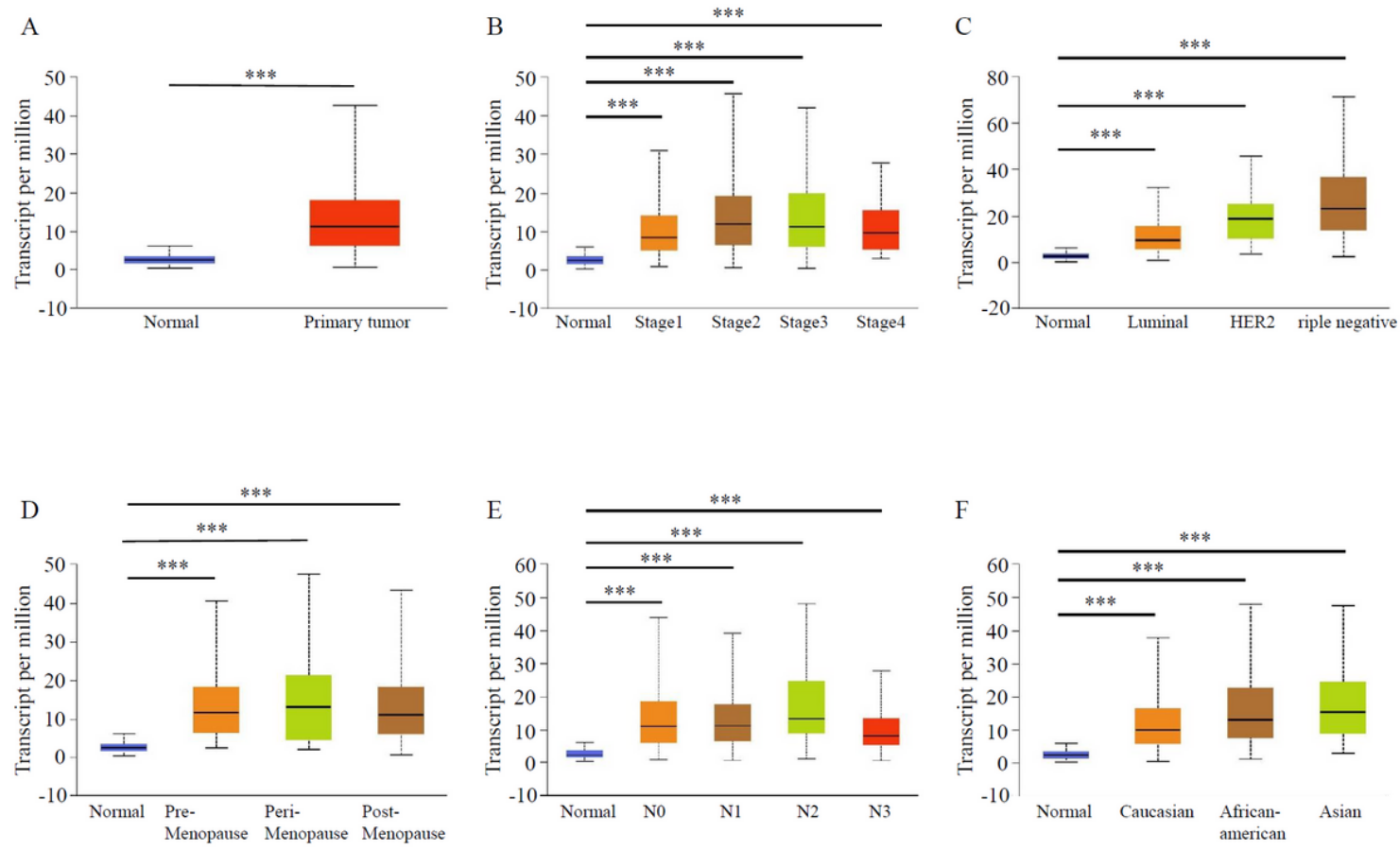

Figure 2

TRIP13 transcription in subgroups of patients with breast carcinoma, stratified based on stage, pathological type and other criteria (UALCAN). (A). Boxplot showing relative expression of TRIP13 in normal and BC samples. (B) Boxplot showing relative expression of TRIP13 in normal individuals or BC patients in stages 1, 2, 3 or 4. (C) Boxplot showing relative expression of TRIP13 in normal individuals or BC patients of luminal, HER2 positive or riple negative. (D) Boxplot showing relative expression of TRIP13 in normal individuals of any age or in BC patients of pre-menopause, peri-menopause and post-menopause. (E) Boxplot showing relative expression of TRIP13 in normal individuals or in BC patients in lymph node stages of N1, N2 or N3. (F) Boxplot showing relative expression of RBM8A in normal individuals of any ethnicity or in BC patients of Caucasian, African-American or Asian ethnicity. Data are mean ± SE. * P < 0.05; **, P < 0.01; ***, P < 0.001.

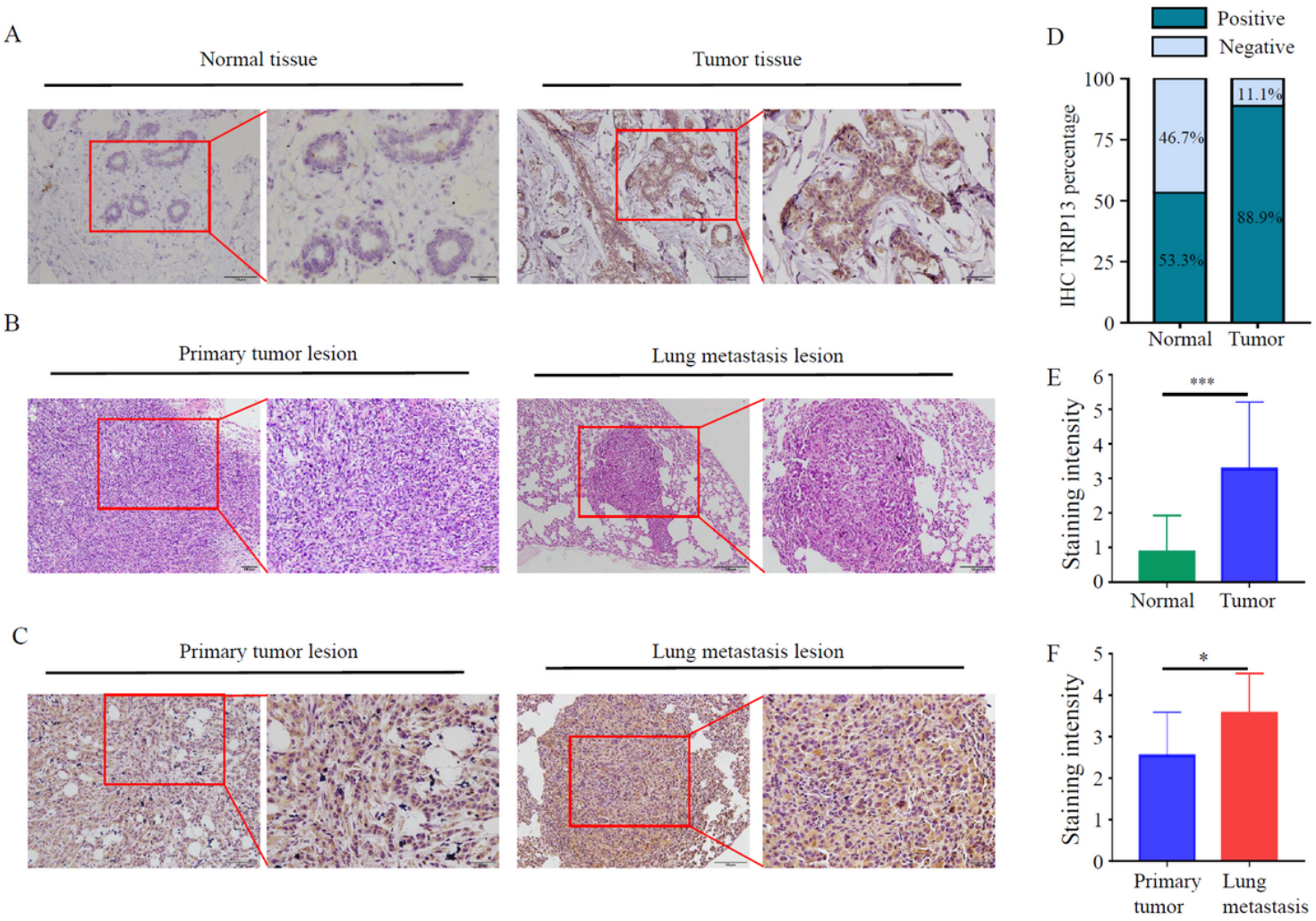

Figure 3

TRIP13 is overexpressed in BC samples, especially in lung metastasis lesion. (A) TRIP13 in adjacent histologically normal tissue and tumor tissue of BC patients. (B) Histopathological diagnosis (H & E staining) of samples from primary lesions and lung metastasis lesion of BALB/C mice 4T1 BC models. (C) TRIP13 in primary lesions and lung metastasis lesion of BALB/C mice 4T1 BC models. (D) Percentage of TRIP13 IHC in BC and matched adjacent normal tissue. (E-F) IHC staining intensity of TRIP13 is shown. The IHC scale bars represent 50 μm and 20 μm. The HE scale bars represent 50 μm and 100 μm, respectively. *P < 0.05; **P < 0.01; ***P < 0.001.

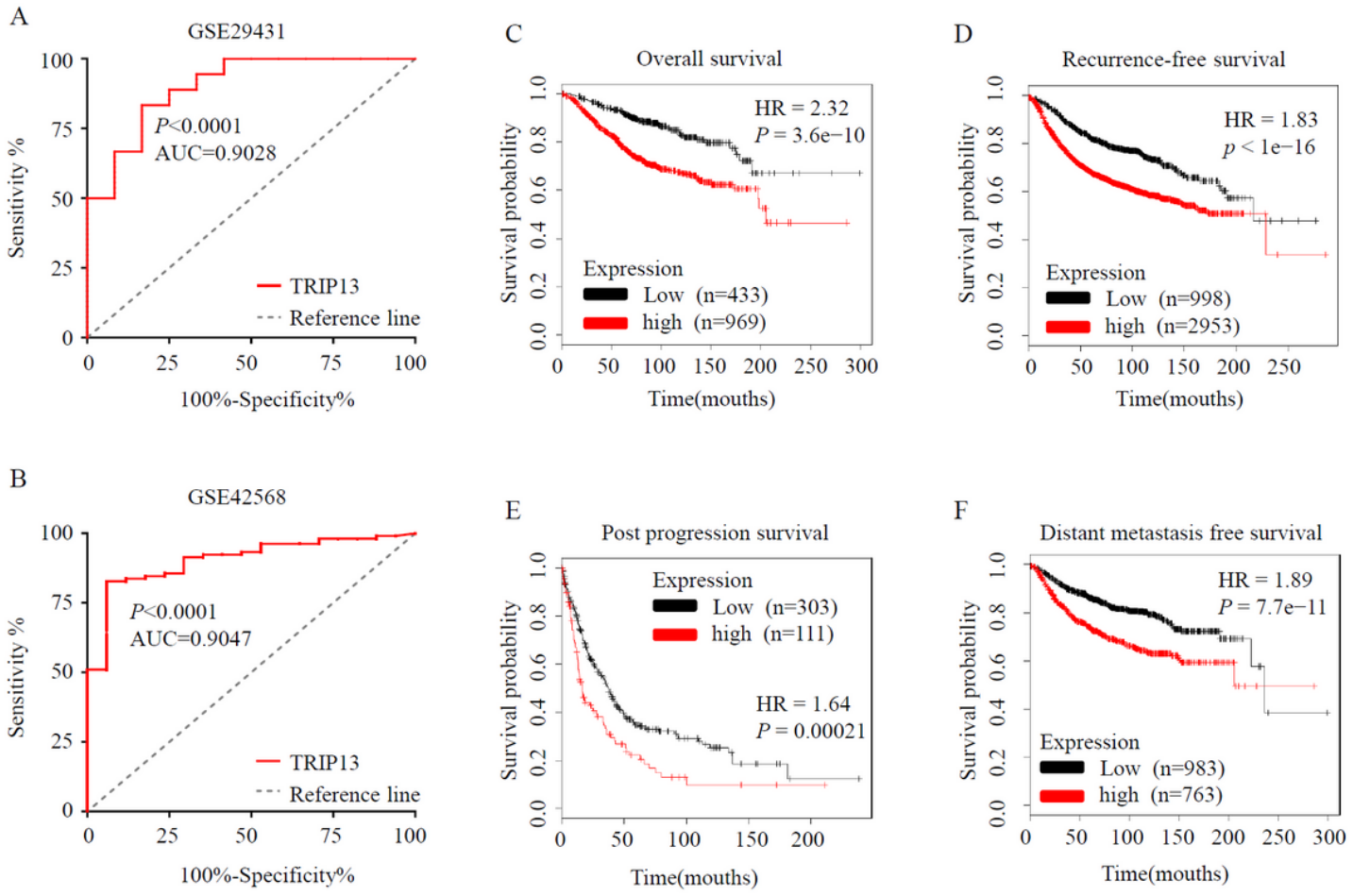

Figure 4

Diagnostic and prognostic value of TRIP13. (A)The AUC of TRIP13 for diagnosing BC in GSE29431 was 0.9028 (P < 0.001) . (B)The AUC of TRIP13 for diagnosing BC in GSE42568 was 0.9047 (P < 0.001). (C) High mRNA levels of TRIP13 were associated with shorter OS in BC patients. (D) High mRNA levels of TRIP13 were associated with shorter RFS in BC patients. (E) High mRNA levels of TRIP13 were associated with shorter PPS in BC patients. (F) High mRNA levels of TRIP13 were associated with shorter DMFS in BC patients.

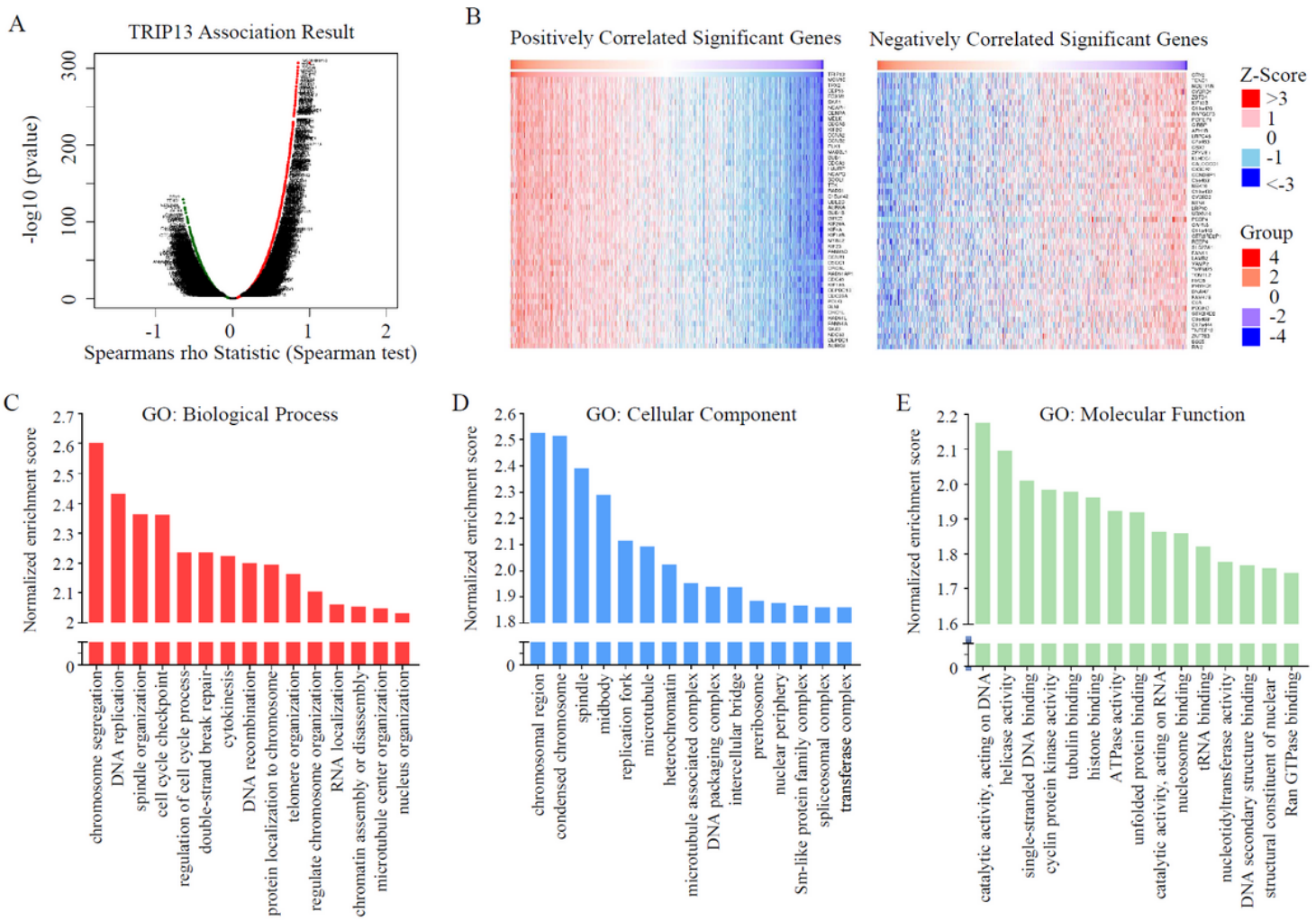

Figure 5

TRIP13 co-expression genes in BC (LinkedOmics) and GO annotations. (A) The global TRIP13 highly correlated genes identified by Pearson test in BC cohort. (B) Heat maps showing top 50 genes positively and negatively correlated with TRIP13 in BC. Red indicates positively correlated genes and blue indicates negatively correlated genes. (C-E) The top 15 GO annotations of TRIP13 in BC, including biological process (C), cellular component (D), and molecular function (E).

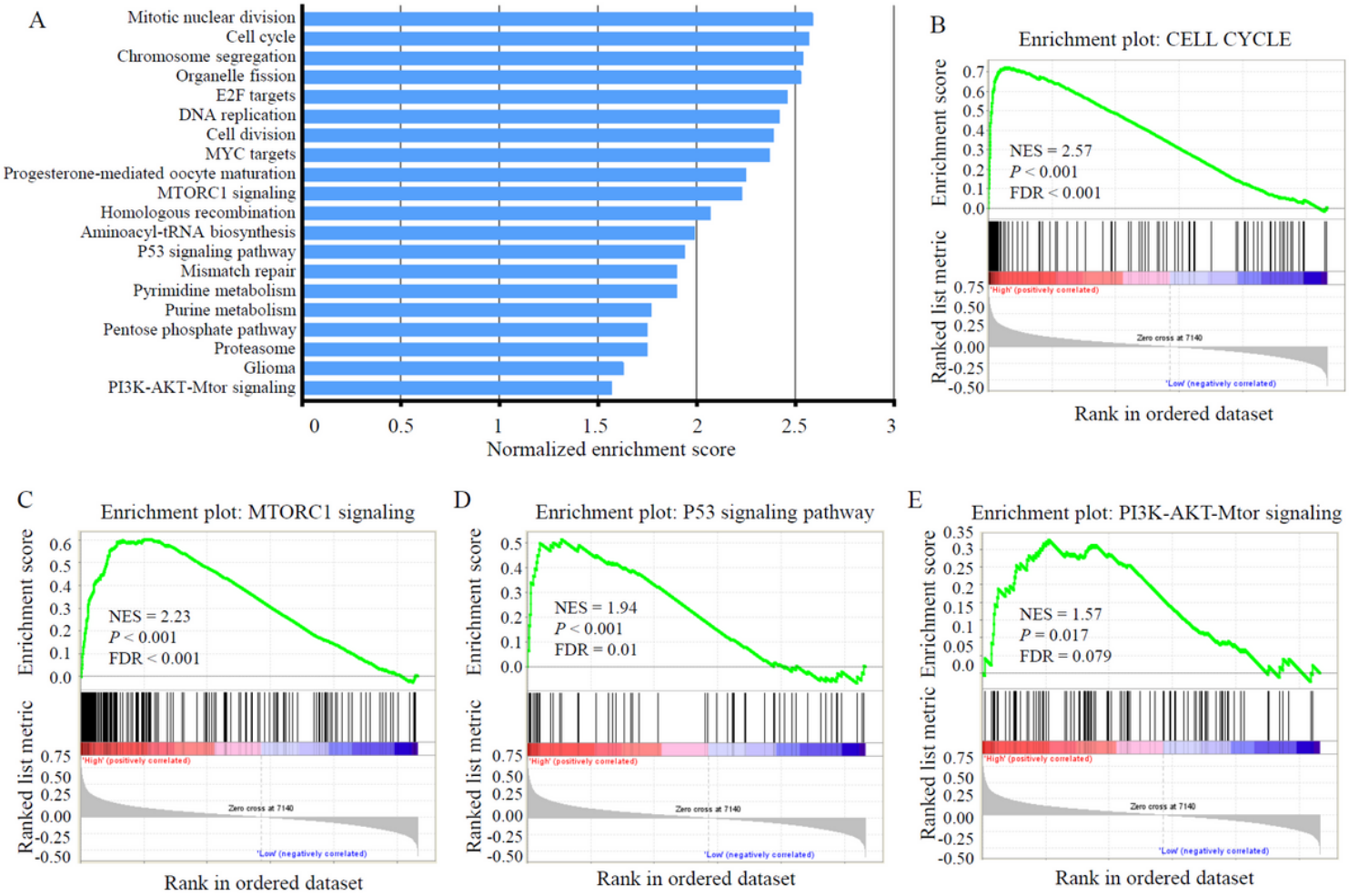

Figure 6

Gene set enrichment analysis reveals potential downstream signaling of TRIP13. (A) the top 20 positively enriched gene sets related to TRIP13. (B) GSEA plots of cell cycle signaling. (C) GSEA plots of MTORC1 signaling. (D) GSEA plots of P53 signaling pathway. (E) GSEA plots of PI3K-AKT-mTOR signaling. NES: normalized enrichment score. Bottom panels show the ranking metrics of each gene. Y-axis: ranking metric values; X-axis: ranks for all genes.

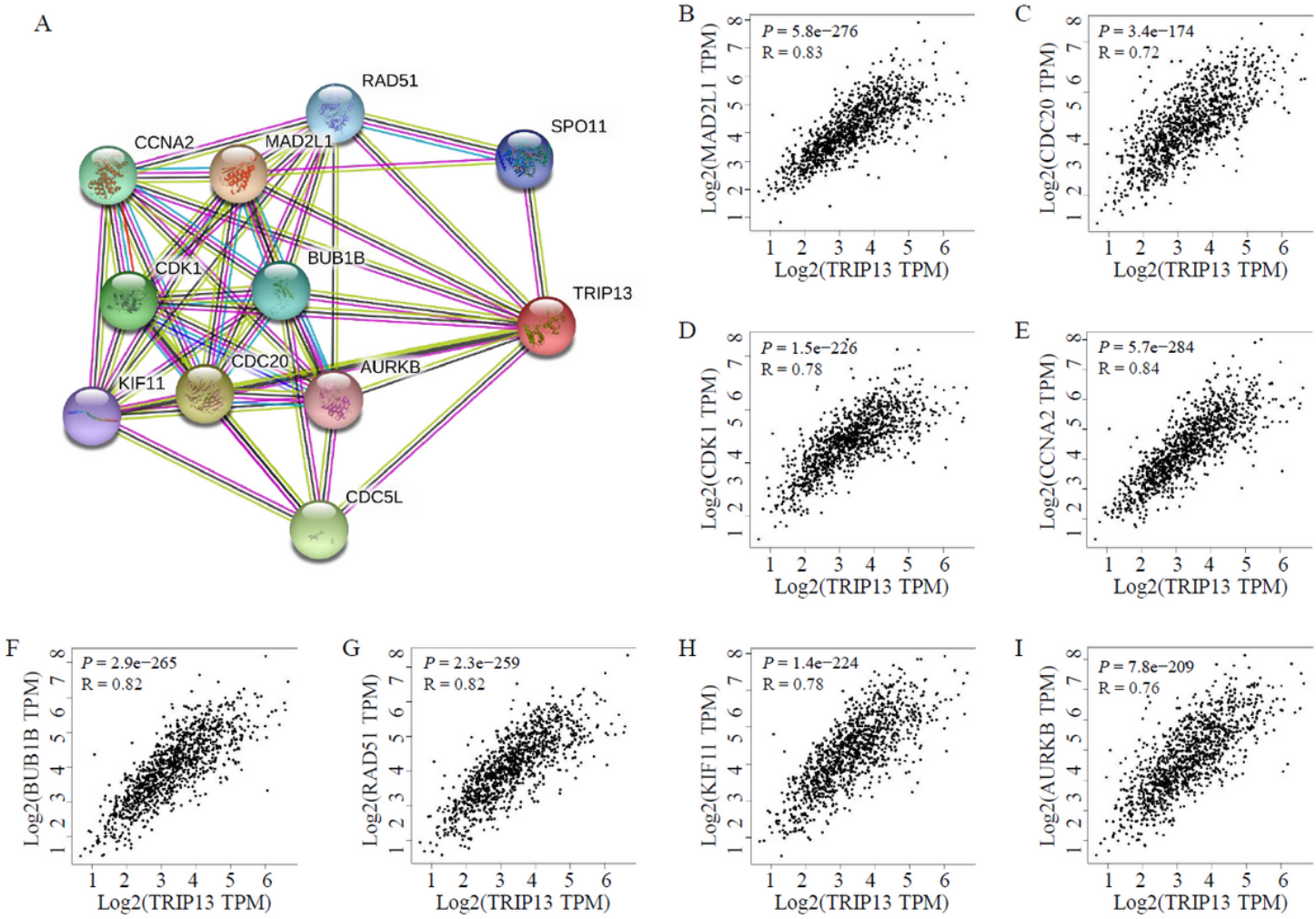

Figure 7

The protein–protein interaction (PPI) of TRIP13. (A)The network of TRIP13 and 10 proteins that significantly interacted with TRIP13 (String). (B-I) Pearson correlation coefficient plots between TRIP13 and hub genes (MAD2L1, CDC20, CDC5L, CDK1, CCNA2, BUB1B, RAD51, SPO11, KIF11 and AURKB).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1.pdf
- SupplementaryFigure2.pdf