Immunoglobulin superfamily member 10 is a novel prognostic biomarker in breast cancer

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Background. Immunoglobulin superfamily member 10 (*IGSF10*), as a member of the immunoglobulin superfamily, is broadly expressed in both gall bladder and ovary. Currently, the role of *IGSF10* in breast cancer remains poorly defined . **Method.** Real-time quantitative polymerase chain reaction (qRT-PCR) and immunohistochemistry were carried out to determine the expression of *IGSF10* in breast cancer cells and tissues. The relationship of *IGSF10* with clinicopathological features and survival outcomes of 700 breast cancer patients in the The Cancer Genome Atlas (TCGA) cohort were analyzed. Gene set enrichment analysis (GSEA) was performed to explore the potential mechanisms and signaling pathways associated with *IGSF10* in breast cancer. **Results.** Our results indicated that *IGSF10* was significantly downregulated in breast cancer compared with normal tissues by using TCGA data, qRT-PCR and immunohistochemistry. The expression of *IGSF10* was significantly associated with age, tumor size, and tumor stage. Moreover, survival analysis showed that low expression of *IGSF10* was significantly associated with poor overall survival (OS) and relapse-free survival (RFS) in breast cancer. Multivariate analysis revealed that *IGSF10* was an independent prognostic factor for OS (HR=1.793, 95% CI: 1.141–2.815, *P*=0.011) and RFS (HR=2.298, 95% CI: 1.317–4.010, *P*=0.003) in breast cancer patients. GSEA demonstrated that *IGSF10* was significantly associated with gene signatures involving DNA repair, cell cycle, glycolysis, mTORC1 signaling pathway, and PI3K/Akt/mTOR signaling pathway. **Conclusion.** This study, for the first time, revealed a clear relationship between *IGSF10* and the tumorigenesis of breast cancer . Further studies are required to gain more insights into the biological role of *IGSF10* in breast cancer.

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Abstract

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Method. Real-time quantitative polymerase chain reaction (qRT-PCR) and immunohistochemistry were carried out to determine the expression of *IGSF10* in breast cancer cells and tissues. The relationship of *IGSF10* with clinicopathological features and survival outcomes of 700 breast cancer patients in the The Cancer Genome Atlas (TCGA) cohort were analyzed. Gene set enrichment analysis (GSEA) was performed to explore the potential mechanisms and signaling pathways associated with *IGSF10* in breast cancer.

Results. Our results indicated that *IGSF10* was significantly downregulated in breast cancer compared with normal tissues by using TCGA data, qRT-PCR and immunohistochemistry. The expression of *IGSF10* was significantly associated with age, tumor size, and tumor stage. Moreover, survival analysis showed that low expression of *IGSF10* was significantly associated with poor overall survival (OS) and relapse-free survival (RFS) in breast cancer. Multivariate analysis revealed that *IGSF10* was an independent prognostic factor for OS (HR=1.793, 95% CI: 1.141–2.815, *P*=0.011) and RFS (HR=2.298, 95% CI: 1.317–4.010, *P*=0.003) in breast cancer patients. GSEA demonstrated that *IGSF10* was significantly associated with gene signatures involving DNA repair, cell cycle, glycolysis, mTORC1 signaling pathway, and PI3K/Akt/mTOR signaling pathway.

Conclusion. This study, for the first time, revealed a clear relationship between *IGSF10* and the tumorigenesis of breast cancer. Further studies are required to gain more insights into the biological role of *IGSF10* in breast cancer.

Introduction

Breast cancer is a common malignancy that seriously threatens the health of women. Approximately 2.1 million newly female breast cancer cases were diagnosed worldwide in 2018,

accounting for one quarter of female cancer cases (Bray et al., 2018). As a heterogeneous disease, a complex interaction between genetic and environmental factors results in the initiation and development of breast cancer (Yang et al., 2019). Despite continuous advances made in surgical techniques, biological drugs and targeted therapies, breast cancer remains an arduous clinical problem (Woolston, 2015). Therefore, identifying breast cancer biomarkers is crucial for understanding tumorigenesis and accurate cancer prognosis, in that biomarkers may assist clinical diagnosis and may serve as potential tumor therapeutic targets in breast cancer (Costa-Pinheiro, Montezuma, Henrique, & Jerónimo, 2015; JR, MA, JT, & medicine, 2012; Qiao et al., 2019).

Immunoglobulin superfamily member 10 (*IGSF10*) is a gene involved in cell differentiation and developmental processes (Thutkawkorapin et al., 2016). The gene encoding *IGSF10* maps to chromosome 3, which contains over 1,100 genes that include a chemokine receptor gene cluster as well as a variety of human cancer related loci. Previous study has revealed that mutations in *IGSF10* delay human puberty (Howard, 2018; Howard et al., 2016). Moreover, mutations in *IGSF10* appear to cause dysregulation of gonadotropin-releasing hormone (GnRH) neuronal migration during embryonic development. Increasing evidence supports that *IGSF10* deficiency may lead to transient GnRH deficiency and reversible congenital hypogonadotropic hypogonadism (Amato et al., 2019; Howard, 2018). In addition, it has been reported that the mutation of *IGSF10* likely contributing to increase cancer risk in rectal and gastric cancer (Thutkawkorapin et al., 2016). Daino and colleagues found that *IGSF10* is significantly down-regulated in alpha-radiation-induced rat osteosarcoma (Daino, Ugolin, Altmeyer-Morel, Guilly, & Chevillard, 2009). The mRNA expression of *IGSF10* was lower in lung cancer than normal tissues and the decreased expression of *IGSF10* was associated with poor prognosis for lung cancer patients (Ling et al., 2020). However, the biological roles of *IGSF10* in the majority of human cancers, especially in breast cancer, have not been investigated and remain largely unknown.

In the present study, we first investigated the *IGSF10* expression based on the public data from The Cancer Genome Atlas (TCGA) database and collected breast cancer tissues. The prognostic significance of *IGSF10* for breast cancer patients was also determined. In addition, potential

mechanisms and signaling pathways, through which *IGSF10* may mediate the progression of breast cancer, were explored by gene set enrichment analysis (GSEA).

Materials & Methods

Cell culture

Human breast cancer cell lines (MDA-MB-231, MCF-7, BT-549, ZR-75-30, SKBR-3, and T47D) were purchased from the American Type Culture Collection (ATCC Manassas, VA, USA) and were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), 1% penicillin and streptomycin (Gibco). MCF10A cells were cultured in a mixture of DME-F12 medium containing epidermal growth factor (20 ng/ml, Sigma-Aldrich, St Louis, MO, USA), cholera toxin (100 ng/ml, Sigma-Aldrich), insulin (0.01 mg/ml, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), hydrocortisone (500 ng/ml, Sigma-Aldrich) and 5% of FBS. All cell lines were cultured in a humidified incubator at 37 °C with 5% CO_2.

Patients and tissue samples of breast cancer

TCGA data was collected as previously described in (Qiu, Li, Zeng, Guan, & Li, 2018). Breast cancer patients only with complete RNA-seq data and fully clinical information including tumor size, lymph node status, TNM stage, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2), and follow-up information were included. In total, we included 700 cases of breast cancer patients in the present study.

Tissue samples of breast cancer were collected as previously described in (Li et al., 2018). Specifically, we collected the tumor and adjacent normal tissues from breast cancer patients treated by primary surgery between 2014 and 2016 at the First Affiliated Hospital of Chongqing Medical University for RT-qPCR and IHC. All specimens were immediately snap-frozen in liquid nitrogen and stored at 80℃ until used for RNA isolation and IHC. This study was approval by the Institutional Ethics Committees of the First Affiliated Hospital of Chongqing Medical University. All patients received an explanation of the study aims and signed informed consent.

RNA isolation and RT-qPCR

As previously described in (Qiu et al., 2018), total RNA was isolated using Trizol reagent (Life Technologics Inc. USA) following manufacture introduction. RNA concentration was determined by spectrophometry with a NanoDrop 2000 (Thermo Scientific, Wilmington, DE, USA). A total of 1 mg RNA was subjected to reverse transcription to cDNA by Reverse Transcription Kit (Promega Inc. USA). RT-qPCR was carried out by ABI 7500 Real-Time PCR System (Applied Biosystems) using Maxima SYBR Green/ROX qPCR Master Mix (MBI Fermentas, St. Leon-Rot, Germany). Thermal cycling conditions were 95℃ for 30s, followed by 5s at 95℃, 1 min at 60℃ for 40 cycles. Relative quantification mRNA expression levels of *IGSF10* were standardized to GAPDH. Primer pairs used were as follows:

Forward primer (*IGSF10*): 5'-TTGGAGTTTGCCTGATGGAAC-3';

Reverse primer (*IGSF10*): 5'-CGCTACCCCAACTTTGTTGAAG-3';

Forward primer (GAPDH): 5'-GGAGCGAGATCCCTCCAAAAT-3';

Reverse primer (GAPDH): 5'-GGCTGTTGTCATACTTCTCATGG-3'.

IHC

The process of IHC was previously described in (Li et al., 2018). Specifically, all specimens were formalin-fixed, paraffin-embedded and sliced into 4 μm sections, which were mounted onto glass slides. The slides were deparaffinized and rehydrated with xylene and a graded ethanol series for 30 min, respectively. Antigen retrieval was performed by microwaving the samples for 20 min in sodium citrate-hydrochloric acid buffer solution at 95°C. After cooling to room temperature, endogenous horseradish peroxidase activity was blocked with 3% hydrogen peroxide. The sections were then washed with phosphate buffered saline (PBS) three times, and then blocked with normal goat serum. Antibodies were added to the sections and incubated overnight at 4°C. After washing with PBS three times, sections were incubated with biotinylated goat anti-mouse IgG, washed, and incubated with streptavidin-biotin-conjugated horseradish peroxidase (HRP). After washing, signals were visualized with diaminobenzidine, and sections were counterstained with

hematoxylin. The anti-IGSF10 rabbit polyclonal antibody (ab197671, 1:100, Abcam) was used. IHC scores were determined according to the staining intensity (0: negative; 1: weak; 2: moderate; 3: strong) and the percentage of positive cells (0: < 5%; 1: 5%–25%; 2: 26%–50%; 3: 51%–75%; 4: > 75%). An overall score was derived by multiplying the intensity and percentage scores.

Bioinformatics analyses

UALCAN is a web portal to perform in-depth analyses of gene expression in various tumor subgroups based on individual clinicopathologic features from the TCGA database. The mRNA expression level of *IGSF10* in different subtypes of breast cancer was evaluated using UALCAN (Chandrashekar et al., 2017).

The mRNA expression of *IGSF10* in different breast cancer datasets was analyzed using Oncomine gene expression array datasets (Rhodes et al.). The cut-off *P*-value and fold change were defined as 0.01 and 2, respectively.

The relationship between the *IGSF10* expression and prognosis in different breast cancer molecular subtypes was analyzed using a Kaplan-Meier plotter (http://kmplot.com/analysis/) (A et al., 2016). The Affymetrix probe set IDs of *IGSF10* is shown as: 230670 at. Patients were divided into high and low expression groups by auto selected cut-off value of the mRNA expression level of *IGSF10*.

GSEA

We performed GSEA (http://software.broadinstitute.org/gsea) to evaluate the association between expression of *IGSF10* and biological processes/pathways following the instructions of the user guide. GESA was performed using a microarray dataset (GSE1456) containing 159 breast cancer samples. Normalized enrichment score (NES) was acquired by analyzing with permutations for 1000 times. A gene set is considered as significantly enriched when a normal *P*-value less than 0.05 and false discovery rate (FDR) less than 0.25.

Additional statistical analyses

All statistical analyses were performed with SPSS version 23.0 software and Graphpad 8.0. The Kaplan-Meier curve was conducted to assess the association between the expression of *IGSF10* and survival time of breast cancer patients. Multivariate analyses for prognosis were carried out by using a Cox proportional hazard regression model. A Student's t test was used for comparison between two groups. Significance was defined as a *P*-value less than 0.05.

Results

1. The expression of *IGSF10* in breast cancer

We first investigated the mRNA expression difference of *IGSF10* through TCGA database. As shown in Figure 1A, the mRNA expression level of *IGSF10* was significantly down-regulated in breast cancer tissues compared with normal tissues. In Oncomine database, the mRNA expression of *IGSF10* was much lower in breast cancer than normal tissues within datasets including TCGA Breast, Karnoub Breast (Karnoub et al., 2007), Zhao Breast (Zhao et al., 2004), Richardson Breast 2 (Richardson et al., 2006), and Finak Breast (Finak et al., 2008) (Table 1). RT-qPCR was performed in 21 paired breast cancer and normal tissues to confirm the expression of *IGSF10* in the database. Consistently, the result showed that the expression of *IGSF10* significantly decreased in breast cancer compared with normal tissues (Figure 1B). IHC was further performed to evaluate the protein expression of *IGSF10* in breast cancer and corresponding normal tissues. The result showed that the protein expression level of *IGSF10* staining was lower in tumor tissues compared with adjacent normal tissues (Figures 1C and 1D). Finally, the mRNA expression levels of *IGSF10* was examined in breast cell lines and founded that the *IGSF10* mRNA expression was significantly decreased in cancer cell lines compared to normal mammary epithelial cell line MCF10A (Figure 1E).

2. The prognostic value of *IGSF10* in breast cancer

To further analyze the clinical correlation and prognostic significance of *IGSF10* in breast cancer, we analyzed the TCGA cohort including 700 breast cancer patients. The results showed that the expression of *IGSF10* was significantly associated with to age ($P<0.001$), tumor size

(*P*=0.003), and TMN stage (*P* =0.03) (Table 2). The association of *IGSF10* expression with overall survival (OS) and relapse-free survival (RFS) were evaluated using Kaplan-Meier survival curves. Patients with high expression level of *IGSF10* were significantly associated with better OS (Figure 2A) and RFS (Figure 2B) than those with low levels of *IGSF10* (*P*<0.05). Subsequently, we used the UALCAN database to further evaluate the prognostic value of *IGSF10* by stratifying patients to different molecular subtypes. Decreased mRNA levels of *IGSF10* were observed in luminal, HER2 positive, and triple-negative breast cancers compared with normal samples (Figure 3A). By using Kaplan-Meier plotter, we found that low expression of *IGSF10* was significantly associated with a worse OS in Basal (hazard radio (HR) =0.44, 95% CI: 0.22–0.86, *P*=0.013), Luminal A (HR=0.47 95% CI: 0.25–0.88, *P*=0.017), and HER2+ (HR=0.28, 95% CI: 0.09–0.81, *P*= 0.012) breast cancer subtypes (Figures 3B–3E). However, there was no significant association between *IGSF10* expression and OS in Luminal B patients (HR=0.61, 95% CI: 0.3–1.23, *P*=0.17, Figure 3D). Multivariate Cox regression analysis showed that *IGSF10* was an independent prognostic factor for OS (HR=1.793, 95% CI: 1.141–2.815, *P*=0.011) and RFS (HR=2.298, 95% CI: 1.317–4.010, *P*=0.003) (Table 3).

3. Potential biological roles and signaling pathways related to *IGSF10*

Potential mechanisms and signaling pathways that may be associated with *IGSF10* in regulating the development of breast cancer were explored using GSEA. Patients were divided into *IGSF10*-high expression group (n=80) and *IGSF10*-low expression group (n=79) based on the median value of *IGSF10* mRNA expression level in the microarray dataset (GSE1456). We found that nine gene sets were enriched in the *IGSF10*-high expression group (Figure 4A). Among the nine gene sets, several cancer-related biological processes including DNA repair (HALLMARK_DNA_REPAIR), cell cycle (HALLMARK_G2M_CKECKPOINT), and glycolysis (HALLMARK_GLYCOLYSIS) were highly enriched in *IGSF10*-high expression group (Figure 4B). PI3K/Akt/mTOR signaling pathway and mTORC1 signaling pathway were also enriched in *IGSF10*-high expression group (Figures 4C and 4D).

Discussion

In recent years, numerous molecular prognostic biomarkers have been developed and validated in cancers, including breast cancer (Nicolini, Ferrari, & Duffy, 2018). In the present study, we described a novel role of *IGSF10* as a tumor suppressor gene in the progression of breast cancer and provided a possible mechanism for its involvement in the development of breast cancer. We demonstrated that the expression of *IGSF10* was significantly downregulated in breast cancer at both mRNA and protein levels and showed prognostic value for breast cancer patients. In addition, the expression of *IGSF10* was closely correlated with age, tumor size, and TMN stage. Multivariate analysis revealed that *IGSF10* was an independent prognostic factor for breast cancer patients. Accordingly, *IGSF10* may serve as a tumor suppressor in breast cancer with potentiality to be targeted in anticancer therapy.

Previous studies have suggested that *IGSF10* may play an important role in tumorigenesis. Ling and colleagues have proved that knockout of *IGSF10* promoted the development of lung cancer and activated the integrin-β1/FAK pathway (Ling et al., 2020). In one family with gastric and colorectal cancer, Thutkawkorapin *et al*. identified 12 new non-synonymous single nucleotide variants, which might contribute to the increased cancer risk, in 12 different genes, including *IGSF10* (Thutkawkorapin et al., 2016). Chang *et al*. identified new mutations in endometrial cancer patients in Taiwan by whole exome sequencing and found that *IGSF10*, as a passenger gene, may be associated with endometrial cancer (Chang, Huang, Yeh, & Chang, 2017). However, to our knowledge, no studies have reported the possible functions and mechanisms of *IGSF10* in breast cancer.

During the past decade, growing evidence has shown clear correlations between the immunoglobulin superfamily members and human diseases. For instance, studies have reported that the loss-of-function mutations in *IGSF1* resulted in an X-linked syndrome of central hypothyroidism and testicular enlargement. *IGSF1* mutations in male patients lead to a late increase in testosterone levels (Howard et al., 2016; Roche et al., 2018; Sun et al., 2012). Significant better overall survival was observed for pediatric mixed-lineage leukemia-rearranged

acute monoblastic leukemia with f(9; 11) (p22; q23) patients with high *IGSF4* expression compared with low *IGSF4* expression (Kuipers et al., 2011). Wang *et al*. proved that *IGSF8* promoted melanoma cell growth and metastasis by negatively regulating TGF-β signaling pathway (Wang, Sharma, Knoblich, Granter, & Hemler, 2015).

In this study, potential biological roles and signaling pathways that may be related to *IGSF10* in breast cancer were analyzed by GSEA. Several biological processes, including DNA repair, cell cycle, and glycolysis, were found to be associated with *IGSF10*. Among these processes, genomic integrity can be maintained through DNA repair pathways. Dysregulation of DNA repair leads to the changes in the genome and causes physiological changes in cells that drive tumor initiation (Jeggo, Pearl, & Carr, 2016; Khanna, 2015; Mouw, Goldberg, Konstantinopoulos, & D'Andrea, 2017). Cell cycle regulates tumor growth and glycolysis modulates tumor microenvironment heterogeneity which is the main cause for cancer survival, progression, metastasis and drug resistance (Jahagirdar et al., 2018). We found that two signaling pathways, mTORC1 and PI3K/Akt/mTOR, were significantly associated with *IGSF10.* In human malignances, mTORC1 and PI3K/Akt/mTOR signaling pathways are usually abnormally activated and promote the development of malignances (Hare & Harvey, 2017). Previous studies have indicated that mTORC1 promotes cell growth by activating key anabolic processes and dysregulation of mTORC1 is the basis of many human cancers (Ben-Sahra & Manning, 2017; Keppler-Noreuil, Parker, Darling, & Martinez-Agosto, 2016). PI3K/Akt/mTOR pathway are associated with various biological processes in breast cancer such as tumorigenesis, cellular transformation, tumor progression, and drug resistance (Guerrero-Zotano, Mayer, & Arteaga, 2016). Therefore, we speculated that *IGSF10* might mechanically regulate the cell growth of breast cancer via mTORC1 signaling pathway and PI3K/Akt/mTOR pathway. However, further studies are needed to elucidate the detailed mechanisms by which *IGSF10* modulates the mTORC1 and PI3K/Akt/mTOR signaling pathways.

Conclusions

In conclusion, we found that *IGSF10* is down-regulated in breast cancer. Low expression of *IGSF10* was significantly associated with poor survival outcomes in breast cancer patients. More importantly, multivariate analysis further revealed that *IGSF10* was an independent prognostic factor for breast cancer patients. In addition, GSEA revealed that *IGSF10* was significantly associated with DNA repair, cell cycle, glycolysis, mTORC1 signaling pathway and PI3K/Akt/mTOR signaling pathways. Together, *IGSF10* may serve as a potential therapeutic target for breast cancer. Future studies are warranted to confirm these findings.

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412

Figure 1

IGSF10 is downregulated in breast cancer.

Figure 1: (A) *IGSF10* is downregulated in BC tissues compared with adjacent normal tissues in TCGA dataset. **(B)** qRT-PCR assay was used to evaluate the mRNA expression levels of *IGSF10* in 21 cases of breast cancer tissues and matched adjacent normal tissues. (C) Representative IHC images of *IGSF10* protein expression in BC and adjacent normal tissues. (D) Histogram shows the IHC score of *IGSF10* in 31 BC cases (IHC score: 3.12 ± 2.04) and 31 normal samples (IHC score: 4.45 ± 2.13). Data are presented as Mean ± SD, unpaired t-test, $*P < 0.05$. (E) qRT-PCR was performed to detect *IGSF10* expression in these cells vs. MCF-10A, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

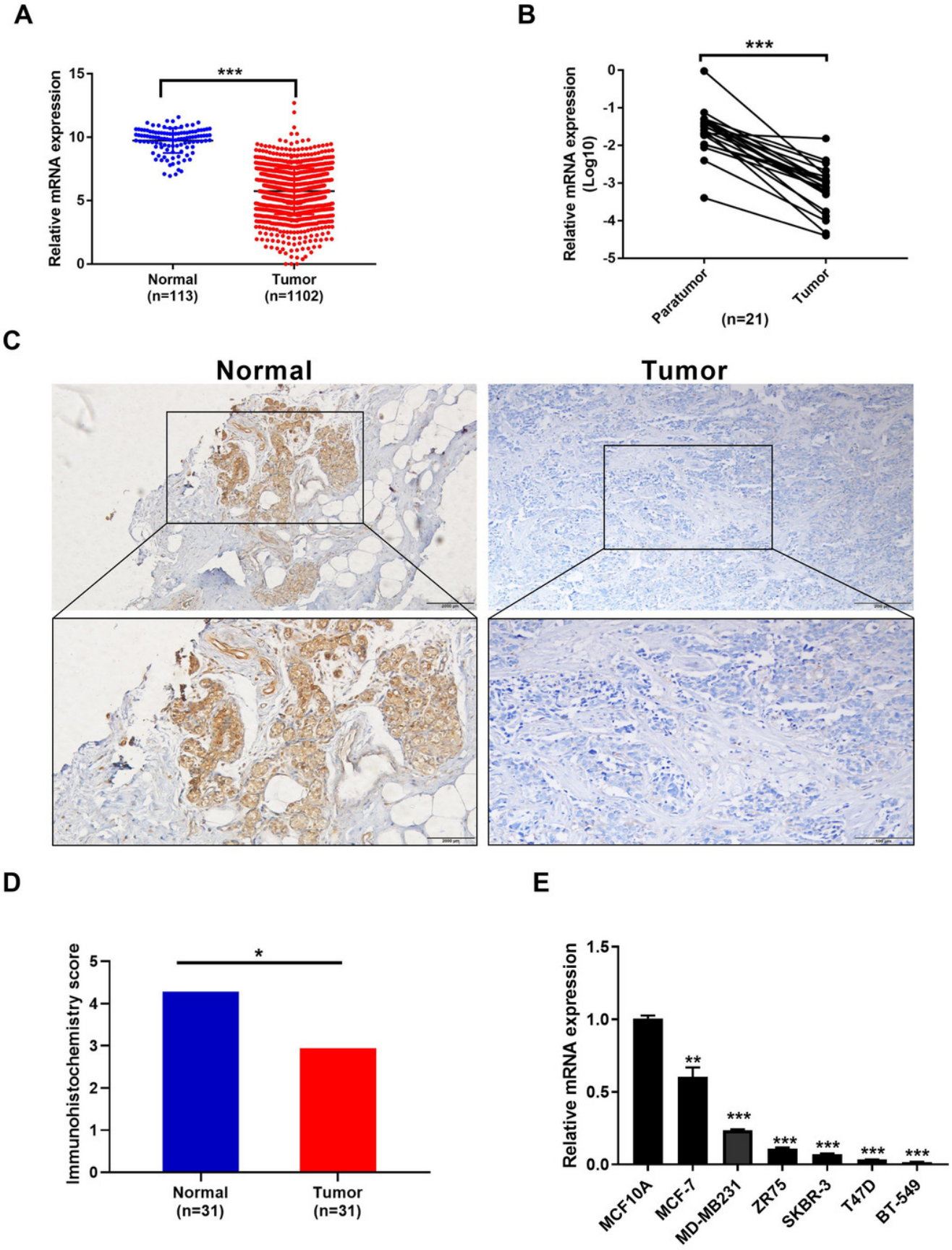
A
Relative mRNA expression
15
10
5
0
***
Normal
(n=113)
Tumor
(n=1102)
B
Relative mRNA expression
(Log10)
0
-1
-2
-3
-4
-5
***
Paratumor
Tumor
(n=21)
C
Normal
Tumor
D
Immunohistochemistry score
5
4
3
2
1
0
*
Normal
(n=31)
Tumor
(n=31)
E
Relative mRNA expression
1.5
1.0
0.5
0.0
**
***
***
***
***
***
MCF10A
MCF-7
MD-MB231
ZR75
SKBR-3
T47D
BT-549

Figure 2

Prognostic values of *IGSF10* in breast cancer patients.

Kaplan-Meier survival curve was plotted with TCGA cohort by stratifying patients into *IGSF10* high and low groups with median expression value. $P < 0.05$ was considered statistical significant. (A) Overall survival curves of breast cancer patients with low expression versus high expression of *IGSF10*. (B) Relapse-free survival curves of breast cancer patients with low expression versus high expression of *IGSF10*.

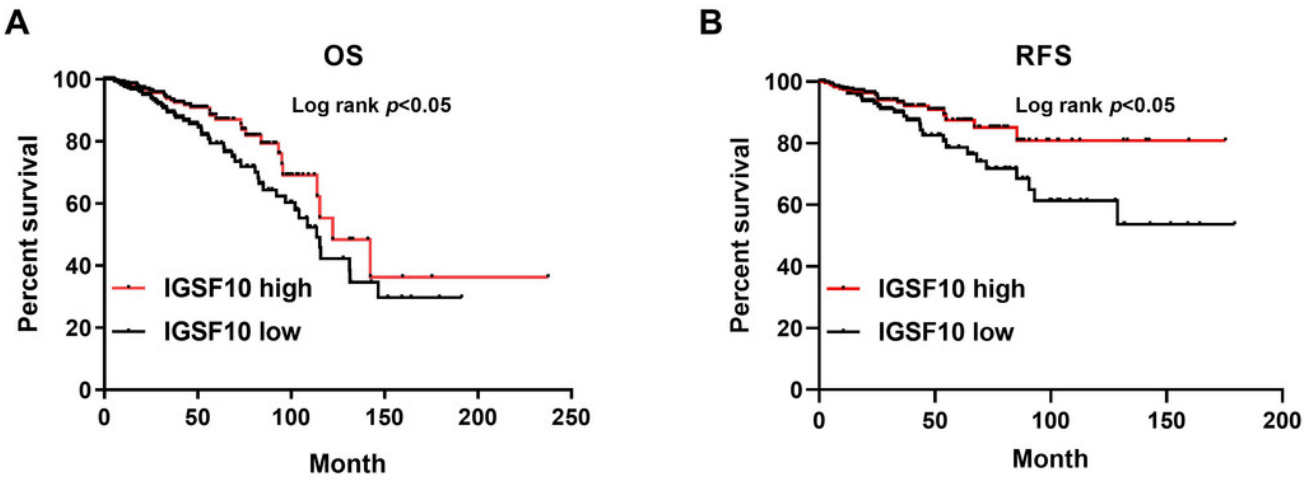

Figure 3

Prognostic values of *IGSF10* in breast cancer patients with different molecular subtypes.

(A) mRNA expression of *IGSF10* in breast cancer patients with different molecular subtypes, including luminal, HER2 positive, and triple negative breast cancer patients. *IGSF10* is plotted for different intrinsic subtypes of breast cancer by using UALCAN. (B) Basal breast cancer; (C) Luminal A breast cancer; (D) Luminal B breast cancer; (E) HER2+ breast cancer. Kaplan-Meier survival curve was generated by Kaplan-Meier plotter (http://kmplot.com/analysis/). $^{**}P < 0.01$.

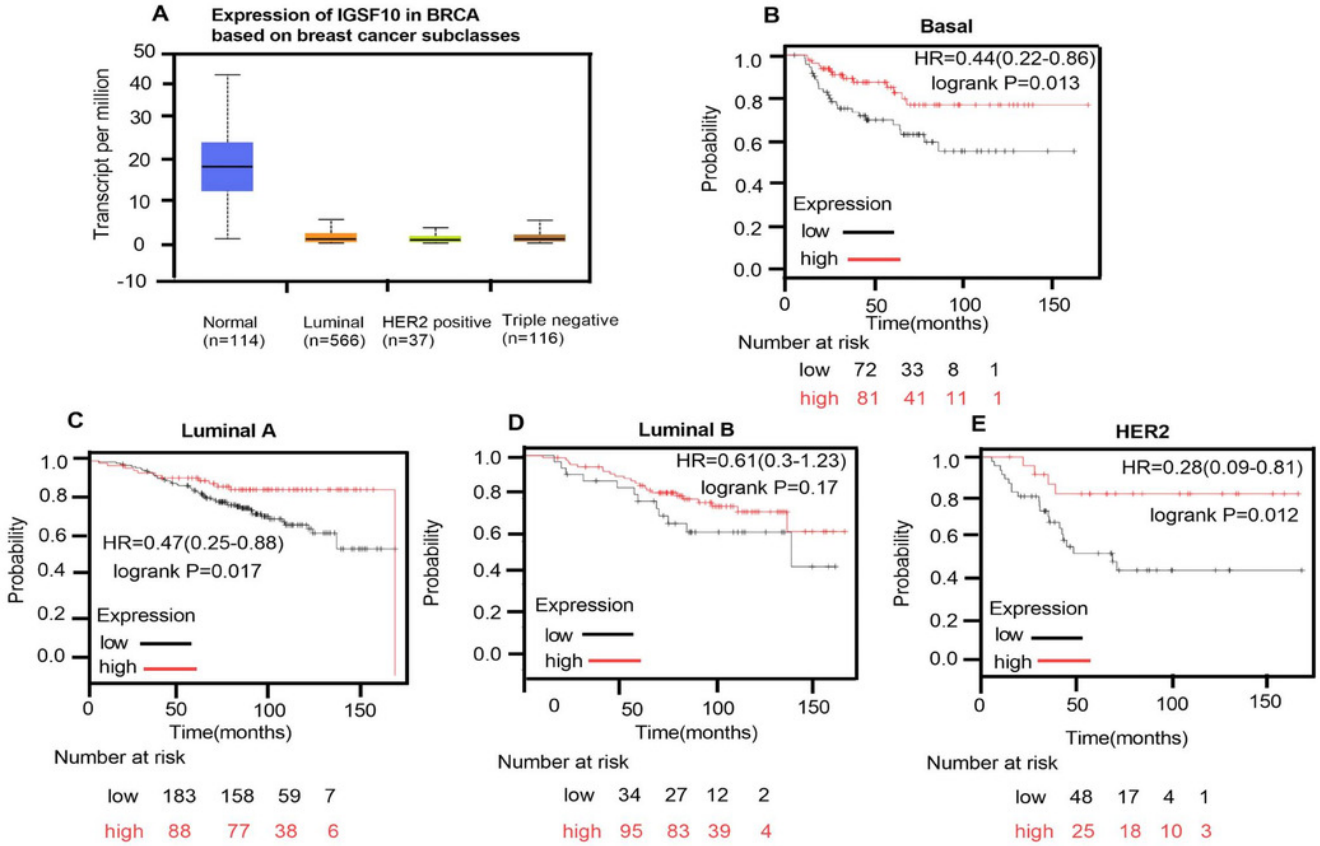

Figure 4

Gene set enrichment analysis (GSEA).

(A) The gene sets that were significantly associated with *IGSF10* with normal *P*-value < 0.05 and false discovery rate (FDR) < 0.25. Gene sets were ranked by normalized enrichment score NES. (B) GSEA enrichment plot showed a significant enrichment of DNA repair, cell cycle, and glycolysis in *IGSF10*-high group. (C) GSEA enrichment plot showed a significant enrichment of PI3K/Akt/mTOR and mTORC1 signaling pathway in *IGSF10*-high group.

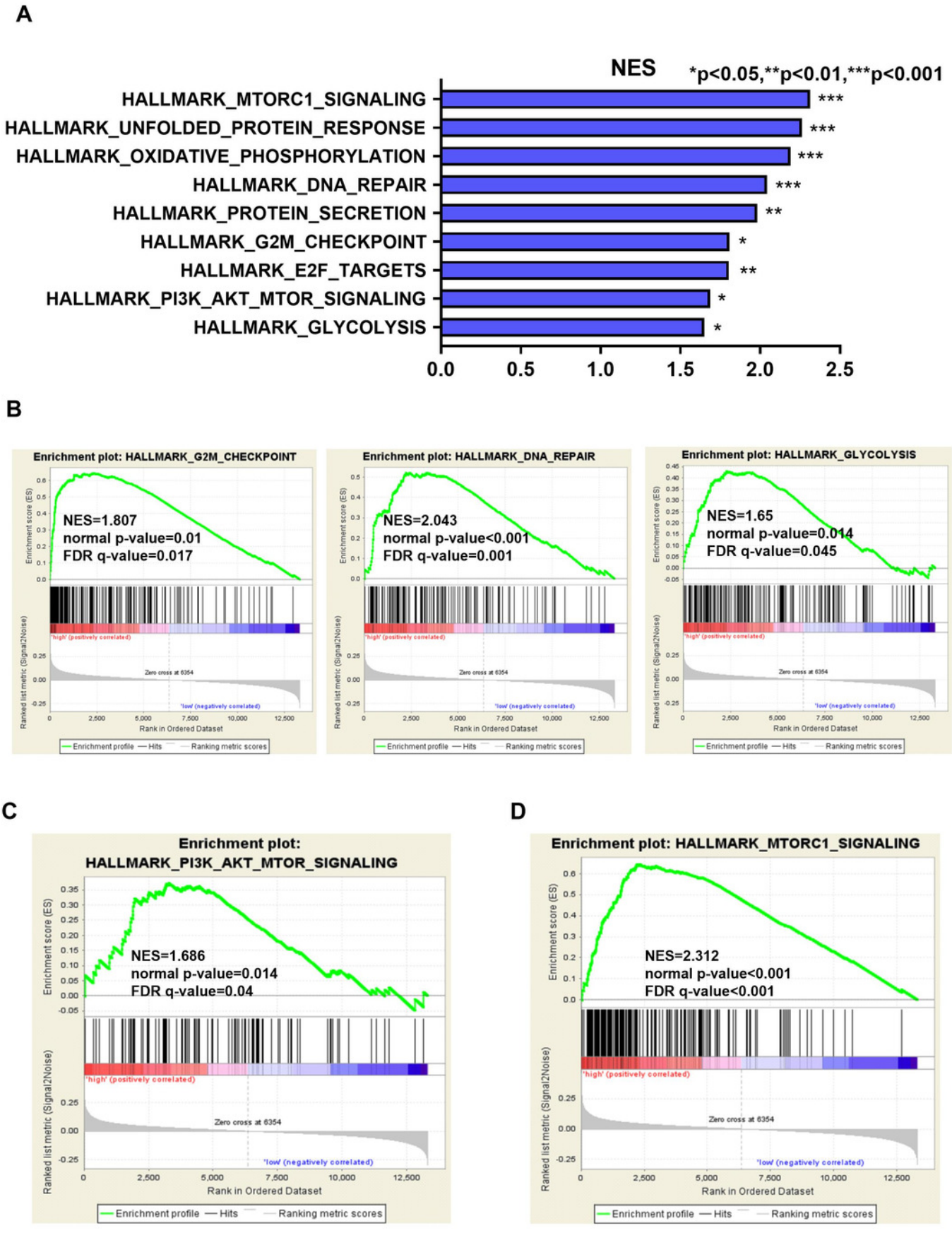
A
NES
*p<0.05,**p<0.01,***p<0.001
HALLMARK_MTORC1_SIGNALING
HALLMARK_UNFOLDED_PROTEIN_RESPONSE
HALLMARK_OXIDATIVE_PHOSPHORYLATION
HALLMARK_DNA_REPAIR
HALLMARK_PROTEIN_SECRETION
HALLMARK_G2M_CHECKPOINT
HALLMARK_E2F_TARGETS
HALLMARK_PI3K_AKT_MTOR_SIGNALING
HALLMARK_GLYCOLYSIS
***
***
***
***
**
*
**
*
*
0.0
0.5
1.0
1.5
2.0
2.5
B
Enrichment plot: HALLMARK_G2M_CHECKPOINT
NES=1.807
normal p-value=0.01
FDR q-value=0.017
Enrichment plot: HALLMARK_DNA_REPAIR
NES=2.043
normal p-value<0.001
FDR q-value=0.001
Enrichment plot: HALLMARK_GLYCOLYSIS
NES=1.65
normal p-value=0.014
FDR q-value=0.045
Enrichment score (ES)
Ranked list metric (Signal2Noise)
'high' (positively correlated)
Zero cross at 6354
'low' (negatively correlated)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores
C
Enrichment plot:
HALLMARK_PI3K_AKT_MTOR_SIGNALING
NES=1.686
normal p-value=0.014
FDR q-value=0.04
D
Enrichment plot: HALLMARK_MTORC1_SIGNALING
NES=2.312
normal p-value<0.001
FDR q-value<0.001

Table 1(on next page)

Analyses of the mRNA expression of IGSF10 in breast cancer

Table 1. Analyses of the mRNA expression of *IGSF10* in breast cancer

Dataset	Normal（cases）	Tumor（cases）	Fold Change	t-Test	*p*-value
TCGA Breast	Breast (61)	Invasive Lobular Breast Carcinoma（36）	-6.845	-15.083	2.86E-23
	Breast (61)	Invasive Breast Carcinoma (76)	-7.060	-16.943	2.91E-35
	Breast (61)	Invasive Ductal Breast Carcinoma (389)	-10.628	-30.383	1.35E-48
Karnoub Breast	Breast (15)	Invasive Ductal Breast Carcinoma (7)	-3.014	-6.149	3.20E-06
Zhao Breast	Breast (3)	Invasive Ductal Breast Carcinoma (38)	-2.306	-11.590	1.27E-08
Richardson Breast 2	Breast (7)	Ductal Breast Carcinoma (40)	-6.421	-10.177	6.42E-06
Finak Breast	Breast (6)	Invasive Breast Carcinoma (53)	-11.035	-20.892	1.08E-20

1

Table 2(on next page)

Clinicopathological features of TCGA breast cancer patients

Table 2. Clinicopathological features of TCGA breast cancer patients

Characteristic	Number of Cases	*IGSF10*		
		High(n)	Low(n)	*P*-value
Age				
< 50	193	125	68	< 0.001*
≥50	507	242	265	
Tumor Size				
T1	183	114	69	0.003*
T2	418	207	211	
T3	75	39	36	
T4	24	7	17	
Lymph node metastasis				
N0	342	181	161	0.865
N1	236	119	117	
N2	85	47	38	
N3	37	20	17	
TMN Stage				
I	124	78	46	0.03*
II	407	203	204	
III	156	82	74	
IV	13	4	9	
ER				
Positive	539	284	255	0.800
Negative	161	83	78	
PR				
Positive	473	249	224	0.870
Negative	227	118	109	
HER-2				
Positive	102	53	49	0.918
Negative	598	314	284	
Triple Negative Breast Cancer				
Yes	119	61	58	0.779
No	581	306	275	

Abbreviation: ER: estrogen receptor, PR: progesterone receptor. $p < 0.05$ was considered statistical significant.

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Table 3(on next page)

Univariate and multivariate Cox regression analysis of IGSF10.

Table 3. Univariate and multivariate Cox regression analysis of *IGSF10*.

Variants	OS						RFS					
	Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
	HR	95%CI	*p*-value	HR	95%CI	*p*-value	HR	95%CI	*p*-value	HR	95%CI	*p*-value
Age (<50 vs. ≥50)	0.597	0.358-0.997	0.049*	0.626	0.367-1.069	0.086	0.768	0.433-0.945	0.041*	0.669	0.373-1.245	0.178
Tumor size (T1/T2 vs. T3/T4)	0.825	0.493-1.380	0.464				0.614	0.322-1.170	0.138			
Lymph node (N0 vs. N1/N2/N3)	0.603	0.384-0.947	0.028*	0.954	0.534-1.704	0.873	0.753	0.443-1.279	0.294			
TNM stage (I/II vs. III/IV)	0.482	0.311-0.747	0.001*	0.538	0.307-0.944	0.031*	0.467	0.359-0.785	0.001*	0.597	0.347-0.842	0.012*
ER (negative vs. positive)	1.197	0.734-1.951	0.471				1.056	0.584-1.909	0.858			
PR (negative vs. positive)	1.489	0.960-2.311	0.076				0.958	0.547-1.680	0.882			
HER2 (negative vs. positive)	1.093	0.563-2.122	0.793				1.388	0.626-3.077	0.419			
IGSF10 (low vs. high)	1.645	1.054-2.569	0.029*	1.793	1.141-2.815	0.011*	2.102	1.222-3.615	0.006*	2.298	1.317-4.010	0.003*

Abbreviation: OS: overall survival; RFS: relapse-free survival; HR: hazard ratio; CI: confidence interval; $p<0.05$ was considered statistical significant

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