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Original Article

TNFSF12 is associated with breast cancer prognosis and immune cell infiltration

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Abstract: Background: Breast cancer (BRCA) is one of the most common cancers in women and is the leading cause of cancer-related deaths in women. TNFSF12, originally a member of the TNF superfamily, is considered a key molecule that is associated with poor prognosis of many cancers. However, its role in progression of BRCA remains unclear. Methods: In this study, the expression profile and clinical information of TNFSF12 across various cancers were obtained from The Cancer Genome Atlas (TCGA) database. Differences in TNFSF12 expression levels between carcinoma and paraneoplastic cancers were compared, and its association with prognosis was examined. Functional enrichment analysis was conducted to explore the potential signaling pathways and biological functions linked with TNFSF12. Moreover, the correlation between TNFSF12 and immune cell infiltration, response to immune checkpoint inhibitors (ICIs), and response to chemotherapy were evaluated. TNFSF12 level in BRCA and normal serum was detected by ELISA. Results: TNFSF12 was lowly expressed in BRCA and is significantly associated with PAM50. TNFSF12 low expression correlates with poor overall survival, particularly among HER2-positive patients. Patients with high level of TNFSF12 expression are usually accompanied with elevated levels of various immune cells, including CD8 T cells, cytotoxic cells, DCs, eosinophils, iDCs, mast cells, neutrophils, NK CD56bright cells, NK cells, pDC, T cells, Tem, and TFH Th17 cells, and exhibit sensitivity to immune checkpoint inhibitors. Functional enrichment analysis indicates significant activation of KRAS signaling, TNFA signaling via NFKB, and epithelial-mesenchymal transition (EMT) in the high TNFSF12 expression group, while MTORC1 signaling, MYC, G2M checkpoint, and E2F targets are inhibited. Furthermore, patients in the low expression group demonstrate higher sensitivity to paclitaxel and rapamycin, whereas those in the high expression group show increased sensitivity to erlotinib and foretinib. ELISA analysis also confirmed a significant decrease of TNFSF12 protein levels in BRCA patients. Conclusion: This study presents a comprehensive analysis of the close correlation between TNFSF12 and prognosis, immune response, as well as the effectiveness of chemotherapeutic agents in BRCA patients.

Keywords: Breast cancer, TNFSF12, immune checkpoints, chemotherapy

Introduction

Breast cancer (BRCA) accounts for approximately 30% of newly diagnosed cancers in women and has become the leading cause of cancer-related death among women worldwide [1, 2]. Different treatment strategies, such as surgical intervention, radiotherapy, chemotherapy, endocrine therapy, and targeted therapy, are available based on BRCA subtype classification [3, 4]. These strategies have improved the 5-year relative survival rate of women with invasive BRCA from 75% to 90% [5]. However, the risk of treatment failure remains high, resulting in recurrence, metastasis, and death [5]. Therefore, understanding the interactions of key molecules during BRCA development and progression is essential to prevent BRCA and identify new therapeutic targets.

TNF superfamily member 12 (TNFSF12), also known as TWEAK, is located on chromosome 17p13.1 and belongs to the TNF superfamily. TNFSF12 is widely distributed in normal tissues

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and can bind to fibroblast growth factor-inducible 14 (Fn14, TNFRSF12A), activating the tumor necrosis factor receptor-associated factor (TRAF) signaling pathway and nuclear factor κB (NF-κB) signaling pathway to perform various functions such as angiogenesis, cell proliferation, apoptosis, fibrosis, epithelial mesenchymal transition (EMT), and immunomodulation [6]. TNFSF12 has been found in different types of tumors, with the levels of expression similar to or lower than those in corresponding non-diseased tissues [7], such as in squamous cervical cancer [8] and endometrial cancer [9]. A study discovered a tendency of lower serum level of TNFSF12 in BRCA patients compared to obese controls [10]. However, the influence of TNFSF12 gene expression on the survival of BRCA and the associated mechanisms are still unknown.

In this study, the predictive significance of TNFSF12 in BRCA and its associated mechanisms were examined. Additionally, the correlation between TNFSF12 expression and immune cell infiltration, as well as their response to immunotherapy and chemotherapy, was identified. Furthermore, potential medications targeting varying levels of TNFSF12 expression were explored. These findings have the potential to enhance treatment strategies and ultimately improve the prognosis of patients with BRCA.

Materials and methods

Data collection and differential expression analysis

TNFSF12 mRNA expression data from 33 tumor tissues and normal tissues, along with corresponding clinical information, were retrieved from the Genotype-Tissue Expression (GTEx) database (https://gtexportal.org/) and The Cancer Genome Atlas (TCGA) database (https://tcga-data.nci.nih.gov/tcga/) [11], and the two databases were merged. Wilcoxon rank sum test was performed to compare the differences in TNFSF12 expression in 33 tumor tissues (TCGA) with that in adjacent paraneoplastic tissues (TCGA) and normal tissues (GTEx). Results were visualized using the R package ggplot2.

UALCAN database was used to study the differences in promoter methylation levels of TNFSF12 between BRCA and normal tissues [12]. β-values indicate promoter methylation levels ranging from 0 to 1, corresponding to unmethylated and fully methylated, respectively.

Diagnostic and prognostic value of TNFSF12

To investigate the diagnostic value of TNFSF12, Receiver Operating Characteristic (ROC) curve analysis was performed. The analysis was executed using the R package pROC and visualized with the R package ggplot2. Additionally, to delve into the prognostic significance of TNFSF12, clinical data encompassing various cancers were gathered from the TCGA database. Patients were stratified into TNFSF12 high and low expression groups. The association between TNFSF12 expression and overall survival (OS) across various cancers was investigated using Cox regression analysis and visualized by R package forest plot. Subsequently, Kaplan-Meier survival curves were plotted using the R packages “survival” and “survminer” to investigate the differential survival outcomes between high and low TNFSF12 expression groups in BRCA patients with common molecular markers [13].

Association between TNFSF12 expression and clinicopathological variables

Differences in TNFSF12 expression between patients with different clinical parameters, including TNM stage, pathologic stage, ER status, PR status, HER2 status and PAM50 typing, were evaluated to elucidate the impact of TNFSF12 in BRCA progression.

Analysis of immune cell infiltration and immune checkpoints

Tumor microenvironment (TME) can be assessed by the stromal score, immune score and estimate score. R package “ESTIMATE” was used to calculate stromal, immune and ESTIMATE scores for each sample based on gene expression [14]. Then, single-sample gene set enrichment analysis (ssGSEA) algorithm was used to calculate per sample infiltration levels of 24 immune cell types [15].

In addition, the expression profiles of common immune checkpoints in BRCA were extracted from the TCGA database to assess the expression differences of immune checkpoint genes

between high and low TNFSF12 expression groups.

Enrichment analysis

Differentially expressed genes (DEGs) between high and low TNFSF12 expression groups were identified using the R package DESeq2, with adjusted *p*-values < 0.05 and |log2-fold-change (FC)| > 1 serving as thresholds. Spearman correlation analysis was employed to assess the correlation between the expression of the top 10 DEGs and TNFSF12. Subsequently, we explored the specific functions and related mechanisms of TNFSF12 in BRCA. GSEA analysis was performed between high and low TNFSF12 expression groups by the "clusterProfiler" package [16]. The main differential signaling pathways associated with BRCA were visualized. In addition, we analyzed the genes involved in these pathways and calculated the correlation between TNFSF12 expression and pathway scores using the R package GSVA.

Correlation of TNFSF12 expression with tumor mutation burden, tumor stemness index and chemotherapy

Spearman's method was used to analyze the correlation of TNFSF12 expression with pan-cancer and different subtypes of BRCA tumor mutation burden (TMB) [17]. Additionally, Kruskal-Wallis test was employed to compare the differences in stemness index between high and low TNFSF12 expression groups in HER2-positive patients. Furthermore, the significant difference in IC50 scores between the high and low TNFSF12 expression groups was assessed to evaluate the therapeutic effect of drugs on BRCA patients using the Wilcoxon test.

Enzyme-linked immunosorbent assay (ELISA)

Serum samples were obtained from 45 patients with BRCA and 30 healthy individuals at the Affiliated Bozhou Hospital of Anhui Medical University. Optical density (OD) readings were taken at 450 nm using a microplate reader, following the guidelines provided by the manufacturer. The concentration of TNFSF12 in each sample was then determined using a standard curve. The assay had a detection range of 15.6 to 1000 pg/mL for human TNFSF12. This study received approval from the Medical Ethics Committee of the Affiliated Bozhou Hospital of Anhui Medical University.

Statistical analysis

All statistical analyses were performed by R software (version 4.2.1) (https://www.r-project.org/). Wilcoxon test or Kruskal-Wallis test was used for difference analysis [18, 19]. Differences were visualized using the R package ggplot2. Correlation analysis was performed using Spearman's correlation method [20, 21]. P < 0.05 were considered statistically significant.

Results

TNFSF12 gene expression is down-regulated in BRCA

To investigate the potential role of TNFSF12 in BRCA progression, TNFSF12 expression levels were analyzed in tumor tissues, adjacent paraneoplastic tissues, and normal tissues using TCGA and GTEx data. Pan-cancer analysis revealed a reduction in TNFSF12 mRNA expression in 11 cancers, including BLCA, BRCA, CESC, COAD, KICH, LUAD, LUSC, PRAD, READ, STAD, and UCEC, compared to paraneoplastic tissues (**Figure 1A**). Moreover, TNFSF12 mRNA expression was reduced in 16 cancers compared to both paraneoplastic and normal tissues, including ACC, BLCA, BRCA, CESC, COAD, ESCA, KICH, LUAD, LUSC, OV, PRAD, READ, STAD, THCA, UCEC, and UCS (**Figure 1B**). TNFSF12 gene expression was significantly lower in BRCA samples than in paraneoplastic and normal breast tissue (P < 0.001) (**Figure 1C**, **1D**). Additionally, TNFSF12 expression was low in 110 paired BRCA and paracancerous tissues (P < 0.001) (**Figure 1E**). ROC curves demonstrated that TNFSF12 expression showed difference between BRCA and normal tissues, with an area under the curve (AUC) of 0.857 (95% confidence interval [CI] = 0.827-0.887) (**Figure 1F**). Furthermore, the promoter methylation analysis showed that TNFSF12 was hypermethylated in BRCA, further confirming the low expression of TNFSF12 in BRCA (**Figure 1G**).

Low TNFSF12 expression is associated with poorer overall survival (OS) in BRCA patients

Cox regression analysis was performed to investigate the correlation between TNFSF12

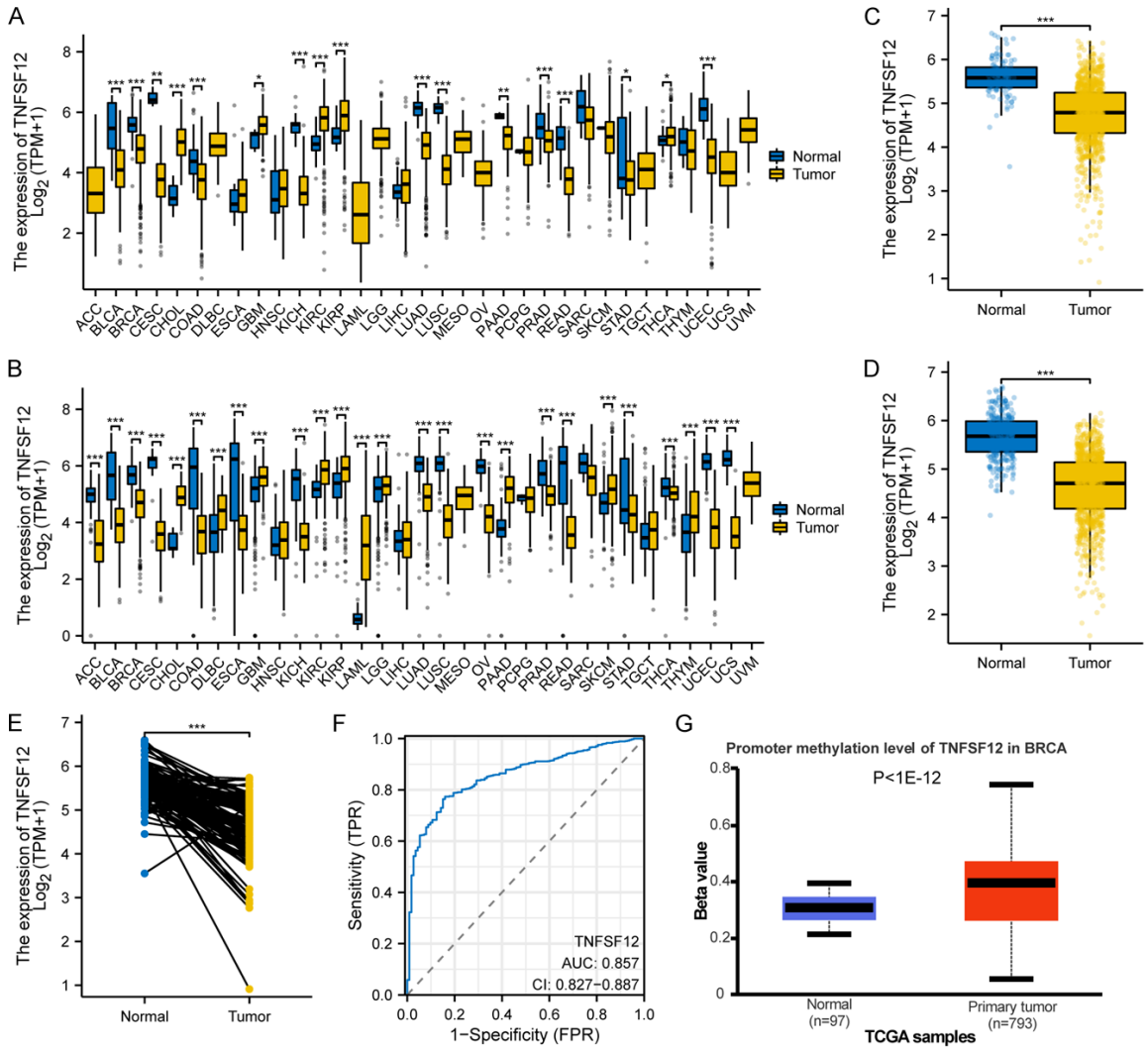


Figure 1. Expression of TNFSF12 in Pan-cancer and BRCA. A. Differential TNFSF12 expression in 33 tumors and adjacent paraneoplastic tissues in TCGA database. B. Differential TNFSF12 expression in 33 tumors and normal tissues in TCGA and GTEx databases. C. Differential expression of TNFSF12 in BRCA and non-matched paraneoplastic tissues in the TCGA databases. D. Differential expression of TNFSF12 in BRCA and non-matched normal tissues in the TCGA and GTEx databases. E. Differential expression of TNFSF12 in paired BRCA and adjacent paraneoplastic tissues in TCGA databases. F. Diagnostic ROC curves in TCGA databases. G. The promoter methylation level of TNFSF12 in BRCA. BRCA, breast cancer; TCGA, The Cancer Genome Atlas; GTEx, Genotype Tissue Expression Project; ROC, receiver operating characteristic. *P < 0.05, **P < 0.01, and ***P < 0.001.

expression and pan-cancer prognosis. The findings revealed that Low TNFSF12 expression was linked to poor prognosis in six types of cancer, including BRCA, KIRP, PCPG, PRAD, SARC, and UCEC (**Figure 2A**). Furthermore, the association between TNFSF12 expression and prognosis was explored in BRCA patients with common molecular markers. The results indicated that Low TNFSF12 expression in patients with HER2 positive resulted in a poor prognosis, whereas TNFSF12 expression had no prognostic significance in patients with other molecular markers (**Figure 2B-G**).

Relationship between TNFSF12 expression and clinicopathologic variables

Figure 3 demonstrated that there were no significant differences in clinicopathologic characteristics, such as pathologic T stage, N stage, M stage, and pathologic stage, between the high and low expression groups of TNFSF12.

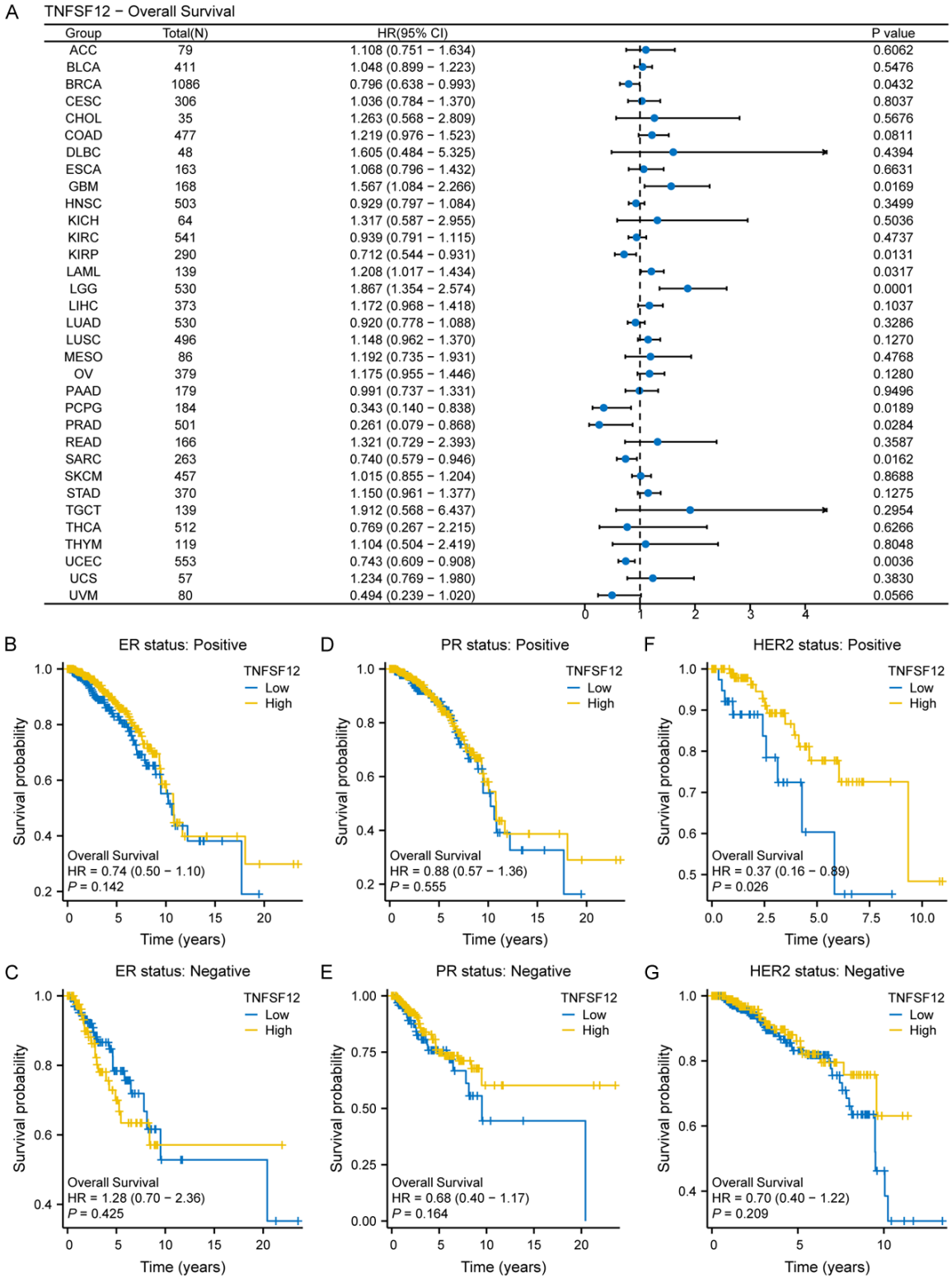


Figure 2. Prognostic values of TNFSF12 expression in BRCA. (A) Forest plots of TNFSF12 expression and OS in pan-cancer. Kaplan-Meier analysis was used to generate survival curves between TNFSF12 expression and OS in breast cancer patients with different molecular subtypes, including (B) ER positive, (C) ER negative, (D) PR positive, (E) PR negative, (F) HER2 positive and (G) HER2 negative. OS, overall survival.

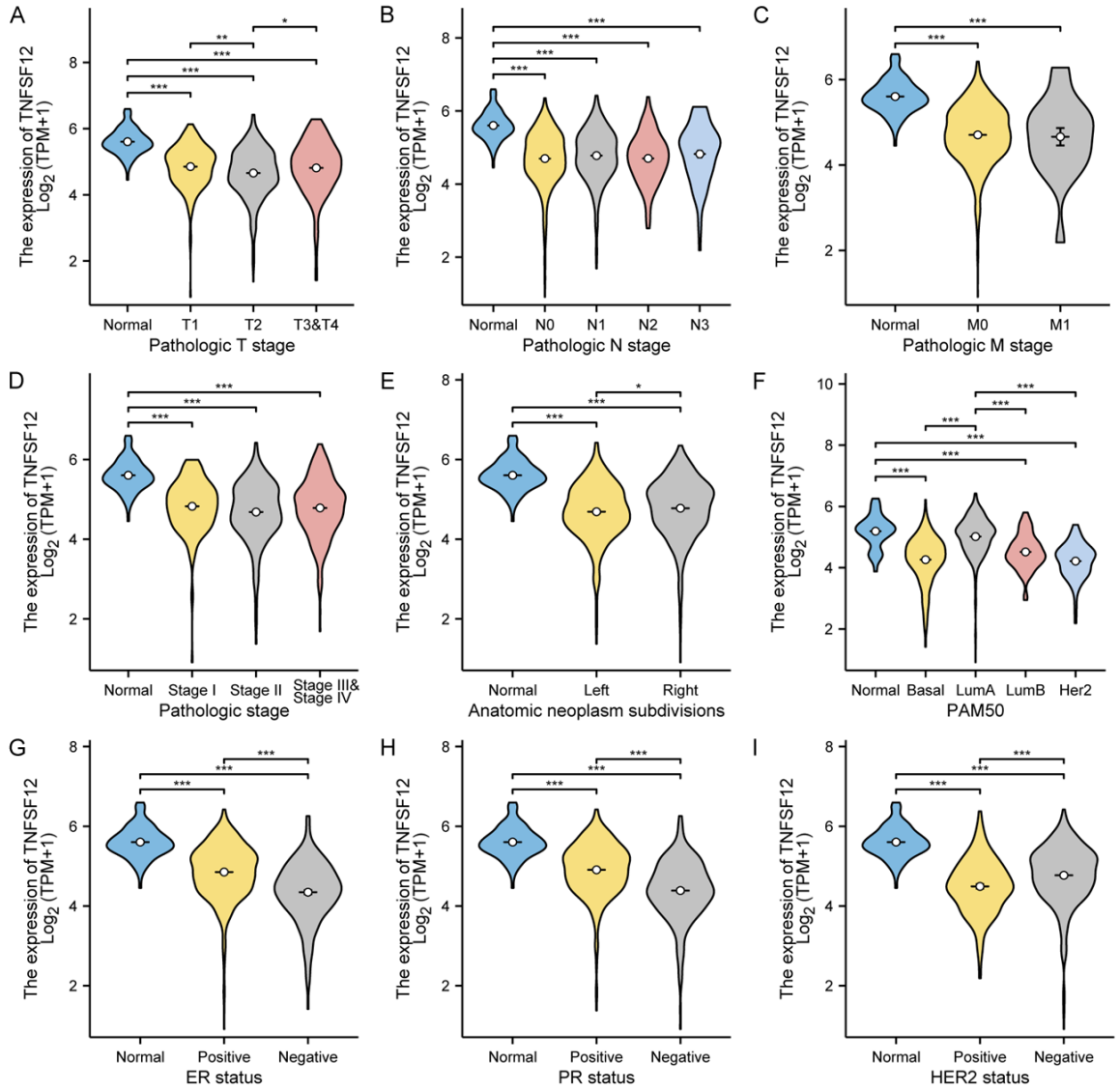


Figure 3. Associations between TNFSF12 expression and clinicopathological characteristics. (A) *T stage,* (B) *N stage,* (C) *M stage*, (D) Pathological stage, (E) Anatomic neoplasm subdivisions, (F) PAM50, (G) *ER status,* (H) *PR status,* (I) *HER2 status.* *P < 0.05, **P < 0.01, and ***P < 0.001.

However, the expression of TNFSF12 was lower in the affected sites on the left side. Notably, variations in TNFSF12 expression were observed among the four main subtypes of BRCA: HER2, Luminal-A (LumA), Luminal-B (LumB), and triple-negative BRCA (TNBC). LumA was the most prevalent subtype with a favorable prognosis and the highest TNFSF12 expression, while HER2 positive patients exhibited the lowest expression. Furthermore, expression analysis with TNFSF12 and different molecular markers in BRCA revealed that TNFSF12 is up-regulated in patients with ER- and PR-positive tumors, but down-regulated in HER2-positive patients compared to negative patients.

Analysis of immune infiltration and immune checkpoints

To investigate the role of TNFSF12 in BRCA, research was further conducted on its relationship with the TME score and immune cell infiltration. A positive correlation was identified between TNFSF12 expression and stromal score, immune score, and estimate score (**Figure 4A-C**). Additionally, the proportions of 24 immune cell types in the TME of BRCA were analyzed using the ssGSEA algorithm. Compared with the low-expression group, higher proportions of CD8 T cells, cytotoxic cells, DC, eosinophils, iDC, mast cells, neutrophils,

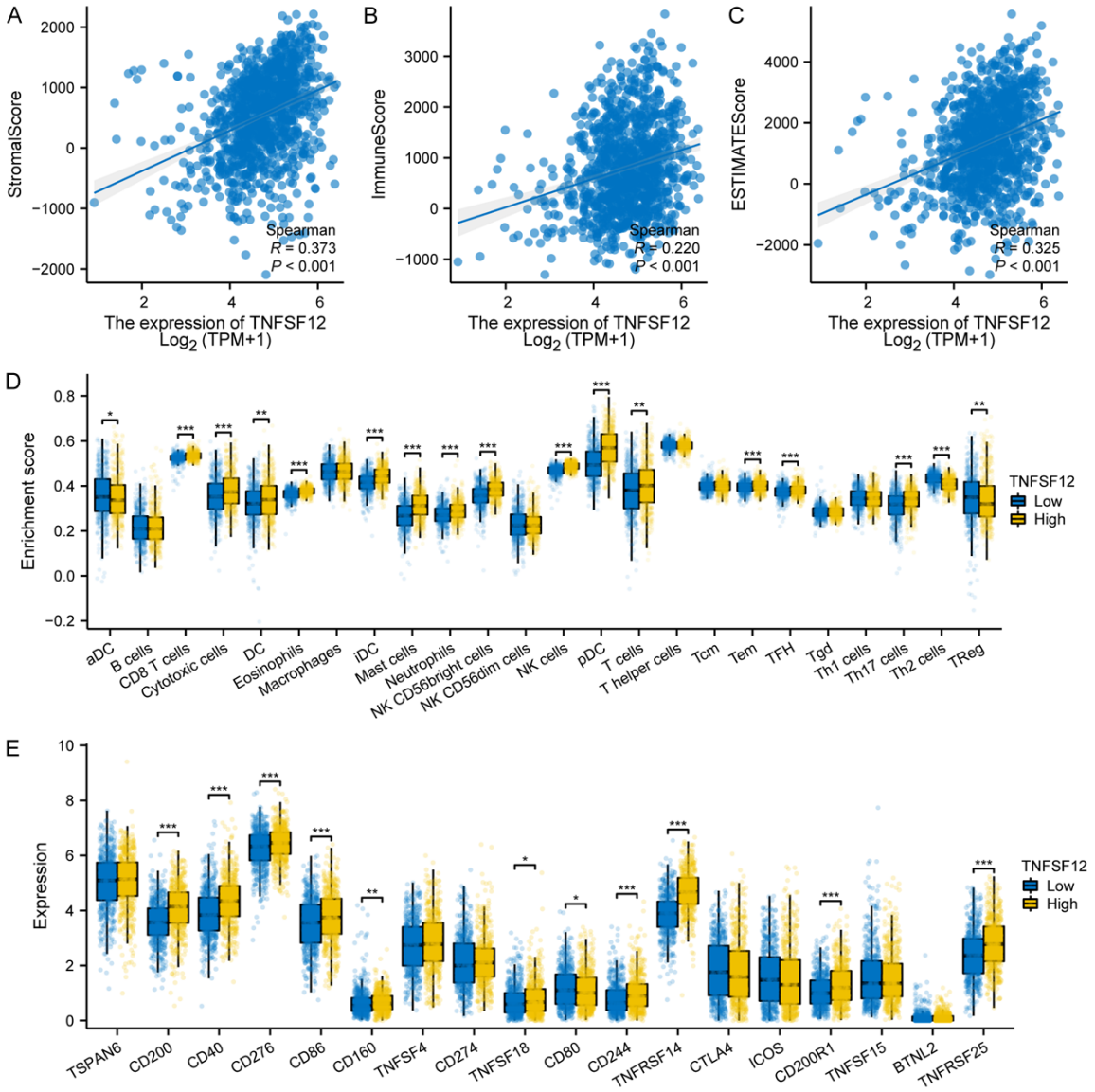


Figure 4. Analysis of immune infiltration and immune checkpoint gene expression between high and low TNFSF12 expression groups. Scatterplot of correlation between TNFSF12 expression and (A) Stromal score, (B) Immune score, and (C) Estimate score. (D) Difference in enrichment scores of 24 immune cells between BRCA patients with low and high TNFSF12 expression. (E) Differential expression of immune checkpoint genes between BRCA patients with low and high TNFSF12 expression. *P < 0.05, **P < 0.01, and ***P < 0.001.

NK CD56bright cells, NK cells, pDC, T cells, Tem, and TFH Th17 cells were detected in the high-expression group. Conversely, aDC, Th2 cells, and TReg cells were underrepresented in the high-expression group (**Figure 4D**).

To evaluate the potential effectiveness of immunotherapy, the expression levels of immune checkpoint genes in both groups were examined. It was observed that CD200, CD40, CD276, CD86, CD160, TNFSF18, CD244, TNFRSF14, CD200R1, and TNFRSF25 genes were more highly expressed in the high-expression group, suggesting that immunotherapy may be beneficial for patients in this high-risk group (**Figure 4E**).

TNFSF12-related molecules and pathways

A total of 1326 genes demonstrated differential expression between the TNFSF12 high- and low-expression groups. This included 275 genes that were up-regulated and 1051 genes that were down-regulated, all with an adjusted

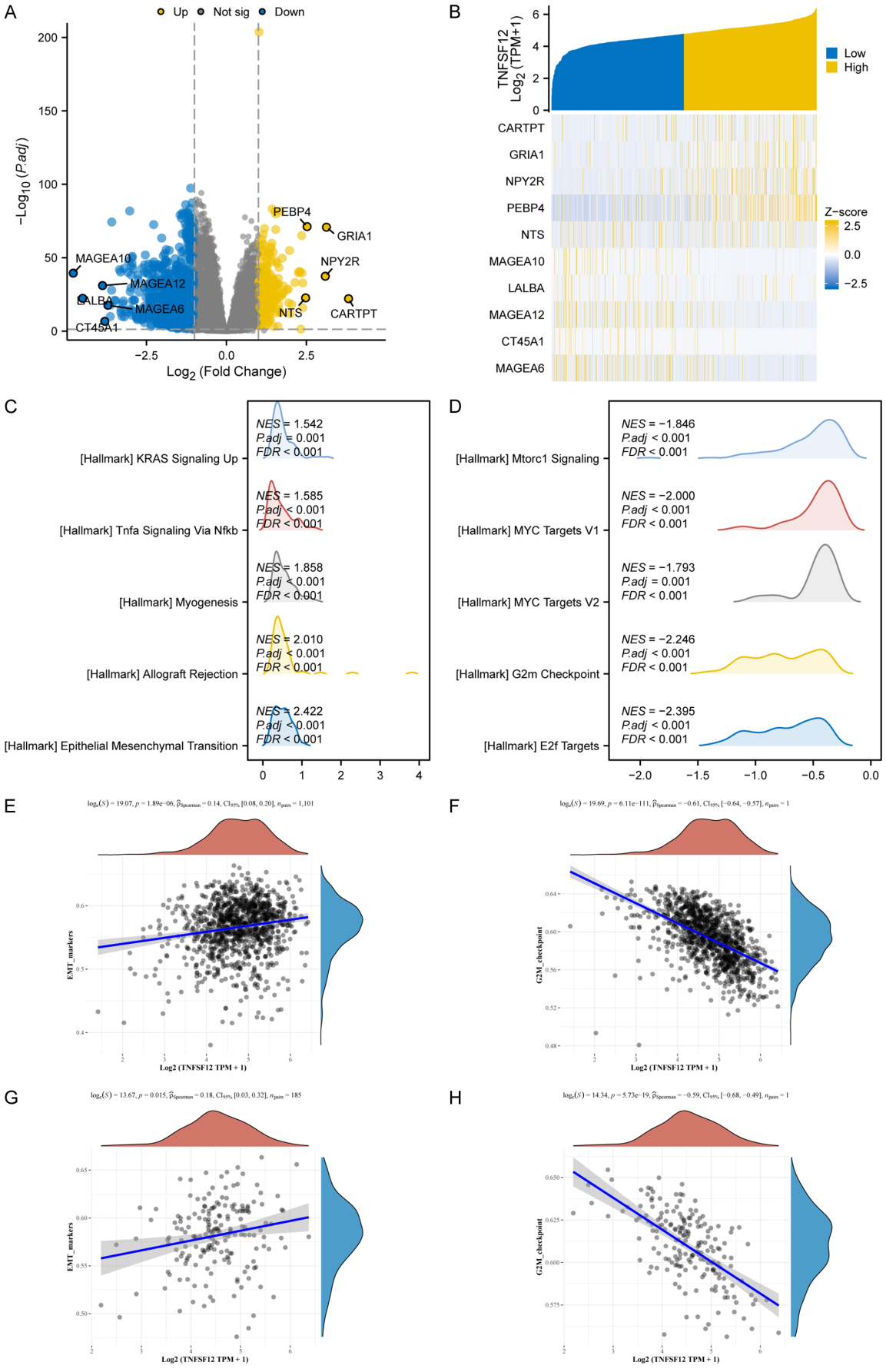

A
Up
Not sig
Down
−Log10 (P.adj)
Log2 (Fold Change)
PEBP4
GRIA1
NPY2R
NTS
CARTPT
MAGEA10
MAGEA12
LALBA
MAGEA6
CT45A1
B
TNFSF12 Log2 (TPM+1)
Low
High
CARTPT
GRIA1
NPY2R
PEBP4
NTS
MAGEA10
LALBA
MAGEA12
CT45A1
MAGEA6
Z−score
2.5
0.0
−2.5
C
[Hallmark] KRAS Signaling Up
NES = 1.542
P.adj = 0.001
FDR < 0.001
[Hallmark] Tnfa Signaling Via Nfkb
NES = 1.585
P.adj < 0.001
FDR < 0.001
[Hallmark] Myogenesis
NES = 1.858
P.adj < 0.001
FDR < 0.001
[Hallmark] Allograft Rejection
NES = 2.010
P.adj < 0.001
FDR < 0.001
[Hallmark] Epithelial Mesenchymal Transition
NES = 2.422
P.adj < 0.001
FDR < 0.001
D
[Hallmark] Mtorc1 Signaling
NES = −1.846
P.adj < 0.001
FDR < 0.001
[Hallmark] MYC Targets V1
NES = −2.000
P.adj < 0.001
FDR < 0.001
[Hallmark] MYC Targets V2
NES = −1.793
P.adj = 0.001
FDR < 0.001
[Hallmark] G2m Checkpoint
NES = −2.246
P.adj < 0.001
FDR < 0.001
[Hallmark] E2f Targets
NES = −2.395
P.adj < 0.001
FDR < 0.001
E
EMT_markers
Log2 (TNFSF12 TPM + 1)
F
G2M_checkpoint
Log2 (TNFSF12 TPM + 1)
G
EMT_markers
Log2 (TNFSF12 TPM + 1)
H
G2M_checkpoint
Log2 (TNFSF12 TPM + 1)

Figure 5. TNFSF12-related DEGs and functional enrichment analysis. (A) Volcano plot of DEGs. Blue and yellow dots represent significantly down-regulated and up-regulated DEGs, respectively. (B) Heatmap of the correlation between TNFSF12 expression and the top 10 DEGs. (C, D) GSEA analysis of the Hallmark gene sets deposited in MSigDB. Correlation between TNFSF12 expression and pathway scores in (E, F) all BRCA patients and (G, H) HER2-positive patients. DEGs, differentially expressed genes; GSEA, gene set enrichment analysis.

P-value < 0.05 and |Log2-FC| > 1 (**Figure 5A**). Additionally, **Figure 5B** illustrated the connection between TNFSF12 and the top 10 DEGs (NTS, PEBP4, GRIA1, CARTPT, NPY2R, MAGEA10, LALBA, MAGEA6, MAGEA12, and CT45A1).

To explore the specific functions and associated mechanisms of TNFSF12 in BRCA, GSEA analysis were employed, and significant activation of KRAS signaling and TNFA signaling via NFKB, as well as Epithelial mesenchymal transition (EMT), were observed in the TNFSF12 high expression group (**Figure 5C**). Conversely, MTORC1 signaling, MYC, G2M checkpoint, and E2F targets were found inactivated (**Figure 5D**). Furthermore, we collected and analyzed the genes involved in these pathways and calculated the correlation between TNFSF12 expression and pathway scores using the R software GSVA package. Our findings revealed a positive correlation between TNFSF12 and the EMT pathway, as well as a negative correlation with the G2M checkpoint in all BRCA patients (**Figure 5E**, **5F**). The above results indicate that TNFSF12 is associated with poor prognosis in HER2-positive patients (**Figure 5G**, **5H**), and our analysis confirmed the correlation between TNFSF12 and the EMT pathway and G2M checkpoint in all BRCA patients.

Correlation of TNFSF12 expression with chemotherapy

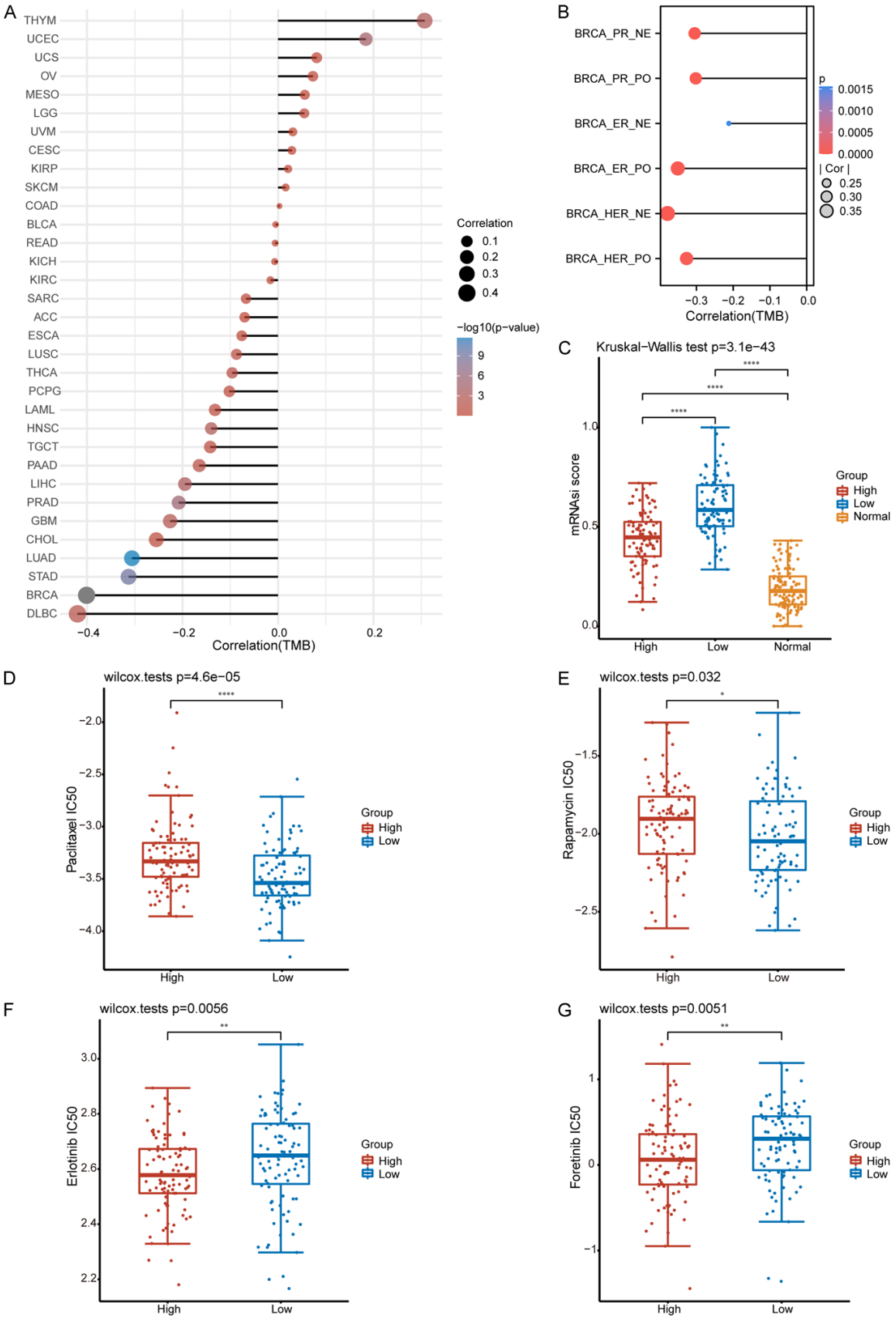
Figure 6A illustrates the correlation between TNFSF12 expression and TMB in various cancers, indicating a negative correlation with DLBC and BRCA, and a positive correlation with THYM and UCEC. Further analysis of BRCA molecular subtypes revealed the top three correlations: ER-positive patients, HER2-negative patients, and HER2-positive patients (**Figure 6B**). Subsequently, stemness in HER2-positive patients was evaluated using the stemness index and was found to be significantly higher in the TNFSF12 low expression group compared to that of the high expression group (**Figure 6C**). Additionally, we investigated the sensitivity of HER2-positive patients in the TNFSF12 high and low expression groups to four chemotherapeutic agents. Notably, patients in the low expression group showed higher sensitivity to Paclitaxel and Rapamycin, whereas patients in the TNFSF12 high expression group exhibited higher sensitivity to Erlotinib and Foretinib (**Figure 6D-G**).

TNFSF12 protein is lowly expressed in the serum of BRCA patients

To investigate the diagnostic potential of TNFSF12 in BRCA patients, we collected plasma samples from 45 BRCA patients and 30 healthy controls for ELISA analysis. The findings revealed a significant reduction of serum TNFSF12 levels in BRCA patients (**Figure 7A**), corresponding well with the low expression levels observed in the TCGA database. Interestingly, while TNFSF12 showed moderate upregulation in HER2-positive BRCA patients, this change was not statistically significant (**Figure 7B**). Notably, TNFSF12 levels appeared elevated in BRCA patients who were both estrogen receptor (ER) and progesterone receptor (PR) positive, among those who were strongly HER2-positive (**Figure 7C**). In **Figure 7D-F**, BRCA patients lacking HER2 expression but strongly positive for ER and PR had a TNFSF12 plasma concentration of 13.77 pg/mL. Conversely, as shown in **Figure 7G-I**, HER2-positive BRCA patients who were negative for ER and PR exhibited a TNFSF12 plasma concentration of 2.37 pg/mL.

Discussion

The prognosis of BRCA can be improved by targeting existing molecular markers such as ER, PR, HER2, Ki67, and grading. However, there are still some patients who experience drug resistance and metastasis, leading to a poor prognosis. Therefore, it is crucial to identify new biomarkers to predict prognosis and improve individualized treatment. Our study analyzed the expression of TNFSF12 in BRCA using the TCGA database and found that TNFSF12 is expressed at lower levels in BRCA compared to normal tissues. Furthermore,


A
THYM
UCEC
UCS
OV
MESO
LGG
UVM
CESC
KIRP
SKCM
COAD
BLCA
READ
KICH
KIRC
SARC
ACC
ESCA
LUSC
THCA
PCPG
LAML
HNSC
TGCT
PAAD
LIHC
PRAD
GBM
CHOL
LUAD
STAD
BRCA
DLBC
−0.4
−0.2
0.0
0.2
Correlation(TMB)
Correlation
0.1
0.2
0.3
0.4
−log10(p−value)
9
6
3
B
BRCA_PR_NE
BRCA_PR_PO
BRCA_ER_NE
BRCA_ER_PO
BRCA_HER_NE
BRCA_HER_PO
−0.3
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Correlation(TMB)
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0.0015
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0.25
0.30
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C
Kruskal−Wallis test p=3.1e−43
mRNAsi score
1.0
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High
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D
wilcox.tests p=4.6e−05
Paclitaxel IC50
−2.0
−2.5
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−3.5
−4.0
High
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wilcox.tests p=0.032
Rapamycin IC50
−1.5
−2.0
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F
wilcox.tests p=0.0056
Erlotinib IC50
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wilcox.tests p=0.0051
Foretinib IC50
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Figure 6. Correlation of TNFSF12 expression with chemotherapy. (A) TNFSF12 was significantly reduced in BRCA patients. (B) TNFSF12 expression was increased in HER2-positive BRCA patients, but not statistically significant. (C) TNFSF12 tended to be elevated in combined ER-positive and PR-positive BRCA patients among strongly HER2-positive patients. (D) Paclitaxel, (E) Rapamycin, (F) Erlotinib and (G) Foretinib in HER2-positive patients with high and low TNFSF12 expression groups. TMB, tumor mutation burden. *P < 0.05, **P < 0.01, and ***P < 0.001.

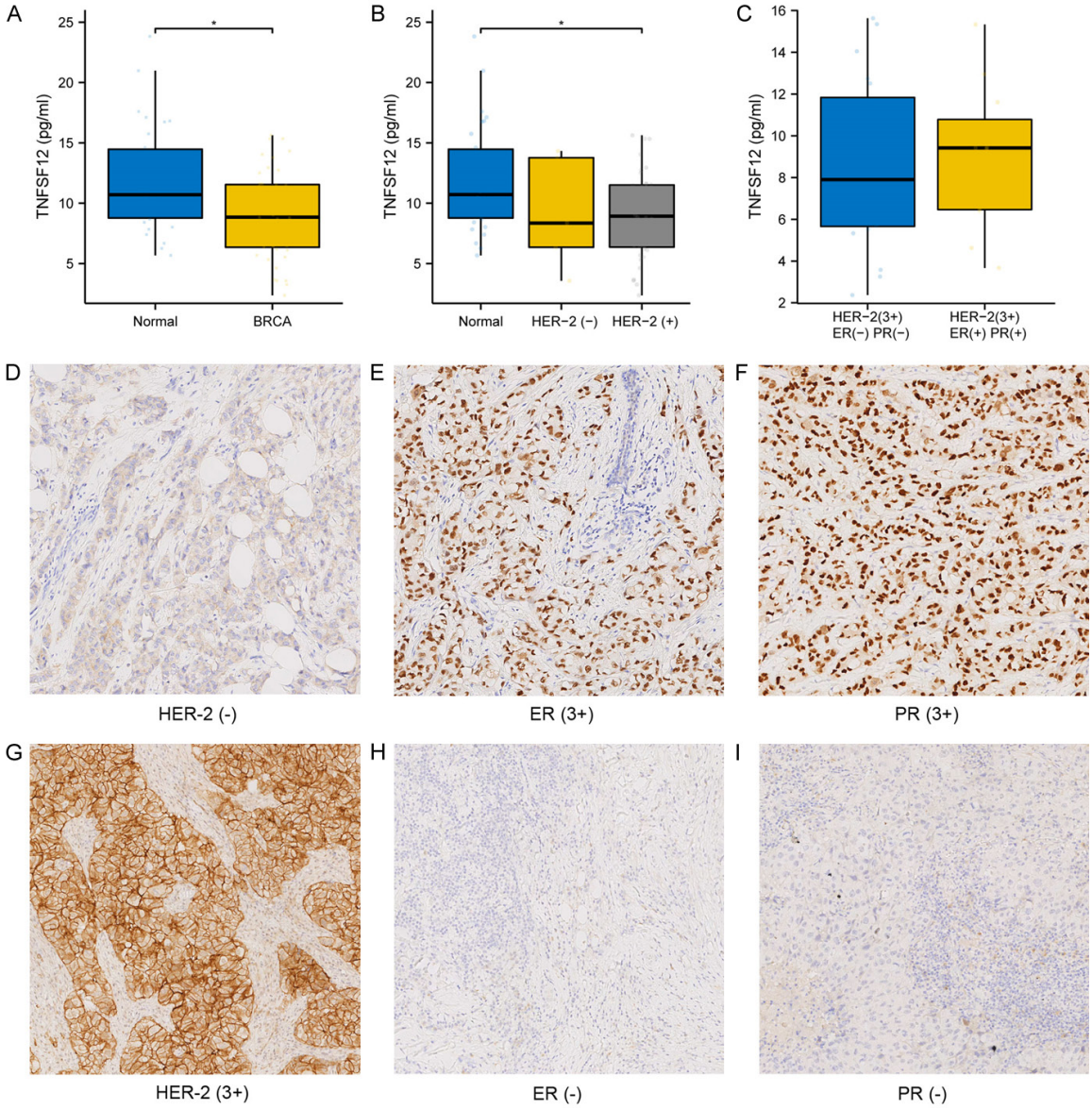

Figure 7. TNFSF12 protein expression in serum of BRCA patients. A. Correlation between TNFSF12 expression and TMB in Pan-Cancer. B. Correlation between TNFSF12 expression and TMB in different molecular subtypes of breast cancer. C. Differences in tumor stemness index between TNFSF12 high and low expression groups in HER2-positive patients and normal samples. D-F. BRCA patients lacking HER2 expression but showing strong positivity for ER and PR had a TNFSF12 plasma concentration of 13.77 pg/mL. G-I. HER2-positive BRCA patients who were negative for ER and PR exhibited a TNFSF12 plasma concentration of 2.37 pg/mL. *P < 0.05.

TNFSF12 is associated with a poor prognosis in BRCA, especially in HER2-positive patients. ELISA analysis also confirmed a significant decrease in TNFSF12 protein levels in BRCA patients.

Tumor cells proliferate in a complex microenvironment comprising of cancer cells, immune cells, and stromal cells [22]. Particularly, malignant tumor cells, such as those found in BRCA, are commonly surrounded by infiltrating

immune cells. These infiltrating immune cells are due to responses to neoadjuvant chemotherapy and immune checkpoint inhibition (ICI) therapy [23]. The group with high TNFSF12 expression levels demonstrated higher proportions of various immune cells, including CD8 T cells, cytotoxic cells, DC, eosinophils, iDC, mast cells, neutrophils, NK CD56bright cells, NK cells, pDC, T cells, Tem, and TFH Th17 cells, compared to the low-expression group. Both pDC and NK cells, as innate immune cells, have been demonstrated to impede the growth of BRCA cells [24, 25]. The presence of CD8+ T cells was associated with a better prognosis for BRCA patients [26]. These findings imply that high level of TNFSF12 expression may influence the advancement and prognosis of BRCA by regulating the level of infiltrating immune cells. Additionally, TNFSF12 expression levels have shown correlation with immune checkpoints, indicating that TNFSF12 might be a useful indicator of the effectiveness of immunotherapy in BRCA patients.

The activation of the tumor necrosis factor receptor-associated factor (TRAF) signaling pathway and the nuclear factor-kappa B (NF-κB) signaling pathway by TNFSF12, through its receptor Fn14, are known to be involved in various functions, including angiogenesis, proliferation, apoptosis, fibrosis, and epithelial-mesenchymal transition. A preclinical study has demonstrated that an antibody targeting TNFSF12 can induce antitumor effects via TWEAKR signaling [27]. It has been shown that TNFSF12 has both anti-invasive and pro-invasive effects, depending on LCN2 expression [28]. However, the specific functions and mechanisms of TNFSF12 in BRCA are not fully understood and require further investigation. In this study, the TNFSF12 high-expression group was shown to activate KRAS signaling and TNFA signaling through NFKB, along with activation of epithelial-mesenchymal transition (EMT). Meanwhile, MTORC1 signaling, MYC, G2M checkpoint, and E2F targets were inhibited. Furthermore, increased sensitivity to paclitaxel and rapamycin was observed in patients in the low-expression group, while patients in the TNFSF12 high-expression group demonstrated higher sensitivity to erlotinib and fosfatinib.

In conclusion, the expression pattern and prognostic role of TNFSF12 in BRCA and other cancers have been comprehensively characterized in this study. TNFSF12 has been identified as a potential biomarker for predicting the efficacy of chemotherapeutic agents and immunotherapies, as well as a novel target for BRCA treatment.

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Disclosure of conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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