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Fibrinogen-Like Protein 2 (FGL2) is a Novel Biomarker for Clinical Prediction of Human Breast Cancer

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Background: Fibrinogen-like protein 2 (FGL2) is a member of the fibrinogen-like protein family and possesses important regulatory functions in both innate and adaptive immune responses. FGL2 is overexpressed in glioma, and its expression level is negatively associated with the prognosis of glioma patients. However, the diagnostic value of FGL2 is unknown in breast carcinoma.


Material/Methods: We comprehensively analyzed the expression pattern of FGL2 in breast cancer. Several online databases – TCGA, Oncomine, GEPIA, Kaplan-Meier plotter, and PrognoScan – were used in this study.

Results: Based on the TCGA dataset and Oncomine database, we found that the expression level of *FGL2* was remarkably lower in breast cancer compared with adjacent normal tissues. Clinical data showed that the expression level of *FGL2* was significantly associated with radiation therapy, PR status, and tumor stage. Bioinformatics analysis of the GEPIA, Kaplan-Meier plotter, and PrognoScan databases showed that lower *FGL2* expression levels were associated with a worse prognosis in breast cancer patients. Furthermore, the expression level of *FGL2* was positively correlated with the immune cell infiltrations in breast cancer, especially those cells with high antitumor activities. GO, KEGG, and GSEA analyses also validated that *FGL2* was closely related to genes involved in the immune response, signal transduction, and T cell receptor signaling pathway in breast cancer.

Conclusions: The results demonstrated that high expression of *FGL2* is a useful marker for breast cancer treatment and appears to be correlated with enhanced antitumor activities in breast cancer patients.

MeSH Keywords: **Fibrinogen • Lymphocytes, Tumor-Infiltrating • Prognosis**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/923531>

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Background

Breast cancer is a common invasive cancer that is the second leading cause of cancer-related deaths in women [1]. It is estimated that more than 268 600 women were diagnosed with breast carcinoma in the USA in 2019 [2]. In women, breast cancer is responsible for 30% of all new cancer diagnoses in the USA. In East Asia, the morbidity rate of breast cancer rapidly increased in the last decade (3). In contrast to Western countries, more than 40% of cases occur in women under 50 years old in East Asia. Thus, breast cancer has become a heavy economic and social burden worldwide. Risk factors of breast cancer include genetic factors, environmental exposure, dietary factors, and increasing age [4]. *BRCA* mutation is correlated with higher morbidity in breast cancer [5]. Other significant gene mutations include *CDH1*, *CHEK2*, *NF1*, *PALB2*, and *PTEN*. A multidisciplinary approach is useful in the treatment of breast cancer [6], and molecular subtypes should be considered in a multidisciplinary setting. The therapeutic targets and clinically relevant biomarkers are HER2, ER and PR in breast cancer. Trastuzumab (Herceptin), a monoclonal antibody to HER2, has been demonstrated to prolong disease-free survival (DFS) in HER2-positive breast cancer patients [8]. Next-generation sequencing (NGS) and mutation analysis may provide new therapeutic targets to benefit breast cancer patients [9].

Fibrinogen-like protein 2 (FGL2) belongs to the fibrinogen-like protein family [10], which is mainly expressed in macrophages, T cells, endothelial cells, and tumor cells. FGL2 is a vital immune regulator of both innate and adaptive responses, with pleiotropic effects [11]. It is usually confirmed to possess multifunctional activities and immune regulatory functions in inflammation, allograft rejection, and abortion. Furthermore, low levels of FGL2 can lead to impaired Treg cell activities [12]. Alterations in FGL2 structure or expression level are correlated with deadly viral infections such as HIV and coronavirus [13,14]. Previous studies have demonstrated that upregulated FGL2 levels were associated with shorter overall survival in glioblastoma (GBM) patients [15]. However, the relationship between breast cancer and FGL2 has not been thoroughly explored.

In our study, we utilized bioinformatics methods to analyze the FGL2 expression pattern in breast cancer. TCGA datasets and the Oncomine database were employed to contrast FGL2 expression in breast cancer and normal adjacent tissues. The TCGA dataset was analyzed to assess the relationship between FGL2 and clinical characteristics of breast cancer patients. We used the online datasets PrognoScan, GEPIA, and Kaplan-Meier plotter to evaluate the prognostic potential of FGL2 in breast cancer, and the TIMER dataset was used to analyze the relationship between FGL2 and immune marker sets and tumor-infiltrating lymphocytes. The STRING website was utilized for FGL2 protein-protein interaction network screening. GO and

KEGG calculates on the DAVID website were used to identify the main biological signatures of FGL2-correlated genes in breast cancer, and the GSEA dataset was used to analyze the biological functions of FGL2. The framework and concise content of this study are shown in Figure 1.

Material and Methods

Patients and samples

The transcriptome profiles were downloaded from TCGA dataset. Multiple samples were used to avoid clinical and race bias in different studies. The expression level of the desired mRNA was normalized by R language for further analysis.

Oncomine database analysis

In this study, the mRNA expression differences of FGL2 between cancers and adjacent normal tissues were investigated with the Oncomine databases. The threshold *P*-value was determined as 0.001. The threshold fold change was determined to be 2.0. The threshold gene rank was the top 10%.

GEPIA database analysis

The prognostic potential of FGL2 in breast cancer was evaluated in the GEPIA database (<http://gepia.cancerpku.cn/>). GEPIA is a new interactive website based on genotype-tissue expression (GTEx) and TCGA data.

PrognoScan database analysis

The prognostic potential of FGL2 was validated in the PrognoScan dataset. Cox *P*-value <0.05 was considered as the threshold value.

Kaplan-Meier plotter dataset

In this study, the association between FGL2 expression and prognosis of breast cancer was tested by Kaplan-Meier plotter. The log-rank *P*-value and hazard ratio (HR) with 95% confidence intervals were also calculated.

TIMER database analysis

The TIMER database was utilized to visualize the correlation of FGL2 expression with immune cell infiltration in breast cancer. The infiltrated immune cells included CD8⁺ T cells, macrophages, B cells, CD4⁺ T cells, neutrophils, and dendritic cells. Furthermore, the associations between FGL2 and gene markers of tumor-infiltration immune cells were investigated with correlation modules.

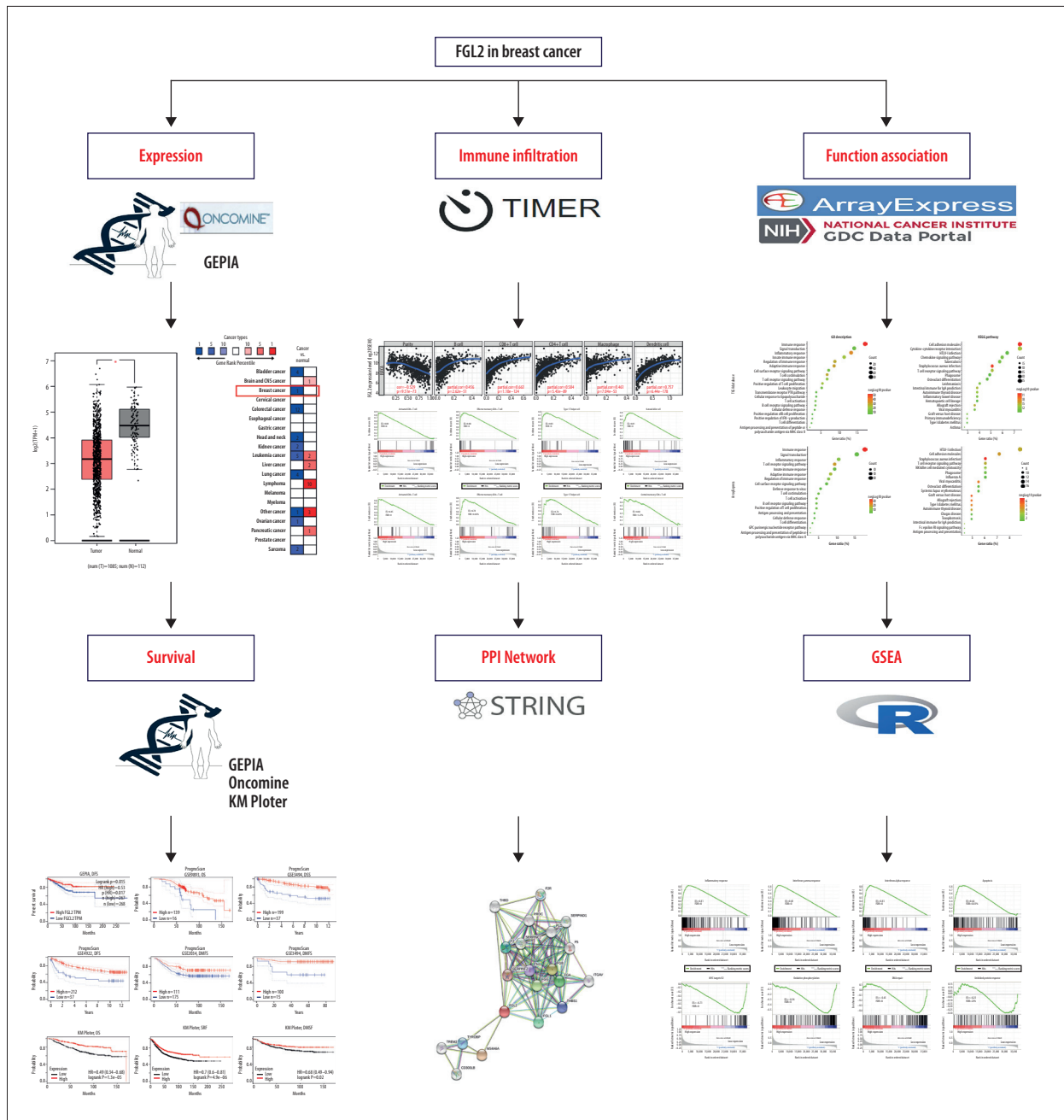


Figure 1. Schematic diagram of the study.

Protein–protein interaction network construction

The STRING database was used to identify the protein–protein interactions of FGL2. The number of edges, number of nodes, average node degree, and PPI enrichment value can be evaluated by the STRING database. Nodes in the network were classified by their corresponding role in biological processes. Edges were classified based on the molecular function of FGL2. The connectivity degree of each protein node was calculated, and the top hub nodes were determined in the PPI network.

Functional enrichment analyses

Biological process (BP) of GO and KEGG analysis were performed in the DAVID website (<https://david.ncifcrf.gov/>). KEGG pathway analysis can be used to understand high-level functions and utilities of the biological system, linking genomic information to higher-order functional information. GO enrichment analysis stores graphical representations of cellular processes, including membrane transport, signal transduction, metabolism, and cell cycle.

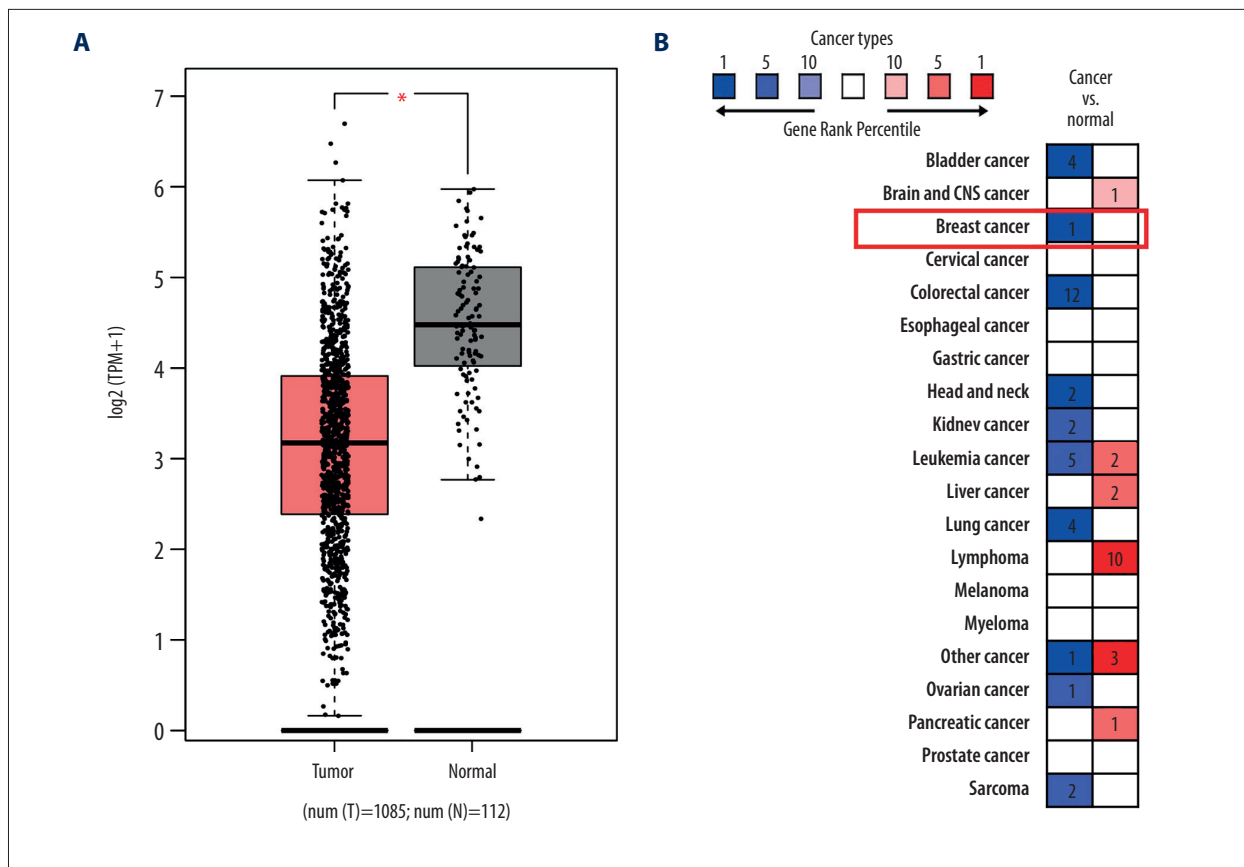


Figure 2. FGL2 expression levels in breast cancer. (A) FGL2 expression levels are significantly lower in breast cancer tissue than levels in adjacent normal tissues in the TCGA database. The left bar represents FGL2 levels in breast cancer tissue. The right bar represents FGL2 levels in matched normal data. (* $P < 0.05$). (B) FGL2 expression in different cancers compared with adjacent normal tissues in the Oncomine database. Threshold (P -value): $1E-4$. Threshold (fold change): 2. Threshold (gene rank): top 10%.

Gene set enrichment analysis (GSEA)

GSEA is a statistical approach to assess whether the genes from particular pathways or other predefined gene sets show concordant differences or statistical significance between biological states. We used the hallmark gene set download from the GSEA website and 28 immune gene sets as the annotate data to analyze the different expression levels of FGL2 enrichment score in different pathways or immune cells. Gene sets with an FDR $< 10\%$ and a nominal P -value < 0.05 were determined as significant.

Statistical analysis

The discrepancies in FGL2 expression were evaluated by t test. Spearman correlation was analyzed by R language. The SPSS 20.0 software was used to measure the data. Log-rank and Cox regression analysis were applied to investigate the prognostic value of FGL2. $P < 0.05$ represented statistical significance.

Results

The mRNA expression levels of FGL2 in breast cancer

Bioinformatics databases were utilized to evaluate the differential expression of FGL2 between breast cancer and adjacent normal tissues. TCGA database indicated that FGL2 was prominently lower in breast invasive carcinoma (BRCA) compared with that in matched normal tissue (Figure 2). The Oncomine database (Study Accession: EGAS00000000083) also showed that FGL2 expression was lower in breast cancer compared with adjacent normal tissue (P -value $< 1.01E-12$, fold change = -2.578 , Gene rank in top 1%). These results indicate that decreased FGL2 expression may be conducive to the tumorigenesis of breast cancer.

The correlation between FGL2 and the clinical characteristics of breast cancer patients

The TCGA database was used to assess the correlation between FGL2 expression and the clinical characteristics in breast

Table 1. Association of FGL2 with the clinical pathological characteristics of breast cancer patients derived from the TCGA database.

Variables	Clinical pathological	Number	Mean±SD	P
Sex	Female	1091	2.89±1.06	0.004
	Male	12	2.00±0.89	
Radiation therapy	No	439	2.77±1.05	0.004
	Yes	553	2.96±1.05	
Age	<60	589	2.89±1.07	0.713
	≥60	513	2.86±1.04	
HER2 status	Negative	290	2.92±1.01	0.496
	Positive	69	2.83±0.95	
ER status	Negative	212	2.87±1.18	0.458
	Positive	727	2.93±1.01	
PR status	Negative	308	2.79±1.13	0.013
	Positive	628	2.98±1.00	
T stage	T1	281	3.02±1.04	0.038
	T2	641	2.83±1.07	
	T3	138	2.93±1.06	
	T4	40	2.58±0.94	
N stage	N0	519	2.88±1.11	0.28
	N1	366	2.85±1.01	
	N2	120	2.95±1.03	
	N3	78	3.07±0.94	
M stage	M0	917	2.87±1.06	0.134
	M1	22	2.53±0.93	
TNM stage	I	182	3.00±1.08	0.055
	II	628	2.83±1.84	
	III	250	2.95±1.00	
	IV	20	2.45±0.95	

cancer. Data of 1104 patients were collected from the TCGA dataset. As shown in Table 1, *FGL2* expression was found to be significantly correlated with sex ($P=0.004$), radiation therapy ($P=0.004$), and PR status ($P=0.013$). Furthermore, *FGL2* expression was also significantly different among tumor stages ($P=0.038$).

Prognostic potential of *FGL2* in breast cancer

Three datasets (the GEPIA dataset, PrognoScan dataset, and KM plotter dataset) were utilized to explore the association between *FGL2* level and breast carcinoma survival rate (Figure 3). In the GEPIA dataset, a lower *FGL2* expression was correlated with a worse prognosis of disease-free survival (DFS) in breast cancer (DFS HR=0.53, log-rank $P=0.015$, cutoff-high=75%). In the PrognoScan database, 5 cohorts were analyzed, and the results showed that lower *FGL2* was associated

with poor prognosis of breast cancer patients (OS HR=0.59, 95% CI=0.35 to 0.99, Cox $P=0.045139$; DSS HR=0.60, 95% CI=0.37 to 0.99, Cox $P=0.043958$; DFS HR=0.73, 95% CI=0.58 to 0.94, Cox $P=0.012526$; DMFS HR=0.67, 95% CI=0.49 to 0.92, Cox $P=0.013025$; DMFS HR=0.29, 95% CI=0.14 to 0.60, Cox $P=0.000852$). Furthermore, the prognostic potential of *FGL2* in breast cancer was also confirmed in the KM plotter dataset. Lower *FGL2* was related to a unfavorable prognosis of breast cancer (OS HR=0.49, 95% CI=0.35 to 0.68, Cox $P=1.3e-05$; RFS HR=0.7, 95% CI=0.60 to 0.81, Cox $P=4.9e-06$; DMFS HR=0.68, 95% CI=0.49 to 0.94, Cox $P=0.02$).

The relationship between *FGL2* and immune status in the breast cancer tumor microenvironment

A higher density of tumor-infiltrating lymphocytes (TILs) is an important parameter leading to better prognoses and

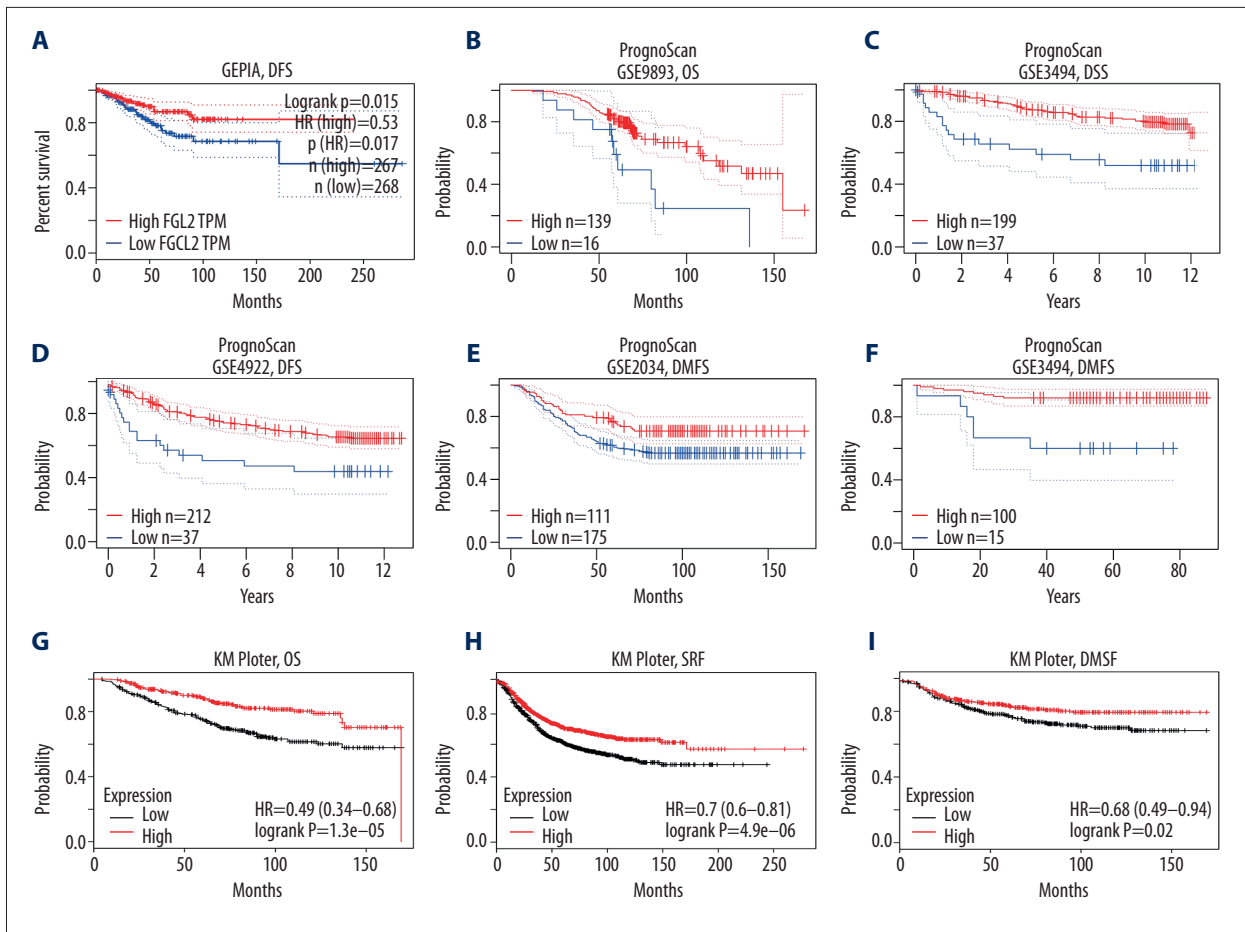


Figure 3. Survival rates for FGL2 in breast cancer. The GEPIA database, PrognScan database, and Kaplan-Meier plotter database were used to analyze the prognostic potential of FGL2 in breast cancer. (A) In the GEPIA database, a lower expression level of FGL2 was associated with a poor prognosis of disease-free survival (DFS) in breast cancer. (B–F) In the PrognScan database, GSE9893, GSE3494, GSE4922, GSE2034, and GSE19615 were used to evaluate overall survival (OS), disease-specific survival (DSS), disease-free survival (DFS), and distant metastasis-free survival (DMFS), respectively. (G–I) The Kaplan-Meier plotter database was used to measure OS, relapse-free survival (RFS), and DMFS related to FGL2 in breast cancer.

therapeutic effects in the treatment of cancer. In this study, the TIMER dataset was utilized to investigate the relationship between *FGL2* and TILs. The results showed that *FGL2* was positively correlated with infiltrating levels of B cells ($r=0.456$, $P=2.62e-51$), CD8⁺ T cells ($r=0.663$, $P=1.10e-124$), CD4⁺ T cells ($r=0.584$, $P=5.43e-89$), macrophages ($r=0.461$, $P=7.04e-53$), and DCs ($r=0.757$, $P=6.44e-178$) in BRCA (Figure 4A). These results suggest that *FGL2* has critical roles in immune cell infiltration in breast cancer.

Further analyses using the GSEA tool demonstrated that *FGL2* was positively correlated with infiltrating levels of antitumor immune cells, such as activated CD8⁺ T cells ($P=0$), effector memory CD8⁺ T cells ($P=0$), type 1 T helper (Th1) cells ($P=0$), natural killer (NK) cells ($P=0$), activated CD4⁺ T cells ($P=0$), effector memory CD4⁺ T cells ($P=0$), natural killer T (NKT) cells ($P=0.003$), and central memory CD4⁺ T cells ($P=0.014$) (Figure 4B).

These results show that higher *FGL2* expression level is correlated with strong antitumor activities in breast cancer.

Apart from TILs, we also investigated the relationship between *FGL2* expression and immune marker sets of diverse immune infiltrating cells. Detailed correlations between *FGL2* and immune marker genes in breast cancer are listed in the Supplementary Tables 1 and 2. Generally speaking, *FGL2* was positively correlated with immune marker sets in the TIMER database (Supplementary Table 1) and GEPIA database (Supplementary Table 2). HLA class II histocompatibility antigens are important factors in the antitumor immune response by presenting tumor-specific antigens to CD4⁺ T cells after phagocytosis of tumors by antigen-presenting cells (APCs). We found that 4 HLA class II genes (*HLA-DPA1*, *HLA-DRA*, *HLA-DPB1*, and *HLA-DQB1*) were positively correlated with *FGL2* expression levels. Class II transcription activator (*CIITA*), which is responsible

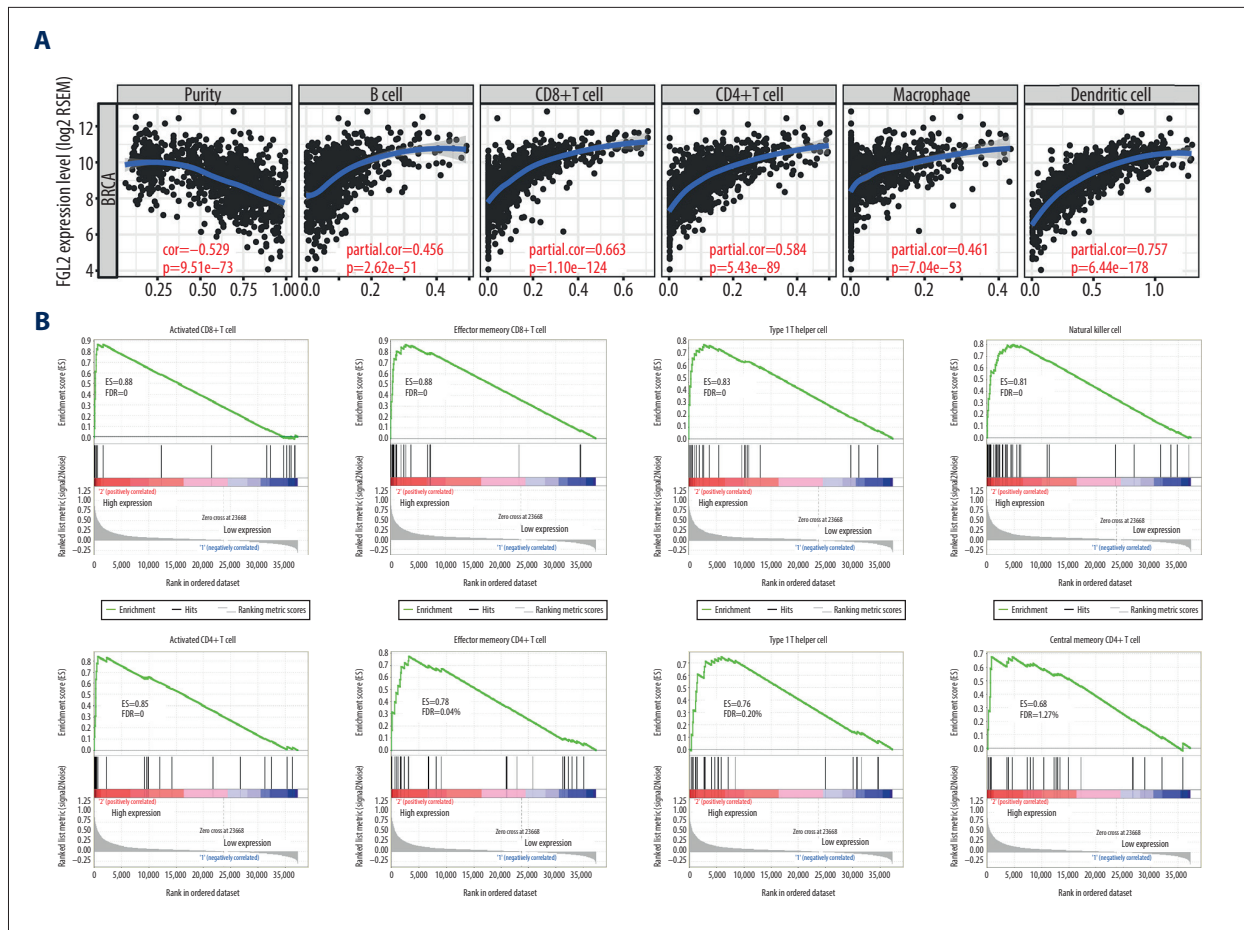


Figure 4. The correlation between *FGL2* expression and immune status in the tumor microenvironment. **(A)** The *FGL2* expression level was positively correlated with infiltrating levels of B cells, CD8⁺ T cells, CD4⁺ T cells, macrophages, neutrophils, and DCs in BRCA. **(B)** The *FGL2* expression level was positively correlated with infiltrating levels of antitumor immune cells, such as activated CD8⁺ T cells, effector memory CD8⁺ T cells, Th1 cells, NK cells, activated CD4⁺ T cells, effector memory CD4⁺ T cells, NKT cells, and central memory CD4⁺ T cells. (ES – enrichment score; FDR – false discovery rate).

for HLA class II gene transcription, is also positively correlated with *FGL2*. Thus, these results showed that *FGL2* can participate in the activities of antigen processing against tumors.

Protein–protein interactions related to *FGL2* in the STRING database

The STRING website was utilized to screen the protein–protein interactions related to *FGL2*. As shown in Figure 5, 21 nodes and 108 edges were filtered in the PPI network complex. Network nodes indicated proteins, and edges indicated the associations between protein and protein. The 10 most significant nodes were: MS4A6A, FGB, FGG, FGA, FGL1, F2, TYROBP, THBS1, FN1, and SERPINF2. In the network nodes, MS4A6A was correlated with *FGL2* (score: 0.801). As a multimeric receptor complex, MS4A6A is involved in signal transduction. The results indicated that *FGL2* contributes to signal transduction in the immune response correlated with MS4A6A.

Functional and pathway enrichment analysis

Functional and pathway enrichment analyses were performed to investigate the main biological signatures of *FGL2* in breast cancer. We analyzed the related genes by Spearman correlation analysis ($|r| > 0.6$ and $P < 0.01$). In total, 479 genes were identified in the TCGA dataset. Then, we performed GO and KEGG analysis in the DAVID website. GO analysis showed that *FGL2*-correlated genes were mainly enriched in immune response, signal transduction, leukocyte migration, T cell costimulation/differentiation/proliferation/activation, B cell receptor signaling pathway, and B cell proliferation (Figure 6A). KEGG pathway analysis showed that *FGL2*-correlated genes were mostly associated with cell adhesion molecules in breast cancer. Additionally, KEGG pathway analysis suggested that *FGL2*-correlated genes were also associated with other canonical immune pathways, including cytokine-cytokine receptor interaction, chemokine signaling pathway, and T cell receptor signaling pathway (Figure 6B).

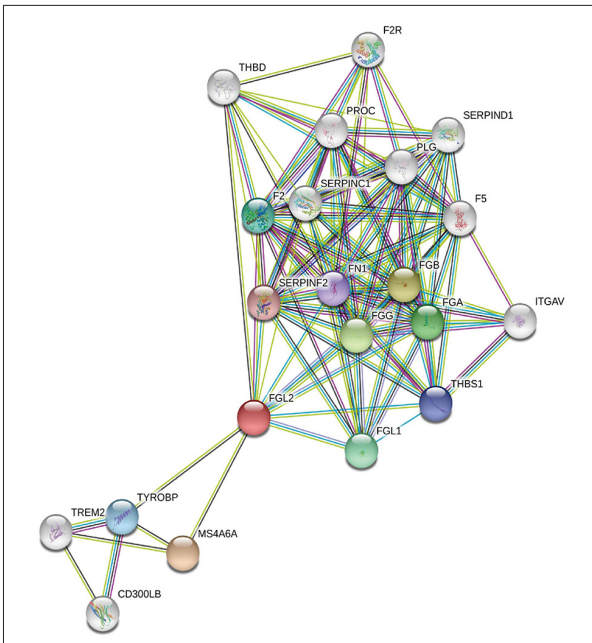


Figure 5. Modular analysis of the protein–protein interaction (PPI) network complex. In the STRING database, 21 proteins were filtered in the network complex. The PPI module consisted of 21 nodes and 108 edges. The average local clustering coefficient was 0.78. The PPI enrichment P-value was less than 1.0e-16. The top 5 predicted functional partners were MS4A6A (score: 0.801), FGB (score: 0.781), FGG (score: 0.776), FGA (score: 0.768), and FGL1 (score: 0.748). The blue and purple edges are considered known interactions. Green, red, and deep blue edges are predicted interactions. Orange, black, and light blue represent text mining, coexpression, and protein homology, respectively.

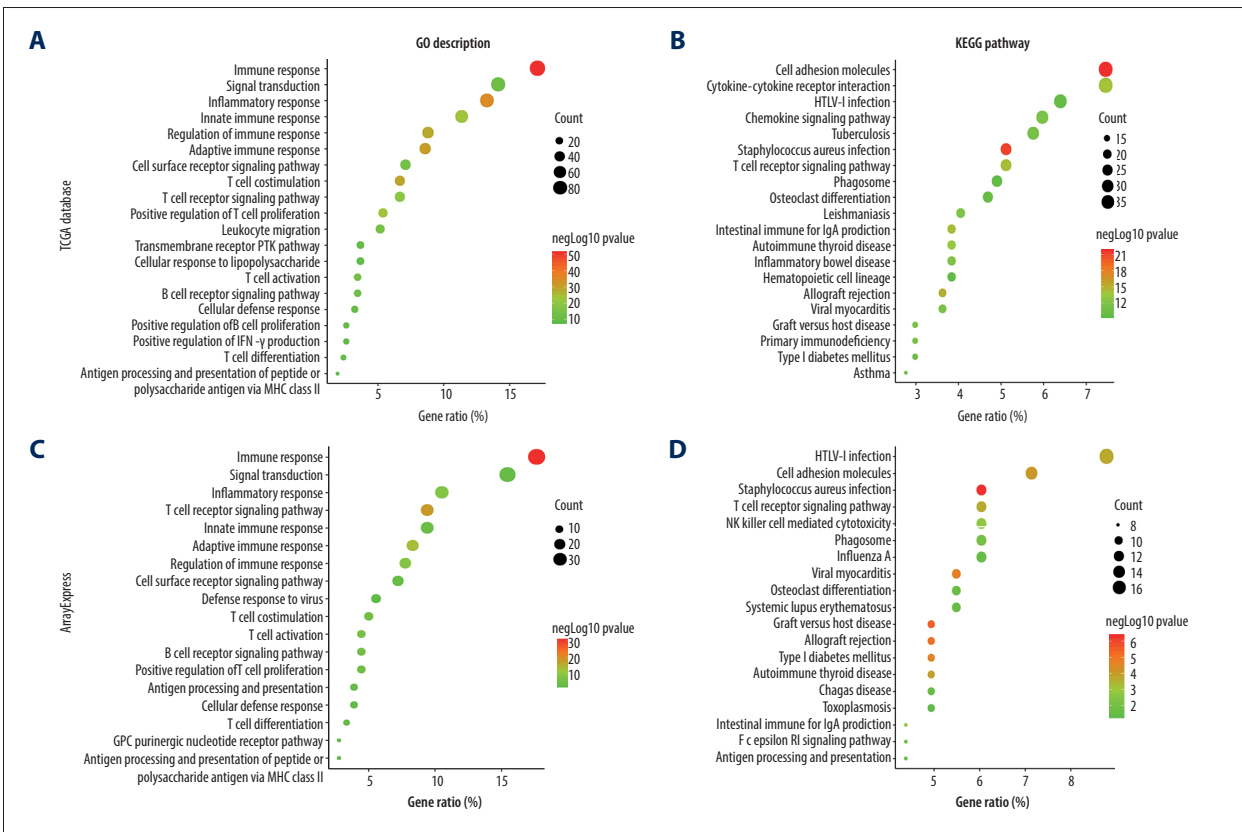


Figure 6. GO analysis and KEGG pathway analysis of FGL2-related genes in breast cancer. (A) GO analysis of FGL2 in breast cancer with the TCGA database. FGL2 was correlated with the immune response, signal transduction, and inflammatory response. (B) KEGG pathway analysis of FGL2 in breast cancer with the TCGA database. FGL2 was correlated with cell adhesion molecules and cytokine-cytokine receptor interactions. (C) GO analysis of FGL2 in breast cancer with the ArrayExpress dataset. FGL2 was correlated with the immune response, signal transduction, and inflammatory response. (D) KEGG pathway analysis of FGL2 in breast cancer with the ArrayExpress dataset. FGL2 was correlated with HTLV-1 infection and cell adhesion molecules.

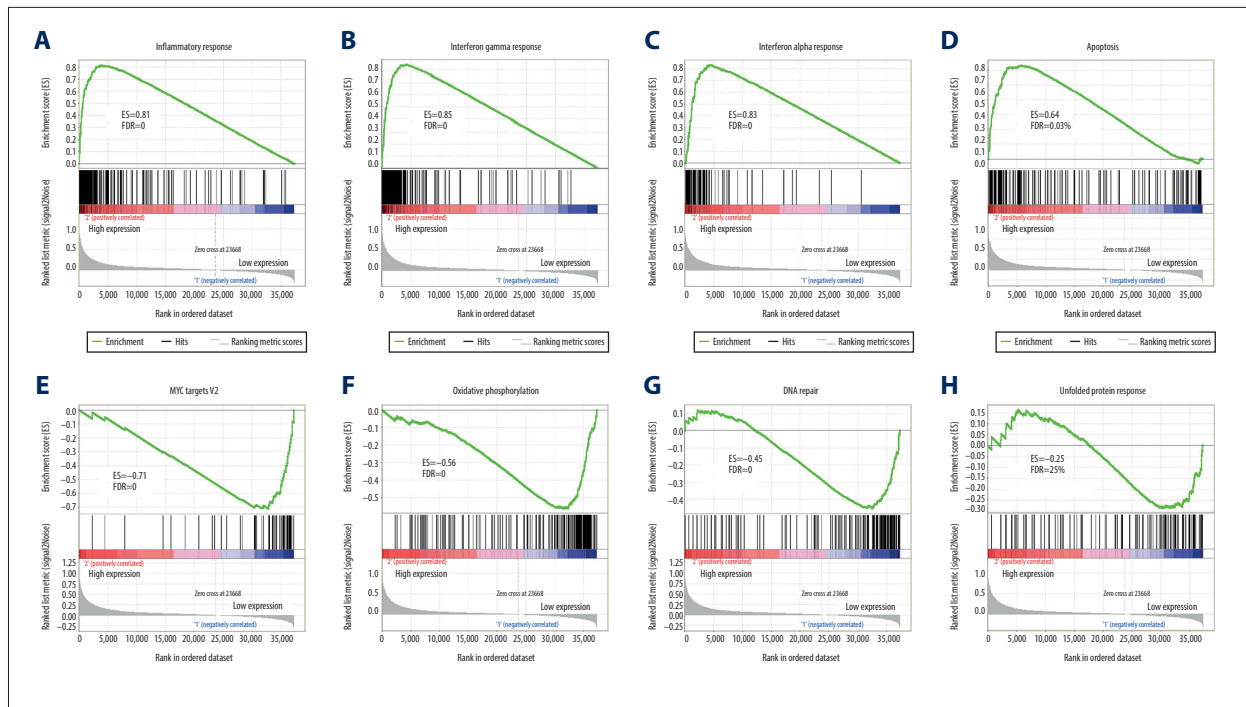


Figure 7. GSEA was used to predict the biological functions of FGL2 in breast cancer. (A) Inflammatory response, (B) Interferon-gamma response, (C) Interferon-alpha response, (D) Apoptosis, (E) MYC targets V2, (F) Oxidative phosphorylation (G) DNA repair, (H) Unfolded protein response. (ES – enrichment score; FDR – false discovery rate).

To validate the results calculated in the DAVID website, we used a microarray dataset to investigate the biological signatures of FGL2 in breast cancer on the website of the European Bioinformatics Institute (EMBL-EBI). In total, 185 genes were found in the microarray meta-dataset of breast cancer (E-MTAB-6703). GO analysis and KEGG pathway analysis showed similar results to TCGA dataset analyses (Figure 6C, 6D). These results demonstrated that FGL2 was positively correlated with immune signaling pathways.

Gene set enrichment analysis (GSEA)

For validation, we used GSEA analysis to predict the biological function of FGL2 in breast cancer. As shown in Figure 7, GSEA suggested that increased *FGL2* expression was positively associated with the inflammatory response in breast cancer. The results also indicated that *FGL2* was positively correlated with inflammatory signaling pathways, including the IFN- γ response, IFN- α response, and apoptosis. *FGL2* was negatively correlated with MYC targets V2, oxidative phosphorylation, DNA repair, and unfolded protein response.

Discussion

FGL2 is a member of the fibrinogen superfamily of proteins, which are mainly expressed by inflammatory cells, including

macrophages, CD4⁺ T, CD8⁺ T, and endothelial cells [10]. FGL2 can oligomerize into a tetramer by forming interchain disulfide bonds. Previous studies have demonstrated that FGL2 exhibits pleiotropic effects in the immune system. Liu et al. found that FGL2 contributes to complement activation and coagulation in virus-induced fulminant hepatitis [16]. Sun et al. showed that FGL2 regulates the functions of T cells in cirrhotic patients with hepatocellular carcinoma [17]. Khattar et al. reported that mice deficient in FGL2 have increased early viral replication of acute viral hepatitis [18]. Owing to the important role of FGL2 in immune regulation, this protein has attracted increasing attention in recent years.

Cancer is a type of disease involving abnormal cell growth correlated with abnormal immune system function. Researchers have focused on the relationship between FGL2 and several types of cancers. Birkhauser et al. found that a mouse renal carcinoma model had elevated expression levels of *FGL2*, *CCL1*, *CXCL9*, and *HMGB1* [19]. These differentially-expressed genes contribute to altered tumor microenvironment in renal carcinoma. Yan et al. reported that FGL2 promotes the development of glioblastoma multiforme (GBM) by inhibiting CD103⁺ dendritic cell differentiation [20]. However, the expression and significance of FGL2 in breast cancer have not been investigated.

In our study, we examined *FGL2* expression in breast cancer using the TCGA and the Oncomine datasets. By utilizing the TCGA

database, we demonstrated that *FGL2* expression was remarkably lower in breast invasive carcinoma (BRCA) compared with normal tissues. The same result was verified in the Oncomine database. We also estimated the correlation between *FGL2* and clinical features of breast cancer. *FGL2* levels were significantly correlated with radiation therapy and PR status. In addition, *FGL2* was associated with tumor (T) stages in breast cancer. These results suggested that *FGL2* has potential as a diagnostic biomarker of breast cancer. In addition, we estimated the correlation between the *FGL2* expression level and survival prognosis of breast cancer with the GEPIA, PrognoScan, and KM plotter datasets. The results showed that lower *FGL2* expression level was correlated with poor breast cancer prognosis, which indicated that low *FGL2* expression can act an independent risk factor for poor survival prognosis of breast cancer patients.

Another aspect in our study is the relationship between *FGL2* and immune status in the tumor microenvironment of breast cancer. Our results demonstrated that *FGL2* level was positively correlated with infiltrating levels of B cells, macrophages, CD8⁺ T cells, CD4⁺ T cells, and DCs in breast cancer. Moreover, *FGL2* expression was positively correlated with infiltrating levels of antitumor cells, such as effector memory CD8⁺ T cells, activated CD8⁺ T cells, Th1 cells, natural killer cells, activated CD4⁺ T cells, effector memory CD4⁺ T cells, natural killer T cells, and central memory CD4⁺ T cells. In brief, these results indicate that *FGL2* is positively correlated with antitumor activities in breast cancer.

Furthermore, we evaluated the protein–protein interaction related to *FGL2* in the STRING database. The PPI network complex showed that *FGL2* was correlated with MS4A6A, FGB, FGG, FGA, FGL1, F2, TYROBP, THBS1, FN1, and SERPINF2. MS4A6A is a member of the membrane-spanning 4A gene family and participates in signal transduction as a factor in a multimeric receptor complex [21]. FGB together with FGG and FGA polymerizes to form an insoluble fibrin matrix, which participates in cell migration [22]. FGL1 is a member of the fibrinogen family of proteins, which also includes *FGL2* [23]. F2 functions include inflammation and wound healing. TYROBP is a transmembrane signaling polypeptide involved in signal transduction [24]. THBS1 mediates cell-to-matrix and cell-to-cell interactions [25]. FN1 is involved in cell adhesion and migration processes [26]. SERPINF2 is a member of the serpin family of serine protease inhibitors. *FGL2* is correlated with signal transduction, cell adhesion, cell-to-cell interactions, and inflammation by interacting with other proteins. We also used GO and KEGG analysis to analyze biological functions and related pathways of *FGL2*-correlated genes in breast cancer. GO analysis indicated that *FGL2*-correlated genes were involved in the immune response, inflammatory response, innate immune response, regulation of immune response, and adaptive immune response in breast cancer. This result showed that *FGL2* is closely correlated with immune activities in breast cancer. In addition, GO analysis in

this study revealed that *FGL2*-correlated genes participate in signal transduction, cell surface receptor signaling pathway, T cell costimulation, T cell receptor signaling pathway, and cell adhesion. MHC class II molecules present antigens to T cells and activate T cells [27]. The biological processes of MHC class II molecules include signal transduction, the cell surface receptor signaling pathway, cell adhesion, T cell costimulation, and the T cell receptor signaling pathway. Therefore, these results are in agreement with the conclusion that *FGL2* is positively correlated with MHC class II for antigens expressed in breast cancer.

Finally, we performed GSEA analysis to investigate the biological functions of *FGL2* associated with breast cancer. *FGL2* was positively correlated with the inflammatory response. Increased *FGL2* enhanced the inflammatory response in breast cancer, including the IFN- γ response, IFN- α response, complement, and apoptosis. IFN- γ is the signature cytokine of Th1 cells and is also produced by NK cells. IFN- γ is necessary for CD4⁺ T cell-mediated antitumor immunity, partially by the upregulation of HLA class II expression. IFN- γ is also responsible for inhibiting angiogenesis in tumors. IFN- α can activate CD8⁺ central memory T cells, CD4⁺ effector memory T cells, and NK cells. IFN- α treatment has moderate success and a clinical response for renal carcinoma, melanoma, and breast cancer. CD8⁺ T cells can induce tumor apoptosis via Fas/FasL interactions. *FGL2* is negatively correlated with the MYC targets V2, oxidative phosphorylation, DNA repair, and unfolded protein response, which are all involved in tumorigenesis.

Conclusions

In summary, we showed that *FGL2* expression was significantly lower in breast cancer compared with adjacent normal tissues and that *FGL2* levels were significantly associated with radiation therapy, PR status, and tumor (T) stage. Lower *FGL2* expression was correlated with poor prognosis of breast carcinoma. *FGL2* was positively correlated with antitumor immune cells infiltration in breast cancer. PPI analysis showed that *FGL2* was related with signal transduction, cell adhesion, cell-to-cell interactions, and inflammation by interacting with other proteins. GO, KEGG, and GSEA analyses also demonstrated that *FGL2* expression was positively associated with activation of CD8⁺ T cells, Th1 cells, NK cells, CD4⁺ T cells, and NKT cells, which may be regulated via the IFN- γ response, IFN- α response, complement, and apoptosis pathways. Our findings indicate that *FGL2* is a promising biomarker for clinical prediction in human breast cancer, and high expression levels of *FGL2* are positively associated with enhanced antitumor activities.

Conflicts of interest

None.

Supplementary Data

Supplementary Table 1. FGL2 was positively correlated with most immune marker sets in the TIMER database.

Description	Gene markers	Cor	P	Description	Gene markers	Cor	P	
T cell (general)	CD3D	0.589	6.66E-94	CTL (cytotoxic T lymphocytes)	CD8A	0.61	2.93E-102	
	CD3E	0.621	3.80E-107		CD8B	0.486	4.19E-60	
	CD2	0.658	2.29E-124		GZMB	0.45	1.12E-50	
B cell	CD19	0.362	2.53E-32	Dendritic cell	HLA-DPB1	0.65	2.23E-120	
	CD79A	0.386	1.37E-36		HLA-DQB1	0.472	2.83E-56	
	CD79B	0.341	1.59E-28		HLA-DRA	0.758	2.83E-186	
	CD22	0.225	6.93E-13		HLA-DPA1	0.774	2.36E-199	
M1 macrophage	INOS	0.003	9.33E-01		DEC-205	0.411	9.88E-42	
	CIITA	0.648	2.39E-119		BDCA-1	0.46	2.96E-53	
	IRF5	0.289	1.49E-20		BDCA-4	0.292	5.93E-21	
	COX2	0.196	4.34E-10		BDCA-2	0.466	8.39E-55	
M2 macrophage	CD163	0.568	6.67E-86		CD11c	0.547	1.03E-78	
	IRF4	0.591	1.22E-94		Th1	CD38	0.555	2.26E-81
	VSIG4	0.476	2.84E-57		T-bet	0.597	3.80E-97	
	MS4A4A	0.655	9.05E-123		STAT4	0.612	4.38E-103	
TAM	CCL2	0.431	2.84E-46		STAT1	0.483	3.35E-59	
	CCL5	0.529	1.02E-72		IFN- γ	0.519	1.19E-69	
	CD68	0.562	9.57E-84		TNF- α	0.224	9.45E-13	
	IL10	0.551	5.84E-80		Th2	GATA3	-0.107	7.48E-04
Neutrophils	CD66b (CEACAM8)	0.031	3.33E-01		IL13	0.184	5.48E-09	
	CD15	0.327	2.91E-26		STAT6	0.044	1.63E-01	
	CD11b (ITGAM)	0.517	6.19E-69		Tfh	BCL6	0.108	6.77E-04
	CCR7	0.49	4.27E-61		CD200	0.358	1.79E-31	
Natural killer cell	NKp46	0.457	1.99E-52		IL21	0.387	7.16E-37	
	NKp44	0.165	1.61E-07		ICOS	0.604	0.09E-100	
	NKp30	0.473	1.32E-56		Th17	STAT3	0.138	1.19E-05
	FCGR3A	0.592	4.66E-95		IL17A	0.16	3.69E-07	
	FCGR3B	0.325	6.52E-26		IL1A	0.295	1.86E-21	
	NKG2A (KLRC1)	0.468	3.30E-55		IL1B	0.444	2.40E-49	
	KIR2DL1	0.277	5.07E-19		CCL20	0.119	1.73E-04	
	KIR2DL3	0.312	7.11E-24		FOXP3	0.493	4.48E-62	
	KIR3DL1	0.337	8.71E-28		Treg	CCR8	0.562	6.60E-84
				TGF β	0.209	2.95E-11		

Supplementary Table 2. FGL2 was positively correlated with most immune marker sets in the GEPIA database.

Description	Gene markers	Cor	P	Description	Gene markers	Cor	P
T cell (general)	CD3D	0.51	0	CTL (cytotoxic T lymphocytes)	CD8A	0.64	0
	CD3E	0.56	0		CD8B	0.44	0
	CD2	0.66	0		GZMB	0.45	0
B cell	CD19	0.23	1.40E-14	Dendritic cell	HLA-DPB1	0.65	0
	CD79A	0.28	0		HLA-DQB1	0.39	0
	CD79B	0.26	0		HLA-DRA	0.74	0
	CD22	0.19	2.00E-10		HLA-DPA1	0.76	0
M1 Macrophage	INOS	-0.021	4.90E-01	DEC-205	0.44	0	
	CIITA	0.54	0	BDCA-1	0.48	0	
	IRF5	0.34	0	BDCA-4	0.39	0	
	COX2	-0.022	4.70E-01	BDCA-2	0.38	0	
M2 Macrophage	CD163	0.45	0	CD11c	0.53	0	
	IRF4	0.44	0	Th1	CD38	0.55	0
	VSIG4	0.46	0		T-bet	0.65	0
	MS4A4A	0.64	0		STAT4	0.64	0
TAM	CCL2	0.35	0		STAT1	0.5	0
	CCL5	0.5	0	IFN-γ	0.47	0	
	CD68	0.53	0	TNF-α	0.15	5.80E-07	
	IL10	0.56	0	Th2	GATA3	-0.16	2.40E-07
Neutrophils	CD66b (CEACAM8)	0.049	1.10E-01		IL13	0.26	0
	CD15	0.41	0	STAT6	0.24	1.80E-15	
	CD11b (ITGAM)	0.31	0	Tfh	BCL6	0.18	3.70E-09
	CCR7	0.2	2.60E-11		CD200	0.43	0
Natural killer cell	NKp46	0.59	0	IL21	0.53	0	
	NKp44	0.02	5.10E-01	ICOS	0.64	0	
	NKp30	0.3	0	Th17	STAT3	0.27	0
	FCGR3A	0.59	0		IL17A	0.097	1.40E-03
	FCGR3B	0.078	1.10E-02		IL1A	0.039	2.00E-01
	NKG2A (KLRC1)	0.35	0		IL1B	0.37	0
	KIR2DL1	0.021	4.90E-01	CCL20	0.019	5.20E-01	
	KIR2DL3	0.32	0	Treg	FOXP3	0.54	0
KIR3DL1	0.34	0	CCR8		0.44	0	
				TGFβ	0.25	0	

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