Multi-Omics Analyses Revealed GOLT1B as a Potential Prognostic Gene in Breast Cancer Probably Regulating the Immune Microenvironment

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As recently reported by The International Agency for Research on Cancer (IARC), breast cancer has the highest incidence of all cancers in 2020. Many studies have revealed that golgi apparatus is closely associated with the development of breast cancer. However, the role of golgi apparatus in immune microenvironment is still not clear. In this study, using RNA-Seq datasets of breast cancer patients from the public database (n = 1080), we revealed that GOLT1B, encoding a golgi vesicle transporter protein, was significantly higher expressed in human breast cancer tissues versus normal tissues. Besides, we verified GOLT1B expression in five breast cancer cell line using our original data and found GOLT1B was significantly up-regulated in MDA-MB-231, MCF-7, SKBR3. Subsequently, we identified GOLT1B as a potential independent prognostic factor for breast cancer. After a multi-omics analysis, we uncovered that the higher expression of GOLT1B in breast cancer tissues versus normal tissues might be due to the amplification of GOLT1B and altered phosphorylation of its potential transcription factors, including JUN and SIN3A. Subsequently, we discovered that GOLT1B potentially regulated the immune microenvironment basing on the finding that its expression was closely related to the tumor microenvironment score and infiltration of immune cells. Moreover, we revealed that GOLT1B might affect the overall survival rates of breast cancer through regulating the immune cell infiltration. Finally, we disclosed the potential pathways involved in the functions of GOLT1B in breast cancer, including metabolism and ECM-receptor interaction pathways. To sum up, we identified GOLT1B as a potential prognostic gene for breast cancer and disclosed its role in regulating the immune microenvironment.

Keywords: GOLT1B, Golgi apparatus, immune microenvironment, breast cancer, prognostic biomarker

INTRODUCTION

The International Agency for Research on Cancer (IARC) has released "the latest data on the global burden of cancer in 2020" (1) showing that the incidence of breast cancer has replaced lung cancer in the first place in the world, accounting for 11.7% of new cancer cases. Nowadays, with the progress of diagnosis and treatment, although the 5-year survival rate of breast cancer has been improved, the mortality rate of patients with advanced breast cancer still reach to more than 70% (2). Nevertheless, distinguishing high-risk patients by prognostic biomarkers can frequently lead to an appropriate individualized treatment and thus reduce the mortality.

Recently, immunotherapy has attracted a lot of attention because of its long-lasting responses to different types of tumors, even advanced tumors (3, 4). However, the effects of immunotherapy in breast cancer are not satisfactory. Consequently, there is a necessary to further explore the mechanism underlying the development of breast cancer and search for key regulatory genes of immune microenvironment. Golgi apparatus is a secretory organelle composed of many flat vesicles in eukaryotic organisms (5). It's mainly involved in biological processes including protein processing, sorting, and transportation. Golgi apparatus is closely related to innate immune signal transduction and subsequent effect response (6). Although the essential role of the golgi apparatus in carcinogenesis has been well characterized, its functions in tumor immune microenvironment are still unclear.

GOLT1B encodes a golgi vesicle transporter that mediates vesicle transport between endoplasmic reticulum and golgi apparatus and is highly conserved in function. One current study has uncovered that the overexpression of GOLT1B can elevate the cell membrane level of DVL2, then activate Wnt/β-catenin pathway, increase the content of nuclear β-catenin, and subsequently induce the process named epithelial-mesenchymal transformation. On the other hand, GOLT1B also promotes the migration and invasion of colorectal cancer *via* inducing T lymphocyte apoptosis (7). Poorer prognosis has been observed in patients with GOLT1B amplifications in lung adenocarcinoma (8). However, little is known about the functions of GOLT1B in tumors. The role of GOLT1B in breast cancer and its functions in immune microenvironment have not been disclosed.

Here, using the multi-platform datasets from the public database, we described the expression, survival correlation, and potential prognostic values of GOLT1B in breast cancer, uncovered GOLT1B potential upstream regulators and relevant pathways, and demonstrated the probable functions of GOLT1B in immune microenvironment.

MATERIALS AND METHODS

Tumor Immune Estimation Resource (TIMER)

TIMER is a comprehensive web resource for systematical evaluations of immune cells in diverse cancers. Differential expression of GOLT1B in 27 tumor tissues versus adjacent normal tissues from the Cancer Genome Atlas (TCGA) was studied using the Diffexp module.

The Human Protein Atlas (HPA)

HPA provides the immunohistochemical results of protein expression in normal tissues and tumor tissues. The protein expression of GOLT1B in normal mammary tissue and breast carcinoma tissues was evaluated using the HPA.

UALCAN

UALCAN is a database for analysis and mining of transcriptome data from TCGA. The mRNA expression of GOLT1B and the relationship between GOLT1B and clinicopathological parameters of breast cancer (gender, tumor stage, lymph node metastatic status, age, ethnicity, and TP53 mutation status) was investigated using UALCAN. Besides, the protein and phosphorylation expression were also analyzed using UALCAN.

Kaplan-Meier Plotter

Kaplan-Meier Plotter database collects the datasets of gene chip and RNA-seq from European Genome-phenome Archive (EGA), TCGA, Gene Expression Omnibus(GEO), and other public databases. The overall survival rates (OS), relapse-free survival rates (RFS), and distant metastasis-free survival rates (DMFS) of GOLT1B in breast cancer were analyzed using Kaplan-Meier Plotter database. Besides, the relationship between GOLT1B expression and the OS of breast cancer patients with different immune infiltration was also investigated using Kaplan-Meier Plotter database.

Gene Expression Profiling Interactive Analysis (GEPIA)

GEPIA is a database analyzing gene expression based on datasets from genotype tissue expression (GTEx) and TCGA. In this study, the expression of GOLT1B in breast carcinoma tissues and mammary tissues was evaluated using the module "Expression DIY" of GEPIA.

cBioPortal

The cBioPortal database is a genomic database characterizing gene mutations in distinct tumors. More than 28,000 samples from different independent studies were included in this database. The type and frequency of GOLT1B mutations in invasive breast cancer were analyzed using the module named "Oncoprint" and "Cancer Types Summary" of cBioPortal.

Linkedomics

Linkedomics is a comprehensive database that contains multi-omics datasets within and across 32 cancer types. In our study, 5720 GOLT1B co-expressed proteins were obtained from the Linkedomics database, and the pathways that GOLT1B involved in were investigated using the module "Gene Set Enrichment Analysis (GSEA)" of Linkedomics.

Bioinformatics

The immune infiltration were investigated using CIBERSORT database following a standard protocol (9). The R package named survival v.2.4.2 was used to analyse the survival rates.

The potential prognostic value was calculated using the R package named Survival and RMS.

Cell Lines and Antibody

The immortalized normal mammary epithelial cell line MCF-10A and breast cancer cell lines MDA-MB-231, MDA-MB-231-Bone, SKBR3, MCF-7, and T47D were derived from the Key Laboratory of Clinical Laboratory diagnostics of Chongqing Medical University. The MCF-10A was cultured with MCF-10A cell-specific medium (Procell, CM-0525, China), MDA-MB-231, MDA-MB-231-Bone, SKBR3, MCF-7, and T47D were cultured with DMEM medium (Gibco, USA) containing 10% fetal bovine serum (Gibco, USA). Cells were placed in a humidified incubator at 37°C with 5% CO_2. The antibody of golt1b for western blotting was purchased from Invitrogen (PA5-103499) and the antibody of actin was purchased from Zoonbi (TE0303).

Statistical Analysis

The significance of differential expression was evaluated using Wilcoxon test. Logarithmic rank method was utilized to calculate the significance of survival analyses. A univariate Cox regression model was used to analyze the hazard ratio (HR) of GOLT1B, and a multivariate Cox regression model was used to determine potential independent prognostic factors. HR and confidence interval were set to 95%. The correlation coefficient between GOLT1B expression and immune infiltration was calculated using the Pearson tests. $p < 0.05$ was considered statistically significant. "*" indicated $p < 0.05$, "**" indicated $p < 0.01$, "***" indicated $p < 0.001$, and "****" indicated $p < 0.0001$.

RESULTS

The Expression of GOLT1B Is Increased in Breast Cancer Patients

To explore the functions of GOLT1B in tumorigenesis, we primarily analyzed the mRNA expression of GOLT1B in 27 human tumors using the datasets from TCGA and TIMER database. The results showed that GOLT1B was up-regulated in 25 types of tumors, such as breast invasive carcinoma (BRCA), adrenal cortical carcinoma (ACC), cholangiocarcinoma (CHOL), esophageal carcinoma (ESCA), clear cell carcinoma of the kidney (KIRC), squamous cell carcinoma of the head and neck (HNSC), liver hepatocellular carcinoma (LIHC), acute myeloid leukemia (LAML). Meanwhile, GOLT1B was significantly down-regulated in endometrial cancer (UCEC) and acute myeloid leukemia (LAML) (**Supplementary Figures S1A, B**). Focusing on breast cancer, we validated GOLT1B expression in breast cancer using GEPIA and UALCAN databases. Shown in **Figures 1A, B**, the mRNA expression of GOLT1B was higher in BRCA tissues than normal breast tissues. Besides, we also investigated the expression of GOLT1B in BRCA tissues and para-cancerous tissues using the datasets from TCGA, and the results showed that GOLT1B mRNA was significantly elevated in BRCA tissues (**Figure 1C**). Moreover, we disclosed that the expression of GOLT1B in BRCA was significantly increased using pair analysis of 112 BRCA tissues versus para-cancerous tissues (**Figure 1D**).

Furthermore, we evaluated the expression of GOLT1B protein in breast cancer tissues and normal tissues using The Human Protein Atlas database. As a result, GOLT1B protein was elevated in breast carcinima versus normal mammary tissues (**Figure 1E**). Next, we further confirm the protein expression level of GOLT1B in breast cancer cell lines using western blotting. The results showed that GOLT1B was higher expressed in MDA-MB-231 (P = 0.0011), SKBR3 (P < 0.0001) and MCF-7 (P < 0.0001) versus the normal mammary epithelial cell line MCF-10A (**Figure 1F**). These results indicated that the expression of GOLT1B was up-regulated in human breast cancer and implied a potentially important role of GOLT1B in cancer progression.

The Correlation Between GOLT1B Expression and Clinical Features of Breast Cancer

To clarify the correlation between the GOLT1B expression and clinical features of breast cancer, we studied GOLT1B mRNA expression in different groups using the UALCAN database. In terms of age, breast cancer patients aged 21-40, 41-60, and 61-80 years expressed higher levels of GOLT1B than healthy people (P < 0.05) (**Supplementary Figure S2A**). For gender, tumor stage and race, there was no significant difference between distinct groups (**Supplementary Figures S2B–D**). In terms of tumor subtypes, compared with luminal breast cancers, triple-negative type showed higher expression of GOLT1B (P < 0.0001), indicating that GOLT1B might be correlated with tumor malignancy (**Supplementary Figure S2E**). In terms of lymph node metastases, GOLT1B was lower expressed in patients classified as N3 than in N0, N1, and N2 patients (P = 0.032, P = 0.033, P = 0.026) (**Supplementary Figure S2F**). Finally, GOLT1B was up-expressed in patients with TP53 mutant than TP53 wild type (P < 0.0001) (**Supplementary Figure S2G**), implying that the high expression of GOLT1B might have a potential association with TP53 mutation.

Increased Expression of GOLT1B Predicts Poor Prognosis in Breast Cancer Patients

Since GOLT1B is potentially associated with the initiation and progression of breast cancer, we explored the relationship between GOLT1B mRNA expression and patient survival using the RNA-Seq datasets from TCGA. Analysis of breast cancer patients (n = 1084) showed that the OS, progression-free survival rate (PFI), and disease-specific survival rate (DSS) were lower in breast cancer patients expressing higher GOLT1B (**Figure 2A**). After analyzing the outcomes using Kaplan Meier database, we also revealed that breast cancer patients (n = 1089) with high expression of GOLT1B exhibited poorer OS, DMFS, and RFS (**Figure 2B**). Furthermore, we investigated the correlation between GOLT1B protein expression and patient survival and revealed that GOLT1B expression was extremely negatively related to patient OS, DMFS, and RFS (**Figure 2C**). Subsequently, to further clarify whether GOLT1B is one potential prognostic gene in human breast cancer, we analyzed other cohorts from prognoScan database and GEO (**Figure 2D**). Consequently, the corrected p values of OS (GSE1456), DSS
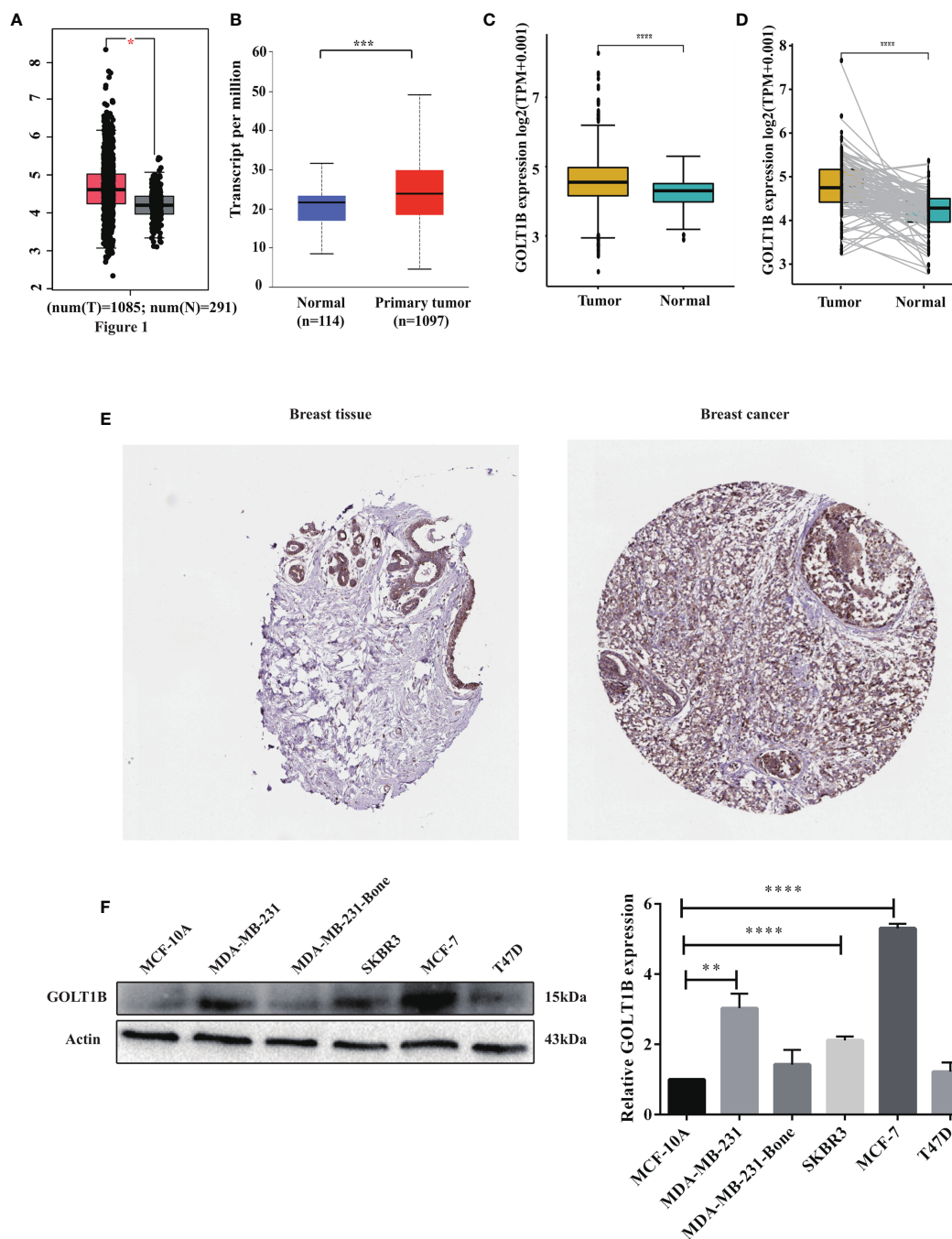
FIGURE 1 | The mRNA and protein expression of GOLT1B was up-regulated in human breast cancer. The expression of GOLT1B in breast cancer tissues versus normal tissues was investigated using the datasets from **(A)** the GEPIA database, **(B)** the UALCAN database, and **(C)** the TCGA database. **(D)** The GOLT1B expression in 112 paired breast cancer tissues and adjacent normal tissues using the datasets from TCGA database. **(E)** The protein expression of GOLT1B in breast cancer tissues and normal breast tissues was evaluated through immunohistochemical tests from The Human Protein Atlas database. **(F)** Western blotting detecting GOLT1B expression in normal mammary epithelial cell line and breast cancer cell lines. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

(GSE1456) and RFS (GSE12276) were all less than 0.05, which additionally provided the possibility of GOLT1B as one prognostic gene in breast cancer. Based on the above results, it is suggested that the mRNA and protein expression of GOLT1B are both closely related to the outcomes of breast cancer. Furthermore, to evaluate the predictive value of GOLT1B, we performed Cox regression on various clinical features and GOLT1B expression. As a result, the univariate and multivariate Cox analyses identified GOLT1B as a
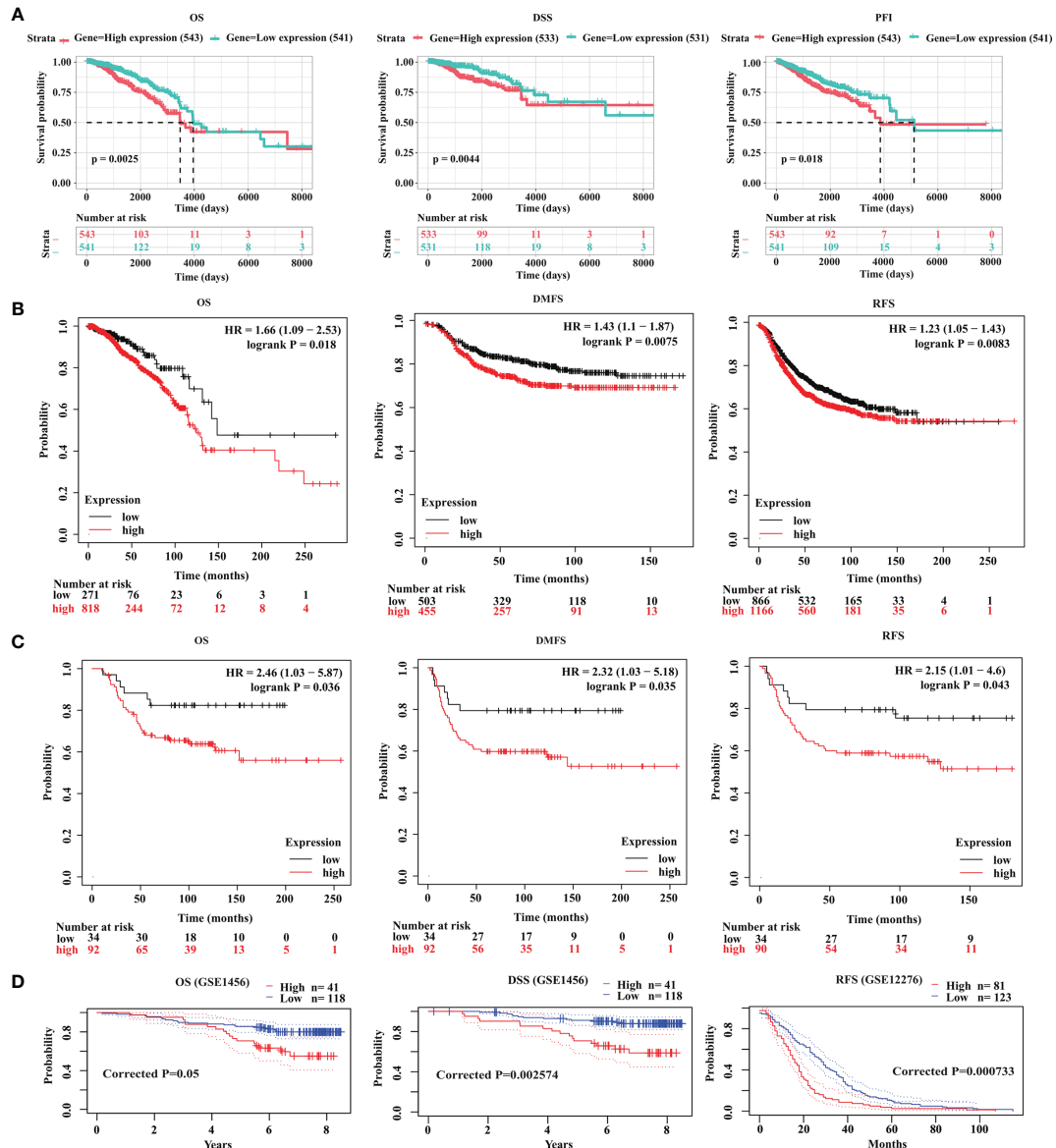
FIGURE 2 | GOLT1B is a potential prognostic factor for breast cancer. **(A)** The correlation between GOLT1B expression and OS, DSS, PFI of breast cancer patients using the datasets downloaded from TCGA database. The correlation between GOLT1B **(B)** mRNA expression and **(C)** protein expression with OS, DMFS, RFS of breast cancer patients as analyzed using the Kaplan-Meier plotter database. **(D)** The correlation between GOLT1B expression and OS, DSS, RFS of breast cancer patients using the datasets from prognoScan and GEO.

potential independent prognostic gene for breast cancer (**Figures 3A, B**), and it could be used in combination with other clinical diagnosis indicators to predict the prognosis of breast cancer (**Figures 3C, D**).

The Potential Mechanisms for the Up-Regulation of GOLT1B in Human Breast Cancer

It's well-known that the expression and activity of transcription factors have a close association with the expression of downstream genes. Therefore, to explore the potential mechanism underlying

the up-regulation of GOLT1B in breast cancer, we performed a multi-omics analysis of the upstream transcriptional factors of GOLT1B. Firstly, we obtained 24 experimentally confirmed transcription factors of GOLT1B in breast tissues using the hTFtarget database (**Supplementary Table S1**). Meanwhile, we analyzed all co-expressed proteins of GOLT1B in breast tumor using the Linkomics database. As a result, 5721 proteins were found to be co-expressed with GOLT1B (**Supplementary Table S1**). The transcriptional factors of GOLT1B, including BRD4, JUN, MAX, and SIN3A, were discovered having a close expression correlation with GOLT1B (correlation coefficient > 0.5
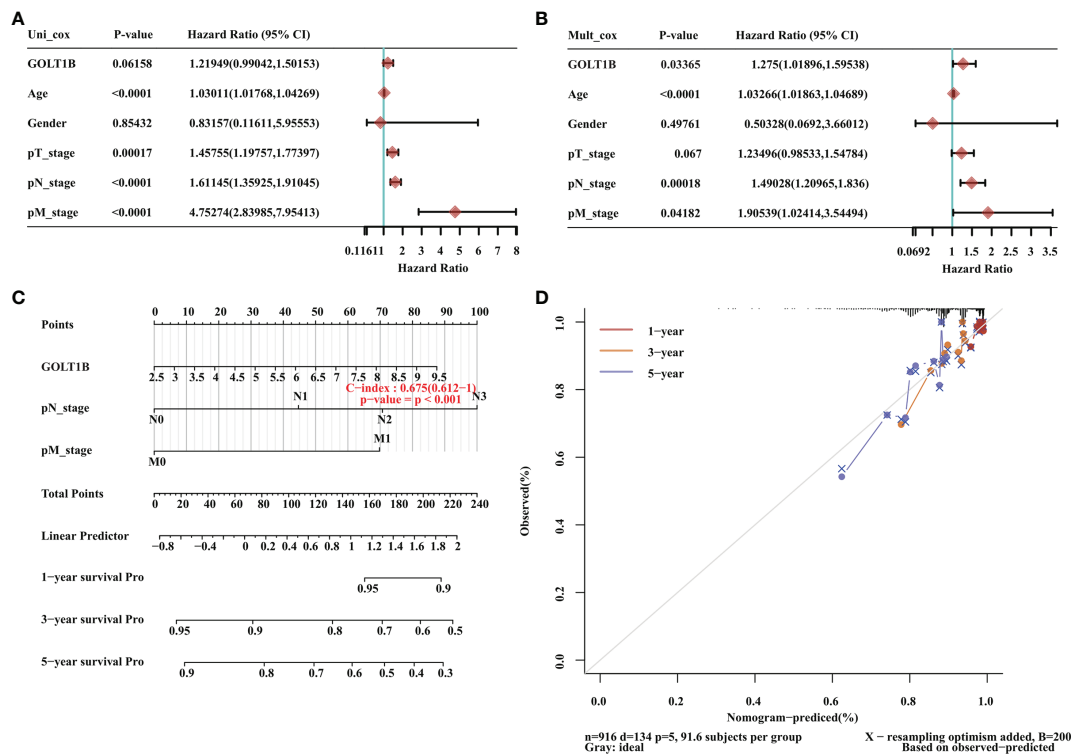
FIGURE 3 | GOLT1B is one potential independent prognostic gene for breast cancer. (A) Univariate Cox analysis revealed that GOLT1B was associated with the risk of breast cancer. (B) Multivariate Cox analysis identified GOLT1B as a potential independent prognostic gene of breast cancer. (C, D) The nomogram showing the function of GOLT1B in the scoring of prognostic risks of breast cancer.

or < -0.5, false discovery rate (FDR) < 0.05). Subsequently, we examined the protein and phosphorylation amount of the four transcriptional factors in breast cancer tissues versus the normal tissues. We observed that the four genes had no significant changes on their protein amount, but the phosphorylation of JUN on Thr 239 and Ser 243, and SIN3A on Ser 940, Ser 1112, and Thr 848 had significant alterations (**Figures 4A–D**). These results suggest that the up-regulation of GOLT1B in breast cancer may be related to the activity alteration of SIN3A and JUN.

On the other hand, we know that mutations may also lead to increased gene expression. Therefore, we investigated the mutation types and frequency of GOLT1B in breast cancer using Cbioportal database (n = 9555). As a result, more than 75% of the patients with GOLT1B mutations were diagnosed with amplifications (**Figures 4E, F**). Besides, we found that the GOLT1B expression was higher in patients with amplifications than the median expression of all mutations (**Figure 4G**). This result indicates that GOLT1B amplifications may be another reason responsible for the increase of GOLT1B expression.

The Functions of GOLT1B in Breast Cancer Are Potentially Associated With the Axis of "Ribosome-Proteasome-Lysosome"

To explore the potential mechanism of GOLT1B in breast cancer, we investigated the influence of GOLT1B on the BRCA signatures.

The results showed that GOLT1B was related to tumor microenvironment (TME) scores, CD8+ T effector cells, immune-checkpoint, antigen-processing-machinery, TME-score-A, mismatch-repair, nucleotide-excision-repair, DNA-damage-response, DNA-replication, base-excision-repair, pan-fibroblast TGFβ response signature (Pan-F-TBRs), epithelial-to-mesenchymal transition 1 (EMT1), EMT2, EMT3, and TME-score-B (**Figure 5A**). Next, to understand the pathways that GOLT1B potentially regulated in breast cancer, we analyzed all co-expressed proteins of GOLT1B using the datasets from the Linkomics database. We obtained 5720 co-expressed proteins, and the top 50 positively-correlated and negatively-correlated genes were shown in **Figures 5B, C**. After analyzing the co-expressed proteins using GSEA, we discovered that GOLT1B was positively correlated with Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, such as proteasome, lysosome, tryptophan and drug metabolism, and pentose phosphate pathway; GOLT1B was negatively correlated with pathways including the ECM-receptor interaction, protein export, and ribosome (**Figure 5D**). It's well-known that ribosomes are responsible for protein synthesis, while proteasomes and lysosomes are related to protein degradation (10–12). Therefore, GOLT1B may be involved in regulating and monitoring protein production in breast cancer, promoting protein synthesis and inhibiting protein degradation.
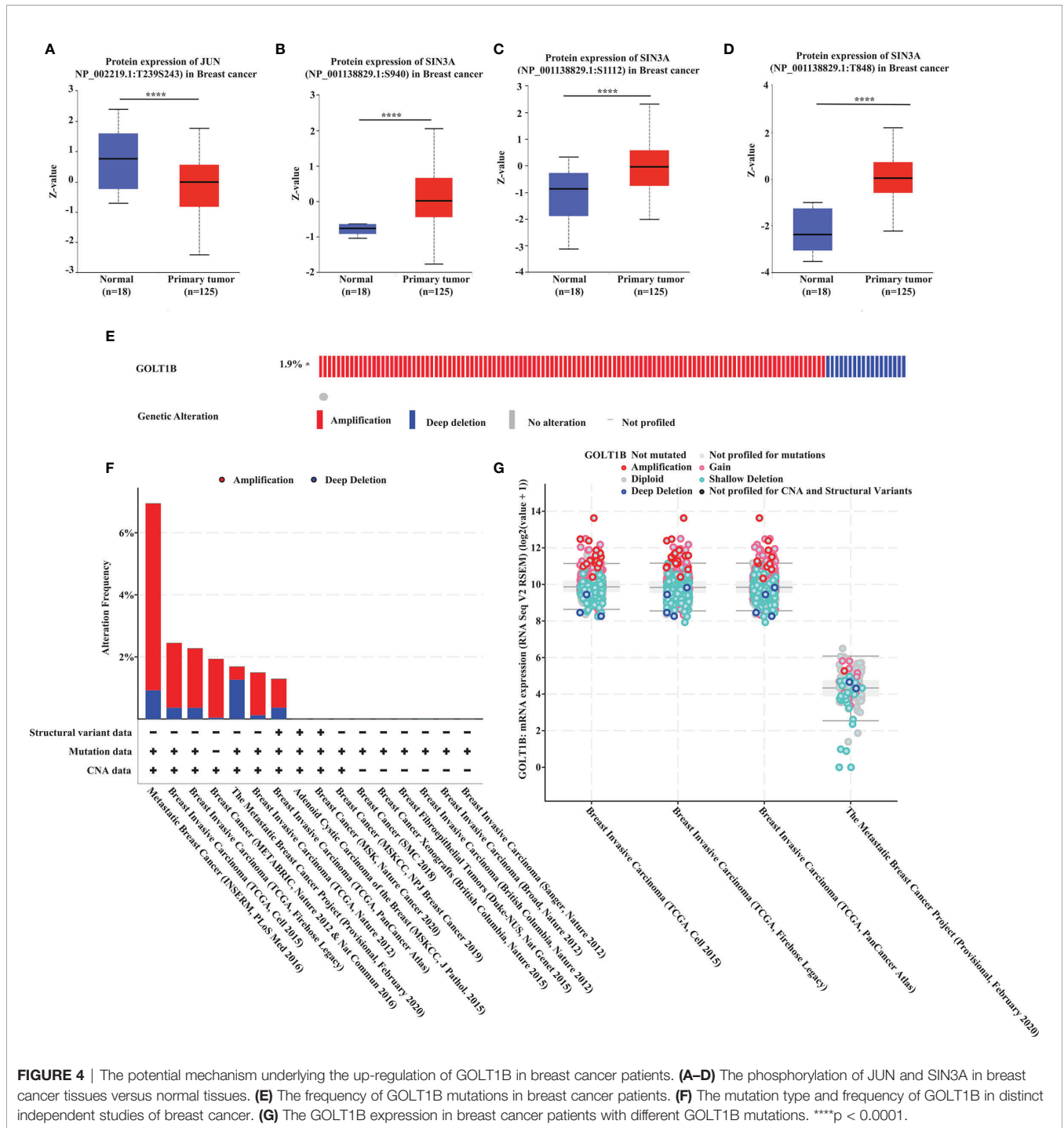
FIGURE 4 | The potential mechanism underlying the up-regulation of GOLT1B in breast cancer patients. **(A–D)** The phosphorylation of JUN and SIN3A in breast cancer tissues versus normal tissues. **(E)** The frequency of GOLT1B mutations in breast cancer patients. **(F)** The mutation type and frequency of GOLT1B in distinct independent studies of breast cancer. **(G)** The GOLT1B expression in breast cancer patients with different GOLT1B mutations. ****p < 0.0001.

GOLT1B Potentially Regulates Immune Microenvironment in Breast Cancer

In the above study, we found that GOLT1B was significantly correlated with TME scores and immune-related signatures including immune-checkpoint, CD8+ T effector cells, and antigen-processing-machinery. To further clarify the correlation between GOLT1B and immune microenvironment in breast cancer, we divided breast cancer patients into GOLT1B

high-expressed and low-expressed groups. We then investigated the correlation between GOLT1B expression and the infiltration of twelve kinds of immune cells, including monocytes, CD8+T cells, CD4+T cells, regulatory T cells, helper T cells, plasma cells, natural killer (NK) cells, neutrophils, macrophages, M0 macrophages, M2 macrophages, and lymphocytes. As a result, the expression of GOLT1B was significantly positively correlated with the infiltration level of four kinds of immune cells, including
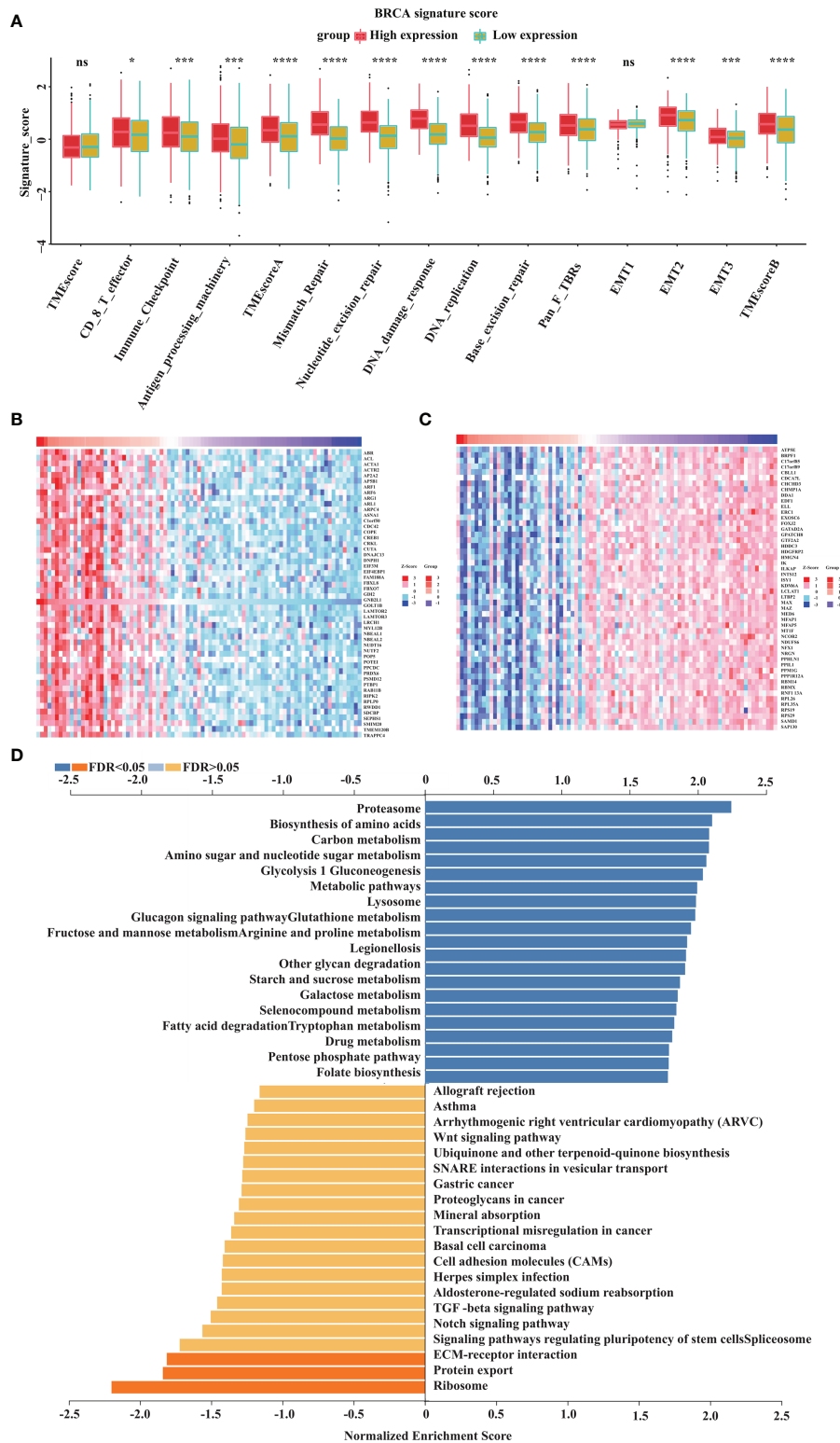
FIGURE 5 | GOLT1B potentially regulates the functions of ribosome, proteasome and lysosome in human breast cancer. **(A)** The correlation between GOLT1B expression and gene signatures. In each group, the scattered dots represent the mean of the signature genes, and the thick lines represent the median value. **(B)** Heat maps of the top 50 genes positively correlated with GOLT1B expression. **(C)** Heat maps of the top 50 genes negatively correlated with GOLT1B expression. **(D)** GSEA analysis showing the positively and negatively correlated pathways of GOLT1B expression. "ns" indicated no statistic significance, *p < 0.05, ***p < 0.001, ****p < 0.0001.

macrophages, M0 macrophages, M2 macrophages, and neutrophils but negatively correlated to monocytes, CD8+T cells, CD4+T cells, regulatory T cells, helper T cells, plasma cells, NK cells, neutrophils (**Figure 6A**). Furthermore, we confirmed the association between GOLT1B and immune infiltration using datasets from CIBERSORT database. The results showed that the expression of GOLT1B was positively correlated with the infiltration of eight kinds of immune cells in breast cancer, including induced regulatory T (iTreg), natural regulatory T (nTreg), macrophages, monocytes, dendritic cells, central memory T cells, regulatory T cells, and type 1 helper T cells, and a significant negative correlation with CD4+T cells, gamma delta T (Tgd), helper follicular T (TFH), mucosal associated invariant T (MAIT), natural killer T (NKT), NK, and CD8+T cells (**Figure 6B**). Subsequently, we revealed that the expression of GOLT1B was positively correlated with some major immune checkpoints, including CD274, TIGIT, and CTLA4 (**Figure 6C**). Furthermore, to confirm that GOLT1B affects tumor progression by regulating the immune microenvironment, we studied the effect of GOLT1B expression on the OS of patients with high/low immune cell infiltration using the Kaplan-Meier Plotter database. As a result, the GOLT1B expression was negatively correlated with the OS in breast cancer patients with decreased infiltration of type 2 helper T cells, but not affect patients with enriched infiltration. Besides, the GOLT1B expression was negatively correlated with the OS in breast cancer patients with enriched infiltration of regulatory T cells, but not affect patients with decreased infiltration (**Figure 6D**). These results demonstrated that GOLT1B was potentially a regulatory factor for the immune infiltration of breast cancer and possibly influences the tumor progression by regulating the immune microenvironment.

DISCUSSION

As one of the most common cancers globally, breast cancer is frequently diagnosed at an advanced stage with poor prognosis, being prone to visceral and bone metastasis (13). Nowadays, the role of golgi apparatus in breast cancer has attracted increasing attention. Some studies demonstrated that golgi somal membrane protein 1 (GOLM1) promoted the proliferation and metastasis of breast cancer cells by regulating matrix metalloproteinase-13 (MMP13) (14). Phosphatidylinositol 4-phosphate in golgi apparatus regulated cell adhesion and invasiveness of breast cancer (15). These studies reveal that golgi apparatus may be a promising target for clinical treatment of breast cancer.

GOLT1B, encoding protein of a golgi transporter, plays an important role in regulating vesicle transport between endoplasmic reticulum and golgi apparatus. One piece of research proclaimed that such vesicle transporters induced cell proliferation in breast cancer (16). Studies have shown that golgi vesicle transporter 1A (GOLT1A) (17, 18), another member of golgi vesicle transporter family, affects tamoxifen sensitivity in breast cancer and promotes cell proliferation in lung cancer.

Besides, high expression of GOLT1B suggests poor prognosis of colorectal cancer, and induces immunosuppression by promoting PD-L2 membrane localization (7). Consistent with the previous report, our study revealed that the expression of GOLT1B was higher in breast cancer tissues than normal tissues (**Figure 1**). Breast cancer patients with high GOLT1B expression had significantly lower survival rates than those patients with low GOLT1B expression (**Figure 2**). GOLT1B was also identified as a potential independent prognostic gene in breast cancer (**Figure 3**). These results all demonstrate that GOLT1B is potentially an oncogene in breast cancer.

The initiation and development of breast cancer are closely related to the interaction between tumor and microenvironment. Breast cancer has a unique immune microenvironment in which vascular endothelial factors are highly expressed, and lymphocytes and tumor-associated macrophages are more infiltrated (19). Based on this, immunotherapy targeting the immune microenvironment has been emerging recently. Nowadays, the immunocheckpoint-targeted therapy, especially targeting programmed death receptor 1/programmed death ligand (PD-1/PD-L1), appears to be a promising treatment for cancer. However, the inhibition efficiency of PD-1/PD-L1 inhibitors on solid tumors is only 10-40%, which means that a large proportion of patients cannot benefit from the treatment (20). Therefore, it is of great significance to search for new immunotherapeutic targets and potential prognostic biomarkers. In our study, we found that GOLT1B was potentially a regulatory gene for the immune microenvironment of breast cancer patients and was closely related to the survival of patients. All these results reveal the potentials of GOLT1B as a drug target or prognostic biomarkers for immunotherapy.

Our study proclaimed that the altered phosphorylation of two potential transcription factors of GOLT1B, JUN and SIN3A, might be responsible for the increased GOLT1B expression in breast cancer. As potential upstream regulators of GOLT1B, JUN and SIN3A have been gained increasing attention in diagnosis and treatment of breast cancer. Many studies have declaimed that JUN is a biomarker and regulatory gene in breast cancer (21–23). In patients with short survival time (< 5 years), the expression of JUN in breast cancer tissue is down-regulated and the risk of recurrence of breast cancer is increased (24). Furthermore, JUN mediates the functions of some important cytokines in breast cancer, such as IL-34, IL-33, and IL-1β (22, 23, 25). On the other hand, SIN3A is a transcriptional suppressor promoting osteolytic destruction in ERα-positive breast cancer (26). In mammary adenocarcinoma cells, SIN3A interacts with STAT3 to silence tumor suppressor gene and inhibit cell survival (27). Besides, SIN3A is not only necessary for the survival and proliferation of breast cancer cells but also essential for maintaining epithelial stability and chemical sensitivity, and is one promising drug target for breast cancer treatment (28).

In the survival and correlation analysis, multiple testing strategies have been employed to ensure the accuracy of the results in our study. We analyzed the FDR of the survival results (**Figure 2**), but found that the FDRs based on the datasets from
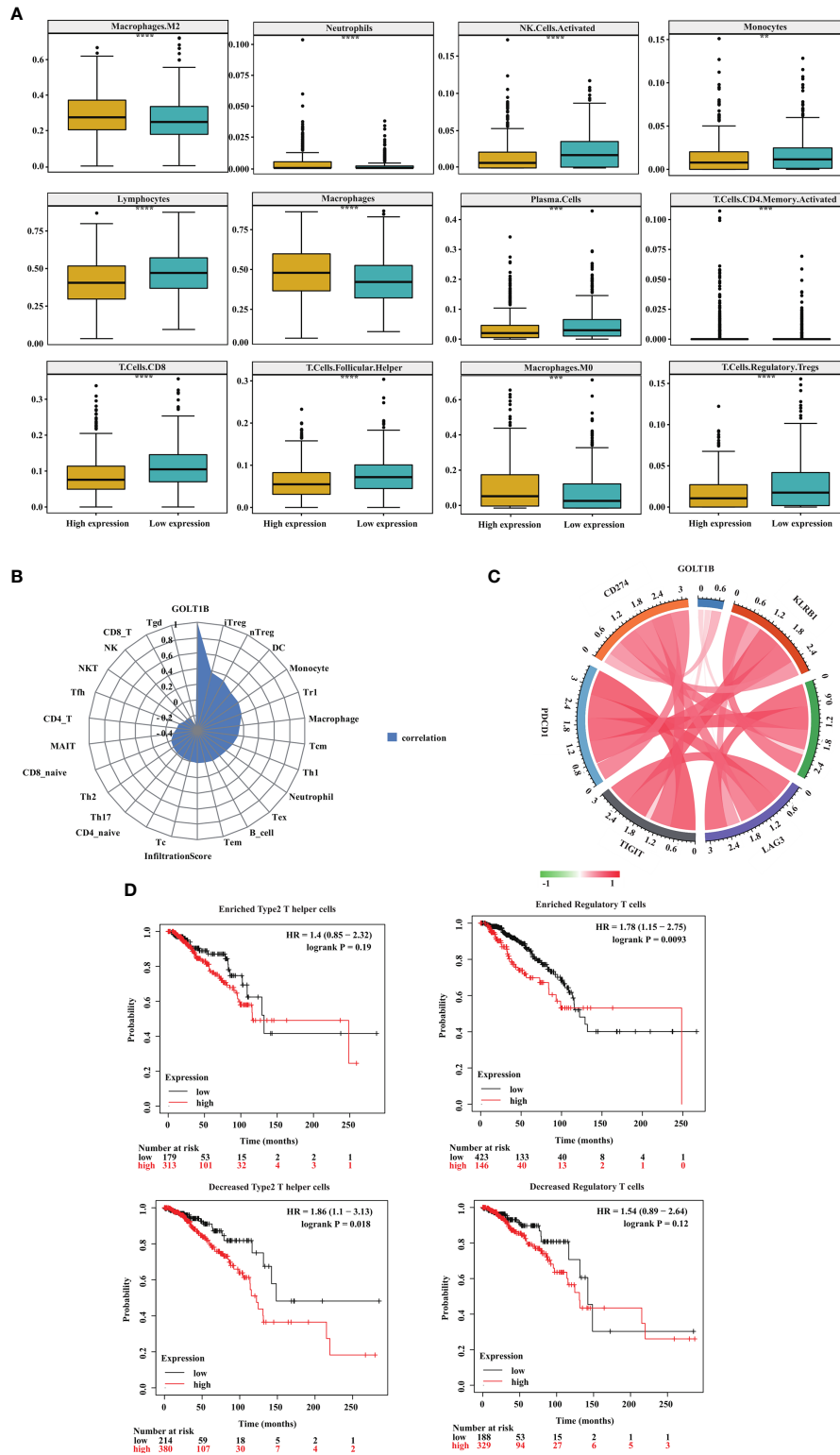
FIGURE 6 | GOLT1B expression is potentially associated with immune infiltration in breast cancer patients. **(A)** The correlation between the GOLT1B expression and infiltration of immune cells in breast cancer was analyzed using the TIMER database. **(B)** The correlation between the GOLT1B expression and infiltration of immune cells in breast cancer was analyzed using the CIBERSORT database. **(C)** The correlation between the expression of GOLT1B and immune checkpoints in human breast cancer. **(D)** The correlation between the expression of GOLT1B and OS of breast cancer patients with different infiltration of immune cells.

TCGA and Kaplan-Meier Plotter database were all greater than 0.5. In fact, we did the FDR correction for all potentially overall-survival-relevant genes (p values < 0.05, n = 2085) in breast cancer, and only found four genes with a FDR < 0.05. Subsequently, to clarify whether GOLT1B is one potential prognostic gene in human breast cancer, we further analyzed other cohorts from prognoScan database and GEO (**Figure 2D**). Consequently, the corrected p values of OS, DSS and RFS were all less than 0.05, supporting a possibility of GOLT1B as one prognostic gene in breast cancer.

Taken together, our study identified GOLT1B as a potential prognostic gene for breast cancer and demonstrated the functions of GOLT1B in immune microenvironment. Our findings in this study proposed a novel potential prognostic biomarker for breast cancer, improved our understanding of the functions of golgi apparatus in tumor immune microenvironment, and provided new opportunities for clinical diagnosis and treatment.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

Study concept and design: WZ and DT. Acquisition of data: WZ and YC. Analysis and interpretation of data: JL. Statistical analysis: WZ, JL, and WC. Drafting of the manuscript: WZ and JL. Critical revision and final approval of the manuscript: all authors. Obtained funding: MT, YD, and WZ. Study supervision: XC and MT. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.805273/full#supplementary-material

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