Original Article

GLUT1/3/4 as novel biomarkers for the prognosis of human breast cancer

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Background: Anomalous expression of glucose transporters (GLUTs) has been observed in a variety of tumor tissues. Although GLUT factors have been shown to have prognostic value for some cancer types, detailed bioinformatics investigation of the factors contributing to the prognostic prediction for patients with breast cancer (BC) has not yet been performed.

Methods: In this study, we examined the transcription levels of *GLUT1*, *GLUT3*, and *GLUT4* and their associations with prognostic clinical data in patients with BC from the ONCOMINE database, using gene expression profiling interactive analysis (GEPIA), Kaplan-Meier (KM) plotter, and cBioPortal online tools.

Results: The transcription level of *GLUT1* was significantly higher in the BC samples than in the normal tissues, whereas the levels of *GLUT3* and *GLUT4* were lower in the BC samples. The expression levels of *GLUT1* and *GLUT3* were associated with the cancer clinical stage. Consistently, survival analysis demonstrated that a high expression level of *GLUT1* was associated with low relapse-free survival (RFS) in patients with BC, whereas high *GLUT3* and *GLUT4* levels predicted a longer RFS in these patients.

Conclusions: Overall, these results suggest *GLUT1* as an effective target of precision therapy, while *GLUT3/4* are novel biomarkers for the prognosis of patients with BC.

Keywords: Bioinformatics; biomarker; breast cancer (BC); glucose transporter (GLUT); relapse-free survival (RFS)

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Introduction

Breast cancer (BC) remains a significant health threat for women despite extensive research and progress in understanding its mechanisms. BC ranks first in incidence and fatality rates among all cancer types in women (1), with 1.7 million new cases diagnosed per year worldwide (2,3). The BC incidence rate increased slightly by 0.3% per year (4). The incidence of BC is higher along with poorer outcomes in developing countries, and the incidence is expected to increase by 55% with a 58% increase in mortality in the next 20 years (5). Therefore, there is an urgent need to identify more reliable prognostic biomarkers and potential drug targets to improve personalized treatments and the prognosis prediction of BC patients.

The metabolic characteristics of tumor cells are distinct from those of normal cells, with tumor cells exhibiting continuous glucose uptake and aerobic glycolysis, i.e., the Warburg effect (6). Glucose transporters (GLUTs) are the most critical rate-limiting enzymes in aerobic glycolysis;

however, different GLUT forms show different affinities for glucose, with the strongest affinities reported for *GLUT1*, *GLUT3*, and *GLUT4*, which also transport glucose at high rates under typical physiological or pathological conditions (7-9). To date, 14 GLUT factors have been identified in mammals, which all belong to the solute carrier 2A (SLC2A) family (10,11). Among them, *GLUT1-4* play roles in the regulation of cellular and systemic glucose homeostasis (12), and *GLUT1/3* expression has been detected in various normal human tissues and malignancies (12-15). A study comprising 118 patients with BC showed that *GLUT1* was expressed in 42% of breast tumors, particularly in high-grade and proliferating tumors (16). Grover-McKay [1998] also found that the expression level of *GLUT1* increased according to the increasing invasiveness of three human BC cell lines (17). A meta-analysis showed that the combination of *GLUT1* and *GLUT3* could be valuable in predicting the malignancy of the cancer (18). *GLUT4* was originally considered to primarily regulate the insulin signaling pathway (19), but was recently found to be amplified in prostate cancer tissues and suggested to play a role in tumorigenesis (20,21). Therefore, *GLUT1/3/4* show promising potential as new biomarkers for improving the prognosis prediction and/or personalized treatment for patients with BC.

Despite numerous studies on the relationship between GLUT factors and cancer, the underlying mechanisms by which they are activated or inhibited in tumorigenesis and the unique roles of GLUT factors in BC have not been completely clarified to date. Therefore, in the present study, we analyzed available transcriptome and clinical data from patients with BC in online public databases, and used bioinformatics approaches to explore the expression patterns, potential functions, and differential prognosis ability of *GLUT1/3/4* with BC.

Methods

Oncomine analysis

We analyzed the expression levels of *GLUT1*, *GLUT3*, and *GLUT4* between various types of tumors and their corresponding normal tissues using the online Oncomine database (22). All datasets met the following criteria: (I) threshold (fold change): 2.0; (II) Student's *t*-test was used to determine the significance level of the difference in the expression of GLUTs between tumor and normal tissues; P<0.01 was considered statistically significant.

Gene expression profiling interactive analysis (GEPIA) dataset

We further utilized GEPIA data to determine the expression patterns of GLUTs specifically in BC. GEPIA includes RNA sequence expression data from 8,587 normal samples and 9,736 tumors collected from the GTEx project and The Cancer Genome Atlas (TCGA) (23). The analyses were conducted according to the instructions on the GEPIA website.

Kaplan-Meier (KM) plotter

We used KM analysis to assess the relationship between *GLUT1/3/4* expression and the prognosis of BC patients (24), including gene expression information and clinical data for 6,234 BC patients. We divided the BC patients into a high expression group and low expression group based on the median GLUT expression level. The associations of GLUT expression with overall survival (OS), relapse-free survival (RFS), and distant metastasis survival (DMFS) were determined according to the KM survival plot; a log-rank test was used to analyze the significance of a KM plot, P<0.05 represented statistical significance.

cBioPortal

The Breast Cancer (METABRIC, Nature 2012 & Nat Commun 2016) dataset (25), including data from 1,904 pathologically reported cases, were utilized to analyze the genomic profiling information (including mutation and mRNA enrichment) of *GLUT1*, *GLUT3*, and *GLUT4* in BC patients with cBioPortal online tools (26,27). In addition, networks were constructed following standard processing procedures to obtain the 50 neighboring genes with the highest frequency of alteration between BC and normal tissues.

TCGA and Gene Expression Omnibus (GEO) dataset analysis

GDC TCGA Breast Cancer (BRCA) (15 datasets) was downloaded from UCSC Xena (http://xena.ucsc.edu/). The matrix data of each TCGA dataset were normalized and converted into TPM. GSE109169 and GSE9574 were downloaded from GEO. The expression level of GLUTs between normal and tumor tissue was analyzed via the Student's *t*-test, P<0.05 represented statistical significance.

Analysis Type By Cancer	Cancer *vs.* normal SLC2A1		Cancer *vs.* normal SLC2A3		Cancer *vs.* normal SLC2A4	
Bladder Cancer	3			2		
Brain and CNS Cancer		1		4		
Breast Cancer	11	1		14		8
Cervical Cancer						
Colorectal Cancer	2		5			2
Esophageal Cancer	2	1	2			1
Gastric Cancer	1		2			1
Head and Neck Cancer	2		2			1
Kidney Cancer	6		3	4		1
Leukemia	3	2	2	12		
Liver Cancer			2			
Lung Cancer	14			6		1
Lymphoma	1	1	3	10		
Melanoma			1	1		
Myeloma				1		
Other Cancer	4		8			1
Ovarian Cancer	1			1		
Pancreatic Cancer	7		3			
Prostate Cancer			1	2		1
Sarcoma			4	1		
Significant Unique Analysis	57	6	35	58		17
Total Unique Analysis	488		462		411	

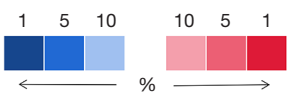

Figure 1 Transcription levels of *GLUT1/3/4* in tumor tissues and normal tissues.

Cell culture

Cells were cultured in Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, USA) with 10% fetal bovine serum (Gibco, USA), 100 U/mL penicillin (Beyotime, China), and 100 mg/mL streptomycin (Beyotime, China). All cell lines were incubated at 37 ℃ at 5% CO_2.

Western blot

Cells were lysed with RIPA buffer (Beyotime, China). Equal amounts of protein in cell lysates were measured by standardization with the BCA Protein Assay Kit (Beyotime, China). Proteins were resolved by SDS-PAGE and transferred to nitrocellulose membranes (Whatman, Dassel, Germany). After blocking with 5% skimmed milk in TBST, membranes were incubated with primary antibodies overnight in 4 ℃, followed by 2 h incubation with HRP-conjugated secondary antibodies. Protein bands were visualized with ECL. Antibodies against *GLUT1/3/4* were purchased from ABclonal (Hubei, China). Antibodies against beta-tubulin were purchased from Cell Signaling Technology (Beverly, MA, USA). The grey of the protein bands was measured using ImageJ software. A two-tailed Student's *t*-test was applied to assess the statistical significance of the difference between two independent groups.

Results

Transcription levels of GLUTs in patients with BC

Comparison of the transcription levels of GLUTs in BC and normal breast tissues from the Oncomine datasets showed different patterns of differential expression depending on the GLUT considered (*Figure 1*). Abundant upregulation of *GLUT1* expression was found in BC samples in 11 datasets (*Figure 1*). In the dataset of Zhao *et al.* (28), the transcription level of *GLUT1* was higher in invasive ductal (ID) and invasive lobular (IL) BC than in normal breast tissue, with fold changes (FC) of 2.800 and 2.075, respectively (*Table 1*). By contrast, the transcription levels of *GLUT3* and *GLUT4* were significantly reduced in 14 and 8 BC patient datasets, respectively (*Figure 1*). In the dataset of Glück *et al.* (29), *GLUT3* expression was down-regulated in ID BC with a FC of -2.102 compared to that of normal tissues, and the dataset of Curtis *et al.* (30) showed down-regulated *GLUT3* expression in all BC types compared with that of breast tissues (FC of –2.753 for ID BC and –2.665 for IL BC) (*Table 1*). In the TCGA Breast dataset, *GLUT4* expression was also down-regulated in all BC types compared to that of breast tissues, with an FC of –5.889 for ID BC and FC of –6.020 for IL BC (*Table 1*).

GLUT transcription levels and clinicopathological parameters of patients with BC

Comparison of the transcription levels of GLUTs between BC samples and non-cancer breast samples in the GEPIA dataset confirmed that the *GLUT1* level was higher in BC tissues, whereas the transcription levels of *GLUT3/4* were lower in the BC samples (*Figure 2*). Moreover, the

Table 1 Transcription levels of glucose transporters (GLUTs) in different types of breast cancer (BC) and normal tissues

GLUTs	Type of BC *vs.* normal tissues	Fold change (FC)	P value	t-statistic	Ref
GLUT1	Invasive ductal breast carcinoma (ID BC)	2.800	1.03E-11	9.276	Zhao *et al.*, 2004
	Invasive lobular breast carcinoma (IL BC)	2.075	6.62E-6	5.631	Zhao *et al.*, 2004
GLUT3	ID BC	–2.753	3.52E-44	–19.000	Curtis *et al.*, 2012
	IL BC	–2.665	5.64E-34	–13.812	Curtis *et al.*, 2012
	Invasive BC	–2.102	0.018	–3.438	Glück *et al.*, 2012
GLUT4	ID BC	–5.889	2.14E-26	–16.643	The Cancer Genome Atlas (TCGA)
	IL BC	–6.020	1.82E-16	–9.886	TCGA

GLUTs, glucose transporters; BC, breast cancer; ID BC, invasive ductal breast carcinoma; IL BC, invasive lobular breast carcinoma; FC, fold change.

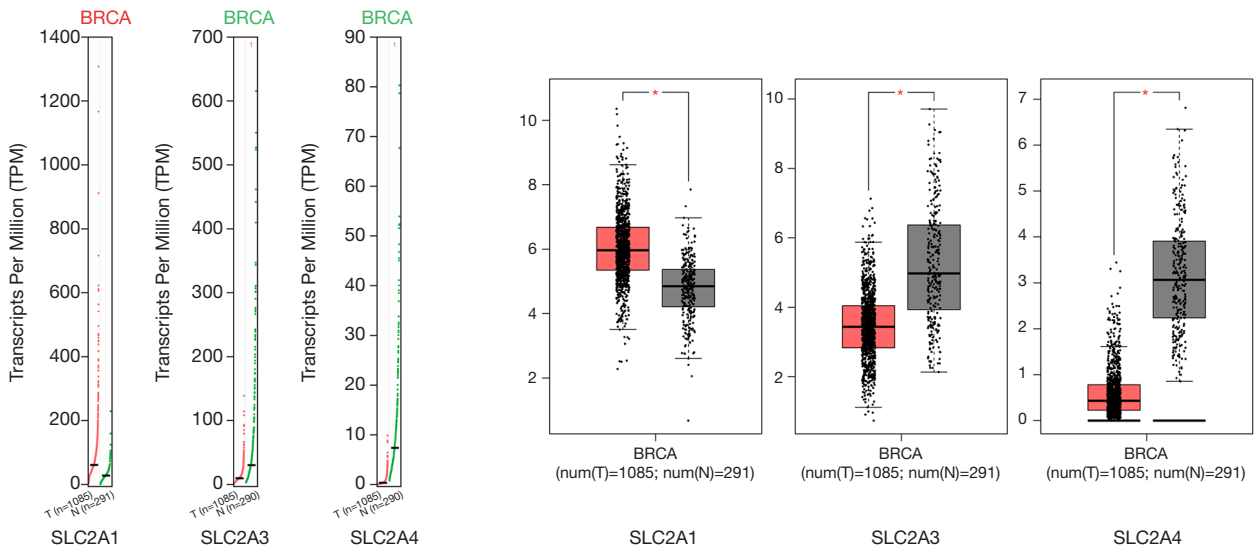

Figure 2 Transcription levels of *GLUT1/3/4* in breast cancer and normal tissues. The profile of transcription levels of *GLUT1/3/4* between breast cancer and normal tissues (A). The boxplot of transcription levels of *GLUT1/3/4* between breast cancer and normal tissues (B), *, P<0.05.

transcription levels of *GLUT1* and *GLUT3* were correlated with clinical cancer stage, whereas the *GLUT4* levels were not (*Figure 3*).

Association of GLUT transcription levels with patient prognosis

Online KM tools indicated that the expression levels of GLUTs could provide reliable predictions of survival in BC patients. KM plotter and the log-rank test suggested that up-regulated *GLUT1* expression and down-regulated *GLUT4* expression were significantly related to a poor OS, RFS, and DMFS in BC patients (P<0.05; *Figure 4*). Moreover, BC patients with a relatively high transcription level of *GLUT3* were predicted to have a high RFS (P<0.05; *Figure 4*).

Predicted functions and pathways of the changes in GLUT factors and their associated gene networks in patients with BC

GLUT expression was altered in 336 of the 1,904 (18%) BC

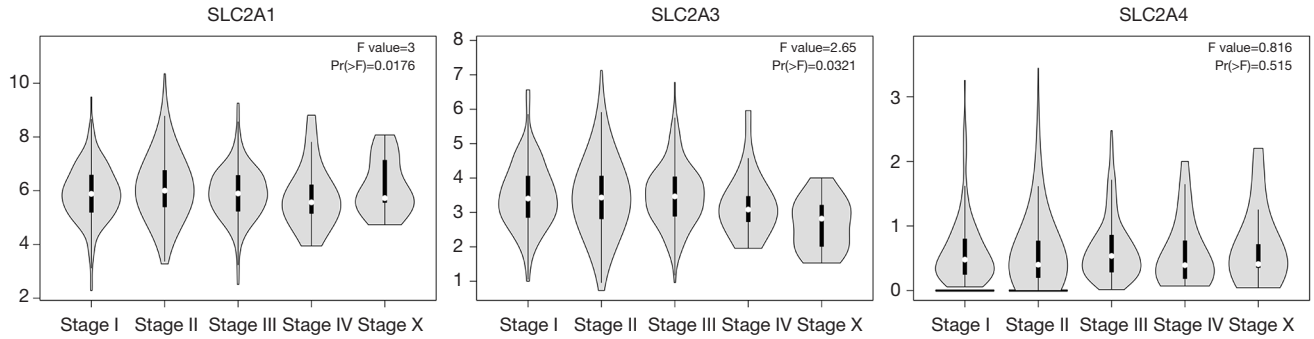

Figure 3 Association between *GLUT1/3/4* transcription and clinical cancer stage in breast cancer patients.

patients in the Breast Cancer (METABRIC, Nature 2012 & Nat Commun 2016) dataset of the cBioPortal database. The highest frequency of mutation was 9.14% (*Figure 5A*), with mutation frequencies in *GLUT1*, *GLUT3*, and *GLUT4* of 8%, 7%, and 5%, respectively (*Figure 5B*). The network showed that the five most frequently altered genes associated with *GLUT* alterations were *MYC*, *YWHAZ*, *ARNT*, *CREBBP*, and *CLTC* (*Figure 5C*).

The GLUTs and 50 neighboring genes were annotated, and the results were visualized using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis in the DAVID tool (31,32). GO analysis showed an enrichment of GLUT mutations in BC in the biological function terms like negative/positive regulation of RNA polymerase II promoter transcription, cellular response to insulin stimulation, and signal transduction (*Figure 6A*), along with other terms of the cytoplasm like cellular exosome, protein binding, protein domain-specific binding, and transcription factor binding (*Figure 6B,C*). KEGG analysis identified 10 pathways associated with the functions of GLUTs mutations in BC (*Figure 6D*), including hsa04110 (cell cycle), hsa04151 (PI3K-AKT signaling pathway), hsa04330 (Notch signaling pathway), and hsa05200 (cancer pathway), which are critical pathways in the tumorigenesis and pathogenesis of BC (*Figure* 7) (33).

Enrichment of mutation and differential expression mRNA between GLUTs

The BC samples showing differences in GLUT expression from normal samples also showed altered mutation frequencies of *TP53*, *PIK3CA*, and *MAP2K4* compared to those of BC samples without altered GLUT expression (*Figure 8A*). Aberrant mutations in both *TP53* and *PIK3CA* have been reported to be associated with the development of BC (34-36).

A total of 80 differentially expressed genes (DEGs: logFC ≥0.5, odds ratio ≤–1.5) between the groups with altered and unaltered GLUT expression were identified (*Figure 8B*). GO analysis of these 80 DEGs showed enrichment in signal transduction, negative regulation of cell proliferation, cellular response to tumor necrosis factor, and cell differentiation, indicating the regulation of these processes by GLUTs in BC (*Figure 8C*). Moreover, extracellular exosome, identical protein binding, structural constituent of the cytoskeleton, and microtubule binding were significantly regulated by these GLUT mutations (*Figure 8D,E*).

The expression of GLUTs between normal tissues and tumors in TCGA and GEO datasets

Comparison of the expression levels of GLUTs between normal breast tissues and BC tissues in the TCGA dataset confirmed that *GLUT1* level was higher in BC tissues, whereas the expression levels of *GLUT3/4* were lower in the BC samples (*Figure 9A*). By analyzing the GEO datasets (GSE109169 and GSE9574), we also found that BC tissues had higher expression levels of *GLUT1*, whereas lower *GLUT3/4* levels were found in the normal breast tissues (*Figure 9B*). Moreover, the expression levels of *GLUT4* were correlated with OS, whereas the *GLUT1/3* levels were not (*Figure 9C*).

Expression of GLUTs between luminal and basal-like BC cell lines

We compared the expression levels of *GLUTs* between luminal (MCF-7/T47D/BT474) and basal-like BC cells (SUM159/BT549/MDA-MB-231) and found that the

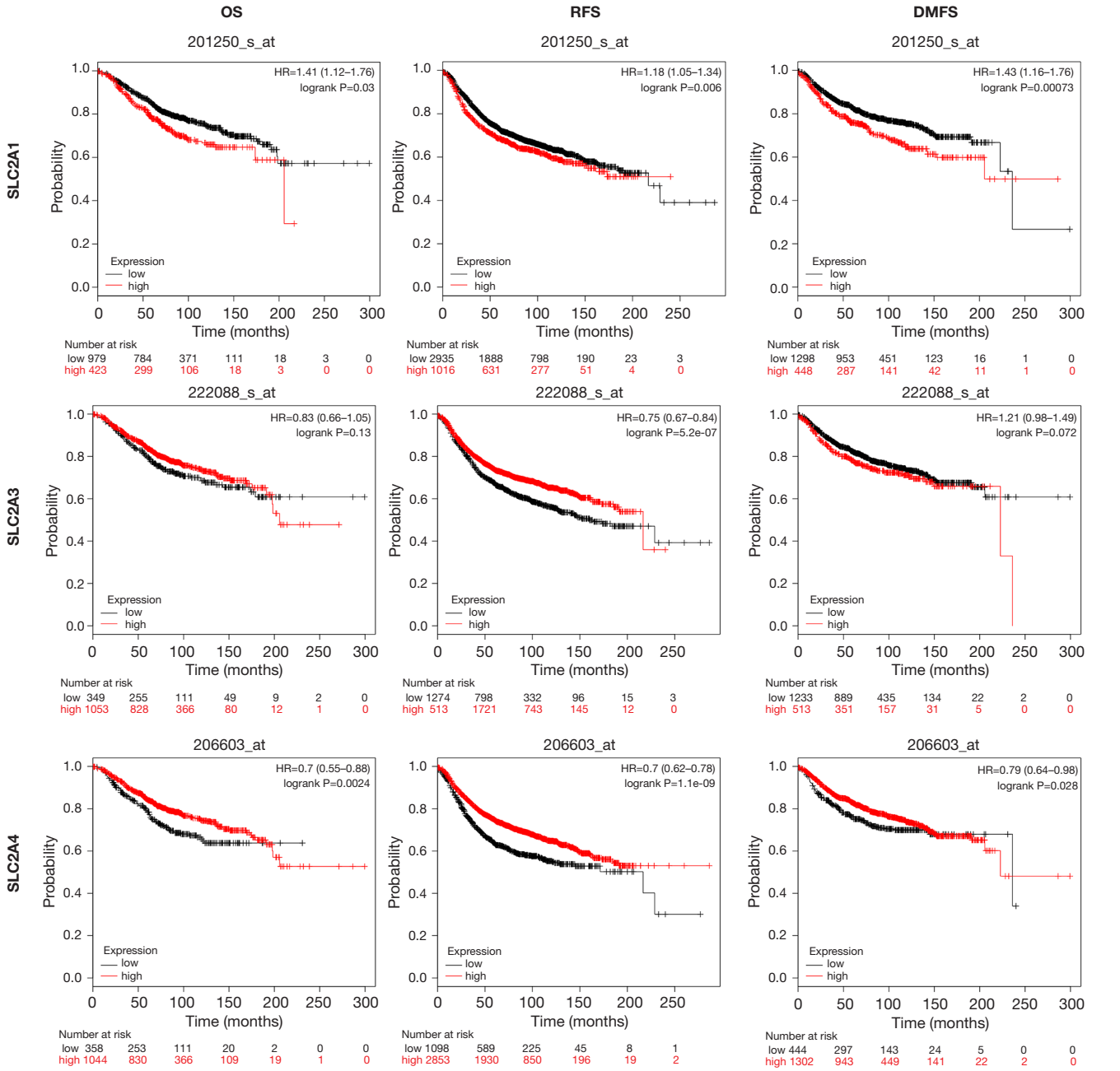

Figure 4 Prognostic prediction in patients with breast cancer according to the transcription levels of *GLUT1/3/4*.

GLUT1 levels were higher in basal-like BC cells and T47D cells, whereas the levels of GLUT3/4 were higher in luminal BC cells (*Figure 10*).

Discussion

Aberrant expression of GLUT has been reported in several types of tumor tissues (9,37-41). Although a role of GLUT in predicting the prognosis has been demonstrated for some cancers (42-44), the detailed associations with BC and underlying mechanisms have remained unclear so far. By the comprehensive analysis of available public data and bioinformatics, we demonstrated clear and distinct patterns of up-regulated *GLUT1* expression, and down-regulated

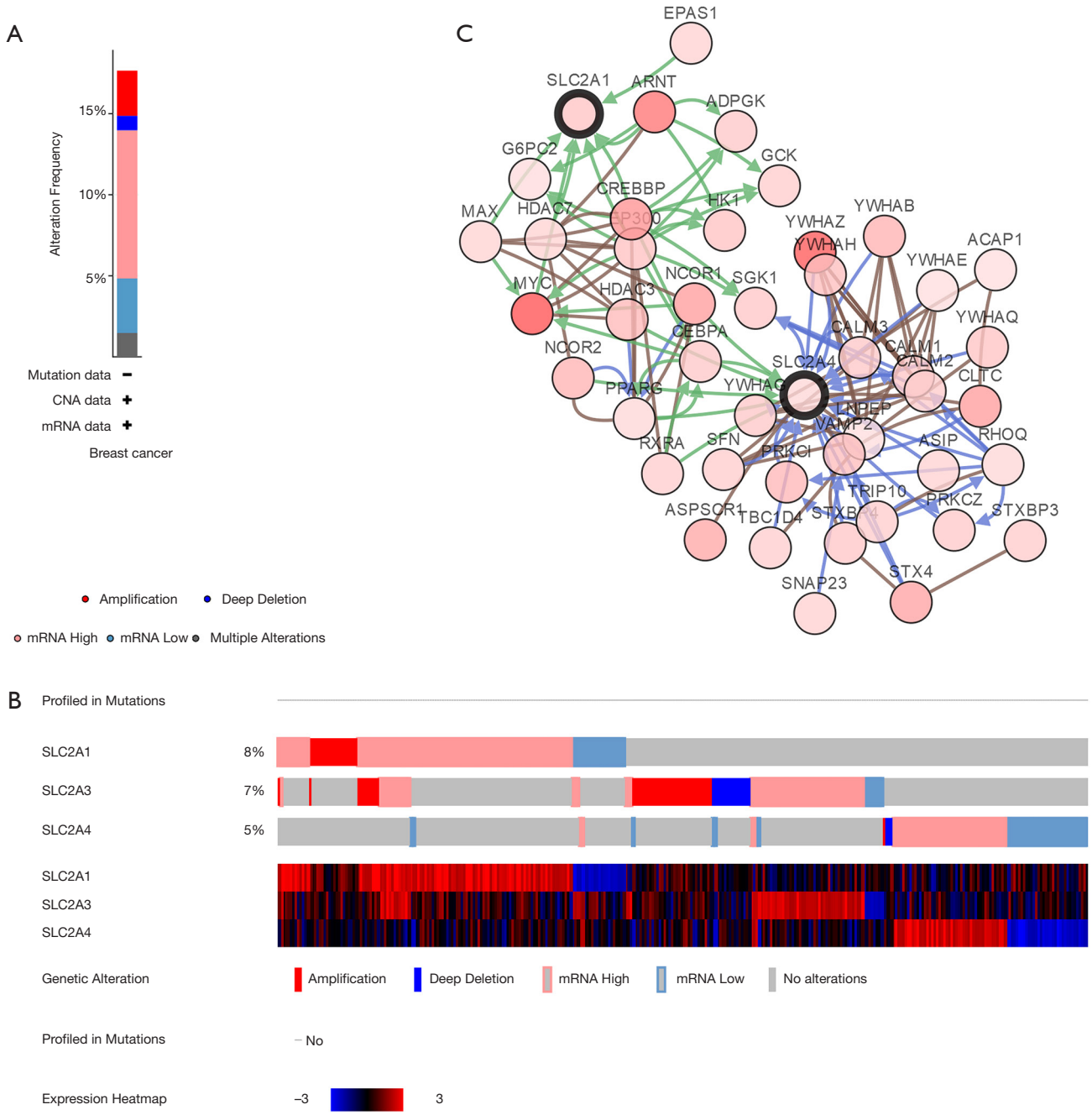

Figure 5 *GLUT1/3/4* mutation analysis and network construction in breast cancer patients.

GLUT3 and *GLUT4* expression in BC, which can help guide personalized treatment and improve the accuracy of prognosis for BC patients.

In line with the present results, *GLUT1* has been reported to be overexpressed in many cancers (15), and was associated with poor survival in BC (16), lung cancer (45,46), oral squamous cell carcinoma (47,48), esophageal cancer (49,50), rectal cancer (51), tongue squamous cell carcinoma (52) and pancreatic cancer (53). *GLUT1* was higher in TNBC cases than in non-TNBC cases (54). Moreover, high expression levels of *GLUT1* were suggested to contribute to the tumorigenesis of BC (16,55), and a recent study

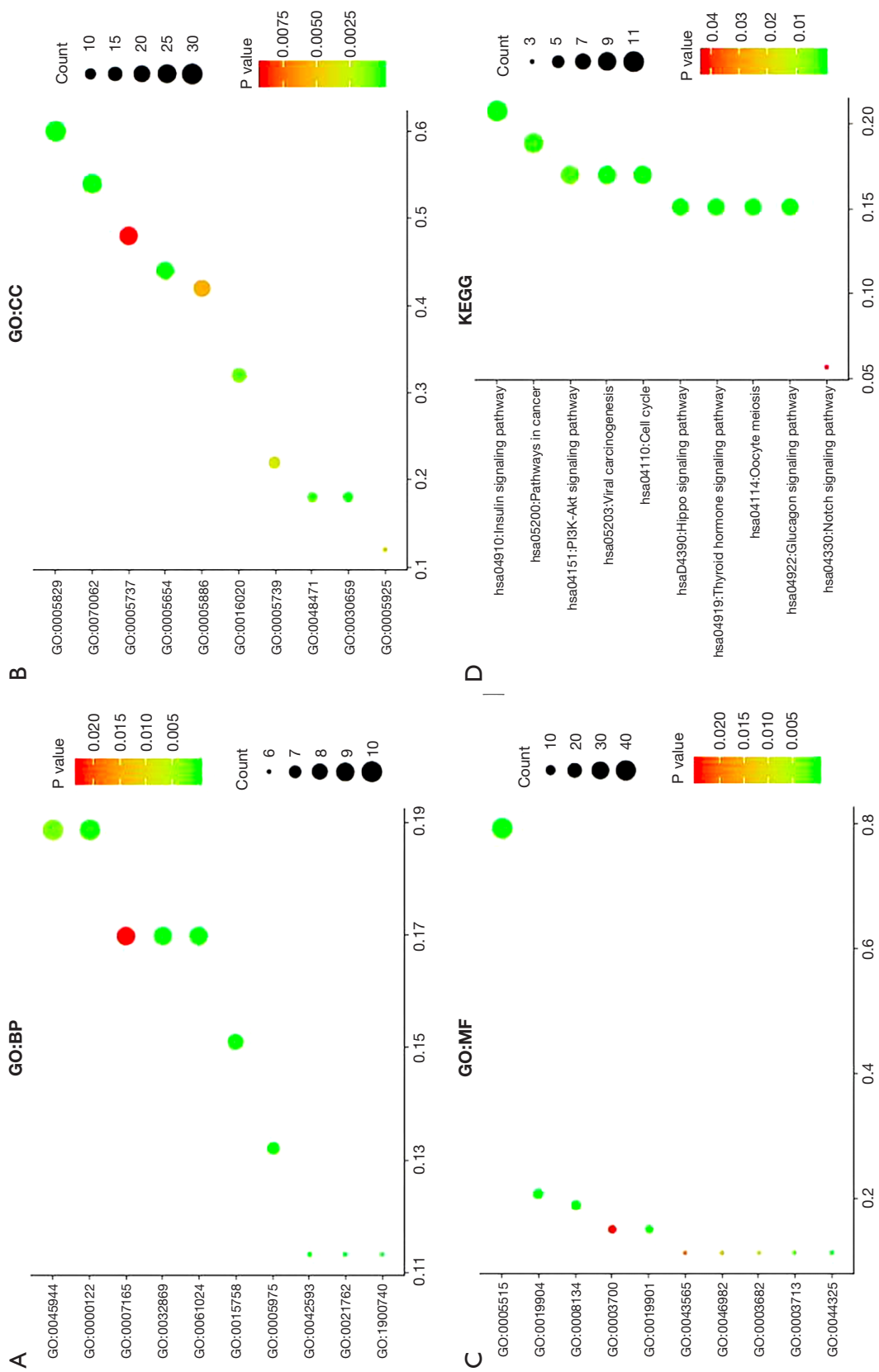

Figure 6 Functions of *GLUT1*, *GLUT3*, and *GLUT4*, and the 50 most frequently mutated neighboring genes predicted by Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis in The Database for Annotation, Visualization and Integrated Discovery (DAVID). GO analysis included biological processes (A), cellular components (B), molecular functions (C). KEGG analysis included KEGG pathways (D).

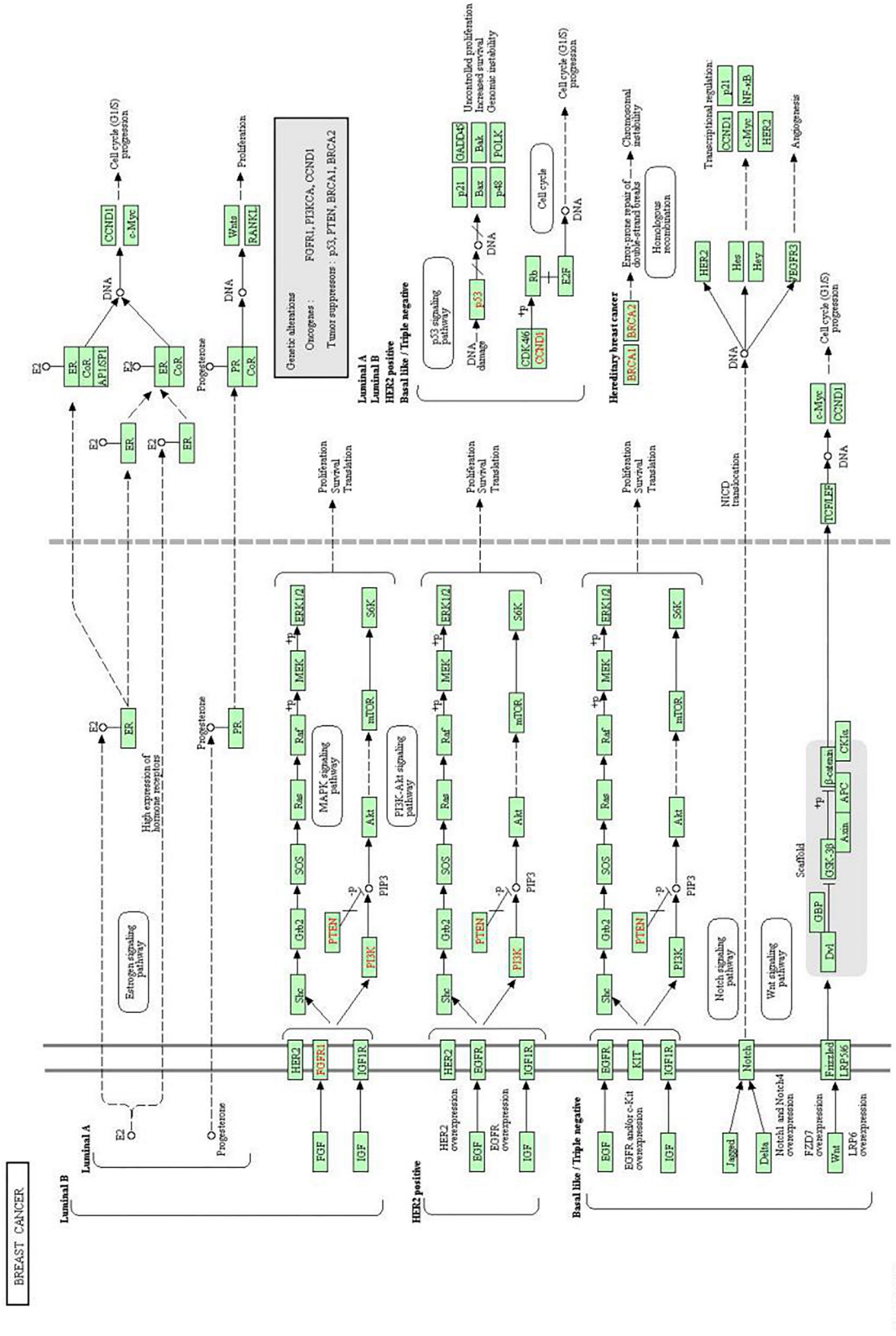

Figure 7 Breast cancer signal pathways regulated by the *GLUT1*, *GLUT3*, and *GLUT4* alteration in breast cancer.

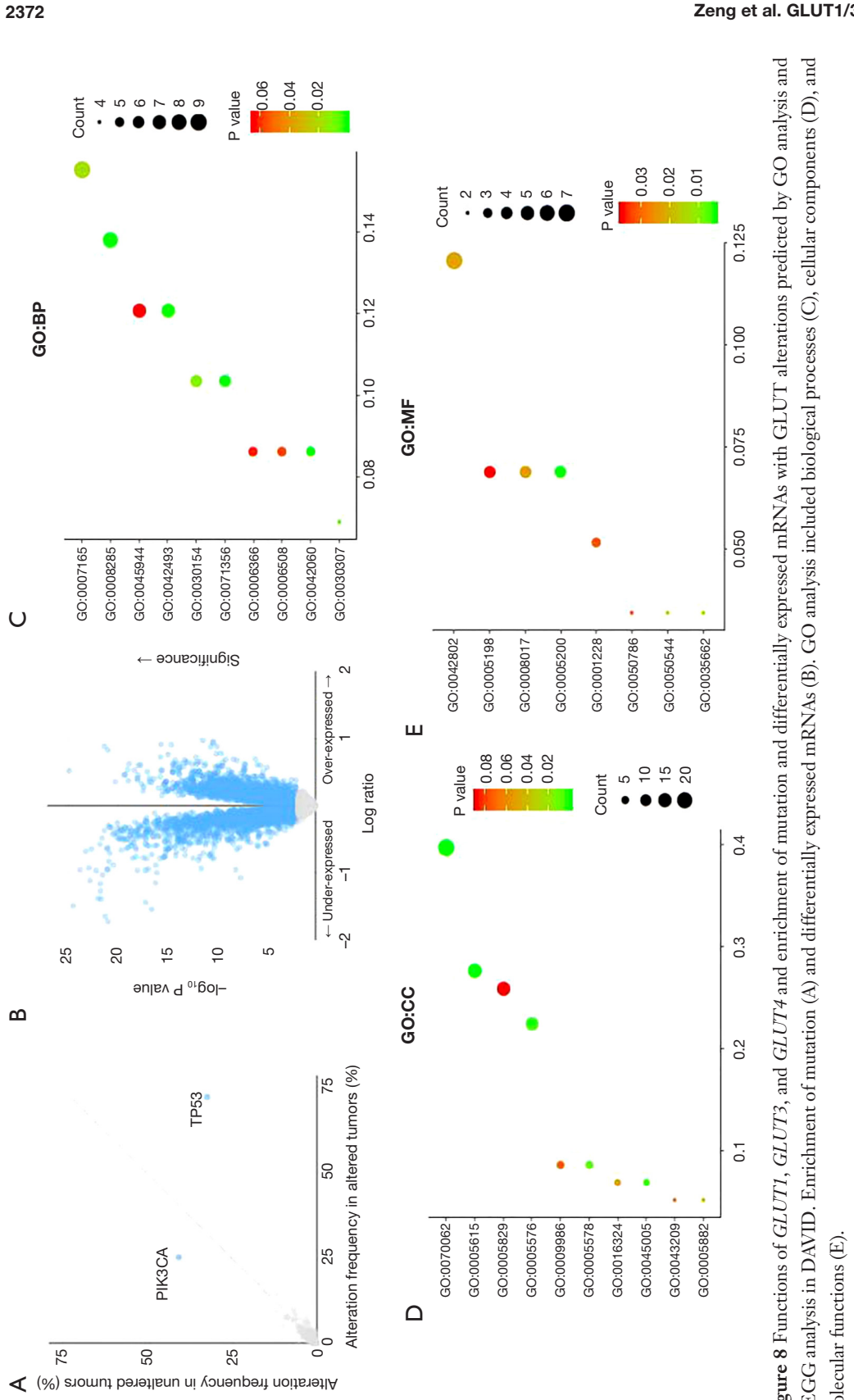

Figure 8 Functions of *GLUT1*, *GLUT3*, and *GLUT4* and enrichment of mutation and differentially expressed mRNAs with GLUT alterations predicted by GO analysis and KEGG analysis in DAVID. Enrichment of mutation (A) and differentially expressed mRNAs (B). GO analysis included biological processes (C), cellular components (D), and molecular functions (E).

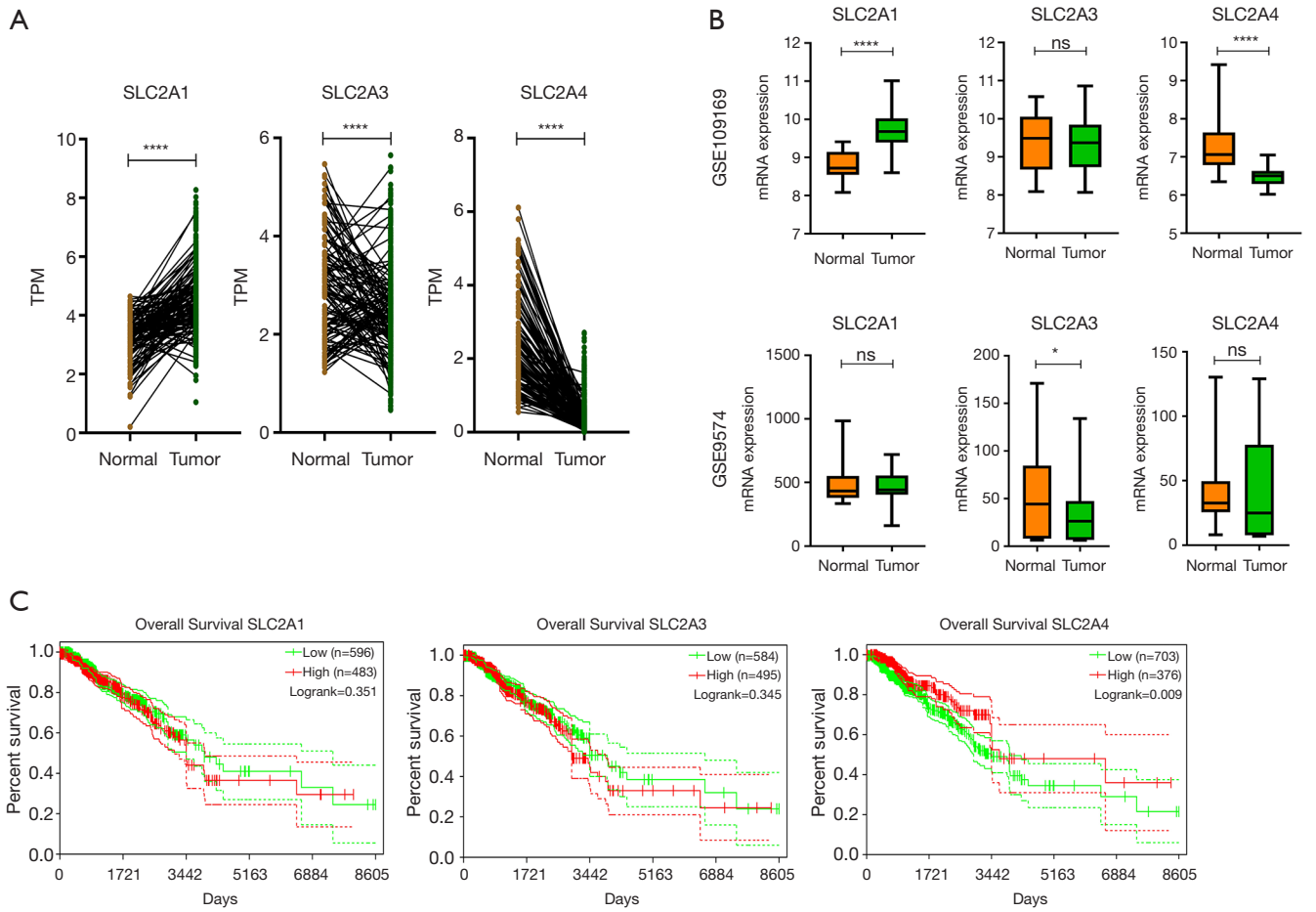

Figure 9 Analysis of *GLUT1/3/4* with The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) datasets. Expression levels of *GLUT1/3/4* between BC and normal breast tissues in TCGA dataset (A), and GEO datasets (C), The relationship between the expression levels of *GLUT1/3/4* and OS in TCGA dataset (B). P<0.05 represented statistical significance, *, P<0.05; ****, P<0.0001; ns means P>0.05.

showed a link between the overexpression of *GLUT1* and the hypoxic condition of the tumor microenvironment (56). The transcription factors c-Myc and sinoculis homeobox 1 (Six1) have been reported to directly activate *GLUT1* transcription (57,58). The analysis of miRNA-mRNA network reveals miR-140-5p as a suppressor of BC glycolysis via targeting *GLUT1* (59). Among colorectal cancer cell lines, those with *KRAS/BRAF* mutations also showed a higher transcription level of *GLUT1* than wild-type cells (60). By contrast, thioredoxin-interacting protein (TXNIP) and p53 repress *GLUT1* transcription (61-64).

GLUT3 has also been reported to be overexpressed in many tumors (65), and was considered a potential marker of poor prognosis in oral squamous cell carcinoma and non-small cell lung cancer (16,47). However, we found an opposite pattern of a reduced transcription level of *GLUT3* in BC than in non-cancer tissues, which was associated with a worse OS and clinical cancer stage.

GLUT4 expression was also found to be down-regulated in BC in the present study, and was a significant predictor of poor OS, RFS, and DMFS. Loss of *GLUT4* induces metabolic reprogramming and impairs viability of BC cells (66). Previous studies have shown diverse patterns of *GLUT4* expression, with overexpression reported in gastric cancer (67,68) and reduced transcription levels in pancreatic cancer (69).

In summary, we have provided new insight to clarify the expression pattern and prognosis-related features of

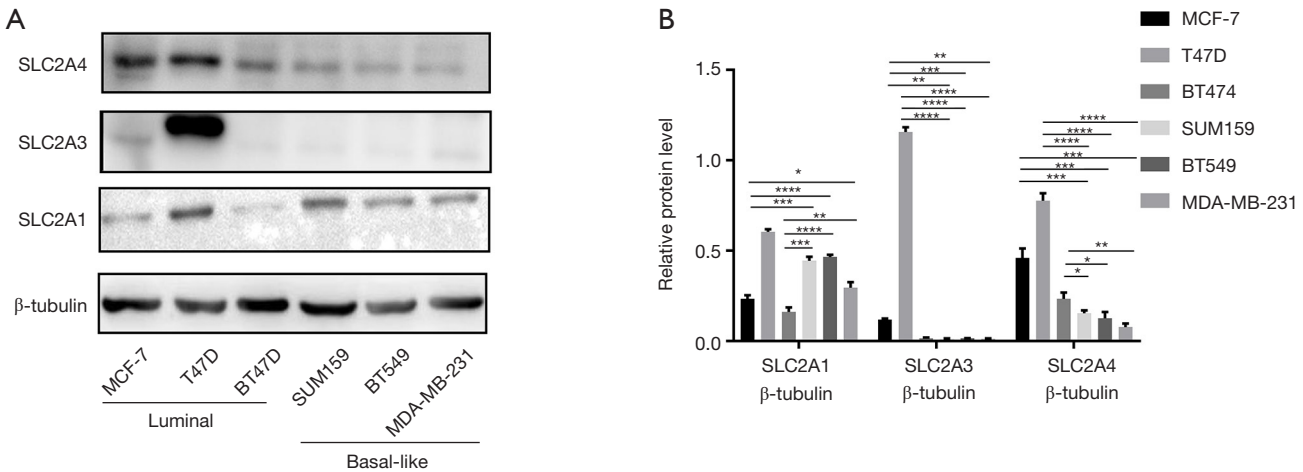

Figure 10 The protein expression levels of *GLUT1/3/4* between luminal and basal-like breast cancer cell lines. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001.

GLUT1, *GLUT3*, and *GLUT4* in BC by integrating the data of online databases. These results demonstrate that the amplified transcription of *GLUT1* might play a critical role in BC tumorigenesis. Moreover, a high transcription level of *GLUT1* can be considered as a reliable marker for discerning high-risk subtypes of BC, suggesting an effective therapeutic target for BC. In addition, the high transcription of *GLUT3/4* can serve as a potential prognostic marker for BC patients, implying a better prognosis.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2020.03.50). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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