



SLC7A5 serves as a prognostic factor of breast cancer and promotes cell proliferation through activating AKT/mTORC1 signaling pathway

Yuan Li¹, Wei Wang², Xue Wu¹, Sunkai Ling¹, Yu Ma¹, Peilin Huang¹

¹Department of Oncology, School of Medicine, Southeast University, Nanjing, China; ²Department of Pathology, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing, China

Contributions: (I) Conception and design: Y Li; (II) Administrative support: P Huang; (III) Provision of study materials or patients: W Wang; (IV) Collection and assembly of data: Y Ma, S Ling; (V) Data analysis and interpretation: Y Li, X Wu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Peilin Huang. Department of Oncology, School of Medicine, Southeast University, Nanjing, China. Email: Seuahl@163.com.

Background: The purpose of our research was to determine if the clinical, pathological, and prognostic functions of *SLC7A5* are the same as those of other molecular breast cancer (BC) subgroups.

Methods: We used the Oncomine and The Cancer Genome Atlas (TCGA) online databases to examine the expression and genetic changes of *SLC7A5* in BC tissues. Immunohistochemical analysis was used to validate the *SLC7A5* protein expression in subtypes of BC, while Kaplan-Meier figures and log-rank tests were used to evaluate the prognostic relevance of *SLC7A5*. Uni- and multivariate Cox regression models were adapted to analyze hazard ratios (HRs) and the independent prognostic factors. We analyzed the alterations of different malignancies of luminal cells by up-regulation of *SLC7A5* in human luminal cell lines MCF-7. *SLC7A5* was overexpressed in luminal cells, and then the AKT, mTOR, and p70-S6K phosphorylation and expression were analyzed by western blot analysis and real-time quantitative polymerase chain reaction (qPCR).

Results: Our results suggested that *SLC7A5* was overexpressed in BC cell lines and in patients' tissues. Elevated *SLC7A5* messenger RNA (mRNA) and *SLC7A5* protein expression was correlated to a worse clinical prognosis ($P < 0.001$) in luminal subtypes of BC. The multivariate analysis suggested that high level of *SLC7A5* expression could be an independent prognostic factor for decreased overall survival (OS). The study also demonstrated that *SLC7A5* overexpression increased proliferation of MCF-7 cells by reducing the cell cycle arrest in G1 phase. Our mechanistic study further indicates that *SLC7A5* enhances the proliferation of the MCF-7 cell by activation of AKT/mTORC1 pathway through phosphorylation.

Conclusions: Our study demonstrated that *SLC7A5* may have a vital function in the biology of BC cells, indicating that *SLC7A5* is a potential prognostic biomarker and may be a valuable therapeutic target in BC patients.

Keywords: Solute carrier family 7 member 5 (*SLC7A5*); breast cancer (BC); prognosis; AKT/mTORC1

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Introduction

According to the 2020 global Cancer Statistics, breast cancer (BC) is the most commonly diagnosed malignancy in females worldwide (1). BC is a typical heterogeneous cancer, which displays significant diversity depending on

the particular molecular signature (2), such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Furthermore, based on the above molecular signatures, BC can be divided into 4 subtypes: the luminal A, luminal B, HER2, and basal-

like subtypes (3). One avenue of cancer research involves examining the molecular profiles of the cancer cells and their possible relationship with the clinical outcomes of the disease process.

The growth of tumor cells relies on amino acids (AAs), as they enhance mammalian targets of rapamycin complex 1 (mTORC1) via nutritional signaling pathways for regulation of protein synthesis and cancer survival (4,5). Studies have suggested that mammalian target of rapamycin complex 1 (mTORC1) plays a role in reprogramming tumor cell metabolism, specifically, in the direct and indirect regulation of AA transport (6).

As a vital member of AA transporters, solute carrier family 7 member 5 (*SLC7A5*) is critical for the sodium-independent transport of large neutral AAs across the membrane (7). This biological process is essential for the rapid growth and proliferation of tumor cells (8). Altered regulation of *SLC7A5* has been found in different cancer types, such as ovarian cancer and non-small cell lung cancer (NSCLC) (9-11).

In our research, we analyzed the gene copy number, messenger RNA (mRNA) expression, and the protein expression of *SLC7A5* to examine its prognostic relevance in BC patients in various molecular subtypes. We found that upregulated *SLC7A5* expression in MCF-7 cells mediates the AKT/mTORC1 signaling pathway, which is critical for the regulation of tumor cell proliferation, indicating that *SLC7A5* might be a potential prognostic parameter and therapeutic target of BC. We present the following article in accordance with the REMARK reporting checklist (available at <http://dx.doi.org/10.21037/atm-21-2247>).

Methods

BC dataset source and preprocessing

We used Oncomine, an online microarray database (<http://www.oncomine.org>) that includes 970 datasets and 84,529 samples to determine *SLC7A5* mRNA expression in various types of cancer tissues and normal tissues (12). Difference of transcriptional expression was compared by Student's *t*-test, and the cutoffs were defined as follows: P level <0.01, fold change >1.5, top gene rank =10%, and a data type of mRNA. Additionally, the expression levels of *SLC7A5* in 32 cancer types were analyzed and compared based on the mRNA sequencing (RNA-seq) data of The Cancer Genome Atlas (TCGA; <http://cancergenome.nih.gov/>) from the Tumor Immune Estimation Resource (TIMER) database (13). Clinical characteristics such as age, race,

tumor-node-metastasis (TNM) stages, and cancer subtypes were obtained with regard to their corresponding clinical patterns. Using the “pROC” package in R (R Foundation of Statistical Computing), we tested the specificity and sensitivity of predictive accuracy of *SLC7A5* with receiver operating characteristic (ROC) analysis and area under the curve (AUC).

Genetic variation of SLC7A5 in BC

The invasive breast carcinoma dataset was downloaded from TCGA on the cBioPortal website (14,15). The levels of mRNA expression were retrieved as RNA-seq by expectation-maximization (RSEM; batch-normalized from Illumina HiSeq_RNASeqV2). For missing values, the gene patterns and clinical signatures were filtered.

Survival analyses

We made Kaplan-Meier plots and used log-rank tests to find the significant relevance between *SLC7A5* expression and prognosis (16). We evaluated the correlation of *SLC7A5* with overall survival (OS) and relapse-free survival (RFS), and calculated hazard ratios (HRs) with 95% confidence intervals (CIs) using uni- and multivariable Cox regression models. Clinical parameters associated with survival were incorporated into the final multivariate prognostic analysis.

Cell lines and cell culture

The human BC cell lines MCF-7 were obtained from the Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital. The cell lines were freshly recovered from a liquid nitrogen. The BC cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, CA, USA) together with the 10% fetal bovine serum (FBS, Gibco, Cappelas, Brazil) in a humidified incubator at a temperature of 37 °C with 5% CO₂.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Women's Hospital of Nanjing Medical University (No.: KY2021054) and informed consent was taken from all individual participants.

Cell transfection

SLC7A5 overexpression lentiviruses were constructed with GV208 by GeneChem Company (Shanghai, China).

MCF-7 cells were seeded into 6-well plates, and lentiviral transfection with experimental constructs or respective controls (1×10^9 TU/mL) was carried out at 60% confluency, following the manufacturer's protocol. After 48–72 hours, infection efficiency was detected and *SLC7A5* expression levels were verified by Western blotting.

Histopathology

The breast cancer tissues were preserved in 10% formalin for histopathology (Solarbio, Beijing, China) and embedded in paraffin and cut into 50- μ m thick cubes. The tissues were then stained with 3,3'-diaminobenzidine (DAB) and subjected to immunohistochemical (IHC) staining with standard methods. All figures of pathological changes were taken using an Olympus BX-145 microscope (Olympus, Japan).

Colony formation assay

Six-well plates were covered with a 0.6% agar layer in a medium with FBS (20%). The cells were prepared in 0.3% agar and seeded in 3 parts. After incubations of the plates at a temperature of 37 °C for 2 weeks, we counted the number of colonies.

Cell cycle analysis

Tumor cells were incubated with 10 or 20 μ mol/L of naringin for 24 hours and fixed in 75% ethanol at 4 °C overnight for the cell cycle analysis. After resuspension in 10 μ g/mL propidium iodide, cell cycle stages were determined using a Coulter Epics XL Flow Cytometry System (Beckman Coulter, Brea, CA, USA).

RT-PCR analysis

TRIzol reagent (Invitrogen, USA) was used to extract the total RNA, and 1 μ g of total RNA was then reverse transcribed. The ABI PRISM 7900 sequence detection system (Applied Biosystems, Foster City, CA, USA) was used to perform RT-PCR. We then conducted PCR amplification in a reaction system containing complement DNA (cDNA) using the forward and reverse primers, SYBR Green qPCR Supermix (Thermo Fisher Scientific, USA), and distilled water. The PCR procedure was carried out for 40 cycles at the temperature of 95 °C for 10 seconds and then at a temperature of 60 °C for

30 seconds. Primer sequences for detection of the mRNA expression were synthesized. We evaluated the relative expression of the amplified RNA samples with the $2^{-\Delta\Delta CT}$ method. All reactions were repeated more than 3 times.

Western blot analysis

We collected whole cell lysates using RIPA lysis buffer (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and calculated the protein concentrations using a BCA Protein Assay Kit (Beyotime, Jiangsu, China). Then, 4–20% sodium dodecyl sulfate polyacrylamide gel electrophoresis (Sigma-Aldrich St. Louis, MO, USA) protein loading buffer was added to the collected 40 μ g protein samples and heated in a boiling water bath for 3–5 minutes to fully denature the protein. After the protein sample was cooled to room temperature, it was directly loaded into the sample well of SDS-PAGE gel for electrophoresis, which was followed by protein electrophoretic transfer to polyvinylidene fluoride membranes (Roche, Indianapolis, IN, USA). After this, we blocked the membranes in 5% milk, and then incubated them with primary antibodies (Abcam, Cambridge, MA, USA) to evaluate *SLC7A5*, p-AKT (Abcam), p-p70-S6K, (Abcam), and p-mTOR (Abcam) expression. Subsequent incubations using the secondary antibody were performed, and GAPDH was used as an internal reference.

Statistical analysis

All clinical parameters are shown with mean \pm standard error of measurement (SEM) and calculated using Student's *t*-test with a P value <0.5 set as the threshold of significance. Experiments were performed in triplicate. Analyses were conducted with R software (version 3.6.1) and Bioconductor packages.

Results

Upregulation of *SLC7A5* expression in BC

The mRNA of *SLC7A5* in different cancers and normal tissues was detected in the Oncomine database. We found an increased level of *SLC7A5* in breast, colorectal, lung, lymphoma, and head and neck cancers (*Figure 1A*). To investigate further *SLC7A5* expression in pancreatic cancer, RNA sequencing from TCGA database was analyzed (2). The various expression patterns in cancer and its expression in the adjacent normal tissues are illustrated in *Figure 1B*.

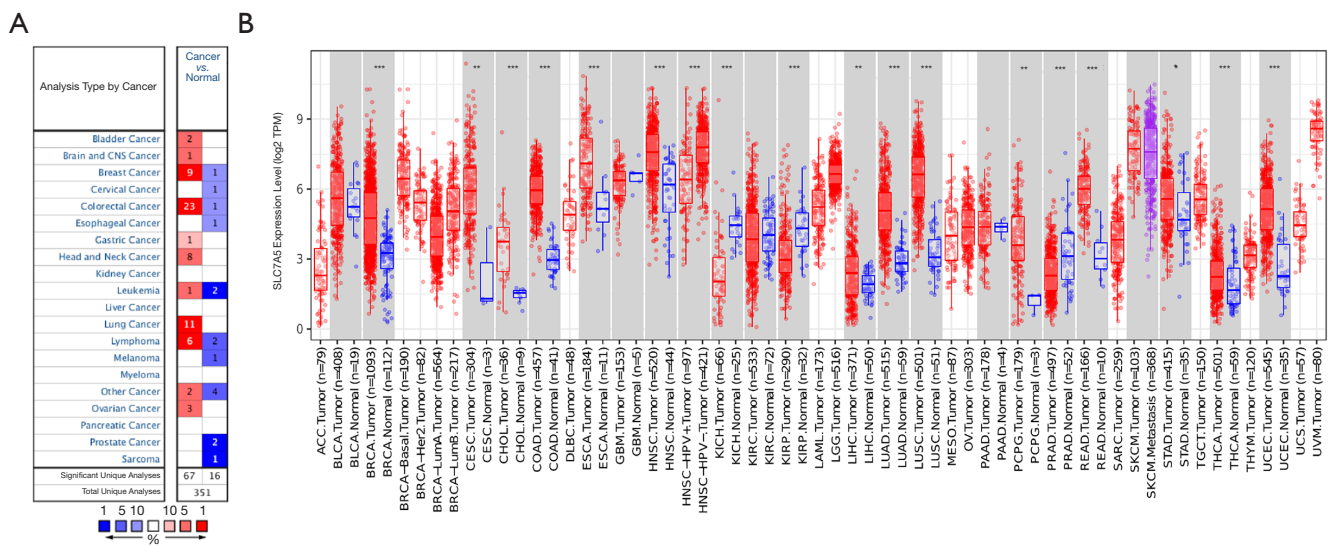


Figure 1 *SLC7A5* expression levels in various cancer types. (A) Higher or lower levels of expression of *SLC7A5* in different cancer tissues in comparison with normal tissues in OncoPrint. The number of data sets for each cancer type is indicated. (B) Human *SLC7A5* expression levels in different cancer types from TCGA data in TIMER. (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). TCGA, The Cancer Genome Atlas; TIMER, Tumor Immune Estimation Resource.

SLC7A5 expression, when compared to its expressions in the normal adjacent tissues, was increased in breast invasive carcinoma, specifically the luminal B, HER2+, and basal-like subtypes, as well as those in colon adenocarcinoma, head and neck squamous carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, rectum adenocarcinoma, and uterine corpus endometrial carcinoma.

We found that high or low expression level of *SLC7A5* protein was found predominantly in invasive BC cells' membrane (Figure 2A). We used polyclonal *SLC7A5* antibody (1:200, Proteintech, USA). The tissue sections were examined by two experienced pathologists using a double-blind method. The difference of positive cells and the intensity of cell staining were considered to score the results of immunohistochemistry. Based on the percentages of positive staining cells, the value obtained by multiplying the 2 scores was the final score for each specimen. We also found the differential expression among the four Intrinsic molecular subtypes (PAM50) of breast cancer subtypes (Figure 2B).

Genetic alterations of *SLC7A5* in BC

Next, we illustrated the genetic alterations distribution with clinical annotation of *SLC7A5* to identify genetic aberrations across the TCGA-BRCA cohort using the

cBioPortal OncoPrint module (Figure 3A). According to the cBioPortal definitions, genetic alterations covered the genomic alterations of number aberrations (CNAs) or mutations. In total, 3% of BRCA samples (29/963) harbored at least 1 alteration event within 0.75% and deep deletions in 2.25% of cases in TCGA datasets. The incidence of *SLC7A5* point mutations was very limited. We also found that amplification of CNAs was related to high mRNA expression while deep deletion of CNAs was associated with low mRNA expression. This analysis was based on the RNA-Seq by Expectation-Maximization values. Furthermore, the amplified CNAs were related to ER-/PR-/HER2-negative status and infiltrating ductal carcinoma, while the deep deleted CNAs were associated with ER-/PR-/HER2-positive status and infiltrating lobular carcinoma (Figure 3B).

Correlation between *SLC7A5* expression with clinicopathological parameters

In the 1,084 BC patients, 541 patients expressed a low level of *SLC7A5* and 542 expressed a high level of *SLC7A5*. There were significant differences in race, T stage, pathologic stage, histological type, ER status, PR status, and PAM50 subtypes among the 2 subgroups in Table 1 (P value > 0.05).

We used the ROC curves to determine the *SLC7A5*

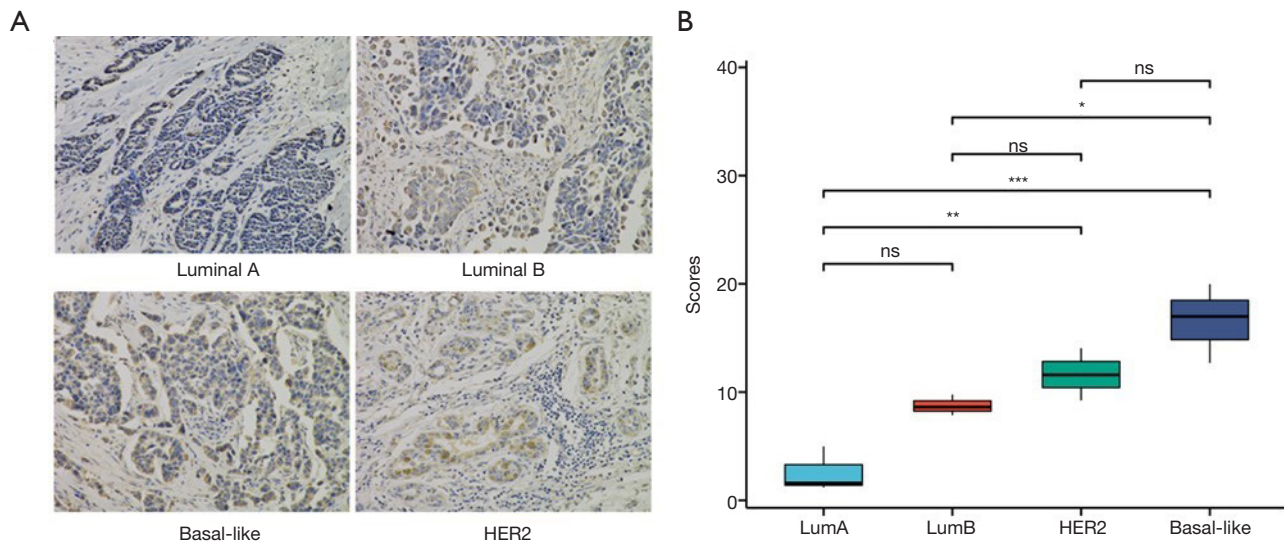


Figure 2 *SLC7A5* expression level in breast cancer subtypes. The staining of 3,3'-diaminobenzidine (DAB) was employed for immunohistochemistry (IHC) expression, magnification 20×20. (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). ns, nonsignificant.

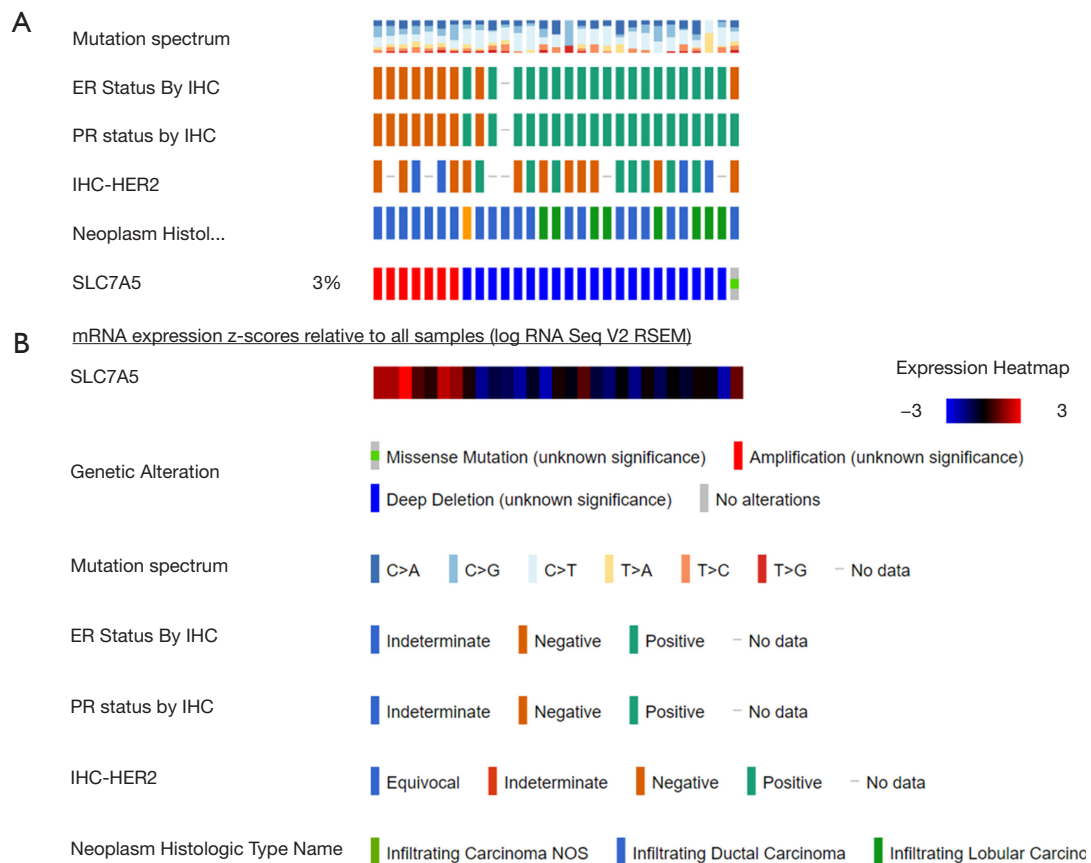


Figure 3 cBioPortal OncoPrint of *SLC7A5* in the invasive breast carcinoma cohort of TCGA dataset. (A) *SLC7A5* gene data were extracted with the cBioPortal to evaluate genetic alteration (amplification, deletion, mutation) of patients of the BRCA cohort. (B) The heatmap shows the mRNA expression levels of the selected samples. TCGA, The Cancer Genome Atlas; BRCA, breast carcinoma.

Table 1 The correlation between the expression of *SLC7A5* and clinicopathological data

Characteristic	Low expression of <i>SLC7A5</i>	High expression of <i>SLC7A5</i>	P value
n	541	542	
Age, n (%)			0.152
≤60	288 (26.6)	313 (28.9)	
>60	253 (23.4)	229 (21.1)	
Race, n (%)			<0.001
Asian	25 (2.5)	35 (3.5)	
Black or African American	57 (5.7)	124 (12.5)	
White	414 (41.6)	339 (34.1)	
T stage, n (%)			<0.001
T1	164 (15.2)	113 (10.5)	
T2	281 (26)	348 (32.2)	
T3	80 (7.4)	59 (5.5)	
T4	15 (1.4)	20 (1.9)	
N stage, n (%)			0.326
N0	255 (24.0)	259 (24.3)	
N1	190 (17.9)	168 (15.8)	
N2	52 (4.9)	64 (6.0)	
N3	34 (3.2)	42 (3.9)	
M stage, n (%)			0.575
M0	441 (81.5)	461 (85.1)	
M1	8 (1.5)	12 (2.2)	
Pathologic stage, n (%)			0.043
Stage I	107 (10.1)	74 (7)	
Stage II	297 (28.0)	322 (30.4)	
Stage III	117 (11.0)	125 (11.8)	
Stage IV	7 (0.7)	11 (1)	
Histological type, n (%)			<0.001
Infiltrating ductal carcinoma	325 (33.3)	447 (45.8)	
Infiltrating lobular carcinoma	160 (16.4)	45 (4.6)	
PR status, n (%)			<0.001
Negative	89 (8.6)	253 (24.5)	
Indeterminate	2 (0.2)	2 (0.2)	
Positive	427 (41.3)	261 (25.2)	

Table 1 (continued)

Table 1 (continued)

Characteristic	Low expression of <i>SLC7A5</i>	High expression of <i>SLC7A5</i>	P value
ER status, n (%)			<0.001
Negative	30 (2.9)	210 (20.3)	
Indeterminate	0 (0)	2 (0.2)	
Positive	488 (47.1)	305 (29.5)	
HER2 status, n (%)			0.082
Negative	284 (39.1)	274 (37.7)	
Indeterminate	4 (0.6)	8 (1.1)	
Positive	66 (9.1)	91 (12.5)	
PAM50, n (%)			<0.001
Normal	23 (2.1)	17 (1.6)	
Lum A	405 (37.4)	157 (14.5)	
Lum B	80 (7.4)	124 (11.4)	
HER2	21 (1.9)	61 (5.6)	
Basal	12 (1.1)	183 (16.9)	
Menopause status, n (%)			0.488
Pre-	107 (11.0)	122 (12.6)	
Peri-	22 (2.3)	18 (1.9)	
Post-	355 (36.5)	348 (35.8)	
Anatomic neoplasm subdivisions, n (%)			0.056
Left	265 (24.5)	298 (27.5)	
Right	276 (25.5)	244 (22.5)	
Radiation therapy, n (%)			0.762
No	217 (22.0)	217 (22.0)	
Yes	283 (28.7)	270 (27.4)	

performance in predicting the outcome of BC subtypes compared with other classical markers (Figure 4). In predicting the outcome of normal or tumor tissues, ER status, and PR status, the predictive ability of the *SLC7A5* variable showed a degree of accuracy (AUC =0.822; AUC =0.826; AUC =0.757) (Figure 4A,B,I), but had a lower accuracy for HER2 status (AUC =0.523) (Figure 4C). In differentiating PAM50 subtypes, such as the presence of luminal A vs. basal-like subtypes, the predictive ability of the *SLC7A5* variables had a high accuracy (AUC =0.929) (Figure 4D), while it had a good degree of accuracy for distinguishing the diverse subtypes of BC: luminal A vs. HER2 (AUC =0.806) (Figure 4E),

HER2 vs. basal-like (AUC =0.783) (Figure 4H), luminal B vs. basal-like (AUC =0.781) (Figure 4G), and luminal A vs. luminal B (AUC =0.730) (Figure 4F).

The prognostic value of *SLC7A5* in BC patients

For the purpose of determining the prognostic relevance of *SLC7A5* in BC, Kaplan-Meier curves were created to evaluate the clinical pattern of *SLC7A5* and its expression levels on survival (OS and RFS) and clinical follow-up period. Log-rank tests were conducted for evaluation of statistical significance. The results suggested that higher levels of *SLC7A5* were correlated with better OS (n=61,

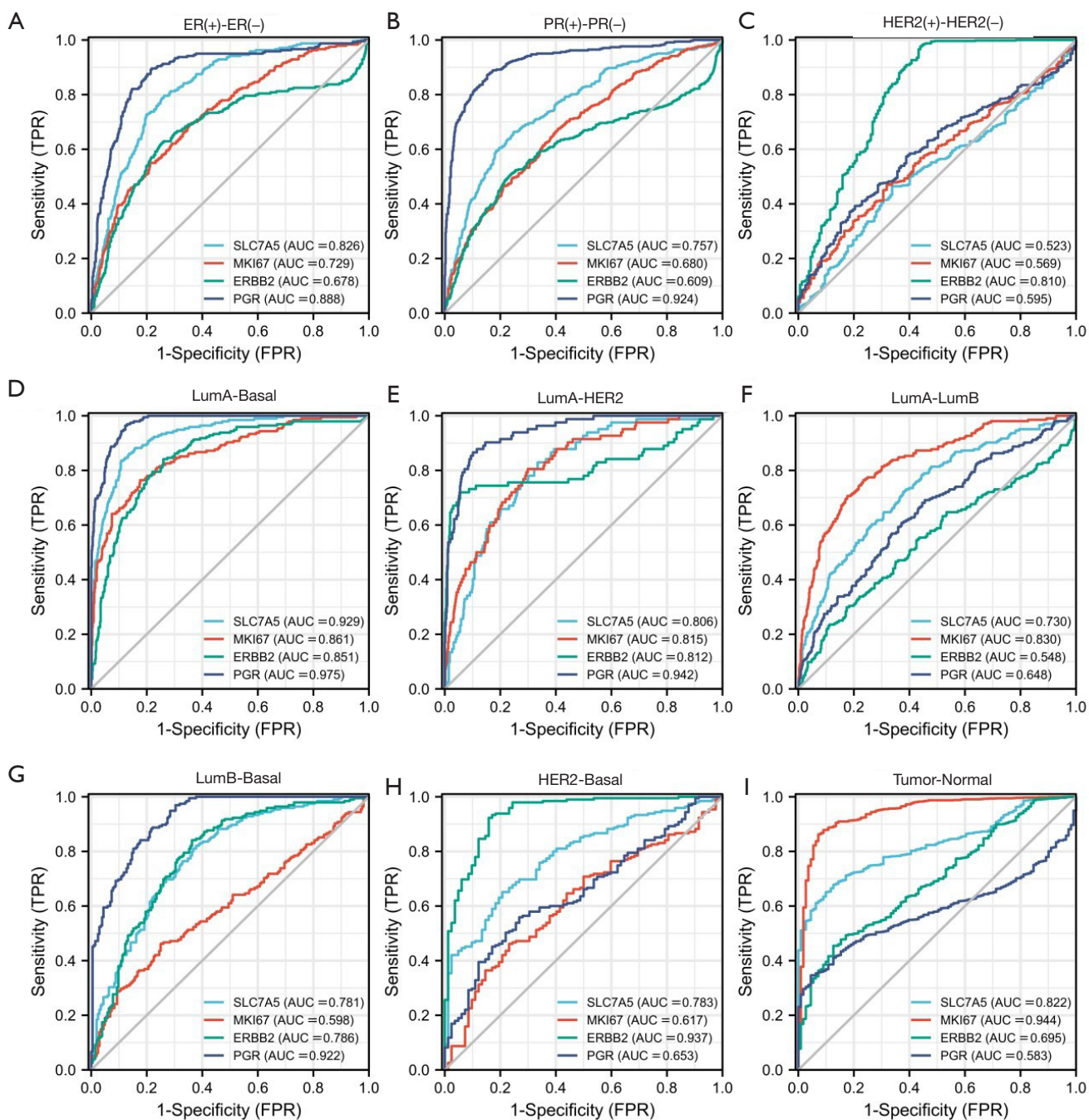


Figure 4 ROC curves comparing the predictive ability of *SLC7A5* to differentiate breast cancer subtypes. ROC, receiver operating characteristic.

$P < 0.001$; *Figure 5*), suggesting that the survival times in patients with a higher expression of *SLC7A5* were significantly shorter than those patients with lower levels of *SLC7A5* expression. We then analyzed *SLC7A5* expression as a significant prognostic parameter in the OS (HR = 1.64,

95% CI, 1.36–1.99, $P < 0.001$) and RFS value (HR = 1.58, 95% CI, 1.42–1.75, $P < 0.001$) of BRCA (*Figure 5A,E*). In the ER-positive subgroup (OS value: HR = 1.95, 95% CI, 1.41–2.7, $P < 0.001$), (RFS value: HR = 1.46, 95% CI, 1.25–1.7, $P < 0.001$) (*Figures 5B,F*), luminal A subtype (OS value: HR = 1.92, 95%

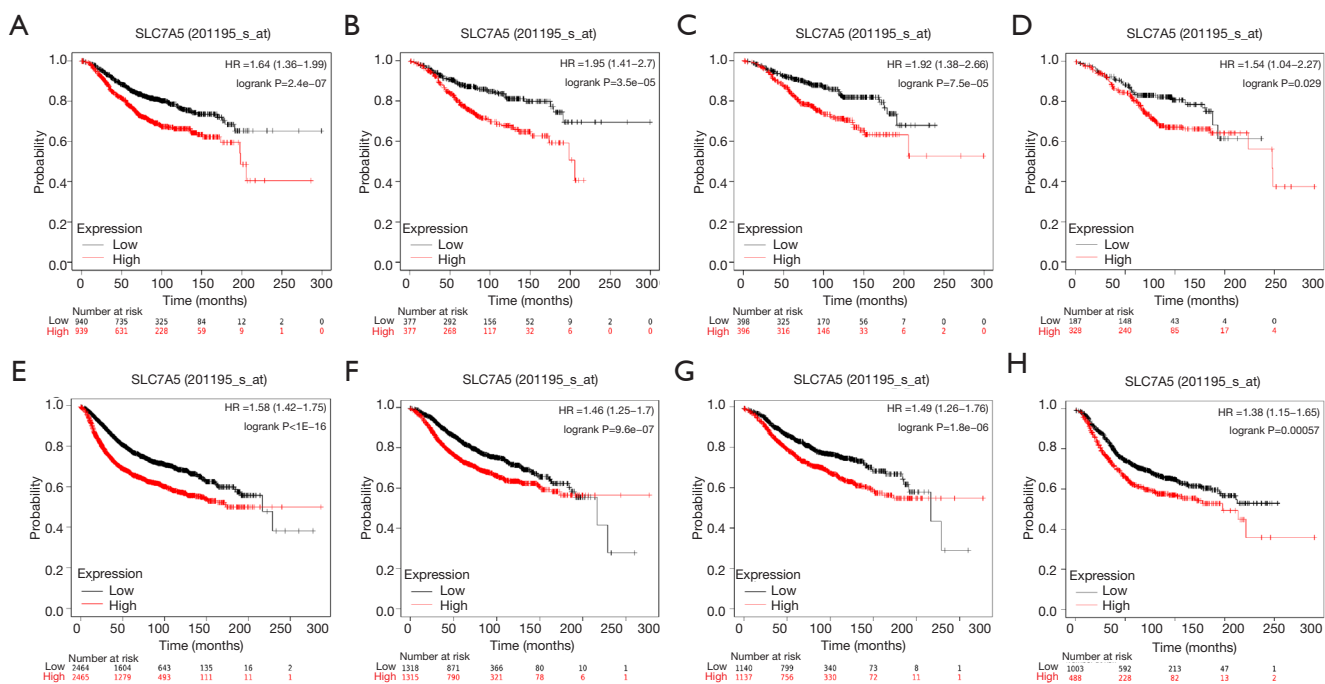


Figure 5 Kaplan-Meier survival curves comparing the high and low expression of *SLC7A5* in different subtypes of breast cancer in Kaplan-Meier Plotter. OS and RFS of breast cancer (A,E), ER+ subtype (B,F), luminal A subtype (C,G), and luminal B subtype (D,H). A red curve represents patients with high expression of *SLC7A5*. Overall survival (OS), relapse-free survival (RFS).

CI, 1.38–2.66, $P < 0.001$), (RFS value, HR =1.49, 95% CI, 1.26–1.76, $P < 0.001$) (Figure 5C,G) and luminal B subtype (OS value: HR =1.54, 95% CI, 1.04–2.27, $P < 0.001$), (RFS value, HR =1.38, 95% CI, 1.15–1.65, $P < 0.001$) (Figure 5D,H).

To examine the risk factors correlated to OS in patients with BC in detail, uni- and multivariate Cox regression models were conducted to determine if a high level of *SLC7A5* expression is prognostic for worse OS. Univariate analyses showed that age, PAM50 subtype, T stage, N stage, radiation therapy, and expression of *SLC7A5* were prognostic factors for OS ($P < 0.05$). Multivariate analyses showed that *SLC7A5* expression was an independent risk factor for tumor prognosis ($P = 0.006$), together with age of patients, T stage, N stage, and radiation therapy ($P < 0.05$). These data suggest that a higher level of *SLC7A5* expression was associated with decreased OS in BC patients (Table 2).

SLC7A5 is upregulated in BC cell lines

We examined the expression of *SLC7A5* in BC cell lines, which indicated that the mRNA and protein expression of *SLC7A5* were markedly increased in the MDA-MB-231, MCF-7, and T47-D cell lines (Figure 6).

SLC7A5 promoted BC cell proliferation

We then investigated the functional roles of *SLC7A5* in BC. *SLC7A5* lentiviruses were introduced into MCF-7 cells and increased expression was detected. The colony formation assay revealed that overexpression of *SLC7A5* led to an increased proliferative ability of MCF-7 cells (Figure 7A,B). To further explore the impact of *SLC7A5* in luminal cell cycle in the control group, we suppressed the expression of *SLC7A5* in MCF-7 cells, and conducted cell cycle evaluation by flow cytometry post transfection. Overexpression of *SLC7A5* led to a lower number of transfected cells in the G1 phase, and a higher number in the S phase in comparison with the control group (Figure 7C,D). These results further suggested that *SLC7A5* is implicated in the tumor growth and proliferation of BC cells.

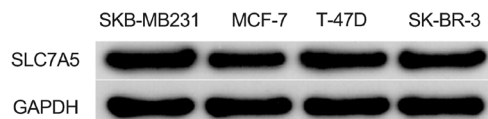
SLC7A5 activated AKT/mTORC1 pathway by phosphorylation

AKT/mTORC1 signaling pathway is known to have a crucial role in multiple tumor types, and suppression of AKT/mTORC1 signaling may limit cancer cell

Table 2 Univariate and multivariate analysis of clinical factors on 1,083 samples with OS

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age (>60 vs. ≤60)	1,082	2.020 (1.465–2.784)	<0.001	2.622 (1.709–4.023)	<0.001
Race (White vs. Asian and Black or African American)	993	0.912 (0.615–1.350)	0.644		
PAM50 (HER2, Basal and Lum B vs. Lum A)	1,042	1.576 (1.136–2.186)	0.006	1.495 (0.954–2.342)	0.080
T stage (T4 and T3 vs. T1 and T2)	1,079	1.608 (1.110–2.329)	0.012	1.703 (1.030–2.816)	0.038
N stage (N3 vs. N0, N1, and N2)	1,063	2.767 (1.610–4.756)	<0.001	2.886 (1.499–5.555)	0.002
Radiation therapy (yes vs. no)	986	0.576 (0.394–0.841)	0.004	0.605 (0.402–0.911)	0.016
TP53 (high vs. low)	1,082	1.360 (0.986–1.875)	0.061	1.002 (0.661–1.519)	0.994
PIK3CA (high vs. low)	1,082	1.258 (0.912–1.736)	0.162		
MYC (high vs. low)	1,082	0.853 (0.620–1.173)	0.329		
<i>SLC7A5</i> (high vs. low)	1,082	1.709 (1.234–2.367)	0.001	1.925 (1.202–3.083)	0.006

OS, overall survival; TP53, tumor protein p53; PIK3CA, phosphatidylinositol 3-kinases.

**Figure 6** *SLC7A5* expression in various BC cell lines. Western blot analysis of *SLC7A5* protein expression level in SKB-MB-231, MCF-7, T47-D, and SK-BR-3 cells.

proliferation. To elucidate the mechanism of *SLC7A5* involved in regulating cell cycle, we examined relevant proteins through western blotting. We transfected cells with *SLC7A5*-overexpressing lentiviruses, and detected the protein expression of p-AKT and p-mTOR (Figure 8). As expected, overexpression of *SLC7A5* increased the levels of p-AKT and p-mTOR but not the total AKT. The overexpression of *SLC7A5* also led to increased p70-S6K mRNA and the phosphorylation of the protein in MCF-7 cells. These results further indicated that *SLC7A5* has a promoting function in the growth of luminal BC cells.

Discussion

BC is a highly heterogeneous disease consisting of various different tumor subtypes (9,17) and variations in terms of cellular, molecular, and biological profiles in its response to treatment effects and clinical behaviors. Moreover, various subtypes exhibit disparate nutritional and metabolic needs.

The ER+/luminal subtype is the most common subtype of BC (18), and tumors in the subgroup are complicated with regard to diagnosis, prognosis, recurrence rate, and mortality (19). Therefore, exploring the biology and the metabolic pathways of BC subclasses could help to identify potential precision therapeutic targets.

Reprogramming of metabolism is a cancer characteristic in which tumor cells alter the metabolism for the purpose of meeting the needs of cellular building blocks and acquiring the necessary energy (20). Oncogenic activities force the dysregulation of metabolic pathways to build cells increased survival and proliferation in the cancer microenvironment (8). As is well known, AA transport systems are very important to maintaining the proliferation of tumor cells, as they supply the AAs needed for protein synthesis and activate nutritional signaling through the mTORC1 pathway. Our research suggests that the AA transporter, *SLC7A5*, is important in BC, especially in ER-positive tumors.

Our study showed that in genetic variation, deletion of *SLC7A5*, rather than its amplification, was significantly correlated to invasive lobular histology. In addition, *SLC7A5* expression level in lobular carcinoma was when compared to that in invasive ductal carcinoma, confirming that CNAs of *SLC7A5* are present in various BC phenotypes.

SLC7A5 is highly expressed in multiple tumor tissues and in different cell lines (21). The present analysis used TCGA populations to identify any significant associations between

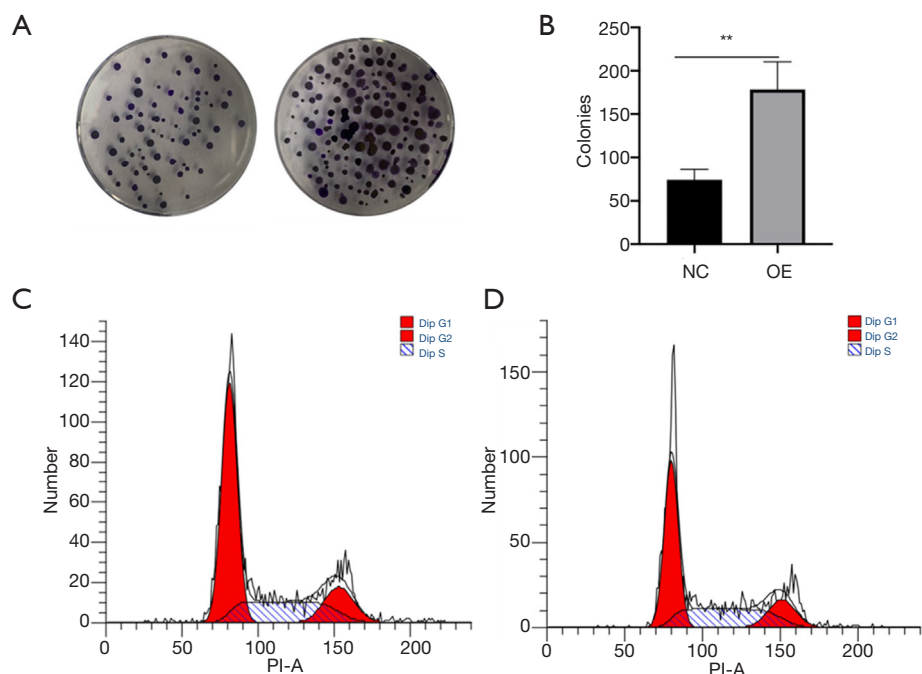


Figure 7 *SLC7A5* is required in regulations of the biological functions of MCF-7 cells. Colony formation assays were conducted to analyze clonogenic ability of MCF-7 cells after transfection, crystal violet staining (A,B). Flow cytometric assays were used to analyze the cell cycle before (C) and after transfection (D). **, $P < 0.01$. NC, negative control; OE, over expression.

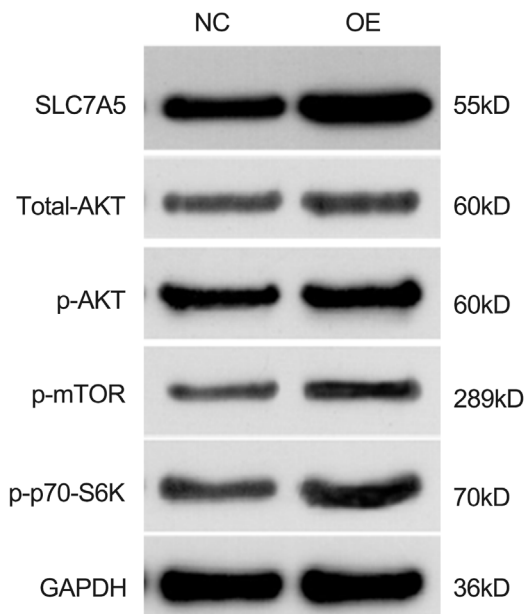


Figure 8 *SLC7A5* activated AKT/mTORC1 signaling pathway. Western blot analysis of phospho-AKT, phospho-mTOR, phospho-p70-S6K, and *SLC7A5* in MCF-7 cells when *SLC7A5* was overexpressed compared with the control group. GAPDH was used as a loading control. NC, negative control; OE, over expression.

the expression level of *SLC7A5* and clinical outcomes and pathological parameters, including PAM50 subtype, T stage, N stage, and radiation therapy. The ability of *SLC7A5* in predicting the outcome of BC subclasses was found to be highly similar to that of Ki-67. This is in accordance with previous publications which suggested that these 2 parameters are significantly correlated to prognosis in patients with tongue cancer (11), neuroendocrine carcinoma of the lung (22), and thymic carcinoma (23), showing that *SLC7A5* is of vital importance in cancer cell proliferation.

SLC7A5 expression was remarkably decreased in low proliferation ER-positive tumors (luminal A subtype) than in luminal B tumors. *SLC7A5* expression was more correlated with worse clinical outcomes, such as shorter OS or RFS, in the ER-positive subgroup than in the ER-negative subgroup. This finding may be explained by different energy and nutritional demands for cell growth, proliferation, and tumor microenvironment (TME). Bartlett *et al.* (24) suggested that *SLC7A5* be one part of the 5-gene Mammostrat[®] immunohistochemistry panel, as it has an increased level of expression. *SLC7A5* was correlated with OS, RFS, and distant metastasis-free survival (DMFS) in ER-positive BC patients undergoing endocrine treatment (25).

SLC7A5 mRNA and protein expression is much higher in the triple-negative BC and HER2-positive tumors but is not correlated with survival (26). The level of *SLC7A5* protein expression has been used as an prognostic factor in ER-positive low-proliferation tumors in our results. We also found the larger sample size of patients with ER-positive tumors has a better potential to reflect a poor prognosis than does a smaller sample size of ER-negative tumors.

An increasing amount of evidence shows that the activation of AKT/mTORC1 pathway is crucial to the cell proliferation process and prevents apoptosis in tumor cells (26). The vital function of *SLC7A5* includes supplying essential AAs for protein synthesis and for regulation of mTORC1 signaling (e.g., leucine). The activation mTORC1 in turn regulates protein translation, cell survival and cell proliferation, and prevents apoptosis in malignancies (27,28). Mechanistically, phosphorylation of AKT and mTORC1 is required to activate the pathway to control motility and phenotype transformation of cells. One study showed that the high expression of *SLC7A5* activated AKT/mTOR signaling pathway in NSCLC (29). In our analysis, *SLC7A5* overexpression significantly elevated the levels of phospho-AKT, phospho-mTOR, phospho-p70-S6K. Up-regulated *SLC7A5* through AKT/mTORC1 signaling pathway, which promoted proliferation of MCF-7 to S phase.

In conclusion, our results suggest that the level of the AA transporter *SLC7A5* expression is increased in breast cancer tissues and cell lines with differential expression in diverse subtypes. We also found that the level of *SLC7A5* expression was correlated with poor prognostic characteristics and worse survival in the ER+/luminal subtypes. Furthermore, overexpression of *SLC7A5* was revealed to be important in the proliferation and cell cycle regulation of the MCF-7 cells through its action of upregulating the expression of phospho-AKT, phospho-mTOR, and phospho-p70-S6K. *SLC7A5* was thus promoting cell proliferation via activation of AKT/mTORC1 pathway through phosphorylation. Therefore, *SLC7A5* is a potential prognostic marker and a potential therapeutic target of the luminal subtype of BC treatment.

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Footnote

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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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Women's Hospital of Nanjing Medical University (No.: KY2021054) and informed consent was taken from all individual participants.

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