Diagnostic and prognostic significance of SLC50A1 expression in patients with primary early breast cancer

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Abstract. There is a lack of validated biomarkers for the diagnosis of early breast cancer (EBC). The current study aimed to determine the diagnostic and prognostic value of solute carrier family 50 member 1 (SLC50A1) in patients with EBC. Therefore, 123 patients with EBC, 30 patients with benign breast disease (BBD) and 26 healthy controls (HCs) were recruited. The serum levels of SLC50A1 in paired sera of 40 postoperative patients were assessed by ELISA. Immunohistochemical staining for SLC50A1 was performed in surgical tissue derived from 83 patients with EBC and 30 patients with BBD. mRNA expression of SLC50A1 and its diagnostic and prognostic value in patients with EBC was evaluated using an RNA-sequencing database. The results showed that serum levels of SLC50A1 in patients with EBC were significantly higher compared with those in patients with BBD and HCs (both P<0.001). Additionally, receiver operating characteristic curve analysis revealed that the serum levels of SLC50A1 distinguished patients with EBC from patients with BBD and HCs with a sensitivity of 76.42% and specificity of 76.79% [area under the curve (AUC)=0.783; P<0.001]. The diagnostic value of SLC50A1 was significantly greater than that of carcinoembryonic (P<0.005) and carbohydrate antigen 15-3 (P<0.029). Furthermore, the number of SLC50A1 positive cells significantly increased in tissue of patients with EBC compared with patients with BBD (P<0.001). A positive association between serum levels of SLC50A1 and its expression in tissue samples was observed in patients with EBC (ρ=0.700; P<0.001). Additionally, bioinformatics analysis verified the diagnostic value of SLC50A1, with an AUC of 0.983 (P<0.001). Multivariate analysis demonstrated that SLC50A1 was an independent prognostic factor in patients with EBC with a hazard ratio of 1.917 (P=0.013). These findings indicated that SLC50A1 may be a potential diagnostic biomarker for primary EBC and that SLC50A1 upregulation may be associated with unfavorable prognosis in patients with EBC.

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Introduction

Breast cancer (BC) is the most common malignant tumor in the world, with an incidence of 2.3 million novel cases in 2020, accounting for 11.7% of all new cancer cases (1). Comprehensive treatment approaches, including surgery, radiotherapy, chemotherapy and targeted therapy and endocrine therapy, have notably improved the 5-year survival rate in patients with early BC (EBC) by >90% (2,3). However, the median survival time of patients with stage IV BC is only 31 months (4). The effective treatment of BC is associated with early diagnosis and regular surveillance. Currently, the screening and diagnosis of BC primarily rely on mammography, ultrasound and magnetic resonance imaging, as well as tissue biopsy when necessary (5,6). Serum tumor biomarkers, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA15-3), have been widely used to monitor BC treatment, recurrence and metastasis (7,8). However, the aforementioned biomarkers are not recommended for diagnosis of EBC due to their low sensitivity and specificity (9). Currently, circulating tumor cells and circulating tumor DNA can be used to evaluate treatment response, recurrence and metastasis in patients with EBC (10-13). However, the aforementioned detection approaches have not been widely used for EBC in clinical practice due to their low sensitivity and high cost (14). Therefore, identifying novel effective biomarkers to improve early diagnosis of EBC is of importance.

The solute carrier (SLC) family is one of the largest families of membrane proteins encoded by the human genome, comprising 65 families with ~400 members (15). The SLC2, SLC5 and SLC50 families are involved in mediating transmembrane transport of glucose (16). Glucose transporters serve a role in the progression of several types of cancer, including pancreatic (17), gastric (18), breast (19) and cervical cancer (20). The serum or tissue protein encoded by

SLC50A1 gene consists of 221 amino acids (molecular weight, 25 kDa) (16,21). Located in the Golgi apparatus, SLC50A1 is involved in efflux of glucose in human intestinal and liver cells as part of the vesicle efflux pathway (22). A previous study demonstrated that SLC50A1 is upregulated in lung adenocarcinoma; to the best of our knowledge, however, whether SLC50A1 is associated with the prognosis of lung cancer has not been elucidated (23). Another study showed that SLC50A1 is associated with high metabolic activity in BC (24); high metabolic activity is a hallmark of several types of cancer, thus indicating that SLC50A1 may be a potential biomarker for BC. This finding has also been verified by previous studies (25,26). To the best of our knowledge, however, the role of SLC50A1 in the diagnosis and prognosis of EBC has not been previously investigated. Therefore, in the present study, expression levels of SLC50A1 in serum and tissue samples were assessed using ELISA and immunohistochemistry (IHC) staining to evaluate its potential value in histopathological and serological diagnosis of EBC. Furthermore, bioinformatics analysis using data from The Cancer Genome Atlas (TCGA) database was performed to determine the association between mRNA expression levels of SLC50A1 with diagnosis and prognosis of EBC.

Materials and methods

Patients. The present study was a prospective observational study. A total of 123 consecutive patients with EBC (age, 20-70 years, with a median age of 54 years, were screened at Cancer Hospital of Shantou University Medical College between January 2020 and February 2021, according to the National Comprehensive Cancer Network guidelines (27). Among patients, 83 underwent surgery, while the remaining 40 patients received neoadjuvant chemotherapy following surgery. The inclusion criteria for patients with EBC were as follows: i) Female patients with newly diagnosed BC; ii) no history of previous malignant or severe disease; iii) no distant metastasis and iv) patients who did not receive antineoplastic therapy prior to diagnosis (Table SI). In addition, 30 patients with benign breast disease (BBD) and 26 healthy controls (HCs) who underwent medical examinations, all of whom were female, aged 20-50 years, with a median age of 42 years and no history of previous malignant or severe disease, were enrolled (Fig. S1). The sample size in the statistical analysis met the requirements of the power test (data not shown). The molecular subtypes of BC were defined according to the 13th St Gallen International Breast Cancer Conference (28). The present study was approved by the Ethics Committee of Cancer Hospital of Shantou University Medical College (approval no. 2019049; Shantou, China). All participants signed an informed consent form and all patient data were anonymized. The study was performed in accordance with the Reporting Recommendations for Tumor Marker Prognostic Studies guidelines (29).

Serum SLC50A1 assay. Serum samples were collected from all patients. Among the 123 patients with EBC, preoperative and 14-day postoperative serum samples were collected from 40 patients. The serum samples obtained from participants fasted for 8 h, were centrifuged at 447.2 x g for 5 min at room temperature and stored at -80˚C. An ELISA kit (Andy Gene Biotechnology Co., Ltd.) was used to measure serum protein levels of SLC50A1, according to the manufacturer's protocol. Each serum sample was repeated three times. Materials and devices are listed in Table SII.

IHC staining. Tumor tissue isolated from 83 patients with EBC and 30 patients with BBD was stained by IHC. Briefly, 4-μm-thick sections were dewaxed, endogenous peroxidase was blocked by adding 3% hydrogen peroxide for 20 min and washed three times using PBS. Diluted 50X EDTA was added and the antigen was repaired at 100˚C for 5 min and 40˚C for 15 min, then washed three times with PBS and blocked using goat serum (Wuhan Boster Biological Technology, Ltd.) for 30 min at 37˚C and incubated with an antibody against SLC50A1 (1:150; Thermo Fisher Scientific, Inc.) at 4˚C overnight. The negative control tissue was treated with PBS. Sections were incubated with the corresponding secondary antibody (goat anti-mouse/rabbit IgG-HRP) at 37˚C for 30 min and then visualized with a 20X 3,3'-diaminobenzidine for 5 min at room temperature. Each section was counterstained with hematoxylin for 30 seconds at room temperature, dehydrated and sealed with neutral balsam at room temperature. Cellular staining in tissue sections were independently evaluated by two pathologists under an optical microscope, and histochemical score (H-score) was used to reflect expression levels of SLC50A1. H-score is a histological scoring system used for the semi-quantification of tissue staining and is expressed as the staining area (0-4) to staining intensity (0-3) ratio (30). H-scores of <6 and ≥ 6 were considered to indicate low and high expression levels, respectively.

Collection of genomic data of patients with EBC from TCGA database. RNA-sequencing data of patients with EBC were extracted from the TCGA-BRCA project of TCGA database (tcga-data.nci.nih.gov/tcga/). Normal tissue samples were obtained from Genome-Tissue Expression (GTEx; gtexportal.org/home/datasets). The clinical data of EBC patients were obtained from TCGA database. All data processing and analysis was performed using R software 3.6.1 (r-project.org/).

Statistical analysis. Serum SLC50A1 levels are expressed as mean ± standard deviation of three independent repeats. A χ^2 or Fisher's exact test was performed to evaluate the association between SLC50A1 expression and clinicopathological features of patients. Mann-Whitney U and Wilcoxon or Kruskal-Wallis H test was used to compare expression levels between groups. Dunn's post hoc test was used with three or more groups. The association between two variables was assessed by Spearman's rank correlation test. The diagnostic value of SLC50A1 was determined using the area under the curve (AUC) by constructing a receiver operating characteristic (ROC) curve. AUC values were compared using the DeLong method (31). Based on the optimal cut-off value determined by ROC curve analysis, the patients were classified into high and low SLC50A1 expression groups. The overall survival (OS) was calculated using the Kaplan-Meier method. Univariate Cox analysis was performed to screen prognostic factors, while multivariate Cox analysis was applied to evaluate independent risk factors. Statistical analysis was performed

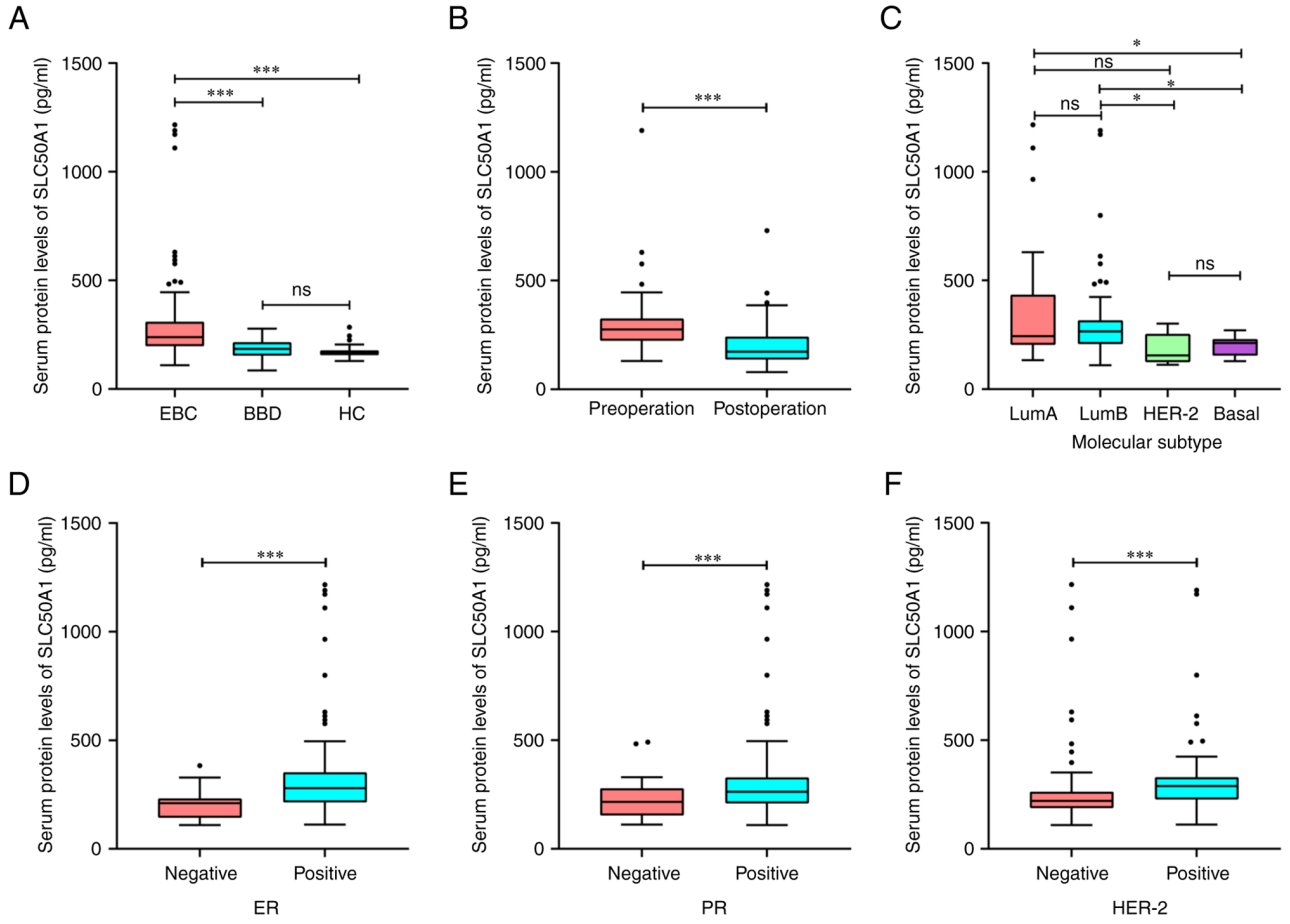

Figure 1. Serum SLC50A1 levels in EBC. (A) Serum SLC50A1 levels were highest in EBC cohort (analyzed using Kruskal-Wallis test). (B) Postoperative serum SLC50A1 levels were significantly lower than preoperative SLC50A1 levels (analyzed using Wilcoxon test). (C) Serum SLC50A1 levels in molecular subtype (analyzed using Kruskal-Wallis test). High SLC50A1 levels were associated with (D) ER, (E) PR and (F) HER-2 status (analyzed using Mann-Whitney U test). *P<0.05; ***P<0.001. SLC50A1, solute carrier family 50 member 1; EBC, early breast cancer; BBD, benign breast disease; ER, estrogen receptor; HC, healthy control; HER-2, human epidermal growth factor receptor 2; Lum A, luminal A; Lum B, luminal B; PR, progesterone receptor; ns, non-significant.

using R software 3.6.1 (r-project.org/) or GraphPad Prism 8.0 (GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

SLC50A1 is upregulated in serum of patients with EBC. To determine protein expression levels of SLC50A1 in serum, ELISA was performed using serum samples from patients with EBC and BBD and HCs. The serum levels of SLC50A1 were notably increased in patients with EBC compared with the other groups (P<0.001; Fig. 1A). The median, 25th and 75th percentile and mean serum levels of SLC50A1 were 238.8, 198.7, 308.3 and 296.5±207.1 pg/ml, respectively, in patients with EBC; 169.9, 157.2, 177.3 and 174.3±33.7 pg/ml, respectively, in patients with BBD and 184.8, 155.5, 214.1 and 179.1±45.3 pg/ml, respectively, for HCs (Table I). The serum levels of SLC50A1 were not significantly different between BBD and HC groups (Fig. 1A). Serum levels of SLC50A1 were significantly lower in postoperative patients compared with preoperative levels (Fig. 1B).

Association between serum SLC50A1 levels and clinicopathological characteristics of patients with EBC. Patients in the high SLC50A1 protein expression group, according to H-score ≥6, exhibited higher serum levels of SLC50A1 compared with those in the low SLC50A1 protein expression group (Table I). Furthermore, serum levels of SLC50A1 were significantly associated with estrogen receptor (ER)-positive BC, progesterone receptor (PR)-positive BC, human epidermal growth factor receptor 2 (HER-2)-positive BC, luminal A subtype and luminal B subtype (Fig. 1C-F). There was no significant association between serum levels of SLC50A1 and age, menopausal status, tumor size, lymph node status, TNM stage and pathological grade (Table I).

Diagnostic value of SLC50A1 serum levels in patients with EBC. To distinguish patients with EBC from HCs or patients with BBD, ROC curve analysis was performed to analyze the predictive value of SLC50A1 levels in serum. Between patients with EBC and HCs and patients with EBC and BBD, the cut-off values for diagnosis of EBC were 188.2 and 221.1 pg/ml, with a sensitivity of 78.86 and 61.79%, specificity of 84.62 and 90% and AUC of 0.792 and 0.774 [95% confidence interval (CI), 0.715-0.869 and 0.698-0.850], respectively (Fig. 2A and B). When patients with BBD and HCs were classified as a non-tumor group, the cut-off value was 197.2 pg/ml, with a sensitivity of 76.42%, specificity of 76.79% and AUC of 0.783 (Fig. 2C).

Table I. Association between clinicopathologic characteristics and serum SLC50A1 levels.

Parameter	Variable	n	Serum SLC50A1 levels, pg/ml		P-value
			Median (interquartile range)	Mean ± SD	
Patient group	Early breast cancer	123	238.8 (198.7-308.3)	296.5±207.1	<0.001
	Benign breast disease	30	169.9 (157.2-177.3)	174.3±33.7	
	Healthy controls	26	184.8 (155.5-214.1)	179.1±45.3	
Operative status	Preoperation	40	275.2 (224.2-324.3)	314.8±176.4	<0.001
	Postoperation	40	173.2 (138.3-241.2)	213.7±124.1	
Age, years	<60	81	245.7 (194.7-309.0)	301.3±211.8	0.680
	≥60	42	230.0 (199.6-303.1)	287.2±199.9	
Menopausal status	Premenopausal	53	261.8 (200.7-305.1)	302.9±212.5	0.639
	Postmenopausal	70	230.0 (197.2-310.6)	291.6±204.3	
Molecular subtype	Basal	15	211.7 (154.8-229.4)	199.5±45.7	0.002
	HER-2	9	155.6 (126.0-253.0)	186.3±70.9	
	Luminal A	24	243.7 (205.4-433.5)	386.3±303.1	
	Luminal B	75	264.8 (208.9-315.9)	300.3±187.6	
Estrogen receptor status	Positive	44	280.2 (214.8-315.2)	348.1±239.2	<0.001
	Negative	79	210.6 (144.7-231.8)	203.7±64.5	
Progesterone receptor status	Positive	79	263.5 (211.0-327.8)	336.1±242.1	0.001
	Negative	44	216.8 (155.6-277.6)	225.3±85.2	
HER-2 status	Positive	56	288.8 (228.8-328.5)	326.4±206.4	<0.001
	Negative	67	220.8 (189.7-261.8)	271.4±205.8	
Tumor size, mm	≤20	45	265.3 (206.6-387.3)	348.2±261.8	0.097
	>20	78	232.1 (186.3-300.8)	266.6±162.2	
Nodal status	Positive	61	244.6 (191.2-307.7)	296.7±213.6	0.889
	Negative	62	236.2 (202.8-308.3)	296.2±202.2	
TNM stage	I	37	238.8 (204.2-353.2)	336.8±248.4	0.613
	II	46	248.4 (196.6-300.9)	265.9±159.1	
	III	40	229.7 (186.5-307.3)	294.2±212.9	
Histological grade	I/II	39	261.8 (204.2-327.6)	267.4±101.8	0.794
	III	42	238.7 (210.6-308.6)	313.7±238.2	
SLC50A1 expression[a]	High	36	314.6 (266.2-440.5)	414.8±264.5	<0.001
	Low	47	211.7 (155.6-242.4)	210.1±58.2	

[a]H-scores of <6 and ≥6 were considered to indicate low and high expression levels, respectively. SLC50A1, solute carrier family 50 member 1; HER-2, human epidermal growth factor receptor 2; SD, standard deviation.

In addition, ROC curves were constructed based on serum levels of CEA and CA15-3 to compare the EBC and BBD groups. The AUC values of CEA and CA15-3 were 0.612 and 0.623, with sensitivity of 54.47 and 30.08% and specificity of 73.33 and 100%, respectively (Fig. 2D and E). AUC value of SLC50A1 was significantly higher compared with that of CEA or CA15-3 (Fig. 2F). These findings indicated that the diagnostic value of SLC50A1 was superior to that of CEA or CA15-3.

Protein expression of SLC50A1 in EBC tissue. SLC50A1 localization was evaluated by staining the membrane and cytoplasm of tumor cells. The results showed that 92.77% (77/83) of EBC and 10% (3/30) of BBD tissue samples exhibited positive staining for SLC50A1 (Fig. 3A-E). The association between protein expression levels of SLC50A1 and clinicopathological characteristics of patients with EBC is shown in Table II. Protein expression levels of SLC50A1 were significantly associated with PR- and HER-2-positive EBC. However, there was no significant association between SLC50A1 expression and age, menopausal status, molecular subtype, ER, tumor size, lymph node status, TNM stage and histological grade (Table II). In addition, Spearman's correlation analysis revealed a moderate positive correlation between expression levels of SLC50A1 between serum and tissue samples derived from patients with EBC (ρ=0.700; Fig. 4).

mRNA expression levels of SLC50A1 in patients with EBC in TCGA database. Data from a total of 901 patients with EBC and 572 healthy individuals were acquired from TCGA and GTEx databases. mRNA expression levels of SLC50A1 were

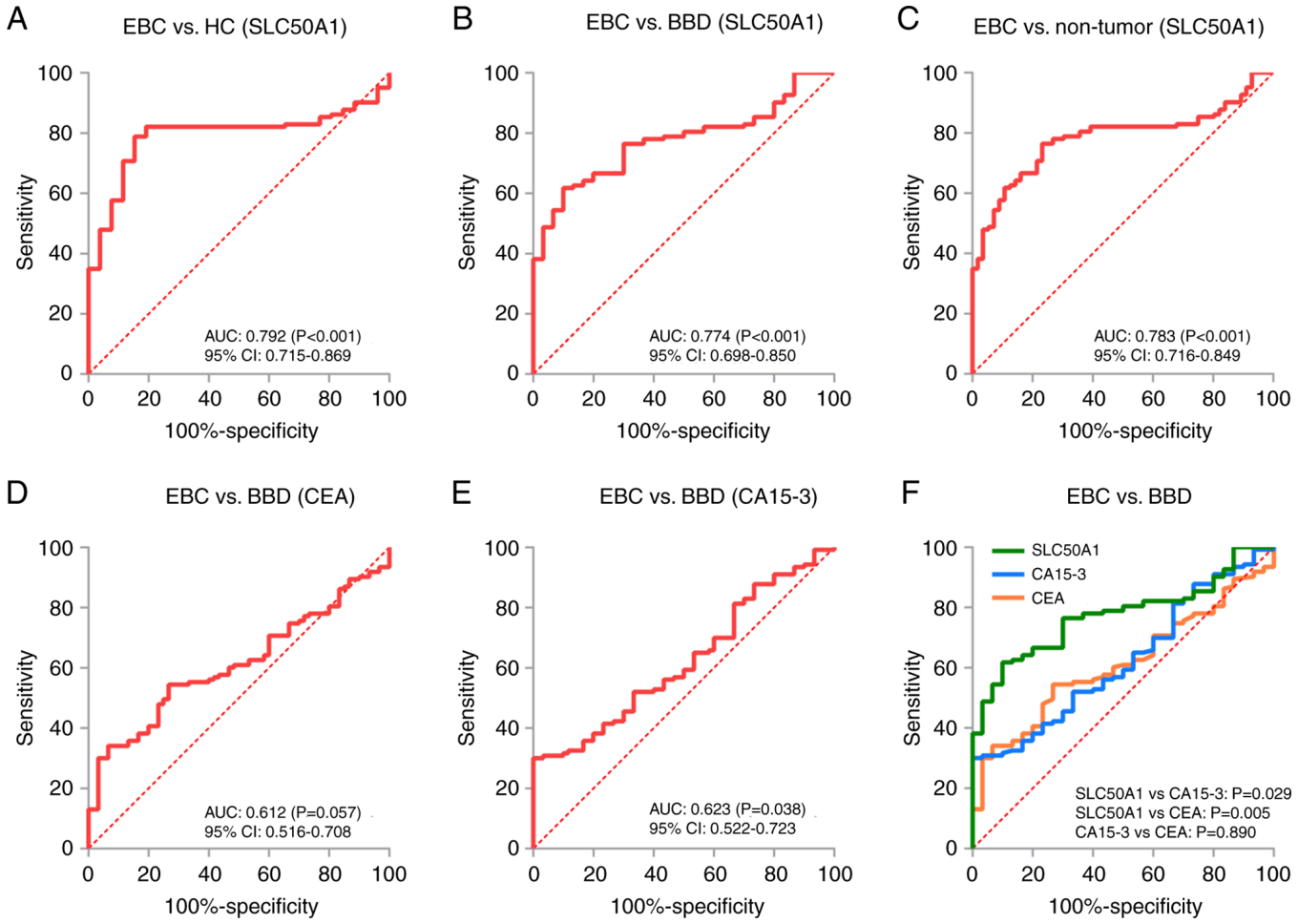

Figure 2. ROC curve analysis of serum SLC50A1, CEA and CA15-3 levels. ROC curve analysis of serum SLC50A1 levels between (A) EBC and HC, (B) EBC and BBD and (C) EBC and the non-tumor group (BBD + HCs). ROC curve analysis of serum (D) CEA and (E) CA15-3 levels between EBC and BBD. (F) ROC curve analysis for serum CEA levels, serum CA15-3 levels and serum SLC50A1 levels between EBC and BBD. ROC, receiver operating characteristic; SLC50A1, solute carrier family 50 member 1; BBD, benign breast disease; CA15-3, carbohydrate antigen 15-3; CEA, carcinoembryonic antigen; EBC, early breast cancer; HC, healthy control; AUC, area under the curve.

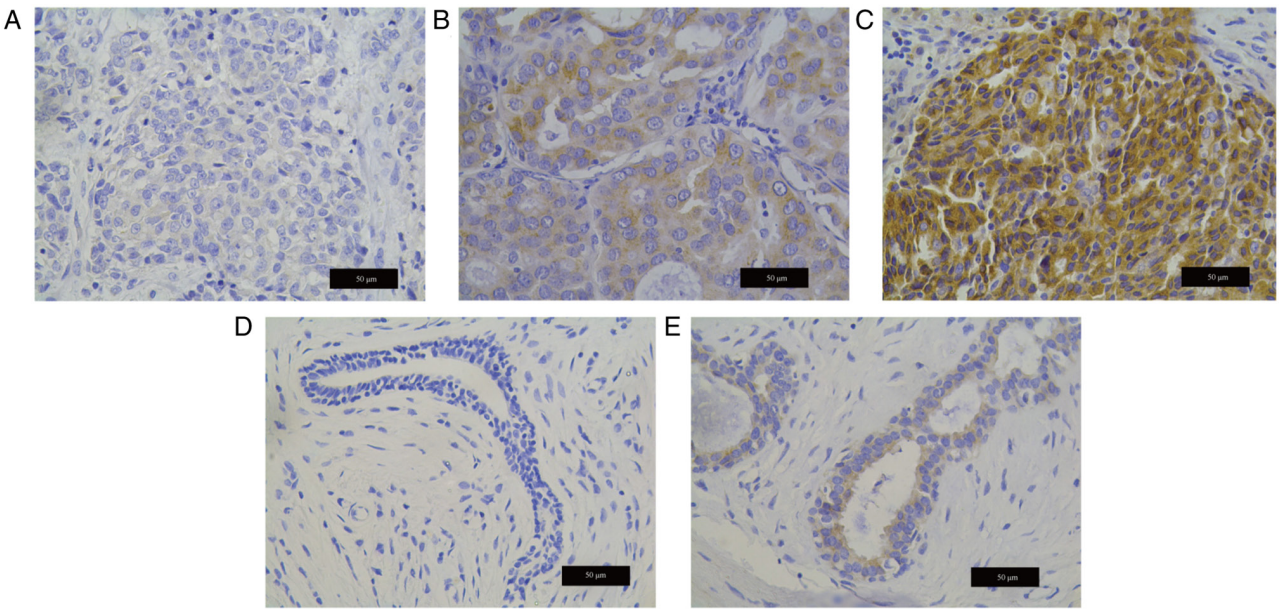

Figure 3. Immunohistochemical staining of SLC50A1 expression. (A) Negative, (B) low and (C) high expression of SLC50A1 in EBC. (D) Negative and (E) positive expression of SLC50A1 in BBD. Original magnification, x400; scale bar, 50 μm. SLC50A1, solute carrier family 50 member 1; BBD, benign breast disease; EBC, early breast cancer.

Table II. Association between clinicopathological characteristics and tissue SLC50A1 expression.

Parameter	Variable	n	SLC50A1 expression		χ^2-value	P-value
			High, n (%)	Low, n (%)		
Age, years	<60	57	26.00 (45.61)	31.00 (54.39)	0.372	0.542
	≥60	26	10.00 (38.46)	16.00 (61.54)		
Menopausal status	Premenopausal	38	18.00 (47.37)	20.00 (52.63)	0.455	0.500
	Postmenopausal	45	18.00 (40.00)	27.00 (60.00)		
Molecular subtype	Basal	11	2.00 (18.18)	9.00 (81.82)	4.936	0.177
	HER-2	5	1.00 (20.00)	4.00 (80.00)		
	Luminal A	19	9.00 (47.37)	10.00 (52.63)		
	Luminal B	48	24.00 (50.00)	24.00 (50.00)		
Estrogen receptor	Positive	32	10.00 (31.25)	22.00 (68.75)	3.117	0.078
	Negative	51	26.00 (50.98)	25.00 (49.02)		
Progesterone receptor	Positive	55	29.00 (52.00)	26.00 (47.27)	5.808	0.016
	Negative	28	7.00 (25.00)	21.00 (75.00)		
HER-2	Positive	33	20.00 (60.61)	13.00 (39.39)	6.623	0.010
	Negative	50	16.00 (32.00)	34.00 (68.00)		
Tumor size, mm	≤20	44	19.00 (43.18)	25.00 (56.92)	0.001	0.970
	>20	39	17.00 (43.59)	22.00 (56.41)		
Nodal status	Positive	25	15.00 (60.00)	10.00 (40.00)	1.737	0.188
	Negative	58	21.00 (36.21)	27.00 (62.79)		
TNM stage	I	37	14.00 (37.84)	23.00 (62.16)	0.846	0.655
	II	40	19.00 (47.50)	21.00 (52.50)		
	III	6	3.00 (50.00)	3.00 (50.00)		
Histological grade	I/II	33	14.00 (42.42)	19.00 (57.58)	0.053	0.974
	III	37	16.00 (43.24)	21.00 (56.76)		
	Unknown	13	6.00 (46.15)	7.00 (53.85)		

Analysis was performed using Fisher's exact test. SLC50A1, solute carrier family 50 member 1; HER-2, human epidermal growth factor-2.

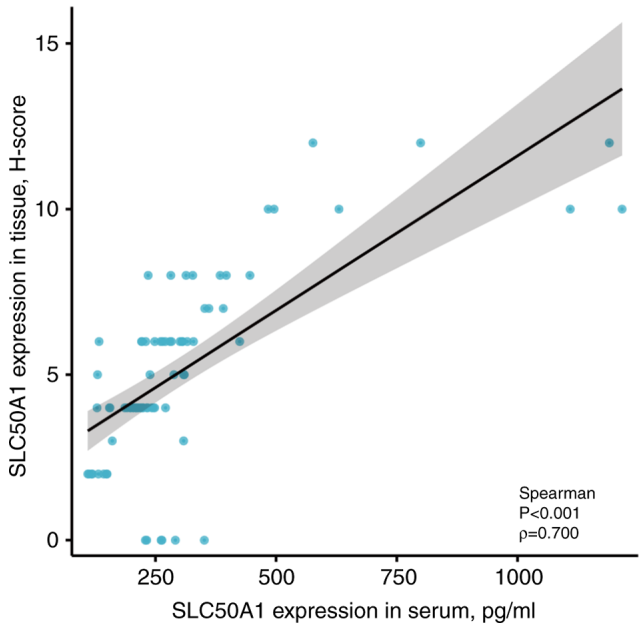

Figure 4. Correlation between SLC50A1 expression levels in serum and tissue. SLC50A1, solute carrier family 50 member 1; H-score, histochemical score.

enhanced in EBC compared with normal tissue (Fig. 5A). No significant differences in SLC50A1 mRNA levels were observed between histological types (Fig. 5B). Consistent with the aforementioned results, significant association was observed between mRNA levels of SLC50A1 and molecular subtype and ER, PR and HER-2 status (Fig. 5C-F). The association between mRNA expression of SLC50A1 with clinicopathological features of patients with EBC from TCGA is shown in Table III. SLC50A1 expression was notably associated with molecular subtype and ER, PR and HER-2 status, T classification and vital status.

Diagnostic value of SLC50A1 mRNA expression in patients with EBC. ROC curves were used to evaluate the association between sensitivity and specificity in patients with EBC from TCGA database vs. HCs from GETx and to determine diagnostic performance. ROC analysis showed that the AUC value was 0.983 (95% CI=0.977-0.989), with a sensitivity of 0.949 and specificity of 0.954 (Fig. 6A). The analysis also demonstrated a notable diagnostic value of SLC50A1 expression in different stages of EBC, with AUC values of 0.972 for stage I, 0.982 for stage II and 0.990 for stage III EBC (Fig. 6B-D).

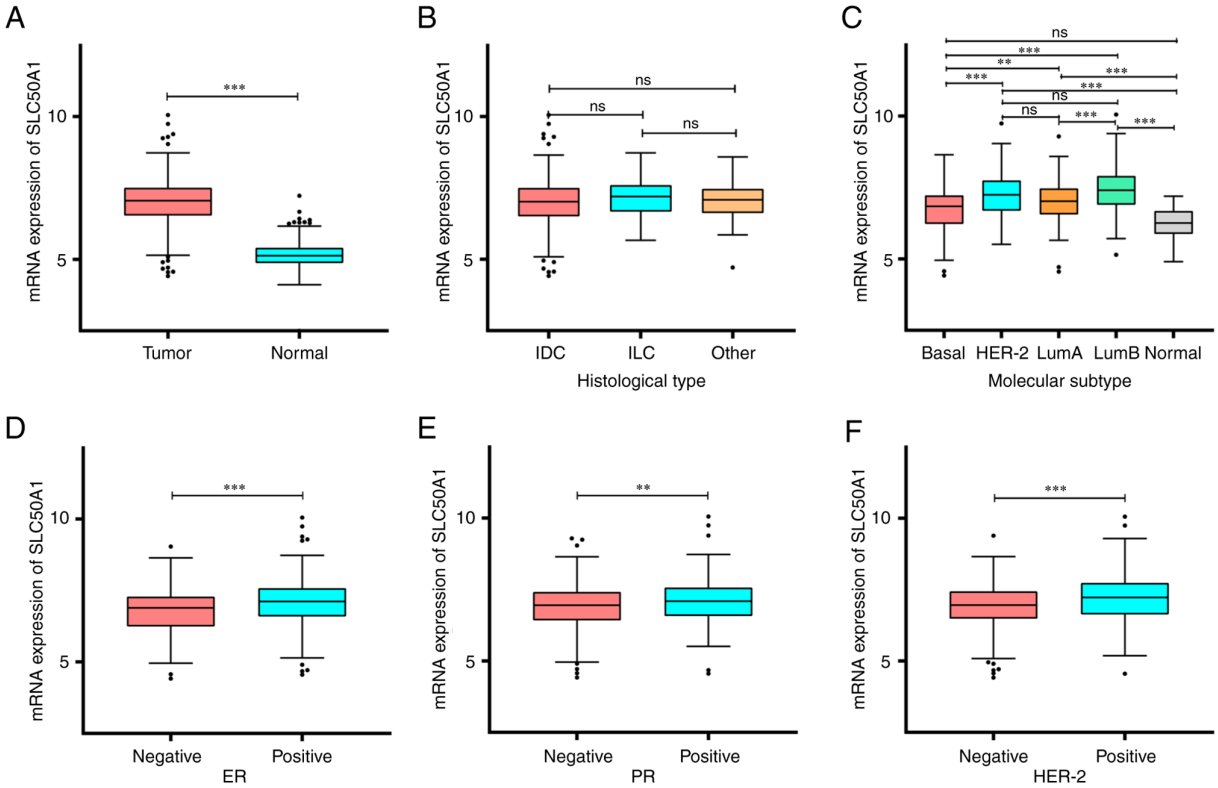

Figure 5. SLC50A1 mRNA expression in EBC from TCGA. (A) SLC50A1 expression was significantly higher in tumor than in normal tissue (analyzed using Mann-Whitney U test). SLC50A1 expression in (B) histological types and (C) molecular subtypes (analyzed using Kruskal-Wallis test). High SLC50A1 expression was associated with (D) ER, (E) PR and (F) HER-2 status (analyzed using Mann-Whitney U test). **P<0.01; ***P<0.001. SLC50A1, solute carrier family 50 member 1; EBC, early breast cancer; ER, estrogen receptor; HER-2, human epidermal growth factor receptor 2; IDC, infiltrating ductal carcinoma; ILC, infiltrating lobular carcinoma; Lum A, luminal A; Lum B, luminal B; PR, progesterone receptor; TCGA, The Cancer Genome Atlas; ns, non-significant.

Prognostic significance of SLC50A1 in EBC. To verify the optimal cut-off values between high and low SLC50A1 expression groups, ROC analysis was performed and a cut-off value of 7.627 was obtained for vital status (Fig. S2). Kaplan-Meier method was used to analyze the association between OS and SLC50A1 expression in patients with EBC from TCGA. Postoperative 3-, 5- and 10-year OS rates in the low SLC50A1 expression group (90.6, 85.0 and 63.4%, respectively) were significantly higher compared with those in the high SLC50A1 expression group (83.4, 73.3 and 49.3%, respectively; Fig. 7). In addition, subgroup analysis showed that high SLC50A1 expression was significantly associated with poor OS in infiltrating lobular carcinoma and ER-positive, HER-2-negative, luminal B and basal-like EBC. Furthermore, univariate and multivariate Cox analysis was performed to assess the potential clinical significance of SLC50A1 expression in EBC. SLC50A1 expression, age, menopausal status, tumor size, nodal status, TNM stage, ER, PR and HER-2 status and histological type were selected as risk factors due to clinical relevance or potential association with poor prognosis. Multivariate Cox analysis revealed that SLC50A1 expression was an independent risk factor for OS, with a hazard ratio of 1.917 (95% CI=1.145-3.211; Table IV).

Discussion

The present study demonstrated that serum levels of SLC50A1 were significantly higher in patients with EBC compared with patients with BBD or HCs. However, no difference was observed between BBD and HC groups. In line with the present study, a previous study comparing protein serum levels of SLC50A1 between 85 patients with BC and 30 HCs revealed that SLC50A1 is upregulated in the serum of patients with BC (26). In addition, the present study showed that the serum levels of SLC50A1 exhibited moderate performance in distinguishing patients with EBC from HCs, with AUC of 0.792, specificity of 84.62% and sensitivity of 78.86%. Similarly, a previous study showed that SLC50A1 differentiates patients with BC from those without BC with a specificity of 100%, sensitivity of 75% and AUC of 0.915 (26). When HCs and patients with BBD were combined, SLC50A1 exhibited an AUC of 0.783, sensitivity of 76.42% and specificity of 76.79% in discriminating patients with EBC. The 85 subjects included in the aforementioned study included 18 patients with BC with distant metastasis; in the present study patients with EBC without distant metastasis were enrolled, which could account for the different results.

CEA and CA15-3 are associated with BC prognosis and have been therefore widely used in clinical surveillance of BC (32,33). However, their use in screening and diagnosis of EBC has not been yet verified (34). The present study showed that the best sensitivity of CEA and CA15-3 in distinguishing EBC from benign lesions was 54.47 and 30.08% with specificity of 73.33 and 100%, respectively. By contrast, the sensitivity and specificity of SLC50A1 in distinguishing EBC and BBD were 61.79 and 90%, respectively, which were significantly higher compared with those observed for CEA and CA15-3.

Table III. Association between clinicopathological characteristics and SLC50A1 expression in samples from patients with early breast cancer from The Cancer Genome Atlas dataset.

			SLC50A1 expression			
Parameter	Variable	n	High, n (%)	Low, n (%)	χ^2-value	P-value
Age, years	<60	492	98.00 (19.92)	394.00 (80.08)	0.053	0.817
	≥60	409	84.00 (20.54)	325.00 (79.46)		
Sex	Female	892	181.00 (20.29)	711.00 (79.71)	0.466	0.495
	Male	9	1.00 (11.11)	8.00 (88.89)		
Menopausal status	Premenopausal	199	47.00 (23.62)	152.00 (76.38)	3.614	0.164
	Perimenopausal	34	4.00 (11.76)	30.00 (88.24)		
	Postmenopausal	581	109.00 (18.76)	472.00 (81.24)		
Histological type	Infiltrating ductal carcinoma	670	135.00 (20.15)	535.00 (79.85)	0.630	0.730
	Infiltrating lobular carcinoma	156	34.00 (21.80)	122.00 (78.20)		
	Other	75	13.00 (17.33)	62.00 (82.67)		
Molecular subtype	Basal	127	10.00 (7.87)	117.00 (92.13)	57.980	<0.001
	HER-2	62	19.00 (30.65)	43.00 (69.35)		
	Luminal A	374	66.00 (17.65)	308.00 (82.35)		
	Luminal B	165	65.00 (39.39)	100.00 (60.60)		
	Normal	22	0.00 (0.00)	22.00 (100.00)		
Estrogen receptor status	Positive	646	150.00 (23.22)	496.00 (76.78)	11.940	<0.001
	Negative	207	25.00 (12.08)	182.00 (87.92)		
Progesterone receptor status	Positive	568	127.00 (22.36)	441.00 (77.64)	14.970	<0.001
	Negative	262	29.00 (11.07)	233.00 (88.93)		
HER-2 status	Positive	152	55.00 (36.18)	97.00 (63.82)	30.740	<0.001
	Negative	632	102.00 (13.74)	530.00 (86.26)		
T classification	T1	238	38.00 (15.97)	200.00 (84.03)	14.720	0.002
	T2	534	103.00 (19.29)	431.00 (80.71)		
	T3	98	33.00 (33.67)	65.00 (66.33)		
	T4	30	8.00 (26.67)	22.00 (73.33)		
N classification	N0	450	95.00 (21.01)	355.00 (78.99)	0.091	0.993
	N1	272	56.00 (20.59)	216.00 (78.31)		
	N2	100	20.00 (20.00)	80.00 (80.00)		
	N3	50	10.00 (20.00)	40.00 (80.00)		
TNM stage	I	161	26.00 (16.15)	135.00 (83.85)	4.010	0.135
	II	533	106.00 (19.89)	427.00 (80.11)		
	III	204	50.00 (24.51)	154.00 (75.49)		
Vital status	Living	775	147.00 (18.97)	628.00 (81.03)	5.219	0.022
	Deceased	126	35.00 (27.78)	91.00 (72.22)		

Analysis was performed using Fisher's exact test. SLC50A1, solute carrier family 50 member 1; HER-2, human epidermal growth factor-2; T, tumor; N, node.

To the best of our knowledge, the localization of SLC50A1 in EBC or BBD tissue has not been previously investigated. Here, IHC staining confirmed that SLC50A1 protein was localized in the cytoplasm and cell membrane and was upregulated in EBC compared with BBD tissue (92.8 vs. 10.0%). In addition, significantly increased levels of SLC50A1 were associated with ER-, PR- and HER-2-positive BC and with luminal A and luminal B molecular subtypes. Additionally, bioinformatics analysis using TCGA database showed that mRNA expression levels of SLC50A1 were significantly higher in EBC compared with normal tissue. Several previous studies have also demonstrated that SLC50A1 is notably upregulated in BC (25,26). Previous bioinformatics analysis using probabilistic integration of cancer genomics data suggested that SLC50A1 may be a potential biomarker for BC development and progression (25). As a class of sugar transporters, SLC50A1 proteins located in the basolateral membrane of human intestinal and hepatic

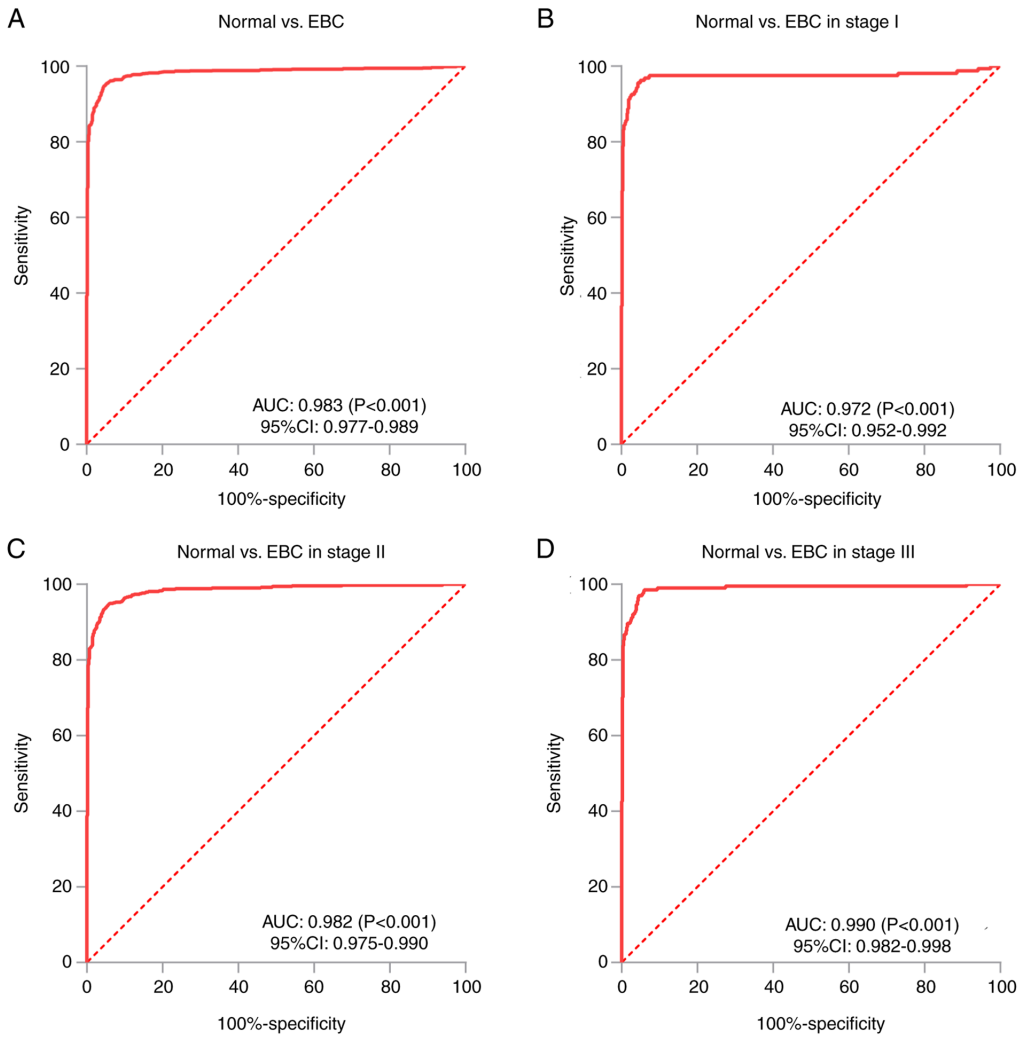

Figure 6. Diagnostic value of SLC50A1 mRNA expression. ROC curve analysis for SLC50A1 expression between (A) EBC and normal tissue, (B) stage I EBC and normal tissue, (C) stage II EBC and normal tissue, and (D) stage III EBC and normal tissue. SLC50A1, solute carrier family 50 member 1; EBC, early breast cancer; ROC, receiver operating characteristic; AUC, area under the curve.

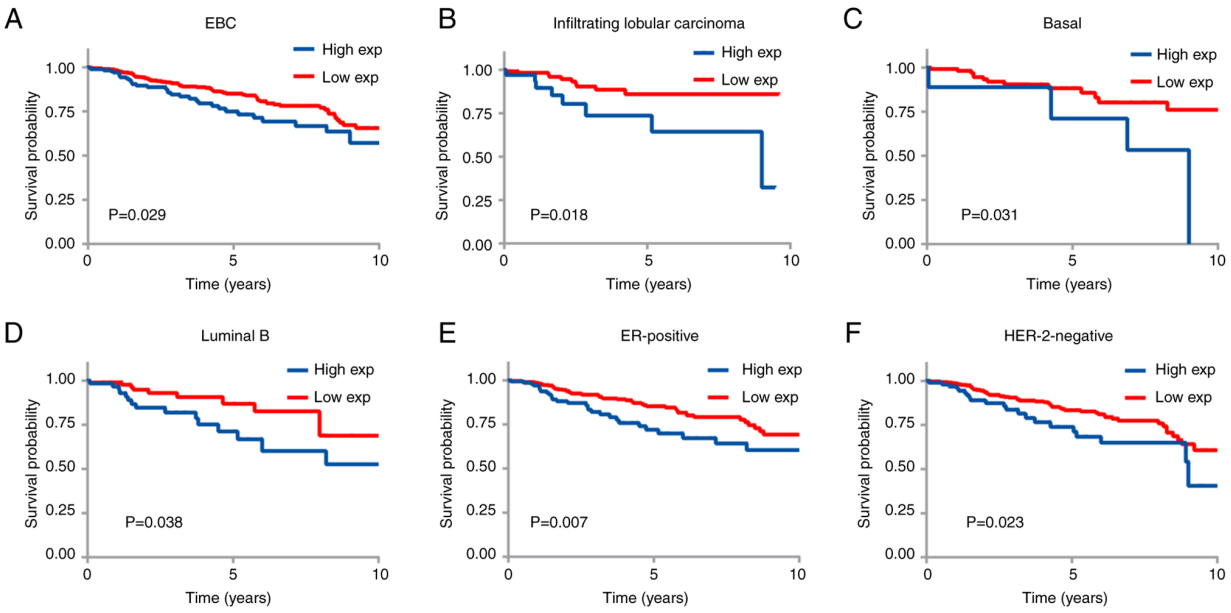

Figure 7. Kaplan-Meier curves of OS. (A) OS curves of patients with EBC. OS curves in patients with (B) infiltrating lobular carcinoma and (C) basal-like, (D) luminal B, (E) ER-positive and (F) HER-2 negative EBC. SLC50A1, solute carrier family 50 member 1; EBC, early breast cancer; ER, estrogen receptor; HER-2, human epidermal growth factor receptor 2; OS, overall survival; exp, expression.

Table IV. Univariate and multivariate Cox regression analysis of prognostic parameters of overall survival in patients with early breast cancer from The Cancer Genome Atlas dataset.

Variable	Univariate P-value	Hazard ratio	Multivariate 95% confidence interval	Multivariate P-value
Age, years (≤60 vs. >60)	0.236	1.415	0.823-2.433	0.209
Menopausal status (premenopausal vs. postmenopausal)	0.089	1.519	0.789-2.927	0.211
Tumor size, cm (≤2 vs. >2)	0.212	1.004	0.461-2.187	0.993
Nodal status (negative vs. positive)	0.101	1.427	0.836-2.436	0.192
TNM stage (I vs. II-III)	0.058	1.261	0.444-3.580	0.663
Estrogen receptor status (negative vs. positive)	0.902	0.993	0.457-2.158	0.986
Human epidermal growth factor receptor 2 status (negative vs. positive)	0.034	1.290	0.743-2.242	0.366
Progesterone receptor status (negative vs. positive)	0.593	0.987	0.495-1.967	0.970
Histological type (infiltrating ductal carcinoma vs. infiltrating lobular carcinoma)	0.804	0.936	0.509-1.723	0.833
Solute carrier family 50 member 1 (low vs. high)	0.029	1.917	1.145-3.211	0.013

cells are considered to mediate excretion of glucose from cells into the bloodstream (22,35,36). By contrast, SLC50A1 provides glucose in the Golgi apparatus of the human mammary gland for synthesis and secretion of lactose (22). To the best of our knowledge, there are no previous reports on the effect of SLC50A1 on growth or metastasis of breast cancer. It was hypothesized that SLC50A1 overexpression in cancerous breast cells provides nutrients for cell proliferation. Wang *et al* (37) demonstrated that the 50% growth inhibitory concentration for bosutinib is significantly decreased in SLC50A1-overexpressing cell lines compared with wild-type ABL-1 breast cancer cell line, thus suggesting that a low concentration of bosutinib inhibits >50% of cells. Therefore, high expression of SLC50A1 may affect treatment efficacy of bosutinib in BC. It was hypothesized that SLC50A1 overexpression may improve the efficacy of targeted therapy against ER-, PR- and HER-2-positive EBC. This should be confirmed in targeted drug sensitivity studies.

The present study showed that the serum levels of SLC50A1 were significantly higher in high compared with low SLC50A1-expressing tissue. A moderate positive association between SLC50A1 levels in serum and tissue was observed. SLC50A1 protein possesses an extracellular N-terminal and a cell membrane C-terminal domain (22). The extracellular domains of certain proteins, such as those of HER-2 and L1 cell adhesion molecule, are shed from the tumor cell membrane by metalloproteinase-mediated cleavage and are detected in the blood (38,39). However, whether SLC50A1 is secreted by tumor cells or shed from the cell surface remains unclear. In addition, serum levels of SLC50A1 were significantly decreased in postoperative patients after a short period, suggesting that the elevated serum levels of SLC50A1 in patients with EBC may originate from the tumor tissue. Therefore, as a potential serological marker, SLC50A1 may be used for early screening of EBC, as well as detection of tumor recurrence. However, further studies are needed to verify its use in clinical practice.

The present study revealed that SLC50A1 overexpression was associated with unfavorable prognosis in patients with EBC. Additionally, multivariate Cox analysis showed that SLC50A1 was an independent prognostic factor in EBC. Wang *et al* (26) also identified SLC50A1 as an independent prognostic marker for patients with high-grade (grade 3) BC, albeit in a small sample size.

The present study has several limitations. Firstly, due to the limited sample size and number of ER-positive tissue samples, SLC50A1 expression in tissue was not shown to be significantly associated with molecular subtype and ER status. Previous studies have reported transcriptome differences, including gene mutations, metabolic pathways and signaling pathways, between Asian and Caucasian women with BC (40,41). All subjects in the present study were Chinese. Therefore, large-sample, multicenter studies with different populations should be conducted. IHC is a semi-quantitative method that cannot fully validate SLC50A1 expression in tissue; this requires further experiments such as reverse transcription-polymerase chain reaction and western blotting. Data downloaded from TCGA demonstrated that the mRNA expression levels of SLC50A1 were significantly higher in patients with EBC compared with HCs and significantly associated with EBC diagnosis and prognosis. The aforementioned results did not fully verify the prognostic and diagnostic value of serum SLC50A1 in EBC but indirectly supported its value at the transcriptome level. Finally, the control group included only patients with BBD and HC and no patients with other

types of cancer were enrolled. Therefore, further studies are needed to determine whether SLC50A1 is a unique biomarker for BC or whether it has a diagnostic value for other types of cancer as well.

In summary, the present study indicated that serum levels of SLC50A1 may serve as a potential diagnostic biomarker for primary EBC. Elevated SLC50A1 was associated with an unfavorable prognosis in EBC. In addition, SLC50A1 may be a potential therapeutic target for EBC. However, further studies are needed to uncover the role of SLC50A1 in glucose transport and other potential underlying molecular mechanisms.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

QZ and JW designed the study and wrote the manuscript. YF and CS performed the experiments and analyzed data. RZ, CH and CC performed experiments, interpreted the data and critically revised the manuscript for important intellectual content. All authors confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Affiliated Cancer Hospital of Shantou University Medical College (Shantou, P.R. China; approval no. 2019049) and informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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