Co-expression Effect of LLCL2 and SLC7A5 to Predict Prognosis in ERα-Positive Breast Cancer

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Research Article

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Abstract

Lethal giant larvae homolog 2 (LLGL2) and solute carrier family 7 member 5 (SLC7A5) have been reported to be involved in resistance to endocrine therapy. This study aimed to assess the effects of LLGL2/SLC7A5 co-expression in predicting prognosis and response to endocrine therapy in ERα-positive breast cancer patients by using LLGL2/SLC7A5 mRNA and protein expression in long-term follow-up invasive breast cancer tissues. We identified that low LLGL2/SLC7A5 mRNA co-expression (LLGL2low/SLC7A5low) was associated with disease-free survival (DFS) compared with other combination groups in all breast cancer patients. In ERα-positive breast cancer patients, LLGL2low/SLC7A5low showed longer DFS and overall survival (OS) compared with LLGL2high/SLC7A5high and a positive trend of longer survival compared with other combination groups. We also observed that LLGL2low/SLC7A5low showed longer survival compared with LLGL2high/SLC7A5high in ERα-positive breast cancer patients receiving adjuvant tamoxifen therapy. Multivariate analysis demonstrated that LLGL2low/SLC7A5low was an independent favorable prognostic factor of both DFS and OS, not only in all breast cancer patients, but also in ERα-positive breast cancer patients. High co-expression of LLGL2 and SLC7A5 protein showed a positive trend of shorter survival. Our study showed that co-expression of LLGL2 and SLC7A5 mRNA is a promising candidate biomarker in early breast cancer patients.

Introduction

Breast cancer remains a leading cause of cancer-related death in women worldwide. About 70% of all breast cancers express estrogen receptor α (ERα) [1,2]. In ERα-positive breast cancers, estradiol is a key regulator of cell growth and survival. Estradiol regulates genes through binding to ERα or indirectly through activating plasma membrane-associated ERα.

Tamoxifen is one of the most common endocrine treatments for breast cancer. Tamoxifen treatment was demonstrated to reduce risk of breast cancer recurrence and death in ERα-positive breast cancer patients [3]. Although endocrine therapy has dramatically improved survival in ERα-positive breast cancer patients, some tumors show *de novo* or acquired drug resistance to endocrine therapy [4–6]. Resistance to endocrine therapies including tamoxifen remains a major challenge in the treatment of ERα-positive breast cancer patients.

Recently, Saito *et al*. reported that lethal giant larvae homolog 2 (LLGL2) functions as a promoter of tumor growth in ERα-positive breast cancer [7]. They reported that the intracellular concentration of leucine decreased in MCF-7 ERα-positive breast cancer cells when *LLGL2* expression was knocked down. They also reported that the proliferation of MCF-7 cells was suppressed when *LLGL2* expression was knocked down, and that excess leucine could rescue the proliferation of *LLGL2*-knockdown cells [7].

LLGL2 is reported to be localized at cell junctions and membranes with a member of the solute carrier (SLC) family, SLC7A5, which is the primary leucine transporter in cells [7,8]. SLC7A5 is a sodium-

independent amino acid transporter that imports leucine [9]. High SLC7A5 expression was reported to be associated with poor prognosis in various cancers including breast cancer [10–14] .

LLGL2 and SLC7A5 are involved in resistance to tamoxifen treatment [7,11,15]. LLGL2 was also reported to function with SLC7A5 at cell junctions and membranes in ERα-positive breast cancer cells, and LLGL2 interacts with SLC7A5 to promote cell proliferation [7]. Therefore, we hypothesized that both LLGL2 and SLC7A5 are required for the efficacy of tamoxifen treatment in ERα-positive breast cancer patients.

In this study, we assessed the effects of LLGL2/SLC7A5 co-expression in predicting prognosis and response to endocrine therapy in ERα-positive breast cancer patients with long-term follow up.

Results

LLGL2 mRNA expression and prognosis of breast cancer patients

We first investigated the association between *LLGL2* mRNA expression level and prognosis of breast cancer patients with long-term follow up. A total of 624 breast cancer tissue samples were subjected to *LLGL2* mRNA expression analysis. The associations between *LLGL2* mRNA expression and clinicopathological characteristics are shown in Supplementary Table S1. Low *LLGL2* mRNA levels were positively associated with larger tumor size (*P*=0.047) and lymph node-negativity (*P*=0.031). Low *LLGL2* mRNA expression was positively associated with longer DFS in all breast cancer patients analyzed in this study (*P*=0.023; Supplementary Fig. S1 online). Furthermore, patients with tumors showing low *LLGL2* mRNA expression showed a tendency towards longer OS (*P*=0.072; Supplementary Fig. S1 online).

Saito *et al.* reported that LLGL2 was involved in prognosis only in ERα-positive breast cancer patients [7]. Therefore, we next investigated the association of *LLGL2* mRNA expression with prognosis according to ERα status. In ERα-positive breast cancer patients, there was a positive correlation between low *LLGL2* mRNA expression and longer DFS and OS (P=0.0009 and P=0.005, respectively), however in ERα-negative breast cancer patients, there was no association between *LLGL2* mRNA expression and prognosis (Supplementary Figure S1). The clinicopathological characteristics of ERα-positive breast cancer patients are shown in Supplementary Table S2. Low *LLGL2* mRNA expression level was positively associated with lymph node negativity (*P*=0.007).

Saito *et al.* also reported that LLGL2 was involved in resistance to tamoxifen in ERα-positive breast cancer patients [7]. Therefore, we investigated the association of *LLGL2* mRNA expression with prognosis in ERα-positive breast cancer patients receiving adjuvant tamoxifen therapy (n=272). As shown in Supplementary Fig. S1, positive associations were found between low *LLGL2* mRNA expression and longer DFS and OS in ERα-positive breast cancer patients receiving adjuvant tamoxifen therapy (*P*=0.016 and *P*=0.018, respectively). Interestingly, no associations were identified between *LLGL2* mRNA expression level and prognosis in ERα-positive breast cancer patients without adjuvant tamoxifen therapy (Supplementary Fig. S1).

We next performed univariate and multivariate Cox regression analyses of clinicopathological factors associated with prognosis using stepwise linear regression in all breast cancer patients (Supplementary Table S3) and in ERα-positive breast cancer patients analyzed in this study (Supplementary Table S4). Although low *LLGL2* mRNA expression was not an independent favorable prognostic factor in all breast cancer patients, we showed that low *LLGL2* was an independent favorable prognostic factor for both DFS and OS in ERα-positive breast cancer patients, as well as nodal status (P=0.012 and P=0.011, respectively).

SLC7A5 mRNA expression and prognosis of breast cancer patients

Next, we investigated the association between *SLC7A5* mRNA expression and prognosis of breast cancer patients. The characteristics of ERα-positive breast cancer patients according to *SLC7A5* mRNA expression are shown in Supplementary Table S5. Low *SLC7A5* mRNA expression was positively associated with favorable prognosis in both DFS and OS in all breast cancer patients analyzed (P=0.002 and P=0.0005, respectively). Low *SLC7A5* mRNA expression was also positively associated with favorable prognosis in both DFS and OS in ERα-positive breast cancer patients (P=0.004 and P=0.004, respectively), but no association was observed in ERα-negative breast cancer patients (Supplementary Fig. S2). As shown in Supplementary Fig. S2, positive associations were identified between low *SLC7A5* mRNA expression and longer DFS in ERα-positive breast cancer patients receiving adjuvant tamoxifen therapy (P=0.014).

Combination of LLGL2 and SLC7A5 mRNA expression and prognosis of breast cancer patients

We then investigated the prognostic impact of the combination of *LLGL2* and *SLC7A5* mRNA expression. Supplementary Table S6 shows the characteristics of breast cancer patients classified by the combination of *LLGL*2 and *SLC7A5* mRNA expression. Low *LLGL2*/*SLC7A5* mRNA co-expression ($LLGL2^{low}$/$SLC7A5^{low}$) was positively associated with lower tumor grade, lymph node negativity, and ERα positivity. As shown in Fig. 1a and b, $LLGL2^{low}$/$SLC7A5^{low}$ was associated with longer survival compared with other combination groups in all breast cancer patients analyzed in this study. The characteristics of ERα-positive breast cancer patients according to *LLGL2*/*SLC7A5* mRNA co-expression are shown in Supplementary Table S7. $LLGL2^{low}$/$SLC7A5^{low}$ was associated with lower grade and lymph node negativity in ERα-positive breast cancer patients. As shown in Fig. 1c and d, $LLGL2^{low}$/$SLC7A5^{low}$ showed longer survival compared with high *LLGL2*/*SLC7A5* mRNA co-expression ($LLGL2^{high}$/$SLC7A5^{high}$) and a positive trend of longer survival compared with other combination groups in ERα-positive breast cancer patients. Then, we investigated the association of prognosis with the combination of *LLGL2* and *SLC7A5* mRNA expression in ERα-positive breast cancer patients receiving adjuvant tamoxifen therapy. As shown in Fig. 1e and f, $LLGL2^{low}$/$SLC7A5^{low}$ showed longer survival than $LLGL2^{high}$/$SLC7A5^{high}$ and a positive trend of longer survival compared with other combination groups in ERα-positive breast cancer patients receiving adjuvant tamoxifen therapy. However, no significant difference was observed between these four combination groups in ERα-positive breast cancer patients who had not received adjuvant tamoxifen therapy (Supplementary Fig. S3).

We performed univariate and multivariate Cox regression analyses of clinicopathological factors associated with prognosis using stepwise linear regression in each group of breast cancer patients. Multivariate analyses demonstrated that *LLGL2*^{low}/*SLC7A5*^{low} was an independent favorable prognostic factor for DFS as well as lymph node negativity and ERα positivity in all breast cancer patients analyzed (Supplementary Table S8). Then, we performed univariate and multivariate analyses in ERα-positive breast cancer patients, which identified *LLGL2*^{low}/*SLC7A5*^{low} as an independent favorable prognostic factor for both DFS and OS, as well as lymph node negativity (Table 1).

Protein expression of LLGL2 and SLC7A5 in breast cancer patients

The expression levels of LLGL2 protein in breast cancer tissue samples were examined by IHC. LLGL2 protein expression was observed in the cytoplasm. Representative images of LLGL2 and SLC7A5 are as shown in Fig. 2a. Immunostaining results were evaluated using the Aperio scanscopeCS2 and eSlide manager application, and H-scores were calculated by this digital pathological system. In this study, a total of 285 consecutive breast cancer tissue samples for which mRNA expression data were available were analyzed for LLGL2 protein expression.

We also evaluated the expression levels of SLC7A5 protein in breast cancer tissue samples. The analysis was performed using the same tissue microarray as the protein expression analysis of LLGL2. SLC7A5 protein expression was observed in the cell membrane (Fig. 2a). Furthermore, SLC7A5 protein expression was observed in 10% of breast cancer tissues. We investigated the association between prognosis and the combination of LLGL2 and SLC7A5 protein expression. The LLGL2^{high}/SLC7A5^{pos} group seemed to show the worst prognosis among the four groups, although there was no statistically significant difference between them (Fig. 2b). The median H-score was used as the cutoff value for LLGL2, and SLC7A5 was divided into two groups based on the presence or absence of staining.

Discussion

In this study, we evaluated the prognostic value of co-expression of LLGL2 and SLC7A5 in primary breast cancer patients with long-term follow up. First, we showed that low *LLGL2* or low *SLC7A5* mRNA expression was an independent favorable prognostic factor in ERα-positive breast cancer patients. Second, we showed that low *LLGL2*/*SLC7A5* mRNA co-expression (*LLGL2*^{low}/*SLC7A5*^{low}) was also an independent favorable prognostic factor both in all breast cancer patients and in ERα-positive breast cancer patients. We also observed that *LLGL2*^{low}/*SLC7A5*^{low} showed longer survival compared with *LLGL2*^{high}/*SLC7A5*^{high} and a positive trend of longer survival compared with other combination groups in ERα-positive breast cancer patients receiving adjuvant tamoxifen therapy.

Saito *et al.* recently reported that LLGL2 promoted leucine uptake and conferred tumor growth and resistance to tamoxifen treatment by increasing the cell surface level of SLC7A5 in ERα-positive breast cancer. They also reported that low *LLGL2* mRNA expression was a favorable prognostic factor in ERα-positive breast cancer [7]. In this study, our data supported the report by Saito *et al* [7]. However, LLGL2 was

originally discovered as a tumor suppressor protein in *Drosophila*. In colorectal cancer and ERα-negative breast cancer, downregulation of LLGL2 was shown to be involved in cancer progression [16–18]. LLGL2 was reported to act as a suppressor for epithelial–mesenchymal transition (EMT), and to interact with the EMT-related transcription factors such as SNAIL and ZEB1 [16,17]. EMT is generally a characteristic of ERα-negative breast cancer [19]. In our study, ERα-negative breast cancer patients with tumors showing high *LLGL2* mRNA expression appeared to show a better prognosis compared with those with tumors showing low *LLGL2* mRNA expression. Therefore, our findings were consistent with previous reports of colorectal cancer and ERα-negative breast cancer [16,18]. Thus, previous reports and our data suggested that the functions of LLGL2 could be completely different between ERα-positive tumors and ERα-negative tumors.

In this study, we showed that low *SLC7A5* mRNA expression was positively associated with favorable prognosis in ERα-positive breast cancer patients but not in ERα-negative breast cancer patients. Our data in this study supported the previous report by Ansari *et al.* [10]. Although SLC7A5 is a systemic L amino acid transporter that carries branch-chain amino acids including leucin, and bulky amino acids including glutamine, which are considered master regulators of the mTORC1 signaling pathway [20,21], the mechanism by which SLC7A5 affects the prognosis of ERα-positive breast cancer patients is not yet fully understood. Recently, Ansari *et al.* reported that enhanced glutamine uptake by SLC family members including SLC7A5 affects the composition of immune cell infiltrates, and might be involved in breast cancer progression [22,23].

In this study, we demonstrated that *LLGL2*^{low}/*SLC7A5*^{low} was an independent favorable prognostic factor not only in all breast cancer patients, but also in ERα-positive breast cancer patients. In ERα-positive breast cancer patients receiving adjuvant tamoxifen therapy, we observed that *LLGL2*^{low}/*SLC7A5*^{low} showed longer survival compared with *LLGL2*^{high}/*SLC7A5*^{high} and a positive trend of longer survival compared with other combination groups. Saito *et al*. reported a novel mechanism by which LLGL2 interacts with its cargo, SLC7A5, in the cytoplasm and transports it to the membrane, increasing SLC7A5 levels on the cell surface in ERα-positive breast cancer. They also reported that knockdown of *LLGL2* decreased cell surface levels of SLC7A5, and that knockdown of *LLGL2* or *SLC7A5* was sufficient to restore tamoxifen sensitivity to tamoxifen-resistant ERα-positive breast cancer cells under low leucine concentration. Our data and that of other groups suggested that co-expression of LLGL2 and SLC7A5 is involved in tamoxifen resistance in ERα-positive breast cancer patients, and that the LLGL2–SLC7A5 axis may be an important determinant of the therapeutic effect of tamoxifen in ER-positive breast cancer.

This study had some limitations. First, this was a retrospective analysis at a single institute using archived materials. For the mRNA analysis, we used samples from surgical specimens that were macro-dissected and cryopreserved immediately after resection. However, we did not confirm the amount of cancer tissue in the cryopreserved samples; therefore, the percentage of cancer cells was likely to vary. A total of 626 consecutive invasive breast cancer tissue samples collected between 1992 and 2008 from the archive of our institute were included in this study, and adjuvant therapies for breast cancer have progressed during in this period. Therefore, we could not eliminate the effects of different adjuvant

therapies in this study. Second, we determined the mRNA cutoff values of *LLGL2* and *SLC7A5* by ROC curve analysis. As results of these analyses, we determined 0.51 and 0.57 as the cut-off levels of relative *LLGL2* and *SLC7A5* mRNA expression, respectively. Because AUC values of 0.51 and 0.57 do not indicate good discriminatory power, the cut-off value for both mRNAs should be re-evaluated using a different dataset in the future. Third, we did not find a positive association between LLGL2/SLC7A5 protein expression and prognosis in this study. The positive rate of SLC7A5 was only 10% in this study. Because long-term follow-up tissues were used in this study, the positive rate of staining might have decreased due to tissue deterioration over time, but the prognosis of cases with staining was poor, which was consistent with the mRNA results of this study.

In Summary, we showed that low *LLGL2/SLC7A5* mRNA co-expression ($LLGL2^{low}$/$SLC7A5^{low}$) was an independent favorable prognostic factor in ERα-positive breast cancer patients, as well as low *LLGL2* or low *SLC7A5* mRNA expression. We also observed that $LLGL2^{low}$/$SLC7A5^{low}$ showed longer survival compared with $LLGL2^{high}$/$SLC7A5^{high}$ and a positive trend of longer survival compared with other combination groups in ERα-positive breast cancer patients receiving adjuvant tamoxifen therapy. Thus, our study showed that the co-expression of *LLGL2* and *SLC7A5* mRNA is a promising candidate biomarker and suggested that the LLGL2–SLC7A5 axis might be a therapeutic target in early breast cancer patients, especially in those receiving adjuvant tamoxifen therapy.

Methods

Patients and samples

A total of 626 consecutive invasive breast cancer tissue samples collected between 1992 and 2008 from the archive of the Department of Breast Surgery, Nagoya City University Hospital, Japan, were included in this study to measure LLGL2 and SLC7A5 mRNA expression. Furthermore, 415 consecutive invasive breast cancer tissues collected between 2000 and 2009 as tissue microarrays were included to evaluate LLGL2 and SLC7A5 protein expression. The tissues were fixed in 10% buffered formalin and embedded in paraffin or snap-frozen in liquid nitrogen immediately after resection and stored at −80°C until RNA extraction. The histological grade was estimated according to the Bloom and Richardson method proposed by Elston and Ellis [17]. Disease-free survival (DFS) was defined as the interval from the date of primary surgery to the earliest occurrence of one of the following: locoregional recurrence, distant metastasis, or death from any cause. Overall survival (OS) was defined as the interval from the date of primary surgery to death from any cause. The median follow-up period was 10.1 years (range, 0.2–215.1 months) and 9.7 years (range, 0.8–215.1 months) for mRNA and protein expression analyses, respectively. Written informed consent for comprehensive research use was obtained from all patients before surgery. This protocol was approved by the institutional review board of Nagoya City University Graduate School of Medical Sciences and conformed to the guidelines of the Declaration of Helsinki.

RNA extraction and quantitative real-time reverse transcription polymerase chain reaction (RT-PCR)

Total RNA extraction and reverse transcription from breast cancer tissues were performed according to the manufacturer's protocol [24], and mRNA expression levels of LLGL2, SLC7A5, and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were measured using TaqMan Gene Expression assays (Thermo Fisher Scientific). Duplex quantitative RT-PCR assays were performed using the StepOnePlus real-time PCR system (Thermo Fisher Scientific). The reaction was analyzed using a FAM-labeled probe for LLGL2 or SLC7A5 (Thermo Fisher Scientific) and a VIC-labeled probe for GAPDH (Thermo Fisher Scientific) as a single assay for each sample. The Composition of amplification reaction mixture and reaction conditions were as previously reported [24] .

We performed a receiver operating characteristic (ROC) curve analysis to determine the cut-off value of LLGL2 and SLC7A5 mRNA (Supplementary Fig. S4 and S5). We used Youden's index (sensitivity + specificity − 1), which corresponded to a point on the ROC curve with the highest vertical distance from the 45° diagonal line, and determined the cut-off values for LLGL2 and SLC7A5 mRNA expression to be 0.82 and 0.54, respectively.

Immunohistochemistry (IHC) of ERα, progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2)

One 4-μm-thick section from each paraffin-embedded specimen was first stained with hematoxylin and eosin to ascertain whether an adequate number of invasive ductal carcinoma cells were present and that the quality of fixation was adequate for immunohistochemical analysis. Serial sections (4-μm-thick) were then prepared from suitable tissue blocks and float-mounted on adhesive-coated glass slides for ERα (Dako Envision FLEX-ER, EP1; Agilent Technologies, Santa Clara, CA, United States), PgR (Dako Envision FLEX-ER, PgR636; Agilent Technologies), and HER2 (HercepTest II; Agilent Technologies) staining. Staining of these hormone receptors was performed using the Autostainer Link 48 (Agilent Technologies). Immunostained specimens were scored after the entire section had been evaluated by light microscopy. ERα and PgR expression was evaluated by the percentage of cells with positive nuclear staining. A positive nuclear staining ratio of ≥1/100 was considered positive. Scoring of HER2 expression was based on the membrane staining pattern and was scored on a scale of 0–3+. Tumors with scores of 0 or 1 were considered negative for HER2 overexpression, and those with a score of 3 were considered positive. Tumors with a score of 2+ were tested for gene amplification by fluorescence in situ hybridization (FISH) using the PathVysion assay (Vysis; Abbott Laboratories, Abbott Park, IL, USA) in accordance with the manufacturer's protocol. A ratio of >2.0 for HER22 gene/chromosome 17 was considered positive. Tumors were considered HER2-positive if immunohistochemical staining was 3+ or positive by FISH.

IHC of LLGL2 and SLC7A5

For the immunohistochemical analysis of LLGL2 and SLC7A5, tissue microarrays on 2-mm-diameter slides were prepared after confirming whether an appropriate number of invasive ductal carcinoma cells were present and whether the fixation quality was suitable for immunohistochemical analysis. The

primary antibodies for LLGL2 and SLC7A5 protein were rabbit monoclonal anti-LLGL2 antibody (Santa Cruz Biotechnology, CA, USA) and rabbit monoclonal anti-SLC7A5/LAT1 antibody (Abcam, Cambridge, UK) at 1:300 and 1:100 dilution, respectively. Immunostaining was performed using the Leica Bond-Max automated system and Leica Refine detection kits (Leica Biosystems).

Tissue microarray slides were scanned at ×20 magnification using an Aperio scanscopeCS2 (Leica Biosystems, San Diego, CA, USA). At least 1,000 tumor cells were evaluated in each tissue core. The protein expression level of LLGL2 was evaluated according to H-score [18] using the eSlide manager application, a digital pathological system (Leica Biosystems). H-score was calculated by classifying the immunostaining intensity into three categories (weak staining, 1; moderate, 2; and strong, 3) and adding the evaluation of the proportion of each staining. The threshold optical density for each staining intensity was defined as 210, 180, and 150, respectively. H-score was assigned using the following formula: [1× (% cells with category 1) + 2 × (% cells with category 2) + 3 × (% cells with category 3)] [18].

SLC7A5 protein expression was localized to the cell membrane, and therefore SLC7A5 immunostaining was evaluated in accordance with the same method employed by the HercepTest (Dako) for HER2 immunostaining, as described above. Scoring of SLC7A5 expression was based on the membrane staining pattern and scored on a scale of 0–3+ [19, 20].

Statistical analysis

The associations of LLGL2 and SLC7A5 mRNA expression with clinicopathological factors were assessed by χ^2 and Fisher's exact probability tests. Survival curves were analyzed using the Kaplan–Meier method and verified by the log-rank test. DFS was censored at the date of last follow up if patients were still relapse-free and alive, and OS was censored at the time when patients were alive. A Cox proportional hazards regression analysis was used for univariate and multivariate analyses of prognostic values using the stepwise variable selection method. The level of statistical significance was set at a P-value of less than 5%. Multiple survival curves were compared by the log-rank test with Bonferroni adjustment. In this study, there were three comparisons: $LLGL2^{high}/SLC7A5^{high}$ vs $LLGL2^{low}/SLC7A5^{low}$; $LLGL2^{high}/SLC7A5^{low}$ vs $LLGL2^{low}/SLC7A5^{low}$; and $LLGL2^{low}/SLC7A5^{high}$ vs $LLGL2^{low}/SLC7A5^{low}$. Therefore, a two-sided P-value of 0.017 (0.05/3) was taken to indicate statistical significance. Missing data points were excluded from the analysis. Statistical calculations were performed with JMP12.2 software (SAS Institute, Inc., Cary, NC, USA).

Abbreviations

ERα, estrogen receptor α; LLGL2, lethal giant larvae homolog 2; SLC7A5, solute carrier family 7 member 5; DFS, disease-free survival; OS, overall survival; ROC, receiver operating characteristic; IHC, immunohistochemistry; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2; OD, optical density.

Declarations

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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.

Ethics declarations

This study was approved by the institutional review board of Nagoya City University Graduate School of Medical Sciences. All tissue samples were provided from a biobank that is maintained by the Department of Breast Surgery, Nagoya City University Graduate School of Medical Sciences and conformed to the guidelines of the Declaration of Helsinki.

Written informed consent for comprehensive research use was obtained from all patients involved in the study.

Consent for publication

All authors have given consent for publication.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

TH and TT contributed to the study conception and design. NK, YW-E, TA, HS, YK, MT, AK, KO, and TT contributed to the acquisition of patient data. TH, YU, and SN performed the mRNA analyses and TH analyzed patient data. TH, MK, and HK evaluated the immunohistological analysis and ST supervised the pathological evaluation. SO performed the statistical analysis and SM supervised these analyses. TH drafted the manuscript. All authors read and approved the final manuscript.

Additional Information

Competing interests: The authors declare no competing interests.

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Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

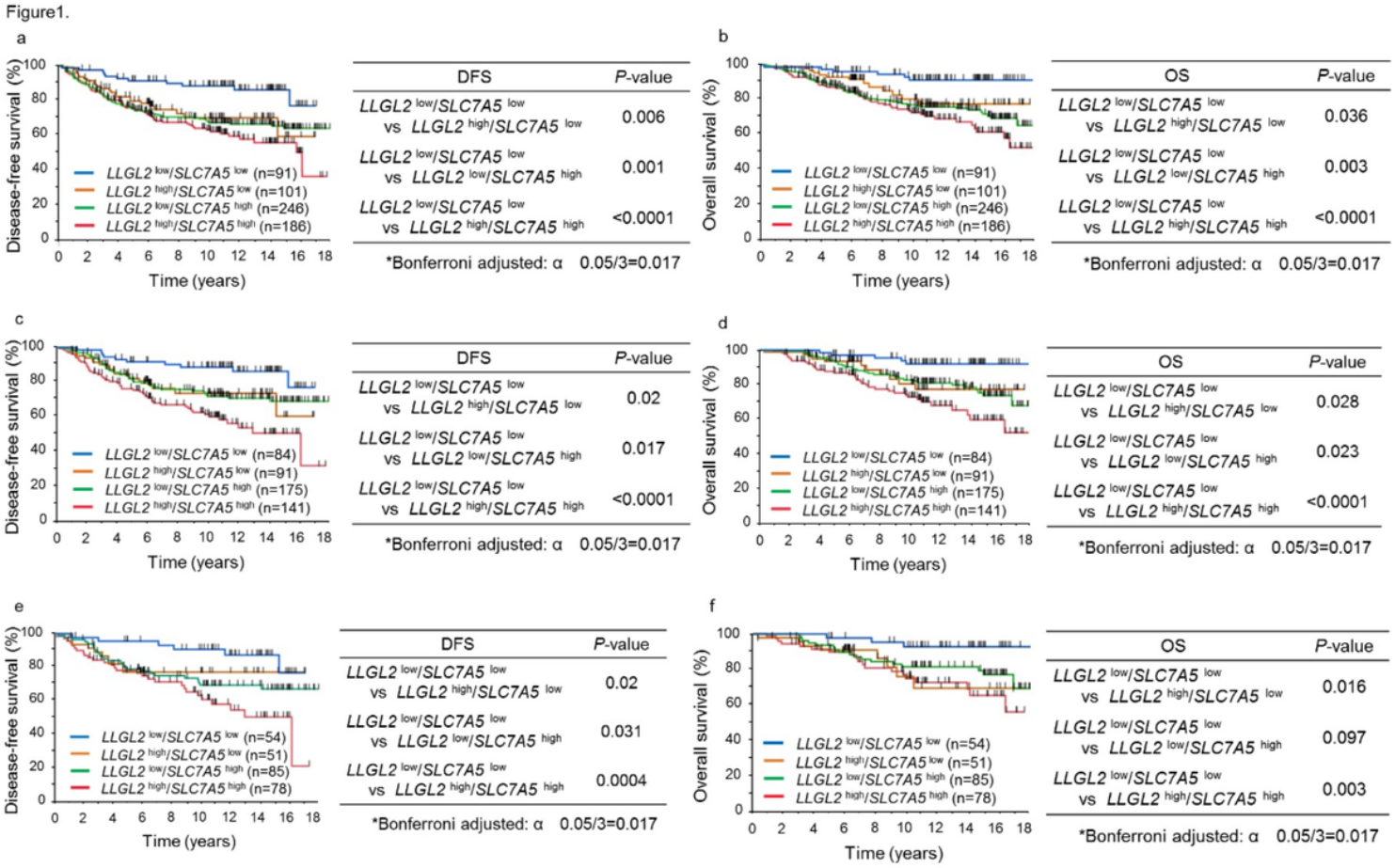

Figure 1

Kaplan–Meier survival curves according to the combination of LLGL2 and SLC7A5 mRNA expression. Graphs show DFS and OS curves, respectively, for all breast cancer patients (a, b), ERα-positive breast cancer patients (c, d), and ERα-positive breast cancer patients receiving adjuvant tamoxifen therapy (e, f).

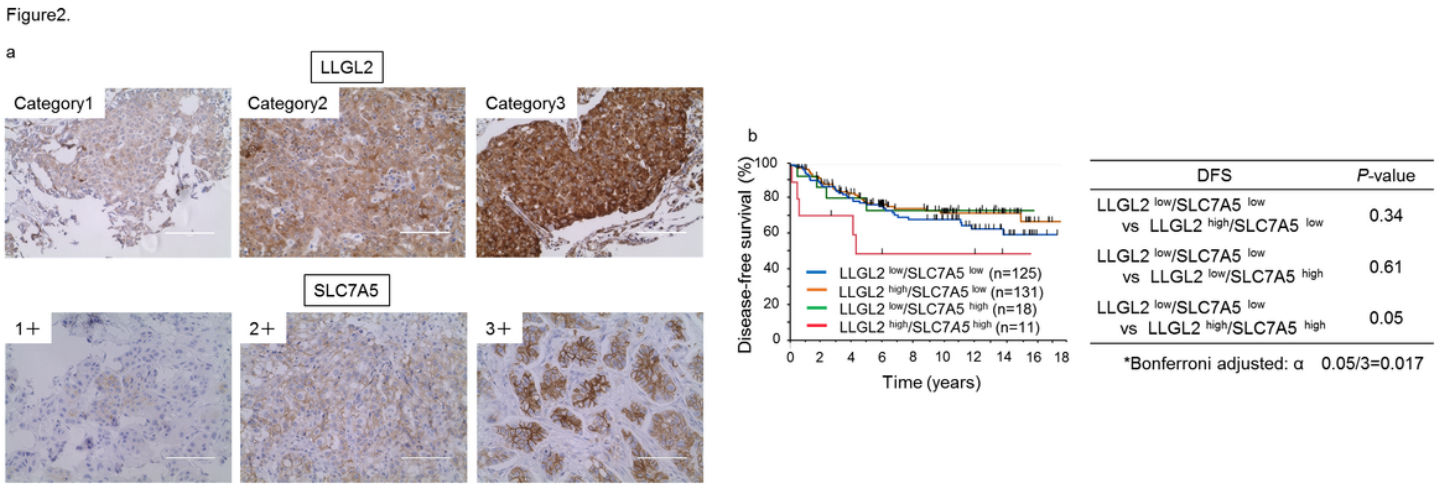

Figure 2

Representative images of LLGL2 and SLC7A5 protein expression in breast cancer tissues (a). Kaplan–Meier survival curves of DFS according to the combination of LLGL2 and SLC7A5 protein expression (b).

Supplementary Files

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- SupplementalTable.doc
- Supplementalfigure.doc
- Table1.png