Received: 12 April 2022 | Accepted: 2 May 2022

DOI: 10.1111/1759-7714.14474

ORIGINAL ARTICLE

WILEY

Galectin-3 enhances trastuzumab resistance by regulating cancer malignancy and stemness in HER2-positive breast cancer cells

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Funding information
Jiangsu Province Science and Technology Project of China, Grant/Award Number: BE2017726; National Natural Science Foundation of China, Grant/Award Numbers: 61871403, 82001422; Natural Science Foundation of Jiangsu Province Youth Project, Grant/Award Number: BK20200274

Abstract

Purpose: The aim of this study was to explore the role of galectin-3 in human epidermal growth factor receptor 2 (HER2)-positive breast cancer cells and the potential mechanism.

Methods: Kaplan–Meier (KM)-plot and The Cancer Genome Atlas (TCGA) databases were used to study the role of galectin-3 in the prognosis of HER2-positive breast cancer. The effects of galectin-3 on cell proliferation, migration, invasion, and colony formation ability in HER2-positive breast cancer cells were examined. The relationship between galectin-3 and important components in the HER2 pathways, including HER2, epidermal growth factor receptor (EGFR), protein kinase B (AKT), and phosphatase and tensin homolog (PTEN), was further studied. Lentivirus and CRISPR/Cas9 were used to construct stable cell lines. Cell counting kit-8 (CCK-8) and apoptosis assays were used to study the relationship between galectin-3 and trastuzumab. The effect of galectin-3 on cell stemness was studied by mammosphere formation assay. The effects of galectin-3 on stemness biomarkers and the Notch1 pathway were examined. Tumorigenic models were used to evaluate the effects of galectin-3 on tumorigenesis and the therapeutic effect of trastuzumab in vivo.

Results: HER2-positive breast cancer patients with a high expression level of *LGALS3* (the gene encoding galectin-3) messenger RNA (mRNA) showed a poor prognosis. Galectin-3 promoted cancer malignancy through phosphoinositide 3-kinase (PI3K)/AKT signaling pathway activation and upregulated stemness by activating the Notch1 signaling pathway in HER2-positive breast cancer cells. These two factors contributed to the enhancement of trastuzumab resistance in cells. Knockout of *LGALS3* had a synergistic therapeutic effect with trastuzumab both in vitro and in vivo.

Conclusions: Galectin-3 may represent a prognostic predictor and therapeutic target for HER2-positive breast cancer.

KEYWORDS
cancer cell stemness, galectin-3, HER2-positive breast cancer, trastuzumab resistance

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INTRODUCTION

Breast cancer is the most common cancer worldwide.[1] Overexpression of human epidermal growth factor receptor-2 (HER2) is observed in ~20%–25% of breast cancers, and HER2 overexpression is significantly associated with poor prognosis in breast cancer patients.[2–4] Trastuzumab is the first developed humanized monoclonal antibody targeting HER2 and it reduces the recurrence and metastasis rate of patients with HER2-positive breast cancer, showing effects on improving prognosis.[5,6] However, most patients with HER2-positive breast cancer relapse or progress within 1 year even treated with trastuzumab combined with chemotherapy.[7,8] Therefore, a better understanding of the mechanisms underlying trastuzumab resistance is required to develop therapeutic strategies to improve patient survival.

The most common mechanisms associated with trastuzumab resistance are the formation of HER2 heterodimers with other ERBB family receptors,[9–11] the heterogeneity of HER2 expression,[12] ERBB nuclear translocation,[13,14] high expression of p95HER2,[15,16] abnormal anti-receptor binding,[17,18] abnormal activation of signaling pathways (phosphoinositide 3-kinase/protein kinase B [PI3K/AKT], Notch1, IGF2/IGF-1R/IRS1) and PIK3CA gene mutations.[19–22] Polarization of M2-type macrophages and reduced infiltration of activated T cells also lead to trastuzumab resistance. In addition, the upregulation of cell stemness in HER2-positive breast cancer contributes to trastuzumab resistance as well.[23] Although several mechanisms of trastuzumab resistance have been proposed,[24–26] the exact mechanisms regarding trastuzumab resistance have not been completely resolved.

Galectin-3 is a β-galactose-containing glycoconjugate-binding lectin that is produced in tumor cells and macrophages.[27] Galectin-3 plays an important role in several types of cancer, such as thyroid cancer, lung cancer, ovarian cancer, and pancreatic cancer.[28–30] Galectin-3 enhances the activity and invasiveness of tumor cells, inhibits apoptosis, and promotes angiogenesis and metastasis.[31–33] Galectin-3 also promotes the M2 polarization of macrophages and increases chemotaxis to recruit more macrophages.[34] Galectin-3 reduces T cell receptor signals and promotes T cell apoptosis, thereby blocking the body's immune response to tumors.[35] Moreover, galectin-3 upregulates stemness in several types of tumors, such as ovarian cancer.[36] Patients with metastatic melanoma and head and neck squamous cell carcinoma benefit from the galectin-3 inhibitor, belapctin, in combination with pembrolizumab.[37]

Although galectin-3 has been shown to exert a tumor-promoting role in a variety of tumors, the role of galectin-3 in HER2-positive breast cancer remains unknown. The purpose of this study was to explore the role of galectin-3 in HER2-positive breast cancer and the potential benefits of galectin-3 inhibition in breast cancer treatment.

METHODS

Cells

The human HER2-positive breast cancer cell lines SKBR3 (trastuzumab-sensitive) and JIMT1 (trastuzumab-resistant) were obtained from the American Type Culture Collection and maintained in McCoy's 5A medium and Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific), respectively. Cells were maintained at 37°C in a humidified incubator with 5% CO_2.

Cell transfection and infection

To overexpress or inhibit *LGALS3* (the gene encoding galectin-3), cells were transfected with small interfering RNA (siRNA) targeting *LGALS3* (Table S1) (Sangon Biotech) in JIMT1 cells or *LGALS3*-expressing plasmid (Genscript) in SKBR3 cells, respectively, using Lipofectamine 3000 (Invitrogen). Lentivirus of *LGALS3* overexpression was custom-designed and provided by Ubigene Biosciences (Figure S1(a)). To generate stable cell lines, SKBR3 cells were infected with *LGALS3* lentivirus and selected with 1 mg/mL puromycin for 10 days. SKBR3-OE was cells stably overexpressing galectin-3, which was constructed by lentiviral transfection (SKBR3-CT as control). To construct the specific guide RNA (gRNA) of *LGALS3*, the following oligonucleotide sequences were used: *LGALS3*-gRNA: 5′-CAT GAT GCG TTA TCT GGG TC-3′ and *LGALS3*-gRNA: 5′-GGC TGG TTC CCC CAT GCG CC-3′ (Figure S1b). JIMT1-KO was cells stably expressing low level of galectin-3, which was constructed by CRISPR/Cas9 (JIMT1-CT as control).

Cell culture and reagents

Recombinant galectin-3 (r-Gal3) was obtained from PeproTech. The galectin 3 inhibitor GB1107 was obtained from MedChemExpress. Trastuzumab was obtained from Roche.

Cell proliferation assay

Cell viability was analyzed by cell counting kit-8 assay (CCK8) (Dojindo Molecular Technologies) in accordance with the manufacturer's instructions. Cells were seeded in 96-well plates at a density of 3000–5000 cells/well with different concentrations of trastuzumab or GB1107. The seeded cells were cultured for 3 days. The cells were then processed using the kit, and optical density values were measured at 450 nm. The amount of formazan produced is proportional to the cell viability. All experiments were repeated at least three times.

Cell invasion assay

A 24-well transwell chamber with a pore size of 8 μm (Corning) was used for experiments, and the inserts were coated with Matrigel (BD Bioscience) in serum-free medium. Transfected cells were trypsinized and transferred to the upper chamber ($3–5 \times 10^5$ cells) in serum-free medium; the bottom chamber included serum containing 10% FBS. Cells were incubated for 24 hours. The non-invading cells on the upper membrane surface were removed with a cotton tip, and the cells that passed through the filter were fixed with 4% paraformaldehyde and stained with crystal violet staining solution (Sangon Biotech). Data presented are representative of three individual wells.

Western blot assays

Total protein was extracted from cells using radioimmunoprecipitation assay (RIPA) buffer (Thermo Scientific) in the presence of Halt Protease and Phosphatase Inhibitor Cocktail (Pierce Chemical). Protein concentrations were measured using a bicinchoninic acid assay (BCA) Protein Assay Kit (Thermo Scientific). Equivalent amounts of protein were mixed with 5 × Lane Marker Reducing Sample Buffer (Thermo Scientific), resolved on a sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred onto Immobilon-P Transfer Membranes (Millipore). The membranes were blocked with 5% non-fat milk in Tris-buffered saline and then incubated with primary antibody, followed by incubation with secondary antibody. The signal was detected using an enhanced chemiluminescence Western blot detection kit (Millipore). The following primary antibodies were used in Western blot assays: galectin-3 (ab76245), β-actin (ab213262), CD44 (ab189524), CD24 (ab179821), CD133 (ab216323), Nanog (ab109250), E-cadherin (ab231303), Notch1 (ab52627), HES1 (ab108937), PTEN (ab267787), HEY1 (ab154077) (Abcam); AKT (469 T1), phospho-AKT (4060 T), PI3K (4257 T), phospho-PI3K (4228 T), ERK1/2 (4695 T), phospho-ERK1/2 (4370 T), HER2 (4290S), phospho-HER2 (2243 T), EGFR (2085 T), and NICD1 (4147 T) (Cell Signaling Technology).

Total RNA and microRNA isolation and RT-qPCR

Total RNA was isolated from cells with an RNeasy PowerLyzer Tissue and Cells Kit (QIAGEN) in accordance with the manufacturer's instructions. First-strand complementary DNA (cDNA) was synthesized with the PrimeScript RT Master Mix (Takara). Real-time polymerase chain reaction (PCR) was performed using an ABI StepOne Plus with TB Green Premix Ex Taq II Tli RNaseH Plus (Takara) by the StepOnePlus Real-Time PCR System (Applied Biosystems). ACTB was used as an internal control. Primer sequences are provided in Table S2.

Immunofluorescence cell staining

Cells were fixed with 4% paraformaldehyde and then incubated in immunol staining blocking buffer (1× PBS/5% BSA/0.3% Triton X-100) for 2 hours to permeabilize the cells and block non-specific protein–protein interactions. Cells were then incubated with anti-galectin-3 antibody (Abcam) or anti-HER2 antibody (Abcam) followed by Alexa Fluor 594-secondary antibody (Proteintech). Nuclei were detected using 4′,6-diamidino-2-phenylindole (DAPI) staining. Cells were then examined using a fluorescence microscope.

Mammosphere culture

Cells were seeded into ultra-low-attachment 6-well plates (40 406) (BEAVER Biomedical Engineering) at a density of 3000 cells per well. Cells were cultured in stem cell culture medium: DMEM/F12 (KeyGEN BioTECH) + 1 × B27 (Gibco, Thermo Fisher Scientific) + 20 ng/mL EGF (Peprotech) + 20 ng/mL bFGF (Peprotech). After 10–14 days, the formed mammospheres were counted manually and images were obtained.

Tumor xenograft model

The animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of Affiliated Jinling Hospital, Medical School of Nanjing University. Standard animal care and laboratory guidelines were followed according to the IACUC protocol. Mice were housed individually in ventilated cages under standard room temperature (22°C) and humidity (55%) in a 12/12 hour light/dark cycle.

Female BALB/c athymic nude mice 5 weeks of age (Gem pharmatech) were randomly grouped (JIMT1-KO, JIMT1-KO + trastuzumab, JIMT1-CT, JIMT1-CT + trastuzumab). JIMT1-KO and JIMT1-CT cells were injected into the mammary fat pads to generate xenograft tumors. When the tumor volumes reached 100 mm^3 (day 10), we injected mice intraperitoneally with trastuzumab (10 mg/kg). The treatment was administered every 5 days for five cycles. The tumor growth was measured every 5 days. When the tumor volumes was over 1000 mm^3 (day 35), mice were sacrificed and tumors were harvested; the wet weight of tumors was recorded. Immunohistochemistry and HE staining were performed by Servicebio.

Clinical analysis

To analyze the relationship between *LGALS3* gene expression and clinical factors, we analyzed clinical data from The Cancer Genome Atlas (TCGA) database. The prognostic survival analysis data were from the Kaplan–Meier (KM)-plot database (http://kmplot.com/).

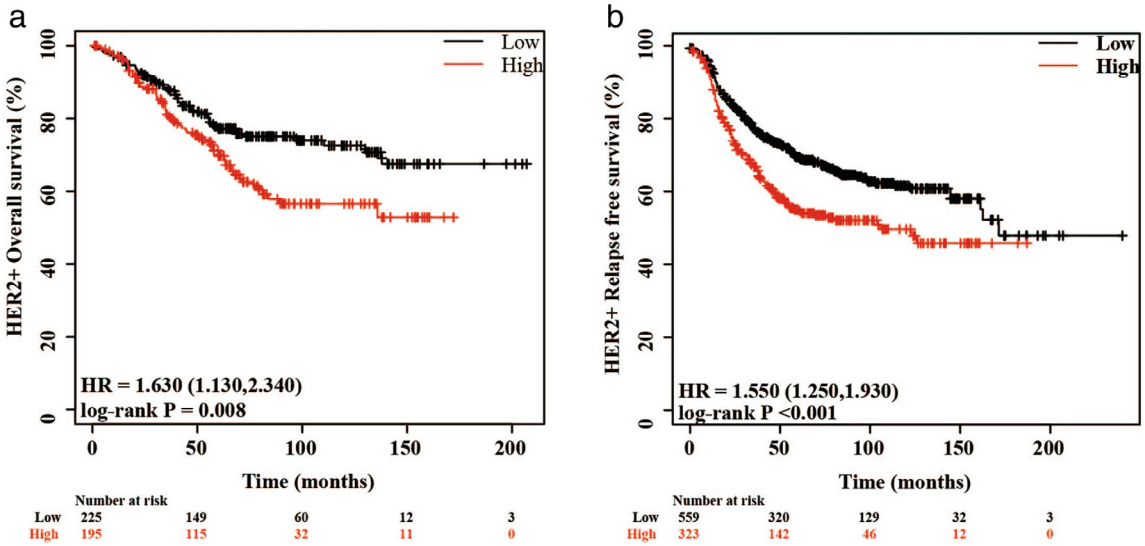

FIGURE 1 High expression level of *LGALS3* was related to poor prognosis in HER2-positive breast cancer. Kaplan–Meier analysis of (a) overall survival and (b) relapse-free survival of HER2-positive breast cancer patients stratified by *LGALS3* mRNA expression level

Statistical analysis

IBMSPSS Statistics 22 (IBM) and Prism 9 (GraphPad Software) were used for statistical analyses. The significance of differences was analyzed using a 2-tailed Student's *t*-test or a χ^2 test. The χ^2 test was used to examine the relationship between *LGALS3* expression level and clinicopathological factors. X-tile software was used to select the cutoff value. The correlations between *LGALS3* expression and overall survival were explored using KM plots and the log-rank test. Multivariate Cox analyses were used to evaluate survival data. Differences were considered significant at $p < 0.05$.

RESULTS

High *LGALS3* messenger RNA expression was related to poor prognosis in HER2-positive breast cancer patients

Analysis of the KM-plot database showed that the prognosis of HER2-positive breast cancer patients with a high *LGALS3* messenger RNA (mRNA) level was worse compared with patients with a low *LGALS3* transcription level, with poor overall survival (OS, $p = 0.008$, hazard ratio [HR], 1.630, 95% confidence interval [CI], 1.130–2.340) and relapse-free survival (RFS, $p < 0.001$, HR, 1.550, 95% CI, 1.250–1.930) (Figure 1(a),(b)).

Expression data and clinical information of a total of 162 patients with HER2-positive breast cancer were collected from the TCGA Database. The relationship between *LGALS3* mRNA expression and clinical factors is shown in Table S3. Patients with a higher tumor stage and larger tumor size had a higher expression level of *LGALS3* ($p < 0.05$). Age, stage, and *LGALS3* mRNA expression were found to be risk factors of prognosis in HER2-positive breast cancer through multivariate Cox analysis (Table S4).

Galectin-3 promoted cancer malignancy of HER2-positive breast cancer cells

To investigate the role of galectin-3 in HER2-positive breast cancer, we first examined a panel of breast cancer cell lines. SKBR3 and JIMT1 cells are HER2-positive breast cancer cell lines. We found that the expression level of *LGALS3* is low in SKBR3 cells and high in JIMT1 cells. The expression level of *LGALS3* in SKBR3 cells was increased after transfecting cells with the pcDNA-*LGALS3* plasmid, which encodes galactin-3, whereas the addition of r-Gal3 did not influence the level of endogenous galectin-3 (Figure 2(a)). Transfecting JIMT1 cells with siRNA targeting *LGALS3* or using GB1107 (an inhibitor of galectin-3) led to decreased expression of galectin-3 (Figure 2(b),(c)). qRT-PCR indicated that the level of *LGALS3* mRNA in cells after plasmid transfection or siRNA was consistent with the expression level of galectin-3 (Figure S2(a),(b)). We found that galectin-3 may affect the expression level of HER2 (Figure 2(a)).

We, next, examined the influence of galectin-3 on the proliferation of HER2-positive breast cancer cells. Endogenous and exogenous galectin-3 promoted the proliferation of SKBR3 cells (Figure 2(d)) while reducing the expression level of galectin-3 or using GB1107 inhibited the proliferative capacity of JIMT1 cells (Figure 2(e)). Moreover, galectin-3 significantly promoted the migration, colony formation, and invasion of HER2-positive breast cancer cells (Figure 2(f)–(h)).

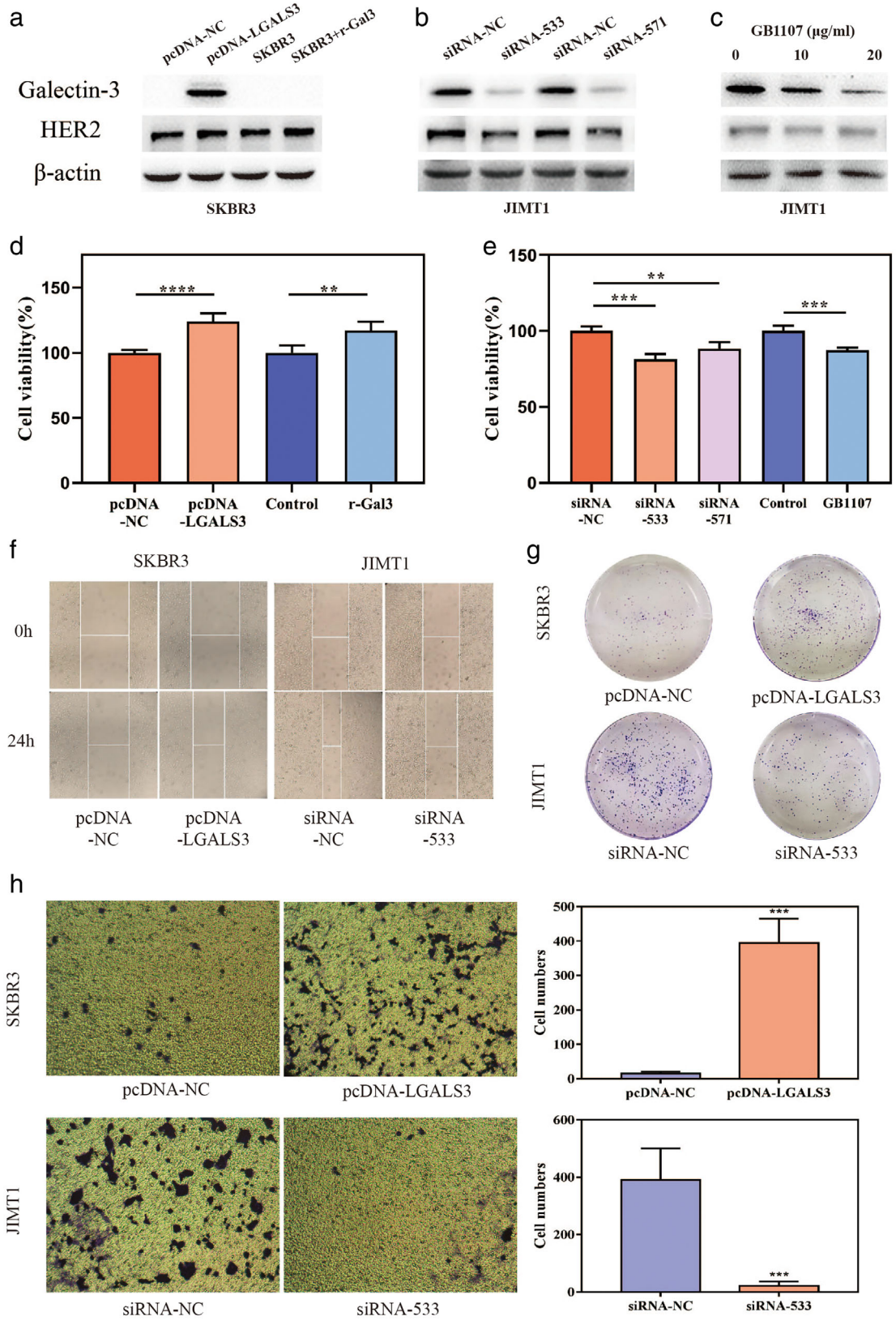

FIGURE 2 Galectin-3 promoted cancer malignancy of HER2-positive breast cancer cells. Western blot analysis of galectin-3, HER2, and β-actin in (a) pcDNA-NC, pcDNA-*LGALS3*, SKBR3, and SKBR3 cells treated with 100 ng/mL r-Gal3 for 3 days; (b) JIMT1 cells transfected with siRNA-NC, siRNA-533, or siRNA-571; and (c) JIMT1 cells treated with 0, 10, or 20 μg/mL GB1107 for 3 days. Cell viability curves for (d) pcDNA-NC, pcDNA-*LGALS3*, SKBR3, and SKBR3 cells treated with 100 ng/mL r-Gal3 for 3 days; and (e) siRNA-NC, siRNA-533, siRNA-571, and JIMT1 cells treated with 10 μg/mL GB1107 for 3 days. (f) Healing assays; (g) colony formation assays; and (h) transwell assays in pcDNA-NC, pcDNA-*LGALS3*, siRNA-NC, and siRNA-533 cells. The significant differences are indicated by asterisk (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$)

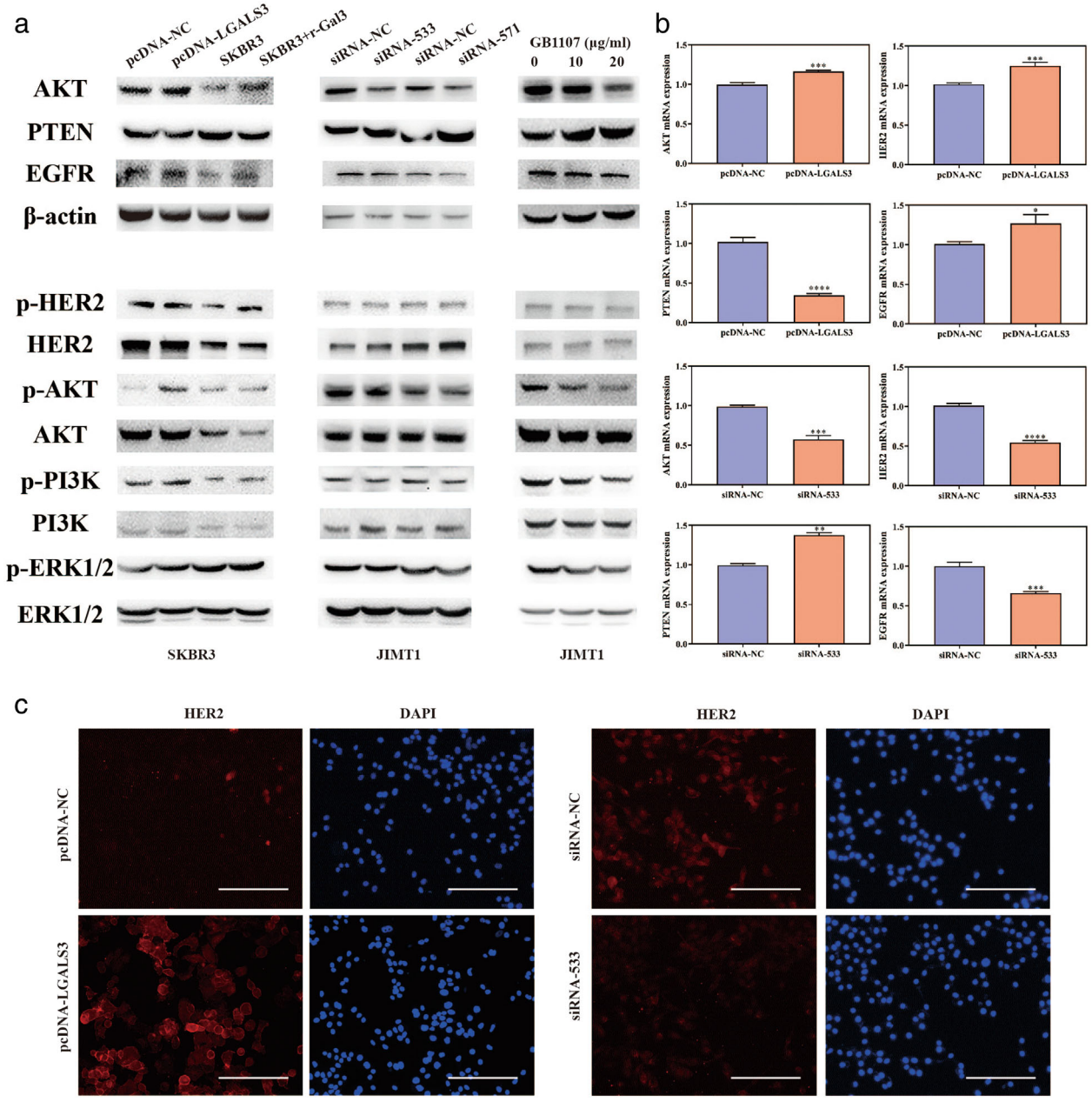

FIGURE 3 Galectin-3 regulated the HER2/EGFR/PI3K/AKT pathway. (a) Western blot analysis of AKT, PTEN, and EGFR (β-actin as a loading control), p-HER2, p-AKT, p-PI3K, and p-ERK1/2. (b) qRT-PCR analysis of *EGFR*, *AKT*, *PTEN*, and *HER2* mRNA; *ACTB* mRNA was used as a loading control. (c) Immunofluorescence images of HER2 (red) and DAPI (blue). Scale bars, 100 μm. The significant differences are indicated by asterisk (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$)

HER2 signaling is associated with the expression level of galectin-3

HER2 is one of four receptors in HER signaling pathway, and phosphorylation of HER2 activates the PI3K/AKT pathway. Notably, we found that the expression level of HER2 was regulated by galectin-3 in HER2-positive breast cancer cells (Figure 2(a)), which might reveal a possible interaction between galectin-3 and HER2. Endogenous or exogenous galectin-3 upregulated the expression level of AKT and downregulated the expression level of PTEN in SKBR3 cells (Figure 3(a)–(c)). After transfection with siRNA or using GB1107, JIMT1 cells showed a lower expression level of AKT and a higher expression level of PTEN than untreated groups (Figure 3(a)–(c)). Furthermore, galectin-3 upregulated the phosphorylation level of HER2, PI3K, and AKT, whereas inhibition of galectin-3 resulted in the opposite result (Figure 3(a)). Galectin-3 could also upregulate EGFR and p-ERK1/2 (Figure 3(a),(b)).

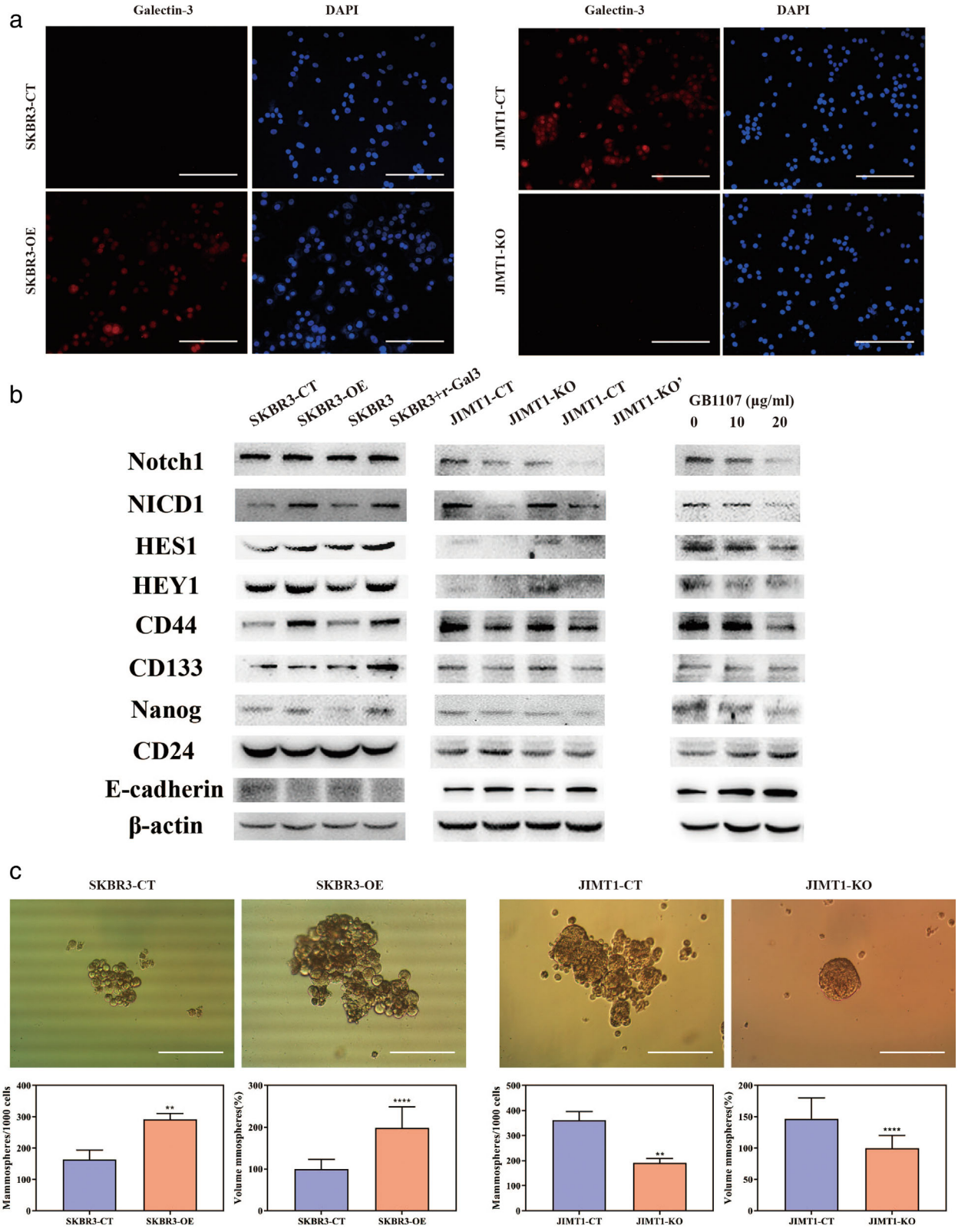

FIGURE 4 Galectin-3 activated Notch1 signaling pathway and promoted the cancer cell stemness of HER2-positive breast cancer cells. (a) Immunofluorescence images of galectin-3 (red) and DAPI (blue) in SKBR3-CT cells, SKBR3-OE cells, JIMT-CT cells, and JIMT-KO cells. (b) Western blot analysis of Notch1 pathway proteins (Notch1, NICD, HES1, and HEY1) and stemness biomarkers (CD24, CD44, CD133, Nanog, and E-cadherin); β-actin was used as a loading control. (c) Mammosphere formation in SKBR3-OE, SKBR3-CT, JIMT1-KO, and JITM1-CT cells. Representative images (top) and quantitative data (bottom) of mammosphere formation are shown. Scale bars, 100 μm. The significant differences are indicated by asterisk (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$)

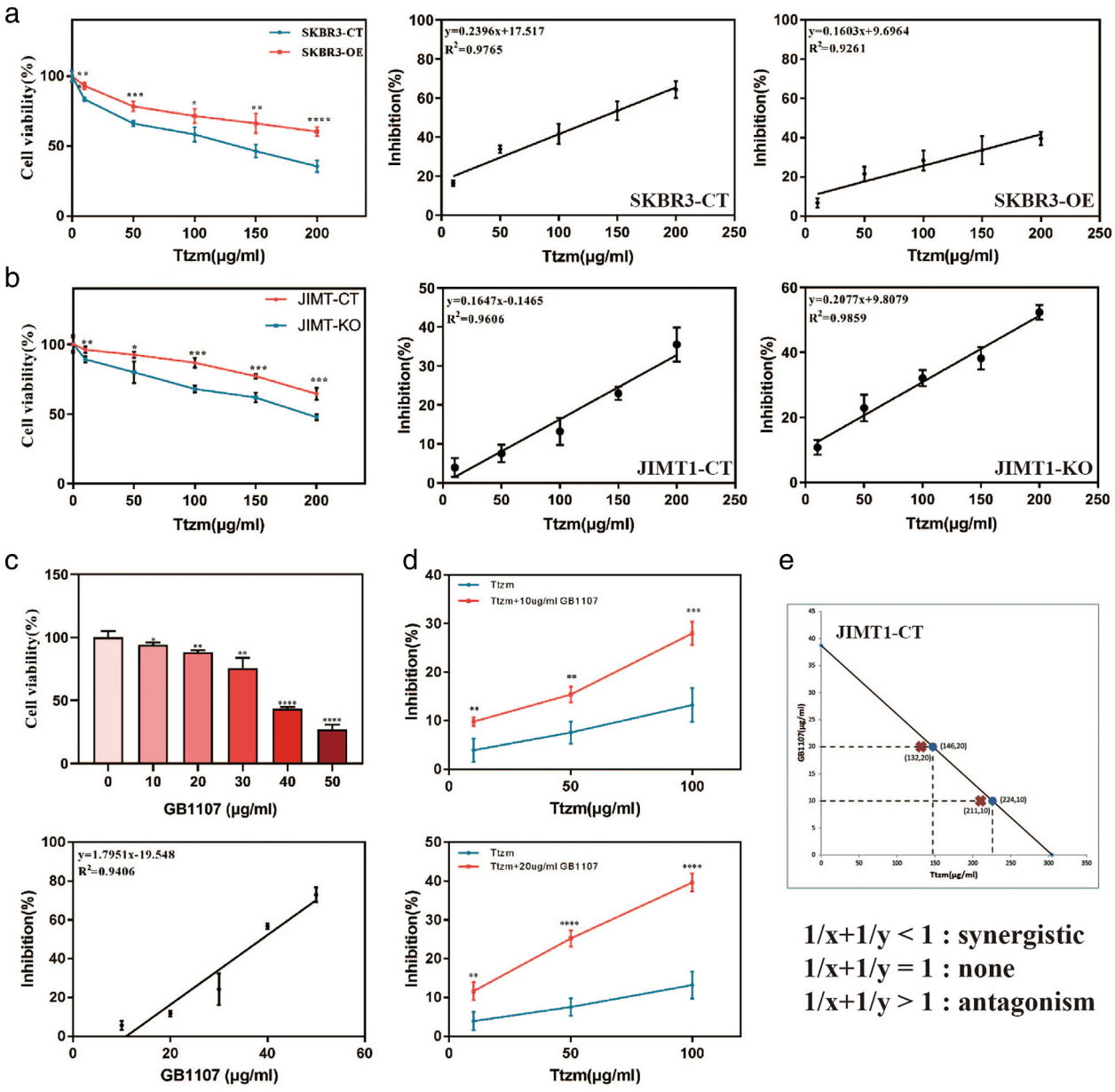

FIGURE 5 Galectin-3 affected the sensitivity of HER2-positive breast cancer cells to trastuzumab. Cell viability curves and inhibition rate of (a) SKBR3-CT and SKBR3-OE cells and (b) JIMT1-CT and JIMT1-KO cells treated with trastuzumab for 72 hours. (c) Cell viability curves and inhibition rate of JIMT1-CT cells treated with GB1107 for 72 hours. (d) Cell viability curves of JIMT1-CT cells treated with GB1107 and/or trastuzumab. (e) Drug interaction analysis chart in JIMT1-CT cells. The significant differences are indicated by asterisk (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$)

Galectin-3 activated the Notch1 signaling pathway and promoted stemness in HER2-positive breast cancer cells

The Notch1 signaling pathway has been reported to be regulated by galectin-3 in several cancers, and its activation promotes cancer cell stemness.[38] However, the relationship between galectin-3 and Notch1 in HER2-positive breast cancer remains unknown. SKBR3-OE cells showed high expression level of galectin-3, whereas JIMT1-KO cells showed low expression level of galectin-3 (Figure 4(a)). We found that galectin-3 overexpression or r-Gal3 addition in SKBR3 cells upregulated the expression level of Notch1, NICD1, HES1, and HEY1, whereas the decrease of galectin-3 caused by knocking down or GB1107 inhibitor in JIMT1 cells downregulated these proteins. These results indicated that galectin-3 activates the Notch1 signaling pathway in HER2-positive breast cancer cells.

CD24, CD44, CD133, Nanog, and E-cadherin are biomarkers for breast cancer stem cells (BCSCs). Because of the activation of Notch1 signaling pathway promoted by galectin-3, the expression levels of CD44, CD133, and Nanog were upregulated, whereas the expression levels of CD24 and E-cadherin were downregulated (Figure 4(b)).

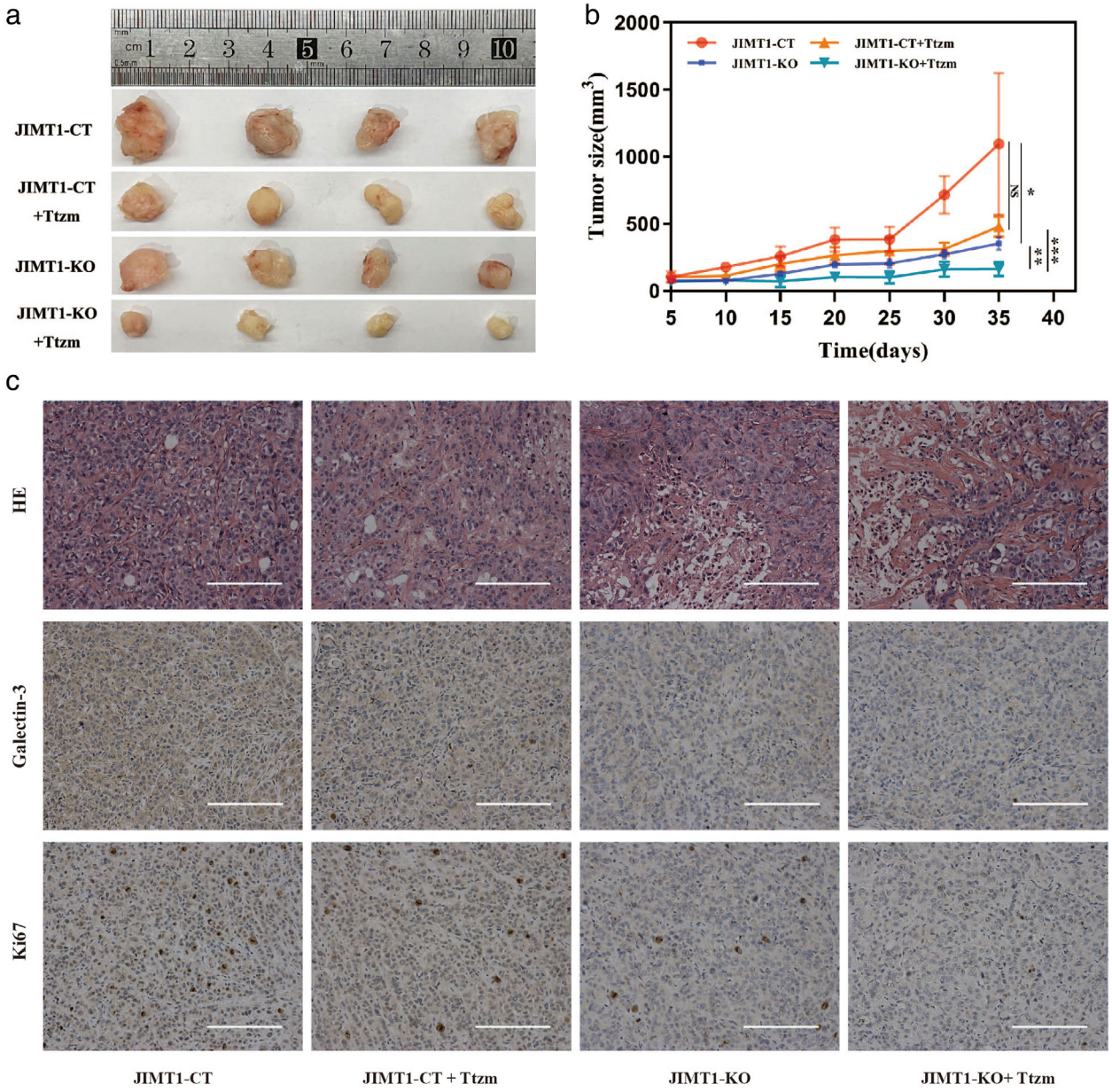

FIGURE 6 Loss of galectin-3 reduced tumor volume and enhanced response to trastuzumab in vivo. (a) Tumors in different groups. (b) The size of tumors was measured every 5 days in different groups. (c) Immunohistochemistry analysis was performed to detect the expression level of galectin-3 and Ki67 in tumors in vivo mouse models. Scale bars, 100 μm. The significant differences are indicated by asterisk (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$)

qRT-PCR results also reflected this tendency (Figure S3). These results showed that galectin-3 could upregulated the stemness of HER2-positive breast cancer cells.

We further evaluated the formation of breast cancer microspheres to explore the impact of galectin-3 on cancer cell stemness. Compared with SKBR3-CT cells, SKBR3-OE cells showed a higher ability of mammosphere formation, including increased size and quantity (Figure 4(c)). Exogenous galectin-3 also increased mammosphere formation in SKBR3 cells (Figure S4(a)). JIMT1-KO cells showed a lower ability of mammosphere formation compared with JIMT1-CT cells (Figure 4(c)). GB1107 also decreased mammosphere formation in JIMT1 cells (Figure S4(b)). Together, these results indicate that galectin-3 significantly increased cancer cell stemness in HER2-positive breast cancer cells.

Inhibition of galectin-3 enhanced the sensitivity of HER2-positive breast cancer cells to trastuzumab

Trastuzumab resistance in HER2-positive breast cancer cells is induced by several mechanisms, including cancer cell

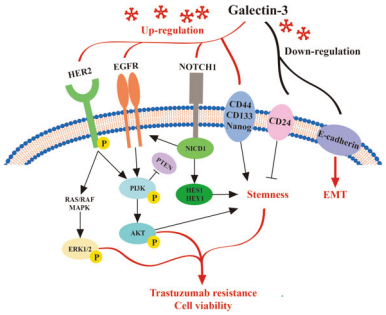

FIGURE 7 Schematic model showed the role of galectin-3 in HER2-positive breast cancer cells

malignancy and stemness. Therefore, we examined whether the expression level of galectin-3 influenced trastuzumab resistance of HER2-positive breast cancer cells. SKBR3-OE cells showed higher cell viability than SKBR3-CT cells after treatment with the same dose of trastuzumab (Figure 5(a)). JIMT1-CT cells also showed higher cell viability than JIMT1-KO cells after treatment with the same dose of trastuzumab (Figure 5(b)). GB1107 decreased the cell viability of JIMT1-CT cells (Figure 5(c)). The cell viability of JIMT1-CT cells treated with GB1107 + trastuzumab was lower than that of cells treated with trastuzumab alone (Figure 5(d)).

The IC_{50} values of trastuzumab and GB1107 in JITM1-CT cells were used to make contour plots. We found that GB1107 and trastuzumab had a synergistic therapeutic effect (Figure 5(e)). Moreover, SKBR3-OE cells showed a lower rate of apoptosis after treatment with trastuzumab compared with SKBR3-CT cells (Figures S4 and S5); whereas JIMT1-KO cells showed a higher rate of apoptosis after treatment with trastuzumab compared with JIMT1-CT cells (Figures S5 and S6).

Loss of galectin-3 reduced tumor volume and enhanced response to trastuzumab in vivo

The size of tumors in the JIMT1-KO group was significantly smaller than that in the JIMT1-CT group (Figure 6(a),(b)). The size of tumors in JIMT1-KO + trastuzumab group was significantly smaller than those in the JIMT1-KO group; there was no statistical significance in the difference in the size of tumors in the JIMT1-CT group and JIMT1-CT + trastuzumab group (Figure 6(a),(b)). HE staining and immunohistochemistry analysis revealed no significant difference in the JIMT1-CT groups with a high expression level of galectin-3 after trastuzumab treatment. However, after trastuzumab treatment in the JIMT-KO group, the necrosis ratio of tumors was significantly increased and the Ki67 expression level was decreased, indicating that knockout of galectin-3 affected the tumor viability and enhanced the sensitivity of JIMT1 cells to trastuzumab in vivo (Figure 6(c)).

DISCUSSION

Studies have shown that galectin-3 promotes the occurrence and development of several types of tumors.[39,40] However, the role of galectin-3 in HER2-positive breast cancer remains unknown. In our study, we found that galectin-3 enhanced the malignancy of HER2-positive breast cancer cells, including stimulation of cell proliferation, migration, colony formation, and invasion. Further investigation showed that galectin-3 upregulated the expression level of HER2 and EGFR and activated the HER2 signaling pathway to promote cancer malignancy. Moreover, upregulation of Notch1 induced by galectin-3 significantly increased cancer cell stemness. Additionally, these proteins had cross-talk with each other, which could further influence the cell viability.[41] These effects of galectin-3 resulted in trastuzumab resistance in HER2-positive breast cancer cells. The inhibition of E-cadherin caused by galectin-3 may reflect changes in epithelial-mesenchymal transition. A schematic model that summarizes our findings is shown in Figure 7.

Trastuzumab is the first-line therapy for patients with HER2-positive breast cancer. However, ~40%–50% of patients treated with trastuzumab develop drug resistance in 1 year, which leads to poor prognosis.[42] Through bioinformatics, we found that the *LGALS3* was a prognostic factor for HER2-positive breast cancer. Furthermore, HER2-positive breast cancer patients with high expression of *LGALS3* mRNA had poor prognosis. HER-2-positive breast cancer patients with higher stage or larger tumor size had a high expression level of *LGALS3* mRNA. Because of the lack of treatment information in the database, we were unable to directly determine the relationship between galectin-3 and trastuzumab resistance in HER2-positive breast cancer patients.

In this study, we found that overexpression of galectin-3 in SKBR3 cells led to trastuzumab resistance and knockout of galectin-3 in JIMT1 cells upregulated cell sensitivity to trastuzumab. Moreover, galectin-3 promoted the proliferation, migration, invasion, and colony formation of HER2-positive breast cancer cells. It was reported that abnormal activation of the HER2/PI3K/ATK pathway contributes to the poor prognosis in HER2-positive breast cancer patients and is also one of the mechanisms of trastuzumab resistance.[10] In our study, we found that galectin-3 upregulated the expression level of HER2, EGFR, and AKT and downregulated the expression level of PTEN. Galectin-3 also increased the phosphorylation level of HER2, PI3K, AKT, and ERK1/2, whereas its inhibition had the opposite effects.

Several recent studies have reported a cross-talk between the HER2/PI3K/AKT pathway and Notch1 pathway, which played an important role in trastuzumab resistance and breast cancer cell stemness.[23,41,43] Many studies have found that trastuzumab cannot eliminate BCSCs that are not drug sensitive, and these BCSCs can lead to the recurrence and metastasis of breast cancer.[44] The enhancement of cancer cell stemness may be one cause of trastuzumab resistance,

and inhibition of stemness may lead to increased drug sensitivity.[45] Some studies showed that galectin-3 promotes cell stemness in several cancers.[38] However, no study had examined the relationship between galectin-3 and stemness in HER2-positive breast cancer. We constructed SKBR3-OE cells and JIMT1-KO cells to evaluate the relationship between galectin-3 and breast cancer stemness. Our cytology experiments showed that either endogenous or exogenous galectin-3 addition significantly increased the population and size of mammospheres in SKBR3 cells. Knocking out of galectin-3 or using GB1107 decreased the population and size of mammospheres in JIMT1 cells. These results indicated that galectin-3 enhances the stemness in HER2-positive breast cancer cells. Moreover, galectin-3 promoted cell viability and inhibited apoptosis in trastuzumab-treated HER2-positive breast cancer cells.

The Notch1 signaling pathway has been shown to promote BCSC survival and self-renewal, which is an important reason for trastuzumab resistance.[22] Notch-1 contributes to trastuzumab resistance by repressing PTEN, which may lead to hyper-activation of ERK1/2 signaling.[46] In addition, trastuzumab resistance may be related to Notch1/AKT/FOXO1 signaling.[47] In our study, we found that galectin-3 upregulated the expression level of Notch1, NICD1, HES1, and HEY1, which may be closely related to cancer cell stemness and induced trastuzumab resistance. We also found that galectin-3 increased the level of p-ERK1/2 and decreased the level of PTEN. Moreover, galectin-3 upregulated the expression level of CSC markers CD44, Nanog, and CD133 and downregulated the level of CD24 and E-cadherin. Several studies have found that the abnormal expression of these markers was related to trastuzumab resistance.[48,49] For example, E-cadherin affects trastuzumab-mediated antibody-dependent cellular cytotoxicity through killer cell lectin-like receptor G1 on natural killer cells.[50] The use of E-cadherin ectodomain-specific monoclonal antibody DECMA-1 inhibited HER2-positive breast cancers by hindering tumor growth and inducing apoptosis via downregulation of key oncogenic pathways involved in trastuzumab resistance.[51] The CD44-hyaluronic acid complex directly affects the binding of HER2 to trastuzumab, whereas the sensitivity of trastuzumab is restored by downregulating CD44.[48]

Our results indicate that inhibition of galectin-3 significantly reverses the trastuzumab resistance of HER2-postive breast cancer cells in vitro and in vivo, suggesting that the galectin-3 inhibitor may be a potential synergist during trastuzumab therapy of HER2-postive breast cancer patients. GB1107 is a potent, selective, and orally active galectin-3 antagonist that inhibits lung adenocarcinoma growth and augments the response to PD-L1 blockade.[52] GB1107 enhances the effects of PD-L1 immune checkpoint inhibitors in increasing the expression level of cytotoxicity and apoptosis effector molecules.[52] Our results showed that GB1107 significantly inhibited cell proliferation of JIMT1 cells and exhibited synergistic effects with trastuzumab. GB1107 also decreased mammosphere formation in JIMT1 cells and regulated CSC markers. Moreover, GB1107 downregulated the PI3K/AKT and Notch1 pathways. These findings indicate that GB1107 may represent an effective drug for HER2-positive breast cancer.

CONCLUSION

We demonstrate that galectin-3 promotes cell proliferation, migration, colony formation, and invasion in HER2-positive breast cancer cells. Galectin-3 activates the HER2 signaling pathway and upregulates EGFR to enhance cancer malignancy of HER2-positive breast cancer cells. Galectin-3 also activates the Notch1 signaling pathway to upregulate the stemness of HER2-positive breast cancer cells. These factors influence the efficacy of trastuzumab in cells. Inhibition of galectin-3 significantly reverses the trastuzumab resistance of HER2-positive breast cancer cells. These results indicate that galectin-3 may represent a prognostic predictor and therapeutic target for HER2-positive breast cancer and suggest the potential use of galectin-3 inhibitor as an effective drug for treatment of HER2-positive breast cancer.

ACKNOWLEDGMENTS

We would like to thank the researchers and study participants for their contributions. We would like to thank TopEdit (www.topeditsci.com) for its linguistic assistance during the preparation of this manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Chen Y, Xu J, Pan W, Xu X, Ma X, Chu Y, et al. Galectin-3 enhances trastuzumab resistance by regulating cancer malignancy and stemness in HER2-positive breast cancer cells. Thorac Cancer. 2022;13(13):1961–73. https://doi.org/10.1111/1759-7714.14474