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ORIGINAL ARTICLE

Transgelin-2 expression in breast cancer and its relationships with clinicopathological features and patient outcome

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Abstract

Background Transgelin-2 is an actin-binding protein that is widely expressed in various tissues and organs of the body, and reportedly may participate in the development and progression of multiple cancers. However, the clinical significance of transgelin-2 still remains controversial. We, therefore, aimed to determine the expression of transgelin-2 in breast cancer as well as its correlation with the tumorigenesis, progression and prognosis of human breast cancer.

Methods We collected tissues of 58 breast cancer patients from our hospital and 1090 samples from The Cancer Genome Atlas (TCGA) database. X-tile software was used to divide the *transgelin-2* mRNA expression level in the database, logistic regression model was used to identify independent factors influencing *transgelin-2* mRNA expression, and then Cox regression and Kaplan–Meier analysis were used to find factors that influence survival of breast cancer.

Results Transgelin-2 was significantly overexpressed in breast cancer tissues from our hospital and receiver operating characteristic (ROC) curve indicated that transgelin-2 may have diagnostic value. Meanwhile, estrogen receptor (ER) was in inverse correlation with transgelin-2 protein and mRNA expression, and transgelin-2 expression was positively correlated with Ki67 in breast cancer tissues. Logistic regression model revealed that TNM stage, ER and progesterone receptor (PR) status were independent factors for *transgelin-2* mRNA expression. Patients with high transgelin-2 mRNA expression showed a poor survival and the trend was statistically significant only in ER-negative patients.

Conclusions Transgelin-2 was expressed significantly higher in breast cancer cells and correlated with some clinicopathological factors. High transgelin-2 expression might predict poor prognosis for ER-negative patients.

Keywords Transgelin-2 · Breast cancer · Target · Tumor progression · Prognosis

Introduction

Breast cancer is the most common malignant tumor in females and the second cause of cancer death among females in more developed countries, while it remains the leading cause of cancer death among females in less developed countries and women younger than 45 years in China [1, 2]. The treatment of breast cancer is a comprehensive process including surgery, radiotherapy, chemotherapy, endocrinotherapy and immunotherapy [3–5]. Chemotherapy is an important part in this treatment that can effectively improve the survival rate of patients. However, the occurrence of multidrug resistance (MDR) in breast cancer greatly reduces the therapeutic efficacy of chemotherapy [4–6]. This situation makes it necessary to identify the mechanisms of MDR in breast cancer and thereby discover appropriate therapeutic agents for overcoming it.

The occurrence of MDR is a complex process involving multiple genes and pathways. A widely accepted mechanism of MDR is that the overexpression of P-glycoprotein and other related transporters such as MDR-related protein (MRP), breast cancer resistant protein (BCRP) and lung resistance-related protein in cancer cells can recognize and catalyze their efflux of diverse anticancer drugs. The increasing resistance to anticancer drug-induced apoptosis is also an important mechanism of MDR. In addition, recent studies have found that changes to the tumor microenvironment

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can promote the MDR of cancer cells, and so more attention should be focused on the MDR properties of tumor stem cells—a small group of self-renewing cancer cells—rather than on the cells that comprise the majority of the tumor population [6, 7].

Transgelin-2 is a member of the calponin family of actin-binding proteins and a homolog of the protein transgelin [8]. It is reported that dysregulation of transgelin-2 may participate in the occurrence of various cancers, including hepatocellular carcinoma (HCC) [9], Barrett's adenocarcinoma [10], colorectal cancer [11], bladder cancer [12], uterine cervical squamous cell carcinoma [13], breast cancer [14], and endometrial cancer [15]. Our group previously used proteomics methods to discover that transgelin-2 was overexpressed in our established human breast cancer paclitaxel-resistant cells (MCF-7/PTX) compared with wild-type breast cancer cells (MCF-7/S) [16]. We subsequently have found transgelin-2 plays an important role in inducing MDR and the invasion and metastasis of breast cancer cells [17–19]. However, there also have some contrasting reports, such as the expression of transgelin-2 being lower at metastatic sites than at the primary tumor in patients with endometrial carcinoma, uterine cervical squamous cell carcinoma and ovarian cancer with brain metastasis [15]. Similar findings have been reported for breast cancer [20] and Barrett's adenocarcinoma [10]. These discrepant observations mean that the precise function of transgelin-2 in breast cancer remains unclear and, therefore, needs further exploration.

The purpose of this study was to investigate the expression of transgelin-2 in breast cancer and clarify its correlation with the tumorigenesis, progression and prognosis of breast cancer.

Materials and methods

Study patients and samples

This study was approved by the Human Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University for the collection of tissues samples. We collected paraffin-embedded breast cancer tissues ($n = 55$) and adjacent non-tumorous tissues ($n = 56$) from 58 patients who underwent surgery in the Department of Breast Surgery, the First Affiliated Hospital of Xi'an Jiaotong University between 2007 and 2014.

Immunohistochemistry

The expression of transgelin-2 was detected by PV two-step immunohistochemistry staining. 3-μm sections were obtained from paraffin-embedded tissues. Immunohistochemistry (IHC) was performed following the manufacturer's instructions. First, the sections were deparaffinized in xylene and rehydrated using alcohol gradiently. Then, antigen retrieval was conducted in a pressure cooker with temperature 121 °C using citrate buffer (pH 6.0) for about 5 min and then cooled to room temperature, washed three times in phosphate buffered saline (PBS) for 3 min each time. Horseradish peroxidase (HRP) was added to block endogenous peroxidase activity for 10 min and washed three times in PBS for 3 min each time before incubating. The sections were then incubated with rabbit anti-*TAGLN2* polyclonal antibody (1:150; Biorbyt, USA) at 37 °C for 60 min, and then washed three times in PBS for 3 min each time. Reaction enhancer was dripped for 20 min in room temperature and then incubated with goat anti-rabbit IgG secondary antibodies for 20 min, followed by washing in PBS anti-rabbit IgG secondary antibodies for 20 min, followed by washing in PBS. Then, the sections were colored by 3,3′diaminobenzidine (DAB) substrate and washed in water. Finally, the sections were counterstained with hematoxylin for 20 s, washed with hydrochloric acid and then ammonia water followed, dehydrated, cleared, and coverslipped with mounting. Goat serum replaced the primary antibody as negative controls to assure the specificity of immunostaining. The staining result of transgelin-2 was determined by combining staining intensity with the percentage of positive staining tumor cells. The staining intensity was scored as follows: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The percentage of positive staining tumor cells was divided as follows: 0 (0–5%), 1 (6–25%), 2 (26–50%), 3 (51–75%), 4 (76–100%). The final score was achieved by multiplying the score of staining intensity and the percentage of positive staining tumor cells ranging from 0 to 12. The expression of transgelin-2 was considered to be negative when the score was 0 and positive when the score was 1–12. Low expression of transgelin-2 was judged if the score was 0–4 and high expression was judged if the score was 5–12 [21].

TCGA database

Transgelin-2 mRNA expression of breast cancer RNA seq data as well as complete clinical pathological and follow-up data of samples in TCGA database ($n = 1090$) were used for analysis, and the data source was breast invasive carcinoma (TCGA, Provisional). The extraction criteria of the database were as follows: breast cancer, large sample size, including mRNA expression data of samples and complete clinical and follow-up data.

Statistical analysis

X-tile software was used to divide the mRNA expression level of *transgelin-2* in the TCGA database and divide into low and high expressions based on its median

expression value; *transgelin-2* mRNA expression was considered expressed low when mRNA expression value was less than 20977.6 and expressed high when expression value was equal or more than 20977.6. All statistics were analyzed by SPSS 19.0. Chi-square test was used to evaluate the different expressions of transgelin-2 in breast cancer tissues and adjacent non-tumorous tissues as well as the correlation of *transgelin-2* mRNA expression with clinicopathologic characteristics; multivariate logistic regression model was performed to find independent influence factors for *transgelin-2* mRNA expression. ROC was applied to estimate the ability of transgelin-2 to recognize breast cancer. Then, correlation between transgelin-2 and Ki67 was analyzed by Spearman correlation analysis. Univariate and multivariate Cox regressions were used to find influence factors for survival of breast cancer. The prognostic value of *transgelin-2* in DFS and OS of breast cancer patients was examined with Kaplan–Meier curve and compared by log-rank test. All tests were considered statistically significant if $P < 0.05$ (two tails).

Results

Expressions of transgelin-2 in adjacent non-tumorous tissues and breast cancer tissues

IHC staining results about transgelin-2 expression in breast cancer tissues and adjacent non-tumorous tissues are shown in Fig. 1 (left); transgelin-2 was strongly expressed in the cytoplasm of breast cancer cells while transgelin-2 expression was not seen in the normal ductal epithelial cells of adjacent non-tumorous tissues. Then, we analyzed all results of tissues; it showed that, just as in Fig. 1 (right), transgelin-2 was significantly overexpressed in breast cancer compared with adjacent non-tumorous tissues ($P < 0.001$). As shown in Supplementary Fig. 1, an ROC curve was constructed to evaluate the diagnostic value of transgelin-2 in distinguishing breast cancer from non-breast cancers. The area under the ROC curve (AUC) was 0.875 (95% confidence interval (CI) = 0.805–0.946, $P < 0.001$) and the cut-off value of the ROC curve was 0.5, indicating that transgelin-2 was overexpressed in breast cancer and suggests it would be useful for identifying breast cancer when IHC score of transgelin-2 was more than 0.5.

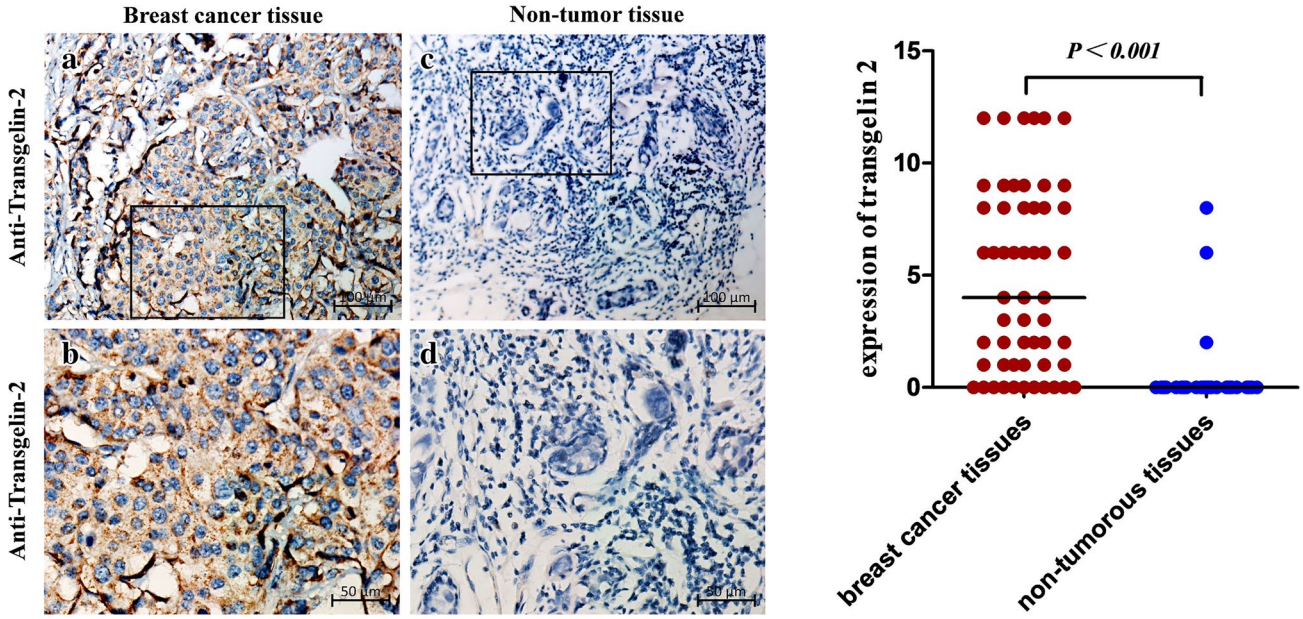

Fig. 1 Transgelin-2 immunoreactivity in breast tissue. Sections of breast cancer tissues stained with antibody to *TAGLN2* and viewed at **a** ×200 magnification and **b** ×400 magnification. Sections of adjacent non-tumorous tissues stained with antibody to *TAGLN2* and viewed at **c** ×200 magnification and **d** ×400 magnification (Left). A scatter gram about transgelin-2 expression in breast cancer tissues and adjacent non-tumorous tissues (Right)

Relationships of transgelin-2 expression with hormone receptors, Her-2 status and Ki67 labeling index

We also explored the relationships between transgelin-2 expression and ER, PR, Her-2 status. As indicated in Table 1, transgelin-2 expression was correlated with ER status but not with PR status or Her-2 status. The 55 breast cancer tissue samples comprised 28 ER-positive and 27 ER-negative samples. The results in Fig. 2 indicate that high expression rate of transgelin-2 was increased in ER-negative breast cancer tissues (25/27, 93%) than in ER-positive breast cancer tissues (19/28, 68%, $P=0.022$). The 55 breast cancer tissue samples could also be categorized into 33 PR-negative and 22 PR-positive samples. Expression of transgelin-2 showed no difference in PR-negative breast cancer tissues (29/33, 88%) and PR-positive breast cancer tissues (15/22, 68%; $P=0.148$), which was also shown in Fig. 2. Ki67 is a non-histone nuclear protein located in proliferative cell nuclei, and so it can be used as a tumor cell proliferation marker [22, 23]. So, here we evaluated the Ki67 labeling index data of 38 breast cancer patients among the 58 patients, for part of patients did not detect the Ki67 labeling index, and the Ki67 labeling index was evaluated by the pathologist of the First Affiliated Hospital of Xi'an Jiaotong University manually under the microscope according to the assessment guidelines in the International Ki67 in Breast Cancer Working Group. Ki67 was judged as high when Ki67 labeling index was equal or more than 30% and low when Ki67 labeling index was less than 30%. As shown in Table 2, our study found high expression rates of transgelin-2 and Ki67 in breast cancer cells, at 41.7% and 61.1%, respectively. A Spearman correlation test revealed a positively correlation between the expression of the two proteins in breast cancer (Spearman correlation coefficient = 0.443, $P=0.007$).

Table 1 The relationships between transgelin-2 expression and hormone receptors and Her-2 status

Hormone receptors and Her-2 status	Cases	Transgelin-2 expression		*P* value
		Low	High	
ER				
Positive	28	9	19	**0.022**
Negative	27	2	25	
PR				
Positive	22	7	15	0.148
Negative	33	4	29	
Her-2				
Positive	27	8	19	0.080
Negative	28	3	25	

Significant *P* value is in bold

ER estrogen receptor, *PR* progesterone receptor, *Her-2* human epidermal growth factor receptor-2

Correlation of transgelin-2 mRNA expression with clinicopathologic characteristics

The above results were obtained from tissues and data that we collected from the First Affiliated Hospital of Xi'an Jiaotong University. We also mined data from TCGA database with *transgelin-2* mRNA expression and complete clinical pathologic and follow-up data of the samples ($n=1090$). We evaluated the correlation between *transgelin-2* mRNA expression and clinicopathologic characteristics using the

Table 2 Correlation of transgelin-2 and Ki67 expression by Spearman correlation test

Protein	Cases	Transgelin-2 expression		*P* value	*R* value
		Low	High		
Ki67 labeling index					
< 30%	14	12	2	**0.007**	0.443
≥ 30%	22	9	13		

Significant *P* value is in bold

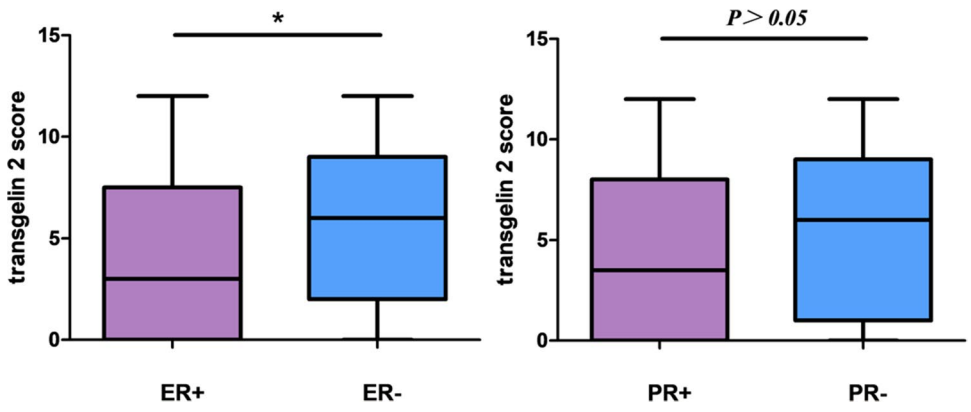

Fig. 2 Histogram of transgelin-2 expression at different ER status and PR status, purple stands for ER-positive or PR-positive, blue stands for ER-negative or PR-negative, $*P<0.05$

Table 3 Relationship between *transgelin-2* mRNA expression of breast cancer tissues and clinicopathological characteristics

Characteristics	Cases	*Transgelin-2*		*P* value
		Low	High	
Age				
< 60	581	455	126	0.216
≥ 60	509	414	95	
TNM stage				
I/II/III	1047	836	211	**0.015**
IV	20	11	9	
Unknown	23	22	1	
Distant metastasis				
M0	914	735	179	**0.028**
M1	22	13	9	
Unknown	154	121	33	
Lymph node metastasis				
N0+N1	873	693	180	0.542
N2+N3	198	161	37	
Unknown				
ER status				
Negative	237	130	107	**< 0.001**
Positive	804	701	103	
Unknown	49	38	11	
PR status				
Negative	339	213	126	**< 0.001**
Positive	699	615	84	
Unknown	52	41	11	
Her-2 status(IHC)				
Negative	562	449	103	0.115
Positive	164	132	42	
Unknown	364	288	76	
Her-2 status(FISH)				
Negative	329	272	57	0.549
Positive	76	65	11	
Unknown	685	532	153	

Significant *P* values are in bold

chi-square test. Table 3 indicates that *transgelin-2* mRNA expression was significantly associated with TNM stage, ER status, PR status and distant metastasis. However, there were no associations between *transgelin-2* mRNA expression and other clinicopathologic characteristics, as shown in Supplementary Fig. 2. Age, ER status, PR status and Her-2 status, lymph node metastasis, TNM stage and distant metastasis were chosen for inclusion in the multivariate logistic regression model to find the independent factors influencing *transgelin-2* mRNA expression. The results in Supplementary Table 1 indicate that TNM stage (OR = 6.363, $P = 0.034$), ER status (OR = 0.234, $P = 0.003$) and PR status (OR = 0.343, $P = 0.022$) influenced *transgelin-2* mRNA expression in breast cancer. The *transgelin-2* mRNA was significantly higher expressed in breast cancer patients with a late TNM stage, as well as in ER-negative patients and PR-negative patients.

Prognostic value of transgelin-2 for breast cancer

As shown in Supplementary Table 2, univariate Cox regression revealed that age, TNM stage, distant metastasis, lymph node metastasis and *Transgelin-2* mRNA expression influenced the OS of breast cancer patients. The subsequent multivariate Cox regression revealed that age, lymph node metastasis, PR status and *Transgelin-2* mRNA expression were independent factors influencing the OS of breast cancer patients. The prognostic value of *transgelin-2* in the DFS and OS of breast cancer patients was examined with the Kaplan–Meier curves and compared using the log-rank test (Fig. 3). Although DFS of patients according to *transgelin-2* mRNA expression showed no difference ($P = 0.164$), OS of patients with low *transgelin-2* mRNA expression (median survival time = 128.98 months) was significantly better than patients with high *transgelin-2* mRNA expression (median survival time = 114.06 months, $P < 0.001$), which indicates that high *transgelin-2* mRNA expression may be a poor

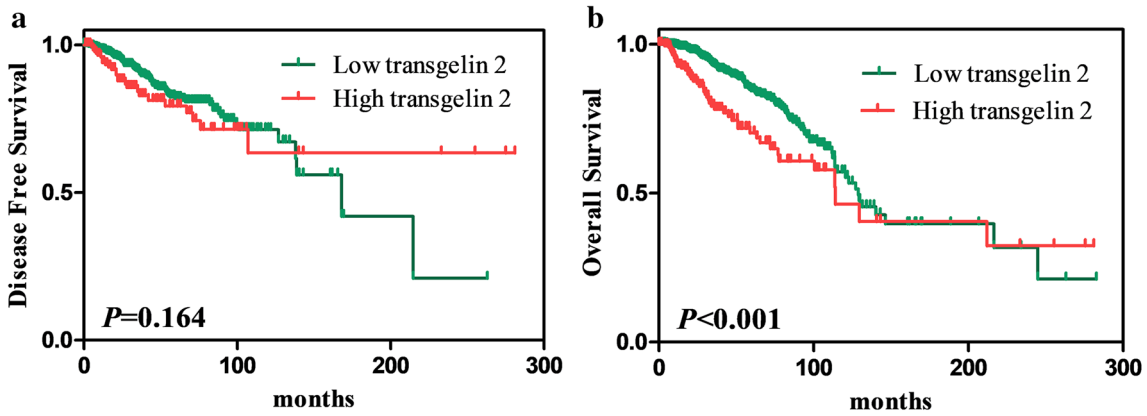

Fig. 3 Kaplan–Meier curves of the association between transgelin-2 mRNA expression and **a** DFS and **b** OS

prognostic marker of breast cancer. Moreover, we also drew Kaplan–Meier curves according to ER status. Interestingly, we discovered that, as shown in Fig. 4, there was no significant difference in patient outcome according to transgelin-2 mRNA expression in ER-positive patients (DFS: $P=0.226$: OS: $P=0.099$). However, DFS and OS in high transgelin-2 mRNA expression group were significantly shorter in ER-negative patients (DFS: $P=0.033$: OS: $P=0.003$).

Discussion

It has been reported that transgelin-2 is upregulated in a variety of cancers. For example, Petra et al. detected increased transgelin-2 expression in MIM-RT cells, which show various characteristics of human HCCs, and found that increased transgelin-2 expression was related to poor OS in HCC patients [9]. Yanbin et al. concluded that a high expression level of transgelin-2 was correlated with lymph node metastasis, distant metastasis, late TNM stage and a poor prognosis in colorectal cancer [11]. However, there remains a study that considered transgelin-2 as a tumor suppressor gene and was negative correlated with lymph node metastasis in MDA-MB-231 cells [20]. It is notable that previous study of our team [19] was in contradiction with the study which considering transgelin-2 was downregulated in MDA-MB-231 high metastasis cells [20]. It may due to the biological difference between the two breast cancer cells for characterization of MCF-7/S and MDA-MB-231 was different. Our study proved that the transgelin-2 was significantly expressed higher in breast cancer tissues than that in adjacent non-tumorous tissues, which was consistent with most of the findings of research into some other types of cancers.

Notably, our study found that transgelin-2 expression was inversely correlated with ER status and positively correlated with Ki67 labelling index in breast cancer tissues. The analysis about TCGA database also showed that *transgelin-2* mRNA expression was significantly associated with TNM stage, ER status, PR status and distant metastasis. Moreover, TNM stage, ER status and PR status were independent factors for *transgelin-2* mRNA expression in breast cancer, suggesting that *transgelin-2* mRNA

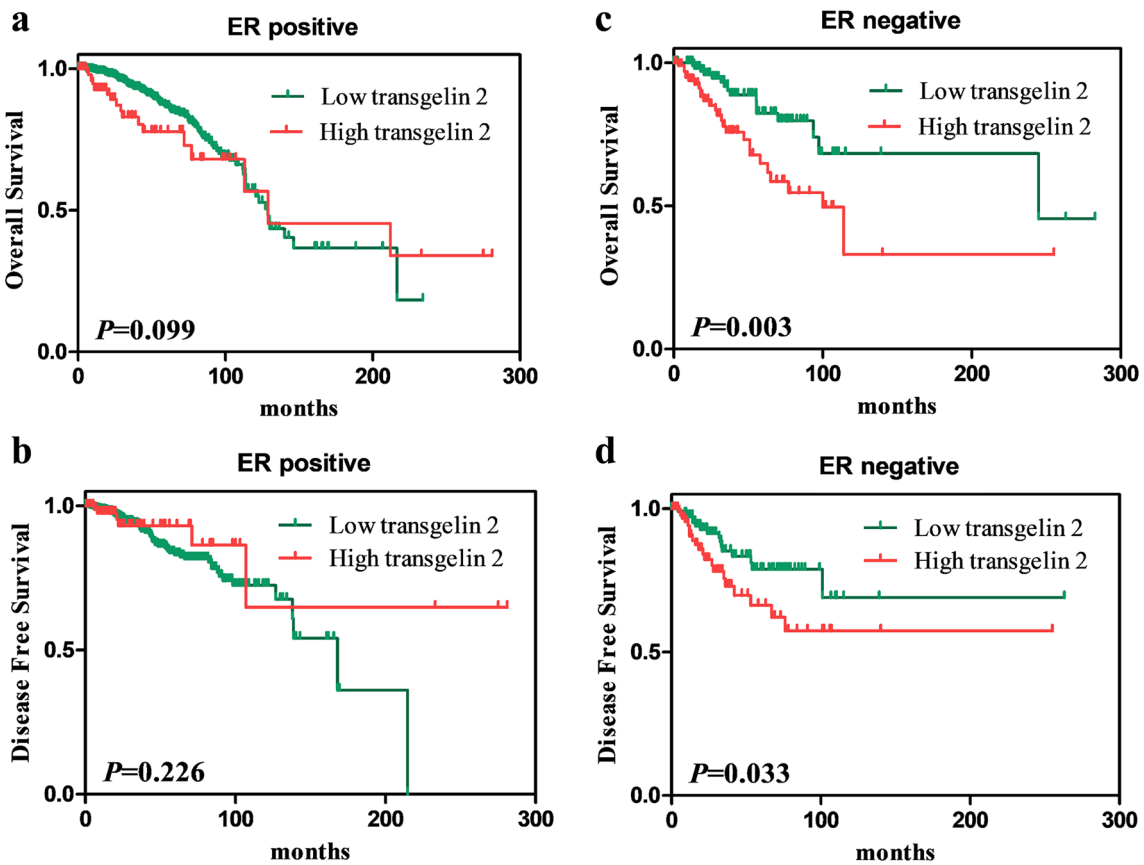

Fig. 4 Kaplan–Meier analysis of the association between transgelin-2 mRNA expression and overall survival (OS) as well as disease-free survival (DFS) in ER+ and ER− breast cancer patients. The overall survival curve and disease-free survival curve of ER+ breast cancer patients in different transgelin-2 status groups (**a**, **b**); the overall survival curve and disease-free survival curve of ER-breast cancer patients in different transgelin-2 status groups (**c**, **d**)

expression is associated with tumor progression. Notably, an inverse correlation between ER and transgelin-2 was consistently observed in both analyses. Therefore, we suggest that ER status should be considered in further studies on the transgelin-2 in breast cancer in the future.

Moreover, our Kaplan–Meier analysis found that high transgelin-2 mRNA expression indicates a poor prognosis of breast cancer patients. The trend was observed only in patients with ER-negative tumors. Considering that transgelin-2 might have a critical role in drug resistance, shown in our previous study, we speculate that transgelin-2 might be a possible candidate as therapeutic target in the future, especially for ER-negative breast cancer.

In conclusion, we found that transgelin-2 was highly overexpressed in breast cancer and relevant to progression. High transgelin-2 expression might predict poor outcome in patients with ER-negative tumors.

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Compliance with ethical standards

Conflict of interest By collecting the conflict of interest disclosure forms of all authors, the authors declare that they have no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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