RESEARCH

Prognostic role of GPER/Ezrin in triple-negative breast cancer is associated with menopausal status

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Abstract

The role of G protein-coupled estrogen receptor 1 (GPER) signaling, including promotion of Ezrin phosphorylation (which could be activated by estrogen), has not yet been clearly identified in triple-negative breast cancer (TNBC). This study aimed to evaluate the prognostic value of GPER and Ezrin in TNBC patients. Clinicopathologic features including age, menopausal status, tumor size, nuclear grade, lymph node metastasis, AJCC TNM stage, and ER, PR and HER-2 expression were evaluated from 249 TNBC cases. Immunohistochemical staining of GPER and Ezrin was performed on TNBC pathological sections. Kaplan–Meier analyses, as well as logistic regressive and Cox regression model tests were applied to evaluate the prognostic significance between different subgroups. Compared to the GPER-low group, the GPER-high group exhibited higher TNM staging ($P = 0.021$), more death ($P < 0.001$), relapse ($P < 0.001$) and distant events ($P < 0.001$). Kaplan–Meier analysis showed that GPER-high patients had a decreased OS ($P < 0.001$), PFS ($P < 0.001$), LRFS ($P < 0.001$) and DDFS ($P < 0.001$) than GPER-low patients. However, these differences in prognosis were not statistically significant in post-menopausal patients (OS, $P = 0.8617$; PFS, $P = 0.1905$; LRFS, $P = 0.4378$; DDFS, $P = 0.2538$). There was a significant positive correlation between GPER and Ezrin expression level ($R = 0.508$, $P < 0.001$) and the effect of Ezrin on survival prognosis corresponded with GPER. Moreover, a multivariable analysis confirmed that GPER and Ezrin level were both significantly associated with poor DDFS (HR: 0.346, 95% CI 0.182–0.658, $P = 0.001$; HR: 0.320, 95% CI 0.162–0.631, $P = 0.001$). Thus, overexpression of GPER and Ezrin may contribute to aggressive behavior and indicate unfavorable prognosis in TNBC; this may correspond to an individual's estrogen levels.

Key Words

- triple-negative breast cancer
- GPER
- Ezrin
- prognosis
- estrogen

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Background

Triple-negative breast cancer (TNBC) is negative for estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth receptor 2 (HER-2), and accounts for 15–20% of all breast cancers (1). Due to this lack of the common therapeutic targets, TNBCs are associated with the worst prognosis and one of the highest risks of metastasis among all subtypes of breast cancer (2, 3).

Further, several population-based studies have shown that TNBC often presents at a younger age despite lack of ER, and older patients may have a better outcome

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when compared with their younger counterparts (4, 5). An epidemiology survey of basal-like breast cancer has revealed that the alterations of hormones throughout a woman's life are linked to the risk of developing TNBC (6). Overall, while these recent studies suggest that estrogen plays a role in the management of TNBC, the mechanism of estrogen involvement remains undefined.

Although TNBCs lack classic nuclear ERs, more evidence demonstrates estrogen could influence cell proliferation and migration elicited by combining steroid hormone receptors such as G protein-coupled estrogen receptor 1 (GPER), ERβ or estrogen-related receptors (7, 8, 9, 10).

GPER, also known as G protein-coupled receptor 30 (GPR30), used to be considered an orphan receptor. Later, two groups independently proved that GPER binds to and is activated by estradiol, and in response, initiates intracellular signaling cascades (10, 11, 12). Although controversies still exist on the specificity of the binding between GPER and estrogen, most studies currently conclude that GPER is driven by estrogen and is compatible with the pattern of ligand–receptor binding (13, 14). Our previous study showed that treatment with 17b-estradiol (E2) in MDA-MB-231 TNBC cells resulted in Ezrin-dependent cytoskeleton rearrangement, and elicited a stimulatory effect on cell migration and invasion. Importantly, we observed that Ezrin phosphorylation, cell migration and invasion activated by E2 could be significantly inhibited by silencing GPER signaling (15), indicating that E2 induces the phosphorylation of Ezrin protein by GPER to mediate important cellular activities in TNBC cells. Moreover, the expression of GPER in TNBC is extensive and its downstream signaling pathway has been proved to be involved in proliferation, metastasis and angiogenesis in TNBC (16, 17). However, the function of GPER in TNBC remains controversial. As reported in some studies, activation of GPER may suppress the epithelial–mesenchymal transition of TNBC cells (18).

Therefore, the current study aimed to determine GPER and Ezrin immunopositivity in a cohort of TNBC patients, to understand the characteristics and prognostic value of GPER, and the possible relationship between these two in TNBC. Furthermore, we investigated a potential association of GPER and Ezrin with the clinicopathological parameters of TNBC.

Materials and methods

Specimen characteristics and ethical approval

FFPE tissue was derived from 249 patients diagnosed with TNBC at our institution from 1999 to 2011. The eligibility criteria were pathologically confirmed TNBC, underwent primary tumor resection after diagnosis and availability of follow-up data. For each case, detailed clinicopathological characteristics including age, menopausal status, tumor size, nuclear grade, lymph node metastasis (LNM) and AJCC TNM stage were routinely abstracted from the medical record and are shown in compliance with the REMARK criteria (19) in Tables 1 and 2. This study was approved by the Ethics Committee of Sun Yat-Sen University Cancer Center, and all patients had provided written informed consent for their data and surgical specimens to be used for research purposes.

Immunohistochemistry

In the histopathological reports, GPER and Ezrin were evaluated with immunohistochemistry. Formalin-fixed paraffin-embedded tumor tissues were conventionally sectioned and then deparaffinized, hydrated and endogenous peroxidase activity was blocked with 3% H_2O_2 for 10 min. Pressure cooker antigen retrieval was carried out in EDTA for GPER (pH = 9.0) and Ezrin (pH = 8.0) for 2 min 30 s to enhance immunoreactivity. Samples were then incubated with a GPER primary antibody (1:100 dilution, rabbit polyclonal, ab39742, Abcam) and Ezrin antibody (1:100 dilution, rabbit polyclonal, NBP2-16396, Novus, CO, USA) overnight at 4°C. After washing with phosphate-buffered saline (PBS), the slides were treated with a peroxidase anti-rabbit secondary antibody (Zhongshan Golden Bridge Biotechnology, Beijing, China) at 37.5°C for 30 min. Subsequently, DAB (Zhongshan Golden Bridge Biotechnology, Beijing, China) was used to stain the slides. Endometrial carcinoma tissue and renal cell carcinoma tissue were used as positive staining control, respectively, which has been demonstrated to express GPER and Ezrin (20, 21). Two positive control tissues exchanged the primary antibody to check for unspecific staining as the negative control (Supplementary Fig. 1, see section on supplementary data given at the end of this article).

IHC scoring

Two independent pathologists blindly scored all the specimens by consensus. The IHC staining was quantified by applying the immunoreactive score (22), which is calculated by multiplying the staining intensity (grade as 0, negative; 1, weak; 2, moderate; and 3, strong staining) and percentage of positively stained cells (0; <5%;

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Table 1 GPER positivity in TNBC as correlated with clinicopathological factor.

	All	GPER-low	GPER-high	*P**
Menopause status				
Pre-menopause	196 (78.7)	140 (79.5)	56 (76.7)	0.619
Post-menopause	53 (21.3)	36 (20.5)	17 (23.3)	
Tumor size (pT, cm)				
pT1	76 (30.5)	48 (27.3)	28 (38.4)	0.113
pT2	147 (59.0)	110 (62.5)	37 (50.7)	
pT3	20 (8.0)	15 (8.5)	5 (6.8)	
pT4	6 (2.4)	2 (1.1)	4 (5.5)	
Nuclear grade				
II	48 (19.3)	34 (19.3)	14 (19.2)	0.980
III	201 (80.7)	142 (80.7)	59 (80.8)	
LNM#				
pN0	133 (53.4)	101 (57.4)	32 (43.8)	0.101
pN1	58 (23.3)	41 (23.3)	17 (23.3)	
pN2	34 (13.7)	21 (11.9)	13 (17.8)	
pN3	24 (9.6)	13 (7.4)	11 (15.1)	
TNM staging#				
I	38 (15.3)	27 (15.3)	11 (15.1)	0.021
IIa	108 (43.4)	80 (45.5)	28 (38.4)	
IIb	40 (16.1)	33 (18.8)	7 (9.6)	
IIIa	34 (13.7)	22 (12.5)	12 (16.4)	
IIIb	5 (2.0)	1 (0.6)	4 (5.5)	
IIIc	24 (9.6)	13 (7.4)	11 (15.4)	
Local treatment				
Mastectomy	84 (33.7)	59 (33.5)	25 (34.2)	0.373
Quadrantectomy	13 (5.2)	7 (4.0)	6 (8.2)	
Mastectomy + RT	149 (59.8)	107 (60.8)	42 (57.5)	
Quadrantectomy + RT	3 (1.2)	3 (1.7)	0 (0)	
Chemotherapy				
Yes	179 (71.9)	127 (72.2)	52 (71.2)	0.882
No	70 (28.1)	49 (27.8)	21 (28.8)	
Death				
Yes	64 (25.7)	31 (17.6)	33 (45.2)	<0.001
No	185 (74.3)	145 (82.4)	40 (54.8)	
Relapse event				
Yes	34 (13.7)	14 (8.0)	20 (27.4)	<0.001
No	215 (86.3)	162 (92.0)	53 (71.6)	
Distant event				
Yes	53 (21.3)	18 (10.2)	35 (47.9)	<0.001
No	196 (78.7)	158 (89.8)	38 (52.1)	

*χ^2 test comparing proportions among GPER-low expression group and GPER-high expression group.
LNM, lymph node metastasis; RT, radiotherapy.

5–24%; 25–49%; 50–74%; 75–100%). An ROC curve of the survival result for all intensity levels was plotted to generate the optimal cut-off value of GPER and Ezrin expression (Supplementary Table 1). The immunoreactive score of IRS = 0.8 was used to distinguish between low and high expression of both GPER and Ezrin.

Statistical analyses

Chi-squared test or Fisher's exact test was used to analyze the association of GPER and Ezrin status with clinicopathological variables. End points including local relapse, distant metastasis, death and any progression were used to calculate the LRFS (local relapse-free survival), DDFS (distant disease-free survival), OS (overall survival) and PFS (progression-free survival). Kaplan–Meier survival analysis and log-rank test were utilized to compare the survival outcome. Multivariate analysis using a Cox proportional hazard model was performed based on variables with a P value of <0.05 from the logistic regression. All significance level of statistics was set at 0.05 except multivariate model which was set at 0.01.

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Table 2 Ezrin positivity in TNBC as correlated to clinicopathological factor.

	All	Ezrin-low	Ezrin-high	*P**
Menopause status				
Pre-menopause	196 (78.7)	134 (79.3)	62 (77.5)	0.747
Post-menopause	53 (21.3)	35 (20.7)	18 (22.5)	
Tumor size (pT, cm)				
pT1	76 (30.5)	48 (28.4)	28 (35.0)	0.166
pT2	147 (59.0)	104 (61.5)	43 (53.8)	
pT3	20 (8.0)	15 (8.9)	5 (6.3)	
pT4	6 (2.4)	2 (1.2)	4 (5.0)	
Nuclear grade				
II	48 (19.3)	37 (21.9)	11 (13.8)	0.128
III	201 (80.7)	132 (78.1)	69 (27.7)	
LNM#				
pN0	133 (53.4)	98 (58.0)	35 (43.8)	0.136
pN1	58 (23.3)	38 (22.5)	20 (25.0)	
pN2	34 (13.7)	20 (11.8)	14 (17.5)	
pN3	24 (9.6)	13 (7.7)	11 (13.8)	
TNM staging#				
I	38 (15.3)	26 (15.4)	12 (15.0)	0.221
IIa	108 (43.4)	78 (46.2)	30 (37.5)	
IIb	40 (16.1)	30 (17.8)	20 (12.5)	
IIIa	34 (13.7)	20 (11.8)	14 (17.5)	
IIIb	5 (2.0)	2 (1.2)	3 (3.8)	
IIIc	24 (9.6)	13 (7.7)	11 (13.8)	
Local treatment				
Mastectomy	84 (33.7)	60 (35.5)	24 (30.0)	0.190
Quadrantectomy	13 (5.2)	6 (3.6)	7 (8.8)	
Mastectomy + RT	149 (59.8)	100 (59.2)	49 (61.3)	
Quadrantectomy + RT	3 (1.2)	3 (1.8)	0 (0)	
Chemotherapy				
Yes	179 (71.9)	118 (69.8)	61 (76.3)	0.292
No	70 (28.1)	51 (30.2)	19 (23.8)	
Death				
Yes	64 (25.7)	29 (17.2)	35 (43.8)	<0.001
No	185 (74.3)	140 (82.8)	45 (56.2)	
Relapse event				
Yes	34 (13.7)	17 (10.1)	17 (21.3)	0.016
No	215 (86.3)	152 (89.9)	63 (78.7)	
Distant event				
Yes	53 (21.3)	15 (8.9)	38 (47.5)	<0.001
No	196 (78.7)	154 (91.1)	42 (52.5)	

*χ^2 test comparing proportions among GPER-low expression group and GPER-high expression group.
LNM, lymph node metastasis; RT, radiotherapy.

Results

Patient characteristics

During the period of study, 249 cases of primitive invasive breast cancer were diagnosed as TNBC for which the clinicopathological characteristics were available. As shown in Tables 1 and 2, all patients in our study (249/249, 100.0%) were assigned as intermediate (G2) or high grade (G3). For TNM grouping, a total of 249 cases were enrolled with 15.2% (38/249) at stage I, 59.5% (148/249) at stage II and 25.3% (63/249) at stage III. Overall median age at diagnosis was 47 years (range, 23–80 years). More than two-thirds (n=196, 78.7%) of patients were in pre-menopause.

Immunoreactivity of GPER and Ezrin in TNBC tissue

Examples of negative (0), weak (1), moderate (2) and strong (3) GPER and Ezrin staining intensity are shown in Figs 1 and 2. GPER and Ezrin staining were detected in the large majority (177/249, 71.1%; 185/249, 74.3%) of TNBC tissue samples. In general, the expression level of GPER and Ezrin were found to be different in cancer nests and adjacent tissues in positive specimens. According to the

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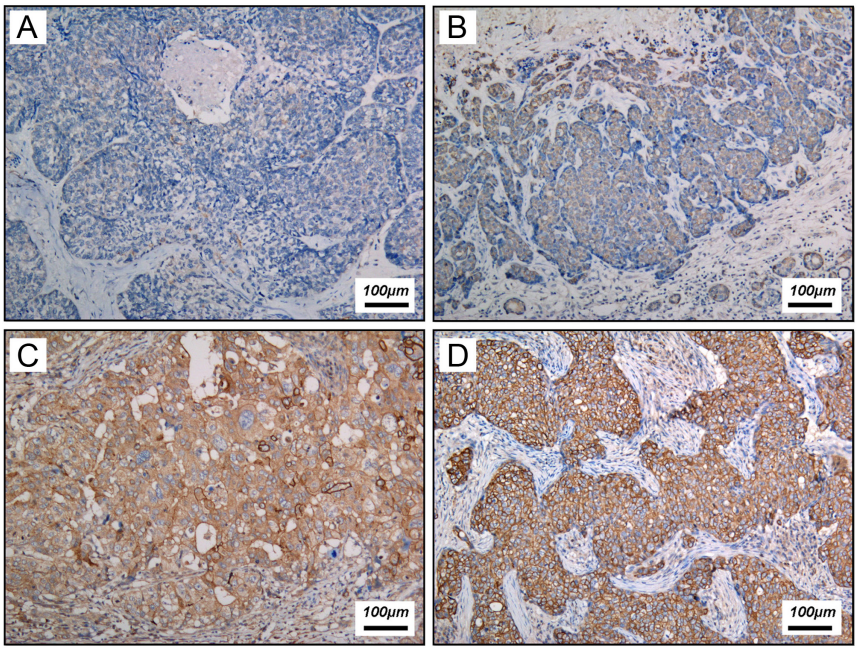

Figure 1
GPER expression in TNBC tissue. Representative IHC figures of GPER grade: (A) negative (0), (B) weak (1), (C) moderate (2) and (D) strong staining (3). Scale bar equals 100 μm.

cut-off IHC score, low and high GPER expression levels were found in 70.7% (176/249) and 29.3% (73/249) of patients, respectively, and 32.1% (80/249) of specimens had high expression of Ezrin (Tables 1 and 2). Among them, 55.4% (138/249) of cases were GPER/Ezrin-low expression and 16.9% (42/249) were GPER/Ezrin-high. Overall, GPER and Ezrin were detected in both cytoplasm and cell membranes of all positively stained sections. The expression level of GPER and Ezrin were found to be different in cancer nests and adjacent tissues (data not shown).

The association of GPER and Ezrin expression with clinicopathological factors

The association of GPER and Ezrin expression with some clinicopathologic variables was assessed. As shown in Tables 1 and 2, high GPER expression was significantly

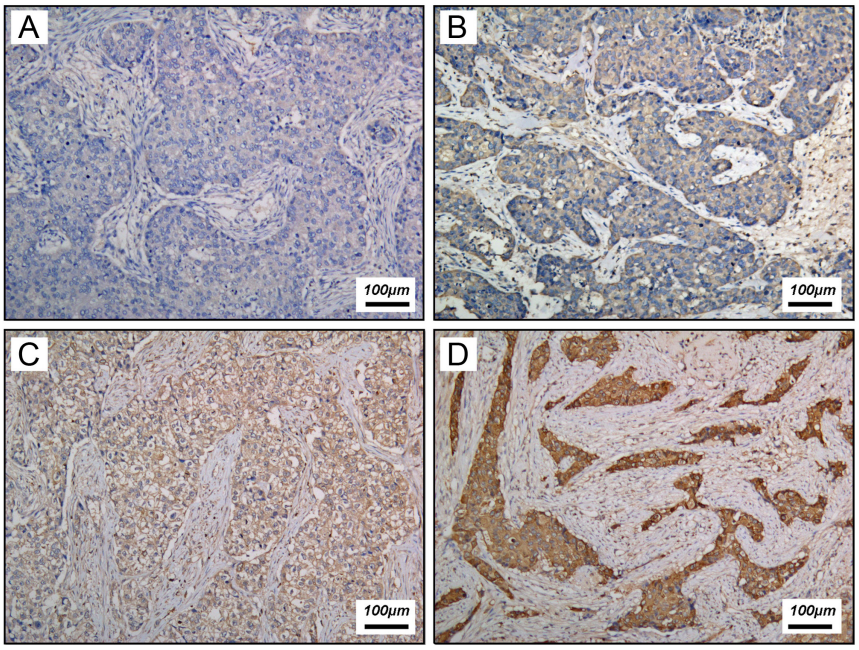

Figure 2
Ezrin expression in TNBC tissue. Representative IHC figures of Ezrin grade: (A) negative (0), (B) weak (1), (C) moderate (2) and (D) strong staining (3). Scale bar equals 100 μm.

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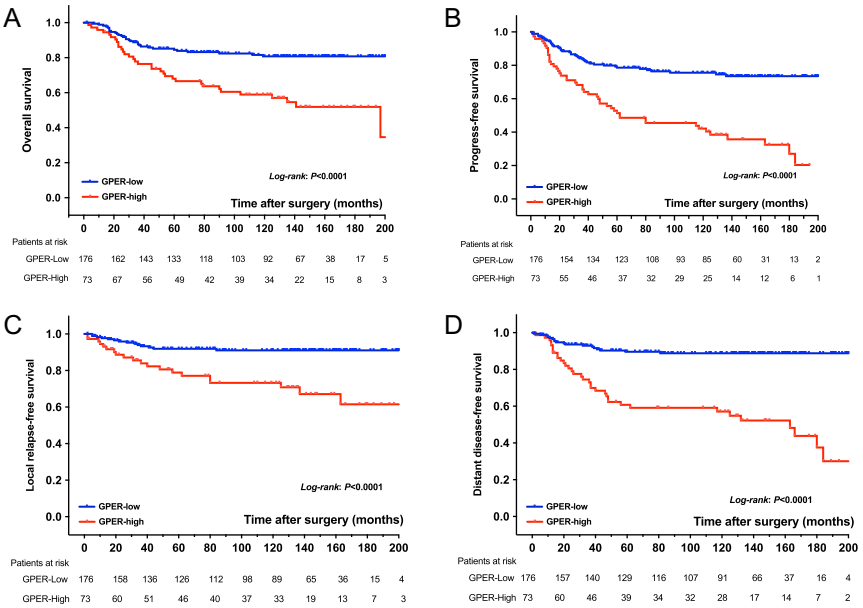

Figure 3
Kaplan–Meier curves comparing survival outcome of TNBC patients with high and low GPER expression. Patients with high GPER expression had a significantly lower OS (A), PFS (B), LRFS (C) and DDFS (D) rate than those with low GPER expression.

associated with high TNM staging (I, 15.1 vs 15.3%; IIa, 38.4 vs 45.5%; IIb, 9.6 vs 18.8%; IIIa, 16.4 vs 12.5%; IIIb, 5.5 vs 0.6%; IIIc, 15.4 vs 7.4%; $P=0.021$), more death (45.2 vs 17.6%; $P<0.001$), relapse (27.4 vs 8.0%; $P<0.001$) and distant events (47.9 vs 10.2%; $P<0.001$). Similarly, a higher ratio of death (45.3 vs 17.2%; $P<0.001$), local relapse (21.3 vs 10.1%; $P=0.016$) and distant metastasis (47.5 vs 8.9%; $P<0.001$) was observed in high Ezrin patients, but not in other variables.

The relationship between GPER, Ezrin and long-term survival outcome

Kaplan–Meier analysis showed that in all 249 cases, patients with high GPER expression in tumors had a significantly lower OS ($P<0.001$), PFS ($P<0.001$), LRFS ($P<0.001$) and DDFS ($P<0.001$) rate than those with low GPER expression (Fig. 3). However, further analysis of patients stratified by menopause status demonstrated that,

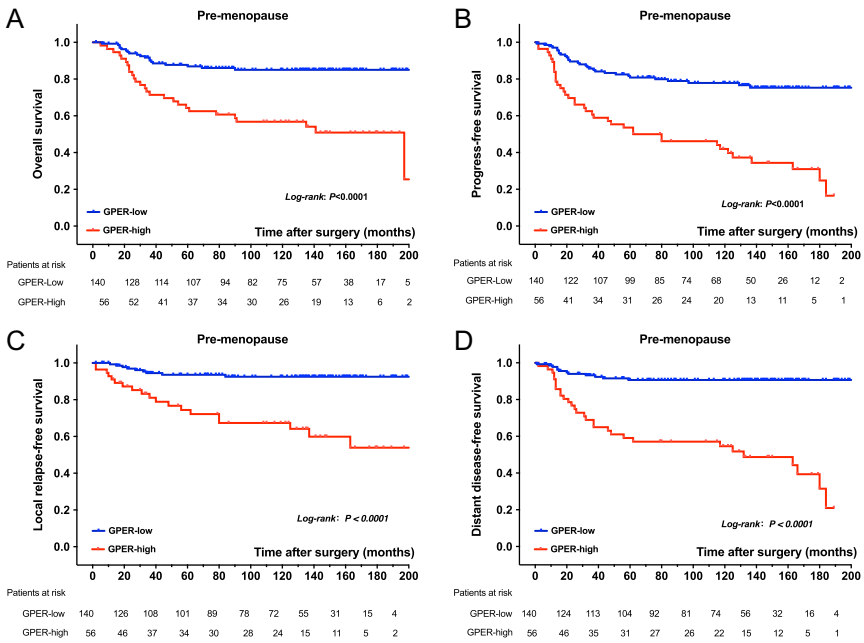

Figure 4
Kaplan–Meier curves comparing survival outcome of pre-menopause TNBC patients with high and low GPER expression. In the pre-menopause subgroup, patients with high GPER expression had a significantly lower OS (A), PFS (B), LRFS (C) and DDFS (D) rate than those with low GPER expression.

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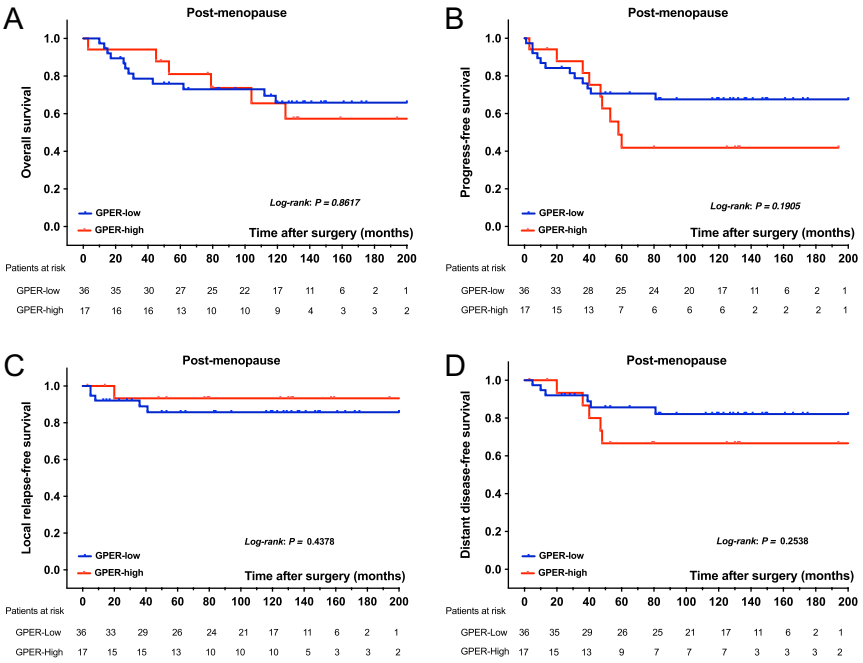

Figure 5
Kaplan–Meier curves comparing survival outcome of post-menopause TNBC patients with high and low GPER expression. In the post-menopause subgroup, there were no significant differences in OS (A), PFS (B), LRFS (C) and DDFS (D) between high and low GPER expression patients.

in the pre-menopause subgroup, the patients with high GPER expression had a significantly lower OS ($P<0.001$), PFS ($P<0.001$), LRFS ($P<0.001$) and DDFS ($P<0.001$) rate than those with low GPER expression (Fig. 4), but no statistically significant difference was observed in terms of OS ($P=0.8617$), PFS ($P=0.1905$), LRFS ($P=0.4378$) and DDFS ($P=0.2538$) rate between the patients with high and low GPER expression in the post-menopause group (Fig. 5). There is no interaction effect of survival outcome between GPER level and menopause status ($P=0.716$; Supplementary Fig. 2).

Spearman correlation showed that there is positive relationship between the full range expression of GPER and Ezrin in our TNBC samples (Table 3, $R=0.508$, $P<0.001$). An effect on survival prognosis by Ezrin, similar to the GPER effect, was observed. The high expression of Ezrin also could significantly decrease the patients' OS ($P<0.001$), PFS ($P<0.001$), LRFS ($P=0.0122$) and DDFS ($P<0.001$) rate (Fig. 6). The statistical differences in the prognostic value of Ezrin disappeared in post-menopause patients (Figs 7 and 8).

Table 3 Positive relationship between expression of GPER and Ezrin.

Ezrin		GPER-low	GPER-high	*R**	*P*
Low	169 (67.9)	138 (78.4)	31 (42.5)	0.508	<0.001
High	80 (32.1)	38 (21.6)	42 (57.5)		

*Spearman's rank correlation coefficient.

By further subgrouping, we found that high co-expression of GPER/Ezrin was significantly linked to the worst OS ($P<0.001$), PFS ($P=0.269$), LRFS ($P=0.001$) and DDFS ($P<0.001$) of patients compared to the low co-expression of GPER/Ezrin group (Fig. 9).

We then used logistic regression and Cox regression to identify the prognostic value of GPER, Ezrin expression and other clinicopathologic variables in univariate and multivariate models. As presented in Table 4, which was adjusted for those risk factors, the multivariate analysis revealed that high GPER still independently predicted poor PFS (HR=0.393; 95% CI 0.246–0.629; $P<0.001$), LRFS (HR=0.329; 95% CI 0.153–0.704; $P=0.004$) and DDFS (HR=0.346; 95% CI 0.182–0.658; $P=0.001$). In addition, Ezrin expression also remained the prognostic factor for DDFS (HR=0.320; 95% CI 0.162–0.631; $P=0.001$).

Discussion

In our study, most cases presented with higher nuclear grades and worse TNM staging, which is characteristic of TNBC, together with a high degree of malignancy and rapid progression (23). Large-scale clinical studies have found that TNBC has a higher risk of recurrence and metastasis, and a worse prognosis in young women than in post-menopausal patients (4). Similar to the literature reported (4, 24), most of our patients are pre-menopausal women.

To the best our knowledge, until now, the clinical relevance of GPER expression in TNBC remains controversial.

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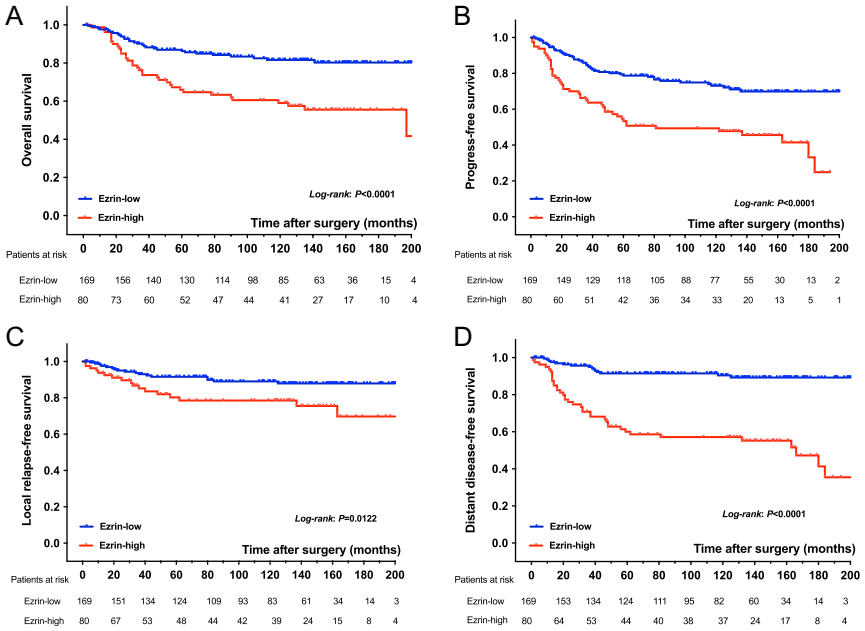

Figure 6
Kaplan–Meier curves comparing survival outcome of TNBC patients with high and low Ezrin expression. Patients with high Ezrin expression had a significantly lower OS (A), PFS (B), LRFS (C) and DDFS (D) rate than those with low Ezrin expression.

Some studies suggest that GPER expression is not associated with or inhibits the progression of TNBC (18, 25). We suspected this phenomenon may be related to the non-specific activity of the GPER-specific agonist G-1 (26). Most studies found that GPER is prevalent in TNBC and associated with young age, and possibly with prognosis (17, 27). Our data also found GPER was mainly located in cancer nests and was significantly correlated with patients' TNM staging and survival outcomes: evidence which links the expression of GPER with TNBC clinical status.

When we focus on the patient's long-term survival, the data showed that the high expression of GPER correlated with high risk of death, local relapse and distant metastasis in TNBC. Further, we noted that GPER expression appears to be lower in younger TNBC patients with better prognosis. Therefore, we divided all patients into pre-menopausal and post-menopausal groups, and then separately studied the effect of GPER expression on outcome. According to the Kaplan–Meier analyses, it is highly likely that the role of GPER in predicting

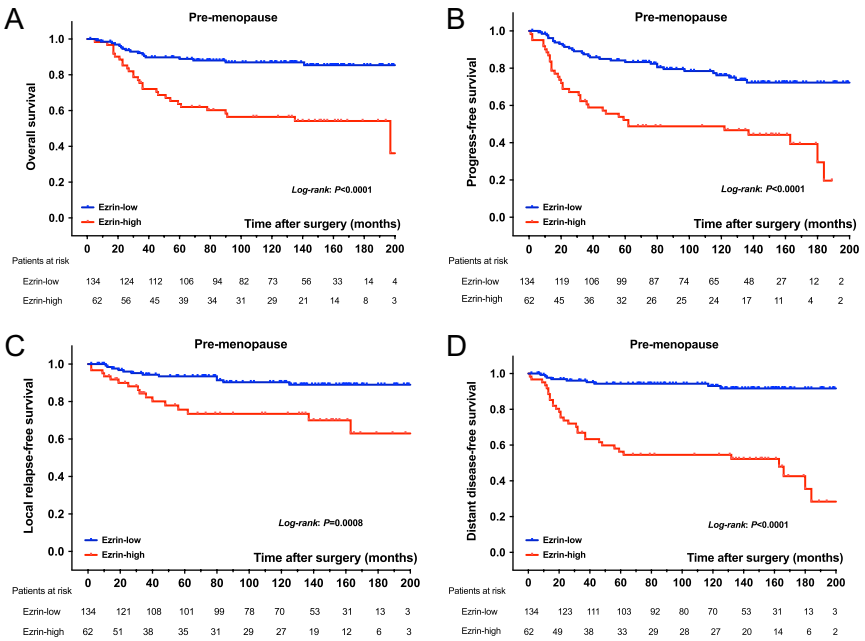

Figure 7
Kaplan–Meier curves comparing survival outcome of pre-menopause TNBC patients with high and low Ezrin expression. In the pre-menopause subgroup, patients with high Ezrin expression had a significantly lower OS (A), PFS (B), LRFS (C) and DDFS (D) rate than those with low Ezrin expression.

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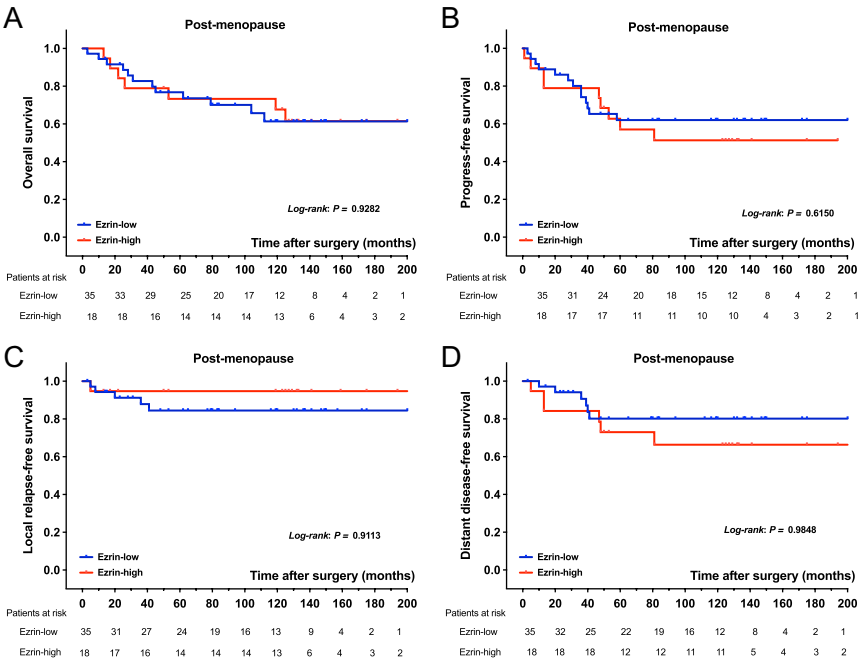

Figure 8
Kaplan–Meier curves comparing survival outcome of post-menopause TNBC patients with high and low Ezrin expression. In the post-menopause subgroup, there were no significant differences in OS (A), PFS (B), LRFS (C) and DDFS (D) between high and low Ezrin expression patients.

the prognosis of TNBC was mediated by estrogen. The mechanism may be related to an estrogen-activated GPER downstream cascade reaction, which promotes tumor cell proliferation, migration and invasion (7, 27, 28).

However, Ezrin belongs to the actin-binding protein family and participates in cell behavior by modulating cytoskeleton rearrangement to promote the formation of membrane protrusions, such as filopodia, lamellipodia (29). Ezrin could be phosphorylated more after 17β-estradiol activates GPER in ER(−) breast cancer cells, such as SK-BR-3 and MDA-MB-231, as previously described (15). In this study, the positive relationship and similar prognostic role between GPER and Ezrin was identified. We hypothesize that for those TNBC women patients who are younger and have a higher GPER expression, high levels of estrogen in the body may promote tumorigenesis by activating signaling pathways downstream of GPER including Ezrin phosphorylation. By conducting a comprehensive analysis of the odds ratio of various clinical factors in the multivariate model, we found that only the DDFS survival rate is affected by both GPER and Ezrin. Although the reason of insufficient sample size can't be excluded, it also provides evidence for GPER to play a role in distant metastasis events.

In conclusion, we propose high GPER expression is a poor maker for young TNBC patients. GPER also might

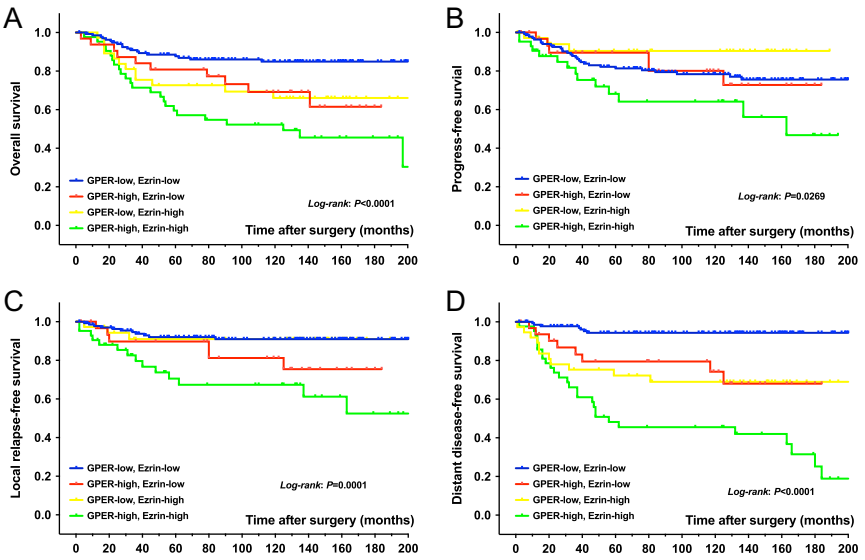

Figure 9
Kaplan–Meier curves comparing survival outcome of TNBC patients with co-expression of GPER/Ezrin. Patients with high co-expression of GPER/Ezrin had significantly lower OS (A), PFS (B), LRFS (C) and DDFS (D) rates than those with low co-expression of GPER/Ezrin group.

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Table 4 Univariate and multivariate survival analyses of clinicopathologic factors, GPER and Ezrin expression.

	Univariate model			Multivariate model			
	*P**	OR	95% CI for OR	*P***	HR	β	95% CI for HR
Any event							
Age	0.836	0.997	0.974–1.022				
Site (left vs right)	0.527	1.182	0.704–1.986				
Nuclear grade (G2 vs G3)	0.001	0.203	0.083–0.500	0.001	0.240	−1.425	0.104–0.566
Tumor size	0.088	1.146	0.980–1.340				
LNM (no vs yes)	<0.001	0.369	0.216–0.629	0.072	0.621	−0.476	0.370–1.043
TNM (I/II vs III)	<0.001	0.187	0.101–0.345	0.002	0.436	−0.831	0.257–0.740
GPER (low vs high)	<0.001	0.173	0.096–0.313	<0.001	0.393	−0.933	0.246–0.629
Ezrin (low vs high)	<0.001	0.274	0.156–0.479	0.141	0.697	−0.361	0.431–1.127
Death							
Age	0.489	1.009	0.983–1.036				
Site (left vs right)	0.634	0.871	0.493–1.538				
Nuclear grade (G2 vs G3)	0.002	0.153	0.046–0.511	0.007	0.201	−1.606	0.063–0.644
Tumor size	0.021	1.215	1.030–1.434	0.023	1.168	0.156	1.022–1.336
LNM (no vs yes)	<0.001	0.218	0.118–0.402	0.064	0.533	−0.629	0.274–1.036
TNM (I/II vs III)	<0.001	0.119	0.063–0.228	0.002	0.359	−1.023	0.190–0.679
GPER (low vs high)	<0.001	0.259	0.142–0.473	0.025	0.528	−0.639	0.301–0.924
Ezrin (low vs high)	<0.001	0.266	0.147–0.483	0.073	0.595	−0.519	0.337–1.336
Relapse event							
Age	0.197	0.977	0.943–1.012				
Site (left vs right)	0.808	1.094	0.530–2.258				
Nuclear grade (G2 vs G3)	0.049	0.230	0.053–0.994	0.050	0.239	−1.433	0.057–1.001
Tumor size	0.950	1.007	0.881–1.250				
LNM (no vs yes)	0.005	0.336	0.158–0.716	0.209	0.569	−0.564	0.236–1.372
TNM (I/II vs III)	<0.001	0.235	0.111–0.498	0.023	0.368	−0.998	0.156–0.871
GPER (low vs high)	<0.001	0.229	0.108–0.485	0.004	0.329	−1.113	0.153–0.704
Ezrin (low vs high)	0.019	0.414	0.199–0.863	0.752	0.884	−0.124	0.410–1.904
Distant event							
Age	0.301	0.985	0.957–1.014				
Site (left vs right)	0.529	0.823	0.448–1.511				
Nuclear grade (G2 vs G3)	0.010	0.201	0.060–0.676	0.005	0.184	−1.695	0.056–0.602
Tumor size	0.075	1.165	0.985–1.379				
LNM (no vs yes)	<0.001	0.310	0.165–0.585	0.070	0.525	−0.644	0.262–1.053
TNM (I/II vs III)	<0.001	0.174	0.090–0.334	0.022	0.449	−0.801	0.227–0.889
GPER (low vs high)	<0.001	0.124	0.063–0.242	0.001	0.346	−1.061	0.182–0.658
Ezrin (low vs high)	<0.001	0.108	0.054–0.214	0.001	0.320	−1.139	0.162–0.631

*Logistic regressive model; **Cox regression model.
CI, confidence interval; HR, hazard ratio; LNM, lymph node metastasis; OR, odds ratio.

provide a new pathway for the prevention and treatment of all types of breast cancer, especially in those ER(+) breast cancer patients who have had a relapse or a drug-resistant event. Further studies on every molecular type of breast cancer could better characterize patients according to the expression of GPER.

Supplementary data
This is linked to the online version of the paper at https://doi.org/10.1530/EC-19-0164.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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