


Cytoplasmic G Protein-Coupled Estrogen Receptor 1 as a Prognostic Indicator of Breast Cancer: A Meta-Analysis

Technology in Cancer Research & Treatment
Volume 21: 1-7
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DOI: 10.1177/15330338221131664
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Abstract

Purpose: To determine whether G protein-coupled estrogen receptor 1 (GPER1) is a suitable biomarker to predict the treatment outcome of breast cancer (BC). **Methods:** A meta-analysis of the literature was performed to clarify the correlation between GPER1 protein expression and BC outcome. The relationship between GPER1 mRNA expression and survival was analyzed using Breast Cancer Gene-Expression Miner (bc-GenExMiner) v4.6 software. **Results:** Six studies involving 2697 patients were included in the meta-analysis. Four studies reported the correlation between GPER1 protein expression and relapse-free survival (RFS) and 4 others reported the impact of GPER1 protein expression on overall survival (OS). The results showed that high GPER1 protein expression was not associated with RFS (hazard ratio [HR] = 1.58; 95% confidence interval [CI] = 0.71-3.48; $P = .26$) or OS (HR = 1.18; 95% CI = 0.64-2.18; $P = .60$). Subgroup analysis suggested that nuclear expression of GPER1 was not associated with OS (HR = 0.91; 95% CI = 0.77-1.08; $P = .30$), but high expression of cytoplasmic GPER1 was significantly associated with longer OS (HR = 0.69; 95% CI = 0.55-0.86; $P = .001$). Furthermore, the association of GPER1 mRNA and OS of BC patients was analyzed using bc-GenExMiner v4.6. Two data sets involving 4016 patients were included in the analysis. The targeted prognostic analysis results showed that high mRNA expression of GPER1 was predictive of better OS in BC patients (HR = 0.71; 95% CI = 0.59-0.86; $P = .0005$), which was remarkably similar to the result of cytoplasmic GPER1. Further subgroup analysis demonstrated that high mRNA expression of GPER1 was predictive of better OS in estrogen receptor (ER)-positive, but not ER-negative or triple-negative BC patients. **Conclusions:** High mRNA and cytoplasmic protein expression of GPER1 were predictive of better OS of BC patients.

Keywords

breast cancer, G protein-coupled estrogen receptor 1, meta-analysis, prognosis, estrogen receptor

Introduction

Breast cancer (BC) has surpassed lung cancer as the most common malignancy in women.¹ Recent progress in individualized precision treatment has improved the outcomes of patients with BC and other malignancies. Nonetheless, BC accounts for more than 400,000 deaths annually worldwide.² Molecular subtyping is widely used to guide and predict treatment outcomes and to reduce mortality associated with BC. BC is a heterogeneous disease that is classified into 5 molecular types based on the expression patterns of estrogen receptor (ER), progesterone receptor, human epidermal growth factor receptor 2, and nuclear protein Ki67.³ However, the prognosis of BC varies in the same molecular subgroup, suggesting that this classification system is insufficient. Thus, novel molecular biomarkers are needed to predict the prognosis of BC.

Transmembrane G protein-coupled estrogen receptor 1 (GPER1) was first identified as an ER in BC in 1997.⁴ Estrogen

promotes the onset and progression of BC. GPER1 mediates the rapid nongenomic activities of estrogen in various types of cancers.^{5, 6} Although countless studies have investigated the prognostic effect of GPER1 in BC, the results have been inconsistent. For example, Martin et al⁷ reported that GPER was predictive of better survival in BC, while Ye et al⁸ found that high expression of GPER1 was predictive of poorer survival. Therefore, the aim of the present study was to comprehensively analyze published data to clarify the relationship between GPER1 protein expression and relapse-free survival (RFS) and overall survival (OS) of BC

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patients. Furthermore, subgroup analysis was performed to determine the subcellular location of GPER1. Also, the prognostic value of GPER1 mRNA was analyzed to validate the role of GPER1 in BC.

Methods

Search Strategy and Selection Criteria

The electronic databases Embase, PubMed, Wan Fang, Chinese Biomedical Literature database, Google Scholar, Medline, Science Direct, Scopus, and China National Knowledge Infrastructure were searched for articles published up to July 2022 using the keywords “breast cancer,” “G protein-coupled estrogen receptor 1,” “GPER,” “GPER1,” “GPR30,” “survival,” and “prognosis.” The abstracts of the retrieved articles were reviewed to eliminate irrelevant studies and only those deemed relevant were included in the meta-analysis. The references of the included articles were also reviewed to identify other relevant studies. The inclusion criteria were (1) studies of female BC patients, (2) that included OS and/or RFS or a survival curve of related data to calculate the hazard ratio (HR); (3) and GPER expression assessment using immunohistochemical (IHC) analysis. The most recently published data of cases from the same institute were included.

Data Extraction

The electronic database search was conducted independently by 2 of the authors and study inclusion was determined by consensus. Before data extraction, the protocol was registered with PROSPERO (registration number: CRD42022347119). The data were extracted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (<https://prisma-statement.org/>).⁹ The following data were extracted from each study: patient number, year of publication, method used for assessment of GPER1 expression, and HR of OS or RFS.

Statistical Analysis

The HR was used for comparative analysis. If not stated in the article, the HR was calculated from the original data and survival curves were generated as described by Tierney et al.¹⁰ and Parmar et al.¹¹ Engauge Digitizer version 2.11 software (<http://sourceforge.net>) was used to analyze Kaplan–Meier curves. Cochran’s test was used to assess the heterogeneity of the studies. A probability (P) value of $< .05$ was considered statistically significant. A random-effect model was used, otherwise a fixed model was used. By convention, $HR > 1$ implied poorer survival was associated with high GPER1 expression. The data extracted from the included studies were combined using RevMan version 5.2 software (<http://www.cochrane.org>).

Results

Description of Studies

Of 453 articles initially retrieved, 41 were discarded because of duplicated data along with 337 that were case reports, review articles,

laboratory studies, or irrelevant to this study (Figure 1). Two studies classified GPER1 expression levels into more than 2 groups and did not provide data of OS or RFS.^{12, 13} One study was unavailable to extract valid data. Finally, 6 studies published from 2011 to 2020 were included for the meta-analysis.^{7, 8, 14–17} In each of the included studies, IHC analysis was performed to assess GPER1 expression based on 3 criteria: (1) nuclear or cytoplasmic staining, which was used in the majority of studies; (2) staining of both the membrane and cytoplasm, which was used only in one study⁸; and (3) separate assessment of nuclear or cytoplasmic staining, which was used in 2 studies.^{7, 15} One study was a prospective analysis of survival, while the others were retrospective. The characteristics of the eligible studies are summarized in Table 1. The cohorts varied from 48 to 782 cases, with a combined total of 2697 cases.

Impact of GPER1 Protein on RFS and OS of BC Patients

Four studies analyzed the correlation between GPER1 expression and RFS in a total of 1320 cases. However, the between-study heterogeneity was significant ($P < .0001$, $I^2 = 87\%$), thus a random-effects model was used for meta-analysis with a pooled HR of 1.58 (95% confidence interval [CI] = 0.71–3.48; $P = .26$, Figure 2a). The results demonstrated that GPER1 protein expression was not associated with RFS of BC patients. Four studies of 2326 patients analyzed the impact of GPER1 protein expression on OS of BC patients (Figure 2b). The heterogeneity was significant among the studies ($P < .00001$; $I^2 = 91\%$), thus a random-effects model was used to combine the HRs. The pooled HR was 1.18 (95% CI = 0.64–2.18; $P = 0.60$). The results suggested that GPER1 protein expression was not associated with OS of BC patients.

Impact of Nuclear and Cytoplasmic GPER Protein on OS of BC Patients

Subgroup analysis was performed based on the subcellular location of GPER1. Two studies including 1482 patients

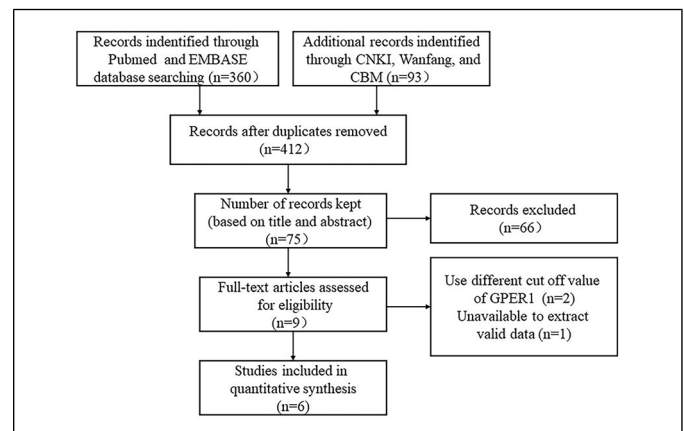
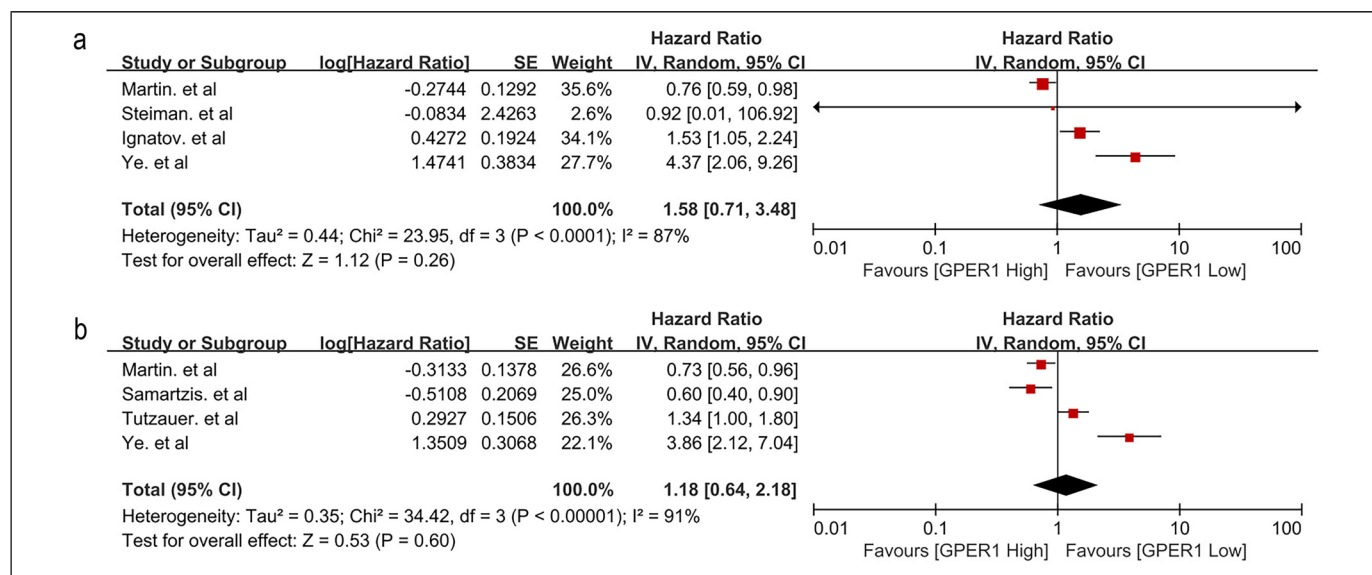


Figure 1. Flow chart of the study selection process.

Table 1. Characteristics of the Six Studies Included in the Meta-analysis of the Prognostic Value of GPER1 in BC.

Reference	Year	Patients number	Patient source	Molecular type	Antibody	Positive	HR estimation	HR (95%CI) of OS	HR (95%CI) of RFS
Ignatov. et al	2011	323	Germany	Hormone receptor positive	SP46779 (Acris)	Cytoplasm or nucleus	Given by author	No	1.53(1.05-2.24)
Steiman. et al	2013	48	USA	TNBC, Hormone receptor positive	NLS 1183 (Novus Biological)	Cytoplasm or nucleus	Survival curve	No	0.92 (0.01-106.92)
Samartzis. et al	2014	782	Switzerland	Unavailable	Ab39742 (Abcam)	Cytoplasm or nucleus	Given by author	Cytoplasm 0.60(0.40-0.9) Nucleus 0.92(0.68-1.25)	No
Martin. et al	2018	700	UK	All	PA5-28647 (Thermo Scientific)	Cytoplasm or nucleus	Cytoplasm : given by author. Nucleus: from survival curve.	Cytoplasm 0.73(0.56-0.99) Nucleus 0.91(0.74-1.12)	0.76(0.59-0.98)
Ye. et al	2019	249	China	TNBC	Ab39742 (Abcam)	Cytoplasm and membranes	Given by author	3.86(2.11-7.04)	4.367(2.06-9.26)
Tutzauer. et al	2020	595	Sweden	Hormone receptor positive	AF5534 (R&D Systems)	Total cellular staining	Given by author	1.3 (0.99-1.8)	No

Abbreviations: HR, hazard ratio; OS, overall survival; RFS, relapse-free survival; TNBC, triple-negative breast cancer.

**Figure 2.** Forest plot of HRs for total GPER1 and the RFS (a) and OS (b) of BC patients. Abbreviations: GPER1, G protein-coupled estrogen receptor 1; RFS, relapse-free survival; OS, overall survival; BC, breast cancer.

evaluated the effect of cytoplasmic expression of GPER1 on OS of BC patients. A fixed model was used to calculate the pooled HR because of the lack of heterogeneity ($P = .43$, $I^2 = 0\%$). The results demonstrated that high cytoplasmic expression of GPER1 was predictive of better OS of BC patients (HR = 0.69; 95% CI = 0.55-0.86; $P = .001$) (Figure 3a). Two studies

including 1482 patients investigated the impact of nuclear GPER1 protein expression on the OS of BC patients. No heterogeneity was detected ($P = .95$, $I^2 = 0\%$), thus a fixed model was used for analysis. The results demonstrated that nuclear expression of GPER1 had no effect on OS of BC patients (HR = 0.91; 95% CI = 0.77-1.08) (Figure 3b).

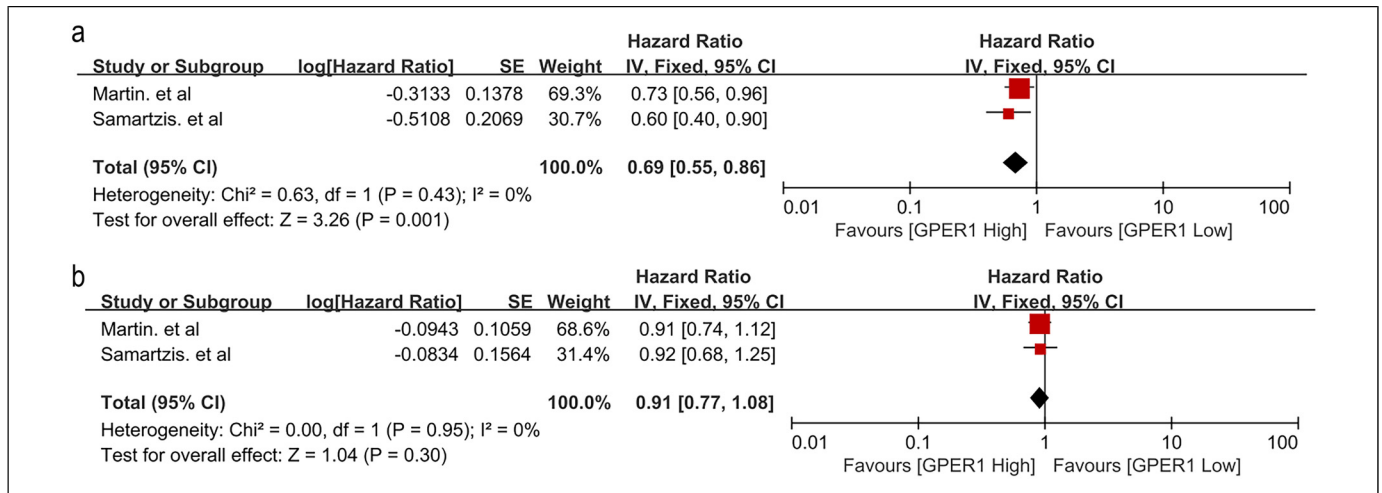


Figure 3. Forest plot of HRs for cytoplasmic (a) and nuclear (b) GPER1 and OS of BC patients. Abbreviations: GPER1, G protein-coupled estrogen receptor 1; OS, overall survival; BC, breast cancer; HR, hazard ratio.

Impact of GPER1 mRNA on the Prognosis of BC

In order to confirm whether GPER1 is a suitable prognostic biomarker in BC, the correlation between GPER1 mRNA expression and OS of BC was analyzed using the statistical mining tool Breast Cancer Gene-Expression Miner v4.6 (<http://bcgenex.ico.unicancer.fr/BC-GEM/GEM-Accueil.php?js=1>) with reference to The Cancer Genome Atlas and SCAN-B RNA-sequence data. A total of 4016 patients were included. The targeted prognostic analysis results showed that the pooled HR was 0.71 (95% CI = 0.59-0.86; $P = .0005$), which was remarkably similar to the result of cytoplasmic expression of GPER1 (Figure 4a). The results demonstrated that high expression of GPER1 mRNA was predictive of better OS of BC patients. Further subgroup analysis showed that high expression of GPER1 mRNA was associated with better OS in ER-positive BC (HR = 0.74; 95% CI = 0.59-0.93; $P = .0083$) (Figure 4b). There was a trend, but without statistical significance, toward an association between high GPER1 mRNA expression and worse OS in ER-negative BC (HR = 1.62; 95% CI = 0.98-2.68; $P = .0601$). High mRNA expression of GPER1 was not associated with OS of triple-negative BC patients (HR = 1.38; 95% CI = 0.65-2.91; $P = .3975$).

Publication Bias

Publication bias, which was assessed using Begg's and Egger's tests, was considered significant at $P < .05$. Four studies that evaluated OS of BC patients potentially had publication bias (Egger's test $P = .048$). Further sensitivity analysis was conducted using the trim and fill method, which conservatively imputes hypothetical negative unpublished studies to mirror the positive studies that cause funnel plot asymmetry. The results showed that no study was trimmed or filled. Further analysis found no association between high GPER1 expression and OS of BC patients. No other publication bias was detected.

Discussion

BC is an estrogen-related malignancy. Estrogen is the "fuel source" for the survival of hormone receptor-positive BC and also involved in the progression of other types of BC, even the triple-negative

subtype. ER α and ER β are located in the nucleus and have been widely studied in BC. GPER1 is a transmembrane ER that was first described in the 1990s. Unlike ER α and ER β , GPER1 can bind and activate various ligands. Reported agonists of GPER1 include tamoxifen,¹⁸ fulvestrant,¹⁹ vitamins,²⁰ and some environmental contaminants.²¹ Although GPER1 is involved in several signaling pathways associated with BC, the usefulness of GPER1 as a prognostic biomarker remains unclear.

The formation of the estrogen/GPER1 complex has been found to activate the mitogen-activated protein kinase/erine/threonine protein kinase-tripartite motif containing 2 signaling axis, the phosphoinositide 3-kinase/protein kinase B signaling pathway, and other signaling cascades associated with cell survival, which enhance the proliferation and migration of BC cells.²²⁻²⁵ Tamoxifen is an agonist of GPER1 and GPER1 is reportedly associated with tamoxifen resistance.²⁶ Ignatov et al¹⁴ reported that GPER1 expression is predictive of shorter RFS. On the other hand, some studies have suggested that GPER1 suppresses the proliferation of BC cells^{27, 28} and that high expression of GPER1 was predictive of better OS of BC patients.⁷ The results of the present meta-analysis of 6 studies revealed that high expression of GPER1 was not associated with PFS or OS of BC patients, which may be associated with the subcellular location of GPER1.

GPER1 activation is reportedly associated with the rapid effects (nongenomic) of estrogen. Therefore, GPER1 is presumed to be located on the plasma membrane.²⁹⁻³¹ Indeed, several studies detected GPER1 in the cellular membrane and reported that GPER1 located in the plasma membrane is critical to the progression of BC and associated with poorer disease-

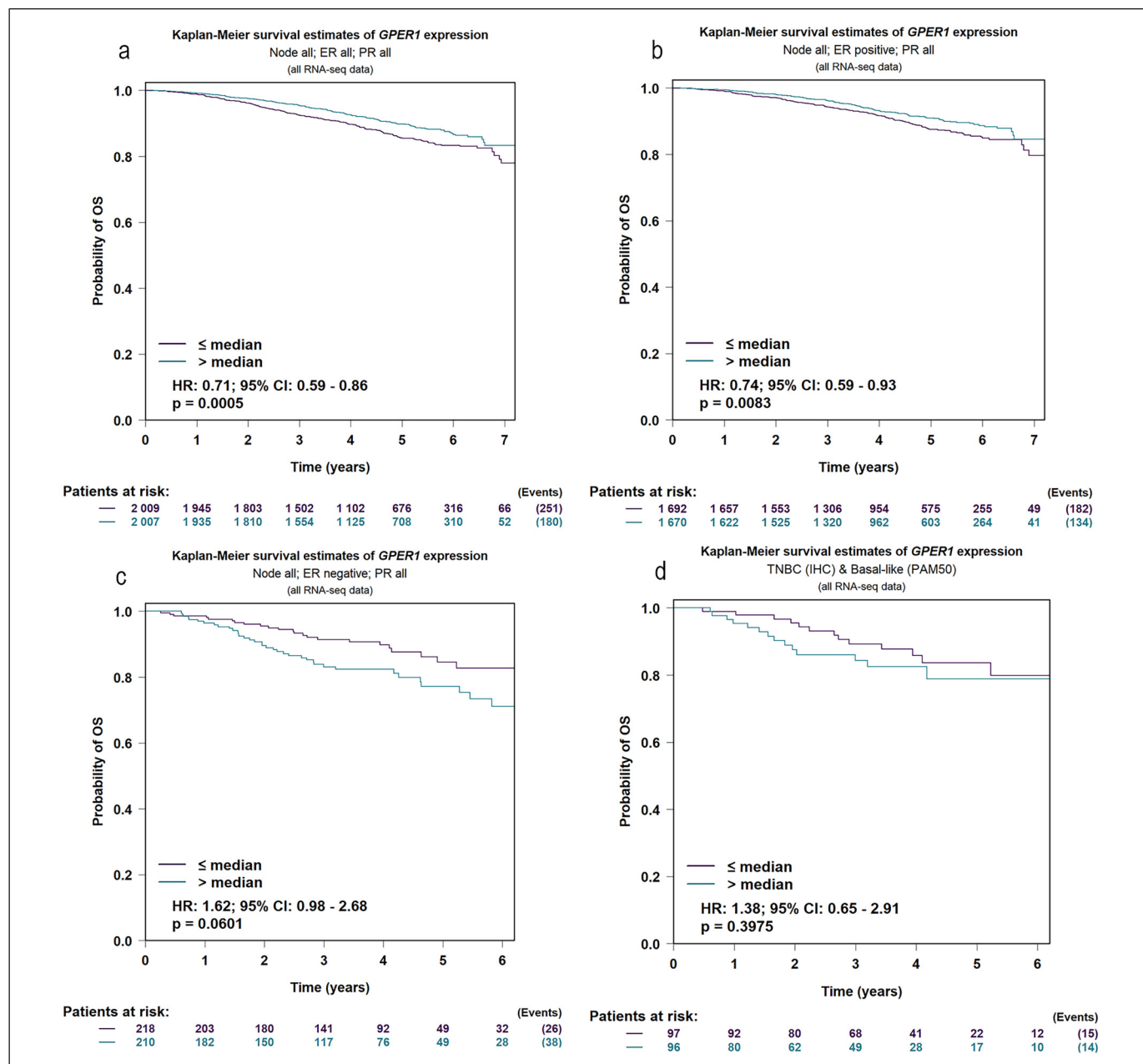


Figure 4. GPER1 mRNA expression and OS of BC patients (a), ER-positive BC (b), ER-negative BC (c), and triple-negative BC (d). Abbreviations: GPER1, G protein-coupled estrogen receptor 1; OS, overall survival; BC, breast cancer; ER, estrogen receptor.

free survival.^{13, 17} However, the expression pattern and subcellular localization of GPER remains controversial. Revankar et al¹⁸ assessed the intracellular and extracellular binding properties of GPER using novel fluorescent estrogen derivatives (E2-Alexas) in monkey kidney fibroblasts (COS-7 cells). Interestingly, confocal microscopy showed that E2-Alexas were predominantly bound to the endoplasmic reticulum rather than the plasma membrane and could also be transported from the plasma membrane to the nucleus.³² Meanwhile, another study reported that the localization of GPER1, as determined by IHC staining, was dependent on the specificity of the antibody and the cell line (predominantly located in the nucleus

of MCF7 cells and the cytoplasm of T47D cells).¹⁵ The distribution of GPER1 varies among cell types and is predominately expressed intracellularly in BC cells with minor amounts on the cell surface.^{18, 33} GPER1 is an atypical G protein-coupled receptor. The location of GPER1 may dynamically change in response to environmental cues. Intracellularly, activated GPER1 is transferred to cell membranes and initiates cellular signaling.^{34, 35} The results of further subgroup analysis demonstrated that cytoplasmic, but not nuclear, GPER1 was predictive of better survival of BC patients. GPER1 mRNA was also associated with better OS of BC patients. Notably, the HR value of cytoplasmic GPER1 protein was similar to that of GPER1

mRNA. The results demonstrated that cytoplasmic, but not nuclear, expression of GPER may reflect the actual biological features of GPER1 in BC. It is reported that GPER1 and ER are functionally related. GPER1 functions as a tumor suppressor and promotes apoptosis and antiproliferation signaling in ER-positive BC cells.^{12, 36} However, GPER1 expression is reportedly correlated with pro-metastasis pathways and promotes migration and invasion of ER-negative BC cells.³⁷ Several studies have noted a different prognostic effect of GPER1 in ER-positive versus ER-negative BC.^{8, 12} Hence, subgroup analysis of the gene expression data was performed to explore the prognostic effect of GPER1 based on ER status. The results showed that high GPER1 mRNA expression was associated with better OS in ER-positive BC patients but not the outcomes of patients with ER-negative or triple-negative BC.

There were several limitations to this study that should be considered when interpreting these results. First, the number of included studies was relatively small. Only 2 studies were included for subgroup analysis of the usefulness of nuclear and cytoplasmic GPER as biomarkers. Hence, more studies with large numbers of patients are needed to reach more reliable conclusions. Second, subgroup analysis to explore the prognostic effect of GPER1 protein in different molecular BC subtypes was not possible because relatively few studies offered concrete data. Third, some studies did not provide HRs. Thus, the HRs were obtained from Kaplan–Meier curves using Engauge Digitizer version 2.11 software. However, the calculated HRs might be less reliable owing to inaccuracies of censored data. Finally, the sources of the primary antibodies against GPER1 differed among the included studies, which may have caused heterogeneity among the studies.

Conclusion

High mRNA and cytoplasmic protein expression of GPER1 are predictive of better OS of BC patients. These results support the use of GPER1 to predict the outcomes of BC patients. Further large-scale prospective cohort studies are needed to confirm the association between GPER1 expression and outcomes of patients with BC.


Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Liaoning Province Science and Technology Project (Applied Basic Research Program) (Grant No. the Joint Program to Shunchao Yan in 2022), the 345 Talent Project of Shengjing Hospital of China Medical University (Grant No. M0313), Shenyang Science and Technology Bureau (Grant No. 21-173-9-69).

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References

- Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71(3):209-249.
- Zhang S, Sun K, Zheng R, et al. Cancer incidence and mortality in China, 2015. *J National Cancer Center.* 2021;1(1):2-11.
- Tsang J, Tse GM. Molecular classification of breast cancer. *Adv Anat Pathol.* 2020;27(1):27-35.
- Carmeci C, Thompson DA, Ring HZ, Francke U, Weigel RJ. Identification of a gene (GPR30) with homology to the G-protein-coupled receptor superfamily associated with estrogen receptor expression in breast cancer. *Genomics.* 1997;45(3):607-617.
- Treeck O, Schüler-Toprak S, Ortmann O. Estrogen actions in triple-negative breast cancer. *Cells.* 2020;9(11):2358.
- Jung J. Role of G protein-coupled estrogen receptor in cancer progression. *Toxicol Res.* 2019;35(3):209-214.
- Martin SG, Lebot MN, Sukkam B, et al. Low expression of G protein-coupled oestrogen receptor 1 (GPER) is associated with adverse survival of breast cancer patients. *Oncotarget.* 2018;9(40):25946.
- Ye S, Xu Y, Li J, Zheng S, Sun P, Wang T. Prognostic role of GPER/ezrin in triple-negative breast cancer is associated with menopausal status. *Endocr Connect.* Jun. 1 2019;8(6):661-671.
- Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *Syst Rev.* 2021;10(1):1-11.
- Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials.* 2007;8(1):16.
- Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med.* 1998;17(24):2815-2834.
- Broselid S, Cheng B, Sjöström M, et al. G protein-coupled estrogen receptor is apoptotic and correlates with increased distant disease-free survival of estrogen receptor-positive breast cancer patients. *Clin Cancer Res.* 2013;19(7):1681-1692.
- Sjöström M, Hartman L, Grabau D, et al. Lack of G protein-coupled estrogen receptor (GPER) in the plasma membrane is associated with excellent long-term prognosis in breast cancer. *Breast Cancer Res Treat.* 2014;145(1):61-71.
- Ignatov A, Ignatov T, Weißenborn C, et al. G-protein-coupled estrogen receptor GPR30 and tamoxifen resistance in breast cancer. *Breast Cancer Res Treat.* 2011;128(2):457-466.
- Samartzis EP, Noske A, Meisel A, Varga Z, Fink D, Imesch P. The G protein-coupled estrogen receptor (GPER) is expressed in two different subcellular localizations reflecting distinct tumor properties in breast cancer. *PLoS one.* 2014;9(1):e83296.
- Steiman J, Peralta EA, Louis S, Kamel O. Biology of the estrogen receptor, GPR30, in triple negative breast cancer. *Am J Surg.* 2013;206(5):698-703.
- Tutzauer J, Sjöström M, Bendahl P-O, et al. Plasma membrane expression of G protein-coupled estrogen receptor (GPER)/G protein-coupled receptor 30 (GPR30) is associated with worse outcome in metachronous contralateral breast cancer. *PLoS one.* 2020;15(4):e0231786.
- Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science.* 2005;307(5715):1625-1630.

19. Thomas P, Pang Y, Filardo EJ, Dong J. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology*. 2005;146(2):624-632.
20. Santolla MF, De Francesco EM, Lappano R, Rosano C, Abonante S, Maggiolini M. Niacin activates the G protein estrogen receptor (GPER)-mediated signalling. *Cell Signal*. 2014;26(7):1466-1475.
21. Thomas P, Dong J. Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: A potential novel mechanism of endocrine disruption. *J Steroid Biochem Mol Biol*. 2006;102(1-5):175-179.
22. Yang W, Tan W, Zheng J, Zhang B, Li H, Li X. MEHP Promotes the proliferation of cervical cancer via GPER mediated activation of Akt. *Eur J Pharmacol*. 2018;824:11-16.
23. Yin H, Zhu Q, Liu M, et al. GPER Promotes tamoxifen-resistance in ER+ breast cancer cells by reduced Bim proteins through MAPK/Erk-TRIM2 signaling axis. *Int J Oncol*. 2017;51(4):1191-1198.
24. Yang K, Yao Y. Mechanism of GPER promoting proliferation, migration and invasion of triple-negative breast cancer cells through CAF. *Am J Transl Res*. 2019;11(9):5858.
25. Yu T, Liu M, Luo H, et al. GPER Mediates enhanced cell viability and motility via non-genomic signaling induced by 17 β -estradiol in triple-negative breast cancer cells. *J Steroid Biochem Mol Biol*. 2014;143:392-403.
26. Ignatov T, Treeck O, Kalinski T, Ortmann O, Ignatov A. GPER-1 expression is associated with a decreased response rate to primary tamoxifen therapy of breast cancer patients. *Arch Gynecol Obstet*. 2020;301(2):565-571.
27. Huang R, Li J, Pan F, Zhang B, Yao Y. The activation of GPER inhibits cells proliferation, invasion and EMT of triple-negative breast cancer via CD151/miR-199a-3p bio-axis. *Am J Transl Res*. 2020;12(1):32.
28. Zhang N, Sun P, Xu Y, et al. The GPER1/SPOP axis mediates ubiquitination-dependent degradation of ER α to inhibit the growth of breast cancer induced by oestrogen. *Cancer Lett*. 2021;498:54-69.
29. MizukaMi Y. In vivo functions of GPR30/GPER-1, a membrane receptor for estrogen: From discovery to functions in vivo. *Endocr J*. 2010;57(2):101-107.
30. Molina L, Figueroa CD, Bhoola KD, Ehrenfeld P. GPER-1/GPR30 a novel estrogen receptor sited in the cell membrane: Therapeutic coupling to breast cancer. *Expert Opin Ther Targets*. Aug 2017;21(8):755-766.
31. Tominna R, Chokr S, Feri M, Chuon T, Sinchak K. Plasma membrane G protein-coupled estrogen receptor 1 (GPER) mediates rapid estradiol facilitation of sexual receptivity through the orphanin-FQ-ORL-1 system in estradiol primed female rats. *Horm Behav*. 2019;112:89-99.
32. Cheng S-B, Graeber CT, Quinn JA, Filardo EJ. Retrograde transport of the transmembrane estrogen receptor, G-protein-coupled-receptor-30 (GPR30/GPER) from the plasma membrane towards the nucleus. *Steroids*. 2011;76(9):892-896.
33. Filardo E, Quinn J, Pang Y, et al. Activation of the novel estrogen receptor G protein-coupled receptor 30 (GPR30) at the plasma membrane. *Endocrinology*. 2007;148(7):3236-3245.
34. Revankar CM, Mitchell HD, Field AS, et al. Synthetic estrogen derivatives demonstrate the functionality of intracellular GPR30. *ACS Chem Biol*. 2007;2(8):536-544.
35. Cheng S-B, Quinn JA, Graeber CT, Filardo EJ. Down-modulation of the G-protein-coupled estrogen receptor, GPER, from the cell surface occurs via a trans-Golgi-proteasome pathway. *J Biol Chem*. 2011;286(25):22441-22455.
36. Weißenborn C, Ignatov T, Poehlmann A, et al. GPER Functions as a tumor suppressor in MCF-7 and SK-BR-3 breast cancer cells. *J Cancer Res Clin Oncol*. 2014;140(4):663-671.
37. Talia M, De Francesco EM, Rigracciolo DC, et al. The G protein-coupled estrogen receptor (GPER) expression correlates with pro-metastatic pathways in ER-negative breast cancer: A bioinformatics analysis. *Cells*. 2020;9(3):622.