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ORIGINAL RESEARCH

PER1 Serves as a Tumor Suppressor in Breast Cancer by Regulating MEK5/ERK5 Signaling Pathway

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Background: Breast cancer (BC) is one of the most frequently diagnosed malignant tumors worldwide. Period circadian protein homolog 1 (PER1) is a primary component of the biorhythm molecular oscillation system. The objective of this study was to elucidate the association between PER1 and clinical BC outcomes and determine the potential effect of PER1 on BC tumor development.

Methods: Immunohistochemical staining for PER1 was performed on 30 normal breast tissue and 172 BC samples. Those BC cases were categorized into two groups to analyze the prognostic significance of PER1 expression. The expression of key proteins in the MEK/ERK pathway (ERK1/2, ERK5, P38, JNK1/2/3) and their phosphorylation levels (p-ERK1/2, p-ERK5, p-P38, and p-JNK1/2/3) were elucidated by western blot test. XMD17-109, a specific ERK5 inhibitor, was used to treat BT-549 and MCF-7 BC cells with knockdown of PER1.

Results: Increased PER1 expression was identified in 26 and 80 normal breast and BC tissues, respectively, whereas low expression was detected in 4 normal and 92 BC tissues. Although no differences were observed in the estrogen receptor (ER), menstrual cycle, TNM, progesterone receptor (PR), and HER-2 stages, age, and tumor size between the two cohorts, both the rate of axillary lymph node metastasis ($P<0.05$) and vascular tumor thrombosis ($P<0.05$) were enhanced in the low cohort. Furthermore, the low-PER1 group had the worst overall survival (HR: 0.44, 95% CI: 0.20–0.96, $P=0.035$) and relapse-free survival (HR: 0.29, 95% CI: 0.13–0.67, $P=0.002$). PER1 overexpression reduced phosphorylation levels of ERK5 in Lenti-blast-PER1-MDA-MB-231 BC cells ($P<0.05$), while PER1 silencing had the opposite effect on the pGenesil-1-PER1-MCF-7 cells ($P<0.05$). Colony formation, 5-ethynyl-2′-deoxyuridine, and Transwell cell migration and invasion assays revealed that XMD17-109 antagonized the enhancement of cell proliferate, migration, and invasion by PER1 knockdown ($P<0.05$).

Conclusion: PER1 plays an anti-tumor role by regulating the MEK5/ERK5 pathway in BC.

Keywords: breast cancer, circadian clock gene, PER1, MEK5/ERK5, tumor suppressor

Background

The International Agency for Research on Cancer published the report "The 2022 Global Cancer Statistics", which indicated that female breast cancer (BC) was the most frequently diagnosed malignant tumor, accounting for 2,308,897 new cases, and 23.8% of all new cancer cases were reported in 2022.[1] In the past few decades, overall BC is survival has increased remarkably, not only because of early detection programs but also because of improved treatment strategies using molecular and genomic diagnostic techniques.[2] However, in 2022, the mortality rate due to BC was as high as 665,684.[1] Patients diagnosed with advanced BC usually have a high relapse rate, low overall survival (OS), and substandard prognosis.[3] Early diagnosis and therapy are the two most critical factors for better prognosis in patients with BC. The molecular mechanism of BC is a multistep procedure involving oncogene activation, inactivation of tumor-suppressing genes, and defects in DNA mismatch repair genes and other tumor-related genes, such as apoptosis,

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proteolysis, cell adhesion, and angiogenesis-related genes.[4,5] Therefore, elucidating the molecular process and novel and efficient bioindices is necessary for the early diagnosis, prognosis, and individualized therapy of patients with BC.[6]

Multiple studies have indicated that disruption of circadian rhythm is markedly linked to the growth, metastasis, and transformation of malignant tumors.[7] As part of the biological molecular oscillation system, period circadian protein homolog 1 (PER1) essentially regulates the body's circadian rhythm and cell cycle, and promotes DNA damage repair.[8–10] Literature suggests that PER1 is aberrantly expressed in multiple tumors and decreased expression of PER1 is positively correlated with the development of cancer transmission.[11,12] PER1 expression is lower in the tissues of various ovarian cancer subtypes and leading to a reduced OS rate.[8] In vivo and in vitro research on gastric cancer has suggested that PER1 silencing disrupts the PER1-HK2 circadian rhythm and reverses trastuzumab resistance.[13] Our previous study found that low PER1 expression in BC tissue enhanced BC cell proliferation, migration, and invasion.[6] However, the association of PER1 with the clinical outcome of BC and its specific mechanism in BC remains unclear.

The mitogen-activated protein kinase (MAPK) pathway is one of the most well understood pathways in cancer biology. Hyperactivation is associated with over 40% of human cancers, including BC.[14–16] Research has shown that the MAPK signaling pathway is positively correlated with circadian rhythm.[17] A study not only revealed the down-regulation of PER1 mRNA levels in stomach adenocarcinoma tissues but also indicated that core circadian clock genes could promote or inhibit certain cancer-linked hallmark pathways, such as apoptosis, DNA damage response, cell cycle, and RAS/MAPK pathways.[18] These findings suggest an inseparable relationship between the PER1 and MAPK pathways.

In this study, the effect of PER1 on clinical outcomes and its potential mechanism of action in BC were elucidated. PER1 expression was detected in 172 BC samples, which were categorized into two groups: high PER1 expression (high-PER1) and low PER1 expression (low-PER1). Next, we assessed the association between PER1, clinicopathological manifestations, and BC patient prognosis. Finally, a series of cell function experiments was performed to elucidate whether PER1 inhibits BC via the MAPK pathway. The results of this study will provide new insights into the pathogenesis and therapeutic targets of BC.

Methods

Patients and Specimens

Not only the ethical board of the Fourth Hospital of Hebei Medical University (No. 2020KY113) but also he ethical board of the First Hospital of Qinhuangdao (No.2019H044) authorized this investigation, and all protocols were conducted in accordance with the Declaration of Helsinki. A total of 172 primary tumor specimens were obtained from women diagnosed with BC who underwent complete resection. The specimens were provided by the Fourth Hospital of Hebei Medical University from December 2010 to November 2013. Thirty normal breast tissue specimens were obtained from the 172 patients. Patients with i) histologically confirmed invasive ductal carcinoma, ii) a complete clinical profile, iii) no other malignant tumor, and iv) no prior tumor treatment such as radiotherapy, chemotherapy, or immunotherapy before surgical resection were included. The oldest and youngest ages were 75 and 25 years, respectively (mean age, 45 years). Formalin-fixed and paraffin-embedded (FFPE) specimens were stained with hematoxylin and eosin (H&E) to elucidate histopathological properties, grading, and TNM staging. Clinicopathological profiles of the patients were acquired from their medical history (Table 1).

Postoperative early adjuvant systemic therapies (chemotherapy, radiotherapy, and hormonal therapy) were administered to 77.3%, 40.6%, and 43.5% of participants, respectively. The participants were followed up at 6-12-month intervals until mortality or at the end of the follow-up period (February 2021). The longest and shortest follow-up time was 124.5 and 11.8 months, respectively, with a mean follow-up time of 111.6 months. The overall survival (OS), was defined as the interval from the end of surgery to the last follow-up date or death, and recurrence-free survival (RFS) here defined as the interval from the end of the surgery's last follow-up date or disease recurrence or metastasis. Both OS and RFS were used as prognostic measures (Table 1).

Table 1 Clinical Manifestations and Prognosis of 172 BC Patient Samples

Group	N	Rate (%)
Age (years)		
≤60	141	81.98
>60	31	18.02
Menopause status		
Pre- Menopause	95	55.23
Post- Menopause	77	44.77
Tumor size (cm)		
≤2	89	51.74
>2	83	48.26
Lymph node metastasis		
Negative	92	53.49
Positive	80	46.51
TNM stage		
I	52	30.23
II	80	46.51
III	40	23.26
TNBC		
Yes	51	29.65
No	121	70.35
HER-2 status		
Negative	142	82.56
Positive	30	17.44
Vascular tumor thrombus		
Negative	109	63.37
Positive	63	36.63
Estrogen receptor		
Negative	78	45.35
Positive	94	54.65
Progesterone receptor		
Negative	88	51.16
Positive	84	48.84
Survival status		
Dead	31	18.02
Alive	124	72.09
Lost	17	9.88
Recurrence and metastasis		
Yes	33	19.18
No	122	70.93
Lost	17	9.88

Immunohistochemistry (IHC)

The FFPE blocks of BC (172) and normal breast (30) tissues were sliced into 4-μm-thick sections, which were de-paraffinized in xylene, rehydrated with gradient alcohol, boiled for 3 min in 0.01 M citrate buffer (pH 6.0) for antigen retrieval, incubated with 3% hydrogen peroxide for 10 min at ambient temperature (AT) to inhibit the action of endogenous peroxidase, and 10% goat serum (Beijing Solarbio Science & Technology Co., Ltd.) for 40 min at AT to prevent nonspecific binding. The sections were then incubated overnight in primary anti-PER1 antibody (OriGene Technologies, Inc.) at 1:100 dilution at 4 °C, warmed for 1 h at AT, rinsed three times with PBS, and incubated in undiluted secondary antibody goat anti-rabbit IgG-HRP (OriGene Technologies, Inc.) at AT for 1 h. For the negative

control, the primary antibody was replaced with PBS. Finally, the specimens were stained with DAB and observed under a light microscope (Leica Microsystems GmbH, Wetzlar, Germany).

The slides were examined by two independent investigators who were blinded to the patients' clinical data. Five random fields from each section were observed under x400 magnification using an optical microscope. Based on the percentage of positive tumor cells and staining intensity, the PER1 expression was scored. The percentage of positive cells was scored as follows: 0 (≤5%), 1 (6–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). The staining intensity was graded as follows: 0 (no staining); 1 (light yellow); 2 (brownish); and 3 (tan). The final score was calculated by multiplying the proportion and the intensity scores. Total score ≥ 4 = high expression and ≤4 = low expression, referring to a previous report.[12]

Cell Culture and Treatment

Three human BC cell lines (including MCF-7 MDA-MB-231 and BT-549) were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). BC cell lines with stable low/high PER1 expression levels (pGenesil-1-PER1-MCF-7 and pGenesil-1-PER1-BT-549/pcDNA3.1-PER1-MCF-7 and Lenti-Blast-PER1-MDA-MB-231) and their control cell lines (pGenesil-1-MCF-7, pGenesil-1-BT-549, pcDNA3.1 -MCF-7 and Lenti-Blast-MDA-MB-231) were constructed for this study. The specific method for constructing stable cell lines can be found in our previously published paper.[6] All cells were propagated at 37°C with 5% CO2 in DMEM (Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS) (GIBCO, USA), 1% penicillin (100 U/mL), streptomycin (0.1 mg/mL) (GIBCO, USA), and screening drugs (200 μg/mL G418 or 10 ug/mL Blasticidin, MCE, USA). 1 μM XMD17-109 (MCE, USA), a specific ERK5 inhibitor, was used to stimulate pGenesil-1-PER1-MCF-7 and pGenesil-1-PER1-BT-549 cells for 48 h, and an equal volume of DMSO (Solarbio, Beijing) was added to the control groups.

Western Blotting

Radioimmunoprecipitation assay buffer with protease, PMSF, and phosphatase inhibitor was used to isolate protein samples, which were quantified using a BCA Protein Assay Kit (Solarbio, Beijing) according to the manufacturer's protocol, separated by electrophoresis in a 10% SDS-PAGE gel, and transferred onto polyvinylidene difluoride (PVDF) membranes before blocking with 1% BSA. The membranes were then incubated with primary PER1 antibodies (1:1000, GTX128974, GENETEX, USA) and β-actin (1:10,000, AC026, Abclonnal, China) at 4°C overnight, followed by incubation with secondary antibodies (1:5000, SA00001-2, Proteintech) for 2h at AT. The protein bands were washed thrice with Tris-Buffered Saline and Tween 20 (TBST) and detected using enhanced chemiluminescence (ECL).

Colony Formation Assay

Log-phase BC cells (700/well) were propagated in 6-well plates for 14 days. The cloned cells were fixed and stained using methanol and 0.1% crystal violet. The cells were counted under a light microscope (Olympus, Japan), and the colony formation rate was measured as the ratio of colonies to inoculated cells.

5-Ethynyl-2′-Deoxyuridine (EdU) Assay

The assay was performed according to the protocol. The percentage of EdU-positive cells with red fluorescence was measured using a fluorescence microscope (Olympus).

Transwell Cell Migration and Invasion Assays

Transwell chambers were used to elucidate the ability of different groups of cells to migrate and invade. Briefly, cells were suspended in serum-free media and propagated (5×10^4/well) in upper transwell chambers. The lower Transwell chambers were filled with 600 μL media augmented with 10% FBS. Transwell invasion and migration assays were performed in a similar manner, except that the upper chambers were laminated with Matrigel (30 μg) (Corning, USA). The cells which infiltrated the bottom surface were fixed via the 4% formaldehyde, then after 24 h were stained with 0.1% crystal violet, and counted from 5 different fields via the laser confocal microscope.

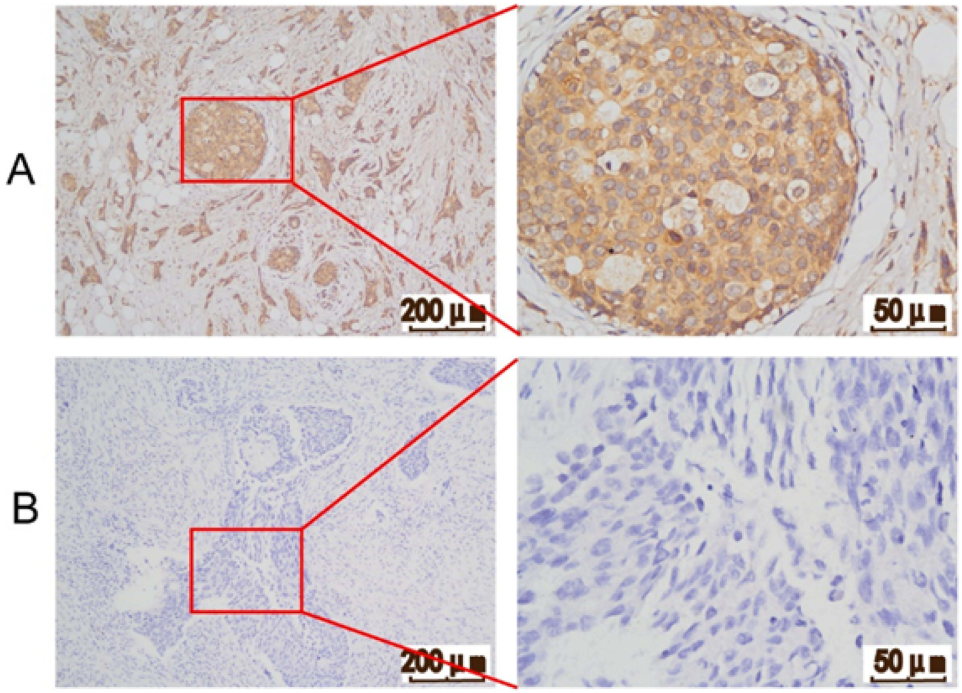

Figure 1 Representative pictures of positive and negative expression of PER1 protein in BC tissues (100× and 400×). (**A**) Positive expression. (**B**) Negative expression.

Statistical Measurement

Statistical analysis was performed using SPSS (22.0) software (SPSS, Chicago, IL, USA). Categorical nominal variables were tested using χ2 or Fisher's exact tests. The Kaplan-Meier (KM) method was used to acquire survival curves, which were then compared using the Log rank test. Multiple group and intergroup comparisons were assessed using single-factor variance analysis and LSD test, respectively. $P < 0.05$.

Results

PER1 Expression in BC and Normal Breast Tissues

To detect PER1 expression in 172 BC and 30 normal breast tissues, immunohistochemistry (IHC) was performed, which revealed that PER1 was primarily located in the cytoplasm, with a small amount in the nucleus (Figure 1A). High PER1 expression was detected in 26 normal and 80 BC tissues, whereas it was low in 4 normal and 92 cancer tissue samples (Figure 1B). Consistent with our previous study, the probability of low PER1 expression in BC tissues was notably lower than that in normal breast tissues ($P<0.05$; Table 2).

Association of PER1 with the Breast Cancer Patient's Clinicopathological Manifestations

Table 3 summarizes the association between PER1 and clinicopathological features of patients with BC. As indicated by statistical analysis, BC patients with low PER1 expression had an increased rate of axillary lymph node metastasis

Table 2 Expression of PER1 in BC and Normal Breast Tissues

Group	N	PER1 Protein Expression			χ^2	*P*
		High	Low	Low Rate (%)		
Cancer	172	80	92	53.49	16.516	0.00
Normal	30	26	4	13.33		

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Table 3 The Relationship Between PER1 and Clinicopathological Parameters of BC Patient Samples

Group	N	PER1 Protein Expression			χ^2	*P*
		High	Low	Low Rate (%)		
Age (years)						
≤60	141	69	72	51.06	1.85	0.17
>60	31	11	20	64.51		
Menopause status						
Pre- Menopause	95	44	51	53.68	0.00	0.95
Post- Menopause	77	36	41	50.23		
Tumor size (cm)						
≤2	89	47	42	47.19	2.94	0.09
>2	83	33	50	60.24		
Lymph node metastasis						
Negative	92	50	42	45.65	4.88	0.03
Positive	80	30	50	62.50		
TNM stage						
I	52	26	26	50.00	0.97	0.62
II	80	38	42	52.50		
III	40	16	24	60.00		
TNBC						
Yes	51	21	30	58.82	0.83	0.36
No	121	59	62	51.24		
HER-2 status						
Negative	142	63	79	55.63	1.51	0.22
Positive	30	17	13	43.33		
Vascular tumor thrombus						
Negative	109	62	47	43.12	12.86	0.00
Positive	63	18	45	71.43		
Estrogen receptor						
Negative	78	37	41	52.56	0.05	0.83
Positive	94	43	51	54.25		
Progesterone receptor						
Negative	88	41	47	53.40	0.00	0.98
Positive	84	39	45	53.57		

(P<0.05) and vascular tumor thrombus (P<0.05); however, there was no substantial association between PER1 expression and other clinical parameters, including progesterone receptor (PR) status, menstrual status, age, TNM stage, tumor size, estrogen receptor (ER) status, and human epidermal growth factor receptor 2 (HER2) status.

Association of PER1 Expression with Breast Cancer Patient's Prognosis

With the help of KM survival analysis was used to evaluate the relationship between PER1 and BC patient's patients was evaluated, and it was indicated that both OS and RFS differed notably between individuals with low and high PER1 expression (P<0.05, Figure 2A). According to the ten-year follow-up data, the RFS and OS probabilities were 68.01% and 74.02% in the low PER1 expression group and 90.89% and 88.34% in high PER1 expression group, respectively (P<0.05). Furthermore, to assess the prognostic importance of PER1 in patient subgroups, stratification according to lymph node and vascular status was performed. KM analysis indicated that low PER1 expression patients had worse prognosis when lymph node metastasis was considered (P<0.05, Figure 2B). The RFS probability of the patients with low and high PER1 expression were 45.15% and 88.53%, respectively (P<0.05). Although no statistical variability in OS

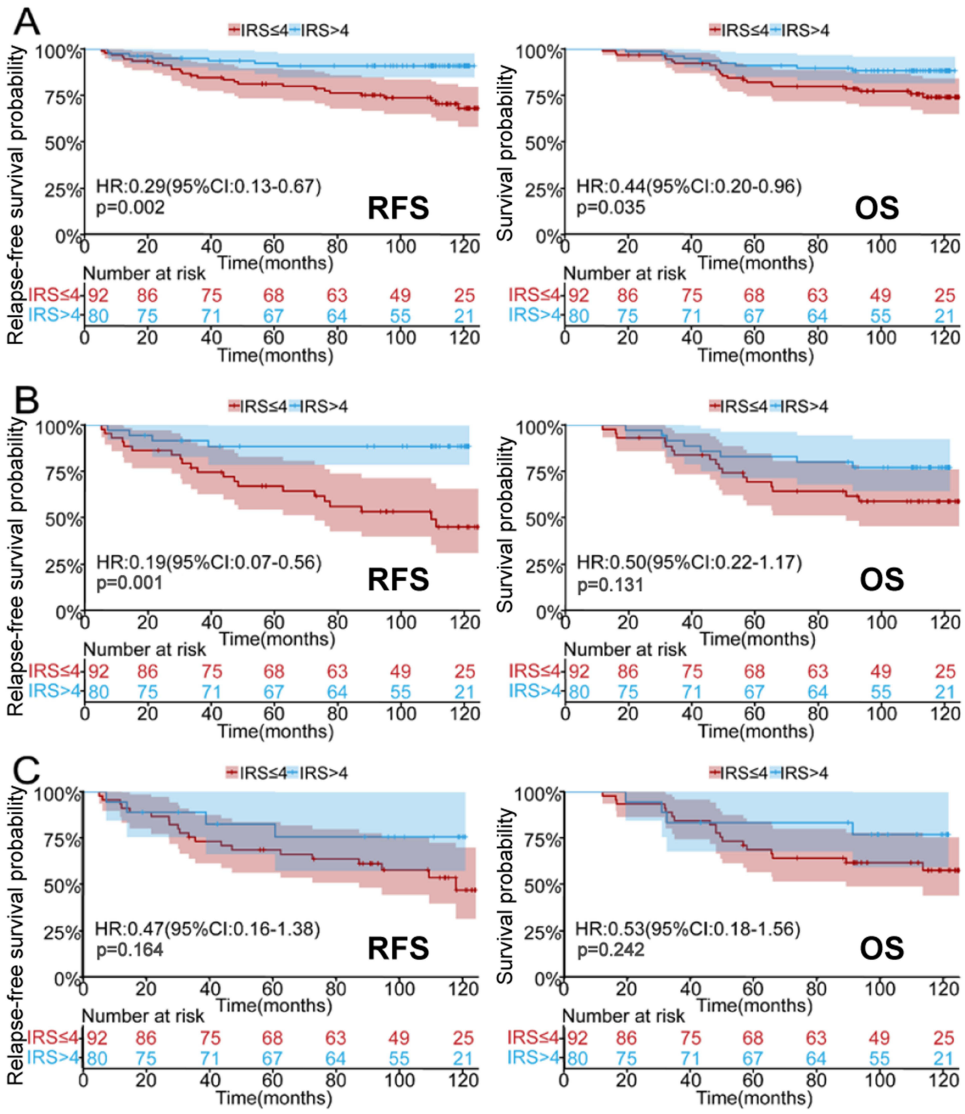

Figure 2 PER1 affects BC patient's prognosis. (**A**) RFS and OS of 172 BC patients based on the ten-year follow-up data. (**B**) RFS and OS in lymph node metastasis subgroup. (**C**) RFS and OS in vascular tumor thrombus subgroup.

was observed between the two groups, patients with low PER1 expression had a lower survival probability in cases of lymph node metastasis (Figure 2B). Moreover, patients with low PER1 expression had an increased recurrence risk and lower survival probability in the vascular tumor thrombus subgroup, although these results were not statistically significant (Figure 2C).

PER1 Suppressing Causes Abnormal Activation of the MEK5/ERK5 Signaling Pathway in BC Cells

GSEA analysis of TCGA dataset showed that MAPK signal transduction pathway related genes were significantly enriched in breast cancer patients with low expression of PER1. The MAPK signaling pathway contains a total of 267 genes, and 93 genes were matched, $P<0.05$ (RDR q-value= 0.009667159). To clarify the association between PER1 and the MEK/ERK pathway, the expression of key proteins in the MEK/ERK pathway, such as ERK1/2, ERK5, P38, and JNK1/2/3, and their phosphorylation levels (p-ERK1/2, p-ERK5, p-P38, and p-JNK1/2/3) were determined using Western blotting. Although the expression of ERK1/2, JNK1/2/3, P38, ERK5, p-P38, p-ERK1/2, and p-JNK1/2/3

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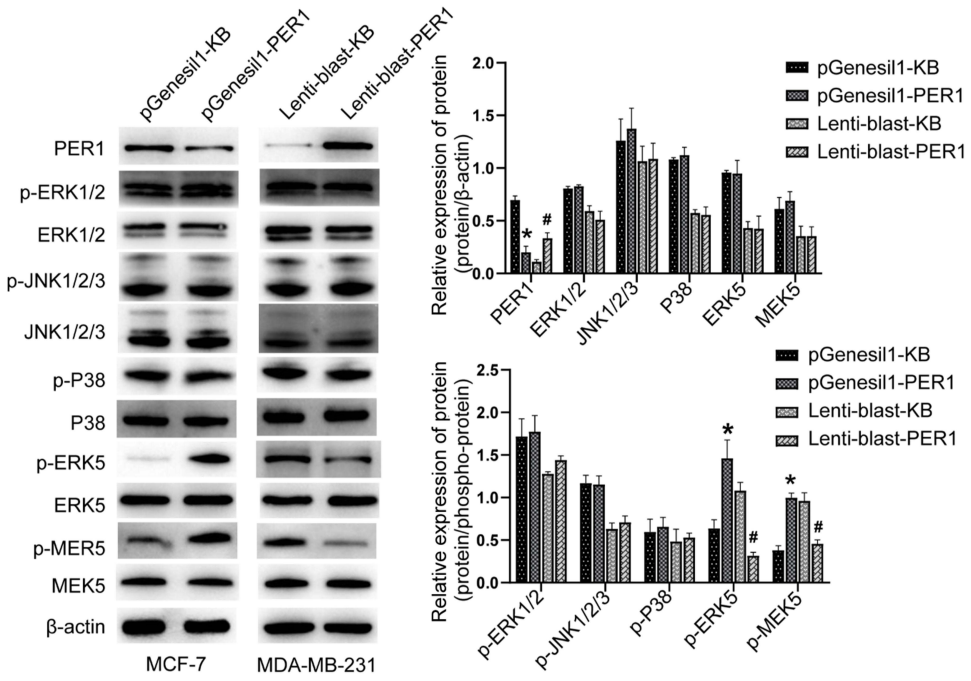

Figure 3 The key protein expression of the MAPK pathway in the breast cancer cell with low and high PER1 expression. *P<0.05 vs pGenesil-1-KB group; #P<0.05 vs Lenti-blast-KB group.

remained unchanged, PER1 overexpression suppressed ERK5 signaling pathways, as evidenced by the reduced phosphorylation levels of ERK5 in Lenti-blast-PER1-MDA-MB-231 BC cells (Figure 3). However, PER1 silencing had the opposite effect on the pGenesil-1-PER1-MCF-7 cells (Figure 3). Furthermore, the effect of PER1 on MEK5 expression, the only upstream factor of ERK5 discovered, which remained unchanged regardless of whether PER1 was overexpressed or silenced; however, p-MER5 showed the same changes as p-ERK5 (Figure 3). Therefore, the regulatory effects of PER1 on the malignant phenotype of BC cells may be associated with abnormal activation of the MEK5/ERK5 pathway.

Effect of XMD17-109 on Activity of MEK5/ERK5 Pathway

To regulate MEK5/ERK5 pathway activity, XMD17-109, a specific ERK5 inhibitor, was used to treat pGenesil-1-PER1-MCF-7 and pGenesil-1-PER1-BT-549 cells (1 μM, 48 h). The expression levels of PER1, MEK5, and p-MEK5 remained unchanged, whereas those of ERK5 and p- ERK5 were reduced markedly (Figure 4).

Inhibition of the MER5/ERK5 Pathway Reverses the Increased Proliferation of BC Cells Caused by PER1 Knockdown

The colony formation assay revealed that PER1 knockdown notably enhanced clone formation rate, whereas XMD17-109 substantially blocked this increase (P<0.05) (Figure 5A). Furthermore, EDU proliferation assay results demonstrated that PER1 knockdown markedly increased the proportion of proliferating cells, as evidenced by the significant elevation in red fluorescence-positive cells (red fluorescence indicating EDU incorporation in actively replicating DNA). Notably, treatment with XMD17-109 effectively attenuated this proliferative effect, resulting in a statistically significant reduction in fluorescence-positive cells compared to the PER1 knockdown group (P<0.05). (Figure 5B). In other words, inactivation of the MEK5/ERK5 pathway reversed the enhancement of cell proliferation caused by PER1 knockdown in BC cells.

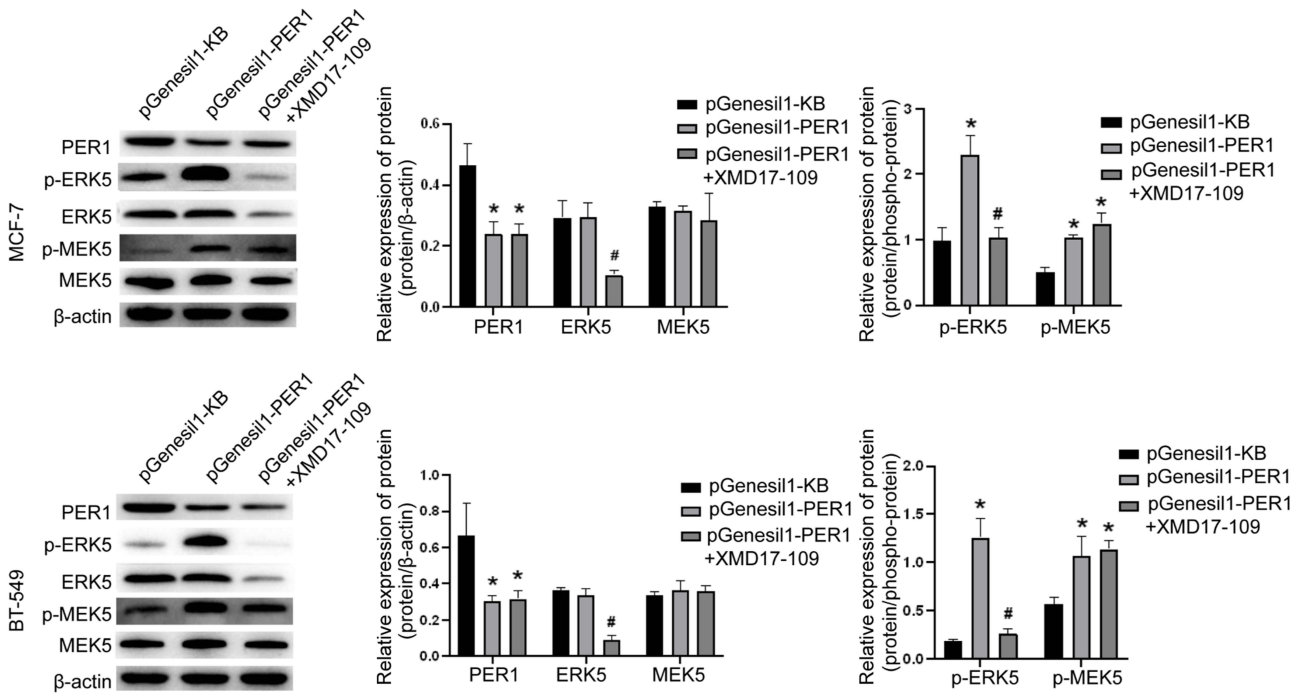

Figure 4 Effects of ERK5 inhibitor XMD17-109 on MEK5/ERK5 pathway activity. *P<0.05 vs pGenesil-1-KB group; #P<0.05 vs pGenesil-1-PER1 group.

Inhibition of the MER5/ERK5 Pathway Reverses Enhancement of Migration and Invasion of BC Cells Caused by PER1 Knockdown

Next, the effect of XMD17-109 on PER1 knockdown BC the migration and invasion was explored. As shown in Figure 6A and B, the pGenesil-1-PER1-MCF-7 group had more migratory and invasive cells than the pGenesil-1-KB-MCF-7 group, and similar results were observed for the pGenesil-1-PER1-BT-549 and pGenesil-1-KB-BT-549 groups. These data imply that blocking the MERK5/ERK5 pathway can antagonize the promotional effect of migration and invasion as a result of PER1 knockdown in BC cells.

Discussion

The mortality of patients with BC is usually ascribed to cell uncontrolled proliferation and metastasis.[8,19,20] Emerging evidence indicates that circadian disruption is linked to tumor incidence and progression by affecting biological processes in cancer cells, including proliferation, DNA repair, metabolism, apoptosis, and stemness.[21] PER1, a key circadian gene, essentially controls mammalian circadian rhythms, the cell cycle, and DNA damage response RNA demethylase.[22] The literature suggests that reduced expression of PER1 promotes cancer occurrence and malignant tumor progression, as PER1 modulates important downstream proteins related to growth, proliferation, and cancer metastasis.[8,13,23–25] PER1 can be utilized as an efficient prognostic bioindex to elucidate immune infiltration in individuals with ovarian cancer.[8] In pancreatic cancer, post-transcriptional activation of PER1 regulated by ALKBH5 reactivates ATM-CHK2-P53/CDC25C signaling, thereby inhibiting cell growth.[22] Consistently, PER1 upregulation and subsequent stimulation of downstream ATM-Chek2 signaling suppress the propagation of multiple human cancer cell lineages by promoting G2/M cell cycle arrest.[26] Furthermore, PER1 expression levels are markedly reduced in BC tissues and cells, and its knockdown promotes cell migration, proliferation, and invasion, indicating its tumor-suppressive activity in BC development and progression.[6] This study suggests that abnormally low expression of PER1 is positively correlated with poor BC differentiation, lymph node metastasis, and vascular tumor thrombosis. Patients with low PER1 expression had poor OS and RFS and a lower 10-year cumulative survival rate than

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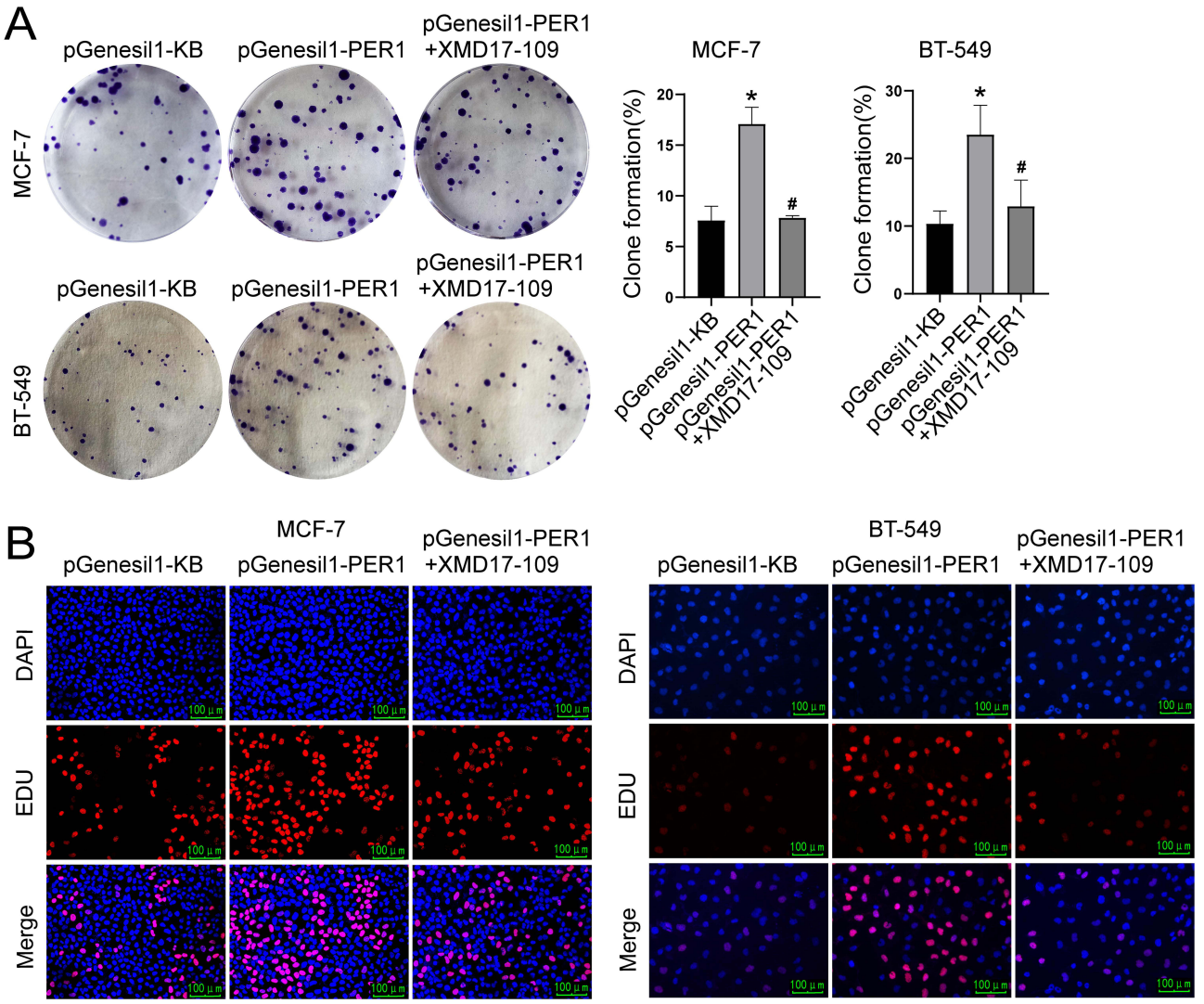

Figure 5 XMD17-109 reverses the enhancement of the proliferative ability in BC cells with low expression of PER1 (100×). (**A**) Colony Formation Assay. (**B**) EdU Assay. *P<0.05 vs pGenesil-1-KB group; #P<0.05 vs pGenesil-1-PER1 group.

those with high PER1 expression, indicating that PER1 downregulation was linked with the progression and malignancy of BC. However, the pathogenesis of PER1 down-regulation in BC tumorigenesis remains unclear.

The MAPK signaling pathway is an essential switch from extracellular signals to intracellular responses. Signaling cascades are altered because genetic and epigenetic changes have been observed in multiple diseases.[16] To elucidate the association between PER1 and the MAPK pathway, changes in the expression of core MAPK pathway factors, such as ERK1/2, P38, JNK1/2/3, ERK5, and their phosphorylation levels after PER1 overexpression or knockdown in BC cells were observed. The expression levels of p-MEK5 and p-ERK5 were negatively correlated with the expression of PER1, which prompted us to focus on the MEK5/ERK5 pathway.

The MEK5/ERK5 pathway belongs to the MAPK family, which comprises a tiered kinase cascade, an important cell proliferation mediator, that induces cell cycle modulators.[27,28] Abnormal activation of MEK5/ERK5 signaling not only increases metastatic risk in various solid cancers but also leads to less favorable survival outcomes.[29] There is clinical evidence that hyperphosphorylation and dysregulated expression of ERK5 are linked to substandard OS rates in BC individuals and chemotherapy resistance.[30,31] Interestingly, an investigation indicated that early phase ERK5 phosphorylation is closely related to circadian rhythm in colon cancer cells.[32] The association between the MEK5/ERK5 pathway, circadian rhythm, and related

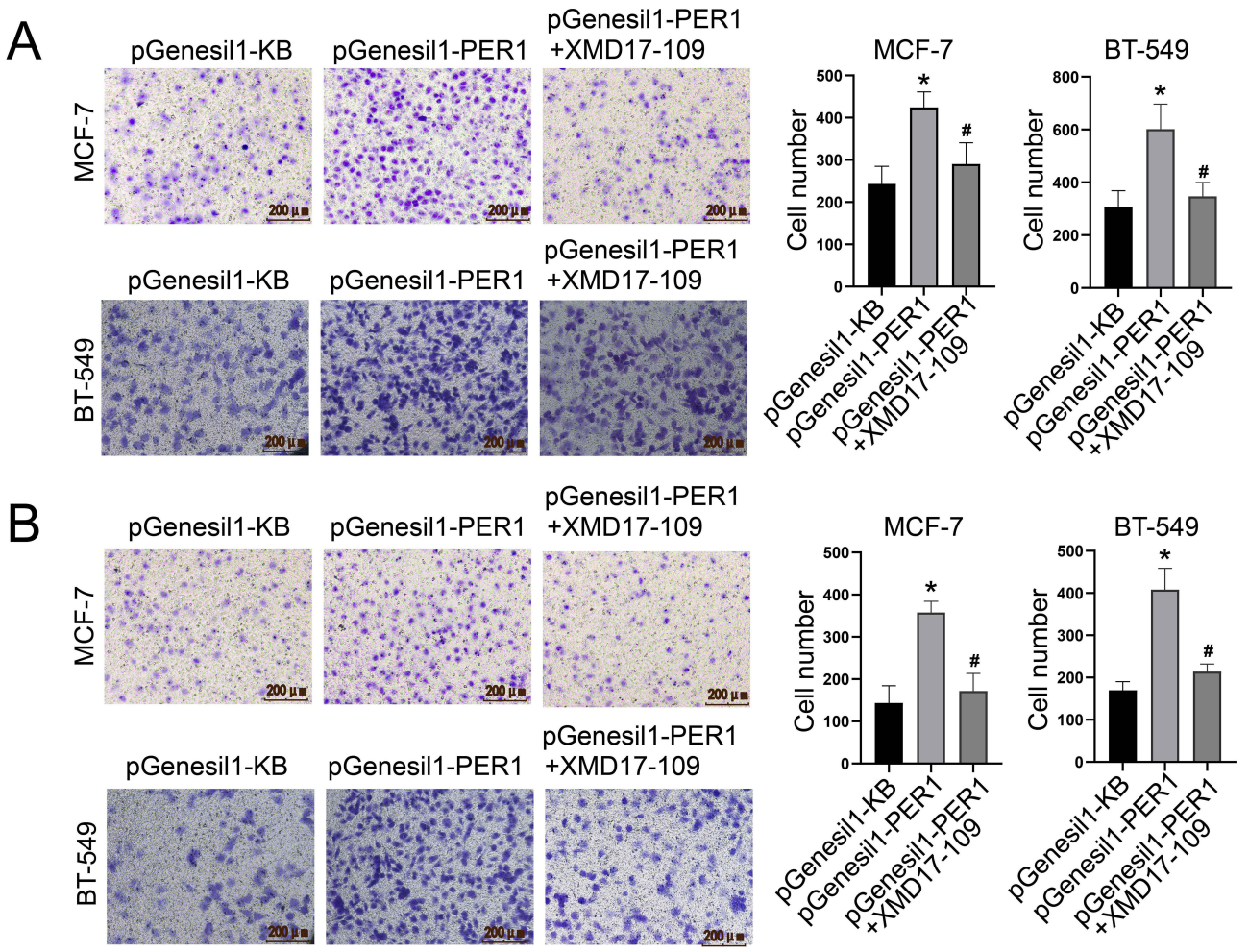

Figure 6 XMD17-109 reverses the increased migration and invasion ability in BC cells with low expression of PER1 (100×). (**A**) Transwell tumor cell migration assay. (**B**) Transwell tumor cell invasion assay. *P<0.05 vs pGenesil-1-KB group; #P<0.05 vs pGenesil-1-PER1 group.

genes remains to be determined. In this study, we found that low PER1 expression activates the MEK5/ERK5 pathway, and inhibition of this pathway can reverse the increase in proliferation, migration, and invasion of BC cells caused by low PER1 expression.

Conclusions

In summary, PER1 loss is associated with substandard clinicopathological manifestations and poor BC prognosis. PER1 knockdown induces the MEK5/ERK5 pathway to promote the malignant phenotype of BC cells. Ultimately, this investigation may help in designing new BC treatment strategies by recovering PER1 loss and inhibiting ERK5.

Abbreviations

BC, Breast cancer; OS, overall survival; PER1, period circadian protein homolog 1; MAPK, mitogen-activated protein kinase; FFPE, formalin-fixed and paraffin-embedded; OS, overall survival; RFS, recurrence-free survival; IHC, immunohistochemistry; AT, ambient temperature; FBS, fetal bovine serum; PVDF, polyvinylidene difluoride; TBST, Tris-Buffered Saline and Tween 20; ECL, enhanced chemiluminescence; EdU, 5-Ethynyl-2′-Deoxyuridine; KM, Kaplan-Meier; PR, progesterone receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2.

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Data Sharing Statement

The data supporting the findings of this study are available upon request from the corresponding author.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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