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ORIGINAL ARTICLE

PER3 plays anticancer roles in the oncogenesis and progression of breast cancer via regulating MEK/ERK signaling pathway

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Abstract

Background: The study aimed at exploring the expression of period circadian regulator 3 (PER3), a major member of the circadian clock gene family, and its biological function in breast cancer.

Methods: PER3-silencing and PER3-overexpression cell lines were established by transfecting with pGenesil1-PER3 and Lentiblast-PER3 vector, respectively.

Results: The results showed that the expression of PER3 was downregulated in breast cancer tissues and cell lines ($p < 0.001$), and its low expression was significantly correlated with advanced tumor stage ($p = 0.031$) and advanced T stage ($p = 0.018$). Cell functional experiments indicated that the silencing of PER3 elevated the ability of breast cancer cells to proliferate, invade, and metastasize in vitro ($p < 0.05$), whereas overexpression of PER3 had an inhibitory effect on these malignant phenotype of breast cancer cells ($p < 0.05$). Moreover, the activation of MEK/ERK signaling pathway was evidently inhibited by silencing of PER3, as evidenced by decreased expression levels of p-MEK and p-ERK1/2 proteins in breast cancer cells ($p < 0.05$). PER3-silencing and PER3-overexpression cells were treated with PD98059 (an inhibitor of MEK/ERK signaling) and TPA (an activator of MEK/ERK signaling), respectively. It was observed that PER3 silencing-mediated malignant phenotype in breast cancer cells was markedly suppressed by PD98059 treatment. Instead, TPA exposure reversed the inhibitory effects of PER3 overexpression on DNA synthesis, proliferation, migration, and invasion of breast cancer cells.

Conclusion: These findings suggested that PER3 function as a tumor suppressor in the development and progression of breast cancer and its anticancer roles might be dependent on the MEK/ERK signaling pathway.

Keywords: Anticancer; Breast cancer; MEK/ERK signaling; Period circadian regulator 3; Tumor suppressor

1. INTRODUCTION

Recent cancer statistics have shown that breast cancer is the most frequently diagnosed malignancy among females around the world, and it is characterized by highly molecular and histological heterogeneity.[1–3] Despite the substantial progress in diagnostic and therapeutic methods, high rates of loco-regional recurrence and distant metastasis are still great challenges for clinical treatment of breast cancer patients.[4,5] The lack of effective molecular hallmarks and prognostic markers has become a key barrier in the improvement of diagnostic and therapeutic strategies. Consequently, it is urgent to explore genetic alterations and molecular mechanisms in the development and progression of breast cancer, which might provide useful diagnostic markers and therapeutic targets.

It has been demonstrated that the circadian rhythm regulated various biological and physiological processes such as cell growth, metabolism, hormone secretion, and gene transcription in human beings.[6–8] Recently, emerging evidence have shown that disturbance of the circadian rhythm plays critical roles in tumorigenesis, progression, and metastasis.[9–11] Period circadian regulator 3 (PER3), a major member of the circadian clock gene family, has been implicated in the control of cell cycle and basic activities of cell such as proliferation and differentiation.[12,13] To date, the genetic alteration of PER3 has been observed in a few human cancers, including prostate cancer, colorectal cancer, and hematopoietic malignancies.[14–18] The previous study reported that the susceptibility of breast cancer induced by carcinogen treatment was markedly increased by the deletion of PER3 in a mouse model.[19] Moreover, gene co-expression analysis in breast cancer tissues and healthy controls revealed that the expression of PER3 was significantly downregulated in cancer samples.[20] These findings suggested that aberrant expression

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and dysfunction of PER3 might contribute to the pathogenesis of breast cancer. However, the biological roles and underlying mechanisms of PER3 in breast cancer were still inadequately understand.

Microarray and RNA-sequencing technologies made comprehensive analysis of specific genes possible. In the current study, we analyzed the expression profile of PER3 in breast cancer samples and its clinical as well as prognostic significance using the public database. Based on the findings of bioinformatic analysis, we hypothesized that PER3 function as a tumor suppressor in the development and progression of breast cancer. For this purpose, altered expression was validated and loss-or-gain of function experiments were conducted to investigate the effect of PER3 expression on biological behaviors of breast cancer cells in vitro. Finally, the molecular mechanisms of PER3 in the pathogenesis of breast cancer was explored. Our findings might provide a valuable information for early diagnosis and therapy of breast cancer patients.

2. METHODS

2.1. Bioinformatic analysis

The expression levels of PER3 mRNA in breast cancer samples and normal samples were analyzed using the Oncomine microarray database (http://www.oncomine.com). The correlations between the expression of PER3 and clinicopathologic parameters of breast cancer were evaluated by Breast cancer gene expression miner (bc-GenExMiner) v4.5, which was an online mining tool of transcriptome data of breast cancer (http://bcgenex.centregauducheau.fr/BC-GEM/GEM-Accueil.php).

2.2. Clinical samples and cell culture

Four matched tumor and adjacent nontumor tissue specimens were obtained from breast cancer patients who underwent surgical resection in our hospital. All patients did not received neoadjuvant chemotherapy, radiotherapy, or anti-HER2 treatment before surgery. Informed consents were obtained from the study participants prior to study commencement, and all protocols were approved by the Ethics Review Board of our hospital (No. 2019H044).

Five cell lines of human breast cancer (MCF-7, MDA-MB-453, MDA-MB-231, SKBR3, and BT-549) and normal breast epithelial cell line MCF-10a were purchased from the National Infrastructure of Cell Line Resource (Beijing, China). All cell lines were routinely cultured in a 37°C humidified incubator with 5% CO_2 and DMEM (GIBCO, USA) or RPMI-1640 medium (GIBCO) with 10% fetal bovine serum (GIBCO) and 100 U/mL streptomycin-penicillin (HyClone, USA) were used for cell culture.

2.3. Plasmid construction and cell transfection

The shRNA oligos of PER3 (5′-GCTTCAGAACACACTTCCAAA-3′) were cloned into the pGenesil-1 vector (pGenesil1- PER3) (Wuhan Genesil Biotechnology, China). The pGenesil-1 vector with nonspecific sequence (pGenesil1-KB) was used as a scramble control. On the other hand, the plasmids with the full-length sequence of PER3 were packaged by the recombinant lentiviral vector (Lenti-blast-PER3), and the negative control was the lentiviral vector with nonsense sequence (Lenti-blast-KB). The procedures of cell transfection were completed in line with the manufacturer's protocols. Briefly, MCF-7 and MDA-MB-231 cells were placed into a six-well plate and cell density was adjusted to 5×10^5 cells/well. Then, pGenesil-1 vector targeted to PER3 was used for transfecting with MCF-7 cell lines for 24 hours, while the lentiviral suspensions with Lenti-blast-PER3 were transfected into MDA-MB-231 cell lines for 24 hours, with a multiplicity of infection (MOI) of 10. Cells in the control group were infected with pGenesil1-KB or Lenti-blast-KB using an equivalent MOI.

The stable PER3-silencing or PER3-overexpression cell lines were screened and established using 800 μg/mL G418 (Solarbio, China) or 10 μg/mL Blasticidin S (Solarbio, China) treatment for at least 2 weeks, respectively.

2.4. qRT-PCR

The extraction of total RNAs from breast cancer specimens and cell lines were implemented by TRIzol Reagent (Takara, Japan). The concentration and purity of RNAs was measured using a Nanodrop spectrophotometer (Takara, Japan) and cDNAs were synthesized by reverse transcription reaction. The PCR amplification reaction was done in a total volume of 20 μL under 95°C for 30 seconds, 40 cycles at 95°C for 5 seconds, and 55°C for 30 seconds, and 72°C for 30 seconds. All qRT-PCR assays were triplicated and the relative expression of PER3 (Primer sequence, F: 5′-GCAGAGGAAATTGGCGGACA-3′; R: 5′-GGTTTATTGCGTCTCTCCGAG-3′) in breast cancer specimens and cells was normalized by 18s mRNA (F: 5′-CCTATTGTCTGTGTTCTTTTCTCTTTATG-3′; R: 5′-AAAGGGCAGGGACGTAGTCA-3′), and the data were quantified by the $2^{-\Delta\Delta CT}$ method.

2.5. Western blot

Breast cancer tissues or cell lines were lysed on the ice for 30 minutes and total proteins were collected using radioimmunoprecipitation (RIPA) buffer (Solarbio, China) containing phosphatase and protease inhibitors. The BCA Protein Assay Kit (Solarbio) was used for measurement of protein concentration. After heat denaturation, 30 μg of protein samples were loaded and then electrophoretically separated by 8% to 12% SDS-PAGE gel. The proteins were electrotransfered onto PVDF membranes (Millipore, USA), followed by blocking with 0.5% BSA (Solarbio) for 60 minutes. Next, the membranes were incubated with primary antibodies against PER3 (1:1000, ab177482, abcam, UK), p-ERK5 (1:1000, No.3372S, CST, USA), ERK5 (1:1000, No. ET1612-7, HUABIO, China), p-p38 (1:1000, No. ARG20124, ARIGOBIO, China), p38 (1:1000, No. ET1702-65, HUABIO), p-JNK (1:1000, No. ET1601-28, HUABIO), JNK (1:1000, No.SA43-06, ARIGOBIO), p-ERK1/2 (1:1000, No. ET1610-13, HUABIO), ERK1/2 (1:1000, No. ET1601-29, HUABIO), p-MEK (1:1000, No. ARG57917, ARIGOBIO), MEK (1:1000, No. ET1603-20, HUABIO), and β-actin (1:10000, No.AC026, abclonal, Korea) overnight at 4°C, and then were treated with the corresponding secondary antibodies (1:5000, No. SA00001-2, Proteintech, China) for 1 hour at room temperature. For further analysis of MEK/ERK signaling pathway, breast cancer cells were additionally treated with 50 μM of its inhibitor (PD98059, APExBIO) and activator (TPA, APExBIO, USA) for 24 hours. Finally, the protein signals were visualized by enhanced chemiluminescence reagent (Affinity Biosciences, USA).

2.6. Colony formation assay

The transfected MCF-7 and MDA-MB-231 cells were collected and seeded into a 6-well plate with 1000 cells/well. The cells were routinely cultured with complete medium for 14 days. 4% paraformaldehyde was used to fix cell colonies and 0.1% crystal violet was applied to stain them at room temperature. The colonies were photographed and counted at five random fields using an inverted microscope (Leica, Germany).

2.7. 5-Ethynyl-2′-Deoxyuridine assay

The effect of PER3 expression on the cell proliferation was further investigated by the 5-Ethynyl-2′-Deoxyuridine (EdU) assay.

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In brief, a total of 5000 transfected cells per well were placed into a 12-well plate. Under the routine culture condition, 10 μM of EdU reagent (Beyotime, China) was added into cell medium for 2 hours. After that, cells were fixed with 4% paraformaldehyde and treated with Apollo 594 working solution, and the nuclear was counterstained with DAPI. The percentage of EdU-positive cells was counted under a fluorescence microscope (Leica).

2.8. Migration and Transwell invasion assays

The migration and Transwell invasion assays were performed in a transwell chamber with 8-μm pore size (Corning, USA). A total of 5×10^4 cells per well were placed into the top chambers and covered with 100 μL serum-free cell suspensions. In contrast, the lower chambers were filled with 600 μL of complete medium with 10% fetal bovine serum. For the Transwell invasion assay, the upper chambers were additionally coated with 30 μg Matrigel (Corning), and other procedures were conducted in line with migration assay. After 24-hour incubation at 37°C with 5% CO_2, migrating or invading cells into the lower chambers were fixed with 4% formaldehyde and stained with 0.1% crystal violet (Solarbio). The cells were counted under at least 5 random fields using a light microscope (Leica). Moreover, the effect of PER3 expression on migration ability of breast cancer cells was also investigated by wound healing assay. A linear wound was artificially generated by a 200-μL pipette tip after cell transfection. Cells were cultured for additional 48 hours and then their migration abilities were observed.

2.9. Statistical analysis

Data processing and statistical analysis were implemented with the SPSS software 22.0 version (IBM Corp, USA). At least three replicates were done for all experiments, and all data were described as mean ± standard deviation (SD). The comparisons of two groups or multiple groups for continuous data were analyzed by Student's t-test or one-way ANOVA test followed by Tukey's post-hoc test, respectively. The statistically significant difference between groups was defined as a two-tailed p value of < 0.05.

3. RESULTS

3.1. The expression of PER3 was downregulated in breast cancer tissues and cell lines

Based on the results of bioinformatic analysis in an online database, we found that the expression level of PER3 mRNA was significantly lower in breast cancer specimens (n = 1109) than in noncancer specimens (n = 103) ($p < 0.001$) (Fig. 1A). The analysis of PER3 mRNA expression in 112 paired breast cancer specimens showed a similar finding, suggesting that PER3

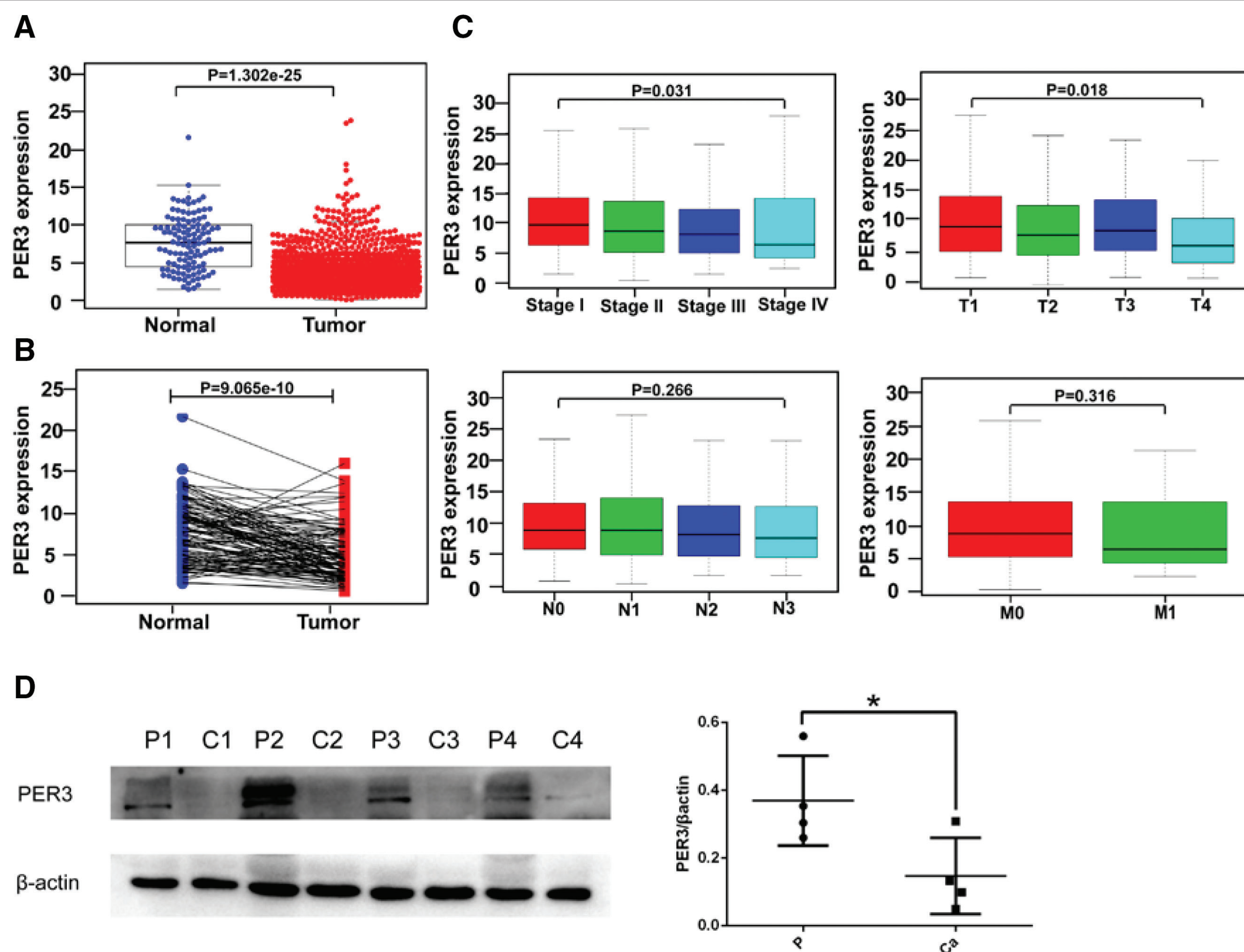

Fig. 1 The expression of period circadian regulator 3 (PER3) in breast cancer samples and its correlations to clinicopathological parameters. A, Comparison of PER3 mRNA expression in 1109 breast cancer samples and 103 noncancer samples. B, Comparison of PER3 mRNA expression in 112 paired breast cancer samples. C, The correlations between PER3 mRNA expression and clinicopathological parameters, including TNM stage, T stage, N stage and M stage. D, The expression of PER3 at both mRNA and protein level were detected in 4 paired breast cancer samples using Western blot analysis.

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was evidently downregulated in breast cancer tissues ($p < 0.001$) (Fig. 1B). Next, we evaluated the clinical significance of PER3 expresssion in breast cancer patients. The results indicated that decreased expression of PER3 was significantly correlated with advanced tumor stage ($p = 0.031$) and advanced T stage ($p=0.018$) (Fig. 1C). In addition, we found that the expression of PER3 was markedly decreased in estrogen receptor (−), PR(−), HER-2(+), Luminal B, triple negative breast cancer (TNBC) and basal-like breast cancer ($p < 0.001$) (Fig. 2). However, there were no correlations between PER3 expression and other clinicopathological parameters such as N stage ($p = 0.266$) and distant metastasis ($p = 0.316$). The correlation between PER3 expression and survival outcome of breast cancer patients was analyzed using the KM-plotter database. The data demonstrated that low expression of PER3 was significantly associated with poor prognosis of breast cancer patients (Supplementary Figure S1, http://links.lww.com/JCMA/A161). To further validate the findings obtained from bioinformatic analysis, we also detected the expression of PER3 in 4 paired samples of breast cancer via Western blot assay. Consistently, the expression of PER3 at both mRNA and protein level were markedly decreased in breast cancer tissues ($p < 0.05$) (Fig. 1D). Based on the above data, we

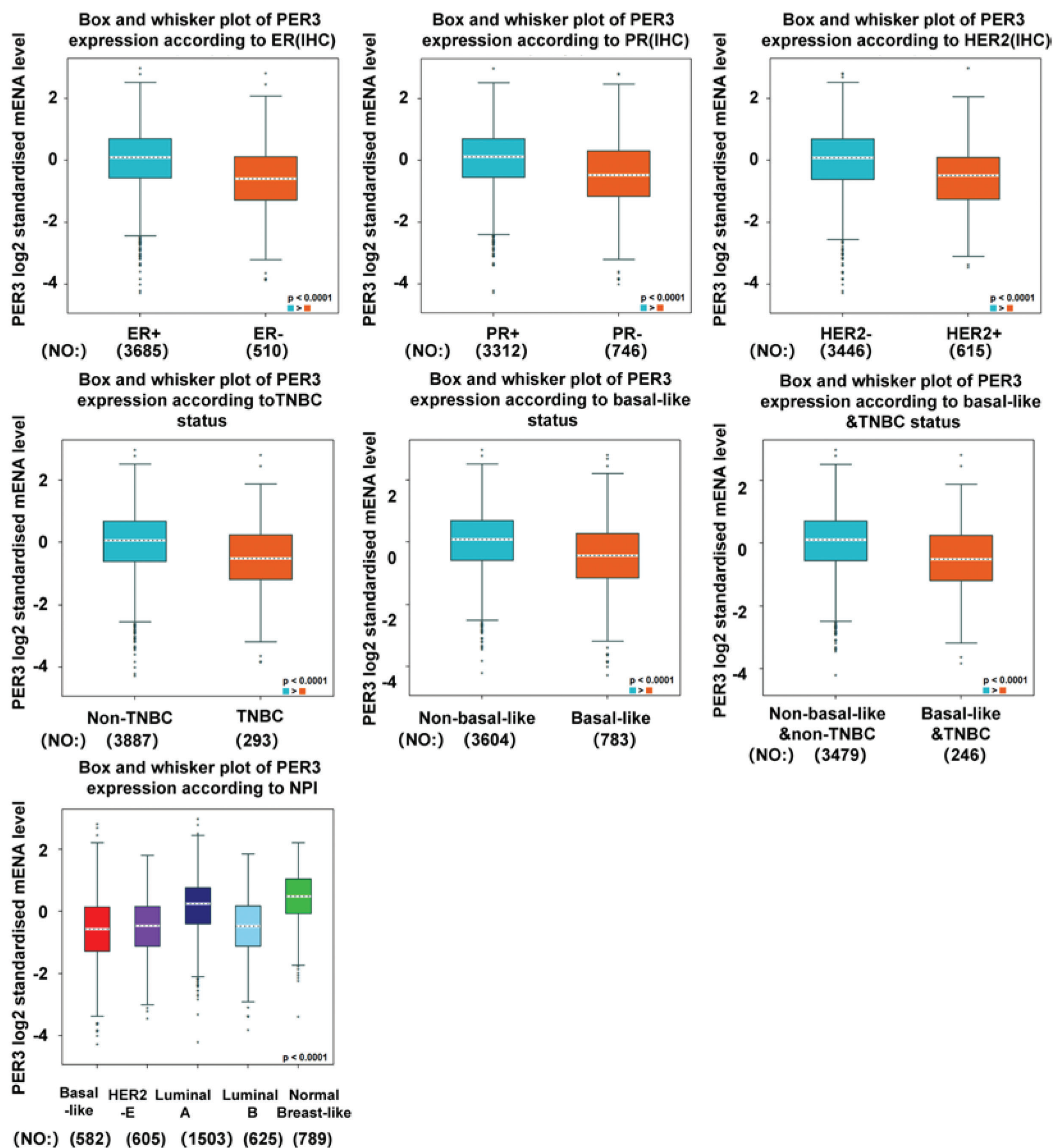

Fig. 2 The relationships between period circadian regulator 3 (PER3) expression and estrogen receptor, PR, HER-2 status as well as molecular subtypes of breast cancer were analyzed using bioinformatic analysis.

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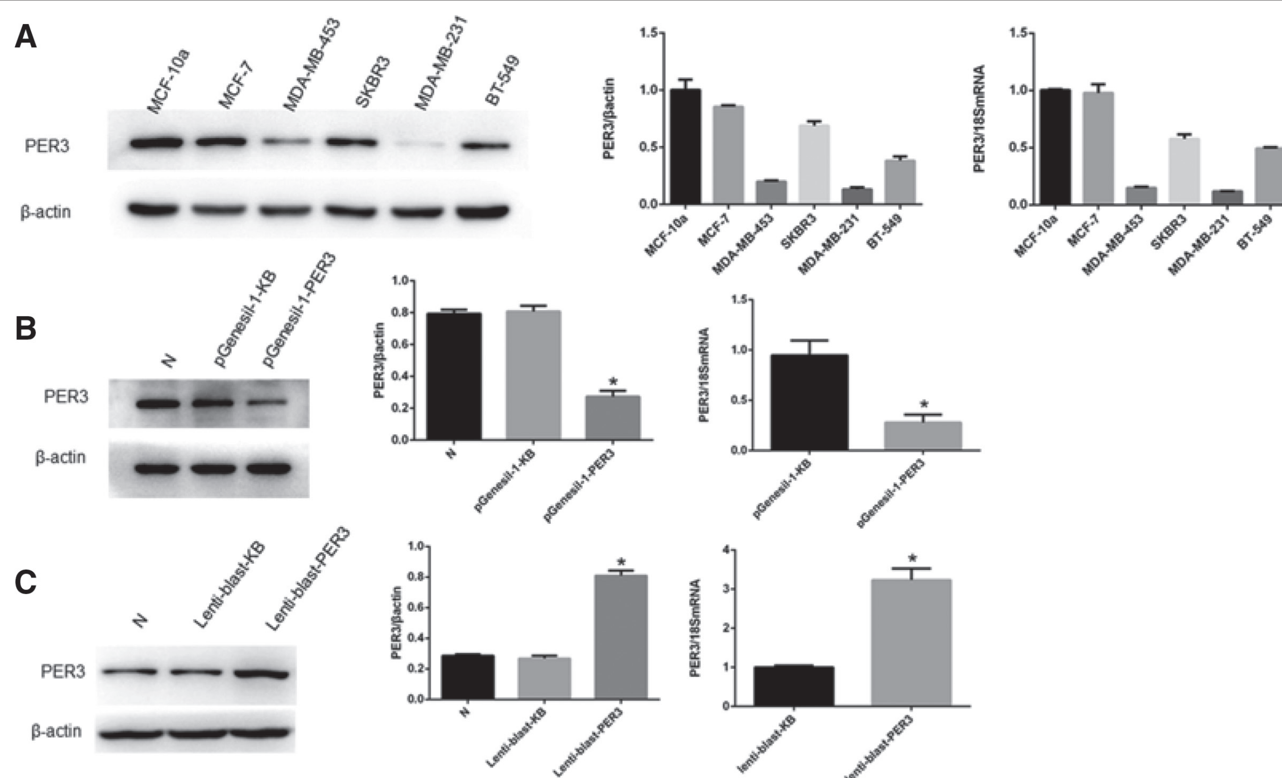

Fig. 3 Construction of period circadian regulator 3 (PER3)-silencing and PER3-overexpression cell line. A, Comparison of PER3 mRNA and protein expression in five breast cancer cell lines and normal breast epithelial cell line MCF-10a using qRT-PCR and Western blot analysis. B, MCF-7 cells were transfected with pGenesil1-PER3 to establish a PER3-silencing cell line, and transfection efficiency was detected by qRT-PCR and Western blot analysis. C, MDA-MB-231 cells were transfected with Lenti-blast-PER3 vector to construct a PER3-overexpression cell line, and transfection efficiency was detected by qRT-PCR and Western blot analysis.

believed that PER3 was abnormally expressed in breast cancer samples and it might be involved in the development and progression of breast cancer.

3.2. PER3 function as a tumor suppressor in the development and progression of breast cancer

The dysregulation of PER3 expression and its correlation to aggressive characteristics urge us to explore the biological function of PER3 in breast cancer. For this purpose, we initially detected the expression of PER3 at both mRNA and protein level among five breast cancer cell lines. Unsurprisingly, the data showed a significant low expression of PER3 in breast cancer cell lines in comparison to normal breast epithelial cell line MCF-10a ($p < 0.05$) (Fig. 3A). Based on the expression level of PER3, MCF-7 and MDA-MB-231 cells were used for subsequent functional experiments. In this study, we established a PER3-silencing cell line by transfecting MCF-7 cells with pGenesil1-PER3. As detected by qRT-PCR and Western blot analysis, the expression of PER3 in MCF-7 cells was effectively downregulated compared to scramble control ($p < 0.05$) (Fig. 3B). On the other hand, we also used Lenti-blast-PER3 vector to upregulate PER3 expression in MDA-MB-231 cells. The transfection efficiency was further confirmed in breast cancer cells ($p < 0.05$) (Fig. 3C).

According to the results of EdU assay, the rate of DNA synthesis in PER3-silencing cells was higher than that of cells in the control group (Fig. 4A). However, the ability of DNA synthesis in PER3-overexpression cells was obviously suppressed (Fig. 4A). Similarly, colony formation assay indicated that the amount of cell colonies in MCF-7 was markedly elevated by the silencing of PER3, but was reduced by overexpression of PER3 ($p < 0.05$) (Fig. 4B). Subsequently, migratory and invasive assays consistently suggested that silencing of PER3 had a promoting effect on these malignant phenotypes of breast cancer cells ($p < 0.05$) (Fig. 5 and Supplementary Fig. S2, http://links.lww.com/JCMA/A161). In contrast, PER3 overexpression markedly inhibited the migratory and invasive abilities of breast cancer cells in vitro ($p < 0.05$) (Fig. 5 and Supplementary Fig. S2, http://links.lww.com/JCMA/A161).

3.3. PER3 regulated cell growth, migration, and invasion in breast cancer via MEK/ERK1 signaling pathway

To better understand the underlying mechanism of PER3 expression in regulating malignant biological behaviors of breast cancer cells, the expressions of key phosphorylation proteins (p-p38, p-ERK5, p-ERK1/2, p-JNK, and p-MEK) in several cancer-related signaling pathways were examined by Western blot. The results demonstrated that the silencing of PER3 evidently elevated the expression levels of p-MEK and p-ERK1/2, but the levels of total MEK and ERK1/2 protein were not altered ($p < 0.05$) (Fig. 6). On the other hand, overexpression of PER3 significantly inhibited the activation of MEK/ERK signaling pathway, as evidenced by reduced levels of p-MEK and p-ERK1/2 expression in breast cancer cells ($p < 0.05$) (Fig. 6). These findings suggested that PER3 affected biological behaviors of breast cancer cells through regulating MEK/ERK signaling pathway.

To further test this hypothesis, we next investigated the effects of blocking or activating MEK/ERK signaling pathway on malignant phenotype of PER3-silencing or PER3-overexpression cells, respectively. Our data showed that PER3 silencing-mediated the alteration of p-MEK and p-ERK1/2 proteins were partially inhibited after PD98059 treatment for 24 hours (50 μM), an inhibitor of MEK/ERK signaling pathway ($p < 0.05$) (Fig. 7A). Conversely,

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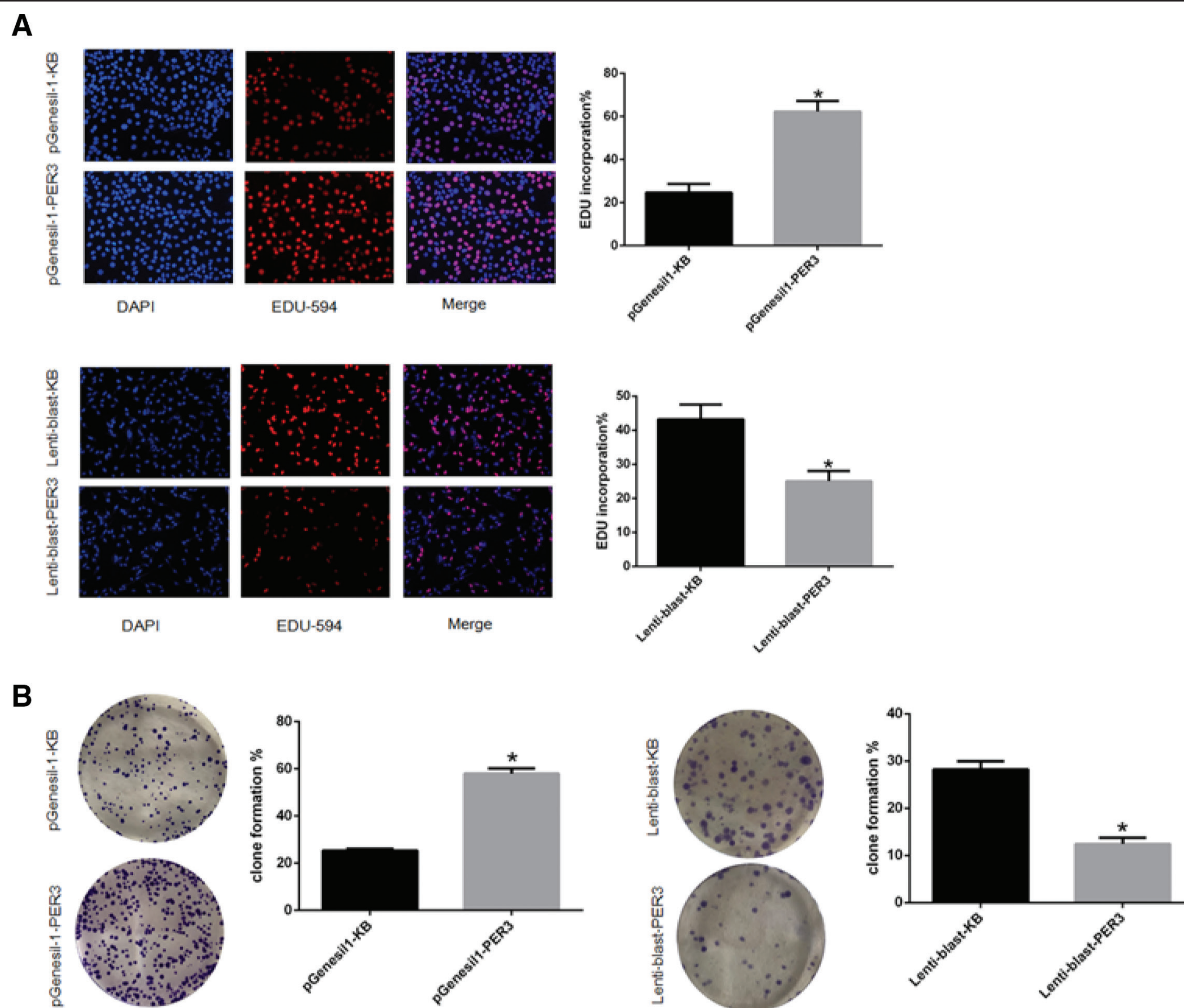

Fig. 4 The effects of period circadian regulator 3 (PER3) expression on biological behaviors of breast cancer cells in vitro. A, 5-Ethynyl-2′-Deoxyuridine (EDU) assay was conducted to investigate the effect of PER3 on DNA synthesis in breast cancer cells. B, Colony formation assay showed that the effect of PER3 on cell growth in breast cancer.

the exposure of TPA (50 μM), an activator of MEK/ERK signaling, increased the expression levels of p-MEK and p-ERK1/2 protein in PER3-overexpression cells ($p < 0.05$) (Fig. 7B).

Interestingly, it was also observed that PER3 silencing-mediated DNA synthesis and cell proliferation was markedly suppressed by PD98059 treatment, while the presence of TPA reversed the inhibitory effects of PER3 overexpression on DNA synthesis and cell proliferation in breast cancer (Fig. 8A B). On the other hand, a significant decrease in the amount of migratory and invasive cells were observed in PER3-silencing cells treated with PD98059, in contrast to those without PD98059 treatment (Fig. 9). Instead, the inhibitory effects of PER3 overexpression on cell migration and invasion were partially abrogated by TPA exposure (Fig. 9). Taken together, these findings illustrated that the anti-tumor roles of PER3 in breast cancer development and progression might be dependent on the regulation of MEK/ERK signaling pathway.

4. DISCUSSION

It is of great importance to identify novel molecular targets for early diagnosis and anticancer treatment of breast cancer. As key regulators of circadian rhythm, circadian clock genes have gained more attention owing to their potential function in carcinogenesis and tumor progression.[21,22] PER3, which is located at 1p36 chromosomal region, is a major member of circadian period family in humans.[19] Epidemiological evidences have shown that genetic alteration of PER3 and gene polymorphism increased susceptibility to various human malignancies including breast cancer.[20,23,24] However, the biological function and underlying molecular mechanisms of PER3 in human cancers are still not clearly elucidated.

In this study, our data indicated that the expression of PER3 is frequently downregulated in breast cancer samples and cell lines. Through correlation analysis of PER3 expression and clinicopathological parameters, we found that low expression of PER3 was significantly correlated with advanced T stage and TNM stage. The differential expression of PER3 in tumor and nontumor tissues has been observed in several human cancers such as colorectal cancer and breast cancer.[15,16,19] Climent et al reported that the expression of PER3 was decreased in breast cancer samples and its deletion was a predictor of breast cancer recurrence, particularly for estrogen receptor-positive patients.[19] Similarly, a recent study uncovered that low expression of PER3 was significantly associated with poorer

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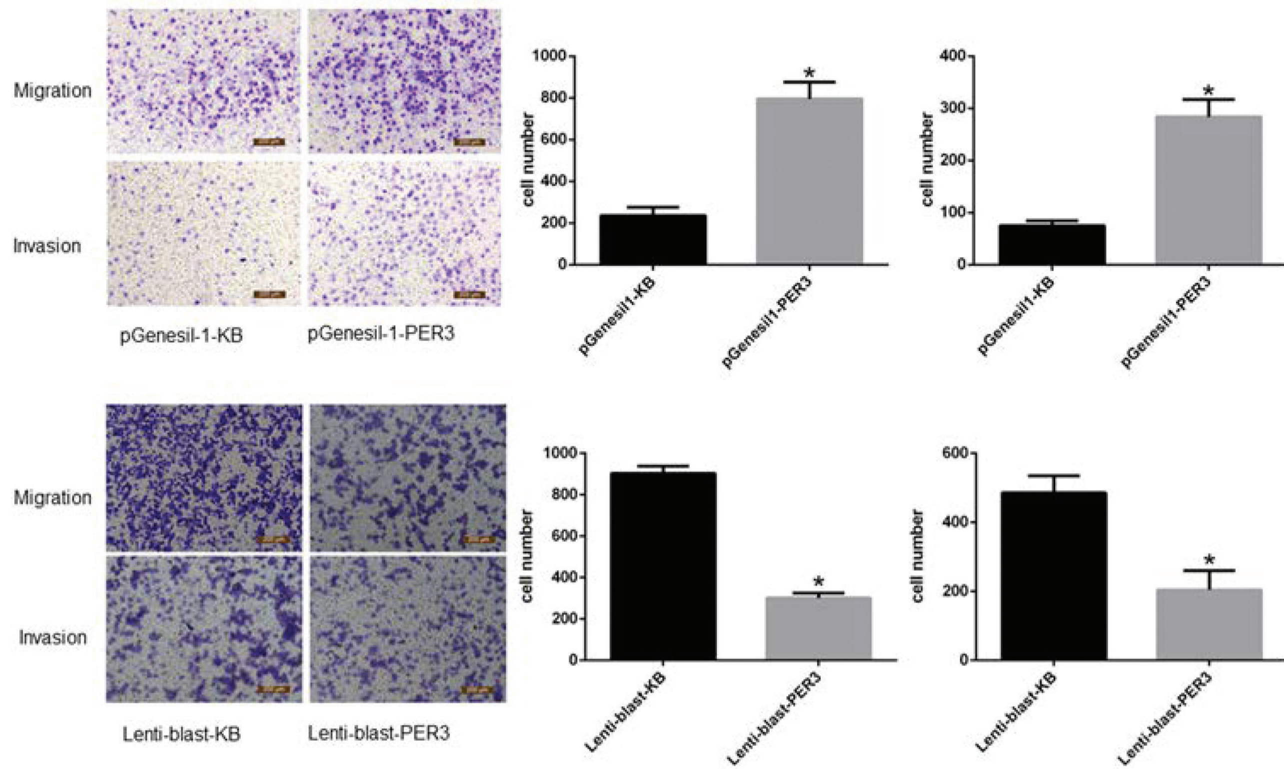

Fig. 5 The effects of period circadian regulator 3 (PER3) on migratory and invasive ability of breast cancer cells. Scale bar, 200 μm.

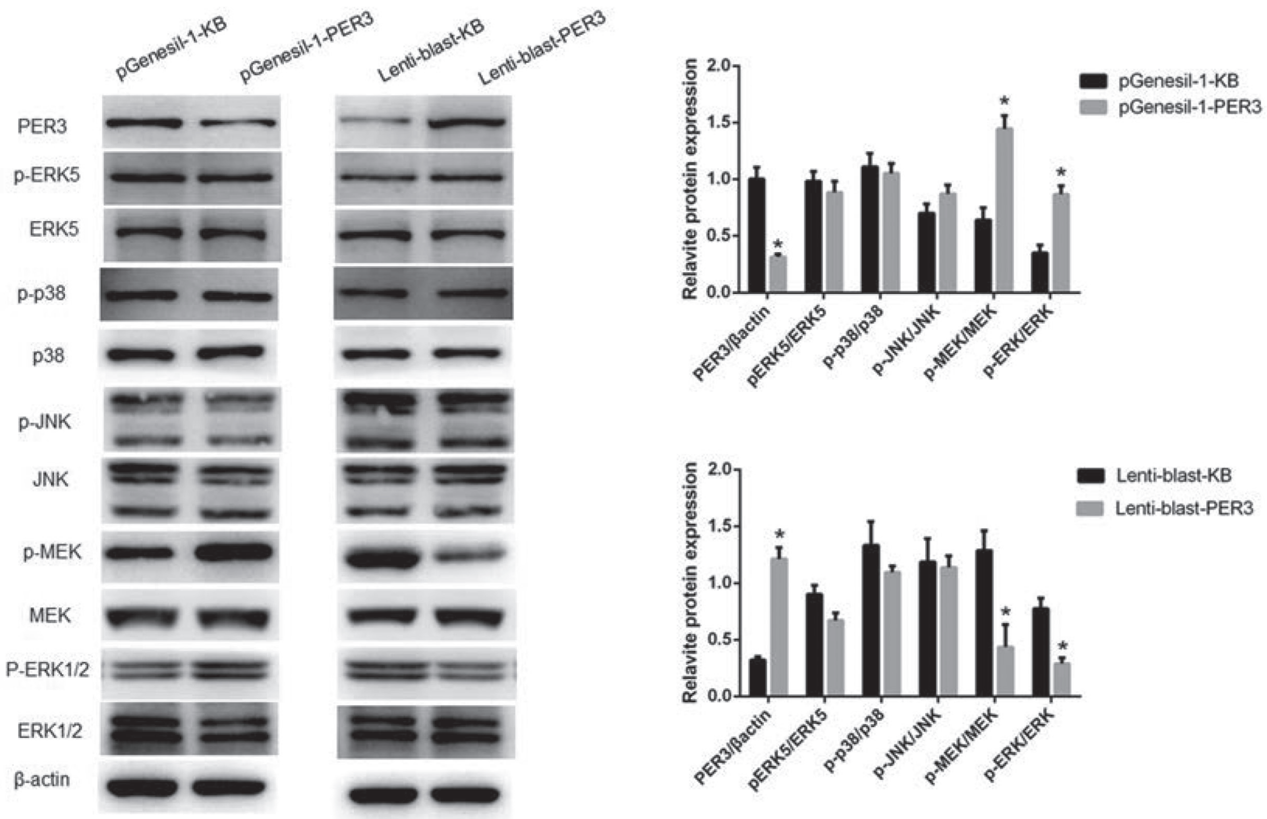

Fig. 6 The effects of period circadian regulator 3 (PER3) on the expression of key proteins in the MAPK signaling pathways.

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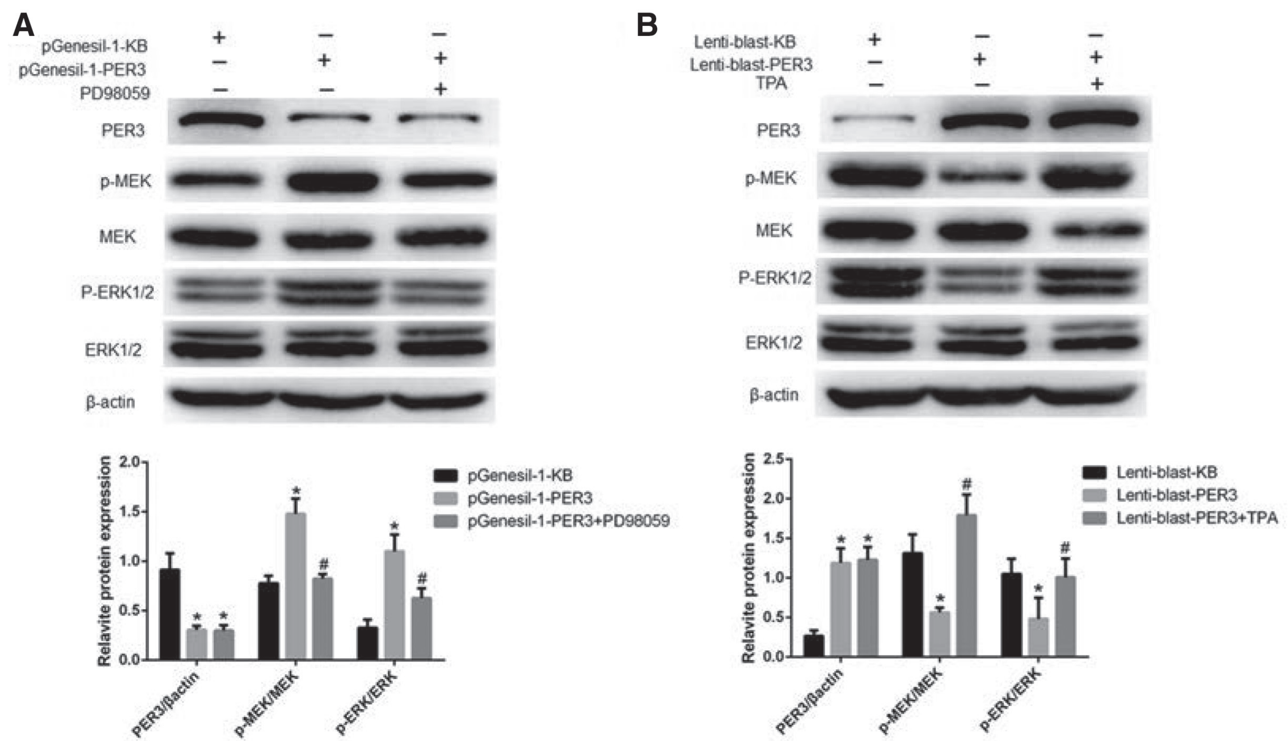

Fig. 7 Period circadian regulator 3 (PER3) regulated cell growth, migration, and invasion in breast cancer via MEK/ERK1 signaling pathway. A, The activation of MEK/ERK signaling pathway in PER3-silencing cells was blocked by PD98059 treatment. B, The inactivation of MEK/ERK signaling pathway in PER3-overexpression cells was reversed by TPA treatment.

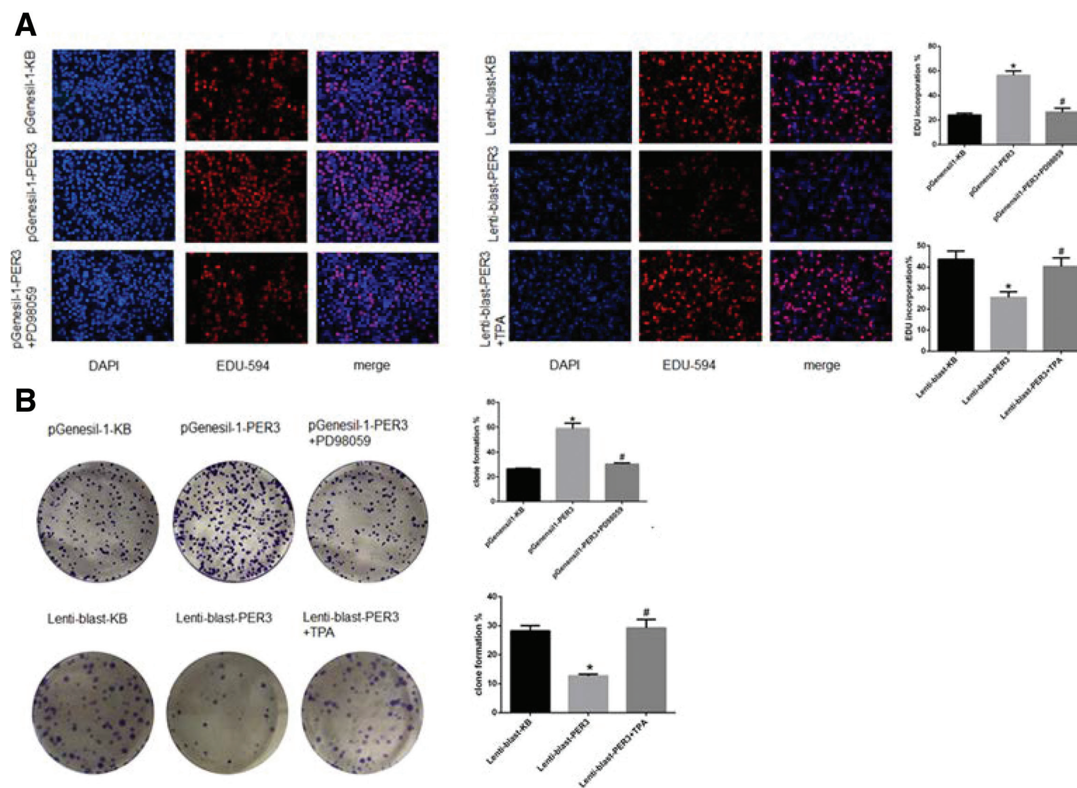

Fig. 8 A, The DNA synthesis mediated by period circadian regulator 3 (PER3) silencing was partially inhibited by PD98059 treatment, while inhibitory effect of PER3 overexpression on DNA synthesis was reversed by TPA treatment. B, The cell proliferation mediated by PER3 silencing was partially inhibited by PD98059 treatment, while inhibitory effect of PER3 overexpression on cell proliferation was reversed by TPA treatment. migration and invasion mediated by PER3 silencing was partially inhibited by PD98059 treatment, while inhibitory effects of PER3 overexpression on migration and invasion were reversed by TPA treatment. Scale bar, 200 μm.

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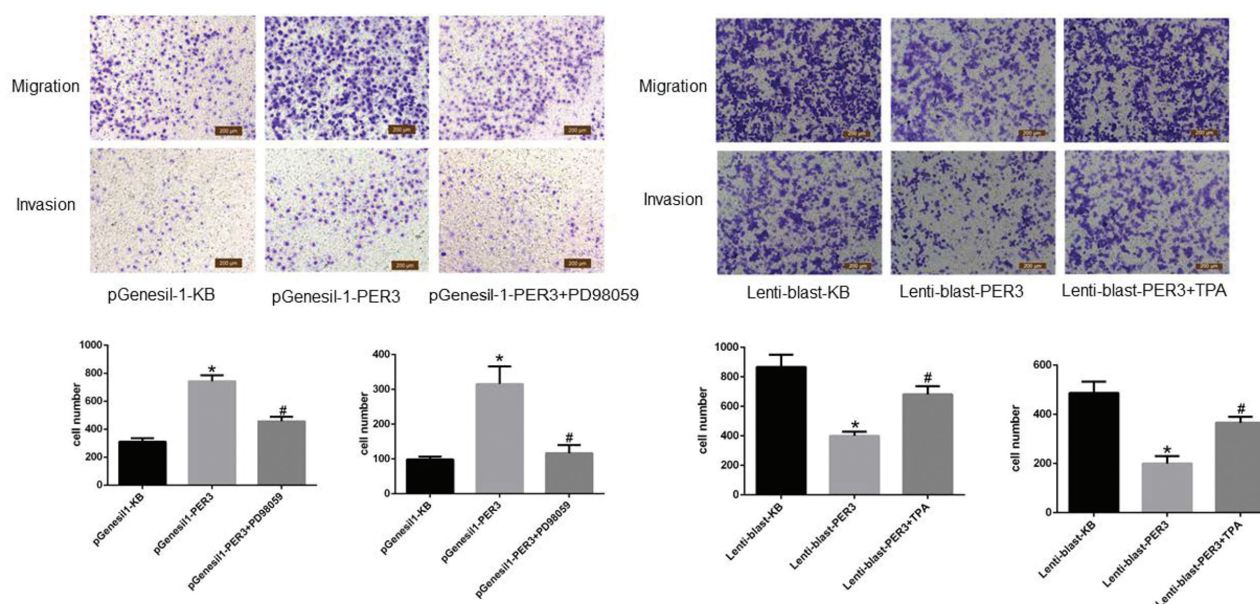

Fig. 9 The migration and invasion mediated by period circadian regulator 3 (PER3) silencing was partially inhibited by PD98059 treatment, while inhibitory effects of PER3 overexpression on migration and invasion were reversed by TPA treatment. Scale bar, 200 μm.

relapse-free survival (RFS) in breast cancer patients with luminal A subtype.[20] These findings supported that PER3 might be involved in regulating oncogenesis and progression of breast cancer.

In view of its aberrant expression and clinical significance, we decided to investigate the biological function of PER3 in breast cancer. The available evidence suggested that upregulation of PER3 suppressed the ability of breast cancer cells to proliferate, invade and metastasize in vitro, implying that it function as a tumor suppressor in breast cancer. These findings were supported by other researches focused on the roles of PER3 in human cancers.[14,16,25] Li et al[14] reported that PER3 negatively regulates tumorigenicity and stemness of cancer cells in vitro through WNT/β-catenin signaling, with a key anti-cancer role in prostate cancer. Likewise, the study by Zhang et al[25] demonstrated that PER3 was lowly expressed in colorectal cancer patients and its overexpression had an inhibitory effect on self-renewal ability and 5-FU chemoresistance of cancer stem-like cells via regulating Notch and β-catenin signal pathways. These findings consistently highlighted the anti-cancer effect of PER3 in human cancers.

It has been shown that MEK/ERK signaling pathway was a crucial regulator in the tumorigenesis and cancer progression and its aberrant activation was frequently observed in breast cancer.[26–28] ERK1/2 was only known downstream target of MEK, with an extremely broad substrate specificity. Phosphorylation activation of ERK1/2 and MEK were essential for key oncogenic processes such as cell survival, proliferation and migration. In the present study, our results showed a significant increase in the expression levels of p-MEK and p-ERK1/2 proteins in response to deletion of PER3, indicating that PER3 might regulate biological behaviors of breast cancer cells via activating the MEK/ERK signaling pathway. This hypothesis was further supported by our data that PD98059 treatment (an inhibitor of MEK/ERK signaling pathway) reversed PER3-mediated proliferation, migration, and invasion in breast cancer cells. All these findings suggested that the anticancer roles of PER3 in breast cancer development and progression might be dependent on the MEK/ERK signaling pathway. However, limitations still exist in this study. First, the off-target effect of TPA could not be excluded completely due to its low selectivity for molecular signaling pathways. Second,the biological function of PER3 in vivo was still unclear. Future research need be carried out to further explore the clinical application of PER3 as a therapeutic target for breast cancer patients. We expect that small-molecule drugs targeted for PER3 could be used as a valuable treatment in future.

In conclusion, first and last, the current research demonstrated that the expression of PER3 was frequently downregulated in breast cancer samples and it exerted anticancer roles in the oncogenesis and progression via regulating the MEK/ERK signaling pathway. Our findings might provide a promising therapeutic target for breast cancer patients.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://links.lww.com/JCMA/A161.

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