Activation of GPER by E2 promotes proliferation, invasion and migration of breast cancer cells by regulating the miR-124/CD151 pathway

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Abstract. Breast cancer is one of the most common malignancies worldwide and is responsible for a high mortality rate. However, the underlying pathological mechanism of breast cancer remains unclear. MicroRNAs (miRNAs/miRs) play critical roles in the progression of breast cancer. Recent studies have reported that miR-124/CD151 participates in the development of breast cancer. However, the exact molecular mechanism of miR-124/CD151 action in 17β-estradiol (E2)-treated breast cancer cells remains unknown. Thus, the present study aimed to investigate miR-124 and CD151 expression levels in MCF-7 cells treated with E2 via reverse transcription-quantitative PCR and western blot analyses. Bioinformatic analysis was performed to predict and identify whether CD151 is a potential target of miR-124. The Cell Counting Kit-8 and colony formation assays were performed to detect proliferation of MCF-7 cells. In addition, the invasive and migratory abilities of MCF-7 cells were assessed via the Transwell and wound healing assays, respectively. The results demonstrated that E2 downregulated miR-124 expression, while upregulating G protein -coupled estrogen receptor (GPER) expression in MCF-7 cells. Following treatment with the GPER antagonist, G15, miR-124 expression was significantly enhanced and E2-induced proliferation, invasion and migration of MCF-7 cells were notably inhibited. In addition, CD151 was confirmed as a direct target of miR-124. CD151 silencing remarkably suppressed the proliferation, invasion and migration of E2-induced MCF-7 cells. Taken together, these results suggest that upregulation of GPER expression induced by E2 promotes proliferation, invasion and migration of breast cancer cells by regulating the miR-124/CD151 pathway. Thus, the results of the present study provide a potential novel method for the treatment and prognosis of breast cancer.

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Introduction

Breast cancer is a lethal malignancy, which has become the second leading cause of mortality in females in the United States (1,2). A previous study has reported that the new cases of breast cancer have an increased incidence of ~30% compared with that noted in other types of cancer in women (3). Despite recent advancements in surgery, chemotherapy, radiation and hormone therapy, the 5-year survival rate of patients with breast cancer is 80-85% (4-6). This is mainly attributed to the lack of molecular mechanism analysis that can explain in detail the pathways responsible for breast cancer formation. Thus, detailed investigation of the pathogenesis of breast cancer is of considerable significance. It has been demonstrated that the prevention of metastasis is a vital factor for the effective reduction of breast cancer (7,8). Estrogen plays a crucial role, not only in the proliferation and initiation of breast cancer, but also in migration (9). Thus, it is essential to unravel the associated molecular mechanisms of breast cancer formation to improve the treatment and prognosis of breast carcinomas.

17β-estradiol (E2) is one of the three main self-producing estrogens and is considered an important hormone in women (10). E2 has a crucial influence on the growth and function of the female reproductive system, and of the mammary gland under physiological conditions (11-13). E2 is involved in the induction of malignant transformation of several types of cancer cells via regulation of the associated biological processes, such as proliferation, invasion and migration (14,15). Most of the biological effects of estrogens are mediated via binding and activation of the classic estrogen receptors (ERs) (16). The G protein -coupled estrogen receptor (GPER) is also denoted as G protein receptor 30 (GPR30) and has been demonstrated to bind to E2 (17). GPER is responsible for interacting with multiple genomic signaling pathways in different types of cells, including breast, endometrial, ovarian and thyroid cancers (18-24). Overexpression of GPER in breast cancer is positively associated with a metastatic phenotype (25). However, the molecular mechanism of proliferation, invasion

and migration of breast cancer resulting from E2 binding to GPER remains largely unknown.

MicroRNAs (miRNAs/miRs) are small non-coding RNA molecules that serve as endogenous regulators of gene expression (26) and are involved in a variety of vital biological processes (27-29). Previous studies have demonstrated that most miRNAs inhibit the metastasis of breast cancer and that their expression levels can be used as metastatic predictors. For example, miR-124 is considered an expression-rich miRNA (30-33), which is usually expressed at low levels and is implicated in hematological malignancies and solid tumors, including breast cancer (34-36). A previous study has reported that miR-124 expression is notably downregulated following treatment with E2 in ER positive breast cancer cells (37). CD151 is a member of tetraspanins that is characterized by 4 hydrophobic domains, and mediates signal transduction events associated with cell proliferation, activation and motility (38). It has been demonstrated that the CD151 protein can accelerate the progression of breast cancer (39). To the best of our knowledge, miR-124 is a negative regulator of breast cancer and it was hypothesized that E2 may regulate miR-124 and CD151 expression levels by GPER, which in turn will affect the proliferation, invasion and migration of breast cancer cells.

In the present study, the potential internal molecular mechanism was investigated with regards to the proliferation, invasion and migration of breast cancer cells. The experiments aimed to investigate the interaction between E2-GPER signaling and alteration in the expression levels of miR-124 and CD151, and the progression of breast cancer cell metastasis. The results of the present study may provide a potential novel approach to the treatment and prognosis of breast carcinomas.

Materials and methods

Cell culture and treatment. The human ER-positive breast cancer cell line, MCF-7, was purchased from Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and stored in liquid nitrogen. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with high glucose, 10% fetal bovine serum (FBS; HyClone; Cytiva) and 1% penicillin-streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.), at 37˚C with 5% CO_2 in a humid environment.

The E2 (5, 10 or 100 nM, Sigma-Aldrich; Merck KGaA)-treated cells were maintained in complete medium with phenol red (Sigma-Aldrich; Merck KGaA) until they reached 60% confluence and subsequently replaced with 2.5% CS-FBS (HyClone; Cytiva) in DMEM containing phenol red-free for 24 h. The dose of E2 was in accordance with previous studies (40,41). Cells were cultured for 24 h at 37˚C in serum-free and phenol-red free DMEM. Subsequently, a certain dose of E2 (5, 10 or 100 nM) was added into the medium. G15 was used as a well-established antagonist of GPER in this experiment (42). To determine whether E2-induced miR-124 downregulation was directly regulated by GPER, MCF-7 cells were pretreated with G15 (100 nM) for 6 h prior to addition of E2.

Cell transfection. MCF-7 cells were incubated for 24 h prior to transfection. miR-124 negative control (mimic-NC; forward, 5'-UUCUCCGAACGUGUCACGUTT-3' and reverse, 5'-ACGUGACAGGUUCGGAGAATT-3'; 50 nM), miR-124 mimic (forward, 5'-UAAGGCACGCGGUGAAUGCCAA-3' and reverse, 5'-CAUUCACCGCGUGCCUUAUU-3'; 50 nM), CD151-small interfering (si)RNA (50 nM) and negative control (siRNA-NC; 50 nM) sequences were synthesized by Shanghai GenePharma, Co., Ltd. Cells were seeded into 6-well plates and cultured until they reached 60-70% confluence. Transfection was performed using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C for 24 h, according to the manufacturer's instructions. Subsequent experiments were performed 24 h post-transfection.

Cell proliferation assay. Cell proliferation was assessed via the Cell Counting Kit-8 (CCK-8) assay (Dojindo Molecular Technologies, Inc.). The cell suspension was seeded into a 96-well plate and cultured for 6 h at 37˚C. Subsequently, cells were incubated with 10 μl CCK-8 solution for 1 h and cell proliferation was measured at a wavelength of 450 nm (A_{450}), using a microplate reader.

Colony formation assay. MCF-7 cells were seeded into 6-well plates at a density of 500 cells/well and cultured at 37˚C in the presence of 5% CO_2 for 7-12 days. Cells were subsequently fixed with 4% paraformaldehyde for 10 min at room temperature and stained with 0.2% crystal violet for 5 min at room temperature. The number of cell colonies were manually counted using ImageJ software (version 1.52r; National Institutes of Health).

Invasion assay. The Transwell assay was performed to assess cell invasion. A total of $1x10^6$ MCF-7 cells were plated in the upper chambers of Transwell plates in serum-free medium (DMEM, Invitrogen; Thermo Fisher Scientific, Inc.). The Transwell membranes were pre-coated with 100 μl cell suspension and Matrigel (BD Biosciences) overnight at 4˚C. DMEM supplemented with 10% FBS was plated in the lower chambers. Following incubation at 37˚C with 5% CO_2 for 24 h, cells that did not pass through the polycarbonate membrane were removed. The invasive cells were fixed with 4% paraformaldehyde for 30 min at room temperature, stained with 1% Giemsa for 15 min at room temperature, rinsed using phosphate-buffered saline (PBS) and air dried. All experiments were performed in triplicate. Stained cells were counted in five randomly selected fields using an inverted light microscope (Olympus Corporation; magnification, x100) and the results were analyzed using ImageJ software (version 1.52r; National Institutes of Health).

Cell migration assay. The cell migratory ability was assessed via the wound healing assay. Briefly, cells were seeded into a 6-well plate ($4x10^5$ cells/well) and incubated at 37˚C until they reached 80% confluence. Subsequently, the cell monolayers were scratched using a plastic scriber. Cells were washed with PBS to elute the debris and the media were replaced with serum-free medium. The average distance that the cells migrated into the wound surface was detected under an inverted light microscope (Olympus Corporation; magnification, x100) after 48 h.

Reverse transcription-quantitative (RT-q)PCR. Total RNA was extracted from MCF-7 cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to

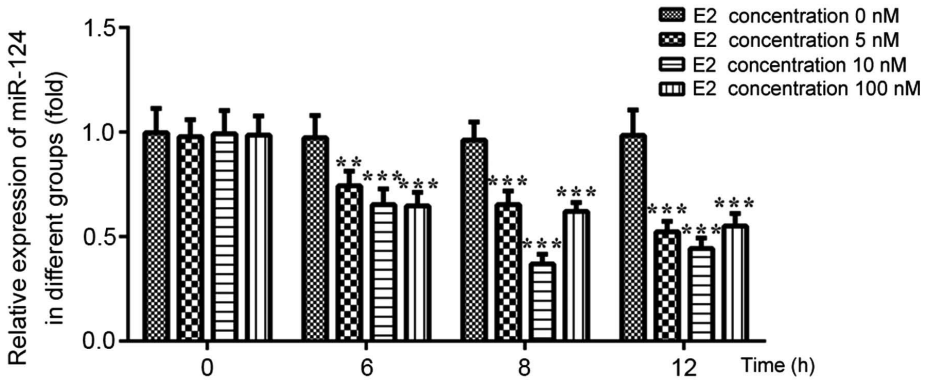

Figure 1. E2 induces miR-124 expression in MCF-7 cells. Reverse transcription-quantitative PCR analysis was performed to detect miR-124 expression in MCF-7 cells treated with different concentrations of E2 at different time points. Data are presented as the mean ± standard deviation (n=3). **P<0.01, ***P<0.001 vs. 0 nM. E2, 17β-estradiol; miR, microRNA.

the manufacturer's instructions. Single-stranded cDNA was synthesized at 42˚C for 30 min using the one-step cDNA synthesis kit (cat. no. 210210; Qiagen, Inc.). The following thermocycling conditions were used: 40 cycles of pre- denaturation at 95˚C for 10 min, denaturation at 95˚C for 30 sec, annealing at 60˚C for 20 sec and extension at 72˚C for 35 sec. The following primer sequences were used: miR-124 forward, 5'-ACGTTGTGTAGCTTATCAGACTG-3' and reverse, 5'-AATGGTTGTTCTCCACACTCTC-3'; CD151 forward, 5'-ACAGCCTACATCCTGGTGGT-3' and reverse, 5'-TTC TCCTTGAGCTCCGTGTT-3'; U6 forward, 5'-ATTGGAACG ATACAGAGAAGATT-3' and reverse, 5'-GGAACGCTTCAC GAATTTG-3'; and GAPDH forward, 5'-ACAACTTTGGTA TCGTGGAAGG-3' and reverse, 5'-GCCATCACGCCACAG TTTC-3'. Relative expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (43).

Western blotting. Anti-GPER rabbit polyclonal anti-body (cat. no. D161727; 1:350), goat anti-rabbit IgG antibody (cat. no. D111018; 1:8,000), anti-matrix metal-loproteinase (MMP)2 (cat. no. D161447; 1:500), anti-MMP9 (cat. no. D162000; 1:500) and anti-GAPDH (cat. no. D110016; 1:500) antibodies were purchased from Sangon Biotech Co., Ltd. Cells were washed with PBS and total proteins were lysed from the cells using SDS lysis buffer (Beyotime Institute of Biotechnology) supplemented with proteinase inhibitor cocktail (Sigma-Aldrich, Merck KGaA). Total protein was quantified using the bicinchoninic acid kit (Beyotime Institute of Biotechnology) and 40 μg protein/lane was fractionated with 8% SDS-PAGE. The separated proteins were subsequently transferred onto polyvinylidene fluoride membranes (EMD Millipore) and subsequently incubated in PBS 0.2% Tween-20 containing 5% skimmed milk powder for 60 min at room temperature to block non-specific binding. Following washing, the blots were incubated overnight at 4˚C with anti-GPER rabbit polyclonal antibody and GAPDH (cat. no. D110016; 1:500; Sangon Biotech Co., Ltd.) was used as the reference protein. Subsequently, the membranes were washed with PBS Tween-20 and incubated with goat anti-rabbit IgG antibody for 2 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence system ChemiDoc MP (Bio-Rad Laboratories, Inc.) and quantified using ImageJ software (version 1.46; National Institutes of Health).

Dual-luciferase reporter assay. The target gene of miR-124 was predicted using the TargetScan database (http://www.targetscan.org/vert_71). A dual-luciferase reporter assay system (Promega Corporation) was used to detect luciferase activities, according to the manufacturer's instructions, using the Envision Multilabel Plate Reader (PerkinElmer, Inc.). The CD151 3'-untranslated region (UTR) was amplified from human cDNA by Shanghai GenePharma Co., Ltd., cloned into a pGL3 luciferase vector (Promega Corporation). The following sequences were used: i) Wild-type (WT) of CD151 3'-UTR forward, 5'-TCTAGAACC CAACTACTGAGCTGAGA-3' and reverse, 5'-TCTAGAGTA CAGCAGTGAACAAAACCA-3'; and ii) mutant type (MUT) of CD151 3'-UTR forward,5'-CTTCTTCCGAGTTTTGCT GCGCACCAATGC-3' and reverse, 5'-AGCAAAACTCGG AAGAAGCTGCCTCTG AGGT-3'. Following seeding into 24-well plates and incubation overnight, cells were co-transfected with miR-124 mimic or mimic-NC for 48 h at 37˚C, using Lipofectamine® 2000. The *Renilla* luciferase activities were normalized to the firefly luciferase activities to set the control samples for the transfection efficiency experiments. All experiments were performed in triplicate.

Statistical analysis. Statistical analysis was performed using GraphPad Prism 6.0 software. All experiments were performed in triplicate and data are presented as the mean ± standard deviation. Unpaired student's t-test was used to compare differences between two groups, while one way ANOVA and Tukey's post hoc test were used to compare differences between multiple groups. P<0.05 was considered to indicate a statistically significant difference.

Results

E2 induces miR-124 expression in MCF-7 cells. MCF-7 cells were treated with E2 at different time points and concentrations and miR-124 expression was detected. The results demonstrated that miR-124 expression significantly decreased following treatment with E2 compared with the control group (P<0.01, P<0.001; Fig. 1). The concentration of E2 was

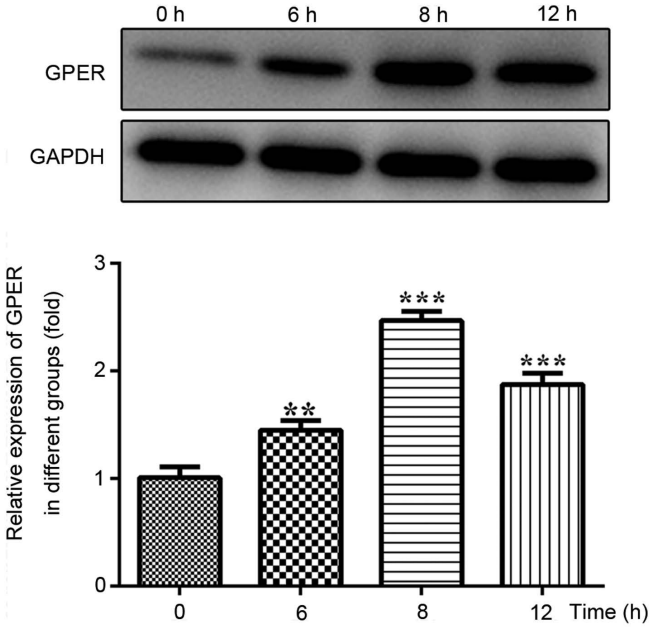

Figure 2. GPER expression is induced by E2. Western blot analysis was performed to detect GPER expression in MCF-7 cells treated with 10 nM E2 at different time points. Data are presented as the mean ± standard deviation (n=3). **P<0.01, ***P<0.001 vs. 0 h. GPER, G protein -coupled estrogen receptor; E2, 17β-estradiol.

selected based on the lowest level of miR-124 expression. The dose-dependent experiments revealed that miR-124 expression considerably decreased in MCF-7 cells following treatment with 10 nM E2 for 8 h compared with the control group. Thus, 10 nM E2 was selected for subsequent experiments, which was in accordance with a previous study (44).

Induction of GPER by E2. MCF-7 cells were treated with 10 nM E2 at different time points (0, 6, 8 and 12 h) and GPER expression was detected. The results demonstrated that GPER expression was upregulated in MCF-7 cells following treatment with E2 for 6, 8 and 12 h, and maximum GPER expression was attained following treatment for 8 h (P<0.001; Fig. 2). Thus, cells treated with E2 for 8 h was selected for subsequent experiments.

GPER mediates the E2-induced decrease in miR-124 expression. The effects of GPER on miR-124 expression were investigated, which were induced by E2 in MCF-7 cells. G15 was used as a well-established antagonist of GPER (41) in this experiment. To determine whether E2-induced miR-124 down-regulation was directly regulated by GPER, MCF-7 cells were pretreated with G15 (100 nM) for 6 h prior to addition of E2. The results demonstrated that miR-124 expression increased in the E2 + G15 group compared with the E2 group (P<0.05; Fig. 3A), suggesting that GPER mediates the E2-induced decrease of miR-124 expression.

GPER mediates the E2-induced proliferation, invasion and migration of MCF-7 cells. Cell proliferation was assessed via the CCK-8 assay. The results indicated that the proliferation of MCF-7 cells in the E2 + G15 group was significantly inhibited compared with the E2 group (P<0.01, P<0.001; Fig. 3B and C). In addition, the results of the Transwell assays demonstrated that the number of invasive cells in the E2 group was significantly higher compared with the control group, the effects of which were reversed following treatment with G15 (P<0.01; Fig. 3D and E). The wound healing assay was performed to assess cell migration. As presented in Fig. 4A and B, the ability of cell migration in the E2 group was notably enhanced compared with the control group, whereas G15 intervention attenuated the effect of E2 on cell migration. The assessment of the expression of migration-associated proteins, including matrix metalloproteinase (MMP)2 and MMP9 exhibited similar results to the cell invasion assay (Fig. 4A-C). Taken together, these results suggest that GPER mediates the E2-induced proliferation, invasion and migration of MCF-7 cells.

CD151 is a direct target of miR-124 in breast cancer cells. The TargetScan database was used to predict the target of miR-124. miR-124 was bound to the 3'-UTR of CD151 (Fig. 5A). Cells were transfected with miR-124 mimic to overexpress miR-124 (Fig. 5B). To validate whether CD151 was regulated by miR-124 in MCF-7 cells, reporter plasmids were generated (WT-CD151 3'-UTR or MUT-CD151 3'-UTR). The results demonstrated that the luciferase activity of CD151 decreased following co-transfection of miR-124 mimic with WT-CD151 3'-UTR in MCF-7 cells compared with the control mimic group. Notably, no significant differences were observed in the luciferase activity following co-transfection of miR-124 mimic with MUT-CD151 3'-UTR in MCF-7 cells (Fig. 5C). To investigate whether CD151 was regulated via the inhibition of miR-124 following GPER induced expression by E2 in MCF-7 cells, western blot and RT-qPCR analyses were performed to detect CD151 expression. In this experiment, MCF-7 cells were treated with 100 nM G15 for 2 h or pre-transfected with miR-124 mimic for 12 h prior to addition of 10 nM E2 for 8 h. The results demonstrated that CD151 protein and mRNA expression levels significantly increased in the E2 group compared with the control group (Fig. 5D and E). However, the effects of E2 on CD151 expression were blocked following pretreatment with miR-124 mimic or G15. Taken together, these results suggest that CD151 is a direct target gene of miR-124, which is consistent with previous findings (26).

Effects of CD151 on the proliferation, invasion and migration of MCF-7 cells following treatment with E2. To investigate the proliferation, invasion and migration of E2-treated MCF-7 cells, cells were transfected with CD151-siRNA. RT-qPCR analysis was performed to verify CD151 knockdown in MCF-7 cells and the results demonstrated that CD151 expression significantly decreased following transfection with CD151-siRNA (Fig. 6A). The effect of CD151 knockdown on cell proliferation was assessed via the CCK-8 assay. The results demonstrated that cell proliferation significantly decreased in the CD151-siRNA group compared with the control group (Fig. 6B and C). The invasive (Fig. 6D and E) and migratory (Fig. 7A and B) abilities of MCF-7 cells following CD151 interference were assessed via the Transwell and wound healing assays, respectively. The results demonstrated that cell invasion and migration significantly decreased following transfection

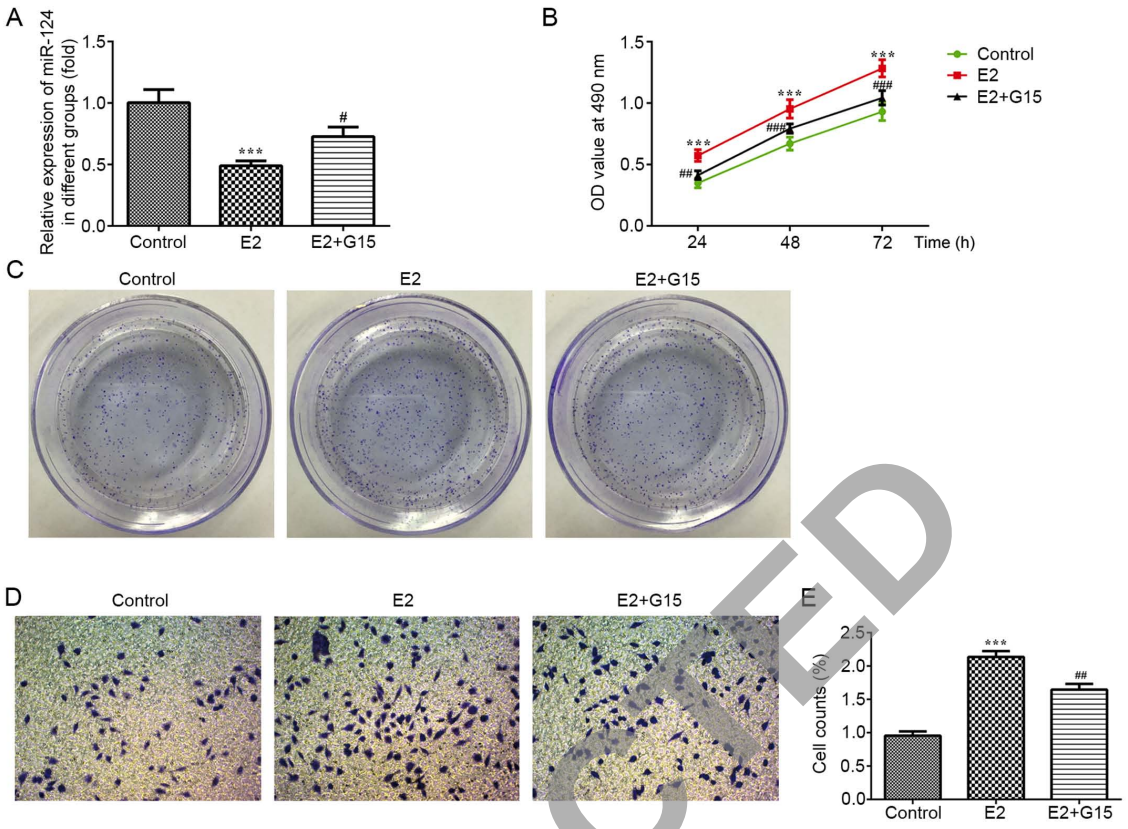

Figure 3. GPER mediates the E2-induced decrease in miR-124 expression and increases the proliferation and invasion of MCF-7 cells. (A) Reverse transcription-quantitative PCR analysis was performed to detect miR-124 expression. Cell proliferation was assessed via the (B) Cell Counting Kit-8 and (C) colony formation assays, respectively (D and E) Cell invasion was assessed via the Transwell assay. Data are presented as the mean ± standard deviation (n=3). $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. E2; ***P<0.001 vs. control. GPER, G protein -coupled estrogen receptor; E2, 17β-estradiol; miR, microRNA; OD, optical density.

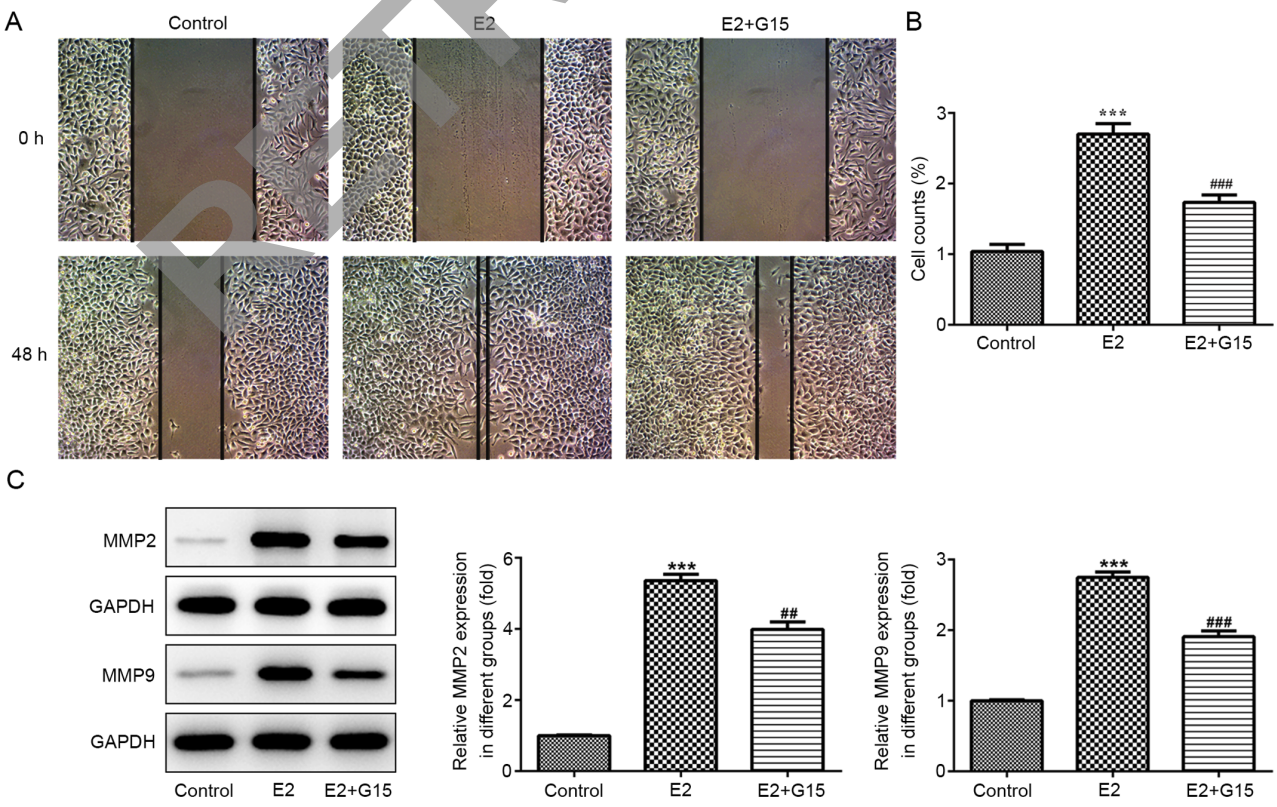

Figure 4. GPER mediates the E2-induced migration of MCF-7 cells. (A and B) Cell migration was assessed via the wound healing assay. (C) Western blot analysis was performed to detect the expression levels of migration-associated proteins. Data are presented as the mean ± standard deviation (n=3). $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. E2; ***P<0.001 vs. control. GPER, G protein -coupled estrogen receptor; E2, 17β-estradiol; MMP, matrix metalloproteinase.

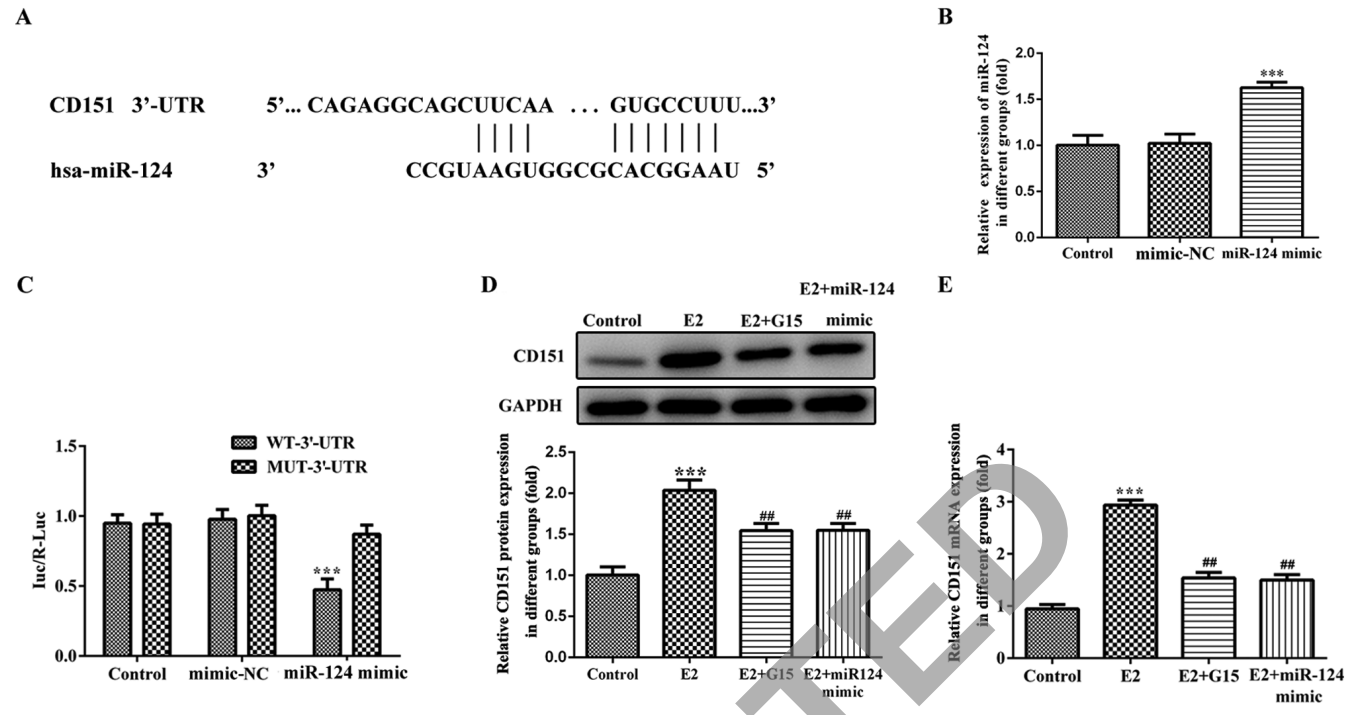

Figure 5. CD151 is a direct target of miR-124. (A) Binding region between CD151 and miR-32-5p was predicted using the TargetScan database. (B) RT-qPCR analysis was performed to detect miR-124 expression following transfection with miR-124 mimic. ***P<0.001 vs. mimic-NC. (C) The dual-luciferase reporter assay was performed to verify the association between miR-124 and CD151. ***P<0.001 vs. MUT-3'-UTR. (D) Western blot and (E) RT-qPCR analyses were performed to detect CD151 expression. Data are presented as the mean ± standard deviation (n=3). ***P<0.001 vs. control; ##P<0.01 vs. E2. miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR; E2, 17β-estradiol; NC, negative control; WT, wild-type; MUT, mutant; UTR, untranslated region.

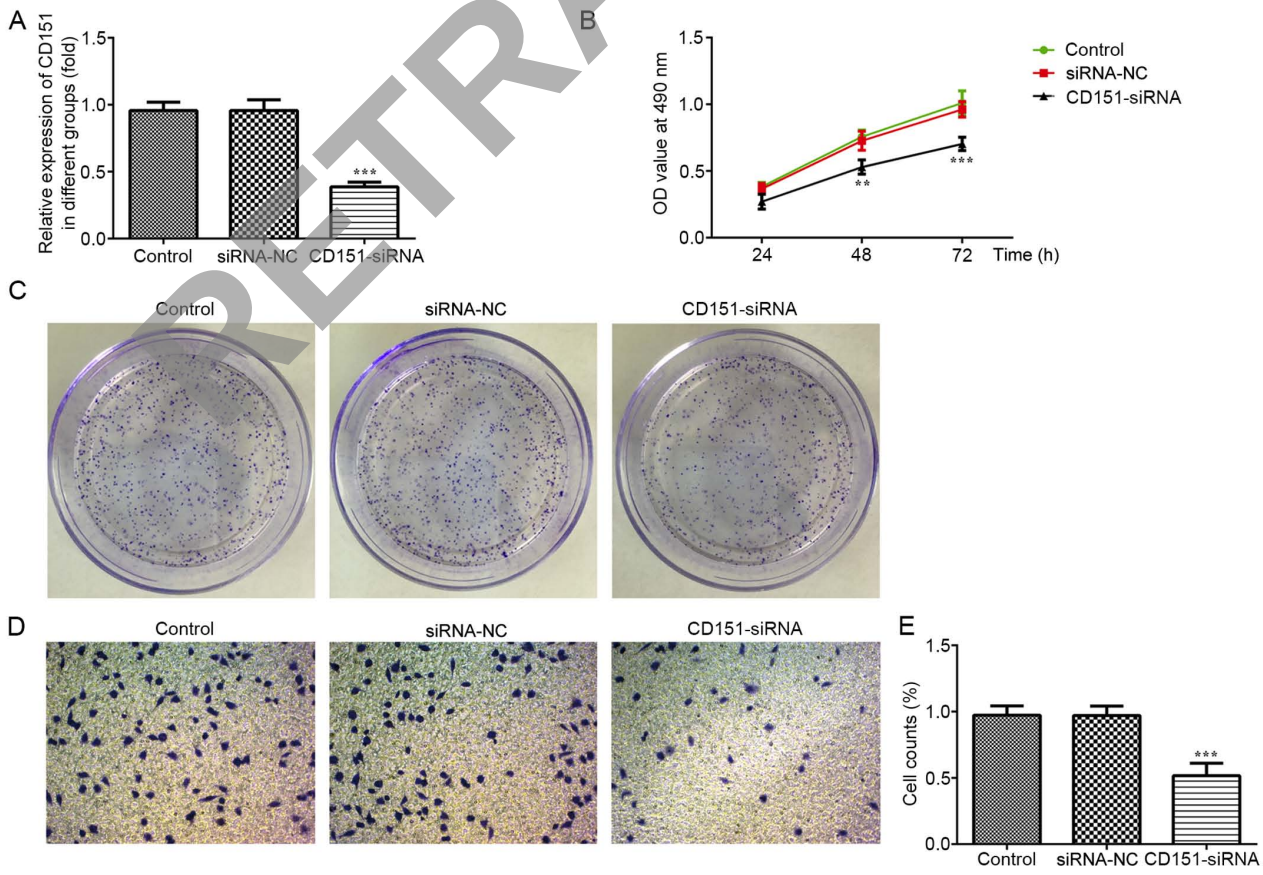

Figure 6. Effects of CD151 interference on the proliferation and invasion of MCF-7 cells treated with E2. (A) Reverse transcription-quantitative PCR analysis was performed to detect CD151 expression. Cell proliferation was assessed via the (B) Cell Counting Kit-8 and (C) colony formation assays, respectively. (Dand E) The Transwell assay was performed to assess cell invasion following CD151 interference. Data are presented as the mean ± standard deviation (n=3). **P<0.01, ***P<0.001 vs. siRNA-NC. E2, 17β-estradiol; si, small interfering; NC, negative control; OD, optical density.

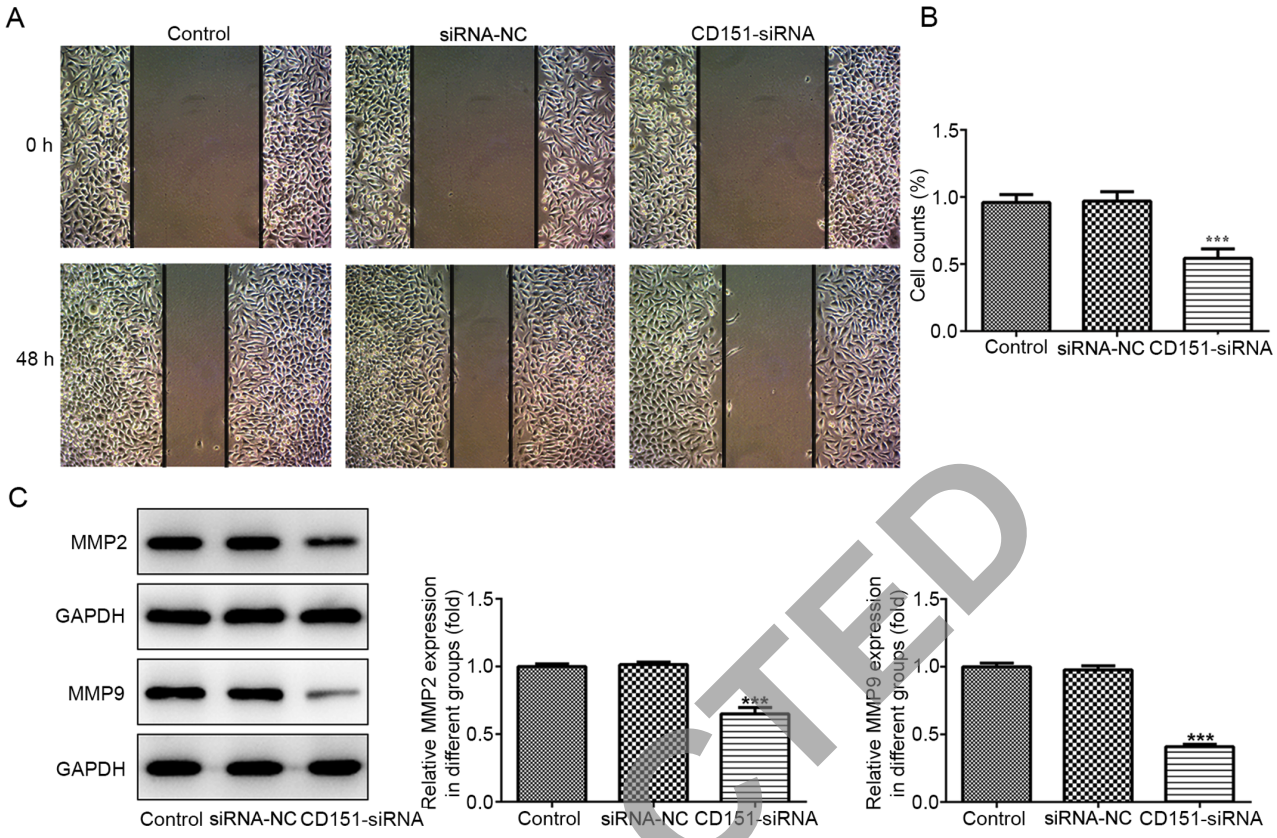

Figure 7. Effect of CD151 interference on the migration of MCF-7 cells treated with E2. (A and B) Cell migration was assessed via the wound healing assay. (C) Western blot analysis was performed to detect the expression levels of migration-associated proteins. Data are presented as the mean ± standard deviation (n=3). ***P<0.001 vs. siRNA-NC. E2, 17β-estradiol; si, small interfering; NC, negative control; MMP, matrix metalloproteinase.

with CD151-siRNA. Collectively, these results suggest that the proliferation, invasion and migration of E2-induced MCF-7 cells are inhibited following CD151 interference.

Discussion

Breast cancer is one of the most common malignancies worldwide that is responsible for a high number of fatalities (45). The number of new breast cancer cases is estimated at 272,400 in China, annually (46). The identification of novel and efficacious methods for early diagnosis and treatment of this disease remains a major challenge. In the present study, the molecular mechanism of the proliferation, invasion and migration of breast cancer cells was investigated.

Estrogens play an important role in breast cancer metastasis (47-49). E2 is one of the typical estrogens that is responsible for the occurrence of 70-80% of human breast cancer and has been generally investigated for its contribution in the development of the human mammary gland (50). It is considered that GPER can mediate estrogenic signals in breast cancer (51). Previous studies have demonstrated that GPER is bound with E2, owing to its high affinity (20,52,53). Thus, it is important to investigate the effects of E2-GPER signaling on the proliferation, invasion and migration of breast cancer cells. A previous study reported that GPER expression is upregulated in patients with breast cancer (54). Another study indicated that E2-treated cancer-associated fibroblasts exhibit a positive feedback behavior, which involves GPER/EGFR/ERK signaling and E2 production, and contributes to the progression of breast cancer (55). Breast cancer progression is associated with overexpression of GPER and PM localization, whereas the lack of GPER is associated with the long-term prognosis of primary breast cancer in PM (56). However, the molecular mechanism underlying the proliferation, invasion and migration of breast cancer cells via E2-GPER signaling remains largely unknown. The results of the present study demonstrated that GPER-E2 signaling decreased miR-124 expression, suggesting that inhibition of this target may be used as a novel treatment in breast carcinoma.

miRNAs are important post-transcriptional regulators of gene expression that play a major role in carcinogenesis (28,29). It has been demonstrated that miR-124 acts as a tumor suppressor that can inhibit the proliferation, invasion and migration of breast cancer cells (37). A previous study reported that miR-124 targets Slug to regulate epithelial-to-mesenchymal transition and metastasis of breast cancer (57). Feng *et al* (58) discovered that downregulation of miR-124 results in breast cancer progression via regulation of long non-coding RNA-MALAT1 and activation of CDK4/E_2F_1 signaling. In addition, another study demonstrated that downregulation of miR-124-3p promotes breast cancer progression by targeting Beclin-1 (59). Thus, it is crucial to investigate the expression levels and regulation of miR-124 in breast cancer. Taken together, the results of the present study demonstrated that E2 inhibited miR-124 expression via the receptor, GPER. Furthermore, E2-GPER signaling promoted CD151 expression by suppressing miR-124 expression.

In conclusion, the present study demonstrated that activation of GPER was mediated by E2 and promoted the proliferation, invasion and migration of breast cancer cells via regulation of the miR-124/CD151 pathway. These findings may provide a novel therapeutic strategy for the treatment and prognosis of breast cancer. However, use of a single breast cancer cell line is a major limitation of the present study. Thus, prospective studies will focus on using different clinical types of breast cancer cells.

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Availability of data and materials

The datasets used and or/analyzed during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

HY and CW designed and performed the experiments. HL drafted the initial manuscript. HY, HL and QW contributed to data collection, analysis and interpretation. QW reviewed and edited the manuscript. HY and QW confirmed the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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