Parthenolide Inhibits Migration and Reverses the EMT Process in Breast Cancer Cells by Suppressing TGF β and TWIST1

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Abstract

Breast cancer metastasis is the leading cause of mortality among breast cancer patients. Epithelial to mesenchymal transition (EMT) is a biological process that plays a fundamental role in facilitating breast cancer metastasis. The present study assessed the efficacy of parthenolide (PTL, *Tanacetum parthenium*) on EMT and its underlying mechanisms in both lowly metastatic, estrogen-receptor positive, MCF-7 cells and highly metastatic triple-negative MDA-MB-231 cells. Cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. Apoptosis was analyzed by FITC (fluorescein isothiocyanate) annexin V apoptosis detection kit. The monolayer wound scratch assay was employed to evaluate cancer cell migration. Proteins were separated and identified by Western blotting. Gene expression was analyzed by quantitative real-time PCR. PTL treatment significantly reduced cell viability and migration while inducing apoptosis in both cell lines. Also, PTL treatment reverses the EMT process by decreasing the mesenchymal marker vimentin and increasing the epithelial marker E-cadherin compared to the control treatment. Importantly, PTL downregulates TWIST1 (a transcription factor and regulator of EMT) gene expression concomitant with the reduction of transforming growth factor beta (TGF β) protein and gene expression in both cell lines. Our findings provide insights into the therapeutic potential of PTL to mitigate EMT and breast cancer metastasis. These promising results demand *in vivo* studies.

Introduction

Breast cancer is a complex and heterogeneous disease and common cancer in females that accounts for the 2nd greatest number of cancer-related deaths among women worldwide [1, 2]. It is estimated that breast cancer is responsible for about 15% of all cancer related deaths among women globally [3]. Recently, increased awareness and early detection have substantially reduced mortality. However, late diagnosis can increase the risk of spreading cancer cells from the primary tumor to neighboring tissues and distant organs, a process known as metastasis. In breast cancer, metastasis is responsible for the majority of deaths[4]. Thus, understanding the molecular basis of metastasis is critical to prevent and reduce breast cancer-related mortality.

The epithelial to mesenchymal transition (EMT) is a biological process that is intimately associated with cancer cell invasiveness and metastasis [5]. Evidence now suggests that in EMT, epithelial cells lose cellular polarity and interaction with the basement membrane while acquiring mesenchymal phenotypes such as cell migration and invasiveness [6]. EMT is also characterized by reduced expression of epithelial cell markers like E-cadherin and higher expression of mesenchymal cell markers such as vimentin and N-cadherin [7]. Substantial evidence suggests that signal transduction pathways including NOTCH and WNT are associated with EMT and contribute to disease progression [8]. EMT is also activated by the cytokine transforming growth factor beta (TGF β) [9] and can be controlled by transcription factors like SNAIL and TWIST [10]. Because of the clear roles of EMT in breast cancer metastasis and recurrence, research targeting EMT related pathways and/or transcription factors to regulate EMT is currently

drawing considerable attention as this may open a new avenue for drug development to treat and prevent metastatic breast cancer.

Phytochemicals have been gaining considerable research interests for their effectiveness and safety to treat different diseases including cancer [11, 12]. Parthenolide (PTL) is a sesquiterpene lactone extracted from the flowers and fruits of the plant *Tanacetum parthenium* (Feverfew). PTL has shown many biological activities including anti-inflammatory and anti-cancer activities. PTL reduced MCF-7 breast cancer stem cell proliferation by regulating the NF- κ B pathway [13]. Berdan et al. reported that PTL regulates breast cancer cell proliferation, survival, and motility by targeting focal adhesion kinase 1 (FAK1) signaling pathway [14]. Carlisi et al. revealed that PTL and its soluble analog dimethylaminoparthenolide (DMAPT) promote cytotoxicity in breast cancer stem-like cells. Additionally, they found that PTL and DMAPT mediated induction of cytotoxicity was associated with increased reactive oxygen species (ROS) production, upregulation of NADPH oxidases, and downregulation of manganese superoxide dismutase and catalase [15]. Moreover, PTL was found to inhibit proliferation, migration, and invasion in pancreatic cancer cells [16]. However, PTL's effects on EMT in both MCF-7 and MB-231 breast cancer cells and its underlying mechanism remains unknown.

The present study evaluated the effects of PTL (2 and 5 μ M) on migration, EMT, and apoptosis in both lowly (MCF-7) and highly (MDA-MB-231) metastatic breast cancer cells. Additionally, we assessed the role of TGF β and EMT-related transcription factors, SNAIL1 and TWIST1, in PTL mediated regulation of EMT in breast cancer cells.

Our results revealed that PTL treatment significantly reduced cell viability and migration as predicted, but also induced apoptosis in both MCF-7 and MB-231 breast cancer cells. Furthermore, PTL treatment reversed EMT by reducing vimentin and increasing E-cadherin. This PTL-mediated reversal of EMT was associated with the suppression of TGF β and TWIST1 gene expression in both cell lines.

Materials And Methods

Cell culture and treatment

MCF-7 (estrogen-receptor positive) and MDA-MB-231 (triple-negative) breast cancer cells were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (11965-118, Life Technologies, Carlsbad, CA, USA) which contained 10% fetal bovine serum (FBS, 10437-028, Thermo Fisher Scientific, Waltham, MA, USA) and 1% penicillin streptomycin, at 37°C in 5% CO₂ incubator. Cells between passages 10 and 15 were used for the experiments. For treatment purposes, MCF-7 and MB-231 cells were serum starved for twelve hours with DMEM without FBS and Penicillin Streptomycin. 70-80% confluent cells were treated with 2 and 5 μ M parthenolide (PTL) (Parthenolide, Item No 70080, Cayman Chemical Company, Ann Arbor, MI, USA) and untreated (control) for 24 hours using DMEM media with 0.5% FBS. Cells were harvested post treatment for RNA isolation and Western Blot Analysis. Key proteins and genes were analyzed by Western blot and qRT-PCR respectively.

MTT assay

Cell proliferation/viability were measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay kit (k299-1000, BioVision Inc., Milpitas, CA, USA). Cells were seeded at 1×10^4 cells/well in 96-well plates and treated (2 μ M or 5 μ M) and untreated (control) PTL for 24 hours. After 24 hours of treatment, the medium was discarded and 50 μ l of serum free medium and 50 μ l of MTT reagent were added into each well. For background control, 50 μ l of MTT reagent were added into a well containing medium only. Plates were incubated at 5% CO₂ and at 37°C temperature for 3 hours. After incubation, 150 μ l of MTT solvent was added into each well. The plate was wrapped in aluminum foil, shaken on a shaker for 15 minutes, and then the absorbance was taken at 590 nm wavelength and proliferation was measured using the data of three independent experiments as previously described [17].

Monolayer wound scratch assay

MCF-7 and MDA-MB-231 cells were plated at 5×10^5 cells/well in 12-well plates. After reaching total confluence, a “scratch” was created using a 10 μ l pipette tip by scraping the monolayer in a neat, straight line. The previous media was discarded, cells were washed with Dulbecco’s phosphate buffered saline (DPBS, 1 ml for each well) and treated with and without PTL (2 and 5 μ M) for 24 hours. Wounds were imaged every 10 min for 24 hours, using a Nikon Eclipse motorized microscope with incubator at 5% CO₂ and 37°C temperature (Nikon Instruments Inc., Melville, NY, USA). The initial and the final wound widths were measured and the migration speed was calculated by taking the difference of the initial and final wound width and dividing them by the total elapsed time [18].

Apoptosis analysis

To detect apoptosis, MCF-7 and MBA-231 cells were plated at 5×10^5 cells/well in a 12 well plate until sub-confluent. Then the cells were treated with and without parthenolide (2 and 5 μ M) for 24 hours. After 24 hours, the medium was removed from the wells and 100 μ l annexin V binding buffer was added along with the stain fluorochrome conjugated annexin V (5 μ l) using the FITC Annexin V Apoptosis Detection Kit I (BD Biosciences, San Jose, CA, USA) following manufacturer’s protocol. Then the cells were incubated for 15 minutes at room temperature and the plate was wrapped with foil for protection from light. The cells were washed again and resuspended with 200 μ l binding buffer and analyzed using a Nikon Eclipse motorized microscope with incubator (Nikon Instruments Inc., Melville, NY, USA).

Cytosolic fraction preparation

Cells were plated at 1×10^6 cells/well in 6 cm plates until sub-confluent, then treated with and without PTL (2 and 5 μ M) for 24 hours. After that, the medium was removed from the wells and washed with cold DPBS (2ml/plate). Then the cell lysate was collected using a scraper after adding 75 μ l of Buffer A (10 mM HEPES, pH 7.5, 10 mM KCL, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 1mM PMSF, 2 μ g/ml each of aprotonin and leupeptin). It was then centrifuged at 12,000 $\times$ g for 1 minute. The supernatant cytoplasmic

extracts were saved. Protein concentration was measured with a protein assay kit (Bio-Rad, Hercules, CA, USA).

Western-blot analysis

Equal amount of proteins (40-60 µg) were separated by electrophoresis on 12% SDS PAGE gels and transferred to PVDF membranes. Membranes were blocked using protein-free Tween 20 blocking buffer (37571, Thermo Scientific) for one hour, and probed using primary and secondary antibodies as described before [19, 20]. The primary antibodies used for the study are vimentin (DSHB, University of Iowa, Iowa City, USA, dilution 1:300) and E-cadherin (701134, Thermo Fisher, dilution 1:200), TGF-β (sc-146, Santa Cruz Biotechnology, Inc., Dallas, TX, USA, dilution 1:300). The secondary antibodies used were (anti-rabbit 0711-625-152, or anti-mouse 115-625-146, 1:5000 dilution, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA). Membranes were scanned and bands quantified using the Odyssey infrared Imaging System (LiCor Biosciences, Lincoln, NE, USA).

Gene expression by quantitative real-time PCR (qRT-PCR)

Both MCF-7 and MBA-231 cells were serum starved for 12 hours with DMEM without FBS and Penicillin Streptomycin and, when the cells were 70-80% confluent, treated with PTL 2 and 5 µM for 24 hours. Total RNA was isolated from treated MCF-7 cells (plated at 5×10^5 cells/well in 6-well plates) using the Qiagen RNeasy Plus kit (74136, Qiagen, Germantown, MD, USA). Reverse transcription was performed using total RNA with the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA). qPCR was performed using primer sets (Sigma-Aldrich, St. Louis, MO, USA) for genes of interest and reference gene and iQ SYBR Supermix (Bio-Rad) following manufacturer's protocol. Total RNA isolation, reverse transcription, and quantitative real-time PCR (qPCR) were performed as previously described [21]. RNA expression data were normalized to levels of reference gene GAPDH using the comparative threshold cycle method.

The primer sequences used are:

Human GAPDH: F- AGCCTCAAGATCATCAGCAATGCC

Human GAPDH: R- TGTGGTCATGAGTCCTTCCACGAT

Human TWIST1: F- GCACCATCCTCACACCTCT

Human TWIST1: R- CTGATTGGCACGACCTCTTG

Human SNAIL1: F- TCGCTGCCAATGCTCATC

Human SNAIL1: R-GGAAGAGACTGAAGTAGAGGAGAA

Human TGFβ: F- GGCCCTGCCCCTACATTT

Human TGF β : R- CCGGGTTATGCTGGTTGTACA

F; Forward, R; Reverse

Statistical analysis

Statistical comparisons between groups were made either using Student's t-test, or one-way ANOVA. All values are reported as mean $\pm$ SEM. Differences were considered to be statistically significant at P values of 0.05 or less.

Results

PTL treatment significantly decreased viability and apoptosis in MCF-7 and MDA-MB-231 breast cancer cells

We first investigated the effects of PTL treatment on cell growth by checking cell viability using the MTT assay. We used both low metastatic MCF-7 (estrogen-receptor positive) and highly metastatic MDA-MB-231 (triple negative) breast cancer cells and used 2 and 5 μ M PTL based on published studies [12, 15]. The results revealed that PTL treatment (2 μ M) did not significantly change cell viability at 0 and 24 hours compared to the control treatment but significantly reduced cell viability after 48 hours ($P < 0.01$, Fig. 1a). In contrast, 5 μ M of PTL was found to more efficiently decrease MCF-7 cell viability after 24 and 48 hours (70% and 85% compared with the control treatment, $P < 0.05$, Fig. 1a). Surprisingly, 5 μ M PTL treatment also exerted a significant reduction of cell viability in MCF-7 cells at 0 hour (Fig. 1a, $P < 0.05$). Treatment of MB-231 cells with 5 μ M of PTL also significantly decreased (30–40%) cell viability at 0, 24 and 48 hours ($P < 0.05$, Fig. 1b). In contrast, 2 μ M of PTL treatment demonstrated a trend to reduce cell viability after 24 and 48 hours though the reduction was not statistically significant when compared to the controls. The results suggest that PTL can inhibit cell growth by reducing cell viability.

To determine the mechanism by which PTL inhibits growth in breast cancer cells, we assessed the effect of PTL on apoptosis. In apoptotic cells, the membrane phospholipid phosphatidylserine translocates from the inner to the outer portion of the membrane and can be detected by annexin V-FITC, a phospholipid-binding protein [22]. To assess the effects of PTL on apoptosis, MCF-7, and MB-231 cells were treated with (2 and 5 μ M) or untreated (control) for 24 hours. FITC Annexin V apoptosis detection kit I (BD Biosciences) was used [23]. Numbers of dead cells were analyzed using a Nikon Eclipse motorized microscope with an incubator (Nikon Instruments Inc.). PTL treatment (2 μ M) showed a trend to induce apoptosis in MCF-7 cells compared to the controls but was not statistically significant. PTL (5 μ M) significantly increased apoptosis after 24 hours of treatment in MCF-7 breast cancer cells ($P > 0.05$, Fig. 1c). In contrast, both 2 and 5 μ M of PTL significantly increased apoptosis in MB-231 cells ($P < 0.05$, $P < 0.01$, Fig. 1d). These results indicate that PTL (5 μ M) treatment is more effective at inducing apoptosis

in both MCF-7 and MB-231 breast cancer cells and may be responsible for the reduction of PTL-mediated cell viability.

PTL treatment inhibited migration of MCF-7 and MB-231 breast cancer cells

In a metastatic condition, breast cancer cells can spread from the primary site of origin to a distant organ. The metastasis of breast cancer cells causes the majority of breast cancer-related deaths. The underlying mechanism of metastasis remains largely unknown. The prerequisite for breast cancer spread and metastasis is the ability of the cells' to migrate and invade the surrounding tissues [24]. We have found that PTL treatment decreased cancer cell viability and increased apoptosis. Next, we evaluated if there is an association of these results to breast cancer cell migration. We treated both breast cancer cells with 2 and 5 μ M PTL for 24 hours. The motility of MCF-7 and MB-231 cells was assessed by the monolayer wound scratch assay. PTL 2 and 5 μ M treatment significantly reduced MCF-7 cell migration when compared to the control treatment ($P < 0.05$, Fig. 2a). Similar results were found with MB-231 cells ($P < 0.05$, Fig. 2b). These observations revealed that PTL mediated induction of apoptosis and reduced cell viability may contribute to the reduced migration of both MCF-7 and MB-231 cells.

PTL treatment reversed the EMT process in MCF-7 and MB-231 cells

Invasion and subsequent migration of cancer cells are often preceded and mediated by an epithelial-to-mesenchymal transition [25]. We found that PTL treatment reduced migration in breast cancer cells. We wanted to evaluate whether PTL-mediated reduction of migration has any influence on EMT. We checked key proteins (vimentin and E-cadherin) implicated in the EMT process by Western blotting. Treatment with 2 μ M of PTL showed a trend in reducing the mesenchymal marker vimentin and modestly increased (not significantly) the epithelial marker E-cadherin in MCF-7 cells compared to the controls (Fig. 3a). However, treatment with 5 μ M of PTL significantly reduced vimentin ($P < 0.01$) and increased E-cadherin ($P < 0.05$) in MCF-7 breast cancer cells (Fig. 3a). In MBA-231 cells, 2 μ M of PTL did not significantly change vimentin expression but increased E-cadherin expression compared to the controls ($P < 0.05$, Fig. 3b). Similar to MCF-7 cells, 5 μ M of PTL significantly decreased vimentin ($P < 0.05$) and induced E-cadherin protein expression ($P < 0.01$) in MBA-231 cells (Fig. 3b). The results suggest PTL (5 μ M) is more efficient at reversing EMT by reducing mesenchymal markers and inducing epithelial markers.

PTL treatment reduced TGF β expression in MCF-7 and MBA-231 cells

TGF β is a cytokine, plays a critical role in migration, EMT activation, and tumor progression [26]. To understand the mechanism of PTL-mediated regulation of migration and the EMT process, we checked TGF β protein and gene expression in breast cancer cells. MCF-7 cells treated with 2 and 5 μ M of PTL significantly reduced TGF β protein expression ($P < 0.05$, Fig. 4a). Similarly, 2 and 5 μ M of PTL significantly reduced TGF β protein expression in MB-231 cells compared to the controls ($P < 0.05$ and $P < 0.01$, Fig. 4b). We also checked the TGF β gene expression in PTL treated MCF-7 and MBA-231 cells. Only PTL at 5 μ M significantly reduced TGF β gene expression in MCF-7 cells ($P < 0.05$, Fig. 4c). Interestingly, TGF β gene expression was significantly reduced by both PTL 2 μ M and PTL 5 μ M treatments in MBA-231 cells

($P < 0.01$, Fig. 4d). These results suggest that PTL treatment may regulate migration and EMT in breast cancer cells by targeting TGF β .

PTL treatment reduced TWIST1 gene expression in MCF-7 and MB-231 cells

Transcription factors TWIST and SNAIL play an essential role in EMT regulation [27, 28]. Accumulating evidence suggests that TGF β activates EMT by inducing SNAIL1 and TWIST1 and in hepatic and prostate cancer cells [29, 30]. To gain further insight into the mechanisms of PTL-mediated reversal of EMT in breast cancer cells, we checked the gene expression of TWIST1 and SNAIL1. PTL treatment (2 and 5 μ M) did not exert any significant changes in SNAIL1 gene expression in MCF-7 and MB-231 breast cancer cells (Fig. 5a and 5b). Only 5 μ M of PTL significantly reduced TWIST1 gene expression in MCF7 breast cancer cells ($P < 0.05$, Fig. 5a). Similarly, TWIST1 gene expression was significantly reduced in MB-231 cells by 5 μ M of PTL treatment ($P < 0.05$, Fig. 5b). The results suggest that PTL may reverse EMT in breast cancer cells by downregulating TWIST1.

Discussion

The present study investigated the anticancer properties and underlying molecular mechanisms of PTL in both low/non metastatic, estrogen-receptor positive, MCF-7 cells and highly metastatic triple-negative MDA-MB-231 cells. We demonstrated here, for the first time, that PTL treatment reverses the EMT process by significantly reducing the mesenchymal marker vimentin and inducing the epithelial marker E-cadherin protein expression in both MCF-7 and MDA-MB-231 cells. The reversal of EMT was concurrent with the reduction of TGF β protein and gene expression, and the EMT inducing transcription factor TWIST1 gene expression.

EMT is a biological process that enhances tumor cell invasiveness and aids tumor cells during intravasation from the primary site, facilitating tumor metastasis [6, 31]. Substantial evidence now suggests that EMT is also linked to cancer drug and chemotherapy resistance in a variety of cancers, including breast cancer [32, 33]. Accumulating evidence also suggests that breast cancer metastasis accounts for most of the breast cancer mortality in women worldwide [4]. Thus, the present findings of the reversal of EMT in breast cancer cells following PTL treatment is significant.

The present study also demonstrated PTL-mediated induction of apoptosis and inhibition of cell viability and migration in both MCF-7 and MBA-231 breast cancer cells and is consistent with previous findings [16, 34]. Cell migration is considered the key early event in the EMT process [6]. The PTL-mediated reduction of migration and induction of apoptosis in both MCF-7 and MB-231 cells may be associated with the reversal of the EMT process. Moreover, the reversal of EMT and inhibition of breast cancer cell migration will eventually prevent breast cancer progression and metastasis.

Understanding the molecular regulation of EMT is critical to prevent metastasis and cancer-related mortality. EMT is also robustly regulated by transcription factors (TFs) [6]. These include SNAIL that represses E-cadherin [35] and TWIST which controls EMT and metastasis [27]. Strong evidence suggests

that these transcription factors regulate EMT by downregulating epithelial markers and upregulating mesenchymal markers [27, 35]. For example, higher TWIST1 expression was found to correlate with lower epithelial marker E-cadherin expression [36]. It was also revealed that TWIST1 is required for intravasation and cell migration while deletion of TWIST1 reduced the number of micrometastases in the lung in a mouse model of breast cancer [36]. Brenot et al. revealed that SNAIL1 promotes breast cancer progression and metastasis by increasing the production of granulocyte-macrophage colony-stimulating factor (GM-CSF) and regulating tumor-associated macrophages (TAMs) function in the mouse model of breast cancer. These results indicate that both TWIST1 and SNAIL1 are crucial to regulate tumor progression by modulating EMT.

PTL was found to reduce vimentin in MCF-7 cells, although the mechanism was unknown [37]. We have observed a PTL-mediated downregulation of TWIST1 gene expression in breast cancer cells. It is thus possible that PTL-mediated suppression of TWIST1 may be linked with PTL-mediated regulation of the EMT process.

TGF β , a cytokine, plays a fundamental role in cancer progression by regulating EMT [26]. Evidence supports that TGF- β acts as a tumor suppressor during the initial stage of tumorigenesis [38]. In contrast, during tumor progression, cancer cells secrete high levels of TGF β , facilitating EMT, invasion, and metastasis [38]. Zhu et al. reported that PTL mitigates TGF β -mediated EMT induction by regulating vimentin, E cadherin and SNAIL in colorectal cancer cells [39]. To gain more insight into the mechanisms of PTL-mediated reversal of EMT and suppression of TWIST1 gene expression in breast cancer, we checked TGF β protein and gene expression in MCF-7 and MB-231 cells. We observed a significant reduction of TGF β protein and gene expression in PTL treated MCF-7 and MB-231 cells. Our results suggest that PTL-mediated inhibition of TGF β , may, in part, be responsible for the reversal of the EMT process in breast cancer cells.

Regarding the mechanism of TGF β -mediated regulation of EMT, studies suggest that TGF β -SMAD signaling plays a significant role [40]. In the SMAD-dependent pathway, TGF β activates the receptor complex and phosphorylates SMAD2 and SMAD3. The phosphorylated SMAD proteins form a complex with SMAD4 and translocate to the nucleus, where they interact with EMT-inducing transcription factors to regulate the expression of target genes implicated in EMT [40, 41]. Studies have shown that inhibition of TGF β -SMAD2 reverses EMT and mitigates breast cancer metastasis [42, 43]. We have demonstrated that PTL treatment significantly reduced the expression of TGF β protein and gene and TWIST1 gene in both MCF-7 and MB-231 cells. It is thus presumed that PTL, by suppressing TGF β may downregulate the transcription factor TWIST1 and its target genes and ultimately reverses EMT in breast cancer. However, we did not evaluate the expression of other proteins in the TGF β signaling pathways, especially the downstream SMAD proteins, in our study. Additionally, TGF β may regulate EMT by modulating other signaling pathways [44–46]. EMT, in vivo, can also be regulated by signals elicited from the stromal cells in the tumor microenvironment [47]. Thus, future studies evaluating the effects of PTL, between the tumor-stroma interaction, will be required for more clarification.

Though this present study reported some important anticancer properties of PTL, it has some limitations. We reported here the downregulation of TGF β after PTL treatment, which may be responsible for the reversal of EMT. However, we do not yet know the underlying molecular details of PTL-mediated regulation of TGF β . Recent studies have shown that microRNAs (miRNAs), long non-coding RNAs (lncRNAs), transcriptional regulators TAZ and YAP, regulate EMT by modulating TGF β signaling [48–50]. It is also not clear about the exact role of estrogen receptors (ERs) in PTL-mediated downregulation of TGF β . Thus, future studies are warranted for clarification.

Altogether, our study revealed that PTL treatment (5 μ M) is effective in reducing migration, decreasing cell viability, and increasing apoptosis in both MCF-7 and MDA-MB-231 breast cancer cells. Importantly, PTL significantly reversed the EMT process by decreasing the mesenchymal signature protein vimentin and increasing the epithelial signature protein E-cadherin in both cancer cells. The reversal of EMT by PTL was associated with the suppression of both TWIST1 and TGF β (Fig. 5c). Thus, PTL might have important therapeutic potential, either alone or in combination, with other existing cancer drugs toward preventing breast cancer aggressiveness and metastasis. This potential warrants future studies in a mouse model of breast cancer metastasis.

Abbreviations

ER α ; estrogen receptor α , EMT; Epithelial to mesenchymal transition, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), TGF β ; transforming growth factor beta, TAZ; Tafazzin, YAP; yes-associated protein, ROS; reactive oxygen species. GM-CSF; granulocyte-macrophage colony-stimulating factor

Declarations

Data availability

Data will be made available from the corresponding author on reasonable request.

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Contributions

Conceptualization: SMR, HBS, FH; Methodology and Data Curation: HBS, JMS, JSK, MTM; Analysis: HBS, JMS, JSK, SMR, MKZ; Writing- Original draft preparation: HBS, SMR; Writing - review and editing: SMR, FH, LSG; All authors read and approved the final manuscript.

Conflict of interest

The authors don't have any conflict of interest.

Ethics approval

Not applicable.

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Figures

Figure 1

Effect of PTL on viability and apoptosis in MCF-7 and MB-231 breast cancer cells. a MCF-7 and b MB-231 cells were treated without (control) and with 2 and 5 µM of PTL for 24-48 hours. Cell viability/proliferation were measured using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay kit (BioVision k299-1000). Data are mean $\pm$ SEM. N=3. * $P < 0.05$ and ** $P < 0.01$ as tested by one-way ANOVA followed by Tukey's post hoc test. All comparisons were considered significant when

P<0.05. Apoptosis was measured by FITC Annexin V Apoptosis Detection Kit I (BD Biosciences) in (c) MCF-7 and (d) MB-231 cells. Representative images are shown. Data are mean $\pm$ SEM and expressed as percent change over control. N=3. *P < 0.05 and **P<0.01 as tested by one-way ANOVA followed by Tukey's post hoc test. All comparisons were considered significant when P<0.05.

Figure 2

Effect of PTL on migration in breast cancer cells. Cell migration was determined by Monolayer wound scratch assay. Representative migration images in (a) MCF-7, and (b) MB-231 cells. Data are mean $\pm$ SEM and expressed as percent change over control. N=3. *P < 0.05 as tested by one-way ANOVA followed by Tukey's post hoc test. All comparisons were considered significant when P<0.05.

Figure 3

PTL reverses EMT in breast cancer cells. Immunoblots and densitometric values of vimentin and E-cadherin in (a) MCF-7 and (b) MB-231 cells. Representative blots are shown. MCF-7 and MB-231 cells were treated without (control) or with 2 and 5 μ M of PTL for 24 hours. Representative blots are shown. Data are mean $\pm$ SEM and expressed as percent change over control. N=3. *P<0.05 and **P<0.01 as tested by one-way ANOVA followed by Tukey's post hoc test. All comparisons were considered significant when P<0.05.

Figure 4

Effects of PTL on TGFβ protein and gene expression. Immunoblots and densitometric values for TGFβ in (a) MCF-7 and (b) MB-231 cells. Representative blots are shown. Data are presented as mean ± SEM. Relative TGFβ gene expression in (c) MCF-7 and (d) MB-231 cells analyzed by qRT-PCR. *P<0.05 and **P<0.01 as tested by one-way ANOVA followed by Tukey's post hoc test. All comparisons were considered significant when P<0.05.

Figure 5

Effects of PTL on SNAIL1 and TWIST1 gene expression. SNAIL1 and TWIST gene expression in (a) MCF-7 and (b) MB-231 cells, analyzed by qRT-PCR. Data are mean $\pm$ SEM. N=3. *P<0.05 as tested by one-way ANOVA followed by Tukey's post hoc test. All comparisons were considered significant when P<0.05. c Model of PTL mediated regulation of EMT in breast cancer cells. PTL treatment increased apoptosis, inhibited migration, and reversed EMT via suppression of TGF β and TWIST1.