RESEARCH ARTICLE

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TMEPAI Confers Paclitaxel Resistance in Triple-Negative Breast Cancer Cells by Promoting AKT Phosphorylation and Its Downstream Cascade

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Abstract

Objective: TMEPAI (transmembrane prostate androgen-induced protein) is one of the proteins associated with the resistance of triple-negative breast cancer (TNBC) to various cytotoxic medicines. However, it has remained uncertain how TMEPAI mechanistically contributes to TNBC resistance to paclitaxel. Thus, this study aimed to investigate the effect and possible mechanism of TMEPAI gene editing via CRISPR-Cas9 on the response of triple-negative breast cancer cells to paclitaxel. **Methods:** The present study was conducted on wild-type triple-negative breast cancer cells (BT-549) and BT-549 cells with TMEPAI knocked out using CRISPR-Cas9. Both cell types underwent treatment with TGF-β, followed by paclitaxel, and were evaluated for cell viability and the expression of cell proliferation, apoptosis, drug efflux transporters, and epithelial-mesenchymal transition markers. **Result:** TMEPAI knock-out cells exhibited a markedly increased susceptibility to paclitaxel, as characterized by decreased viability and elevated expression of pro-apoptotic genes (*Bax, caspase-3, caspase-9*), as well as a reduction in anti-apoptotic markers (*Bcl-2*). The presence of TMEPAI perpetuated the phosphorylation of AKT (pAKT/AKT), elevated the expression of drug efflux transporters (particularly P-glycoprotein and MRP-1), and facilitated epithelial-mesenchymal transition (EMT), as evidenced by increased levels of Snail, Zeb1, and Twist. All these effects were diminished in TMEPAI-knock-out triple-negative breast cancer cells. **Conclusion:** TMEPAI appears to facilitate paclitaxel resistance in triple-negative breast cancer cells by promoting cell survival signaling, inhibiting apoptosis, enhancing drug efflux, and initiating epithelial-mesenchymal transition (EMT). Targeting TMEPAI may be a viable approach to overcoming resistance and improving treatment outcomes in triple-negative breast cancer cells.

Keywords: AKT phosphorylation- efflux transporters- epithelial-mesenchymal transition- paclitaxel

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Introduction

Triple-negative breast cancer (TNBC) is the most aggressive type of breast cancer, with a poor prognosis and the highest mortality rate compared to other types of breast cancer. This type of breast cancer also has limited therapeutic options, including cytotoxic/chemotherapy agents such as doxorubicin, paclitaxel, and cisplatin [1, 2]. Additionally, the emergence of breast cancer resistance to the previously mentioned cytotoxic drugs is exceedingly rapid, allowing disease recurrence. Numerous studies have explored TNBC resistance to cytotoxic drugs and chemotherapy to address this issue [3–5].

The emergence of chemoresistance in TNBC is multifaceted, resulting from the intricate interactions among the tumor microenvironment, epigenetics, drug efflux mechanisms, and cancer stem cells. Modifications of many signaling pathways regulate these interactions. Furthermore, the considerable variability of TNBC, underscored by the presence of many molecular markers, poses a substantial challenge to effective treatment [6, 7].

TMEPAI (transmembrane prostate androgen-induced) is one of the proteins associated with the resistance of triple-negative breast cancer (TNBC) to various cytotoxic medicines. TMEPAI is a protein considered to act as a converter for TGF-β from a tumor suppressor to a tumor promoter [8–10]. TMEPAI is overexpressed in lung, colon, pancreatic, breast, and kidney cancer cells, inducing tumorigenic activity [10]. The importance of TMEPAI in tumorigenic activity in cancer remains a topic of debate

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[10]. TMEPAI is reportedly elevated in about two-thirds of individuals with triple-negative breast cancer [11]. Elevated TMEPAI expression correlates with reduced survival rates and unfavorable prognosis in TNBC patients [11, 12].

Regarding TMEPAI's role in drug resistance, the suppression of TMEPAI in prostate cancer enhances the proliferation of cancer cells, thereby conferring sensitivity to the androgen antagonists bicalutamide and enzalutamide [13, 14]. Our prior research has also shown that TNBC with a knocked-out TMEPAI gene exhibits enhanced cellular sensitivity to doxorubicin. Additional analyses demonstrated that TNBC cells with elevated TMEPAI levels demonstrate reduced sensitivity to paclitaxel. However, it has remained uncertain how the mechanism of TMEPAI involvement contributes to TNBC resistance to paclitaxel. Thus, this study aimed to investigate the effect and possible mechanism of TMEPAI gene editing via CRISPR-Cas9 on the response of triple-negative breast cancer cells to paclitaxel.

Materials and Methods

Cell Culture and Drug Treatment

Triple-negative breast cancer cells, BT-549 wild-type cells, were obtained from the ATCC and maintained in the Laboratory of Experimental Pathology, University of Tsukuba. BT-549 TMEPAI-knock-out cells were established and described previously by our group [14, 15]. Maintenance of the cells was performed in Dulbecco's Modified Eagle Medium DMEM (Invitrogen), supplemented with 10% Fetal Bovine Serum/FBS (Gibco), 10 µg/mL of insulin, 100 units/mL of penicillin G, and 0.1 mg/mL streptomycin sulfate (Wako). The cells were incubated in a CO_2 incubator at 37°C. The experiments were conducted on cells at 70% confluency under starvation in 1% Fetal Bovine Serum (Gibco) in tissue culture dishes (Corning). Cells were initially exposed to 2 ng/mL recombinant human TGF-β (Wako) for 24 hours to induce TMEPAI. Subsequently, the combination of 2 ng/mL TGF-β and 12 nM paclitaxel (Wako) (treatment), or 2 ng/mL TGF-β and vehicle (control), was administered for an additional 24 hours. Those concentrations were determined based on the IC50 value in BT-549 cells. Following harvesting and cell counting, these cells were processed to isolate RNA and protein for downstream analysis. All treatments were conducted in triplicate, with independent experiments performed in duplicate.

RT-PCR

RNA from cell lysates was extracted using the Total RNA Mini Kit for Cultured Cells (Geneaid). Concentrations of RNA were measured with a Nanodrop 200 UV-Vis Spectrophotometer. Reverse transcription of 100 ng of total RNA into complementary DNA was performed in the presence of ReverTra Ace (Toyobo). For the quantitative RT-PCR, 100 ng of equal cDNA was used, performed on the LightCycler® 480 System from Roche, with Thunderbird® SYBR qPCR Mix from Toyobo. The mRNA relative expression was determined using $2^{-\Delta\Delta CT}$ and the reference gene β-actin. Primer sequences were obtained from the previous study [15].

Western Blotting

Equal amounts of cell lysates were analyzed by SDS-PAGE with antibodies for β-actin, phosphorylated SMAD3, total SMAD3, phosphorylated AKT, total AKT, PI3K, Vimentin, E-Cadherin, and MRP-1 (Cell Signaling). Primary antibodies used were as follows: AKT (Cell Signaling Technology, CST #4685), phospho-AKT (Ser473) (CST #3787), β-Actin (CST #4967), GAPDH (CST #2118), *caspase-3* (CST #9662), Cleaved *caspase-3* (CST #9664), and TMEPAI (kindly provided by the Experimental Pathology Laboratory, University of Tsukuba). Equal amounts of proteins loaded (50 µg) were denatured with buffer Laemmli, separated on 8–12.5% sodium dodecyl sulfate-polyacrylamide (SDS_PAGE) gels by electrophoresis, and transferred to membranes with nitrocellulose (Bio-Rad). Membranes were blocked for 1 hour or 2 hours for the phosphorylated proteins in 5% skim milk in Tris-buffered saline with Tween-20, then incubated overnight at 4°C with primary antibodies diluted 1:1000 in Tris-buffered saline containing 0.01% Tween-20 (TBST). Afterward, the membranes were washed and incubated with 1:4000 dilutions of secondary antibody at room temperature for 1 hour. Protein expression was then visualized by enhanced chemiluminescence (ImmunoStar, Wako) and analyzed using a Gel Documentation System (UVTech).

Statistical Analysis

Comparison across four treatment groups was analyzed by one-way ANOVA, followed by the post-hoc Tukey test. The results are expressed as mean ± SD, and a value of $p < 0.05$ was considered statistically significant.

Results

TMEPAI weakened the paclitaxel's cytotoxic effect

Paclitaxel showed its cytotoxic effect in both wild-type and knock-out cells by suppressing cell viability. However, in the absence of TMEPAI, the cells were more sensitive to paclitaxel as compared to the wild-type cells (Figure 1).

TMEPAI contributes to the failure of paclitaxel in reducing the cell proliferation marker Ki-67

Cell proliferation was measured by determining the mRNA expression of *Ki-67* in this study. Higher proliferation was observed in the TMEPAI wild-type cells compared with the knock-out cells after paclitaxel treatment, as evidenced by increased mRNA expression of *Ki-67* (Figure 2).

Higher expression of AKT phosphorylation in wild-type TMEPAI cells after paclitaxel treatment

BT-549 wild-type cells showed a significantly greater ratio of phosphorylated AKT to total AKT expression as compared to BT-549 TMEPAI knock-out cells. Both pAKT and total AKT were normalized to *GAPDH*. The full blot of GAPDH, pAKT, and AKT was presented in the Supplementary Material (Figure S1, Figure S2, Figure S3). Paclitaxel treatment results in a more pronounced

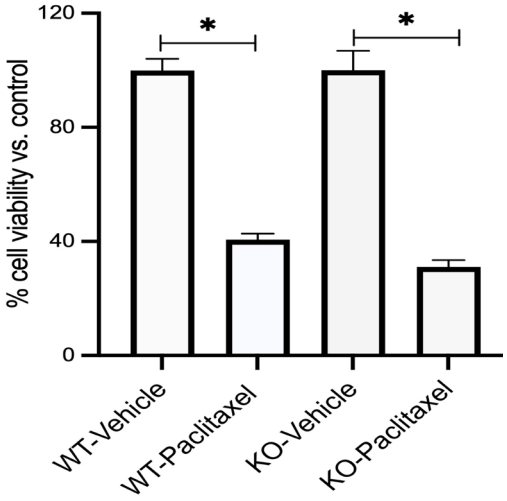

Figure 1. Paclitaxel Decreased Cell Viability more Prominently in the Absence of TMEPAI. Cells were treated with TGF-β 2 ng/mL and vehicle (control), or TGF-β 2 ng/mL and Paclitaxel 12 nM. The experiments were conducted three times in duplicate. Data are presented as mean ±SD). Differences between groups were analyzed with one-way ANOVA, followed by the post-hoc Tukey test. (*) refers to a significant difference at $p < 0.05$. WT-Vehicle: BT 549 wild-type cells treated with TGF-β 2 ng/mL and vehicle; WT-Paclitaxel: BT 549 wild-type cells treated with TGF-β 2 ng/mL and Paclitaxel 12 nM; KO-vehicle: BT-549 knock-out cells treated with TGF-β 2 ng/mL and vehicle; KO-paclitaxel: BT-549 knock-out cells treated with TGF-β 2 ng/mL and 12 nM paclitaxel.

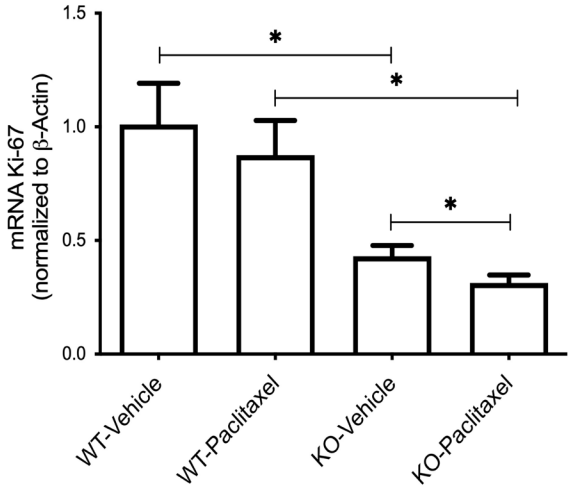

Figure 2. Paclitaxel was more Effective in Reducing Ki-67 mRNA Expression in TMEPAI Knock-Out Cells as Compared to the Wild-Type Cells. Cells were treated with TGF-β 2 ng/mL and vehicle (control), or TGF-β 2 ng/mL and Paclitaxel 12 nM. The experiments were conducted three times in duplicate. Data are presented as mean ±SD). Differences between groups were analyzed with one-way ANOVA, followed by the post-hoc Tukey test. (*) refers to a significant difference at $p < 0.05$. WT-Vehicle: BT 549 wild-type cells treated with TGF-β 2 ng/mL and vehicle; WT-Paclitaxel: BT 549 wild-type cells treated with TGF-β 2 ng/mL and Paclitaxel 12 nM; KO-vehicle: BT-549 knock-out cells treated with TGF-β 2 ng/mL and vehicle; KO-paclitaxel: BT-549 knock-out cells treated with TGF-β 2 ng/mL and 12 nM paclitaxel.

reduction in AKT phosphorylation in TMEPAI knock-out cells compared to wild-type cells (Figure 3). The full blot TMEPAI expression in thewild-type and knock-out cells is presented in the Supplementary Material Figure S4.

TMEPAI weakened the effects of paclitaxel by altering the expression of apoptosis markers

The presence of TMEPAI was found to reduce the apoptotic effect of paclitaxel. In TMEPAI knock-out cells, there was a significant elevation in the mRNA expression of the pro-apoptotic gene (*Bax*) and a reduced expression of the anti-apoptotic gene (*Bcl-2*) in comparison to wild-type TNBC cells. (Figure 4). The net effect was demonstrated by the ratio of *Bax* to Bcl2 mRNA expression. Our finding is further corroborated by the mRNA expression levels of *caspase-3* and *caspase-9*, as

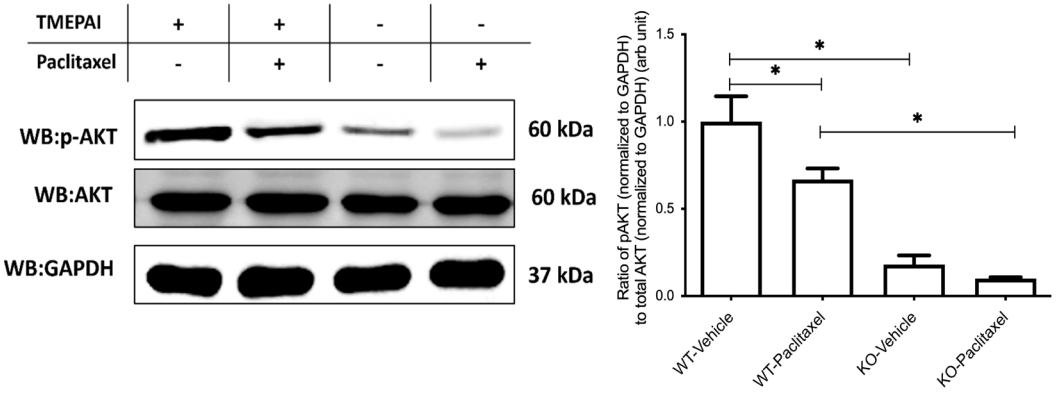

Figure 3. Marked reduction of pAKT/AKT expressions after paclitaxel treatment in the absence of TMEPAI compared to wild-type cells. Cells were treated with TGF-β 2 ng/mL and vehicle (control), or TGF-β 2 ng/mL and Paclitaxel 12 nM. The experiments were conducted three times in duplicate. Data are presented as mean ±SD). Differences between groups were analyzed with one-way ANOVA, followed by the post-hoc Tukey test. (*) refers to a significant difference at $p < 0.05$. WT-Vehicle: BT 549 wild-type cells treated with TGF-β 2 ng/mL and vehicle; WT-Paclitaxel: BT 549 wild-type cells treated with TGF-β 2 ng/mL and Paclitaxel 12 nM; KO-vehicle: BT-549 knock-out cells treated with TGF-β 2 ng/mL and vehicle; KO-paclitaxel: BT-549 knock-out cells treated with TGF-β 2 ng/mL and 12 nM paclitaxel.

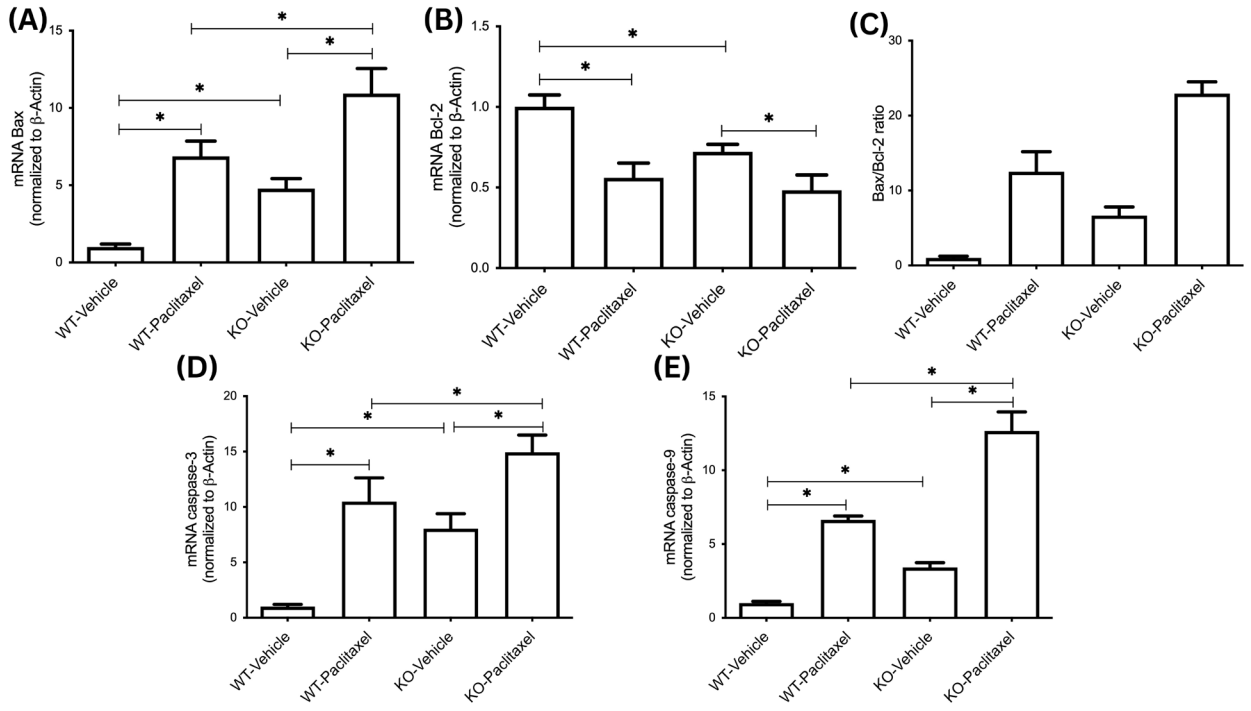

Figure 4. Paclitaxel effect on apoptotic genes (A) Bax; (B) Bcl-2; (C) Ratio of Bax/Bcl-2; (D) Caspase-3; (E) Caspase-9 in the TMEPAI knock-out vs. wild-type cells. Cells were treated with TGF-β 2 ng/mL and vehicle (control), or TGF-β 2 ng/mL and Paclitaxel 12 nM. The experiments were conducted three times in duplicate. Data are presented as mean ±SD). Differences between groups were analyzed with one-way ANOVA, followed by the post-hoc Tukey test. (*) refers to a significant difference at $p < 0.05$. WT-Vehicle: BT 549 wild-type cells treated with TGF-β 2 ng/mL and vehicle; WT-Paclitaxel: BT 549 wild-type cells treated with TGF-β 2 ng/mL and Paclitaxel 12 nM; KO-vehicle: BT-549 knock-out cells treated with TGF-β 2 ng/mL and vehicle; KO-paclitaxel: BT-549 knock-out cells treated with TGF-β 2 ng/mL and 12 nM paclitaxel.

well as the presence of cleaved *caspase-3* protein (Figure 5). The full blot of *caspase-3* and cleaved *caspase-3* is presented in Supplementary Material Figure S5 and Figure S6. Treatment with paclitaxel can increase the ratio of *Bax* to *Bcl-2* in wild-type cells. However, the absence of TMEPAI doubled the efficacy of paclitaxel in causing apoptosis.

TMEPAI influenced the elevation of mRNA drug efflux transporter in paclitaxel-treated TNBC cells

Overexpression of drug efflux transporters has been associated with unfavorable outcomes, decreased survival rates, and resistance to chemotherapy in TNBC. This study investigated the mRNA expression of drug efflux transporters, including P-glycoprotein, MRP-1, and BCRP,

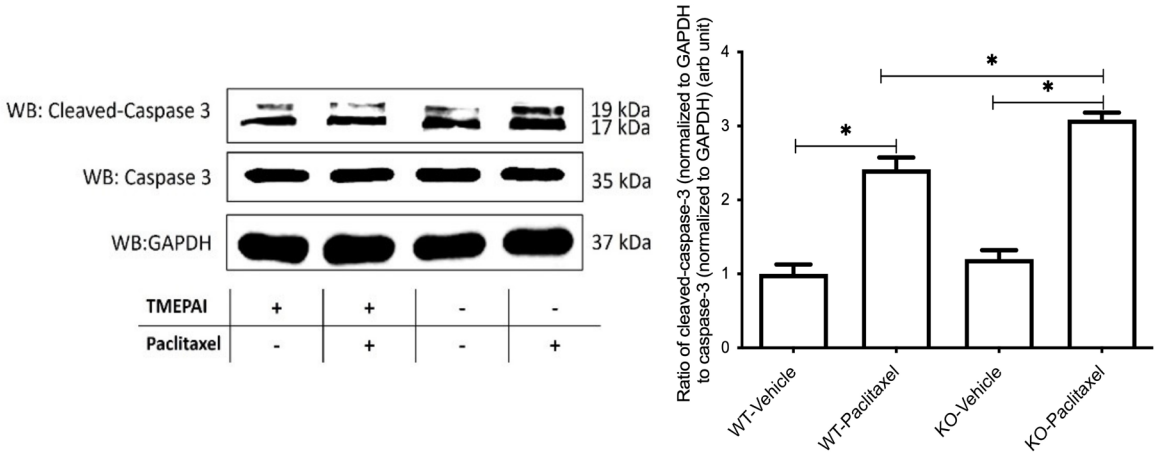

Figure 5. The Effect of Paclitaxel on Cleaved Caspase-3 was more Pronounced in the Absence of TMEPAI. Cells were treated with TGF-β 2 ng/mL and vehicle (control), or TGF-β 2 ng/mL and Paclitaxel 12 nM. The experiments were conducted three times in duplicate. Data are presented as mean ±SD). Differences between groups were analyzed with one-way ANOVA, followed by the post-hoc Tukey test. (*) refers to a significant difference at $p < 0.05$. WT-Vehicle: BT 549 wild-type cells treated with TGF-β 2 ng/mL and vehicle; WT-Paclitaxel: BT 549 wild-type cells treated with TGF-β 2 ng/mL and Paclitaxel 12 nM; KO-vehicle: BT-549 knock-out cells treated with TGF-β 2 ng/mL and vehicle; KO-paclitaxel: BT-549 knock-out cells treated with TGF-β 2 ng/mL and 12 nM paclitaxel.

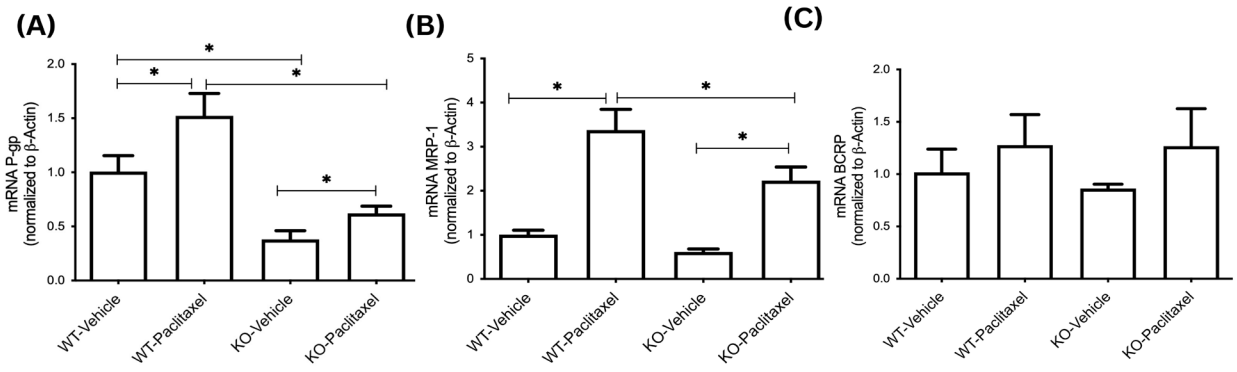

Figure 6. TMEPAI enhanced mRNA expression level of drug efflux transporters (A) P-gp, (B) MRP-1, and (C) BCRP after paclitaxel treatment. Cells were treated with TGF-β 2 ng/mL and vehicle (control), or TGF-β 2 ng/mL and Paclitaxel 12 nM. The experiments were conducted three times in duplicate. Data are presented as mean ±SD). Differences between groups were analyzed with one-way ANOVA, followed by the post-hoc Tukey test. (*) refers to a significant difference at $p < 0.05$. WT-Vehicle: BT 549 wild-type cells treated with TGF-β 2 ng/mL and vehicle; WT-Paclitaxel: BT 549 wild-type cells treated with TGF-β 2 ng/mL and Paclitaxel 12 nM; KO-vehicle: BT-549 knock-out cells treated with TGF-β 2 ng/mL and vehicle; KO-paclitaxel: BT-549 knock-out cells treated with TGF-β 2 ng/mL and 12 nM paclitaxel.

in WT and KO cells after paclitaxel treatment (Figure 6). TMEPAI notably increased mRNA expression of drug efflux transporters, in particular P-glycoprotein (P-gp) and multidrug resistance-associated protein 1 (MRP-1). Treatment with paclitaxel led to an increase in these efflux transporters in both wild-type and knock-out cells. However, the elevation of the efflux transporters after paclitaxel treatment was slightly less than in wild-type cells.

TMEPAI augmented the mRNA expressions of epithelial-mesenchymal transition markers after paclitaxel treatment.

Epithelial-mesenchymal transition (EMT) is a well-established mechanism that contributes to anticancer resistance, including resistance to paclitaxel. This study investigated the mRNA expression levels of EMT markers: snail, zeb-1, and twist (Figure 7). Our study demonstrated that the presence of TMEPAI facilitates the increase in the three markers following paclitaxel treatment.

Discussion

To date, taxanes continue to be the primary treatment for triple-negative breast cancer. Nonetheless, their clinical efficacy is constrained by the development of chemoresistance [16, 17]. Our findings show that TMEPAI contributes to the development of doxorubicin and paclitaxel resistance in TNBC [14]. In a previous study of doxorubicin treatment in triple-negative breast cancer, TMEPAI enhanced chemoresistance by modulating apoptosis and epithelial-mesenchymal transition (EMT) through the upregulation of PI3K/AKT pathways. Moreover, TMEPAI induced the augmentation of drug efflux transporter expressions [15]. In the present study, we aimed to elucidate the role of TMEPAI in contributing to chemoresistance during paclitaxel treatment.

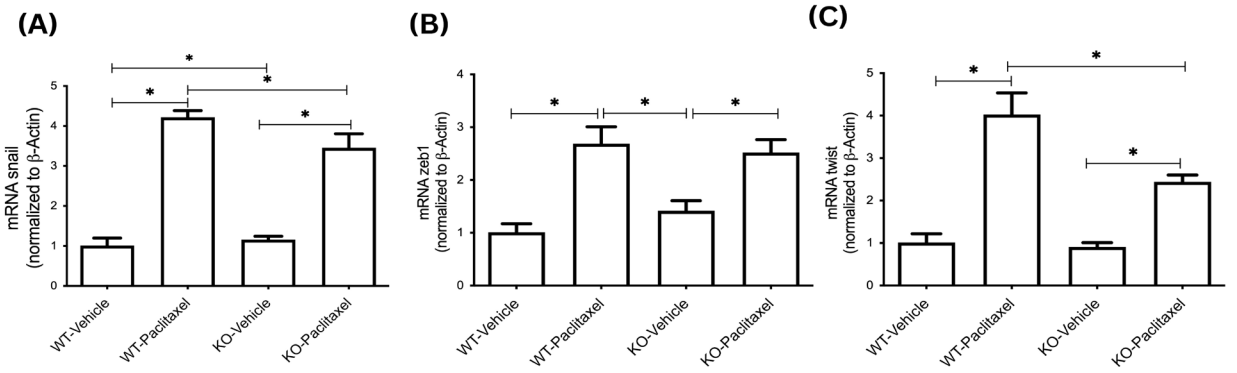

Figure 7. TMEPAI induced mRNA expression level of EMT markers (A. snail; B. zeb-1; C. twist) after paclitaxel treatment. Cells were treated with TGF-β 2 ng/mL and vehicle (control), or TGF-β 2 ng/mL and Paclitaxel 12 nM. The experiments were conducted three times in duplicate. Data are presented as mean ±SD). Differences between groups were analyzed with one-way ANOVA, followed by the post-hoc Tukey test. (*) refers to a significant difference at $p < 0.05$. WT-Vehicle: BT 549 wild-type cells treated with TGF-β 2 ng/mL and vehicle; WT-Paclitaxel: BT 549 wild-type cells treated with TGF-β 2 ng/mL and Paclitaxel 12 nM; KO-vehicle: BT-549 knock-out cells treated with TGF-β 2 ng/mL and vehicle; KO-paclitaxel: BT-549 knock-out cells treated with TGF-β 2 ng/mL and 12 nM paclitaxel.

TMEPAI is known to drive cancer cell proliferation by participating in the dysregulation of the NF-κB pathway. TMEPAI recruits the ubiquitin ligase Nedd4, which causes ubiquitination and subsequent degradation of Iκ-Bα [18, 19]. Furthermore, the process led to the transcriptional upregulation of key proliferative genes, such as cyclin D1 and c-Myc, which in turn promote cancer cell proliferation and resistance to chemotherapy [14, 20]. In accordance with the known pathway, our results showed that in the presence of TMEPAI, cell viability and proliferation markers, *Ki-67*, were significantly elevated. Some studies have suggested that high *Ki-67* levels in cancer indicate the activation of the PI3K/AKT/mTOR pathway, which promotes cell growth [21, 22].

The activation of the PI3K/AKT/mTOR signaling pathway in breast cancer was associated with worse disease-free survival (DFS) and overall survival (OS), suggesting its role in promoting aggressive tumor phenotypes and anticancer drug resistance, particularly in patients with exon 9 PI3KCA mutations [23]. Its pathways were reported to be overactivated in approximately 70% of breast cancer cases, including TNBC. It promotes down-signaling, such as mTORC1 activation, which contributes to cell proliferation and survival. The mTOR activation facilitates tumor growth, drives drug resistance, and enhances resistance to chemotherapy, such as paclitaxel. Activation of the PI3K/AKT pathway is associated with paclitaxel resistance in TNBC, as it reduces apoptosis and promotes an aggressive tumor behavior [24]. As demonstrated in our results, the presence of TMEPAI reinforces AKT activation. TMEPAI appears to diminish the ability of paclitaxel to suppress AKT phosphorylation, maintaining partial activation of this pro-survival signaling pathway. This signaling reduces the effectiveness of paclitaxel in inducing apoptosis. By maintaining suppression of the *Bax*/*Bcl-2* ratio and reducing cleaved *caspase-3* activation (pro-apoptotic pathways), as well as mRNA expression of *caspase-3* and *caspase-9*, TMEPAI prevents TNBC cells from paclitaxel-induced apoptosis. These results suggest that TMEPAI limits the caspase-mediated apoptosis, contributing to reduced cell death in response to paclitaxel.

AKT hyperactivation in breast cancer may also influence drug resistance by affecting the expression and functionality of drug transporters, especially ABC transporters [22]. Drug-resistant TNBC cells secrete extracellular vesicles (EVs) enriched with high levels of drug efflux transporters, including MDR1, MRP1, and BCRP. These EVs are strongly associated with patients who exhibit poor response to neoadjuvant chemotherapy, such as paclitaxel [25]. Paclitaxel works by stabilizing microtubules, thereby preventing cell division and inducing apoptosis. However, efflux transporters move paclitaxel out of cells, so it can't reach therapeutic levels to disrupt microtubule function. The higher the efflux activity, the lower the intracellular drug concentration, directly correlating with increased resistance [26, 27]. EVs facilitate cell-to-cell communication by transferring drug-resistant traits, specifically the overexpression of these transporters, to transform sensitive cells into resistant cells [25]. Although EVs were not assessed in the current work, our findings revealed that TMEPAI significantly upregulates the mRNA expression of drug efflux transporters, including P-glycoprotein and MRP-1, with a modest increase in BCRP.

In contrast, knocking out TMEPAI from cells led to a marked reduction in the expression of these transporters. These results highlight the critical role of TMEPAI in modulating drug resistance mechanisms and suggest its potential as a predictive biomarker for paclitaxel resistance. Moreover, incorporating TMEPAI expression status into therapeutic strategies could enable personalized treatment approaches, improving TNBC management.

Moreover, our current study researched the downstream epithelial-mesenchymal transition (EMT) markers in the interaction between TMEPAI and the PI3K/AKT pathway under TGF-β induction. EMT itself is highly relevant to TNBC, playing a critical role in both drug resistance and metastasis and even serving as a prognostic factor [28–30]. Interaction with NEDD4 not only impacts proliferation but also mediates the effect of TMEPAI on suppressing the classical TGF-β signaling pathway by facilitating the E3 ubiquitin ligase-dependent degradation of Smad2/3. However, in the case of TNBCs, it plays a dual function by suppressing PTEN, thereby leading to the activation of the PI3K/AKT pathway, while also promoting EMT and inducing drug resistance, thus forming the basis of both tumor progression and therapeutic resistance in cancer cells [15, 19, 31]. More specifically, TMEPAI-induced EMT represses the typical epithelial markers such as E-cadherin upon the increased expression of key transcriptional factors that represent the mesenchymal phenotype, including Snail, Twist, or ZEB1, and promotes the mesenchymal markers vimentin and N-cadherin [31]. Therefore, TMEPAI inhibition may weaken such effects by reducing TGF-β-driven EMT and PI3K/AKT activation, thereby sensitizing the cells to chemotherapeutic agents like paclitaxel. Furthermore, combination therapies involving PI3K/AKT inhibitors and inhibitors of the TGF-β pathway may exhibit a synergistic effect in inhibiting EMT and resistance mechanisms. Coupling EMT markers with the monitoring of TMEPAI and PI3K/AKT activity could subsequently provide the basis for a personalized therapeutic approach that offered better management of TNBC.

Reflecting on our findings, we propose a mechanism of the role of TMEPAI in driving EMT under paclitaxel treatment (Figure 8). Our study showed that TMEPAI significantly upregulates key EMT transcription factors, including Snail, Zeb1, and Twist, in the wild-type cells under paclitaxel treatment. Conversely, the expression of these EMT markers was pronouncedly suppressed in TMEPAI-knock-out cells treated with paclitaxel compared to wild-type cells. It highlights the fact that TMEPAI indeed promotes EMT. This TMEPAI-driven EMT likely contributes to paclitaxel resistance, as the EMT process encourages the acquisition of a stem-like phenotype characterized by high survival, migration, and invasion capacities, as well as chemoresistance. More importantly, the low expression of EMT markers in the TMEPAI-knock-out cells suggests that inhibiting TMEPAI may suppress EMT and increase sensitivity to paclitaxel.

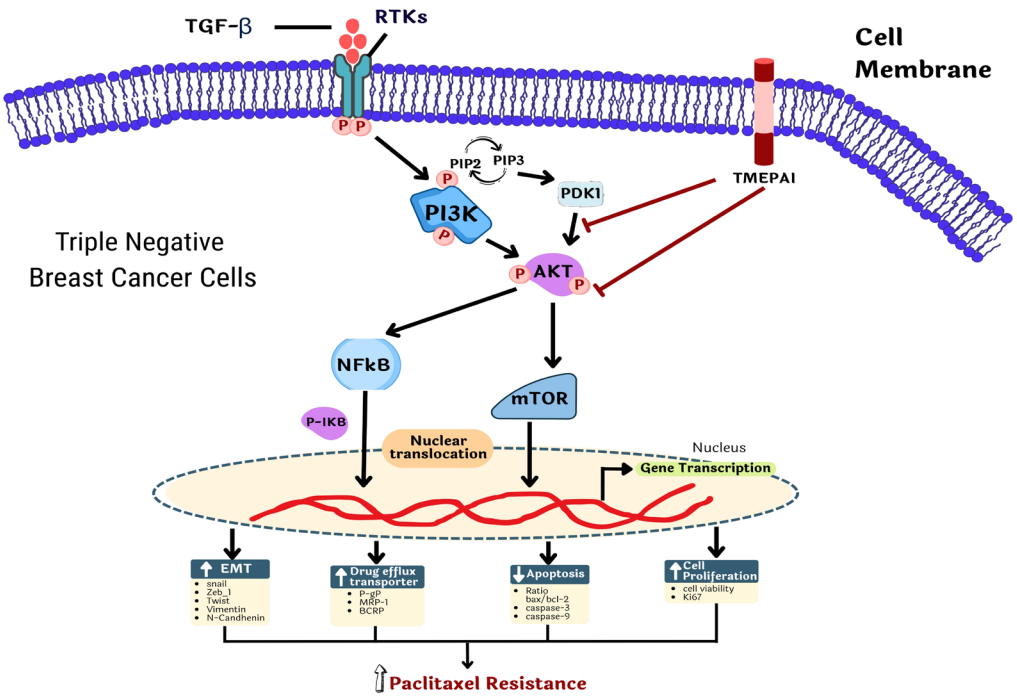

Figure 8. The Proposed Mechanism by which TMEPAI Enhances Paclitaxel Resistance in TNBC via PI3K/AKT Signaling Pathways. TGF-β induces PI3K activation and AKT phosphorylation through receptor tyrosine kinase (RTKs). The downstream signaling cascade leads to increased expression of EMT markers, upregulation of drug efflux transporters, inhibition of apoptosis, and enhanced cell proliferation. TMEPAI suppresses the inhibitory effects of PI3K and AKT, respectively, intensifying AKT-mediated gene transcription and NF-κB nuclear translocation. These processes collectively enhance paclitaxel resistance, emphasizing TMEPAI as a critical target for therapeutic intervention in TNBC. TGF-β: tumor growth factor β; RTKs: receptor tyrosine kinases; PI3K: Phosphoinositide 3-Kinase; PIP2: phosphatidylinositol 4,5-biphosphate; PIP3: phosphatidylinositol 4,5-triphosphate; AKT: protein kinase B; PDK1: phosphoinositide-dependent kinase-1; PTEN: phosphate and tensin homolog; NF-kB: nuclear factor Kappa B; IkB: inhibitor of Kappa B; mTOR: mechanistic target of rapamycin; EMT:epithelial-mesenchymal transition; P-gP: p-glycoprotein; MRP-1: multidrug resistance-associated protein-1; BCRP: breast cancer resistance protein; Bax: Bcl-2 associated resistance protein; Bcl-2: B cell lymphoma 2; TMEPAI: transmembrane prostate androgen-induced protein; TNBC: triple negative breast cancer

These findings further confirm that TMEPAI may be a potent therapeutic target in TNBC, whose inhibition could block EMT and enhance the efficacy of paclitaxel, providing a potential strategy to circumvent the problem of drug resistance.

We understand that using just one triple-negative breast cancer cell line is considered a limitation of our study. Different cell lines may have distinct signaling pathways and treatment responses, which could impact the generalizability of our results. The BT-549 cell line was chosen due to our previous findings that the BT-549 knock-out cell line showed better sensitivity to doxorubicin and paclitaxel. In our earlier study, we have also successfully produced another triple-negative breast cancer cell line, Hs587T [32]. However, Hs587T knock-out cells showed a slow-growing property, which makes it hard to study further. Thus, in the present study, a single-cell model analysis was conducted for signaling pathway analysis. Further study is necessary to validate our findings by incorporating additional cell lines from various tissue origins or molecular profiles, which can serve as the basis for the external validity of our results.

Another limitation of our study is the lack of investigation into the role of EVs in mediating TMEPAI-driven drug resistance. Hence, a gap remains in how EVs contribute to the mechanisms of paclitaxel resistance in TNBC. Our results have yet to be validated through in vivo studies involving either animal models or clinical samples, which would further increase their clinical relevance. The subsequent research also needs to explore the analyses of EVs secreted by TNBC cells regarding the transfer of drug-resistant properties and how TMEPAI contributes to EV-mediated intercellular communication. Animal model-based in vivo studies are suggested to confirm this and provide a conclusion regarding the therapeutic value of coadministration of TMEPAI inhibition with paclitaxel in TNBC. Furthermore, clinical trials investigating the expression of TMEPAI in patients and its correlation with therapeutic response may provide important translational insights. Combination therapies targeting the pathways involved in TMEPAI, PI3K/AKT signaling, and the TGF-β pathway may elicit a synergistic reaction, thereby overcome EMT-driven resistance and refining therapeutic modalities for the effective management of TNBC.

In conclusion, the present study identifies TMEPAI as a critical factor in driving chemoresistance and tumor progression in TNBC following treatment with paclitaxel. TMEPAI enhances resistance mechanisms

through AKT phosphorylation, the overexpression of drug efflux transporters such as P-glycoprotein, MRP-1, and BCRP, and transcription factors involved in the epithelial-to-mesenchymal transition. Consequently, this reduces apoptosis and diminishes the cytotoxic effects of paclitaxel. These findings suggest that targeting TMEPAI could represent a promising therapeutic strategy, evidenced by the significant reduction in expression of EMT markers and drug resistance characteristics observed in TMEPAI-knock-out cells.

Author Contribution Statement

All authors participated in the idea and design of the research. ML and BWK conducted material preparation, data collection, and analysis. The first draft of the text was composed by ML and BWK. All writers reviewed and approved the final manuscript.

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Approval

This research is part of a dissertation that has been approved by the Committee of the Doctoral Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia.

Data Availability

The data contributing to the conclusion of this manuscript are available upon request to the corresponding Author.

Conflict of Interest

The Author of this manuscript declares no conflict of interest.

References

1. Yin L, Duan JJ, Bian XW, Yu SC. Triple-negative breast cancer molecular subtyping and treatment progress. Breast Cancer Res. 2020;22(1):61. https://doi.org/10.1186/s13058-020-01296-5.
2. Zagami P, Carey LA. Triple negative breast cancer: Pitfalls and progress. NPJ Breast Cancer. 2022;8(1):95. https://doi.org/10.1038/s41523-022-00468-0.
3. Kesireddy M, Elsayed L, Shostrom VK, Agarwal P, Asif S, Yellala A, et al. Overall survival and prognostic factors in metastatic triple-negative breast cancer: A national cancer database analysis. Cancers (Basel). 2024;16(10). https://doi.org/10.3390/cancers16101791.
4. Obidiro O, Battogtokh G, Akala EO. Triple negative breast cancer treatment options and limitations: Future outlook. Pharmaceutics. 2023;15(7). https://doi.org/10.3390/pharmaceutics15071796.
5. El Hejjioui B, Lamrabet S, Amrani Joutei S, Senhaji N, Bouhafa T, Malhouf MA, et al. New biomarkers and treatment advances in triple-negative breast cancer. Diagnostics (Basel). 2023;13(11). https://doi.org/10.3390/diagnostics13111949.
6. Aziz MH, Ahmad A. Epigenetic basis of cancer drug resistance. Cancer Drug Resist. 2020;3(1):113-6. https://doi.org/10.20517/cdr.2020.06.
7. Nedeljković M, Damjanović A. Mechanisms of chemotherapy resistance in triple-negative breast cancer-how we can rise to the challenge. Cells. 2019;8(9). https://doi.org/10.3390/cells8090957.
8. Amalia R, Abdelaziz M, Puteri MU, Hwang J, Anwar F, Watanabe Y, et al. Tmepai/pmepa1 inhibits wnt signaling by regulating β-catenin stability and nuclear accumulation in triple negative breast cancer cells. Cell Signal. 2019;59:24-33. https://doi.org/10.1016/j.cellsig.2019.03.016.
9. Puteri MU, Watanabe Y, Wardhani BWK, Amalia R, Abdelaziz M, Kato M. Pmepa1/tmepai isoforms function via its py and smad-interaction motifs for tumorigenic activities of breast cancer cells. Genes Cells. 2020;25(6):375-90. https://doi.org/10.1111/gtc.12766.
10. Itoh S, Itoh F. Tmepai family: Involvement in regulation of multiple signalling pathways. J Biochem. 2018;164(3):195-204. https://doi.org/10.1093/jb/mvy059.
11. Haque MA, Abdelaziz M, Puteri MU, Vo Nguyen TT, Kudo K, Watanabe Y, et al. Pmepa1/tmepai is a unique tumorigenic activator of akt promoting proteasomal degradation of phlpp1 in triple-negative breast cancer cells. Cancers (Basel). 2021;13(19). https://doi.org/10.3390/cancers13194934.
12. Abdelaziz M, Watanabe Y, Kato M. Pmepa1/tmepai knockout impairs tumour growth and lung metastasis in mda-mb-231 cells without changing monolayer culture cell growth. J Biochem. 2019;165(5):411-4. https://doi.org/10.1093/jb/mvz022.
13. Wardhani BW, Puteri MU, Watanabe Y, Louisa M, Setiabudy R, Kato M. Decreased sensitivity of several anticancer drugs in TMEPAI knockout triple-negative breast cancer cells. Medical journal of Indonesia. 2019 Aug 9;28(2):110-5.
14. Wardhani BW, Puteri MU, Watanabe Y, Louisa M, Setiabudy R, Kato M. Tgf-β-induced tmepai attenuates the response of triple-negative breast cancer cells to doxorubicin and paclitaxel. J Exp Pharmacol. 2020;12:17-26. https://doi.org/10.2147/jep.S235233.
15. Wardhani BWK, Louisa M, Watanabe Y, Setiabudy R, Kato M. Tgf-β-induced tmepai promotes epithelial-mesenchymal transition in doxorubicin-treated triple-negative breast cancer cells via smad3 and pi3k/akt pathway alteration. Breast Cancer (Dove Med Press). 2021;13:529-38. https://doi.org/10.2147/bctt.S325429.
16. Abouzeid HA, Kassem L, Liu X, Abuelhana A. Paclitaxel resistance in breast cancer: Current challenges and recent advanced therapeutic strategies. Cancer Treat Res Commun. 2025;43:100918. https://doi.org/10.1016/j.ctarc.2025.100918.
17. Alalawy AI. Key genes and molecular mechanisms related to paclitaxel resistance. Cancer Cell Int. 2024;24(1):244. https://doi.org/10.1186/s12935-024-03415-0.
18. Li Y, Zhang Y, Li L, Zhang M, Song N, Zhao Q, et al. Tmepai promotes degradation of the nf-κb signaling pathway inhibitory protein iκbα and contributes to tumorigenesis. Int J Biol Macromol. 2023;235:123859. https://doi.org/10.1016/j.ijbiomac.2023.123859.
19. Singha PK, Pandeswara S, Geng H, Lan R, Venkatachalam MA, Saikumar P. Tgf-β induced tmepai/pmepa1 inhibits canonical smad signaling through r-smad sequestration and promotes non-canonical pi3k/akt signaling by reducing pten in triple negative breast cancer. Genes Cancer. 2014;5(9-10):320-36. https://doi.org/10.18632/genesandcancer.30.
20. Pavitra E, Kancharla J, Gupta VK, Prasad K, Sung JY, Kim J, et al. The role of nf-κb in breast cancer initiation,

growth, metastasis, and resistance to chemotherapy. Biomed Pharmacother. 2023;163:114822. https://doi.org/10.1016/j.biopha.2023.114822.
21. Zhang W, Zhou Q, Wei Y, Da M, Zhang C, Zhong J, et al. The exosome-mediated pi3k/akt/mtor signaling pathway in cervical cancer. Int J Clin Exp Pathol. 2019;12(7):2474-84.
22. Miricescu D, Totan A, Stanescu S, II, Badoiu SC, Stefani C, Greabu M. Pi3k/akt/mtor signaling pathway in breast cancer: From molecular landscape to clinical aspects. Int J Mol Sci. 2020;22(1). https://doi.org/10.3390/ijms22010173.
23. Lv W, Du C, Zhang Y, Wu F, Jin Y, Chen X, et al. Clinicopathological characteristics and prognostic analysis of pik3ca mutation in breast cancer patients in northwest china. Pathol Res Pract. 2022;238:154063. https://doi.org/10.1016/j.prp.2022.154063.
24. Zhang HP, Jiang RY, Zhu JY, Sun KN, Huang Y, Zhou HH, et al. Pi3k/akt/mtor signaling pathway: An important driver and therapeutic target in triple-negative breast cancer. Breast Cancer. 2024;31(4):539-51. https://doi.org/10.1007/s12282-024-01567-5.
25. Kim MW, Lee H, Lee S, Moon S, Kim Y, Kim JY, et al. Drug-resistant profiles of extracellular vesicles predict therapeutic response in tnbc patients receiving neoadjuvant chemotherapy. BMC Cancer. 2024;24(1):185. https://doi.org/10.1186/s12885-024-11822-9.
26. Liu XY, Jiang W, Ma D, Ge LP, Yang YS, Gou ZC, et al. Sytl4 downregulates microtubule stability and confers paclitaxel resistance in triple-negative breast cancer. Theranostics. 2020;10(24):10940-56. https://doi.org/10.7150/thno.45207.
27. Gao W, Sun L, Gai J, Cao Y, Zhang S. Exploring the resistance mechanism of triple-negative breast cancer to paclitaxel through the scrna-seq analysis. PLoS One. 2024;19(1):e0297260. https://doi.org/10.1371/journal.pone.0297260.
28. Zhang M, Meng M, Liu Y, Qi J, Zhao Z, Qiao Y, et al. Triptonide effectively inhibits triple-negative breast cancer metastasis through concurrent degradation of twist1 and notch1 oncoproteins. Breast Cancer Res. 2021;23(1):116. https://doi.org/10.1186/s13058-021-01488-7.
29. Zhang B, Zhao R, Wang Q, Zhang YJ, Yang L, Yuan ZJ, et al. An emt-related gene signature to predict the prognosis of triple-negative breast cancer. Adv Ther. 2023;40(10):4339-57. https://doi.org/10.1007/s12325-023-02577-z.
30. Ribatti D, Tamma R, Annese T. Epithelial-mesenchymal transition in cancer: A historical overview. Transl Oncol. 2020;13(6):100773. https://doi.org/10.1016/j.tranon.2020.100773.
31. Singha PK, Pandeswara S, Geng H, Lan R, Venkatachalam MA, Dobi A, et al. Increased smad3 and reduced smad2 levels mediate the functional switch of tgf-β from growth suppressor to growth and metastasis promoter through tmepai/pmepa1 in triple negative breast cancer. Genes Cancer. 2019;10(5-6):134-49. https://doi.org/10.18632/genesandcancer.194.
32. Wardhani BW, Puteri MU, Watanabe Y, Louisa M, Setiabudy R, Kato M. TMEPAI genome editing in triple negative breast cancer cells. Med J Indones. 2017 May 16;26(1):14-8.