AKT1 and *CTNNB1* mutations as drivers of paclitaxel resistance in breast cancer cells

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Abstract. Breast cancer (BC) is the most prevalent cancer type in the world, with increasing incidence rates. Drug resistance is a notable factor that limits the effectiveness of BC therapy. Paclitaxel (PTX), a chemotherapeutic agent belonging to the taxane class, is commonly used in BC; however, its efficacy is often compromised by drug resistance, which is primarily attributed to genetic alterations. The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) and wingless-type MMTV integration site family/β-catenin signaling pathways are involved in essential cellular processes, such as proliferation, apoptosis and maintenance of homeostasis. Dysregulated activation of these pathways is strongly associated with carcinogenesis and drug resistance. In the present study, the potential effects of *AKT1* (E17K/E49K/L52R) and catenin β-1 (*CTNNB1*; S33P/T41A/S45F) mutations on PTX resistance in BC were investigated *in vitro* using site-directed mutagenesis, transient transfection, MTS assay and western blot analyses. The results of the present study indicated that *AKT1*-E17K/E49K and *CTNNB1*-S45F/T41A mutations induced PTX resistance compared with *AKT1*-wild-type (WT) and *CTNNB1*-WT in MCF-7 cells, respectively. In MDA-MB-231 cells, all three *AKT1* mutations (E17K/E49K/L52R) triggered PTX resistance compared with *AKT1*-WT, while none of the *CTNNB1* mutations exhibited such an effect. In conclusion, *AKT1* mutations may serve as a biomarker for PTX resistance in both estrogen receptor (ER)(+)/progesterone receptor (PR)(+)/HER2(-) and triple negative BC, while *CTNNB1* mutations may be a potential biomarker for PTX resistance in ER(+)/PR(+)/HER2(-) BC.

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Abbreviations: BC, breast cancer; PTX, paclitaxel; PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B; WT, wild-type

Key words: AKT1, *CTNNB1*, mutation, breast cancer, drug resistance, paclitaxel

Introduction

According to global cancer statistics for 2022, breast cancer (BC) is the second most frequently diagnosed cancer type, with ~2.3 million new cases (1). As a natural consequence of the growing and aging of the world population, the burden of BC is expected to exceed 3 million new cases and 1 million mortalities in the next two decades (2). BC comprises a group of diseases, exhibiting different clinical behaviors (treatment response/resistance, prognosis and metastatic potential) between subtypes, which reflects its heterogeneous nature (3). Although there are numerous approaches such as hormonal, targeted drugs and chemotherapy in BC treatment, drug resistance is a common phenomenon that limits the treatment efficacy. However, the mechanisms underlying drug resistance are complex and not yet fully understood (4).

The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway is key for numerous cellular processes, including cell cycle progression, proliferation and apoptosis (5). Dysregulation of this pathway is associated with tumor progression and drug resistance in BC. Mutations in genes involved in the PI3K/AKT pathway, such as *PI3K*, *AKT1/2/3* and *PTEN*, may disturb the regulation of tumor growth, angiogenesis and cell survival, contributing to cancer development (6). As a result, this pathway is a promising target for overcoming drug resistance in BC (7). AKT1, a serine/threonine kinase encoded by the *AKT1* gene, is an important signaling protein that serves a key role in the PI3K/AKT pathway (8). AKT1 phosphorylates several targets, such as the mammalian target of rapamycin (mTOR), matrix metalloproteinases, cyclin-dependent kinases and vascular endothelial growth factor, to promote cell survival (9). During the AKT1 activation process, PI3K is primarily activated through the binding of ligands, including growth factors, hormones and cytokines, to receptors. Activated PI3K leads to the formation of phosphatidylinositol-(3,4,5)-trisphosphate (PIP3) from phosphatidylinositol-(4,5)-bisphosphate, facilitating the recruitment of AKT1 to the plasma membrane. The pleckstrin homology (PH) domain is key for the activation of AKT1, which binds to PIP3 via its PH domain and is subsequently phosphorylated by 3'-phosphoinositide-dependent protein kinase 1 and mTOR complex 2 (10). Missense mutations in the PH domain of AKT1, including *AKT1*-E17K (49G>A), -E49K (145G>A) and -L52R (155T>G), have been demonstrated to increase AKT1 activity (11-13). The *AKT1*-E17K and -L52R

mutations have also been observed in patients with BC (11,14). While the *AKT1*-E49K mutation has been reported in bladder cancer and pulmonary sclerosing hemangioma (12,15), to the best of our knowledge, its presence in BC has not been reported.

The canonical wingless-type MMTV integration site family (WNT) pathway (WNT/β-catenin) is an evolutionarily conserved signaling cascade that regulates key biological processes, including cell fate determination, organogenesis, tissue homeostasis and pathological conditions (such as cancer, osteoporosis and coronary artery disease). Dysregulation of the WNT/β-catenin pathway is associated with carcinogenesis through multiple mechanisms of action (16). In BC, various components of the WNT/β-catenin pathway undergo alteration due to factors such as mutations, amplifications and post-transcriptional/-translational modification. These alterations lead to changes in the localization of β-catenin within the cell (17). The phosphorylation status of cytosolic β-catenin is key to the WNT/β-catenin pathway (18). When the pathway is inactivated, β-catenin binds to the destruction complex that includes axis inhibition protein (AXIN), adenomatous polyposis coli, casein kinase 1 (CK-1) and glycogen synthase kinase 3β (GSK-3β). β-catenin is sequentially phosphorylated by CK-1 on Ser45 and then by GSK-3β on Thr41, Ser37 and Ser33. This phosphorylation leads to ubiquitination and proteasomal degradation of β-catenin (19). In physiological/pathological conditions such as embryogenesis and carcinogenesis, the WNT/β-catenin pathway is activated by binding of WNT family members (WNT1, WNT2, WNT3a, etc.) to the Frizzled (FZD) receptor and its coreceptor, low-density lipoprotein receptor-related protein (LRP) 6 or LRP5. The formation of the WNT-FZD-LRP6/LRP5 complex mediates the assembly of the destruction complex around the receptor, resulting in inhibition of destruction complex-mediated β-catenin phosphorylation and hence accumulation of β-catenin in the cytosol. This accumulation triggers the translocation of β-catenin to the nucleus, where it initiates the expression of target genes essential for cell survival, proliferation, differentiation and migration (20).

Mutations in the catenin β-1 (*CTNNB1*) gene, which encodes β-catenin, are observed in numerous types of cancer and are predominantly all localized in exon 3. The mutations are primarily found in the phosphorylation sites of β-catenin (N-terminal region; S33, S37, T41 and S45), leading to resistance to phosphorylation. As a result, β-catenin accumulates in the cell, eventually translocating to the nucleus (21). Missense mutations in the phosphorylation sites of β-catenin include *CTNNB1*-S33P (97T>C), -T41A (121A>G) and -S45F (134C>T). The presence of these mutations is associated with an increase in cellular proliferation (22-29). *CTNNB1*-T41A and -S45F have been reported in BC (30), whereas the presence of *CTNNB1*-S33P remains unclear.

Paclitaxel (PTX) is a microtubule-stabilizing agent from the taxane class of plant alkaloids that is commonly used in the treatment of BC. PTX induces apoptosis in cancer cells through various mechanisms of action, including activation of antitumor immunity, the regulation of mitochondrial function, cell cycle arrest, downregulation of the PI3K/AKT pathway and production of reactive oxygen species (31,32). Despite its potent apoptotic effects via these mechanisms, the efficacy of PTX is limited, primarily due to the development of drug resistance (33).

Understanding the molecular mechanisms underlying PTX resistance and identifying predictive biomarkers for PTX resistance in BC treatment is key for improving therapeutic outcomes. To the best of our knowledge, no established biomarker exists to predict PTX resistance. Therefore, the present study explored the potential effects of *AKT1* (E17K/E49K/L52R) and *CTNNB1* (S33P/T41A/S45F) mutations, which are known to enhance AKT1 activity (11-13) and β-catenin stability (21), on PTX resistance in *in vitro* models of BC. The results of the present study may serve as a guide for clinical studies associated with PTX resistance.

Materials and methods

Site-directed mutagenesis. The *AKT1* and *CTNNB1* mutations were created using a site-directed mutagenesis kit (cat. no. 200519; Agilent Technologies, Inc.) on the pCMV6 vectors with C-terminal Myc-DDK tag carrying *AKT1* (cat. no. RC220361; OriGene Technologies, Inc.) and *CTNNB1* (cat. no. RC208947; OriGene Technologies, Inc.) following the manufacturer's protocol. Mutagenic primers were designed using the specific design program accessible online at agilent.com/genomics/qcpd and synthesized by Integrated DNA Technologies, Inc. (Table I).

Transformation and plasmid isolation. *Escherichia coli* cells (cat. no. C3030-03; Invitrogen; Thermo Fisher Scientific, Inc.) were used to transform mutant and wild-type (WT) *AKT1*/*CTNNB1* pCMV6 vectors, as well as an empty pCMV6 vector (EV) (cat. no. PS100001; OriGene Technologies, Inc.), following the manufacturer's protocol based on the heat-shock method (34). The transformed cell mixtures were plated onto lysogeny broth (LB) agar (cat. no. L3147; Sigma-Aldrich; Merck KGaA) containing 25 μg/ml kanamycin (KAN) (cat. no. 60615; Sigma-Aldrich; Merck KGaA) in Petri dishes and incubated at 37˚C overnight. Since the vectors contained the KAN-resistance gene, the cells transformed with these vectors proliferated and formed colonies on LB agar containing KAN. Then the colonies containing transformed cells were collected by a loop and cultured in LB at 37˚C for 8 h (cat. no. L3522; Sigma-Aldrich; Merck KGaA). The DNA was purified using an isolation kit (cat. no. 12123; Qiagen, Inc.), according to the manufacturer's instructions. The DNA samples were sequenced using next-generation sequencing by RefGen Gene Research and Biotechnology Company to verify the efficiency of the transformation.

Cell culture. MCF-7 (cat. no. HTB-22) and MDA-MB-231 (cat. no. HTB-26) BC cell lines were obtained from the American Type Culture Collection. MCF-7 cells were maintained in EMEM (cat. no. 320-026-CL; Wisent Biotechnology) supplemented with 10% fetal bovine serum (FBS; cat. no. FBS-11A; Capricorn Scientific GmbH), 100 U/ml penicillin, 100 μg/ml streptomycin (cat. no. 450-201-EL; Wisent Biotechnology) and 0.01 mg/ml recombinant human insulin (cat. no. I9278; Sigma-Aldrich; Merck KGaA). MDA-MB-231

Table I. Mutagenic primers used to generate *AKT1* and *CTNNB1* mutations.

Mutation	Primer sequence (5'-3')
AKT1	
E17K (Glu17Lys)	Forward, GCACAAACGAGGGAAGTACATCAAGAC
49G>A	Reverse, GTCTTGATGTACTTCCCTCGTTTGTGC
E49K (Glu49Lys)	Forward, GATGTGGACCAACGTAAGGCTCCCCTCAAC
145G>A	Reverse, GTTGAGGGGAGCCTTACGTTGGTCCACATC
L52R (Leu52Arg)	Forward, CGTGAGGCTCCCCGCAACAACTTCTCTG
155T>G	Reverse, CAGAGAAGTTGTTGCGGGGAGCCTCACG
CTNNB1	
S33P (Ser33Pro)	Forward, CAGTCTTACCTGGACCCTGGAATCCATTC
97T>C	Reverse, GAATGGATTCCAGGGTCCAGGTAAGACTG
T41A (Thr41Ala)	Forward, CATTCTGGTGCCACTGCCACAGCTCCTTCTC
121A>G	Reverse, GAGAAGGAGCTGTGGCAGTGGCACCAGAATG
S45F (Ser45Phe)	Forward, CTACCACAGCTCCTTTTCTGAGTGGTAAAG
134C>T	Reverse, CTTTACCACTCAGAAAAGGAGCTGTGGTAG

CTNNB1, catenin β-1; AKT, protein kinase B.

cells were maintained in RPMI-1640 (cat. no. 350-007-CL; Wisent Biotechnology) supplemented with 10% FBS and 100 U/ml penicillin/100 µg/ml streptomycin. The cells were cultured in a humidified incubator with 5% CO_2 at 37˚C. The cell lines were passaged four times prior to use, with transfection performed at passage four. Following transfection, the cells were passaged a fifth time prior to drug treatment. To minimize cellular stress and ensure more reliable results, a cell scraper was used during passaging.

Plasmid DNA transfection. Transient transfection of the plasmid DNA (5 µg) into the cells was performed using the MegaTran 2.0 polymer-based plasmid DNA transfection agent (cat. no. TT210003; OriGene Technologies, Inc.) following the manufacturer's recommendations. MCF-7 ($3x10^6$) and MDA-MB-231 ($1.5x10^6$) cells were seeded in Petri dishes at 37˚C for 24 h. Subsequently, the cells were transfected at 37˚C for 17-18 h. Afterward, the medium containing the transfection complex was removed, fresh medium was added and the cells were incubated at 37˚C for 24 h to allow for proliferation. At the end of 24 h, the cells were collected to perform subsequent experiments. The EV served as the transfection control.

Cell viability assay. The viability of cells was analyzed using MTS (cat. no. G111; Promega Corporation). MCF-7 ($5x10^4$) and MDA-MB-231 ($1x10^4$) cells were seeded into 96-well plates. PTX (cat. no. T7402; Sigma-Aldrich; Merck KGaA) was dissolved in DMSO and serially diluted (1:1) to obtain 16 different PTX doses (100-0.003 µg/ml). The cells were exposed to PTX at 37˚C for 24, 48 and 72 h. DMSO was used as the negative control. A total of 20 µl MTS/phenazine methosulfate (cat. no. P9625; Sigma-Aldrich; Merck KGaA) mixture (20:1, volume:volume) was added and the plates were maintained for 1-4 h. The absorbance values were determined at 490 nm and cell death was determined using the following formula: Cell death (%)=[1-(absorbance of the sample/absorbance of the control)] x100.

Western blot analysis. MCF-7 and MDA-MB-231 cells were lysed with RIPA [50 mM Tris-HCl (pH 8), 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate and 0.1% SDS] and the concentration of the total protein was assessed by the bicinchoninic acid protein assay kit (cat. no. BCA1; Sigma-Aldrich; Merck KGaA). A total of 20 µg/lane total protein was loaded onto the Any kD Mini-PROTEAN tris-glycine eXtreme (TGX) precast gels (cat. no. 4569034; Bio-Rad Laboratories, Inc.) and transferred onto a polyvinylidene difluoride membrane (cat. no. 1704272; Bio-Rad Laboratories, Inc.). The membrane was blocked in Tris-buffered saline with 0.1% Tween 20 containing 5% non-fat dry milk at room temperature for 1 h, and was incubated with anti-Myc-DDK (1:2,000; cat. no. TA50011; OriGene Technologies, Inc.) and anti-β-actin (1:10,000; cat. no. M01263; Boster Biological Technology) primary antibodies for 12 h at 4˚C. The anti-Myc-DDK antibody was used due to the C-terminal Myc-DDK tag on pCMV6 vectors carrying the *AKT1* and the *CTNNB1* genes. The membrane was incubated with secondary antibodies (1:10,000; anti-mouse IgG-HRP; cat. no. sc-2005; Santa Cruz Biotechnology, Inc.; 1:20,000; anti-rabbit IgG-HRP; cat. no. ab205718; Abcam) for 1 h at room temperature. The membrane was incubated with a chemiluminescence substrate (cat. no. 1705060; Bio-Rad Laboratories, Inc.) and imaged using Fusion FX (Vilber Lourmat Sté). The immunoreactive protein (IRP) bands were quantified using ImageJ 1.59 software (National Institutes of Health).

Statistical analysis. The experiments were performed in triplicate. Statistical analysis was performed using SPSS Statistics version 21 (IBM Corp.). The normality of the variables was evaluated using the Shapiro-Wilk test and all

variables were found to be normally distributed. Descriptive statistics, including the mean and the standard deviation, were calculated. To compare differences among >2 groups, one-way ANOVA was performed followed by Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Western blot analysis of AKT1/CTNNB1-WT and mutant proteins. BC cells were transfected with the EV and vectors carrying *AKT1*/*CTNNB1*-WT and mutations, followed by western blot analysis. As expected, no IRP band was observed for the EV. The presence of IRP bands in the samples from *AKT1*/*CTNNB1*-WT and mutant-transfected cells confirmed the success of the transfection process (Fig. 1). IRP levels of *AKT1*-E17K/E49K/L52R were significantly lower compared with those of *AKT1*-WT in both cell lines (P<0.05). *AKT1*-L52R mutant IRP levels were significantly lower than those of *AKT1*-E17K and -E49K (P<0.05; Fig. 1A and B). However, no significant changes were observed between the IRP levels of *CTNNB1*-WT/S33P/T41A/S45F (P>0.05; Fig. 1C and D).

Impact of AKT1 mutations on PTX resistance in BC cells. BC cells were incubated with PTX for 24, 48 and 72 h. The PTX responses were similar in both EV-transfected and non-transfected BC cells (P>0.05; Tables SI-SVI), indicating that the EV did not have an effect.

PTX induced cell death in EV-transfected MCF-7 cells at 24, 48 and 72 h; however, PTX induced cell death in MCF-7 cells transfected with *AKT1*-WT only at 72 h. The death of *AKT1*-WT-transfected MCF-7 cells was significantly decreased compared with EV-transfected MCF-7 cells at all exposure times (P<0.05; Tables SI-III). These findings reveal that *AKT1*-WT is associated with PTX resistance in MCF-7 cells.

PTX exhibited no cytotoxic effects on the cells transfected with *AKT1* mutations at 24 and 48 h. Following 72 h, death was observed in the cells carrying *AKT1*-L52R, while PTX remained ineffective in cells expressing *AKT1*-E17K and -E49K mutations. In addition, the death of cells expressing *AKT1*-E17K and -E49K was significantly lower compared with that of cells expressing *AKT1*-WT (P<0.05); however, no significant difference was noted between *AKT1*-L52R and *AKT1*-WT (P>0.05; Tables SI-III). These findings indicate that *AKT1*-E17K and -E49K conferred enhanced resistance to PTX in MCF-7 cells compared with *AKT1*-WT (Fig. 2A).

In both EV-transfected and *AKT1*-WT-transfected MDA-MB-231 cells, PTX induced death at 24, 48 and 72 h. In *AKT1*-WT-transfected cells, cell death was reduced compared with EV-transfected cells at 48 and 72 h (P<0.05; Tables SIV-VI). These results indicated that *AKT1*-WT was associated with PTX resistance in MDA-MB-231 cells, which was similar to the findings noted in MCF-7 cells.

The effects of *AKT1* mutations on the PTX response in MDA-MB-231 cells were also evaluated. The PTX-induced death was significantly higher in cells carrying *AKT1*-WT compared with those harboring *AKT1*-E17K/E49K/*AKT1*-L52R (P<0.05; Tables SIV-VI). These results indicated that *AKT1*-E17K/E49K/L52R induced higher PTX resistance in MDA-MB-231 cells than *AKT1*-WT (Fig. 2B).

Impact of CTNNB1 mutations on PTX resistance in BC cells. BC cells were incubated with PTX for 24, 48 and 72 h, and cell death was evaluated. The similar PTX responses in both EV-transfected and non-transfected BC cells confirmed there was no effect exerted by EV on cell death (P>0.05; Tables SVII-XII).

PTX induced death in transfected MCF-7 cells with *CTNNB1*-WT at 24, 48 and 72 h. The death of MCF-7 cells carrying *CTNNB1*-WT was significantly lower compared with that of EV-transfected MCF-7 cells (P<0.05; Tables SVII-IX). These findings revealed that *CTNNB1*-WT was associated with PTX resistance in MCF-7 cells. Furthermore, the effects of *CTNNB1* mutation on the PTX resistance in MCF-7 cells were assessed. PTX exhibited cytotoxic effects on cells expressing *CTNNB1*-S45F and -T41A only at 72 h. PTX-induced death was observed in cells transfected with *CTNNB1*-S33P at 24, 48 and 72 h (Tables SVII-IX). PTX-induced cell death of *CTNNB1*-WT was higher compared with that of *CTNNB1*-S45F and -T41A (P<0.05); however, no significant difference (P>0.05) was noted between *CTNNB1*-WT and -S33P (Fig. 3A). These results indicated that *CTNNB1*-T41A and -S45F display higher PTX resistance compared with *CTNNB1*-WT in MCF-7 cells.

In MDA-MB-231 cells, PTX induced different death in cells transfected with *CTNNB1*-WT. The death of cells expressing *CTNNB1*-WT was significantly decreased compared with that of EV-transfected cells (P<0.05; Tables SX-XII). These findings suggest that *CTNNB1*-WT was associated with PTX resistance in MDA-MB-231 cells.

The impact of *CTNNB1* mutations on PTX resistance in MDA-MB-231 cells was also evaluated (Tables SX-XII). The death levels of the cells expressing *CTNNB1*-S33P/S45F/T41A were comparable with those of cells expressing *CTNNB1*-WT (P>0.05; Fig. 3B). These findings demonstrated that *CTNNB1*-S33P/S45F/T41A mutations did not confer additional PTX resistance in MDA-MB-231 cells compared with *CTNNB1*-WT.

Discussion

BC is a heterogeneous group of diseases with different morphology and molecular structures, exhibiting varying biological behaviors and prognoses. Given the heterogeneous nature of BC, the primary challenge in its treatment is drug resistance. The identification of the molecular mechanisms underlying drug resistance and potential predictive biomarkers are key for overcoming this (35,36). The majority of the commonly used predictive biomarkers in BC treatment are estrogen receptor (ER)α, progesterone receptor (PR) and epidermal growth factor 2 (HER2). ERα and PR are used to guide endocrine therapy, while HER2 is used to determine the potential efficacy of anti-HER2 targeted therapies (37).

In the present study, cell lines (MCF-7 and MDA-MB-231) with different genetic profiles were used. The MCF-7 cell line represents a typical luminal subtype of BC characterized by ER(+)/PR(+)/HER2(-) (38). By contrast, the MDA-MB-231 cell line is a typical example of triple negative (TN)BC [ER(-)/PR(-)/HER2(-)] (39). The molecular pathophysiology of TNBC remains poorly understood and the primary strategy of the treatment is taxane-based chemotherapy. By contrast,

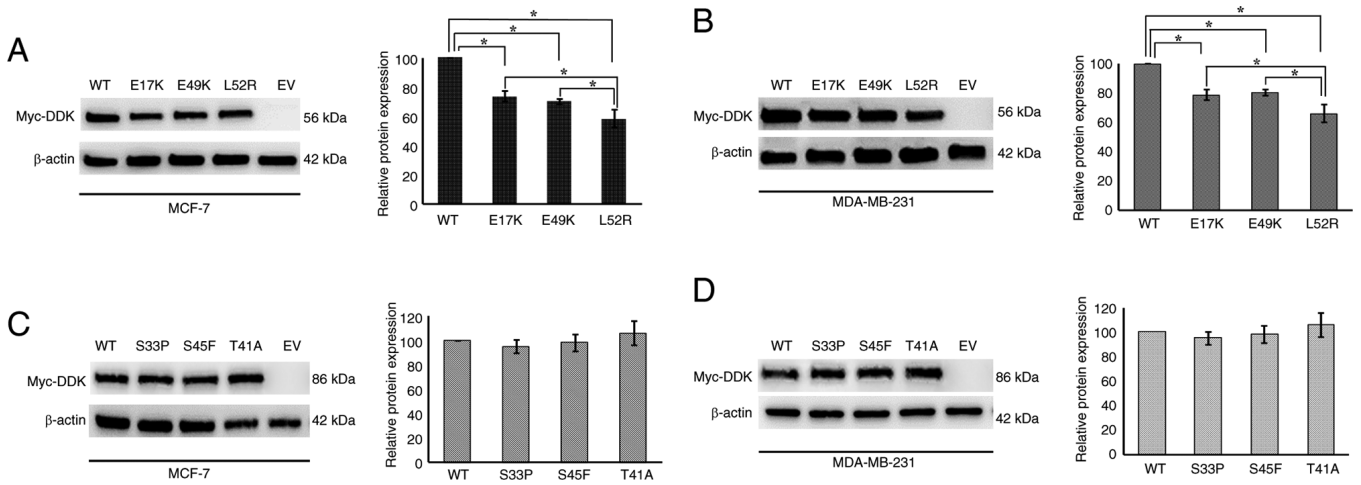

Figure 1. IRP bands in breast cancer cells. IRP bands and levels of AKT1 WT and mutants in (A) MCF-7 and (B) MDA-MB-231 cells. IRP bands and levels of β-catenin WT and mutants in (C) MCF-7 and (D) MDA-MB-231 cells. Anti-Myc-DDK monoclonal antibody was used for AKT1 and β-catenin detection. *P<0.05. EV, empty vector; IRP, immunoreactive protein; WT, wild-type; AKT1, protein kinase B.

endocrine therapy is the primary treatment strategy for ER(+)/HER2(-) BC; however, resistance is a common issue in patients with advanced BC, leading to recurrence. To decrease the risk of recurrence, taxane-based chemotherapy is often recommended. Nevertheless, the response rates are generally lower in ER(+)/HER2(-) patients with BC compared with those with HER2(+) or TNBC (40,41).

PTX is a taxane-class chemotherapeutic agent commonly used in BC treatment; however, its effectiveness is limited by drug resistance. The underlying mechanisms of PTX resistance remain unclear. To date, mutations in genes such as multidrug resistance 1, β-tubulin and p53 have been implicated in PTX resistance (33). In addition, elevated PI3K/AKT signaling in BC contributes to PTX resistance via specific mechanisms, such as promoting cell survival, enhancing anaerobic metabolism and inhibiting apoptosis (42). Genetic alterations in the PI3K/AKT pathway are frequently observed in BC, including gain-of-function mutations in *PIK3CA* and *AKT1*, as well as mutations in *PIK3R1*, *AKT2*, *AKT3* and *PTEN*. These alterations highlight the key role of this pathway in breast carcinogenesis (11,43,44).

AKT1-E17K/E49K/L52R are the PH domain mutations resulting in AKT1 activation in a PI3K-independent manner (11-13). The elevated levels of PI3K/AKT signaling due to these mutations lead to enhanced cell survival and oncogenic transformation (12,13,45-48). In the present study, western blot analysis indicated that *AKT1*-E17K/E49K/L52R protein levels were lower compared with those of *AKT1*-WT in both cell lines. Also, *AKT1*-L52R protein levels were lower than those of *AKT1*-E17K and -E49K. The decreased levels of mutant proteins may be due to several factors, including altered protein stability, improper folding or differential interactions with cellular components (such as chaperones and downstream signaling partners). These mutations may affect the overall structural integrity or function of the protein, resulting in a decreased expression level. Additionally, the L52R mutation may disrupt critical interactions within the AKT1 protein, affecting its stability or trafficking within the cell.

The potential effects of *AKT1*-E17K/E49K/L52R mutations on PTX resistance in BC cells were also investigated *in vitro*, as well as their impact on protein activity. *AKT1*-E17K and -E49K mutations conferred increased PTX resistance in MCF-7 cells compared with *AKT1*-WT. In MDA-MB-231 cells, all three *AKT1* mutations (E17K/E49K/L52R) were associated with increased PTX resistance. In our previous study, the association between *AKT1*-E17K/E49K/L52R mutations and cetuximab, 5-fluorouracil, 7-ethyl-10-hydroxy-camptothecin (SN-38), oxaliplatin and irinotecan resistance in colorectal cancer cells was identified (49). The present study indicated similarities to those observed in colorectal cancer regarding drug resistance. Studies performed on patients with BC harboring an *AKT1*-E17K mutation have reported that the use of AKT inhibitors alone (50,51) or in combination with PTX (52) or fulvestrant (53) increases the response to treatment. The findings of the present study regarding the *AKT1*-E17K mutation and PTX resistance in BC are consistent with those reported in the literature (49-53). To the best of our knowledge, the association between *AKT1*-E49K and -L52R mutations and drug resistance has not been previously reported in BC.

In addition to the PI3K/AKT pathway, the WNT/β-catenin pathway is associated with BC. Elevated levels of nuclear and/or cytoplasmic β-catenin are commonly observed in BC tissue and are associated with poor clinical outcomes and chemoresistance (54-57). Genetic and epigenetic alterations in the WNT/β-catenin pathway lead to enhanced transcription of WNT-targeted genes (such as AXIN2, cyclin D1, epidermal growth factor receptor and myelocytomatosis viral oncogene homolog) in BC (58).

CTNNB1-S33P/T41A/S45F mutations (in exon 3) decrease ubiquitination-mediated proteolysis of β-catenin, causing its nuclear accumulation and activation (22-24,27,28). Therefore, in the present study, the association between PTX resistance and the WNT/β-catenin pathway was explored. The results indicate that *CTNNB1*-S45F and -T41A induce PTX resistance, whereas *CTNNB1*-S33P did not affect the PTX response in MCF-7 cells. By contrast,

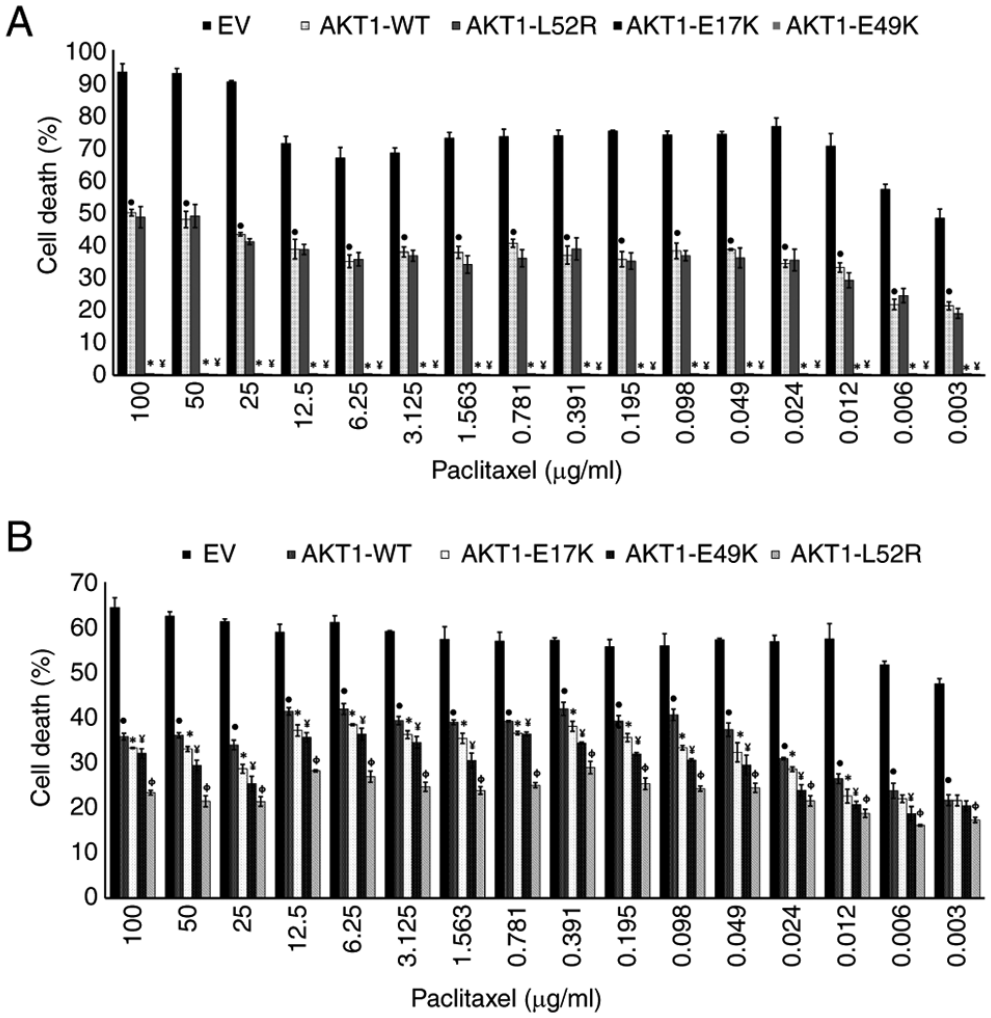

Figure 2. Effect of *AKT1*-WT and mutations on PTX resistance in breast cancer cells (72 h). PTX-induced death in (A) MCF-7 and (B) MDA-MB-231 cells transfected with *AKT1*-WT and mutations. *P<0.05 vs. respective EV. ¥P<0.05, ΦP<0.05 vs. respective WT. PTX, paclitaxel; WT, wild-type; AKT1, protein kinase B; EV, empty vector.

CTNNB1-S33P/T41A/S45F mutations did not cause changes in PTX response in MDA-MB-231 cells. The varying effects of *CTNNB1*-T41A and -S45F mutations on PTX resistance may be attributed to the different genetic profiles of these cell lines. To the best of our knowledge, the relationship between *CTNNB1*/S33P/T41A/S45F mutations and drug resistance has rarely been investigated (49). A comprehensive analysis of patients with BC performed by Ozcan (59) identified *CTNNB1* as a reliable biomarker for predicting the response to taxane-based chemotherapy in ER(+)/HER2(-) BC. The findings of the present study regarding MCF-7 are consistent with previous studies (49,59).

Yoo *et al* (60) reported that while high mutation rates in *TP53*, *PIK3CA*, *BRCA2* and *ATM* serine/threonine kinase genes are observed in patients with TNBC, *CTNNB1* mutations are rare. Furthermore, Geyer *et al* (61) reported that activation of the WNT/β-catenin pathway is notably associated with the TNBC phenotype with poor clinical outcomes but is not triggered by the *CTNNB1* exon 3 mutations. The aforementioned studies support the findings of the present study on the association between *CTNNB1* mutation and PTX response in MDA-MB-231 cells and suggest that *CTNNB1* mutations do not drive PTX resistance in TNBC. Therefore, the activation of the WNT/β-catenin pathway in TNBC may not be linked to *CTNNB1* exon 3 mutations, or this activation could be driven by mutations in other exons of *CTNNB1* or genes associated with the WNT/β-catenin pathway.

In conclusion, to the best of our knowledge, the present study is the first to demonstrate the association of *AKT1* and *CTNNB1* mutations with PTX resistance in BC cells. *AKT1*-E17K/E49K and *CTNNB1*-S45F/T41A mutations may serve as predictive biomarkers for PTX resistance in patients with BC and ER(+)/PR(+)/HER2(-), while *AKT1*-E17K/E49K/L52R mutations may be used as indicators of PTX resistance in patients with TNBC. The emergence of resistance to cancer therapies highlights the necessity for personalized treatment approaches.

Although the association between *AKT1*/*CTNNB1* mutations and PTX resistance has been established in the present study, the underlying molecular mechanisms, particularly the roles of these mutations in PTX resistance via the PI3K/AKT and WNT/β-catenin pathways, remain unclear. To the best of our knowledge, the phosphorylation status of AKT1 and β-catenin, as well as their downstream

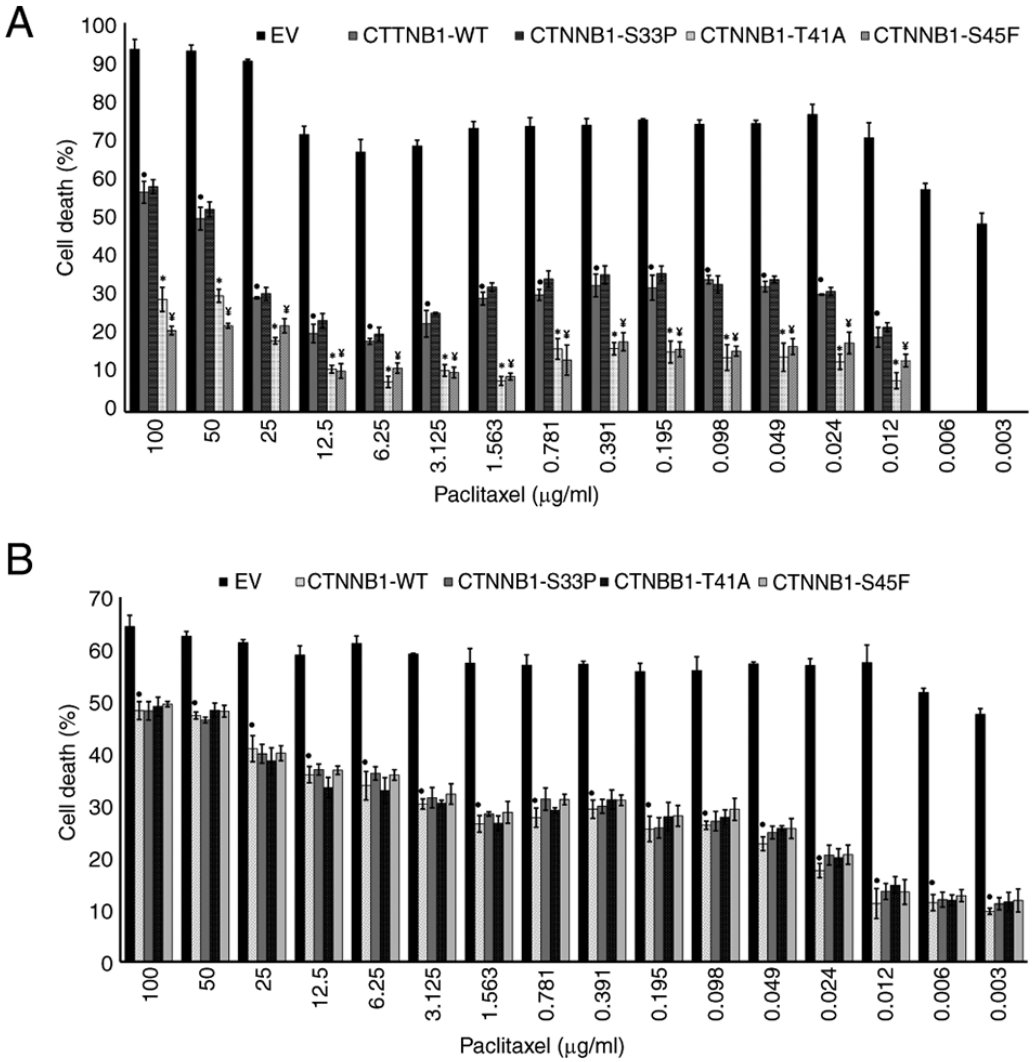

Figure 3. Effects of *CTNNB1* WT and mutations on PTX resistance in breast cancer cells (72 h). (A) PTX-induced cell death in (A) MCF-7 and (B) MDA-MB-231 cells transfected with *CTNNB1*-WT and mutations. *P<0.05 vs. respective EV. ¥P<0.05 and ¥P<0.05 vs. respective WT. CTNNB1, catenin β-1; PTX, paclitaxel; WT, wild-type, EV, empty vector.

effectors such as mTOR and GSK-3β, have not been investigated. Furthermore, considering the highly heterogeneous nature of BC, the use of only MCF-7 and MDA-MB-231 cell lines limits the generalizability of the present findings. Finally, the absence of combination therapy involving PTX and other widely used chemotherapeutics, and the lack of *in vivo* experiments are key limitations of the present study. Future studies should incorporate additional HER2(+) BC cell lines, such as BT474 and SK-BR-3, to represent the various BC subtypes. Future studies should also include gene silencing/overexpression experiments, protein-protein interaction assays and phosphorylation-specific analyses to explore the roles of these mutations in PTX resistance via the PI3K/AKT and WNT/β-catenin pathways. To validate the clinical implications of the *in vitro* results, future studies should also incorporate *in vivo* xenograft tumor models.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

GAU and PAS designed the study, analyzed and interpreted data and wrote the manuscript. GAU and GHC performed the experiments. GAU and PAS confirm the authenticity of all the raw data. All authors 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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