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Silencing dishevelled-1 sensitizes paclitaxel-resistant human ovarian cancer cells *via* AKT/GSK-3β/β-catenin signalling

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Abstract

Objectives: Expression of dishevelled-1 (DVL1) has recently been linked to cancer progression, however, its role in resistance to cancer therapy is unclear. In this study, we aimed to explore the function of DVL1 in paclitaxel-resistant human ovarian cancer cells.

Materials and methods: The MTT assay was used to assess effects of DVL1 silencing on sensitivity of cells that were otherwise resistant to paclitaxel (Taxol). Western blotting and immunofluorescence staining were used to examine effects of DVL1 on AKT/GSK-3β/β-catenin signalling.

Results: Dishevelled-1 was found to be over-expressed in a paclitaxel-resistant cell line derived from human ovarian cancer cell line A2780 (A2780/Taxol line) as well as parental A2780 cells. Down-regulation of DVL1 (using the inhibitor 3289-8625 or siRNA (siDVL1) against DVL1) sensitized A2780/Taxol cells to paclitaxel. Over-expression of DVL1 in A2780 cells increased protein levels of P-gp, BCRP and Bcl-2, which are known targets of β-catenin. Silencing DVL1 in A2780/Taxol cells also reduced levels of these proteins, and led to accumulation of β-catenin. In addition, DVL1 aberrantly activated AKT/GSK-3β/β-catenin signalling. Inactivation of AKT signalling attenuated DVL1-mediated inhibition of GSK-3β and accumulation of β-catenin, in both A2780 and A2780/Taxol cells.

Conclusions: Taken together, these results suggest that silencing DVL1 sensitized A2780/Taxol cells to paclitaxel, by down-regulating AKT/GSK-3β/β-catenin signalling, providing a novel strategy for chemosensitization of ovarian cancer to paclitaxel-induced cytotoxicity.

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Introduction

Ovarian cancer remains one of the most lethal gynaecological malignancies, and is a leading cause of cancer-related deaths in females (1–3). Although advances in treatment of ovarian cancer have been made over recent decades, prognosis for patients with advanced ovarian tumours remains poor, due to chemoresistance (4,5). Paclitaxel (Taxol) is currently used as the first-line chemotherapeutic agent for several types of cancer, including ovarian carcinoma. However, it frequently induces drug resistance, which leads to treatment failure (6–8). Understanding the mechanisms involved and thus overcoming drug resistance, are critical to ovarian cancer treatment.

Mechanisms of chemoresistance are complex, and differ between cancers. The most common conflict with anticancer drugs is due to over-expression of one or more energy-dependent ATP binding cassette (ABC) transporters in cancer cells, such as P-glycoprotein (P-gp), multidrug resistance-associated protein and breast cancer resistance protein (BCRP), which eject anticancer drugs from cells, and thereby reduce their cytotoxic effects (9–12). Other proteins that affect apoptosis, growth factor and cytokine signalling, and cell cycle behaviour, also play important roles in drug resistance (13–16). It has been reported that paclitaxel resistance is associated with altered cell signalling, including AKT and Wnt/β-catenin pathways (17,18). The aim of the current study was to examine the role of dishevelled

(DVL), a critical regulator of Wnt/β-catenin, in paclitaxel-resistant ovarian cancer cells.

Dishevelled, a key link that bridges receptors and downstream components of the Wnt signalling pathway, inhibits activation of GSK-3β and degradation of β-catenin. This increases translocation of β-catenin to the nucleus, where it interacts with the transcription factor, T-cell factor (TCF)/lymphoid enhancer factor (LEF), to induce expression of target genes, including *P-gp*, *BCRP* and *Bcl-2* (19–23). DVL not only regulates canonical Wnt/β-catenin signalling but also mediates crosstalk between Wnt/β-catenin and other signalling molecules such as AKT (24,25). Recently, DVL1, a DVL homologous protein (DVL1–3), has been identified in many tumours, and has been suggested to be responsible for tumour growth, progression and metastasis (26–28). However, its effect on drug resistance in cancer chemotherapy has remained unknown.

The current study demonstrated that DVL1 was overexpressed in the paclitaxel-resistant human ovarian cancer cell line A2780/Taxol compared to parental A2780 cells. Silencing DVL1 reduced expression of P-gp, BCRP and Bcl-2, and increased sensitivity of A2780/Taxol cells to paclitaxel. Furthermore, overexpressing or silencing DVL1, promoted or inhibited AKT/GSK-3β/β-catenin signalling, respectively. These results suggest that silencing DVL1 seemed to sensitize paclitaxel-resistant ovarian cancer cells by down-regulating the AKT/GSK-3β/β-catenin pathway; this provides a novel strategy for improving chemosensitivity of ovarian cancer.

Materials and methods

Reagents

RPMI-1640, foetal bovine serum (FBS), penicillin and streptomycin were obtained from HyClone Laboratories (Logan, UT, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) and dimethyl sulphoxide (DMSO) were purchased from Sigma-Aldrich (St Louis, MO, USA). Paclitaxel was purchased from the National Institutes for Food and Drug Control (Beijing, China). DVL inhibitor compound 3289-8625 was obtained from Merck (Darmstadt, Germany) and AKT inhibitor MK-2206 was purchased from Selleck Chemicals (Houston, TX, USA). siDVL1 (target sequence, 5′-GGAGGAGATCTTTGATGAC-3′) and siControl (non-specific siRNA) were from Genepharma (Shanghai, China). cDNA encoding DVL1 was cloned into pcDNA3.1 to generate DVL1 expression vector pcDNA3.1-DVL1. Rabbit antibodies anti-P-gp, -BCRP, -Bcl-2, -GSK-3β, -phospho-GSK-3β (Ser-9), -AKT and -phospho-AKT (Ser-473) were obtained from Cell Signaling Technology (Danvers, MA, USA). Mouse antibodies anti-DVL1, -β-catenin and -GAPDH were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Cell culture

A2780 human ovarian cancer cells and their paclitaxel-resistant derivative A2780/Taxol cells were obtained from KeyGEN Biotech Co. (Nanjing, Jiangsu, China). Cells were cultured in RPMI-1640 supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin sulphate, and incubated at 37 °C with 5% CO_2. For A2780/Taxol cells, 0.3 μmol/l of paclitaxel was included in the medium to maintain paclitaxel-resistance.

Transient transfection

A2780/Taxol cells were cultured in 96- or 6-well plates overnight, and were then transfected with siDVL1, siControl, pcDNA3.1-DVL1 or pcDNA3.1 empty vector, using Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Seventy-two hours after transfection, cells were collected and analysed using western blotting or MTT assays.

Drug sensitivity assay

Drug sensitivity was assessed using the MTT assay. Briefly, cells (5×10^3/well) were seeded in 100 μl RPMI 1640 with 5% FBS in 96-well plates. Twenty-four hours after incubation, they were either treated with 100 μM DVL inhibitor compound 3289-8625 or transfected with siDVL1 for 24 h, and were then treated with a range of concentrations of paclitaxel, for an additional 48 h. Then, MTT was added to each well at final concentration of 0.5 mg/ml. After 4 h, media were discarded, and formazan blue that forms in live cells was dissolved in 150 μl of DMSO. Absorbance at 490 nm was measured using an Ultra Microplate Reader (Bio-Tek Instruments, Winooski, VT, USA). Paclitaxel concentrations that achieved 50% growth inhibition (IC_{50}) were calculated from survival curves using the Bliss method (29).

Western blotting

After the indicated treatments, cells were washed three times in PBS, lysed in RIPA buffer (Beyotime, Nantong, China) on ice for 30 min, and centrifuged at 15 000 ***g*** for 15 min. Supernatants were collected, and

protein concentrations were determined using a BCA protein assay kit (Beyotime, Nantong, China). Forty micrograms of protein were then analysed using western blotting following standard protocols. An ECL chemiluminescent detection system (Thermo Scientific, Barrington, IL, USA) was used to develop immunoreactive bands, which were then visualized using a Bio-Rad Molecular Imager (Hercules, CA, USA). Relative protein levels (means ± SD) from three separate experiments were determined by densitometry using Image J software (National Institutes of Health, Bethesda, MD, USA) according to the manufacturer's instructions.

Immunofluorescence staining

Cells were cultured on confocal dishes, and treated as indicated. They were washed three times in PBS, fixed in 4% paraformaldehyde for 20 min, then permeabilized using 0.3% Triton X-100 for 10 min. After blocking with 10% goat serum for 2 h at room temperature, cells were incubated with anti-β-catenin antibodies at 4 °C overnight, followed by Alexa Fluor 488 secondary antibodies. Cells were counterstained with 5 mg/ml DAPI for 10 min, and images were acquired using a Zeiss confocal microscope.

Statistical analysis

All experiments were repeated in triplicate, and results are expressed as means ± SD, $n = 3$. Data were analysed using two-tailed unpaired Student's *t*-test between any two groups. One-way ANOVA analysis of variance was used to assess difference of means between groups. All analyses were performed using GraphPad Prism Software Version 5.0 (GraphPad Software Inc, La Jolla, CA, USA); level of significance was set at a $P < 0.05$.

Results

Silencing DVL1 enhanced sensitivity of A2780/Taxol ovarian cancer cells to paclitaxel

Dishevelled-1 plays an important role in cancer progression (26,30), however, up to now its role in cancer chemoresistance has remained unknown. To determine whether DVL1 is related to paclitaxel-induced resistance of ovarian cancer, its expression was compared in paclitaxel-resistant A2780/Taxol and parental A2780 ovarian cancer cells. Western blotting showed that DVL1 was overexpressed in A2780/Taxol cells (Fig. 1a), suggesting that it might be involved in resistance of A2780/Taxol cells to paclitaxel. Next, effects of DVL on paclitaxel cytotoxicity was examined in A2780/Taxol cells treated with or without the DVL small molecule inhibitor compound, 3289-8625. As shown in Fig. 1b and 1c, MTT assay revealed that IC_{50} for paclitaxel was significantly ($P < 0.05$) greater in A2780/Taxol cells (1.79 μM) compared to A2780 cells (0.18 μM), indicating that A2780/Taxol cells were more resistant than were A2780 cells, to paclitaxel. In the presence of compound 3289-8625, IC_{50} of A2780/Taxol cells was significantly reduced, to 0.33 μM ($P < 0.05$). Similarly, silencing DVL1 using siDVL1 also sensitized A2780/Taxol cells to paclitaxel, and reduced IC_{50} of A2780/Taxol (0.49 μM) cells significantly compared to siControl (1.43 μM; $P < 0.05$) (Fig. 1d,e). These results show that overexpression of DVL1 was related to paclitaxel resistance in A2780/Taxol cells, and that down-regulating DVL1 enhanced cytotoxicity of paclitaxel in otherwise resistant ovarian cancer cells.

DVL1 positively regulated expression of P-gp, BCRP and Bcl-2

Next, expression of drug resistance-related and anti-apoptotic proteins, including P-gp, BCRP and Bcl-2, was compared in A2780 and A2780/Taxol cells to assess the role of DVL1 in resistance of ovarian cancer cells to paclitaxel. Protein expression levels of P-gp, BCRP and Bcl-2 were up-regulated significantly in A2780/Taxol cells compared to A2780 cells (Fig. 2a). As DVL1 was also overexpressed in A2780/Taxol cells, we assessed whether it would regulate expression of P-gp, BCRP and Bcl-2. A2780 cells were transfected with pcDNA3.1-DVL1, and western blotting then revealed that levels of P-gp, BCRP and Bcl-2 were significantly increased ($P < 0.05$) compared to control-transfected cells (Fig. 2b).

To confirm that increased expression of P-gp, BCRP and Bcl-2 was mediated by DVL1, we inhibited DVL1 in A2780/Taxol cells using compound 3289-8625, and then examined expression of P-gp, BCRP and Bcl-2. As expected, treatment with compound 3289-8625 reduced expression of P-gp, BCRP and Bcl-2 (Fig. 2c). Similarly, down-regulation of DVL1 using siDVL1 also reduced expression of P-gp, BCRP and Bcl-2. Moreover, siDVL1-induced reduction in P-gp, BCRP and Bcl-2 expression was rescued by overexpressing DVL1 (Fig. 2d). Collectively, these results suggest that DVL1 up-regulated expression of P-gp, BCRP and Bcl-2, mediating paclitaxel resistance in ovarian cancer.

DVL1 increased accumulation and nuclear translocation of β-catenin

Previous studies demonstrated that *P-gp, BCRP* and *Bcl-2* are β-catenin target genes (19–21,31–33).

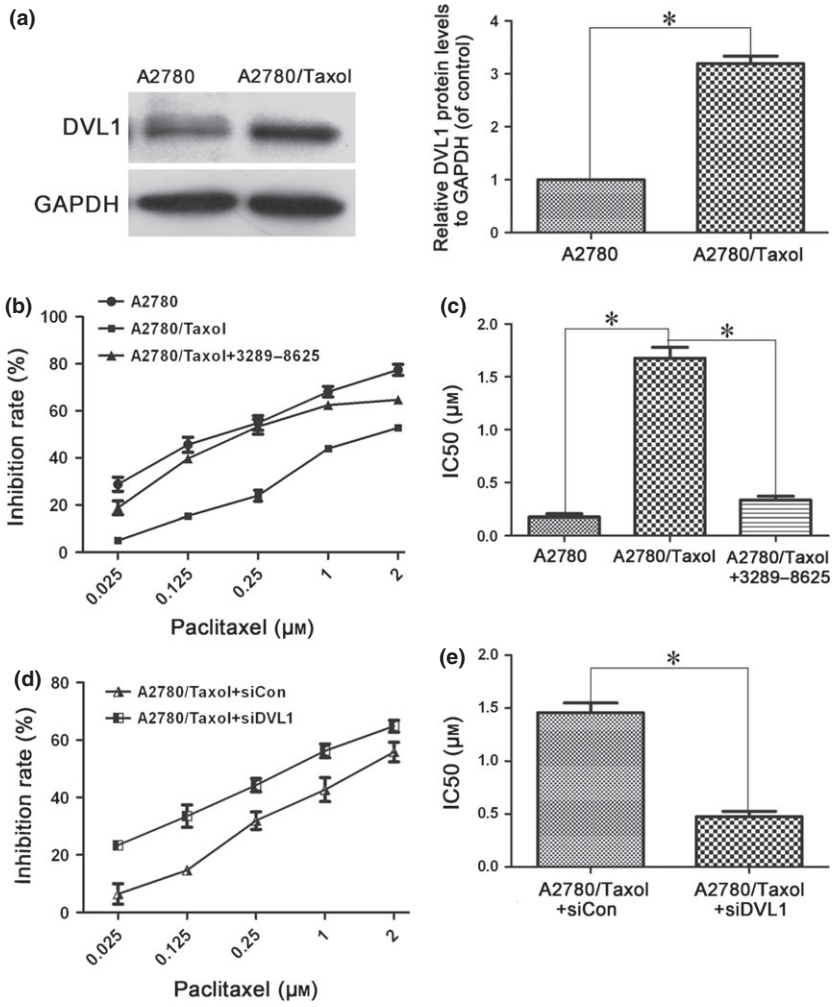

Figure 1. Silencing DVL1 enhanced the sensitivity of A2780/Taxol ovarian cancer cells to paclitaxel. (a) Western blotting of DVL1 expression in A2780 and A2780/Taxol cells. GAPDH was used as the internal control. The left panel shows representative Western blots, and the right panel shows quantitation of the Western blots to show the relative DVL1 expression normalized to GAPDH control. (b) The drug sensitivity of A2780, A2780/Taxol and compound 3289-8625-treated A2780/Taxol cells was assessed using MTT assays to measure cell viability. (c) The IC_{50} of paclitaxel in A2780, A2780/Taxol and compound 3289-8625-treated A2780/Taxol cells. The IC_{50} of paclitaxel was calculated from the survival curves generated in (b) using the Bliss method. (d) The drug sensitivity of A2780/Taxol cells transfected with siDVL1 or siControl. (e) The IC_{50} of paclitaxel in transfected A2780/Taxol cells, which was calculated from the survival curves in (d) using the Bliss method. Each experiment was performed in triplicate. $^*P < 0.05$.

Thus, effects of DVL1 on expression and subcellular translocation of β-catenin were investigated. β-catenin protein levels were increased by overexpression of DVL1 in A2780 cells (Fig. 3a), and reduced by treatment with compound 3289-8625 in A2780/Taxol cells (Fig. 3b). Also, β-catenin protein levels were down-regulated in siDVL1-transfected A2780/Taxol cells, which were rescued by overexpression of DVL1 (Fig. 3c). These results indicate that DVL1 increased accumulation of β-catenin in ovarian cancer cells. To confirm this, we demonstrated that β-catenin levels were higher in A2780/Taxol compared to A2780 cells, and that silencing DVL1 reduced β-catenin levels significantly (Fig. 3d). Immunofluorescence staining of A2780 cells revealed that β-catenin was localized primarily in cell membranes and cytoplasm, and that little was found in the nucleus. Conversely in A2780/Taxol cells, more cytoplasmic accumulation and nuclear translocation were observed. However, β-catenin levels in both cytoplasm and nucleus were reduced in A2780/Taxol cells transfected with siDVL1 (Fig. 3e). Taken together, these results show that DVL1 increased accumulation and nuclear translocation of β-catenin.

DVL1 inhibited GSK3β by activating AKT

Activated AKT can inactivate GSK-3β *via* phosphorylation at Ser-9/21. Inactivation of GSK-3β prevents phosphorylation and degradation of β-catenin, leading to its accumulation and nuclear translocation (34,35). To explore molecular mechanisms underlying DVL1-regulated β-catenin accumulation, we assessed effects of DVL1 on AKT and GSK-3β activity. As shown in Fig. 4a, overexpression of DVL1 significantly up-regulated phosphorylation of AKT at Ser-473 and GSK-3β at Ser-9 in A2780 cells. In contrast, levels of phosphorylated AKT (Ser-473) and GSK-3β (Ser-9) were reduced in compound 3289-8625-treated and siDVL1-transfected A2780/Taxol cells. reduced phosphorylation induced by DVL1-silencing was rescued by DVL1-overexpression

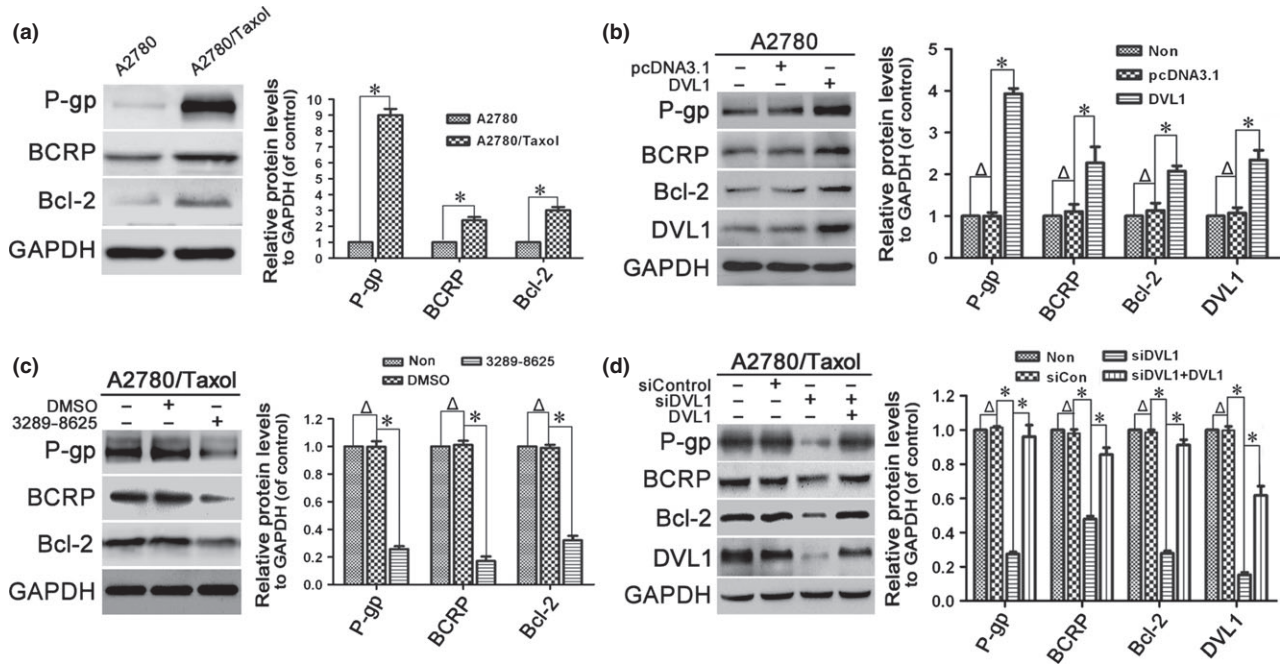

Figure 2. Effect of DVL1 on drug resistance-related proteins. Western blotting for the drug resistance-related proteins P-gp, BCRP and Bcl-2 in (a) A2780 and A2780/Taxol cells, (b) A2780 cells transfected with pcDNA3.1-DVL1 or pcDNA3.1 for 72 h, (c) A2780/Taxol cells treated with or without 100 μM compound 3289-8625 for 72 h, and (d) A2780/Taxol cells transfected with siControl, siDVL1 or siDVL1 plus pcDNA3.1-DVL1 for 72 h. GAPDH was used as the internal control. The left panels show representative Western blots, and the right panels show relative quantitation of the blots after normalization to GAPDH. In B and D, DVL1 blots were included to confirm either the overexpression of DVL1 from pcDNA3.1-DVL1 or the silencing of DVL1 by siDVL1. Each experiment was performed in triplicate. $^{\Delta}P > 0.05$; $^{*}P < 0.05$.

(Fig. 4b,c). These data suggest that DVL1 increased AKT and inhibited GSK-3β activity. Furthermore, inactivation of AKT using inhibitor MK-2206 attenuated DVL1-mediated inhibition of GSK-3β and accumulation of β-catenin in A2780 and A2780/Taxol cells (Fig. 4d, e). These results imply that DVL1 inhibited GSK3β by activating AKT to increase accumulation of β-catenin.

Discussion

Paclitaxel (Taxol) is a front-line chemotherapeutic agent used to treat a number of human carcinomas, including ovarian cancer (36,37). However, it frequently induces drug resistance, which is a major obstacle to successful clinical treatment of ovarian cancer (8,38). Thus, understanding mechanisms contributing to drug resistance is urgent for development of efficient therapeutic strategies and overcoming chemoresistance. Cancer-derived cell lines, in which paclitaxel resistance is induced by intermittent drug treatment, are used commonly as models to explore this phenomenon. Thus, stable paclitaxel-resistant ovarian cancer cell line A2780/Taxol was used in the present study and was compared to its parental cell line A2780. A2780/Taxol cells exhibited strong resistance to paclitaxel, with an IC_{50} value that was almost 10-fold of that of A2780 cells (Fig. 1b,c).

Accumulating evidence has shown that altered activity of the Wnt/β-catenin signalling pathway, responsible for a diverse set of biological processes (such as cell proliferation, differentiation, migration, survival/apoptosis and stem cell self-renewal), is involved in development of paclitaxel-resistance in cancers (17,39). In addition, abnormal activation of Wnt/β-catenin signalling has been implicated in development of a broad spectrum of tumours, including ovarian cancer (40,41). DVL is a key regulator of Wnt/β-catenin signalling, and relays Wnt signals from their receptors to downstream effectors. Upon Wnt stimulation, DVL is recruited to the cell surface receptor frizzled (FZ), resulting in disassembly of β-catenin destruction complex (that contains GSK-3β), with inhibition of GSK-3β catalytic activity and β-catenin degradation. This leads to cytoplasmic accumulation and nuclear translocation of β-catenin, where it ultimately activates transcription of target genes (22,42). Notably, DVL overexpression can be sufficient to promote Wnt/β-catenin pathway activity in the absence of Wnt stimulation by inhibiting GSK-3β activity (43,44). It has been reported that DVL is involved in development of several cancers (27,45). However,

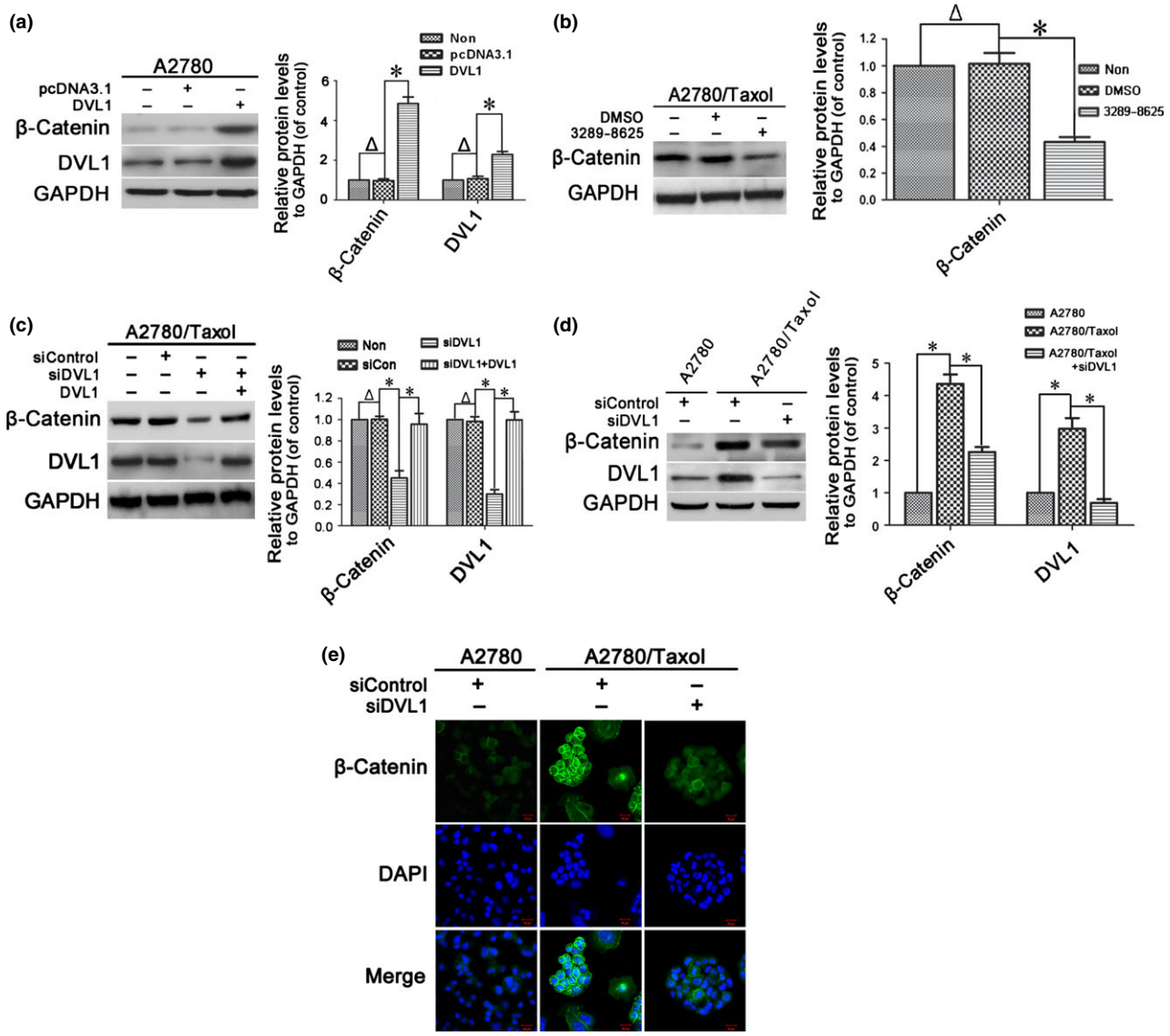

Figure 3. The effect of DVL1 on the accumulation and nuclear translocation of β-catenin. (a–d) Western blotting for β-catenin in (a) A2780 cells transfected with pcDNA3.1-DVL1 or pcDNA3.1 for 72 h, (b) A2780/Taxol cells treated with or without 100 μM compound 3289-8625 for 72 h, (c) A2780/Taxol cells transfected with siControl, siDVL1 or siDVL1 plus pcDNA3.1-DVL1 for 72 h, and (d) A2780 cells transfected with siControl and A2780/Taxol cells transfected with siControl or siDVL1 for 72 h. GAPDH was used as the internal control. Left panels show representative Western blots, and right panels show the relative quantification after normalization to GAPDH. In (a, c and d), DVL1 blots were included to confirm either the overexpression of DVL1 from pcDNA3.1-DVL1 or the silencing of DVL1 by siDVL1. Each experiment was performed in triplicate. $^{\Delta}P > 0.05$; $^{*}P < 0.05$. (e) Immunofluorescence analysis of the cellular distribution of β-catenin in the cytoplasm and nucleus of A2780 and A2780/Taxol cells transfected with siControl or siDVL for 72 h. Green, β-catenin; blue, nuclear DNA (Scale bar = 20 μm).

mechanisms by which DVL plays a role in cancer chemoresistance has remained unknown. For this purpose the current study examined the relationship between DVL and paclitaxel-resistance in ovarian cancer.

Data acquired revealed that DVL1 was overexpressed in A2780/Taxol compared to A2780 cells (Fig. 1a), indicating that DVL1 was related to paclitaxel resistance in ovarian cancer. To determine the role of DVL1 in paclitaxel-resistance, DVL1 function was blocked using the small molecule inhibitor compound 3289-8625, a cell-permeable amidobenzanilide compound that disrupts FZ-DVL interaction by targeting the PDZ domain of DVL (46,47). Compound 3289-8625 increased sensitivity of A2780/Taxol cells to paclitaxel, and reduced the IC_{50} value of the drug (Fig. 1b,c). To further examine the role of DVL1 in chemoresistance, its expression was down-regulated using siDVL1. Consistent with the above observations, siDVL1 increased sensitivity of

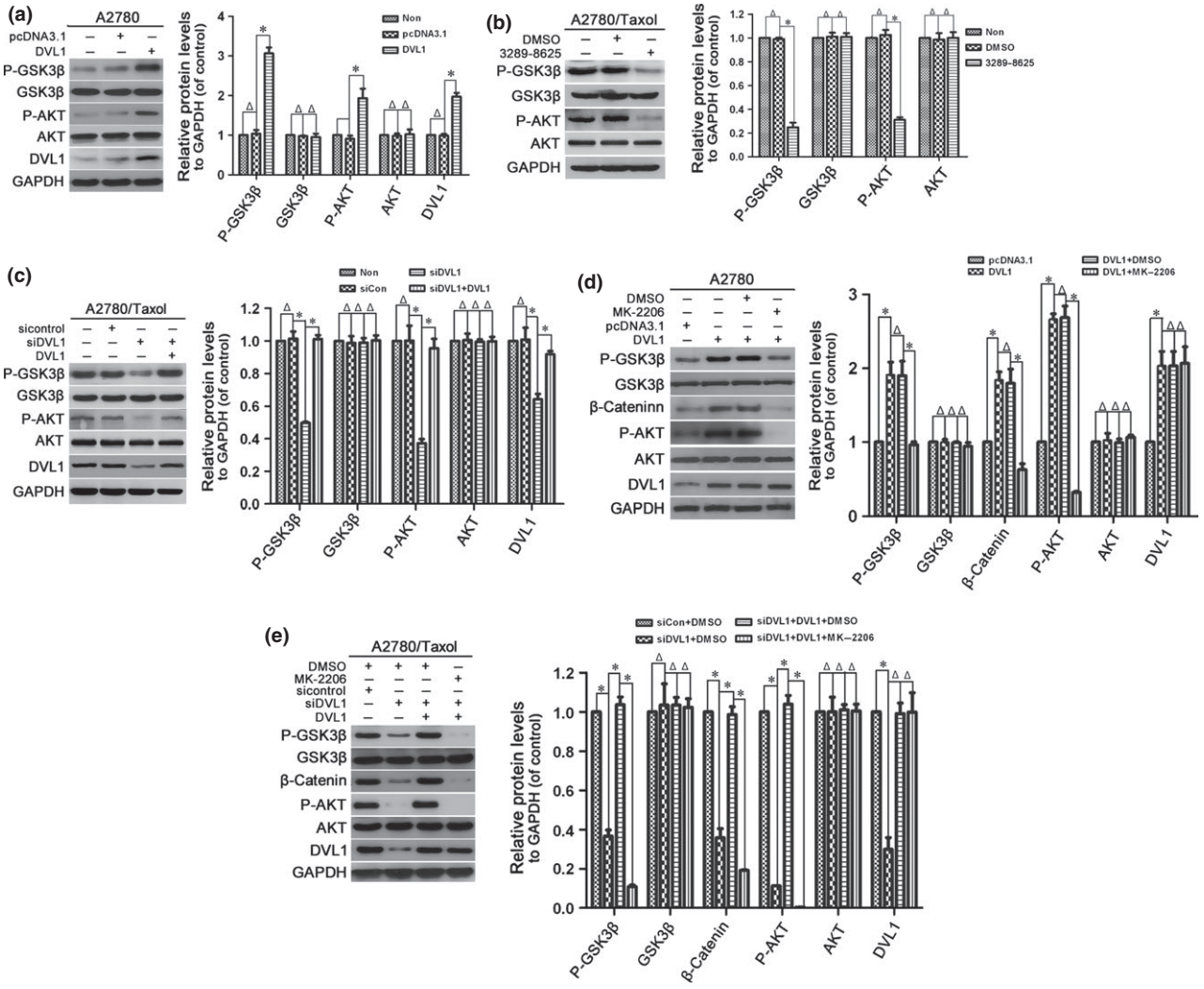

Figure 4. The effect of DVL1 on AKT/GSK-3β/β-catenin signalling. Western blotting for phospho-GSK-3β (Ser-9), GSK-3β, phospho-AKT (Ser-473), AKT and DVL1 (when pcDNA3.1-DVL1- or siDVL-transfected cells were included) in (a) A2780 cells transfected with pcDNA3.1-DVL1 or pcDNA3.1 for 72 h, (b) A2780/Taxol cells treated with or without compound 3289-8625 for 72 h, (c) A2780/Taxol cells transfected with siControl, siDVL1 or siDVL1 plus pcDNA3.1-DVL1 for 72 h, (d) A2780 cells transfected with pcDNA3.1 or pcDNA3.1-DVL1 for 72 h, and then treated with the AKT inhibitor MK-2206 for 5 h, and (e) A2780/Taxol cells transfected with siControl, siDVL1 or siDVL1 plus pcDNA3.1-DVL1 for 72 h, and then treated with MK-2206 for 5 h. GAPDH was used as the internal control. Left panels show representative Western blots, and right panels show the relative quantification after normalization to GAPDH. Each experiment was performed in triplicate. $^{\Delta}P > 0.05$; $^{*}P < 0.05$.

A2780/Taxol cells to paclitaxel and rreduced the IC_{50} value (Fig. 1d,e). These data imply that DVL1 is positively correlated to resistance of ovarian cancer to paclitaxel, and that down-regulation of DVL1 restores sensitivity of ovarian cancer to paclitaxel.

We then focused on proteins involved in drug resistance. Membrane transporters in the ABC protein superfamily can reduce intracellular drug concentrations and cytotoxicity *via* drug efflux. Overexpression of these proteins is positively correlated with resistance to drugs, including paclitaxel (10,48,49). Anti-apoptotic proteins are also related to drug resistance. Abnormal up-regulation of anti-apoptotic proteins such as Bcl-2 also contributes to drug resistance (50). The currently acquire data demonstrated that two important ABC transporter proteins, P-gp and BCRP, and anti-apoptotic protein Bcl-2 were overexpressed in A2780/Taxol cells compared to A2780 (Fig. 2a). In addition, levels of these proteins were increased when DVL1 was up-regulated (Fig. 2b), but reduced when DVL1 was down-regulated using compounds 3289-8625 or siDVL1 (Fig. 2c,d). Meanwhile, reduction in siDVL1 was reversed by ectopic DVL1 expression (Fig. 2d). These data indicate that silencing DVL1 enhanced sensitivity of A2780/Taxol

cells to paclitaxel by down-regulating P-gp, BCRP and Bcl-2.

P-gp, *BCRP* and *Bcl-2* are target genes of the Wnt/β-catenin pathway. The role of β-catenin in drug resistance of mixed-lineage leukaemia stem cells and cholangiocarcinoma has been reported previously (51,52). In the current study, we demonstrated that β-catenin was overexpressed in A2780/Taxol paclitaxel-resistant human ovarian cancer cells compared to parental A2780 cells (Fig. 3d), showing that β-catenin was involved in resistance of ovarian cancer cells to paclitaxel. As DVL1 was also overexpressed in A2780/Taxol cells (Fig. 1a) and recent studies suggested that DVL might be sufficient to activate Wnt/β-catenin signalling (43,44), we thought that altered DVL1 protein levels could modulate expression of β-catenin. Our hypothesis was confirmed by results revealing that DVL1 positively regulated accumulation and nuclear translocation of β-catenin in A2780 and A2780/Taxol cells (Fig. 3), implying that DVL1 regulated expression of P-gp, BCRP, and Bcl-2 by activating β-catenin.

β-Catenin is activated after an event triggered by DVL-mediated inactivation of GSK-3β (19,22). In the current study, overexpression and silencing of DVL1 inhibited or promoted GSK-3β activity, respectively (Fig. 4a–c). Two mechanisms have been proposed to account for DVL-mediated inhibition of GSK-3β: inhibition of catalytic activity of GSK-3β by serine phosphorylation (22,53,54), and disruption of the interaction between GSK-3β and β-catenin (55). However, the process whereby DVL stimulates serine phosphorylation of GSK-3β is not well understood. It has been reported that activated AKT (phosphorylated at Thr-308 and Ser-473) phosphorylates GSK-3β at Ser-9/21, inhibiting its activity (56). As both DVL and AKT regulate GSK-3β activity, these signalling cascades could converge at GSK-3β to regulate β-catenin. Here, we demonstrated that overexpression of DVL1 enhanced AKT activity (Fig. 4a), whereas inhibiting DVL1 using compound 3289-8625 or siDVL1 reduced it (Fig. 4b,c). Conversely, inactivation of AKT using MK-2206 attenuated DVL1-mediated inhibition of GSK-3β and accumulation of β-catenin (Fig. 4d, e). These results show that DVL1 inhibited GSK-3β by activating AKT to increase accumulation of β-catenin.

To the best of our knowledge, this is the first study demonstrating roles for DVL1 in ovarian cancer resistant to paclitaxel. DVL1 was overexpressed in paclitaxel-resistant human ovarian cancer cells, where it inhibited GSK-3β activity by activating AKT. This increased accumulation of β-catenin and enhanced expression of P-gp, BCRP and Bcl-2. Silencing DVL1 sensitized paclitaxel-resistant human ovarian cancer cells by down-regulating AKT/GSK-3β/β-catenin signalling, providing a novel insight into chemosensitization of ovarian cancer to paclitaxel-induced cytotoxicity.

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