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Research Article

Inhibition of disheveled-2 resensitizes cisplatin-resistant lung cancer cells through down-regulating Wnt/β-catenin signaling

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ABSTRACT

Cisplatin (CDDP) is currently recommended as the front-line chemotherapeutic agent for lung cancer. However, the resistance to cisplatin is widespread in patients with advanced lung cancer, and the molecular mechanism of such resistance remains incompletely understood. Disheveled (DVL), a key mediator of Wnt/β-catenin, has been linked to cancer progression, while the role of DVL in cancer drug resistance is not clear. Here, we found that DVL2 was over-expressed in cisplatin-resistant human lung cancer cells A549/CDDP compared to the parental A549 cells. Inhibition of DVL2 by its inhibitor (3289-8625) or shDVL2 resensitized A549/CDDP cells to cisplatin. In addition, over-expression of DVL2 in A549 cells increased the protein levels of BCRP, MRP4, and Survivin, which are known to be associated with chemoresistance, while inhibition of DVL2 in A549/CDDP cells decreased these protein levels, and reduced the accumulation and nuclear translocation of β-catenin. In addition, shβ-catenin abolished the DVL2-induced the expression of BCRP, MRP4, and Survivin. Furthermore, our data showed that GSK3β/β-catenin signals were aberrantly activated by DVL2, and inactivation of GSK3β reversed the shDVL2-induced down-regulation of β-catenin. Taken together, these results suggested that inhibition of DVL2 can sensitize cisplatin-resistant lung cancer cells through down-regulating Wnt/β-catenin signaling and inhibiting BCRP, MRP4, and Survivin expression. It promises a new strategy to chemosensitize cisplatin-induced cytotoxicity in lung cancer.

1. Introduction

Lung cancer is one of the most prevalent cancers around the world with over one million new cases diagnosed annually [1,2]. It continues to be associated with the highest incidence and mortality rates of all cancers [3]. The treatment advances in lung cancer have been made over the last decades, such as surgical resection, chemotherapy and radiation. Although surgical resection remains one method of choice to cure lung cancer, many patients at diagnosis present unresectable tumors in advanced disease stages with extensive local/regional tumor invasion or distal metastasis [4,5]. Therefore, chemotherapy and/or radiation play a critical role in clinical treatment of lung cancers. Cisplatin (CDDP)-based chemotherapy has been recommended as the first-line therapy for advanced lung cancer [6]. Unfortunately, the chemotherapy efficacy is far from satisfactory due to emergence of drug resistance in cancer cells [7,8]. In this case, understanding the mechanisms of resistance and overcoming the major obstacle in lung cancer chemotherapy is critical for successful therapy of lung cancer.

The reasons for chemoresistance in cancer are complex and may be associated with several mechanisms. One well-studied mechanism is the reduced accumulation of cellular chemotherapeutics. In the last decades, many studies have focused on various ATP binding cassette (ABC) transporters, which increase drug efflux to reduce the drug accumulation in cancer cells, such as ABCG2 (breast cancer resistance protein, BCRP), ABCC subfamily (multidrug resistance-associated protein, MRP), ABCB1 (multidrug resistance protein 1, MDR1/P-gp). A growing body of evidences also suggest that drug resistance is not only mediated by efflux-related proteins, but also by over-expression of anti-apoptotic proteins, such as Survivin and Bcl-2. It has been reported that cisplatin resistance is involved in aberrant cell signaling including PI3K-AKT, and Wnt/β-catenin signaling pathway [9,10]. In present

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study, we aimed to examine the role of a key regulator of Wnt/β-catenin, disheveled (DVL), in cisplatin-resistant lung cancer.

The Wnt/β-catenin signaling pathway controls a vast array of biological process including cell proliferation, differentiation, apoptosis, migration, polarity establishment and stem cell self-renewal [11]. Abnormal Wnt/β-catenin signaling leads to a diverse range of human diseases, such as Alzheimer's disease, polycystic kidney disease, osteoporosis, schizophrenia, metabolic disease, and various cancers [12]. In the absence of Wnt signaling, β-catenin is contained within the so-called "destruction complex" of axin, adenomatous polyposis coli (APC) and glycogen synthase kinase 3β (GSK3β); β-catenin is phosphorylated by GSK3β and subsequently degraded in a ubiquitin-dependent manner in cytoplasm [13]. Under the activation of Wnt signaling, the binding of Wnt to its frizzled receptor results in depolymerization of the "destruction complex", and subsequent phosphorylation of an inhibitory serine residue (Ser9) on GSK3β that causes catalytic inactivation, β-catenin is not phosphorylated. Instead, the free β-catenin is allowed to accumulate in cytoplasm and translocate into cell nucleus, where it associates with the transcription factors lymphoid enhancer factor (LEF)/T cell factor (TCF) to trigger expression of Wnt target genes including ABC transporters and Survivin [14,15]. Many studies have showed that DVL is an important link bridging the receptors and downstream components of Wnt signaling. Notably, regardless of Wnt stimulation, DVL over-expression can activate Wnt/β-catenin pathway and turn on the transcription of the target genes [16–18]. Recently, DVL2, one of DVL homology proteins (DVL1-3), has been linked to tumor growth, metastasis and invasion [19–21]. However, the relationship between DVL2 and cancer chemoresistance has not yet been reported. Therefore, the roles and related mechanisms of DVL2 in lung cancer resistance to cisplatin were investigated in the present study.

2. Materials and methods

2.1. Chemicals and reagents

Cisplatin (CDDP), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and Lithium chloride (LiCl, a GSK3β inhibitor) were purchased from Sigma-Aldrich (St Louis, MO, USA). The culture medium RPMI-1640, penicillin, and streptomycin were purchased from HyClone Laboratories (Logan, UT, USA). Fetal bovine serum (FBS) was purchased from TransGen Biotech (Peking, China). The compound 3289-8625 (DVL inhibitor) was purchased from Merck (Darmstadt, Germany). All compounds which were solubilized in DMSO with the working concentrations of DMSO in the assay were less than 0.5% (v/v). Medium containing 0.5% DMSO was used as the control. shDVL2 (target sequence, 5′-GGAAGAAATTTCAGATGAC-3′) and shRNA negative control (shNC) were gained from genechem (Shanghai, China). pCMV5-3XFlag DVL2 (WT) was a gift from Jeff Wrana (Addgene plasmid # 24,802) [22]. Lipofectamine 3000 transfection reagent was purchased from Invitrogen (Carlsbad, CA, USA). RIPA (Radioimmunoprecipitation) lysis buffer and BCA protein assay kit were purchased from Beyotime Biotechnology (Nantong, China). Primary antibodies against BCRP, MRP4, Survivin, GSK3β, phospho-GSK3β (Ser-9) were purchased from Cell Signaling Technology (Danvers, MA, USA). Primary antibodies against DVL2, β-catenin, and GAPDH were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Horseradish peroxidase-conjugated secondary antibody was obtained from ZSGB-bio (Peking, China).

2.2. Cell culture

The cisplatin-resistant human lung cancer cells A549/CDDP and its parent cells A549 were selected in the present study and purchased from Shanghai Bogoo Biotechnology (Shanghai, China). They were maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 100 μg/mL streptomycin and 100units/mL penicillin under a humidified 5% CO2 atmosphere at 37 °C in incubator. Cisplatin (10 μM) was added in the medium to maintain resistance of A549/CDDP cells.

2.3. Western blotting analysis

Cells were harvested into RIPA lysis buffer after washing three times with PBS. Protein concentrations were determined using a BCA protein assay kit, and equal amounts of protein (80 μg/sample) were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred to PVDF membranes. After blocked with 5% non-fat milk at room temperature for 2 h, membranes were incubated with the primary antibody overnight at 4 °C, and then incubated with a horseradish peroxidase-conjugated secondary antibody for 2 h at room temperature. An ECL chemiluminescent detection system was used to develop the immunoreactive bands, which were then visualized using a Bio-Rad Molecular Imager [23]. GAPDH was used as the internal control.

2.4. MTT assays

MTT assays were used to assess sensitivity of lung cancer cells to cisplatin. Cells (4×10^3/well) were incubated in 96-well plates with 100 μL RPMI-1640 medium and 5% FBS. After incubated for 12 h, cells were either treated with 100 μM 3289-8625 or transfected with shDVL2 for 24 h, and then treated with various concentrations of cisplatin (0, 45, 90, 135, 200, 250 μM) for an additional 48 h. Then, MTT was added to each well at a final concentration of 0.5 mg/mL. After 4 h, the medium was discarded, and 150 μL of DMSO was added in each well. The absorbance at 490 nm was measured using an Ultra Microplate Reader (Bio-Tek Instruments, Winooski, VT, USA). The inhibition ratio and IC50 (cisplatin concentrations that achieved 50% growth inhibition) were calculated from the survival curves using the Bliss method.

2.5. Cell transfection and cotransfection

Cells were seeded in 96- or 6-well plates overnight to be 70–90% confluent, and then transfected with shDVL2, shNC, pCMV5-DVL2, or pCMV5 empty vector using Lipofectamine 3000 transfection reagent according to the manufacturer's instructions. Specially, A549/CDDP cells were cotransfected with shDVL2 plus pCMV5-DVL2. A549/CDDP cells were seeded in 6-well plates to be 70–90% confluent at cotransfection. Firstly, 3.75 μL Lipofectamine™ 3000 Reagent was diluted in 125 μL Opti-MEM™ Medium, and master mix of 1 μg shDVL2 and 1 μg pCMV5-DVL2 was prepared by diluting the DNA in 125 μL Opti-MEM™ Medium, then 4 μL P3000™ Reagent was added into the master mix, mixed well. Next, the diluted DNA was added into the diluted Lipofectamine™ 3000 Reagent, incubated for 10–15 min at room temperature, and then the DNA-lipid complex was added into cells directly. shβ-catenin and pCMV5-DVL2 were also cotransfected into A549 cells following the same procedures. 24 or 72 h after transfection, cells were used for MTT assays or western blotting.

2.6. Immunofluorescence staining

Cells were cultured on confocal dishes, and treated as indicated. Cells were washed three times with phosphate buffer

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solution (PBS), fixed with 4% paraformaldehyde for 20 min, permeabilized using 0.2% Triton X-100 for 20 min, and blocked with 10% goat serum for 2 h at room temperature. Cells were then incubated with mouse monoclonal anti-β-catenin antibodies diluted 1:50 in blocking buffer overnight at 4 °C. After washed with PBS, followed by incubation with Alexa Fluor 488 secondary antibodies diluted 1:1000 in blocking buffer for 2 h at room temperature. After washed with PBS, cells were counterstained with 5 mg/mL DAPI for 10 min. The immunofluorescence staining images were obtained on a Zeiss confocal microscope [24].

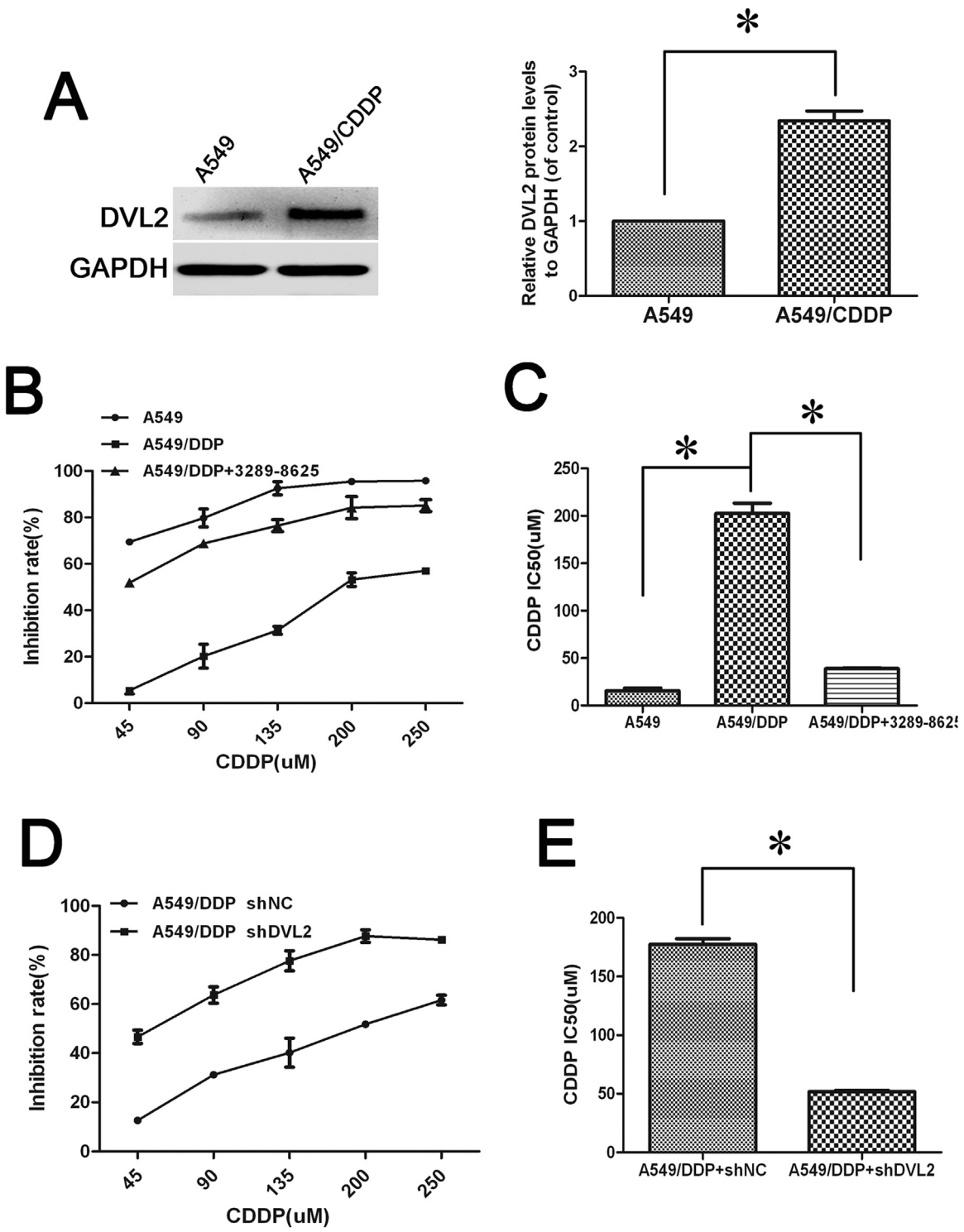

Fig. 1. Inhibiting DVL2 improved the sensitivity of A549/CDDP lung cancer cells to cisplatin. (A) A549 and A549/CDDP cells were lysed to detect expression of DVL2. The left panel showed representative western blotting, and the right panel showed the relative DVL2 expression normalized to GAPDH control. (B) The sensitivity of A549, A549/CDDP and 3289-8625-treated A549/CDDP cells to cisplatin was determined by MTT assays. (C) The IC50 of cisplatin in A549, A549/CDDP, and compound 3289-8625-treated A549/CDDP cells. (D) The drug sensitivity of A549/CDDP cells transfected with shDVL2 or shDVL2. (E) The IC50 in A549/CDDP cells transfected with shDVL2 or shDVL2. Each experiment was performed in triplicate. *, $P < 0.05$.

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2.7. Statistical analysis

All values were reported as mean ± SD of three independent experiments unless otherwise specified. Data were analyzed by two-tailed unpaired Student's *t*-test between two groups and by One-Way ANOVA followed by Bonferroni test for multiple comparison involved. Statistical analysis was carried out using SPSS 16.0 for Windows. The cut-off for significance was set to $p < 0.05$.

3. Results

3.1. Inhibiting DVL2 resensitized A549/CDDP lung cancer cells to cisplatin

To date, the role of DVL2 in cancer chemoresistance remains unclear. Therefore, the expression of DVL2 in cisplatin resistant lung cancer cells was firstly examined. Western blotting revealed that the expression of DVL2 protein was obviously up-regulated in cisplatin-resistant lung cancer cells A549/CDDP as compared with their parental cells A549 (Fig. 1A), indicating that DVL2 might be involved in resistance of A549/CDDP to cisplatin. Next, the effect of DVL on cisplatin cytotoxicity was determined in A549/CDDP cells treated with or without the DVL inhibitor 3289-8625. As shown in Fig. 1B and C, MTT assays showed that the IC50 for cisplatin was significantly ($P < 0.05$) greater in A549/CDDP cells (202.5 μM) compared to A549 cells (15.5 μM), suggesting that A549/CDDP cells were more resistant to cisplatin than A549 cells. The IC50 of A549/CDDP cells treated with 3289-8625 were decreased to 38.8 μM ($P < 0.05$). Similarly, silencing DVL2 by shDVL2 also made A549/CDDP cells more sensitive to cisplatin, and significantly reduced the IC50 of A549/CDDP cells (51.8 μM) compared with shNC (177.4 μM; $P < 0.05$) (Fig. 1D and E). Above results suggested that the over-expression of DVL2 was positively correlated with cisplatin resistance in A549/CDDP cells, and that inhibiting DVL2 can improve the cytotoxicity of cisplatin in lung cancer.

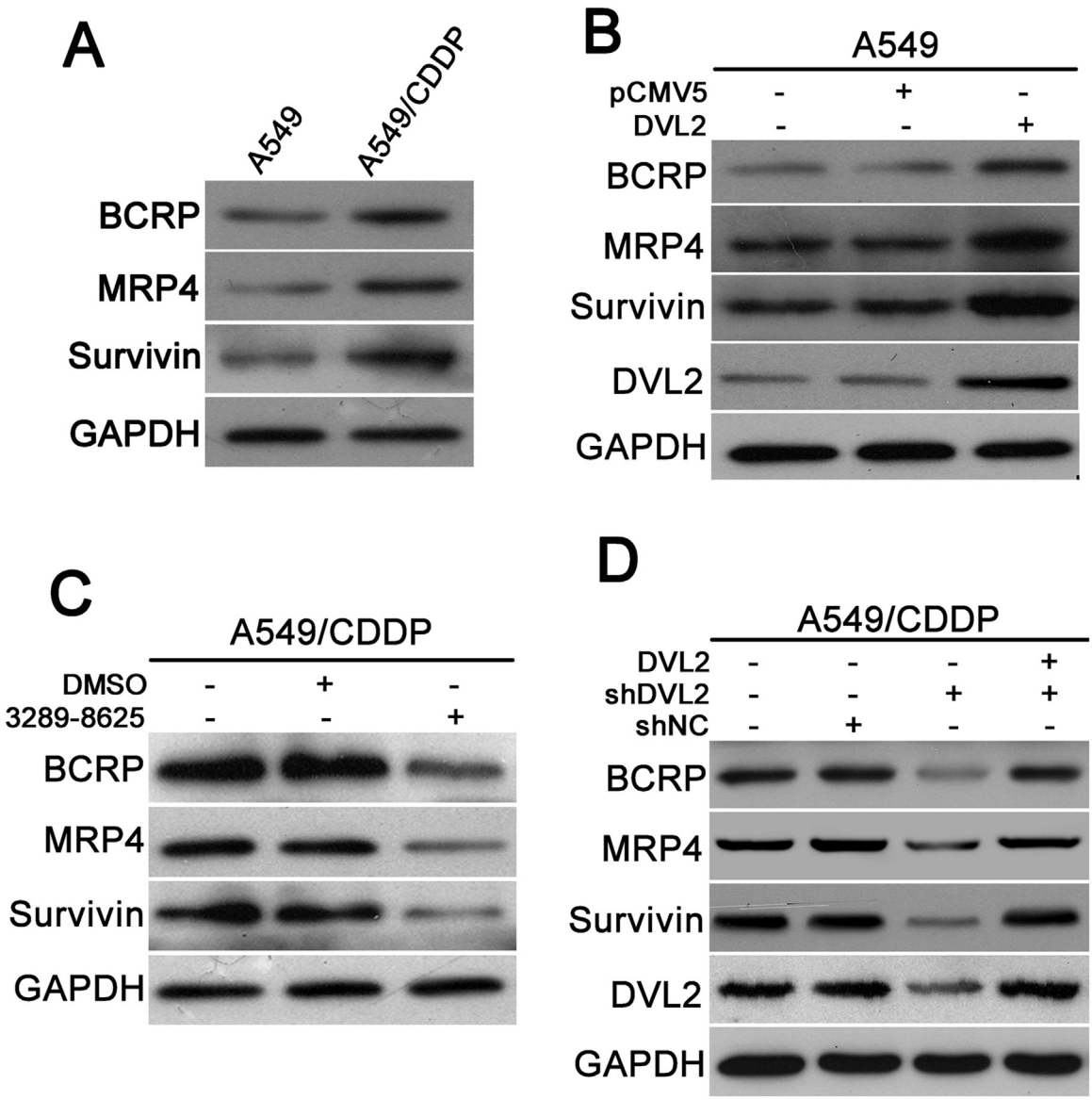

Fig. 2. Effect of DVL2 on the expression of BCRP, MRP4 and Survivin. (A) Untreated A549 and A549/CDDP cells, (B) A549 cells transfected with pCMV5-DVL2 or pCMV5 for 72 h, (C) A549/CDDP cells treated with or without 100 μM compound 3289-8625 for 72 h, and (D) A549/CDDP cells transfected with shNC, shDVL2, or shDVL2 plus pCMV5-DVL2 for 72 h, were lysed to detect the protein levels of BCRP, MRP4, Survivin and DVL2 using Western blotting. In each case, the blot was representative of immunoblots resulting from three separate experiments.

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3.2. DVL2 positively controlled the expression of drug-resistance related proteins

To further analyze the role of DVL2 in the resistance of lung cancer cells to cisplatin, the protein expression of BCRP, MRP4 and Survivin were measured in A549 and A549/CDDP cells. The protein levels of BCRP, MRP4 and Survivin were significantly increased in A549/CDDP cells compared to A549 cells (Fig. 2A). Because DVL2 was also over-expressed in A549/CDDP cells, we assessed whether DVL2 controlled the expression of BCRP, MRP4 and Survivin. The recombinant vectors pCMV5-DVL2 were transfected into A549 cells, and western blotting showed that the expression of BCRP, MRP4 and Survivin was up-regulated by DVL2 over-expression compared with control group (Fig. 2B).

To confirm that the up-regulation of BCRP, MRP4 and Survivin was mediated by DVL2, we inhibited DVL2 in A549/CDDP cells using compound 3289-8625, and then examined the expression of BCRP, MRP4 and Survivin. As expected, the expression of BCRP, MRP4 and Survivin were decreased by treatment with compound 3289-8625 (Fig. 2C). Similarly, the expression of BCRP, MRP4 and Survivin were also reduced by silencing DVL2 using shDVL2. Moreover, shDVL2 and pCMV5-DVL2 were cotransfected into A549/CDDP cells, the shDVL2-reduced expression was rescued by over-expressing DVL2 (Fig. 2D). Collectively, these results suggested that DVL2 up-regulated the expression of BCRP, MRP4 and Survivin to increase resistance of lung cancer to cisplatin.

3.3. β-catenin was crucial for DVL2-regulated expression of BCRP, MRP4 and Survivin

Accumulating evidences have shown that BCRP, MRP4 and Survivin are target genes of β-catenin [25–27]. Therefore, we examined the effect of DVL2 on β-catenin, and role of β-catenin in DVL2-regulated expression of BCRP, MRP4 and Survivin. The protein level of β-catenin was increased by over-expression of DVL2 in A549 cells (Fig. 3A), and decreased by treatment with compound 3289-8625 in A549/CDDP cells (Fig. 3B). Also, the protein level of β-catenin was reduced by silencing DVL2 in A549/CDDP cells transfected with shDVL2, which was restored by over-expression of DVL2 in A549/CDDP cells cotransfected with shDVL2 and

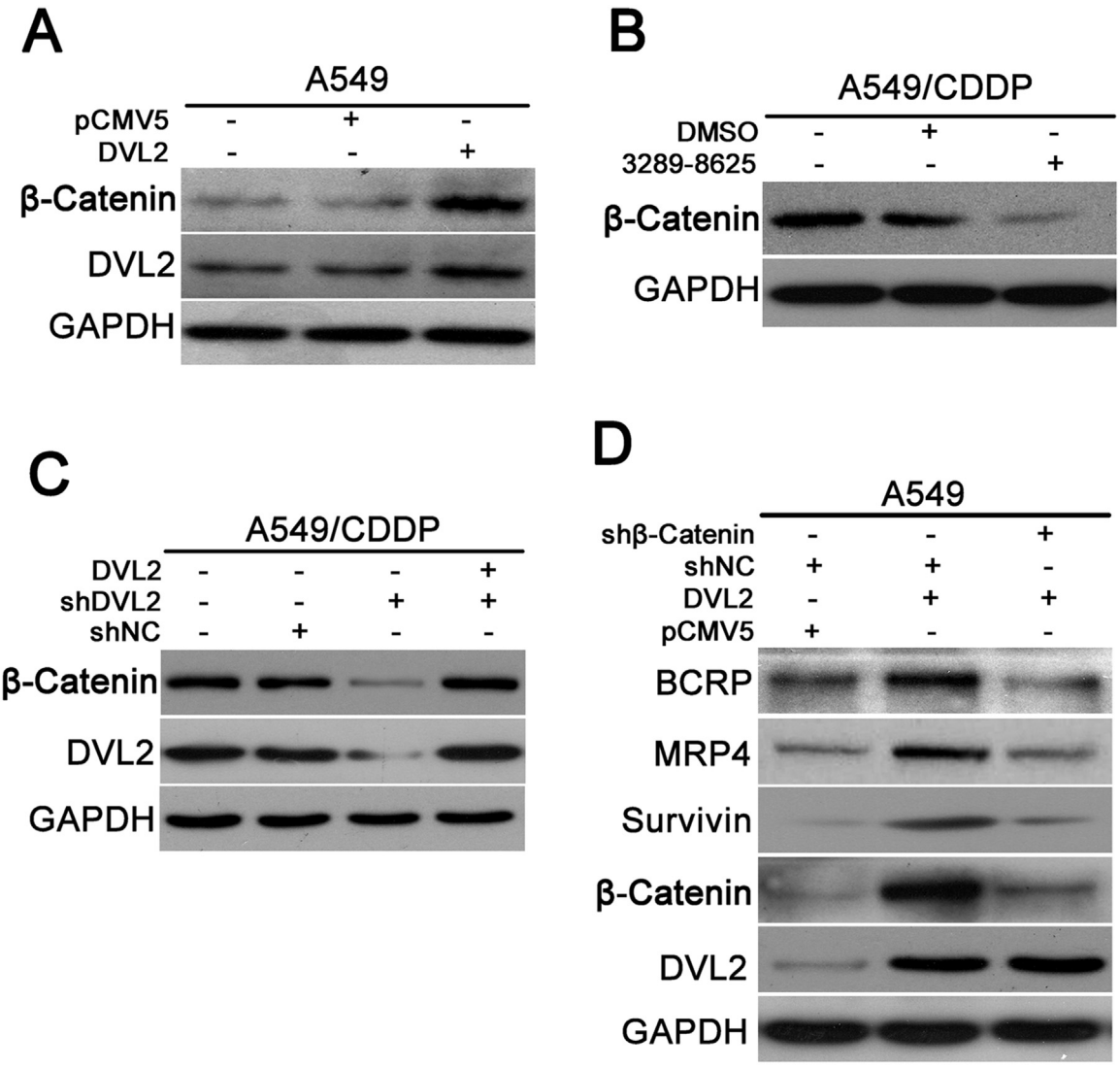

Fig. 3. β-catenin mediated DVL2-induced up-regulation of BCRP, MRP4 and Survivin. (A) A549 cells transfected with pCMV5-DVL2 or pCMV5 for 72 h, (B) A549/CDDP cells treated with DMSO or 100 μM compound 3289-8625 for 72 h, (C) A549/CDDP cells transfected with shNC, shDVL2, or shDVL2 plus pCMV5-DVL2 for 72 h, and (D) A549 cells transfected with pCMV5, pCMV5-DVL2, shNC or shβ-catenin plus pCMV5-DVL2 for 72 h, were lysed to detect β-catenin, DVL2, BCRP, MRP4 and Survivin using Western blotting, as indicated. In each case, the blot is representative of immunoblots resulting from three separate experiments.

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pCMV5-DVL2 (Fig. 3C). These results suggested that DVL2 can increase accumulation of β-catenin in lung cancer cells. Furthermore, silencing β-catenin using shRNA abolished DVL2-induced up-regulation of BCRP, MRP4 and Survivin in A549 cells co-transfected with shβ-catenin and pCMV5-DVL2 (Fig. 3D). Taken together, these results suggested that DVL2 up-regulated the expression of BCRP, MRP4 and Survivin via β-catenin.

3.4. DVL2 enhanced the nuclear translocation of β-catenin

A series of studies have verified that nuclear translocation of β-catenin can activate its target gene expression. To further determine whether DVL2 regulate distribution of β-catenin in cells, then the subcellular localization of β-catenin in A549 and A549/CDDP cells were checked by use of immunofluorescence staining. The results revealed that more cytoplasmic accumulation and nuclear translocation of β-catenin were observed in A549/CDDP cells compared to A549 cells. Also, over-expression of DVL2 obviously triggered the nuclear translocation of β-catenin in A549 cells. While, silencing DVL2 notably reduced nuclear β-catenin levels in A549/CDDP cells (Fig. 4). These results suggested that DVL2 enhanced the nuclear translocation of β-catenin in lung cancer cells.

3.5. DVL2 up-regulated β-catenin via inhibiting GSK3β

Previous studies revealed that the inactivation of GSK3β can improve β-catenin stability, leading to accumulation and nuclear translocation of β-catenin [28]. To investigate the molecular mechanism underlying DVL2 regulated β-catenin, we assessed whether DVL2 affect the activity of GSK3β. As shown in Fig. 5A, the DVL2 over-expression increased the phosphorylation of GSK3β at Ser-9 significantly in A549 cells. In contrast, the level of phosphorylated GSK3β (Ser-9) was reduced by treatment with compound 3289-8625 and shDVL2 in A549/CDDP cells. The shDVL2-induced down-regulation of phosphorylated GSK3β (Ser-9) was rescued by DVL2 over-expression in A549/CDDP cells co-transfected with shDVL2 and pCMV5-DVL2 (Fig. 5B and C). These results suggested that DVL2 can inhibit the activity of GSK3β. Moreover, the inactivation of GSK3β using the inhibitor LiCl abolished the shDVL2-induced down-regulation of β-catenin (Fig. 5D). These results suggested that DVL2 inhibited GSK3β activity to enhance the accumulation of β-catenin.

4. Discussion

Cisplatin-based chemotherapy is a vital treatment regimen for clinical management of various cancers including lung cancer [29,30]. However, the development of drug resistance to cisplatin limits the chance of successful chemotherapy, and is considered as a major challenge in cancer therapy [31,32]. Thus, it is very important to understand the underlying mechanisms responsible for cisplatin resistance and develop efficient therapeutic strategies to overcome chemoresistance. Cancer cells become resistant to drugs through various complex mechanisms. Cancer-derived drug resistant cell lines are commonly used as models to clarify these mechanisms [33]. Therefore, the stable cisplatin-resistant lung cancer cell line A549/CDDP and their parental cell line A549 were used in present study to explore mechanism of resistance to cisplatin in lung cancer. A549/DDP cells exhibited stronger resistance to cisplatin with an IC50 value that was almost 13-fold greater than that observed in A549 cells (Fig. 1B).

Growing evidences have shown that abnormal activation of Wnt/β-catenin signaling is involved in the development and drug resistance of a series of cancers such as lung cancer [34,35]. Under Wnt stimulation, a critical regulator of Wnt/β-catenin signaling DVL is recruited to cell surface receptor frizzled (FZ) and relays

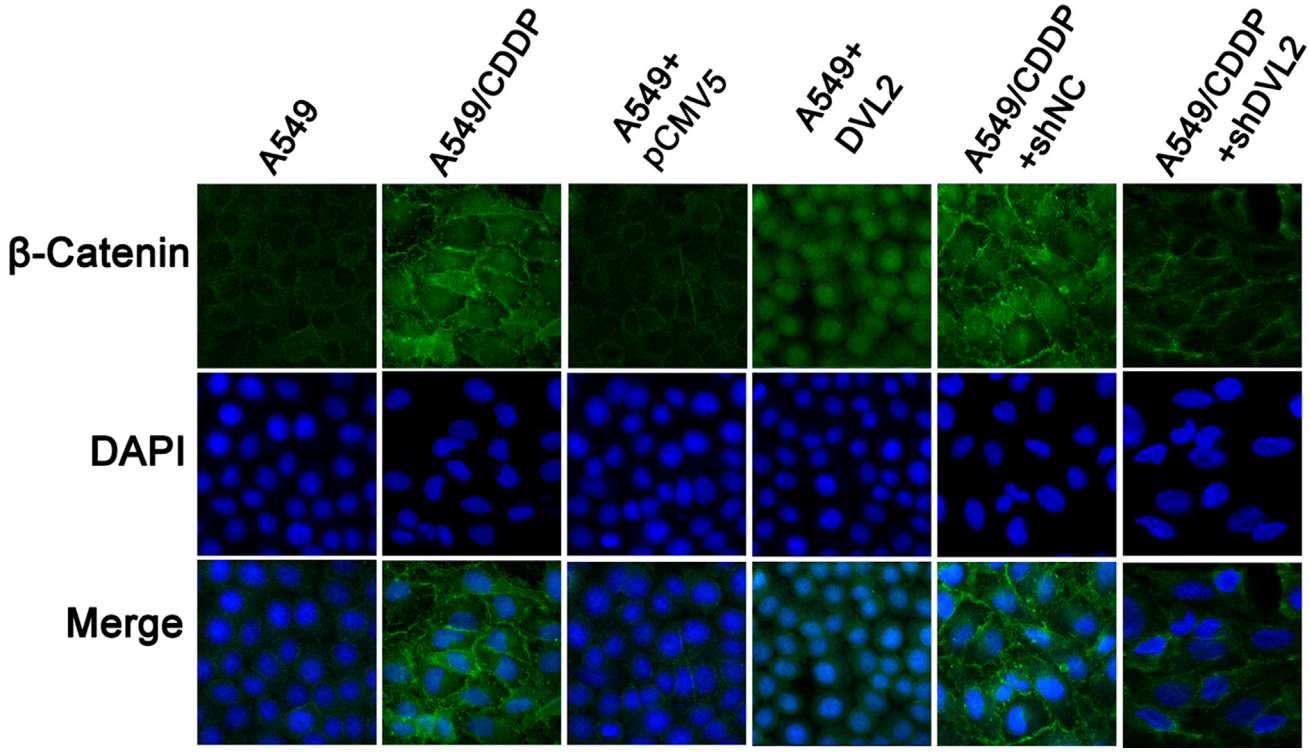

Fig. 4. The effect of DVL2 on nuclear translocation of β-catenin. Immunofluorescence staining was used to detect subcellular localization of β-catenin in A549 and A549/CDDP cells. The cells were transfected with pCMV5, pCMV5-DVL2, shNC or shDVL2 for 72 h, as indicated. Green, β-catenin; blue, nuclear DNA.

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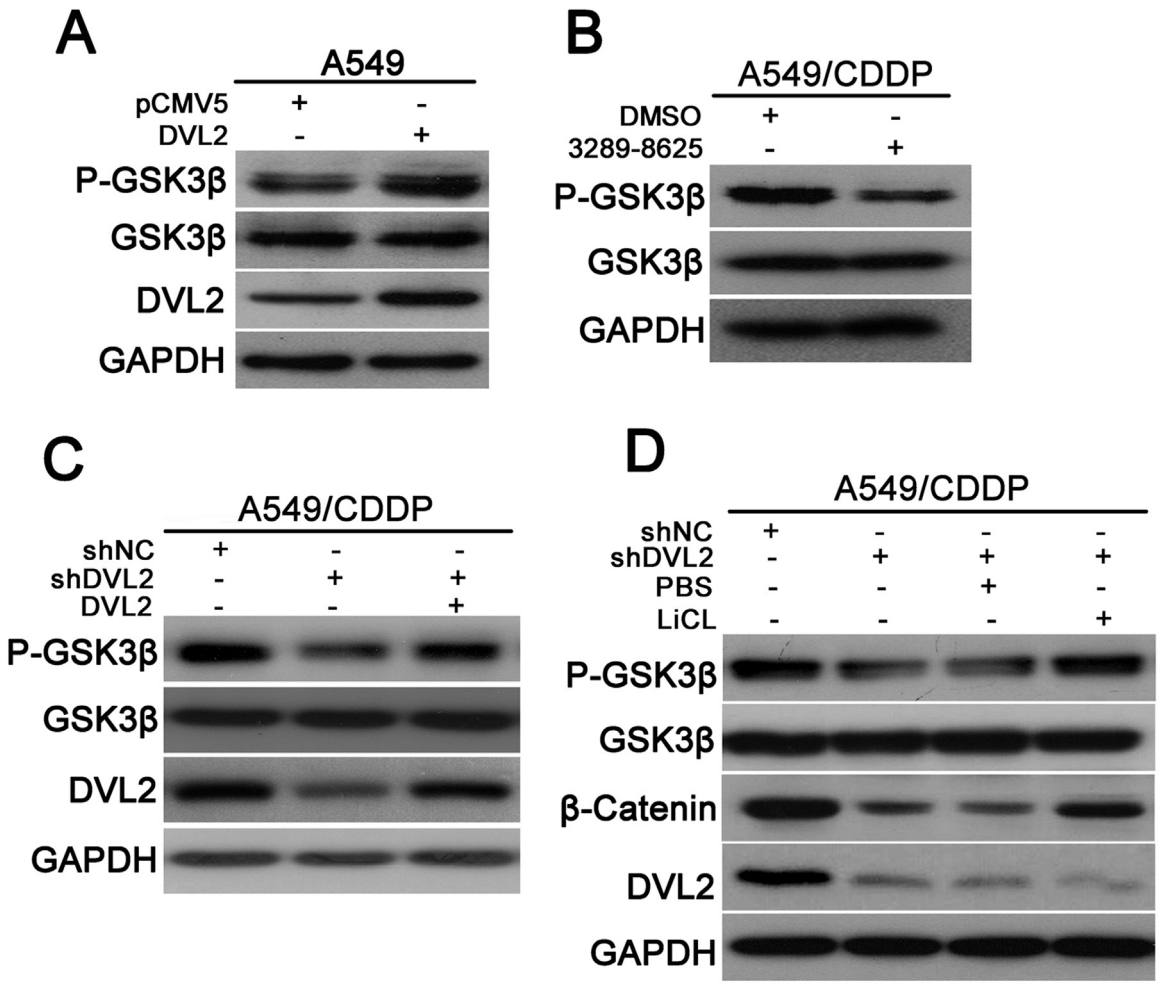

Fig. 5. The role of GSK3β in DVL2-regulated accumulation of β-catenin. (A) A549 cells transfected with pCMV5 or pCMV5-DVL2 for 72 h, (B) A549/CDDP cells treated with or without 100 μM compound 3289-8625 for 72 h, (C) A549/CDDP cells transfected with shNC, shDVL2, or shDVL2 plus pCMV5-DVL2 for 72 h, and (D) A549/CDDP cells transfected with shNC, shDVL2 or shDVL2 plus treated with 20 nM LiCl for 72 h, were lysed to detect the expression of phospho-GSK-3β (Ser-9), GSK-3β, β-catenin and DVL2 using Western blotting, as indicated. PBS as the negative control. In each case, the blot is representative of immunoblots resulting from three separate experiments.

Wnt signals from their receptors to the downstream effectors to activate Wnt/β-catenin signaling [36]. Interestingly, if DVL is expressed at a high level, it can also efficiently activate the Wnt/β-catenin signaling, independently of Wnt stimulation [37,38]. Elevated DVL2 expression can block the formation of β-catenin "destruction complex" and inhibit activity of GSK-3β [16]. This results in cytoplasmic accumulation and nuclear translocation of β-catenin, ultimately triggering expression of Wnt/β-catenin target genes [39]. It has been reported that DVL is related to development of several cancers [40–42]. However, the roles and mechanisms of DVL in cancer chemoresistance are still not elucidated. Therefore, the present study aimed to explore relationship between DVL and cisplatin-resistance in lung cancer.

Our results revealed that DVL2 was over-expressed in A549/CDDP in contrast with A549 cells (Fig. 1A), suggesting that DVL2 was related to resistance of lung cancer to cisplatin. To examine the role of DVL2 in the resistance, the function of DVL2 was pharmacologically inhibited by its inhibitor (3289-8625), which is a cell-permeable aminobenzanilide compound that disrupts FZ-DVL interaction by targeting the PDZ domain of DVL [43]. It was found that the sensitivity of A549/CDDP cells to cisplatin was elevated by 3289-8625 treatment and the IC_{50} value was notably reduced (Fig. 1B and C). To further examine roles of DVL2 in the resistance, DVL2 expression was silenced by shDVL2. Consistent with the above observations, the sensitivity of A549/CDDP cells to cisplatin was promoted by shDVL2 and the IC_{50} value was also remarkably decreased (Fig. 1D and E). These data suggested that DVL2 positively regulated the resistance of lung cancer to cisplatin, and inhibition of DVL2 resensitized lung cancer to cisplatin.

We then analyzed the mechanism underlying inhibition of DVL2 resensitized cisplatin-resistant lung cancer. Analysis of resistance mechanisms focused on membrane transporters in the super family of ABC proteins which can decrease the intracellular drug concentrations and cytotoxicity via drug efflux, such as BCRP and MRP [44]. The over-expression of these proteins has been demonstrated to be responsible for resistance to drugs including cisplatin [45,46]. Previous studies have also proved that up-regulation of anti-apoptotic genes including Survivin contributes to the drugs resistance [47]. Here, our data demonstrated that the expression of BCRP, MRP4 and Survivin were stronger in A549/CDDP cells in contrast with A549 (Fig. 2A), suggesting that BCRP, MRP4 and Survivin were involved in resistance of lung cancer to cisplatin. In addition, these protein levels were up-regulated when DVL2 was over-expressed, but down-regulated when DVL2 was inhibited using compound 3289-8625 or silenced by shDVL2. Meanwhile, the shDVL2-induced down-regulation of these proteins was reversed by DVL2 over-expression (Fig. 2B–D). Above data suggested that inhibition of DVL2 improved the sensitivity of A549/CDDP cells to cisplatin via down-regulating BCRP, MRP4 and Survivin. However, the mechanism of DVL2-mediated the

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expression of BCRP, MRP4 and Survivin remains unknown.

Previous studies have demonstrated that BCRP, MRP4 and Survivin are target genes of Wnt/β-catenin signaling [26,48]. So we examined whether DVL2 regulated the expression of β-catenin in A549 and A549/CDDP cells. The present study proved that the expression of β-catenin was positively regulated by DVL2 (Fig. 3A–C). Therefore, we hypothesized that DVL2 can modulate the expression of BCRP, MRP4 and Survivin via β-catenin. Our hypothesis was confirmed by the result that silencing β-catenin abolished DVL2-mediated the expression of BCRP, MRP4 and Survivin (Fig. 3D). It is known that the nuclear translocation of β-catenin is necessary for expression of its targets genes and a marker of activating Wnt/β-catenin [49]. Considering that DVL2 is potent enough to activate Wnt/β-catenin signaling independently of Wnt stimulation [37,38], we examined whether altered DVL2 protein levels affect the nuclear translocation of β-catenin. Immunofluorescence staining revealed that DVL2 positively regulated the accumulation and nuclear translocation of β-catenin in A549 and A549/CDDP cells (Fig. 4). Taken together, above data suggested that DVL2 mediated the expression of BCRP, MRP4 and Survivin by activating β-catenin. Previous studies showed that activated β-catenin is allowed to translocate into cell nucleus, then it associates with nuclear transcription factor TCF/LEF to trigger expression of Wnt/β-catenin target genes [14]. Inhibiting the binding of TCF to its specific DNA-binding sites can significantly decrease the transcription of BCRP, MRP4 and Survivin, suggesting that BCRP, MRP4 and Survivin are downstream genes of β-catenin/TCF [26,50]. Furthermore, the functional TCF binding sites in the promoter regions of BCRP and Survivin have been identified, confirming that BCRP and Survivin are direct downstream genes of β-catenin/TCF [50–52]. However, there is no report of the presence of TCF binding sequences in the promoter regions of MRP4. Therefore, it should be further investigated whether MRP4 is a direct downstream gene of β-catenin/TCF.

The activation of β-catenin is regulated by “destruction complex” of which GSK3β is a critical component. The inactivation of GSK3β leads to release of dephosphorylated β-catenin from “destruction complex”, which allows β-catenin to accumulate in the cytoplasm and migrate to the nucleus [53]. In the present study, over-expression of DVL2 increased phosphorylation of serine residue (Ser9) on GSK3β, whereas inhibition of DVL2 decreased the phosphorylation GSK3β and accumulation of β-catenin (Fig. 5). Because the phosphorylation of serine residue (Ser9) on GSK3β leads to its catalytic inactivation [54], our data showed that DVL2 can inhibit activation of GSK3β, suggesting that DVL2 up-regulated accumulation of β-catenin via inhibiting GSK3β. It was reported that the regulation of GSK3β on β-catenin will be lost in some cancers such as colon cancer [55,56]. Therefore, we examined whether GSK3β can modulate DVL2-regulated accumulation of β-catenin in lung cancer cells. The inactivation of GSK3β using LiCl abolished the shDVL2-induced down-regulation of β-catenin (Fig. 5D). These results confirmed that DVL2 inhibited GSK-3β activity to increase the accumulation of β-catenin in lung cancer cells.

In summary, this is the first study demonstrating roles of DVL2 in cisplatin-resistant human lung cancer. DVL2 was over-expressed in cisplatin-resistant lung cancer cells, where it inhibited GSK3β activity. This activated β-catenin and increased the expression of BCRP, MRP4 and Survivin. The inhibition of DVL2 resensitized cisplatin-resistant lung cancer cells through down-regulating Wnt/β-catenin signaling, providing a new perspective to chemosensitize cisplatin-induced cytotoxicity in lung cancer.

Competing interests

The authors have no competing interests.

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