Cancer Cell International

RESEARCH

Open Access

Proapoptotic role of CDK1 in overcoming paclitaxel resistance in ovarian cancer cells in response to combined treatment with paclitaxel and duloxetine

Gyeongmi Kim[1,2], Se-Kyeong Jang[1], Se Hee Ahn[1], Selim Kim[1,2], Chan Sub Park[3], Min-Ki Seong[3], Hyun-Ah Kim[3], Seunghee Bae[4], Jae Ho Lee[4], Hyunggee Kim[2], Hyeon-Ok Jin[5] and In-Chul Park[1*]

Abstract

Background Paclitaxel resistance and recurrence are major obstacles in ovarian cancer, which is the leading cause of death among gynecologic cancers. During cancer cell progression, cyclin-dependent kinase 1 (CDK1) drives cells through the G2 phase and into mitosis. In this study, we demonstrated that CDK1 played a crucial role in switching paclitaxel-resistant ovarian cancer cells from mitotic arrest to apoptosis following combined treatment with paclitaxel and duloxetine, an antidepressant known as a serotonin-norepinephrine reuptake inhibitor (SNRI).

Methods Cell viability was assessed by MTT assay. Apoptotic cell death and mitochondrial membrane potential (MMP) were detected by flow cytometry. Protein expression levels were explored using western blotting. Mitochondrial and cytosolic fractionation were performed to determine the mitochondrial localization of proteins. Immunofluorescence was used to detect protein expression levels and localization.

Results Combined treatment with paclitaxel and duloxetine induced apoptotic cell death in paclitaxel-resistant ovarian cancer cells. We suggested that combined treatment of these drugs induced CDK1 activation and increased mitochondrial localization of activated CDK1, which caused phosphorylation of the antiapoptotic Bcl-2 and Bcl-xL proteins. Selective CDK1 inhibitors blocked Bcl-2 and Bcl-xL phosphorylation induced by paclitaxel and duloxetine, and strongly suppressed apoptotic cell death. Furthermore, we demonstrated that S6K is a potential upstream mediator of the proapoptotic activation of CDK1.

Conclusion Taken together, switching CDK1 to a proapoptotic role through the combination of paclitaxel and duloxetine could overcome paclitaxel resistance in ovarian cancer cells, providing promising therapeutic strategies for treating paclitaxel-resistant ovarian cancer.

Keywords Apoptosis, Bcl-2, CDK1, Duloxetine, mTORC1/S6K, Ovarian cancer, Paclitaxel resistance

*Correspondence:
In-Chul Park
parkic@kirams.re.kr
[1]Division of Fusion Radiology Research, Korea Institute of Radiological and Medical Sciences, Seoul 01812, Korea
[2]Department of Biotechnology, College of Life Science and Biotechnology, Korea University, Seoul 02841, Korea
[3]Department of Surgery, Korea Cancer Center Hospital, Korea Institute of Radiological and Medical Sciences, Seoul 01812, Korea
[4]Department of Cosmetics Engineering, Konkuk University, Seoul 05029, Korea
[5]KIRAMS Radiation Biobank, Korea Institute of Radiological and Medical Sciences, Seoul 01812, Korea

Background

Ovarian cancer is a malignant tumor and leading cause of death among gynecological malignancies, accounting for 5% of cancer-related deaths in women [1]. Paclitaxel blocks progression at the G_2/M phase by stabilizing the microtubule and is currently one of the most common anticancer agents for the treatment of primary and relapsed ovarian cancer [2, 3]. However, even among patients who have achieved a complete response, the majority still relapse due to acquired resistance [3]. Therefore, there is an urgent need to explore the molecular mechanisms underlying acquired resistance and to determine ways to overcome these obstacles.

Cyclin-dependent kinase 1 (CDK1), also called CDC2, is a key protein kinase that drives cells through the G_2 phase and mitosis in the normal cell cycle [4]. CDK1 activation requires binding to cyclin B1, phosphorylation at T161 residue, and dephosphorylation at Y15 [5, 6]. Inactivation of CDK1 during mitotic exit is regulated through the ubiquitin-proteasomal degradation of cyclin B1 [5, 6]. Generally, cells with suppressed CDK1 activity tend to arrest in the G_2 phase, whereas cells with elevated CDK1 activity tend to enter mitosis [7, 8]. Although the conventional cell cycle-dependent activation of CDK1 is well established, unconventional biological functions of CDK1 have also been identified through the phosphorylation of proteins independent of the cell cycle [9]. The involvement of CDK1 activity during apoptotic cell death was first identified in lymphoma cells [10]. The CDK1/cyclinB1 complex can localize or translocate to the mitochondria, where this complex plays an important role in the regulation of apoptosis [11]. While CDK1 stimulates apoptotic cell death by phosphorylating Bcl-2 family members, it also has been reported that CDK1 inhibits apoptosis by phosphorylating the caspase-9 and BIRC5 proteins [12, 13]. Therefore, the contrasting role of CDK1 in apoptosis needs to be investigated and could be an important achievement in identifying new therapeutic strategies.

Duloxetine is utilized as an antidepressant that acts by inhibiting the reuptake of serotonin and norepinephrine, thereby alleviating depression and anxiety in patients [14]. Duloxetine, the brand name Cymbalta, was already approved for medical use in both the USA and the European Union in 2004, and it has since gained significant recognition [15]. Recently, the therapeutic scope of duloxetine for cancer patients has extended beyond its conventional use as an antidepressant targeting mood disorders and cancer-related pain [16–18]. Emerging evidence suggests that duloxetine may play a preventive role in drug-induced neurotoxicity [19]. However, the direct effects of duloxetine on cancer cells are not well understood, and its potential therapeutic effects and mechanisms in cancer need to be investigated.

In the present study, we provide evidence showing CDK1 activation in paclitaxel-resistant ovarian cancer cells treated with paclitaxel and duloxetine. The activation of CDK1 is responsible for the phosphorylation of antiapoptotic Bcl-2 and Bcl-xL proteins, which results in the activation of the mitochondrial apoptotic cell death pathway. Furthermore, we found that inhibition of mTORC1/S6K is an important regulator of the proapoptotic role of CDK1 in cells, treated with paclitaxel and duloxetine. In conclusion, switching the CDK1 activation to proapoptotic roles via drug combination could overcome paclitaxel resistance in ovarian cancer, suggesting that the proapoptotic activation of CDK1 could be a promising therapeutic strategy for treating paclitaxel resistance in ovarian cancer.

Methods

Cell culture and reagents

HEYA8, HEYA8-MDR, SKOV3 and SKOV3-TR ovarian cancer cells were provided by Dr Anil K. Sood (The University of Texas MD Anderson Cancer Center, Houston, TX, USA) [20]. The cells were cultured in RPMI1640 medium (#LM011-01, Welgene, Gyeongsan-si, Gyeongsangbuk-do, Republic of Korea) supplemented with 10% fetal bovine serum (#26140-079, Gibco; Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C and 5% CO_2. HEYA8-MDR and SKOV3-TR cells were maintained with 50 nM paclitaxel (#10461, Cayman Chemical Company, Ann Arbor, MI, USA) to sustain the paclitaxel resistance. Duloxetine was purchased from Tocris Bioscience (#4798, Bristol, UK). Ro-3306 (#S7747), LY2584702 (#S7698), and RAD001 (#S1120) were purchased from Selleck Chemicals (Houston, TX, USA). Avotaciclib (#HY-137432) and PF4708671 (#HY-112683) were purchased from MedChemExpress (Monmouth Junction, NJ, USA).

Cell viability assay

Cell viability was assessed by measuring the mitochondrial conversion of thiazolyl blue tetrazolium bromide (MTT; #M2128, Merck KGaA, Darmstadt, Germany). Cells were seeded in culture medium in 6-well plates at a density of 10 000 cells per well and treated with each drug for 48 h. The cells were incubated in 1 mg/ml MTT solution at 37 °C for 1 h. The supernatant was removed, and 2-propanol (#I9516, Sigma–Aldrich, Merck KGaA) was added to dissolve the formazan crystals. The proportion of converted MTT was calculated by measuring the absorbance at 595 nm. The results were calculated as the percentage of cell viability relative to the untreated control. Each experiment was repeated 3 times.

Mitochondrial membrane potential (Δψm)

To measure mitochondrial membrane potential, cells were stained with tetramethylrhodamine ethyl ester (TMRE) according to the manufacturer's instructions (#HY-D0985A, MedChemExpress). Briefly, the cells were seeded in culture medium in 6-well plates at a density of 10 000 cells per well and treated with each drug for 24 h. The cells were trypsinzed and stained with 100 nM TMRE and incubated for 30 min at 37℃. After incubation, the cells were analyzed using Cytoflex (Beckman Coulter, Brea, CA, USA) flow cytometer. As a positive control, 50 μM carbonyl cyanide m-chlorophenylhydrazone (CCCP; #M34152, Invitrogen, MA, USA) was pretreated for 1 h before analysis.

Apoptosis assay

To measure apoptotic cell death, cells were stained with Annexin V-FITC and propidium iodide according to the manufacturer's instructions (#ab14085, Abcam, Cambridge, UK). Briefly, the cells were seeded in culture medium in 6-well plates at a density of 10 000 cells per well and treated with each drug for 48 h. The cells were collected by centrifugation and resuspended in 500 μl of 1 × Binding Buffer with 5 μl of Annexin V-FITC and 5 μl of propidium iodide. After incubation for 5 min in the dark, the cells were analyzed using Cytoflex flow cytometer.

Isolation of mitochondrial and cytosolic fractions

Mitochondrial and cytosolic fractions were isolated from cells using a Mitochondria/Cytosol Fractionation Kit (#ab65320, Abcam). According to the manufacturer's instructions, the collected cells (500 000 cells) were washed with PBS and homogenized in 1 × Cytosol Extraction Buffer. Homogenized cells were centrifuged at 1 000 × g for 10 min at 4 °C, after which the supernatant was collected. To isolate the cytosolic fractions, the samples were centrifuged again at 10 000 × g for 30 min at 4 ℃. Cytosolic fractions were collected from the supernatant. The pellets were resuspended in 1 × Mitochondria Extraction Buffer and collected as mitochondrial fractions.

Western blot analysis

For western blotting, the drug-treated cells were rinsed in cold PBS and lysed with 1 × lysis buffer (#9803, Cell Signaling Technology, Beverly, MA, USA) containing protease/phosphatase inhibitor (#78440, Thermo Fisher Scientific). The protein concentration was determined using Bradford reagent (#5000006, Bio-Rad Laboratories, Hercules, CA, USA). The proteins from the total lysates were resolved by 6–15% SDS–PAGE and transferred to nitrocellulose membranes, after which the membranes were incubated with primary antibodies at 4 ℃ and horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. The following antibodies were used: Cleaved PARP (#9541, Cell Signaling Technology), p-Bcl-2 (S70) (#2827, Cell Signaling Technology), Bcl-2 (#A19693, ABclonal, Wuhan, Hubei, China), p-Bcl-xL (S62) (#AP0314, ABclonal), Bcl-xL (#2764, Cell Signaling Technology), p-CDK1 (Y15) (#9111, Cell Signaling Technology), p-CDK1 (T161) (#9114, Cell Signaling Technology), CDK1 (#9116, Cell Signaling Technology), Cyclin B1 (#sc-245, Santa Cruz Biotechnology), COX IV (#4850, Cell Signaling Technology), p-mTOR (S2448) (#2971, Cell Signaling Technology), mTOR (#2983, Cell Signaling Technology), p-S6K (T389) (#9205, Cell Signaling Technology), S6K (#9202, Cell Signaling Technology), p-S6 (S240/244) (#5364, Cell Signaling Technology), S6 (#2217, Cell Signaling Technology), β-Actin (#A5316, Sigma–Aldrich), Goat anti-Rabbit IgG-heavy and light chain Antibody HRP Conjugated (#A120-101P, Bethyl Laboratories Inc., Montgomery, TX, USA), and Goat Anti-Mouse IgG (H+L) HRP (#A1012S, ACE Biolabs, Foshan, Guangdong, China). The blots were exposed to X-ray film. β-Actin was used as the loading control.

Immunofluorescence

Cells were grown on coverslips in 12-well plates, fixed with 4% PFA for 20 min and then permeabilized with 0.3% Triton X-100 for 20 min. The cells were blocked with 1% BSA and 5% FBS in PBS, incubated with primary antibodies overnight at 4 ℃, and then incubated with fluorescence-labeled secondary antibody for 1 h at room temperature. Staining for mitochondria was performed using MitoBright LT Green (#MT10, Dojindo, Kumamoto, Japan) before PFA fixation. The following antibodies were used: Cdk1/Cdc2 (phospho-T161) (#A29577, Antibodies.com, Cambridge, UK), CDK1 (#9116, Cell Signaling Technology), and Bcl-xL (#2764, Cell Signaling Technology), Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 647 Conjugate) (#4414, Cell Signaling Technology), and Rabbit anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 (#A11059, Invitrogen).

Statistical analysis

The data are presented as the mean±standard deviation (SD) of three independent experiments. Statistical differences were determined by one-way ANOVA followed by Tukey's test using GraphPad Prism software (version 8.0.1, San Diego, CA, USA). All values were considered significant at $p<0.05$ ($p<0.05$:* $p<0.01$:** $p<0.001$:***).

Results

Duloxetine sensitizes HEYA8-MDR cells to paclitaxel through the phosphorylation of antiapoptotic Bcl-2 family proteins

First, we confirmed the paclitaxel resistance in HEYA8-MDR cells by treating both HEYA8 and HEYA8-MDR cells with different concentrations of paclitaxel for 48 h. The results revealed a considerable decrease in cell viability in HEYA8 cells treated with 10 nM and 100 nM paclitaxel, while there was no observable effect in HEYA8-MDR cells (Fig. S1A). Additionally, the level of apoptotic cleaved PARP was increased in a dose-dependent manner upon paclitaxel treatment in HEYA8 cells, but there was no such response in HEYA8-MDR cells (Fig. S1B). Thus, we verified that compared with parental HEYA8 cells, HEYA8-MDR cells were highly resistant to paclitaxel.

Antidepressants are commonly used to alleviate somatic symptoms, anxiety, and nervousness in cancer patients. Recent reports have suggested unexpected anticancer effects for some antidepressants, involving the regulation of cellular mechanisms related to apoptosis and proliferation [21–23]. Therefore, we investigated the anticancer effects of serotonin and norepinephrine reuptake inhibitors (SNRIs), including duloxetine, venlafaxine, desvenlafaxine, and milnacipran, in HEYA8-MDR cells. Among the four different antidepressants tested, duloxetine effectively decreased cell viability and sensitized HEYA8-MDR cells to paclitaxel (Fig. 1A). Microscopy images revealed that combined treatment with paclitaxel and duloxetine induced cell death in HEYA8-MDR cells (Fig. 1B). Furthermore, the proportions of cells in early and late apoptosis were significantly increased under the combined treatment of paclitaxel and duloxetine (Fig. 1C).

Previous reports have highlighted that during mitotic arrest, the phosphorylation of Bcl-2 impedes its binding with other Bcl-2 family members, including Bax and Bak, ultimately inducing apoptotic cell death [24, 25]. It has been identified that Bcl-2 phosphorylation plays an important role in overcoming paclitaxel resistance in SKOV3-TR ovarian cancer cells [26]. Moreover, the attenuated antiapoptotic function of phosphorylated Bcl-xL, which leads to apoptotic cell death, has also been reported in H1299 human NSCLC cells [27]. To investigate the relevance of Bcl-2 family proteins and their phosphorylation in combined treatment-induced apoptotic cell death, we examined the protein levels of Bcl-2 family members and their phosphorylation. Combined treatment with paclitaxel and duloxetine significantly induced the PARP cleavage and phosphorylation of Bcl-2 and Bcl-xL in HEYA8-MDR cells (Fig. 1D). These results suggest that the apoptosis triggered by the combined treatment of paclitaxel and duloxetine is mediated through the phosphorylation of Bcl-2 and Bcl-xL.

Inhibition of CDK1 reverses the apoptotic cell death induced by combined treatment with paclitaxel and duloxetine in HEYA8-MDR cells

CDK1 is a potential kinase for Bcl-2 phosphorylation and has an important role in switching cells to apoptosis during mitotic arrest [25, 28]. To evaluate whether CDK1 is a valuable kinase for Bcl-2 and Bcl-xL phosphorylation, we treated with Ro-3306, a selective ATP-competitive inhibitor of CDK1, in HEYA8-MDR cells treated with paclitaxel and duloxetine. Microscopy images showed that inhibition of CDK1 using Ro-3306 dramatically inhibited the combined treatment-induced cell death (Fig. 2A). Additionally, Ro-3306 treatment effectively rescued the apoptotic cell death that occurred in response to duloxetine and paclitaxel (Fig. 2B). We further verified this effect by using another CDK1 inhibitor, avotaciclib (Fig. 2C). Immunoblot analysis revealed that the phosphorylation of CDK1 at the T161 residue, which is required for CDK1 activation, was increased by the combined treatment, but the phosphorylation of CDK1 at the Y15 residue, which is required for CDK1 inactivation was decreased by the combined treatment (Fig. 2D). Cyclin B1 levels were also increased when CDK1 was activated and decreased by Ro-3306 treatment (Fig. 2D). The increased phosphorylation of CDK1 at the T161 residue was decreased by Ro-3306 treatment (Fig. 2D). Moreover, the increased PARP cleavage and phosphorylation of Bcl-2 and Bcl-xL were reversed by CDK1 inhibition (Fig. 2E). Thus, these results show that CDK1 is a key mediator of Bcl-2 and Bcl-xL phosphorylation and subsequent apoptosis in response to combined treatment with paclitaxel and duloxetine.

Combined treatment with paclitaxel and duloxetine increases mitochondrial activated CDK1 in HEYA8-MDR cells

Anti-apoptotic Bcl-2 family proteins, such as Bcl-2 and Bcl-xL, help maintain mitochondrial membrane potential (MMP) and preserve mitochondrial stability, thereby inhibiting apoptosis through mitochondrial pathways [29]. Therefore, we examined whether MMP was affected by the combined treatment of paclitaxel and duloxetine. Our results showed that the combined treatment significantly reduced MMP, with CCCP used as a positive control for MMP reduction (Fig. 3A). Next, we investigated the cellular localization of activated CDK1 following treatment with paclitaxel and duloxetine. The combined treatment led to a robust increase in activated phosphorylation of CDK1 (T161) throughout the cell, including in the cytosol, nucleus, and mitochondria (Fig. 3B). Sustained activation of CDK1 can translocate to

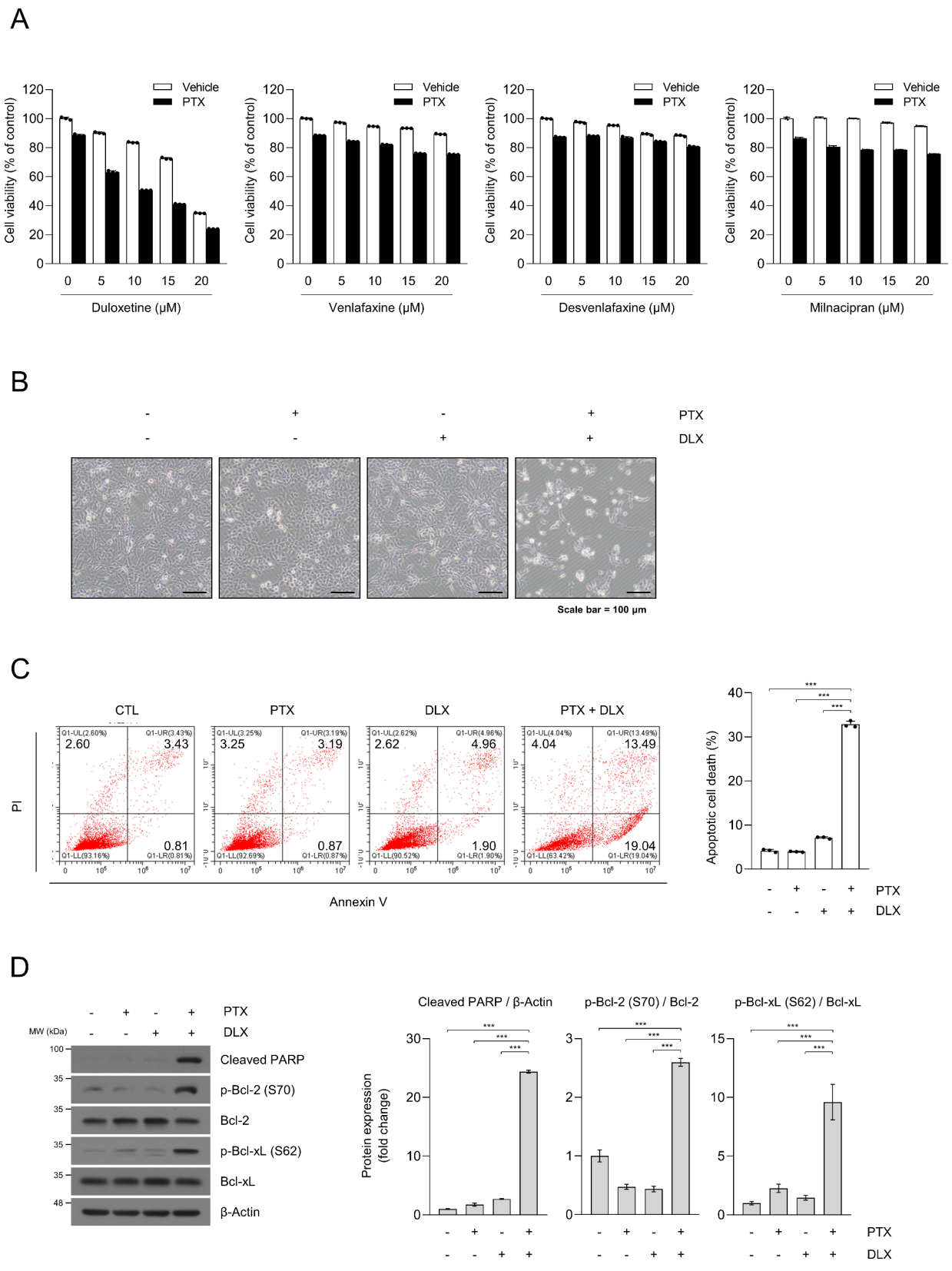

Fig. 1 (See legend on next page.)

(See figure on previous page.)

Fig. 1 Combined treatment with duloxetine and paclitaxel induces apoptosis via phosphorylation of the antiapoptotic Bcl-2 family in HEYA8-MDR cells. (**A**) HEYA8-MDR cells were treated with antidepressants (duloxetine, venlafaxine, desvenlafaxine, and milnacipran) and 100 nM paclitaxel for 48 h. Cell viability was measured by MTT assay. (**B**, **C**) The cells were treated with 100 nM paclitaxel and 15 μM duloxetine for 48 h. (**B**) Microscopic observations of HEYA8-MDR cells treated with paclitaxel and duloxetine. (**C**) Apoptotic cell death was determined by Annexin V-FITC/PI staining using flow cytometry. A statistical graph of apoptotic cells is shown. (**D**) The cells were treated with 100 nM paclitaxel and 15 μM duloxetine for 12 h. The expressions of apoptotic cleaved PARP and antiapoptotic Bcl-2 family members were analyzed by immunoblot analysis. The relative optical densities were quantified using ImageJ software. The data are presented as the mean ± SD of the percentage or the fold change relative to the control ($n = 3$, *** $p < 0.001$). PTX, paclitaxel; DLX, duloxetine

the mitochondria, where it phosphorylates Bcl-2 family proteins, thereby disrupting their anti-apoptotic functions and promoting apoptosis [11, 28, 30]. Therefore, we investigated the interaction between CDK1 and Bcl-xL using confocal microscopy. The result showed that colocalization of CDK1 and Bcl-xL was increased after the combined treatment in HEYA8-MDR cells (Fig. S2). To further confirm the increase in activated CDK1 in the mitochondria, we fractionated cytosol and mitochondrial components. In the mitochondrial fraction, phosphorylation of CDK1 at T161 was increased, while phosphorylation at Y15 was decreased following the combined treatment (Fig. 3C). Therefore, these results suggest that combined treatment with paclitaxel and duloxetine increases activated CDK1 in the mitochondria, which might interact with Bcl-2 family proteins in HEYA8-MDR cells.

Inhibition of mTORC1/S6K sensitizes HEYA8-MDR cells by phosphorylation of the antiapoptotic Bcl-2 family

Activation of the mTORC1/S6K signaling pathway is well known to affect cancer cell proliferation, survival and progression [31, 32]. Moreover, we demonstrated that inhibition of the mTORC1/S6K pathway can sensitize SKOV3-TR ovarian cancer cells to paclitaxel in previous research [26]. Treatment with duloxetine alone inhibited the phosphorylation of mTOR, S6K and the downstream substrate S6 in a dose-dependent manner in HEYA8-MDR cells (Fig. 4A). Additionally, the phosphorylation levels of mTOR, S6K and S6 were more strongly decreased by combined treatment with paclitaxel and duloxetine than by treatment with duloxetine alone (Fig. 4B). To investigate whether the mTORC1/S6K pathway plays a crucial role in paclitaxel resistance in HEYA8-MDR cells, we treated cells with selective S6K inhibitors (PF4708671 and LY2584702). S6K inhibitors effectively inhibited the phosphorylation of S6, a substrate for S6K, and sensitized the cells to paclitaxel (Fig. 4C, Fig. S3). Furthermore, a selective mTOR inhibitor, RAD001, inhibited the phosphorylation of S6K and sensitized cells to paclitaxel (Fig. 4D). Immunoblot analysis revealed that combined treatment with PF4708671 and paclitaxel induced PARP cleavage and phosphorylation of Bcl-2 and Bcl-xL (Fig. 4E).

We further investigated whether S6K inhibitor could enhanced the combined effect of duloxetine and paclitaxel on overcoming paclitaxel resistance in HEYA8-MDR cells. Treatment with duloxetine and PF4708671 sensitized the cells to paclitaxel more effectively (Fig. 4F). Moreover, PARP cleavage and the phosphorylation of Bcl-2 and Bcl-xL were amplified by treatment with duloxetine and PF4708671 (Fig. 4G). Thus, these results suggest that mTORC1/S6K signaling plays an important role in the sensitizing effect of duloxetine to paclitaxel in HEYA8-MDR cells.

CDK1 mediates the inhibition of S6K and paclitaxel-induced apoptotic cell death in HEYA8-MDR cells

We found that combined paclitaxel and duloxetine treatment-induced apoptosis in HEYA8-MDR cells was mediated by CDK1 activation (Fig. 2). Because paclitaxel and duloxetine treatment inhibited the mTORC1/S6K signaling pathway and induced apoptosis, we investigated whether the sensitizing effect of mTORC1/S6K inhibition on HEYA8-MDR cells was mediated by CDK1. Treatment with PF4708671 and paclitaxel markedly induced the phosphorylation of CDK1 at the T161 residue, the accumulation of Cyclin B1, but the combination treatment decreased the phosphorylation of CDK1 at the Y15 residue (Fig. 5A). Additionally, the inhibition of CDK1 by Ro-3306 treatment effectively reversed PF4708671- and paclitaxel-induced cell death (Fig. 5B). Inhibition of CDK1 using Ro-3306 or avotaciclib significantly reduced the proportions of apoptotic cells (Fig. 5C, D). Ro-3306 treatment inhibited the phosphorylation of CDK1 at T161, which was induced by combined treatment with PF4708671 and paclitaxel (Fig. 5E). Moreover, Ro-3306 inhibited the combined-induced PARP cleavage and phosphorylation of Bcl-2 and Bcl-xL (Fig. 5F). Furthermore, PF4708671 further increased the activation of CDK1 induced by duloxetine and paclitaxel treatment in HEYA8-MDR cells (Fig. 5G). These results indicate that sensitization to paclitaxel by inhibition of S6K is mediated by CDK1 activation and that CDK1 is a key mediator in overcoming paclitaxel resistance in HEYA8-MDR cells.

Duloxetine sensitizes SKOV3-TR cells to paclitaxel by phosphorylating the antiapoptotic Bcl-2 family in a CDK1-dependent manner

To explore whether duloxetine sensitizes other ovarian cancer cell types to paclitaxel, we investigated the

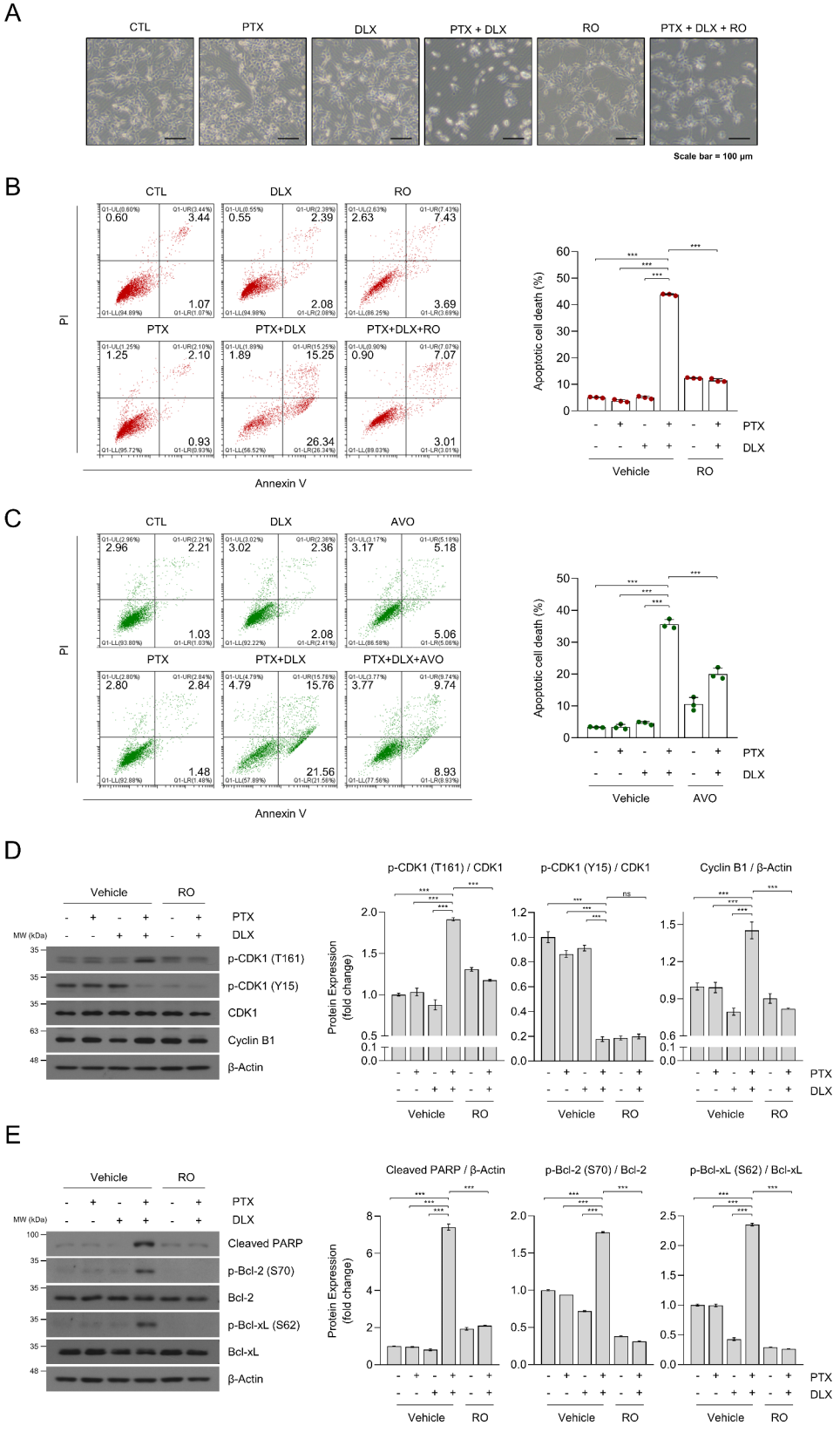

Fig. 2 (See legend on next page.)

(See figure on previous page.)

Fig. 2 Inhibition of CDK1 reverses duloxetine- and paclitaxel-induced apoptosis in HEYA8-MDR cells. (**A-C**) HEYA8-MDR cells were treated with 100 nM paclitaxel and 15 μM duloxetine in combination with 10 μM Ro-3306 or 1 μM avotaciclib for 48 h. (**A**) Cells were visualized via microscopy. (**B**, **C**) Apoptotic cell death were determined by Annexin V-FITC/PI staining using flow cytometry. Statistical graphs of apoptotic cells were shown. (**D**, **E**) The cells were treated with 100 nM paclitaxel and 15 μM duloxetine in combination with 10 μM Ro-3306 for 12 h. (**D**) The expression levels of CDK1 and cyclin B1 were detected by immunoblot analysis. (**E**) The expression levels of apoptotic cleaved PARP and the antiapoptotic Bcl-2 family were detected by immunoblot analysis. The relative optical densities were quantified using ImageJ software. The data are presented as the mean ± SD of the percentage or the fold change relative to the control ($n = 3$, *** $p < 0.001$; ns, not significantly different). PTX, paclitaxel; DLX, duloxetine; RO, Ro-3306; AVO, avotaciclib

effect of duloxetine on SKOV3-TR cells, which also exhibit paclitaxel resistance. Indeed, we observed that similar to HEYA8-MDR treatment, duloxetine treatment sensitized SKOV3-TR cells to paclitaxel (Fig. 6A). Furthermore, duloxetine decreased the phosphorylation of mTOR, S6K, and its substrate S6 in SKOV3-TR cells (Fig. 6B). Next, we tested whether combined paclitaxel and duloxetine treatment-induced apoptosis is dependent on CDK1-mediated Bcl-2 and Bcl-xL phosphorylation in SKOV3-TR cells. Importantly, Ro-3306 treatment alleviated the combined treatment-induced apoptosis, suggesting that CDK1 mediates cell death caused by paclitaxel and duloxetine treatment (Fig. 6C). There was a significant increase in the phosphorylation of CDK1 at T161 and decrease in the phosphorylation of CDK1 at Y15 by the combined treatment, and Ro-3306 effectively reversed this effect (Fig. 6D). Cyclin B1 accumulation was also dependent on CDK1 activation (Fig. 6D). In addition, the level of apoptotic cleaved PARP and the level of phosphorylated Bcl-2 and Bcl-xL were increased in response to combined paclitaxel and duloxetine treatment and were restored by Ro-3306 (Fig. 6E). Overall, the combination of paclitaxel and duloxetine can overcome paclitaxel resistance in different types of ovarian cancer cells via mTORC1/S6K- and CDK1-mediated Bcl-2 and Bcl-xL phosphorylation.

Duloxetine enhances paclitaxel sensitivity in parental ovarian cancer cells

To investigate whether duloxetine enhances paclitaxel sensitivity in parental ovarian cancer cells, we treated duloxetine with paclitaxel in HEYA8 cells. Duloxetine

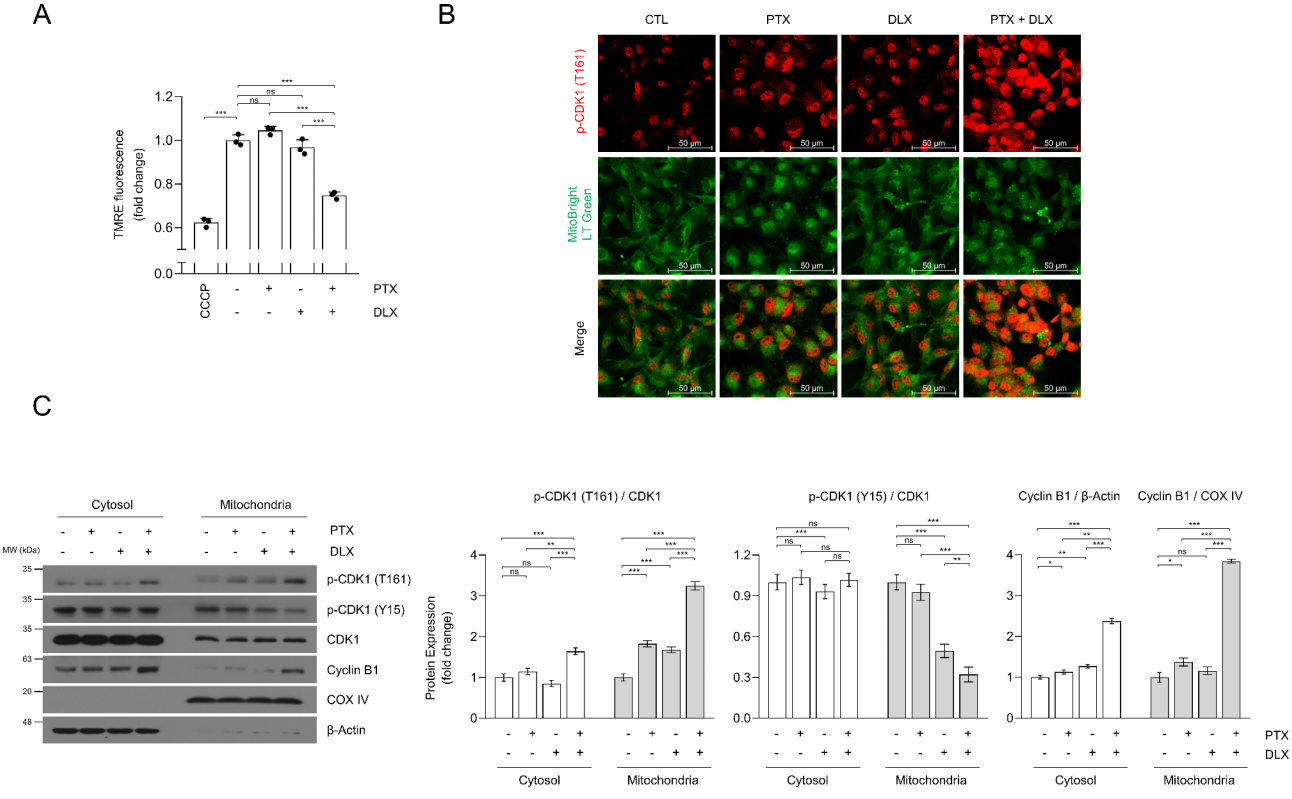

Fig. 3 Combined treatment with paclitaxel and duloxetine increases the mitochondrial localization of activated CDK1 in HEYA8-MDR cells. (**A**) HEYA8-MDR cells were treated with 100 nM paclitaxel and 15 μM duloxetine for 24 h. Mitochondrial membrane potential was assessed by TMRE fluorescence and analyzed by flow cytometry. A statistical graph of TMRE fluorescence is shown. CCCP at 50 nM served as a positive control. (**B**, **C**) HEYA8-MDR cells were treated with 100 nM paclitaxel and 15 μM duloxetine for 12 h. (**B**) The mitochondrial localization of p-CDK1 (T161) was detected using confocal microscopy. (**C**) Cytosolic and mitochondrial expressions of CDK1 and cyclin B1 were detected by immunoblot analysis. The data are presented as the mean ± SD of the fold change relative to the control ($n = 3$, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significantly different). PTX, paclitaxel; DLX, duloxetine

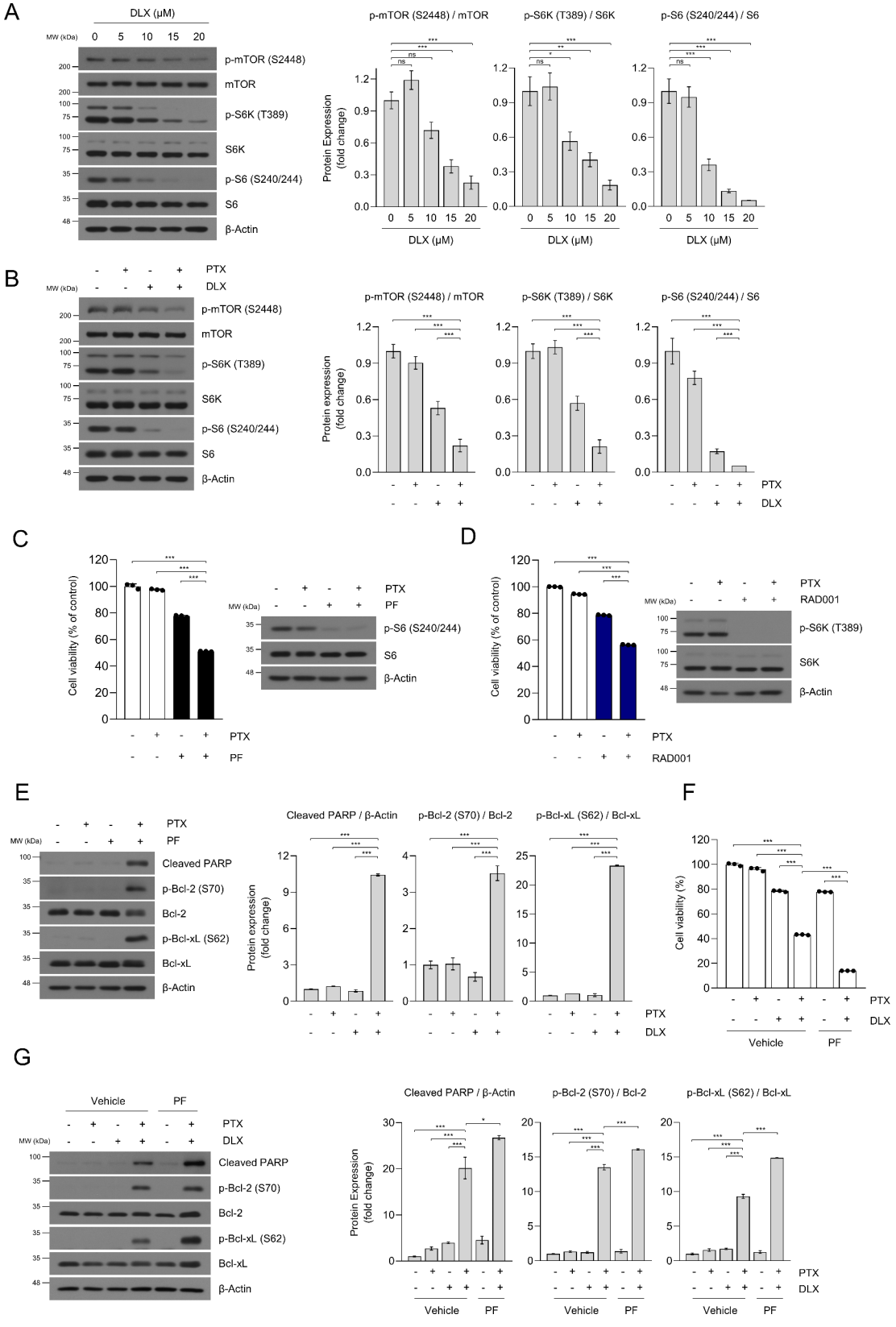

Fig. 4 (See legend on next page.)

(See figure on previous page.)

Fig. 4 Inhibition of mTORC1/S6K sensitizes HEYA8-MDR cells by phosphorylation of the antiapoptotic Bcl-2 family. (**A**) HEYA8-MDR cells were treated with the indicated concentrations of duloxetine for 12 h. The expression levels of mTOR, S6K and S6 were detected by immunoblot analysis. (**B**) The cells were treated with 100 nM paclitaxel and 15 μM duloxetine for 12 h. The expression levels of mTOR, S6K and S6 were detected by immunoblot analysis. (**C**) The cells were treated with 100 nM paclitaxel and 10 μM PF4708671 for 48 h. Cell viability was measured by MTT assay. The expression levels of S6 were detected by immunoblot analysis. (**D**) The cells were treated with 100 nM paclitaxel and 5 μM RAD001 for 48 h. Cell viability was measured by MTT assay. The expression levels of S6K were detected by immunoblot analysis. (**E**) The cells were treated with 100 nM paclitaxel and 10 μM PF4708671 for 12 h. The expressions of apoptotic cleaved PARP and antiapoptotic Bcl-2 family members were analyzed by immunoblot analysis. (**F**) The cells were treated with 100 nM paclitaxel and 15 μM duloxetine in combination with 10 μM PF4708671 for 48 h, and cell viability was measured by MTT assay. (**G**) The cells were treated with 100 nM paclitaxel and 15 μM duloxetine in combination with 10 μM PF4708671 for 12 h. The expression levels of apoptotic cleaved PARP and the antiapoptotic Bcl-2 family were detected by immunoblot analysis. The relative optical densities were quantified using ImageJ software. The data are presented as the mean ± SD of the percentage or the fold change relative to the control ($n = 3$, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significantly different). PTX, paclitaxel; DLX, duloxetine; PF, PF4708671

treatment increased paclitaxel sensitivity in HEYA8 cells, as demonstrated by a reduction in cell viability (Fig. 7A). Additionally, duloxetine significantly decreased phosphorylation of mTOR, S6K, and S6 proteins, and this phosphorylation was further suppressed with combined duloxetine and paclitaxel treatment (Fig. 7B). We observed that combined treatment with paclitaxel and duloxetine induced apoptotic cell death, which was attenuated by Ro-3306 treatment (Fig. 7C). The combination also increased CDK1 phosphorylation at the T161 activation site, while decreasing phosphorylation at the Y15 inactivation site (Fig. 7D).

Similarly, in another parental ovarian cancer cell line SKOV3, duloxetine enhanced paclitaxel sensitivity, as shown by reduced cell viability, and inhibited phosphorylation of mTOR, S6K, and S6 (Fig. S4A, B). Furthermore, consistent with observations in HEYA8 cells, this combination treatment induced apoptotic cell death, which was attenuated by Ro-3306 treatment in SKOV3 cells (Fig. S4C). The combination treatment also resulted in increased phosphorylation of CDK1 at T161 and decreased phosphorylation at Y15 in SKOV3 cells (Fig. S4D). Collectively, these findings suggest that duloxetine enhances the sensitivity of parental ovarian cancer cells to paclitaxel treatment.

Discussion

Despite ongoing efforts in the treatment of ovarian cancer, chemotherapy resistance remains a major hurdle for patients [33, 34]. Our study demonstrated the effectiveness of duloxetine treatment in overcoming paclitaxel resistance in two distinct types of ovarian cancer cells, HEYA8-MDR and SKOV3-TR. Duloxetine treatment inhibited mTORC1/S6K signaling, and combined treatment with paclitaxel resulted in the activation of CDK1 and increased mitochondrial localization of activated CDK1. This led to the phosphorylation of the antiapoptotic proteins Bcl-2 and Bcl-xL, subsequently triggering apoptotic cell death (Fig. 7E). The use of selective CDK1 inhibitors, Ro-3306 or avotaciclib, completely blocked the Bcl-2 and Bcl-xL phosphorylation and apoptotic cell death induced by combined paclitaxel and duloxetine treatment. Furthermore, our investigation revealed that S6K acts as an upstream mediator of CDK1 activation. Treatment with S6K inhibitors (PF4780671 and LY2584702) along with paclitaxel induced Bcl-2 and Bcl-xL phosphorylation and apoptotic cell death. Inhibiting S6K amplified the CDK1 activation and apoptotic cell death induced by combined paclitaxel and duloxetine treatment. In summary, our study proposes novel therapeutic strategies utilizing duloxetine to address paclitaxel resistance in ovarian cancer.

Several strategies have been proposed for the repurposing of existing drugs for the treatment of different diseases, commonly referred to as "drug repurposing" [35, 36]. Drug repurposing has many advantages compared to de novo drug discovery because it enhances cost and time efficiency, leading to a reduction in the number of clinical trials needed for the drug to successfully reach the market [36]. In this paper, we revealed new indications for duloxetine, which can be a promising drug for paclitaxel-resistant ovarian cancer. Duloxetine effectively inhibited the mTORC1/S6K signaling pathway, and under paclitaxel treatment, duloxetine activates CDK1, leading to subsequent antiapoptotic Bcl-2 and Bcl-xL phosphorylation in paclitaxel-resistant ovarian cancer cells. These findings highlight the multifaceted therapeutic potential of duloxetine, suggesting that it is a valuable and promising drug candidate for treating paclitaxel-resistant ovarian cancer. This understanding of duloxetine's mechanisms of action broadens its potential applications in various cancer treatment, extending beyond its established role as an antidepressant.

P-glycoprotein (P-gp), also known as multidrug resistance protein 1 (MDR1), is a membrane transporter that actively effluxes chemotherapeutic agents out of cancer cells, reducing intracellular drug accumulation and thereby diminishing therapeutic efficacy [37]. Overexpression of P-gp is a hallmark of multidrug resistance (MDR) and is frequently observed in various cancer cells, including ovarian cancer cells resistant to multiple chemotherapy agents [38]. It has been reported that some antidepressants, including duloxetine, fluoxetine, paroxetine, and sertraline, exhibit inhibitory effects on P-gp,

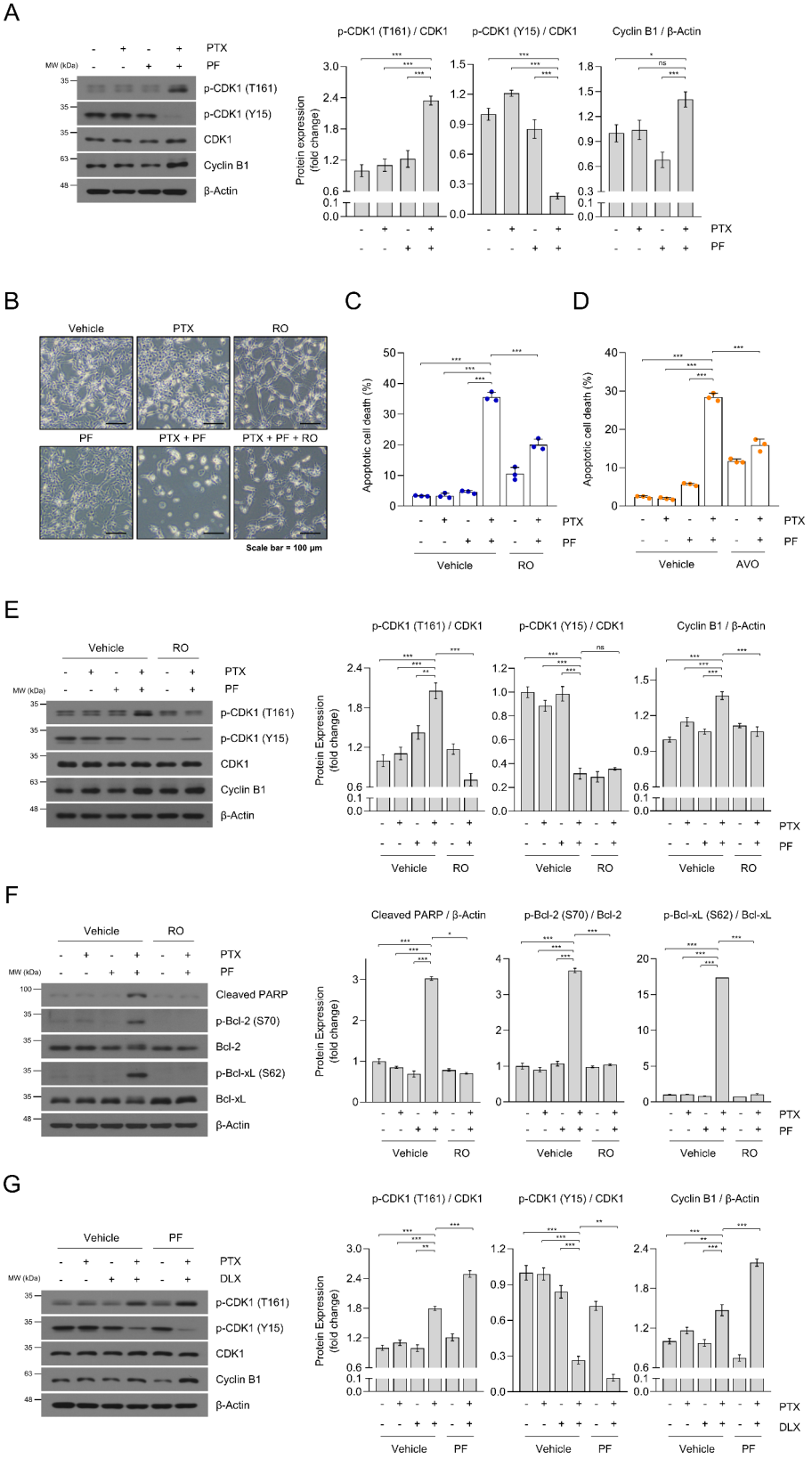

Fig. 5 (See legend on next page.)

(See figure on previous page.)

Fig. 5 Inhibition of S6K and paclitaxel treatment-induced apoptosis is mediated by CDK1 activation. (**A**) HEYA8-MDR cells were treated with 100 nM paclitaxel and 10 μM PF4780671 in combination with 10 μM Ro-3306 for 12 h. The expression levels of CDK1 and cyclin B1 were detected by immunoblot analysis. (**B**-**D**) The cells were treated with 100 nM paclitaxel and 10 μM PF4708671 in combination with 10 μM Ro-3306 or 1 μM avotaciclib for 48 h. (**B**) Cells were visualized via microscopy. (**C**, **D**) Apoptotic cell death were determined by Annexin V-FITC/PI staining using flow cytometry. Statistical graphs of apoptotic cells were shown. (**E**, **F**) The cells were treated with 100 nM paclitaxel, 10 μM PF4780671 and 10 μM Ro-3306 for 12 h. (**E**) The expression levels of CDK1 and cyclin B1 were detected by immunoblot analysis. (**F**) The expression levels of apoptotic cleaved PARP and the antiapoptotic Bcl-2 family were detected by immunoblot analysis. (**G**) The cells were treated with 100 nM paclitaxel and 15 μM duloxetine in combination with 10 μM PF4708671 for 12 h. The expression levels of CDK1 and cyclin B1 were detected by immunoblot analysis. The relative optical densities were quantified using ImageJ software. The data are presented as the mean ± SD of the percentage or the fold change relative to the control ($n = 3$, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significantly different). PTX, paclitaxel; DLX, duloxetine; PF, PF4708671; RO, Ro-3306; AVO, avotaciclib

thereby reversing multidrug resistance [39–42]. In our study, we demonstrated that duloxetine could overcome paclitaxel resistance in paclitaxel-resistant ovarian cancer cells with high MDR1 expression. Interestingly, we also found that even in parental HEYA8 and SKOV3 parental ovarian cancer cells, which exhibit very low MDR1 expression, duloxetine effectively sensitized the parental cells to paclitaxel treatment (Fig. 7 and Fig. S4, 5). These results suggest that duloxetine may enhance paclitaxel sensitivity through mechanisms beyond MDR1 inhibition, extending its potential role in chemotherapy to improve treatment efficacy.

CDK1, widely recognized as a central regulator of mitosis, forms an active heterodimer with cyclin B1 to orchestrate cell division [4–6]. However, recent studies have uncovered an additional role for CDK1 beyond cell cycle regulation [9, 10, 43, 44]. During mitotic arrest, activated CDK1 translocates to the mitochondria, where it phosphorylates key mitochondrial proteins [30, 45, 46]. Specifically, CDK1 interacts with antiapoptotic Bcl-2 family proteins, such as Bcl-2, Bcl-xL, and Mcl-1, at the mitochondrial membrane, leading to their inactivation and induction of apoptosis [9, 24, 25]. The CDK1/cyclin B1 complex has been reported to phosphorylate Bcl-2 at S70 and Bcl-xL at S62, disrupting their antiapoptotic functions during mitotic arrest [11, 28]. In line with these findings, we observed that the combined treatment with paclitaxel and duloxetine increased the phosphorylation of CDK1 at its activation site, as well as the phosphorylation of Bcl-2 and Bcl-xL, leading to the apoptotic cell death. Furthermore, the activated phosphorylation of CDK1 was significantly increased in the mitochondrial fraction. We also found that inhibition of CDK1 attenuated the phosphorylation of Bcl-2 and Bcl-xL, effectively reducing apoptotic cell death. These findings suggest that CDK1-mediated phosphorylation of Bcl-2 and Bcl-xL may play an important role in inducing apoptosis, providing a potential mechanism for overcoming paclitaxel resistance in ovarian cancer cells.

We proposed that inhibition of mTORC1/S6K signaling in combination with paclitaxel activates CDK1 in paclitaxel-resistant ovarian cancer cells. mTORC1/S6K signaling is known to regulate cell growth, protein biogenesis, and glucose homeostasis [31, 32]. A previous report suggested that S6K phosphorylates CDK1 at S39 residue, inducing G2/M mitotic arrest in mouse embryonic fibroblasts [47]. Although an interaction between S6K and CDK1 regarding cell cycle has been reported, the proapoptotic effects of this interaction remain poorly understood. Moreover, the role of the mTORC1/S6K pathway, which acts as an upstream mediator of CDK1 and induces the proapoptotic phosphorylation of Bcl-2 family proteins, remains unclear. In the present study, combined treatment with S6K inhibitors and paclitaxel activated CDK1 and subsequently induced Bcl-2 and Bcl-xL phosphorylation. These findings suggest that inhibition of mTORC1/S6K signaling may mediate CDK1 activation, contributing to apoptotic pathways. However, given the complexity of cellular signaling, further investigations are needed to clarify the interaction between mTORC1/S6K signaling and CDK1, as well as the potential contributions of other signaling pathways.

In clinical settings, plasma concentrations of duloxetine generally range from approximately 24.6 ng/mL to 110 ng/mL (roughly 0.08 to 0.37 μM), depending on the dose and dosing regimen [48]. While the concentrations of duloxetine used in this study exceed standard therapeutic levels, the investigation into its underlying anticancer pathways could contribute to future therapeutic strategies. To enhance clinical applicability, further studies are needed to explore structural modifications or formulation changes that could improve its efficacy at clinically achievable concentrations. Furthermore, as resistant cancer types frequently occur in advanced-stage patients with limited therapeutic options, these findings may support further investigation into the potential application of duloxetine in such contexts.

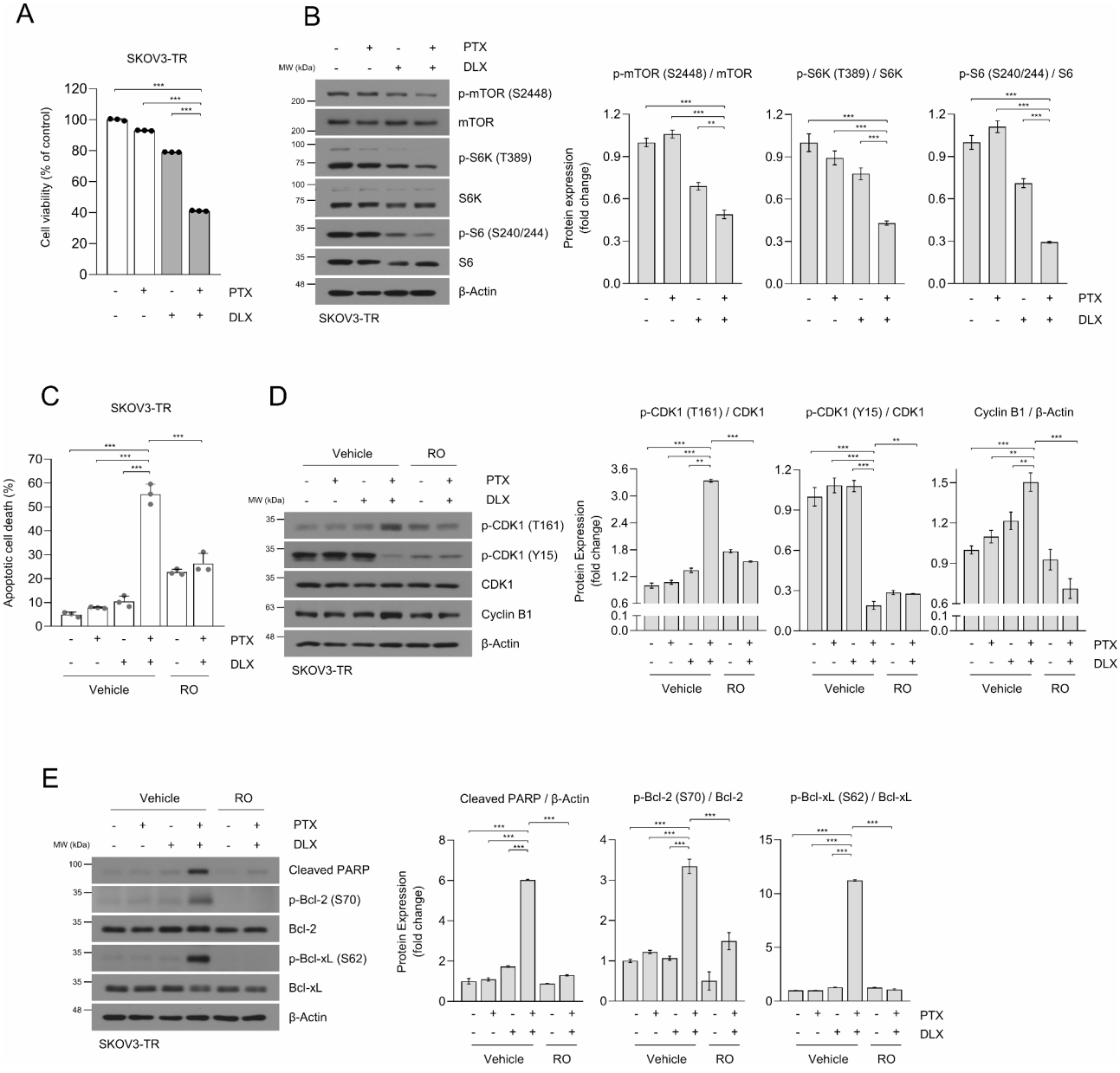

Fig. 6 Treatment with duloxetine overcomes paclitaxel resistance in SKOV3-TR cells via phosphorylation of the antiapoptotic Bcl-2 family in a CDK1-dependent manner. (**A**) SKOV3-TR cells were treated with 100 nM paclitaxel and 15 μM duloxetine for 48 h. Cell viability was measured by MTT assay. (**B**) The cells were treated with 100 nM paclitaxel and 15 μM duloxetine for 12 h. The expression levels of mTOR, S6K and S6 were detected by immunoblot analysis. (**C**) The cells were treated with 100 nM paclitaxel and 15 μM duloxetine in combination with 10 μM Ro-3306 for 48 h. Apoptotic cell death was determined by Annexin V-FITC/PI staining using flow cytometry. A statistical graph of apoptotic cells was shown. (**D**, **E**) The cells were treated with 100 nM paclitaxel and 15 μM duloxetine in combination with 10 μM Ro-3306 for 12 h. (**D**) The expression levels of CDK1 and cyclin B1 were detected by immunoblot analysis. (**E**) The expression levels of apoptotic cleaved PARP and the antiapoptotic Bcl-2 family were detected by immunoblot analysis. The relative optical densities were quantified using ImageJ software. The data are presented as the mean ± SD of the percentage or the fold change relative to the control ($n = 3$, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significantly different). PTX, paclitaxel; DLX, duloxetine; RO, Ro-3306

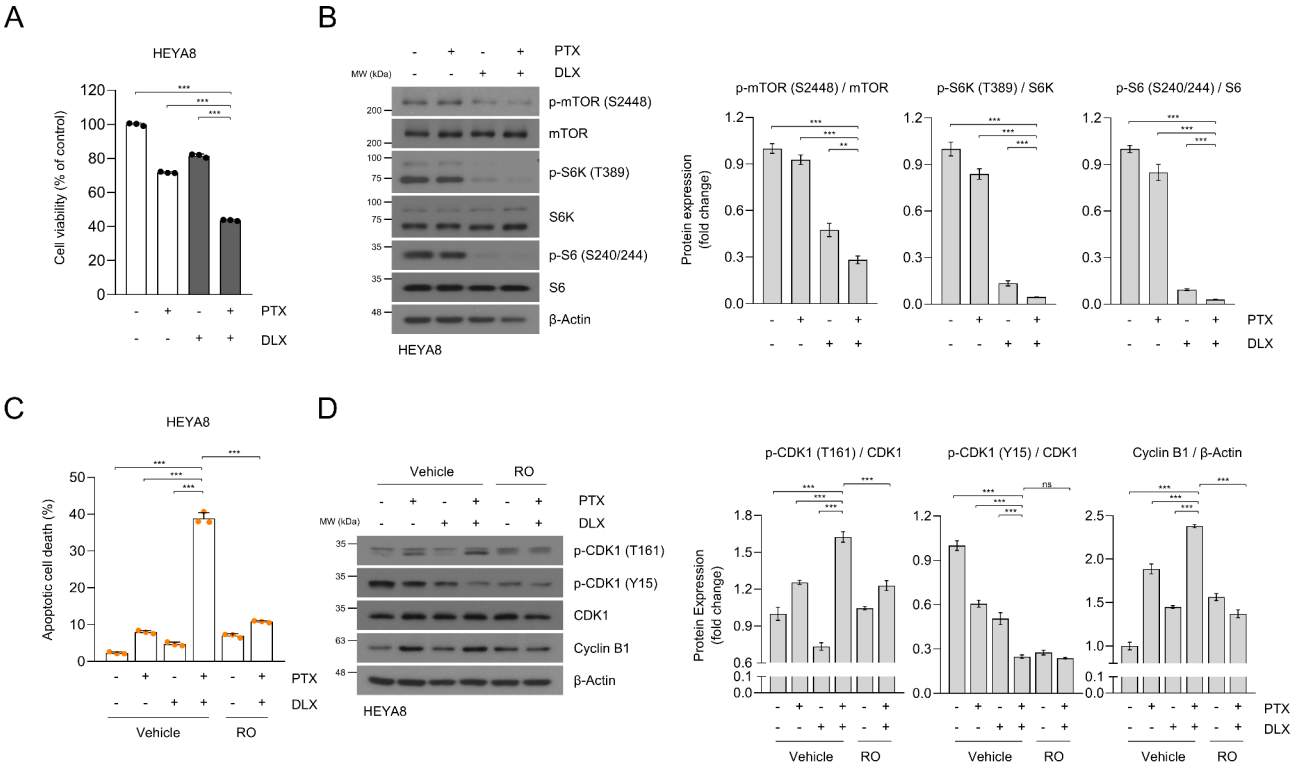

Fig. 7 Treatment with duloxetine enhances sensitivity of paclitaxel in parental ovarian cancer cells. (**A**) HEYA8 cells were treated with 5 nM paclitaxel and 15 μM duloxetine for 48 h. Cell viability was measured by MTT assay. (**B**) HEYA8 were treated with 5 nM paclitaxel and 15 μM duloxetine for 12 h. The expression levels of mTOR, S6K and S6 were detected by immunoblot analysis. (**C**) HEYA8 were treated with 5 nM paclitaxel and 15 μM duloxetine in combination with 10 μM Ro-3306 for 48 h. Apoptotic cell death was determined by Annexin V-FITC/PI staining using flow cytometry. A statistical graph of apoptotic cells was shown. (**D**) HEYA8 were treated with 5 nM paclitaxel and 15 μM duloxetine in combination with 10 μM Ro-3306 for 12 h. The expression levels of CDK1 and cyclin B1 were detected by immunoblot analysis. The relative optical densites was quantified using ImageJ software. The data are presented as the mean ± SD of the percentage or the fold change relative to the control ($n = 3$;; ** $p < 0.01$; *** $p < 0.001$; ns, not significantly different). (**E**) Proposed mechanism of duloxetine-mediated sensitization of ovarian cancer cells to paclitaxel. PTX, paclitaxel; DLX, duloxetine; RO, Ro-3306

Conclusions

In conclusion, this study suggests novel therapeutic approaches utilizing duloxetine, an already approved drug for medical use, to overcome paclitaxel resistance in ovarian cancer cells. Moreover, these findings provide promising insights into potential clinical applications, highlighting the intricate molecular mechanisms underlying drug resistance of ovarian cancer cells.

Abbreviations

Bak	Bcl2 antagonist/Killer
Bax	Bcl2 associated X
Bcl-xL	B-cell lymphoma-extra large
Bcl2	B-cell lymphoma 2
BIRC5	Baculoviral IAP repeat containing 5
BSA	Bovine serum albumin
CDK1/CDC2	Cell cycle-dependent kinase 1
COX IV	Cytochrome c oxidase subunit 4
FBS	Fetal bovine serum

mTOR	Mechanistic target of rapamycin
mTORC1	Mechanistic target of rapamycin complex 1
PARP	Poly (ADP-ribose) polymerase
PBS	Phosphate buffered saline
PFA	Paraformaldehyde
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
S6K	Ribosomal protein S6 kinase
S6	Ribosomal protein S6

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12935-024-03607-8.

Supplementary Material 1

Acknowledgements
Not applicable.

Author contributions
I.-C.P. developed the concept and designed the study. G.K., S.-K.J., S.H.A. and S.K. carried out the experiments. S.B., J.H.L., H.K., C.S.P., M.-K.S., H.-A.K. and H.-O.J. provided technical support and conceptual advice. G.K. and I.-C.P. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding
This research was supported by grants from the Korea Institute of Radiological and Medical Sciences (KIRAMS), funded by the Ministry of Science and ICT (MSIT) (grant no. 50531 – 2024 and grant no. 50548 – 2024) and from the National Research Foundation (NRF) of Korea, funded by the MIST (grant no. NRF-2022R1F1A1063450 and NRF-2023R1A2C1003833), Republic of Korea.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 13 June 2024 / Accepted: 6 December 2024
Published online: 19 December 2024

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. Cancer J Clin. 2022;72(1):7–33.
2. Pazdur R, Kudelka AP, Kavanagh JJ, Cohen PR, Raber MN. The taxoids: paclitaxel (Taxol®) and docetaxel (Taxotere®). Cancer Treat Rev. 1993;19(4):351–86.
3. Adams M, A'hern R, Calvert A, Carmichael J, Clark P, Coleman R, Earl H, Gallagher C, Ganesan T, Gore M. Chemotherapy for ovarian cancer–a consensus statement on standard practice. Br J Cancer. 1998;78(11):1404.
4. Santamaría D, Barrière C, Cerqueira A, Hunt S, Tardy C, Newton K, Cáceres JF, Dubus P, Malumbres M, Barbacid M. Cdk1 is sufficient to drive the mammalian cell cycle. Nature. 2007;448(7155):811–5.
5. MURRAY AW. Cyclin synthesis and degradation and the embryonic cell cycle. J Cell Sci. 1989;1989(Supplement12):65–76.
6. Solomon MJ, Lee T, Kirschner MW. Role of phosphorylation in p34cdc2 activation: identification of an activating kinase. Mol Biol Cell. 1992;3(1):13–27.
7. Aaltonen K, Amini R-M, Heikkilä P, Aittomäki K, Tamminen A, Nevanlinna H, Blomqvist C. High cyclin B1 expression is associated with poor survival in breast cancer. Br J Cancer. 2009;100(7):1055–60.
8. Kao H, Marto JA, Hoffmann TK, Shabanowitz J, Finkelstein SD, Whiteside TL, Hunt DF, Finn OJ. Identification of cyclin B1 as a shared human epithelial tumor-associated antigen recognized by T cells. J Exp Med. 2001;194(9):1313–24.
9. Massacci G, Perfetto L, Sacco F. The cyclin-dependent kinase 1: more than a cell cycle regulator. Br J Cancer. 2023;129(11):1707–16.
10. Shi L, Nishioka WK, Th'ng J, Bradbury EM, Litchfield DW, Greenberg AH. Premature p34cdc2 activation required for apoptosis. Science. 1994;263(5150):1143–5.
11. Terrano DT, Upreti M, Chambers TC. Cyclin-dependent kinase 1-mediated Bcl-xL/Bcl-2 phosphorylation acts as a functional link coupling mitotic arrest and apoptosis. Mol Cell Biol. 2010;30(3):640–56.
12. Allan LA, Clarke PR. Phosphorylation of caspase-9 by CDK1/cyclin B1 protects mitotic cells against apoptosis. Mol Cell. 2007;26(2):301–10.
13. Connor O DS, Grossman D, Plescia J, Li F, Zhang H, Villa A, Tognin S, Marchisio PC, Altieri DC. Regulation of apoptosis at cell division by p34cdc2 phosphorylation of survivin. Proc Natl Acad Sci. 2000;97(24):13103–7.
14. Westanmo AD, Gayken J, Haight R. Duloxetine: a balanced and selective norepinephrine-and serotonin-reuptake inhibitor. Am J health-system Pharm. 2005;62(23):2481–90.
15. Wright A, VanDenBerg C. Duloxetine in the treatment of generalized anxiety disorder. Int J Gen Med 2009:153–62.
16. Yang Y-H, Lin J-K, Chen W-S, Lin T-C, Yang S-H, Jiang J-K, Chang S-C, Lan Y-T, Lin C-C, Yen C-C. Duloxetine improves oxaliplatin-induced neuropathy in patients with colorectal cancer: an open-label pilot study. Support Care Cancer. 2012;20:1491–7.
17. Torta R, Leombruni P, Borio R, Castelli L. Duloxetine for the treatment of mood disorder in cancer patients: a 12-week case-control clinical trial. Hum Psychopharmacology: Clin Experimental. 2011;26(4–5):291–9.
18. Kajiwara I, Sano M, Ichimaru Y, Oshima Y, Kitajima O, Hao H, Masamune A, Kim J, Ishii Y, Ijichi H. Duloxetine improves cancer-associated pain in a mouse model of pancreatic cancer through stimulation of noradrenaline pathway and its antitumor effects. Pain. 2020;161(12):2909–19.
19. Velasco R, Besora S, Argyriou AA, Santos C, Sala R, Izquierdo C, Simó M, Gil-Gil M, Pardo B, Jiménez L. Duloxetine against symptomatic chemotherapy-induced peripheral neurotoxicity in cancer survivors: a real world, open-label experience. Anticancer Drugs. 2021;32(1):88–94.
20. Halder J, Landen CN Jr, Lutgendorf SK, Li Y, Jennings NB, Fan D, Nelkin GM, Schmandt R, Schaller MD, Sood AK. Focal adhesion kinase silencing augments docetaxel-mediated apoptosis in ovarian cancer cells. Clin Cancer Res. 2005;11(24):8829–36.
21. Yuan S-Y, Cheng C-L, Ho H-C, Wang S-S, Chiu K-Y, Su C-K, Ou Y-C, Lin C-C. Nortriptyline induces mitochondria and death receptor-mediated apoptosis in bladder cancer cells and inhibits bladder tumor growth in vivo. Eur J Pharmacol. 2015;761:309–20.
22. Zhang Z, Du X, Zhao C, Cao B, Zhao Y, Mao X. The antidepressant Amitriptyline shows potent therapeutic activity against multiple myeloma. Anticancer Drugs. 2013;24(8):792–8.
23. Pula G, Pistilli A, Montagnoli C, Stabile AM, Rambotti MG, Rende M. The tricyclic antidepressant Amitriptyline is cytotoxic to HTB114 human leiomyosarcoma and induces p75NTR-dependent apoptosis. Anticancer Drugs. 2013;24(9):899–910.
24. Eichhorn J, Sakurikar N, Alford S, Chu R, Chambers T. Critical role of anti-apoptotic Bcl-2 protein phosphorylation in mitotic death. Cell Death Dis. 2013;4(10):e834–834.
25. Scatena CD, Stewart ZA, Mays D, Tang LJ, Keefer CJ, Leach SD, Pietenpol JA. Mitotic phosphorylation of Bcl-2 during normal cell cycle progression and taxol-induced growth arrest. J Biol Chem. 1998;273(46):30777–84.
26. Kim G, Jang S-K, Kim YJ, Jin H-O, Bae S, Hong J, Park I-C, Lee JH. Inhibition of glutamine uptake resensitizes paclitaxel resistance in SKOV3-TR ovarian cancer cell via mTORC1/S6K signaling pathway. Int J Mol Sci. 2022;23(15):8761.
27. Jin H-O, Seo S-K, Woo S-H, Lee H-C, Kim E-S, Yoo D-H, Lee S-J, An S, Choe T-B, Kim J-I. A combination of sulindac and arsenic trioxide synergistically induces apoptosis in human lung cancer H1299 cells via c-Jun NH2-terminal kinase-dependent Bcl-xL phosphorylation. Lung Cancer. 2008;61(3):317–27.
28. Zhou L, Cai X, Han X, Xu N, Chang DC. CDK1 switches mitotic arrest to apoptosis by phosphorylating Bcl-2/Bax family proteins during treatment with microtubule interfering agents. Cell Biol Int. 2014;38(6):737–46.
29. Harris M, Thompson C. The role of the Bcl-2 family in the regulation of outer mitochondrial membrane permeability. Cell Death Differ. 2000;7(12):1182–91.
30. Wang Z, Fan M, Candas D, Zhang T-Q, Qin L, Eldridge A, Wachsmann-Hogiu S, Ahmed KM, Chromy BA, Nantajit D. Cyclin B1/Cdk1 coordinates

mitochondrial respiration for cell-cycle G2/M progression. Dev Cell. 2014;29(2):217–32.
31. Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. Nat Rev Mol Cell Biol. 2009;10(5):307–18.
32. Zou Z, Tao T, Li H, Zhu X. mTOR signaling pathway and mTOR inhibitors in cancer: progress and challenges. Cell Bioscience. 2020;10(1):1–11.
33. Cornelison R, Llaneza DC, Landen CN. Emerging therapeutics to overcome chemoresistance in epithelial ovarian cancer: a mini-review. Int J Mol Sci. 2017;18(10):2171.
34. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. Nat Rev Cancer. 2013;13(10):714–26.
35. Krishnamurthy N, Grimshaw AA, Axson SA, Choe SH, Miller JE. Drug repurposing: a systematic review on root causes, barriers and facilitators. BMC Health Serv Res. 2022;22(1):1–17.
36. Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, Doig A, Guilliams T, Latimer J, McNamee C. Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discovery. 2019;18(1):41–58.
37. Seelig A. A general pattern for substrate recognition by P-glycoprotein. Eur J Biochem. 1998;251(1–2):252–61.
38. Nielsen D, Skovsgaard T. P-glycoprotein as multidrug transporter: a critical review of current multidrug resistant cell lines. Biochim et Biophys Acta (BBA)-Molecular Basis Disease. 1992;1139(3):169–83.
39. Peer D, Dekel Y, Melikhov D, Margalit R. Fluoxetine inhibits multidrug resistance extrusion pumps and enhances responses to chemotherapy in syngeneic and in human xenograft mouse tumor models. Cancer Res. 2004;64(20):7562–9.
40. Weiss J, Dormann S-MG, Martin-Facklam M, Kerpen CJ, Ketabi-Kiyanvash N, Haefeli WE. Inhibition of P-glycoprotein by newer antidepressants. J Pharmacol Exp Ther. 2003;305(1):197–204.
41. Drinberg V, Bitcover R, Rajchenbach W, Peer D. Modulating cancer multidrug resistance by sertraline in combination with a nanomedicine. Cancer Lett. 2014;354(2):290–8.
42. Ruike Z, Junhua C, Wenxing P. In vitro and in vivo evaluation of the effects of duloxetine on P-gp function. Hum Psychopharmacology: Clin Experimental. 2010;25(7–8):553–9.
43. Castedo M, Perfettini J, Roumier T, Kroemer G. Cyclin-dependent kinase-1: linking apoptosis to cell cycle and mitotic catastrophe. Cell Death Differ. 2002;9(12):1287–93.
44. Golsteyn RM. Cdk1 and Cdk2 complexes (cyclin dependent kinases) in apoptosis: a role beyond the cell cycle. Cancer Lett. 2005;217(2):129–38.
45. Candas D, Qin L, Fan M, Li J-J. Experimental approaches to study mitochondrial localization and function of a nuclear cell cycle kinase, Cdk1. J Visualized Experiments. 2016;2016(108):53417.
46. Wijnen R, Pecoraro C, Carbone D, Fiuji H, Avan A, Peters GJ, Giovannetti E, Diana P. Cyclin dependent kinase-1 (CDK-1) inhibition as a novel therapeutic strategy against pancreatic ductal adenocarcinoma (PDAC). Cancers. 2021;13(17):4389.
47. Amar-Schwartz A, Hur VB, Jbara A, Cohen Y, Barnabas GD, Arbib E, Siegfried Z, Mashahreh B, Hassouna F, Shilo A. S6K1 phosphorylates Cdk1 and MSH6 to regulate DNA repair. Elife. 2022;11:e79128.
48. Knadler MP, Lobo E, Chappell J, Bergstrom R. Duloxetine: clinical pharmacokinetics and drug interactions. Clin Pharmacokinet. 2011;50:281–94.

Publisher's note