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Original Article

Targeting Notch-1 reverses cisplatin chemosensitivity in ovarian cancer cells by upregulation of PUMA

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Abstract: Aim: Cisplatin (DDP) is the first-line chemotherapeutic therapeutics for ovarian cancer treatment. Its early response is usually effective, the majority of patients will ultimately recur with chemotherapy-resistant cancer. However, the mechanisms of DDP chemoresistance remain unclear. The aim of this work is to explore the potential mechanism for development of chemoresistance in ovarian cancer. Methods: SKOV3 and SKOV3^{DDP} cells was treated with 3 μg/ml of cisplatin for 24-72 h. Using siRNAs and inhibitors to examine the relation between p53, Notch-1 and PUMA expression exposure to cisplatin, and the effect on cisplatin chemosensitivity of ovarian cancer cells. A subcutaneously implanted tumor model of SKOV3 and SKOV3^{DDP} cells in nude mouse was used to observe the effects of Notch-1 knockdown in combination with cisplatin on tumorigenesis. Results: Our data revealed that p53, Notch-1 and PUMA protein expression were induced by DDP. In the early, DDP-induced p53-dependent PUMA expression contributes to DDP-induced apoptosis of SKOV3 cells. In the later, DDP-induced p53 enhanced expression of Notch1, further downregulated PUMA expression, and weaken p53-induced apoptosis and cell growth inhibition. Knockdown of Notch-1 expression sensitized SKOV3 and SKOV3^{DDP} cells to DDP treatment in cultured cell lines and xenograft nude mice cell model. Furthermore, our data demonstrated that Notch1 was overexpressed in SKOV3^{DDP} cells, and the expression of Notch1 correlated with chemoresistance of SKOV3 cells. Conclusions: P53/Notch-1/PUMA axis has an important role in the development of chemoresistance in ovarian cancer. Notch1 has an anti-cancer role in ovarian cancer. Thus, inhibition of Notch1 function reverses cisplatin chemosensitivity in ovarian cancer cells, which provides a logical approach for effective cancer therapy.

Keywords: Ovarian cancer, chemoresistance, cisplatin, P53, Notch-1, PUMA

Introduction

Epithelial ovarian carcinoma (EOC) remains the most lethal gynecologic malignancy [1]. Currently, chemotherapy in combination with surgical debulking is the preferred treatment option and derivatives of cisplatin (DDP) are first-line chemotherapeutic therapeutics [2]. While early response is usually effective, the majority of patients will ultimately recur with chemotherapy-resistant cancer and succumb to disease [2]. Therefore, the control of drug-resistance against DDP is one of the important issues in the improved treatment of ovarian cancer. To date, the mechanisms by which tumor cells develop resistance to DDP remain incompletely understood.

p53 is a short lived protein, which is activated (phosphorylation) by DNA damage signal. The activated p53 in turn activates its downstream signals, and regulates cell cycle progression, DNA repair and apoptosis. p53 can regulate cisplatin induced cell death by several mechanisms like: Degradation of flice-like inhibitory protein (FLIP), direct binding and counteracting the antiapoptotic function of B-cell lymphoma-extra-large (Bcl-xL), over expression of phosphatase and tensin homolog (PTEN) and inhibition of AMPK [3]. PUMA (p53 upregulated modulator of apoptosis), a BH3-only Bcl-2 family protein, plays an essential role in p53-dependent and p53-independent pathway induced apoptosis by various stimuli [4, 5]. Cisplatin could activate p53 dependent PUMA upregulation in ovarian cancer cells and act on the mitochondria and cause the release of mitochondrial death proteins [6-8]. Although DDP induced apoptosis in EOC cells, only low levels (<10%) of apoptosis were detected in EOT cells

following DDP treatment [8]. This might be due to simultaneous induction of the antiapoptotic protein in addition to p53 and PUMA.

The Notch signaling pathway, a highly evolutionarily conserved pathway, which is activated by its ligands, in both invertebrate and vertebrate development, plays a key role in cell differentiation, survival, and proliferation [9]. When the Notch receptor is activated, the receptor-ligand then triggers a second Notch extracellular domain cleavage by a metalloproteinase ADAM, which in turn downregulates ligand activity [10]. Up to date, only four Notch genes have been identified in mammals (Notch-1 to 4) [11]. All the Notch receptors are very similar in structures, although there are some subtle differences in their cytoplasmic domains and extracellular [9].

The function of Notch signaling in tumorigenesis could be either oncogenic or anti-proliferative, and the function could be context dependent. Notch signaling has been shown to be anti-proliferative in a limited number of tumor types, including skin cancer, human hepatocellular carcinoma, medullary thyroid, cervical cancer, and small cell lung cancer [12-16]. However, most of the studies have shown the Notch pathway has been shown to be activated in multiple tumors, including EOC [17], and as aoncogenic function of Notch in many human carcinomas [18-20]. Recently, Notch pathway has been reported to be involved in intrinsic resistance and acquired drug-resistance, and knockdown of Notch-1 could reverse chemosensitivity [21]. The mechanism for this potentiation of chemosensitivity reversion by Notch inhibition may be related to downregulation of pro-survival pathways and upregulation of anti-survival pathways [22].

Recently, it has found that activated p53 could upregulate the expression of Notch1 inhuman cancer cell lines. Moreover, inhibition of Notch1 activity after genotoxic stress by an inhibitor of Notch signaling increased susceptibility to apoptosis [23], suggesting that Notch-1 plays anti-apoptosis effect. However, the molecular processes that mediate antiapoptotic activity of Notch-1 are not completely understood. Meurette et al. has found that activation of Notch-1 signalling in breast cancer cells caused resistance to p53-dependent apoptosis induced by DNA damage, followed by decreased pro-apoptotic PUMA and NOXA accumulation [24], suggesting that activation of p53-dependent Notch-1 signalling could result in PUMA inactivation.

In the present study, we demonstrate that knockdown of Notch-1 could induce apoptosis and reverses cisplatin chemosensitivity in ovarian cancer cells via a direct interaction amongp53/Notch-1/PUMA axis. Our results suggest that DDP treatment early activates p53-dependent PUMA upregulation and induces cell apoptosis. DDP treatment later activates p53-dependent Notch-1 signal, resulting in PUMA inactivation and apoptosis inhibition.

Materials and methods

Ethics statement

All studies involving mice were approved by the Institutional Animal Care and Treatment Committee of People's hospital of Laiwu, Shandong.

Cell lines and culture

The human ovarian cancer cell line SKOV3 were purchased from the Cell Bank of the Chinese Academy of Science (Shanghai, China). Cisplatin (DDP)-resistant SKOV3 cell ($SKOV3^{DDP}$) were purchased from shybio.biomart (ATCC, Shanghai, China). Both cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) in 5% CO_2/95% air at 37°C. *In vitro* assays were performed at 60-70% cell density.

Antibodies and agents

Cisplatin [cis-diammine-dichloroplatinum (II)] was acquired from Sigma-Aldrich. Anti-Notch-1, Anti-PUMA, Anti-P53 and Anti-β-actin antibodies were purchased from Santa Cruz Biotechnology (Shanghai, China). Notch-1 siRNA, PUMAsiRNAs, p53 siRNA and Notch 1 shRNA Plasmid (h) were purchased from Santa Cruz Biotechnology (Guangzhou, China).

Transfection with siRNAs

Lipofectamine 2000 (Invitrogen) kits were used for transfections of siRNAs, respectively, according to the manufacturer's instructions. Briefly, SKOV3 and $SKOV3^{DDP}$ cells were seeded in six-well plates (density 2.5×10^5 cells/well) in antibiotic free media, and transfected with

50 nM Notch-1 siRNAs or PUMAsiRNAs or p53 siRNA or scramble siRNA or Notch 1 shRNA-plasmid in serum-free medium for 5-8 h. The culture medium was then replaced with fresh medium for 48 h. Cells were then treated accordingly. For stable siRNA transfection, 24 hs after Notch-1 siRNAs or Notch 1 shRNA transfection, the cells were split into 96-well plates and subjected to the G418 (1 mg/ml) selection for 2-3 weeks.

Cisplatin treatment

The length of treatment for all experiments consisted of 24-72 h. SKOV3 and SKOV3^{DDP} cells were first plated and cultured with 10% FBS in RPMI 1640 media for 24 hours before initiation of treatment to allow cells to attach to the plate. The next day, the media was replaced with 0.1% FBS in RPMI 1640 containing DDP 3 μM concentrations for 24-72 h. To determine the effect of Notch-1, PUMA and p53 on DDP-induced apoptosis and growth inhibition, SKOV3 and SKOV3^{DDP} cells were transfected with siRNA targeting Notch-1 or PUMA or p53 24 h before DDP treatment about. Cells were then treated accordingly.

Cell survival assay

After exposure to various treatments, cells were seeded in 96-well plates at a density of 3 × 10^3 cells/well in DMEM containing 10% FBS. After they had adhered, cells were assessed for growth using an MTT assay. Briefly, 20 μl of MTT solution (5 mg/ml in PBS) was added into triplicate wells and cells were incubated for 4-6 h in an incubator. Absorbance at 490 nm was read with a microplate reader.

Apoptosis assay

Apoptosis induction was quantified by Annexin V/PI double staining followed by flow cytometry. Annexin V/PI double staining was performed using an apoptosis detection kit (Biovision, Mountain view, CA) following the manufacturer's instruction. Briefly, after exposure to various treatments, cells were gently detached by brief trypsinization, and then washed with ice cold PBS. After another wash with binding buffer, cells were suspended in 300 μL binding buffer containing Annexin V and propidium iodide, and incubated for 5 min at room temperature. Early apoptotic cells were identified as Annexin V positive/PI negative cells, while late apoptotic/necrotic cells were identified as Annexin V positive/PI positive cells using a BD LSR II cell analyzer.

Western blotting

Cell lysates were homogenized by sample buffer (100 mM Tris-HCl (pH 6.8), 2% Sodium Dodecyl Sulfate (SDS), 0.002% bromophenol blue, 20% glycerol, 10% β-mercaptoethanol (all from Sigma-Aldrich). Those were subjected to SDS-PAGE and transferred onto nitrocellulose membranes (Amersham Biosciences). The following primary antibodies were used for immunodetection: anti-β-actin, Notch-1, P53 and PUMA and the Western Blot Substrate kit (Pierce) were used to detect chemiluminescence.

Xenograft models

Immunodeficient female mice, 4 to 6 weeks old, were purchased from the Shanghai Animal Center. Stable Notch-1 shRNA or control shRNA transfected SKOV3 and SKOV3^{DDP} cells (5 × 10^6) were injected subcutaneously into 6-week-old female athymic nude mice. On days 6-8 and 16-18, cisplatin (3 mg/kg/d) was injected intraperitoneally into the mice. Tumor growth was monitored every 3 days with calipers to calculate tumor volume according to the formula [length × width^2]/2. Six weeks later, primary tumor masses were excised, fixed in 4% paraformaldehyde, and embedded in paraffin. Sections (5 μm thick) were prepared and stained with hematoxylin and eosin.

Terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL) analysis

TUNEL was performed with an In situ Cell Death Detection Kit (Roche). Cell apoptosis was quantified by determining the percentage of positively stained cells for all of the nuclei in 20 randomly chosen fields/section at 200× magnification. Slides of the apoptosis studies were quantified in a blind manner by two independent reviewers two different times.

Immunohistochemistrical staining

Tissue sections were deparaffinized and rehydrated. The slides were autoclaved in 10 mm sodium citrate buffer for antigen retrieval and

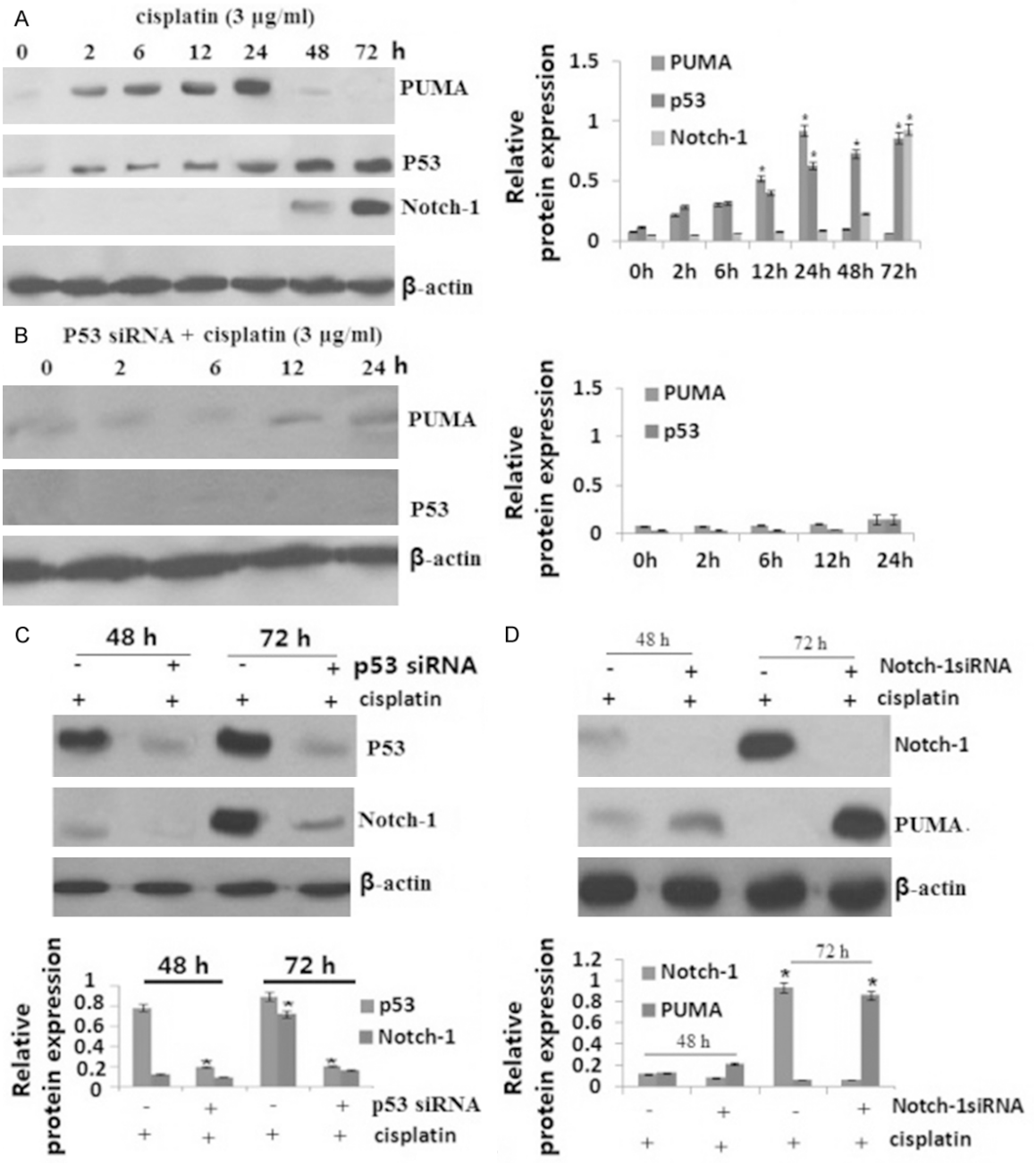

Figure 1. p53-dependent induction of PUMA by cisplatin treatment. A. SKOV3 ovarian cancer cells was treated with 3 μg/ml of cisplatin for 24, 48 and 72 hs. PUMA, P53 and Notch-1 protein expression was detected by western blot assay. B. SKOV3 cells were transfected with p53 siRNA for 24 h, then treated with 3 μg/ml of cisplatin for 24 hs. PUMA and P53 protein expression was detected by western blot assay. C. SKOV3 cells were transfected with p53 siRNA for 24 h, then treated with 3 μg/ml of cisplatin for 48-72 hs. Notch-1 and P53 protein expression was detected by western blot assay. D. SKOV3 cells were transfected with Notch-1 siRNA for 24 h, then treated with 3 μg/ml of cisplatin for 48-72 hs. Notch-1 and PUMA protein expression was detected by western blot assay. $^{*}P<0.05$, statistically significant compared with control (Student's t test).

blocked in 5% BSA in PBS at RT for 1 h. The sections were incubated with primary antibody (PUMA and Notch-1) overnight at 4°C followed by incubation with biotin-conjugated secondary antibodies for 1 h at RT. The sections were incubated with an Avidin-Biotin complex for 45 min, followed by diaminobenzidine (DAB) staining. The DAB-stained preparations were

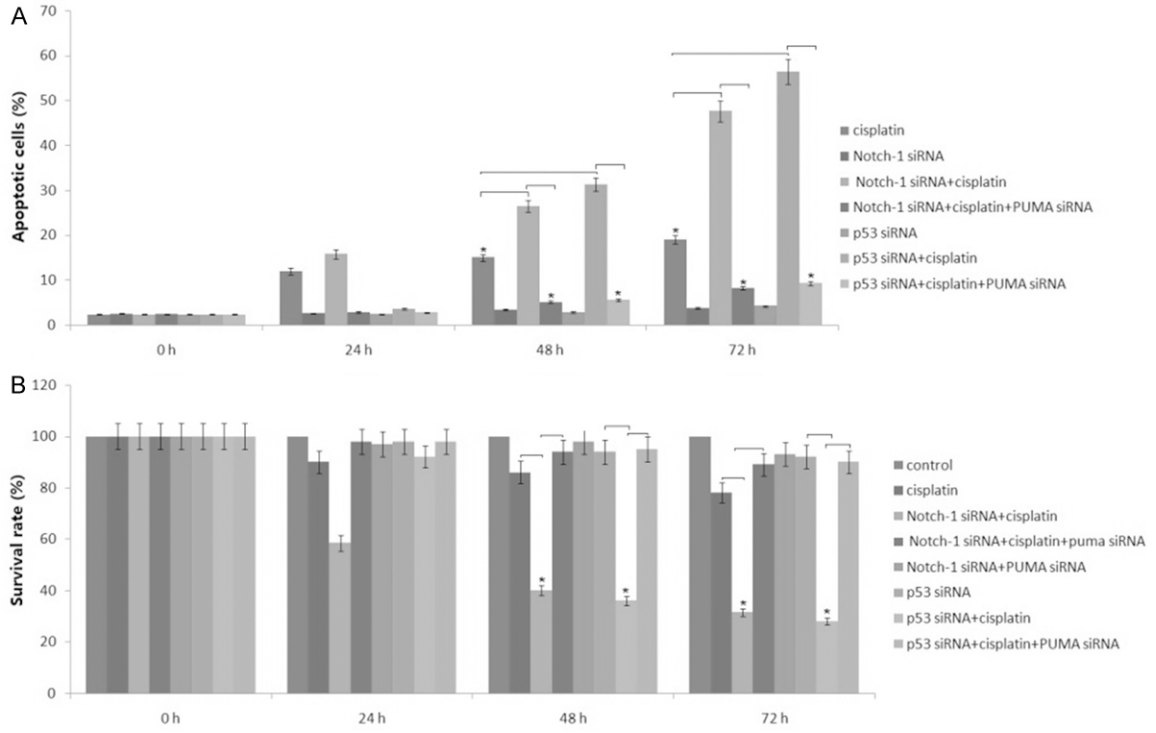

Figure 2. Effect of PUMA oncisplatin-induced cell apoptosis and growth of SKOV3 cells. A. Analysis of apoptosis using annexin V/propidium iodide (PI) staining; B. Analysis of cell survival using MTT; $^{*}P<0.01$, statistically significant compared with control (Student's t test).

visualized with a TE-2000U bright-field optical microscope.

Statistics

Data was expressed as means ± S.E. Statistical analysis was performed by ANOVA or Student's t-test (two-tailed) using SPSS 17.0 software. A *P*-value of <0.05 was considered to be statistically significant.

Results

Early p53-dependent induction of PUMA by cisplatin treatment

SKOV3 ovarian cancer cells was treated with 3 μg/ml of cisplatin. PUMA protein was induced by cisplatinat 2 h, with the peak level of *PUMA* protein at 24 h. After treatment of cisplatin for 24 h, PUMA protein expression was decreased, and undetectable after 48 h (**Figure 1A**). We next detected p53 protein expression in the SKOV3 cells after cisplatin treatment. The results showed that P53 protein was induced gradually with cisplatin treatment, and reach the peak at 48-72 h (**Figure 1A**).

We also detected Notch-1 protein expression in the SKOV3 cells after cisplatin treatment. The results showed that Notch-1 protein began to induce by cisplatinat 48 h, and reach the peak at 72 h (**Figure 1A**).

To determine whether p53 is involved in PUMA induction, SKOV3 cells were transfected with p53 siRNA to inhibit p53 expression, then treated with 3 μg/ml of cisplatin for 24 h, only to find that induction of PUMA protein was blocked in the present of cisplatin (**Figure 1B**).

p53-dependent induction of Notch-1 by cisplatin

SKOV3 ovarian cancer cells was treated with 3 μg/ml of cisplatin for 24-72 h. Notch-1 protein was induced by cisplatinat 48 h, and reached the peak level of *PUMA* protein at 72 h (**Figure 1A**). To determine whether p53 is involved in Notch-1 induction, SKOV3 cells were transfected with p53 siRNA to inhibit p53 expression, then treated with 3 μg/ml of cisplatin for 48-72 h, only to find that induction of Notch-1 protein was blocked in the present of cisplatin (**Figure 1C**).

Activated P53/Notch-1 signal inhibits cisplatin-induced PUMA upregulation

To determine whether PUMA was inhibited by cisplatin-activated Notch-1, SKOV3 cells were transfected with Notch-1 siRNA to inhibit Notch-1 expression, then treated with 3 μg/ml of cisplatin for 72 h, only to find that PUMA protein was significantly induction in the present of cisplatin (**Figure 1D**). The above data collectively indicate that p53-dependent induction of PUMA by cisplatinis blocked by cisplatin-induced Notch-1 activation.

Knockdown of Notch-1 induced PUMA-dependent growth inhibition and apoptosisin SKOV3 cells

18% of apoptosis was detected in SKOV3 cells following cisplatin treatment for 24 h-72 h (**Figure 2A**). Knockdown of *Notch-1* by siRNA alone did not affect cell apoptosis (P<0.01, **Figure 2A**) and cell growth (P<0.01, **Figure 2B**) of SKOV3 cells, but led to a significant increase in cisplatin-induced apoptosis and growth inhibition at 48-72 hs (P<0.01, **Figure 2A**, **2B**). However, the effect of *Notch-1* knockdown on cisplatin-induced apoptosis was much reduced and growth inhibitionin was decreased in the PUMA siRNA/SKOV3 cells (P<0.01, **Figure 2A**, **2B**). The control siRNA has no effect on cisplatin-induced apoptosis at 48-72 hs (data not shown). In addition, knockdown of *p53* by siRNA inhibited cisplatin-induced apoptosis at 24 h (P<0.05), and led to a significant increase in cisplatin-induced apoptosis and growth inhibition at 48-72 hs (P<0.01, **Figure 2A**, **2B**). However, the effect of *p53* knockdown on cisplatin-induced cell apoptosis and growth was much reduced in the PUMA siRNA/SKOV3 cells (P<0.01, **Figure 2A**, **2B**). These results suggest that PUMA induction represents a novel mechanism mediating cisplatin-induced apoptosis and growth inhibition, and that activation of p53-dependent Notch-1 signal can compromise cisplatin-induced and PUMA-mediated apoptosis and growth inhibition.

Knockdown of Notch-1 induced growth inhibition and PUMA-dependent apoptosisin SKOV3^{DDP} cells

Notch-1 and p53 protein was overexpressed, and PUMA was less expressed in the SKOV3^{DDP} cells by western blot assay. Knockdown of Notch-1 (**Figure 3A**) or p53 (**Figure 3B**) by siRNA induced PUMA protein expression in the SKOV3^{DDP} cells. In addition, knockdown of p53 blocked Notch-1 protein in SKOV3^{DDP} cells (**Figure 3B**). However, knockdown of Notch-1 did not affect p53 protein in SKOV3^{DDP} cells (**Figure 3A**). The above data indicate that PUMA was p53/Notch-1 dependent regulation in the SKOV3^{DDP} cells.

Knockdown of Notch-1 reverses cisplatin chemosensitivity of SKOV3^{DDP} cells by upregulation of PUMA

SKOV3^{DDP} cells were transfected with Notch-1 siRNA or control siRNA for 48 h, then treated with 3 μg/ml of cisplatin for 24-72 h. Analysis of apoptosis using annexin V/propidium iodide (PI) staining showed that knockdown of Notch-1 alone induced apoptosis of SKOV3^{DDP} cells (P<0.05, **Figure 3C**). However, when PUMA protein was knockdown by siRNA (**Figure 3C**), knockdown of Notch-1 did not affect apoptosis of SKOV3^{DDP} cells (**Figure 3C**).

Although SKOV3^{DDP} cells were resistant to cisplatin treatment, knockdown of Notch-1 significantly enhanced cisplatin-induced apoptosis (**Figure 3D**). However, when PUMA protein was knockdown by siRNA, knockdown of Notch-1 blocked cisplatin-induced apoptosis (**Figure 3D**). MTT assay also showed that knockdown of Notch-1 significantly enhanced cisplatin-induced cell growth inhibition (data not shown). However, when PUMA protein was knockdown by siRNA, knockdown of Notch-1 blocked cisplatin-induced cell growth inhibition (data not shown). The above data indicate that knockdown of Notch-1 reverses cisplatin chemosensitivity in SKOV3^{DDP} cells by upregulation of PUMA.

Knockdown of siRNA in combination cisplatin yields significantly better antitumor efficacy compared with the single-agent treatment

Next, to run a pilot efficacy study to combine the Notch-1 sliencing and cisplatin treatment, mice bearing SKOV3^{DDP} or SKOV3 cells (Notch-1 siRNA or control siRNA) tumors were treated with PBS or cisplatin. There was tumor growth inhibition observed in mice that had Notch-1 siRNA alone (~35%) or cisplatin alone (~8%) or the combination treatment (~60%) in SKOV3^{DDP} cells. In addition, there was tumor growth inhi-

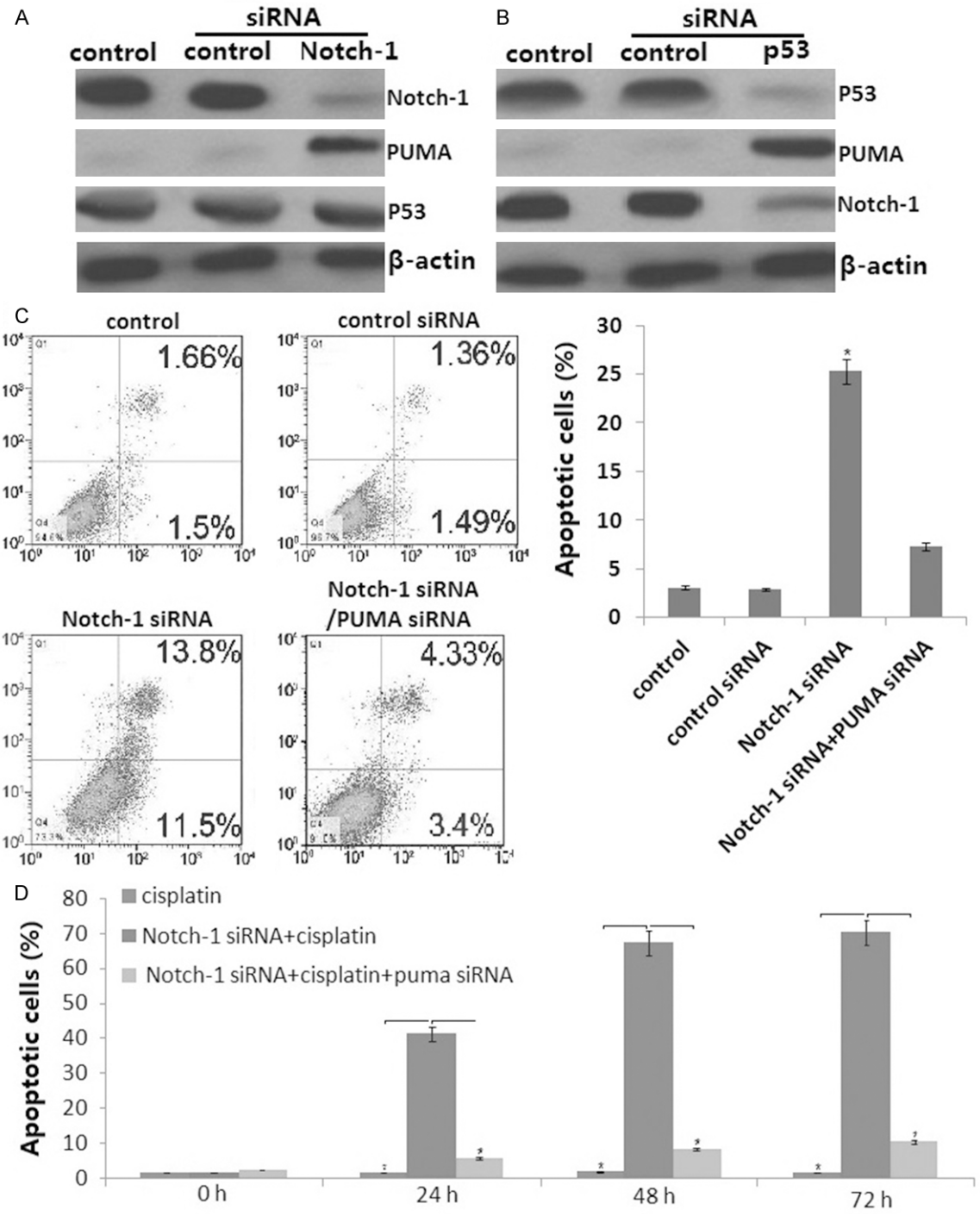

Figure 3. Knockdown of Notch-1 reverses Cisplatin chemosensitivity of SKOV3^{DDP} cells. A. SKOV3^{DDP} cells were transfected with Notch-1 siRNA for 72 h, Notch-1, p53 and PUMA was detected by western blot assay. B. SKOV3^{DDP} cells were transfected with P53 siRNA for 48 h, Notch-1, p53 and PUMA was detected by western blot assay. C. SKOV3^{DDP} cells were transfected with Notch-1 siRNA for 72 h, cell apoptosis was detected by FCM assay, $P<0.05$. D. SKOV3^{DDP} cells were transfected with Notch-1 siRNA or/and PUMA siRNA for 48 h, and then treated with 3 μg/ml of cisplatin for 24-72 h, cell apoptosis was detected by FCM assay, $^{*}P<0.05$; $^{**}P<0.01$.

bition observed in mice that had Notch-1 siRNA alone (~10%) or cisplatin alone (~20%) or the combination treatment (~45%) in SKOV3 cells. The combination group showed significantly better growth inhibition compared with PBS or either of the single-agent treatment on day 42

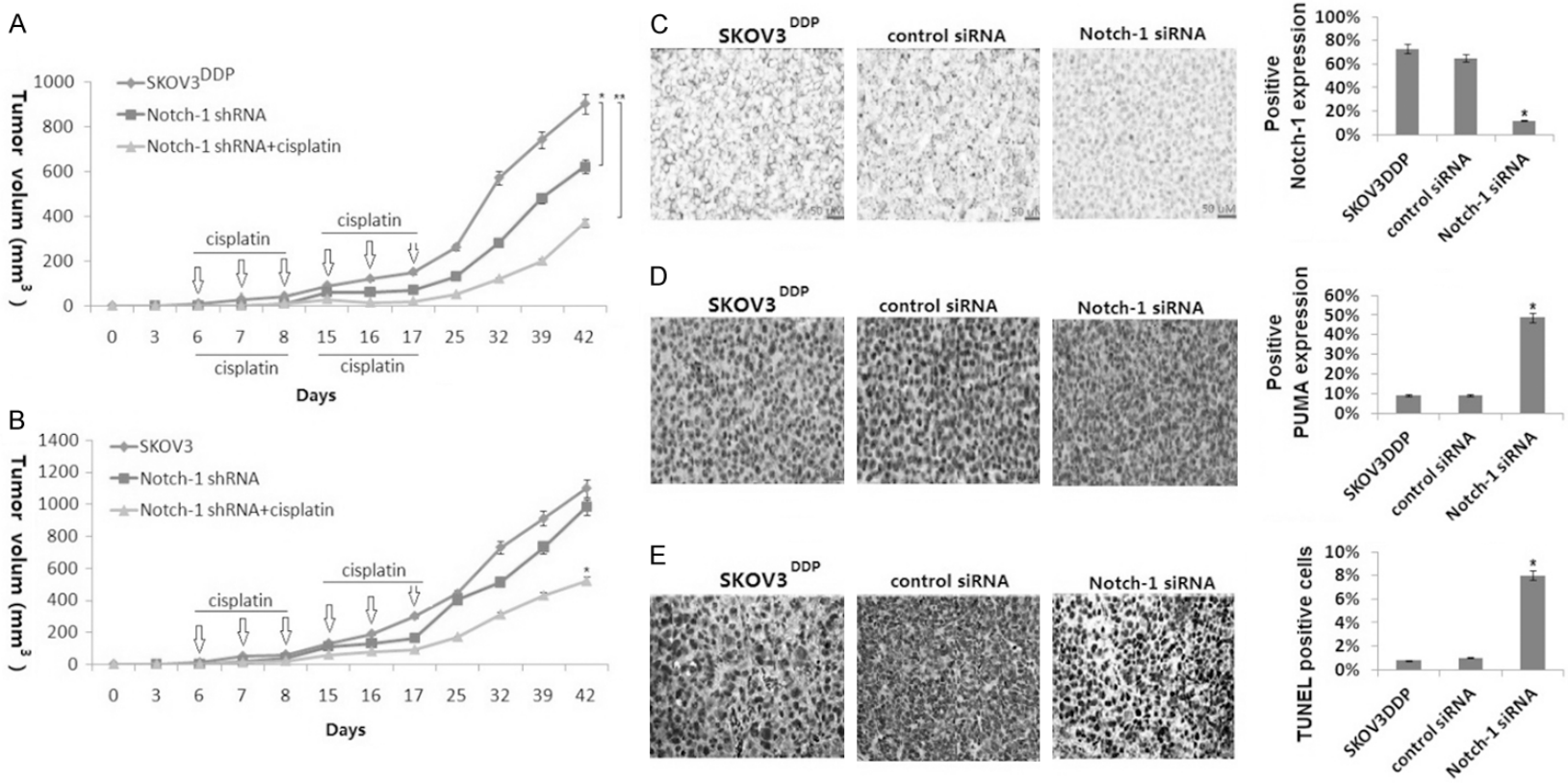

Figure 4. Inhibition of tumor formation after combined Notch-1 sliencing and cisplatin treatment. 6 weeks after the beginning of treatment, mice from each group (n=6) were killed; their tumors were recovered. A, B. Tumor (SKOV3^{DDP} cells and SKOV3 cells) growth curve. C. Representative immunohistochemistrical staining for Notch-1 in SKOV3^{DDP} tumor. D. Representative immunohistochemistrical staining for PUMA in SKOV3^{DDP} tumor. E. Representative TUNEL staining for apoptosis. $^{*}P$<0.05 and $^{**}P$<0.01.

in both of the cells (P<0.01, respectively) (**Figure 4A**, **4B**).

Immunohistochemistrical staining assay showed that Notch-1 was knockdown and PUMA was induced in the groups of combination treatment in the SKOV3^{DDP} cells. (**Figure 4C**, **4D**). Analyzing tissue sections from tumors revealed that combination treatment had significantly increased apoptosis in the tumors assessed by TUNEL staining in both of the cells (P<0.01, respectively) (**Figure 4E**). Notch-1 siRNA has the same results as that to Notch-1 and PUMA expression, as well as cell apoptosis in SKOV3 cells (data not show).

Discussion

Inhibition of cell proliferation and inducing apoptosis by p53 is largely attributable to its ability to transcriptionally activate the expression of genes that encode proteins, which determine cell fate [25, 26]. Depending on cellular context, wild-type p53 limits cell proliferation in response to DNA damage and other cellular stresses by inducing cell cycle arrest, apoptosis, or senescence [27, 28].

In our study, we found that treatment of SKOV3 cells with DDP induced apoptosis and growth inhibition of SKOV3 cells at 24 h. Furthermore, p53 and PUMA expression was significantly increased. Knockdown of PUMA blocked DDP induced cell apoptosis at 24 h. In addition, knockdown of p53 blocked DDP induced cell apoptosis and PUMA upregulation at 24 h. We therefore suggested that DDP treatment activated p53 dependent PUMA, which plays an important role in DDP-induced apoptosis and growth inhibition of SKOV3 cells. DDP induced apoptosis (<20%) in SKOV3 cells at 24 h, no significant change was found at 48-72 h compared to the apoptosis at 24 h. Furthermore, PUMA was significantly increased at 24 h and undetectable at 48 h following DDP treatment. We suggested that DDP might simultaneously induce some antiapoptotic protein after 24 h in addition to p53 and PUMA.

Notch-1 signaling plays a pivotal role in cell survival, in a manner that can deeply influence the final outcome in tumor development. Recently, Notch1 gene has been identified as a direct transcriptional target of p53 [29]. In primary human keratinocytes, knock-down of p53 results in down-modulation of Notch1 expression [30]. Conversely, increased p53 levels leads to Notch1 up-regulation in normal keratinocytes and, to a substantial greater extent, in SCC cells [31]. In the present study, we found that Notch-1 was induced at 48 h after DDP treatment, and reached the peak at 72 h. knockdown of p53 expression in SKOV3 cells reduced the expression levels of Notch1 protein in response to DDP treatment. In addition, knockdown of Notch-1 increased susceptibility to apoptosis induced by DDP treatment of cells. These observations provide support for the idea that p53-mediated upregulation of Notch1 expression in SKOV3 cells counteracts p53-mediated proapoptotic functions.

It has recently found that activation of Notch-1 signalling decreased pro-apoptotic PUMA expression and resulted in resistance to p53-dependent apoptosis induced by DNA damage [24]. In the present study, Notch-1 was significantly increased and PUMA was significantly decreased at 72 h after DDP treatment. Knockdown of Notch-1 expression in SKOV3 cells enhanced the expression levels of PUMA protein in response to DDP treatment. In addition, knockdown of PUMA decreased susceptibility to apoptosis of Notch-1 silenced SKOV3 cells. Based on our observation and accumulating evidence, we could concluded that Notch1 gene is atranscriptional target of p53, and PUMA is atranscriptional target of Notch-1. Activation of Notch1 expression inhibited PUMA activation, and decreased p53-dependent apoptosis.

We further observed that Notch-1 was overexpressed in the SKOV3^{DDP} cells. Knockdown of Notch-1 restored the susceptibility of SKOV3^{DDP} cells to DDP treatment via PUMA upregulation. In addition, p53 was also overexpressed in the SKOV3^{DDP} cells. Knockdown of p53 decreased Notch-1 expression and increased the PUMA expression, and restored the susceptibility of SKOV3^{DDP} cells to DDP treatment. We therefore suggested that inhibition of Notch1 function could reverse cisplatin chemosensitivity in ovarian cancer cells.

In the present study, we found that early chemosensitivity of ovarian cancer cells to cisplatin is associated with p53-dependent PUMA upregulation. Later chemoresistance of ovarian cancer cells to cisplatin is associated with

p53-dependent Notch-1 activation, resulting in PUMA inactivation and counteracting p53-mediated proapoptotic functions. Overexpression of Notch-1 is associated with chemoresistance of ovarian cancer cells to cisplatin. Thus, inhibition of Notch1 function reverses cisplatin chemosensitivity in ovarian cancer cells, which provides a logical approach for effective cancer therapy.

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Disclosure of conflict of interest

None.

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