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Original Article

Sam68 overexpression desensitizes of ovarian cancer cells to cisplatin via promoting Cyclin D1b

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Abstract: Multiple oncoproteins have been identified to correlate with the chemoresistance in epithelial ovarian cancer (EOC). Src-associated in mitosis (Sam68) is a signaling marker in the transduction and activation of RNA family, and is also indicated to be oncogenic in multiple types of malignancies. However, it has not been recognized to contribute to the progression, recurrence and chemoresistance of EOC. This study was designed to investigate the expression of Sam68 in EOC OVCAR-3 and SK-OV-3 cells post the treatment with Cisplatin or Paclitacxel. And then we overexpressed Sam68 in SK-OV-3 cells to re-evaluate the cell sensitivity to Cisplatin via the colony forming assay, CCK-8 assay and apoptosis analysis. In addition, the expression of Cyclin D1a and D1b was examined in the abovementioned SK-OV-3 cells. Results demonstrated that Sam68 was downregulated by the treatment with Cisplatin or Paclitacxel in both cells. However, the Cisplatin-caused colony reduction and the growth reduction were markedly ameliorated by the Sam68 overexpression. Moreover, the Sam68 overexpression inhibits the cisplatin-promoted cellular viability reduction and apoptosis induction in SK-OV-3 cells. In addition, the downregulation of Cyclin D1b in the Cisplatin-treated EOC cells was also significantly blocked by the Sam68 overexpression. In conclusion, this study found the downregulation of Sam68 by Cisplatin treatment, and the Sam68 overexpression desensitizes ovarian cancer cells to cisplatin probably via promoting Cyclin D1b. It implies that Sam68 might be an effective target to optimize the chemosensitivity of EOC.

Keywords: Sam68, ovarian cancers, cisplatin-resistance, cyclin D1b

Introduction

Epithelial ovarian cancer (EOC) is the most deadly gynecological malignancy for women [1], usually being diagnosed at an advanced stage when the prognosis is poor. And the median overall survival of EOC patients was only 2 to 4 year long, under current therapeutic approaches [2]. The poor prognosis of EOC is mainly caused by the progressive chemoresistance, particularly platinum resistance [3, 4]. Even the novel combinations [5] of the rare kinds of agents [6, 7], do not improve the progression-free survival (PFS) or overall survival (OS) of EOC patients [8]. And multiple oncoproteins have been identified to correlate with such chemoresistance in EOC. Overexpressed DJ-1 implicates a poor prognosis in ovarian cancer and predicts the earlier development of platinum resistance [3], probably via inhibiting apoptotic pathways [9]. The serum Annexin A3 level has also been recognized to be a potential predictor of platinum resistance in EOC patients [10]. In addition, microRNAs such as miRNA-1307 are upregulated in the chemoresistant EOC tissues, independent of menopause, tumor differentiation state, clinical stage, and lymph node metastasis of EOC patients [11]. Therefore, the identification of chemoresistance-associated markers would be clinically useful.

Src-associated in mitosis (Sam68) is a signal transduction and activation of RNA family, with a KH RNA-binding domain [12]. Sam68 has been recognized to involve in such cellular processes as RNA metabolism, cell cycle progression and apoptosis [12-14]. Moreover, Sam68 is also indicated to be oncogenic in multiple types of malignancies such as breast cancer [15], lung cancer [16], cervical cancer [17], glio-

blastoma [13] and prostate cancer [18]. Sam68 promotes the conversion of G1/S or G2/M phase [14, 15, 19], by modulating cell cycle- or apoptosis-associated markers such as Cyclin D1 and Bcl-xl [20, 21]. Moreover, Sam68 could promote esophageal squamous cell carcinoma cell proliferation via the activation of Akt/GSK-3β pathway [22]. However, there has not been recognition about the oncogenic role of Sam68 in EOC, particularly in the chemoresistance of EOC.

In the present study, we have investigated the expression of Sam68 in EOC cell lines, OVCAR-3 and SK-OV-3 cells, subject to Cisplatin or Paclitacxel. Then we upregulated the Sam68 expression in SK-OV-3 cells, and examined the influence of the upregulated Sam68 on the sensitivity of SK-OV-3 cells to Cisplatin *in vitro*. Our study recognized the desensitizing effect of Sam68 in EOC cells to Cisplatin.

Materials and methods

Cell lines, cell culture and cell treatment

The human EOC cell line OVCAR-3 was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). And the OVCAR-3 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Carlsbad, CA, USA), and were supplemented with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) and 1% streptomycin/penicillin (Sigma-Aldrich, St. Louis, MO, USA) in an incubator with 5% CO_2 under 37°C. The medium was changed at alternate days and cells were split before they reached more than 90% confluence. The SK-OV-3 EOC cell line was provided by the cell resource center of Chinese academy of medical sciences (Beijing, China) and was grown in McCoy's 5A medium (Hyclone, Pittsburgh, PA, USA) supplemented with 10% FBS and with 1% streptomycin/penicillin. The cells were also incubated at 37°C, with 5% CO_2. Cisplatin was purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA), was dissolved in DMEM or McCoy's 5A medium (Invitrogen, Carlsbad, CA, USA) with a concentration of 1 mM and was stored at -80°C before use. To overexpress Sam68 in EOC SK-OV-3 cells, the coding sequence of Sam68 or EGFP (as control) was amplified via PCR, were cloned intro the pcDNA3.1(+) vector (Invitrogen, Carlsbad, CA, USA) and were confirmed via sequencing. Then the SK-OV-3 cells with more than 85% confluence were transfected with the recombinant Sam68-pcDNA3.1(+) or EGFP-pcDNA3.1(+) with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

RNA isolation and quantitative real-time PCR (qRT-PCR)

Cellular RNA was extracted from OVCAR-3 or SK-OV-3 cells with TRIzol reagent (Life Technologies, Grand Island, NY, USA) according to the product's protocol. Reverse transcription PCR was performed using a PrimeScript RT-PCR kit (TaKaRa, Dalian, China). And the qRT-PCR was performed on the Applied Biosystems 7900HT machine (Applied Biosystems, Carlsbad, CA, USA). And the following primers were used for the qRT-PCR analysis of Sam68 and β-actin. Forward primer for Sam68: 5'-cct gcc cga act cat ggc cg-3', and reverse primer for Sam68: 5'-caa ttt ctg ccg tca gca gc-3'. Forward primer for β-actin: 5'-cgg gac ctg act gac tac ct-3', and reverse primer for β-actin: 5'-gct cgg ccg tgg tgg tga ag-3'. Forward primer for Cyclin D1a or D1b (common for D1a and D1b): 5'-cca gag tga tca agt gtg ac-3', reverse primer for Cyclin D1a: 5'-caa gga gaa tga agc ttt ccc tt-3', reverse primer for Cyclin D1b: 5'-ggg aca tca ccc tca ctt ac-3'. β-actin was used as the internal control.

Western blotting assay

Cellular protein samples were extracted from OVCAR-3 or SK-OV-3 cells with Nuclear/Cytosol Fractionation Kit (BioVision, San Diego, CA, USA), were separated via subjecting to 10% SDS-PAGE electrophoresis and were transferred onto polyvinylidene fluoride hydrophobic membrane (Millipore, Bedford, MA, USA). Western blotting was performed by using rabbit polyclone antibody against human Sam68 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), against human caspase 3 (Sigma-Aldrich, St. Louis, MO, USA), against Poly (ADP-ribose) polymerase (PARP) (Abcam, Cambridge, UK), against Cyclin D1a or D1b (NeoMarkers, Fremont, CA, USA), or against β-actin antibody (Sigma-Aldrich, St. Louis, MO, USA). Horseradish peroxidase-linked goat anti-rabbit IgG (Pierce, Rockford, IL, USA) and the Enhanced chemiluminescence (Thermo Scientific, Rockford, IL, USA) were utilized to Sam68 level in cancer tissues or OVCAR-3 cells.

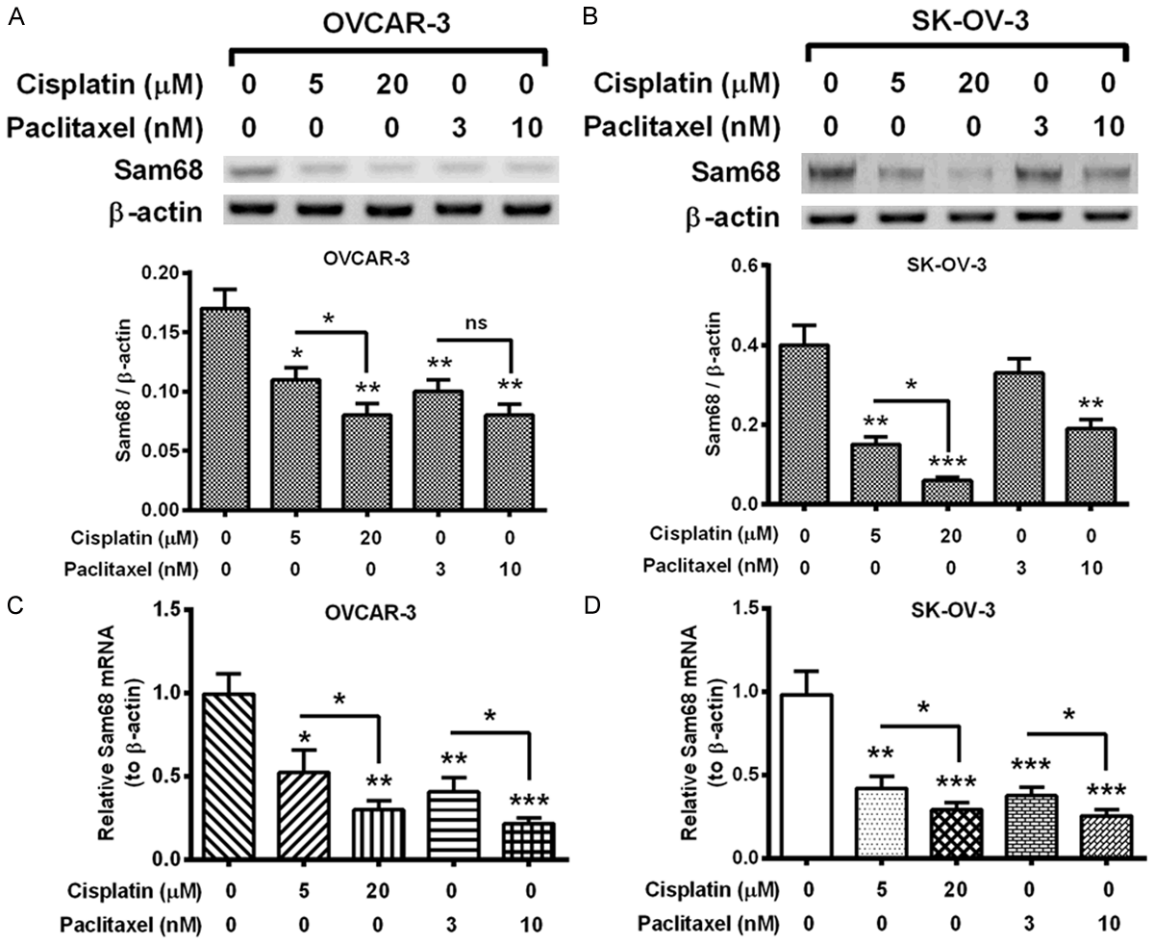

Figure 1. mRNA and protein levels of Sam68 in the EOC cells subject to the treatment with Cisplatin or Paclitaxel. A and B: Western blot analysis of Sam68 in the OVCAR-3 and SK-OV-3 cells, which were treated with 5 or 20 μM Cisplatin or with 3 or 10 nM Paclitaxel for 24 hours, with β-actin as internal control; C and D: Relative mRNA level of Sam68 to β-actin in the OVCAR-3 and SK-OV-3 cells, which were treated with 5 or 20 μM Cisplatin or with 3 or 10 nM Paclitaxel for 24 hours. All experiments were independently performed in triplicate. And statistical significance was showed as *P < 0.05, **P < 0.01, ***P < 0.001.

Colony forming assay and CCK-8 assay

To assay the influence of Cisplatin treatment on the proliferation of SK-OV-3 (Sam68 Up) or SK-OV-3 (Ctrl Up), the colony formation assay and the CCK-8 assay were performed. For cell colony formation assay, 600 SK-OV-3 cells were incubated in 12-well plates, and then were treated with 20 μM Cisplatin and were inoculated for 2 days; the cells were stained with crystal violet (0.005%) for 20 minutes and recorded the colony numbers by imaging J software. For CCK-8 assay, 85% confluent SK-OV-3 cells were treated 20 μM Cisplatin for 12, 24 or 48 hours, and then were incubated in CCK-8 (DOJINDO, Kumamoto, Japan). The 450 nm absorbance of each cell well was detected after visual color occurrence.

Cell viability assay

Cellular viability of SK-OV-3 (Sam68 Up) or SK-OV-3 (Ctrl Up) cells post treatment was determined by MTT assay. The SK-OV-3 cells which seeded in 96-well plates, with 85% confluence, were treated with cisplatin for 24 or 48 hours. Then the medium was replaced with 50 μL 1× MTT solution for incubation for 2 hours at 37°C. The MTT solution was updated with 150 μL DMSO was added to dissolve the precipitate completely at room temperature. The optical density was then measured at 570 nm using a spectrophotometer. The cell viability was

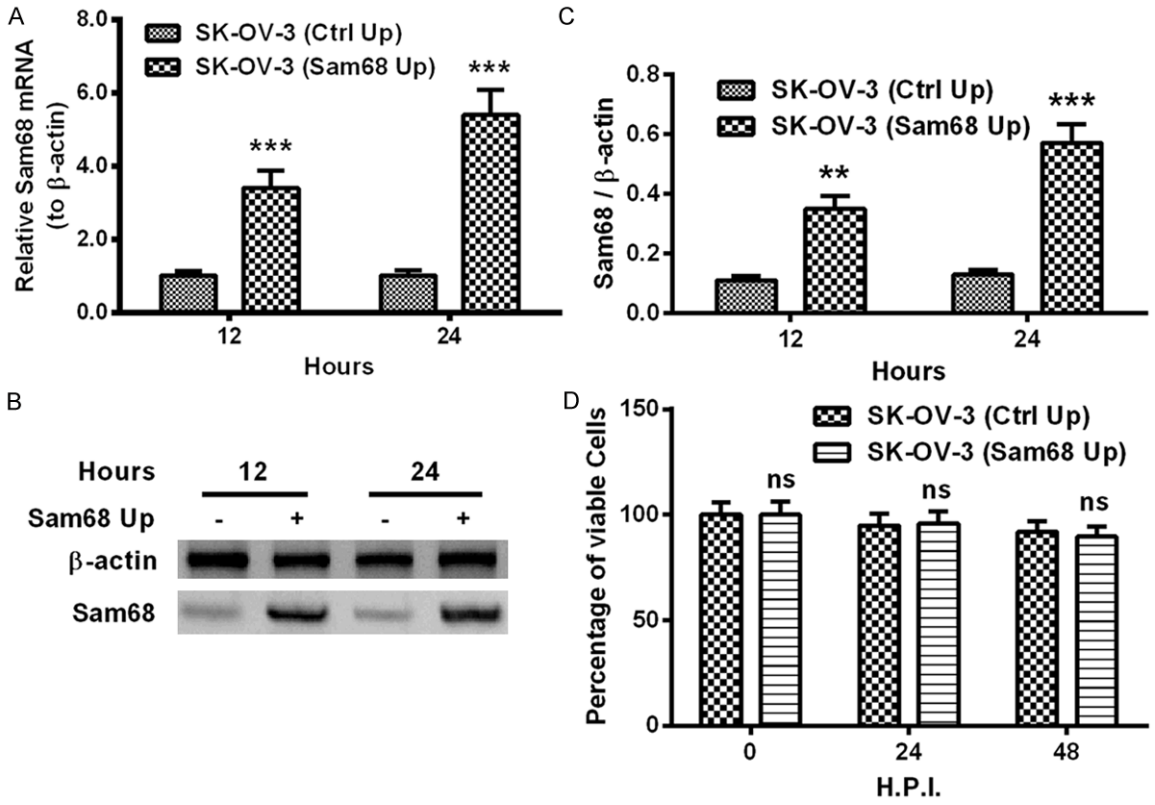

Figure 2. Construction of Sam68-overexpressed SK-OV-3 cells. (A) Quantitative analysis of Sam68 mRNA level in SK-OV-3 cells, which were transfected with Sam68-pcDNA3.1(+) or EGFP-pcDNA3.1(+) for 12 or 24 hours; (B and C) Western blot analysis (B) and relative protein level (C) of Sam68 in the SK-OV-3 cells, which were transfected with Sam68-pcDNA3.1(+) or EGFP-pcDNA3.1(+) for 12 or 24 hours; (D) MTT assay for the cellular viability of the Sam68-pcDNA3.1(+)- or EGFP-pcDNA3.1(+)-transfected SK-OV-3 cells at 0, 12 or 24 hour post inoculation. Each data was averaged for triple independent results, and the statistical significance was considered when $P < 0.05$ or less. **$P < 0.01$, ***$P < 0.001$, ns: no significance.

expressed as relative viable cells (%) to control SK-OV-3 cells.

Apoptosis analysis

Apoptosis of SK-OV-3, SK-OV-3 (Sam68 Up) or SK-OV-3 (Ctrl Up) cells post Cisplatin treatment was determined by flow cytometry using the Annexin V-FITC Apoptosis Detection Kit (Abcam, Cambridge, UK) according to the product's manual. In brief, SK-OV-3 cells were treated with 10 μM cisplatin for 24 or 48 hours and then were harvested and resuspended in binding buffer. Cells were mixed with annexin VFITC and PI for an incubating for 15 min in the dark. The apoptotic cells were assayed with a flow cytometry.

Statistical evaluations

Results were presented as mean ± SE. the analysis of the relative mRNA or protein level, of the colony number, cellular viability or of the apoptotic cell number was analyzed using the Student's t test.

Results

Downregulation of Sam68 by Cisplatin and Paclitacxel in EOC cells

To investigate the possible role of Sam68 in the chemosensitivity of EOC cells, we treated EOC OVCAR-3 and SK-OV-3 cells with Cisplatin or Paclitacxel, and then examined the Sam68 expression in the Cisplatin- or Paclitacxel-treated OVCAR-3 and SK-OV-3 cells. Western blotting assay demonstrated that the Sam68 expression was significantly downregulated in protein level in the OVCAR-3 cells, which were treated with 5 or 20 μM Cisplatin ($P < 0.05$ for 5 μM or $P < 0.01$ for 20 μM, **Figure 1A**), dose-dependently ($P < 0.05$ for 5 vs 20 μM). And the

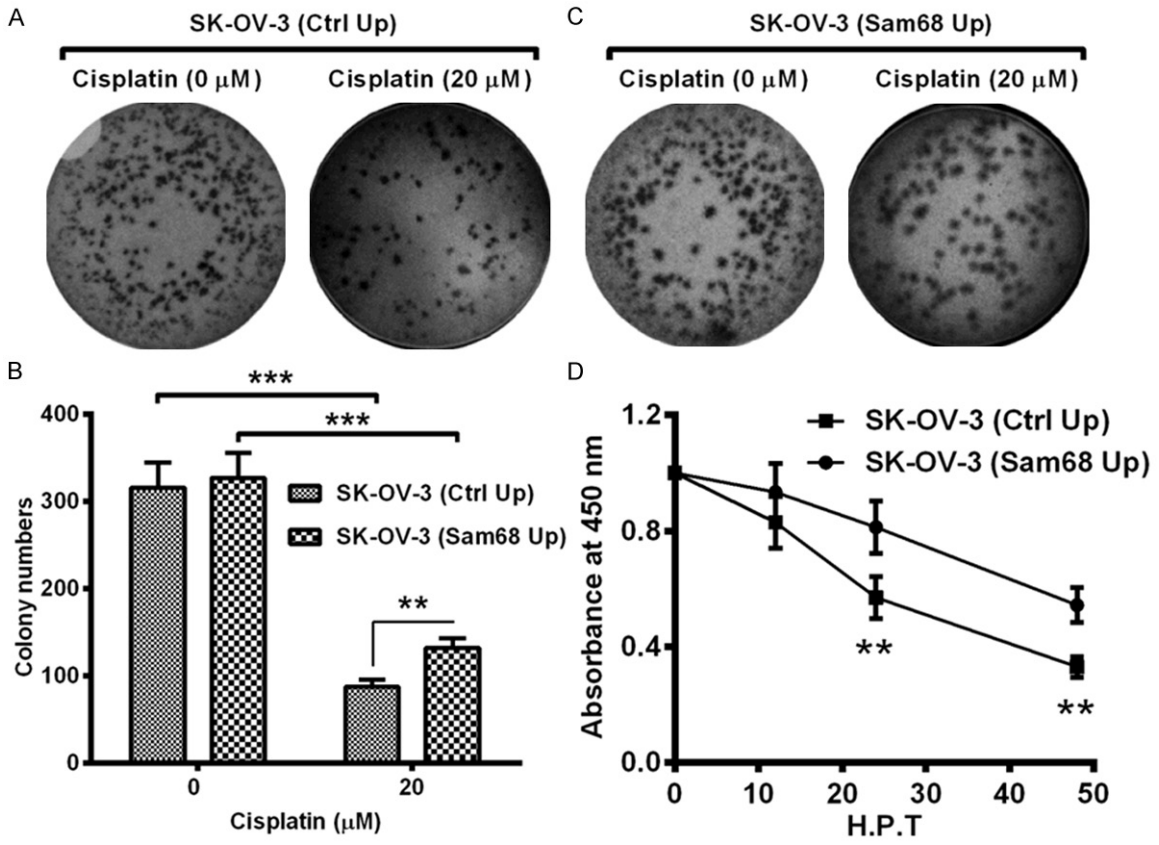

Figure 3. Colony forming by SK-OV-3 cells, with or without Sam68 overexpression. A: Representative images of the colony forming in the SK-OV-3 cells, which were transfected with EGFP-pcDNA3.1(+) (Ctrl Up) and were treated with 0 or 20 μM Cisplatin; B: Counting of colonies which were formed by the SK-OV-3 (Ctrl Up) cells or by the Sam68-pcDNA3.1(+)-transfected SK-OV-3 (Sam68 Up) cells, treated with 0 or 20 μM Cisplatin; C: Representative images of the colony forming in the SK-OV-3 (Sam68 Up) cells, which were treated with 0 or 20 μM Cisplatin; D: CCK-8 assay of the SK-OV-3 (Ctrl Up) or SK-OV-3 (Sam68 Up) cells, post the treatment with 20 μM for 12, 24 or 48 hours. All experiments were independently performed in triplicate. And statistical significance was showed as $^{**}P < 0.01$, $^{***}P < 0.001$.

Paclitacxel treatment with 3 or 10 nM also markedly downregulated the Sam68 level in OVCAR-3 cells ($P < 0.01$ for either 3 or 10 nM, **Figure 1A**). We then repeated such examination of the chemotherapy-reduced Sam68 in SK-OV-3 cells. It was indicated in **Figure 1B** that both agents also downregulated the Sam68 level in SK-OV-3 cells ($P < 0.01$ for 5 μM or $P < 0.001$ for 20 μM in the Cisplatin-treated cells; $P < 0.05$ for 3 nM or $P < 0.01$ for 10 nM in the Paclitacxel-treated cells).

In addition, we also examined the mRNA level of Sam68 in the OVCAR-3 or SK-OV-3 cells, which were treated with Cisplatin or Paclitacxel. As shown in **Figure 1C** and **1D** that the Sam68 mRNA level was also significantly downregulated by either agent in OVCAR-3 or SK-OV-3 cells ($P < 0.05$, $P < 0.01$ or $P < 0.001$), also with a dose-dependence ($P < 0.05$). Taken together, we confirmed the downregulation by the treatment with Cisplatin or Paclitacxel in EOC OVCAR-3 or SK-OV-3 cells.

Manual upregulation of Sam68 in SK-OV-3 cells

To investigate the role of Sam68 in the chemotherapy and the chemo-sensitivity of EOC cells, we manipulated the Sam68 expression in SK-OV-3 cells with a eukaryotic expression vector. The coding sequence of Sam68 or EGFP (as control) was amplified via PCR, were cloned intro the pcDNA3.1(+) vector and were confirmed via sequencing. Then the recombinant Sam68-pcDNA3.1(+) or EGFP-pcDNA3.1(+) was transfected into SK-OV-3 cells. It was demonstrated in **Figure 2A** that the Sam68 mRNA level was significantly upregulated in the SK-OV-3 cells, 12 or 24 hours post the Sam68-

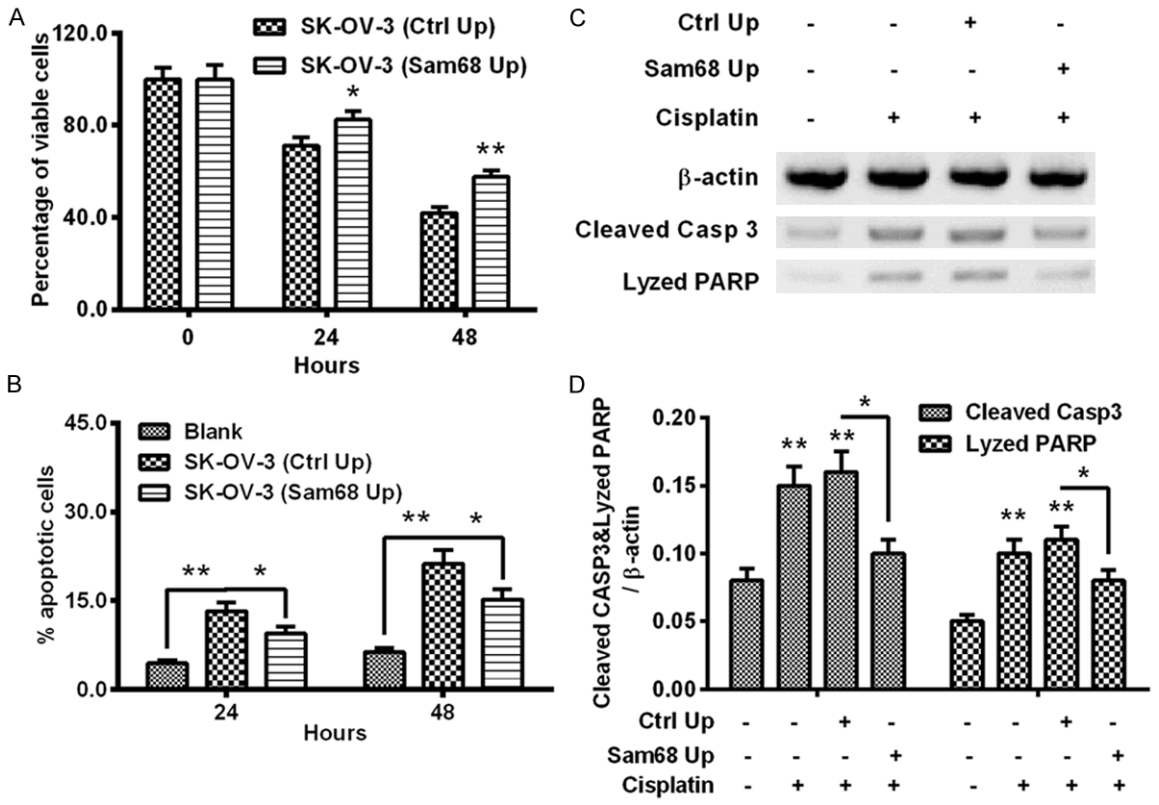

Figure 4. Apoptosis induction and the expression of apoptosis-associated markers in the cisplatin-treated SK-OV-3 (Sam68 Up) cells. A: MTT assay of SK-OV-3 (Ctrl Up) or SK-OV-3 (Sam68 Up) cells, which were treated with 20 μM Cisplatin for 0, 24 or 48 hours; B: Apoptosis induction in the blank SK-OV-3, SK-OV-3 (Ctrl Up) or SK-OV-3 (Sam68 Up) cells, either of which were treated with 20 μM cisplatin for 24 or 48 hours. C: Western blot analysis of cleaved caspase 3 (Cleaved Casp 3, active form of caspase 3) and lysed PARP, which were lysed by the active caspase 3; D: Relative level of Cleaved Casp 3 and lysed PARP to β-actin in the Cisplatin-treated SK-OV-3 (Ctrl Up) or SK-OV-3 (Sam68 Up) cells. All experiments were performed in triplicate. H. P. T.: Hour post treatment. Statistically significant was showed as ns: no significance, *$P < 0.05$ or **$P < 0.01$.

pcDNA3.1(+) transfection (Sam68 Up) ($P < 0.001$ respectively), compared to the EGFP-pcDNA3.1(+) transfection (Ctrl Up). And the western blotting assay also indicated that the Sam68 in protein level was also markedly upregulated in "Sam68 Up" SK-OV-3 cells (**Figure 2B** and **2C**) ($P < 0.01$ for 12 and $P < 0.001$ for 24 hours post transfection). In addition, we examined the influence of the Sam68 overexpression on the viability of SK-OV-3 cells. It was indicated in **Figure 2D** that there was no significant difference in the cellular viability between the "Sam68 Up" and "Ctrl Up" SK-OV-3 cells.

Sam68 overexpression inhibits the cisplatin-induced growth reduction of SK-OV-3 cells

To examine the regulation of the overexpressed Sam68 on the cytotoxicity of Cisplatin in SK-OV-3 cells, we evaluated the proliferation of the two groups ("Sam68 Up" and "Ctrl Up") SK-OV-3 cells, in the presence of Cisplatin. The colony forming assay indicated that the treatment with 20 μM Cisplatin significantly reduced the colony number in both groups of SK-OV-3 cells ($P < 0.001$ respectively, **Figure 3A** and Column 3 vs Column 1 in **Figure 3B** for "Ctrl Up" SK-OV-3 cells, **Figure 3C** and Column 4 vs Column 2 in **Figure 3B** for "Sam68 Up" SK-OV-3 cells). However, such Cisplatin-caused colony reduction was markedly less in the group of "Sam68 Up" SK-OV-3 cells; more colonies were observed in the group of "Sam68 Up" SK-OV-3 cells ($P < 0.05$, Column 4 vs Column 3 in **Figure 3B**). In addition, CCK-8 assay was performed to observe the inhibitory action of Cisplatin on "Sam68 Up" and "Ctrl Up" SK-OV-3 cells. It was indicated in **Figure 3D** that there was marked difference in the growth curve between the

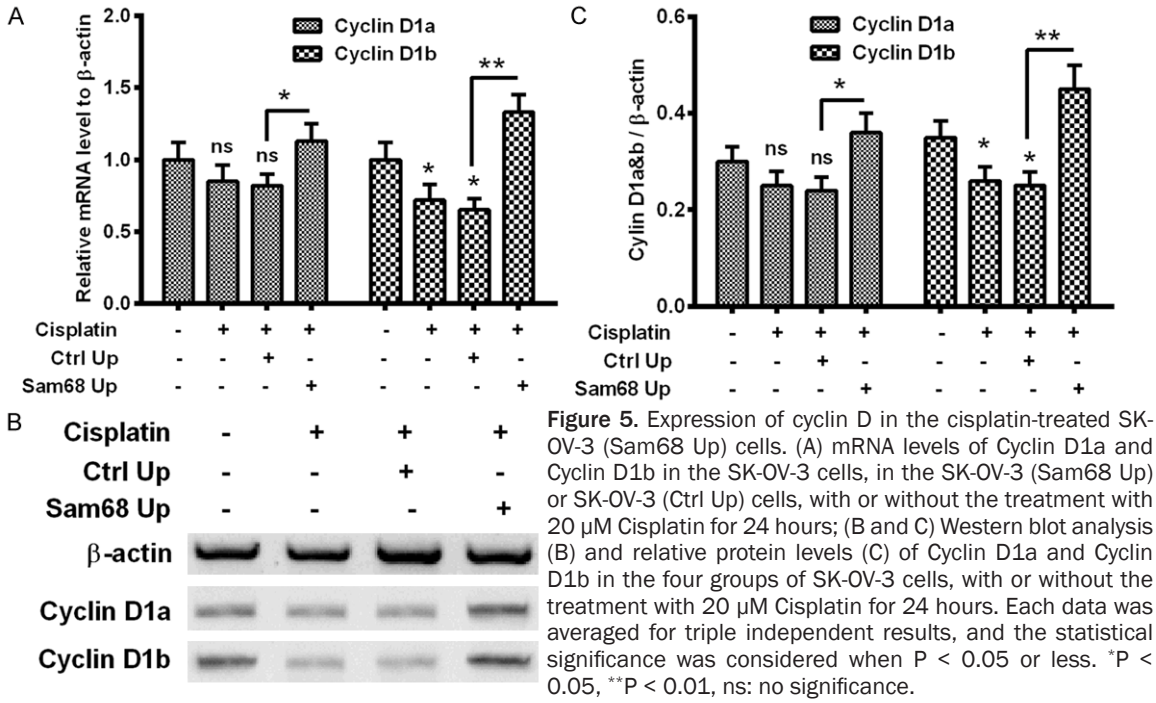

Figure 5. Expression of cyclin D in the cisplatin-treated SK-OV-3 (Sam68 Up) cells. (A) mRNA levels of Cyclin D1a and Cyclin D1b in the SK-OV-3 cells, in the SK-OV-3 (Sam68 Up) or SK-OV-3 (Ctrl Up) cells, with or without the treatment with 20 μM Cisplatin for 24 hours; (B and C) Western blot analysis (B) and relative protein levels (C) of Cyclin D1a and Cyclin D1b in the four groups of SK-OV-3 cells, with or without the treatment with 20 μM Cisplatin for 24 hours. Each data was averaged for triple independent results, and the statistical significance was considered when $P < 0.05$ or less. $^{*}P < 0.05$, $^{**}P < 0.01$, ns: no significance.

"Sam68 Up" and "Ctrl Up" SK-OV-3 cells, the inhibition by Cisplatin treatment on the growth of SK-OV-3 cells was more effective in the "Sam68 Up" group than in the "Ctrl Up" group ($P < 0.01$ respectively at 24 or 48 hours post treatment (H.P.T.).

Sam68 overexpression reduces the cytotoxicity of cisplatin in SK-OV-3 cells

To further investigate the regulatory role of Sam68 on the cytotoxicity of cisplatin in SK-OV-3 cells, we measured the viability and the apoptosis levels in the cisplatin-treated "Sam68 Up" or "Ctrl Up" SK-OV-3 cells. The MTT assay result demonstrated that the Cisplatin treatment with 20 μM induced significant cellular viability reduction of both groups of SK-OV-3 cells. However, the cellular viability reduction was markedly less in the "Sam68 Up" group than in the "Ctrl Up" group ($P < 0.05$ for 24 H.P.T. and $P < 0.01$ for 48 H.P.T.). Moreover, the cisplatin-induced apoptosis of SK-OV-3 cells was also markedly inhibited by the Sam68 overexpression ($P < 0.05$ for 24 or 48 H.P.T, **Figure 4B**). In addition, the western blot analysis confirmed the inhibition by Sam68 overexpression on the Cisplatin-induced apoptosis-associated markers, such as cleaved caspase 3 and lyzed PARP (by the cleaved caspase 3) ($P < 0.05$ for 24 or 48 H.P.T, **Figure 4C** and **4D**). Therefore, Sam68 overexpression reduced the cytotoxicity of Cisplatin in SK-OV-3 cells.

Sam68 overexpression upregulates the expression of cyclin D in the cisplatin-treated SK-OV-3 cells

It has been indicated that Sam68 expression positively correlates with levels of Cyclin D1b, but not D1a, in human prostate carcinomas [23, 24]. And the overexpressed Sam68 binds to Cyclin D1 mRNA and directly affects alternative splicing of Cyclin D1 mRNA to increase the level of Cyclin D1b [25]. In order to explore the possible mechanism underlining the regulation by Sam68 in the chemosensitivity to Cisplatin in SK-OV-3 cells, we determined the expression of both Cyclin D1a and Cyclin D1b in SK-OV-3 (Sam68 Up) cells, with or without Cisplatin treatment. **Figure 5A** demonstrated that the treatment with 20 μM Cisplatin did not markedly regulated the mRNA of Cyclin D1a mRNA in SK-OV-3 or SK-OV-3 (Ctrl Up) cells, though the Cyclin D1a mRNA was significantly higher in SK-OV-3 (Ctrl Up) than in the SK-OV-3 (Sam68 Up) group ($P < 0.05$, Column 4 vs Column 1 in **Figure 5A**), whereas the Cyclin D1b mRNA was significantly downregulated by the Cisplatin treatment in both types of cells ($P < 0.05$ respectively for Column 6 or 7 vs Column 5 in **Figure 5A**). And such downregulation was also

reversed by the Sam68 overexpression, the Cyclin D1b mRNA was markedly lower in the Cisplatin-treated SK-OV-3 (Ctrl Up) cells than in the Cisplatin-treated SK-OV-3 (Ctrl Up) ($P < 0.01$, Column 8 vs Column 7 in **Figure 5A**). Then we analyzed the expression of Cyclin D1a and Cyclin D1b in protein levels by western blotting assay. **Figure 5B** indicated that the downregulation of Cyclin D1a in protein level was neither significant in the Cisplatin-treated SK-OV-3 cells, whereas the Cyclin D1b in protein level was significantly downregulated by the Cisplatin treatment in the blank SK-OV-3 or SK-OV-3 (Ctrl Up) cells ($P < 0.05$ respectively for Column 6 or 7 vs Column 5 in **Figure 5C**). Moreover, the there was a significant upregulation of either Cyclin D1a or Cyclin D1b by the Sam68 overexpression ($P < 0.05$ for Cyclin D1a, Column 4 vs Column 3, $P < 0.01$ for Cyclin D1b, Column 8 vs Column 7, in **Figure 5C**). Taken together, the Sam68 overexpression markedly promoted the Cyclin D1, particular Cyclin D1b in ovarian cancer SK-OV-3 cells.

Discussion

Given the low response to chemotherapy of EOC, it is urgent to identify novel biomarkers for therapeutic response and molecular targets to increase sensitivity to chemotherapy. In the present study, we found in the Cisplatin- or Paclitaxel-treated EOC OVCAR-3 and SK-OV-3 cells, the downregulation of Sam68, which is an oncogenic marker in multiple malignancies [15-17], via by modulating cell cycle or apoptosis [14, 15, 19, 20]. Both mRNA and protein levels of Sam68 were markedly downregulated in either OVCAR-3 and SK-OV-3 cells by the Cisplatin treatment. And followed results demonstrated that the overexpression of Sam68 inhibits the Cisplatin-induced growth reduction of SK-OV-3 cells. Both the colony forming assay and the CCK-8 assay found that the Cisplatin-caused colony reduction and the growth reduction were markedly ameliorated by the Sam68 overexpression. Moreover, the Sam68 overexpression reduces the cytotoxicity of cisplatin in SK-OV-3 cells. The cellular viability and the apoptosis levels in the cisplatin-treated "Sam68 Up" or "Ctrl Up" SK-OV-3 cells were significantly different. The cellular viability reduction was markedly less in the "Sam68 Up" SK-OV-3 cells; the Cisplatin-induced apoptosis and the Cisplatin-upregulated apoptosis-associated markers, such as cleaved caspase 3 and lysed PARP, were also markedly reduced in the "Sam68 Up" SK-OV-3 cells.

Cyclin D1 was identified as independent predictors of progression-free survival (PFS), overall survival (OS) and chemosensitivity of EOC, and to predict the poor prognosis of advanced serous EOC [26]. Overexpression of cyclin D1 was significantly associated with first-line chemosensitivity of EOC cells [26]. And various anti-tumor agents or tumor suppressors sensitized EOC cells to Cisplatin. miR-211 suppresses epithelial ovarian cancer proliferation and cell-cycle progression by targeting Cyclin D1 [27]. Sohlh2 inhibits ovarian cancer cell proliferation by upregulation of p21 and downregulation of cyclin D1 [28]. OVCA1 inhibits the proliferation of epithelial ovarian cancer cells by decreasing cyclin D1 [29]. Therefore, Cyclin D1 is one of key targets for the chemoresistance of EOC and other types of cancers.

Cyclin D1b, as an isoform of Cyclin D1, has been proposed to have higher oncogenic potential than cyclin D1a [30-32]. Aberrant Cyclin D1b expression has been confirmed to confer resistance to therapeutic treatment breast cancer [33], and is associated with poor prognosis in patients [34]. The deregulation of Cyclin D1b had also been confirmed in various other cancer types [35, 36], and was also shown to enhance cell invasiveness and growth of bladder cancer cells [37]. In particular, the change in the Cyclin D1b/cyclin D1a ratio is marked in prostate carcinomas [23, 24]. And the splicing regulator Sam68 modulates the ratio of cyclin D1 isoform expression, in favor of Cyclin D1b in prostate carcinomas [20], with a positive correlation between Sam68 and Cyclin D1b expression in prostate carcinoma patients. In the present study, we confirmed the downregulation of Cyclin D1b in the Cisplatin-treated EOC cells in both mRNA and protein levels. However, such downregulation of Cyclin D1b was significantly blocked by the Sam68 overexpression in EOC SK-OV-3 cells. Therefore, we speculated that Cyclin D1b might mediate the desensitization by Sam68 of EOC cells to Cisplatin.

In conclusion, the present study found the downregulation of Sam68 by Cisplatin treatment, and the Sam68 overexpression desensitizes ovarian cancer cells to cisplatin probably via promoting Cyclin D1b. It implies that Sam68

might be an effective target to optimize the chemosensitivity of EOC.

Disclosure of conflict of interest

None.

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