



# Recombinant adenovirus-mediated overexpression of PTEN and KRT10 improves cisplatin resistance of ovarian cancer *in vitro* and *in vivo*

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**ABSTRACT.** Drug resistance is a major cause of treatment failure in ovarian cancer patients, and novel therapeutic strategies are urgently needed. Overexpression of phosphatase and tensin homolog (PTEN) has been shown to preserve the cisplatin-resistance of ovarian cancer cells, while cisplatin-induced keratin 10 (KRT10) overexpression mediates the resistance-reversing effect of PTEN. However, whether overexpression of PTEN or KRT10 can improve the cisplatin resistance of ovarian cancer *in vivo* has not been investigated. Therefore, we investigated the effects of adenovirus-mediated PTEN or KRT10 overexpression on the cisplatin resistance of ovarian cancer *in vivo*. Recombinant adenoviruses carrying the gene for PTEN or KRT10 were constructed. The effects of overexpression of PTEN and KRT10 on cisplatin resistance of ovarian cancer cells were examined using the 3(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) and TdT-mediated dUTP nick-end labeling (TUNEL) assays *in vitro*. Subcutaneously transplanted

nude mice, as a model of human ovarian cancer, were used to test the effects of PTEN and KRT10 on cisplatin resistance of ovarian cancer *in vivo*. The MTT assay showed that recombinant adenovirus-mediated overexpression of KRT10 and PTEN enhanced the proliferation inhibition effect of cisplatin on C13K cells. Recombinant adenovirus-mediated overexpression of KRT10 and PTEN also increased the cisplatin-induced apoptosis rate of C13K cells. Furthermore, recombinant adenovirus-mediated overexpression of KRT10 and PTEN enhanced the inhibitory effect of cisplatin on C13K xenograft tumor growth. Thus, recombinant adenovirus-mediated overexpression of KRT10 and PTEN may improve the cisplatin resistance of ovarian cancer *in vitro* and *in vivo*.

**Key words:** Cisplatin; Cisplatin-induced keratin 10; Ovarian cancer; Multi-Drug Resistance; Phosphatase and tensin homolog

## INTRODUCTION

Ovarian cancer, one of the most common gynecologic malignancies, has the highest mortality rate among all gynecologic malignancies. Currently, surgery and platinum-based chemotherapy are the primary methods for treating ovarian cancer. Although 75-80% of ovarian cancer patients respond to chemotherapy initially, more than 80% of patients who undergo chemotherapy display drug resistance, and even multi-drug resistance (MDR), ultimately leading to poor prognosis. This suggests that MDR is a common cause of chemotherapy failure in ovarian cancer (Stordal et al., 2007). Therefore, developing targeted treatment methods for reversing MDR is very important for treating ovarian cancer.

Phosphatase and tensin homolog (PTEN), a tumor suppressor gene, plays a crucial role in cell apoptosis, cell cycle arrest, and cell migration (Li et al., 1997; Dahia, 2000). Chemotherapy drugs, such as cisplatin, can induce the apoptosis of ovarian cancer cells by enhancing PTEN activity (Yan et al., 2006; Wan et al., 2007; Li et al., 2011; Singh et al., 2013). These studies indicate that overexpression of PTEN improves the MDR of ovarian cancer (Steelman et al., 2008; Wu et al., 2008; Singh et al., 2013). In our previous study, we showed that overexpression of PTEN reversed cisplatin resistance in the ovarian cancer cell line C13K (Wu et al., 2008). Further examination demonstrated that cisplatin-induced keratin 10 (KRT10) overexpression in C13K/PTEN ovarian cancer cells mediated the resistance-reversing effect of PTEN. However, whether overexpression of PTEN or KRT10 also improves the cisplatin resistance of ovarian cancer *in vivo* has not been investigated. Therefore, in the present study, we first constructed recombinant adenoviruses carrying *PTEN* or *KRT10* genes and then investigated their effects on the cisplatin resistance of ovarian cancer *in vivo*.

## MATERIAL AND METHODS

### Cell culture

The cisplatin-resistant ovarian cancer cell line C13K was cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 U/mL strep-

tomycin at 37°C, 5% CO<sub>2</sub>, and 95% humidity. The three-dimensional (3D) ovarian cancer culture model was prepared according to a previous study (Smith et al., 2011).

### Construction of recombinant adenoviruses

*PTEN* and *KRT10* genes were inserted into the pacAd5-CMV-IRES-GFP vector to prepare shuttle vectors. HEK293 cells (2 x 10<sup>6</sup> cells/well) were seeded in 6-cm dishes in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS and incubated at 37°C with 5% CO<sub>2</sub> overnight. The medium was replaced before transfection. The cells were transfected at 80-90% confluence with pacAd5-9.2-100 and shuttle plasmids pacAd5-CMV-IRES-GFP/*KRT10* or pacAd5-CMV-IRES-GFP/*PTEN* using Lipofectamine 2000 (Invitrogen; Carlsbad, CA, USA) according to manufacturer instructions. Twelve days post-transfection, recombinant adenoviruses carrying *PTEN* (AD-*PTEN*) and *KRT10* (AD-*KRT10*) genes were harvested. All recombinant adenoviruses were amplified in HEK293 cells and purified using double cesium chloride density gradient ultracentrifugation. Titers of the adenoviral stocks were determined using a plaque assay on 293 cells.

### 3(4,5-Dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) assay

Ovarian cancer cells were seeded into 96-well plates at a density of 1 x 10<sup>4</sup> cells/well in RPMI-1640 supplemented with 10% FBS. Cells were treated with or without the indicated concentrations of cisplatin as: 0, 2.5, 5, 7.5, 10, 12.5 and 15 µM. After 24 h, 10 µL 5 mg/mL MTT solution was added to each well. The plates were incubated at 37°C for 4 h; the supernatant was discarded and 100 µL dimethyl sulfoxide was added to each well and mixed thoroughly before reading on a microplate reader.

### TdT-mediated dUTP nick-end labeling (TUNEL) assay

After cisplatin treatment for 24 h, the cells were fixed with 4% paraformaldehyde and embedded with paraffin using standard methods. The TUNEL assay was performed according to fluorescein *in situ* cell death detection kit manufacture instructions (Roche, Basel, Switzerland). Nuclei were stained using 4'-diamidino-2-phenylindole. Four different fields from each section were analyzed. The number of TUNEL-positive cells was divided by the total number of ovarian cancer cells to determine the apoptosis rate.

### Establishment of a subcutaneously transplanted nude mice model of human ovarian cancer

C13K cells (4 x 10<sup>6</sup> cells/animal) were injected subcutaneously into nude mice to produce implanted tumors. Tumor volumes were measured using a slide caliper every 3 days according to the formula: volume = (larger diameter) x (smaller diameter)<sup>2</sup> / 2 (Liu et al., 2008). Two weeks later, AD-*KRT10*, AD-*PTEN*, and negative adenovirus were injected into the tumors. Twenty-four hours later, 5 mg/kg cisplatin was injected through the tail vein. Tumor volumes were measured using a slide caliper every 3 days until the 16th day after cisplatin administration.

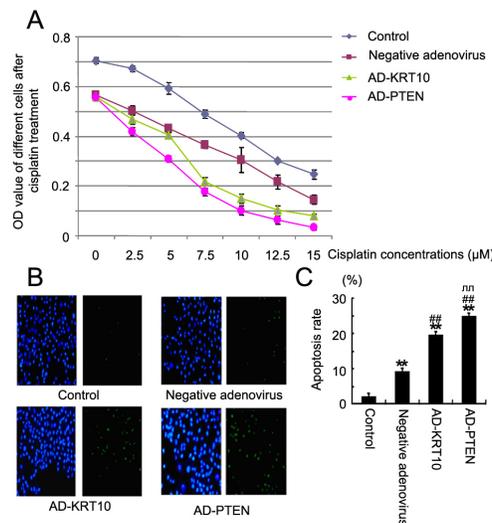
## Statistical analysis

All results are reported as means  $\pm$  standard deviation (SD). Statistical significance was determined using SPSS 17.0 for Windows (SPSS, Inc., Chicago, IL, USA). One-way analysis of variance was performed for multiple comparisons followed by Fisher's least-square deconvolution *post-hoc* comparisons. Differences were considered significant when  $P < 0.05$ .

## RESULTS

### Recombinant adenoviruses carrying *PTEN* or *KRT10* genes enhanced the chemosensitivity of C13K cells to cisplatin

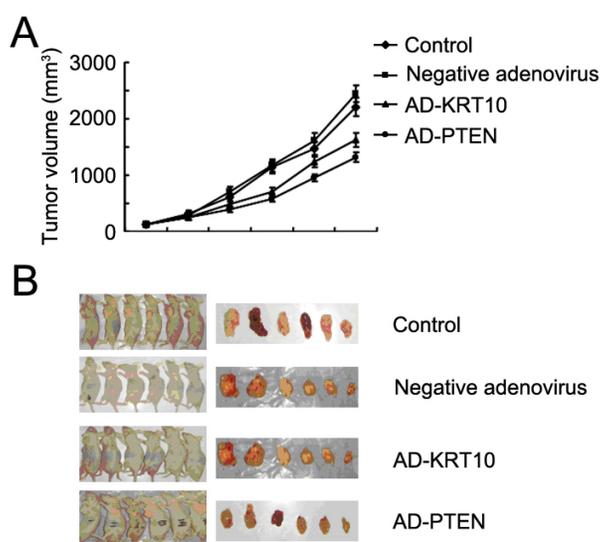
To investigate whether adenovirus expressing *PTEN* or *KRT10* could improve the chemosensitivity of ovarian cancer cells to cisplatin, C13K cells were infected with AD-*PTEN* or AD-*KRT10* and then seeded in a 3-D culture environment on a 96-well plate. After treatment with cisplatin for 48 h, the MTT assay was performed. As shown in Figure 1A, both AD-*PTEN* and AD-*KRT10* significantly enhanced the cytotoxicity of cisplatin toward C13K cells compared to control adenovirus-infected cells. AD-*PTEN*-infected C13K cells showed higher cisplatin sensitivity than AD-*KRT10*-infected C13K cells. Furthermore, the TUNEL assay showed that the apoptosis rates of AD-*PTEN*- and AD-*KRT10*-infected C13K cells were higher than that of control adenovirus-infected cells after cisplatin treatment (Figure 1B). A significant difference was also noted between AD-*PTEN*- and AD-*KRT10*-infected C13K cells (Figure 1C).



**Figure 1.** Recombinant adenovirus-mediated *KRT10* and *PTEN* overexpression enhanced the sensitivity of C13K cells to cisplatin. **A.** *KRT10* and *PTEN* overexpression enhanced the proliferation inhibitory effect of cisplatin on ovarian cancer cells. **B.** *KRT10* and *PTEN* overexpression enhanced cisplatin-induced apoptosis of C13K cells determined by TUNEL staining. **C.** Apoptosis rate of C13K cells induced by cisplatin. C13K cells were infected with AD-*KRT10*, AD-*PTEN*, or negative adenovirus, and these cells were seeded into 96- and 6-well plates in a 3-D microenvironment. The MTT or TUNEL assay was performed after cisplatin treatment for 48 or 24 h, respectively. Data are reported as means  $\pm$  SD ( $N = 3$ ). \*\* $P < 0.01$ , compared with control group. ## $P < 0.01$ , compared with negative adenovirus group. ## $P < 0.01$ , compared with AD-*KRT10* group.

### Recombinant adenoviruses carrying *PTEN* or *KRT10* genes improved the anti-ovarian cancer effect of cisplatin *in vivo*

To confirm the results obtained *in vitro*, we tested the *in vivo* effects of AD-PTEN and AD-KRT10. Tumor growth was slower in the AD-PTEN and AD-KRT10 groups than in the control adenovirus group. As shown in Figure 2A and B, 6 weeks post-cisplatin treatment, tumor volumes were much lower in the AD-PTEN and AD-KRT10 groups than in the control adenovirus group. Similarly to the *in vitro* results, AD-PTEN showed a more powerful effect in strengthening the anti-ovarian cancer effect of cisplatin compared to AD-KRT10.



**Figure 2.** Recombinant adenovirus-mediated KRT10 and PTEN overexpression enhanced the *in vivo* antitumor efficacy of cisplatin. BALB/c nude mice bearing C13K xenograft tumors were injected with AD-KRT10, AD-PTEN, and negative adenovirus and treated with cisplatin 24 h later. The growth curves of subcutaneous xenografts of C13K are shown (left panel). Representative tumors were removed and photographed (right panel).

## DISCUSSION

Resistance to platinum-based chemotherapy is a major cause of treatment failure in human ovarian cancer. In previous studies, we showed that overexpression of PTEN can increase the sensitivity of human ovarian cancer cells to cisplatin. We also identified KRT10 as a downstream molecule of PTEN in C13K ovarian cancer cells after cisplatin treatment. In the current study, we showed that recombinant adenovirus-mediated PTEN or KRT10 overexpression improved the sensitivity of ovarian cancer cells to cisplatin both *in vitro* and *in vivo*, indicating that overexpression of PTEN and KRT10 may be a therapeutic method for the resistance of platinum-based chemotherapy in ovarian cancer.

PTEN and KRT10 have been reported to play important roles in cell cycle regulation, tumor cell apoptosis, and the regulation of cell adherence, migration, and differentiation (Cantley and Neel, 1999; Paramio et al., 1999; Reichelt et al., 2004). In addition, PTEN plays an important role in the reversal of MDR (Hou et al., 2010; Cassinelli et al., 2013). Overex-

pression of PTEN can enhance the chemosensitivity of multiple cancer cells (Han et al., 2010; Li et al., 2013). In our previous study, overexpression of PTEN in C13K ovarian cancer cells improved the sensitivity to cisplatin, but not to paclitaxel. Furthermore, KRT10, which is present in numerous epithelial cells and tissues (Waters et al., 2009; Kobayashi et al., 2012), was identified as a downstream protein of PTEN in C13K/PTEN cells. In this study, we found that overexpression of PTEN or KRT10 mediated by recombinant adenovirus improved the anti-ovarian cancer effect of cisplatin *in vitro* and *in vivo*. These data suggest that PTEN and KRT10 are potential therapeutic targets for reversing the cisplatin resistance of ovarian cancer cells.

### Conflicts of interest

The authors declare no conflict of interest.

### ACKNOWLEDGMENTS

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