Down-regulation of survivin enhances paclitaxel-induced Hela cell apoptosis


Abstract. – OBJECTIVE: Paclitaxel is one of the common anticancer drugs in the treatment of cervical cancer, while the mechanism of restraining and killing cancer cells is still unclear. This study aimed to investigate the molecular mechanism of paclitaxel in regulating proliferation and apoptosis of cervical cancer Hela cells. MATERIALS AND METHODS: Paclitaxel at 2 μmol/L was used to treat Hela cells for 48 h. MTT assay and flow cytometry were applied to test Hela cells proliferation and apoptosis respectively. Western blot was adopted to determine the expression of survivin. SiRNA was performed to suppress survivin protein expression in Hela cells. RESULTS: Paclitaxel restrained Hela cells growth and induced apoptosis. Also, paclitaxel treatment significantly reduced survivin protein expression in Hela cells. Moreover, survivin siRNA transfection further promoted Hela cells apoptosis after intervention by 2 μmol/L paclitaxel. CONCLUSIONS: Down-regulation of survivin promoted paclitaxel-induced apoptosis of cervical cancer Hela cells.

Key Words: Paclitaxel, Survivin, Cervical cancer, Hela, Apoptosis.

Introduction

Cervical cancer is a common reproductive system tumor in female with a higher morbidity rate. The pathogenesis of cervical cancer is very complicated and is thought to be influenced by viral infections, sexual behavior, and delivery times. Also, genetic factors affect the development of cervical cancer. Although the curative effect of combined therapy is significant, it has numerous shortcomings and side effects, such as bleeding. At present, a variety of treatment strategies have been developed in clinical practice. However, how to improve the accuracy and success rate of cervical cancer treatment is a big challenge in the medical field. Targeted therapy is the first choice in clinic. The curative effect of current molecular targeting anti-apoptotic proteins, such as survivin and apollon on cervical cancer, is still not satisfactory. Therefore, it is urgently required to explore more effective molecular targets for the treatment of cervical cancer in clinic. Paclitaxel is a kind of important first-line anticancer drug for the treatment of cervical cancer. Paclitaxel catalyzes the rapid synthesis of exciton protein, which binds to the microtubules, leading to stabilization and prevention of microtubule depolymerization. Paclitaxel can inhibit mitosis, promote apoptosis, as well as inhibit tumor cell migration through directly or indirectly suppressing the expression and activity of myosin and actin. After injected to the blood, paclitaxel is quickly decomposed into smaller albumin-paclitaxel polymer, and further binds to albumin GP60 receptor on the surface of vascular endothelial cells. Then, the albumin-paclitaxel is transported out of the blood vessel through endocytosis and aggregates around the tumors to regulate tumor formation. It was showed that the concentration of abraxane was 1.33-fold higher in the tumor tissue, while half in normal tissue compared with general solvent-based paclitaxel. Current guidelines recommend single use of abraxane in the treatment of metastatic breast cancer, pancreatic cancer, and other solid tumors. Up to now, however, the molecular mechanism of paclitaxel in inhibiting and eliminating cervical cancer cells remains poorly understood. Anti-apoptosis protein survivin is a kind of protein kinase involving in apoptosis, necroptosis, autophagy, and NF-κB signaling pathway. It was speculated...
that survivin may play an important role in Hela cells apoptosis\cite{9}. However, the exact mechanism by how survivin induces Hela cells apoptosis remains unclear. The aim of the present study was to investigate the specific regulatory mechanism of survivin on Hela cells apoptosis.

**Materials and Methods**

**Reagents**

Cell apoptosis detection kit was purchased from Beyotime (Shanghai, China). MTT, lipofectamine reagent escort\textsuperscript{TM}, and rabbit anti-human survivin primary antibody were purchased from Sigma-Aldrich (St. Louis, MO, USA). Survivin siRNA and control siRNA were designed and synthesized by GenePharma (Shanghai, China). The sequences were 5’GCGATGATTGGTTATCGT3’ and 5’AAAATAGGTCATCATGTT3’, respectively. Dulbecco’s Modified Eagle Medium (DMEM) was provided by Beijing Dingguo Changsheng Biotechnology Co. Ltd (Beijing, China).

**Cell Culture**

Hela cells were purchased from ATCC. The cells were resuscitated and routinely cultured. Paclitaxel at 2 μmol/L or dimethyl sulfoxide (DMSO) was applied to treat the cells for 48 h.

**Lipofectamine Escort\textsuperscript{TM} Transfection**

The cells were seeded and transfected using survivin siRNA and Lipofectamine reagent Escort\textsuperscript{TM} according to the manual.

**Flow Cytometry**

Cell membrane potential specific dye TMRE and cell membrane phosphatidylserine specific dye FITC-Annexin V were applied to test the apoptosis of cervical cancer cell line by flow cytometry.

**MTT Assay**

The cells were seeded into 96-well plate at a density of 10,000 cells/well for analysis of cell proliferation by methylthiazolyl tetrazolium bromide (MTT) assay.

**Caspase-3 Activity Detection**

The cells were cracked on ice for 10 min and added into the 96-well plate. Then, the chromophoric substrate was added to the plate for measuring the caspase-3 activity.

**Western Blot**

The cells were treated with lysis buffer and separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Primary antibodies for survivin and actin were applied for detection.

**Statistical Analysis**

All data analysis was performed with SPSS18.0 software (SPSS Inc., Chicago, IL, USA). Data were represented as mean ± standard deviation (SD). Student’s *t*-test was performed for comparing the difference between two groups. One-way ANOVA and Dunnet post hoc test were performed to assess the statistical significance among multiple treatment groups. A significant difference was depicted as *p* < 0.05.

**Results**

**Paclitaxel Inhibited Hela Cells Proliferation**

The impacts of paclitaxel on Hela cell survival and proliferation were tested by MTT assay. As shown in Figure 1, paclitaxel significantly suppressed Hela cell growth (*p* < 0.05), suggesting it affects the proliferation of Hela cells.

**Paclitaxel Reduced Cell Membrane Potential of Hela Cells**

Mitochondrial membrane potential of Hela cells was evaluated by flow cytometry after paclitaxel treatment. It was found that paclitaxel obviously declined mitochondrial membrane potential of Hela cells (*p* < 0.05) (Figure 2), indicating that paclitaxel may induce the apoptosis of Hela cells.

**Paclitaxel-induced Hela Cell Apoptosis**

To confirm whether paclitaxel induces apoptosis of Hela cells, we measured the phosphati-
Dysglycemic exposure of Hela cells after paclitaxel treatment. Results showed that after paclitaxel intervention, Hela cells exhibited markedly increased phosphatidylserine exposure ($p < 0.05$) (Figure 3), revealing that paclitaxel induces Hela cell apoptosis.

**Paclitaxel Activated Caspase-3 in Hela Cells**

Considering paclitaxel-induced apoptosis of Hela cells, caspase-3 activity was also measured. As shown in Figure 4, caspase-3 activity in Hela cells was obviously enhanced after paclitaxel treatment.

**Paclitaxel Down-regulated Survivin Protein Expression in Hela Cells**

Western blot was adopted to assess survivin protein expression in Hela cells. It was revealed that survivin protein level was down-regulated in Hela cells after treated with paclitaxel (Figure 5).

**Inhibition of Survivin Promoted Paclitaxel-induced Hela Cells Apoptosis**

As shown in Figure 6, survivin level in Hela cells was suppressed after survivin siRNA transfection, whereas, caspase-3 activity was enhanced in survivin siRNA transfected Hela cells treated with paclitaxel, revealing that down-regulation of survivin facilitated paclitaxel-induced Hela cells apoptosis.

**Discussion**

Paclitaxel is an important anti-cancer drug in clinic. In this study, we discussed the impacts of paclitaxel on the growth and apoptosis of cervical cancer cell line Hela. Firstly, we explored the influence of paclitaxel on cervical cancer Hela cells growth and survival and showed paclitaxel significantly decreased the proliferation of Hela cells, which is consistent with previous study. To evaluate the effect of
Survivin mediates paclitaxel effect in Hela cells. Paclitaxel on Hela cells apoptosis, flow cytometry and caspase-3 activity detection revealed that Hela cells exhibited mitochondrial membrane potential reduction and phosphatidylyserine exposure, suggesting that paclitaxel-induced Hela cells apoptosis possibly through activation of caspase-3, which was in accordance with previous studies. However, va-
rious apoptosis indexes and detection methods showed that under the similar concentrations of paclitaxel (2 μmol/L), Hela cells presented a higher sensitivity compared with other cancer cells. It may be caused by the fact that different cells have the diverse sensitivity to the same chemical drug. Apoptosis pathway mainly activates caspase-8, while mitochondria-mediated intrinsic apoptosis pathway may activate caspase-3/7\textsuperscript{19}. In this study, paclitaxel activated caspase-3 of Hela cells, suggesting that the apoptosis of Hela cells induced by paclitaxel was possibly through mitochondria-mediated intrinsic apoptosis pathway, which was in agreement of previous investigations\textsuperscript{20,21}. As an inhibitor of apoptosis protein, survivin plays a critical role in apoptosis, necroptosis, autophagy and NF-κB signaling pathway\textsuperscript{16}. Our results revealed that paclitaxel treatment significantly increased the apoptotic rate of Hela cells with down-regulation of survivin, indicating that knockdown of survivin contributes to cancer therapy. Based on our data, several investigations could be carried out in the future. Clinical cervical cancer specimens in different stages could be collected to detect survivin expression to evaluate whether survivin is associated with clinical stages. Different types of paclitaxel and similar drugs could be adopted to clarify the specific target and mechanism of paclitaxel on cervical cancer, thus providing more valuable information for clinical practice. However, future studies are required to confirm our findings using survivin knockout mice.

**Conclusions**

Paclitaxel-treated Hela cells display reduced proliferation and increased apoptosis. Moreover, down-regulation of survivin facilitates paclitaxel-induced apoptosis of cervical cancer cell line Hela cells.

**Conflict of interest**

The authors declare no conflicts of interest.

**References**


