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ORIGINAL ARTICLE

FOXO3a Reverses the Cisplatin Resistance in Ovarian Cancer

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Objective. Ovarian cancer is one of the most serious disease in female reproductive system. Platinum is the first-line drug for the treatment of ovarian cancer, while the resistance of platinum drug in clinical hindered the relief ovarian cancer. Our previous study found that decreased FOXO3a might be a poor prognosis in human ovarian cancer. In this research, we study whether FOXO3a was involved in the mechanism of platinum drug resistance.

Methods. The CCK-8 and FACS analysis were used to monitor the survival of ovarian cancer, and the FOXO3a expression was detected by western-blot.

Results. We found that FOXO3a expression upregulated significantly in A2780 compared with A2780/DDP cells with the treatment of platinum. Moreover, overexpression of FOXO3a in ovarian cancer inversed the platinum resistance in ovarian cancer.

Conclusion. These observations reminded that the role of FOXO3a might be one of the critical mechanisms in developing platinum drug resistance in ovarian cancer.

Key Words: FOXO3a, Platinum drug resistance, Ovarian cancer.

Introduction

Ovarian cancer is one of the most serious disease in female reproductive system. Due to the not obvious early symptoms, most patients with ovarian cancer are diagnosed at the advanced stage (1). The primary treatment is cytoreductive surgery, followed by chemotherapy, however, long-term survival remains poor, due to recurrent disease and emerging drug resistance. Platinum is the first-line drug for the therapy of ovarian cancer, while the resistance of platinum drug in clinical hindered the relief of ovarian cancer. Therefore, it is important to study the molecular mechanisms of platinum resistance for improving the curative effect of clinical treatment of ovarian cancer, which is an urgent problem needing to solve (2,3).

More and more studies showed that the tumor is genetic correlation disease, multitudinous genes were involved in the occurrence and development of ovarian cancer. Therefore, we suspect that the resistance of platinum drug might be related with the apoptosis gene. Some pivotal moleculars lead to the cancer occurrence, progress of ovarian cancer, cell proliferation, differentiation. What's more, the less apoptosis of cells will promote tumor cell malignant transformation by escaping apoptosis (4,5). The block of cell apoptosis induced by these abnormal expression of apoptosis genes is the key factors of platinum drug resistance (6). Thus the mechanism of platinum drug resistance might be the block of cell apoptosis. The tumor cells escape of apoptosis makes them exempting from the killing effect of chemotherapy drugs, therefore, the study on the molecular mechanism of cell apoptosis is particular important (7,8). Our previous study found that the expression of FOXO3a was downregulated in ovarian cancer. Therefore, we speculate whether the regulation of FOXO3a was associated with platinum drug resistance.

FOXO3a is a member of Forkhead O transcription factors which activate or repress multiple genes such as Bim

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and FasL including apoptosis (9,10). In breast cancer, FOXO3a overexpression has been shown to inhibit tumor growth *in vitro* and *in vivo* (11,12). FOXO3a is localized in the nucleus, where it target the transcriptional genes. Upon stimulation with growth factors FOXO3a is phosphorylated, which accelerates the nuclear exclusion of FOXO3a, thereby inhibiting its ability to bind DNA (13,14).

In this research, we study whether FOXO3a was involved in the mechanism of platinum drug resistance. Our results found that the platinum drug could not induce the apoptosis of the platinum drug resistance-ovarian cancer cells compared to the control ovarian cancer cells. Furthermore, the expression of FOXO3a was downregulated in the drug resistance ovarian cancer. Finally, overexpression of FOXO3a could reverse the platinum drug resistance in ovarian cancer. These results suggested that the molecular mechanism of platinum drug resistance might be the downregulation of FOXO3a, which could provides direction for the treatment of platinum drug resistance in ovarian cancer.

Material and Methods

Cell Culture

The ovarian cancer cells A2780 and A2780/DDP were obtained from the ATCC (Manassas, VA) and cultured in RPMI-1640 (Hyclone) supplemented with 10% fetal bovine serum, 100 units/ml penicillin (Sigma), and 100 μg/ml streptomycin (Sigma) in 5% CO2 at 37°C.

Cell Apoptosis Analysis

The A2780, A2780/DDP cells were treated with cisplatin or carboplatin (10 μmol/L) (Sigma). After 24 h, cells were trypsinized, fixed with methanol, and their nuclei were labeled with annexin V (Sigma) and PI (Sigma) as described (15,16). After that, the annexin V and PI positive nuclei were gated and analyzed in a FACS/Calibur Flow Cytometer.

Cell Count Kit Assay

To evaluate the effect of cisplatin on cell growth of the variety of ovarian cancer cells, cells were seeded on a 96 well cell culture cluster (Corning Inc., Corning, NY) at a concentration of 2 x 10^4/well of 100 μl. Twenty-four hours later, each well was incubated with cisplatin (10 μmol/L) at 24 h. Cell numbers were measured colorimetrically using the Cell Counting Kit (Dojindo, Kumamoto, Japan) by Immuno-Mini NJ-2300 (NJ InterMed, Tokyo, Japan) at a test wavelength of 450 nm.

Western Blot Analysis

The cells were promptly homogenized in a homogenization buffer containing 1 M Tris-HCl pH 7.5, 1% Triton X-100, 1% NP-40 (nonidet p-40), 10% sodium dodecyl sulfate (SDS), 0.5% Sodium Deoxycholate, 0.5 M EDTA, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM PMSF, then centrifuged at 10,000g for 30 min to collect the supernatant. The protocol of the western blot was conducted as our previous study (15−20). Antibodies used were as follows: anti-GAPDH (cell signal technology), anti-FOXO3a (cell signal technology).

Statistical Analysis

Experimental data were presented as mean ± SD of at least three independent experiments. The Student's *t*-test was used to assess the comparisons between groups. The statistical significance level was set as $*p < 0.05$.

Results

The Influence of the Platinum Drugs on the Growth of Ovarian Cancer

We first divided the ovarian cancer cells into different groups, A2780, A2780/DDP cells with the treatment of cisplatin and carboplatin. The CCK-8 kit analysis results showed that cisplatin and carboplatin induced the inhibited growth of A2780, while cisplatin and carboplatin could not inhitited the growth A2780/DDP cells (Figure 1A). The above results suggested that the similar results of cisplatin and carboplatin on the ovarian cancer cells. In the latter study, we use the cisplatin as the platinum in our experimentation. The FACS analysis confirmed the same results cisplatin induced the cell apoptosis in A2780 cells (Figure 1B), while cisplatin could not induced apoptosis in A2780/DDP cells (Figure 1C). The quantitative of the apoptosis cells were in Figure 1D. These results suggested that platinum drugs could inhibit growth of A2780 cells, while it could not inhibit the growth A2780/DDP cells.

The Expression of FOXO3a in the Platinum Treated Ovarian Cancer

We detected the expression of FOXO3a with the concentration of cisplatin treated different ovarian cancer. The results found that the FOXO3a expression upregulated obviously in A2780 cells, while FOXO3a expression upregulated slightly in A2780/DDP cells (Figure 2). These results hinted that FOXO3a might exert important role in platinum drug resistance in ovarian cancer cells.

Overexpression of FOXO3a Prevented the Platinum Drug Resistance in Ovarian Cancer

To further study the significance of FOXO3a in the platinum drug resistance in ovarian cancer, we transfected FOXO3a to the ovarian cancer cells. The Figure 3A showed that successful overexpression of FOXO3a in ovarian

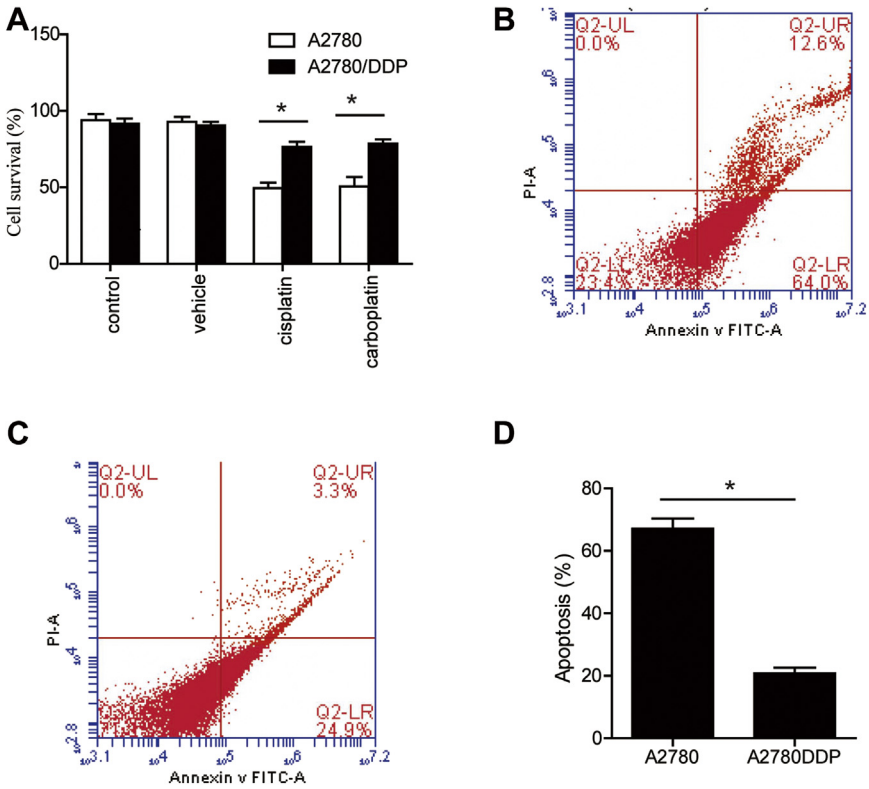

Figure 1. The influence of the platinum drugs on the growth of ovarian cancer. (A). The percentages of cell viability were detected by Cell Count Kit-8. The different cells were treated with cisplatin or carboplatin (10 μmol/L). Results shown are the means of three independent experiments; bars, means ± SD. (B–C). The A2780 cells (B) and A2780/DDP cells (C) were treated with cisplatin (10 μmol/L) for 24 h. After that, the cells were respectively harvested for analysis using PI and annexin v staining for apoptosis by flow cytometry (FCM). (D). The quantitative of the Figure B and (C).

cancer cells. We then stimulated these cells with cisplatin. The CCK-8 analysis found that overexpression of FOXO3a decreased the cell survival in A2780/DDP cells in FOXO3a transfected cells than vector transfected cells (Figure 3B). Furthermore, the FACS analysis also showed that overexpression of FOXO3a increased the cell apoptosis A2780/DDP cells (Figure 3C and D) than the control (Figure 1C). These findings demonstrated that overexpression of FOXO3a might inverse the platinum drug resistance in ovarian cancer.

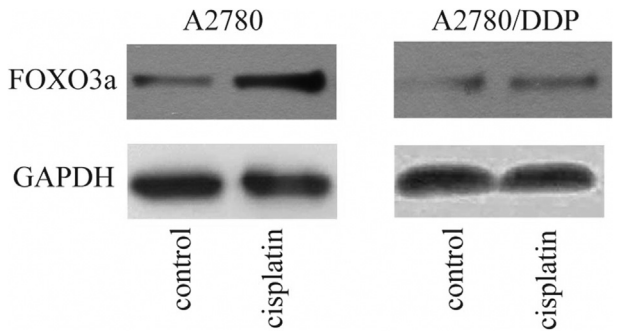

Figure 2. The expression of FOXO3a in the platinum treated ovarian cancer. The cells were treatment with or without cisplatin (10 μmol/L) for 24 h. The FOXO3a expression was assessed by western blot. The data were collected from three independent experiments. GAPDH was as a loading control.

Discussion

Our results found that FOXO3a was downregulated in platinum drug resistance-ovarian cancer. Furthermore, FOXO3a expression upregulated in A2780 while FOXO3a expression does not upregulated in A2780/DDP cells with the treatment of cisplatin. Moreover, overexpression of FOXO3a in ovarian cancer inversed the platinum resistance ovarian cancer. These observations are also consistent with the role of FOXO3a is one of the critical mechanisms in developing platinum drug resistance in ovarian cancer.

The primary surgical cytoreduction plus adjuvant platinum-based chemotherapy is the most important therapy in the current treatment for patients with advanced disease. However, approximately 75% of patients will relapse (21). The key problem of the treatment in ovarian cancer is progressive chemoresistance, particularly platinum resistance. Lots of studies suggested that FOXO3a was the one of the identified candidate in the progression of ovarian cancer. Functionally, FOXO3a and p53 has the striking similarities in regulate cell cycle and apoptosis. Furthermore,

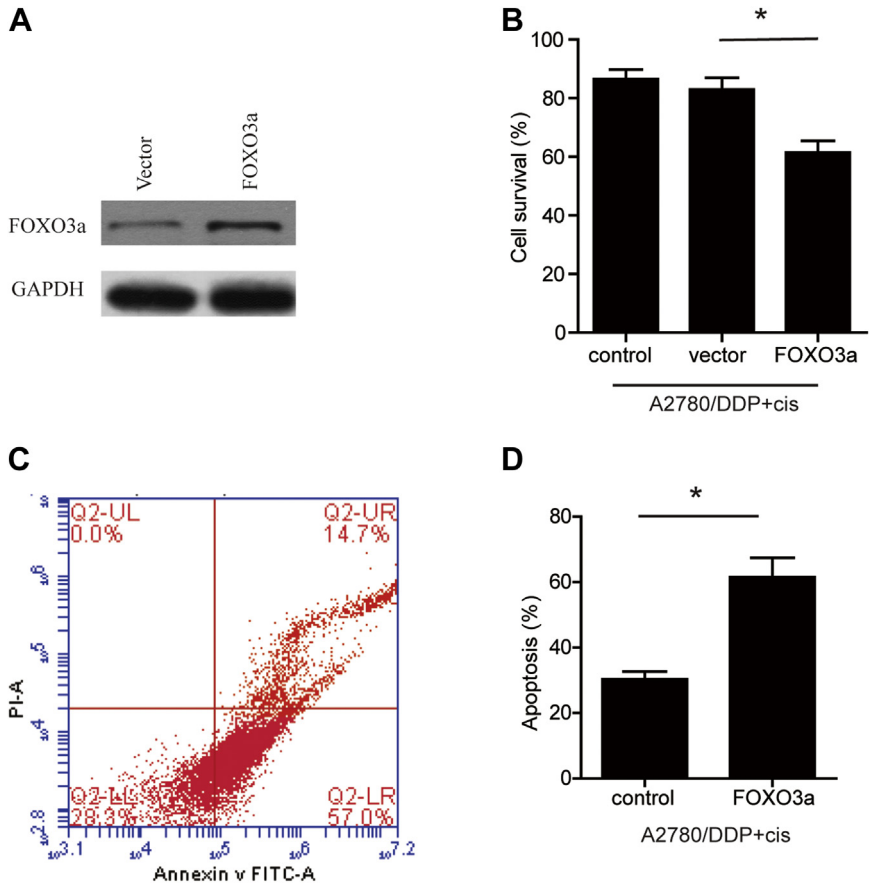

Figure 3. Overexpression of FOXO3a prevented the platinum drug resistance in ovarian cancer. (A). The FOXO3a was assessed by western blot after the transfection of FOXO3a. (B). The percentages of cell viability were detected by Cell Count Kit-8. All the cells were treated with cisplatin (10 μmol/L) after the transfection of FOXO3a or vector control. (C). The cells were treated with cisplatin (10 μmol/L) after the transfection of FOXO3a. The cells were harvested for analyzed PI and annexin v staining for apoptosis by flow cytometry (FCM). (D). The quantitative of the flow cytometry results after the transfection of FOXO3a or vector control.

these two transcription factors share many downstream targets. In the tumors, FOXO3a frequently downregulated by posttranslational modifications, while p53 always mutation in the cancer. The FOXO3a exert its role in initiating apoptotic programs and upregulating proapoptotic genes, while downregulating antiapoptotic genes. In several malignancies such as colorectal cancer, FOXO3a responses to cisplatin treatment, and silencing endogenous FOXO3a impairs cytotoxicity of this chemotherapeutic agent (22—24). The next study was on the mechanism of cisplatin drug resistance. FOXO3a is a important regulator in cell cycle and apoptosis in tumor. Whether FOXO3a was involved in the cisplatin drug resistance. Our results found that FOXO3a expression upregulated in A2780 cells while FOXO3a expression does not upregulated in A2780/DDP cells with the treatment of cisplatin, suggesting that FOXO3a might involved in the cisplatin drug resistance.

Whether FOXO3a signal pathway is a critical target for ovarian cancer chemointervention remains a question. The fact that FOXO3a overexpression upregulated the sensitive of platinum drug in ovarian cancer. In this context, FOXO3a overexpression should increase cell death in ovarian cancer cells and sensitized cells to death induced by platinum drug. In conclusion, our results found that FOXO3a might be the important factor which inverse the platinum resistance ovarian cancer.

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References

1. Bell D, Berchuck A, Birrer M, et al. Integrated genomic analyses of ovarian carcinoma. Nature 2011;474:609−615.
2. Batista L, Gruosso T, Mechta-Grigoriou F. Ovarian cancer emerging subtypes: role of oxidative stress and fibrosis in tumour development and response to treatment. Int J Biochem Cell Biol 2013;45: 1092−1098.
3. Chock KL, Allison JM, Shimizu Y, et al. BRCA1-IRIS overexpression promotes cisplatin resistance in ovarian cancer cells. Cancer Res 2010; 70:8782−8791.
4. Bosserman L, Prendergast F, Herbst R, et al. The microculture-kinetic (MiCK) assay: the role of a drug-induced apoptosis assay in drug development and clinicalcare. Cancer Res 2012;72:3901−3905.
5. Anand P, Kunnumakkara AB, Harikumar KB, et al. Modification of cysteine residue in p65 subunit of nuclear factor-kappaB (NF-kappaB) by picroliv suppresses NF-kappaB-regulated gene products and potentiates apoptosis. Cancer Res 2008;68:8861−8870.
6. Djavaheri-Mergny M, Amelotti M, Mathieu J, et al. NF-kappaB activation represses tumor necrosis factor-alpha-induced autophagy. J Biol Chem 2006;281:30373−30382.
7. Choudhary S, Sood S, Wang HC. Synergistic induction of cancer cell death and reduction of clonogenic resistance by cisplatin and FK228. Biochem Biophys Res Commun 2013;436:325−330.
8. Rai V, Touré F, Chitayat S, et al. Lysophosphatidic acid targets vascular and oncogenic pathways via RAGE signaling. J Exp Med 2012;209:2339−2350.
9. Kops GJ, Dansen TB, Polderman PE, et al. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. Nature 2002;419:316−321.
10. Onuma H, Vander Kooi BT, Boustead JN, et al. Correlation between FOXO1a (FKHR) and FOXO3a (FKHRL1) binding and the inhibition of basal glucose-6-phosphatase catalytic subunit gene transcription byi nsulin. Mol Endocrinol 2006;20:2831−2847.
11. Hu MC, Lee DF, Xia W, et al. InB kinase promotes tumorigenesis through inhibition of forkhead FOXO3a. Cell 2004;117: 225−237.
12. Yang JY, Zong CS, Xia W, et al. ERK promotes tumorigenesis by inhibiting FOXO3a via MDM2-mediated degradation. Nat Cell Biol 2008;10:138−148.
13. Liu L, Rajareddy S, Reddy P, et al. Infertility caused by retardation of follicular development in mice with oocyte-specific expression of Foxo3a. Development 2007;134:199−209.
14. Huang H, Tindall DJ. Dynamic FoxO transcription factors. J Cell Sci 2007;120:2479−2487.
15. He S, Lu M, Xue W, et al. Phosphorylated p27Kip1 on Thr157 is an important prognosis in human hepatocellular carcinoma *in vivo* and *in vitro*. Med Oncol 2011;28:94−104.
16. Fei M, Lu M, Wang Y, et al. Arsenic trioxide-induced growth arrest of human hepatocellular carcinoma cells involving FOXO3a expression and localization. Med Oncol 2009;26:178−185.
17. Lu M, Fei M, Cheng C, et al. Mutant p27(Kip1) and its potential effect as hepatocellular gene therapy. Arch Med Res 2008;39:573−581.
18. Lu M, Ma J, Xue W, et al. The expression and prognosis of FOXO3a and Skp2 in human hepatocellular carcinoma. Pathol Oncol Res 2009; 15:679−687.
19. Lu M, Xiang J, Xu F, et al. The expression and significance of pThr32-FOXO3a in human ovarian cancer. Med Oncol 2012;29:1258−1264.
20. Lu M, Zhao Y, Xu F, et al. The expression and prognosis of FOXO3a and Skp2 in human ovarian cancer. Med Oncol 2012;29:3409−3415.
21. Bookman MA, Brady MF, McGuire WP, et al. Evaluation of new platinum-based treatment regimens in advanced-stage ovarian cancer: a Phase III Trial of the Gynecologic Cancer Intergroup. J Clin Oncol 2009;27:1419−1425.
22. Blanchard Z, Paul BT, Craft B, et al. BRCA1-IRIS inactivation overcomes paclitaxel resistance in triple negative breast cancers. Breast Cancer Res 2015;17:5.
23. Fang L, Wang H, Zhou L, et al. FOXO3a reactivation mediates the synergistic cytotoxic effects of rapamycin and cisplatin in oral squamous cell carcinoma cells. Toxicol Appl Pharmacol 2011;251:8−15.
24. Gao J, Yang X, Yin P, et al. The involvement of FoxO in cell survival and chemosensitivity mediated by Mirk/Dyrk1B in ovarian cancer. Int J Oncol 2012;40:1203−1209.