Inhibition of Heat Shock Protein Hsp90 Reverses Chemotherapy Resistance of Ovarian Cancer

Mingbo Wu¹, Ye Zhao¹, HouYi Huang¹, Mengju Jiang¹, Lianzhi Peng¹, Linpeng Li¹, Jie Yang¹, Li Feng¹, Xiuhui Gu², Jing Liu¹, Guodan Zeng¹, He Wei¹,*, Minhui Li²*, Kun Zhang¹*

¹School of Biomedical Sciences, Chengdu Medical College, Chengdu, China
²School of Basic Medical Sciences, Chengdu Medical College, Chengdu, China
*These authors contributed equally to this work

Abstract

Aim: To explore the mechanism of Hsp90 in chemotherapy resistance of paclitaxel in ovarian cancer.

Methods: MTT were used to detect the effect of BIIB021 on ovarian cancer resistant cells (A2780/Taxol) to paclitaxel, half inhibitory concentration and flow cytometry were used to detect apoptosis. Western blotting was performed to detect MRP1, MDR1, Bcl2 and Survivin protein expression in A2780 and A2780/Taxol treated with Hsp90 inhibitor BIIB021 or transfected pcDNA3.1-Hsp90 and siHsp90.

Results: Inhibition of Hsp90 enhanced the inhibitive rates of paclitaxel to A2780/taxol, reduced the IC50, and the expression of MRP1, MDR1, Bcl-2 and Survivin. Apoptosis was significantly increased in A2780/taxol cells treated with BIIB021 and paclitaxel. Furthermore, overexpression of Hsp90 increased MRP1, MDR1, Bcl-2 and Survivin expression while interference of Hsp90 reduced the expression of above.

Conclusions: Hsp90 could enhance the chemotherapy sensitivity to paclitaxel in ovarian cancer by inhibiting the expression of Bcl2, MDR1, survivin and MRP1.

Keywords: Heat shock protein 90; Chemotherapy resistance; Paclitaxel; Ovarian cancer

Introduction

Ovarian cancer is the third most common female genital malignancies, second to only cervical cancer and corpus carcinoma, and is the main cause of genital malignancy-related deaths in females, greatly threatening women’s life[1]. Currently, treatment for ovarian cancer mainly includes surgical resection, chemotherapy and radiotherapy[2-3]. Paclitaxel is a commonly used anti-cancer drug, often as the first-line drug for chemotherapy. Paclitaxel show superior efficacy not only for ovarian cancer, but also for breast cancer, lung cancer, head and neck cancer, and lymphoma[4-5]. However, long-term chemotherapy often leads to resistance to the drug and fails at last. Chemotherapy with paclitaxel also faces the problem of drug resistance; the efficacy of paclitaxel gradually decreases as the chemotherapy proceeds. So, it is urgent to understand the mechanism of drug resistance in ovarian cancer and to reverse this process in treatment of ovarian cancer.

Drug resistance of tumors is a multifactorial, complex, multi-stage process, varying between different drugs and multiple tumor types. Extensive studies have revealed that Multidrug Resistance-Associated Protein 1 (MRP1), an ATP-dependent transporter, is associated with multidrug resistance of cancer cells through intracellular localization[6]. MRP1 cannot only export drug molecules outside of the cell membrane, but concentrate drugs into intracellular vesicles, thus isolating the drugs from their targets[7]. In addition, abnormal expression of Multidrug Resistance (MDR) genes has as well been found to be one of the major causes of chemotherapy failure. MDR1 encodes a...
P-glycoprotein (P-gp), which belongs to the ATP Binding Cassette (ABC) family of transporters, and has the capacity to pump out drugs in an ATP-dependent manner. MDR1 over expression causes increased exclusion of intracellular drugs, which lowers intracellular drug concentration and leads to drug resistance of the tumor cells[9]. Moreover, abnormal expression of anti-apoptotic proteins is also closely correlated with chemotherapy resistance of the tumors. Studies have shown that Survivin, an important member of the inhibitors of apoptosis protein (IAP) family, plays a vital role in the development and progression of various malignancies, actually, Survivin is highly expressed in almost all malignant tumors, and is thus considered as a key oncogene in tumorigenesis. Studies on prostate cancer reveal that Survivin inhibits apoptosis of prostate cancer cells, and is greatly involved in the development and progression of prostate cancer[9]. Bcl2 is also an important member of the anti-apoptotic proteins, and plays a crucial role in survival of breast cancer, colorectal cancer, prostate cancer, and leukemic cells. Studies have shown that suppression of Bcl2 induces proliferation inhibition and apoptosis in breast cancer cells[10]. And further studies have revealed that up regulation of Survivin and Bcl2 can lead to drug resistance in cancers, including ovarian cancers, brain tumors, and gastric cancers[11,12].

90kD heat shock protein (Hsp90) is a highly conserved member of the Heat Shock Protein (HSP) family, as a molecular chaperone, Hsp90 takes part in cell proliferation, differentiation, and apoptosis. Studies have shown that Hsp90 is over expressed in gastric cancer, breast cancer, and liver cancer, and is involved in their malignant behaviors[13,14]. Tumors with high Hsp90 expression show resistance to paclitaxel, doxorubicin and other drugs[15], resulting in lower sensitivity to chemotherapy[16]. However, the mechanism how Hsp90 induces resistance has yet to be not completely clarified. So, in this study, we intend to explore the role of Hsp90 in resistance of ovarian cancer to paclitaxel as well as the underlying mechanism.

In this study, inhibition of Hsp90 function with BIIB021 significantly enhanced inhibition ratio of A2780/Taxol by paclitaxel, lowering its IC50, and reduced expression of the drug resistance protein MRP1 and MDR1, the anti-apoptotic protein Bcl2 and Survivin, significantly enhancing paclitaxel-induced apoptosis of the A2780/Taxol cells. Further studies showed that over expressing of Hsp90 enhanced protein expression of MRP1, MDR1, Bcl2 and Survivin, while interfering of Hsp90 reduced expression of these proteins. These results indicate that Hsp90 is closely related to chemotheraphy resistance of ovarian cancer, and suppression of Hsp90 may reduce resistance of ovarian cancer cells to paclitaxel by down regulation of MRP1, MDR1, Bcl2 and Survivin. The role of Hsp90 in ovarian cancer provides a new avenue to address chemotheraphy resistance of ovarian cancer.

Materials

Human ovarian cancer cells A2780 and its paclitaxel-resistant derivative cells A2780/Taxol were purchased form KeyGen Bitotech Co. (Nanjing, Jiangsu, China); antibodies were purchased from Santa Cruz (Dallas, Texas, USA); Annexin-V/PI apoptosis detection kit and BCA protein assay kit were purchased from Beyotime (Nantong, China); pcDNA3.1-HA, pcDNA3.1-HA-Hsp90 were stored in our lab. siNC (siRNA negative control) and siHsp90 (sense strand: 5’ GGA AAG AGC UGC AUA UUA ATT; antisense strand 5’ UUA AUU UGC AGC UCU UUC TT) were purchased from genepharma (Shanghai, China).

Methods

Cell culture

A2780 and A2780/Taxol cells were cultured in RPMI-1640 supplemented 1% penicillin/streptomycin sulphate, 10% FBS, and incubated at 37°C with 5% CO₂. When cell confluency reached 80% to 90%, cells were incubated with 0.25% Trypsin and then passaged.

MTT assay

A2780/Taxol cells (3 × 10³/well) were seeded in 100 μl RPMI 1640 with 5% FBS and without or without 1 μmol/L BIIB021 in 96-well plate for 24 hours, and then cells were cultured in medium with paclitaxel (0, 0.1, 0.2, 0.4, 0.8, 1.6, 2.0 μmol/L) for additional 48 hours. MTT assay was performed to examine the sensitivity of A2780/Taxol cells to paclitaxel. Paclitaxel concentrations that achieved 50% growth inhibition (IC50) were calculated from survival curves using the Bliss method.

Cell transient transfection

The confluent A2780 cells (80%) were transfected with pcDNA3.1 or pcDNA3.1-HA-Hsp90 using Lipofectamine 3000 transfection reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer’s instructions, and the confluent A2780/Taxol cells (50%) were then transfected with siNC or siHsp90 using Lipofectamine 3000 transfection reagent. 72 hours after transfection, cells were collected and analyzed using western blotting.

Western Blotting

Cells after treatment were washed three times in PBS, lysed in RIPA buffer. Then supernatants were collected, and protein concentrations were determined using a BCA protein assay kit. Equal quantities of protein were analyzed using Western blotting following standard protocols. For immunodetection, membranes were blocked overnight and incubated overnight at 4°C with primary antibody against goat monoclonal anti-Hsp90, rabbit monoclonal anti-HA, mouse monoclonal anti-MRP1, rabbit monoclonal anti-MDR1, rabbit monoclonal anti-Bcl2, rabbit monoclonal anti-Survivin and rabbit monoclonal anti-GAPDH prepared in blocking buffer. Membranes were then incubated with their respective horseradish peroxidase (HRP)-conjugated secondary antibodies. Immunoreactive bands were visualized using an ECL chemiluminescent detection kit and a Bio-Rad Molecular Imager (Hercules, CA, USA).

Flow cytometry analysis (FACS)

FACS was carried out to analyze cell apoptosis. Briefly, A2780/Taxol cells were seeded in RPMI 1640 with 5% FBS in 12-well plate (2 × 10⁵ cells/well) the cells was then treated with medium with different concentration of BIIB021 (0, 0.5, 1.0 μmol/L) for 24 hours, and followed by the treatment with paclitaxel (0.4, 0.8 μmol/L) for additional 48 hours. Adherent A2780/Taxol cells were detached from the culture plate. Cells (10⁵ cells/ml) were then incubated with Annexin V and Propidium Iodide (PI) for 20 minutes at 4°C and analyzed using a flow cytometer.
Silencing Hsp90 improves chemosensitivity of ovarian cancer

(Sc Bioscience; San Jose, CA, USA).

Statistical Analysis

Results from at least three independent experiments were expressed as mean ± SEM. Statistical significance was evaluated using a two-tail t-test for comparison between two groups. One-way ANOVA analysis of variance was used to assess a difference of means between groups. All analyses were performed using Graph Pad Prism Software Version 5.0 (Graph Pad Software Inc, La Jolla, CA, USA). A value of P < 0.05 was considered statistically significant.

Results

Inhibiting Hsp90 increased the sensitivity of ovarian cancer to paclitaxel

To examine whether Hsp90 is involved in ovarian cancer resistance to paclitaxel, its protein level was compared between paclitaxel-resistant ovarian cancer cells A2780/Taxol and parental cells A2780. Western blotting showed that the protein expression of Hsp90 was significantly increased in A2780/Taxol compared with A2780 (Figure 1A and B), suggesting that it plays an important role in resistance of ovarian cancer to paclitaxel. Next, BIIB021, a specific inhibition regent of Hsp90, was used to examine the effect of Hsp90 on drug sensitivity of A2780/Taxol cells to paclitaxel. MTT assay showed that BIIB021 significantly increased inhibition rate of paclitaxel in A2780/Taxol cells (Figure 1C). Compared with control, the IC50 of paclitaxel in A2780/Taxol cells was decreased from 1.38 μmol/L to 0.5 μmol/L (Figure 1 D). These results suggested that inhibiting Hsp90 resensitized ovarian cancer to paclitaxel.

Inhibition of Hsp90 down-regulates the expression of drug resistance related proteins and anti-apoptosis proteins

The whole proteins from A2780 and A2780/Taxol were collected by RIPA lysis buffer. Compared with A2780 cells, the resistance-related protein MRP1 and MDR1 expression were significantly increased in A2780/Taxol cells. Moreover, the anti-apoptosis protein Bcl2 and Survivin were also over expressed in A2780/Taxol cells in contrast with A2780 cells (Figure 2 A). Further, BBIB021, a small molecular inhibitor targeted Hsp90, was employed to check the effect of Hsp90 on these protein expressions. The results of western blotting showed that the resistance-related protein MRP1 and MDR1 expression were gradually decreased by BBIB021 in a dosage dependent manner, and the anti-apoptosis protein Bcl2 and Survivin were also reduced as the same manner (Figure 2 B), suggesting that inhibiting Hsp90 can suppress these proteins expression.

BBIB021 enhanced paclitaxel-induced apoptosis of A2780/Taxol cells

The paclitaxel-induced apoptosis of A2780/Taxol cells was detected by Flow cytometry. The results showed the apoptosis rate of each individual group was that: group A was 2.2%, group B was 3.8%, group C was 6.6%, Group D was 3.7%, Group E was 14%, group F was 12.2%, which was significantly higher in contrast with group D; group G was 29.4% which was significant higher in contrast with D; group H was 43.6% which was significant higher in contrast with E (Figure 3). These results suggested that inhibition of Hsp90 significantly increased the apoptosis of paclitaxel induced A2780/Taxol cells.
Silencing Hsp90 improves chemosensitivity of ovarian cancer

**Discussion**

Surgical resection and chemotherapy are the major treatments for ovarian cancer, but unfortunately, chemotherapy against ovarian cancer often fails at last due to multidrug resistance. Thus, to elucidate the mechanism of chemotherapy resistance is the key to successful treatment. Hsp90 is chaperone with high conserved structure, and it’s involved in proliferation, differentiation, and apoptosis of cells. Studies have shown that abnormal activation of Hsp90 is associated with development and progression of various cancers. Moreover, sustained high expression of Hsp90 directly mediates the resistance of cancer cells to chemotherapy\[17,18\]. This study proposes to investigate the effect and mechanism of Hsp90 on paclitaxel resistance of ovarian cancer.

To further clarify the mechanism of how Hsp90 mediates drug resistance is not entirely clear, which may vary among different drugs.

To further clarify the mechanism of how Hsp90 mediates drug resistance to paclitaxel, we examined the expression of multidrug resistance associated protein (MRP1), multidrug resistance protein (MDR1), anti-apoptotic proteins (Bcl2 and Survivin), since drug resistance of tumor cells is often correlated with abnormal efflux of drugs and ectopic expression of anti-apoptotic proteins. MRP1 and MDR1 are key members of the ABC transporter family, which lower intracellular drug concentration and impair cytotoxicity of chemotherapeutic drugs by promoting drug efflux\[21\].

In this study, we found that expressions of both MRP1 and MDR1 are significantly higher in ovarian cancer pacl-

NA3.1-HA-Hsp90 were transfected into A2780 cells for 72 hours. The Hsp90 expression was increased after transfection with HA-Hsp90 compared with control, suggested that the over expression of Hsp90 was successfully performed. Moreover, the expressions of MRP1, MDR1, Bcl2 and Survivin were increased as Hsp90 increased (Figure 4A). Further, siNC and siHsp90 were also transfected into A2780/Taxol cells compared with control (Figure 4B). Above results suggested that Hsp90 can control positively the expression of MRP1, MDR1, Bcl2 and Survivin.

**Figure 3:** The effect of Hsp90 on paclitaxel-induced apoptosis of A2780/Taxol cells

A) 2780/Taxol cells were treated with paclitaxel, BIIB021 as indicated, A) A2780/Taxol cells treated with DMSO for 72 h, B) 0.5 μmol/L BIIB021 for 72 h, C) 1.0 μmol/L BIIB021 for 72 h, D) 0.4 μmol/L paclitaxel for 48 h, E) 0.8 μmol/L paclitaxel for 48 h, F) 0.5 μmol/L BIIB021 for 24 h and then 0.4 μmol/L paclitaxel for 48 h, G) 1.0 μmol/L BIIB021 for 24 h and then 0.4 μmol/L paclitaxel for 48 h, H) 1.0 μmol/L BIIB021 for 24 h and then 0.8 μmol/L paclitaxel for 48 h. The paclitaxel-induced apoptosis of A2780/Taxol cells were analyzed with Annexin V/PI double staining and flow cytometry assay. Each experiment was performed in triplicate. Δ, P < 0.05.

**Figure 4:** The expression of Hsp90 regulated the protein levels of MRP1, MDR1, Bcl2 and Survivin.

A) A2780 cells were transfected with pcDNA3.1-HA-Hsp90 or pcDNA3.1-HA-Hsp90 for 72 h before the cells were harvested. The cells lysates were used for Western blotting analysis with anti-MRP1, anti-MDR1, anti-Bcl2 and anti-Survivin, anti-HA, anti-GAPDH. B) A2780/Taxol cells were transfected with siNC, siHsp90 for 72 h and then harvested for Western blotting analysis as indicated.

**Hsp90 positively regulated the expressions of MRP1, MDR1, Bcl2 and Survivin**

To further confirm the regulation of MRP1, MDR1, Bcl2 and Survivin by Hsp90. After pcDNA3.1-HA and pcDNA3.1-HA-Hsp90 were transfected into A2780 cells for 72 hours. The Hsp90 expression was increased after transfection with HA-Hsp90 compared with control, suggested that the over expression of Hsp90 was successfully performed. Moreover, the expressions of MRP1, MDR1, Bcl2 and Survivin were increased as Hsp90 increased (Figure 4A). Further, siNC and siHsp90 were also transfected into A2780/Taxol cells compared with control (Figure 4B). Above results suggested that Hsp90 can control positively the expression of MRP1, MDR1, Bcl2 and Survivin.
taxel-resistant cells A2780/Taxol than their parental A2780 cells (Figure 2A), suggesting that paclitaxel resistance of ovarian cancer involves MRPI and MDR1, and efflux of paclitaxel by these proteins. Bcl2 is an anti-apoptotic protein that inhibits programmed cell death, and it’s believed to play an important role in cell proliferation, differentiation, and drug resistance of breast cancer, colorectal cancer, prostate cancer, leukemia and other malignancies[22-24]. Similarly, Survivin is also an important member of the inhibitor of apoptosis (IAP) protein family, participates in development, progression, and drug resistance of tumors. Survivin is found to be up regulated in various malignancies, for example, Survivin is highly expressed in gastric cancer, and it promotes malignancy of gastric cancer by inhibiting apoptosis and proliferation of gastric cancer cells, and promoting angiogenesis[25]. It was found in this study, that both Bcl-2 and Survivin are significantly up regulated in the paclitaxel-resistant ovarian cancer cell line A2780/Taxol compared to the paclitaxel-sensitive ovarian cancer cell line A2780 (Figure 2A), suggesting that high expression levels of Bcl2 and Survivin are also involved in resistance of ovarian cancer to paclitaxel. So ovarian cancer may gain drug resistance through up regulation of both drug transporters that promote efflux of drugs (MRPI and MDR1) and anti-apoptotic proteins (Survivin and Bcl2).

Since Hps90, MRPI, MDR1, Bcl2 and Survivin are all over expressed in paclitaxel-resistant ovarian cancer cells, we suspected that Hsp90 may regulate MRPI, MDR1, Bcl2 and Survivin expression during the development of resistance to paclitaxel. So we tested the effect of Hsp90 on expression of these proteins in the paclitaxel-resistant A2780/Taxol cells. It was found that inhibition of Hsp90 function with BIIB021 reduced MRPI and MDR1 expression in a dose-dependent manner. Moreover, BIIB021 also decreased the anti-apoptosis protein Bcl2 and Survivin expression (Figure 2B). Furthermore, BIIB021 significantly enhanced paclitaxel-induced apoptosis of ovarian cancer cells (Figure 3), suggesting that inhibiting Hsp90 may improve efficacy of paclitaxel by weakening the anti-apoptosis pathways. Above results suggested that inhibiting Hsp90 may enhance sensitivity of ovarian cancer cells to paclitaxel by down-regulating MRPI, MDR1, Bcl2 and Survivin expression.

To further confirm a regulation of MRPI, MDR1, Bcl2 and Survivin expression by Hsp90, we over expressed Hsp90 in the paclitaxel-sensitive A2780 cells, and silenced Hsp90 expression in the paclitaxel-resistant cells by siRNA. It was found that over expressing HSP90 up regulated MRPI, MDR1, Bcl2 and Survivin expression (Figure 4A), while silencing Hsp90 down regulated MRPI, MDR1, Bcl2 and Survivin expression (Figure 4B). These results also suggest that Hsp90 enhances paclitaxel-resistance of ovarian cancer cells by up-regulating MRPI, MDR1, Bcl2 and Survivin expression, and silencing Hsp90 may restore sensitivity of ovarian cancer cells to chemotherapy by down-regulating these proteins.

Taken together, our results demonstrated that Hsp90 is closely related chemotherapy resistance of ovarian cancer, and inhibiting Hsp90 can enhance sensitivity of ovarian cancer to paclitaxel by down regulation of MRPI, MDR1, Bcl2 and Survivin expression, providing a new avenue to reverse the chemotherapy resistance of ovarian cancer and improve efficacy of paclitaxel in cancer cells.

Acknowledgments
This work was supported by National Natural Science Foundation of China (Grant no. 81301919), Application and Basic Project of Science and Technology Department of Sichuan Province (Grant no. 2016JY0184), Scientific Research Foundation of the Education Department of Sichuan Province (Grant no. 14ZA0230), Natural Science Foundation of Chengdu Medical College (Grant no. 13Z092,15Z106, 16Z145), State Undergraduate Innovative Experiment Program (Grant no. 201513705019, 508-2019054) and Sichuan Province Undergraduate Innovative Experiment Program (Grant no. 201313705026, 508-2019054), and all support is gratefully acknowledged.

Conflict of Interest: The authors have no relevant conflicts of interest, personal or financial, to disclose.

References
Silencing Hsp90 improves chemosensitivity of ovarian cancer

Pubmed

Pubmed | Crossref | Others

Pubmed | Crossref | Others

Pubmed | Crossref | Others

Pubmed | Crossref | Others

Pubmed | Crossref | Others

Pubmed | Crossref | Others

Pubmed | Crossref | Others

Pubmed | Crossref

Crossref | Others

Pubmed | Others

Pubmed | Crossref | Others

Pubmed | Crossref | Others

Pubmed | Crossref | Others

Pubmed | Crossref

Pubmed | Crossref | Others