MDR1 and MDR3 Genes and Drug Resistance to Cisplatin of Ovarian Cancer Cells

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Summary: To investigate the relationship between MDR1 and MDR3 gene and drug resistance to cisplatin of ovarian cancer cells. Two siRNAs (MDR1, MDR3) which specifically targeted MDR1 and MDR3 genes were transfered into A2780/DDP cells. Then double staining with Annexin-V-FITC/PI was used to detect cell apoptosis by the flow cytometry (FCM). A2780/DDP cell viability was determined by MTT. MDR1 and MDR3 mRNA were assessed by RT-PCR. Caspase-3 protein was detected by Western blotting. Transfection of MDR1 and MDR3 siRNA into A2780/DDP cells failed to reverse the drug-resistance of A2780/DDP cells to cisplatin (P>0.05). No significant difference in the apoptosis efficiency was observed between the MDR1 and MDR3 siRNA, pSuppressor-Neo vector transfection cells and untreated cells (P>0.05). In the presence of cisplatin of different concentrations, the viability of A2780/DDP cells was not significantly decreased after the transfection. No changes in MDR1 and MDR3 mRNA were found in MDR1 and MDR3 siRNA-transfected A2780/DDP cells. As compared with pSuppressorNeo and untreated groups, no significant difference existed in the expression of MDR1 and MDR3 mRNA (P>0.05). The expression of caspase-3 protein in MDR1 and MDR3 siRNA transfected A2780/DDP cells was not significantly increased. It is concluded that multidrug resistance induced by cisplatin in ovarian carcinoma cell lines is not due to overexpression of MDR1 and MDR3 gene. The drug resistance of ovarian carcinoma cells to cisplatin is not mediated by P-glycoprotein.

Key words: RNA interference; P-glycoprotein; cisplatin; A2780/DDP cells; apoptosis

The P-glycoprotein (p-gp) that mediates the active efflux of chemotherapeutic drugs plays a critical role in the development of drug-resistance of cancer cells. However, p-gp expression was negative in many drug-resistant cells and in fact, a lot of studies showed that in cancer cells resistant to cisplatin the p-gp expression negative, suggesting that different cells respond to drugs differently. The drug resistance is an involved process. The purpose of this study is to examine two genes of human p-gp family, MDR1 and MDR3, by employing RNAi technique, in order to explore the relationship between the expression of MDR1 and MDR3 genes and the drug resistance of A2780/DDP cell line to cisplatin.

1 MATERIAL AND METHODS

1.1 Materials
A2780/DP and cisplatin-sensitive A2780 cells were purchased from Guangxi Medical University, Nanning, China. pSuppressorNeo plasmid, pSuppressorNeo MDR1 and MDR3 siRNA plasmid were gift of Dr Duan, Department of Hematology/Oncology, Massachusetts General Hospital, Boston, USA. Lipofect2000™ was bought from Gibco Co., USA. Annexin V-FITC/PI apoptosis detection kit was from Beijing Biosea Biotechnology Co., Ltd., China. The PCR-amplified product for β-actin, MDR1 and MDR3 were 838 bp, 304 bp and 177 bp in length respectively.

1.2 Methods
1.2.1 Cell Culture and Plasmid Transfection
A2780/DDP cell was cultured in DMEM complete culture medium without cisplatin for at least one week. For transfection, 2.5×10⁵ cells mixed with OPTI-MEM culture medium (containing no antibody and FBS) were seeded into a well of a 6-well plate and incubated at 37°C for 24 h. Three groups were set up: pSuppressor-Neo plasmid (serving as negative control), pSuppressorNeo MDR1siRNA plasmid, pSuppressorNeo MDR3 siRNA plasmid groups. Lipofect2000-mediated method was used by following the manufacturer’s instruction. The cells that were 90% confluent in were transfected with 5 μg pSuppressorNeo plasmid, pSuppressorNeo MDR1siRNA plasmid, pSuppressorNeo MDR3 siRNA plasmid groups. Lipofect2000-mediated method was used by following the manufacturer’s instruction. The cells that were 90% confluent in were transfected with 5 μg pSuppressorNeo plasmid, pSuppressorNeo MDR1siRNA plasmid, pSuppressorNeo MDR3 siRNA plasmid groups. The ratio of plasmid to Lipofect2000 was 1:2. After a 6-h transfection, the medium was replaced by complete culture medium. Cells were continuously cultured until harvest for analysis.

1.2.2 Flow Cytometric Analysis of Apoptosis
Apoptotic cells were quantified by Annexin V-FITC/PI assay. After a 48-h transfection, cells were collected and centrifuged for 5 min at 1000 g to remove culture medium, and then cells were washed in PBS and centrifuged for another 5 min at 1000 g. Cell suspension (100 μL) were treated with 5 μL Annexin V-FITC and 5 μL PI for 30 min in the dark, and cell nuclei were analyzed by using a flow cytometer (Becton Dickinson FACScan, California, USA).
1.2.3 Cell Viability Assay The suspended cells (1×10⁴/mL) were cultured in 96-well plates with 10000 cells in each well at 37°C in 5% CO₂ in an incubator. After the cells were cultured for 24 h, cisplatin of various concentrations (used for the experimental group) and complete medium (used for blank control) were added into 3 wells of each group, with 100 µL for each well. The cells were cultured for another 48 h, and then MTT (5 mg/mL and 10 µL for each well) was added into each well, which was followed by addition of DMSO 4 h later (200 µL for each well) to measure the OD₅₇₀ values after storage for over 4 h. The cell viability was determined by cell cytotoxicity and calculated by cell multiplication inhibition tests.

1.2.4 Reverse Transcription PCR Total cellular RNA was extracted from cells with TRIzol reagent (Gibco BRL, USA) and quantified by UV absorbance spectrophotometry. For the β-actin (forward: 5′-ATCTGGCACCACACCTTCTACAAGCTGCG-3′; reverse: 5′-CGCTATGCTGCTGACATCTGC-3′), the PCR-amplified product was 838 bp; MDR1 primer (forward: 5′-ATCCTGGCTGCAGATGAGTC-3′; reverse: 5′-GCCCTTGCTGCTGACATCTGC-3′), the PCR product was 304 bp; MDR3 primer (forward: 5′-CATGCTTCTGAGCTGCTGACATCTGC-3′; reverse: 5′-TGCAATTAGCCAACCTGGTT-3′), the PCR product was 177 bp. Amplification cycles for MDR1 and β-actin were: 95°C for 3 min, then 30 cycles at 95°C for 35 s, 57°C for 35 s, 72°C for 45 s, followed by 72°C for 5 min. Amplification cycles for MDR3 were: 94°C for 2 min, then 30 cycles at 94°C for 30 s, 64°C for 35 s, 69°C for 60 s, followed by 72°C for 5 min. Aliquots of the PCR product were electrophoresed on 1.8% agarose gels. And PCR fragments were visualized by UV illumination after stained by ethidium bromide. The results of PCR analysis represented the average of three individual experiments.

1.2.5 Modulation of Caspase-3 Expression by siRNA Expression Vector After a transfection for 48 h, 20 µg of total protein was fractionated by 10% PAGE gels and transblotted onto nitrocellulose-ECL membranes. Membranes were blocked overnight with 2% casein prior to incubation with 1:1000 caspase-3 antibody (Santa Cruz Co., USA) at 18°C for 3 h. After washing, bound antibody was detected by using 1:5000 sheep anti-rabbit antibody linked to horseradish peroxidase and bound complexes were detected by using enhanced chemiluminescence. The results of Western blot analysis represented the average of three individual experiments.

1.3 Statistical Analysis Data were expressed as X±s. The statistical significance of differences in mean values was assessed with Student’s t-test by using SPSS 12.0 statistic software. A P<0.05 was considered to be statistically significant.

2 RESULTS

2.1 Quantification of Apoptotic Cells with Annexin V-FITC Assay No significant changes were observed in the early and late apoptosis in A2780/DDP cells after MDR1 and MDR3 siRNA transfection (fig.1). There was no significant difference in cell apoptosis between the pSuppressorNeo group and the untreated transfection groups (P>0.05).

2.2 Cytotoxicity of the Drugs and Determination of Cellular Growth Statistical analysis showed that the cell viability of A2780/DDP cells after MDR1 and MDR3 siRNA transfectant, was not decreased significantly at 20–100 µmol/L of cisplatin (fig. 2). There was no significant difference in cell viability between the pSuppressorNeo group and the untreated groups (P>0.05).

2.3 Inhibition of the MDR1 and MDR3 Gene mRNA Expression by siRNA Vectors The expression of MDR1 and MDR3 in cisplatin-