Knockdown of MDR1 Increases the Sensitivity to Adriamycin in Drug Resistant Gastric Cancer Cells

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Abstract

Gastric cancer, one of the most frequently occurring malignancies in the world, development of multiple drug resistance (MDR) to chemotherapy is known as the major cause of treatment failure for gastric cancer. Multiple drug resistance 1/P-glycoprotein (MDR1/p-gp) contributes to drug resistance via ATP-dependent drug efflux pumps and is overexpressed in many solid tumors including gastric cancer. To investigate the role of MDR1 knockdown on drug resistance reversal, we knocked down MDR1 expression using shRNA in drug resistant gastric cancer cells and examined the consequences with regard to adriamycin (ADR) accumulation and drug-sensitivity. Two shRNAs efficiently inhibited mRNA and protein expression of MDR1 in SGC7901-MDR1 cells. MDR1 knockdown obviously decreased the ADR accumulation in cells and increased the sensitivity to ADR treatment. Together, our results revealed a crucial role of MDR1 in drug resistance and confirmed that MDR1 knockdown could reverse this phenotype in gastric cancer cells.

Keywords: Gastric cancer cell - MDR1 - drug resistance - adriamycin

Materials and Methods

Cell culture and transfection

Human gastric cancer cell line SGC7901 and drug resistance cell line SGC7901-MDR1 were used in the present study. These cells were maintained in RPMI-1640

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medium supplemented with 10-15% heat-inactivated fetal bovine serum, glutamine, and antibiotics at 37 °C in 5% CO2. The transfections were performed using the LipofectamineTM 2000 (Invitrogen, USA) following the manufacturer’s instruction.

Antibodies and regents
Anti-MDR1 and anti-β-actin rabbit polyclonal antibodies (pAb) were purchased from Santa Cruz Biotechnology (USA). Horseradish peroxidase (HRP)-linked goat anti-rabbit IgG and HRP-linked goat anti-mouse IgG were purchased from Zhongshan Goldenbridge Biotechnology (China). G418 and ADR were brought from Invitrogen (USA) and Sigma (USA) respectively. 3- (4, 5-Dimethylthiazol-2-yl) -2, 5- diphenyltetrazolium bromide (MTT) was purchased from Beyotime (China).

Western blots
Western blot analysis was performed as described (Wang et al., 2013). In brief, whole cell lysates were extracted from cells suspended in radio immune precipitation buffer supplemented with PMSF. The lysates were resolved by electrophoresis on polyacrylamide gels containing 0.1% SDS (SDS-PAGE) and then transferred to 0.45 μm PVDF membranes. The membranes were blocked with 5% non-fat milk in Tris Buffer Saline Tween 20 (TBST) buffer. The blots were incubated with the appropriate primary antibody diluted by TBST and then exposed to the appropriate second antibody conjugated with horseradish peroxidase. The bands on the membrane were visualized and captured using the ECL reagent (Beyotime, China) and X-ray films (Kodak, USA).

MTT assay
Cells were treated with ADR at different concentrations for 48 h. 20 μl MTT (5 mg/mL) was added to each well and incubated for an additional 4 h at 37 °C. The purple-blue MTT formazan precipitate was dissolved in 100 μl of DMSO. The activity of the mitochondria, reflecting cellular growth and viability, was evaluated by measuring the optical density at 570 nm.

Data analysis
Statistical significance was determined using the Student’s t-test and a p value of <0.05 was regarded as statistically significant. The standard errors were demonstrated by the bar in the figures.

Results
Establishment of MDR1 knockdown cell line.
Many studies have reported the elevated MDR1 and low sensitivity to chemotherapy drugs in gastric cancer cells (Yamauchi et al., 1992; Li et al., 2011). Since SGC7901 cells express low level of MDR1, we established a drug resistance subline of SGC7901, SGC7901-MDR1, which stably expresses MDR1 in high level. SGC7901-MDR1 cells showed low sensitivity to ADR (data not shown), so it is used as a drug resistance gastric cancer cell line. Two GFP-labeled shRNAs were used to knockdown the expression of MDR1 in SGC7901-MDR1 cells to investigate the roles of MDR1 in drug resistance. G418 screening and FACS sorting were performed after transfection with GFP-positive cells for 48 h and incubated for an additional 4 h at 37 °C. The purple-blue MTT formazan precipitate was dissolved in 100 μl of DMSO. The activity of the mitochondria, reflecting cellular growth and viability, was evaluated by measuring the optical density at 570 nm.

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Figure 2. mRNA and Protein Expression of MDR1 Were Decreased in MDR Knockdown Cell Lines. (A) mRNA and (B) protein of MDR1 in SGC7901, SGC7901-MDR1 (S-MDR1), S-MDR1 sh control, S-MDR1 sh MDR#1, S-MDR1 sh MDR#2 cell lines were detected by Western blot analysis. Analysis of β-actin mRNA or protein was included as a loading control.

Knockdown of MDR1 increased the sensitivity to ADR. shRNAs inhibit the expression of MDR1 in SGC7901-MDR1 cells and the drug resistance of MDR1 knockdown cells was evaluated. SGC7901, SGC7901-MDR1, SGC7901-MDR1 sh control, -sh MDR1#1, and -sh MDR1#2 cells were treated with or without ADR for 90 min. Fluorescent cells were measured by FACS and the specific ADR-positive cells were counted (Figure 3A and B). High percentage of ADR cells was observed in SGC7901 cells, whereas MDR1-expressed cell lines, SGC7901-MDR1 and SGC7901-MDR1 sh control, showed low ADR uptake. ADR-positive cells were increased significantly after MDR1 knockdown in SGC7901-MDR1 cells. Half inhibitory concentration (IC50) of ADR was measured in MDR1 knockdown cells to confirm the sensitivity. Two MDR1 knockdown cell lines showed high sensitivity to ADR, whereas SGC7901-MDR1-sh control cells were more sensitive to the drug. These results indicated that MDR1 expression could induce drug resistance and MDR1 knockdown inhibit drug resistance in SGC7901-MDR1 cells.

Discussion

In the present study, we investigated the consequence of MDR1 knockdown in drug resistance cell line. Our results demonstrated that MDR1 knockdown reverse the drug resistance to ADR in SGC7901-MDR1 cells, which express MDR1 and are resistant to anticancer drugs. These findings indicated the important role of MDR1 expression in multiple drug resistance in gastric cancer cells and that MDR1 knockdown could be used as a strategy to overcome MDR.

Many drug resistance cell lines including SGC7901/VCR (Zhang et al., 2012), SGC7901/DDP (Song et al., 2012), SGC7901/ADM (Huang et al., 2011) have been established to investigate the mechanisms of MDR and to develop the strategies to overcome the MDR in gastric cancer. However, these cell lines were resistant to certain drugs and always lose the property of drug resistance after a few passages. Thus, it’s necessary to use a new and stable drug resistance cell line. In the present study, we used SGC7901-MDR1 cells, which express stably MDR1 in high level and are resistant to ADR. SGC7901 cells rarely express MDR1 and are sensitive to ADR (Figure 2 and 3), so SGC7901-MDR1 cells are a better model of...
gastric cancer in vitro. Our finding confirms that MDR1 expression could induce drug resistance and MDR1 knockdown could reverse this action.

Many studies showed that knockdown of MDR1 reduce drug resistance of several cancer cells including yolk sac carcinoma L1 cells (He et al., 2011), human colon cancer stem cells (Liu et al., 2009), HePG2/MDR1 cells (Pan et al., 2009), human uterine sarcoma cells (Hua et al., 2005), and Caco-2 cells (Hilgendorf et al., 2005). Decreased MDR1 expression induces an increased accumulation of anticancer drugs, resulting in the increased cytotoxicity to cancer cells. We utilized two shRNAs to suppress the expression of MDR1 and the MDR1 mRNA and protein were reduced significantly in MDR1 knockdown cell lines. In MDR1 knockdown cells, the decreased accumulation of ADR was observed, indicating that the ability of pumping ADR out was inhibited by decreased MDR1.

In summary, two MDR1 knockdown cell lines were established using two efficient shRNAs of MDR1. MDR1 expression was significantly decreased in these cells. MDR1 knockdown reduced the accumulation of ADR in gastric cancer cells and increased the sensitivity to ADR treatment. MDR1 knockdown might be a potential and efficient strategy to overcome MDR in gastric cancer cells.

Acknowledgements

We are most grateful to Prof. Hui Wang, director of Research Center for Immunology, for supplying experiment space. This research is supported by the grant from the Open Project Program of Key Subject of Xinxiang Medical University (Grant no. ZD200945) and Programs for Science and Technology Development of Henan Province (Grant no.122102310197).

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