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Original Paper

miR-100 Reverses Cisplatin Resistance in Breast Cancer by Suppressing HAX-1

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Key Words
miR-100 • HAX-1 • Cisplatin • Resistance • Breast

Abstract
Background/Aims: Breast cancer (BC) is the most common cancer in women worldwide. Despite great advancements in cancer therapy in recent years, surgery and chemotherapy are still the mainstays of BC treatment. However, cancer cells usually develop mechanisms to evade cell death induced by chemotherapy. Thus, strategies are needed to reverse the chemoresistance of cancer cells. ***Methods:*** We established cisplatin-resistant BC models in MDA-MB-231 and MCF-7 cells through long-term exposure to cisplatin. Quantitative reverse transcription PCR was used to examine the expression of microRNA (miR)-100. MTT cell viability assays were performed to determine cell viability. Regulation of hematopoietic cell-specific protein 1-associated protein X-1 (HAX-1) targeted by miR-100 was confirmed by western blotting and luciferase reporter assays. The mitochondrial membrane potential and apoptosis were measured by flow cytometry. Release of cytochrome c from the mitochondria into the cytoplasm, HAX-1 expression, and activation of caspase-9 and caspase-3 were detected by western blotting. ***Results:*** A clear decrease in miR-100 expression was observed in cisplatin-resistant MDA-MB-231 and MCF-7 cells (MDA-MB-231/R and MCF-7/R). Overexpression of miR-100 increased the sensitivity of MDA-MB-231/R and MCF-7/R cells to cisplatin treatment and promoted cisplatin-induced mitochondrial apoptosis by targeting HAX-1. ***Conclusions:*** MiR-100 targeted HAX-1 to increase the chemosensitivity of BC by mediating the mitochondrial apoptosis pathway.

Introduction

Breast cancer (BC) is the most common cancer in women worldwide. It causes high mortality because of metastatic spread of the cancer cells to vital organs [1, 2]. Currently,

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surgical resection and chemotherapy are still the main treatment strategies for this disease [3, 4]. However, many patients with BC exhibit a poor response to systematic chemotherapy [5, 6]. Thus, there is an urgent need to develop strategies that reverse the chemoresistance of BC cells.

Cisplatin is an effective broad-spectrum platinum-based anticancer drug used for the treatment of various cancers, including BC. Its mechanism of action involves binding to DNA nucleobases and inducing DNA damage by forming platinum–DNA adducts that trigger apoptosis in cancer cells [7, 8]. However, continuous exposure to cisplatin can cause cancer cells (including BC cells) to develop mechanisms to survive cisplatin treatment [9–11]. Thus, novel interventions are required to reverse chemoresistance to cisplatin in BC treatment.

MicroRNAs (miRNAs) are a class of endogenously expressed and non-coding small RNAs that suppress gene expression by binding to the 3′ untranslated region (3′ UTR) of target miRNAs. Studies have reported that dysregulation of miRNAs is responsible for tumorigenesis and cancer development, because miRNAs participate in various biological processes, including cell proliferation, differentiation, and apoptosis [12–14]. Moreover, dysregulation of miRNAs induces cisplatin resistance in many cancers, including BC [15, 16]. Therefore, targeting dysregulated miRNAs may be a potential strategy for improving the efficiency of cisplatin therapy.

In this study, we investigated the ability of miRNA miR-100, a potential tumor suppressor, to reverse cisplatin resistance in BC.

Materials and Methods

Cell culture

Human breast cancer cell lines MDA-MB-231 and MCF-7 were purchased from American Type Culture Collection (Manassas, VA) and cultured at 37°C in Dulbecco's modified Eagle's medium (Gibco, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS; Gibco) in a humidified 5% CO_2 incubator. To establish cisplatin-resistant BC models, MDA-MB-231 and MCF-7 cells were exposed to increasing concentrations of cisplatin (Sigma-Aldrich, Darmstadt, Germany) for a long term. Briefly, MDA-MB-231 and MCF-7 cells were initially treated with 1 μM cisplatin for 2 months, and the cisplatin concentration was increased every 3 weeks by 0.2 μM to a final concentration of 5 μM.

Quantitative reverse transcription real time PCR

Total RNA was extracted from MDA-MB-231, MDA-MB-231/R, MCF-7, and MCF-7/R cell lines using TRIzol® reagent (Invitrogen, Carlsbad, CA). For analysis of miR-100 expression, total RNA was reverse transcribed using stem-loop reverse transcription primers and the PrimeScript RT Reagent Kit according to the manufacturer's protocol (Takara Bio Inc., Otsu, Japan). The miR-100 reverse transcription primer (Ribobio, Guangzhou, China) had the following sequence: 5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCATACCTA-3′. SYBR® Premix Ex Taq II Reagent (Takara) was used for PCR amplification of miR-100 using the Applied Biosystems 7500 RT Thermocycler (Thermo Fisher Scientific, Waltham, MA). The relative expression of miR-100 was normalized to U6 snRNA and determined according to $2^{-\Delta\Delta Ct}$ analysis [17].

Transfection

For knockdown of hematopoietic cell-specific protein 1-associated protein X-1 (HAX-1), HAX-1 small interfering RNA (siRNA) was purchased from Santa Cruz Biotechnology (Dallas, TX). To overexpress HAX-1, recombinant pcDNA3.1 plasmid (Invitrogen) containing the HAX-1 open reading frame was used. For transfection, 2 μg/mL HAX-1 vector, 50 pmol/mL HAX-1 siRNA, 50 pmol/mL miR-100 mimics (5′-CAAGCUUGUAUCUAUAGGUAUG-3′, GenePharma Co., Ltd., Shanghai, China) and 50 pmol/mL negative control oligonucleotide (NCO, 5′-CUUAUGAGAUCACGUAGUAUGU-3′; GenePharma Co., Ltd.) were transfected into BC cells using Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's instructions.

Luciferase reporter assay

HAX-1 3′ UTR containing the putative miR-100 binding site was cloned downstream of the firefly luciferase gene in the pMIR-REPORT™ miRNA Expression Reporter Vector (Thermo Fisher Scientific) and

designated pMIR-HAX-1. To perform the luciferase reporter assay, cells were incubated in 48-well plates overnight at 37°C. Then, cells were co-transfected with pMIR-HAX-1 and Renilla Luciferase pRL-TK vectors (Promega Corp., Madison, WI) and miR-100 mimics using Lipofectamine 2000. At 48 h post-transfection, the luciferase activities were measured using the Dual Luciferase Reporter Assay System (Promega Corp.) according to the manufacturer's instructions.

Cell viability assay

BC cells were seeded in 96-well plates at a density of 5×10^3 cells per well overnight at 37°C. Subsequently, the cells were treated with different concentrations of cisplatin, carboplatin, and oxaliplatin for 48 h. Cell viability was evaluated using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay as previously described [18]. The absorbance was read at 570 nm using a microplate reader. The half maximal inhibitory concentration (IC_{50}) of cisplatin, carboplatin, and oxaliplatin was calculated according to the cell viability curve of BC cells.

Western blot analysis

Cells were lysed in RIPA lysis buffer (Cell Signaling Technology [CST], Inc., Beverly, MA). Subsequently, 50 μg total proteins were separated by 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane (EMD Millipore, Billerica, MA). Then, the membranes were incubated with primary antibodies (anti-HAX-1, GAPDH, cytochrome c, cytochrome c complex IV [COXIV], caspase-9, and caspase-3; CST) overnight at 4°C. Next, the membranes were incubated with appropriate horseradish peroxidase-conjugated secondary antibodies for 2 h followed by detection with an enhanced chemiluminescence detection kit (Pierce Biotechnology, Rockford, IL).

Mitochondrial membrane potential and apoptosis detection

For detection of the mitochondrial membrane potential ($\Delta\Psi_m$), cells were collected and stained with JC-1 (Molecular Probes, Thermo Fisher Scientific) as an indicator [19] before analysis of $\Delta\Psi_m$ by flow cytometry (Becton Dickinson, Franklin Lakes, NJ) according to the manufacturer's instructions. To measure the apoptotic rate, cells were collected and stained with annexin V/propidium iodide (Sigma-Aldrich). Subsequently, cell apoptosis was analyzed by flow cytometry and annexin V-positive cells were considered to be apoptotic BC cells.

Tumor growth in nude mice

Lentivirus expressing the miR-100 precursor (LV-miR-100) was generated using a lentiviral-based system (Genechem Co., Shanghai, China) according to the manufacturer's instructions. For tumorigenesis assays, MDA-MB-231/R cells were transfected with empty lentivirus or LV-miR-100 before inoculation into the flank of BALB/c, nu/nu). After xenografts reached 0.5 cm in diameter, mice were treated with intraperitoneal injection twice a week (5 mg/kg) until euthanasia (28 days post-injection). The animal care and experimental protocols were approved by the Animal Care Committee of Shandong Provincial Hospital Affiliated to Shandong University (Jinan, China).

Statistical analysis

Data are represented as the mean ± standard deviation, and were obtained from three independent experiments. For comparive analyses, one-way analysis of variance and the Bonferroni post-hoc test were used to determine differences. Statistical analysis was performed using SPSS 14.0 software (SPSS Inc., Chicago, IL). $P<0.05$ was considered statistically significant.

Results

Decrease of miR-100 in cisplatin-resistant BC cells

To investigate the potential role of miR-100 in cisplatin resistance in BC, we first established cisplatin-resistant BC models in MDA-MB-231 and MCF-7 cell lines (MDA-MB-231/R and MCF-7/R, respectively). As shown in Fig. 1A, MDA-MB-231/R and MCF-7/R cells exhibited significant cisplatin resistance compared with their parental MDA-MB-231

and MCF-7 cells, respectively. Next, we detected changes in miR-100 expression in these BC cell lines, and found that miR-100 expression was decreased in both cisplatin-resistant BC models (Fig. 1B). These results suggested that the decrease in miR-100 expression was associated with cisplatin resistance in BC.

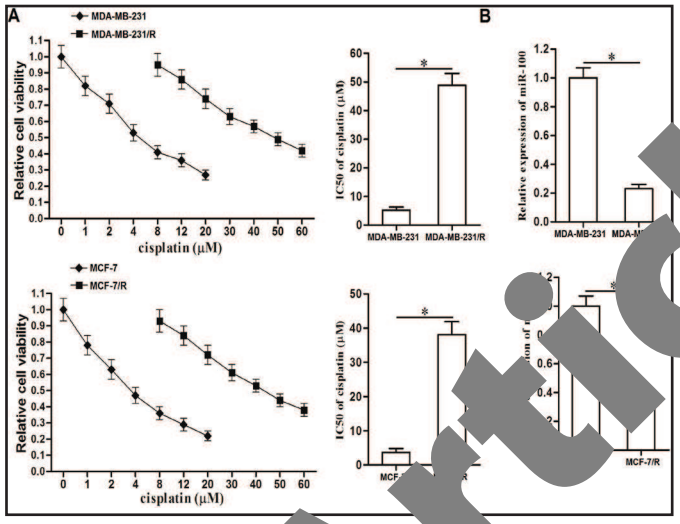


Fig. 1. Expression of miR-100 and cisplatin resistant BC cells. A: After treatment with different concentrations of cisplatin (0–60 μM), the viability of MDA-MB-231, MDA-MB-231/R, MCF-7, and MCF-7/R cells was detected using MTT assays. The IC_{50} of cisplatin against MDA-MB-231, MDA-MB-231/R, MCF-7, and MCF-7/R cells was calculated according to the cell viability curves (*P<0.05). B: Quantitative real-time transcription PCR analysis was performed to detect the expression of miR-100 in MDA-MB-231, MDA-MB-231/R, MCF-7, and MCF-7/R cell lines (*P<0.05).

Recovery of miR-100 increased the sensitivity of cisplatin-resistant BC cells to cisplatin treatment

Because miR-100 expression was decreased in cisplatin-resistant BC cells, we transfected MDA-MB-231/R and MCF-7/R cells with miR-100 mimics to restore miR-100 levels in both cell lines (Fig. 2A). Results of the MTT assay showed that restoration of miR-100 expression significantly increased the sensitivity of MDA-MB-231/R and MCF-7/R cells to cisplatin treatment (Fig. 2B). To confirm the importance of miR-100 in cisplatin-induced toxicity against BC cells, we knocked down miR-100 expression in MDA-MB-231 and MCF-7 cells using miR-100 antisense oligonucleotide (anti-miR-100) (Fig. 2C). Knockdown of miR-100 in MDA-MB-231 and MCF-7 cells clearly decreased cisplatin sensitivity to these BC cells (Fig. 2D). These results suggested that restoration of miR-100 reversed cisplatin resistance in BC cells.

MiR-100 targets HAX-1 in BC

To explore the mechanism by which miR-100 reversed cisplatin resistance, we performed a series of analyses to identify the molecular target of miR-100 in BC. Data from TargetScan, a public miRNA prediction database, showed that the HAX-1 gene contained a seed region paired with miR-100 in the 3′ UTR of its mRNA (Fig. 3A). Previous studies have reported that HAX-1 overexpression decreases apoptosis and promotes the survival of BC cells; thus, we focused on the relationship between miR-100 and HAX-1 in BC [20, 21]. Western blot analysis showed that MDA-MB-231/R and MCF-7/R cells expressed clearly higher levels of HAX-1 compared to their parental MDA-MB-231 and MCF-7 cells (Fig. 3B). These data together with that shown in Fig. 1B led us to speculate that HAX-1 overexpression in cisplatin-resistant BC cells may have been induced by downregulation of miR-100 in these cells. For confirmation, we transfected the BC cells with miR-100 before detection of HAX-1 protein expression. We observed that miR-100 expression decreased HAX-1 levels in MDA-MB-231/R, MCF-7/R, MDA-MB-231/R, and MCF-7/R cells (Fig. 3C). The results of the luciferase reporter assay showed that co-transfection with miR-100 decreased the luciferase activities of pMIR-plasmid carrying HAX-1 3′ UTR (Fig. 3D). Taken together, these data confirmed that miR-100 targets HAX-1 in BC. In addition, overexpression of HAX-1 in cisplatin-resistant BC cells may be induced by decreasing miR-100 levels.

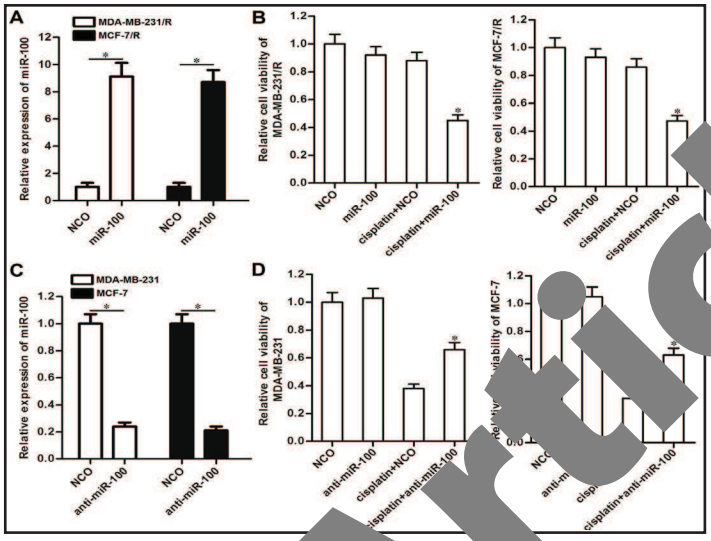


Fig. 2. Effects of miR-100 on decreasing cisplatin resistance in BC. A: Transfection with miR-100 mimics (50 pmol/mL) increased the cellular level of miR-100 in MDA-MB-231/R and MCF-7/R cells (*P<0.05). B: Transfection with miR-100 mimics (50 pmol/mL) increased the sensitivity of MDA-MB-231/R and MCF-7/R cells to cisplatin (10 μM) treatment (*P<0.05 vs. cisplatin + negative control oligonucleotide [NCO] group). C: Transfection with anti-miR-100 (50 pmol/mL) decreased the cellular level of miR-100 in MDA-MB-231 and MCF-7 cells (*P<0.05). D: Transfection with anti-miR-100 mimics (50 pmol/mL) decreased the sensitivity of MDA-MB-231 and MCF-7 cells to cisplatin treatment (*P<0.05 vs. cisplatin + NCO group).

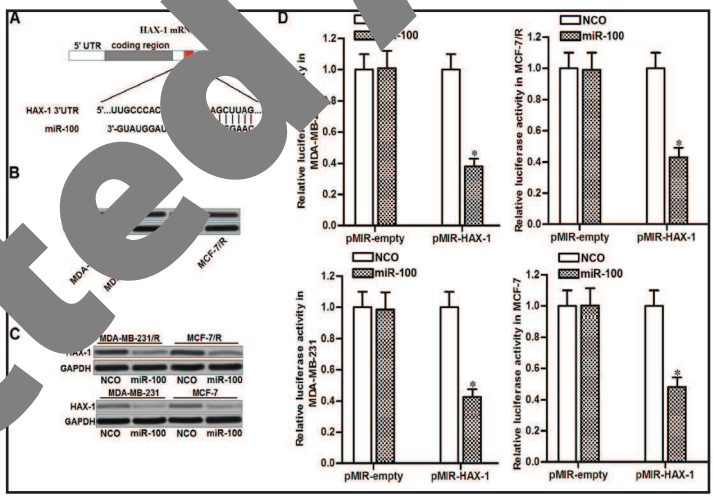


Fig. 3. miR-100 targeted HAX-1 in BC. A: Seed region of HAX-1 3′ UTR paired with miR-100. B: Protein expression of HAX-1 in MDA-MB-231, MDA-MB-231/R, MCF-7, and MCF-7/R cell lines. C: Effects of miR-100 mimics (50 pmol/mL) on inhibiting HAX-1 expression in MDA-MB-231/R and MCF-7/R cells. After co-transfection with miR-100 and pMIR-plasmid carrying HAX-1 3′ UTR in MDA-MB-231/R and MCF-7/R cells, luciferase activities were measured using the Luciferase Reporter Assay System according to the manufacturer's instructions (*P<0.05 vs. NCO group).

miR-100 reversed cisplatin resistance in BC cells by suppressing the HAX-1 pathway

To explore the importance of HAX-1 in the induction of cisplatin resistance in BC, we performed gain-of-function and loss-of-function experiments. As shown in Fig. 4A, transfection with the HAX-1 eukaryotic expression vector abolished the effects of miR-100 on decreasing HAX-1 expression. Meanwhile, transfection with HAX-1 siRNA clearly suppressed HAX-1 expression in MDA-MB-231/R and MCF-7/R cells. Overexpression of HAX-1 partially "rescued" MDA-MB-231/R and MCF-7/R cells co-treated with cisplatin and miR-100. Conversely, knockdown of HAX-1 using specific siRNA enhanced the cytotoxicity of cisplatin against MDA-MB-231/R and MCF-7/R cells, similar to miR-100 (Fig. 4B). Both HAX-1 siRNA and miR-100 enhanced cisplatin-induced apoptosis in MDA-MB-231/R and MCF-7/R cells. However, miR-100-mediated cisplatin-induced apoptosis could be inhibited by HAX-1 overexpression in these cisplatin-resistant BC cells (Fig. 4C). Taken together, these results indicate that the miR-100/HAX-1 axis is an important pathway for determining the sensitivity of cisplatin-resistant BC cells to cisplatin-induced apoptosis. Restoration of miR-100 may represent a potential approach for reversing cisplatin resistance in BC.

MiR-100 increased the sensitivity of cisplatin-resistant BC cells to cisplatin-induced mitochondrial apoptosis

The overexpression of HAX-1 inhibits mitochondrial outer membrane permeabilization (MOMP) in cancer cells [22]. Therefore, we investigated the role of the miR-100/HAX-1 axis in cisplatin-induced apoptosis in cisplatin-resistant BC cells. Flow cytometry analysis showed that cisplatin treatment induced a slight decrease of $\Delta\Psi_m$ in MDA-MB-231/R and MCF-7/R cells. However, combination with miR-100 clearly promoted the cisplatin-induced collapse of $\Delta\Psi_m$ (Fig. 5A). Furthermore, co-treatment with miR-100 was promoted release of cytochrome c in cisplatin-treated MDA-MB-231/R and MCF-7/R ce (Fig. 5B). The results showe that combination treat nt with cisplatin and mi 00 significantly increase the expression of caspase- caspase-3, whic optotic executors in D 31/R and MCF-7 R cel Fig C). In addition over ssion of HAX-1 i ted the apoptotic ay DA-MB-231/R CF-7/R cells co-treated with n and miR-100. Taken together, these results demonstrated that expression miR-100 increased the sensitivity of cisplatin-resistant BC cells to cisplatin-induced mit hondrial apoptosis by suppressing the HAX-1 pathway.

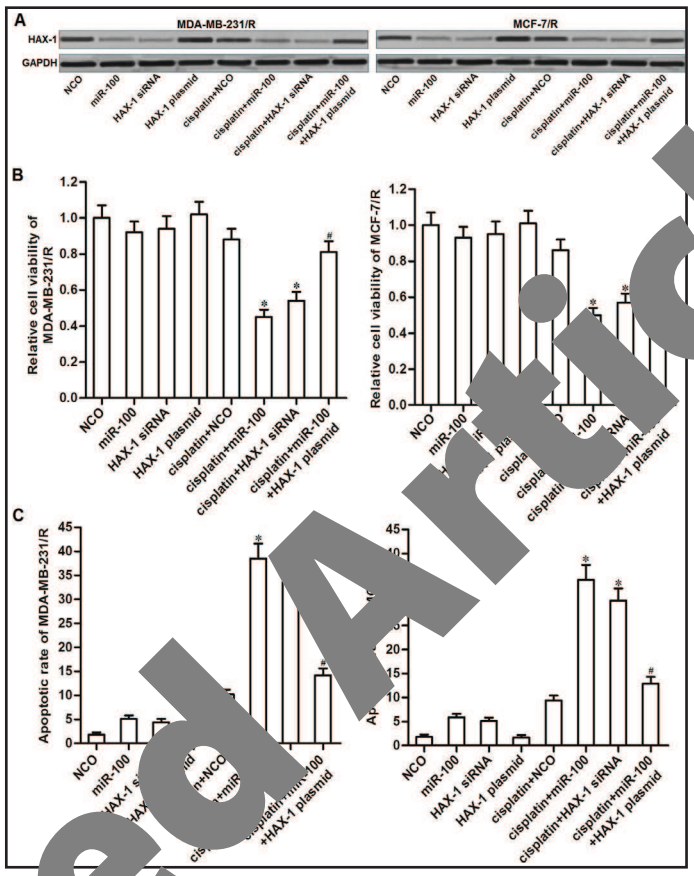


Fig. -10 HAX-1 axis determined the sensitivity of cisplatin-esist lls to cisplatin. A: Effects of HAX-1 plasmid (2 μg/L) and HAX-1 siRNA (50 pmol/mL) on changes in HAX-1 protein on in MDA-MB-231/R and MCF-7/R cells. B: Effects of HAX-1 plasmid (2 μg/mL) and HAX-1 siRNA (50 pmol/mL) on changes n sensitivity of MDA-MB-231/R and MCF-7/R cells to cisplatin (10 μM) treatment (*P<0.05 vs. cisplatin + NCO group; #P<0.05 vs. cisplatin + miR-100 group). C: Effects of HAX-1 plasmid (2 μg/mL) and HAX-1 siRNA (50 pmol/mL) on changes in sensitivity of MDA-MB-231/R and MCF-7/R cells to cisplatin-induced (10 μM) apoptosis (*P<0.05 vs. cisplatin + NCO group; #P<0.05 vs. cisplatin + miR-100 group).

Expression of miR-100 improved the anti-tumor effects of cisplatin in cisplatin-resistant BC models in vivo

To investigate the effects of exogenous miR-100 on improving the anti-tumor effects of cisplatin in cisplatin-resistant BC model *in vivo*, MDA-MB-231/R cells transfected with empty lentivirus (LV-empty) or lentivirus carrying miR-100 precursor (LV-miR-100) were inoculated into nude mice. Mice inoculated with LV-empty-transfected MDA-MB-231/R cells exhibited obvious resistance to cisplatin treatment. However, mice transfected with miR-100-overexpressed MDA-MB-231/R cells showed more sensitivity to cisplatin treatment (Fig. 6A). After euthanasia, the tumor tissues were resected to detect miR-100 and HAX-1

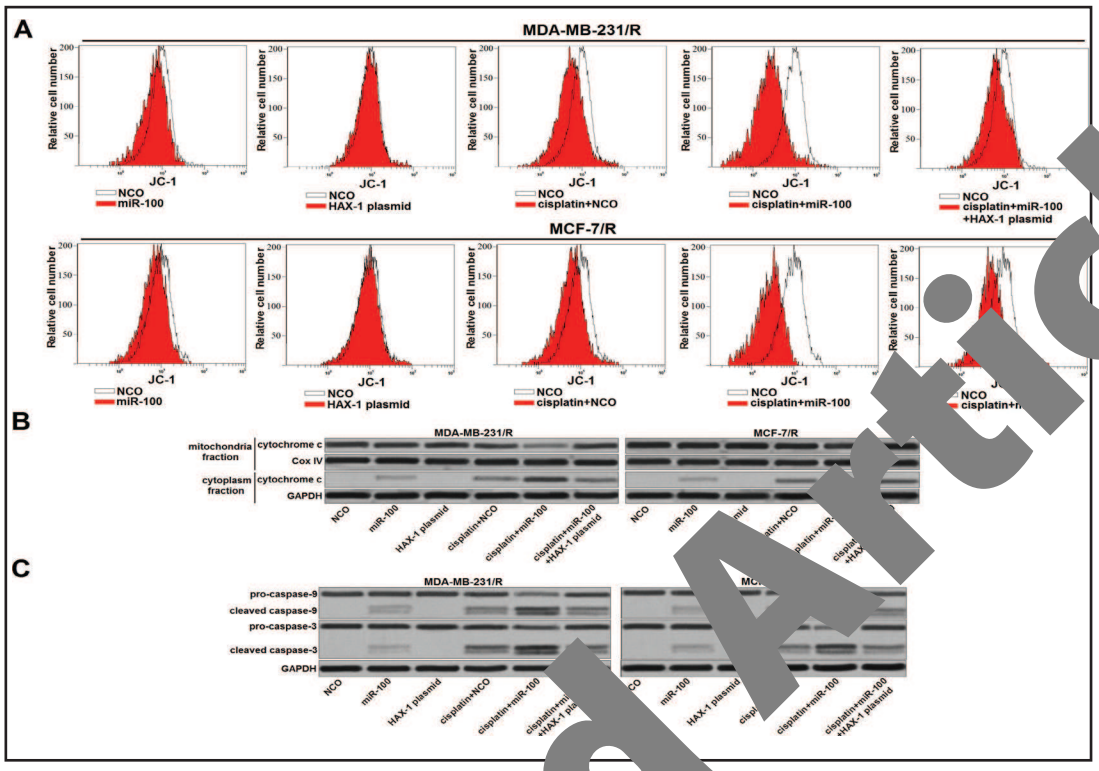
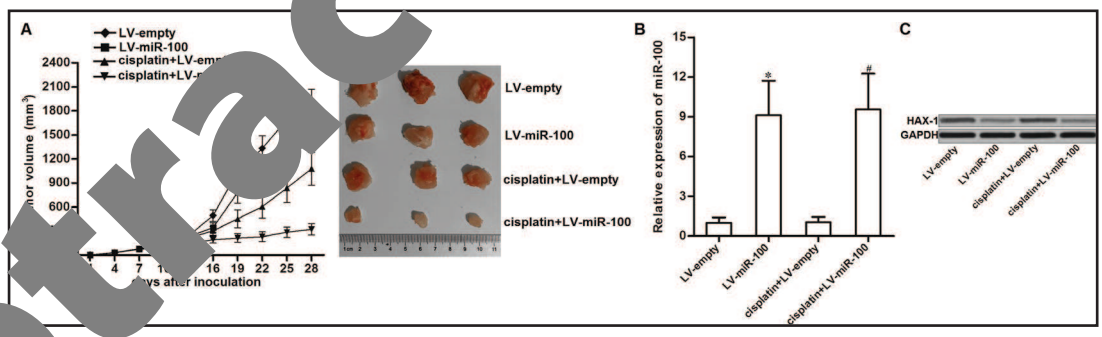


Fig. 5. miR-100 sensitized cisplatin-induced mitoc rial a tosis in cisplatin-resistant BC cells. A: Flow cytometry analysis was performed to d t th MDA-MB-231/R and MCF-7/R cells after treatment with cisplatin (10 μM), miR-100 mic pmol/mL), and HAX-1 expression vector (2 μg/mL). B: Western blot analysis was performed to th lease of cytochrome c from the mitochondria into the cytoplasm of MDA-MB-231/ CF hat were treated with cisplatin (10 μM), miR-100 mimics (50 pmol/mL) and HAX-1 sion vector (2 μg/ml). C: The cleavage of caspase-9 and caspase-3 was evaluated by western blot alysi



g. 6. Expression of miR-100 improved the anti-tumor effects of cisplatin on cisplatin-resistant BC models in . A: Nude mice (n=24) were inoculated with LV-empty-transfected or LV-miR-100-transfected MDA- 231/R cells before treatment with 5 mg/kg cisplatin twice a week. Tumor volumes were detected every 3 days until euthanasia (28 days post-injection). B: Quantitative reverse transcription PCR assays were performed to detect the expression of miR-100 in tumor tissues resected from tumor-bearing mice (*P<0.05 vs. LV-empty group; #P<0.05 vs. cisplatin + LV-empty group). C: Western blot analysis was performed to evaluate the protein expression of HAX-1 in tumor tissues resected from tumor-bearing mice.

expression. Tissues from mice inoculated with LV-miR-100-transfected cells had significantly higher levels of miR-100 than tissues from mice inoculated with LV-empty-transfected cells (Fig. 6B). Conversely, the protein expression of HAX-1 in mice inoculated with LV-

miR-100-transfected MDA-MB-231/R cells was obviously inhibited (Fig. 6C). These results demonstrated that expression of miR-100 improved the anti-tumor effects of cisplatin in cisplatin-resistant BC by suppressing HAX-1 expression *in vivo*.

Expression of miR-100 reversed the resistance of MDA-MB-231/R and MCF-7/R cells to platinum-based chemotherapy drugs

The results of the MTT assay showed that cisplatin-resistant MDA-MB-231 and MCF-7 cells exhibited significant resistance to carboplatin (Fig. 7A) and oxaliplatin (Fig. 7B). However, miR-100 expression in these cisplatin-resistant BC cells reversed the resistance to platinum-based chemotherapy drugs and decreased the IC_{50} of carboplatin (Fig. 7C) and oxaliplatin (Fig. 7D) to MDA-MB-231/R and MCF-7/R cells. These results indicated that expression of miR-100 was able to reverse the resistance of BC cells to platinum-based chemotherapy.

Discussion

Recent studies have indicated that changing miRNA expression is an important mechanism by which cancer cells develop chemoresistance to various anti-cancer drugs. Among the dysregulated miRNAs, miR-100 downregulation has been found in multiple cancers. Because miR-100 reportedly inhibits cancer development and promotes cancer cells apoptosis, it is considered to be a potential tumor suppressor [23–26]. In addition, some studies have reported that the absence of miR-100 is associated with a poor prognosis in cancer patients [27–29]. Therefore, restoration of miR-100 expression may be an efficient therapeutic strategy for cancer.

In this study, we focused on the potential role of miR-100 in cisplatin resistance in BC. We observed the significant decrease of miR-100 expression in cisplatin-resistant MDA-

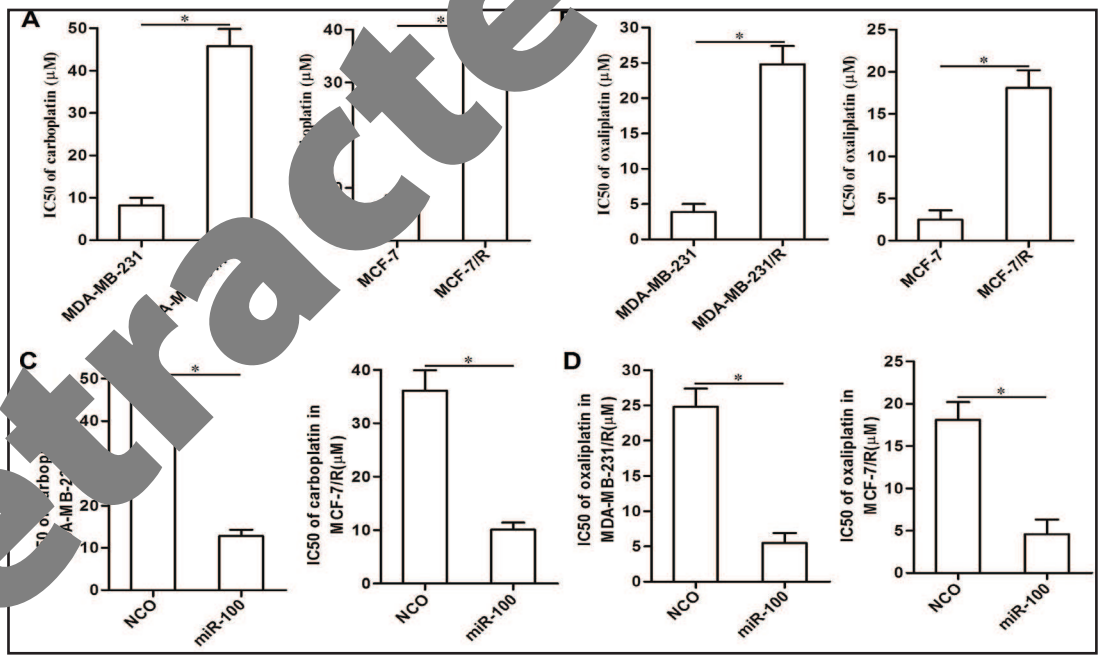


Fig. 7. Effects of miR-100 on reversing the resistance of cisplatin-resistant BC cells to carboplatin and oxaliplatin. A: IC_{50} of carboplatin to MDA-MB-231, MDA-MB-231/R, MCF-7, and MCF-7/R cell lines (*P<0.05). B: IC_{50} of oxaliplatin to MDA-MB-231, MDA-MB-231/R, MCF-7, and MCF-7/R cell lines (*P<0.05). C: Effects of miR-100 mimics (50 pmol/mL) on decreasing the IC_{50} of carboplatin to MDA-MB-231/R and MCF-7/R cell lines (*P<0.05). D: Effects of miR-100 mimics (50 pmol/mL) on decreasing the IC_{50} of oxaliplatin to MDA-MB-231/R and MCF-7/R cell lines (*P<0.05).

MB-231 and MCF-7 BC cell lines. Interestingly, restoration of miR-100 in these cisplatin-resistant BC cells reversed cisplatin resistance both *in vitro* and *in vivo*. Thus, restoring miR-100 expression in cisplatin-treated cells may be a potential approach for reversing the chemoresistance of BC cells.

In cisplatin-induced cytotoxicity against cancers, induction of apoptosis pathway is an important mechanism [30, 31]. Among the cellular proteins that regulate the apoptosis pathway of cancer cells, HAX-1 is a key anti-apoptotic protein located in the mitochondria [32]. Previous studies have shown that HAX-1 maintains the $\Delta\Psi_m$ and suppresses MOMP [33, 34]. Therefore, cellular HAX-1 inhibits mitochondrial apoptosis in cancer cells. Recent studies have demonstrated that overexpression of HAX-1 is responsible for chemoresistance in a variety of cancers including esophageal squamous carcinoma, laryngeal cancer, and breast cancer [35–37]. HAX-1 is considered a potential target for improving the efficiency of chemotherapy. In this study, the overexpression of HAX-1 in cisplatin-resistant BC cells led to induction of cisplatin resistance in BC cells. Thus, inhibiting HAX-1 expression may represent a potential strategy for enhancing chemosensitivity to cisplatin.

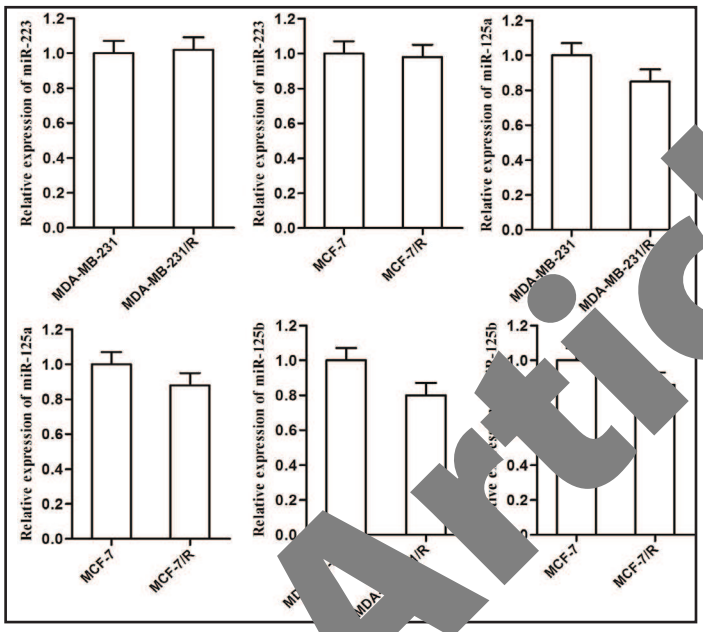


Fig. 8. Relative expression of miR-223, miR-125a, and miR-125b in MDA-MB-231, MDA-MB-231/R, MCF-7 and MCF-7/R cell lines.

Previous studies have reported that HAX-1 can be regulated by miRNAs such as miR-125a, miR-125b, and miR-223 [, 38, 39]. However, we did not observe a significant difference in miR-223 expression between cisplatin-resistant BC cells and their parental cells. Meanwhile, the reduction of miR-125a (reduced by 13.5%) and miR-125b (reduced by 16.9%) expression was observed in BC cells became cisplatin-resistant (Fig. 8). Mechanistically, our data showed that HAX-1 overexpression was induced by decrease of miR-100 in cisplatin-resistant BC cells. Restoration of miR-100 was able to suppress the aberrant expression of HAX-1 in cisplatin-resistant BC cells, thereby resensitizing these cells to cisplatin-induced mitochondrial apoptosis through suppression of HAX-1 expression.

Development of cisplatin resistance in cancer cells usually induces resistance to platinum derivatives [40]. In our study, we also observed the resistance of cisplatin-resistant BC cells to carboplatin and oxaliplatin. However, combination treatment with miR-100 reversed the resistance to these platinum-based anti-tumor drugs.

Taken together, the results of this study provide strong evidence that the miR-100/HAX-1 axis is associated with chemoresistance to platinum-based anti-tumor drugs in BC. Combination treatment with miR-100 may represent an effective approach for improving the anti-tumor effects of platinum-based chemotherapeutic drugs in BC.

Acknowledgements

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Disclosure Statement

The authors declare no conflict of interests.

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