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Suppression of HAX-1 induced by miR-325 resensitizes bladder cancer cells to cisplatin-induced apoptosis

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Abstract. – OBJECTIVE: MicroRNA-325 (miR-325) is a tumor suppressor in some cancers. However, the role of miR-325 in determining the chemosensitivity to cancer cells is still not clear. The aim of this study was to investigate the effect of miR-325 on reversing the cisplatin resistance of bladder cancer.

MATERIALS AND METHODS: Cisplatin-resistant 5637 and T24 bladder cancer cell lines (5637/R and T24/R) were established through long term exposure of them to cisplatin. 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) assays were performed to evaluate the viability of 5637, 5637/R, T24, and T24/R cells. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was used to examine the expression of miR-325 in these cell lines. The regulatory mechanism was confirmed by Western blot analysis and Luciferase reporter assays. After treatment with miR-325 and cisplatin, mitochondrial membrane potential (MMP) and apoptosis were measured using flow cytometry. Expression of hematopoietic cell-specific protein 1-associated protein X-1 (HAX-1) and activation of caspase-9, caspase-7, and caspase-3 were detected by Western blotting.

RESULTS: We found the downregulation of miR-325 in 5637/R and T24/R cells compared to their parental 5637 and T24 cells. Moreover, overexpression of miR-325 in cisplatin-resistant bladder cancer cells was found to increase the cytotoxicity of cisplatin to them. However, transfection with HAX-1 plasmid can abolish the effect of miR-325 on cisplatin. We, then, showed that overexpression of miR-325 suppressed the expression of HAX-1. Thus, miR-325 promoted the mitochondria collapse and cisplatin-induced apoptosis in bladder cancer cells.

CONCLUSIONS: Downregulation of miR-325 is responsible for the development of cisplatin resistance in bladder cancer. Overexpression of miR-325 may represent a novel strategy to reverse the chemoresistance of bladder cancer.

Key Words:
Bladder cancer, Resistance, MiR-325, HAX-1, Apoptosis.

Introduction

Bladder cancer is the most common type of malignant cancer involving the urinary system in the world[1,2]. At initial diagnosis, approximately 30% of patients are diagnosed with muscle-invasive bladder cancer. For these patients, platinum-based chemotherapy is still a standard and major treatment approach. However, a large proportion of patients benefit little from chemotherapy because of the chemoresistance[3-5].

Cisplatin is the commonly used chemotherapeutic drug for bladder cancer treatment[6,7]. Cisplatin in bladder cancer cells damages the cellular DNA by forming the cross-link with DNA. As the apoptosis initiating signal, DNA damage induces apoptotic cell death of cancer cells[8,9]. However, bladder cancer cells usually develop mechanisms to evade the cisplatin-induced apoptosis. As one of the important strategies, bladder cancer cells usually overexpressed the anti-apoptotic proteins to antagonize the cisplatin-induced cell death[10,11]. It is urgent to develop strategies to impair the chemoresistance of bladder cancer cells.

MicroRNAs (miRNAs) are endogenous, small and non-coding RNA molecules. They function as gene suppressors through binding with targeted mRNAs at the 3' untranslated region (3' UTR)[12,13]. As miRNAs regulate about 60% of human genes, they are involved in various physiological processes including proliferation, metabolism, survival and apoptosis. Therefore, dysregulation of miRNAs in cancer cells is responsible for tumor development and drug resistance[14-18]. In the present study, we showed that miR-325 was downregulated in the cisplatin-resistant bladder cancer cells. However, overexpression of miR-325 was found to be able to increase the cisplatin sensitivity of these cells.

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Materials and Methods

Cell Culture

Human bladder cancer cell lines 5637 and T24 were cultured under the condition recommended by the vendor American Type Culture Collection (ATCC; Rockville, Manassas, VA, USA). Cells were cultured in 5% CO_2 incubator at 37°C. To acquire the cisplatin-resistant bladder cancer cell lines, 5637 and T24 cells were gradually exposed to increasing concentrations of cisplatin from 0.5 μM to 2 μM. The acquired cisplatin-resistant 5637 and T24 were named as 5637/R and T24/R.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Relative expression of miR-325 was measured by using qRT-PCR analysis. Briefly, cellular total RNAs were extracted by using the TRIzol reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instruction. Next, the extracted RNAs were used to synthesize the cDNAs by using One Step PrimeScript miRNA cDNA Synthesis Kit (TaKara Bio, Inc., Otsu, Shiga, Japan). Finally, the expression of miR-325 was measured by using SYBR Premix Ex Taq (TaKaRa, Bio, Inc., Otsu, Shiga, Japan) according to the manufacturer's instruction. The primer sequence for miR-325 amplification is as follows: 5'-TTTATTGAGGACCTCCTATCAA-3'.

Transfection

Recombinant pcDNA3.1 plasmid carrying HAX-1 open reading frame, hsa-miR-325 mimic (5'-UUUAUUGAGGACCUCCUAUCAA-3'), hsa-miR-325 antisense-oligonucleotide (miR-325 inhibitor, 5'-UUGAUAGGAGGUCCUCAAUAAA-3'), negative control oligonucleotide (NCO, 5'-CCAAAUGCAAUCUGCUUUGUAU-3') (GenePharma Co. Ltd, Shanghai, China) and HAX-1 siRNA (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were transfected into cells by using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction.

Cell Viability Assay

After transfection, cells were seeded into 96-well plates overnight. Next, the adherent bladder cancer cells were treated with platinum-based chemotherapeutic drugs (cisplatin, carboplatin and oxaliplatin) followed by incubation for 48 h. After cisplatin treatment, 20 μl of 5 mg/ml MTT reagent (Sigma-Aldrich, St. Louis, MO, USA) was added into each well for another 4 h incubation. To evaluate the cell viability, the absorbance at 570 nm for each well was read on an enzyme-linked immunosorbent assay (ELISA) microplate reader (Sunrise Microplate Reader, TECAN, Switzerland). 50% inhibiting concentration (IC50) of cisplatin to bladder cancer cells was measured according to the cell viability curve.

Luciferase Reporter Assay

A fragment of HAX-1 3' UTR was cloned into the downstream of firefly Luciferase gene in the pMIR-REPORT™ miRNA Expression Reporter Vector (Thermo Fisher Scientific, Inc, Waltham, MA, USA). For Luciferase reporter assay, cells were plated into 48-well plates overnight. Next, bladder cancer cells were co-transfected with miR-325 (or NCO, 50 pmol/ml), recombinant pMIR reporter plasmid (2 μg/ml) and Renilla Luciferase pRL-TK vectors (100 ng/ml, Promega, Madison, WI, USA) for 48 h. After incubation, Luciferase activities were measured by using the Dual-Luciferase Reporter assay system (Promega, Madison, WI, USA) according to the manufacturer's instruction. Firefly Luciferase was normalized to the *Renilla* Luciferase.

Western Blot Analysis

Protein expression in bladder cancer cells was detected by Western blot analysis. Briefly, cellular total proteins were extracted using radio immunoprecipitation assay (RIPA) buffer (Cell Signaling Technologies, Danvers, MA, USA). Next, 50 μg of the extracted proteins were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by transference to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The resulting membranes were then blocked in 5% non-fat milk before incubation with different primary antibodies purchased from Santa Cruz Biotechnology (anti-human HAX-1) and Cell Signaling (including anti-human glycerophosphate dehydrogenase (GAPDH), cytochrome c, caspase-9, caspase-7, and caspase-3) overnight. Subsequently, the membranes were washed and probed with horseradish peroxidase-conjugated antibodies. Signals were detected by using an enhanced chemiluminescence detection kit (ECL; Pierce, Rockford, IL, USA). In addition, to detect the released cytochrome c in cytoplasm, cellular mitochondria were separated by using Mitochon-

dria/Cytosol Fraction Kit (BioVision, Milpitas, CA, USA) according to the manufacturer's instruction.

Flow Cytometry Analysis

Mitochondrial membrane potential (MMP) and cell apoptosis were detected by flow cytometry. For evaluation of MMP, cells were stained with 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolyl carbocyanine iodide (JC-1, Molecular Probes; Waltham, MA, USA) as an indicator. Cells emitted red fluorescence were considered as the cells with high MMP[19]. For measurement of cell apoptosis, cells were staining with Annexin V-FITC Apoptosis Detection Kit (Sigma-Aldrich, St. Louis, MO, USA) according to their manufacturer's instruction. The Annexin V-positive cells were considered as the apoptotic cells.

Statistical Analysis

All data were represented as the mean ± standard deviation (SD). Data were carried out by at least three independent experiments. For comparison analysis, two-tailed Student's *t*-tests were used to estimate the statistical differences between two groups. One-way analysis of variance (ANOVA) and Bonferroni's post-hoc test were used to determine the differences between three or more groups. Statistical analysis was performed by using SPSS 15.0 software (SPSS Inc, Chicago, IL, USA). Values of $p<0.05$ were considered significant.

Results

MiR-325 is Downregulated in Cisplatin-Resistant Bladder Cancer Cells

To study the cisplatin resistance of bladder cancer, we established the cisplatin-resistant 5637 and T24 cell lines through gradual exposure to increasing concentrations of cisplatin. Results of MTT assays showed that IC50 of cisplatin to the established 5637/R cells was remarkably lower than their parental 5637 cells (Figure 1A). Similarly, cisplatin showed lower cytotoxicity to T24/R rather than the T24 cells (Figure 1B). Next, we compared the expression of miR-325 between cisplatin-resistant bladder cancer cells and their parental cell lines. We found that expression level of miR-325 was significantly decreased in cisplatin-resistant bladder cancer cells compared to their parental 5637 and T24 cells (Figure 1C). Change of miR-325 expression may be associated with cisplatin resistance in 5637/R and T24/R cells.

Decrease of MiR-325 Expression is Responsible for Cisplatin Resistance of 5637/R and T24/R

To investigate the role of miR-325 in cisplatin-induced cytotoxicity in 5637/R and T24/R, we transfected these cells with miR-325 mimics to overexpress it (Figure 2A). Of note, we found that overexpression of miR-325 significantly increased the sensitivity of 5637/R and T24/R to cisplatin-induced cytotoxicity (Figure 2B). To confirm the effect of miR-325 on regulating the cisplatin sensitivity of bladder cancer cells, we transfected the parental 5637 and T24 cells with miR-325 inhibitor (Figure 2C). We, then, found that absence of miR-325 decreased the cytotoxicity of cisplatin against 5637 and T24 cells (Figure 2D). Taken together, we suggested that expression of miR-325 was associated with cisplatin sensitivity. Decrease of miR-325 expression was responsible for cisplatin resistance in bladder cancer.

HAX-1 is the Target of MiR-325 in Bladder Cancer

To explore the downstream gene of miR-325 in bladder cancer, public databases of TargetScan, miRanda, and PicTar were used to search the potential target of miR-325. All of the three miRNA databases showed that HAX-1 mRNA 3' UTR contained highly conserved sequences which were complementarily paired with miR-325 (Figure 3A). Furthermore, by contrast to the downregulation of miR-325 in cisplatin-resistant bladder cancer cells (Figure 1C), 5637/R and T24/R cells expressed significantly higher level of HAX-1 compared to the 5637 and T24 cells, respectively (Figure 3B). We thus inferred that HAX-1 may be the target of miR-325 in bladder cancer. To prove the miR-325/HAX-1 axis, we detected the HAX-1 expression after changing the cellular level of miR-325 in bladder cancer cells. We observed that overexpression of miR-325 in 5637/R and T24/R cells can decrease the expression of HAX-1 (Figure 3C). By contrast, transfection with miR-325 inhibitor increased the protein level of HAX-1 in 5637 and T24 (Figure 3D). It indeed showed that cellular miR-325 determined the expression HAX-1. To detect the miR-325/HAX-1 axis in bladder cancer, we performed Luciferase reporter assays. As shown in Figure 3E, miR-325 significantly decreased the Luciferase

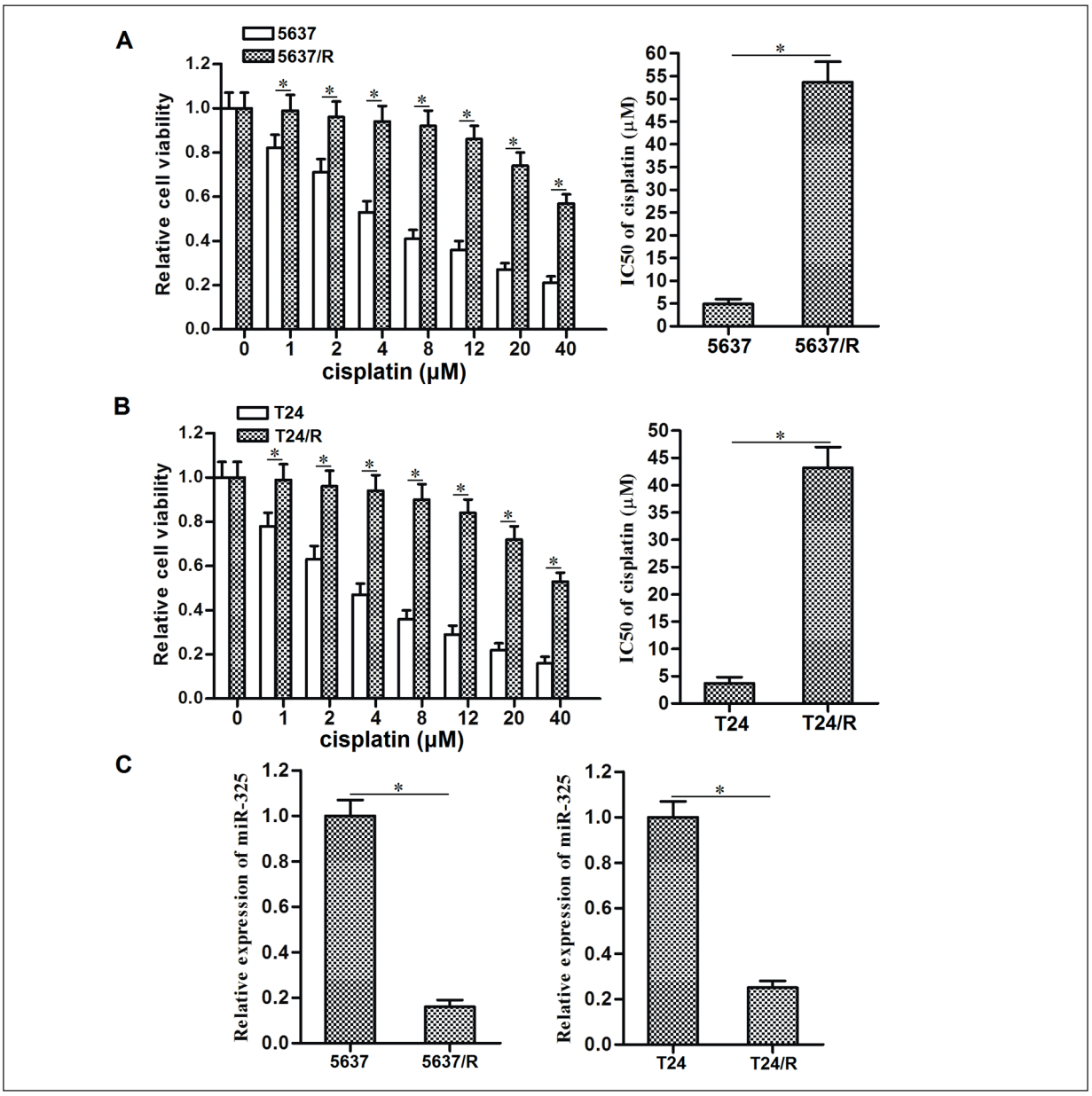

Figure 1. MiR-325 is downregulated in cisplatin-resistant bladder cancer cells. **A,** Cell viability of 5637 and 5637/R cells after treatment with different concentrations (0-40 μM) of cisplatin. IC50 of cisplatin to 5637 and 5637/R was measured according to the cell viability curve. *p<0.05. **B,** Cell viability of T24 and T24/R cells after treatment with different concentrations (0-40 μM) of cisplatin. IC50 of cisplatin to T24 and T24/R was measured according to the cell viability curve. *p<0.05. **C,** QRT-PCR analysis was performed to measure the expression of miR-325 paired with 5637/5637/R and T24/T24/R. *p<0.05.

activities in 5637/R, T24/R, 5637 and T24. Taken together, we demonstrated that HAX-1 was the target of miR-325 in bladder cancer.

Overexpression of MiR-325 Attenuates the Cisplatin Resistance of 5637/R and T24/R Through Suppression of HAX-1

To explore the role of miR-325/HAX-1 axis in regulating the cisplatin sensitivity in 5637/R and T24/R, we performed gain-of-function and loss-of-function experiments on HAX-1 through transfection with HAX-1 siRNA and HAX-1 plasmid. The transfection efficiency of HAX-1 siRNA and HAX-1 plasmid in 5637/R and T24/R was shown in Figure 4A. Similarly, with miR-325, we found that HAX-1 siRNA increased the cisplatin-induced cytotoxicity against 5637/R and T24/R cells. On the con-

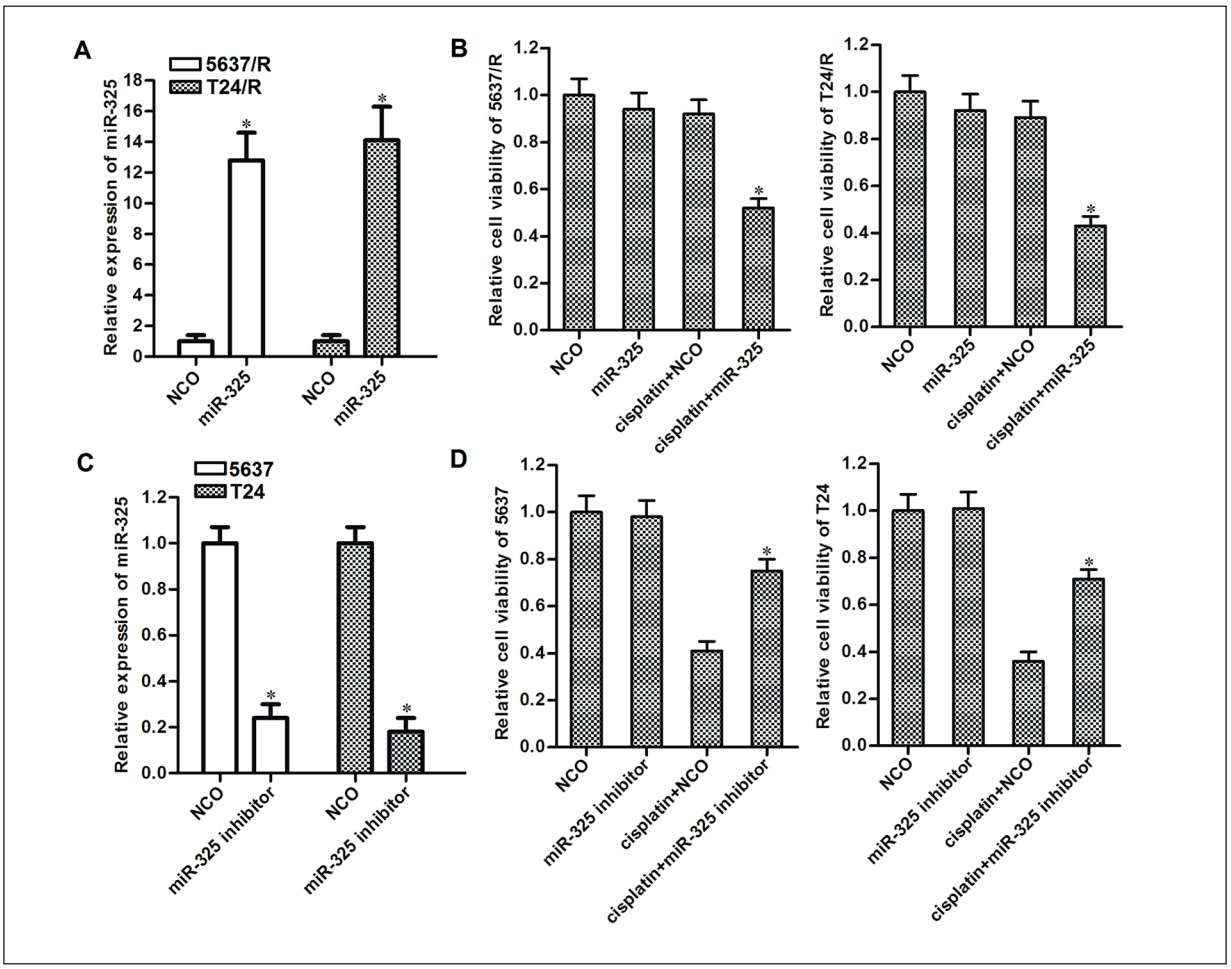

Figure 2. Role of miR-325 in regulating the cisplatin sensitivity in bladder cancer cells. **A,** Effect of miR-325 mimics transfection in 5637/R and T24/R cells. *p<0.05 *vs.* NCO group. **B,** MiR-325 enhanced the cytotoxicity of cisplatin against 5637/R and T24/R cells. *p<0.05 *vs.* cisplatin+NCO group. **C,** Transfection efficiency of miR-325 inhibitor in 5637 and T24 cells. *p<0.05 *vs.* NCO group. **D,** MiR-325 inhibitor induced cisplatin resistance in 5637 and T24 cells. *p<0.05 *vs.* NCO group.

trary, overexpression of HAX-1 by using HAX-1 plasmid abolished the effect of miR-325 on reducing the drug resistance of 5637/R and T24/R cells (Figure 4B). These data indicated that overexpression of miR-325 can attenuate the cisplatin resistance of 5637/R and T24/R through suppression of HAX-1. We next investigated the role of HAX-1 in changing the sensitivity of routine 5637 and T24 cells to cisplatin treatment. The transfection efficiency of HAX-1 siRNA and HAX-1 plasmid in 5637 and T24 was shown in Figure 4C. We observed that overexpression of HAX-1 markedly induced resistance to cisplatin in 5637 and T24, similarly with miR-325 inhibitor. By contrast, knockdown of HAX-1 by using HAX-1 siRNA increased the cytotoxicity of cisplatin to 5637 and T24 cells which were transfected with miR-325 inhibitor (Figure 4D). We demonstrated that miR-325/HAX-1 axis regulated the cisplatin resistance in bladder cancer.

MiR-325/Axis Regulates Sensitivity of 5637/R and T24/R cells to Cisplatin-Induced Mitochondrial Apoptosis

To study the role of miR-325/HAX-1 axis in regulating the cisplatin-dependent mitochondrial apoptosis in 5637/R and T24/R, the mitochondrial membrane potential (MMP) was tested. We found that the cisplatin-dependent MMP collapse can be expanded by overexpression of miR-325. However, co-transfection with HAX-1 plasmid significantly inhibited the effect of miR-325 on the MMP of 5637/R and T24/R cells (Figure 5A). Furthermore, we showed that miR-325 promoted

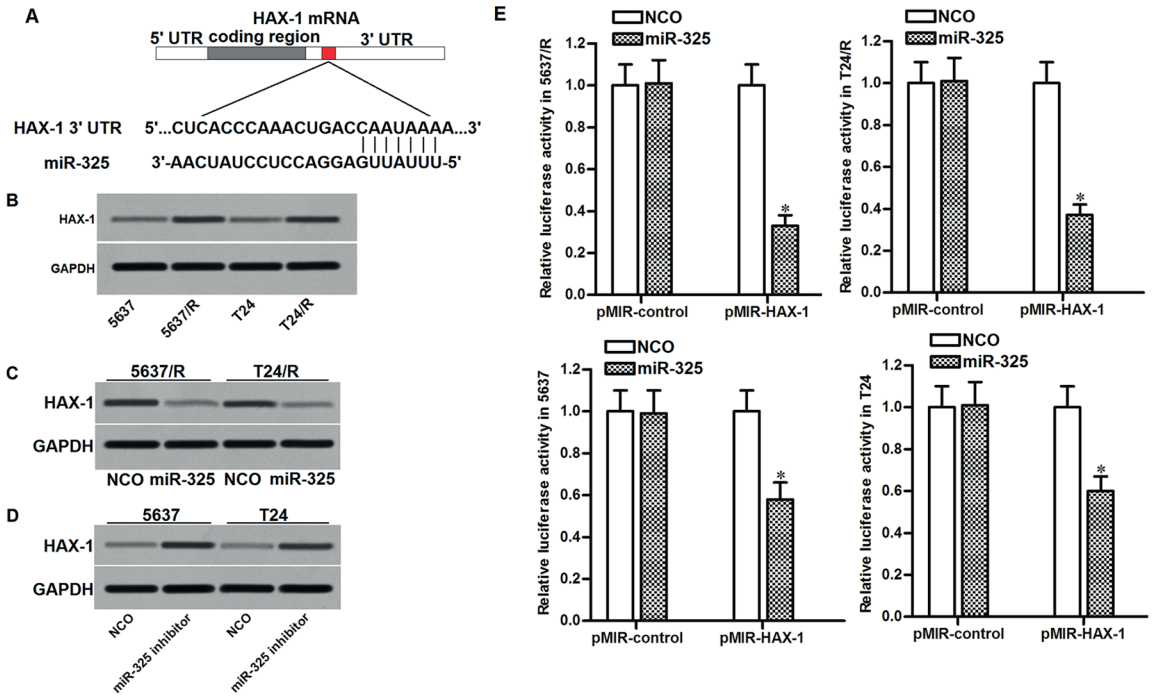

Figure 3. HAX-1 is the target of miR-325 in bladder cancer. **A,** TargetScan, miRanda and PicTar miRNA databases showed the putative miR-325 binding sites on 3' UTR of HAX-1 mRNA. **B,** Protein expression of HAX-1 in 5637, 5637/R, T24 and T24/R cells. **C,** Effect of miR-325 on changing the HAX-1 expression in 5637/R and T24/R cells at the protein level. **D,** Effect of miR-325 inhibitor on changing the HAX-1 expression in 5637 and T24 cells at the protein level. **E,** Luciferase activities in 5637, 5637/R, T24 and T24/R cells were measured by using Dual-Luciferase Reporter Assay System according to the manufacturer's instruction. *$p<0.05$ *vs.* NCO group.

release of cytochrome c from mitochondria into cytosol of 5637/R and T24/R through the HAX-1 pathway (Figure 5B). These data indicated that overexpression of miR-325 can enhance the damage of mitochondria in cisplatin-treated 5637/R and T24/R cells. As the downstream of mitochondrial pathway, we observed that overexpression of miR-325 strongly increased the cleavage of caspase-9, Caspase-7, caspase-3 (Figure 5C) and the final cell apoptosis (Figure 5D) in the cisplatin-treated 5637/R and T24/R. We demonstrated that miR-325 can increase the sensitivity of 5637/R and T24/R cells to cisplatin-induced mitochondrial apoptosis through suppression of HAX-1.

Overexpression of MiR-325 Attenuated the Cross Resistance of Carboplatin and Oxaliplatin in 5637/R and T24/R

To study the effect of miR-325/HAX-1 axis on regulating the cross resistance of some other platinum-based drugs, we treated the 5637/R and T24/R cells with carboplatin and oxaliplatin. We found that IC50 of carboplatin and oxaliplatin to 5637/R and T24/R cells was significantly higher compared to their parental 5637 and T24 cells, respectively (Figure 6A). It indicated that both 5637/R and T24/R cells showed cross resistance to platinum-based drugs. Next, we found that overexpression of miR-325 significantly increased the cytotoxicity of carboplatin (Figure 6B) and oxaliplatin (Figure 6C) to 5637/R and T24/R cells. However, co-transfection with HAX-1 plasmid inhibited the effect of miR-325 on reversing the resistance to carboplatin (Figure 6B) and oxaliplatin (Figure 6C). Taken together, we demonstrated that overexpression of miR-325 can attenuate the cross resistance of platinum-based drugs to bladder cancer.

Discussion

Despite cisplatin is commonly used for treatment of bladder cancer, drug resistance remains a major obstacle that restricts its clinical appli-

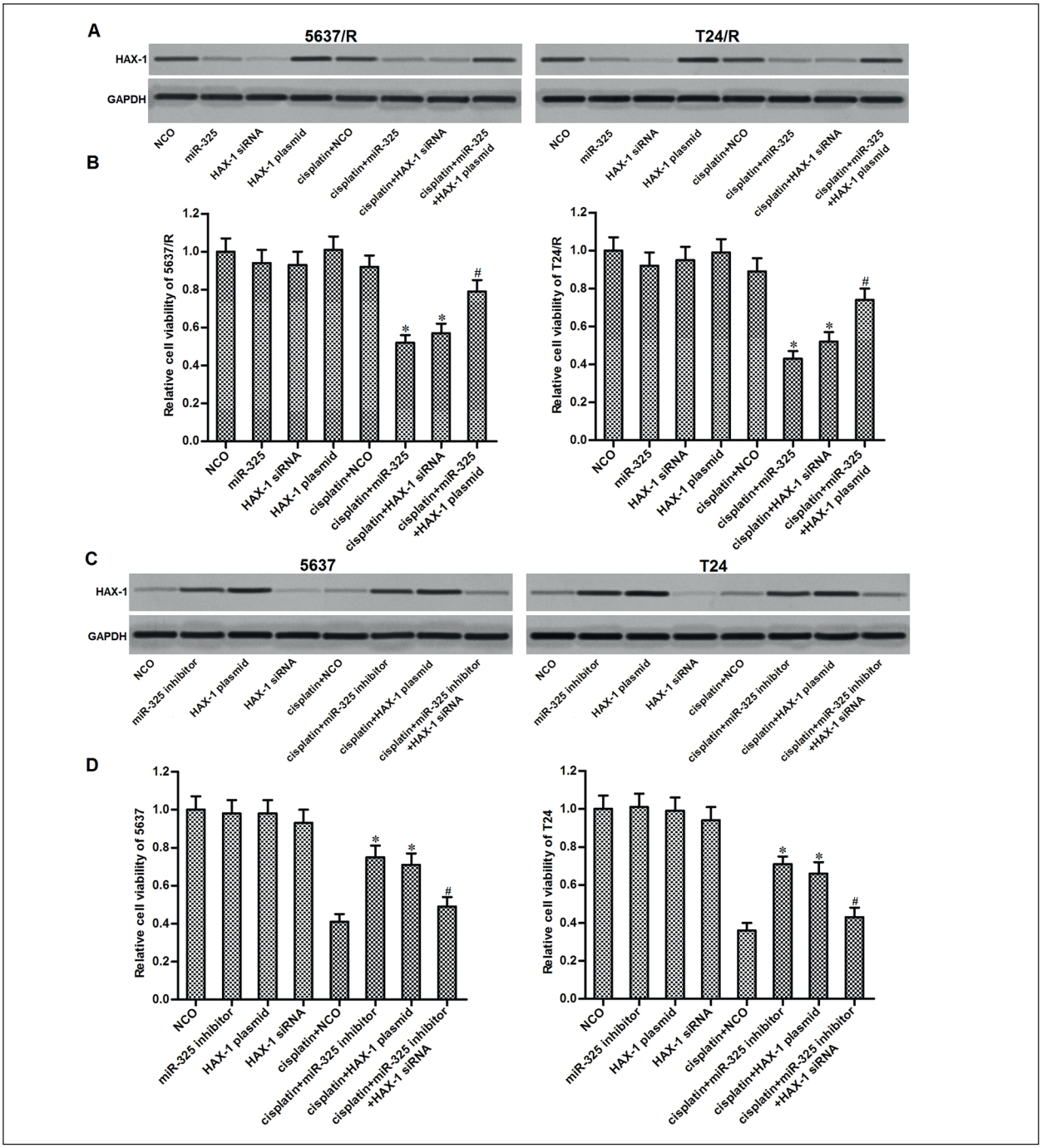

Figure 4. Role of miR-325/HAX-1 axis in regulating the cisplatin sensitivity in bladder cancer. **A,** Effect of miR-325, HAX-1 siRNA and HAX-1 plasmid transfection in 5637/R and T24/R cells. **B,** Effect of miR-325, HAX-1 siRNA and HAX-1 plasmid on regulating the cisplatin-induced cytotoxicity in 5637/R and T24/R cells. *p<0.05 *vs.* cisplatin+NCO group. $^{\#}p$<0.05 *vs.* cisplatin+miR-325 group. **C**, Effect of miR-325 inhibitor, HAX-1 siRNA and HAX-1 plasmid transfection in 5637 and T24 cells. **D,** Effect of miR-325 inhibitor, HAX-1 siRNA and HAX-1 plasmid on regulating the cisplatin-induced cytotoxicity in 5637 and T24 cells. *p<0.05 *vs.* cisplatin+NCO group. $^{\#}p$<0.05 *vs.* cisplatin+miR-325 inhibitor group.

cation. Recently, studies[20-22] have demonstrated that dysregulation of miRNAs in cancer cells is closely associated with development of drug resistance. Therefore, correcting the miRNA dysregulation may represent a promising strategy for attenuating the chemoresistance in bladder

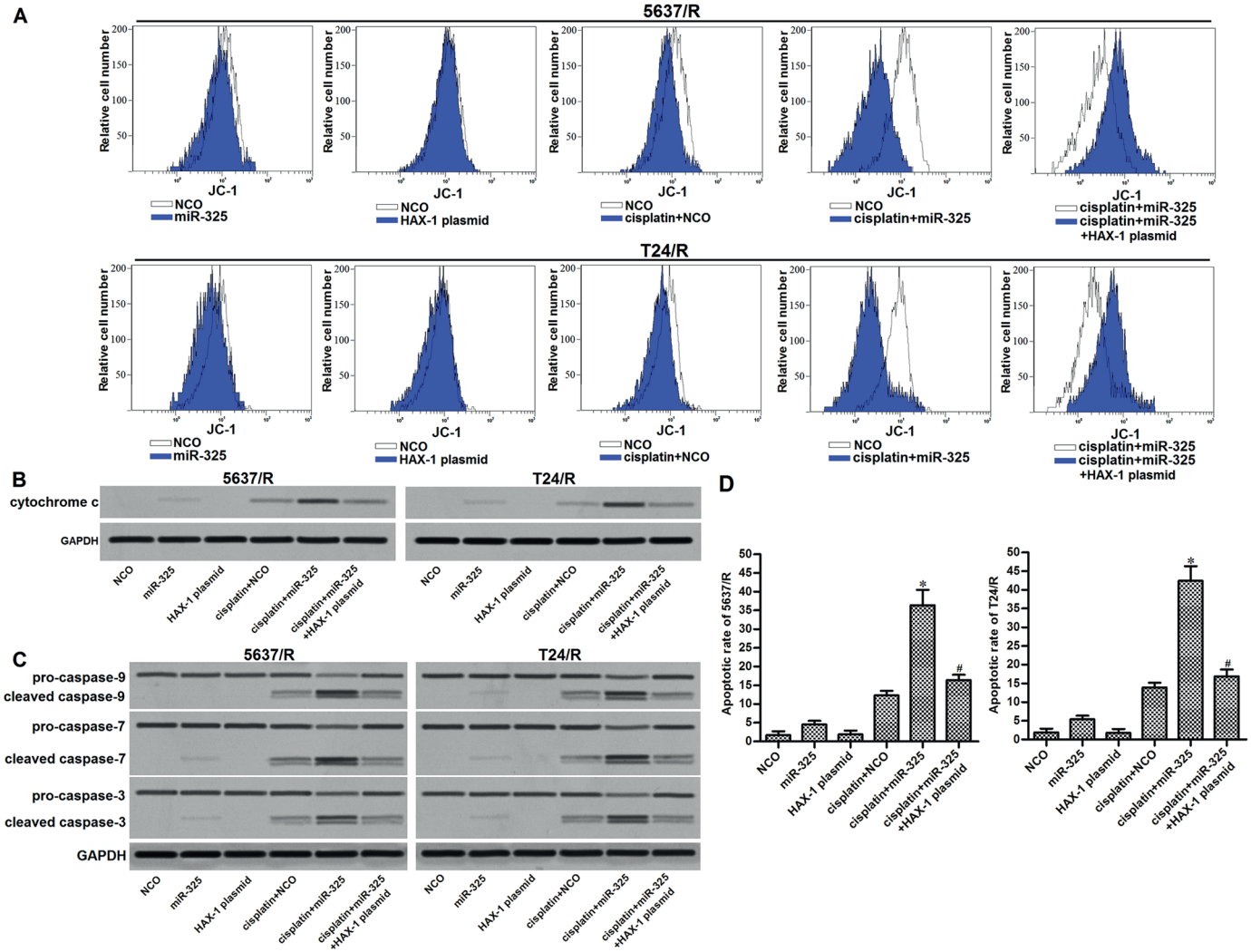

Figure 5. Role of miR-325/HAX-1 axis in regulating the mitochondrial apoptosis in cisplatin-treated 5637/R and T24/R cells. **A,** Effect of miR-325, cisplatin and HAX-1 plasmid on changing the mitochondrial membrane potential (MMP) of 5637/R and T24/R cells. **B,** Mitochondria derived cytochrome c in cytosol of 5637/R and T24/R was detected by Western blot analysis. **C,** Effect of miR-325, cisplatin and HAX-1 plasmid on changing the cleavage of caspase-9, -7, and -3 in 5637/R and T24/R cells. **D,** Measurement of apoptotic rate of 5637/R and T24/R cells after treatment with miR-325, cisplatin and HAX-1 plasmid. *$p<0.05$ *vs.* cisplatin+NCO group. $^{\#}p<0.05$ *vs.* cisplatin+miR-325 group.

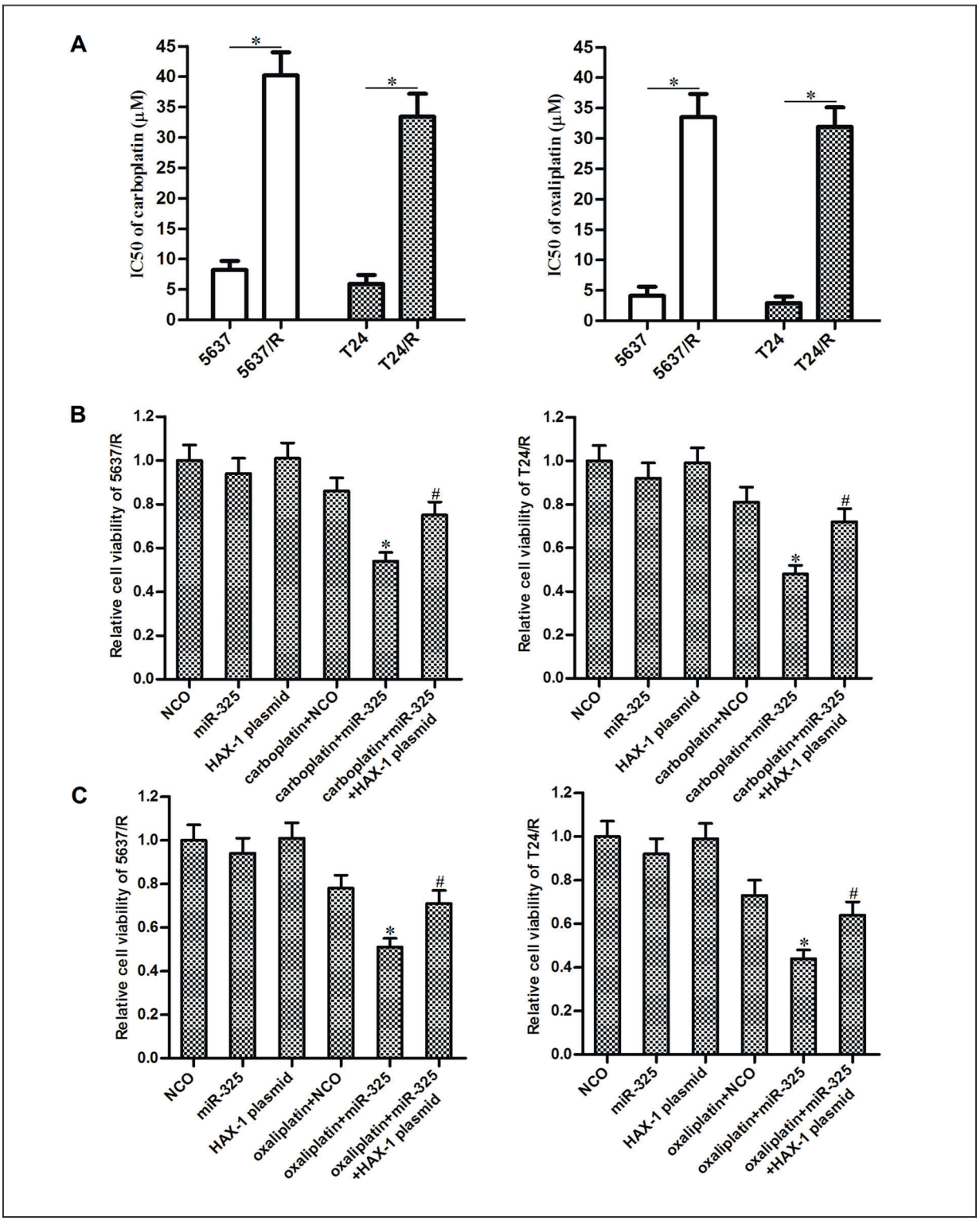

Figure 6. Overexpression of miR-325 attenuated the cross resistance of carboplatin and oxaliplatin in 5637/R and T24/R. **A,** IC50 of carboplatin and oxaliplatin to 5637/R and T24/R cells. *$p<0.05$ *vs.* cisplatin+NCO group. **B,** Effect of miR-325 and HAX-1 plasmid on changing the sensitivity of 5637/R and T24/R cells to carboplatin. *$p<0.05$ *vs.* carboplatin+NCO group. $^{\#}p<0.05$ *vs.* carboplatin+miR-325 group. **C,** Effect of miR-325 and HAX-1 plasmid on changing the sensitivity of 5637/R and T24/R cells to oxaliplatin. *$p<0.05$ *vs.* oxaliplatin+NCO group. $^{\#}p<0.05$ *vs.* oxaliplatin+miR-325 group.

cancer. Among the cancer-related miRNAs which are usually dysregulated in cancer cells, miR-325 has been reported to act as a tumor suppressor and usually downregulated in cancers including lung cancer, hepatocellular carcinoma and bladder cancer[23-25]. However, the role of miR-325 in regulating the cisplatin sensitivity in bladder cancer remains unclear. We should explore the potential effect of miR-325 on attenuating the cisplatin resistance in bladder cancer.

As long-term use of cisplatin usually induces significant drug resistance in bladder cancer cells, we established the cisplatin-resistant bladder cancer models on 5637 and T24 cell lines through continuous exposure to cisplatin. We found that the expression level of miR-325 in cisplatin-resistant 5637 and T24 cells was significantly decreased compared to the routine 5637 and T24 cells. Moreover, our data showed that recovery of miR-325 in these cisplatin-resistant bladder cancer cells can resensitize them to platinum-based drugs. In conclusion, we proved that dysregulation of miR-325 was associated with drug resistance to cisplatin in bladder cancer. Overexpression of miR-325 can attenuate the resistance of bladder cancer to platinum-based chemotherapeutic drugs.

Uptake of cisplatin in cancer cells induces DNA damage to initiate the apoptosis pathway[26,27]. However, cell apoptosis is regulated by multiple proteins in cancer cells. Among these apoptosis-related proteins, hematopoietic cell-specific protein 1-associated protein X-1 (HAX-1) is one of the key members that functions as an anti-apoptotic protein[28,29]. HAX-1 is mainly localized at mitochondria. It functions to inhibit mitochondria collapse and loss of mitochondrial membrane potential (MMP). Therefore, HAX-1 decreased the release of cytochrome c and the subsequent activation of caspase-9, -7 and -3[30,31]. Previous studies have demonstrated that overexpression of HAX-1 is usually observed in multiple human cancers. Furthermore, overexpression of HAX-1 is found to be responsible for formation of resistance to many chemotherapeutic drugs including cisplatin[32-34]. Thus, HAX-1 has become a potential therapeutic target in the chemotherapy.

Dysregulation of miRNAs and their targeted genes induce cisplatin resistance in cancers[35,36]. In this research, we provided evidence that bladder cancer cells developed cisplatin resistance through downregulation of miR-325 and upregulation of HAX-1. We then proved that the effect of miR-325 on attenuating the cisplatin resistance of bladder cancer was dependent on the suppression of HAX-1. In the apoptosis pathway caused by cisplatin treatment, miR-325 targeted HAX-1 to promote the collapse of mitochondria and the following release of cytochrome c and activation of caspases. In final, recovery of miR-325 resensitized the cisplatin-resistant bladder cancer cells to cisplatin-induced apoptosis. On the other hand, we found that the cisplatin-resistant bladder cancer cells exhibited cross-resistance to other platinum-based drugs. However, intervention through miR-325/HAX-1 axis was able to decrease the cross-resistance to these platinum-based drugs.

Conclusions

Cisplatin resistance is partially induced by downregulation of miR-325 in bladder cancer. We provide evidence that overexpression of miR-325 can decrease the cisplatin resistance of bladder cancer cells. Mechanically, miR-325 suppresses the expression of HAX-1 to promote the mitochondrial apoptosis dependent by cisplatin. Therefore, miR-325/HAX-1 axis may represent a novel strategy for the treatment of bladder cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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