European Review for Medical and Pharmacological Sciences 2019; 23: 7256-7265

Long non-coding RNA ROR regulated ABCB1 to induce cisplatin resistance in osteosarcoma by sponging miR-153-3p

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Abstract. – OBJECTIVE: Osteosarcoma (OS) is a common cancer among adolescences worldwide. Cisplatin is widely used to treat cancer, but many patients acquire chemoresistance over time. LncRNA regulator of reprogramming (ROR) has been reported to be associated with many malignancies, including OS. However, the function of ROR in cisplatin resistance and the biological mechanism of ROR remain unclear in OS.

PATIENTS AND METHODS: The levels of ROR, miR-153-3p, and ABCB1 in cisplatin-resistant OS tissues and cells were detected by qRT-PCR and/or Western blot assay. The interactions of miR-153-3p, ROR, and ABCB1 were predicted by miRcode Tools and StarBase v2.0 online database, respectively, and validated by Dual-Luciferase reporter assay. The cell viability in different concentrations of DDP, cell proliferative capacity, migrated cells, and invaded cells were detected by MTT assay and transwell assay, respectively.

RESULTS: The levels of ROR and ABCB1 were both drastically increased, and miR-153-3p was apparently decreased in relapsed OS tissues, MG63/DDP, and U2OS/DDP cells. MiRcode Tools and StarBase v2.0 predicted that miR-153-3p had complementary sequences with ROR and ABCB1 3'UTR, and following Dual-Luciferase reporter assay validated that miR-153-3p was a direct target of ROR and ABCB1. Moreover, ABCB1 overexpression alleviated the inhibitory effects on cell viability in different concentrations of DDP, cell proliferative capacity, migrated cells, and invaded cells in MG63/DDP and U2OS/DDP cells transfected with sh-ROR. ROR regulated ABCB1 expression in MG63/DDP and U2OS/DDP cells by sponging miR-153-3p.

CONCLUSIONS: We found that ROR or ABCB1 knockdown can increase the cisplatin sensitivity of MG63/DDP and U2OS/DDP cells. ROR contributed to cisplatin resistance in OS via miR-153-3p/ABCB1 axis, unraveling a new regulatory network of chemoresistance in cisplatin-resistant OS cells and may provide a therapeutic target for OS patients.

Key Words:
LncRNA ROR, MiR-153-3p, ABCB1, Cisplatin resistance, Osteosarcoma.

Introduction

Osteosarcoma (OS) is a malignant tumor of bone among teenagers[1]. Conventional treatment for OS patients is surgery combined with multidrug chemotherapy such as doxorubicin (Dox), cisplatin (DDP), ifosfamide, and methotrexate[2]. However, chemotherapy often becomes less effective over time due to the multidrug resistance (MDR)[3]. Meanwhile, the survival time of recurrent OS patients with drug resistance is approximately one year[4-6]. Thus, it is urgent to find novel therapeutic targets and explore the mechanism of cisplatin resistance for OS patients.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs with >200 nt in length, which have been reported[7] to regulate some oncogenes expression at the transcriptional and posttranscriptional stage. Aberrant expression of lncRNA has been documented to MDR in diverse diseases. For example, a report[8] on ovarian cancer manifested that lncRNA SNHG15 was highly expressed in cisplatin-resistant A2780 and SKOV3 cells. Hu et al[9] indicated that lncRNA NEAT1 was apparently increased in OS tissues, and NEAT1 overexpression enhanced DDP resistance. LncRNA regulator of reprogramming (ROR) was also reported to be associated with cisplatin resistance in various types of tumors. Some analyses[10] in non-small-cell lung cancer manifested

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that ROR was highly expressed in human A549/DDP cell lines, and ROR silencing restrained cell proliferation, migration, and invasion. However, the biological of ROR in cisplatin resistance of OS is still unclear.

MicroRNAs (miRNAs) are a class of non-coding RNAs with about 22 nt in length, and function as tumor suppressors to affect tumor progression[11]. MiRNA also has been reported to participate in MDR process. For instance, miR-181b overexpression inhibited cell proliferation and induced cell apoptosis in non-small cell lung cancer A549/DDP cells, indicating that miR-181b enhanced the sensitivity of NSCLC cells to DDP[12]. Many researches[13-15] have indicated that ATP binding cassette subfamily B member 1 (ABCB1, also named as MDR1) could reduce sensitive to chemicals by the P-gp protein to pump out the intracellular drugs. Many non-coding RNAs have been confirmed to involve in the process of drug resistance in cancers by regulating ABCB1 expression. For example, a research[16] in ovarian cancer demonstrated that miR-186 promoted sensitivity to paclitaxel and cisplatin by targeting ABCB1. However, the mechanism of miR-153-3p and ABCB1 in cisplatin resistance of OS is still barely documented.

In this work, we revealed that ROR and ABCB1 were upregulated and miR-153-3p was downregulated in cisplatin-resistant OS tissues and cells. Further function and mechanism analyses unraveled that ROR contributed to cisplatin resistance by regulating ABCB1 via miR-153-3p in OS. This novel regulatory network may provide a promising therapeutic method for OS patients with DDP resistance.

Patients and Methods

Tissue Samples

The study was approved by the Ethics Committee of Weinan Central Hospital, and informed consent was provided for all OS patients or their guardians. Twenty-five relapsed OS tissues and primary OS tissues were collected from Weinan Central Hospital. All tissues were frozen in liquid nitrogen and stored at -80°C until further used.

Cell Culture and Transfection

The two human osteosarcoma cell lines MG63 and U2OS were bought from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cells were cultivated in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (BIOSUN, Shanghai, China) containing 10% fetal bovine serum (FBS, Thermo Fisher Scientific, Waltham, MA, USA) in a humidified incubator with the parameter of 37°C and 5% CO_2. Cisplatin-resistant OS cells (MG63/DDP and U2OS/DDP) were established from the parental cell lines MG63 and U2OS using an intermittent stepwise selection protocol over 6 months, ending with exposure to 1.2 mg/mL cisplatin[17]. The small hairpin RNA (shRNA) targeting ROR (sh-ROR) and its mock (sh-NC), shRNA targeting ABCB1 (sh-ABCB1) and its matched control (sh-NC) were synthesized and then inserted into the pLL3.7 lentiviral vector (Addgene, Watertown, MA, USA). The miR-153-3p mimics (miR-153-3p) and its negative control (miR-NC), ROR overexpression vector (ROR) and empty vector (pcDNA) were purchased from GenePharma (Shanghai, China). The transfection was conducted using Lipofectamine 2000 Reagent (Invitrogen, Carlsbad, CA, USA) according to the manual.

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

The total RNA from cells was extracted using miRNeasy Mini Kit (Qiagen, Valencia, CA, USA), and the concentration of RNA samples was detected using NanoDrop 2000c (Thermo Fisher Scientific, Waltham, MA, USA). Reverse transfection was conducted using Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland). Following quantitative PCR was performed using FastStart Universal SYBR Green Master (Roche) and analyzed using Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) with the method of $2^{-\Delta\Delta Ct}$. The primers used in this study were list as follows: ROR (Forward, 5'-CTTGATGGCATTGTCGCTAA-3', Reverse, 5'-TCCAGTGGCTGTGCTAGATG-3'); miR-153-3p (Forward, 5'-GGGTTGCATAGTCACAAAAG-3', Reverse, 5'-TTTGGCACTAGCACATT-3'); ABCB1 (Forward, 5'-AGACATGACCAGGTATGCCTAT-3', Reverse, 5'-AGCCTATCTCCTGTCGCATTA-3); GAPDH (Forward, 5'-TGTTCGTCATGGGTGTGAAC-3', Reverse, 5'-ATGGCATGGACTGTGGTCAT-3') and U6: (Forward, 5'-CTCGCTTCGGCAGCACA-3', Reverse, 5'-AACGCTTCACGAATTTGCGT-3').

Western Blot

The protein from cells was extracted using RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific, Waltham, MA, USA). The protein

samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Subsequently, the membranes were blocked in skim milk, and incubated with primary antibody and secondary antibody in order. RapidStep ECL Reagent (Millipore, Billerica, MA, USA) was used to detect the chemiluminescence intensity. All the antibodies were bought from Abcam (Cambridge, MA, USA).

Cell Proliferation Assay

3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) was used to detect the cell proliferative capacity. The cells were suspended in a 96-well plate with Roswell Park Memorial Institute-1640 (RPMI-1640) medium containing 10% fetal bovine serum (FBS) and incubated for 0, 24, 48, 72 h. After incubated with MTT for 4 h, dimethyl sulfoxide (DMSO) was added in the well to dissolve the formazan. The colorimetric analysis at 490 nm was assessed using a spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Transwell Assay

The cell migration experiments were performed using a 6-well transwell system (Corning, Tewksbury, MA, USA). For cell migration, MG63/DDP and U2OS/DDP cells were suspended in serum-free medium in the upper chamber, the lower chamber was supplemented with RPMI-1640 medium containing 10% fetal bovine serum (FBS). After incubated for 24 h, the cells that migrated the upper chambers were fixed in methanol for 15 min and stained with 0.1% crystal violet for 10 min. The cells were counted under a light microscope. For cell invasion, the protocol was similar with that in cell migration, while the difference regarded the upper chamber, which was coated with matrigel matrix (Bedford, MA, USA) in the invasion assay.

Luciferase Reporter Assay

MiRcode Tools (http://mircode.org) and StarBase v2.0 (http://starbase.sysu.edu.cn) online databases were used to predict the interactions of miR-153-3p, ROR, and ABCB1. ROR and ABCB1 3'UTR were amplified and then inserted into psiCHECK2 plasmid (HanBio Biotechnology, Shanghai, China), named WT-ROR and ABCB1 3'UTR-WT, respectively. The Luciferase reporter WT-ROR (MUT-ROR) or ABCB1 3'UTR-WT (ABCB1 3'UTR-MUT) and miR-153-3p (miR-NC) were co-transfected into MG63/DDP and U2OS/DDP cells. The Luciferase activity of WT-ROR (MUT-ROR) or ABCB1 3'UTR-WT (ABCB1 3'UTR-MUT) reporter was detected using Dual-Luciferase® Reporter Assay system (Promega, Madison, WI, USA). Renilla Luciferase activities function as an internal reference to normalize the firefly Luciferase activities.

Statistical Analysis

All of the data were performed using GraphPad Prism 7 (GraphPad Inc., La Jolla, CA, USA). All quantitative data were repeated for at least three times and were presented as the mean ± standard deviation (SD). The differences between the two groups were analyzed by the Student's *t*-test. The statistical significance was considered at $p<0.05$.

Results

ROR is Markedly Increased in Cisplatin-Resistant OS Tissues and Cells, and ROR Knockdown Increases the Cisplatin Sensitivity of MG63/DDP and U2OS/DDP Cells

To explore the role of ROR in the cisplatin resistance of OS, the ROR relative expression was detected in the OS tissues and cells. The qRT-PCR results indicated that ROR was strikingly upregulated in relapsed OS tissues compared to that in the primary OS tissues (Figure 1A). Also, ROR was remarkably increased in MG63/DDP and U2OS/DDP cells in comparison to that in the MG63 and U2OS cells (Figure 1B). The qRT-PCR results validated the knockdown efficiency demonstrated by the markedly decrease in MG63/DDP and U2OS/DDP cells related to that in MG63 and U2OS cells (Figure 1C). With the raised concentration of DDP, the cell viability was significantly reduced in MG63 and U2OS cells transfected with sh-ROR in contrast to that in sh-NC (Figures 1D and 1E). These data indicated that ROR was significantly upregulated in the relapsed OS tissues, MG63/DDP, and U2OS/DDP cells, and ROR correlated to cisplatin resistance in OS.

ROR Silencing Inhibits Cell Proliferation, Migration, and Invasion in MG63/DDP and U2OS/DDP Cells

Based on the above results, the possible effect of ROR in MG63/DDP and U2OS/DDP cells was

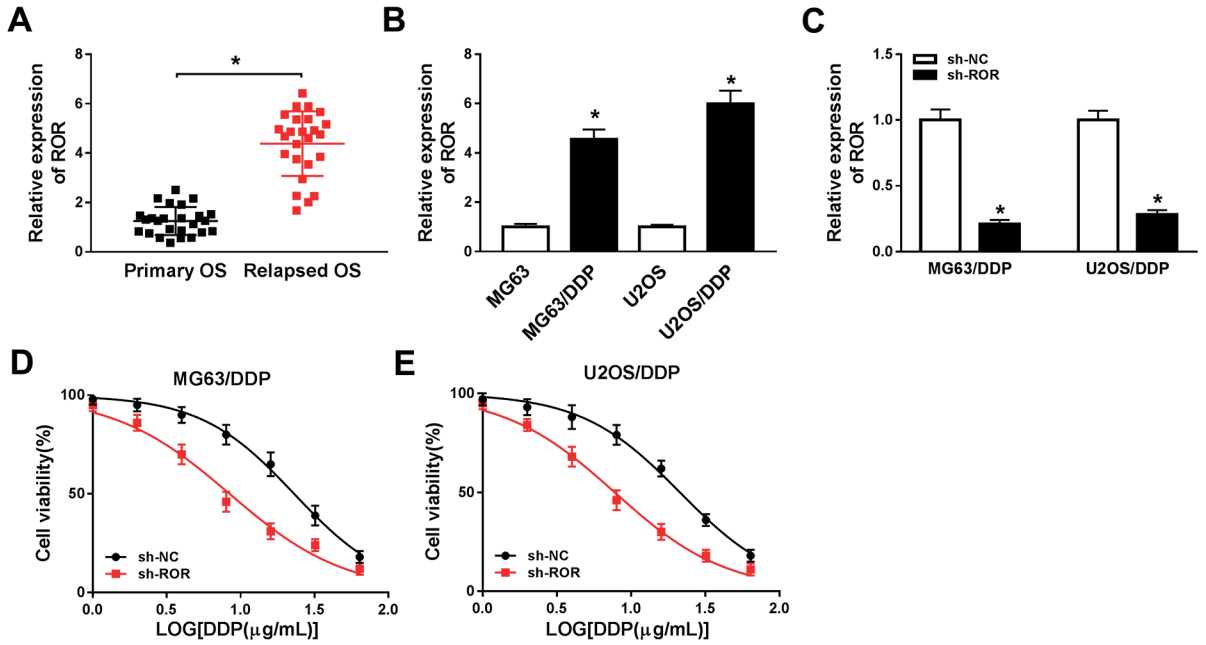

Figure 1. ROR is markedly increased in the relapsed OS tissues, MG63/DDP, and U2OS/DDP cells, and ROR knockdown increases the cisplatin sensitivity of MG63/DDP and U2OS/DDP cells. The relative expression of ROR in the relapsed OS tissues ***(A)***, MG63/DDP, and U2OS/DDP cells ***(B)*** and their matched controls were measured by qRT-PCR. ***(C)*** The knockdown efficiency was verified by qRT-PCR. The cell viability of MG63/DDP ***(D)*** and U2OS/DDP cells ***(E)*** transfected with sh-ROR or sh-NC in different concentrations were assessed by MTT assay. $p<0.05$.

further explored. The MTT assay showed that the cell proliferative capacity was apparently decreased in MG63/DDP and U2OS/DDP cells with the transfection of si-ROR (Figures 2A and 2B). The transwell assay exhibited that migrated cells and invaded cells, which were both downregulated in sh-ROR-transfected MG63/DDP and U2OS/DDP cells related to that in the sh-NC group (Figures 2C and 2D). Taken together, the depletion of ROR restrained cell proliferation, migration, and invasion in MG63/DDP and U2OS/DDP cells.

ABCB1 is Strikingly Upregulated in Cisplatin-Resistant OS Tissues and Cells, and ABCB1 Silencing Represses Cell Proliferation, Migration, and Invasion in MG63/DDP and U2OS/DDP Cells

To investigate the function of ABCB1 in the relapsed OS, ABCB1 was knocked down in MG63/DDP and U2OS/DDP cells. Firstly, the qRT-PCR and Western blot assay results presented that the mRNA and protein levels of ABCB1 were evidently increased in the relapsed OS tissues, MG63/DDP, and U2OS/DDP cells compared to that in their matched controls (Figures 3A and 3C-3E). Meanwhile, the level of ABCB1 was positive linear correlated with the level of ROR (Figure 3B). The Western blot assay confirmed that the knockdown efficiency indicated by the downregulation of ABCB1 protein level in sh-ABCB1-transfected MG63/DDP and U2OS/DDP cells (Figure 3F). Moreover, the MTT assay displayed that cell viability was apparently reduced in sh-ABCB1-transfected MG63/DDP and U2OS/DDP cells with raised concentration of DDP in comparison with that in the sh-NC group (Figures 3G and 3H). In addition, the MTT and the transwell assay results presented that cell proliferative capacity, migrated cells, and invaded cells were decreased in MG63/DDP and U2OS/DDP cells transfected with sh-ABCB1 compared to that in the sh-NC group, respectively (Figures 3I and 3L). These results demonstrated that ABCB1 was highly expressed in the relapsed OS tissues, MG63/DDP and U2OS/DDP cells, and ABCB1 knockdown suppressed cell proliferation, migration, and invasion in MG63/DDP and U2OS/DDP cells.

ABCB1 Overexpression Relieves the Inhibitory Effects on Cell Proliferation, Migration and Invasion in MG63/DDP and U2OS/DDP Cells by Targeting ROR

To further illustrate the function of ROR and ABCB1 in the cisplatin resistance of OS, sh-ROR and ABCB1 were co-transfected into MG63/DDP and U2OS/DDP cells. The Western blot assay showed that the protein level of ABCB1 was notably decreased in sh-ROR-transfected MG63/DDP and U2OS/DDP cells compared to that in the sh-NC treatment, while ABCB1 in ROR overexpression group presented the opposite trend (Figures 4A and 4B). Furthermore, the MTT assay showed that ABCB1 overexpression alleviated the inhibitory effects on cell viability in different concentrations of DDP and cell proliferative capacity in MG63/DDP and U2OS/DDP cells induced by sh-ROR (Figures 4C-4F). The transwell assay indicated that the overexpression of ABCB1 attenuated the inhibitory effects on the migrated cells and invaded cells in MG63/DDP and U2OS/DDP with the transfection of si-ROR (Figures 4G and 4H). These data manifested that the overexpression of ABCB1 mitigated the inhibitory effects on cell proliferation, migration, and invasion in MG63/DDP and U2OS/DDP cells caused by sh-ROR.

MiR-153-3p Interacts with ROR and ABCB1, and ROR Regulates ABCB1 Expression in MG63/DDP and U2OS/DDP Cells by Sponging MiR-153-3p

To illustrate the mechanism of the cisplatin resistance in OS, miRcode Tools and StarBase v2.0 online databases were used to predict the putative target of ROR and ABCB1, respectively. Firstly, we found that miR-153-5p was dramatically decreased in relapsed OS tissues, MG63/DDP, and U2OS/DDP cells in contrast to that in their matched controls (Figures 5A and 5B). MiRcode Tools and StarBase v2.0 online databases showed

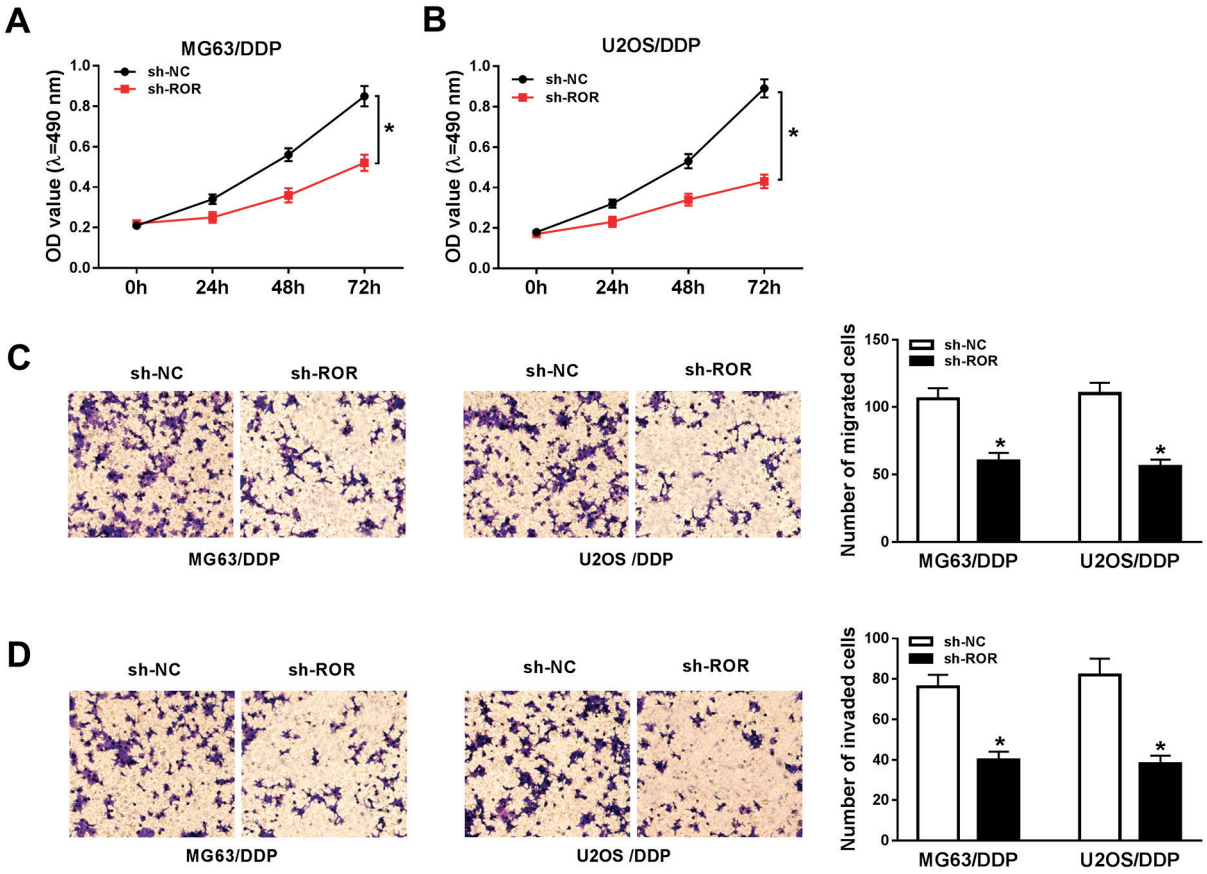

Figure 2. ROR silencing inhibits cell proliferation, migration, and invasion in MG63/DDP and U2OS/DDP cells. The cell proliferative capacity ***(A*** and ***B)***, migrated cells (100×) ***(C)***, and invaded cells (100×) ***(D)*** in MG63 and U2OS cells incubated in 2 μg/mL DDP, and transfected with sh-ROR or sh-NC were measured by MTT assay and transwell assay, respectively. $p<0.05$.

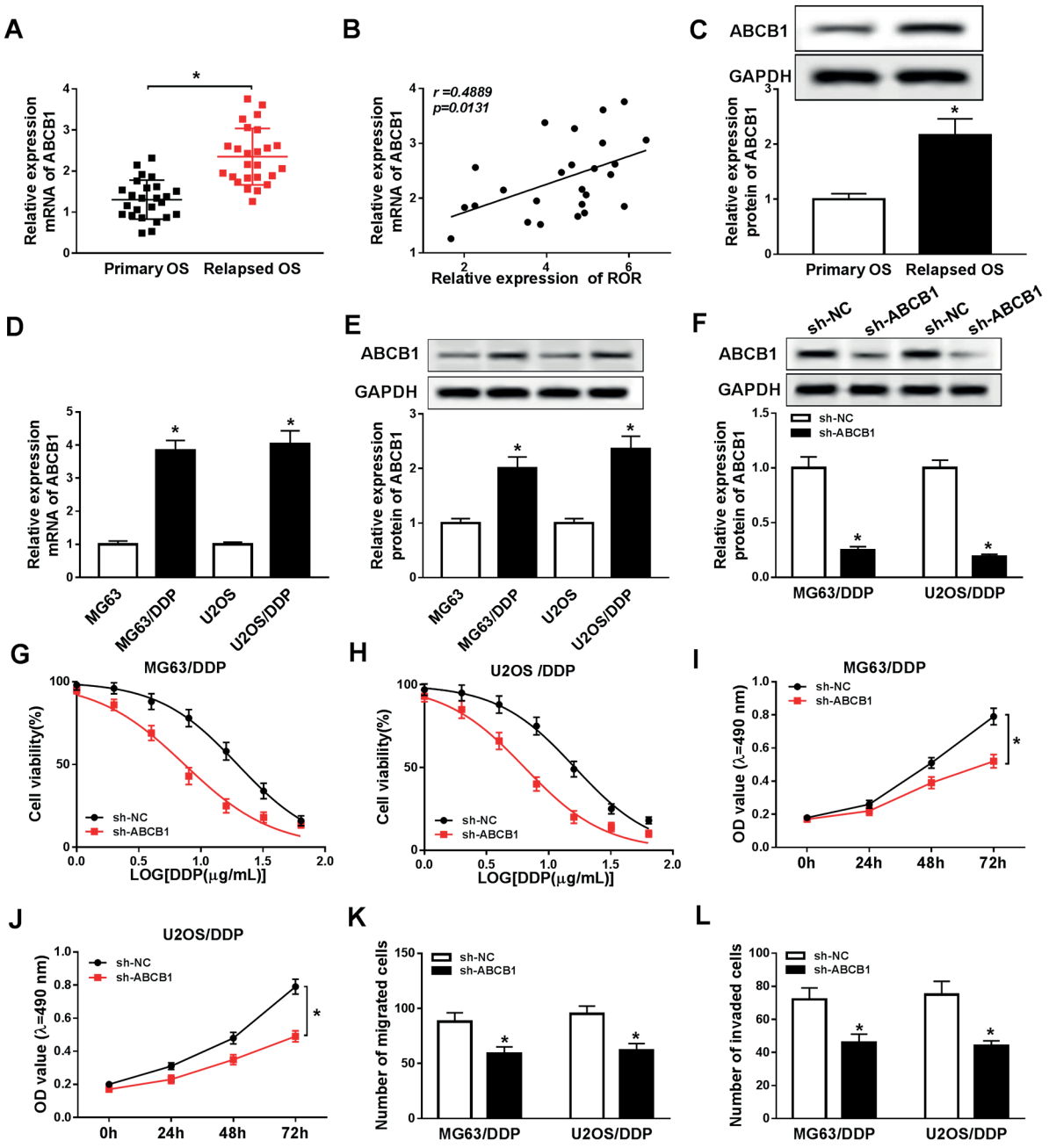

Figure 3. ABCB1 is strikingly upregulated in the relapsed OS tissues, MG63/DDP, and U2OS/DDP cells. ABCB1 silencing represses cell proliferation, migration, and invasion in MG63/DDP and U2OS/DDP cells. The mRNA level of ABCB1 in relapsed OS ***(A)***, MG63/DDP, and U2OS/DDP cells ***(D)*** and their controls were measured by qRT-PCR. The protein level of ABCB1 in relapsed OS tissues ***(C)***, MG63/DDP, and U2OS/DDP cells ***(E)*** and the knockdown efficiency ***(F)*** were detected by Western blot. The correlation between ROR1 and ABCB1 mRNA ***(B)*** in the relapsed OS tissues was detected by qRT-PCR (r=0.4889, p=0.0131).The cell viability in different concentrations of DDP ***(G*** and ***H)***, the proliferative capacity ***(I*** and ***J)***, the migrated cells ***(K)***, and the invaded cells ***(L)*** were all significantly deduced in MG63/DDP and U2OS/DDP cells transfected with sh-ABCB1 or sh-NC, respectively. p<0.05.

that miR-153-3p had complementary sequences with ROR and ABCB1 (Figure 5C). Following Dual-Luciferase reporter assay indicated that the transfection of miR-153-3p resulted in the down-regulation of Luciferase activity of WT-ROR and ABCB1 3'UTR-WT reporter in MG63/DDP and

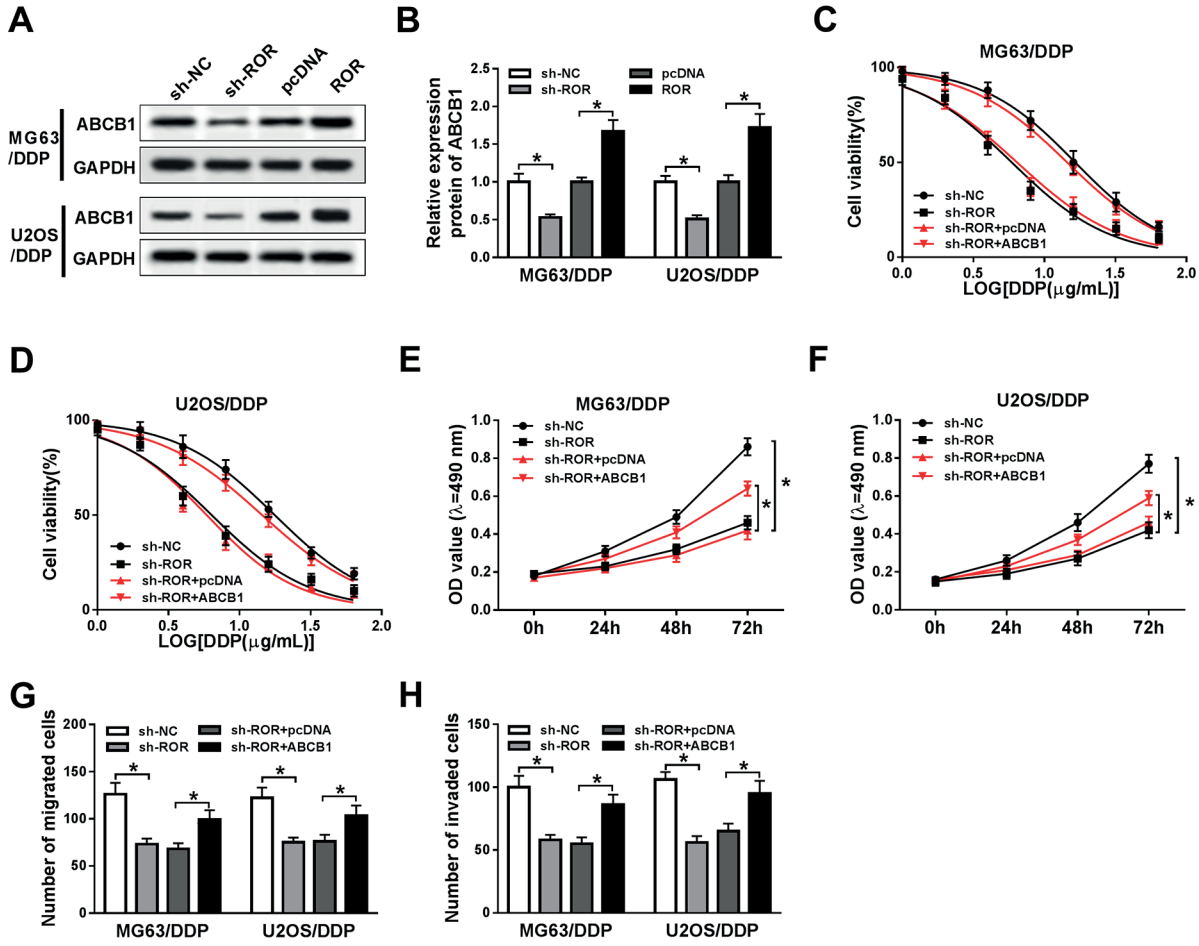

Figure 4. ABCB1 overexpression relieves the inhibitory effects on cell proliferation, migration, and invasion in MG63/DDP and U2OS/DDP cells by targeting ROR. (**A** and **B**) The protein level of ABCB1 in MG63/DDP and U2OS/DDP cells transfected with sh-ROR, ROR or their matched controls were measured by Western blot. The cell viability in different concentrations of DDP (**C** and **D**), cell proliferative capacity (**E** and **F**), the migrated cells (**G**), and the invaded cells (**H**) in MG63/DDP and U2OS/DDP cells transfected with sh-ROR, sh-ROR+ABCB1 or their matched controls were detected by MTT assay and transwell assay, respectively. $p<0.05$.

U2OS/DDP cells, but the Luciferase activity of MUT-ROR and ABCB1 3'UTR-MUT reporter had no change (Figures 5D-5G). Moreover, the protein level of ABCB1 was conspicuously downregulated in MG63/DDP and U2OS/DDP cells, while ROR overexpression blocked the inhibitory effect (Figures 5H and 5I). Taken together, miR-153-3p was a direct target of ROR, ABCB1, and ROR that regulated ABCB1 expression in MG63/DDP and U2OS/DDP cells by targeting miR-153-3p.

Discussion

Cisplatin has been used to function as an antineoplastic drug in various types of tumors[18]. Many researchers[19] proved that lncRNA plays a vital role in drug resistance of diverse diseases. In this work, we aimed at exploring the mechanism and function of ROR in the cisplatin resistance in OS. The results indicated that ROR knockdown can enhance the cisplatin sensitive for OS cells. Functional and mechanistic studies revealed that ROR contributed to cisplatin resistance of OS cells by regulating ABCB1 via miR-153-3p.

LncRNA ROR has been documented to be aberrantly expressed in drug resistance human cancers. For example, an investigation[20] on lung adenocarcinoma (LAD) revealed that ROR was markedly upregulated in docetaxel-resistant LAD cells, and ROR overexpression promoted cell proliferation and enhanced docetaxel resistance in

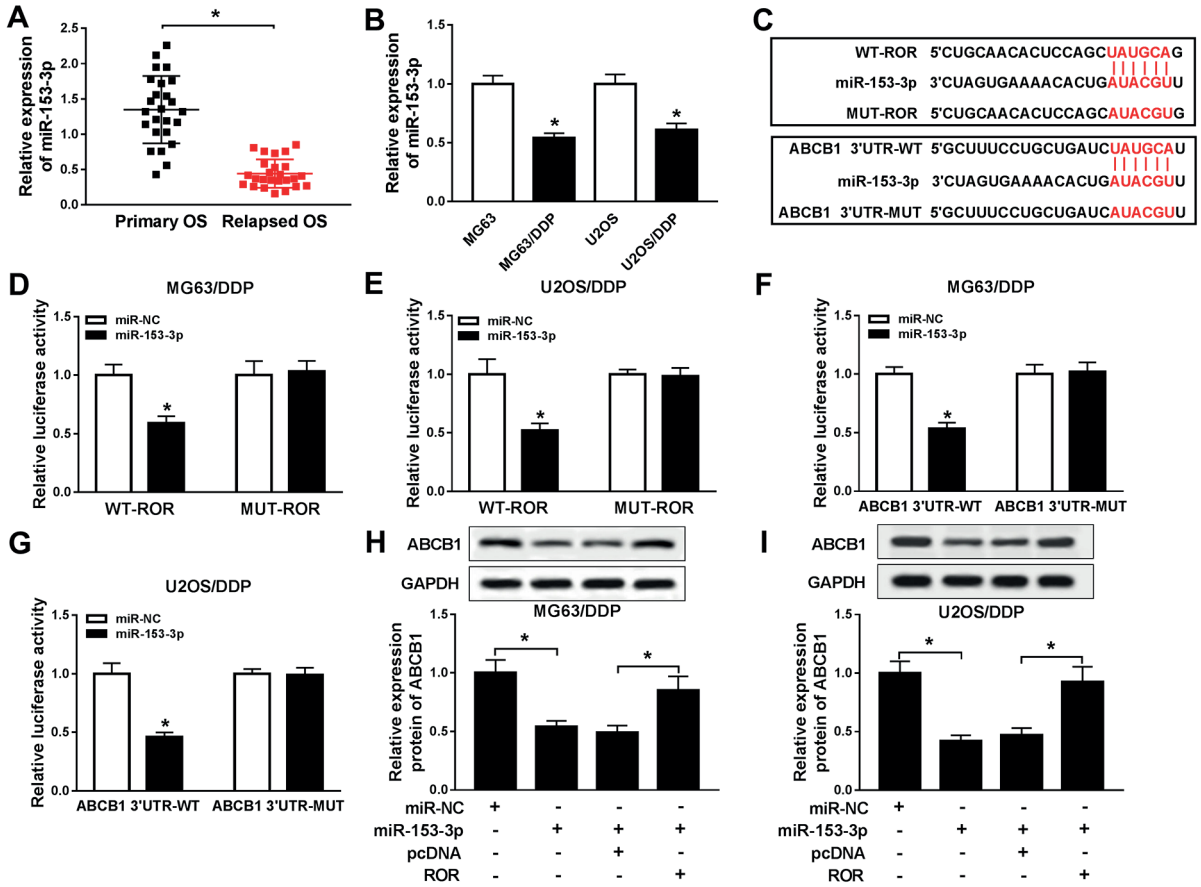

Figure 5. MiR-153-3p negatively interacts with ROR and ABCB1, and ROR regulates ABCB1 expression in MG63/DDP and U2OS/DDP cells by sponging miR-153-3p. The level of miR-153-3p in the relapsed OS tissues ***(A)***, MG63/DDP, and U2OS/DDP cells ***(B)*** and their matched controls were measured by qRT-PCR. ***(C)*** The interactions of miR-153-3p, ROR, and ABCB1. ***(D-G)*** The Luciferase activities of the Luciferase reporter of WT-ROR (MUT-ROR) and ABCB1 3'UTR-WT (ABCB1 3'UTR-MUT) in MG63/DDP and U2OS/DDP cells transfected with mi-153-3p mimics or miR-NC were assessed by Dual-Luciferase reporter assay. The protein level of ABCB1 in MG63/DDP ***(H)*** and U2OS/DDP cells ***(I)*** transfected with miR-NC, miR-153-3p, miR1-153-3p+pcDNA, or miR1-153-3p+ROR were measured by Western blot. * $p<0.05$.

LAD cells. Shi et al[10] in non-small-cell lung cancer manifested that ROR was highly expressed in human A549/DDP cell lines, and ROR silencing restrained cell proliferation, migration, and invasion. In the present investigation, we found that ROR was apparently high expressed in the relapsed OS tissues, MG63/DDP, and U2OS/DDP cells. ROR silencing repressed cell viability in different concentrations of DDP in MG63/DDP and U2OS/DDP cells, and manifested that ROR knockdown can enhance the cisplatin sensitive for MG63/DDP and U2OS/DDP cells. Moreover, the depletion of ROR inhibited cell proliferation, migration, and invasion in MG63/DDP and U2OS/DDP cells. In brief, lncRNA ROR was involved in the cisplatin resistance of osteosarcoma.

Some reports have demonstrated that the abnormal expression of ABCB1 was found in drug resistance in diverse diseases, including OS. For instance, Tian et al[21] indicated that the level of ABCB1 expression was notably upregulated in the ovarian cancer tissues and cells, and ABCB1 overexpression decreased cell sensitivity to cisplatin of HO8910PM and SKOV-3 cells. Han and Shi[22] showed that ABCB1 was apparently upregulated in methotrexate resistance osteosarcoma cells, and the depletion of ABCB1 suppressed cell proliferation and invasion. In this study, ABCB1 was distinctly increased in the relapsed OS tissues, MG63/DDP, and U2OS/DDP cells. Also, ABCB1 knockdown enhanced the cisplatin sensitive for MG63/DDP and U2OS/DDP

cells. ABCB1 interference inhibited cell proliferation, migration, and invasion in MG63/DDP and U2OS/DDP cells. The level of ABCB1 was positively linear correlated to ROR. In addition, the overexpression of ABCB1 attenuated the inhibitory effects on cell proliferation, migration, and invasion in MG63/DDP and U2OS/DDP cells with the transfection of sh-ROR. These results revealed that ROR promoted cell proliferation, migration, and invasion in MG63/DDP and U2OS/DDP cells by targeting ABCB1.

MiR-153-3p has been identified as a tumor suppressor and has been discovered to be involved in drug resistance in many malignant tumors. Liu et al[23] documented that the relative expression of miR-153 was conspicuously reduced in pancreatic cancer tissues, and miR-153 mimics decreased the gemcitabine resistance in Capan-2-GR, Panc1-GR, and As-Pc-1-GR cells. An investigation[24] in acute myeloid leukemia demonstrated that miR-153-3p mimics decreased the level of X-linked inhibitor of apoptosis protein (XIAP) in doxorubicin-resistant THP-1 AML cells, indicating that miR-153-3p increased the doxorubicin sensitive of acute myeloid leukemia cells. In this work, miR-153-3p was drastically downregulated in the relapsed OS tissues, MG63/DDP, and U2OS/DDP cells. The Dual-Luciferase reporter assay verified that miR-153-3p was the direct target of ROR and ABCB1. ROR overexpression mitigated the inhibitory effect on ABCB1 expression induced by miR-153-3p mimics in MG63/DDP and U2OS/DDP cells. Based on the above results, we found that ROR contributed to cisplatin resistance in osteosarcoma by regulating ABCB1 expression *via* miR-153-3p.

Conclusions

LncRNA ROR and ABCB1 were dramatically upregulated, and miR-153-3p was strikingly downregulated in the relapsed OS tissues, MG63/DDP, and U2OS/DDP cells. Further research indicated that ROR contributes to cisplatin resistance in osteosarcoma via miR-153-3p/ABCB1 axis. This may provide a promising therapy strategy for OS with cisplatin resistance.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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