Role and mechanisms of microRNA-503 in drug resistance reversal in HepG2/ADM human hepatocellular carcinoma cells

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Abstract. Hepatocellular carcinoma is one of the most common malignancies worldwide and drug resistance is a major cause of treatment failure. In order to investigate the effects and mechanisms of microRNA-503 (miR-503) in the reversal of Adriamycin® (ADM) resistance in the drug-resistant HepG2/ADM hepatocellular cancer cell line, an ADM-resistant HepG2/ADM cell line was established using continuous drug exposure. HepG2/ADM cells overexpressing miR-503 were further established. HepG2/ADM cells overexpressing miR-503 demonstrated an enhanced sensitivity to ADM. Furthermore, miR-503 overexpression was found to increase intracellular rhodamine-123 levels and the rate of apoptosis, block the cell cycle at G_0/G_1-phase and significantly decrease intracellular superoxide dismutase and glutathione levels. The expression of a number drug resistance-related proteins, including multi-drug resistance 1, multi drug resistance-associated protein 1, DNA excision repair protein ERCC-1, survivin and B-cell lymphoma 2, was significantly downregulated by miR-503 overexpression, as indicated by western blotting and a quantitative polymerase chain reaction. By contrast, levels of RhoE were increased. In addition, the phosphorylation of Akt was decreased and expression of cyclin-dependent kinase 1 was decreased by miR-503 overexpression. Furthermore, the secretion of transforming growth factor-β, interleukin (IL)-6 and IL-8 was downregulated, and the transcriptional activities of nuclear factor κ-light-chain-enhancer of activated B cells and activating protein-1 were significantly reduced. In conclusion, miR-503 was observed to reverse ADM resistance in HepG2/ADM cells by inhibiting drug efflux, downregulating the expression of drug resistance-related proteins, blocking the cell cycle and promoting cell apoptosis.

Introduction

Hepatocellular carcinoma is one of the most common malignancies globally. Liver cancer causes ~560,000 mortalities each year, with half of these lethal cases reported in China (1). Hepatocellular carcinoma exhibits a subtle onset and its early symptoms are often undetectable. Therefore, the majority of patients with hepatocellular carcinoma are diagnosed in the middle or advanced stages of the disease. Patients with advanced stage hepatocellular carcinoma are not suitable candidates for surgical resection; therefore, they rely on alternative treatments, including hepatic artery intervention therapy, systematic drug therapy or organ transplantation. In recent years, the optimization of available treatment options and the development of a variety of targeted drugs have significantly enhanced the efficacy of current treatment and the survival of patients with liver cancer. However, certain problems remain, including liver cancer cell drug resistance (2). Adriamycin® (ADM) is an anti-tumor drug that belongs to the anthraquinone tetracyclic family. ADM inhibits DNA synthesis and transcription through targeting topoisomerase II, preventing cell growth and ultimately leading to tumor cell apoptosis. ADM is a key component of the transcatheter arterial chemoembolization and systemic chemotherapy programs (3). During long-term chemotherapy, liver cancer cells often develop resistance to ADM, causing a significant decrease in treatment efficacy. Therefore, investigating the mechanism underlying tumor cell drug resistance and developing novel methods to reverse drug resistance are of clinical significance for improving the treatment outcome and survival rate of patients. Previous studies have identified several mechanisms by which tumor cells develop drug resistance, including reducing drug concentration, increasing drug efflux through ATP-binding cassette (ABC) transport proteins, enhancing anti-cancer drug clearance through the glutathione (GSH) system and downregulating the rate of apoptosis through the modulation of apoptotic pathways (4-7).

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microRNAs (miRNAs) are endogenous non-protein-coding small RNAs, with a length of between 18 and 24 nucleotides. miRNAs bind to complementary sequences in target mRNAs, causing degradation of the target mRNA or gene silencing, thereby regulating gene expression at a post-transcriptional level (8). Evidence suggests that an association may exist between the expression of certain miRNAs and cancer. These studies have shown that, through regulating the expression of multiple target genes, a single miRNA molecule is capable of controlling cell growth and apoptosis and the migration and invasion of tumor cells, as well as tumor cell sensitivity to drug therapies (9,10).

The role of miRNA-503 (miR-503) in cancer treatment has attracted significant attention. Studies have shown that the expression of miR-503 is decreased in oral and liver cancer (11,12), but increased in parathyroid and adrenal cortical carcinomas (13,14). Furthermore, in patients with adrenal carcinoma, an increase in miR-503 was correlated with reduced overall survival (13). In the HCCLM3 hepatoma cell line, miR-503 has been reported to arrest the cell cycle at G_1-phase and inhibit cell migration and invasion (11). However, further studies are required to elucidate the role of miR-503 in drug resistance in liver cancer cells.

Materials and methods

Cell culture and stable cell line construction. The present study was approved by the ethics committee of Tianjin Medical University Cancer Institute and Hospital (Tianjin, China). The HepG2 human hepatocellular carcinoma cell line was purchased from the Cell Bank of the Chinese Academy of Science (Shanghai, China). Cells were cultured in medium containing 90% Eagle's Minimal Essential Medium and 10% fetal bovine serum at 37˚C in 5% CO_2 with saturated humidity. Trypsin-EDTA (0.25%) was used for cell digestion and passage. Cells in the logarithmic growth phase were used for all experiments. HepG2 cells were treated with low-dose ADM (IC_{50}, 1/10). Following the stabilization of the cell growth rate, the concentration of ADM was gradually increased in order to generate an ADM-resistant HepG2/ADM cell line.

miR-503 negative control miRNA mimics (Ago-miR-NC, 5'-GCTAGCCTGGACCACCCAGACCAACCTCGAGCA GAGGGCTTACTATCATTCTTCG-3') and stabilized miRNAs (Ago-miR-503, 5'-GTCGTATCCAGTGCAGGG TCCGAGGTGCACTGGATACGACCTGCAG-3') were purchased from Guangzhou RiboBio Co., Ltd., (Guangzhou, China) and transfected into cells using Lipofectamine® 2000. The cell lines overexpressing miR-503 were established and further assessed using quantitative polymerase chain reaction (qPCR) analysis. The cells were divided into four experimental groups: i) HepG2 cells (parent group), ii) HepG2/ADM cells (control group), iii) HepG2/ADM negative control miRNA mimic-transfected cells as a negative control (Ago-miR-NC group) and iv) HepG2/ADM miRNA-transfected cells (Ago-miR-503 group).

MTS detection of the effect of miR-503 on tumor cell drug resistance. Cells were cultured on 96-well plates at a density of $2x10^4$/ml with 100 μl medium/well overnight, followed by the addition of 0, 0.1, 1, 10 and 100 μg/ml ADM for 48 h. A total of 100 μl RPMI-1640 containing 0.5 mg/ml MTS was then added and the cells were cultured for a further 4 h. Optical density (OD) values were detected at a wavelength of 490 nm using an enzyme-labeled meter. The growth inhibitory rate of ADM was calculated using the following formula: Rate of inhibition rate = (1 - OD value of the experimental group/OD value of the control group) x 100%. Inhibition curves for different concentrations of ADM were plotted using drug concentration as the horizontal axis and inhibitory rate as the vertical axis. IC_{50} values were calculated using the following formula: Reversal fold (RF) = IC_{50} (without miR-503)/IC_{50} (with miR-503).

Flow cytometry to assess rhodamine-123 (Rh-123) concentration, apoptosis and cell cycle in HepG2/ADM cells
Rh-123 concentration. Cells were cultured in six-well plates at a density of $3x10^6$/ml in 100 μl medium/well overnight. The cells were then collected and washed, followed by the addition of 10 μM Rh-123 dye solution for 1 h. The cell density was adjusted to $1x10^6$/ml and the fluorescence intensity of Rh-123 excited at 488 nm was detected using flow cytometry in order to measure the intracellular concentration of Rh-123.

Cell cycle distribution. Cells were cultured in six-well plates at a density of $3x10^6$/ml in 100 μl medium/well overnight. Cells were then collected and washed, prior to the addition of 100 μl RNase-A and propidium iodide (PI) for 30 min. The cell density was adjusted to $1x10^6$/ml and the fluorescence intensity of PI excited at 488 nm was detected using flow cytometry to assess the cell cycle distribution of the cells.

Detection of apoptosis. Cells were cultured in six-well plates at a density of $3x10^6$/ml in 100 μl medium/well overnight, followed by treatment with 3 μg/ml ADM for a further 24 h. Cells were then collected, washed, and PI/Annexin V-fluorescein isothiocyanate (FITC) was added to the cultured cells for 15 min. The cell density was adjusted to $1x10^6$/ml and the fluorescence intensity of Annexin V-FITC and PI excited at 488 nm was detected using flow cytometry to assess cell apoptosis.

Biochemical assays to detect superoxide dismutase (SOD) and GSH activity. Cells were lysed and SOD activity was detected using the nitroblue tetrazolium method. GSH activity was examined by measuring the absorbance of the yellow 5-thio-2-nitrobenzoic acid and glutathione disulfide generated from the 5,5'-dithiobis-(2-nitrobenzoic acid) reaction. The experiments were performed in accordance with the kit manufacturer's instructions (Beyotime Institute of Biotechnology, Haimen, China) and absorbance was measured using a microplate reader.

Liquidchip assay to detect drug resistance-related cytokines. Cells were cultured in six-well plates at a density of $3x10^6$/ml in 100 μl medium/well overnight and then incubated with fresh serum-free culture medium for a further 24 h. The supernatant was collected and the levels of transforming growth factor (TGF)-β, interleukin (IL)-6 and IL-8 were determined using liquidchip assay (EMD Millipore, Billerica, MA, USA) according to the manufacturer's instructions.

Western blot analysis to detect the protein expression of multidrug resistance genes in HepG2/ADM cells. Cells were lysed and total proteins were extracted. Total protein was quantified using the bicinchoninic acid method. A total of 80 μg protein was separated using 12% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were then blocked using 10% milk and incubated with the following monoclonal antibodies: Anti-multidrug resistance 1 (MDR1) (diluted 1:600), -multidrug resistance-associated protein 1 (MRP 1) (diluted 1:600), -DNA excision repair protein ERCC-1 (ERCC1) (diluted 1:600), -RhoE (diluted 1:500), -survivin (diluted 1:800), -B-cell lymphoma-2 (Bcl-2) (diluted 1:500), -phosphorylated cyclin-dependent kinase 1 (P-CDK1) (diluted 1:400) and -phosphorylated Akt (diluted 1:400), all from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), and anti-β-actin (diluted 1:5,000) from Sigma-Aldrich (St. Louis, MO, USA) at 4°C overnight. The PVDF membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies at room temperature for 2 h. Immunoreactive bands were detected using an enhanced chemiluminescence kit following washing. β-actin was used as an internal control.

qPCR analysis to detect the mRNA expression of multidrug resistance genes in HepG2/ADM cells. Total RNA was extracted using TRIzol® reagent and qPCR was performed using a commercial kit and the following primers: MDR1, 5'-AAAAAGATCAACTCGTACCACTC-3' (forward), 5'-GCACAAAATACACCAACAA-3' (reverse); MRP1, 5'-ACTTCCACATCTGCTTCGTCAGTG-3' (forward), 5'-ATTCAGCCACAGGAGGTAGAGAGC-3' (reverse); ERCC1, 5'-GGGAATTTGGCGACGTAATTC-3' (forward), 5'-GCGGAGGCTGAGGAACAG-3' (reverse); RhoE, 5'-CCTCCACGTTGATTCGACTGTT-3' (forward), 5'-TGTAAAAGCCGTACGTTGCGGT-3' (reverse); survivin, 5'-GCATGGGGTGCCCCGACGTTG-3' (forward), 5'-GCTCCGGCCAGAGGCCTCAA-3' (reverse); Bcl-2, 5'-ACGGGGTGAACTGGGGGGAGGA-3' (forward), 5'-TGTTTGGGGCAGGCATGTTGACTT-3' (reverse); and β-actin, 5'-TGAGCGCGGCTACAGCTT-3' (forward), 5'-TCCTTAATGTCACGCACGATTT-3' (reverse). The PCR conditions were as follows: Denaturation at 94°C for 3 min followed by 40 cycles of amplification at 95°C for 5 sec, 65°C for 35 sec and 72°C for 60 sec, then a final extension for 5 min at 72°C. β-actin was used as a internal control.

Double fluorescent reporter gene assay to detect the transcriptional activity of NF-κB and AP-1. Cells were cultured in six-well plates at a density of 3×10^6/ml in 100 μl medium/well overnight. A total of 0.1 μg pGL4.74 [hRluc/TK] and 0.1 μg pGL 4.32 [luc2P/NF-kB-RE/Hypro] or pGL 4.44 [luc2P/AP-1-RE/Hygro] plasmids (Jinxing BioTech, Tianjin, China) were then added into each well and transfected into the cells according to the manufacturer's instructions. The cells were then cultured for 24 h, washed and changed into fresh medium. A Dual-Glo™ Luciferase assay system (Promega Corporation, Madison, WI, USA) was used to detect the activity of the two fluorescent enzymes.

Statistical analysis. Experimental data are presented as the mean ± standard deviation. SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA) was used to conduct the statistical
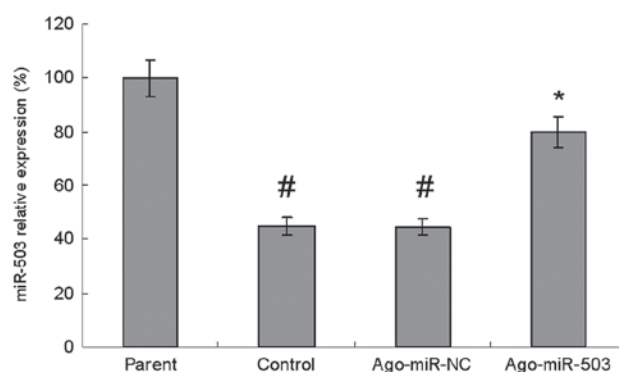

Figure 1. miR-503 expression in HepG2 and HepG2/ADM cells, as determined using quantitative polymerase chain reaction analysis. Values are presented as the mean ± standard deviation (n=5). #P<0.05 versus the parent group; *P<0.05 versus the control group. ADM, Adriamycin; miR-503, microRNA-503; NC, negative control.

analysis of data. One-way analysis of variance was performed to compare the results. A value of P<0.05 was considered to indicate a statistically significant difference.

Results

Establishment of the miR-503 overexpressing HepG2/ADM cell line. miR-503 expression in HepG2 and HepG2/ADM cells was measured using qPCR. miR-503 expression was decreased in the HepG2/ADM cells compared with that in the parent group, while in the miR-503-overexpressing HepG2/ADM cells miR-503 expression was increased relative to the expression in the control and Ago-miR-NC groups (Fig. 1).

miR-503 increases ADM sensitivity, promotes apoptosis, arrests the cell cycle and increases intracellular Rh-123 concentrations in HepG2/ADM cells. HepG2/ADM cells were established through long-term culture in media containing ADM. MTS assay revealed that HepG2/ADM cells showed enhanced resistance to ADM compared with the parent group. IC_{50} values were calculated to assess ADM resistance. The ADM resistance of the HepG2/ADM cell line (IC_{50}, 11.2 μg/ml) was found to be 62.2-fold that of the parent HepG2 cell line (IC_{50}, 0.18 μg/ml).

The MTS assay results further revealed that the sensitivity of the Ago-miR-503-transfected cells to ADM was significantly increased compared with that of the control and Ago-miR-NC groups. Based on the IC_{50} value of Ago-miR-503, which was 3.3 μg/ml, the RF was 3.4. An increase in apoptosis and cell cycle arrest at G_0/G_1-phase were also observed in the Ago-miR-503 group as compared with the control and Ago-miR-NC groups. Furthermore, the intracellular Rh-123 content was found to be increased in the Ago-miR-503 group compared with that in the control and Ago-miR-NC groups, suggesting that miR-503 reversed ADM resistance through increasing intracellular drug concentration (Fig. 2).

miR-503 decreases the expression of MDR1, MRP1, ERCC1, survivin and Bcl-2, decreases the activity of SOD and GSH, and increases the expression of RhoE in HepG2/ADM cells. In order to investigate the mechanism underlying the
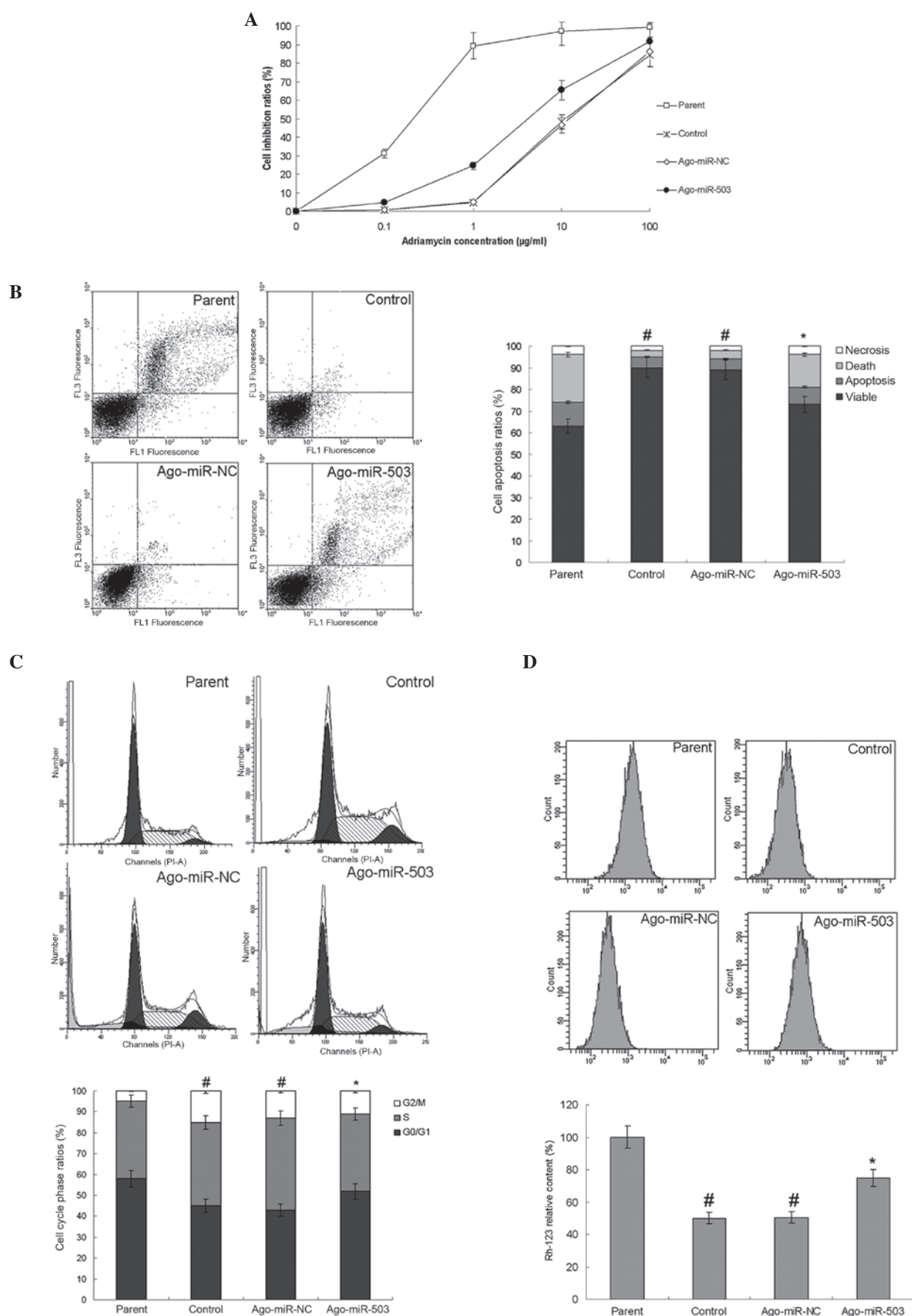
Figure 2. Effect of miR-503 on the reversal of ADM resistance in human hepatocellular carcinoma HepG2/ADM cells. (A-D) Effect of miR-503 on (A) ADM sensitivity, determined using MTS assay (n=5); (B) apoptosis, determined using flow cytometry (n=3); (C) cell cycle, determined using flow cytometry (n=3) and (D) intracellular Rh-123 concentrations, determined using flow cytometry (n=3), in HepG2/ADM cells. Values are presented as the mean ± standard deviation. #P<0.05 versus the parent group; *P<0.05 versus the control group. ADM, Adriamycin; Rh, rhodamine; miR-503, microRNA-503; NC, negative control.
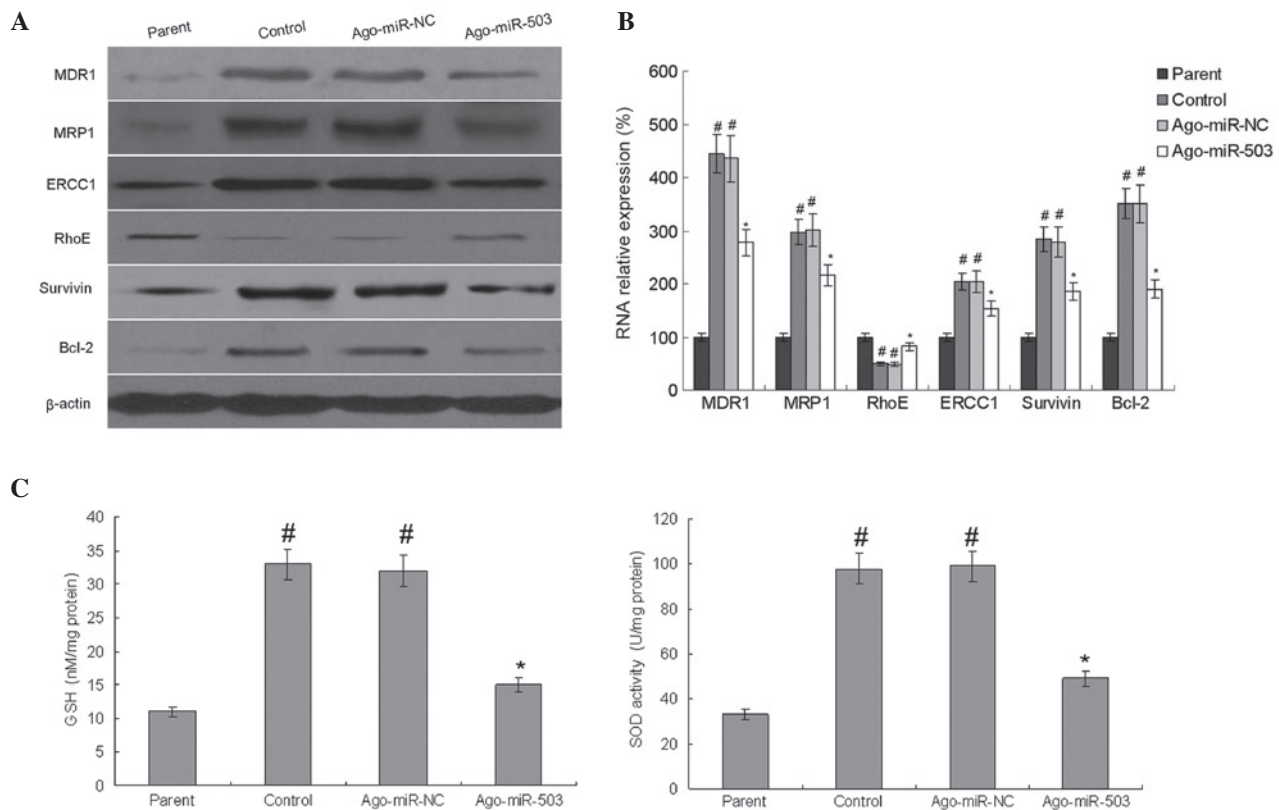
Figure 3. Effect of miR-503 on the expression of drug resistance genes in human hepatocellular carcinoma HepG2/ADM cells. (A and B) Effect of miR-503 on (A) protein and (B) mRNA expression of drug resistance genes in HepG2/ADM cells, determined using western blot analysis and quantitative polymerase chain reaction analysis, respectively. β-actin expression was used as an internal control. (C) Effect of miR-503 on SOD and GSH activity in HepG2/ADM cell lines, determined using biochemical assay. Values are presented as the mean ± standard deviation (n=5). #P<0.05 versus the parent group; *P<0.05 versus the control group. ADM, Adriamycin; MDR1, multidrug resistance 1; MRP1, multidrug resistance-associated protein 1; ERCC1, DNA excision repair protein ERCC-1; Bcl-2, B-cell lymphoma-2; SOD, superoxide dismutase; GSH, glutathione; miR-503, microRNA-503; NC, negative control.
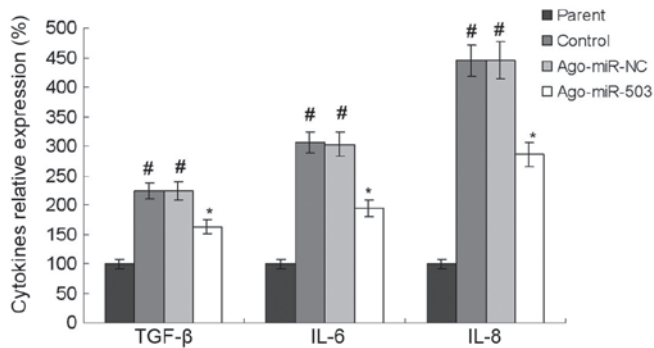

Figure 4. Effect of miR-503 on the production of cytokines associated with ADM resistance in human hepatocellular carcinoma HepG2/ADM cell lines. The effect of miR-503 on the expression of TGF-β and IL-6 and -8 in HepG2/ADM cell lines was determined using liquidchip assay. Values are presented as the mean ± standard deviation (n=5). #P<0.05 versus the parent group; *P<0.05 versus the control group. ADM, Adriamycin; TGF-β, transforming growth factor-β; IL, interleukin; miR-503, microRNA-503; NC, negative control.

miR-503-induced reversal of ADM resistance and promotion of apoptosis in HepG2/ADM cells, the expression of relevant genes was detected. The expression of MDR1 and MRP1, two multidrug resistance proteins, was observed to be significantly reduced in the Ago-miR-503 cells. The activity of the small detoxifying molecules SOD and GSH was also found to be

significantly reduced. Furthermore, the expression of ERCC1, a protein involved in repairing DNA damage, and survivin and Bcl-2, which inhibit apoptosis, was also observed to be significantly downregulated by miR-503. Of note, miR-503 was found to increase RhoE expression in cells in the Ago-miR-503 group as compared with those in the control and Ago-miR-NC groups. qPCR revealed that the regulatory role of miR-503 on the aforementioned proteins was achieved through transcriptional regulation (Fig. 3).

miR-503 inhibits the secretion of TGF-β and IL-6 and -8 in HepG2/ADM cells. Cytokines have an important role in the initiation, development and progression of tumors (15). In the present study, the expression of three cytokines secreted by HepG2 cells, TGF-β and IL-6 and -8, was analyzed. Compared with the control and Ago-miR-NC groups, the expression of TGF-β and IL-6 and -8 in the Ago-miR-503 group was significantly decreased (Fig. 4).

miR-503 inhibits Akt phosphorylation, increases CDK1 expression and inhibits the transcriptional activity of NF-κB and AP-1 in HepG2/ADM cells. In order to study the molecular mechanism underlying miR-503-induced G_0/G_1-phase arrest in HepG2/ADM cells, the phosphoinositide 3-kinase (PI3K)/Akt and cyclin/CDK1 signaling pathways, which are closely associated with the cell cycle, were analyzed. Western blot analysis revealed that Akt phosphorylation was decreased
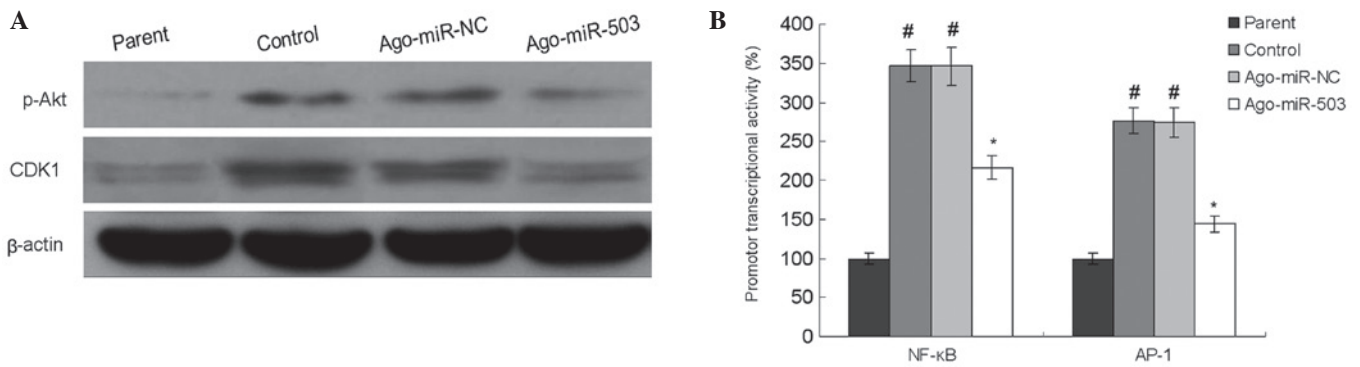
Figure 5. Effect of miR-503 on ADM resistance-related signaling molecules in HepG2/ADM human hepatocellular carcinoma cells. (A) Effect of miR-503 on Akt phosphorylation in HepG2/ADM cells, determined using western blot analysis. β-actin was used as an internal control. (B) Effect of miR-503 on the transcriptional activity of NF-κB and AP-1 in HepG2/ADM cells, determined using double fluorescent reporter gene assay. Values are presented as the mean ± standard deviation (n=5). #$P<0.05$ versus the parent group; *$P<0.05$ versus the control group. ADM, Adriamycin; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; AP-1, activator protein 1; CDK1, cyclin-dependent kinase 1; p-Akt, phosphorylated Akt; NC, negative control; miR-503, microRNA-503.

in the Ago-miR-503 group, indicating that the PI3K/Akt pathway was inhibited. The expression of CDK1 was also decreased, indicating that the cyclin/CDK1 signaling pathway was also inhibited by miR-503. In order to elucidate the molecular mechanism underlying the regulation of cytokine secretion by miR-503, gene reporter systems were used to evaluate the transcriptional activity of NF-κB and AP-1. The transcriptional activities of these two transcription factors were significantly decreased (Fig. 5).

Discussion

Increasing drug efflux and reducing drug concentration are major mechanisms by which tumor cells develop resistance to chemotherapeutic drugs. ABC family transmembrane transporter proteins, including MDR1 and MRP1, pump drugs out of cells and are often overexpressed in drug-resistant tumor cells (16). Upon miR-503 transfection, flow cytometry revealed that the concentration of intracellular Rh-123 was increased. This finding indirectly indicates that the mechanism by which miR-503 reverses drug resistance in HepG2/ADM cells may be associated with reducing drug efflux. Furthermore, miR-503 was found to decrease the protein and mRNA expression of MDR1 and MRP1, providing evidence for the above hypothesis.

In addition to MDR1 and MRP1, other molecules may also be involved in ADM resistance in tumor cells. ERCC1 repairs DNA damage induced by chemotherapeutic drugs (17); therefore, its expression may be associated with HepG2/ADM cell drug resistance. Western blot analysis revealed that ERCC1 protein expression was high in HepG2/ADM cells, but decreased significantly following miR-503 treatment. This decrease in expression was associated with reduced mRNA expression. SOD and GSH act as detoxifying molecules in tumor tissues and reduce the toxicity of the chemotherapeutic drugs (18,19). The present study showed that the SOD and GSH levels in HepG2/ADM cells were significantly increased compared with those in the normal HepG2 cells. Subsequent to reversing the drug resistance in HepG2/ADM cells using miR-503, the SOD and GSH levels decreased, indicating that these two molecules may also have a role in the drug resistance mechanisms of tumor cells. RhoE is an atypical member of the Rho GTPase family and is present in a constitutively

active state for GTP-binding. RhoE expression has recently been found to be decreased in certain major liver cancer cell lines and tissues; this decreased expression may in turn promote tumor invasion and metastasis (20). In the present study miR-503 was found to increase the expression of RhoE, indicating that miR-503 plays an inhibitory role in tumor invasion and metastasis.

Survivin is a member of the anti-apoptotic gene family. This gene exhibits certain tumor-specific characteristics, and is solely expressed in tumors and embryonic tissues. Survivin can inhibit tumor apoptosis and promote proliferation and angiogenesis. Therefore, it is considered to be a highly valuable therapeutic target for tumor treatment (21,22). In the present study, miR-503 was found to decrease survivin expression, which is consistent with the observation that miR-503 increased the rate of tumor cell apoptosis. Bcl-2 has important functions in apoptosis and inhibits tumor cell apoptosis. The overexpression of Bcl-2 has been found to be associated with drug resistance in numerous tumor cells (23,24). In the non-small-cell cisplatin-resistant A549/CDDP cell line, miR-503 has been reported to reduce the expression of Bcl-2 and reverse drug resistance (25). In the present study Bcl-2 expression was observed to be significantly increased in HepG2/ADM cells, indicating that the anti-apoptotic effect of Bcl-2 may be one of the mechanisms by which drug resistance develops in tumor cells. In the present study, miR-503 transfection significantly reduced Bcl-2 expression in HepG2/ADM cells, which is consistent with the enhanced apoptosis detected using flow cytometry. This may be one of the important mechanisms underlying the miR-503-induced reversal of drug resistance in tumor cells.

In the present study, the overexpression of miR-503 was found to arrest HepG2/ADM cells at G_0/G_1-phase. CDK1 is an important regulatory protein in the cell cycle and has an essential role in G_0/G_1- to S-phase progression (26). In tumor cells, checkpoint cyclins often become abnormally regulated by CDKs, resulting in uncontrolled tumor cell proliferation (26,27). The present study found that miR-503 decreased CDK1 expression. In HepG2/ADM cells, miR-503 may regulate CDK1 through the same mechanism. PI3K/Akt is another signaling pathway that plays an important role in regulating the cell cycle (28). In the present study, miR-503 was observed to

inhibit Akt phosphorylation and, in turn, inhibit the activity of the PI3K/Akt signaling pathway, thereby inhibiting the proliferation of tumor cells and reversing tumor cell drug resistance.

Cytokines have an important role in the occurrence and development of liver cancer (29,30). TGF-β and IL-6 and -8 expression was observed in normal HepG2 cells; however, in HepG2/ADM cells, the concentration of these cytokines was significantly increased. These cytokines may help liver cancer cells to regulate their microenvironment, enhance their proliferation, invasion and metastasis, and ultimately survive in the challenging environment (30). The present study showed that miR-503 downregulated the expression of TGF-β and IL-6 and -8. NF-κB and AP-1 are two important transcription factors that have key roles in cell proliferation, apoptosis and the secretion of inflammatory cytokines in normal and tumor cells (31,32). The present study indicated that miR-503 may negatively regulate the activity of NF-κB and AP-1. Although the specific mechanism requires further investigation, this negative regulation may be one of the mechanisms responsible for the miR-503-induced reversal of drug resistance in HepG2/ADM cells.

In conclusion, we considered miR-503 as a potential target for the reversal of drug resistance in human hepatocellular carcinoma. The mechanisms underlying this miR-503-induced reversal of drug resistance may involve the inhibition of drug efflux, the downregulation of drug resistance-associated gene expression, the prevention of cell cycle progression and the promotion of cell apoptosis.

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