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RESEARCH ARTICLE

miR-103/107 modulates multidrug resistance in human gastric carcinoma by downregulating Cav-1

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Abstract MicroRNAs (miRNAs) are a class of non-protein-coding small RNAs with the capacity to regulate fundamental biological processes essential for cancer initiation and progression. In the present study, we analyzed miRNA expression levels between multidrug-resistant gastric carcinoma cell line SGC7901/ADR and its parent cell line SGC7901 using a miRNA microarray. MiR-103/107 was downregulated compared with parental SGC7901 cells. Overexpression of miR-103/107 sensitized SGC7901/ADR cells to doxorubicin (DOX), as demonstrated by in vitro and in vivo drug sensitivity assay. We further confirmed that miR-103/107 inhibited P-gp function in gastric cancer SGC7901/ADR cells. Finally, we verified that caveolin-1 (Cav-1), a critical component of lipid rafts, was a target of miR-103/107.

Keywords Gastric carcinoma · miR-103/107 · Multidrug resistance · Caveolin-1

Introduction

Gastric cancer is one of the most common malignancies worldwide. Chemotherapy can improve survival rates and quality of life in patients with unresectable gastric cancer [1]. However, despite considerable advances in drug discovery, the effectiveness of chemotherapy is seriously limited by drug resistance [2]. The molecular genetic basis of resistance to cancer therapeutics is complex and involves multiple processes such as drug transport, drug metabolism, DNA repair, and apoptosis. Traditionally, therapeutic targets and modulators are focused at the DNA level, and changes in messenger RNA (mRNA) and protein expression levels have been investigated to clarify the mechanisms responsible for pharmacologic responses [3–8]. Expression profiling has usually been performed at the mRNA level, but mRNA and encoded protein levels are not necessarily proportional, and the expression of noncoding RNAs has recently been suggested to contribute to the lack of proportionality between mRNA and encoded proteins [9].

MicroRNAs (miRNAs) are small noncoding RNAs of 21–25 nucleotides in length that negatively modulate protein expression [10]. One strand of the mature double-stranded miRNA is incorporated into the RNA-induced silencing complex, which downregulates target mRNAs either by degradation or translational inhibition [11]. miRNAs play important roles in the normal regulation of cell proliferation and apoptosis. Moreover, altered miRNA expression is implicated in cancers. Emerging evidence has shown that knock-down or re-expression of specific miRNAs by synthetic antisense oligonucleotides or miRNA precursors or mimics can modulate drug resistance [12–17]. For example, miR-200c expression was downregulated in multidrug-resistant (MDR) human breast cancer cells (MCF-7/ADR), and upregulation of miR-200c by transfection with miR-200c mimics enhanced their chemosensitivity to epirubicin [13]. miR-1915 inhibits Bcl-2 and modulates MDR by increasing drug sensitivity in human colorectal carcinoma cells [14]. miR-181a was downregulated in a MDR leukemia cell line (K562/A02), while overexpression of miR-181a sensitized K562/A02 cells to daunorubicin by targeting Bcl-2 [15]. In contrast, miR-21 was upregulated in glioblastoma cells (U251), and miRNA-21 inhibitor sensitized U251 cells to chemotherapeutic drugs [16]. Knock-

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down of miR-203 upregulated SOCS3 expression and enhanced cisplatin chemosensitivity in breast cancer cells [17]. Overall, these reports suggest that miRNAs play a significant role in drug resistance.

However, the role of miR-103/107 in drug resistance in gastric cancer remains unknown. We therefore compared miR-103/107 expression levels in the drug-resistant human gastric carcinoma cell line SGC7901/ADR and the parental SGC7901 cell line. We investigated its relationship with doxorubicin (DOX) sensitivity and examined the potential role of caveolin-1 (Cav-1) in miR-103/107-mediated regulation of drug resistance in human gastric cancer cells.

Materials and methods

Reagents

Antibodies against P-gp and β-actin were purchased from Santa Cruz Biotechnology Inc. (CA, USA). Antibodies specific to caveolin-1 were purchased from Cell Signaling Technology (Beverly, MA, USA). Doxorubicin (DOX), verapamil, 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl-tetrazolium bromide (MTT), and other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Cell lines and culture

Human gastric adenocarcinoma cell line SGC7901 was obtained from the Academy of Military Medical Science (Beijing, China), and its DOX-selected P-gp overexpressing derivative cell line, SGC7901/ADR, was granted by the Institute of Gastroenterology, Xijing Hospital, Fourth Military Medical University. To maintain the drug-resistant phenotype, DOX (with final concentration of 1 μg/mL) was added to the culture media for SGC7901/ADR cells. All cell lines were cultured in RPMI 1640 supplemented with 10 % fetal bovine serum at 37 °C in the presence of 5 % CO_2.

miRNA microarray analysis

Prior to experimentation, SGC7901 and SGC7901/ADR cells were cultured 1 week without DOX. Total RNA from SGC7901 and SGC7901/ADR cell lines was isolated with Trizol reagent (Invitrogen, Carlsbad, CA), and the integrity of these total RNAs was assessed using an Agilent 2100 bioanalyzer. miRNA fraction was further purified using a mirVana™ miRNA isolation kit (Ambion, Austin, TX). The isolated miRNAs from the two cell lines were then labeled with Hy3 using the miRCURY™ Array Labeling kit (Exiqon, Vedbaek, Denmark) and hybridized respectively on a miRCURY™ LNA microRNA Array (v 8.0; Exiqon). Microarray images were acquired using a GenePix 4000B scanner (Axon Instruments, Union City, CA) and processed and analyzed with GenePix Pro 6.0 software (Axon Instruments) and Excel.

Quantitative real-time PCR analysis for miRNA

Total RNA from the cultured cells was isolated with Trizol reagent (Invitrogen, Carlsbad, CA), and the concentration of the total RNA was quantified by measuring the absorbance at 260 nm. The expression of mature miRNAs was assayed using stem-loop reverse transcription (RT) followed by real-time PCR analysis [18]. All reagents for the stem-loop RT were obtained from Applied Biosystems (Foster City, CA). The PCR primers used were as follows: Cav-1 forward 5′ TCA ACC GCG ACC CTA AAC ACC 3′, Cav-1 reverse 5′ TGA AAT AGC TCA GAA GAG ACA T 3′ (561 bp); GAPDH forward 5′ TGG TAT CGT GGA AGG ACT CAT GAC 3′, GAPDH reverse 5′ AGT CCA GTG AGC TTC CCG TTC AGC 3′ (198 bp). Quantitative real-time PCR was performed using SYBR Premix Ex Taq II (TaKaRa) and measured in a LightCycler 480 system (Roche, Basel, Switzerland). U6 or GAPDH was used as the internal control. The $2^{-\Delta\Delta Ct}$ method was used to calculate the fold change of the RNA expression of one sample compared to the calibration sample [19].

Oligonucleotide transfection

The miR-103/107 mimics or inhibitor and corresponding negative control were designed and synthesized by RiboBio (Guangzhou, China). Target cells were transfected with miR-103/107 mimics, inhibitor, or the corresponding negative controls at a final concentration of 50 nM (mimics) or 200 nM (inhibitor) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Cells were collected 48 h after transfection.

In vitro drug sensitivity assay

The sensitivity of gastric cancer cells to anticancer drugs was evaluated using a colony-forming assay and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay as described previously [20, 21]. Twenty-four hours after, transfection cells were seeded into 96-well plates (5×10^3 cells/well) for next step experiment. After cellular adhesion, freshly prepared anticancer drug DOX was added with the different concentrations as previously described [21]. Forty-eight hours after the addition of the drug, cell viability was assessed by MTT assay. The absorbance at 490 nm (A490) of each well was read on a spectrophotometer. The concentration at which the drug produced 50 % inhibition of growth (IC_{50}) was estimated by the relative survival curve.

Three independent experiments were performed in quadruplicate.

Determination of intracellular DOX concentrations

The effect of miR-103/107 on the intracellular accumulation of DOX in SGC7901/ADR cell lines was measured by flow cytometry as previously described [21, 22]. Briefly, cells were seeded onto six-well plates (1×10^6 per well) and cultured overnight at 37 °C. After addition of DOX (final concentration 5 μg/mL), cells were cultured for a further 1 h. Cells were then either harvested (DOX uptake) or cultured in drug-free RPMI 1640 medium for a further 1 h (DOX retention), followed by harvesting. Cells were washed with PBS, and the mean fluorescence intensity of intracellular DOX was determined using flow cytometry at an excitation wavelength of 488 nm and an emission wavelength of 575 nm. The DOX releasing index of cells was calculated according to the formula: releasing index=(accumulation value−retention value)/accumulation value.

Luciferase reporter assay

The full-length 3′ untranslated regions and coding sequences of Cav-1 were cloned into the psiCheck2 dual luciferase reporter vector (Promega). Potential miR-103/107 sites in genes were identified by alignment with the complementary miR-103/107 sequence. Mutagenesis was performed using the QuickChange Multi Site-Directed Mutagenesis Kit (Agilent Technologies). Cells in 24-well plates were transfected with either 400 ng of luciferase reporter psiCheck2 control or 500 ng psiCheck2 plus Cav-1 3′ untranslated region (UTR) constructs using Lipofectamine 2000 (Invitrogen), according to the manufacturer's instructions. Cotransfection of miR-103/107 mimic and antagomiR at 50 nM was also performed. All transfection experiments were performed in triplicate. Luciferase activity was assayed at 48 h post-transfection, using a dual luciferase reporter assay system (Promega).

Western blot analysis

Cells were extracted and proteins were quantified as described previously [22]. Aliquots (50 μg) of each lysate were separated by electrophoresis on SDS-PAGE gels and transferred to nitrocellulose membranes. Membranes were blocked with 5 % non-fat milk in TBST (10 mM Tris-HCl pH 7.4, 100 mM NaCl, 0.5 % Tween-20) for 2 h at room temperature and incubated overnight at 4 °C in 5 % non-fat milk in TBST containing P-gp, Cav-1, or β-actin antibodies. Membranes were washed and incubated with peroxidase-conjugated second antibodies for 1 h. After extensive washing with TBST, proteins were visualized using the enhanced chemiluminescence reagent (SuperSignal Western Pico Chemiluminescent Substrate; Pierce, Rockford, IL). Final images were analyzed using NIH Image J software.

Tumor xenograft studies

We subcutaneously injected 5×10^6 SGC7901/ADR cells resuspended in 50 μL of phosphate-buffered saline into the right flank of 4-week-old BALB/C athymic mice (Shanghai SLAC Laboratory Animal Co., Ltd). Mice were housed and maintained under specific pathogen-free conditions. All animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of China Medical University. When mice had palpable tumors, the mice were randomly assigned, to avoid treatment bias, to treatment groups (n=6 mice per group). DOX (2 mg/kg) was given intraperitoneally every other day for 28 days. For agomiR treatment, agomiR-103 or agomiR-NC (RiboBio, Guangdong, China) was directly injected intratumorally at the dose of 1 nmol (diluted in 20 μL phosphate-buffered saline) per mouse every 4 days for seven times. Tumor volumes were calculated as length×(square of width)/2. After the initial treatment, the tumor size was determined every day. Mice were killed by cervical dislocation under anesthesia. Investigators were blinded to the treatment groups.

Statistical analysis

Data are presented as the mean±standard deviation, and differences were determined using the Student's t test. A P value of less than 0.05 was considered statistically significant. All means were calculated from at least three independent experiments.

Results

miR-103/107 was downregulated in MDR gastric cancer cells

In order to determine whether miRNAs are involved in the development of MDR in human gastric cancer cells, we performed profile analysis of miRNA expression in SGC7901/ADR compared with parental SGC7901 cells. Thirteen of miRNAs had shown obvious differential expression between SGC7901/ADR cells and their parent cells (Table 1, fold change ≥2.5).

To verify the results obtained by microarray profiling, we performed quantitative real-time polymerase chain reaction (RT-PCR) in the two cell lines. As consistent with the microarray data, quantitative RT-PCR analysis demonstrated that miR-103/107 was significantly downregulated in SGC7901/ADR cells compared with SGC7901 cells, with decreased fold changes of 0.32±0.04 and 0.45±0.09, respectively (Fig. 1a, b).

Table 1 miRNAs differentially expressed in SGC7901 and parental SGC7901 cells

miRNA	Upregulation or downregulation in SGC7901/ADR
hsa-miR-10a	Up
hsa-miR-1254	Up
hsa-miR-145	Up
hsa-miR-17	Up
hsa-miR-297	Up
hsa-miR-338-5p	Up
hsa-miR-519b-5p	Up
hsa-miR-523	Up
hsa-miR-103	Down
hsa-miR-107	Down
hsa-miR-1224-5p	Down
hsa-miR-30e	Down
hsa-miR-30b	Down
hsa-miR-361-3p	Down
hsa-miR-424	Down
hsa-miR-487b	Down
hsa-miR-520f	Down
hsa-miR-587	Down
hsa-miR-593	Down
hsa-miR-628	Down
hsa-miR-675	Down
hsa-miR-760	Down
hsa-miR-92b	Down

miR-103/107 regulated DOX resistance in human gastric cancer cells in vitro

To explore the role of miR-103/107 in gastric cancer, SGC7901/ADR drug-resistant cells were transfected with miR-103/107 mimics or miRNA mimic control, and parental SGC7901 cells were transfected with anti-miR-103/107 or anti-miR control. DOX sensitivity was greatly increased in cells transfected with miR-103/107 mimics compared with miRNA mimic control-transfected cells, demonstrated by MTT assay (Fig. 2a). In contrast, DOX resistance was increased in SGC7901 cells transfected with anti-miR-103/107 compared with control-transfected cells (Fig. 2b). Treatment of SGC7901/ADR cells with miR-103/107 mimics and DOX also significantly reduced the colony-forming ability of the cells (Fig. 2c). These results suggest that miR-103/107 might regulate DOX resistance in human gastric cancer cells.

miR-103/107 reduced DOX efflux by P-glycoprotein downregulation

To determine if miR-103/107 affected drug efflux in gastric cancer cells, we measured the DOX-release index in SGC7901/ADR cells transfected with miR-103/107 mimics. DOX efflux was clearly lower in miR-103/107-transfected cells (Fig. 3a). Consistent with these results, RT-PCR demonstrated decreased mdr1 levels in miR-103/107-transfected SGC7901/ADR cells (Fig. 3b), and reduced P-gp expression in SGC7901/ADR cells transfected with miR-103/107 was also validated by Western blot (Fig. 3c). To further investigate the mechanism of miR-103/107-mediated reversal of MDR in gastric cancer, we treated transfected cells with the P-gp inhibitor verapamil. DOX release was reduced in miR-103/107 mimic-transfected SGC7901/ADR cells, but was not decreased further in the same cells incubated with verapamil (Fig. 3d). This indicates that P-gp downregulation only partly accounted for miR-103/107-induced reversion of DOX efflux.

miR-103/107 modulated MDR by targeting Cav-1

Cav-1 is a critical component of lipid rafts and has been identified as a direct target gene of miR-103/107 [23]. As a specialized domain in the plasma membrane, lipid rafts have

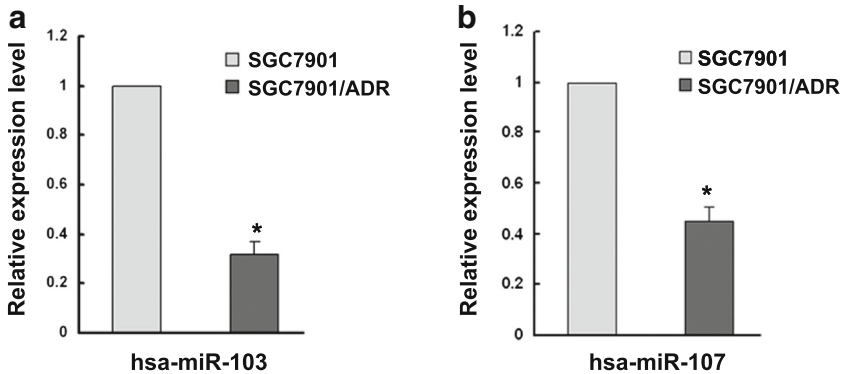

Fig. 1 Expression of miR-103/107 in SGC7901/ADR and SGC7901 cell lines. **a**, **b** Real-time quantification of miR-103/107 by RT-PCR showed that miR-103/107 was downregulated in SGC7901/ADR compared with SGC7901 cells. Triplicate assays were performed for each RNA sample, and the amount of miR-103/107 was normalized relative to U6 snRNA. Results represent the means of the values. *Bars* indicate SD. *$P<0.05$ (significant difference between groups)

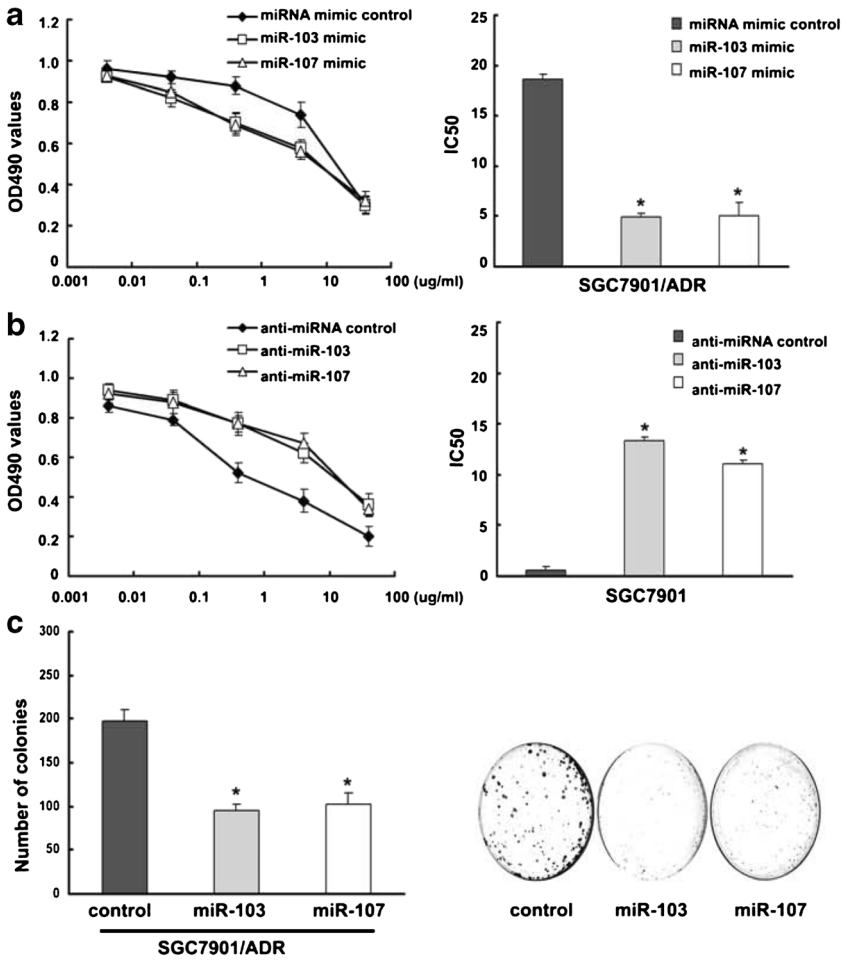

Fig. 2 miR-103/107 regulated DOX resistance of human gastric cancer cells in vitro. **a** SGC7901/ADR cells transfected with miR-103/107 mimics exhibited greatly reduced resistance to DOX. **b** SGC7901 cells transfected with anti-miR-103/107 exhibited greatly enhanced resistance to DOX. **c** SGC7901/ADR cells were treated with 5 μg/mL DOX as a control or combined with transfection with miR-103/107 mimics for 6 h, and their colony-forming abilities were analyzed. Colonies were counted (*left*) and captured (*right*). Colonies ≥100 μm in diameter were counted under a microscopic at ×10 magnification. Results represent the means of values from three independent experiments. *Bars* indicate SD. **P*<0.05 (significant difference between groups)

been shown to be essential for channel formation by P-gp and to play important roles in modulating P-gp transport activity [24–27]. The decreased expression of miR-103/107 in SGC7901/ADR cells compared with parental SGC7901 cells was concurrent with the overexpression of Cav-1 protein (Fig. 4a), but not mRNA levels (data not shown). We transfected SGC7901/ADR cells with miR-103/107 mimics and control miRNA mimics and measured the changes in Cav-1 expression levels. Cav-1 protein levels in SGC7901/ADR cells were significantly reduced 72 h after transfection with miR-103/107 mimics, compared with miRNA mimic control-transfected cells (Fig. 4b).

To assess whether miR-103/107 directly regulates Cav-1 expression, the target sequence of Cav-1 3′ UTR (wt 3′ UTR) or the mutant sequence (mt 3′ UTR) was cloned into a luciferase reporter vector. SGC7901/ADR cells were then transfected with wt or mt 3′ UTR vector and miR-103/107 mimics. The results showed a significant decrease in luciferase activity when compared with miR control (Fig. 4c). The activity of mt 3′ UTR vector was unaffected by a simultaneous transfection with miR-103/107 (Fig. 4c). Moreover, cotransfection with anti-miR-103/107 and wt 3′ UTR vector in SGC7901/ADR cells led to an increase in luciferase activity (Fig. 4c). Taken together, all these results strongly suggested that Cav-1 is a direct target of miR-103/107 in SGC7901/ADR cells.

Effect of miR-103/107 on tumor growth in vivo combined with DOX

To further demonstrate the role of miR-103/107 in chemosensitization, we used miR-103/107 agomiR, a cholesterol-conjugated 2′-*O*-methyl-modified miR-103/107 that has suitable pharmacokinetic properties for in vivo studies. Mice were treated with vehicle alone, DOX plus miR-103/107 agomiR, or DOX plus scramble agomiR. DOX plus scramble agomiR had no statistical effects on tumor growth. However, in mice treated with DOX plus miR-103/107 agomiR, tumor growth was statistically significantly delayed, with a 49 % (*P*<0.01) and 58 % (*P*<0.01) reduction in tumor volume at day 35 compared with vehicle or DOX plus scramble agomiR, respectively (Fig. 5a, c). Moreover, tumor weights after tumor cell infection with DOX plus miR-103/107 agomiR were statistically significantly lower than the vehicle or DOX plus scramble agomiR, respectively (DOX plus miR-103/107 agomiR: mean tumor weight=50.8 mg,

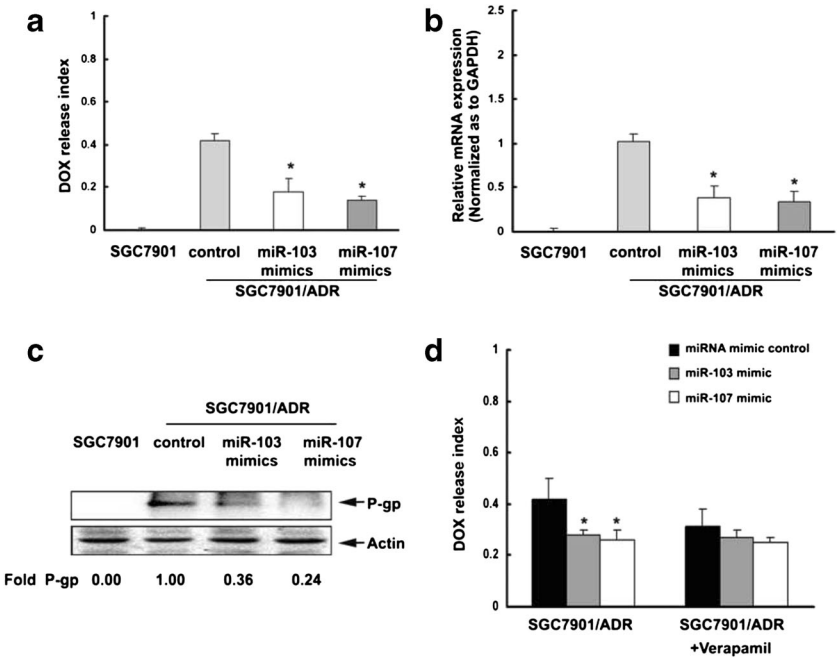

Fig. 3 miR-103/107 decelerated DOX efflux through P-gp downregulation. **a** DOX-release index of miR-103/107-transfected SGC7901/ADR cells was measured after incubation with 5 μg/mL DOX. DOX-release index was calculated as described in "Materials and Methods." **b** Expression of mdr1 in SGC7901/ADR was examined by qRT-PCR after transfection with miR-103/107 mimics. GAPDH mRNA level was used as an internal control. **c** Expression of P-gp in SGC7901/ADR cells was examined by Western blot after transfection with miR-103/107 mimics. Actin was used as an internal control. **d** SGC7901/ADR cells were transfected with miR-103/107 mimics and incubated with 5 μg/mL DOX after incubation with verapamil (10 μmol/L) or negative control for 24 h, and the DOX-release index was calculated as that in the "Materials and Methods." Data are shown as the mean±SEM (n=3) of one representative experiment. Similar results were obtained in three independent experiments

95 % CI=46.7 to 89.2 mg; vehicle alone: mean tumor weight=166.7 mg, 95 % CI=148.1 to 202.6 mg; DOX plus scramble agomiR: mean tumor weight=181.6 mg, 95 % CI=153.3 to 217.4 mg) (Fig. 5b, d). Moreover, treatment with DOX plus miR-103/107 agomiR did not result in any obvious signs of toxicity such as weight loss over the course of the treatment (Fig. 5e). These data support the notion that miR-103/107 is a potent DOX sensitizer in vivo.

Mice were killed after 28 days of treatment. Tumors overexpressing miR-103/107 had a lower level of caveolin-1 protein than control tumors by IHC. Furthermore, miR-103/107 overexpressing tumors showed lower Ki67 staining, which indicates that miR-103/107 overexpressing tumors have lower proliferative potential (Fig. 5f). Therefore, these data suggest that miR-103/107-dependent downregulation of caveolin-1 plays a positive role in the treatment of gastric cancer.

Discussion

Acquired resistance to classical chemotherapeutics is the most frequent cause of treatment failure in gastric cancer chemotherapy [2]. DOX is used in many cancer therapies, either as a single agent or in combination with other agents. Several recent studies have demonstrated an involvement of miRNAs in the development of drug resistance in cancer [12–15, 18]. The results of the current study provide the first evidence to suggest that miR-103/107 might regulate DOX resistance in human gastric cancer cells by targeting Cav-1.

In humans, the classical causes of drug resistance involve overexpression of the MDR1 gene product, P-gp, which correlates with poor prognosis in many cancer types [28]. Inhibition of P-gp function or expression can reverse P-gp-mediated MDR and improve the efficacy of chemotherapy [29]. The current study demonstrated downregulation of miR-103/107 in SGC7901/ADR cells and significant downregulation of P-gp by miR-103/107 transfection. DOX release was also decreased in miR-103/107 mimic-transfected SGC7901/ADR cells. These results indicate that miR-103/107 reduces the efflux of cytotoxic drugs by downregulating the expression of the ABC transporter P-gp, and that modulation of miR-103/107 expression could alter the sensitivity of gastric cancer cells to DOX. Notably, we treated transfected cells with the P-gp inhibitor verapamil. DOX release was reduced in miR-103/107 mimic-transfected SGC7901/ADR cells, but was not decreased further in the same cells incubated with verapamil. This indicates

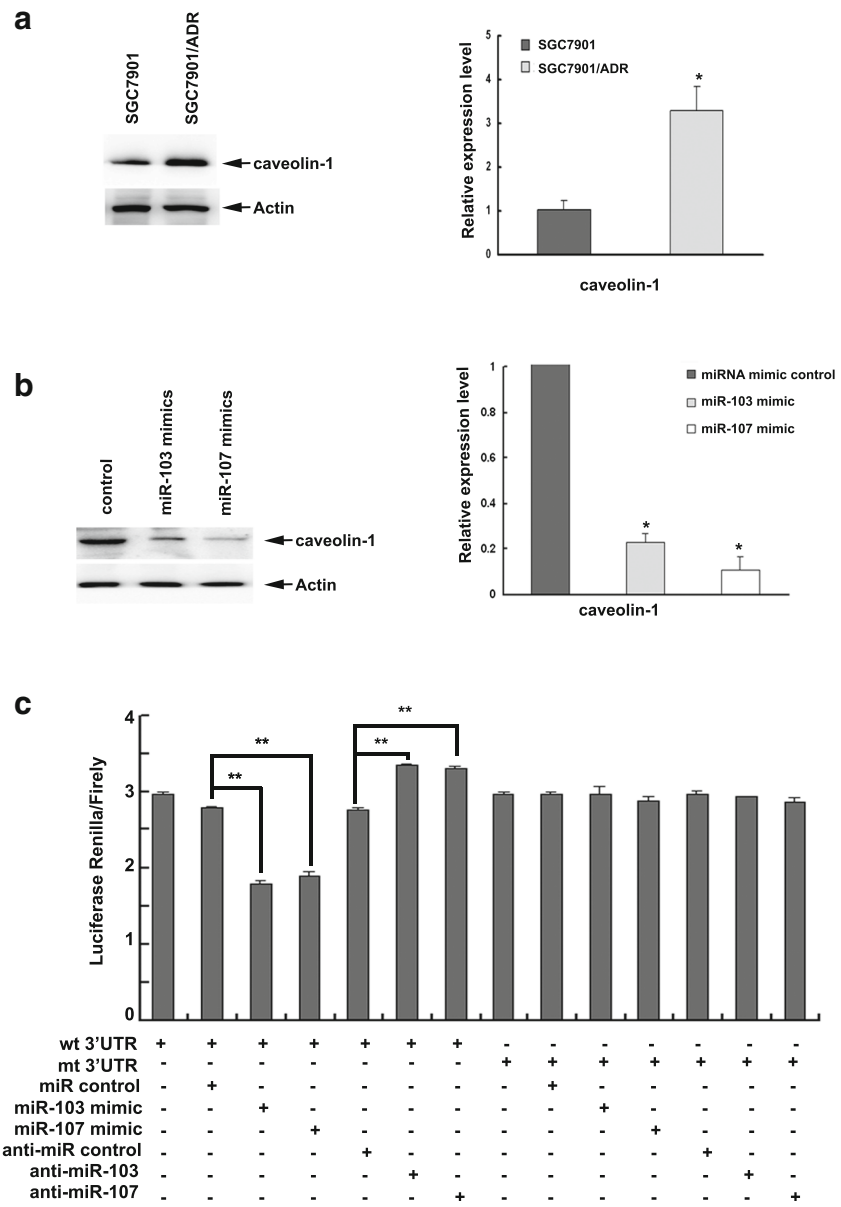

Fig. 4 miR-103/107 modulated DOX resistance by repressing Cav-1 protein expression. **a** Caveolin-1 protein was overexpressed in SGC7901/ADR cells compared with parental SGC7901 cells. Representative Western blot is attached beside the graph. **b** Caveolin-1 protein levels were significantly reduced inn SGC7901/ADR cells at 72 h after transfection with miR-103/107 mimics compared with miRNA mimic control-transfected cells, as demonstrated by Western blot. Representative Western blot is attached beside the graph. Results represent the mean±SD from three independent experiments. **c** Dual luciferase assay was performed in SGC7901/ADR cells transfected with luciferase construct alone or cotransfected with miR-103/107 mimics and miR-103/107 antagomiRs. Firefly luciferase construct containing mutant target site of the Cav-1 3′ UTR was generated and transfected as indicated. Firefly luciferase activity was normalized to Renilla luciferase activity for each sample. The results shown represent the mean±SE from three independent experiments. (*$P<0.05$; **$P<0.001$)

that P-gp downregulation only partly accounted for miR-103/107-induced reversion of DOX efflux, suggesting that P-gp downregulation only partly accounts for the miR-103/107-induced deceleration of drug efflux.

Lipid rafts are small, heterogeneous, highly dynamic, sterol- and sphingolipid- enriched domains. Caveolae are a subset of lipid rafts characterized by flask-like invaginations of the plasma membrane and distinguished from bulk lipid rafts by the presence of Cav-1 [27]. Caveolin-1 interacts with P-gp and modulates P-gp transport activity [30, 31], indicating that caveolae represent the platform for P-gp channel formation. Trajkovski et al. reported that Cav-1 acted as the functional target of miR-103/107 during the regulation of insulin sensitivity [23]. We therefore investigated the effect of miR-103/107 on Cav-1 expression in MDR gastric cancer cells. Compared with parental SGC7901 cells, the decreased expression of miR-103/107 in SGC7901/ADR cells was concurrent with Cav-1 protein overexpression. Furthermore, we demonstrated a decreased expression of Cav-1 in miR-103/107-transfected SGC7901/ADR cells. Given that reduced Cav-1 expression could affect P-gp drug transport activity to reverse DOX chemoresistance, we suggest that miR-103/107 increases drug susceptibility by regulating P-gp function. Several recent studies confirmed that downregulation of Cav-1 by short interfering RNA increased the sensitivity of acquired drug-resistant cells toward DOX [30] and paclitaxel [31]. Ho et al. also reported that Cav-1 expression was associated with a poor prognosis and with drug resistance in

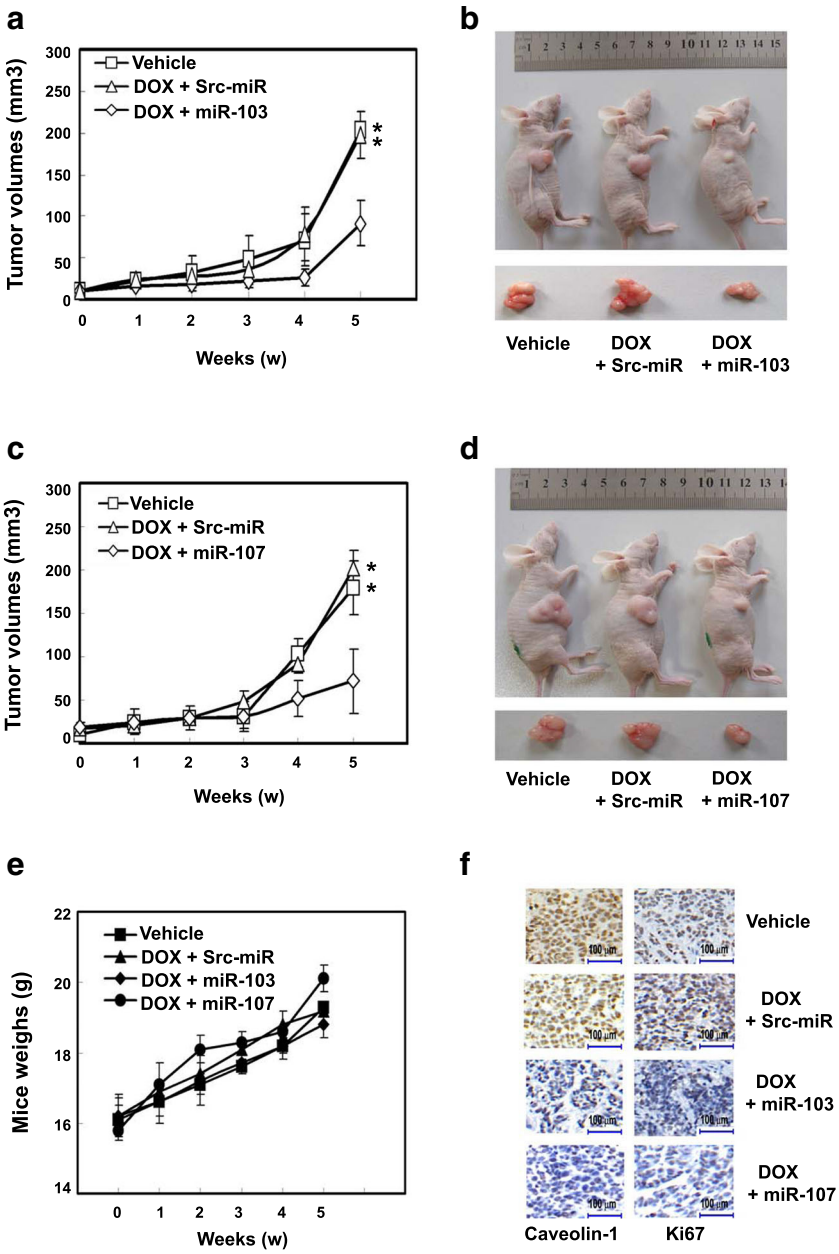

Fig. 5 Effect of miR-103/107 on tumor growth in vivo combined with DOX. **a** Growth curves of SGC7901/ADR subcutaneous xenograft tumors treated with vehicle, DOX (2 mg/kg, intraperitoneally every other day) plus agomiR-103/107 (1 nmol, intratumoral injection, every 4 days), or DOX (2 mg/kg, intraperitoneally every other day) plus agomiR-scramble-NC (1 nmol, intratumoral injection, every 4 days) are shown. Tumor volumes were calculated as length×(square of width)/2; $n=6$ per group (*$P<0.01$, two-sided Student's t test). **b** The gross morphology of tumors (*upper panel*) and the final xenograft tumor weights (*lower panel*) measured on day 35 after tumor cell injection (*$P<0.01$, two-sided Student's t test). **c** The changes in weights of mice treated as indicated above were plotted (*$P<0.01$, two-sided Student's t test). **d** The immunohistochemistry analyses for caveolin-1 and Ki67 staining were carried out on SGG7901/ADR xenograft tumor sections collected from mice treated with the indicated treatments. Representative staining are shown. *Scale bars*=100 μm. Data in **a**–**d** are the mean values ±95 % confidence intervals. *scr-miR*, agomiR-scramble NC; *DOX*, doxorubicin

patients with advanced non-small cell lung cancer after gemcitabine-based chemotherapy [32]. These results suggest that miR-103/107 may also inhibit drug efflux by regulating Cav-1 expression.

In conclusion, the results of this study demonstrate that miR-103/107 is downregulated in MDR gastric cancer cell lines. Moreover, miR-103/107 increases the sensitivity of gastric cancer cells to the anticancer agent DOX by inhibiting drug efflux and downregulating P-gp expression. Importantly, Cav-1 was confirmed as a functional target of miR-103/107 in gastric cancer cells, demonstrating that miR-103/107 may reverse MDR by downregulating Cav-1 expression. These results enhance our understanding of the molecular network underlying MDR in gastric cancer.

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Conflicts of interest None

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