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ORIGINAL ARTICLE

miR-200bc/429 cluster modulates multidrug resistance of human cancer cell lines by targeting BCL2 and XIAP

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Abstract
Purpose MicroRNAs (miRNAs) are short non-coding RNA molecules, which post-transcriptionally regulate genes expression and play crucial roles in diverse biological processes. Recent studies have shown that dysregulation of miRNAs might modulate the resistance of cancer cells to anti-cancer drugs, yet the modulation mechanism is not fully understood. We aimed to investigate the possible role of miRNAs in the development of multidrug resistance (MDR) in human gastric and lung cancer cell lines.
Methods miRNA Quantitative real-time PCR was used to detect the different miRNA expression levels between drug resistant and parental cancer cells. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to test the drug-resistant phenotype changes in cancer cells via over or downregulation of miRNAs. Dual-luciferase activity assay was used to verify the target genes of miRNAs. Western blot analysis and apoptosis assay were used to elucidate the mechanism of miRNAs on modulating drug resistance in cancer cells.
Results miR-200bc/429 cluster was downregulated, while BCL2 and XIAP were upregulated in both MDR SGC7901/VCR (vincristine) and A549/CDDP (cisplatin) cells, compared with the parental SGC7901 and A549 cells, respectively. Overexpression of miR-200bc/429 cluster sensitized SGC7901/VCR and A549/CDDP cells to anti-cancer drugs, respectively. Both BCL2 and XIAP 3′-UTR reporters constructed in MDR cells suggested that BCL2 and XIAP were the common target genes of the miR-200bc/429 cluster. Enforced miR-200bc/429 cluster expression reduced BCL2 and XIAP protein level and sensitized both MDR cells to VCR-induced and CDDP-induced apoptosis, respectively.
Conclusions Our findings first suggest that miR-200bc/429 cluster could play a role in the development of MDR in both gastric and lung cancer cell lines, at least in part by modulation of apoptosis via targeting BCL2 and XIAP.

Keywords miR-200bc/429 cluster · Multidrug resistance · BCL2 · XIAP

Abbreviations

miRNAs	MicroRNAs
MDR	Multidrug resistance
VCR	Vincristine
CDDP	Cisplatin
ADR	Adriamycin
5-Fu	5-Fluorouracil
VP-16	Etoposide

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Introduction

Cancer is one of the leading causes of death globally. Chemotherapy now is still one of the major treatment methods for cancer. Unfortunately, drug resistance of cancer cells either intrinsic or acquired continues to be a dominating obstacle to the successful clinical cancer therapy [1]. Previous studies have indicated that the cytological basis of resistance to cancer therapeutics was complex, involving multiple processes, such as drug transport, drug metabolism, DNA synthesis and repair, cell survival and apoptosis, etc. [2–4]. Recently, studies have showed that both genetic changes including mutations, translocations, deletions and amplification of genes or promoter regions and epigenetic changes including aberrant DNA methylation, histone modifications, and non-coding RNA expression might form the underlying molecular mechanisms of drug resistance of cancer cells. Especially, the epigenetic changes, which do not necessarily require a stable heritable genetic alteration, might play an more important role in acquired drug resistance of cancer cells, which is closely relevant to clinical practice [5–7].

MicroRNAs (miRNAs) are a class of small non-coding RNAs of 18–24 nucleotides, which regulate protein expression of specific mRNA by either translational inhibition or mRNA degradation [8]. miRNAs may play important roles in the regulation of gene expression for development, proliferation, and apoptosis. Because many of the same biological processes are relevant to cancer chemoresistance, miRNAs are therefore thought to be associated with drug resistance; exactly, recent studies have suggested that drug-induced dysregulation of miRNA function might modulate the resistance of cancer cells to anti-cancer agent's therapy [9–12]. Emerging evidence has shown that knock-down or re-expression of specific miRNAs by synthetic anti-sense oligonucleotides or miRNAs precursors or mimics could modulate drug resistance [13–20]; for instance, miR-21 was upregulated in various chemoresistance cancer cells, such as breast cancer and glioblastoma cells [13–15], downregulating miR-21 via transfection of anti-miR-21 oligonucleotide sensitized cells to undergo apoptosis [14, 15]; blocking miR-140 could sensitize colon cancer stem-like chemoresistance cells and osteosarcoma cells to anti-cancer drugs via targeting HDAC4 [16]; let-7a was overexpressed in doxorubicin-resistant human squamous carcinoma A431 cells, down-regulation of let-7a increased the doxorubicin-induced apoptosis through targeting caspase-3 [17]; overexpression of miR-181s via transfection of miR-181s mimics could increase multidrug-resistant human lung cancer cell line A549/CDDP (cisplatin) to CDDP-induced apoptosis [18]; miR-15b, miR-16, miR-181b, and miR-497 were down-regulated in multidrug-resistant gastric cancer cell line SGC7901/VCR (vincristine), overexpression of above four miRNAs could sensitize cells to anti-cancer drugs via targeting antiapoptotic gene BCL2 [18–20], etc.

In this study, we reported that miR-200bc/429 cluster was downregulated in both multidrug-resistant human gastric cancer cell line SGC7901/VCR and multidrug-resistant human lung cancer cell line A549/CDDP, compared with the parental SGC7901 and A549 cell lines, respectively. We demonstrated that miR-200bc/429 cluster might play an important role in the development of MDR in human gastric and lung cancer cell lines by targeting the anti-apoptotic genes BCL2 and XIAP.

Materials and methods

Cell culture

Human gastric adenocarcinoma cell line SGC7901 (obtained from Academy of Military Medical Science, Beijing, China) and its multidrug-resistant variant SGC7901/VCR (obtained from the State Key Laboratory of Cancer Biology and Institute of Digestive Diseases, Xijing Hospital, Fourth Military Medical University, Xian, China), human lung cancer cell line A549 and its multidrug-resistant variant A549/CDDP (both obtained from Biosis Biotechnology Company, Shanghai, China) were all cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (Gibco BRL, Grand Island, NY) in a humidified atmosphere containing 5% CO2 at 37°C. To maintain the MDR phenotype, vincristine (VCR, with final concentration of 1 μg/ml) and CDDP (with final concentration of 4 μg/ml) were added to the culture media for SGC7901/VCR and A549/CDDP cells, respectively.

Quantitative real-time PCR analysis for miRNA

The RNA preparation was as described above. The concentration and purity of the RNA samples were determined spectroscopically. Expression of mature miRNA was assayed using stem-loop RT followed by real-time PCR analysis [20]. The SYBR and U6 gene were used for detecting the gene amplification and normalizing the each sample, respectively. EzOmics^{TM} miRNA qPCR Detection Primer Set (Catalog No.BK1010) and EzOmics^{TM} One-Step qPCR Kit (Cat No. BK2100), which were purchased from Biomics Biotechnologies Co., Ltd (Nantong, China), were used for quantitative real-time PCR analysis for miR-200bc/429 cluster and U6 snRNA, respectively. The fold change for miRNA from SGC7901/VCR cells and A549/CDDP cells relative to each control SGC7901 and A549 cells was calculated using the $2^{-\Delta\Delta Ct}$ Method [21], where $\Delta\Delta Ct = \Delta Ct$ SGC7901/VCR$-\Delta Ct$ SGC7901 or

$\Delta\Delta Ct = \Delta Ct$ A549/CDDP$-\Delta Ct$ A549 and $\Delta Ct = Ct$ miRNA$-$Ct U6 snRNA. PCR was performed in triplicate.

In vitro drug sensitivity assay

SGC7901/VCR, A549/CDDP, SGC7901, and A549 cells were plated in 6-well plates (6 × 105 cells/well); 100 nM of the miR-200bc/429 cluster mimics or 100 nM miRNA mimic control were transfected in SGC7901/VCR and A549/CDDP cells, while 100 nM of the miR-200bc/429 cluster inhibitors or 100 nM miRNA inhibitor control were transfected in SGC7901 and A549 cells, using lipofectamine 2000 (Invitrogen, Long Island, NY, USA), according to the manufacturer's protocol, respectively. The miR-200bc/429 cluster mimics, miRNA mimic control, 2′-*O*-methyl (2′-*O*-Me)-modified miR-200bc/429 cluster inhibitors, and miRNA inhibitor control were chemically synthesized by Shanghai GenePharma Company (Shanghai, China). The sequence of each was shown in supplementary data 1A and 1B.

Twenty-four hours after, transfection cells were seeded into 96-well plates (5 × 103 cells/well) for next step experiment. After cellular adhesion, freshly prepared anti-cancer drugs including vincristine (VCR), 5-fluorouracil (5-Fu), cisplatin (CDDP), etoposide (VP-16), and adriamycin (ADR) were added with the final concentration being 0.01, 0.1, 1, and 10 times of the human peak plasma concentration for each drug, as previously described [20]. The peak serum concentrations of various anti-cancer drugs are 0.5 μg/ml for VCR, 10 μg/ml for 5-Fu, 2.0 μg/ml for CDDP, 10 μg/ml for VP-16, and 0.4 μg/ml for ADR [22, 23]. Forty-eight hours after the addition of drugs, cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. The absorbance at 490 nm (A490) of each well was read on a spectrophotometer. The concentration at which each drug produced 50% inhibition of growth (IC50) was estimated by the relative survival curve. Three independent experiments were performed in quadruplicate.

Dual-luciferase activity assay

The 3′UTR of human BCL2 and XIAP cDNA containing the putative target sites for the miR-200bc/429 cluster (sequence shown in supplementary data 2) was chemically synthesized and inserted at the XbaI site, immediately downstream of the luciferase gene in the pGL3-control vector (Promega, Madison, WI) by Biomics Biotechnologies Co., Ltd (Nantong, China), respectively.

Twenty-four hours before transfection, cells were plated at 1.5 × 105 cells/well in 24-well plates. 200 ng of pGL3-BCL2-3′-UTR or pGL3-XIAP-3′-UTR plus 80 ng pRL-TK (Promega) was transfected in combination with 60 pmol of the miR-200bc/429 cluster mimics or miRNA mimic control using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol as described, respectively [20]. Luciferase activity was measured 24 h after transfection, using the Dual Luciferase Reporter Assay System (Promega). Firefly luciferase activity was normalized to renilla luciferase activity for each transfected well. Three independent experiments were performed in triplicate.

Western blot analysis

SGC7901/VCR and A549/CDDP cells were plated in 6-well plates (6 × 105 cells/well); Seventy-two hours after the transfection of miRNA mimics, cells were harvested and homogenized with lysis buffer. Total protein was separated by denaturing 10% SDS–polyacrylamide gel electrophoresis. Western blot analysis was performed as described [20]. The primary antibodies for BCL2, XIAP, β-actin, GAPDH, and α-tubulin were purchased from Cell signaling Technology, Bioworld Technology and Santa Cruz Biotechnology, respectively. Protein levels were normalized to GAPDH, β-actin, or α-Tubulin. Fold changes were determined.

Apoptosis assay

Cells were plated in 6-well plates (6 × 105 cells/well). Twenty-four hours after the transfection of miRNA mimics, as described above, SGC7901/VCR and A549/CDDP cells were treated by VCR and CDDP, with final concentration of 5 μg/ml and 20 μg/ml, respectively. Forty-eight hours after the treatment of VCR and CDDP, flow cytometry was used to detect apoptosis of the transfected SGC7901/VCR and A549/CDDP cells by determining the relative amount of AnnexinV-FITC-positive, PI-negative cells as previously described [20], respectively.

Statistical analysis

Each experiment was repeated at least 3 times. Numerical data were presented as mean ± SD. The difference between means was analyzed with Student's t test. All statistical analyses were performed using SPSS11.0 software (Chicago, IL). Differences were considered significant when $P < 0.01$.

Results

miR-200bc/429 cluster is downregulated in both SGC7901/VCR and A549/CDDP cells, compared with SGC7901 and A549 cells, respectively

In our previous study [20], the miRNA microarray analysis between SGC7901/VCR and the parental SGC7901 cells

showed that miR-200bc/429 cluster was significantly downregulated in SGC7901/VCR cells, respectively.

Quantitative real-time PCR for miR-200bc/429 cluster further verified that miR-200bc/429 cluster was significantly downregulated in both SGC7901/VCR and A549/CDDP cells, compared with SGC7901 and A549 cells, respectively. In SGC7901/VCR cell line, the expression level of miR-200bc/429 cluster was significantly downregulated 5.40 ± 0.58-fold, 2.78 ± 0.44-fold, and 7.62 ± 1.89-fold compared with SGC7901 cell line; in A549/CDDP cell line, the expression level of miR-200bc/429 cluster was significantly downregulated 4.10 ± 1.86-fold, 4.41 ± 1.13-fold, and 9.18 ± 6.01-fold compared with A549 cell line, respectively (Table 1).

miR-200bc/429 cluster modulates MDR of SGC7901/VCR and A549/CDDP cell lines

In both SGC7901/VCR and A549/CDDP cells, MTT assay verified that those transfected with miR-200bc/429 cluster mimics exhibited greatly enhanced sensitivity to VCR, CDDP, VP-16, and ADR, but not to 5-Fu compared with the miRNA mimic control-transfected cells, respectively (Fig. 1a, b), while in both SGC7901 and A549 cells, those transfected with miR-200bc/429 cluster inhibitors exhibited greatly enhanced resistance to VCR, CDDP, VP-16, and ADR, but not to 5-Fu compared with the miRNA inhibitor control transfected cells, respectively (Fig. 1c, d). These results showed that miR-200bc/429 cluster might modulate MDR of SGC7901/VCR and A549/CDDP cell lines.

The anti-apoptotic BCL2 and XIAP are the common target genes of miR-200bc/429 cluster

TargetScan 5.1 (http://www.targetscan.org) predicted that BCL2 and XIAP were the common target genes of the miR-200bc/429 cluster conserved between different species (supplementary data 3). To explore whether BCL2 and XIAP were the common target genes of the miR-200bc/429 cluster, we constructed two luciferase reporter vectors with the putative BCL2 and XIAP 3′ UTR target sites for the miR-200bc/429 cluster downstream of the luciferase gene (pGL3-BCL2-3′-UTR and pGL3-XIAP-3′-UTR), respectively. Luciferase reporter vectors together with the miR-200bc/429 cluster mimics or the miRNA mimic control were transfected into SGC7901/VCR and A549/CDDP cells, respectively. In both SGC7901/VCR and A549/CDDP cells, a significant decrease in relative luciferase activity was noted when pGL3-BCL2-3′-UTR or pGL3-XIAP-3′-UTR was co-transfected with the miR-200bc/429 cluster mimics, but not with the miRNA mimic control, respectively (Fig. 2), suggesting that BCL2 and XIAP were the common target genes of the miR-200bc/429 cluster.

Table 1 miR-200bc/429 cluster downregulated in drug-resistant cells by qRT-PCR analysis

miRNA	Fold change	
	SGC7901/VCR versus SGC7901	A549/CDDP versus A549
miR-200b	−5.40 ± 0.58*	−4.10 ± 1.86*
miR-200c	−2.78 ± 0.44*	−4.41 ± 1.31*
miR-429	−7.62 ± 1.89*	−9.18 ± 6.01*

* $P < 0.01$

miR-200bc/429 cluster modulates MDR by repressing BCL2 and XIAP protein expression

Interestingly, in our study, the decreased expression of miR-200bc/429 cluster in SGC7901/VCR and A549/CDDP cells was concurrent with the overexpression of BCL2 and XIAP protein, compared with the parental SGC7901 and A549 cells, respectively (Fig. 3a). Since BCL2 and XIAP were both anti-apoptotic protein and the targets of the miR-200bc/429 cluster, we hypothesized that the miR-200bc/429 cluster might modulate multidrug resistance of cancer cells via inhibiting the BCL2 and XIAP protein expression.

To testify our hypothesis, we transfected the miR-200bc/429 cluster mimics and the control miRNA mimic into SGC7901/VCR and A549/CDDP cells to detect the BCL2 and XIAP expression level changes, respectively. In both SGC7901/VCR and A549/CDDP cells, 72 h after the transfection, Western blot showed significantly decreased BCL2 and XIAP protein level in all miR-200bc/429 cluster mimics-transfected cells compared with the miRNA mimic control-transfected cells (Fig. 3b).

These results suggested that miR-200bc/429 might modulate multidrug resistance of cancer cells at least in part by inhibiting the BCL2 and XIAP protein expression.

miR-200bc/429 cluster sensitizes SGC7901/VCR and A549/CDDP cells to VCR- and CDDP-induced apoptosis, respectively

The formation of drug resistance in various cancer cells was relevant to a reduced rate of drug-induced apoptosis, which was caused by, at least in some cases, over-expressing of anti-apoptotic proteins, such as BCL2, IAPs, BCL-XL [18–20, 24, 25], etc. Since the miR-200bc/429 cluster might modulate multidrug resistance of cancer cells at least in part by repressing the BCL2 and XIAP protein expression, considering the well-characterized role of

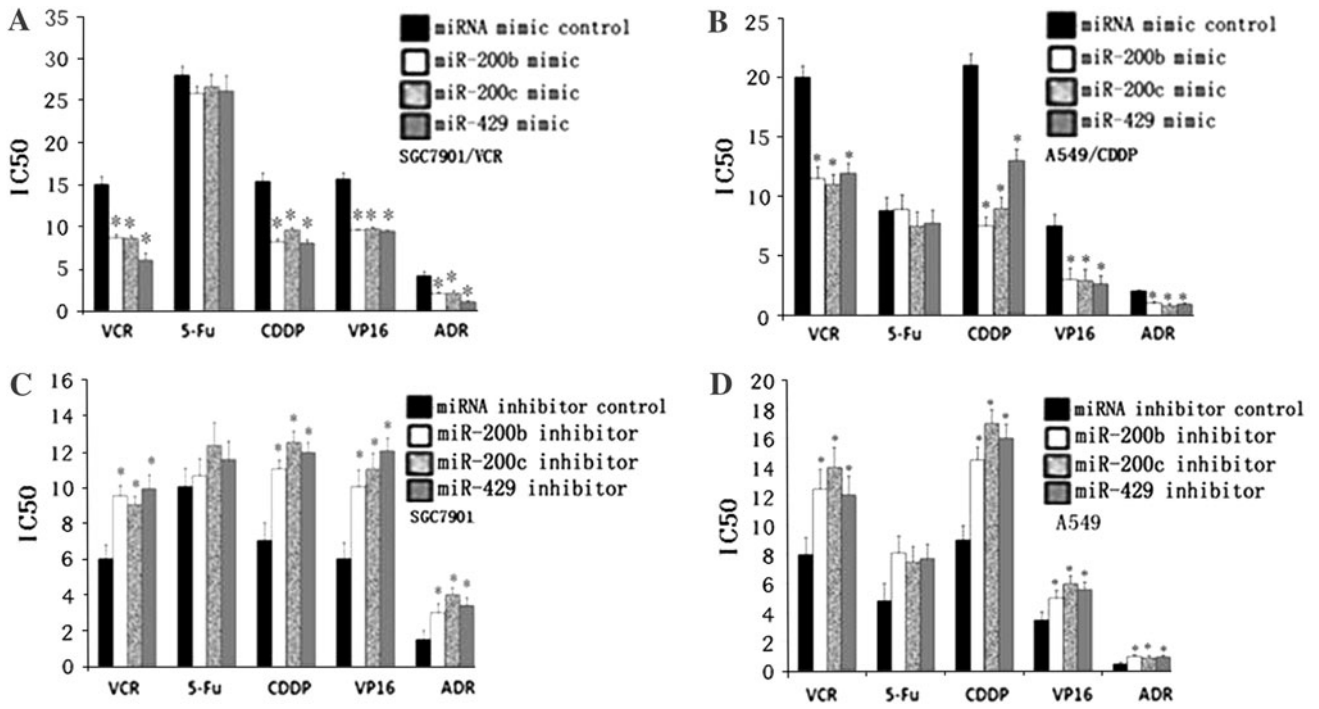

Fig. 1 miR-200bc/429 cluster modulates MDR of human gastric and lung cancer cell lines (**a** and **b**). In both SGC7901/VCR and A549/CDDP cells, those transfected with miR-200bc/429 cluster mimics exhibited greatly enhanced sensitivity to VCR, CDDP, VP-16, and ADR, but not to 5-Fu, compared with the miRNA mimic control-transfected cells, respectively (**c** and **d**). In both SGC7901 and A549 cells, those transfected with miR-200bc/429 cluster inhibitors exhibited greatly enhanced resistance to VCR, CDDP, VP-16, and ADR, but not to 5-Fu, compared with the miRNA inhibitor control transfected cells, respectively. $*P < 0.01$

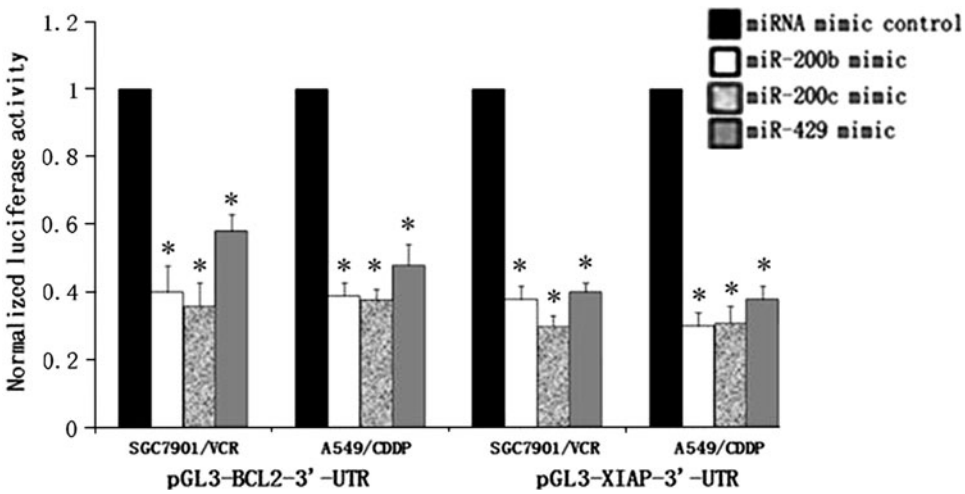

Fig. 2 Dual-luciferase assay performed in SGC7901/VCR and A549/CDDP cells suggested that BCL2 and XIAP are the common target genes of the miR-200bc/429 cluster. In both SGC7901/VCR and A549/CDDP cells, a significant decrease in relative luciferase activity was noted when pGL3-BCL2-3′-UTR or pGL3-XIAP-3′-UTR was co-transfected with the miR-200bc/429 cluster mimics, but not with the miRNA mimic control. $*P < 0.01$

BCL2 and XIAP in apoptosis and drug resistance, we suggested a hypothesis that miR-200bc/429 cluster might play a role in the formation of MDR at least in part by modulation of apoptosis by targeting BCL2 and XIAP.

To verify this hypothesis, we analyzed VCR- and CDDP-induced apoptosis after transfection of SGC7901/VCR and A549/CDDP cells with the miR-200bc/429 cluster mimics and the miRNA mimic control, respectively. In both SGC7901/VCR cells and A549/CDDP cells, a marked increase in apoptosis, as assessed by flow cytometry, was found in the miR-200bc/429 cluster mimics-transfected cells after VCR and CDDP treatment, compared with the miRNA mimic control-transfected cells, respectively (Fig. 4).

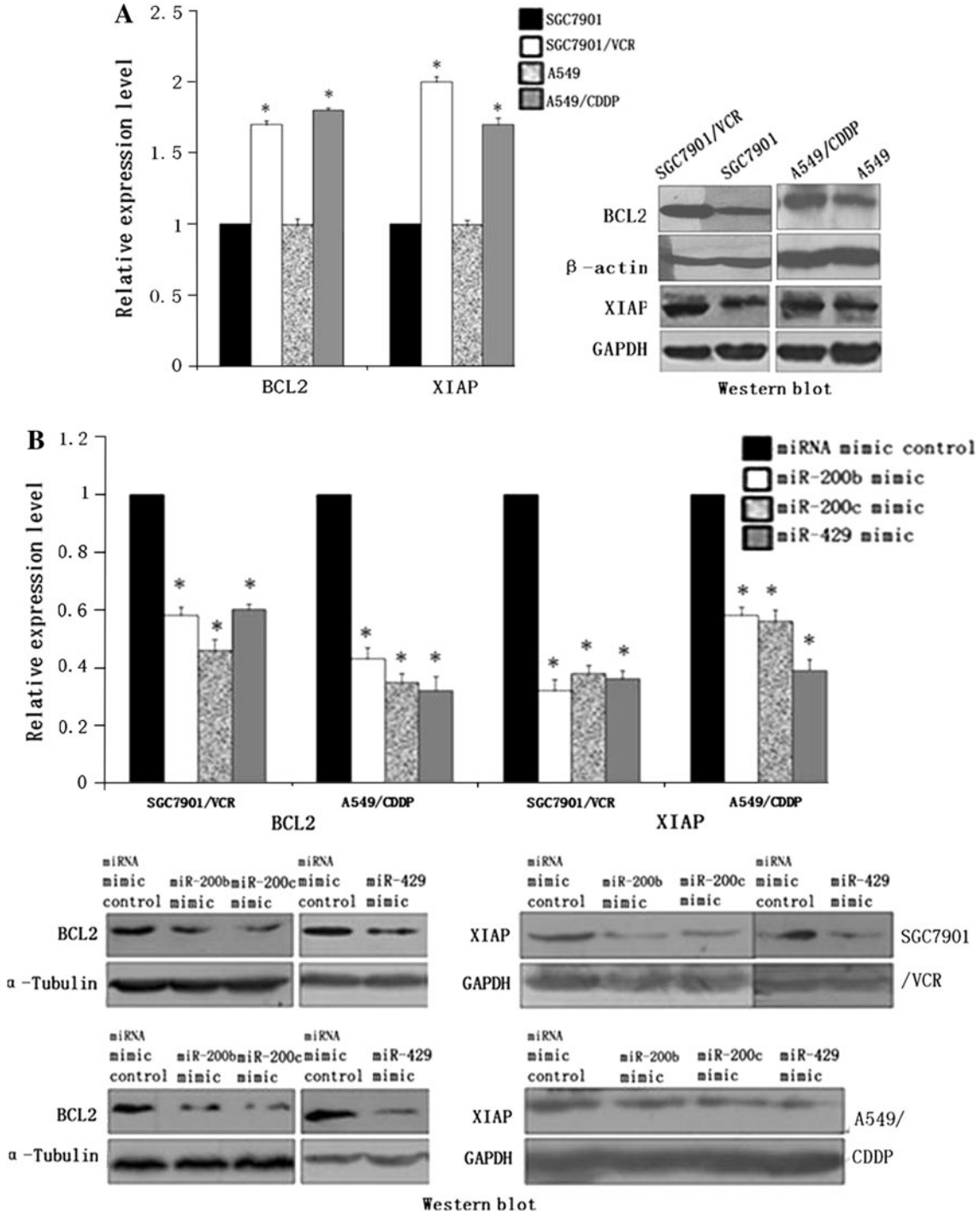

Fig. 3 **a** The anti-apoptotic BCL2 and XIAP protein were overexpressed in SGC7901/VCR and A549/CDDP cells, compared with the parental SGC7901 and A549 cells, respectively. Representative Western blots were attached beside the graphs. **b** In both A549/CDDP and SGC7901/VCR cells, 72 h after the transfection, Western blot demonstrated significantly decreased BCL2 and XIAP protein level in all miR-200bc/429 cluster mimics-transfected cells compared with the miRNA mimic control-transfected cells,. Representative Western blots were attached under the graphs. The results shown represent the mean ± SD from 3 independent experiments. $^{*}P < 0.01$

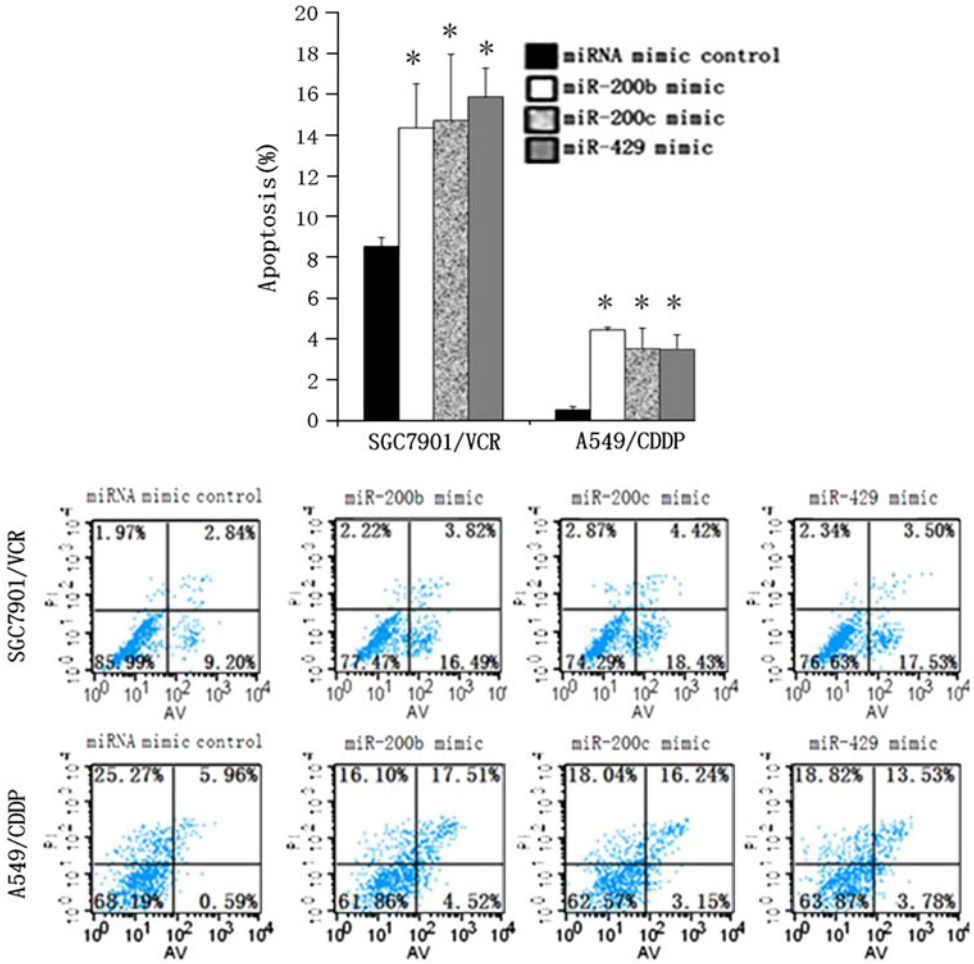

Fig. 4 miR-200bc/429 cluster mimics sensitize SGC7901/VCR and A549/CDDP cells to VCR- and CDDP-induced apoptosis, respectively. In SGC7901/VCR cells, apoptosis evaluated by flow cytometry showed a marked increase of apoptosis in miR-200bc/429 cluster mimics-transfected cells after VCR treatment, compared with the miRNA mimic control-transfected cells. In A549/CDDP cells, apoptosis evaluated by flow cytometry showed a marked increase of apoptosis in miR-200bc/429 cluster mimics-transfected cells after CDDP treatment, compared with the miRNA mimic control-transfected cells. Representative flow cytometry report was attached under the graphs. The results shown represent the mean ± SD from 3 independent experiments. $^{*}P < 0.01$

Discussion

One of the major mechanisms of drug resistance in cancer cells is the abnormal apoptosis pathway [3, 4]. Recently, extensive studies have established that miRNAs could modulate drug resistance in cancer cells at least in part via this mechanism [17–20]. In our study, we found that the anti-apoptotic protein BCL2 and XIAP were upregulated, while the miR-200bc/429 cluster was downregulated in both SGC7901/VCR and A549/CDDP cells, compared with SGC7901 and A549 cells, respectively. The mechanistic connection of miR-200bc/429 cluster dysregulation with the development of multidrug resistance in SGC7901/VCR and A549/CDDP cells was proven by the correlation between exogenous overexpression of miR-200bc/429 cluster and corresponding alternation in the protein levels of its targets BCL2 and XIAP, which play an important role in the formation of cancer cells drug resistance.

The human miR-200 family consists of paralogs located on two chromosomal regions. miR-200a, miR-200b, and miR-429 are located on chromosome 1p36; miR-200c and miR-141 are located on 12p13. Alternatively, based on the seed region (nucleotides 2–7), they can be divided into two sequence clusters: miR-200b, 200c, and 429 (miR-200bc/429 cluster), as well as miR-200a and 141 (miR-200a/141 cluster). The miR-200bc/429 cluster differs from the miR-200a/141 cluster with regard to only the fourth nucleotide (U to C) in the seed region [26]. Recently, reduced expression of the miR-200 family was shown to be associated with tumor metastasis by inducing epithelial-to-mesenchymal transition of tumor cells via upregulating its target protein zing finger E-box binding homeobox 1 (ZEB1) and Smad interacting protein 1 (SIP1) [27–30]. Exactly, recent two studies also shown that upregulation of the miR-200 family could lead to the reversal of epithelial-to-mesenchymal transition in gemcitabine-resistant pancreatic cancer cells and bladder cancer cells [31, 32].

Although the effect of the two sequence clusters of human miR-200 family on the epithelial-to-mesenchymal transition of tumor cells was well-known, the function of the miR-200 family on cell apoptosis was rarely studied; the study by Uhlmann et al. demonstrated that only miR-200bc/429 cluster could increase apoptosis of breast cancer cells via targeting phospholipase C gamma 1 (PLCG1), but not the miR-200a/141 cluster, which suggested that the different fourth nucleotide in the seed region of the two

clusters may cause different biological function [26]. Moreover, recent study by Igor P. Pogribny et al. found that the miR-200bc/429 cluster was downregulated in cisplatin-resistant breast cancer cell line MCF-7/CDDP, compared with the parental MCF-7 cell line, suggesting that miR-200bc/429 cluster might play a role in acquired drug resistance of breast cancer cell line [33]. Our study also found that miR-200bc/429 cluster was downregulated in human drug-resistant gastric and lung cancer cell lines, compared with their each parental cell lines. Recent few studies suggested that the aberrant DNA methylation of the promoter region of either the miR-200ab/429 cluster or the miR-200c/141 cluster maybe one of the important underlying mechanisms of the alterative expression level of the miR-200 family [34–37]. In addition, our results was in concordance with the studies by S Uhlmann et al.; we also found that exogenous overexpression of miR-200bc/429 cluster sensitized SGC7901/VCR and A549/CDDP cells to VCR- and CDDP-induced apoptosis, at least in part via targeting the antiapoptotic BCL2 and XIAP, respectively.

However, because a miRNA could target more than one gene, the function of miRNAs may still exist cell-type specificity; for instance, studies have showed that the function of miR-200b seems to be conflicting in different cancer cell types, and miR-200b was reported to be significantly downregulated in cisplatin-resistant human breast cancer (MCF-7/CDDP) cell line and docetaxel-resistant human non-small cell lung carcinoma cells (SPC-A1/docetaxel) [33, 38]; upregulating of miR-200b could increase apoptosis of breast cancer cells [26], while miR-200b was highly overexpressed in malignant cholangiocytes, and inhibition of miR-200b increased sensitivity of cells to gemcitabine-induced apoptosis [39]. One possible explanation for this is that the contrary function of miR-200b on regulating apoptosis in different cell types may rely on the balance of the expression level between its target inhibiting and promoting apoptosis genes [10].

Interestingly, in our study, miR-200bc/429 cluster might have the same effect on enhancing the sensitivity of both cells to VCR, CDDP, ADR, VP-16, but not to 5-Fu; a possible explanation for this phenomenon is that 5-Fu might promote apoptosis mainly via the Fas/FasL pathway, at least in some cell types, such as colon carcinoma cells [40], so that factors which affect the mitochondrial release of cytochrome c (e.g., BCL2 and XIAP overexpression) will have little influence on the cell response to 5-Fu-induced apoptosis [19].

In summary, the findings we reported here suggested the first evidence that has-miR-200bc/429 cluster might be involved in the formation of MDR in human gastric and lung cancer cell lines, respectively. has-miR-200bc/429 cluster might modulate the resistance of gastric and lung cancer cell lines to some anti-cancer drugs, at least in part, via targeting BCL2 or XIAP expression. Our study might have implications for chemotherapy, whose efficiency was mainly impeded by the establishment of drug resistance. Therapeutic methods targeting the MDR-related miRNAs, such as hsa-miR-200bc/429 cluster, might be another promising way to enhance treatment effect. However, it should be noted that our data were derived from cell lines that have been removed from their in vivo context and could not be considered accurate surrogates for clinical tumors. Thus, future studies to analyze the roles of hsa-miR-200bc/429 cluster in vivo and in clinical context were needed.

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