Original Article

Clinical significance and correlation of miR-200c and P-gp expression in gastric cancer and the effects on multidrug resistance

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Background: Poor prognosis is common in gastric cancer patients due to multidrug resistance (MDR)-induced recurrence and metastasis. In the present study, we investigated the expression of microRNA (miR)-200c in gastric cancer tissues and cell lines and its relationship with the expression of the drug resistant gene *ABCB1*, which encodes P-glycoprotein (P-gp).

Methods: The basic characteristics of 102 patients with gastric cancer were reviewed. Real time-polymerase chain reaction (PCR), immunohistochemistry, and Western blot were employed to detect the expression levels of miR-200c and P-gp in gastric carcinoma tissues and cell lines. The correlation of miR-200c messenger RNA (mRNA) level with clinicopathological characteristics and P-gp protein expression were analyzed. SGC7901/vincristine (VCR) cells were transfected with miR-200c mimics or a specific small interfering RNA (siRNA) targeting the *ABCB1* gene. The methyl thiazolyl tetrazolium (MTT) assay and flow cytometry were used to determine the role of miR-200c and *ABCB1* on the viability and apoptosis of gastric carcinoma cell lines.

Results: The level of miR-200c in carcinoma tissues was significantly lower than that in adjacent tissues, and the expression level of P-gp in carcinoma tissues was obviously higher than that in adjacent tissues ($P<0.01$, $P=0.029$). The expression levels of miR-200c and P-gp were associated with the malignant characteristics of gastric cancer, and patients with high expression of miR-200c or negative expression of P-gp had a better prognosis ($P=0.006$, $P=0.022$). MiR-200c negatively regulated the *ABCB1* gene in gastric cancer cell lines. MiR-200c overexpression and *ABCB1* down-regulation increased the sensitivity of SGC7901/VCR cells to VCR and reversed MDR by promoting cell apoptosis.

Conclusions: The expression level of miR-200c decreases in gastric carcinoma tissues and drug-resistant gastric cancer SGC7901/VCR cells. Overexpression of miR-200c may enhance the sensitivity of SGC7901/VCR cells to VCR by regulating the expression of P-gp.

Keywords: Gastric carcinoma; microRNA (miR)-200c; SGC-7901; P-glycoprotein (P-gp); multidrug resistance (MDR)

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Introduction

Gastric cancer is one of the deadliest malignant tumors and its incidence is relatively higher in China than that in western countries (1). Chemotherapy is the main curative method for advanced gastric cancer. However, poor prognosis is common in gastric cancer patients due to multidrug resistance (MDR)-induced recurrence and metastasis (2). Therefore, finding a potential target to reverse gastric cancer MDR is important for elucidating the mechanism of tumor MDR and enhancing the sensitivity of tumor cells to chemotherapeutic agents. Multiple causes are associated with the chemoresistance of tumors. One of the major mechanisms is that overexpressed adenosinetriphosphate (ATP)-binding cassette (ABC) drug transporters induce the extrusion of anticancer agents from the tumor cells and decrease the sensitivity of the tumor cells to chemotherapeutic agents (3).

P-glycoprotein (P-gp), a 170 kDa transmembrane drug transporter encoded by ATP-binding cassette sub-family B member 1 (*ABCB1*), plays an important role in drug efflux-based MDR in cancers (4). Tumor cells can overexpress P-gp after long-term exposure to chemotherapeutic agents due to the induction and amplification of the *ABCB1* gene. P-gp has the ability to bind and transport a wide range of structurally diverse molecules, leading to decreased drug accumulation inside the tumor cells and consequently a reduced therapeutic effect (5).

MicroRNAs (miRNAs) are small single-stranded noncoding RNAs with 21–25 nucleotides. MiRNAs regulate many important pathophysiological processes by silencing their target genes after binding to the 3'-untranslated regions of the messenger RNAs (mRNAs) via translation inhibition or mRNA degradation (6,7). Aberrant expression of miR-200c has been found in multiple malignant tumors including melanoma (8), endometrial cancer (9), epithelial ovarian cancer (10), and breast cancer (11). Current evidence indicates that miR-200c can inhibit cell proliferation, invasion, metastasis, epithelial-to-mesenchymal transition (EMT), and MDR in various cancers (12-14). Specifically, Li *et al.* (15) reported lower expression of miR-200c in gastric cancer tissues, and miR-200c could suppress the proliferation, invasion, and metastasis of gastric cancer cells (16). However, the effect and mechanism of miR-200c in the MDR of gastric cancer remain to be elucidated.

Currently, the factors that regulate the MDR of gastric cancer remain poorly understood, and no evidence has been revealed with the hypothesis that miR-200c regulates MDR by targeting P-gp. Thus, we designed the current study to clarify the potential function of the miR-200c and P-gp in gastric cancer and MDR is regulated based on above functions. This finding has clinical significance for the diagnosis and treatment of gastric cancer. We present the following article in accordance with the MDAR reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-167/rc).

Methods

Patients and tissue samples

A total of 102 pairs of gastric cancer tissues and 34 pairs of adjacent normal tissues (>5 cm from the cancerous tissues) were collected from patients in the Shanxi Provincial People's Hospital (China) from April 2012 to October 2013. The inclusion criteria were as follows: (I) The tissue specimens were all confirmed by pathological examination as gastric adenocarcinoma; (II) the patients did not receive any anti-tumor treatments including chemotherapy or immunotherapy before surgery; (III) the clinical characteristics and other medical data of the patients were complete. The exclusion criteria were as follows: (I) Patients received other anti-tumor treatments before surgery; (II) patients disapproved of the collection of samples; (III) patients could not tolerate the operations; (IV) patients complicated by other tumors or serious systemic disease. All the specimens were frozen in liquid nitrogen within 30 min after surgery and then stored at –80 ℃ until RNA extraction. Other tissues used for immunohistochemistry were fixed and embedded in paraffin. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The current study was approved by the Medical Ethics Committee of the Shanxi Provincial People's Hospital (China) (No. 2019-88). All the patients signed written informed consent for the use of the specimens.

Cell culture

Human gastric epithelial GES-1 cells, human gastric cancer SGC-7901 cells, and the vincristine (VCR)-resistant human gastric carcinoma cell line SGC7901/VCR were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured with RPMI1640 medium containing 10% fetal bovine serum (FBS, Gibco, San Diego, CA, USA) in an incubator with 5% CO_2 and 95% air and maintained at 37 ℃. The density of SGC7901/

Table 1 Sequence of the primers used for qRT-PCR

Genes	Primer pair sequences
miR-200c	F: 5'-TCGTCTTACCCAGCAGTGTT-3'
	R: 5'-CTCCATCATTACCCGGCAGT-3'
U6	F: 5'-AGAAGATTAGCATGGCCCCT-3'
	R: 5'-ATTTGCGTGTCATCCTTGCG-3'
ABCB1	F: 5'-GGGATGGTCAGTGTTGATGGA-3'
	R: 5'-GCTATCGTGGTGGCAAACAATA-3'
GAPDH	F: 5'-CTGGGCTACACTGAGCACC-3'
	R: 5'-AAGTGGTCGTTGAGGGCAATG-3'

qRT-PCR, quantitative real time reverse transcription-polymerase chain reaction.

VCR cells was adjusted to 2×10^5/mL and cells were seeded into 6-well plates for transfection experiments. The cells were transfected with miR-200c mimics, negative control oligonucleotide (miR-NC), *ABCB1* small interfering RNA (siRNA), and negative control siRNA (siRNA-NC) (all from GenePharma, Shanghai, China) using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA). The subsequent experiments were performed after culture for 48 h.

Quantitative real time reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted from the gastric cancer tissue samples and cultured cancer cells with Trizol reagent (Gibco) using the one step method. RNA (1 μg) was converted to cDNA with reverse transcriptase. The qRT-PCR was performed using the ABI 7500 PCR instrument (Applied Biosystems, Grand Island, NY, USA). The expression of miR-200c was normalized by miRNA U6 and *ABCB1* mRNA was normalized by glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The Hairpin-it™ miRNAs qPCR Quantitation Kit (for miR-200c) and U6 small nuclear RNA (snRNA) Real-time PCR Normalization Kit were from GenePharma. Primers were synthesized by Sangon Biotech (Shanghai, China), and the sequences are listed in *Table 1*. The relative expression levels of miR-200c and *ABCB1* mRNA were calculated by the $2^{-\Delta\Delta Ct}$ method.

Immunohistochemistry

The expression of P-gp protein was detected in the gastric cancer tissues and adjacent normal tissues using the immunohistochemical method. The paraffin-embedded tissues were cut into 5-μm slices and dehydrated by ethanol. Antigen retrieval was performed on tissue slices by microwave heating. Primary mouse anti-human P-gp antibody (1:200, Abcam, Cambridge, MA, USA) was added onto tissue slices at 4 ℃ overnight. Phosphate-buffered saline (PBS) served as a negative control. Incubation with HRP-conjugated secondary antibody (1:2,000) and diaminobenzidine (DAB) staining were then performed following the instructions of the manufacturer. The immunohistochemical scores of P-gp were evaluated by 2 independent pathologists. Disagreements were resolved by discussions or consultation with a third-party pathologist. Cells were randomly selected from 5 fields (×200) in each slice for scoring, which was calculated by multiplying staining intensity (colorless=0, light yellow=1, light brown=2, and brown=3) and the percentage of positive cells (negative=0, ≤25%=1, 25–50%=2, 50–75%=3, and >75%=4). The final score of P-gp staining was determined as <5 for negative and ≥5 for positive.

Western blot

The total proteins of tissue and cells were extracted with RIPA buffer (Beyotime, Shanghai, China) containing protease inhibitor. Proteins were electrophoresed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to a polyvinylidene fluoride (PVDF) membrane. Nonfat dry milk (10%) was used to block the non-specific reactivity for 1 h. The PVDF membrane was incubated with primary antibodies for Bax, Bcl-2, and caspase-3 (all 1:1,000, Abcam) at 4 ℃ overnight. The membrane was washed in TBST 3 times and then incubated with corresponding secondary antibodies for 1 h. Membranes were washed and an ECL Detection Kit (Amersham, Arlington Heights, IL, USA) was used to detect the signals, which were then recorded by a gel imaging system. The gray value was measured by Image J software.

Luciferase reporter assay

To determine whether *ABCB1*, the encoded gene of P-gp, is a potential target of miR-200c, both wild-type and mutant-type *ABCB1* mRNA 3'-untranslated region (3'UTR) were cloned into the pGL3-basic luciferase vector (Promega, Madison, WI, USA). The luciferase reporter

vector containing wild-type or mutant-type sequences of *ABCB1* mRNA was transfected into SGC7901/VCR cells with miR-200c mimic or its negative control using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The luciferase activity was determined using the dual-luciferase reporter system (Promega, Madison, WI, USA) at 48 h after transfection.

VCR sensitivity assay

Human gastric cancer SGC-7901 cells and VCR-resistant SGC7901/VCR cells were treated with different concentrations of VCR (0.1, 0.2, 0.5, 1, 2, 5, and 10 μM/L, Sigma, St. Louis, MO, USA). The cell viability was detected using the methyl thiazolyl tetrazolium (MTT) assay 48 h after treatments. The value of IC_{50} was calculated according to the dose-effect curve of the anticancer drug to evaluate its sensitivity to the cells. The SGC7901/VCR cells were transfected with miR-200c mimics, miR-NC, *ABCB1* siRNA, and siRNA-NC. Then, the cells were treated with different concentrations of VCR for 48 h and the IC_{50} values were determined.

Cell apoptosis assay

The SGC7901/VCR cells transfected with miR-200c mimic, miR-NC, *ABCB1* siRNA, and siRNA-NC were treated with 0.1 μM/L VCR for 48 h. The cells were then harvested using pancreatic enzyme and re-suspended in cold PBS. Cell apoptosis was detected with the Alexa Fluor® 488 Annexin V/Dead Cell Apoptosis Kit (Invitrogen, Carlsbad, CA, USA). The cells were incubated with Annexin V and propidium iodide (PI) in binding buffer for 15 min according to the instructions of the manufacturer. The fluorescence emissions of stained cells were measured at 530 nm and 575 nm using 488 nm excitation of a flow cytometer.

Statistical analysis

Statistical analysis was performed using SPSS 19.0 software (SPSS, Chicago, IL, USA). Continuous variables are presented as mean ± SD. Two independent groups were compared by the *t* test if the data were normally distributed. More groups were compared using one-way ANOVA followed by post hoc multiple comparisons. A P value <0.05 was considered statistically significant.

Results

Expression of miR-200c and P-gp in gastric cancer tissue

A total of 102 gastric cancer specimens and 34 adjacent normal tissue specimens were analyzed for the expression levels of miR-200c and P-gp. The results of qRT-PCR indicated that the relative expression of miR-200c was significantly lower in gastric cancer tissues compared with that in adjacent normal tissues (0.39±0.07 *vs.* 0.61±0.07, P<0.01, *Figure 1A*). Using immunohistochemical analysis, the positive expression rate of P-gp was 51.0% (52/102) in gastric cancer tissues. This rate decreased to 29.4% (10/34) in adjacent normal tissues, indicating that the expression of P-gp was significantly higher in gastric cancer tissues than in adjacent normal tissues (P =0.029, *Figure 1B*). The hematoxylin and eosin (HE) and immunohistochemical staining of the normal and gastric cancer tissues are shown in *Figure 1C*. Notably, the expression of P-gp was up-regulated in gastric cancer tissues even without induction of chemotherapeutic agents.

Relationship between the expression levels of miR-200c and P-gp and clinicopathological characteristics

There were 55 males and 47 females gastric cancer patients, with an average age of 55.7 years (ranging from 31 to 78 years). The basic characteristics of the patients including age, gender, tumor size, TNM stage, and whether the patients had lymph node or distant metastasis were retrospectively analyzed.

In gastric cancer tissues, the expression of miR-200c was not significantly different between patients with different ages (≤55 *vs.* >55 years), gender, and tumor size (≤5 *vs.* >5 cm). Patients at TNM III–IV stage had significantly decreased expression of miR-200c compared with those at TNM I–II stage (P<0.001). Furthermore, significantly decreased expression of miR-200c was also found in patients with lymph node metastasis and distant metastasis compared to those with no metastasis (P=0.008 and P=0.010, respectively, *Table 2*).

The positive expression rate of P-gp was also not significantly different between patients with different ages (≤55 *vs.* >55 years), gender, and tumor size (≤5 *vs.* >5 cm). Patients at TNM III–IV stage had a significantly increased expression rate of P-gp compared with those at TNM I–II stage (P=0.025). Furthermore, significantly increased expression rates of P-gp were also found in patients with

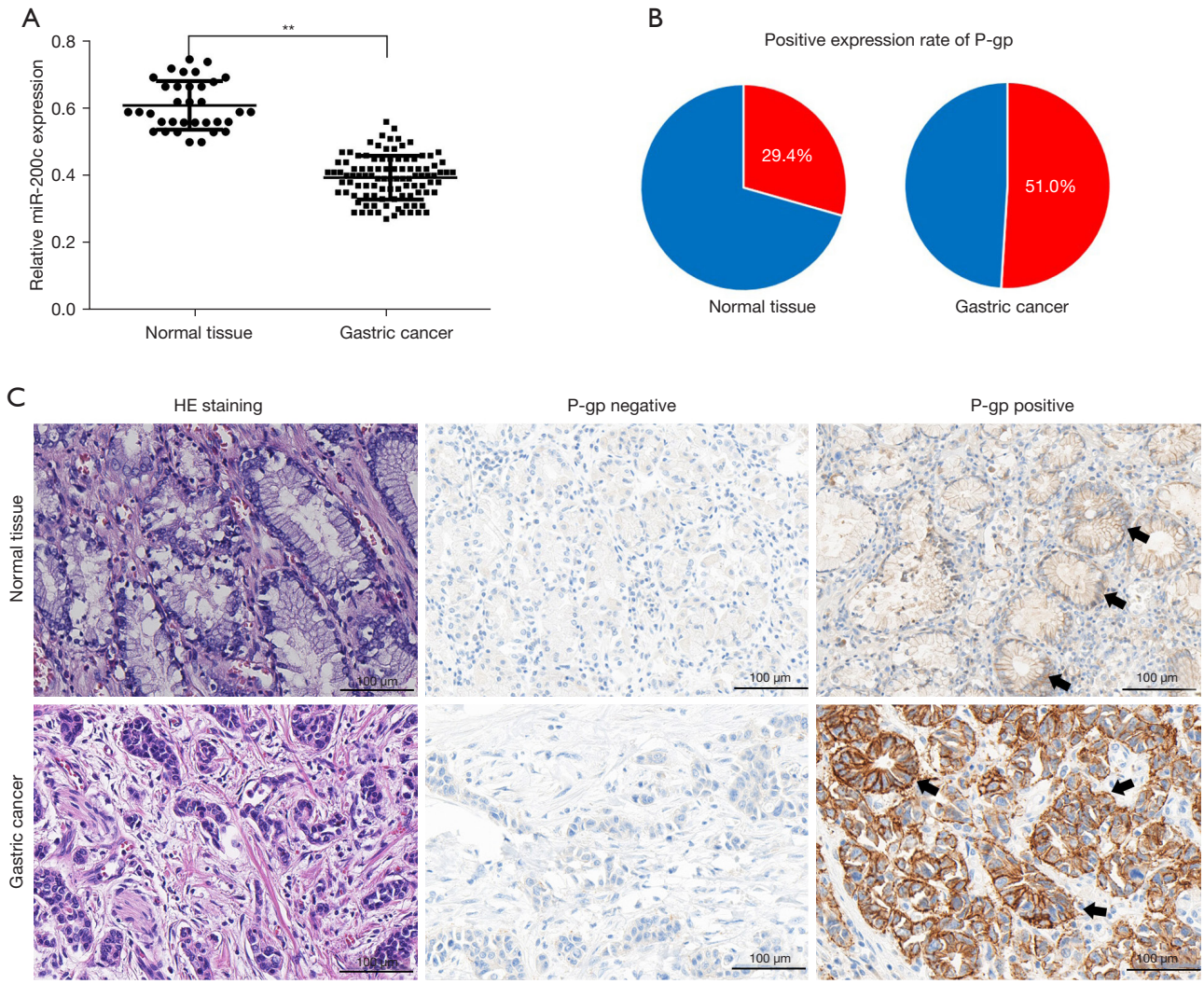

Figure 1 Expression levels of miR-200c and P-gp in gastric cancer tissues. (A) The relative expression of miR-200c was significantly lower in gastric cancer tissues compared with that in adjacent normal tissues (0.39±0.07 *vs.* 0.61±0.07, P<0.01). (B) The positive expression rate of P-gp was significantly higher in gastric cancer tissues than that in adjacent normal tissues (P=0.029). (C) The HE and immunohistochemical staining of the normal and gastric cancer tissues. Arrows show the expression of P-gp, up-regulated in gastric cancer tissues. **, P<0.01 *vs.* normal tissue. P-gp, P-glycoprotein; HE, hematoxylin and eosin.

lymph node metastasis and distant metastasis compared to those with no metastasis (P=0.005 and P=0.003, respectively, *Table 2*). These data suggest that the expression levels of miR-200c and P-gp were associated with the malignant characteristics of gastric cancer.

In P-gp-negative gastric cancer tissue specimens, the relative expression of miR-200c was 0.41+0.06. This value significantly decreased to 0.38+0.06 in P-gp-positive gastric cancer tissue specimens (P=0.003). Spearman rank correlation analysis indicated a significant negative correlation between the expression of miR-200c and P-gp (r_s=–0.265, P=0.007, *Figure 2A*).

We defined 58 cases with a lower expression of miR-200c (relative expression ≤0.40) as the low-expression group or miR-200c (low). The other 44 cases with relative expression >0.40 were included in the high-expression group or miR-200c (high). The overall survival curve indicated that the patients with high expression of miR-200c had a better prognosis than the miR-200c-low patients (P=0.006, *Figure 2B*). Moreover, patients with negative expression of

Table 2 Relationship between the expression levels of miR-200c and P-gp in gastric cancer tissues and clinicopathological characteristics

Basic characteristics	n	miR-200c	t	P	P-gp		χ^2	P
		Relative expression			Negative	Positive		
Age (years)								
≤55	50	0.40±0.07	1.354	0.179	28	22	1.912	0.234
>55	52	0.39±0.06			22	30		
Gender								
Male	55	0.40±0.06	1.425	0.157	28	27	0.171	0.696
Female	47	0.38±0.07			22	25		
Tumor size								
≤5 cm	67	0.39±0.07	0.126	0.900	36	31	1.735	0.188
>5 cm	35	0.39±0.07			14	21		
TNM stage								
I–II	23	0.45±0.06	5.351	<0.001	16	7	5.016	0.025
III–IV	79	0.38±0.06			34	45		
Lymph node metastasis								
No	28	0.42±0.07	2.723	0.008	20	8	7.755	0.005
Yes	74	0.38±0.06			30	44		
Distant metastasis								
No	76	0.40±0.07	2.644	0.010	44	32	9.398	0.003
Yes	26	0.37±0.05			6	20		

P-gp, P-glycoprotein; TNM, tumor node metastasis.

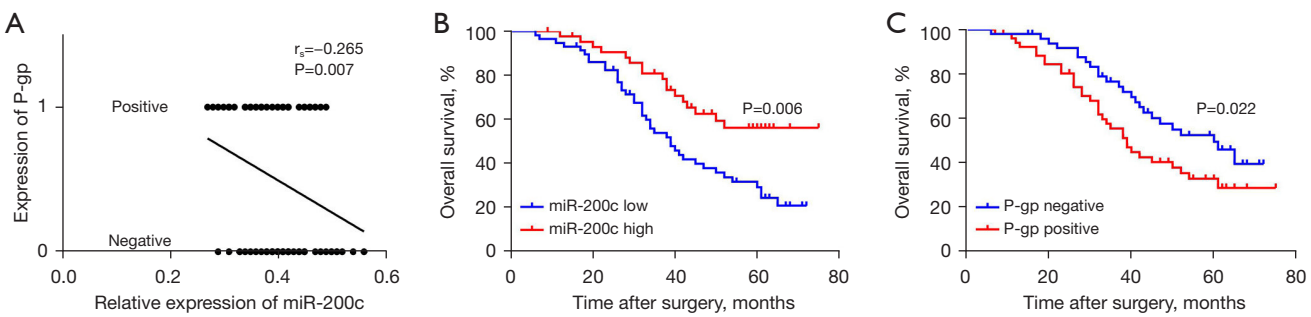

Figure 2 Relationship between the expression levels of miR-200c and P-gp and the prognosis of patients with gastric cancer. (A) Spearman rank correlation analysis indicated a significant negative correlation between the expression of miR-200c and P-gp (r_s=–0.265, P=0.007). (B) Overall survival curve according to the expression level of miR-200c. Patients with high expression of miR-200c had a better prognosis than the miR-200c-low patients (P=0.006). (C) Overall survival curve according to the expression level of P-gp. Patients with negative expression of P-gp had a better prognosis than the P-gp-positive patients (P=0.022). P-gp, P-glycoprotein.

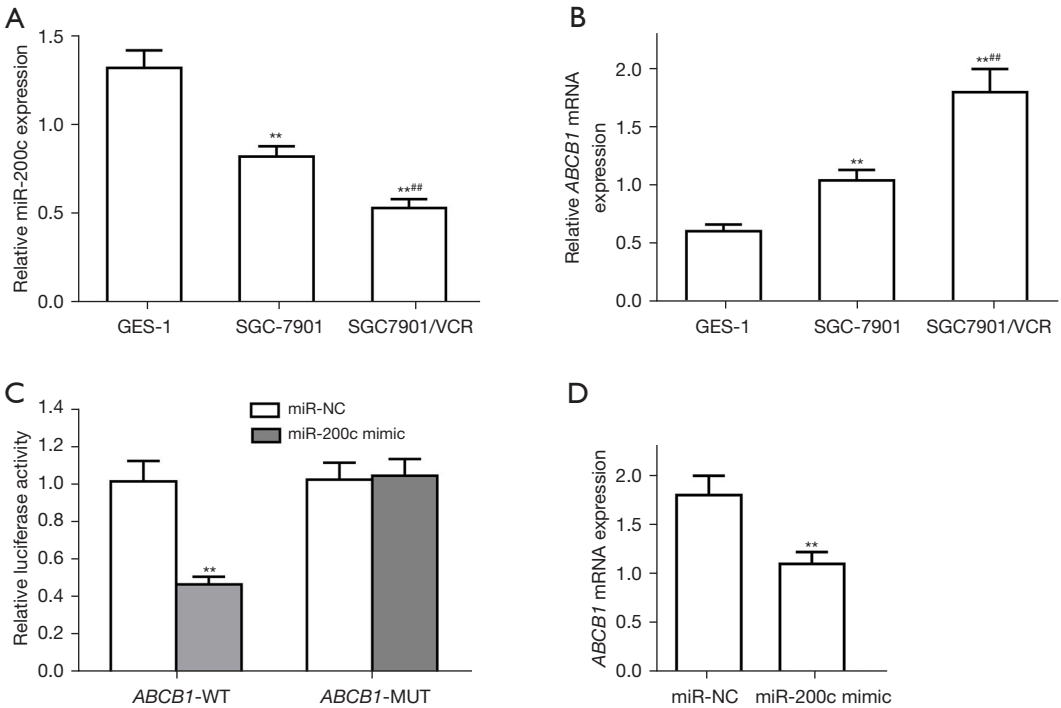

Figure 3 MiR-200c negatively regulates the *ABCB1* gene in gastric cancer cell lines. (A) Expression of miR-200c was significantly decreased in gastric cancer SGC-7901 cells and VCR-resistant SGC7901/VCR cells compared with that in gastric epithelial GES-1 cells (both P<0.01). A lower level of miR-200c was found in SGC7901/VCR cells compared with that in SGC-7901 cells (P<0.01). (B) The expression of *ABCB1* mRNA was significantly increased in SGC-7901 cells and SGC7901/VCR cells compared with that in gastric epithelial GES-1 cells (both P<0.01), and a higher level of *ABCB1* mRNA was found in SGC7901/VCR cells compared with that in SGC-7901 cells (P<0.01). **, P<0.01 *vs.* GES-1; ##, P<0.01 *vs.* SGC-7901. (C) The results of the dual-luciferase reporter assay indicated that miR-200c mimic, rather than its negative control, significantly suppressed the luciferase activity of *ABCB1* mRNA 3'UTR wild-type (P<0.01). However, both miR-200c mimic and its negative control could not inhibit the luciferase activity of *ABCB1* mRNA 3'UTR mutant-type (P>0.05). **, P<0.01 *vs.* miR-NC. (D) Transfection of miR-200c mimic significantly inhibited *ABCB1* mRNA expression compared with transfection of miR-negative control (P<0.01). **, P<0.01 *vs.* miR-NC. VCR, vincristine; UTR, untranslated region; NC, negative control; WT, wild-type; MUT, mutant-type.

P-gp had a better prognosis than the P-gp-positive patients (P=0.022, *Figure 2C*).

MiR-200c negatively regulates the ABCB1 gene in gastric cancer cell lines

The level of miR-200c was significantly decreased in gastric cancer SGC-7901 cells and VCR-resistant SGC7901/VCR cells compared with that in gastric epithelial GES-1 cells (both P<0.01). Lower expression of miR-200c was found in SGC7901/VCR cells compared with that in SGC-7901 cells (P<0.01, *Figure 3A*). In addition, the expression of *ABCB1* mRNA was significantly increased in SGC-7901 cells and SGC7901/VCR cells compared with that in gastric epithelial GES-1 cells (both P<0.01), and a higher level of *ABCB1* mRNA was found in SGC7901/VCR cells compared with that in SGC-7901 cells (P<0.01, *Figure 3B*).

To determine whether miR-200c regulates the expression of the *ABCB1* gene, a dual-luciferase reporter assay was performed. The results indicated that miR-200c mimic, rather than its negative control, significantly suppressed the luciferase activity of *ABCB1* mRNA 3'UTR wild-type (P<0.01). However, both miR-200c mimic and its negative control could not inhibit the luciferase activity of *ABCB1* mRNA 3'UTR mutant-type (P>0.05, *Figure 3C*). Furthermore, we detected *ABCB1* mRNA expression after the transfection of miR-200c mimic or its negative control in SGC7901/VCR cells. The results of qRT-PCR showed

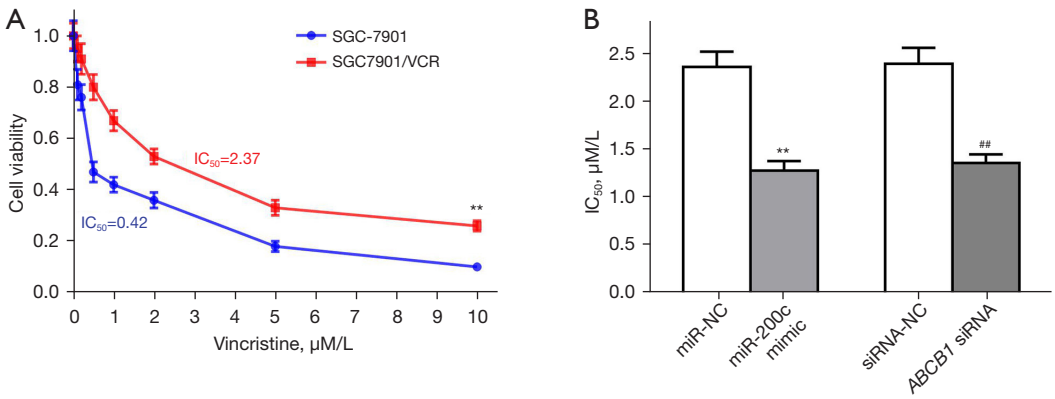

Figure 4 MiR-200c overexpression and *ABCB1* down-regulation increase the sensitivity of SGC7901/VCR cells to vincristine. (A) The SGC-7901 cells and SGC7901/VCR cells were treated with different concentrations of vincristine (0.1, 0.2, 0.5, 1, 2, 5, and 10 μM/L) for 48 h. The value of IC_{50} was calculated according to the results of cell viability and the dose-effect curve of vincristine. The results showed that the IC_{50} values of vincristine in SGC7901 and SGC7901/VCR cells were 0.42 and 2.37 μM/L, respectively. **, P<0.01 *vs.* SGC-7901. (B) The effects of miR-200c overexpression and *ABCB1* down-regulation on the sensitivity of SGC7901/VCR cells to vincristine were evaluated. The IC_{50} value was significantly lower in SGC7901/VCR cells transfected with miR-200c mimic compared with that in cells transfected with miR-NC (P<0.01). In addition, the IC_{50} value was also significantly decreased in SGC7901/VCR cells transfected with *ABCB1* siRNA compared with that in cells transfected with siRNA-NC (P<0.01). **, P<0.01 *vs.* miR-NC; ##, P<0.01 *vs.* siRNA-NC. VCR, vincristine; IC_{50}, half maximal inhibitory concentration; NC, negative control.

that transfection of miR-200c mimic significantly inhibited *ABCB1* mRNA expression compared with transfection of miR-NC (P<0.01, *Figure 3D*). These data suggest that *ABCB1* is a direct target gene of miR-200c.

MiR-200c overexpression and ABCB1 down-regulation increase the sensitivity of SGC7901/VCR cells to VCR

To determine the sensitivity of different gastric cancer cell lines to the chemotherapeutic agent, SGC-7901 cells and SGC7901/VCR cells were treated with different concentrations of VCR (0.1, 0.2, 0.5, 1, 2, 5, and 10 μM/L) for 48 h. The value of IC_{50} was calculated according to the results of cell viability and the dose-effect curve of VCR. The results showed that the IC_{50} values of VCR in SGC7901 and SGC7901/VCR cells were 0.42 and 2.37 μM/L, respectively (*Figure 4A*). Then, we evaluated the effects of miR-200c overexpression and *ABCB1* down-regulation on the sensitivity of SGC7901/VCR cells to VCR. The results indicated that the IC_{50} value was significantly lower in SGC7901/VCR cells transfected with miR-200c mimic compared with that in cells transfected with miR-NC (P<0.01). In addition, the IC_{50} value was also significantly decreased in SGC7901/VCR cells transfected with *ABCB1* siRNA compared with that in cells transfected with siRNA-NC (P<0.01, *Figure 4B*).

MiR-200c overexpression and ABCB1 down-regulation reverse MDR by promoting cell apoptosis

To determine whether cell apoptosis was induced after miR-200c overexpression or *ABCB1* down-regulation, we detected the apoptosis rates of SGC7901/VCR cells using flow cytometry. Consistent with the result of increased sensitivity of SGC7901/VCR cells to VCR, the apoptosis rate was significantly higher in SGC7901/VCR cells transfected with miR-200c mimic compared with that in cells transfected with miR-NC (P<0.01). In addition, the apoptosis rate was also significantly increased in SGC7901/VCR cells transfected with *ABCB1* siRNA compared with that in cells transfected with siRNA-NC (P<0.01, *Figure 5A,5B*).

To determine the effects of miR-200c overexpression and *ABCB1* down-regulation on apoptosis in SGC7901/VCR cells, several cell apoptosis-related proteins were detected using Western blotting. The results indicated that the expression levels of Bax and caspase-3 were significantly increased in SGC7901/VCR cells transfected with miR-200c mimic compared with those in cells transfected with miR-NC (both P<0.01), and the expression of Bcl-

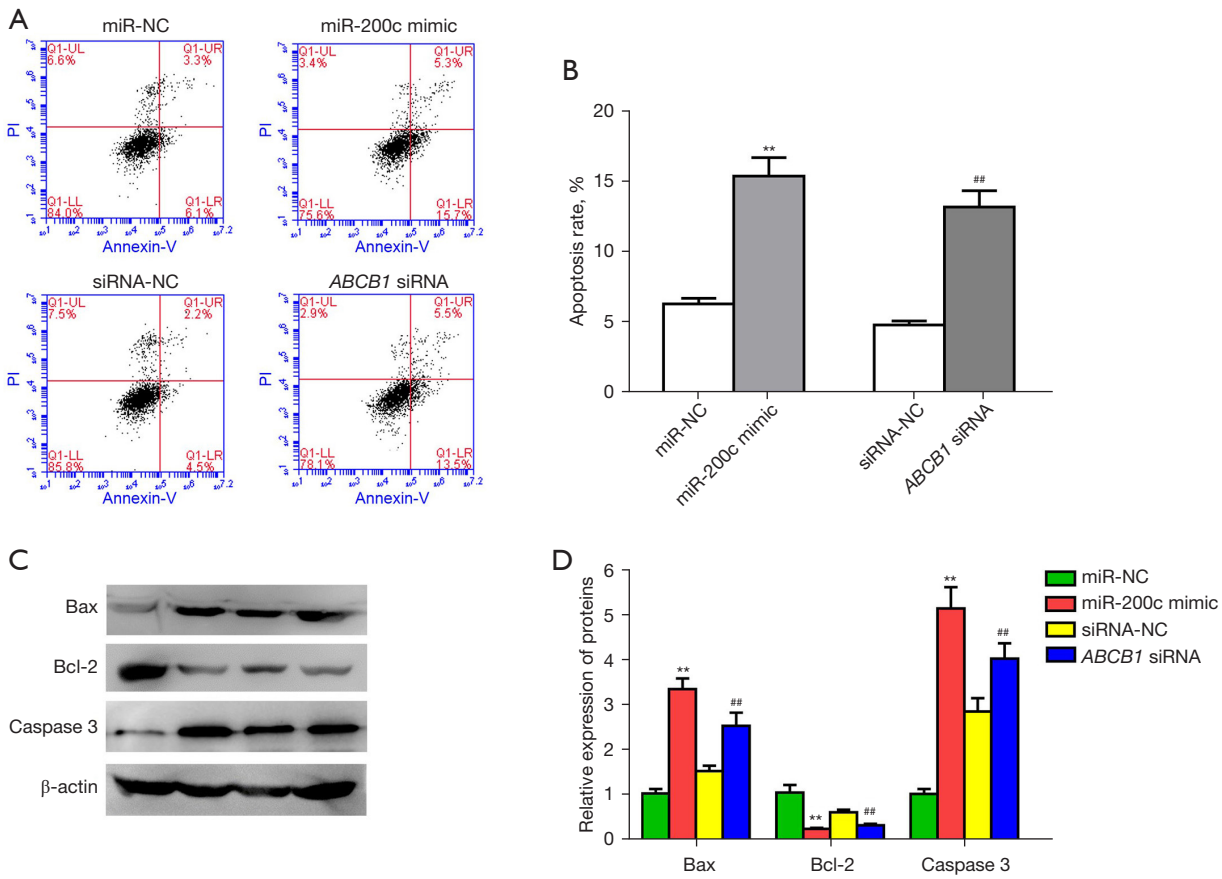

Figure 5 MiR-200c overexpression and *ABCB1* down-regulation reverse MDR by promoting cell apoptosis. (A,B) The apoptosis rates of SGC7901/VCR cells were detected using flow cytometry. The apoptosis rate was significantly higher in SGC7901/VCR cells transfected with miR-200c mimic compared with that in cells transfected with miR-NC (P<0.01). In addition, the apoptosis rate was also significantly increased in SGC7901/VCR cells transfected with *ABCB1* siRNA compared with that in cells transfected with siRNA-NC (P<0.01). (C,D) Several cell apoptosis-related proteins were detected using Western blot. The expression levels of Bax and caspase-3 were significantly increased in SGC7901/VCR cells transfected with miR-200c mimic compared with those in cells transfected with miR-NC (both P<0.01), and the expression of Bcl-2 was significantly decreased in SGC7901/VCR cells transfected with miR-200c mimic compared with that in cells transfected with miR-NC (P<0.01). Furthermore, the expression levels of Bax and caspase-3 were also significantly increased in SGC7901/VCR cells transfected with *ABCB1* siRNA compared with those in cells transfected with siRNA-NC (both P<0.01), and the expression of Bcl-2 was significantly decreased in SGC7901/VCR cells transfected with *ABCB1* siRNA compared with that in cells transfected with siRNA-NC (P<0.01). **, P<0.01 *vs.* miR-NC; ##, P<0.01 *vs.* siRNA-NC. MDR, multidrug resistance; VCR, vincristine; NC, negative control.

2 was significantly decreased in SGC7901/VCR cells transfected with miR-200c mimic compared with that in cells transfected with miR-NC (P<0.01). Furthermore, the expression levels of Bax and caspase-3 were also significantly increased in SGC7901/VCR cells transfected with *ABCB1* siRNA compared with those in cells transfected with siRNA-NC (both P<0.01), and the expression of Bcl-2 was significantly decreased in SGC7901/VCR cells transfected with *ABCB1* siRNA compared with that in cells transfected with siRNA-NC (P<0.01, *Figure 5C,5D*). These data indicated that miR-200c overexpression and *ABCB1* down-regulation reversed MDR by promoting cell apoptosis.

Discussion

Gastric cancer has one of the highest morbidity and

mortality rates of all malignancies worldwide. Chemotherapy plays an important role in the comprehensive treatment strategy of gastric cancer (17). The 5-year survival rate of patients has gradually increased due to the continuous development of chemotherapy drugs and the improvement of chemotherapy regimens. However, there is still a lack of specific chemotherapy drugs, and the therapeutic effect of chemotherapy drugs is seriously affected by MDR, resulting in a high death rate of gastric cancer. MDR occurring in gastric cancer is a result of the mutual regulation and interaction of multiple related genes and signaling pathways (18). The mechanism of MDR in gastric cancer is complicated, and studies on the reversal of MDR in gastric cancer have not made breakthroughs unless the key regulatory molecules are first discovered. Therefore, the potential causes of MDR and finding molecular targets to reverse MDR in gastric cancer remain hot topics in the field of cancer research.

With the deepening of research on miRNAs, more studies have shown that miRNAs are closely related to the occurrence and development of tumors (19). Many studies have confirmed that the miR-200 family plays a certain role in the occurrence and development of various tumors, including pancreatic cancer (20), breast cancer (21), non-small cell lung cancer (22), and colorectal cancer (23), among others. MiR-200c, the most studied miRNA in the miR-200 family, inhibits the invasion and metastasis of multiple cancers (24,25).

In this study, the relative expression of miR-200c was significantly lower in gastric cancer tissues than that in matched normal tissues. In addition, the expression of miR-200c was associated with some malignant characteristics of gastric cancer including TNM stage, lymph node metastasis, and distant metastasis. In cultured gastric cancer cells, the level of miR-200c was significantly decreased compared with that in gastric epithelial cells. This level was markedly decreased in VCR-resistant SGC7901/VCR cells. These data suggest that miR-200c plays an important role in the progression, prognosis, and drug resistance of gastric cancer. Furthermore, multiple targeted genes of miR-200c have been found in a variety of cancers. MiR-200c inhibits the growth and metastasis of endometrial and renal carcinoma cells by targeted regulation of BMI-1 (25,26). In gastric cancer, miR-200c could suppress the proliferation, migration, and invasion of cancer cells via regulating the ZEB2 protein (6). These results indicate that miR-200c regulates the occurrence and development of tumors through a variety of pathways.

P-gp is a classical MDR-related protein that increases the efflux of cytotoxic drugs. A previous study showed that P-gp was involved in the resistance to chemotherapeutic agents both in gastric cancer tissues and cell lines (27). Increased expression of P-gp was found after gastric cancer cells were transfected with miR-19a/b, which mediates MDR via the PTEN signaling pathway (28). In the current study, the positive expression rate of P-gp was significantly higher in gastric cancer tissues than that in adjacent normal tissues. Interestingly, increased expression of P-gp in cancer tissue is not a result of chemotherapeutic agent induction. It seems that the cancer cells are prepared to resist drugs in advance. Our findings in cultured cells also support this speculation that the expression of *ABCB1* mRNA progressively increases in gastric epithelial GES-1 cells, SGC-7901 cells, and SGC7901/VCR cells.

After we discovered that P-gp expression was also associated with the malignant characteristics of gastric cancer including TNM stage, lymph node metastasis, and distant metastasis, a significant negative correlation between the expression of miR-200c and P-gp was found in gastric cancer tissues. In addition, patients with high expression of miR-200c or negative expression of P-gp had a better prognosis. Therefore, we hypothesized that miR-200c played its role in gastric cancer via regulating the expression of P-gp. A dual-luciferase reporter assay was used to determine whether miR-200c regulated the expression of the *ABCB1* gene. The results indicate that *ABCB1* is a direct target gene of miR-200c, and miR-200c could significantly inhibit the expression of *ABCB1* mRNA.

Furthermore, the current study indicated that miR-200c up-regulation or *ABCB1* down-regulation increased the sensitivity of the SGC7901/VCR cell line to VCR. This result was reached by the calculation of IC_{50} values in SGC7901/VCR cells with different treatments. To determine whether apoptotic signals were implicated in this process, we observed the apoptosis rates of SGC7901/VCR cells after miR-200c up-regulation or *ABCB1* down-regulation. The results indicated that both miR-200c up-regulation or *ABCB1* down-regulation could increase the apoptosis rate of SGC7901/VCR cells via the regulation of apoptosis-related proteins including Bax, caspase-3, and Bcl-2. These data suggest that miR-200c increases the chemotherapy sensitivity of gastric cancer through apoptotic signaling.

Actually, multiple miRNAs play important roles in cancer MDR through complicated molecular mechanisms. Xia *et al.* (29) reported that MiR-15b and miR-16 modulate

MDR by modulation of apoptosis via targeting BCL2 in human gastric cancer cells. In ovarian cancer, miR-214 induces cell survival and cisplatin resistance by targeting the 3'UTR of PTEN (30). These miRNAs play important roles in the regulation of tumor development and are potential targets for cancer intervention. These MDR-related miRNAs including miR-200c play important roles in the tumor development and are potential targets for cancer intervention. Our finding of miR-200c-P-gp regulation may provide a new reference for gastric cancer chemotherapy strategy. Further experimental studies are required for identification of the signaling pathways might miR-200c be involved in reversing MDR. In addition, the role of miR-200c *in vivo* should be investigated using transgenic mice. The current study is a retrospective analysis, which is likely to cause some deviations in the results. It needs to be further confirmed by a multicenter clinical trial in the future.

Conclusions

The expression level of miR-200c decreases in gastric cancer tissues and drug-resistant gastric cancer SGC7901/VCR cells. Overexpression of miR-200c may enhance the sensitivity of SGC7901/VCR cells to VCR by regulating the expression of P-gp. MiR-200c and P-gp mediate chemotherapy sensitivity via the apoptotic signaling pathway in gastric cancer.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-167/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The current study was approved by the Medical Ethics Committee of the Shanxi Provincial People's Hospital (China) (No. 2019-88). All the patients signed written informed consent for the use of the specimens.

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