miR-15b and miR-16 modulate multidrug resistance by targeting BCL2 in human gastric cancer cells

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microRNAs are endogenous small noncoding RNAs that regulate gene expression negatively at posttranscriptional level. This latest addition to the complex gene regulatory circuitry revolutionizes our way to understanding physiological and pathological processes in the human body. Here we investigated the possible role of microRNAs in the development of multidrug resistance (MDR) in gastric cancer cells. microRNA expression profiling revealed a limited set of microRNAs with altered expression in multidrug-resistant gastric cancer cell line SGC7901/VCR compared to its parental SGC7901 cell line. Among the downregulated microRNAs are miR-15b and miR-16, members of miR-15/16 family, whose expression was further validated by qRT-PCR. In vitro drug sensitivity assay demonstrated that overexpression of miR-15b or miR-16 sensitized SGC7901/VCR cells to anticancer drugs with far reduction in level of these using antitumor oligonucleotide con-ferred SGC7901 cells MDR. The downregulation of miR-15b and miR-16 in SGC7901/VCR cells was concurrent with the upregula-tion of Bcl-2 protein. Enforced mir-15b or miR-16 expression reduced Bcl-2 protein level and the luciferase activity of a BCL2 3’ untranslated region-based reporter construct in SGC7901/VCR cells, suggesting that BCL2 is a direct target of miR-15b and miR-16. Moreover, overexpression of miR-15b or miR-16 could sensitize SGC7901/VCR cells to VCR-induced apoptosis. Taken to-gether, our findings suggest that miR-15b and miR-16 could play a role in the development of MDR in gastric cancer cells at least in part by modulation of apoptosis via targeting BCL2.

Key words: microRNA; multidrug resistance; BCL2; apoptosis; gastric cancer

Gastric cancer ranks as the second leading cause of cancer death worldwide, probably accounting for about 10% of newly diagnosed cancers although the incidence and mortality rates have generally declined during the past decades. Chemotherapy remains the primary treatment for both resectable and advanced gastric cancer as to improving overall survival and quality of life for patients. In many cases, however, therapies fail because of multidrug resistance (MDR) of cancer cells either intrinsic or acquired after an initial round of treatment. MDR was initially thought to arise from molecular changes inhibiting the drug-target interaction, represented by overexpression of drug efflux pumps such as P-glycoprotein (P-gp, a product of MDR-1 gene) or intra-cellular detoxifiers such as antioxidants (e.g., glutathione) in drug-resistant tumor cells. More recently, other factors acting downstream of the initial drug-induced insult have been proposed to play an important role in the development of MDR such as enhanced DNA repair activity, defective apoptosis pathway, etc. The MDR mechanisms in gastric cancer cells have been extensively explored, yet have not been fully characterized. Evidences so far suggest that mechanisms responsible for MDR in gastric cancer are likely to be multifaceted and extremely intricate.

microRNAs (miRNAs) are a newly discovered class of small noncoding RNAs encoded by the genomes of a wide range of multicellular organisms. Primary microRNA transcripts are transcribed by RNA polymerase II, which then undergo sequential processing by 2 RNaseIII enzymes, Drosha and Dicer to yield ~22-nucleotide mature form. Mature miRNAs are incorporated into the RNA-induced silencing complex (RISC) and then target the 3’ untranslated region (3’UTR) of a specific mRNA by base pairing, leading to translational repression or mRNA degradation. Accounting for about 1% of all expressed human genes, miRNAs are predicted to regulate the expression of up to 1/3 of human protein-coding genes, which implies the potential influence of miRNAs on almost every genetic pathway. Indeed, miRNAs have been shown to play crucial roles in diverse biological processes, such as development, differentiation, apoptosis and proliferation.

Recently, accumulating evidence has indicated that altered miRNA level resulted from mutation or aberrant expression is correlated with various human cancers. It is suggested that the abnormally expressed miRNAs in human cancers target transcripts of essential protein-coding genes involved in tumorigenesis, including oncogenes and tumor-suppressor genes. While the roles of miRNAs in cancer development are being extensively investigated and become increasingly well defined, their involvement in tumor cell response to chemotherapy has also been suggested by a few reports. It was found that suppression of miR-21 using antisense oligonucleotide sensitized MCF7 cells to anticancer drug topotecan. In addition, it was shown that gemcitabine and 5-FU treatment could alter the expression of a surprising number of diverse miRNAs in cholangiocarcinoma tumor cells and colon cancer cells, respectively. Moreover, the modulation of some miRNAs increases the sensitivity of cholangiocarcinoma tumor cells to gemcitabine in vitro. More recently, the effect of miRNAs on chemotherapy was systematically studied as part of the Molecular Targets Program aimed at elucidating molecular targets and understanding mechanisms of chemosensitivity and chemoresistance. The expression levels of miRNAs in the NCI-60 (a panel of 60 diverse human cancer cell lines) were measured and analyzed in combination with other molecular profiling data sets. Comparison of miRNA expression patterns and potency patterns of the 3,089 chemical compounds showed significant correlations, suggesting that miRNAs may play a role in chemoresistance. However, gastric cancer cell line was not included in the panel and there have been little available data on the potential role of miRNAs in the chemoresistance of gastric cancer.

In our study, we reported that a limited set of miRNAs were differentially expressed in a multidrug-resistant human gastric cancer cell line SGC7901/VCR and its parental cell line SGC7901. miR-Abbreviations: ADR, Adriamycin; CDDP, cisplatin; CLL, chronic lym-phocytic leukemia; 5-FU, 5-fluorouracil; MDR, multidrug resistance; miRNA, microRNA; MMC, mitomycin C; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; UTR, untranslated region; VCR, vincristine; VP-16, etoposide.

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miR-15b and miR-16, among the downregulated miRNAs in SGC7901/VCR cells, were demonstrated to play a role in the development of MDR in gastric cancer cells by targeting the antiapoptotic gene BCL2.

Material and methods

Cell lines and cultures

Human gastric adenocarcinoma cell line SGC7901 (obtained from Academy of Military Medical Science, Beijing, China) and its multidrug-resistant variant SGC7901/VCR (established and maintained in our laboratory) were 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 (with final concentration of 1 μg/ml) was added to the culture media for SGC7901/VCR cells.

miRNA microarray analysis

Prior to experimentation, SGC7901/VCR cells were cultured 1 week without vincristine. Total RNA from SGC7901 and SGC7901/VCR cell lines was isolated with Trizol reagent (Invitrogen, Carlsbad, CA) and miRNA fraction was further purified with a mirVana™ miRNA isolation kit (Ambion, Austin, TX).

miRNA microarray analysis

miRNA microarray analysis was performed on SGC7901/VCR cells to analyze the expression of miRNAs. This involved the isolation of total RNA from both cell lines, followed by a hybridization step using a microarray containing known miRNAs. The hybridization was performed on a glass slide, and the expression levels of each miRNA were quantified using a scanner. The data was then analyzed using bioinformatic tools to identify differentially expressed miRNAs.

Quantitative RT-PCR for miRNA

Quantitative RT-PCR was performed to confirm the microarray results. Total RNA was extracted from the cells using the mirVana™ miRNA isolation kit. The miRNA was reverse transcribed using the miRCURY™ LNA microRNA Array protocol (Exiqon, Vedbaek, Denmark) and hybridized against a set of miRNA probes on a microarray chip. The relative expression levels of each miRNA were calculated using the Ct method, taking into account the efficiency of reverse transcription and the expression levels of the housekeeping gene U6.

Western blot analysis

SGC7901/VCR cells were plated in 6-well plates (3 × 10^5 cells/well) and transfected with 200 pmol of miR-15b or miR-16 precursor or negative control using siPORT NeoFx. After 72 hr of culturing, cells were harvested and homogenized with lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% SDS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.02% sodium azide, 100 μg/ml PMSF, 1 μg/ml aprotinin). Eighty micro gram of total protein extract was separated on 12% SDS-PAGE gels and transferred to nitrocellulose membranes (Millipore, Bedford, MA). After blocking with 5% nonfat dry milk in TBS, the membrane was probed with primary monoclonal antibody specific to Bcl-2 (1:200, Santa Cruz Biotechnology, CA) or β-actin (1:5000, Sigma, St Louis, MO) which was used as an internal control for protein loading.

In vitro drug sensitivity assay

miRNA precursors, antisense inhibitors or controls were all purchased from Ambion. SGC7901 and SGC7901/VCR cells were plated in 6-well plates (4 × 10^5 cells/well) in antibioticfree medium and transfected with 200 pmol oligonucleotides using siPORT neoFX (Ambion) in accordance with the manufacturers’ procedures. Twenty-four hours after transfection, cells were seeded into 96-well plates (8 × 10^3 viable cells/well) and allowed to attach overnight. After cellular adhesion, freshly prepared anticancer drugs including vincristine (VCR), adriamycin (ADR), 5-fluorouracil (5-FU), cisplatin (CDDP), mitomycin C (MMC) and etoposide (VP-16) were added with the final concentration being 0.01, 0.1, 1 and 10 times of the human peak plasma concentration for each drug. The peak serum concentrations of various anticancer drugs are 0.4 μg/ml for ADR, 10 μg/ml for 5-FU, 2.0 μg/ml for CDDP, 1.0 μg/ml for MMC, 0.5 μg/ml for VCR and 10 μg/ml for VP-16.26,27 After 72 hr, cell viability was assessed using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium 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 triplicate.

Luciferase activity assay

The 3’UTR of human BCL2 cDNA containing the putative target site for miR-15b and miR-16 was amplified by PCR using the primers: 5’-CTAGTCTAGAGCCGCTCAGGAAC AGAA-3’ and 5’-CTAGTCTAGAGCGCTCAGGAAC AGAA-3’. The PCR products were cloned into pGL3-control vector 24 and inserted into the Xba I site, immediately downstream of the luciferase gene in the pGL3-control vector (Promega, Madison, WI). A mutant version with a deletion of 5 bp from the site of perfect complementarity was also generated by using the QuikChange II Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). Wild type and mutant inserts were confirmed by sequencing. Twenty-four hours before transfection, cells were plated at 1.5 × 10^5 cells/well in 24-well plates. 200 ng of pGL3-bcl2-3’-UTR or pGL3-bcl2-3’-UTR plus 80 ng pRL-TK (Promega) were transfected alone or in combination with 40 pmol of miR-15b/miR-16 precursors or precursor control using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. Luciferase activity was measured 24 hr 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.
TABLE II – miRNAs DIFFERENTIALLY EXPRESSED IN SGC7901/VCR CELL LINE AND SGC7901 CELL LINE

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Upregulation or downregulation in VCR</th>
<th>Chromosome location1</th>
<th>Putative targets associated with MDR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>let-7a</td>
<td>Down</td>
<td>9q22.2; 11q24.2; 22q13.3</td>
<td>RAS, ABCC5</td>
</tr>
<tr>
<td>miR-15b</td>
<td>Down</td>
<td>3q25.33</td>
<td>BCL2, CHK1</td>
</tr>
<tr>
<td>miR-16</td>
<td>Down</td>
<td>3q25.33; 13q14.2</td>
<td>BCL2, CHK1</td>
</tr>
<tr>
<td>miR-17-5p</td>
<td>Down</td>
<td>13q31.3</td>
<td>MCL1, STAT3</td>
</tr>
<tr>
<td>miR-20a</td>
<td>Down</td>
<td>13q31.3</td>
<td>MCL1, STAT3</td>
</tr>
<tr>
<td>miR-23b</td>
<td>Down</td>
<td>9q22.32</td>
<td>CCND1</td>
</tr>
<tr>
<td>miR-106a</td>
<td>Down</td>
<td>Xq26.2</td>
<td>MCL1, STAT3</td>
</tr>
<tr>
<td>miR-106b</td>
<td>Down</td>
<td>7q22.1</td>
<td>MCL1, STAT3</td>
</tr>
<tr>
<td>miR-190a</td>
<td>Down</td>
<td>17q21.32; 12q13.13</td>
<td>CDKN1B</td>
</tr>
<tr>
<td>miR-302b</td>
<td>Up</td>
<td>4q25</td>
<td></td>
</tr>
<tr>
<td>miR-320</td>
<td>Down</td>
<td>8p21.3</td>
<td>MCL1, CHK1</td>
</tr>
<tr>
<td>miR-492</td>
<td>Up</td>
<td>12q22</td>
<td></td>
</tr>
</tbody>
</table>

1From miR Base Sequences (http://microrna.sanger.ac.uk). –2miRNA putative targets predicted using the algorithm TargetScan (http://www.targetscan.org).

Results

miRNAs are expressed differentially in multidrug-resistant gastric cancer cell line

To determine whether miRNAs are involved in the development of MDR in gastric cancer cells, we performed a comprehensive miRNA profiling of multidrug-resistant gastric cancer cell line SGC7901/VCR and its parental cell line SGC7901. It was shown that 10 of 342 human miRNAs examined were downregulated more than 2-fold in SGC7901/VCR cells compared to SGC7901 cells. In contrast, only 2 miRNAs were upregulated more than 2-fold in SGC7901/VCR cells (Table II). These miRNAs may play an important role in the development of MDR in gastric cancer cells. Interestingly, some of the deregulated miRNAs are from the same family and may produce synergistic effects in mediating the MDR phenotype by performing similar functions. Among them are miR-15b and miR-16 of miR-15/16 family, both downregulated in SGC7901/VCR cells. They were selected for further study because miR-15a and miR-16 have already been shown to promote apoptosis by negatively regulating BCL2 in chronic lymphocytic leukaemia (CLL) cells. To verify the results obtained by microarray profiling, we performed quantitative RT-PCR analysis of miR-15b and miR-16 expression in the 2 cell lines. In accordance with the microarray data, quantitative RT-PCR showed decreased miR-15b and miR-16 levels in SGC7901/VCR cells compared to its counterpart (Figs. 1a and 1b).

miR-15b and miR-16 modulate MDR phenotype of gastric cancer cells

To investigate whether miR-15b and miR-16 have a direct function in MDR development or are simply differentially modulated in MDR gastric cancer cells, we used gain-of-function and loss-of-function approaches in SGC7901/VCR and SGC7901 cells, which respectively express relatively low and high levels of miR-15b and miR-16, and observed the effects on MDR phenotype thereafter. MiT assay revealed that SGC7901/VCR cells transfected with miR-15b or miR-16 precursor exhibited greatly enhanced sensitivity to VCR, ADR, VP-16 and CDDP, but not to 5-FU and MMC compared to those transfected with scrambled oligonucleotides, as indicated by significantly decreased IC50 values (Fig. 2a). On the other hand, suppression of miR-15b or miR-16 level in SGC7901/VCR cells by transfecting the specific inhibitor of miR-15b or miR-16 led to decreased sensitivity of SGC7901 cells to VCR, ADR, VP-16 and CDDP (Fig. 2b). The above data indicate that modulation of miR-15b or miR-16 expression could alter the MDR phenotype of gastric cancer cells.

BCL2 is a target of posttranscriptional repression by miR-15b and miR-16

miR-15a, miR-15b and miR-16 share the identical 9 nucleotides of the 5’ “seed” region which are complementary to bases 2528–2536 of the BCL2 3’UTR (Fig. 3a), thus they all potentially target
miR-15b and miR-16 sensitize SGC7901/VCR cells to VCR induced apoptosis

The development of drug resistance in various cancer cells has been linked to a reduced susceptibility to drug-induced apoptosis, which was shown to be a consequence, at least in some cases, of overexpression of antiapoptotic proteins, such as Bcl-2 and Bcl-XL. Considering the well-characterized role of BCL2 in apoptosis and drug resistance, we suggested a hypothesis that miR-15b and miR-16 play a role in the development of MDR at least in part through the posttranscriptional regulation of BCL2 expression. This hypothesis was supported by both in silico and experimental studies.

In silico analysis showed that BCL2 expression was decreased in SGC7901/VCR cells compared to SGC7901 cells, indicating a potential role for miR-15b and miR-16 in the regulation of BCL2 expression. To confirm this, we transfected SGC7901/VCR cells with specific precursor or control miRNA precursor. Western Blot analysis revealed a decrease in Bcl-2 protein level in miR-15b or miR-16 precursor-transfected cells compared to control miRNA precursor-transfected group. In contrast, RT-PCR analysis showed no significant difference in the mRNA level of BCL2 in these cells.

To further investigate the mechanism of this regulation, we constructed a luciferase reporter vector with the putative BCL2 3′-UTR target site for miR-15b and miR-16 downstream of the luciferase gene (pGL3-Bcl2-3′UTR) and a mutant version thereof (pGL3-mutBcl2-3′UTR). Luciferase reporter vector alone or together with miR-15b or miR-16 precursor or control miRNA were transfected into SGC7901/VCR cells that weakly expressed miR-15b and miR-16. A significant decrease in relative luciferase activity was noted when pGL3-Bcl2-3′UTR was cotransfected with miR-15b or miR-16 precursor but not with scrambled oligonucleotides. As expected, this suppression was abolished by deleting part of the perfectly complementary sequences in the BCL2 3′-UTR (pGL3-mutBcl2-3′UTR) which disrupts the interaction between miR-15b/miR-16 and BCL2.

Taken together, these results demonstrate that miR-15b and miR-16 directly inhibit BCL2 expression at posttranscriptional level through its 3′UTR.
by modulation of apoptosis via targeting BCL2. To confirm this hypothesis, we evaluated the effects of enforced miR-15b and miR-16 expression on VCR-induced apoptosis in SGC7901/VCR cells. A marked increase in apoptosis, as assessed by flow cytometry, was observed in miR-15b or miR-16 precursor-transfected SGC7901/VCR cells after VCR treatment compared to precursor control-transfected cells (Figs. 4a and 4b). In addition, caspase-3 and caspase-7 (key executioners of apoptosis) enzymatic activities were significantly increased by 72 hr posttransfection in cells transfected with miR-15b or miR-16 precursor relative to cells transfected with control oligonucleotides (Fig. 4c).

**Discussion**

Recently much research effort has been intensely focused on studying the role of altered miRNA expression in human malignancies. miRNA expression signatures seem to hold great promise in tumor characterization and could be potential diagnostic and prognostic markers for cancer diagnosis and treatment. In addition, approaches to interfering with miRNA function are considered to offer novel therapeutic opportunities for cancer. Our present data demonstrating the involvement of miRNAs in the development of MDR in gastric cancer cells might add to the evidences that miRNAs could also serve as potential targets for chemosensitizing strategies.

The multidrug-resistant cell line SGC7901/VCR was derived from human gastric adenocarcinoma cell line SGC7901 by stepwise selection using VCR as inducing reagent. It has been widely employed as an *in vitro* model for the study of MDR in gastric cancer. Advances in the understanding of pathogenesis of MDR in gastric cancer have benefited from the emerging of novel genomics and proteomics approaches. Dozens of differentially expressed mRNAs and proteins have been identified between SGC7901/VCR cell line and its parental SGC7901 cell line by using subtractive hybridization, differential display, and 2-DE. These data have yielded a wealth of novel insights into the molecular mechanisms of MDR in gastric cancer. Nonetheless, analysis and characterization of all of these molecules is challenging because of the lack of correlation among each other. Here we intended to approach MDR at miRNA level, a new layer of the
miR-20a and miR-106a. It is likely that these miRNAs well characterized cancer association such as let-7a, miR-16, miR-

described in SGC7901/VCR cells.

In our study, a subset of miRNAs was found to be differentially expressed in SGC7901/VCR cells and its parental SGC7901 cells. We located the chromosomal sites of the deregulated miRNAs and correlated them with the loci of chromosomal abnormalities identified in SGC7901/VCR cells by our previous study. It was found that the chromosomal site of miRNA-106b gene (7q22.1) resides within a region (7q22) where deletions are frequently detected in SGC7901/VCR cells. Whether there is a causal relationship between chromosome 7q22 deletion and the downregulation of miR-106b in SGC7901/VCR cells needs further investigation. As to other loci of chromosomal abnormalities, no differentially expressed miRNA genes were found to locate therein. It seems that the cytogenetic alteration resulted from drug selection may not be the major cause for altered miRNA expression in SGC7901/VCR cells.

Among the differentially expressed miRNAs are some with well characterized cancer association such as let-7a, miR-16, miR-17-5p, miR-20a and miR-106a. It is likely that these miRNAs modulate cell behaviors common to carcinogenesis and drug resistance. Indeed, some “oncomirs” have been shown to play critical roles in the cancer biology underlying chemoresistance. For example, miR-21, overexpressed in many different human cancers, has been reported to have anti-apoptotic properties in glioblastoma, cholangiocarcinoma and breast cancer cells. Not surprisingly, inhibition of miR-21 was shown to increase the sensitivity to topotecan and gemcitabine in breast cancer cells and cholangiocarcinoma cells, respectively. Moreover, it is suggested that miR-21 may regulate BCL2 expression indirectly in MCF-7 cells, which may be partly responsible for increased apoptosis and drug sensitivity after miR-21 inhibition. miR-15a and miR-16, often deleted or downregulated in a majority of B-cell CLL, have been shown to promote apoptosis by targeting BCL2. So far, the tumor-suppressor role of this cluster has only been manifested in various leukaemias but not in cancers that originate from other tissues. In SGC7901/VCR cells, we observed the downregulation of miR-16 as well as miR-15b, a homologue of miR-15a with 4 nucleotides difference at the 3' end. Since miR-15b, miR-15a and miR-16 potentially regulate the same target genes and BCL2 has been widely accepted as a culprit of drug resistance, we wondered if miR-15b and miR-16 play a role in the development of MDR by targeting BCL2.

The downregulation of miR-15b and miR-16 in SGC7901/VCR cells is of functional significance since we demonstrated that modulation of their expression could alter the sensitivity of cells to chemotherapeutic drugs. It is generally accepted that the mitochondrial pathway plays a predominant role in chemotherapy-induced apoptosis. BCL2 is well acknowledged as a critical regulator of the mitochondrial pathway by diminishing cytochrome c release, which leads to inhibition of apoptosis. Our present work reveals that miR-15b and miR-16 directly regulate BCL2 expression, thereby modulating the susceptibility of cancer cells to chemotherapeutic drug-induced apoptosis. This explains the altered sensitivity of gastric cancer cells to VCR, ADR, VP-16 and CDDP after modified expression of miR-15b and miR-16, but it is worthy noting that cytotoxicity was not modulated with 5-FU and MMC. A possible explanation for this phenomenon could be that 5-FU and MMC trigger apoptosis of gastric cancer cells through a pathway where BCL2 is not necessarily involved. In support of our postulation, there have been some evidences which strongly suggest that 5-FU induces apoptosis through the Fas/FasL pathway, at least in some cell types. It is such that factors that affect the mitochondrial release of cytochrome c (e.g., BCL2 overexpression) will have little impact on the cell response to drug-induced apoptosis. The mechanism of MMC-induced apoptosis remains controversial as to different cell context. It was indicated that MMC-induced apoptosis in SNU-16 human gastric adenocarcinoma cells was mediated by caspase-8, 9, and caspase-3 activation independently of Fas/FasL interactions. While in cervical carcinoma cells, MMC was reported to induces apoptosis via caspase-8 and -3 processing in a Fas/FasL-dependent manner. In addition, a
recent study demonstrated that MMC could significantly induce caspase-8 promoter activity in hepatoma cells, leading to increased caspase-8 enzymatic activity and apoptosis. Thus, it is likely that BCL2 may not or only partially be involved in MMC-induced apoptosis in SGC7901 and its derivative cell lines. Our study may have implications for cancer chemotherapy whose efficacy is often impeded by the development of MDR. Therapeutic strategies targeting the MDR-related miRNAs, such as miR-15b and miR-16, may be more promising than those targeting single proteins considering the fact that miRNAs function through the regulation of multiple genes and have global impact on diverse cellular processes. However, it should be noted that our data are derived from cell lines which have been removed from their in vivo context and cannot be considered accurate surrogates for clinical tumors. Thus, future studies to assess the roles of these miRNAs in vivo and in clinical context are warranted.

In summary, the findings we reported here presented the first evidence that miRNAs may be involved in the development of MDR in gastric cancer cells. miR-15b and miR-16 could modulate the sensitivity of gastric cancer cells to some anticancer drugs, at least in part, through regulating BCL2 expression. Our data provide a novel insight into the mechanisms of MDR in gastric cancer and maybe useful for the future development of chemo-sensitizing strategy through manipulating miRNA expression.

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References


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