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Original Article

miR-181a sensitizes a multidrug-resistant leukemia cell line K562/A02 to daunorubicin by targeting BCL-2

Hao Li, Lulu Hui, and Wenlin Xu*

Department of Central Laboratory, The Affiliated People's Hospital, Jiangsu University, Zhenjiang 212001, China
*Correspondence address. Tel: +86-511-88915583; Fax: +86-511-88915583; E-mail: xuwl0511@yahoo.com

The aim of this study was to investigate whether miR-181a could modulate the sensitivity of the leukemia drug-resistant cell line K562/A02 to the chemotherapeutic agent daunorubicin (DNR), and explore the mechanism of miR-181a on the DNR sensitivity of K562/A02 cells. MicroRNA microarray and stem-loop reverse transcription-polymerase chain reaction were used to detect the expression of miR-181a. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay was performed to quantify the effect of miR-181a on K562 cells growth and viability. Apoptotic cells were quantitatively detected using Annexin V/FITC and PI apoptosis detection kit. BCL-2 protein expression was measured by western blot. Luciferase reporter vector with the putative *BCL-2* 3′ untranslated region was constructed to explore whether *BCL-2* was a direct target gene of miR-181a. BCL-2 siRNA was transfected into the cell to explore the relationship between BCL-2 and DNR resistance. The miR-181a expression level was lower in the K562/A02 cells than in the K562 cells ($P < 0.05$). K562 cells that were transfected with miR-181a inhibitor had a significantly higher survival than K562 cells, and K562/A02 cells that were transfected with the miR-181a mimic had a significantly lower survival than K562/A02 cells ($P < 0.05$). miR-181a could enhance DNR-induced apoptosis in K562/A02 cells. BCL-2 siRNA transfected K562/A02 cells had decreased survival compared with the K562/A02 control group. In conclusion, miR-181a could play a role in the development of DNR resistance in K562/A02 cells and the over-expression of miR-181a could sensitize K562/A02 cells to DNR by targeting BCL-2.

Keywords miR-181a; apoptosis; BCL-2

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Introduction

Chemotherapy is an important therapeutic option for most cancer patients including leukemia patients. Daunorubicin (DNR) is a chemotherapeutic that belongs to the anthracycline family, and DNR-based chemotherapy has become one of the most effective antileukemia strategies[1,2]. However, the failure of the curative treatment of leukemia patients often occurs as a result of acquired drug resistance of leukemia cells to chemotherapeutic agents. Accumulating studies indicate that there are several major mechanisms of drug resistance in cancer cells. The most cited mechanisms for the acquisition of multidrug resistance are the expression of energy-dependent transporters that eject anticancer drugs from cells [3]. Furthermore, a number of proteins, including K-ras, COX-2, cyclin D1, BCL-2, and Survivin, play critical roles in drug resistance to conventional chemotherapeutics [4–7]. In addition, the major cell survival signaling pathway receptors and downstream proteins have been reported to be involved in drug resistance such as the epidermal growth factor receptor [8–12]. Moreover, recent studies have shown that cancer stem cells and epithelial–mesenchymal transition-type cells could play critical roles in drug resistance [13–16]. Finally, recent studies have demonstrated that microRNAs (miRNAs) are involved in the regulation of drug resistance [17]. Researchers have carried out many studies on drug resistance reversal in recent years and have developed three generations of drug resistance reversal agents, termed as I, II, and III [18]. However, these agents failed to be applied in clinic because of their severe side effects or poor pharmacokinetics *in vivo* [19]. Therefore, increasing the drug sensitivity is a key step toward improving therapy for cancer patients.

Currently, extensive studies have indicated that the acquisition of drug resistance by cancer cells may also be modulated via the changes in miRNA levels [20–24]. miRNAs are short non-coding RNA molecules that post-transcriptionally regulate gene expression. miRNAs bind to the 3′ untranslated region (3′ UTR) of mRNA, and either repress its translation or result in the degradation of the target mRNA [25]. There are over 700 miRNAs in humans [26], and ~30% of all genes expression are regulated by

miRNAs. Si *et al*. [27] have found that suppressing the expression of miR-21 with antisense oligonucleotides could sensitize MCF7 cells to anticancer drug topotecan. Other studies have indicated that miR-21 contributes to drug resistance in solid tumors through several pathways [28–31]. Moreover, in multidrug-resistant gastric cancer cell line SGC7901/VCR, miR-15b and miR-16 were down-regulated, compared with its parental SGC7901 cell line. Up-regulating miR-15b and miR-16 could sensitize SGC7901/VCR cells to VCR-induced apoptosis via targeting *BCL-2* [23]. Collectively, these reports suggest a role of miRNAs in drug resistance. Further in-depth research is needed to fully understand this role and to find novel treatment strategies for cancer drug resistance.

In this study, we reported that miR-181a was down-regulated in multidrug-resistant human leukemia cell line K562/A02 compared with the parental K562 cell lines. We demonstrated that miR-181a may play a role in the development of drug resistance in human leukemia cell lines by targeting the anti-apoptotic gene *BCL-2*.

Materials and Methods

Cell lines and cell culture

The human chronic myeloid leukemia cell line K562, and its mutidrug-resistant counterpart K562/A02 were obtained from Shanghai Institute of Cell Biology, China Academy of Sciences (Shanghai, China). Cells were all cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (Gibco BRL, Grand Island, USA) in a humidified atmosphere containing 5% CO_2 at 37°C. To maintain the multidrug resistance phenotype, doxorubicin was added to the culture media for K562/A02 cells at the final concentration of 1 μg/ml. The cells were cultured for 2 weeks in drug-free medium prior to their use in the experiments.

miRNA microarray assay

Total RNAs from K562/A02 and K562 cell lines were isolated with Trizol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. The concentration of total RNA was quantified by measuring the absorbance at 260 nm. MiRNA fraction was further purified using a mirVana^{TM} miRNA isolation kit (Ambion, Austin, USA). The isolated miRNAs from K562/A02 and K562 cells were then labeled with Hy3 using the miRCURY^{TM} array labeling kit (Exiqon, Vedbaek, Denmark) and hybridized, respectively, on a miRCURY^{TM} LNA miRNA array (V 8.1, Exiqon) as described [32]. Microarray images were acquired using a Genepix 4000B scanner (Axon Instruments, Union City, USA), processed, and analyzed with Genepix Pro 6.0 software (Axon Instruments). Three RNA samples of K562 and K562/A02 cells were analyzed individually. Intensity values were transformed into log 2 scale, and fold changes were given in log 2 scale. A *t*-test was performed between K562 and K562/A02 cells, and statistical significance was considered at $P < 0.05$.

Real-time quantification of miRNAs by stem-loop reverse transcription-polymerase chain reaction

Total RNA was extracted from the K562 or K562/A02 cells using Trizol (Invitrogen), and the concentration of total RNA was quantitated by measuring the absorbance at 260 nm. The expression of mature miRNAs was assayed using stem-loop reverse transcription (RT) followed by real-time polymerase chain reaction (PCR) analysis as previously described [33]. All reagents for stem-loop RT were obtained from Applied Biosystems (Foster City, USA). The relative amount of each miRNA was normalized to U6 snRNA. The fold change for each miRNA from K562/A02 cells relative to the control (K562 cells) was calculated using the $2^{-\Delta\Delta CT}$ method [34]. PCR was performed in triplicate. The primers used for stem-loop RT-PCR for miR-181a are listed in **Table 1**.

miRNA transfection assay

The miR-181a mimic, miR-181a inhibitor, and negative control miRNA mimic were chemically synthesized by Shanghai GenePharma Company (Shanghai, China). The sequence of the miR-181a mimic, miR-181a inhibitor, and negative control miRNA mimic were shown in **Table 2**. K562 cells and K562/A02 cells were plated in six-well

Table 1 The primers used for stem-loop RT-PCR for miR-181a

Primer	Sequence
U6 RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACGATT
U6 forward	CCTGCGCAAGGATGAC
U6 reverse	GTGCAGGGTCCGAGGT
miR-181a RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACTCAC
miR-181a forward	GAACATTCAACGCTGTCG
miR-181a reverse	GTGCAGGGTCCGAGGT

Table 2 The sequence of the control mimic and the miR-181a mimic, inhibitor

miRNA mimics		Sequence
hsa-miR negative control mimic	5′ to 3′	UUCUCCGAACGUGUCACGUTT
hsa-miR-181a mimic	5′ to 3′	AACAUUCAACGCUGUCGGUGAGU
hsa-miR-181a inhibitor	5′ to 3′	UCACCGACAGCGUUGAAUGUUUU

plates (6×10^5 cells/well) and transfected with 100 nM of the miR-181a mimic, miR-181a inhibitor, or negative control miRNA mimic using Lipofectamine 2000 (Invitrogen, Long Island, USA) according to the manufacturer's protocol.

Cell viability assay

The colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, St Louis, USA) assay was performed to quantify the effect of miR-181a on K562 cells growth and viability. The transfected cells and untransfected cells were seeded into 96-well plates in RPMI-1640 medium containing 10% fetal bovine serum. After 24 h, the cells were treated with serial dilutions of DNR. Following 68 h of treatment, 20 μl of 5 mg/ml MTT in phosphate-buffered saline (PBS) was added to each well for an additional 4 h of incubation. The blue formazan precipitate was dissolved in 150 μl of dimethylsulfoxide and agitated for 15 min. Absorbance in each well was read at 490 nm by an automated microplate reader (Bio-Rad, Hercules, USA).

Apoptosis assay

The surface exposure of phosphatidylserine in apoptotic cells was quantitatively detected using Annexin V/FITC and PI apoptosis detection kit (Becton Dickinson, Franklin Lakes, USA). Twenty-four hours after the transfection as described above, K562/A02 cells were treated by DNR, with final concentration of 0.1 μM. Forty-eight hours after the treatment of DNR, flow cytometry was used to detect apoptosis of the K562/A02 cells by determining the relative amount of AnnexinV-FITC-positive, PI-negative cells as previously described [35].

Quantitation of *BCL-2* mRNA levels

Real-time PCR was used to quantify mRNA expression levels of *BCL-2* as previously described [36].

Western blot assay

Immunoblots were performed as described previously with some modifications [37]. The cells were washed twice with PBS, which contain 1 mM phenyl-methylsulfonyl fluorid, and lysed with 25 μl of complete lysis M (Roche, Basel, Switzerland) as described in the product manual. Twenty-five micrograms of protein lysate was separated on 8%–12% gel and subsequently transferred to a polyvinylidene difluoride membrane (Bio-Rad) as described. The membranes were blocked with 5% milk and incubated with primary antibody overnight at 4°C. The primary antibodies for BCL-2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Abcam Ltd (Cambridge, USA). The secondary antibodies were purchased from Beyotime Ltd (Haimen, China). Band detection via enzyme-linked chemiluminescence was performed according to the manufacturer's protocol (ECL; Pierce Biotechnology Inc., Rockford, USA). Protein levels were normalized to GAPDH. Fold changes were determined.

Dual luciferase activity assay

A 566 bp segment from the 3′ UTR of the *BCL-2* gene was amplified by PCR from human genomic DNA and then cloned into the *Xho*I and *Not*I sites in the psi-CHECK2 vector (Promega, Madison, USA). The following primer sets were used to generate specific fragments: BCL-3-UTR forward, 5′-GCCACAAGTGAAGTCAACA-3′, BCL-3-UTR reverse, 5′-ACAGGCACAGAACATCCAG-3′. We also generated a mutant 3′ UTR of the *BCL-2* genes with 3 bp substitutions from the site of perfect complementarity by using the QuikChange XL site-directed mutagenesis kit (Stratagene, La Jolla, USA). The sequence of mutant *BCL-2* 3′ UTR segments contained 5′-aaaccctgtg***GCC*** ctatcctgccaa-3′ (the three italic bold nucleotides are mutated). K562/A02 cells were plated at 0.5×10^5 cells per well in 24-well plates. The following day cells were co-transfected with 800 ng Luciferase vector, including the 3′ UTR of *BCL-2*, and miR-181a mimic or mimic control at a final concentration of 50 nM by using Lipofectamine 2000 (Invitrogen). Luciferase assays were performed by using the dual luciferase reporter assay system (Promega) 48 h after transfection.

siRNA transfection assay

SignalSilence® Bcl-2 siRNA kit was purchased from Cell Signaling Technology, Inc. (Beverly, USA), and the transfection was performed according to the manufacturer's protocol. The cells were prepared for next experiments 48 h after transfection. The transfection efficiency was evaluated by FCM by calculating the percentage of

fluorescein-labeled cells. The transfection efficiency was ~75%.

Statistical analysis

All the experiments were repeated in triplicate. The results were calculated using SPSS version 12.0 software (SPSS, Chicago, USA), presented as mean ± standard deviation, and compared using analysis of variance. Statistical significance was defined as $P < 0.05$.

Results

miR-181a is down-regulated in the DNR-resistant K562/A02 cell line

Our miRNA profiling studies indicated that 4 miRNAs were up-regulated (miR-21, miR-221, miR-155, and miR-99a) and 10 miRNAs were down-regulated (miR-98, miR-181a, let-7f, mir-29c, miR-30b, miR-30c, miR-183, miR-221, miR-222, and miR-224) in the K562/A02 cells compared to the parental K562 cells. We selected miR-181a to further validate the array results because previous studies have shown that miR-181a is related to drug resistance [38,39]. Furthermore, we confirmed the difference of miR-181a expression between K562/A02 cells and K562 cells by real-time PCR analyses. The results showed that the miR-181a expression level was lower in the K562/A02 cells than in the K562 cells (**Fig. 1** , $P < 0.05$), indicating that miR-181a may be associated with DNR resistance in K562 cells.

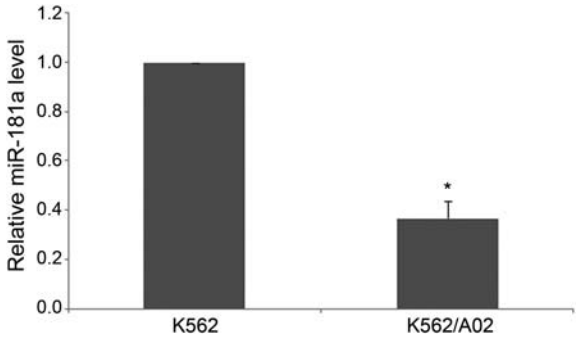

Figure 1 The expression of miR-181a Real-time quantification of miR-181a by stem-loop RT-PCR showed that miR-181a was down-regulated in K562/A02 cell lines, compared with K562 cell lines, respectively. The relative amount of miR-181a was normalized to U6 snRNA. Data were shown as fold changes of miR-181a levels in K562/A02 cell lines relative to K562 cell lines, respectively. *$P < 0.05$.

Knockdown of miR-181a renders the K562 cells resistant to DNR

To directly test the relationship between miR-181a and chemoresistance in the K562 cells, we knock down the miR-181a in K562 cell line. The K562 cells were transfected with miR-181a inhibitor or negative control mimic, treated with various doses of DNR. The expression of miR-181a was shown in **Fig. 2(A)**. The cell viability results showed that the inhibition of miR-181a was significantly associated with the increased survival of K562 cells [**Fig. 2(B)**]. When treated with DNR at the concentration of 0.01 μM or higher, the K562 cells transfected with miR-181a inhibitor showed a significantly higher survival than K562 cells ($P < 0.05$). This result suggests that down-regulation of miR-181a contributes to DNR resistance in the K562 cells.

Over-expression of miR-181a partially sensitizes the K562/A02 cells to DNR

We further investigated the effects of miR-181a on DNR-induced cytotoxicity in K562/A02 cells. The cells

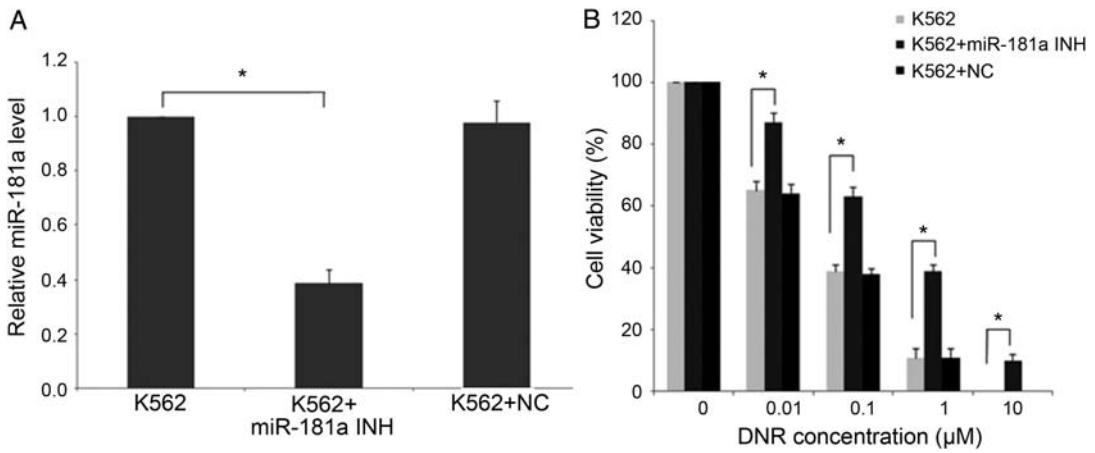

Figure 2 miR-181a confers DNR resistance in K562 cells (A) The K562 cells were transfected with miR-181a inhibitor (miR-181a INH) or negative control (NC). The miR-181a expression was measured by stem-loop RT-PCR. (B) The untransfected K562 cells and transfected K562 cells were subsequently treated with various doses of DNR. Cell viability was determined using an MTT assay. The error bar shows the standard deviation for three independent experiments. *$P < 0.05$.

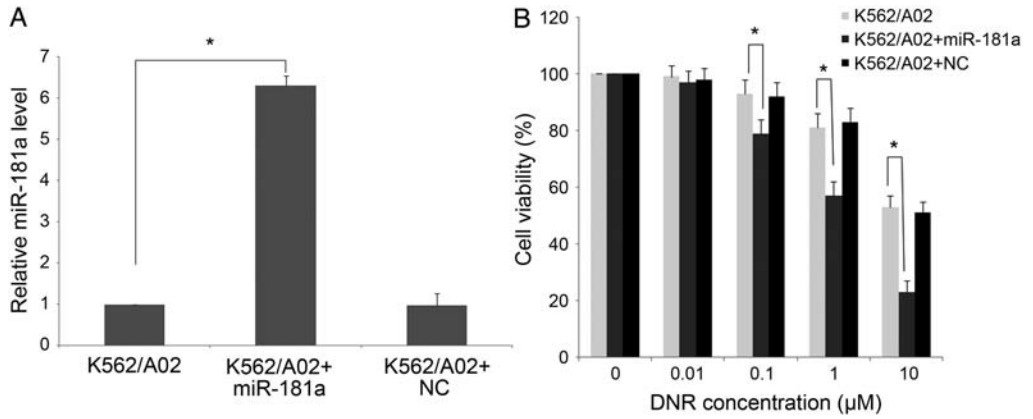

Figure 3 Over-expression of miR-181a partially sensitizes the K562/A02 cells to DNR (A) K562/A02 cells were transfected with miR-181a mimic (K562/A02 + miR-181a) or negative control (K562/A02 + NC). miR-181a expression was quantified by stem-loop RT-PCR. (B) The untransfected K562/A02 cells and transfected K562/A02 cells were subsequently treated with various doses of DNR. Cell viability was determined using MTT assay. The error bar shows the standard deviation for three independent experiments. $*P < 0.05$.

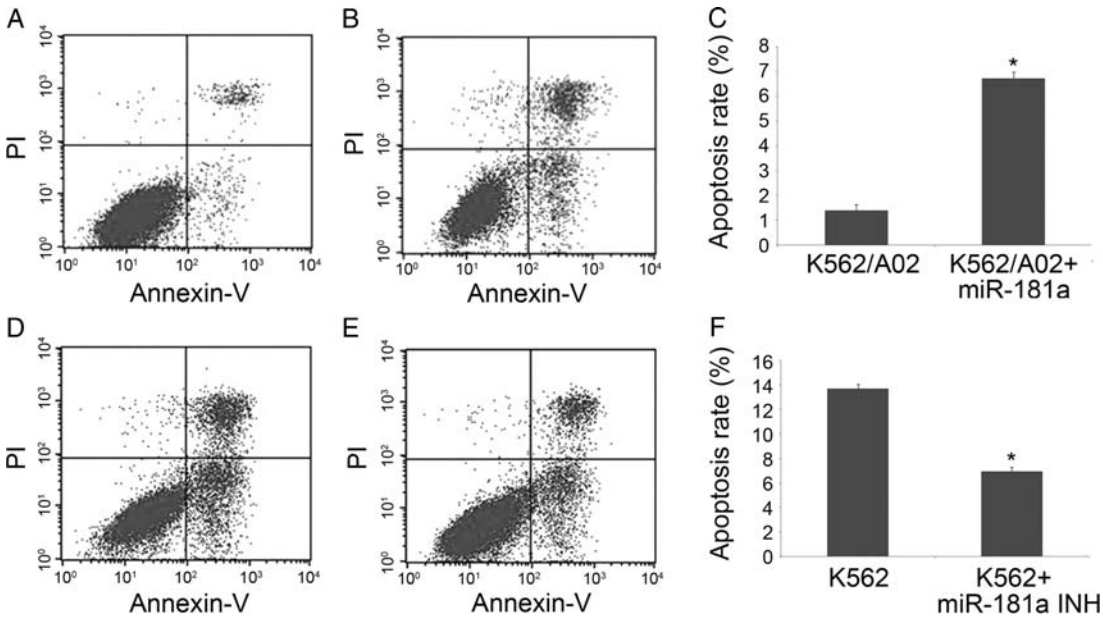

Figure 4 Association between miR-181a- and DNR-induced apoptosis (A–C) Apoptotic cells was quantitatively detected using Annexin V/FITC and PI apoptosis detection kit. K562/A02 cells transfected with miR-181a mimic (K562/A02 + miR-181a) showed a marked increase in apoptosis when treated with 0.1 μM DNR, compared with the K562/A02 cells. (D–F) K562 cells transfected with miR-181a inhibitor (K562 + miR-181a INH) showed a marked decrease in apoptosis after 0.1 μM DNR treatment, compared to the K562 cells. The results represent the mean ± standard deviation from three independent experiments. $*P < 0.05$.

were transfected with either the miR-181a mimic or a negative control, incubated with various doses of DNR. As shown in **Fig. 3(A)**, the miR-181a mimic effectively increased the expression of miR-181a ($P < 0.05$). When treated with DNR at the concentration of >0.1 μM, K562/A02 cells transfected with the miR-181a mimic had a significantly lower survival than K562/A02 cells [**Fig. 3(B)**, $P < 0.05$], which suggests that increasing miR-181a expression enhances DNR sensitivity in K562/A02 cells.

Association between miR-181a and DNR-induced apoptosis

The regulation of miR-181a on the cytotoxicity of DNR toward K562 and K562/A02 cells was also evaluated by quantification of apoptotic cells. As shown in **Fig. 4**, 0.1 μM DNR treatment resulted in 1.5% of apoptosis in K562/A02 cells, and the percentage of apoptosis was 6.7% when transfected with miR-181a mimic [**Fig. 4(A–C)**]. After treatment of 0.1 μM DNR, the apoptosis rate is

13.2% in K562 cells and 6.3% in K562 cells transfected with miR-181a inhibitor [**Fig. 4(D–F)**]. The result suggested that miR-181a was associated with DNR-induced apoptosis in K562 cells.

miR-181a modulates BCL-2 protein expression

TargetScan 5.1 (http://www.targetscan.org) was used for prediction of miR-181a target genes. The sequence alignment of human miR-181a indicates that BCL-2 is one of the potential targets of miR-181a. Since BCL-2 is a pro-survival protein, we hypothesized that miR-181a might sensitize K562/A02 cells to DNR by repressing the BCL-2 protein expression. At first, we measured the difference of BCL-2 protein levels between K562 cells and K562/A02 cells. Western blot results showed that the BCL-2 protein level was higher in K562/A02 cells than in K562 cells [**Fig. 5(A,B)**]. Furthermore, in miR-181a mimic-treated K562/A02 cells, BCL-2 was significantly decreased after 72 h transfection [**Fig. 5(C,D)**]. Quantitative RT-PCR showed that there were no significant differences at *BCL-2* mRNA levels between these cell lines [**Fig. 6(A,B)**]. The results suggested that miR-181a could modulate BCL-2 protein expression in K562/A02 cells.

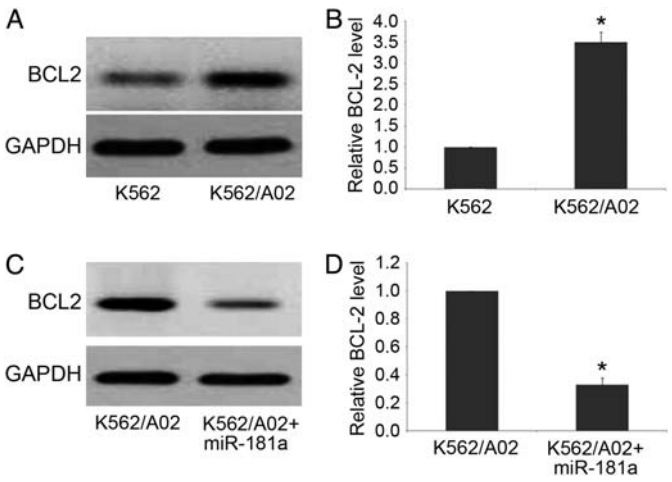

Figure 5 miR-181a modulates BCL-2 protein expression (A, B) The expression of BCL-2 in K562 cells and K562/A02 cells. (C, D) The BCL-2 protein level in K562/A02 cells transfected with miR-181a mimic (K562/A02 + miR-181a). The results represent the mean ± standard deviation from three independent experiments. $*P < 0.05$.

BCL-2 is a direct target gene of miR-181a

To explore whether *BCL-2* is a direct target gene of miR-181a, we constructed a luciferase reporter vector with the putative *BCL-2* 3′ UTR target site for the miR-181a downstream of the luciferase gene (*BCL-2*-3′ UTR). The sequence alignment between miR-181a and the targeted *BCL*-2 3′ UTR is shown in **Fig. 7(A)**. Luciferase reporter vector together with the miR-181a mimic or the control miRNA mimic were transfected into K562/A02 cells. In K562/A02 cells, a significant decrease of the relative luciferase activity was noted when *BCL*-2-3′ UTR was cotransfected with the mature miR-181a mimic compared with the control miRNA mimic, respectively. The similar results were not observed in K562/A02 cells transfected with mutant *BCL*-2-3′ UTR vector [**Fig. 7(B)**]. The results suggest that there is a target site of miR-181a in the *BCL*-2 3′ UTR.

BCL-2 plays a key role in K562 DNR resistance

To explore the relationship between BCL-2 and DNR sensitivity, we transfected BCL-2 siRNA or a scrambled siRNA into K562/A02 cells, followed by treatment with various concentrations of DNR. BCL-2 siRNA effectively reduced the BCL-2 protein level [**Fig. 8(A,B)**]. Furthermore, K562/A02 cells that were pre-treated with BCL-2 siRNA had decreased survival when compared with K562/A02 cells [**Fig. 8(C)**]. More importantly, the K562/A02 cells that were treated with BCL-2 siRNA had increased a survival pattern that is similar to cells with miR-181a over-expression, suggesting that miR-181a confers DNR resistance via regulating BCL-2 in the K562/A02 cells.

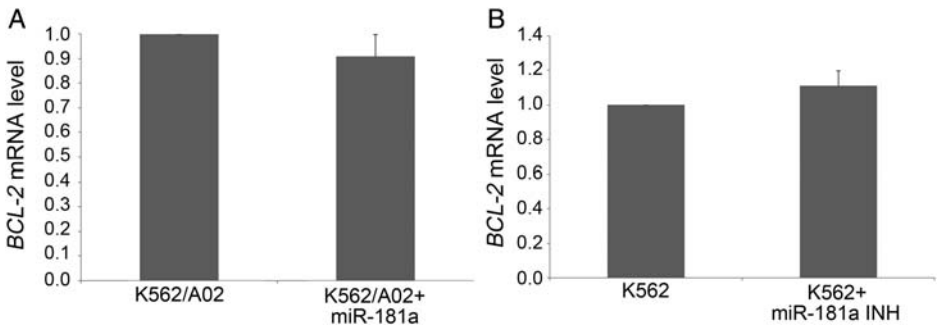

Figure 6 Quantitation of *BCL-2* mRNA levels Real-time PCR was used to quantify mRNA expression of *BCL-2*. (A) *BCL-2* mRNA level in K562/A02 cells and miR-181a mimic (K562/A02 + miR-181a) transfected K562/A02 cells. (B) *BCL-2* mRNA level in K562 cells and miR-181a inhibitor (K562 + miR-181a INH) transfected K562 cells.

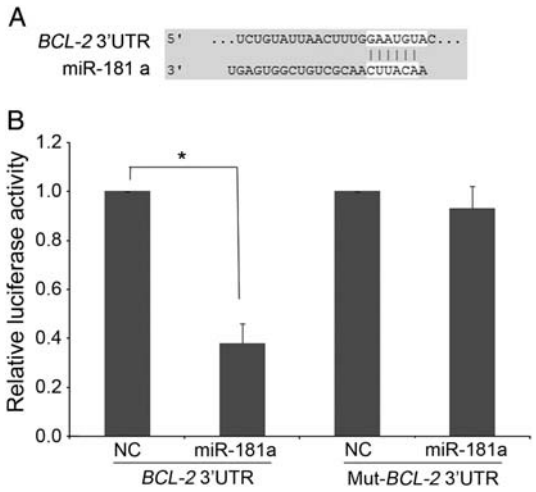

Figure 7 BCL-2 is a direct target gene of miR-181a (A) The sequence alignment between miR-181a and the targeted *BCL-2* 3′ UTR. (B) The luciferase reporter assay results demonstrated significantly decreased *BCL-2*-3′ UTR relative luciferase activity in miR-181a mimic transfected K562/A02 cells compared with negative control transfected K562/A02 cells. $*P < 0.05$.

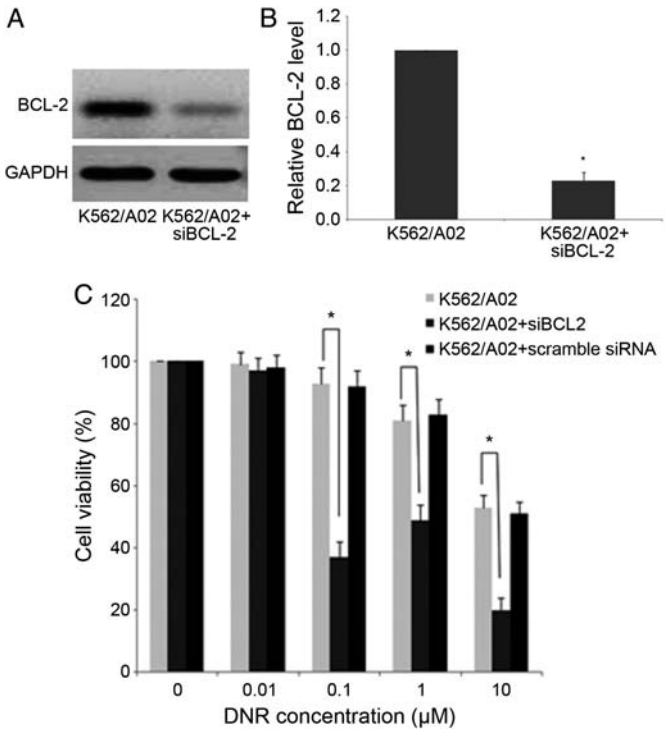

Figure 8 BCL-2 plays a key role in K562 DNR resistance (A, B) Western blots of BCL-2 protein levels transfected with Bcl-2 siRNA in K562/A02 cells. (C) K562/A02 cells were transfected with BCL-2 siRNA or scrambled siRNA, and were subsequently treated with various doses of DNR. Cell viability was determined using an MTT assay. The error bars indicate the standard deviation.$*P < 0.05$.

Discussion

The defective apoptosis pathway was thought as one major mechanism of drug resistance in cancer cells [40,41]. Recently, according to the increased number of findings miRNAs could modulate drug sensibility of cancer cells, at least in part [20–24]. In this study, we found that miR-181a was involved in the resistance of leukemia cell line K562 to DNR. The over-expression of miR-181a in DNR-resistant K562/A02 cells could enhance cytotoxicity of DNR through decreasing BCL-2 expression.

miRNAs regulate protein expression through degrading or repressing the translation of target mRNAs. Evidence from recent studies shows that miRNAs are associated with chemoresistance of cancer cells. However, little is known about how they affect the sensibility of a tumor to the cytotoxic agent. To determine whether miRNAs were involved in the response to chemotherapy in leukemia, miRNA microarrays were used to compare the relative levels of cellular miRNAs between DNR-sensitive K562 cells and DNR-resistant K562/A02 cells. The results showed 4 miRNAs were up-regulated and 10 were down-regulated in K562/A02 cells compared with K562 cells. We were interested in miR-181a because it was involved in the apoptosis. QRT-PCR was used to confirm the expression changes of miR-181a. The down-regulation of miR-181a was confirmed as a >2-fold change in K562/A02 cells.

Function research of miR-181s was first focused on hematopoietic lineage differentiation in mouse. Recent studies by Shi *et al*. [42] and Fanini and Faabbri [43] showed that miR-181a and miR-181b may serve as tumor suppressors in human acute monocytic leukemia (AML) and human glioma cells, respectively. Increasing miR-181a expression induced apoptosis of AML blasts and over-expression of miR-181a and miR-181b also induced apoptosis of human glioma cells. The alignment with TargetScan 5.1 indicates that BCL-2 is one of the potential targets of miR-181a. Previous studies have found that anti-apoptotic BCL-2 contributes to the survival and chemoresistance of quiescent leukemia $CD34^+$ cells [44]. DNR-induced apoptosis can be blocked by BCL-2 over-expression in DNR-sensitive $CD34^-$ U937 cells [45]. Conversely, suppressing BCL-2 expression with siRNA could enhance DNR-induced apoptosis in DNR-resistant $CD34^+$ KG1a and Kasumi-1 cells [45]. These results suggest that high levels of BCL-2 expression could contribute to DNR resistance.

In this study, we first verified the difference of miR-181a expression by miRNA microarray and real-time PCR analyses in K562/A02 cells and K562 cells. The results showed that the miR-181a expression level was lower in the K562/A02 cells than in the K562 cells. The sequence alignment of human miR-181a indicates that Bcl-2 is one of the potential targets of miR-181a. Next, we found knockdown of miR-181a rendered the K562 cells resistant to DNR and over-expression of miR-181a

partially sensitized the K562/A02 cells to DNR. So, we hypothesized that miR-181a might sensitize K562/A02 cells to DNR by repressing the BCL-2 protein expression. Then we transfected miR-181a mimic into K562/A02 cells, and we found that the percentage of apoptosis was increased, while the BCL-2 protein level was decreased, which indicated BCL-2 was involved in K562 DNR resistance.

After 0.1 μM DNR treatment, K562/A02 cells transfected with BCL-2 siRNA had a significantly low survival [**Fig. 7(C)**] than the cells with miR-181a over-expression [**Fig. 3(B)**]. These results suggest that the other regulation molecular may exist for modulating the expression of BCL-2. The previous study has shown that the down-regulation of BCL-2, due to miR-15a and miR-16-1 activity, could trigger apoptosis [46]. BCL-2 has been found to be one of miR-195 targets suggesting that miR-195 probably exerts its role by targeting BCL-2 [47]. Another miRNA, miR-143, has also been described to directly target BCL-2 [48]. In this study, we found low miR-181a levels to be one of the causes of the high expression of BCL-2 in K562/A02 cells. The regulatory mechanism of BCL-2 expression should be further researched.

In summary, our results showed that leukemia cells with decreased miR-181a expression and elevated BCL-2 protein expression were more resistant to DNR than the control cells. This study provides a novel mechanism for understanding leukemia drug resistance.

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