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miRNA143 Induces K562 Cell Apoptosis Through Downregulating BCR-ABL

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Data Collection B
Statistical Analysis C
Data Interpretation D
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Background: Leukemia seriously threats human health and life. MicroRNA regulates cell growth, proliferation, apoptosis, and cell cycle. Whether microRNA could be treated as a target for leukemia is still unclear and the mechanism by which microRNA143 regulates K562 cells needs further investigation.

Material/Methods: miRNA143 and its scramble miRNA were synthesized and transfected to K562 cells. MTT assay was used to detect K562 cell proliferation. Flow cytometry and a caspase-3 activity detection kit were used to test K562 cell apoptosis. Western blot analysis was performed to determine breakpoint cluster region-Abelson (BCR-ABL) expression. BCR-ABL overexpression and siRNA were used to change BCR-ABL level, and cell apoptosis was detected again after lipofection transfection.

Results: miRNA143 transfection inhibited K562 cell growth and induced its apoptosis. miRNA143 transfection decreased BCR-ABL expression. BCR-ABL overexpression suppressed miRNA143-induced K562 cell apoptosis, while its reduction enhanced miRNA143-induced apoptosis.

Conclusions: miRNA143 induced K562 cell apoptosis through downregulating BCR-ABL. miRNA143 might be a target for a new leukemia therapy.

MeSH Keywords: Amlodipine • Apoptosis Inducing Factor • Genes, abl

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Background

Leukemia is a type of malignant tumor in the blood; it is the fifth greatest cause of death among all cancers. Leukemia seriously threatens human life and health [1]. Therefore, investigating the molecular mechanism of leukemia has both theoretical significance and practical value for leukemia detection, prevention, treatment, and prognosis.

Recent studies suggested that the etiology of leukemia is complex and includes genetic factors, chemicals, radiation, and viruses. The current treatment includes chemotherapy, radiotherapy, molecular targeted therapy, stem cell transplantation, and immunotherapy [2]. These methods play important roles in leukemia treatment, but they all have shortcomings and insufficiencies. Due to the complexity of leukemia etiology, there is no unified rule on treatment. Recent studies concluded that molecular targeted therapy has obvious advantages and is effective in all types of leukemia. Target selection is undoubtedly important but is difficult. In this study, we attempted to find potential targets to provide a theory for leukemia treatment.

MicroRNA (miRNA) is an important member of the small RNA family. miRNA can regulate cell growth, proliferation, survival, death, and cell cycle. It also plays a role in signaling pathways [3]. miRNA143 may have a regulating role through targeting transcription factors and cytokines directly or indirectly, but the specific mechanism still needs investigation.

Research proved that miRNA143 level is decreased in multiple types of cancers such as breast cancer, lung cancer, leukemia, and bile duct carcinoma [3–5]. miRNA143 can inhibit cholangiocarcinoma cell growth and promote cell death. In this study, we selected K562 cells to study the effect of miRNA143 on K562 cell growth, proliferation, and apoptosis [6].

Breakpoint cluster region-Abelson (BCR-ABL) is a constitutively activated tyrosine kinase that is produced by the Philadelphia (Ph) chromosome. This protein is present in almost all people with chronic myeloid leukemia (CML), and in 20% of people with acute lymphoblastic leukemia (ALL) [7,8]. Although BCR-ABL participates in cell cycle regulation [7], whether it is involved in the miRNA-induced regulation of K562 cells remains unclear.

In this study, we investigated the effect of miRNA143 on K562 cell growth, proliferation, and apoptosis, as well as its related mechanism. Targeting miRNA143 might be a new method for leukemia treatment.

Material and Methods

Reagents and cell line

The K562 cell line was bought from the American Type Culture Collection (ATCC). The MTT kit (3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide) was purchased from Beijing Dingguo Science and Technology Co., LTD. BCR-ABL plasmid was from the plasmid library in our laboratory. BCR-ABL, β-actin primary antibody, and HRP-tagged IgG secondary antibody were bought from Beijing Dingguo Science and Technology Co., LTD. DMEM and fetal bovine serum were from Beyotime. Caspase-3 activity detection kit and apoptosis detection reagent FITC-Annexin-V (used for detecting phosphatidylserine eversion) were from Beijing Dingguo Science and Technology Co., LTD. MiRNA143 and scramble miRNA were designed and synthesized by Genepharma.

Cell culture

K562 cells were cultured in DMEM containing 10% FBS and maintained in an incubator at 37°C and 5% CO_2 [9].

MTT assay

K562 cell viability was detected by MTT assay [10]. We seeded 1×10^4 cells in 6-well plates and cultured them for 36 h. Ten mg/ml of MTT reaction fluid was added to the cells and cultured for 12 h. Then, the cells were added to 200 µl DMSO for 20 min and detected at 490 nm [11].

Colony formation assay

K562 cell colony formation ability was detected as reported elsewhere [12]. K562 cells in each group were diluted to 500 cells, 250 cells, 100 cells, 50 cells, and 0 cells. Then the cells were seeded in soft agar plates and cultured for 12 days. When the cells in the largest dilution formed clones, 100% methanol solution was added to the soft agar for 15 min. Finally, the cells were stained with Giemsa for 15 min. Four parallel experiments were run for each sample. Five regions (2×4 cm) were randomly selected on each plate to count the number of clones. The maximum number was considered as the standard. The relative clone formation ability was calculated as the ratio of the number of clones for each sample to the maximum number. The experiments were independently repeated 3 times.

Cell transfection

K562 cells in 12-well plates were added to 0.5 ml DMEM for 24 h. BCR-ABL siRNA (sequence: 5-ATTAGGTTGTTACGCTTCC-3 and 5-TGGCCTGATAAGAGTTCTC-3) at 200 nM was mixed with lipo2000 for 15 min. The mixture was added to the cells for

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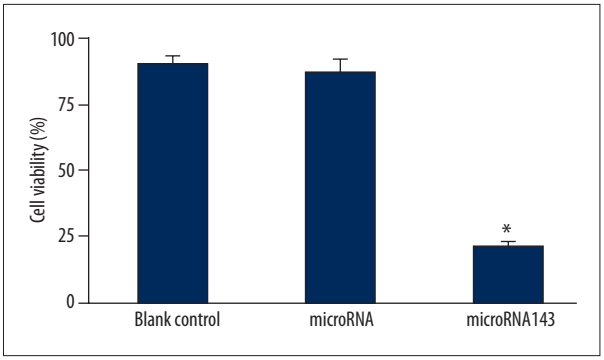

Figure 1. MiRNA143 inhibits K562 cell growth. * P<0.05, compared with normal control.

12 h, and then the cells were collected for Western blot analysis and cell apoptosis detection [13].

Using previously reported methods [14,15], the cells were collected and detected by flow cytometry. The cells were collected and centrifuged at 800 rpm for 10 min. Then, the cells were resuspended in FITC-Annexin V reaction fluid at 50:1:250 for 30 min in the dark. Cell apoptosis was detected by flow cytometry.

Western blot analysis

Western blot analysis was performed according to the conventional method. BCR-ABL and β-catin primary antibodies, together with secondary antibody, were used for protein level detection [16].

Caspase-3 activity assay

Caspase-3 activity was detected according to the manual [17].

Statistical analysis

All statistical analyses were performed using SPSS16.0 software [18]. All data are presented as mean ± standard deviation. One-way ANOVA was used for data comparison. P<0.05 was considered as a significant difference.

Results

MiRNA143 inhibits K562 cell growth

As shown in Figure 1, MTT assay showed that, compared with the control group, 2-μg miRNA143 transfection significantly decreased K562 cell viability (P=0.034). There was no significant difference in the transfection efficiency (P>0.05, Figure 2). Under the same conditions, K562 cells growth showed no significant difference between scramble miRNA transfection group and normal control. Therefore, we omitted the untransfected group in the following studies.

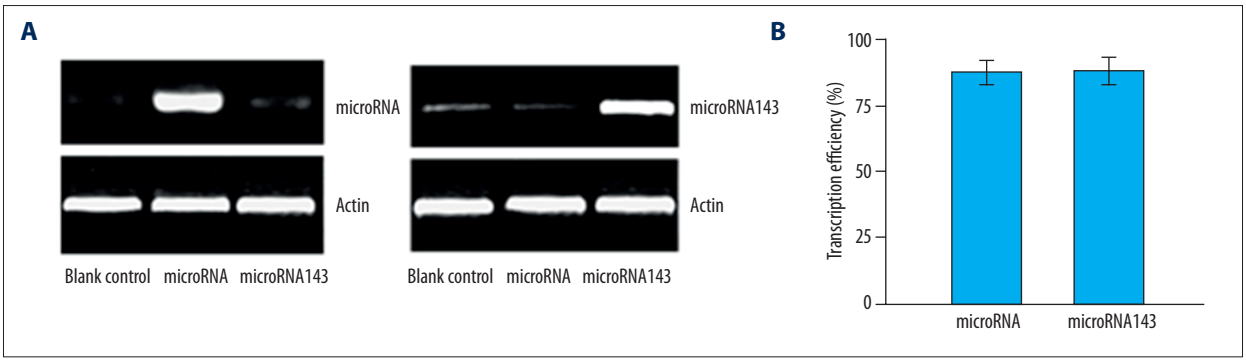

Figure 2. (**A, B**) Transfection efficiency of microRNA and microRNA143.

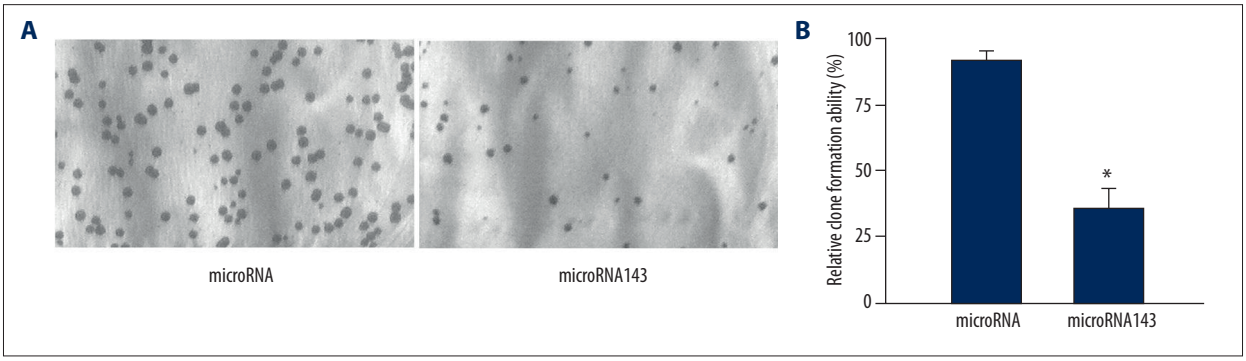

Figure 3. (**A, B**) MiRNA143 suppresses K562 cell proliferation. * P<0.05, compared with normal control.

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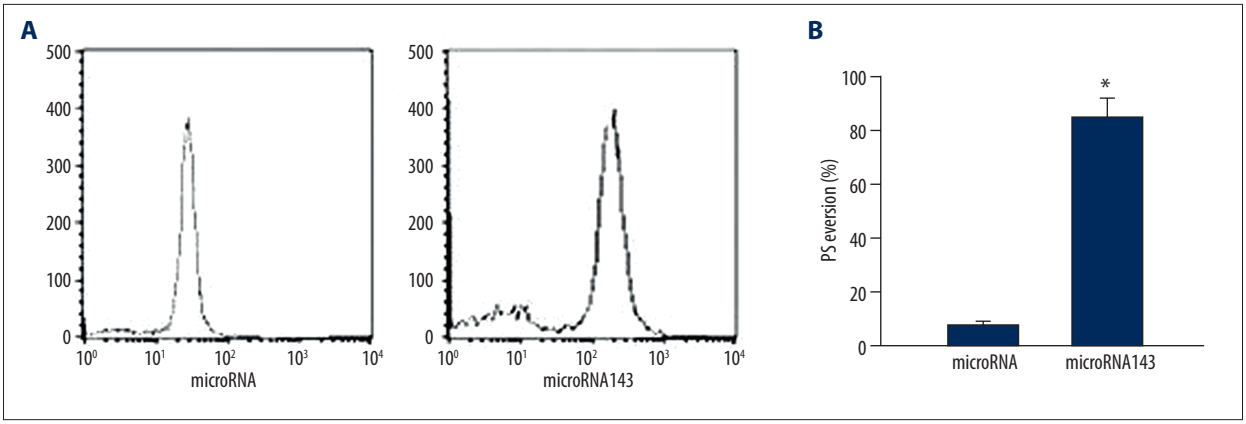

Figure 4. (**A, B**) MiRNA143 induces K562 cell apoptosis. * P<0.05, compared with normal control.

MiRNA143 suppresses K562 cell proliferation

Colony formation assay revealed that 2-μg miRNA143 transfection obviously reduced the clone formation ability of K562 cells compared with scramble miRNA transfection (P=0.031) (Figure 3).

MiRNA143 induces K562 cell apoptosis

Flow cytometry indicated that 2-μg miRNA143 transfection markedly elevated the phosphatidylserine eversion percentage in K562 cells compared with control (P=0.0019) (Figure 4).

MiRNA143 induces caspase-3 activation in K562 cells

Caspase-3 activity detection showed that 2-μg miRNA143 transfection obviously increased caspase-3 activity in K562 cells compared with control (P=0.031) (Figure 5).

MiRNA143 decreases BCR-ABL protein level

Western blot analysis showed that 2-μg miRNA143 transfection significantly decreased BCR-ABL protein level in K562 cells compared with control (Figure 6).

BCR-ABL knockdown promotes miRNA143 induced K562 cell apoptosis

As shown in Figure 7, flow cytometry revealed that after BCR-ABL siRNA treatment, the percentage of 2-μg miRNA143 transfection-induced K562 cells phosphatidylserine eversion increased significantly compared with control (P=0.011).

BCR-ABL overexpression inhibits miRNA143-induced K562 cell apoptosis

As shown in Figure 8, flow cytometry showed that after BCR-ABL overexpression, the percentage of 2-μg miRNA143 transfection-induced K562 cells phosphatidylserine eversion declined markedly compared with control (P=0.012).

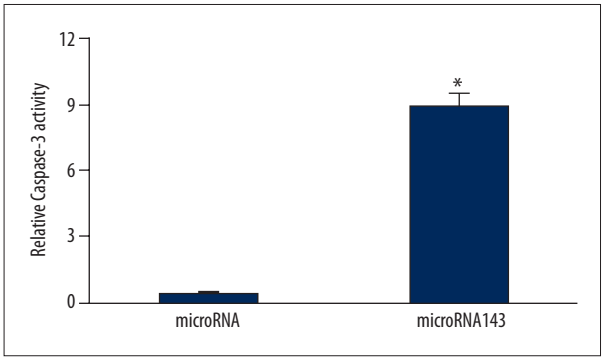

Figure 5. MiRNA143 induces caspase-3 activation in K562 cells. * P<0.05, compared with normal control.

MiRNA 143 reduces BCR-ABL mRNA

As shown in Figure 9, PCR analysis showed that the level of BCR-ABL mRNA was significantly decreased compared with the microRNA group and control (P<0.05), indicating that mir143 might regulate the intracellular level of BCR-ABL through other transcription factors.

Discussion

miRNAs are known to play important roles in the regulation of cell growth [19], proliferation [20], apoptosis [21], survival, and signal transduction [22]. Recent studies have suggested that miRNAs are also involved in cancer development and are thus important for the diagnosis, treatment, and prognosis of the disease [23]. In this study, the regulatory effect of miRNA143 in K562 cells was investigated. Our results showed that miRNA143 transfection inhibited K562 cell growth and induced cell apoptosis, suggesting that miRNA143 might be a new target for leukemia therapy [24].

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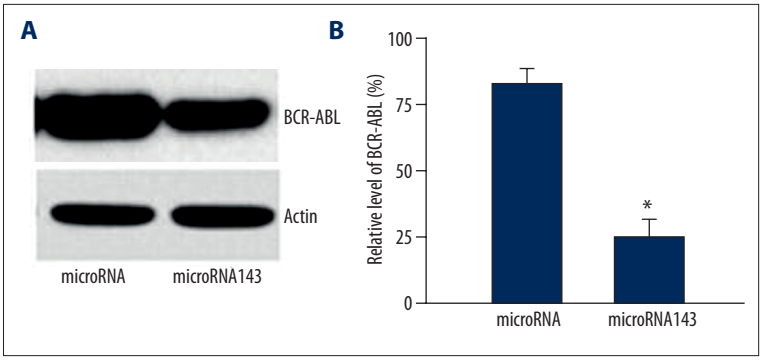

Figure 6. (**A, B**) MiRNA143 decreases BCR-ABL protein level. * P<0.05, compared with normal control.

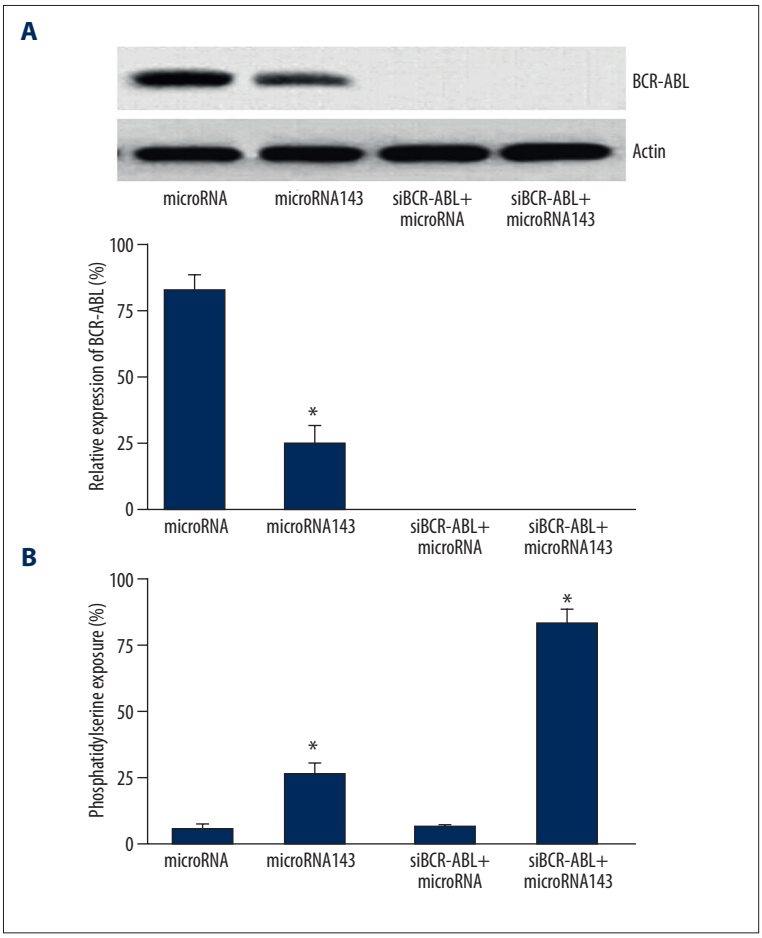

Figure 7. (**A, B**) BCR-ABL knockdown promotes miRNA143-induced K562 cell apoptosis. * P<0.05, compared with normal control.

The BCR-ABL gene is located on the long arm of chromosome 9 in humans, with 12 exons [7]. Previous studies have shown that BCR-ABL is related to leukemia by participating in the cell cycle [7,8]. Our study further explored the molecular mechanism of miRNA143-induced apoptosis of K562 cells. Western blot results showed that BCR-ABL protein expression was decreased markedly in K562 cells transfected with miRNA143. Moreover, BCR-ABL siRNA induced more obvious apoptosis in miRNA143-transfected cells, whereas BCR-ABL overexpression significantly inhibited cell apoptosis, indicating that miRNA143 induced the apoptosis of K562 cells through downregulating BCR-ABL expression. Therefore, BCR-ABL-targeted intervention might be a new approach for the treatment of leukemia [25].

This study has 3 limitations. The first is the lack of leukemia specimens from patients at various clinical stages. Western blot analysis could be used to detect BCR-ABL protein expression level in leukemia and normal tissues [26] to test the results in this research [27]. The second limitation is the lack of leukemia specimens from patients receiving various chemotherapy drugs. Western blot detection of BCR-ABL protein in leukemia and normal tissues can further clarify their relationship [28].

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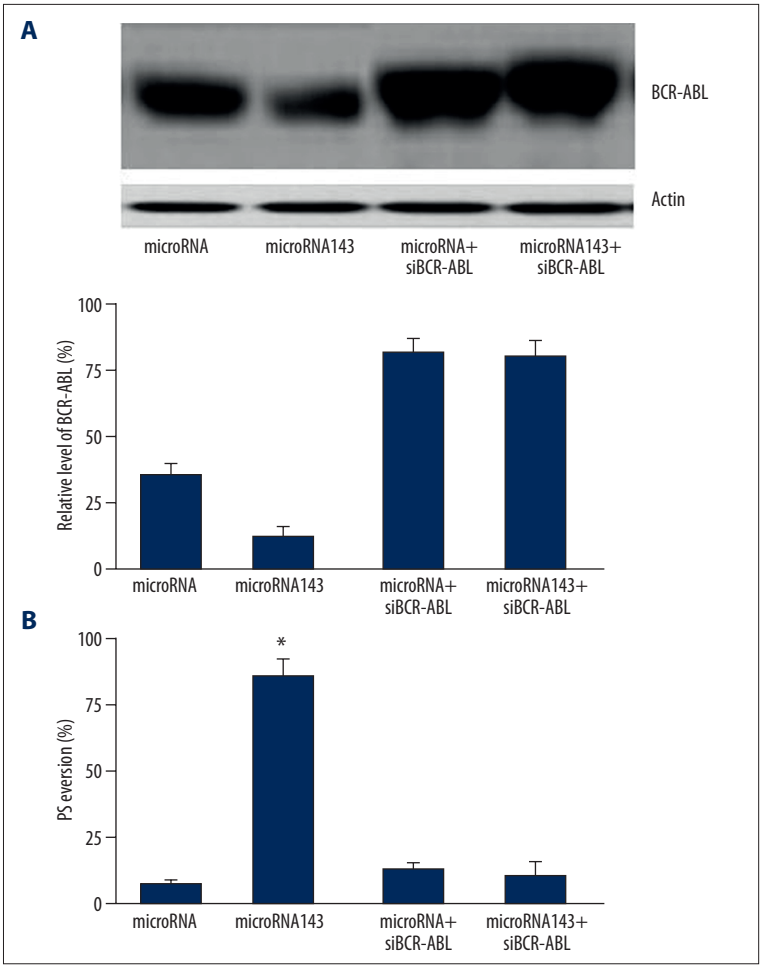

Figure 8. (**A, B**) BCR-ABL overexpression inhibits miRNA143-induced K562 cell apoptosis. * P<0.05, compared with normal control.

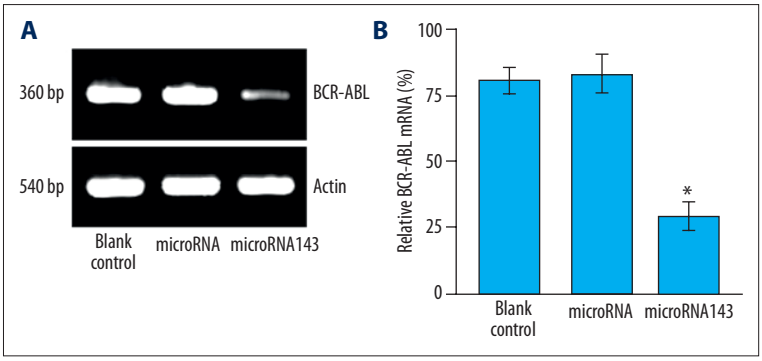

Figure 9. (**A, B**) microRNA143 transfection reduces BCR-ABL mRNA level. * P<0.05, compared with normal control.

The third limitation is the lack of a rat animal model using miRNA143 treatment to investigate the therapeutic effect of miRNA143 in leukemia.

Conclusions

In this preliminary study, we identified multiple miRNAs that induce the apoptosis of K562 cells. Because mir143 had the highest effect, we further investigated the relevant mechanism, finding that mir143 regulated the expression of BCR-ABL through other transcription factors. In future research, we plan to study which transcription factors mediate the regulatory effect of mir143. In addition, the expression correlation between gene expression and mir143 needs to be investigated through a large-scale dataset, such as TCGA LAML data.

In summary, our results suggest that miRNA143 induces K562 cell apoptosis through downregulating BCR-ABL, indicating that miRNA143 might be a new target for leukemia therapy.

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