

# Overexpression of microRNA-143 inhibits growth and induces apoptosis in human leukemia cells

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**Abstract.** microRNA-143 (miR-143) has been suggested to be a tumor suppressor, yet its role in hematological tumors has not been determined. Thus, we aimed to explore the expression and function of miR-143 in leukemia cells. miR-143 expression was assessed in bone marrow samples from 63 leukemia patients and 15 healthy controls using q-PCR, and its correlation with DNMT3A expression was determined. In addition, after lentiviral-mediated miR-143 overexpression, K562 cell proliferation was evaluated using CCK-8 analysis; cell cycle progression and apoptosis were determined using flow cytometry. The expression of Bcl-2 and pro-caspase-3 and -9 was assessed by q-PCR and western blot analysis, respectively. Leukemia patients had significantly lower relative miR-143 expression than healthy controls ( $P=0.004$ ), and the expression levels of miR143 and DNMT3A were negatively correlated ( $r=-0.663$ ,  $P=0.001$ ). Overexpression of miR-143 decreased DNMT3A mRNA and protein expression, and significantly reduced K562 cell proliferation at 72 and 96 h (both  $P\leq 0.018$ ). In addition, reduced colony formation and cell cycle progression were observed upon miR-143 overexpression. Flow cytometric analysis revealed that the early apoptosis rate was higher in the miR-143 group than the rate in the NC group. Bcl-2 mRNA expression and pro-caspase-3 and -9 protein expression were reduced in the miR-143-expressing

cells. These findings suggest that miR-143 plays an important role in leukemia cell proliferation and apoptosis, possibly through silencing of DNMT3A. Further studies are necessary to determine the prognostic value and therapeutic potential of targeting miR-143.

## Introduction

Leukemia encompasses a group of diseases with varying presentations, prognoses and treatments. For example, acute lymphoblastic leukemia (ALL) is the most common type of leukemia in young children; treatment with chemotherapy and radiotherapy results in an 85% survival rate in this population (1). ALL also affects older adults  $\geq 65$  years of age with 30-70% achieving remission (2). The incidence of acute myelogenous leukemia (AML) is greatest in male adults and less common in children. Chemotherapy results in an overall 5-year survival rate of 40% (3). The incidence of chronic lymphocytic leukemia (CLL) is also highest in adult males  $>55$  years of age. Although incurable, the 5-year survival rate is  $\sim 79\%$  (4). Furthermore, chronic myelogenous leukemia (CML) occurs mainly in adults, and the 5-year survival rate is  $\sim 93\%$  (5).

In 2001, estimates indicate that almost 256,000 individuals worldwide developed leukemia and  $\sim 209,000$  succumbed to the disease (6). Moreover, the incidence of leukemia in developed nations is almost double that observed in less developed areas (7). Despite improvements in patient survival, the exact causes of leukemia remain unknown (8). For example, exposure to ionizing radiation, pesticides and formaldehyde has been linked to the development of leukemia (8). In addition, aberrant microRNA (miRNA) expression has also been implicated in the pathogenesis of leukemia (9).

miRNAs are non-coding single-stranded RNA molecules of 19-25 nucleotides in length that silence target gene expression through binding of the 3'UTR, resulting in degradation of mRNA or translation inhibition. In addition to regulating target gene expression, some miRNAs may serve as oncogenes or tumor-suppressor genes and may be important for genetic diagnosis, prognostic determination, and targeted therapy of hematological tumors (10,11). For example, miR-15a/miR-16-1 (11), miR-29 (12), miR-203 (13), and miR-181a (14) may exert antitumor effects in hematological tumors, whereas miR-155 (15), miR-9 and let-7a (16) may

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**Abbreviations:** AO/EB, acridine orange/ethidium bromide; AML, acute myeloid leukemia; ANOVA analysis of variance; DNMT3A, DNA methyltransferase 3A; FBS, fetal bovine serum; miRNA, microRNA; MOI, multiplicity of infection; scr control, scrambled negative control; SD, standard deviation

**Key words:** apoptosis, hsa-miR-143, DNA methyltransferase, DNMT3A, leukemia

Table I. Primer sequences used for the PCR reactions.

Gene	Primer sequence (5'-3')	Amplicon size (bp)
<i>miR-143</i>	F: TGT AGT TTT CGG AGT TAG TGT CGC GC R: CCT ACG ATC GAA AAC GAC GCG AAC G	56
<i>U6</i>	F: GTT TTG TAG TTT TTG GAG TTA GTG TTG TGT R: CTC AAC CTA CAA TCA AAA ACA ACA CAA ACA	96
<i>DNMT3A</i>	F: TAT TGA TGA GCG CAC AAG AGA GC R: GGG TGT TCC AGG GTA ACA TTG AG	111
<i>DNMT3B</i>	F: GAC TTG GTG ATT GGC GGA A R: GGC CCT GTG AGC AGC AGA	270
<i>DNMT1</i>	F: CCG AGT TGG TGA TGG TGT GTA C R: AGG TTG ATG TCT GCG TGG TAG C	324
<i>Bcl-2</i>	F: AGA GGT CAC GGG GGC TAA T R: CCA GGT AAC AAA ACC CCA CA	60

confer carcinogenic effects in lymphoma possibly through inhibition of apoptosis. Furthermore, in acute myeloid leukemia, miR-125b-2 is highly expressed (17). In addition, miRNAs may increase the sensitivity of tumor cells to chemotherapeutics (18); they may also regulate the tumor microenvironment, promoting the infiltration and metastasis of tumor cells (19).

Notably, miR-143 may also possess antitumor activity (10), and its expression is reduced in malignant tumors, such as prostate cancer (18), B-cell chronic lymphocytic leukemia, Burkitt's lymphoma (20), nasopharyngeal cancer (21), esophageal adenocarcinoma (22), gastric cancer (23), lung cancer (24), osteosarcoma (25) and colon cancer (26,27). Reduced miR-143 expression in non-small cell lung cancer was associated with smoking status (24). miR-143 levels were significantly lower in esophageal adenocarcinoma as compared to Barrett's esophagus, suggesting a possible role in disease progression (22).

miR-143 was also found to suppress the proliferation of prostate (18) and gastric (23) cancer cells and enhance their chemosensitivity, possibly through suppression of its target gene, *KRAS* (18). In nasopharyngeal cancer, miR-143 dysregulation was inversely correlated with genes involved in vascular endothelial growth factor (VEGF) signaling as well as cell cycle progression (21). In addition, the antiproliferative effects of miR-143 may be mediated through suppression of its target genes, including ERK5 (20,23) and Akt (23) or induction of apoptosis by targeting Bcl-2 (25). Moreover, miR-143 may regulate epigenetic modification via silencing DNA methyltransferase 3A (DNMT3A) expression (26). However, the mechanism by which miR-143 exerts its effects on hematological tumors is less clear.

Therefore, the present study examined the hypothesis that expression of miR-143 may impact leukemia cell growth through altered DNMT3A expression. miR-143 expression was assessed in bone marrow samples of leukemia patients and healthy controls, and its correlation with DNMT3A expression was determined. The effects of miR-143 on the growth,

colony formation, cell cycle progression and apoptosis were also determined in CML K562 cells (28). miR-143 expression was reduced in leukemia patients and was negatively associated with DNMT3A expression. Lentiviral-mediated miR-143 overexpression inhibited K562 cell proliferation, transition from the G1 to S phase and Bcl-2 expression. These data support the notion that identification of miRNAs and their targets in cancer progression may provide novel therapeutic strategies for the diagnosis and treatment of leukemia.

## Materials and methods

**Patients and cell lines.** Bone marrow cells were collected from 63 patients with leukemia, who were hospitalized in the Affiliated Union Hospital of Fujian Medical University from March 2009 to December 2010. The patients consisted of 37 males and 26 females with a mean age of 36.7±17.7 years (range, 10-77 years). These patients were diagnosed with either AML or ALL, according to the French-American-British (FAB) classification criteria (29). Patients who underwent chemotherapy or radiotherapy prior to the study were excluded. In addition, 15 bone marrow donors without malignant hematological diseases served as normal controls. The present study was approved by the Institutional Review Board of the Affiliated Union Hospital, and informed consent was obtained from all patients and healthy controls.

The AML (HL-60, NB4 and U937), CML (K562), acute erythroleukemia (HEL), T lymphocytic leukemia (Jurkat and CEM), B-cell lymphoma (CA46, Raji cells of Burkitt's lymphoma) and multiple myeloma (U266) cells were provided by the Institute of Hematological Diseases in Fujian Province. All cell lines were maintained in RPMI-1640 medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS; Sijiqing Biotech, Hangzhou, China) and 2 mM L-glutamine at 37°C in 5% CO<sub>2</sub>. Cells were passaged every two days, and those in logarithmic growth were used for the subsequent analyses. The peripheral blood mononuclear cells from healthy subjects served as a negative control.

**Lentiviral-mediated miR-143 expression.** The PCR primers used to amplify the *miR-143* gene are listed in Table I and were synthesized by Invitrogen (Carlsbad, CA, USA). PCR was performed with an ABI 7500 fluorescence quantitative thermal cycler (Applied Biosystems, Foster City, CA, USA) and Platinum SYBR-Green I qPCR SuperMix-UDG kit (Invitrogen), according to the manufacturer's instructions. U6 snRNA served as controls for PCR analysis. PCR reactions were sequentially denatured at 50°C for 2 min and at 95°C for 2 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 30 sec. At the end of the PCR amplification, melting curve assays were performed to ensure the purity of the amplicon. The resultant PCR product was inserted into the pMAGic 7.1-GFP Puro lentivirus vector, which underwent splicing by *XhoI* and *BamHI*. After transformation into competent bacteria, the positive recombinant plasmids were extracted, and the *miR-143* gene was confirmed by sequencing performed by Shanghai SBO Medical Biotechnology (Shanghai, China).

The recombinant plasmids were transfected into 293T cells using Lipofectamine 2000 (Invitrogen). After 48 h, the supernatant was collected and concentrated to determine the viral titer by multiplicity of infection (MOI). The resultant miR-143-expressing lentivirus was used to infect human chronic granulocytic leukemia K562 cells, and the monoclonal cells were screened with puromycin for one week.

**Real-time PCR analysis.** Bone marrow (5 ml) was collected by aspiration using a syringe with the anticoagulant, heparin. Total RNA was extracted from mononuclear cells using TRIzol (Invitrogen). cDNA was isolated by reverse transcription using a kit following the manufacturer's instructions (Fermentas, Lafayette, CO, USA). PCR primers for miR-143, DNMT3A, DNMT3B and DNMT1, Bcl-2, U6 snRNA are listed in Table I. U6 snRNA served as an internal reference. The relative mRNA expression levels of miR-143, DNMT3A and Bcl-2 were determined using the  $2^{-\Delta\Delta C_t}$  method.

**Western blot analysis.** Protein was extracted using lysis solution containing protease inhibitors (all from Xiamen Lulong Biotech Development Co., Ltd., Fujian, China). Total proteins (50  $\mu$ g) were separated by 10% SDS-PAGE (Bio-Rad Laboratories, Hercules, CA, USA). The proteins were then transferred onto nitrocellulose membranes, which were blocked with skim milk (Xiamen Lulong Biotech Development, Co.), and then incubated with the following primary antibodies at 4°C overnight:  $\beta$ -actin (Millipore, Billerica, MA, USA), DNMT3B (Abcam, Cambridge, MA, USA), DNMT3A and DNMT1 (Cell Signaling Technology, Boston, MA, USA), procaspase-3 and procaspase-9 (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Zhongshan, China). After washing with TBST, the membranes were incubated with secondary antibodies (Golden Bridge Biotechnology) and developed using ECL (Xiamen Golden Biotechnology). Following visualization, the bands were scanned and analyzed by a gel image analysis scanner (Gel Doc 1000; Bio-Rad Laboratories).

**Cell proliferation and colony formation analyses.** K562 cells were seeded into 96-well plates at a density of  $2 \times 10^4$  cells/100  $\mu$ l. In each plate, there were three groups (three wells

per group): blank control group (no cells and medium), control group (K562 transfected with lentivirus expressing scrambled vectors), and the experimental group (K562 cells transfected with lentivirus expressing miR-143). Cell proliferation was determined after 0, 24, 48, 72 and 96 h using the Cell Counting Kit-8 (CCK-8; Beyotime Institute of Biotechnology, Beijing, China). After 2-3 h, the optical density (OD) was measured with a microplate reader at 450 nm using the following formula:  $OD_{\text{experiment}} - OD_{\text{blank}}$ . This experiment was performed three times.

To assess the colony formation, K562 cells transfected with the control scrambled lentivirus or the miR-143-expressing lentivirus were seeded into 24-well plates (500  $\mu$ l/well; 200 cells/well; three wells/group), which were pre-coated with methyl cellulose solution (500  $\mu$ l/well). The methyl cellulose was dissolved in sterilized RPMI-1640 at a final concentration of 1.6%. After sterilization at a high pressure (10 pounds for 20 min), the methyl cellulose solution was stored at 4°C for use. After 10-14 days, the colonies were counted under an inverted microscope (Nikon, Tokyo, Japan). A colony was defined as an aggregate of >40 cells.

**Analysis of the cell cycle.** K562 cells ( $1 \times 10^6$ ) were collected and mixed with 2 ml of lysis buffer (Becton-Dickinson, Franklin Lakes, NJ, USA). After incubation in the dark for 10 min,  $1 \times 10^6$  cells were counted by flow cytometry followed by sequential addition of solutions A-C (Becton-Dickinson) following the manufacturer's instructions.

**Analysis of apoptosis.** K562 cells ( $1 \times 10^6$ ) were collected and independently treated with acridine orange/ethidium bromide (AO/EB) fluorescent dye and bisbenzimidazole (Hoechst 33258) (both from Xiamen Lulong Biotech), following the manufacturer's instructions. Apoptotic cells were observed under a fluorescence microscope (Nikon 2000) at an excitation wavelength of 352 nm and emission wavelength of 461 nm.

Apoptotic cells were also assessed after Annexin V and FITC/propidium iodide (PI) staining. In brief,  $1-5 \times 10^5$  cells were collected and resuspended in binding buffer (Beyotime Institute of Biotechnology). A mixture of Annexin V-FITC and PI from the apoptosis kit (Beyotime Institute of Biotechnology) was added to the cell suspension followed by incubation in the dark at room temperature for 5-15 min. Flow cytometry was performed at an excitation wavelength of 488 nm and emission wavelength of 530 nm.

**Statistical analysis.** Continuous variables were expressed as mean with standard deviation. For comparisons between healthy controls and leukemia patients, or normal control (NC) and miR-143 groups, independent two sample t-tests were performed. For comparisons among the various cell lines, one-way analysis of variance (ANOVA) was used. When a significant difference between groups was apparent, multiple comparisons were performed using the Bonferroni procedure with type-I error adjustment. The relationship between relative miR-143 expression and DNMT3A was evaluated using the Pearson's correlation coefficient test. SAS software package, version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for the statistical analysis. All statistical assessments were evaluated at a two-sided  $\alpha$  level of 0.05.

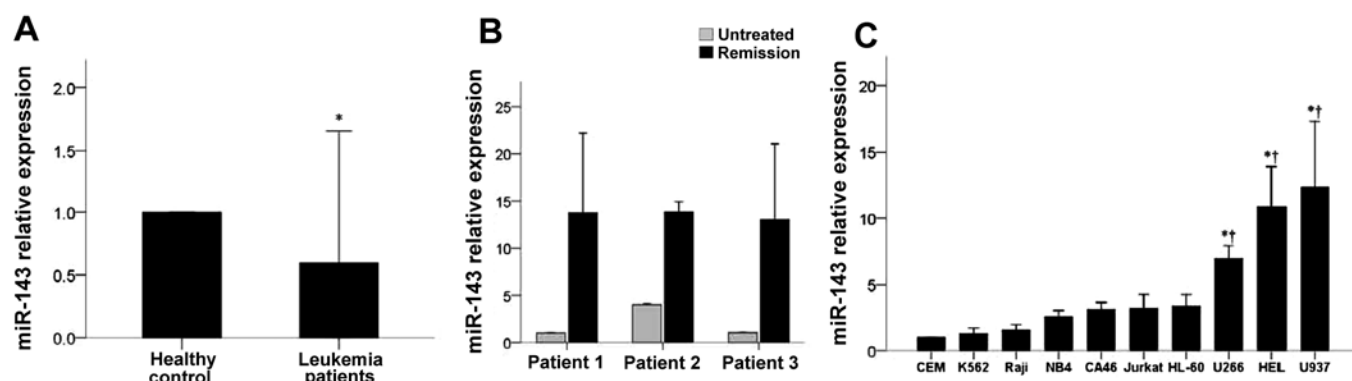


Figure 1. Expression of miR-143 in the bone marrow cells of leukemia patients and in human hematologic tumor cell lines using RT-PCR analysis. (A) miR-143 levels when determined in bone marrow cells from leukemia patients and healthy control patients. (B) miR-143 levels in 3 leukemia patients before and after complete remission. (C) miR-143 expression in 10 human cell lines derived from hematologic malignancies. \* $P < 0.05$ , indicates a significant difference from (A) the healthy control or (C) CEM cells. † $P < 0.05$  indicate a significant difference from the K562 cells.

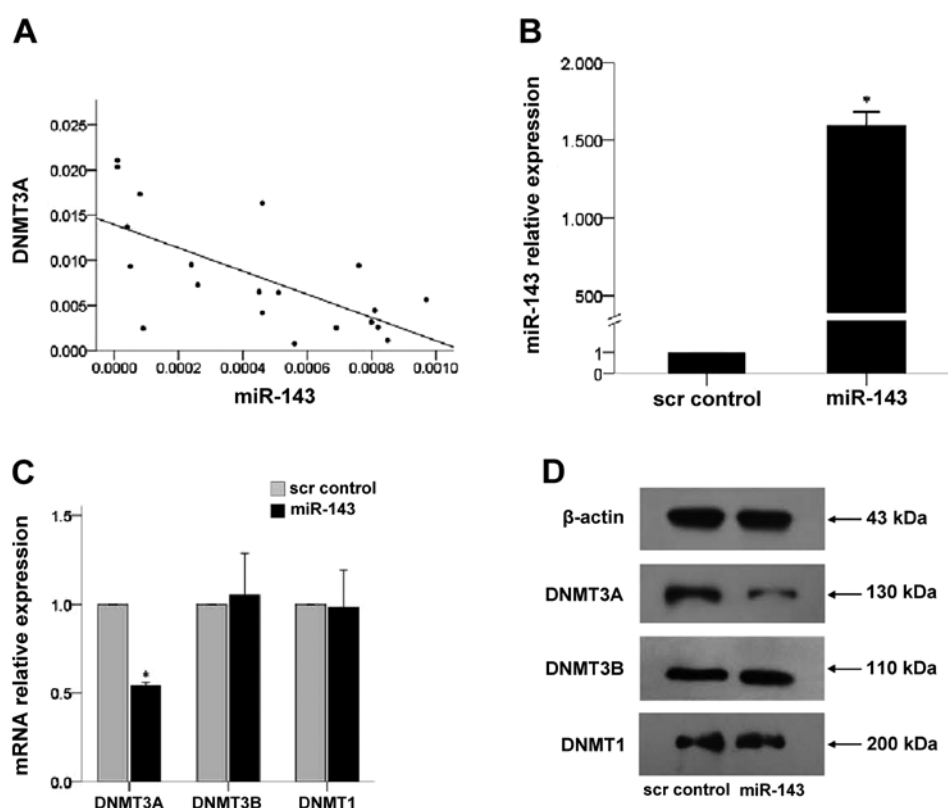


Figure 2. Effect of miR-143 on DNMT3A expression. (A) Correlation of miR-143 with DNMT3A expression in the bone marrow cells of leukemia patients. (B) Lentiviral-mediated miR-143 overexpression in K562 cells. (C) mRNA and (D) protein expression of DNMT was determined in K562 cells using RT-PCR and western blot analysis, respectively, in the normal control (NC) and miR-143-overexpressing K562 cells. \* $P < 0.05$ , indicates a significant difference from the NC group.

## Results

**Expression of miR-143 in bone marrow cells from leukemia patients and in hematologic tumor cell lines.** As shown in Fig. 1A, bone marrow cells from 63 leukemia patients had significantly lower miR-143 expression as compared to the bone marrow samples of 15 healthy controls ( $P = 0.004$ ). In 3 patients with disease remission, higher relative miR-143 expression was observed as compared to the levels prior to treatment (Fig. 1B). Furthermore, miR-143 levels in 10 different hemo-

logic tumor cell lines were quantified (Fig. 1C). No significant differences were observed among the CEM, K562, Raji, NB4, CA46, Jurkat and HL-60 cell lines, while U266, HEL and U937 expressed significantly higher levels of miR-143 when compared to the levels in the CEM and K562 cells (all  $P \leq 0.002$ ). Since K562 cells expressed relatively lower levels of miR-143, they were used for the subsequent experiments.

**Effects of miR-143 on DNMT3A expression.** Since miR-143 may regulate epigenetic modification via silencing its target,

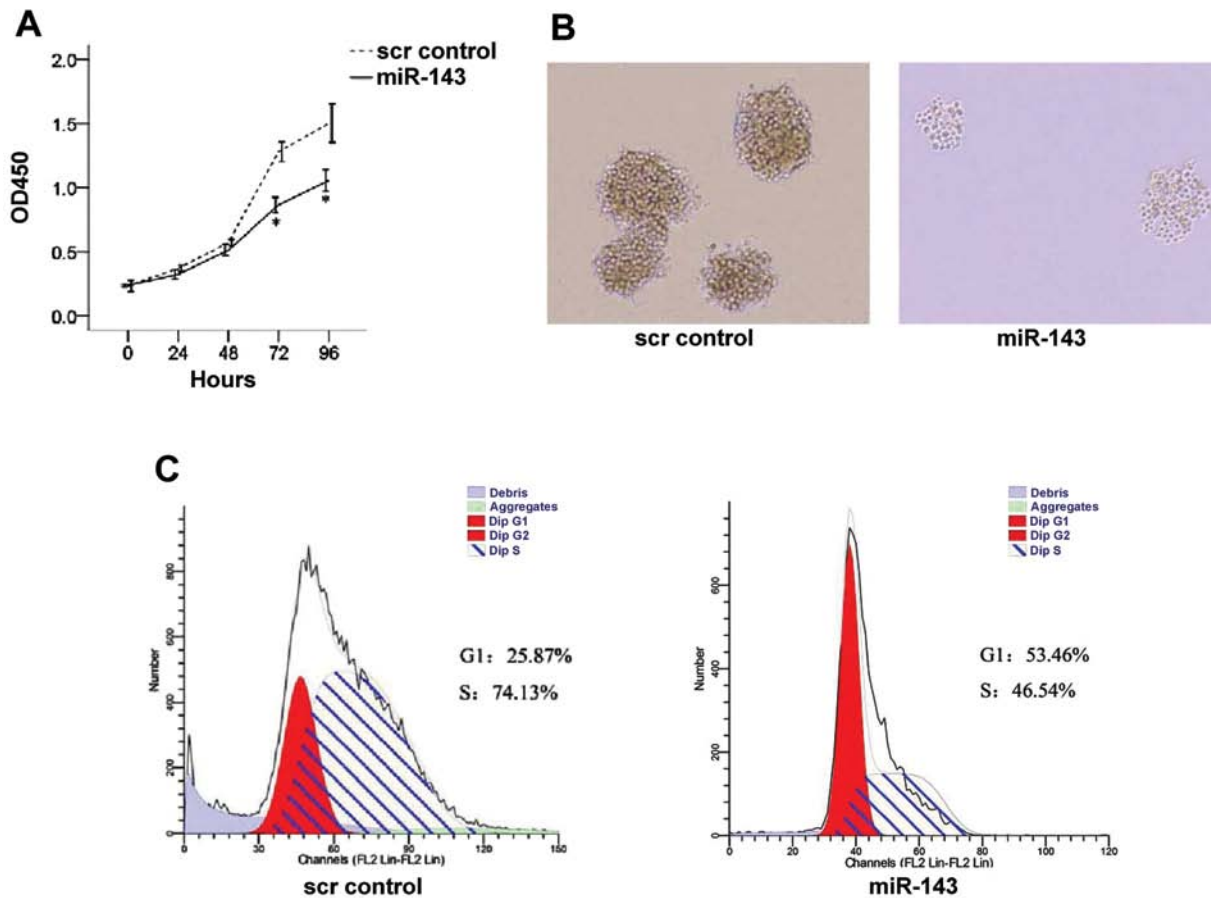


Figure 3. Effects of miR-143 on (A) cell proliferation, (B) colony formation, and (C) cell cycle distribution in normal K562 cells and miR-143-overexpressing K562 cells. \* $P < 0.05$ , indicates a significant difference from the corresponding NC group.

DNMT3A (27), the correlation between miR143 and DNMT3A levels was assessed in 20 leukemia patient samples. As shown in Fig. 2A, the expression levels of miR143 and DNMT3A were negatively correlated ( $r = -0.663$ ,  $P = 0.001$ ).

The effects of miR-143 expression were determined in K562 cells after lentivirus-mediated overexpression in the cells. As shown in Fig. 2B, the expression of miR-143 in K562 cells infected with lenti-miR-143 was 1,594 times higher than that observed in the scrambled negative control (scr control) group ( $P = 0.024$ ). Overexpression of miR-143 decreased DNMT3A mRNA and protein expression ( $P < 0.001$ ; Fig. 2C and D, respectively). However, no changes in DNMT3B and DNMT1 expression were observed.

**Effects of miR-143 on K562 proliferation, colony formation and cell cycle.** As shown in Fig. 3A, overexpression of miR-143 significantly reduced K562 cell proliferation at 72 and 96 h as compared to the scr control group (both  $P \leq 0.018$ ). In addition, reduced colony formation was observed upon miR-143 expression (Fig. 3B). Furthermore, cell cycle progression in scr control and miR-143-overexpressing K562 cells was analyzed by flow cytometry. A greater proportion of cells in the G1 phase was observed in the miR-143-expressing K562 cells than the proportion in the scr control group (53.46 vs. 25.87%,  $P = 0.001$ ; Fig. 3C); the scr control group had a greater proportion of cells in the S phase (74.13 vs. 46.54%,  $P = 0.001$ ; Fig. 3C).

**Effects of miR-143 on K562 cell apoptosis.** After staining with AO/EB, apoptotic K562 cells were observed by microscopy. As shown in Fig. 4A (left panel), most cells in the scr control group were bright green with only a few orange-red necrotic cells. Upon miR-143 expression, the number of apoptotic K562 cells increased (Fig. 4A, right panel). These results were confirmed by Hoechst 33258 staining, which revealed no apoptotic cells in the scr control group (Fig. 4B, left panel). However, in the miR-143-transfected cells, the nuclei were dense, and apoptotic bodies in several cells were observed (Fig. 4B, right panel). In addition, flow cytometric analysis revealed that the early apoptosis rate was higher in the miR-143 group as compared to the scr control group (84.9 vs. 5.1%,  $P < 0.001$ ; Fig. 4C).

The effects of miR-143 on the expression of the anti-apoptotic protein, Bcl-2, was next determined. As shown in Fig. 4D, Bcl-2 mRNA expression was reduced in the miR-143-expressing K562 cells ( $P < 0.001$ ; Fig. 4D). In addition, western blot analysis revealed that protein expression of pro-caspase-3 and pro-caspase-9 was reduced in the miR-143-expressing cells (Fig. 4E).

## Discussion

In the present study, reduced miR-143 expression was observed in the bone marrow cells of leukemia patients, which is consistent with the findings of Batliner *et al* (30). In addi-

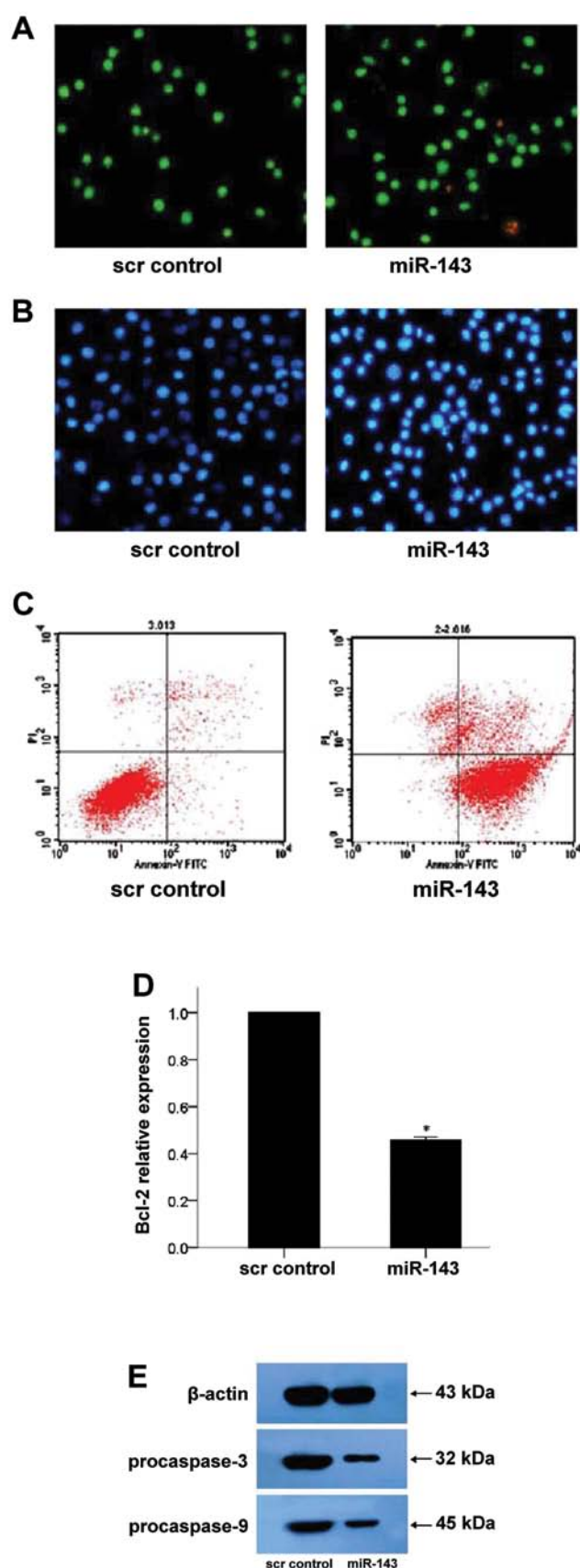


Figure 4. Effect of miR-143 on K562 cell apoptosis. Apoptosis of the normal control (NC) and miR-143-overexpressing K562 cells was evaluated using (A) AO/EB staining, (B) Hoechst 33258 staining, and (C) flow cytometry after Annexin V and PI staining. The effects of miR-143 expression on (D) Bcl-2 mRNA expression and (E) pro-caspase protein expression were determined using RT-PCR and western blot analysis, respectively. \* $P < 0.05$ , indicates a significant difference from the NC group.

tion, miR-143 reduced the expression of its target, DNMT3A, and its overexpression reduced K562 cell proliferation, colony formation and cell cycle progression. Furthermore, increased apoptosis was observed upon miR-143 overexpression.

Although miR-143 expression was found to be downregulated in colon cancer, its association with clinicopathological features of colorectal cancer patients is inconsistent (27,31,32). Whereas Wang *et al* (32) reported that miR-143 was not associated with clinicopathological features, such as age, gender and TNM stage, Slaby *et al* (31) observed that decreased miR-143 was associated with tumor diameter. In addition, Calin *et al* (11) observed a microRNA signature in chronic lymphoid leukemia patients that was correlated with patient prognosis and disease pathogenesis. In the present study, miR-143 expression was markedly lower in the bone marrow samples of leukemia patients than in healthy subjects, and its expression increased with disease remission in 3 patients. Further studies are necessary to determine whether miR-143 expression is related to disease progression, prognosis or other clinicopathological features.

miR-143 overexpression was previously found to inhibit the proliferation and migration of prostate cancer cells, silencing the *KRAS* gene to inhibit protein kinase signaling and increasing the chemosensitivity of cancer cells to docetaxel (18). In addition, miR-143 levels were negatively associated with Raji cell proliferation (20). These results are consistent with those of the present study in which miR-143 overexpression inhibited K562 cell proliferation, cell cycle progression and colony formation. Although the mechanisms underlying the effects of miR-143 on cell proliferation are unclear, we speculate that miR-143 might act on ERK5 (33), ErbB3 and K-ras (34) growth factor receptor signaling to arrest the cell cycle. Further studies are necessary to determine whether miR-143 inhibits the growth factor receptor signaling pathway to suppress cancer cell proliferation.

In addition to reduced cell proliferation, increased apoptosis was observed in K562 cells exhibiting miR-143 overexpression, which is consistent with Zhang *et al* (25). We postulate that miR-143 inhibits Bcl-2 expression and activates procaspase-3 and procaspase-9 and thereby the endogenous mitochondrial pathway, which promotes cell apoptosis. There is also evidence indicating that Bcl-2 may be a target gene of miR-143 (25). In addition, miR-143 may target ERK5 during Fas-induced apoptosis (35). However, the specific mechanism by which miR-143 expression induces apoptosis requires further research.

DNA methyltransferase plays important roles in regulating chromatin structure and gene expression via modifying DNA methylation. DNMT3A directly influences the expression of various oncogenes and tumor-suppressor genes (36). Although the mechanisms underlying the effects of these DNA methyltransferases are largely unclear, their abnormal expression has been reported in numerous types of cancers (37,38), and their inhibition is being explored as a potential therapy (38). In the present study, miR-143 reduced DNMT3A expression, which is in agreement with a previous study (26). In addition, miR-143 levels were negatively correlated with DNMT3A levels. miR-143 may bind to the 3'-UTRs of the *DNMT3A* gene to inhibit its activity. Alternatively, an indirect inhibition of DNMT1 by miR-29b via Sp1 has also been shown (12).



The mechanism by which miR-143 inhibits DNMT3A will be assessed in further studies. In addition, the effects of reduced DNMT3A expression on global DNA methylation and re-expression of genes regulated by promoter methylation, including *p15<sup>INK4b</sup>* and *ESR1* (12), will also be assessed in further studies.

The small number of patients assessed in the present study represents a study limitation. In addition, the present study did not assess the mechanism by which miR-143 expression is decreased in leukemia patients. Since p73 regulates miR-143 transcription during neutrophil differentiation (30), its expression and activity should be determined in leukemia patients. Furthermore, the effects of miR-143 overexpression and inhibition need to be analyzed in an *in vivo* model of leukemia. Finally, as the study aimed to assess the expression and function of miR-143 in leukemia cells, specific leukemia subtypes (e.g., AML, ALL, CML and CLL) were not individually examined. Therefore, further study is required to assess the role of miR-143 among the various subtypes.

In conclusion, taken together, miR-143 inhibited cell proliferation and induced apoptosis in K562 cells, which may be mediated through silencing of DNMT3A expression *in vitro*. Furthermore, miR-143 expression was significantly reduced in the bone marrow samples of leukemia patients. Further studies are warranted to elucidate the possible therapeutic potential and prognostic value of miR-143 in cancer.

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