Long non-coding RNA SNHG14 affects the proliferation and apoptosis of childhood acute myeloid leukaemia cells by modulating the miR-193b-3p/MCL1 axis

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Abstract. The purpose of the present study was to determine the biological function and associated regulatory mechanism of small nucleolar RNA host gene 14 (SNHG14) in childhood acute myeloid leukaemia (AML). SNHG14 expression was measured via RT-qPCR in bone marrow tissues from 57 patients with AML and 57 healthy donors. The clinicopathological features of AML patients with low and high SNHG14 expression were analysed. AML cell viability and apoptosis were assessed using MTT and flow cytometry analyses. The starBase online database, and RNA-binding protein immunoprecipitation and dual luciferase reporter gene assays were employed to analyse the interactions among SNHG14, microRNA (miR)-193b-3p and MCL1 apoptosis regulator BCL2 family member (MCL1). SNHG14 was found to be overexpressed in the bone marrow tissues of patients with AML. The French-American-British classification and cytogenetics were significantly different between patients with high and low expression of SNHG14. Silencing SNHG14 decreased AML cell proliferation and facilitated apoptosis. SNHG14 functioned as a sponge for miR-193b-3p, and miR-193b-3p decreased the viability and accelerated the apoptosis rate of AML cells. In addition, miR-193b-3p targeted MCL1. Furthermore, silencing SNHG14 resulted in the sponging of miR-193b-3p to regulate cell viability, apoptosis, and MCL1 expression in AML. SNHG14 silencing decreased the viability and promoted apoptosis of AML cells by modulating the miR-193b-3p/MCL1 axis.

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Abbreviations: AML, acute myeloid leukaemia; RIP, RNA-binding protein immunoprecipitation; DLR, dual luciferase reporter gene; PVDF, polyvinylidene fluoride; lncRNAs, long non-coding RNAs; SNHG14, small nucleolar RNA host gene 14; miRNAs, microRNAs; ceRNAs, competitive endogenous RNA; NBM, normal marrow tissues

Key words: acute myeloid leukaemia, small nucleolar RNA host gene 14, microRNA-193b-3p, MCL1 apoptosis regulator BCL2 family member, apoptosis

Introduction

Childhood acute myeloid leukaemia (AML) is a common malignancy in children, accounting for one-third of all childhood cancer types (1). AML, a common type of leukaemia, is caused by the uncontrolled proliferation, apoptosis, and differentiation of haematopoietic cells in the bone marrow and other haematopoietic tissues (2). AML is known for its high rates of morbidity, recurrence and mortality (3). Although there are many treatment options available, most patients with AML relapse and succumb to remission, and the prognosis remains unsatisfactory (4). Therefore, to develop more effective monitoring and treatment strategies, it is imperative to identify novel biomarkers that can be used to improve the diagnosis and prognosis of AML.

Long non-coding RNAs (lncRNAs) have been reported to function as important regulators of biological and pathological processes in diverse human cancers, including AML (5-7). For example, myocardial infarction associated transcript knockdown was shown to repress the proliferation and promote the apoptosis of AML cells by regulating microRNA (miR)-495 (8). Silencing long intergenic non-protein coding RNA (LINC)00152 was found to inhibit proliferation, induce apoptosis and enhance cycle arrest in AML cells by sponging miR-193a (9). Small nucleolar RNA host gene (SNHG)5 knockdown increases the sensitivity of AML cells to chemotherapy by targeting the miR-32/DNAJ heat shock protein family (hsp40) member B9 axis (10). SNHG14 has been reported to induce oncogenic function by regulating the proliferation, migration, invasion, and chemical resistance ability of various malignant tumour types, including non-small cell lung (11), cervical (12) and gastric (13) cancer. Recently, Wang *et al* (14) reported

that SNHG14 functions as an antitumour gene in glioma. However, the biological function of SNHG14 in AML and the potential mechanism of its action remain unclear.

miRNAs have been identified to function as oncogenic or tumour-suppressive genes, regulating diverse biological processes in cancer, (15) and it has been well documented that they are related to the occurrence and development of AML (16). miR-193b-3p has been shown to function as an antitumour gene in numerous malignant tumour types, including ovarian (17), gastric (18) and breast (19) cancer. Additionally, a recent study indicated that miR-193b serves an anticancer role in homeobox A9/meis homeobox 11-induced leukaemia *in vivo* (20). Notably, increasing lines of evidence support a novel mechanism by which lncRNAs act as competitive endogenous RNAs (ceRNAs) to mediate the expression and functions of specific miRNAs involved in tumour progression (21). For example, Wang *et al* (22) demonstrated that silencing lncRNA linc00152 impeded the progression of gastric cancer by modulating miR-193b-3p. Xie *et al* (23) reported that SNHG14 contributes to the tumorigenesis of breast cancer by sponging miR-193a-3p. Nevertheless, the regulatory mechanism involved in the interaction between SNHG14 and miR-193b-3p in AML remains unclear.

In the present study, first, SNHG14 expression in bone marrow tissues from patients with AML was measured. Next, the effect of SNHG14 on AML cell viability and apoptosis was evaluated. Furthermore, the potential mechanism involved in the interaction among SNHG14, miR-193b-3p and MCL1 apoptosis regulator BCL2 family member (MCL1) in AML cells was explored. The results of the current study may aid in better understanding the pathogenesis of AML and provide a promising therapeutic target for AML treatment.

Materials and methods

Patients and specimens. A total of 57 children with AML (28 male, 29 female; 10 months-14 years old) were identified after screening potential participants at the East Hospital of Shouguang People's Hospital (Shouguang, China) between January 2017 and January 2018. The inclusion criteria included a first-time diagnosis and no history of antitumour treatment. The exclusion criteria included the presence of other malignant tumours and/or organ dysfunctions. AML was diagnosed according to the World Health Organization classification of tumours of haematopoietic and lymphoid tissues (Version 2008) (24). A total of 57 age- and sex-matched children with normal bone marrow morphology, and without malignancy who were examined at the hospital at the same time were enrolled as the control group. The clinical characteristics of patients with AML and controls listed in Table I. The bone marrow samples were obtained by puncture and were stored at -80˚C until use. Informed consent was obtained from the legal guardians of children <18 years old. This study was approved by the Ethics Committee of the East Hospital of Shouguang People's Hospital in accordance with the Declaration of Helsinki.

Cell culture and transfection. Human normal bone marrow $CD34^+$ cells and AML cell lines (MV-4-11, AML-193, HL-60, and KG-1 cells) were obtained from the American Type Culture Collection and cultured in Dulbecco's modified Eagle's medium (DMEM; HyClone; GE Healthcare) containing 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C. The small interfering RNA targeting SNHG14 (si-SNHG14) (5'-CAGCAUAUGUAAGUGGAACUCAGAA-3'), corresponding negative control (si-NC) (5'-AUCUUCAUUGGCACCGAACGUGUCACGUUU-3'), miR-193b-3p mimics (5'-AACUGGCCCUCAAAGUCCCGCU-3'), miR-193b-3p inhibitor (5'-AGCGGGACUUUGAGGGCCAGUU-3'), miR-NC (5'-UUUGUACUACACAAAAGUACUG-3'), pcDNA-SNHG14, pcDNA-NC, and pcDNA-MCL1 were all purchased from Shanghai GenePharma Co., Ltd. MV-4-11 and AML-193 cells were transfected with the aforementioned oligonucleotides (50 nM) or plasmids (50 ng) using Lipofectamine® 3000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Following an incubation period of 48 h at 37˚C, the cells were utilised in the subsequent experiments.

Reverse transcription-quantitative (RT-q)PCR. Total RNA was isolated from the bone marrow samples and cells using an miRNeasy Mini kit (Qiagen GmbH). A PrimeScript™ IV 1st strand cDNA Synthesis Mix (Takara Bio, Inc.) was used to reverse transcribe the isolated RNA (2 μg) into cDNA according to the manufacturer's protocol. qPCR analyses were conducted using an SYBR Green PCR Master mix (Qiagen GmbH) on an ABI 7500HT Fast Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc). The reaction conditions were as follows: 95˚C for 3 min, and 40 cycles at 95˚C for 10 sec, 60˚C for 20 sec and 72˚C for 34 sec. The melting curve had a single peak. The $2^{-\Delta\Delta Cq}$ method was used to analyse the relative mRNA expression levels (25). GAPDH or U6 was utilised as an internal control. The primer sequences were shown as follows: SNHG14 forward, 5'-GGGTGTTTACGTAGACCAGAACC-3' and reverse, 5'-CTTCCAAAAGCCTTCTGCCTTAG-3'; miR-193b-3p forward, 5'-TCTACAGTGCACGTGTCTCCAG-3' and reverse, 5'-ACCTGCGTAGGTAGTTTCATGT-3'; MCL1 forward, 5'-GGACATCAAAAACGAAGACG-3' and reverse, 5'-GCAGCTTTCTTGGTTTATGG-3'; GAPDH forward, 5'-GCACCGTCAAGGCTGAGAAC-3' and reverse, 5'-AGGGATCTCGCTCCTGGAA-3'; U6 forward, 5'-AGTACCAGTCTGTTGCTGG-3' and reverse, 5'-TAATAGACCCGGATGTCTGGT-3'.

Target predicting. The targets of SNHG14 and miR-193b-3p were predicted by a starBase online database (starbase.sysu.edu.cn/agoClipRNA.php?). miR-193-3p was identified as a target of SNHG14, and MCL1 was identified as a target of miR-193b-3p.

RNA-binding protein immunoprecipitation (RIP). The target association between SNHG14 and miR-193b-3p was analysed using the RIP assay with a Magna RIP RNA-binding protein immunoprecipitation kit (#17-700, EMD Millipore) in accordance with the manufacturer's instructions. Briefly, MV-4-11 and AML-193 cells were lysed in NP-40 lysis buffer containing 1% protease inhibitor (Sigma-Aldrich; Merck KGaA). After 10 min of centrifugation at 200 g and 4˚C, 100 μl supernatant was conjugated with human anti-protein argonaute-2 (ab186733, 1:1,000, AGO2) antibody (Abcam) for

Table I. Clinical characteristics of patients with AML and healthy controls.

Characteristics	AML	Healthy controls
Age, years old[a]	8±5[a]	9±5[a]
Sex, n		
Male	28	28
Female	29	29
FAB classification, n		
M0	0	-
M1	13	-
M2	16	-
M3	11	-
M4	7	-
M5	5	-
M6	1	-
M7	4	-
Bone marrow blasts (non-M3, n=46), % [mean ± standard deviation (range)]	54.5±22.9 (30.3-97.8)	-
Cytogenetics, n		
Favourable	15	-
Intermediate	33	-
Unfavourable	9	-

[a]Mean ± standard deviation; range, 10 months-14 years old. AML, acute myeloid leukaemia; FAB, French-American-British.

10 h at 4˚C. Mouse IgG (M8642, 1:1,000, EMD Millipore) was used as a negative control. After Coprecipitated RNA was isolated and measured using RT-qPCR for direct binding as aforementioned.

Dual luciferase reporter gene (DLR) assay. The wild-type (wt) or mutated (mut) fragments of SNHG14 (SNHG14 wt/mut) and MCL1 (MCL1 wt/mut) were cloned into a pmiRGLO vector (Promega Corporation). To investigate the association among SNHG14, miR-193b-3p, and MCL1, MCL1 wt/mut or SNHG14 wt/mut was co-transfected into MV-4-11 and AML-193 cells with miR-NC/miR-193b-3p mimics using Lipofectamine® 3000 reagent. After transfection for 48 h at 37˚C, the Dual-Luciferase® Reporter assay system (Promega Corporation) was used to measure the luciferase activity. *Renilla* luciferase activity was measured as the internal control.

MTT assay. MV-4-11 and AML-193 cells ($1x10^4$ cells/well) were seeded in 96-well plates and cultured for 24, 48, 72 or 96 h. Cells were then incubated with 20 μl MTT (0.5 mg/ml) for 4 h at 37˚C, followed by the addition of 150 μl dimethyl sulfoxide to terminate the reaction. The absorbance at 450 nm (A450) was measured using a microplate reader.

Flow cytometry analysis. The cell apoptosis assay (MV-4-11 and AML-193 cells) was performed using an Annexin V-FITC/PI Apoptosis Detection kit (Sigma-Aldrich; Merck KGaA) according to the manufacturer's protocol. Briefly, the cells were double-stained with 5 μl Annexin V/FITC and 10 μl PI. The cells were then incubated for 15 min at 25˚C in the dark. The stained cells were detected using a flow cytometer (FACSAria II, Becton Dickinson, Franklin Lakes, NJ, USA) with CellQuest software (v1.5, Becton Dickinson).

Western blot analysis. Total proteins were isolated from MV-4-11 cells using RIPA lysis buffer (Thermo Fisher Scientific, Inc.) and quantified using a BCA Protein assay kit (Thermo Fisher Scientific, Inc.). The proteins (30-50 μg/lane) were separated using 10% SDS-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene membrane. Following blocking with 5% non-fat milk for 2 h at 25˚C, the membrane was incubated with rabbit anti-MCL1 (1:5,000; cat. no. ab246684; Abcam) and rabbit anti-β-actin (1:10,000; cat. no. 4970; Cell Signaling Technology, Inc.) primary antibodies at 4˚C overnight. Then, the membrane was incubated with HRP-conjugated goat anti-rabbit IgG (A0545, 1:10,000; Sigma-Aldrich) for 1 h at room temperature. Finally, the protein bands were visualised using enhanced chemiluminescent substrate reagent kit (WP20005, Thermo Fisher Scientific, Inc.), and quantified using ImageJ software (v1.5, National Institutes of Health). β-actin was used as an internal control.

Statistical analysis. Each experiment was repeated at least three times. SPSS 22.0 statistical software (SPSS, Inc.) and GraphPad Prism v7.01 (GraphPad Software, Inc.) were used for all statistical analyses. Data are presented as the mean ± standard deviation. Student's t-test was used to compare significant differences between two groups and one-way ANOVA followed by Tukey's post hoc test was applied when analysing >two groups. Spearman's correlation analysis was performed to evaluate the correlation between SNHG14 and miR-193b-3p expression. $P<0.05$ was considered to indicate a statistically significant difference.

Results

SNHG14 is overexpressed in the bone marrow tissues of patients with AML. To investigate the role of SNHG14, the gene expression of SNHG14 was measured first. SNHG14 was found to be significantly overexpressed in AML bone marrow tissue compared with the level in normal marrow tissue ($P<0.001$; Fig. 1A). The AML bone marrow tissues were divided into two groups according to the expression of SNHG14: High expression, SNHG14 expression level ≥median and low expression, SNHG14 expression level <median. The clinicopathological features of the patients with AML are shown in Table II. The analysis of the data revealed that the French-American-British classification ($P=0.348$) and cytogenetics ($P<0.001$) were significantly different between the high and low expression groups (Table II). In addition, SNHG14 gene expression was significantly upregulated in AML cell lines (MV-4-11, AML-193, HL-60 and KG-1) compared with that in human normal bone marrow $CD34^+$ cells ($P<0.01$; Fig. 1B). MV-4-11 and AML-193 cells, which had relatively

Table II. Clinicopathologic characteristics of AML patients with low and high expression of SNHG14.

Characteristics	No. of patients			P-value
	Total	Low SNHG14 expression	High SNHG14 expression	
Age, years				0.681
≤7	26	12	14	
8-14	31	16	15	
Sex				0.896
Male	28	14	14	
Female	29	14	15	
Leukocytes, μl				0.661
>10,000	35	18	17	
≤10,000	22	10	12	
Bone marrow blasts (non-M3, n=46), %				0.203
30-55	26	14	12	
≥55	20	7	13	
FAB classification				0.042[a]
M1-6	53	28	25	
M7	4	0	4	
Cytogenetics				<0.001[b]
Favourable	15	15	0	
Intermediate	33	13	20	
Unfavourable	9	0	9	

[a]P<0.05 and [b]P<0.001 between low and high groups. AML, acute myeloid leukaemia; SNHG14, small nucleolar RNA host gene 14; FAB, French-American-British.

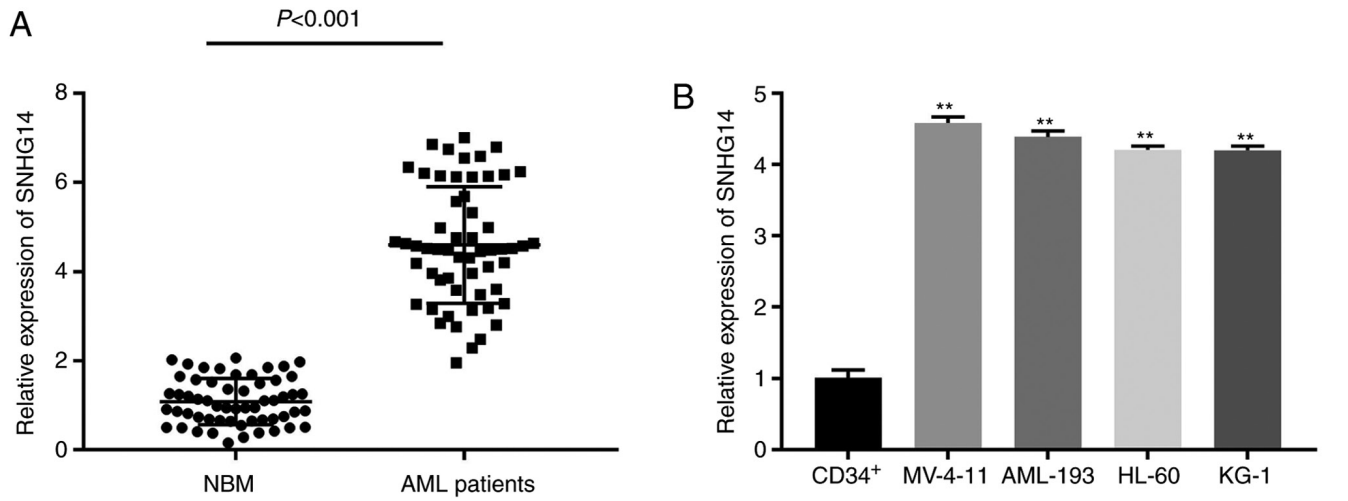

Figure 1. SNHG14 gene expression is upregulated in bone marrow tissues of patients with AML and AML cell lines. (A) Relative expression of SNHG14 in 57 AML bone marrow tissues and NBM. (B) Relative expression of SNHG14 in AML cell lines and human normal bone marrow CD34+ cells. **P<0.01 vs. CD34+ cells. The $2^{-\Delta\Delta Cq}$ method was used to analyse the relative mRNA expression level using GAPDH as an internal control. AML, acute myeloid leukaemia; NBM, normal marrow tissues; SNHG14, small nucleolar RNA host gene 14.

higher SNHG14 gene expression, were utilised in the subsequent experiments.

Silencing SNHG14 inhibits the proliferation and facilitates the apoptosis of AML cells. To explore the effect of SNHG14 on AML progression, SNHG14 gene expression was knocked down by transfection of MV-4-11 and AML-193 cells with SNHG14 siRNA. RT-qPCR analysis revealed that SNHG14 expression was significantly reduced in the si-SNHG14-1 and si-SNHG14-2 groups compared with that in the blank control

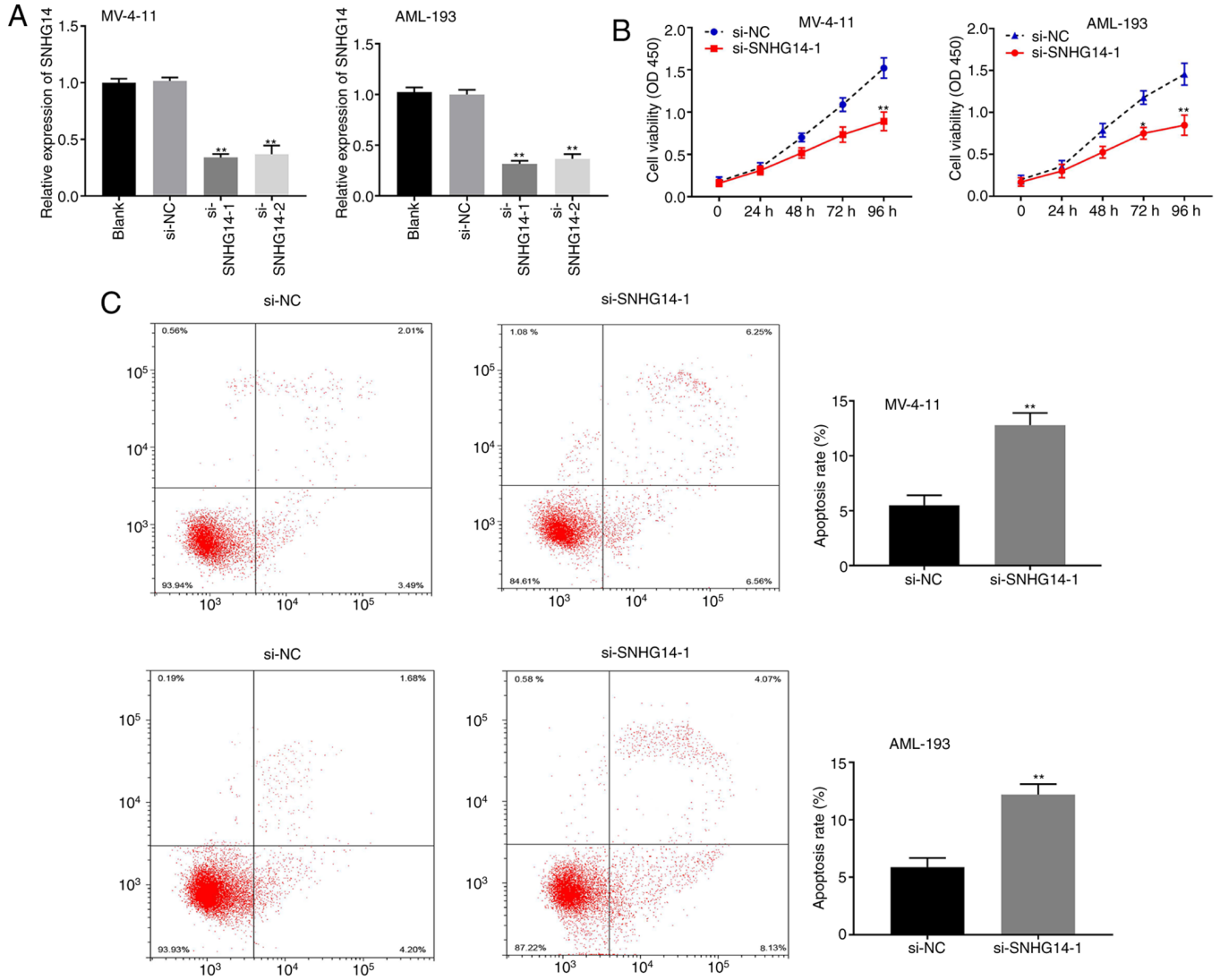

Figure 2. Silencing of SNHG14 inhibits the proliferation and facilitates the apoptosis of AML, acute myeloid leukaemia. (A) Relative expression of SNHG14 in transfected MV-4-11 and AML-193 cells was measured by RT-qPCR. **P<0.01 vs. blank control. The $2^{-\Delta\Delta Cq}$ method was used to analyse the relative mRNA expression level using GAPDH as an internal control. (B) The viability of transfected MV-4-11 and AML-193 cells was detected by MTT assay. **P<0.01 vs. si_NC. (C) The apoptosis rate of transfected MV-4-11 and AML-193 cells was detected by flow cytometry. **P<0.01 vs. si-NC. SNHG14, small nucleolar RNA host gene 14; si, small interfering RNA; NC, negative control; OD, optical density.

group (P<0.01; Fig. 2A). si-SNHG14-1 was utilised in the subsequent tests as it resulted in markedly lower SNHG14 expression compared with si-SNHG14-2. Cell viability was significantly lower in the si-SNHG14-1 group compared with that in the si-NC group for both cell lines (both P<0.05; Fig. 2B). Conversely, the apoptosis rate was significantly higher in the si-SNHG14-1 group compared with the si-NC group (P<0.01; Fig. 2C).

SNHG14 acts as a sponge for miR-193b-3p in AML cells. The starBase online database was used to predict the potential binding site between miR-193-3p and SNHG14 (Fig. 3A). As illustrated in Fig. 3B, SNHG14 knockdown significantly elevated miR-193-3p expression in MV-4-11 and AML-193 cells compared with the si-NC and blank control groups (all P<0.01). The results of the RIP assay indicated that the expression of SNHG14 and miR-193-3p were significantly enriched in the AGO2 immunoprecipitate compared with that in the IgG immunoprecipitate in both cell lines (all P<0.01; Fig. 3C). The DLR assay revealed that miR-193-3p mimics significantly decreased the luciferase activity of SNHG14 wt but did not affect that of SNHG14 mut in MV-4-11 and AML-193 cells (P<0.01; Fig. 3D). As shown in Fig. 3E, miR-193-3p expression was markedly decreased in bone marrow tissues from patients with AML compared to that in the NBM group (P<0.01). Additionally, a negative correlation was observed between the expression of miR-193-3p and SNHG14 (r=-0.4085; P=0.0016; Fig. 3F). miR-193-3p expression was significantly decreased in AML cell lines compared with that in human NBM $CD34^+$ cells (P<0.01; Fig. 3G). Together, these results indicate that SNHG14 functions as a sponge for miR-193b-3p in AML cells.

miR-193b-3p suppresses the viability and induces the apoptosis of AML cells. As indicated in Fig. 4A, miR-193b-3p expression

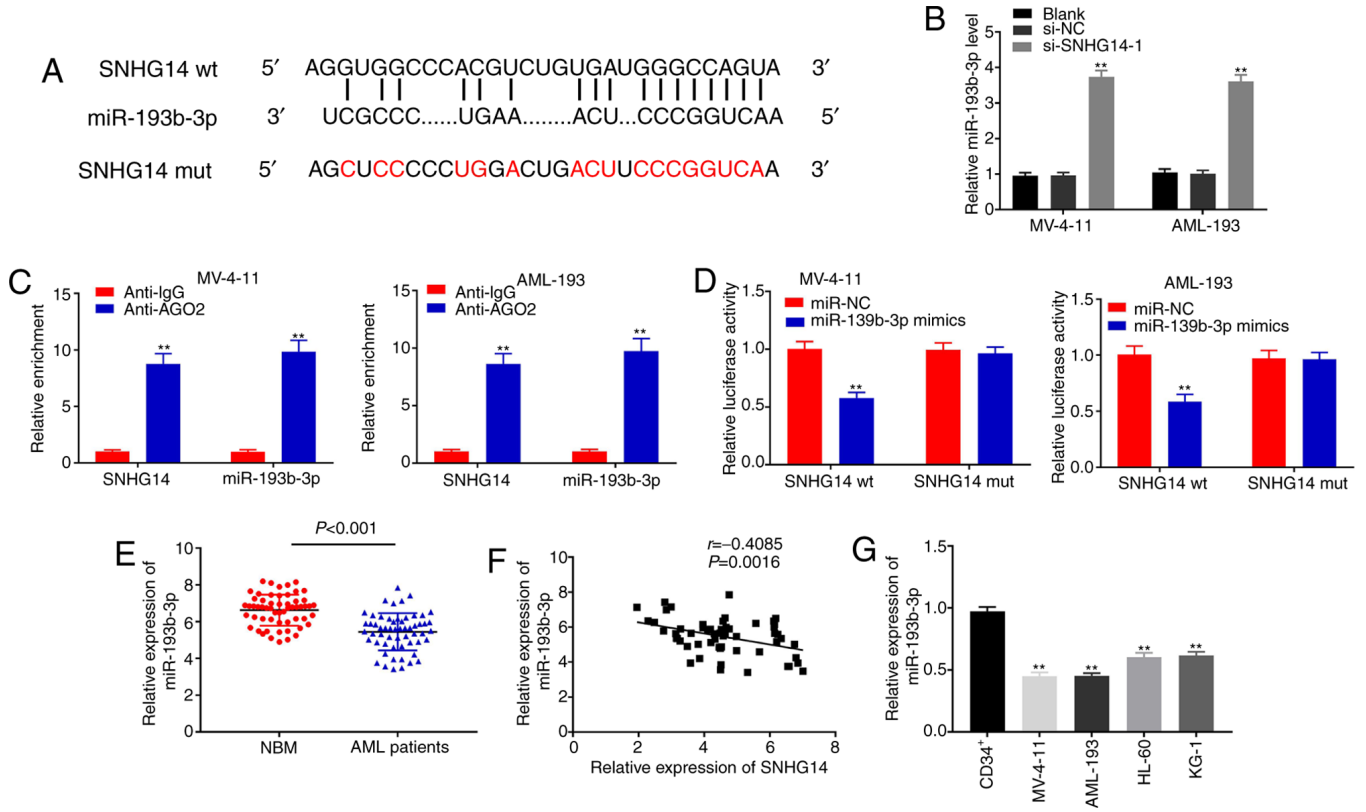

Figure 3. miR-193b-3p is a target of SNHG14 in AML cells. (A) Starbase was used to predict the binding site between SNHG14 and miR-193b-3p. (B) Relative expression of miR-193b-3p in MV-4-11 and AML-193 cells following SNHG14 silencing. **P<0.01 vs. blank control. (C) The target association between SNHG14 and miR-193b-3p was determined using an RNA immunoprecipitation assay. **P<0.01 vs. Anti-IgG. (D) The target association between SNHG14 and miR-193b-3p was determined using a dual luciferase reporter gene assay. **P<0.01 vs. miR-NC. (E) Relative expression of miR-193b-3p in 57 AML bone marrow tissues and NBM was detected by RT-qPCR. (F) Spearman's correlation analysis was performed to evaluate the correlation between SNHG14 and miR-193b-3p expression. (G) Relative expression of miR-193b-3p in AML cell lines and human normal bone marrow CD34+ cells. **P<0.01 vs. CD34+ cells. The $2^{-\Delta\Delta Cq}$ method was used to analyse the relative mRNA expression level using U6 as an internal control. SNHG14, small nucleolar RNA host gene 14; miR, microRNA; si, small interfering RNA; NC, negative control; wt, wildtype; mut, mutated; NBM, normal marrow tissues; AGO2, protein argonaute-2; AML, acute myeloid leukaemia.

was markedly enhanced in the miR-193b-3p mimics group but was markedly decreased in the miR-193b-3p inhibitor group compared with that in the blank control group in both cell lines (both P<0.01). The MTT analysis revealed that miR-193b-3p mimics markedly reduced the viability and promoted the apoptosis of MV-4-11 and AML-193 cells compared with that in the miR-NC group (both P<0.01; Fig. 4B and C).

miR-193b-3p targets MCL1. The starBase online database was used to first predict the possible binding site between miR-193b-3p and MCL1 (Fig. 5A). As shown in Fig. 5B, the DLR assay revealed that miR-193b-3p mimics significantly reduced the luciferase activity of MCL1 wt (P<0.05), whereas there was no notable change in the MCL1 mut in both cell lines. The expression of MCL1 was markedly elevated in bone marrow tissues from patients with AML compared with that in the NBM group (P<0.001; Fig. 5C). As shown in Fig. 5D and E, miR-193b-3p expression was negatively correlated with MCL1 (r=-0.4012; P=0.002), whereas SNHG14 expression was positively correlated with MCL1 (r=0.4322; P<0.001). Finally, the relative expression of MCL1 was significantly higher in all AML cell lines compared with that in CD34+ cells (P<0.01; Fig. 5F). Together, these results indicate that miR-193b-3p targets MCL1 and that MCL1 is highly expressed in AML.

Silencing SNHG14 inhibits the viability and induces the apoptosis of AML cells by modulating the miR-193b-3p/MCL1 axis. The western blotting results indicated that the protein expression of MCL1 was notably decreased in the miR-193b-3p mimics + pcDNA-NC group compared with that in the miR-NC + PCDNA-NC group, and the decrease in MCL1 expression caused by miR-193b-3p was partially rescued by the overexpression of SNHG14 (P<0.05; Fig. 6A). To further investigate the underlying mechanism involved in the interaction among SNHG14, miR-193b-3p, and MCL1 in AML progression, MV-4-11 cells were co-transfected with si-SNHG14-1 and pcDNA-MCL1 or miR-193b-3p inhibitors. As shown in Fig 6B, MV-4-11 cell viability was significantly decreased in the si-SNHG14-1 group, and the decrease in cell viability induced by SNHG14 knockdown was recovered by transfection with pcDNA-MCL1 or miR-193b-3p inhibitors (P<0.05). Conversely, MV-4-11 cell apoptosis was markedly enhanced in the si-SNHG14-1 group, and pcDNA-MCL1 or miR-193b-3p inhibitors partially eliminated the increase in cell apoptosis caused by SNHG14 knockdown (P<0.05; Fig. 6C). In summary, these results indicate that SNHG14 may act as ceRNA to modulate AML cell viability and apoptosis via the miR-193b-3p/MCL axis.

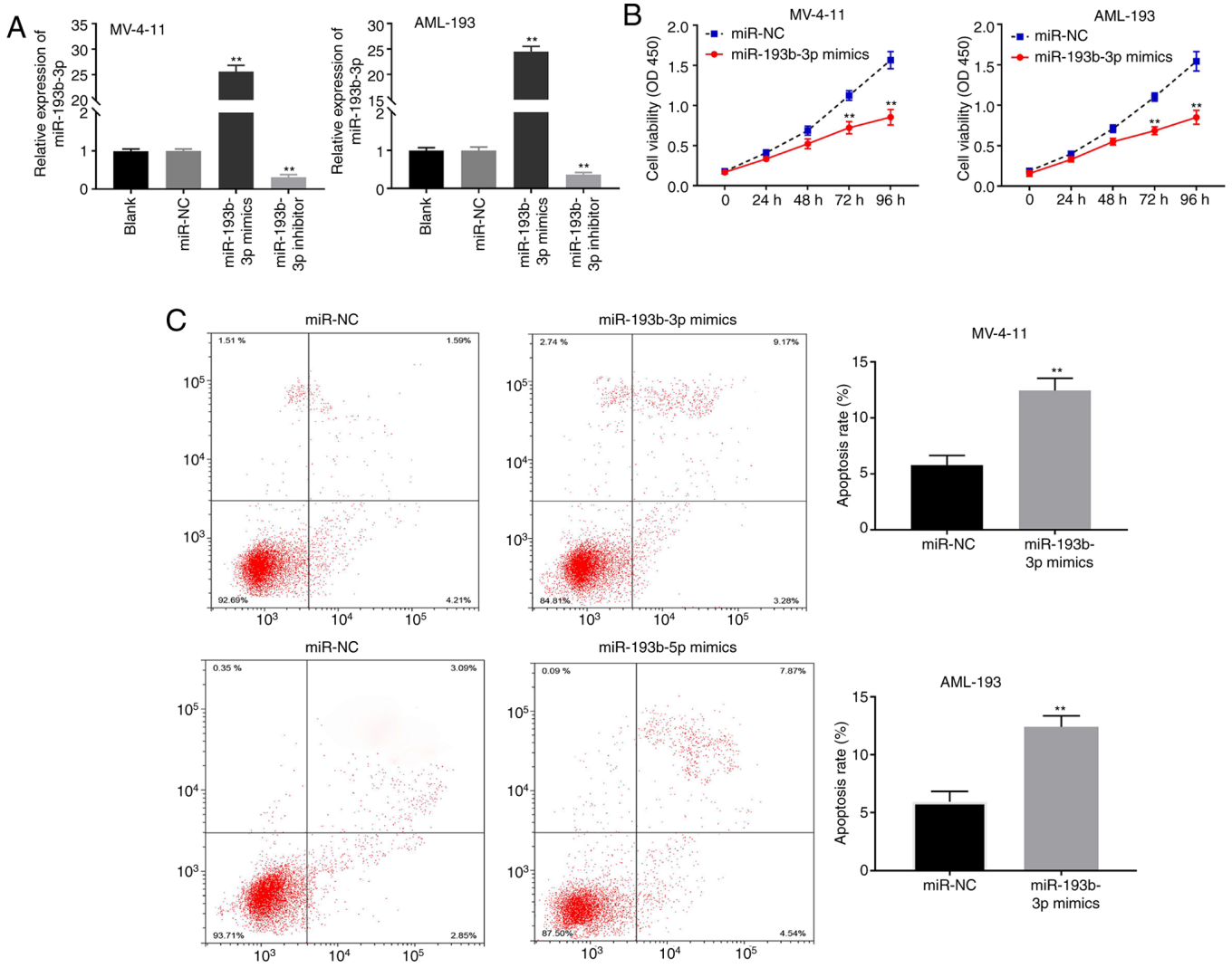

Figure 4. miR-193b-3p inhibits the viability and induces the apoptosis of AML cells. (A) Relative expression of miR-193b-3p in transfected MV-4-11 and AML-193 cells. **P<0.01 vs. blank control. The $2^{-\Delta\Delta Cq}$ method was used to analyse the relative mRNA expression level using U6 as an internal control. (B) The viability of transfected MV-4-11 and AML-193 cells was detected by MTT assay. (C) The apoptosis of transfected MV-4-11 and AML-193 cells was detected by flow cytometry. **P<0.01 vs. miR-NC. miR, microRNA; NC, negative control; OD, optical density.

Discussion

Numerous lines of evidence indicate that lncRNAs are involved in the biological and pathological processes of AML. For example, UCA1 and LINC00662 are overexpressed in AML tissues, and their knockdown has been shown to decrease cell proliferation and promote cell apoptosis in AML (26,27). In the present study, SNHG14 was found to be overexpressed in bone marrow tissues from patients with AML and AML cell lines, suggesting that SNHG14 may be associated with the development of AML. Previous studies have shown that SNHG14 functioned as an oncogenic gene in the progression of various human cancer types, including gastric cancer (13), colorectal cancer (28), cervical cancer (29) and hepatocellular carcinoma (30). Notably, SNHG14 silencing decreased the proliferation and facilitated the apoptosis of AML cells in the present study. These findings demonstrate that SNHG14 may function as an oncogenic gene in AML progression, which is consistent with previous data on the role of SNHG14 in tumours.

Numerous studies have shown that SNHG14 is involved in various types of human cancers via acting as a competitive ceRNA for different miRNAs. For example, SNHG14 accelerates the progression of cervical cancer by sponging miR-206 to regulate the expression of YWHAZ (29). SNHG14 facilitates the development of gastric cancer by targeting miR-145 to modulate SOX9 expression (13). The results of the present study demonstrated that SNHG14 acted as a sponge for miR-193b-3p and that the expression of miR-193b-3p in AML cells was negatively modulated by SNHG14, further clarifying the mechanism by which SNHG14 affects the progression of AML. miR-193b-3p has been reported to be underexpressed in tumour tissues and identified as an antitumour gene in human malignancies (17,18,31). Notably, Bhayadia *et al* (20) found that miR-193b-3p was underexpressed in patients with AML. Furthermore, Mets *et al* (32) revealed that miR-193b-3p acts as a tumour suppressor in T-cell AML. Consistent with previous studies, a significant decrease in miR-193-3p expression in patients with AML was observed in the present study. Additionally, these results demonstrated that miR-193b-3p

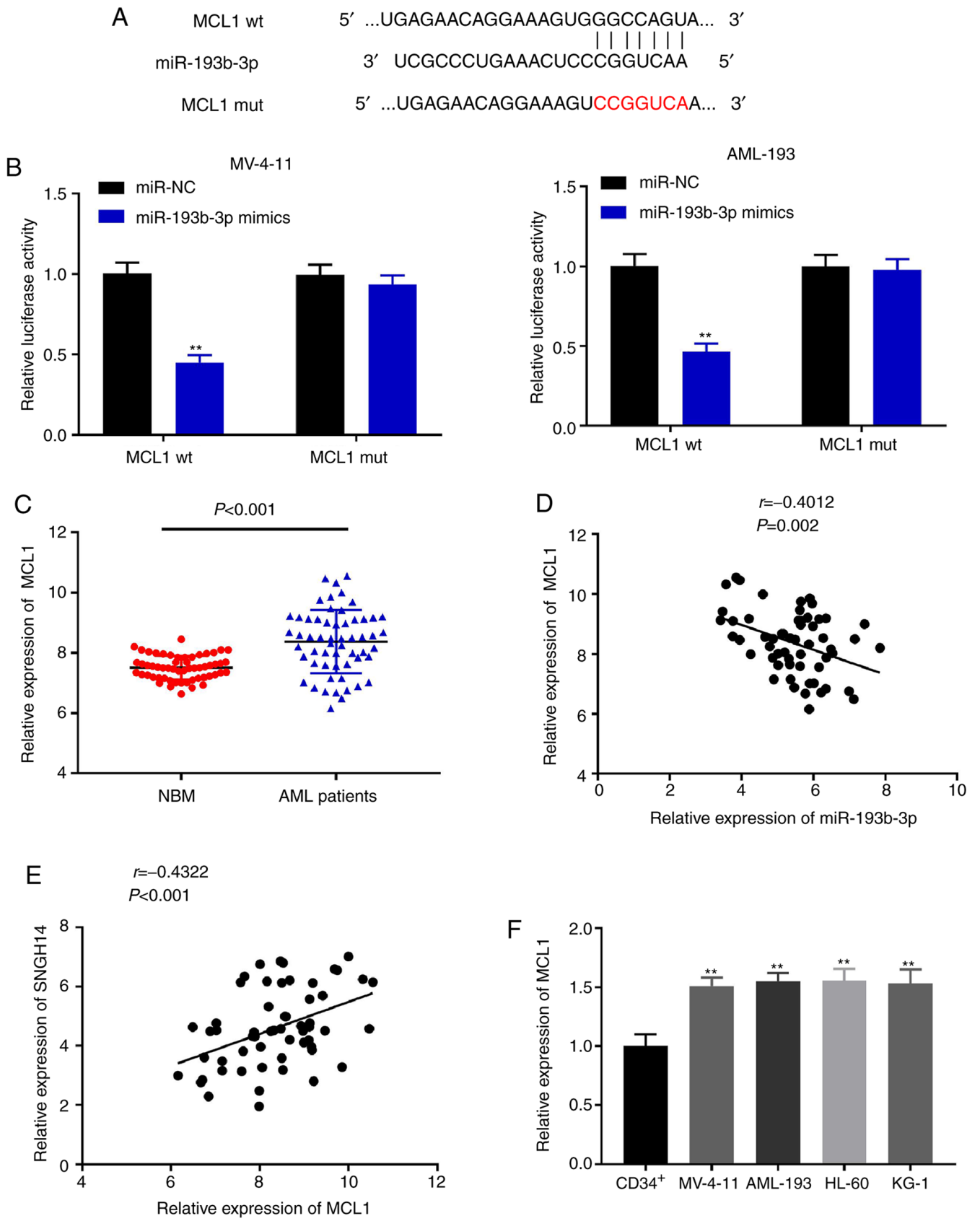

Figure 5. miR-193b-3p targets MCL1 in AML cells. (A) TargetScan was used to predict the binding site between miR-193b-3p and MCL1. (B) A dual luciferase reporter gene assay was employed to verify the target association between miR-193b-3p and MCL1. **P<0.01 vs. miR-NC. (C) Relative expression of MCL1 in 57 AML bone marrow tissues and NBM was detected by RT-qPCR. (D) Spearman's correlation analysis was performed to evaluate the correlation between MCL1 and miR-193b-3p expression. (E) Spearman's correlation analysis was performed to evaluate the correlation between MCL1 and SNHG14 expression. (F) Relative expression of miR-193b-3p in AML cell lines and human normal bone marrow CD34+ cells. **P<0.01 vs. CD34+ cells. The $2^{-\Delta\Delta Cq}$ method was used to analyse the relative mRNA expression level using GAPDH as an internal control. SNHG14, small nucleolar RNA host gene 14; miR, microRNA; NC, negative control; wt, wildtype; mut, mutated; NBM, normal marrow tissues; AML, acute myeloid leukaemia; MCL1, MCL1 apoptosis regulator BCL2 family member.

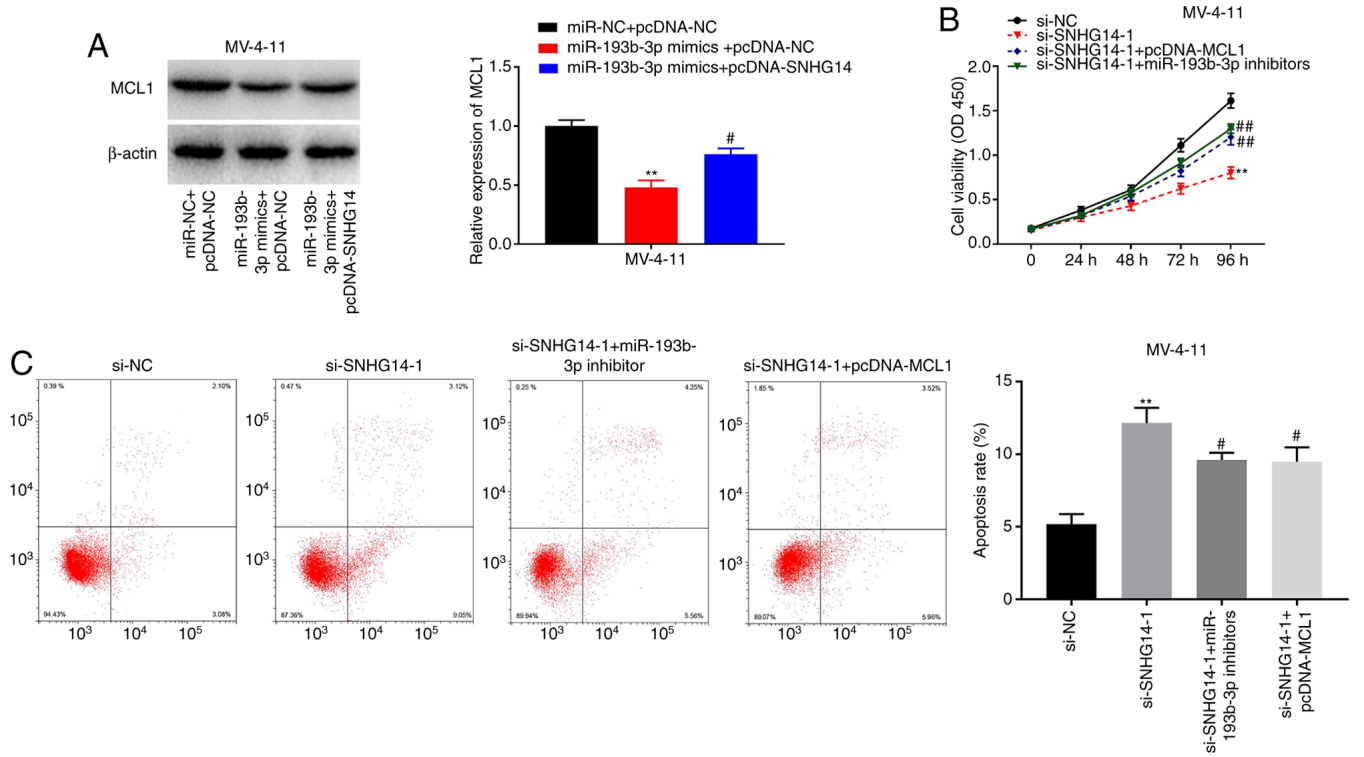

Figure 6. Silencing of SNHG14 inhibits the viability and induces the apoptosis of AML cells through regulating miR-193b-3p/MCL1 axis. (A) Western blot analysis was performed to detect the protein expression of MCL1 in transfected MV-4-11 cells. **P<0.01 vs. miR-NC+pcDNA-NC; $^{\#}$P<0.05 vs. miR-193b-3p mimics+pcDNA-NC. (B) The viability of transfected MV-4-11 cells was detected by MTT assay. **P<0.01 vs. si-NC; $^{\#\#}$P<0.01 vs. si-SNHG14-1. (C) The apoptosis of transfected MV-4-11 cells was detected by flow cytometry. **P<0.01 vs. si-NC; $^{\#}$P<0.05 vs. si-SNHG14-1. SNHG14, small nucleolar RNA host gene 14; miR, microRNA; NC, negative control; MCL1, MCL1 apoptosis regulator BCL2 family member; si, small interfering RNA; pcDNA, plasmid; OD, optical density.

inhibited the viability and induced the apoptosis of AML cells, suggesting that miR-193b-3p might function as an anti-tumour gene in AML. Xie *et al* (23) reported that SNHG14 promotes the proliferation and invasion of breast cancer cells by sponging miR-193a-3p. Similarly, the current findings indicate that SNHG14 might exert its oncogenic function in AML progression via sponging miR-193b-3p.

In the present study, starBase and DLR assays were performed and demonstrated that MCL1 was a direct target of miR-193b-3p. MCL1 has been reported to be overexpressed in numerous human cancer types, including malignant melanoma (33) liver cancer (34) and ovarian adenocarcinoma (35). In this study, MCL1 was highly expressed in AML bone marrow tissues and cells. Previous studies have shown that several miRNAs are involved in the progression of human cancer by targeting MCL1. For example, miR-107 impedes the progression of cervical cancer by directly targeting MCL1 (36). miR-125b impedes cell proliferation and invasion by negatively regulating MCL1 expression in gastric cancer (37). Notably, Huang *et al* (38) demonstrated that LINC00152 accelerated the cell proliferation rate in gastric cancer by modulating miR-193a-3p and its target gene MCL1. In the present study, the data indicated that miR-193b-3p targets MCL1, and the antitumour effect of SNHG14 knockdown on AML cells was rescued by overexpressing MCL1 or inhibiting miR-193b-3p, suggesting that SNHG14 acts as a ceRNA, modulating AML progression via the miR-193b-3p/MCL axis.

In conclusion, SNHG14 was found to be overexpressed in bone marrow tissues from patients with AML and AML cell lines. Furthermore, the silencing of SNHG14 decreased the proliferation and facilitated the apoptosis of AML cells. Additionally, miR-193b-3p was identified as a target of SNHG14, and suppressed the viability and increased the apoptosis rate of AML cells. More importantly, miR-193b-3p targeted MCL1, and the effects of SNHG14 knockdown on AML cell viability and apoptosis were abrogated by miR-193b-3p inhibition or MCL1 overexpression. In summary, the results of the current study indicate that silencing SNHG14 decreased the viability and induced the apoptosis of AML cells by modulating the miR-193b-3p/MCL1 axis, which suggests that SNHG14/miR-193b-3p/MCL1 may be a promising target for AML treatment.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Authors' contributions

XW and WL made substantial contributions to conception and design, acquisition of data, and analysis and interpretation of data. YC and LZ took part in drafting the article and revising it critically for important intellectual content, they are responsible for the experimental operation and the analysis of the experimental data, software application processing, the whole project management. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the East Hospital of Shouguang People's Hospital in accordance with the Declaration of Helsinki. Informed consents were obtained from legal guardians of children <18 years old.

Patient consent for publication

Informed consent was obtained for the release of patient data.

Competing interests

The authors declare that they have no competing interests.

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