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MiR-10b regulates the proliferation and apoptosis of pediatric acute myeloid leukemia through targeting HOXD10

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Abstract. – OBJECTIVE: To investigate the role of miR-10b in the proliferation and apoptosis of acute myeloid leukemia (AML), and to explore the underlying mechanism.

PATIENTS AND METHODS: The expression level of miR-10b in clinical AML cases and cell lines was detected by quantitative reverse transcription polymerase chain reaction (qRT-PCR). The interaction between miR-10b and homeobox D10 (HOXD10) was confirmed by qRT-PCR, Western blotting and Luciferase assay. The effect of miR-10b on biological functions of AML cell line (HL60) was analyzed in vitro. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay and colony formation assay were used to detect the proliferation and colony formation ability of AML cells, respectively. Meanwhile, flow cytometry and TUNEL staining were applied to measure cell cycle and apoptosis of AML cells, respectively.

RESULTS: miR-10b was significantly up-regulated in AML cases and cell lines. The potential target genes of miR-10b were analyzed by three public databases. Results showed that HOXD10 was a direct target of miR-10b. QRT-PCR, Western blotting and luciferase assay confirmed the regulatory effect of miR-10b on HOXD10. Overexpression of miR-10b accelerated the proliferation and colony formation ability of AML cells. Meanwhile, miR-10b overexpression decreased the percentage of AML cells in the G0/G1 phase when compared with S phase, and suppressed the apoptosis of AML cells. However, the addition of HOXD10 could reverse the effects of miR-10b.

CONCLUSIONS: MiR-10b could regulate the proliferation, colony formation, cell cycle and apoptosis of AML cells through targeting HOXD10, indicating that miR-10b might be a potential therapeutic target for the treatment of AML.

Key Words:
MiR-10b, Pediatric, Acute myeloid leukemia (AML), Homeobox D10 (HOXD10), HL60.

Introduction

Leukemia is a type of neoplastic disease originated from malignant clonal transformation of hematopoietic progenitor cells. It's characterized by an unlimited proliferation of leukemic cells in the bone marrow and other hematopoietic tissues. Meanwhile, leukemia ranks first among all childhood malignant tumors. Pediatric acute leukemia (AL), accounting for over 90% of childhood leukemia, includes acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Pediatric AML, whose incidence rate ranks next to ALL, is a kind of highly malignant and heterogeneous leukemia. The occurrence and development of pediatric AML is an uncontrolled process involving multiple genes and steps, which is related to myeloid cells[1]. The treatment response and prognosis of this disease are influenced by several factors. In the past few decades, the remission rate of induction chemotherapy for pediatric AML is close to 90%, and the 5-year survival rate of these patients has gradually increased to 55%-65%[2]. However, events of treatment failure and relapse still occur from time to time[3,4]. Molecular or genetic abnormalities have been hotspots of research in recent years. However, there are certain defects in traditional cytogenetic evaluation. Therefore, it is of great importance to explore the significance of novel molecular characteristics in the treatment and prognosis of AML.

In 1993, Lee et al[5] discovered the first micro-ribonucleic acid (miRNA) lin-4 in nematodes. They also proved that the small molecule acted on corresponding messenger RNA (mRNA) and inhibited its synthesis in the manner of complementary base pairing[6]. Since 2000, however, scientists have come to realize that similar

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conditions exist not only in nematodes but also in mammals[7]. Nowadays, it has been shown that such endogenous, small, noncoding RNAs with a length of about 18-25 nt are highly conserved and may regulate the expression of more than 60% of total human genes. Meanwhile, their post-transcriptional levels can affect mRNAs by suppressing translation or interfering with the stability of mRNAs[8]. Around 50% of genes encoded by miRNAs are located in fragile sites or related genomic regions of cancers, which exert the activities of "oncogenes" or "suppressor genes". Aberrant expression of miRNAs is closely correlated to the occurrence of diseases[9,10]. By regulating specific upstream and downstream target genes, miRNAs not only participate in the formation of leukemia, but also affect its treatment response and prognosis[11-14]. A majority of studies on leukemia-associated miRNAs are still focused on adults. However, the role of miRNAs in pediatric AML has not been fully elucidated.

MiRNA-10b (miR-10b), a member of the miRNA family, is located in the homeobox (HOX) gene cluster of 2p31.1[15]. As a special type of transcriptional regulator, miR-10b participates in the proliferation and differentiation of normal tissues. Researches have also demonstrated that miR-10b plays an extremely important role in the occurrence, development, metastasis and invasion of diseases including non-small cell lung cancer (NSCLC)[16], Huntington's disease[17], chronic stress depression[18], and nasopharyngeal carcinoma[19]. However, few studies have investigated the role of miR-10b in the development of pediatric AML.

In this study, we found that the expression level of miR-10b was significantly decreased in AML tissues. Moreover, the biological function of miR-10b in AML cells was further studied.

Patients and Methods

Clinical Cases and Cells

Bone marrow specimens were collected from 65 newly diagnosed AML children treated in the Department of Hematology at People's Hospital of Changle County. A total of 30 children with idiopathic thrombocytopenic purpura (ITP) were included in this study as controls. The study was approved by the Ethics Committee of Wendeng Orthopedics Hospital of Shandong Province. Informed consents of their parents were obtained before the study.

Human AML cell line HL60 and human embryonic kidney cell line HEK-293T were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). All cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA) complemented with 10% fetal bovine serum (FBS), 100 μg/mL streptomycin and 100 IU/mL penicillin (Gibco, Rockville, MD, USA) in a 37°C, 5% CO_2 incubator.

Luciferase Reporter Gene Assay

In TargetScan, miRDB and microRNA websites, we found that HOXD10 was a target gene of miR-10b. The binding sequence of miR-10b at the 3'-UTR of HOXD10 was mutated by using a point mutation kit (Agilent Technologies, Santa Clara, CA, USA). Subsequently, mutated HOXD10 (Mut-type) and non-mutant HOXD10 (WT-type) were connected with the pGL3-Basic luciferase reporter vector (Promega, Madison, WI, USA). PGL3-Basic vector with mutant HOXD10 was transfected into HL60 cells after lentivirus intervention on 24-well plates. According to the procedure of the Luciferase Reporter Gene Assay Kit, the same treatment was performed for the pGL3-Basic vector connected with the non-mutant HOXD10. Luciferase activity was then detected by a multi-function microplate reader.

Cell Transfection

MiR-10b mimics and si-HOXD10 were synthesized and transfected into AML cell line (HL60) to analyze the biological function of miR-10b. Next, three groups were established to elucidate the potential correlation between miR-10b and HL60 cells, including: the NC group (negative control), the miR-10b mimics (HL60 cells transfected with miR-10b mimics) group and the mimics + HOXD10 (HL60 cells transfected with miR-10b mimics and si-HOXD10) group. All the stuff was purchased from RiboBio (Guangzhou, China). Cell transfection was performed according to the instructions of lipofectamine RNAiMAX (Life Technologies, Gaithersburg, MD, USA).

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

The expression levels of miR-10b and HOXD10 in bone marrow specimens of AML cases and HL60 cells were detected *via* quantitative reverse transcription polymerase chain reaction (qRT-PCR). Total RNA was extracted in accordance with the manufacturer's protocol of

TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). SYBR green qPCR assay was used to measure the expression level of HOXD10. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. TaqMan miRNA assay (Applied Biosystems, Foster City, CA, USA) was used to measure the expression level of miR-10b normalized to miRNA U6. Primers used in this study were as follows: HOXD10, F: 5'-TCGGCCGAGGTCCCTTCGTA-3', R: 5'-TGGAGCTGCCTTCGGGGCTA-3'; miR-10b, F: 5'-TACCCTGTAGAACCGAATTTGT-3', R: 5'-GTGCAGGGTCCGAGGT-3'; U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Western Blot

Radio-immunoprecipitation assay (RIPA) lysate (Santa Cruz, Santa Cruz, CA, USA) was employed to extract total protein of HL60 cells. The concentration of extracted protein was measured by the bicinchoninic acid (BCA) reagent kit (Merck, Billerica, MA, USA). A total of 20 μg extracted protein were separated on polyacrylamide gels and transferred to nitrocellulose membranes. After blocking with the blocking buffer (TBS with 0.05% Tween 20, pH 7.6 with 5% skimmed milk), the membranes were washed with PBS and incubated with anti-HOXD10 and anti-β-GADPH (1:1000 dilution. Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight. Then the membranes were sealed with 5% skim milk at room temperature for 2 h. After washing for 3 times, the membranes were then incubated with electrochemiluminescence (ECL) (Millipore, Billerica, MA, USA) for luminescence generation. The proteins were visualized and detected, and the grey level of each protein was normalized to GADPH. The results were analyzed *via* the Image-J software.

Cell Proliferation

When cells grew to the logarithmic growth phase, they were collected and diluted into 1×10^6 cell suspension. Then the cells were seeded into 96-well plates with 5×10^3/100 μL per well. The wells only added with medium were used as blank controls. Cell viability was determined *via* MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay (Sigma-Aldrich, St. Louis, MO, USA). A total of 15 μL MTT reagents (500 μg/mL) was first added into each well for incubation for 2 h. Subsequently, the absorbance was measured by an enzyme-labeled spectrophotometer, followed by zero setting with blank controls.

Colony Formation Assay

HL60 cells were digested with trypsin, and the single cell suspension was then prepared. Next, 6×10^3 cells were cultured in a 60-mm culture dish for 14 d. Subsequently, bacterial colonies were fixed, stained with 0.5% crystal violet for 15 min, and washed for 3 times. 10 randomly selected fields were observed under a light microscope, and the number of colonies was counted (cell groups consisting of more than 50 cells were taken as one cell colony). Finally, the differences in the number of cell colonies in each group were compared. The experiment was repeated three times.

Cell Cycle

A single cell suspension was prepared 48 h after cell transfection for each group, respectively. The number of AML cells in different cell phases was measured using the cell cycle staining kit (Multi Sciences Biotech Co., Ltd., Hangzhou, China) by flow cytometry. The rate of cells in the G0/G1 or S phases was presented in the results.

Cell Apoptosis

The apoptosis of HL60 cells was detected by TUNEL assay according to the manufacturer's instructions (Roche, Basel, Switzerland). Horseradish peroxidase-mediated diaminobenzidine reaction was used to visualize TUNEL-positive cells, followed by counterstaining. Randomly selected fields were photographed at a magnification of 200×. The apoptosis index was used to measure the degree of cell apoptosis.

Statistical Analysis

Prism 6.02 Software (La Jolla, CA, USA) was used for statistical analysis. Student's *t*-test or *F*-test was used for comparison between two groups. All *p*-values were two-sided, and $p < 0.05$ were considered statistically significant.

Results

The Expression of miR-10b was Reduced in AML Cases and Cells

To explore the role of miR-10b in AML, we detected the expression level of miR-10b in bone

marrow specimens of AML and ITP cases, respectively. QRT-PCR results indicated that the expression of miR-10b was significantly higher in AML cases than that of ITP cases (Figure 1A). The same results were obtained at the cellular level (Figure 1B). Taken together, we believed that miR-10b might play a regulatory role in the progression of AML.

HOXD10 was a Direct Target of miR-10b

To investigate the putative and potential target of miR-10b, we searched three publicly available algorithms, including TargetScan, miRDB and microRNA websites. Results showed that the HOXD10 was predicted as a target gene of miR-10b (Figure 2A). Therefore, HOXD10 caught our attention and was implemented to our further study. Firstly, we detected the expression level of HOXD10 in AML bone marrow specimens and cell lines by qRT-PCR. Results indicated that HOXD10 was obviously down-regulated in AML tissues and cells when compared with controls (Figure 1C, 1D). Then, we established luciferase reporter vectors containing wild- or mutant-type miR-10b seed sequences of the HOXD10 3'-UTR. We found that transfection of miR-10b mimics significantly decreased the luciferase activity of wide-type HOXD10 3'-UTR reporter gene. However, miR-10b overexpression had no effect on mutant-type (Figure 2B), suggesting that the expression of HOXD10 could be regulated by miR-10b. Together, these results indicated that miR-10b might be correlated with HOXD10 during the progression of AML.

MiR-10b Decreased the Expression Level of HOXD10

Three groups were established in HL60 cells for similar experiments, including the miR-NC group, the miR-10b mimics group and the mimics + HOXD10 group.

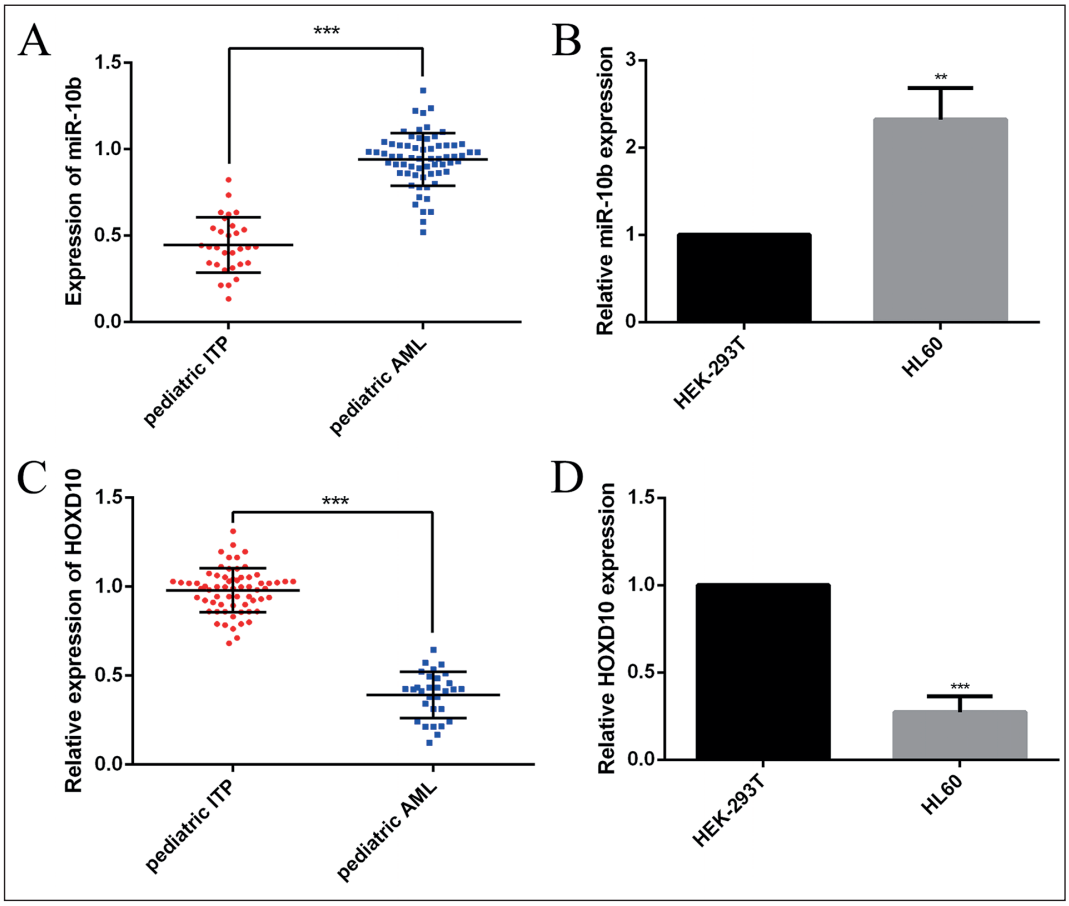

Figure 1. The expression levels of miR-10b and HOXD10 in AML bone marrow specimens and cells. ***A,*** and ***C,*** Difference in the expression of miR-10b and HOXD10 between AML and ITP bone marrow specimens. ***B,*** and ***D,*** Difference in the expression of miR-10b and HOXD10 between human AML cell line HL60 and human embryonic kidney cell line HEK-293T. (**$p < 0.01$, ***$p < 0.001$).

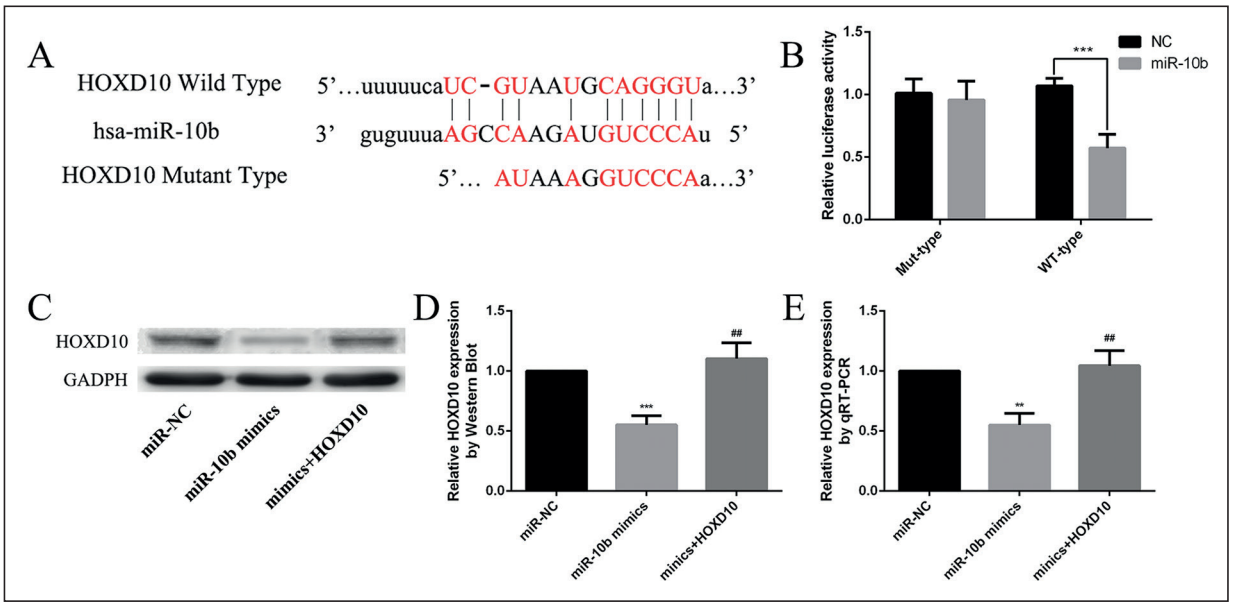

Figure 2. HOXD10 was a direct and functional target of miR-10b. HL60 cells were transfected with miR-10b mimics and inhibitor, respectively. ***A,*** Diagram of putative miR-10b binding sites of HOXD10. ***B,*** Relative activities of luciferase reporters (***$p < 0.001$). ***C,*** Protein expression of HOXD10. ***C,*** The protein expression level of HOXD10 was detected by Western blot. ***D,*** The mRNA expression level of HOXD10 was measured by qRT-PCR. All data were presented as means ± standard deviations. (**$p < 0.01$, ***$p < 0.001$ *vs.* NC group; $^{\#\#}p < 0.01$ *vs.* Mimics group).

QRT-PCR results showed that the expression level of HOXD10 was significantly decreased by miR-10b up-regulation in HL60 cells (Figure 3A). These data further illustrated the regulatory effect of miR-10b on the expression of HOXD10.

MiR-10b Promoted the Proliferation of HL60 Cells

MTT assay was performed to detect the proliferation rates of HL60 cells. Results suggested that the proliferation rate of HL60 cells was obviously accelerated by miR-10b mimics transfection. In contrast, the growth of AML cells was found remarkably encouraged in the mimics+HOXD10 group (Figure 3B). Moreover, colonies formed by HL60 cells transfected with miR-10b mimics were obviously higher in number and size than those of control cells (Figure 3C, 3D).

The Influence of miR-10b on the Cell Cycle of HL60 Cells

We then explored whether miR-10b had an effect on cell cycle. Surprisingly, we found that overexpression of miR-10b significantly decreased the percentage of cells in the G0/G1 phase when compared with the S phase. However, an opposite effect was found after HOXD10 intervention. These results suggested that miR-10b could encourage HL60 cells from crossing the node between G0/G1 phase and S phase, as well as entering the S phase for division and proliferation (Figure 3E, 3F).

MiR-10b Suppressed the Apoptosis of HL60 Cells

The apoptosis rates of HL60 cells were detected by TUNEL assay. As shown in Figure 3G and 3H, after transfection with miR-10b mimics, the percentage of apoptotic cells was obviously decreased. However, the apoptotic total rate was obviously higher in the miR-NC group and the mimics+HOXD10 group. The results indicated the anti-apoptosis effect of miR-10b.

Discussion

Acting as oncogenes or suppressor genes, miRNAs exert a crucial supervisory role in the human body. Meanwhile, altered expression levels of miRNAs can trigger multiple diseases including tumor. Therefore, they are a fairly ideal marker of diseases. Small RNAs are also extensively involved in the regulation of the human hematopoietic system, which may regulate the

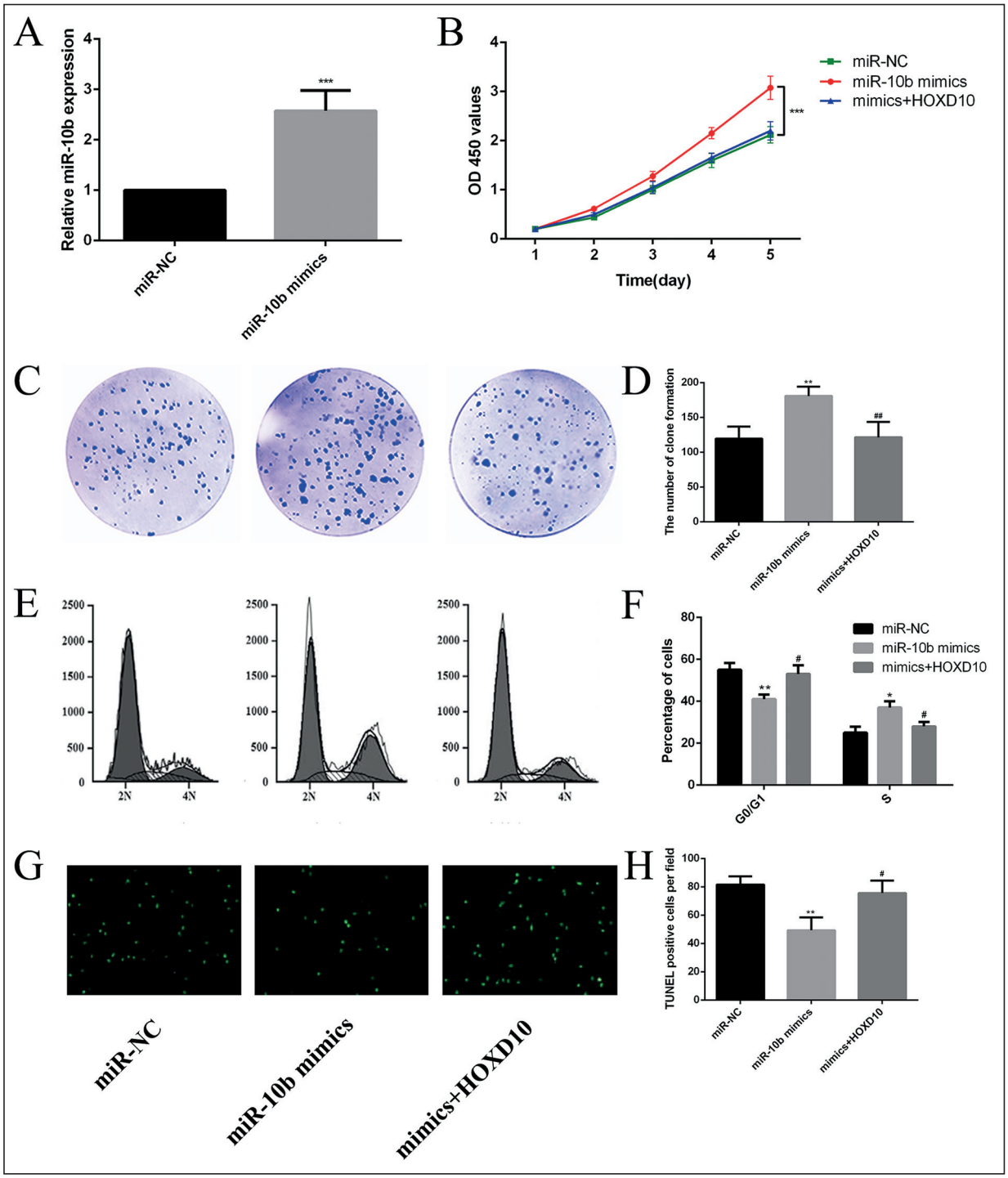

Figure 3. MiR-10b inhibited the biological function of AML cells. ***A,*** The changes of miR-10b expression after cell transfection was analyzed by qRT-PCR. ***B,*** Cell proliferation of AML cells was detected by MTT assay. ***C,*** and ***D,*** Assessment of colony formation assay. ***E,*** and ***F,*** Flow cytometry was applied to detect the cell cycle phases of AML cells. ***G,*** and ***H,*** TUNEL staining was used to measure the apoptosis of ALL cells. All data were presented as means ± standard deviations. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs.* NC group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ *vs.* Mimics group).

proliferation, apoptosis, differentiation, migration and other vital life processes at the post-transcriptional level through binding to the 3'-UTR of target mRNAs[8]. Although current researches on the function of miRNAs in leukemia treatment are still in the initial stage, a growing amount of

evidence has illustrated that the prospect of miRNAs in the diagnosis and treatment of leukemia cannot be ignored. However, a great challenge for bioinformatics is how to accurately predict miRNA markers for leukemia. Previous studies have found that miR-10b is abnormally expressed in relevant diseases. Therefore, we detected the expression level of miR-10b in the bone marrow tissues of 65 initially diagnosed AML patients by qRT-PCR in this study. It was discovered that the expression of miR-10b in pediatric patients initially diagnosed with AML was significantly higher than that of controls.

Since miR-10b has been verified to be highly expressed in AML, the key is how to screen its downstream target genes. In this study, a potential target gene HOXD10 was obtained by integrating several commonly used target gene prediction software. HOXD10 belongs to the HOX gene family. It not only controls the development and differentiation of normal cells, but also exerts an inhibitory effect on a variety of diseases such as breast cancer[20], prostate cancer[21], gliomas[22], and gastric cancer[23]. Luciferase assay revealed that miR-10b bound to the 3'-UTR of wild-type HOXD10, which inhibited the transcription of HOXD10. As a result, the fluorescence intensity was obviously weakened. However, mutant HOXD10 could not bind to miR-10b effectively, and the inhibitory effect disappeared. It has been proved that HOXD10 is a direct target gene of miR-10b. Subsequently, the expression level of HOXD10 in AML cells and tissues was detected, and the results were consistent with our expectations. In cells, HOXD10 exhibited the opposite expression model as miR-10b. However, in bone marrow tissues, the expression of HOXD10 in childhood AML patients was significantly lower than in ITP patients. However, AML children were confirmed to have a high expression of miR-10b. We, then, transfected miR-10b mimics into membranous cells (HL60 cells) to increase the expression of miR-10b. Results showed that there was a significantly negative regulatory relationship between miR-10b and HOXD10 at both mRNA and protein levels. Therefore, based on the above observations, we believed that HOXD10 was a direct or indirect target gene of miR-10b, which could also be regulated by miR-10b.

Subsequently, we further preliminarily explored the biological function of miR-10b and its target gene HOXD10 *in vitro*. As mentioned previously, with unlimited proliferation of leukemic cells as the characteristic manifestation, the leukemic cell line HL60 was transfected with liposomes. Results demonstrated that overexpression of miR-10b could prominently accelerate the proliferation and differentiation of leukemic cells and inhibit cell apoptosis. Nevertheless, miR-10b could arrest HL60 cells in the G0/G1 phase by up-regulating HOXD10 expression. Moreover, the functions of enhancing cloning, proliferation and anti-apoptosis of miR-10b were restrained markedly. All these results further proved that miR-10b could regulate the biological function of AML cells by targeting HOXD10.

As a research hotspot in recent years, the function of miRNAs has been explored in multiple biological fields, such as the occurrence, development, differentiation and drug resistance of pediatric AML and other diseases. MiRNAs are involved in the biological regulation of diseases by interference with corresponding target genes, thereby affecting the process of diseases. In the near future, these abnormally expressed miRNAs as well as their target genes, may not only act as powerful biomarkers for early diagnosis and prognosis evaluation, but also can become new target and direction for the treatment of pediatric AML according to the ideas proposed by Garzon[24].

Conclusions

We found that miR-10b could regulate the proliferation, colony formation, cell cycle and apoptosis of AML cells through targeting HOXD10, indicating miR-10b might be a potential therapeutic target for the treatment of AML.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Manola KN. Cytogenetics of pediatric acute myeloid leukemia. Eur J Haematol 2009; 83: 391-405.

2) Harrison CJ, Hills RK, Moorman AV, Grimwade DJ, Hann I, Webb DK, Wheatley K, de Graaf SS, van den Berg E, Burnett AK, Gibson BE. Cytogenetics of childhood acute myeloid leukemia: United Kingdom Medical Research Council Treatment trials AML 10 and 12. J Clin Oncol 2010; 28: 2674-2681.

3) Pui CH, Carroll WL, Meshinchi S, Arceci RJ. Biology, risk stratification, and therapy of pediatric acute leukemias: an update. J Clin Oncol 2011; 29: 551-565.

4) Creutzig U, van den Heuvel-Eibrink MM, Gibson B, Dworzak MN, Adachi S, de Bont E, Harbott J, Hasle H, Johnston D, Kinoshita A, Lehrnbecher T, Leverger G, Mejstrikova E, Meshinchi S, Pession A, Raimondi SC, Sung L, Stary J, Zwaan CM, Kaspers GJ, Reinhardt D. Diagnosis and management of acute myeloid leukemia in children and adolescents: recommendations from an international expert panel. Blood 2012; 120: 3187-3205.

5) Lee RC, Feinbaum RL, Ambros V. The C. Elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993; 75: 843-854.

6) Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. Elegans. Cell 1993; 75: 855-862.

7) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

8) Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215-233.

9) Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006; 6: 857-866.

10) Alvarez-Garcia I, Miska EA. MicroRNA functions in animal development and human disease. Development 2005; 132: 4653-4662.

11) Chen YL, Zhang ZX, Shou LH, Di JY. Regulation of DNA methylation and tumor suppression gene expression by miR-29b in leukemia patients and related mechanisms. Eur Rev Med Pharmacol Sci 2018; 22: 158-165.

12) Zhang X, Zeng J, Zhou M, Li B, Zhang Y, Huang T, Wang L, Jia J, Chen C. The tumor suppressive role of miRNA-370 by targeting FoxM1 in acute myeloid leukemia. Mol Cancer 2012; 11: 56.

13) Favreau AJ, McGlauflin RE, Duarte CW, Sathyanarayana P. MiR-199b, a novel tumor suppressor miRNA in acute myeloid leukemia with prognostic implications. Exp Hematol Oncol 2015; 5: 4.

14) Firatligil B, Biray AC, Baran Y. MiR-17 in imatinib resistance and response to tyrosine kinase inhibitors in chronic myeloid leukemia cells. J BUON 2013; 18: 437-441.

15) Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nat Genet 2007; 39: 673-677.

16) Huang J, Sun C, Wang S, He Q, Li D. MicroRNA miR-10b inhibition reduces cell proliferation and promotes apoptosis in non-small cell lung cancer (NSCLC) cells. Mol Biosyst 2015; 11: 2051-2059.

17) Hoss AG, Labadorf A, Latourelle JC, Kartha VK, Hadzi TC, Gusella JF, MacDonald ME, Chen JF, Akbarian S, Weng Z, Vonsattel JP, Myers RH. MiR-10b-5p expression in Huntington's disease brain relates to age of onset and the extent of striatal involvement. BMC Med Genomics 2015; 8: 10.

18) Jiang Y, Zhu J. Effects of sleep deprivation on behaviors and abnormal hippocampal BDNF/miR-10B expression in rats with chronic stress depression. Int J Clin Exp Pathol 2015; 8: 586-593.

19) Wang W. Study of miR-10b regulatory mechanism for epithelial-mesenchymal transition, invasion and migration in nasopharyngeal carcinoma cells. Oncol Lett 2017; 14: 7207-7210.

20) Vardhini NV, Rao PJ, Murthy PB, Sudhakar G. HOXD10 expression in human breast cancer. Tumour Biol 2014; 35: 10855-10860.

21) Mo RJ, Lu JM, Wan YP, Hua W, Liang YX, Zhuo YJ, Kuang QW, Liu YL, He HC, Zhong WD. Decreased HoxD10 expression promotes a proliferative and aggressive phenotype in prostate cancer. Curr Mol Med 2017; 17: 70-78.

22) Sun L, Yan W, Wang Y, Sun G, Luo H, Zhang J, Wang X, You Y, Yang Z, Liu N. MicroRNA-10b induces glioma cell invasion by modulating MMP-14 and uPAR expression via HOXD10. Brain Res 2011; 1389: 9-18.

23) Lin Z, Luo M, Chen X, He X, Qian Y, Lai S, Si J, Chen S. Combined detection of plasma ZIC1, HOXD10 and RUNX3 methylation is a promising strategy for early detection of gastric cancer and precancerous lesions. J Cancer 2017; 8: 1038-1044.

24) Garzon R, Marcucci G, Croce CM. Targeting microRNAs in cancer: rationale, strategies and challenges. Nat Rev Drug Discov 2010; 9: 775-789.