Abnormal regulation of microRNAs and related genes in pediatric β-thalassemia

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Abstract
Background: MicroRNAs (miRNAs) participate in the reactivation of γ-globin expression in β-thalassemia. However, the miRNA transcriptional profiles of pediatric β-thalassemia remain unclear. Accordingly, in this study, we assessed miRNA expression in pediatric patients with β-thalassemia.

Methods: Differentially expressed miRNAs in pediatric patients with β-thalassemia were determined using microRNA sequencing.

Results: Hsa-miR-483-3p, hsa-let-7f-1-3p, hsa-let-7a-3p, hsa-miR-543, hsa-miR-433-3p, hsa-miR-4435, hsa-miR-329-3p, hsa-miR-92b-5p, hsa-miR-6747-3p and hsa-miR-495-3p were significantly upregulated, whereas hsa-miR-4508, hsa-miR-20a-5p, hsa-let-7b-5p, hsa-miR-93-5p, hsa-let-7i-5p, hsa-miR-6501-5p, hsa-miR-221-3p, hsa-let-7g-5p, hsa-miR-106a-5p, and hsa-miR-17-5p were significantly downregulated in pediatric patients with β-thalassemia. After integrating our data with a previously published dataset, we found that hsa-let-7b-5p and hsa-let-7i-5p expression levels were also lower in adolescent or adult patients with β-thalassemia. The predicted target genes of hsa-let-7b-5p and hsa-let-7i-5p were associated with the transforming growth factor β receptor, phosphatidylinositol 3-kinase/AKT, FoxO, Hippo, and mitogen-activated protein kinase signaling pathways. We also identified 12 target genes of hsa-let-7a-3p and hsa-let-7f-1-3p and 21 target genes of hsa-let-7a-3p and hsa-let-7f-1-3p, which were differentially expressed in patients with β-thalassemia. Finally, we found that hsa-miR-190-5p and hsa-miR-1278-5p may regulate hemoglobin switching by modulation of the B-cell lymphoma/leukemia 11A gene.

Conclusion: The results of the study show that several microRNAs are dysregulated in pediatric β-thalassemia. Further, the results also indicate toward a critical role of let7 miRNAs in the pathogenesis of pediatric β-thalassemia, which needs to be investigated further.

KEYWORDS
B-cell lymphoma/leukemia 11A, let7 microRNA, microRNA sequencing, pediatric β-thalassemia, γ-globin reactivation

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1 | INTRODUCTION

β-Thalassemia is one of the most common genetic disorders of blood.1,2 There are three subtypes of β-thalassemia, that is, β-thalassemia minor, intermedia, and major.3 β-Thalassemia minor is caused by a deficiency in one β-globin gene, and patients usually have no symptoms or suffer from mild anemia. By contrast, β-thalassemia intermedia or β-thalassemia major is caused by double heterozygotes or homozygotes of the β-globin gene.4,5 Patients with β-thalassemia have major fetal health issues at birth and require lifelong blood transfusions and iron chelation treatments.6-8 However, current treatments for β-thalassemia major are associated with severe side effects,9,10 and alternative therapeutic approaches are still being developed.11-13 Therefore, strategies for the prenatal diagnosis of β-thalassemia are urgently needed, particularly in regions with a high prevalence of β-thalassemia.

In human developmental processes, β-like hemoglobin is switched from fetal γ-globin to adult β-globin at the time of birth.14,15 The absence of or reduction in β-globin in β-thalassemia may reactivate γ-globin expression and fetal hemoglobin (HbF) synthesis.16,17 Understanding the molecular mechanisms of the reactivation of fetal γ-globin expression in adult erythroid cells will provide novel therapies for patients with β-thalassemia.18 B-cell lymphoma/leukemia 11A (BCL11A) is a major suppressor of γ-globin19-21 and a therapeutic target of β-thalassemia.22 BCL11A can bind to the distal promoter regions of HbF and represses its expression.23,24 Moreover, BCL11A is a target of Krueppel-like factor 1 (KLF1), and inhibiting KLF1 expression is associated with repression of γ-globin.25,26 In erythroid cells, zinc finger and BTB domain-containing protein 7A (ZBTB7A) also blocks the expression of HbF,27 and KLF1 directly drives ZBTB7A expression by binding to its promoter regions.28,29 HBS1-like translational GTPase-MYB proto-oncogene (MYB) also plays a critical role in regulating HbF expression.30-33

MicroRNAs (miRNAs) regulate globin gene switching through post-transcriptional mechanisms.34,35 For example, hsa-miR-15a and hsa-miR-16 target the MYB transcription factor to elevate γ-globin expression.36,37 Moreover, hsa-miR-210, hsa-miR-30a, and hsa-miR-486-3p regulate γ-globin gene expression through the post-transcriptional regulation of BCL11A expression.38-40 Importantly, let7 miRNAs have also been implicated in the developmental progression of fetal and adult human erythroblasts.41 In K-562 cells, hsa-miR-26b specifically activates the transcription factor GATA1 to increase the expression of γ-globin.42 With the development of sequencing technology, more differentially expressed genes, long noncoding RNAs, and miRNAs have been identified in patients with β-thalassemia.43,44

Because of the different expression profiles of pediatric and adult blood cells,45 we hypothesized that pediatric and adult β-thalassemia may have different molecular characteristics. Differentially expressed miRNAs in adolescent or adult patients with β-thalassemia had been reported in a previous study.46 However, the miRNA expression profiles in pediatric β-thalassemia were unclear. Accordingly, in this study, we determined the miRNA expression profiles modulated in pediatric patients with β-thalassemia. Our analysis suggested that abnormal regulation of transcriptional networks mediated by let7 miRNAs was critical for the pathogenesis of pediatric β-thalassemia.

2 | MATERIALS AND METHODS

2.1 | Study participants

This study was approved by the institutional ethics committee of our hospital (approval no. 201, 2018). 5 ml peripheral blood was collected from five pediatric patients with β-thalassemia and five healthy controls in Fujian Maternity and Child Health Hospital, Fujian, China. The red cells were lysed using PAXgene Blood RNA Kit. The remaining mononuclear cells were used for further RNA isolation. The information and clinical conditions of the participants were also collected.

2.2 | Total RNA isolation

Total RNA from mononuclear cells was isolated using a miRNeasy Mini Kit (Qiagen) according to the manufacturer’s protocol. Briefly, mononuclear cells were lysed using lysis reagent, and 140 μl chloroform was added. The upper aqueous phase was then mixed with 100% ethanol, and the mixture was transferred to the column, washed, and eluted with RNase-free water.

2.3 | MicroRNA library construction and sequencing

Total RNA was used to prepare the miRNA sequencing library. After linker ligation, CDNA synthesis, and polymerase chain reaction (PCR) amplification, 135–155-bp PCR amplification fragments were selected. The library was denatured into single-stranded DNA, captured on an Illumina flow cell, amplified into clusters, and sequenced for 51 cycles using an Illumina NextSeq 500 sequencer (Illumina).

2.4 | Data processing

After sequencing, Solexa Chastity software was used for quality control. The linkers were removed using Cutadapt,47 leaving tags with lengths greater than or equal to 15 as the trimmed reads. We used miRDeep2 software to quantify known miRNAs.48 Counts per million reads (CPM) were used to represent the expression levels of miRNAs. The differentially expressed miRNAs between pediatric patients with β-thalassemia and healthy controls were determined using edgeR (version 3.32.1, http://bioconductor.org/packages/release/bioc/html/edgeR.html) in R statistics software,49 based on an absolute fold change greater than 1.5, p value less than 0.05, and CPM greater than or equal to 1.
2.5 | Volcano plot and Venn diagram plot
Volcano plots and Venn diagrams were generated using Fancy Volcano Plot and Wonderful Venn in TBtools software (version x32_1_064, https://github.com/CJ-Chen/TBtools), respectively.\textsuperscript{30}

2.6 | Heatmap presentation
Unsupervised heatmaps were generated using "pheatmap" package (version 1.0.12, https://cran.r-project.org/web/packages/pheatmap/) in R statistics software.

2.7 | Prediction of the target genes of miRNAs
The targets of miRNAs were predicted using miRDB (http://mirdb.org/\textsuperscript{51} and TargetScan Human 7.2 (http://www.targetscan.org/vert_72/\textsuperscript{52}) online tools. Target genes were predicted in both miRDB and TargetScan and were selected for further analyses.

2.8 | Biological process annotations and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway analysis
The enriched biological processes and KEGG signaling pathways were determined using the Database for Annotation, Visualization, and Integrated Discovery Web site (version 6.8; https://david.ncifcrf.gov).\textsuperscript{53,54} Statistical significance was set at $p < 0.05$.

2.9 | Gene Expression Omnibus (GEO) data collection
The gene expression matrix from patients with β-thalassemia and normal controls was deposited in the GSE56088 dataset from the GEO Web site (www.ncbi.nlm.nih.gov/geo). Microarray expression was analyzed using R software (version 3.5.0; https://www.r-project.org/).

2.10 | Analysis of the let7 miRNA-associated transcriptional network
A network of let7 miRNAs and predicted target genes was created using Cytoscape (http://www.cytoscape.org/).

2.11 | Statistical analysis
Box plots were generated using GraphPad Prism software. Statistical analysis was performed using Student’s t tests. Statistical significance was set at $p < 0.05$.

3 | RESULTS
3.1 | MiRNA expression profiling of pediatric β-thalassemia
Peripheral blood samples from five children diagnosed with β-thalassemia in our hospital and five healthy children were collected to identify differentially expressed miRNAs. The clinical characteristics of β-thalassemia and normal individuals are shown in Table 1. The mean age of the β-thalassemia and normal individuals was 3 years and two-six years, respectively. Compared with normal individuals, the mean hemoglobin, red blood cell, and hematocrit in the β-thalassemia patients were significantly lower. Moreover, the mean corpuscular hemoglobin level was also decreased in β-thalassemia patients. However, the mean corpuscular volume and platelet count were not significantly different between the β-thalassemia and normal cohorts.

Next, we performed miRNA sequencing to identify the miRNA expression profile of pediatric β-thalassemia. In total, 530 miRNAs were identified; among these, 111 miRNAs were upregulated, whereas 85 miRNAs were downregulated in patients with β-thalassemia. Three hundred and 330 miRNAs showed no differential expression in pediatric β-thalassemia and normal cohorts, respectively (Figure 1A). All differentially expressed miRNAs were further illustrated using a heatmap. The results showed that these miRNAs could clearly distinguish normal individuals from patients with β-thalassemia (Figure 1B).

\begin{table}[h]
\centering
\caption{Clinical characteristics of patients with β-thalassemia and normal individuals}
\begin{tabular}{|l|c|c|c|}
\hline
 & Control & β-thalassemia & p Value \\
\hline
\text{Sex (male/female)} & 3/2 & 3/2 & \\
\text{Age (years)} & 3.00 ± 1.22 & 2.6 ± 1.18 & 0.67 \\
\text{Hemoglobin (g/L)} & 124.6 ± 8.56 & 59 ± 5.34 & <0.001 \\
\text{Red blood cell (10^{12}/L)} & 4.47 ± 0.18 & 2.86 ± 0.49 & <0.001 \\
\text{Hematocrit (%)} & 36.24 ± 1.54 & 21.1 ± 4.68 & <0.001 \\
\text{Mean corpuscular volume (fl)} & 80.44 ± 3.05 & 73.92 ± 8.22 & 0.14 \\
\text{Mean corpuscular hemoglobin (pg)} & 27.88 ± 1.52 & 23.8 ± 2.87 & 0.02 \\
\text{Mean corpuscular hemoglobin concentration (g/L)} & 346.6 ± 18.09 & 302.4 ± 47.37 & 0.09 \\
\text{Platelet count (10^9/L)} & 258.4 ± 84.24 & 275.2 ± 141.33 & 0.82 \\
\hline
\end{tabular}
\label{table1}
\end{table}
Some miRNAs have been reported to be abnormally regulated in β-thalassemia. For example, hsa-miR-210 regulates γ-globin expression through the transcription factor BCL11A,38 and miR-15a and miR-16-1 elevate γ-globin expression through the transcription factor MYB.36,37 Additionally, hsa-miR-503 has been shown to be downregulated in patients with β-thalassemia.35 Consistent with these results, we found that hsa-miR-2100-3p, hsa-miR-15a-5p, hsa-miR-16-5p, and hsa-miR-503-5p were all downregulated in pediatric patients with β-thalassemia compared with that in normal individuals (Figure 1C). These results suggested that our miRNA sequencing data were accurate.

### 3.2 Top 10 miRNAs up- or downregulated in pediatric β-thalassemia

Based on fold changes, we further evaluated the top 10 miRNAs up- or downregulated in pediatric patients with β-thalassemia. Interestingly, let7 microRNAs were particularly altered in these patients. We found that hsa-let-7f-1-3p and hsa-let-7a-3p were significantly upregulated in pediatric patients with β-thalassemia (Table 2). By contrast, hsa-let-7b-5p, hsa-let-7i-5p, and hsa-let-7g-5p were significantly downregulated in these patients (Table 3). These results highlighted the abnormal regulation of let7 microRNAs in pediatric β-thalassemia.

Notably, hsa-miR-483-3p, hsa-miR-543, hsa-miR-433-3p, hsa-miR-4435, hsa-miR-329-3p, hsa-miR-92b-5p, hsa-miR-6747-3p, and hsa-miR-495-3p were upregulated (Table 2), whereas hsa-miR-4508, hsa-miR-20a-5p, hsa-miR-93-5p, hsa-miR-6501-5p, hsa-miR-221-3p, hsa-miR-106a-5p, and hsa-miR-17-5p were downregulated in pediatric β-thalassemia (Table 3). Most of these miRNAs had not previously been reported to be involved in the pathology of β-thalassemia.

### 3.3 Downregulation of let7 miRNAs in pediatric and adult patients with β-thalassemia

Differentially expressed miRNAs in adolescent or adult patients with β-thalassemia have been reported in a previous study.34 By integrating these datasets with our current results, we determined the unique and common miRNAs associated with pediatric and adult β-thalassemia. Four miRNAs, that is, hsa-miR-29b-3p, hsa-miR-192-5p, hsa-miR-215-5p, and hsa-miR-150-5p, were upregulated in pediatric and adult patients with β-thalassemia (Figure 2A). Five let7 microRNAs, that is, hsa-let-7b-5p, hsa-let-7i-5p, hsa-let-7f-5p, hsa-let-7e-5p, and hsa-let-7d-5p, were downregulated in pediatric and adult patients with β-thalassemia (Figure 2B). Moreover, three miRNAs, that is, hsa-miR-125b-5p, hsa-miR-130a-3p, and hsa-miR-130b-3p, were both altered in pediatric and adult patients with β-thalassemia (Figure 2B). These results partially validated our analysis and again suggested that let7 microRNAs were critical to the pathology of β-thalassemia.
Transcriptional networks mediated by let7 microRNAs

MiRNAs may regulate the expression of γ-globin through post-transcriptional silencing of target genes.\textsuperscript{34,35} Therefore, using miRDB and TargetScan online tools, we next identified the target genes of hsa-let-7a-3p and hsa-let-7f-1-3p, which were upregulated in pediatric patients with β-thalassemia. Interestingly, we found that hsa-let-7a-3p target genes were also predicted to be the target genes of hsa-let-7f-1-3p. Using miRDB, 1092 genes were predicted to be targets of hsa-let-7a-3p and hsa-let-7f-1-3p. In total, 238 genes were predicted to be targets of hsa-let-7a-3p and hsa-let-7f-1-3p.

Overlapping the results from miRDB and TargetScan, we identified 142 genes that were targets of hsa-let-7a-3p and hsa-let-7f-1-3p (Figure 3A). The connections of hsa-let-7a-3p and hsa-let-7f-1-3p with their target genes are shown in Figure 3B.

Similarly, the target genes of hsa-let-7b-5p and hsa-let-7i-5p, which were downregulated in pediatric and adult patients with β-thalassemia, were identified using the miRDB and TargetScan online tools. The target genes of hsa-let-7b-5p and hsa-let-7i-5p were quite similar. We identified 326 genes as targets of hsa-let-7a-3p and hsa-let-7f-1-3p (Figure 4A). The connections between hsa-let-7b-5p and hsa-let-7i-5p and their target genes were also demonstrated (Figure 4B).
FIGURE 3 Transcriptional network mediated by hsa-let-7a-3p and hsa-let-7f-1-3p. (A) Venn diagram showing common targets of hsa-let-7a-3p and hsa-let-7f-1-3p, predicted by miRDB and TargetScan. (B) Transcriptional networks of hsa-let-7a-3p, hsa-let-7f-1-3p, and their target genes.
Transcriptional network mediated by hsa-let-7b-5p and hsa-let-7i-5p. (A) Venn diagram showing common targets of hsa-let-7b-5p and hsa-let-7i-5p, predicted by miRDB and TargetScan. (B) Transcriptional networks of hsa-let-7b-5p, hsa-let-7i-5p, and their target genes.
3.5 Biological processes and signaling pathways associated with the target genes of let7 miRNAs

Next, the biological processes and signaling pathways associated with the target genes of hsa-let-7a-3p and hsa-let-7f-1-3p were determined. We found that targets of hsa-let-7a-3p and hsa-let-7f-1-3p were involved in the cellular response to interleukin-1, protein K11-linked ubiquitination, mesoderm development biological processes (Figure 5A), pancreatic secretion, and tuberculosis signaling pathways (Figure 5B). However, how these biological processes and signaling pathways are involved in the pathology of β-thalassemia is not clear.

The targets of hsa-let-7b-5p and hsa-let-7i-5p were involved in the negative regulation of translation, positive regulation of cell migration, regulation of cytokine biosynthesis, transforming growth factor (TGF) β receptor signaling pathway, and somatic stem cell population maintenance (Figure 5C). TGFβ is an important cytokine involved in cell migration and negatively regulates erythrocyte differentiation and maturation in the early stages of erythropoiesis. In addition, somatic stem cell population maintenance is associated with changes in genes in adult β-thalassemia.

The targets of hsa-let-7b-5p and hsa-let-7i-5p were associated with the phosphatidylinositol 3-kinase (PI3K)/AKT, FoxO, Hippo, and mitogen-activated protein kinase (MAPK) signaling pathways.

**Figure 5** Biological processes and signaling pathways associated with target genes of let7 miRNAs. (A) Biological processes associated with common targets of hsa-let-7a-3p and hsa-let-7f-1-3p. (B) Functional pathway enrichment analysis of common targets of hsa-let-7a-3p and hsa-let-7f-1-3p. (C) Biological processes associated with common targets of hsa-let-7b-5p and hsa-let-7i-5p. (D) Functional pathway enrichment analysis of common targets of hsa-let-7b-5p and hsa-let-7i-5p.
(Figure 5D). FOXO3 is a downstream transcription factor of the PI3K/AKT signaling pathway and is also involved in the FoxO signaling pathway.\(^{59}\) Previous results have shown that the PI3K/AKT signaling pathway and FOXO3\(^{61,62}\) are important regulators of erythroid maturation during erythropoiesis. The MAPK signaling pathway also participates in the development of erythropoiesis.\(^{63}\) KEGG pathway enrichment analysis identified several other pathways involved in the regulation of \(\gamma\)-globin expression and the development of erythropoiesis, including the Hippo signaling pathway, RAS signaling pathway, and transcriptional dysregulation (Figure 5D).

3.6 | Differentially expressed target genes of let7 miRNAs in \(\beta\)-thalassemia

In a previous study, differentially expressed genes between patients with \(\beta\)-thalassemia and healthy controls were studied; the data were deposited in the GSE56088 dataset. Using this dataset, we determined the expression of the targets of let7 miRNAs. BLC11A is a critical transcription factor that regulates hemoglobin switching and is a target of let7 miRNAs.\(^{64}\) First, we showed that the expression of BLC11A was significantly downregulated in patients with \(\beta\)-thalassemia compared with that in normal individuals in the GSE56088 dataset (Figure 6A).

We also identified 12 target genes of hsa-let-7a-3p and hsa-let-7f-1-3p, which were differentially expressed in patients with \(\beta\)-thalassemia (Figure 6B). GP1BA and ST8SIA4 were downregulated, whereas FOXN2, PLAG1, LEMD3, LRPC34, AP1S3, RAB11FIP2, NRBF2, RAB2A, PUM2, and TAOK1 were upregulated in patients with \(\beta\)-thalassemia. Additionally, 21 target genes of hsa-let-7b-5p and hsa-let-7i-5p were differentially expressed in patients with \(\beta\)-thalassemia (Figure 6C). STOX2, LIMD1, RBMS1, KCNQ4, GNPTAB, HMGAI1, SCD, IGF2BP2, STK40, FOXP2, and UTRN were downregulated, whereas FIGN, FRMD4B, PRPF38B, IGF2BP3, STARD3NL, SOCS4, C5orf51, TMPPE, E2F6, and FNDC3A were upregulated in patients with \(\beta\)-thalassemia.

3.7 | Hsa-miR-190-5p and hsa-miR-1278-5p may regulate hemoglobin switching by modulation of BCL11A

BLC11A is a critical transcription factor that regulates hemoglobin switching.\(^{19-21}\) Finally, we attempted to identify novel microRNAs regulating hemoglobin switching via modulation of BCL11A expression. Using miRDB, we found that two miRNAs, that is, hsa-miR-190-5p and hsa-miR-1278-5p, targeted BCL11A. In particular, hsa-miR-190-5p was perfectly matched to the two 3′ untranslated regions (UTRs) of BCL11A (Figure 7A). In addition, hsa-miR-1278-5p targeted the 3′ UTRs of BCL11A (Figure 7A). Moreover, hsa-miR-190-5p and hsa-miR-1278-5p were both downregulated in patients with \(\beta\)-thalassemia compared with those in normal controls (Figure 7B).
We further identified the target genes of hsa-miR-190-5p and hsa-miR-1278-5p. In addition to BCL11A, hsa-miR-190-5p also targeted ZNF99, FNDC3A, ORC4, PFDN4, ZNF382, EPC2, PHF20L1, ASAP2, WDR44, ZNF529, NHLRC2, ZFC3H1, CHMP7, YTHDF3, and TAPBP genes (Figure 7B). Furthermore, hsa-miR-190-5p also targeted NRBF2, ZRANB2, PAX8, DNAJB14, TIPARP, LHX6, IGF2BP2, and STK40 (Figure 7C). Interestingly, IGF2BP2 and STK40 were also target genes of hsa-let-7a-3p and hsa-let-7f-1-3p (Figure 6B). However, the functions of hsa-miR-190-5p and hsa-miR-1278-5p in hemoglobin switching and β-thalassemia need to be studied in greater detail.

4 | DISCUSSION

β-Thalassemia is a heterogeneous disease, and the clinical manifestations of β-thalassemia in pediatric and adult patients may be different.\(^{45,66}\) Because of the high hematopoietic stem cell repopulating capacity in children and the impaired functions of the bone marrow niche during aging, pediatric patients with β-thalassemia have a superior therapeutic response to hematopoietic stem cell gene therapy than adult patients with β-thalassemia.\(^{47}\) Therefore, pediatric and adult β-thalassemia may have different molecular characteristics. In this study, we showed that the miRNAs associated with pediatric and adult β-thalassemia were quite different. Only four miRNAs were upregulated, and eight miRNAs were downregulated in both pediatric and adult patients with β-thalassemia. These differences were partially due to the different cohorts and approaches; however, we also revealed that five let7 miRNAs, that is, hsa-let-7b-5p, hsa-let-7i-5p, hsa-let-7f-5p, hsa-let-7e-5p, and hsa-let-7d-5p, may be involved in the reactivation of γ-globin expression and HbF synthesis in pediatric and adult patients with β-thalassemia.

Consistent with these observations, reports have shown that the LIN28B/let7 axis directly regulates BCL11A expression to promote hemoglobin switching.\(^{48,65}\) Targeted inhibition of hsa-let-7a...
and hsa-let-7b reactivated the expression of HbF in erythroid cells.\textsuperscript{41} However, we showed that hsa-let-7a was upregulated in pediatric β-thalassemia, whereas hsa-let-7b was downregulated in pediatric β-thalassemia. The functions of hsa-let-7i-5p, hsa-let-7f-5p, hsa-let-7e-5p, and hsa-let-7d-5p in the regulation of γ-globin expression or HbF synthesis in erythroid cells have not been reported. Furthermore, we identified 21 target genes of hsa-let-7b-5p and hsa-let-7i-5p, which were differentially expressed in patients with β-thalassemia. We also found that the target genes of hsa-let-7b-5p and hsa-let-7i-5p were associated with the PI3K/AKT, FoxO, Hippo, and MAPK signaling pathways. How those pathways involved in the pathology of β-thalassemia should be further studied.

BCL11A,\textsuperscript{19-21} KLF1,\textsuperscript{29} and MYB\textsuperscript{31,32} are transcription factors that play important roles in hemoglobin switching. Hsa-miR-210 and hsa-let-7b-5p regulate γ-globin expression through BCL11A.\textsuperscript{38} Additionally, \textit{mir-15a} and \textit{mir-16-1} elevate γ-globin expression through the transcription factor MYB.\textsuperscript{36,37} Our results showed that hsa-miR-210, hsa-let-7b-5p, \textit{mir-15a}, and \textit{mir-16-1} were all downregulated in β-thalassemia. We believe that our data could help to identify more miRNAs associated with the BCL11A transcription factor. Indeed, our findings showed that two miRNAs, that is, hsa-miR-190-5p and hsa-miR-1278-5p, may regulate hemoglobin switching by targeting BCL11A. However, the functions of these miRNAs should be studied further.

To the best of our knowledge, this is the first study to identify differentially expressed miRNAs, particularly in pediatric β-thalassemia. Our results suggest that let7 miRNAs and their target genes are abnormally dysregulated in pediatric β-thalassemia. However, there were some limitations to the integrated analysis of the different datasets. Because of differences in cohorts and approaches, our analysis could not fully reveal the miRNA profiles associated with pediatric and adult β-thalassemia. In addition, identification of differentially expressed miRNA target genes in patients with β-thalassemia using the GSE56088 dataset may also have some bias. In our subsequent studies, we will collect a large cohort of β-thalassemia cases comprising patients of different ages and perform miRNA sequencing and mRNA sequencing simultaneously. Additionally, the functions of hsa-let-7i-5p, hsa-let-7f-5p, hsa-let-7e-5p, and hsa-let-7d-5p in the regulation of γ-globin expression will be studied in greater detail.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

HW performed the data analysis and wrote the manuscript. MH collected the blood samples. SY, YL, and YH helped with the collection of blood samples. HL and LP designed the study and supervised the work.

DATA AVAILABILITY STATEMENT

The data generated during the current study are available from the corresponding author upon reasonable request.

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