

# Identification of potential therapeutic target genes, key miRNAs and mechanisms in acute myeloid leukemia based on bioinformatics analysis

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**Abstract** The study was aimed to explore the underlying mechanisms and identify the potential target genes and key miRNAs for acute myeloid leukemia (AML) treatment by bioinformatics analysis. The microarray data of GSE9476 were downloaded from Gene Expression Omnibus database. A total of 64 samples, including 26 AML and 38 normal samples, were used to identify differentially expressed genes (DEGs) between AML and normal samples. The functional enrichment analysis was performed, and protein–protein interaction (PPI) network of the DEGs was constructed by Cytoscape software. Besides, the target miRNAs for DEGs were identified. Totally, 323 DEGs were identified, including 87 up-regulated and 236 down-regulated genes. Not only up-regulated genes but also down-regulated genes were related to hematopoietic-related functions. Besides, down-regulated genes were also enriched in primary immunodeficiency pathway. Tumor necrosis factor (*TNF*), interleukin 7 receptor (*IL7R*), lymphocyte-specific protein tyrosine kinase (*LCK*), CD79a molecule and immunoglobulin-associated alpha (*CD79A*) were identified in these functions. *TNF* and *LCK* were hub nodes in PPI networks. miR-124 and miR-181 were important miRNAs in this study. The hematopoietic-related functions and primary immunodeficiency pathway may be associated

with AML development. Genes, such as *TNF*, *IL7R*, *LCK* and *CD79A*, may be potential therapeutic target genes for AML, and miR-124 and miR-181 may be key miRNAs in AML development.

**Keywords** Acute myeloid leukemia · Differentially expressed genes · Bioinformatics analysis

## Introduction

Acute myeloid leukemia (AML) is one of the most common hematopoietic malignancies [1]. It is a heterogeneous clonal disorder of hematopoietic progenitor cells, characterized by uncontrolled proliferation of white blood cells that gradually replace normal hematopoiesis in the bone marrow. Approximately 6500 new cases (almost children and adolescents) in the USA of AML are diagnosed annually [2]. Its incidence increases progressively with age [3]. The 5-year survival rate of AML is less than 15 % [1]. Therefore, an improved understanding mechanism on the pathogenesis of AML would supply new insights for the diagnosis and treatment of AML.

Numerous studies in molecular biology have been done to decipher the pathogenesis of AML. The carcinogenesis of AML is a process of accumulation of genetic changes in hematopoietic cells growth, proliferation differentiation and apoptosis [3, 4]. For example, over-expression of KIAA1524 (*CIP2A*) is related to active cell proliferation and arrest cell differentiation [4]. It has been reported that the phosphoinositide 3-kinase (PI3-kinase) signaling pathway plays an important role in the regulation of tumor cell survival and proliferation [5]. The work of Grandage et al. [6] found that blocking PI3-kinase had direct antileukemic effect and that it was useful in the treatment of AML.

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Besides, miR-424 and miR-155 deregulation was involved in the pathogenesis of AML with nucleophosmin-1 and fms-related tyrosine kinase 3 mutations, respectively [7]. Although many factors have been detected in AML, the pathogenic mechanisms of AML are still not clearly demonstrated. It is lack of potential target genes for AML treatment.

The microarray data (GSE9476) were analyzed with microarray analysis to identify the differentially expressed genes (DEGs) between AML and normal samples by Stirewalt et al. [8]. In this study, we downloaded these data and used different methods to identify the DEGs. The functional analysis was performed, and protein–protein interaction (PPI) network was constructed. What is more, the target miRNAs for DEGs were identified. The purpose of this study was to explore the underlying mechanisms and identify the potential target genes and key miRNAs in the AML development.

## Data and methods

### Affymetrix microarray data

The gene expression profile data of GSE9476 based on the platform of GPL96 (Affymetrix Human Genome U133A Array) (Affymetrix Inc., Santa Clara, CA, USA) were downloaded from Gene Expression Omnibus (GEO) database in National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov/geo/>), which was deposited by Stirewalt et al. [8]. The datasets available in this analysis contained 64 samples, including leukemic blasts from 26 AML patients and normal hematopoietic cells from 38 healthy donors.

### Data preprocessing and differential expression analysis

The probe IDs were converted into corresponding gene names based on the annotation information on the platform. When multiple probes corresponded to a same gene, the average expression value was calculated to represent the gene expression level. The raw microarray data were normalized with the PreprocessCore package (<http://master.bioconductor.org/packages/release/bioc/html/preprocessCore.html>) [9] in R. The limma package (<http://master.bioconductor.org/packages/release/bioc/html/limma.html>) [10] in R was used to identify DEGs between AML and normal samples. Then,  $\log_2$ -fold change ( $\log_2FC$ ) was calculated to identify genes with expression-level differences. Only DEGs with  $|\log_2FC| > 1.0$  and adjusted  $P$  value  $< 0.05$  were selected.

### Functional enrichment analysis

Gene Ontology (GO) database (<http://geneontology.org/>) [11] is a set of a large number of gene annotation terms. Kyoto Encyclopedia of Genes and Genomes (KEGG) knowledge database (<http://www.kegg.jp/>) [12] is applied to identify the significantly enriched metabolic or signal pathways for target genes. Database for Annotation, Visualization and Integrated Discovery (DAVID) (<http://david.abcc.ncifcrf.gov/>) [13] is a tool that provides a comprehensive set of functional annotation for large list of genes. GO categories and KEGG pathway enrichment analyses were performed for DEGs using DAVID, respectively.  $P$  value  $< 0.05$  was the cutoff criterion for the functional enrichment analysis.

### Protein–protein interaction (PPI) network construction

Search Tool for the Retrieval of Interacting Genes (STRING, <http://www.string-db.org/>) [14] is an online database, which collects comprehensive information of proteins. The STRING online tool was applied to analyze the interactions of protein pairs, and only the interaction with confidence score  $> 0.4$  was selected as significant. PPI network was constructed with Cytoscape software (<http://cytoscape.org/>) [15].

### Prediction of target miRNAs for DEGs

Web-based Gene Set Analysis Toolkit (WebGestalt, <http://genereg.ornl.gov/webgestalt/>) [16] is an integrated system for exploring large sets of gene. WebGestalt was used to identify miRNAs corresponding to DEGs. MiRNAs with DEGs number  $> 2$  and raw  $P$  value  $< 0.05$  were identified as target miRNAs. The gene–miRNA network was visualized with Cytoscape software.

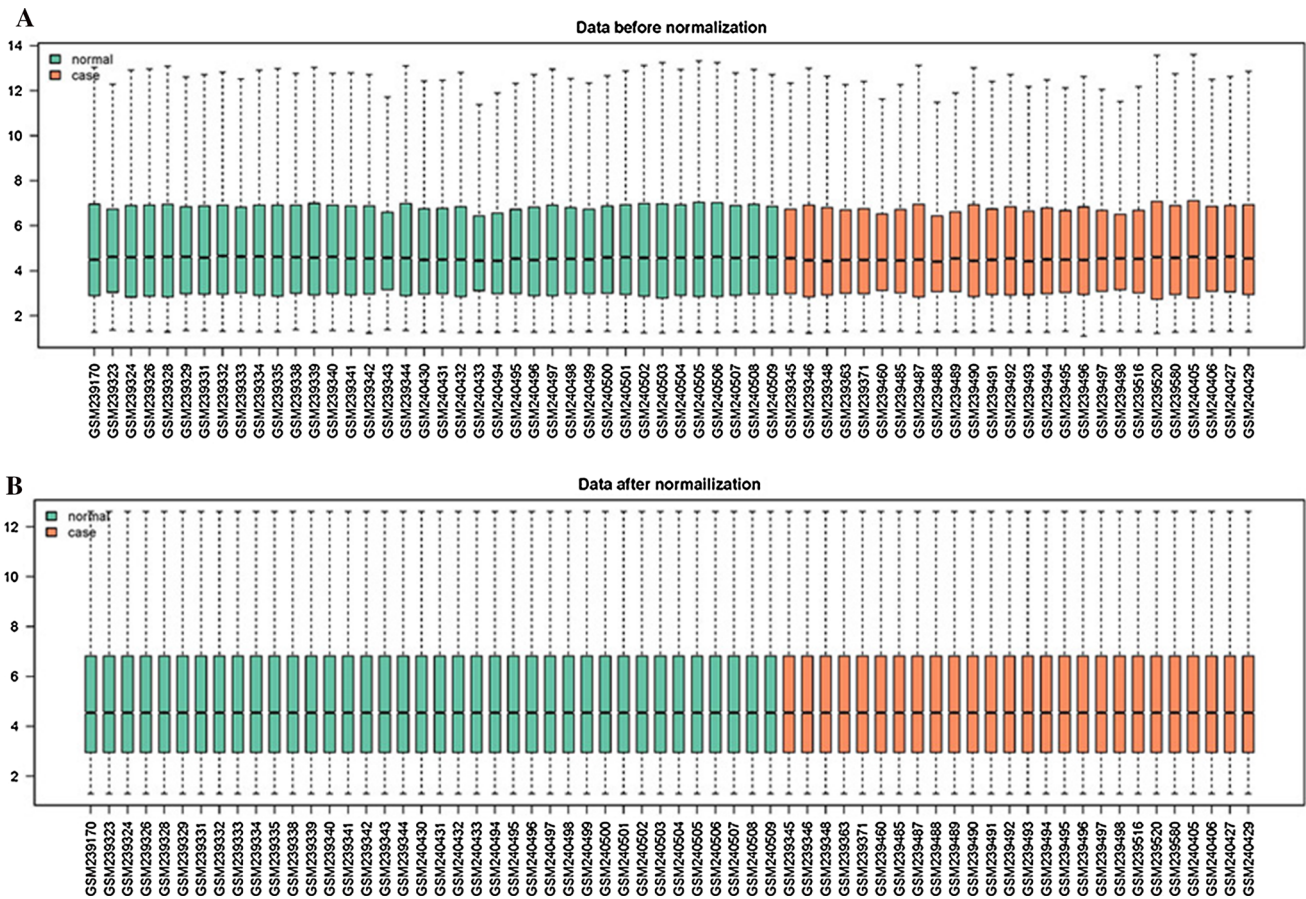
## Results

### Identification of DEGs

As shown in Fig. 1, the raw expression data were normalized after preprocessed. A total of 323 DEGs were identified between AML and normal samples, including 87 up-regulated genes and 236 down-regulated genes.

### Functional enrichment analysis

The top five GO terms enriched by up- and down-regulated genes are shown in Table 1, respectively. The up-regulated genes were mainly enriched in GO biological process (BP) terms of defense response, hemopoiesis and inflammatory



**Fig. 1** Box plots of data normalization. The *x*-coordinate represents samples; the *y*-coordinate represents gene expression values. The green boxes represent the normal samples, and the yellow boxes represent the AML samples

**Table 1** The top five GO terms enriched by up- and down-regulated genes

Category	Term	Count	<i>P</i> value
<i>Up-regulated genes</i>			
GOTERM_BP_FAT	GO:0006952 ~ defense response	14	2.17E-05
GOTERM_BP_FAT	GO:0030097 ~ hemopoiesis	8	2.75E-04
GOTERM_BP_FAT	GO:0042742 ~ defense response to bacterium	6	3.41E-04
GOTERM_BP_FAT	GO:0006954 ~ inflammatory response	9	3.52E-04
GOTERM_BP_FAT	GO:0048534 ~ hemopoietic or lymphoid organ development	8	4.94E-04
<i>Down-regulated genes</i>			
GOTERM_BP_FAT	GO:0030097 ~ hemopoiesis	19	7.46E-09
GOTERM_BP_FAT	GO:0048534 ~ hemopoietic or lymphoid organ development	19	3.36E-08
GOTERM_BP_FAT	GO:0002520 ~ immune system development	19	8.37E-08
GOTERM_CC_FAT	GO:0005886 ~ plasma membrane	84	1.57E-07
GOTERM_CC_FAT	GO:0044459 ~ plasma membrane part	54	7.93E-06

Count: enriched gene number in the category

BP biological process, CC cellular component

response. The most enriched GO terms of down-regulated genes were relevant with BP terms of hemopoiesis, hemopoietic or lymphoid organ development and immune system development.

The KEGG pathways of up- and down-regulated genes are shown in Table 2, respectively. The up-regulated genes were mainly enriched in hematopoietic cell lineage, systemic lupus erythematosus and AML. DEGs including

**Table 2** KEGG pathway enrichment analysis of up- and down-regulated genes

Term	Count	P value
<i>Up-regulated genes</i>		
hsa04640:Hematopoietic cell lineage	4	0.012428
hsa05322:Systemic lupus erythematosus	4	0.018128
hsa05221:Acute myeloid leukemia	3	0.042637
<i>Down-regulated genes</i>		
hsa05340:Primary immunodeficiency	6	2.68E-04
hsa04640:Hematopoietic cell lineage	6	0.014677
hsa00860:Porphyrin and chlorophyll metabolism	4	0.017681
hsa04650:Natural killer cell-mediated cytotoxicity	7	0.024373
hsa04060:Cytokine–cytokine receptor interaction	10	0.031833
hsa04660:T cell receptor signaling pathway	6	0.03527
hsa04062:Chemokine signaling pathway	8	0.037916

Count: enriched gene number in the KEGG term

tumor necrosis factor (*TNF*) and fms-related tyrosine kinase 3 (*FLT3*) were identified in the significantly function of hematopoietic cell lineage. Additionally, down-regulated genes were related to primary immunodeficiency, hematopoietic cell lineage and porphyrin and chlorophyll metabolism. The down-regulated genes including lymphocyte-specific protein tyrosine kinase (*LCK*) and CD79a molecule, immunoglobulin-associated alpha (*CD79A*) were enriched in primary immunodeficiency pathway. Down-expression of interleukin 7 receptor (*IL7R*) was identified in hematopoietic cell lineage pathway.

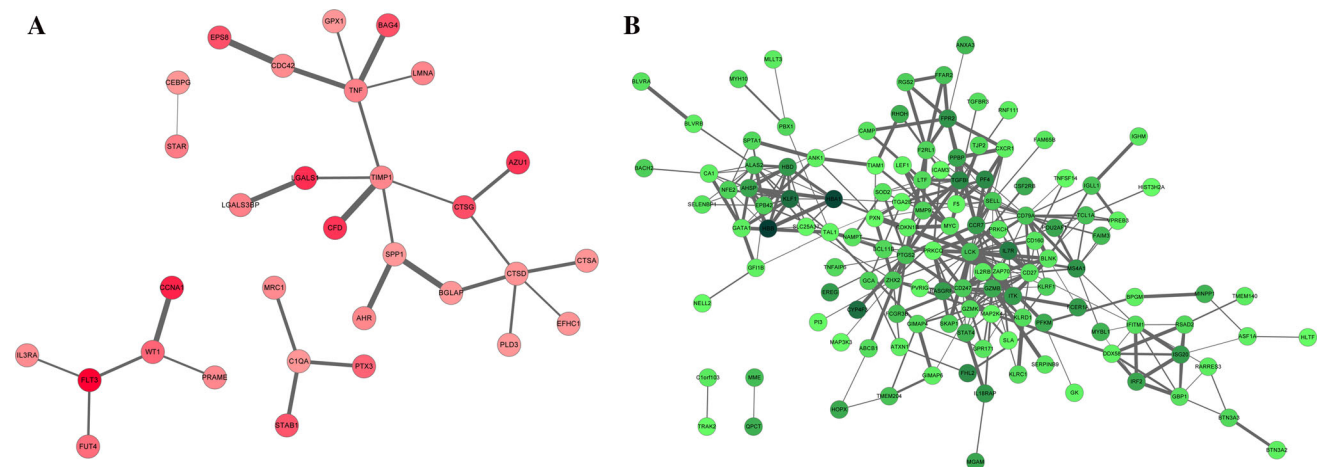
### PPI network construction

The PPI networks of up- and down-regulated genes are shown in Fig. 2, respectively. The up-regulated network

was constructed with 31 nodes and 28 edges (A). The proteins *TNF* (degree = 6), *TIMP1* metalloproteinase inhibitor 1 (*TIMP1*, degree = 6) and cathepsin D (*CTSD*, degree = 6) were hub nodes in this network. The down-regulated PPI network was constructed with 129 nodes and 316 edges (B). *LCK* (degree = 26), granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1) (*GZMB*, degree = 17), *CD79A* (degree = 17), chemokine (C–C motif) receptor 7 (*CCR7*, degree = 15) and *CD27* molecule (*CD27*, degree = 15) were hub proteins in this network.

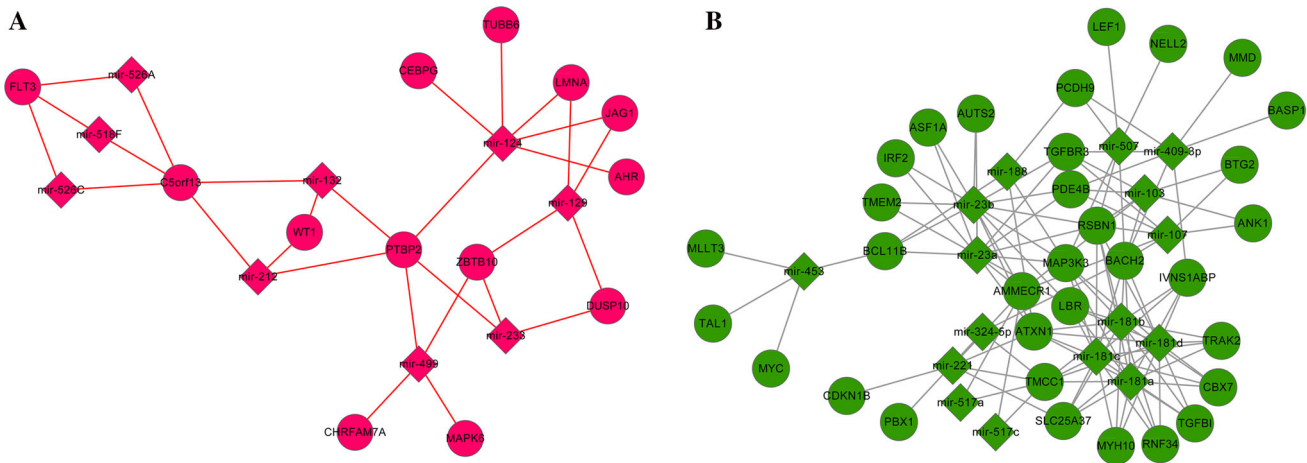
### Prediction of target miRNAs for DEGs

The target miRNAs for up- and down-regulated genes are presented in Fig. 3, respectively. In the up-regulated



**Fig. 2** PPI networks of DEGs. **a** PPI network of up-regulated genes. **b** PPI network of down-regulated genes. Nodes stand for proteins, and edges represent interactions between two proteins. The thickness of lines represents degree of interaction between nodes. Thicker line

indicates stronger interaction. The shades of color represent expression value difference of DEGs between AML and normal samples. The deeper the color, the greater the different expression value



**Fig. 3** Target gene-miRNA networks. **a** Gene-miRNA network of up-regulated genes. **b** Gene-miRNA network of down-regulated genes. Red node stands for up-regulated genes, and green node stands for down-regulated genes. Diamond stands for miRNA

gene-miRNA network, the miR-124 ( $n = 6$ ) and miR-499 ( $n = 4$ ) regulated the most up-regulated genes (A). Polypyrimidine tract-binding protein 2 (*PTBP2*) was regulated by 4 miRNAs, such as miR-124A and miR-499. Besides, miR-181 ( $n = 13$ ) and miR-23 ( $n = 12$ ) families regulated the most DEGs in down-regulated gene-miRNA network (B). Gene transmembrane and coiled-coil domain family 1 (*TMCCI*) was the hub node and was regulated by 8 miRNAs, including miR-181 family and miR-221.

**Discussion**

AML is one of the most common and deadly forms of hematopoietic malignancies [1]. Understanding the molecular mechanism of AML is of critical importance for management policy. In this study, the gene expression profile data of GSE9476 were downloaded from GEO database to identify DEGs between AML and normal samples using bioinformatics analysis. Totally, 323 DEGs including 87 up- and 236 down-regulated genes were selected. The functional enrichment analysis results showed that not only up-regulated genes but also down-regulated genes were related to hemopoiesis and hematopoietic cell lineage functions. Genes *TNF* and *IL7R* were identified in these functions. Besides, down-regulated genes, *LCK* and *CD79A*, were enriched in primary immunodeficiency pathway. *TNF* and *LCK* were hub nodes in PPI networks. miR-124 and miR-181 were important miRNAs in this study, and *TMCCI* was regulated by miR-181 family. These DEGs, their related functions and miRNAs may be involved in AML progress.

The hematopoietic-related functions (hemopoiesis and hematopoietic cell lineage pathway) were the significant functions in this study. *TNF* and *IL7R* were identified in

these functions. *TNF* is a monocyte-derived cytotoxin that has been implicated in tumor regression and cell apoptosis [17]. It has been reported that *TNF* induces potent growth inhibition of myeloid progenitor cells, and it is a potential growth stimulator of human multipotent hematopoietic cells [18]. *TNF* exerts proliferative effects on human AML blasts, and the effects are mediated by both p55 and p75 TNF-receptor (*TNFR*) in AML cells [19]. In this study, *TNF* was up-regulated gene in AML samples, which was consistent with a previous study that Cimino et al. [20] reported that serum *TNF* level was significantly increased in AML patients. It was hub node with high degree of 6 in the up-regulated PPI network, and *TNF* was enriched in hematopoietic functions, suggesting that hematopoietic-related gene *TNF* may play an important role in AML development. Additionally, *IL7R* was also identified in hematopoietic functions. *IL7R* is a receptor for *IL7* and is a protein found on the surface of cells. *IL7* belongs to interleukin family. *IL11* (another member of the interleukin family) is a stromal factor that has a number of hematopoietic-related effects cooperating with *IL3* and stem cell factor [21]. It has been reported that *IL7R* mutations may contribute to the pathogenesis of acute leukemias [22]. In our study, *IL7R* was down-regulated gene in AML samples, suggesting that it may play an important role in AML carcinogenesis. Our results showed that *TNF* and *IL7R* may be associated with AML progress and that hematopoietic-related functions may play a key role in the pathogenesis of AML.

A primary immunodeficiency pathway was identified as an important function associated with the progression of AML. *LCK* and *CD79A* were identified in this pathway. *LCK* is involved in primary T cell immunodeficiency [23], and it is a member of the Src family of tyrosine kinases. Members of the Src family of tyrosine kinases are signaling

intermediates that can regulate cell metabolism, proliferation, differentiation and migration in many liquid cancers including AML [24]. The work of Dos Santos et al. [25] indicated that silencing Lyn (a member of the Src family of tyrosine kinases) expression by small interfering RNA in AML cells strongly inhibited proliferation. However, the evidence concerning the impact of *LCK* in AML is rare. In this study, down-expressed *LCK* was enriched in the primary immunodeficiency pathway and was hub node in PPI network, suggesting that *LCK* may play an important role in AML progression by regulating primary immunodeficiency pathway. In addition, *CD79A* is a B lymphocyte antigen receptor. It is associated with B cell-mediated immunodeficiency [26]. *CD79A* is a marker for B cell neoplasms including AML in routinely processed tissue samples [27]. Cruse et al. [28] reported that the aberrant expression of *CD79A* portended a poor prognosis in AML. Down-expressed *CD79A* was hub node in PPI network in this study. It suggested that primary immunodeficiency pathway may affect the progress of AML by inhibiting the expression of *LCK* and *CD79A*.

Apart from DEGs and their functions, miRNAs such as miR-124 and miR-181 family may be important for the development of AML. It has been reported that miR-124 reduces the retinoblastoma protein phosphorylation [29], as well as its role as a tumor suppressor. The work of Vázquez et al. [30] found that the expression of MDS1 and EVI1 complex locus (*EVI1*) in AML patients was associated with decreased expression of miR-124. The *EVI1* gene codes for a transcription factor involved in the development and progression of high-risk AML [31]. Furthermore, miR-181 was reported that its expression was used for molecular risk assessment in AML patients [32]. In this study, *TMCCI* was the target gene of members of the miR-181 family. *TMCCI* was down-expressed in AML samples, and its expression was inversely correlated with the expression level of miR-181. Therefore, it indicated that miR-181 was over-expressed in AML. It has been reported that high expression of miR-181 may reduce the aggressiveness of AML [32]. Taking these factors into account, we speculated that miR-124 and miR-181 may be key regulators in AML development.

In conclusion, our study shows that hematopoietic-related functions and primary immunodeficiency pathway may be closely associated with AML development. Genes such as *TNF*, *IL7R*, *LCK* and *CD79A* may be potential therapeutic target genes for AML. miR-124 and miR-181 may be key miRNAs in AML development. However, further studies are still required to confirm our results.

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**Conflict of interest** None.

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