 Investigating the microRNA-mRNA regulatory network in acute myeloid leukemia

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Abstract. Acute myeloid leukemia (AML) is a common myelogenous malignancy in adults that is often characterized by disease relapse. The pathophysiological mechanism of AML has not yet been elucidated. The present study aimed to identify the crucial microRNAs (miRNAs/miRs) and target genes in AML, and to uncover the potential oncogenic mechanism of AML. miRNA and mRNA expression-profiling microarray datasets were downloaded from the Gene Expression Omnibus database. Differential expression analysis was performed and a regulatory network between miRNAs and target genes was constructed. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses were used to predict the biological functions of the differentially expressed genes. Reverse transcription-quantitative polymerase chain reaction analysis was employed to verify the expression levels of miRNAs and target genes in AML patient samples. A total of 86 differentially expressed miRNAs and 468 differentially expressed mRNAs between AML and healthy blood samples were identified. In total, 47 miRNAs and 401 mRNAs were found to be upregulated, and 39 miRNAs and 67 mRNAs were found to be downregulated in AML. A total of 223 miRNA-target genes pairs were subjected to the construction of a regulatory network. Differentially expressed target genes were significantly enriched in the Wnt signaling pathway (hsa04310), melanogenesis (hsa04916) and pathways in cancer (hsa05200). Significantly differentially expressed miRNAs and target genes, including hsa-miR-155, hsa-miR-192, ANXA2, FZD3 and PLAG1 may be associated with the development of AML via the involvement of the Wnt signaling pathway, melanogenesis and other cancer-associated signaling pathways.

Introduction

Leukemia is one of the 10 leading causes of cancer-associated mortality in China; in 2011 there were 27,907 mortalities in men and 19,708 mortalities in women from leukemia (1). The four types of Leukemia are acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia (AML) and chronic myeloid leukemia. AML accounts for ~80% of cases of acute leukemia in adults (2).

AML is a highly heterogeneous leukemia associated with excessive progenitor cell proliferation and a differentiation block for cell-cycle arrest. AML is often caused by karyotypic abnormalities, including chromosomal translocations, deletions and inversions (3,4). Etiological factors driving AML development remain unclear, but lifestyle and environmental exposures, including obesity and smoking, are reported to be associated with the disease (5).

The French-American-British (FAB) and World Health Organization (WHO) systems are the two main AML classification systems. The FAB system classifies AML into subtypes M0-M7 according to the cell type from which AML develops and the degree of maturation of the cells (6). According to the 2008 WHO Classification, AML are classified into six subgroups: AML with recurring genetic abnormalities, AML with myelodysplasia-related changes, therapy-related myeloid neoplasms, not otherwise specified AML, myeloid proliferations related to down syndrome and blastic plasmacytic dendritic cell neoplasms, with diagnosis performed according to morphology, cytochemistry, immunophenotype, genetics and clinical features (7).

Karyotypic abnormalities and genetic mutations are associated with AML progression and prognosis. Translocation of chromosomes 15 and 17 [t(15;17)], t(8;21) or inversion of chromosome 16 is predictive of a relatively good prognosis (8), whereas deletion of chromosome 7, deletion of 5q or >3 chromosomal abnormalities is predictive of a poor prognosis in AML patients (9,10). Fms-like tyrosine kinase 3-internal

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duplication (FLT3-ITD) and nucleophosmin (NPM1) are the two most commonly mutated genes in AML patients. Mutations to NPM1 occur in 50% of AML patients, whereas mutations to FLT3-ITD occur in 30%. FLT3-ITD, KIT proto-oncogene receptor tyrosine kinase and brain and acute leukemia, cytoplasmic gene mutations have a negative impact on AML prognosis (11,12), while NPM1 and CCAAT/enhancer binding protein-α have a positive impact on prognosis (12-14).

At present, the pathogenic mechanism of AML is unclear. Acute promyelocytic leukemia (APL) is an M3 subtype of AML according to the FAB classification system. Overexpression of microRNA (miRNA/miR)-125a decreases APL NB4 cell proliferation, the inhibition of cell cycle progression and the promotion of cell apoptosis by targeting the ErbB pathway in APL (15). miR-150 expression induces the myeloid differentiation of human acute leukemia cells and normal hematopoietic progenitors. In AML patient samples and cell lines, miR-150 expression is low or absent, which contributes to the blocking of myeloid differentiation in acute leukemia cells (16).

The aim of the present study was to identify featured target genes of significantly differentially expressed miRNAs in AML by comparing AML samples with healthy ones, and analyzing the correlation of miRNA-target genes. Candidate target genes identified by these approaches may provide the groundwork for the elucidation of the mechanism of AML. However, further investigation of the potential function of these genes in the treatment of AML is required.

Materials and methods

Transcriptomics datasets. In the Gene Expression Omnibus (GEO; http://ncbi.nlm.nih.gov/geo/) (17), only the studies comparing AML and healthy blood were assessed. A total of 6 studies were assessed in which the global profile of gene expression was measured in AML patients' blood samples, with accession numbers GSE48558, GSE35008, GSE35010, GSE24395, GSE17054 and GSE51908. The details of studies, including the platform, number of cases, controls, year and author, were extracted and assessed.

Data processing and identification of differentially expressed miRNAs and mRNAs. Raw expression datasets were downloaded from the GEO and the raw datasets were preprocessed by log₂ transformation and Z-score normalization. Limma, which is a linear model for microarray data analysis, was utilized to analyze the differentially expressed miRNAs and mRNAs between the AML and healthy control samples (18). A false discovery rate (FDR) of <0.05 was set as the threshold of differentially expressed miRNAs and mRNAs.

miRNA target gene prediction. Targets genes for differentially expressed miRNAs were predicted via miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/). Over 50,000 miRNA-target interactions in the miRTarBase database have been validated by experiments such as reporter assays, western blotting or microarray experiments with overexpression or knockdown of miRNAs (19,20).

Construction of regulatory miRNA-mRNA networks. The miRNA-mRNA interaction network of differentially expressed miRNA and mRNA was visualized using Cytoscape (http://cytoscape.org) (21). This software presents the regulation between miRNA and mRNA as two-dimensional network with nodes and edges, which represent miRNA-target gene associations.

Functional enrichment analysis of the differentially expressed target genes. To obtain the functions of differentially expressed target genes, Gene Ontology (GO) terms (22) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (23) pathways were enriched using GOEAST (http://omicslab.genetics.ac.cn/GOEAST) (24) and GeneCodis (http://genecodis.cnb.csic.es/analysis), respectively (25). P<0.01 and FDR <0.05 were set as the thresholds of significance for GO terms and KEGG pathway analysis.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The blood samples were collected from 3 males with AML treated in Qilu Hospital of Shandong University (Shandong, China) in 2015, with a mean age of 45.6 years. In addition, 3 normal blood samples were also included with corresponding gender and age. Total RNA of fresh blood samples were extracted by TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions. Use of these samples was approved by the Ethics Committee of Qilu Hospital of Shandong University (Jinan, China). The SuperScript III Reverse Transcription kit (Invitrogen; Thermo Fisher Scientific, Inc.) was used to synthesize the cDNA according to the manufacturer's instructions. RT-qPCR was performed using Power SYBR Green PCR Master mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) on the Applied Biosystems 7500 (Applied Biosystems; Thermo Fisher Scientific, Inc.). The RT-qPCR cycling conditions were 1 cycle of 95˚C for 10 min, followed by 45 cycles of 95˚C for 15 sec and 60˚C for 60 sec. The miRcute miRNA First-Strand cDNA kit (Tiangen Biotech Co., Ltd., Beijing, China) and the miRcute miRNA qPCR Detection kit (Tiangen Biotech Co., Ltd.) were used for miRNA expression level detection. The RT-qPCR cycling conditions for miRNA were 1 cycle of 94˚C for 2 min, followed by 45 cycles of 94˚C for 20 sec and 60˚C for 34 sec. U6 small nuclear RNA and β-actin was used as internal controls for miRNA and mRNA detection, respectively. The relative expression of target genes was calculated using the 2ΔΔCq method (26). At least three independent experiments were performed. The PCR primers used were as follows: hsa-miR-155 forward, 5'-TAAAGCTAATCGTGATAGGGTT-3' and reverse, GTGCAAGGGTCCAGGT; hsa-miR-192 forward, 5'-TGACCTATAGATTGACAGCC-3' and reverse, GTGCAAGGGTCCAGGT; frizzled class receptor 3 (FZD3) forward, 5'-TCTCCCTCTAGGCTGGCAGTATTACCC-3' and reverse, 5'-GCAGGGTCCAGGT-3' and reverse, 5'-TCCACCACAAGGTACGGCAGC-3'.

Statistical analysis. RT-qPCR experimental data was expressed as the mean ± standard deviation. Statistical significance was evaluated using an unpaired Student's t-test. P<0.05 was considered to indicate a statistically significant difference.
Results

Differentially expressed miRNAs and mRNAs in AML. A total of 5 mRNA and 1 miRNA expression profiles datasets, including 137 AML and 84 healthy samples were downloaded from the GEO, normalized and processed (Table I) (27-31). Differentially expressed genes between AML and normal samples, including 86 miRNAs and 468 mRNAs, were screened with a threshold of FDR<0.05. Of the 86 miRNAs, 47 were upregulated and 39 were downregulated in AML samples compared with the normal samples; of the 468 mRNAs, 401 were upregulated and 67 were downregulated. The top 10 upregulated and downregulated miRNAs are shown in Table II (the full list of differentially expressed miRNAs and mRNAs is not shown).

Construction of miRNA‑mRNA regulatory networks. The miRTarBase database was used to predict the target genes of the 47 upregulated and 39 downregulated miRNAs in AML; 223 miRNA‑target gene pairs, including 31 differentially expressed miRNAs and 153 target genes, were visualized using Cytoscape software (Fig. 1). A total of 55 differentially expressed miRNAs, including hsa‑miR‑29b‑3p and hsa‑miR‑194, were not displayed in the network, as the 55 differentially expressed miRNAs were not available in miRTarBase database (data not shown). hsa‑miR‑26b, hsa‑miR‑192, hsa‑miR‑21, hsa‑miR‑181a and hsa‑miR‑155 regulated 43, 25, 26, 15 and 11 targets, respectively, and displayed the highest connectivity. Pleomorphic adenoma gene 1 (PLAG1), high‑mobility group AT‑hook 2, RUN‑domain‑containing 3B, transmembrane protein 2, TNF‑α induced protein 3 and family with sequence similarity 3 member C, which were regulated by 7, 5, 4, 4, 4 and 4 miRNAs, respectively, were the mRNAs with the highest connectivity (Fig. 1).

Functional analysis of miRNA target genes. GO classification and KEGG pathway analyses were used to obtain the biological functions of miRNA target genes, including biological process, cellular component, molecular function and signaling pathway. The threshold of GO classification was set as P<0.01. Negative regulation of blood coagulation (GO:0030195, P=1.83x10^-24), negative regulation of hemostasis (GO:1900047, P=1.83x10^-24) and negative regulation of coagulation (GO:0050819, P=2.65x10^-23) were the most significantly enriched target genes of biological processes; sarcolemma (GO:0042383, P=1.85x10^-29), Schmidt‑Lanterman incisure (GO:0043220, P=1.80x10^-25) and myelin sheath adaxonal region (GO:0035749, P=5.91x10^-25) were the most significantly enriched target genes of the cellular component; and phospholipase inhibitor activity (GO:0004859, P=1.14x10^-44), lipase inhibitor activity (GO:0055102, P=3.76x10^-44) and calcium‑dependent phospholipid binding (GO:0005544, P=5.77x10^-44) were the most significantly enriched target genes of the molecular function (Table III).

Table I. Characteristics of mRNA and miRNA expression profiling of the acute myeloid leukemia.

|     | Author, year | Gene expression omnibus ID | Platform | Samples, H:P | (Refs.) |
|-----|--------------|----------------------------|----------|--------------|--------|
| A   | Civin et al, 2013 | GSE48558 | GPL6244 [HuGene-1.0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene version)] | 49:18 | (27) |
|     | Barreyro et al, 2012 | GSE35008 | GPL6244 [HuGene-1.0-st] Affymetrix Human Gene 1.0 ST Array | 16:12 | (28) |
|     | Barreyro et al, 2012 | GSE35010 | GPL6244 [HuGene-1.0-st] Affymetrix Human Gene 1.0 ST Array | 16:15 | (28) |
|     | Kikushige et al, 2010 | GSE24395 | GPL6106 Sentrix Human-6 v2 Expression BeadChip | 5:12 | (29) |
|     | Majeti et al, 2009 | GSE17054 | GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array | 4:9 | (30) |

B, miRNA expression profiling

|     | Author, year | Gene expression omnibus ID | Platform | Samples, H:P | (Refs.) |
|-----|--------------|----------------------------|----------|--------------|--------|
|     | Tan et al, 2013 | GSE51908 | GPL8786 [miRNA-1_0] Affymetrix miRNA Array | 47:18 | (31) |

H, healthy subject; P, AML patient; miRNA, microRNA.
were the most significantly enriched pathways in KEGG analysis, with the criteria of FDR<0.05 (Table IV).

**RT-qPCR validation of differentially expressed miRNAs and target genes.** To validate the microarray analysis data, the levels of significant differentially expressed miRNA and target genes were quantified by RT-qPCR in three AML blood samples and three normal blood samples. hsa-miR-155 was significantly (P<0.05) upregulated in AML compared with that in the normal samples, and the target gene ANXA2 was significantly downregulated in AML (Fig. 2A). FZD3 was significantly upregulated in the three AML samples compared with the normal samples (P<0.01; Fig. 2B). The present study identified hsa-miR-192 as a downregulated miRNA in AML, although the expression level was not found to be significantly different in AML by RT-qPCR validation (Fig. 2C).

**Discussion**

In the present study, hsa-miR-155 was one of the five miRNAs with the highest connectivity with target genes, targeting 11 differentially expressed mRNAs (Fig. 1), and was significantly upregulated in AML. In the present study, ANXA2 was predicted as a putative target gene of hsa-miR-155. RT-qPCR validated that hsa-miR-155 was significantly upregulated and ANXA2 was significantly downregulated in AML (Fig. 2A), which is in accordance with the bioinformatics analysis. The fact that hsa-miR-155 was upregulated in AML was consistent with the results of a previous study (32). Mounting evidence identifies hsa-miR-155 as having an oncogenic role, generating AML; overexpression of hsa-miR-155 causes myeloproliferation with cell cell-cycle arrest (33,34). High expression of hsa-miR-155 is associated with a poor outcome in AML patients, which has been observed in numerous AML patients via sequencing studies and miRNA expression analyses (35-37). Additionally, hsa-miR-155 is reported to contribute to the metastasis of various solid tumors, including colorectal carcinoma (38), oral squamous cell carcinoma (39) and renal cell carcinoma (40). ANXA2 is a target gene of hsa-miR-155 and its downregulation is associated with a poor AML patient prognosis, based on gene expression profile analysis (41). hsa-miR-155 upregulation and ANXA2 downregulation may be potential biomarkers for the clinical evaluation of AML prognosis.

Through KEGG analysis, FZD3 was found to be enriched in four signaling pathways, including the Wnt signaling pathway, melanogenesis, pathways in cancer and basal cell carcinoma. The Wnt signaling pathway was the most significantly enriched pathway in AML (Table IV). Higher expression of FZD3 was detected in three AML patients compared with that in the normal control, as determined by RT-qPCR (Fig. 2B), which was consistent with the bioinformatics analysis. FZD3 is a member of the frizzled gene family, which also includes FZD1 and FZD7, and functions as
a receptor for the canonical Wnt/β-catenin signaling pathway. Overactivation of the Wnt signaling pathway contributes to tumorigenesis (42,43). According to the present study, the Wnt signaling pathway was essential for AML progression and oncogenicity. CXXC finger protein 5, which is frequently deleted in AML, inhibits the Wnt pathway and leukemic cell proliferation (44). Activation of the Wnt/β-catenin pathway mediates transformation of AML progenitor cells and results in impaired myelomonocytic differentiation (45,46). The FZD3/Wnt signaling pathway may therefore be important in AML pathogenesis.

In the present study, hsa-miR-192 was the most significantly downregulated miRNA and regulated 25 target genes in AML (Fig. 1). miR-192 downregulation is associated with cell cycle progression, cell growth, apoptosis and proliferation of solid tumors (47,48). Overexpression of miR-192 induces apoptotic death in bladder cancer cells, increases the proportion of cells in the G0/G1 phase and decreases the proportion of cells in the S phase compared with a control (47). Curcumin is a traditional Chinese medicine extracted from turmeric that inhibits non-small cell lung cancer cell (NSCLC) cell proliferation and induces NSCLC cell apoptosis through the upregulation of miR-192-5p and the suppression of the phosphoinositide-3 kinase/protein kinase B signaling pathway (47,48). In the present study, hsa-miR-192 was downregulated in AML (Fig. 2C), suggesting that it may also serve a key role in AML cell apoptosis and proliferation. PLAG1 was targeted by 7 miRNAs, meaning it had the highest connectivity of the mRNAs in the miRNA-mRNA network (Fig. 1). The PLAG family consists of 3 members (PLAG1, PLAGL1 and PLAGL2), each with a highly conserved zinc finger structure that allows them to function as transcription factors to recognize DNA and/or RNA (49). PLAG1 serves an oncogenic role in AML, cooperating with CBF-SMMHC to induce AML tumorigenesis (50). The results of the present study revealed that PLAG1 was upregulated in AML.

In summary, a miRNA-mRNA regulatory network was constructed based on differentially expressed miRNAs and target genes in AML. In this network, a number of miRNAs and target genes that may play important roles in AML, such as hsa-miR-155, hsa-miR192, ANXA2, FZD3 and PLAG1, were identified. These results indicated that the Wnt signaling pathway, melanogenesis and pathways in cancer may be involved in the pathogenesis of AML. An miRNA-target gene regulatory network was constructed in AML using bioinformatic tools. A number of miRNAs and mRNAs that are potentially important for AML tumorigenesis were identified. However, the mechanism behind the associations between miRNA, mRNA and miRNA-mRNA involved in AML progression and development requires further investigation.

Table II. Significantly differentially expressed miRNAs (top 10).

| miRNA          | P-value | Log (fold-change) |
|----------------|---------|-------------------|
| **Upregulated miRNAs** |         |                   |
| hsa-miR-432    | 9.93x10^{-12} | 1.66          |
| hsa-miR-126    | 7.44x10^{-10} | 1.57          |
| hsa-miR-10a    | 4.35x10^{-8}  | 1.55          |
| hsa-miR-130a   | 3.39x10^{-11} | 1.54          |
| hsa-miR-34a    | 2.05x10^{-14} | 1.43          |
| hsa-miR-181d   | 2.32x10^{-13} | 1.3           |
| hsa-miR-181a*  | 6.65x10^{-10} | 1.3           |
| hsa-miR-551b*  | 3.27x10^{-8}  | 1.17          |
| hsa-miR-501-5p | 1.17x10^{-8}  | 1.08          |
| hsa-miR-125b   | 6.04x10^{-5}  | 1.06          |
| **Downregulated miRNAs** |       |                   |
| hsa-miR-192    | 6.74x10^{-7}  | -1.12         |
| hsa-miR-29b-1* | 2.75x10^{-8}  | -1.1          |
| hsa-miR-194    | 1.66x10^{-5}  | -1.1          |
| hsa-miR-31     | 2.98x10^{-3}  | -1.05         |
| hsa-miR-26b    | 6.59x10^{-8}  | -0.971        |
| hsa-miR-628-3p | 6.31x10^{-4}  | -0.755        |
| hsa-miR-30e    | 2.84x10^{-6}  | -0.715        |
| hsa-miR-29b    | 1.53x10^{-4}  | -0.664        |
| hsa-miR-200c   | 3.06x10^{-3}  | -0.635        |
| hsa-miR-21     | 3.96x10^{-3}  | -0.605        |

Figure 2. Verification of miRNA and target gene expression levels in AML and normal controls, as determined by reverse transcription-quantitative polymerase chain reaction. (A) hsa-miR-155 and ANXA2 expression levels in AML patients and healthy controls. (B) FZD3 expression levels in AML patients and healthy controls. (C) hsa-miR-192 expression levels in AML patients and healthy controls. *P<0.05, **P<0.01. miRNA/miR, microRNA; AML, acute myeloid leukemia; ANXA2, annexin A2; FZD3, frizzled class receptor 3; CON, healthy control patient blood samples; LEU, AML patient blood samples.
Table III. GO annotation of differentially expressed microRNA target genes in acute myeloid leukemia samples (top 15).

| GO ID      | GO Term                                           | Count | P-value         |
|------------|---------------------------------------------------|-------|-----------------|
| Biological process                                                                                       |
| GO:0030195 | Negative regulation of blood coagulation          | 21    | 1.83x10^{-24}  |
| GO:190047  | Negative regulation of hemostasis                | 21    | 1.83x10^{-24}  |
| GO:0050819 | Negative regulation of coagulation               | 21    | 2.65x10^{-23}  |
| GO:0042730 | Fibrinolysis                                      | 17    | 1.90x10^{-22}  |
| GO:0040023 | Establishment of nucleus localization             | 16    | 2.55x10^{-22}  |
| GO:0051961 | Negative regulation of nervous system development | 14    | 2.70x10^{-22}  |
| GO:0051964 | Negative regulation of synapse assembly           | 14    | 2.70x10^{-22}  |
| GO:0030198 | Extracellular matrix organization                 | 35    | 6.31x10^{-21}  |
| GO:0043062 | Extracellular structure organization              | 35    | 6.58x10^{-21}  |
| GO:0051241 | Negative regulation of multicellular organismal process | 40   | 2.08x10^{-20}  |
| GO:0001525 | Angiogenesis                                      | 35    | 2.86x10^{-20}  |
| GO:0060252 | Positive regulation of glial cell proliferation   | 15    | 3.64x10^{-20}  |
| GO:0030320 | Cellular monovalent inorganic anion homeostasis   | 14    | 3.68x10^{-20}  |
| GO:0030644 | Cellular chloride ion homeostasis                 | 14    | 3.68x10^{-20}  |
| GO:0055064 | Chloride ion homeostasis                          | 14    | 3.68x10^{-20}  |
| Cellular component                                                                                       |
| GO:0042383 | Sarcolemma                                        | 33    | 1.85x10^{-29}  |
| GO:0043220 | Schmidt-Lanterman incisure                        | 18    | 1.80x10^{-25}  |
| GO:0035749 | Myelin sheath adaxonal region                     | 17    | 5.91x10^{-25}  |
| GO:0043218 | Compact myelin                                    | 18    | 2.95x10^{-23}  |
| GO:0005925 | Focal adhesion                                    | 30    | 1.69x10^{-21}  |
| GO:0005924 | Cell-substrate adherens junction                  | 30    | 3.04x10^{-21}  |
| GO:0030055 | Cell-substrate junction                           | 30    | 1.30x10^{-20}  |
| GO:0070161 | Anchoring junction                                | 32    | 1.09x10^{-17}  |
| GO:0005912 | Adherens junction                                 | 31    | 1.73x10^{-17}  |
| GO:0043209 | Myelin sheath                                     | 18    | 2.16x10^{-15}  |
| GO:0019897 | Extrinsic to plasma membrane                      | 18    | 2.01x10^{-13}  |
| GO:0019898 | Extrinsic to membrane                             | 18    | 4.02x10^{-10}  |
| GO:0030054 | Cell junction                                     | 40    | 1.61x10^{-09}  |
| GO:0014704 | Intercalated disc                                 | 14    | 2.10x10^{-09}  |
| GO:0044291 | Cell-cell contact zone                            | 14    | 3.13x10^{-09}  |
| Molecular function                                                                                      |
| GO:0004859 | Phospholipase inhibitor activity                  | 29    | 1.14x10^{-44}  |
| GO:0055102 | Lipase inhibitor activity                         | 29    | 3.76x10^{-43}  |
| GO:0005544 | Calcium-dependent phospholipid binding            | 35    | 5.77x10^{-41}  |
| GO:0030234 | Enzyme regulator activity                         | 79    | 1.58x10^{-23}  |
| GO:0004857 | Enzyme inhibitor activity                         | 43    | 1.15x10^{-22}  |
| GO:0005509 | Calcium ion binding                               | 65    | 2.23x10^{-20}  |
| GO:0005546 | Phosphatidylinositol-4,5-bisphosphate binding     | 18    | 2.28x10^{-20}  |
| GO:0005543 | Phospholipid binding                              | 53    | 7.37x10^{-19}  |
| GO:1901981 | Phosphatidylinositol phosphate binding            | 19    | 2.81x10^{-17}  |
| GO:0008289 | Lipid binding                                     | 56    | 1.54x10^{-15}  |
| GO:0043548 | Phosphatidylinositol 3-kinase binding             | 14    | 6.81x10^{-14}  |
| GO:0008092 | Cytoskeletal protein binding                       | 51    | 1.35x10^{-13}  |
| GO:0017137 | Rab GTPase binding                                | 17    | 1.55x10^{-13}  |
| GO:0004713 | Protein tyrosine kinase activity                  | 25    | 1.43x10^{-12}  |
| GO:0035091 | Phosphatidylinositol binding                       | 22    | 2.75x10^{-11}  |

GO, Gene Ontology.
Table IV. KEGG pathway enrichment analysis of differentially expressed microRNA target genes in acute myeloid leukemia (top 15).

| KEGG ID   | KEGG term                        | Count | FDR     | Genes                                                                 |
|-----------|----------------------------------|-------|---------|-----------------------------------------------------------------------|
| hsa04310  | Wnt signaling pathway            | 4     | 8.70x10^-4 | FZD7, PLCB4, FZD1, FZD3                                               |
| hsa04916  | Melanogenesis                    | 4     | 8.70x10^-4 | FZD7, PLCB4, FZD1, FZD3                                               |
| hsa05200  | Pathways in cancer               | 8     | 1.60x10^-3 | FZD7, AKT3, FZD1, LAMC1, FZD3, PTK2, ARNT2, PLD1                      |
| hsa05146  | Amoebiasis                       | 4     | 2.65x10^-3 | PLCB4, LAMC1, PTK2, COL5A                                             |
| hsa05222  | Small cell lung cancer           | 3     | 2.90x10^-3 | AKT3, LAMC1, PTK2                                                     |
| hsa04010  | MAPK signaling pathway           | 6     | 3.04x10^-3 | DUSP16, RASGRP1, RPS6KA6, RAPGEF2, AKT3, CACNB2                      |
| hsa05217  | Basal cell carcinoma             | 3     | 3.59x10^-3 | FZD7, FZD1, FZD3                                                      |
| hsa04724  | Glutamatergic synapse            | 4     | 4.75x10^-3 | SLC1A6, PLCB4, TRPC1, PLD1                                            |
| hsa04530  | Tight junction                   | 4     | 5.01x10^-3 | JAM2, MYH10, AKT3, MPDZ                                               |
| hsa04630  | Jak-STAT signaling pathway       | 4     | 8.08x10^-3 | IL15, AKT3, MPL, SPRED1                                               |
| hsa04060  | Cytokine-cytokine receptor interaction | 5   | 9.00x10^-3 | IL15, BMRP1B, MPL, IL1RAP, ACVR2A                                    |
| hsa04660  | T-cell receptor signaling pathway | 3    | 1.57x10^-2 | RASGRP1, AKT3, PDK1                                                  |
| hsa04510  | Focal adhesion                   | 4     | 1.61x10^-2 | AKT3, LAMC1, PTK2, COL5A                                              |
| hsa04722  | Neurotrophin signaling pathway    | 3     | 2.02x10^-2 | RPS6KA6, AKT3, PDK1                                                  |
| hsa05145  | Toxoplasmosis                    | 3     | 2.12x10^-2 | AKT3, LAMC1, PDK1                                                   |

KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate.

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