SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 14: 3981-3988, 2017

Investigating the microRNA-mRNA regulatory network in acute myeloid leukemia

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Received November 27, 2015; Accepted May 25, 2017

DOI: 10.3892/ol.2017.6686

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 genes, including hsa-miR-155, hsa-miR-192, annexin A2 (*ANXA2*), frizzled class receptor 3 (*FZD3*), and pleomorphic adenoma gene 1 (*PLAG1*), may serve essential roles in AML oncogenesis. Overall, 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.

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Key words: acute myeloid leukemia, differential expression, microRNA, target genes, regulatory network, reverse transcription-quantitative polymerase chain reaction

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

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 $\log_2$ 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 targeted 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., Bejing, 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 $2^{-\Delta\Delta Cq}$ method (26). At least three independent experiments were performed. The PCR primers used were as follows: hsa-miR-155 forward, 5'-TAATGCTAATCG TGATAGGGGT-3' and reverse, GTGCAGGGTCCGAGGT; hsa-miR-192 forward, 5'-TGACCTATGAATTGACAGCC-3' and reverse, GTGCAGGGTCCGAGGT; frizzled class receptor 3 (*FZD3*) forward, 5'-TCTCCTCTTAGCTGGCAT TATATCC-3' and reverse, 5'-GCAGCGTTCTTGTATCCA CGTT-3'; and Annexin A2 (*ANXA2*) forward, 5'-AGAATC ATGGTCTCCCGCAGTG-3' and reverse, 5'-TCCACCACA CAGGTACAGCAGC-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.

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

A, mRNA expression profiling

Author, year	Gene expression omnibus ID	Platform	Samples, H:P	(Refs.)
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.

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 genes 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-1* 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, $P=3.76x10^{-43}$) and calcium-dependent phospholipid binding (GO:0005544, $P=5.77x10^{-41}$) were the most significantly enriched target genes of the molecular function (Table III).

In total, 148 of the 153 differentially expressed miRNA target genes were enriched in the KEGG database. The Wnt signaling pathway ($FDR=8.70x10^{-4}$), melanogenesis ($FDR=8.70x10^{-4}$) and pathways in cancer ($FDR=1.60x10^{-3}$)

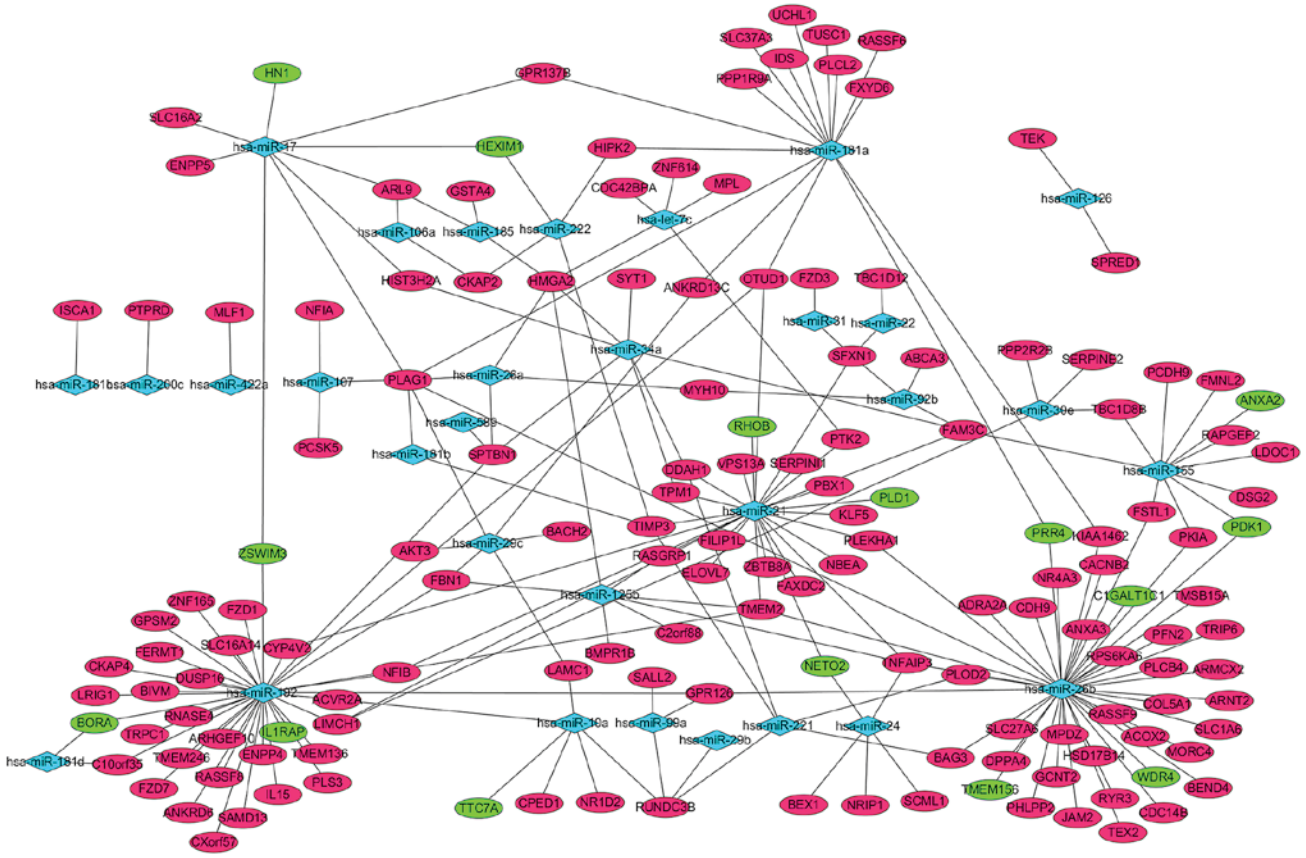

Figure 1. miRNA-target gene regulatory network of acute myeloid leukemia. Circular nodes represent target genes and diamond nodes represent miRNAs. Green nodes represent downregulation, red nodes represent upregulation. Solid lines indicate regulatory associations between the miRNAs and target genes. miRNA/miR, 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

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	$2.84x10^{-4}$	-0.715
hsa-miR-29b	$1.53x10^{-4}$	-0.664
hsa-miR-200c	$3.06x10^{-5}$	-0.635
hsa-miR-21	$3.96x10^{-3}$	-0.605

miRNA/miR, microRNA.

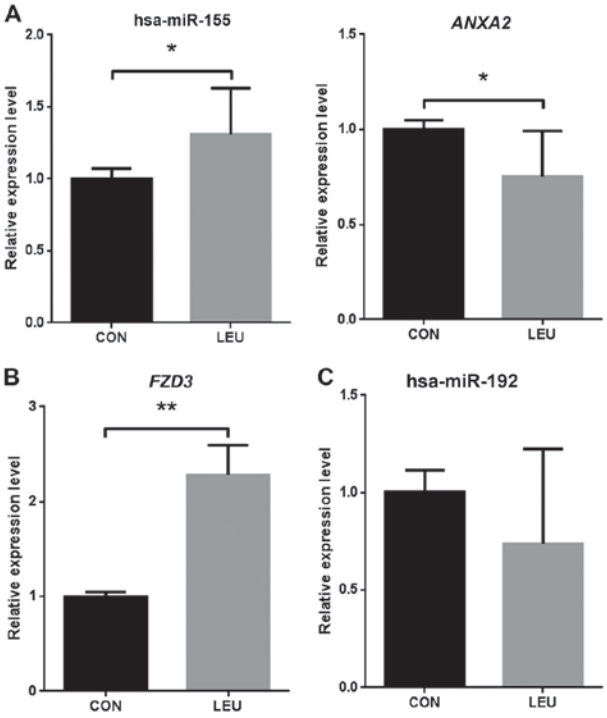

Figure 2. Verification of miRNA and target gene expression levels in AML and normal controls, as determined by reverse transcription-quantitative polymerase chain reaction. (A) hsa-miR-155 and *ANXA2* expression levels in AML patients and healthy controls. (B) *FZD3* expression levels in AML patients 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.

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 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	1.83×10^{-24}
GO:1900047	Negative regulation of hemostasis	21	1.83×10^{-24}
GO:0050819	Negative regulation of coagulation	21	2.65×10^{-23}
GO:0042730	Fibrinolysis	17	1.90×10^{-22}
GO:0040023	Establishment of nucleus localization	16	2.55×10^{-22}
GO:0051961	Negative regulation of nervous system development	14	2.70×10^{-22}
GO:0051964	Negative regulation of synapse assembly	14	2.70×10^{-22}
GO:0030198	Extracellular matrix organization	35	6.31×10^{-21}
GO:0043062	Extracellular structure organization	35	6.58×10^{-21}
GO:0051241	Negative regulation of multicellular organismal process	40	2.08×10^{-20}
GO:0001525	Angiogenesis	35	2.86×10^{-20}
GO:0060252	Positive regulation of glial cell proliferation	15	3.64×10^{-20}
GO:0030320	Cellular monovalent inorganic anion homeostasis	14	3.68×10^{-20}
GO:0030644	Cellular chloride ion homeostasis	14	3.68×10^{-20}
GO:0055064	Chloride ion homeostasis	14	3.68×10^{-20}
Cellular component			
GO:0042383	Sarcolemma	33	1.85×10^{-29}
GO:0043220	Schmidt-Lanterman incisure	18	1.80×10^{-25}
GO:0035749	Myelin sheath adaxonal region	17	5.91×10^{-25}
GO:0043218	Compact myelin	18	2.95×10^{-23}
GO:0005925	Focal adhesion	30	1.69×10^{-21}
GO:0005924	Cell-substrate adherens junction	30	3.04×10^{-21}
GO:0030055	Cell-substrate junction	30	1.30×10^{-20}
GO:0070161	Anchoring junction	32	1.09×10^{-17}
GO:0005912	Adherens junction	31	1.73×10^{-17}
GO:0043209	Myelin sheath	18	2.16×10^{-15}
GO:0019897	Extrinsic to plasma membrane	18	2.01×10^{-13}
GO:0019898	Extrinsic to membrane	18	4.02×10^{-10}
GO:0030054	Cell junction	40	1.61×10^{-09}
GO:0014704	Intercalated disc	14	2.10×10^{-09}
GO:0044291	Cell-cell contact zone	14	3.13×10^{-09}
Molecular function			
GO:0004859	Phospholipase inhibitor activity	29	1.14×10^{-44}
GO:0055102	Lipase inhibitor activity	29	3.76×10^{-43}
GO:0005544	Calcium-dependent phospholipid binding	35	5.77×10^{-41}
GO:0030234	Enzyme regulator activity	79	1.58×10^{-23}
GO:0004857	Enzyme inhibitor activity	43	1.15×10^{-22}
GO:0005509	Calcium ion binding	65	2.23×10^{-20}
GO:0005546	Phosphatidylinositol-4,5-bisphosphate binding	18	2.28×10^{-20}
GO:0005543	Phospholipid binding	53	7.37×10^{-19}
GO:1901981	Phosphatidylinositol phosphate binding	19	2.81×10^{-17}
GO:0008289	Lipid binding	56	1.54×10^{-15}
GO:0043548	Phosphatidylinositol 3-kinase binding	14	6.81×10^{-14}
GO:0008092	Cytoskeletal protein binding	51	1.35×10^{-13}
GO:0017137	Rab GTPase binding	17	1.55×10^{-13}
GO:0004713	Protein tyrosine kinase activity	25	1.43×10^{-12}
GO:0035091	Phosphatidylinositol binding	22	2.75×10^{-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	8.70×10^{-4}	*FZD7*, *PLCB4*, *FZD1*, *FZD3*
hsa04916	Melanogenesis	4	8.70×10^{-4}	*FZD7*, *PLCB4*, *FZD1*, *FZD3*
hsa05200	Pathways in cancer	8	1.60×10^{-3}	*FZD7*, *AKT3*, *FZD1*, *LAMC1*, *FZD3*, *PTK2*, *ARNT2*, *PLD1*
hsa05146	Amoebiasis	4	2.65×10^{-3}	*PLCB4*, *LAMC1*, *PTK2*, *COL5A1*
hsa05222	Small cell lung cancer	3	2.90×10^{-3}	*AKT3*, *LAMC1*, *PTK2*
hsa04010	MAPK signaling pathway	6	3.04×10^{-3}	*DUSP16*, *RASGRP1*, *RPS6KA6*, *RAPGEF2*, *AKT3*, *CACNB2*
hsa05217	Basal cell carcinoma	3	3.59×10^{-3}	*FZD7*, *FZD1*, *FZD3*
hsa04724	Glutamatergic synapse	4	4.75×10^{-3}	*SLC1A6*, *PLCB4*, *TRPC1*, *PLD1*
hsa04530	Tight junction	4	5.01×10^{-3}	*JAM2*, *MYH10*, *AKT3*, *MPDZ*
hsa04630	Jak-STAT signaling pathway	4	8.08×10^{-3}	*IL15*, *AKT3*, *MPL*, *SPRED1*
hsa04060	Cytokine-cytokine receptor interaction	5	9.00×10^{-3}	*IL15*, *BMPR1B*, *MPL*, *IL1RAP*, *ACVR2A*
hsa04660	T-cell receptor signaling pathway	3	1.57×10^{-2}	*RASGRP1*, *AKT3*, *PDK1*
hsa04510	Focal adhesion	4	1.61×10^{-2}	*AKT3*, *LAMC1*, *PTK2*, *COL5A1*
hsa04722	Neurotrophin signaling pathway	3	2.02×10^{-2}	*RPS6KA6*, *AKT3*, *PDK1*
hsa05145	Toxoplasmosis	3	2.12×10^{-2}	*AKT3*, *LAMC1*, *PDK1*

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

Acknowledgements

The present study was supported by a grant from the Program of Jining Science and Technology Development Plan (grant no, 2015-57-102).

References

1. Chen W, Zheng R, Zeng H and Zhang S: The updated incidences and mortalities of major cancers in China, 2011. Chin J Cancer 34: 507-507, 2015.
2. Cripe LD: Adult acute leukemia. Curr Probl Cancer 21: 1-64, 1997.
3. Marcucci G, Haferlach T and Döhner H: Molecular genetics of adult acute myeloid leukemia: Prognostic and therapeutic implications. J Clin Oncol 29: 475-486, 2011.
4. Deguchi K and Gilliland DG: Cooperativity between mutations in tyrosine kinases and in hematopoietic transcription factors in AML. Leukemia 16: 740-744, 2002.
5. Finn L, Sproat L, Heckman MG, Jiang L, Diehl NN, Ketterling R, Tibes R, Valdez R and Foran J: Epidemiology of adult acute myeloid leukemia: Impact of exposures on clinical phenotypes and outcomes after therapy. Cancer Epidemiol 39: 1084-1092, 2015.
6. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR and Sultan C: Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol 33: 451-458, 1976.
7. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellström-Lindberg E, Tefferi A and Bloomfield CD: The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: Rationale and important changes. Blood 114: 937-951, 2009.
8. Marcucci G, Mrózek K, Ruppert AS, Maharry K, Kolitz JE, Moore JO, Mayer RJ, Pettenati MJ, Powell BL, Edwards CG, *et al*: Prognostic factors and outcome of core binding factor acute myeloid leukemia patients with t(8;21) differ from those of patients with inv(16): A Cancer and Leukemia Group B study. J Clin Oncol 23: 5705-5717, 2005.
9. Stone RM: Prognostic factors in AML in relation to (ab)normal karyotype. Best Pract Res Clin Haematol 22: 523-528, 2009.
10. Schanz J and Haase D: Cytogenetic features in myelodysplastic syndromes. 2014.
11. Care RS, Valk PJM, Goodeve AC, Abu-Duhier FM, Geertsma-Kleinekoort WM, Wilson GA, Gari MA, Peake IR, Löwenberg B and Reilly JT: Incidence and prognosis of c-KIT and FLT3 mutations in core binding factor (CBF) acute myeloid leukaemias. Br J Haematol 121: 775-777, 2003.
12. Langer C, Radmacher MD, Ruppert AS, Whitman SP, Paschka P, Mrózek K, Baldus CD, Vukosavljevic T, Liu CG, Ross ME, *et al*: High BAALC expression associates with other molecular prognostic markers, poor outcome, and a distinct gene-expression signature in cytogenetically normal patients younger than 60 years with acute myeloid leukemia: A Cancer and Leukemia Group B (CALGB) study. Blood 111: 5371-5379, 2008.
13. Preudhomme C, Sagot C, Boissel N, Cayuela JM, Tigaud I, de Botton S, Thomas X, Raffoux E, Lamandin C, Castaigne S, *et al*: Favorable prognostic significance of CEBPA mutations in patients with de novo acute myeloid leukemia: A study from the acute leukemia French association (ALFA). Blood 100: 2717-2723, 2002.
14. Döhner K, Tobis K, Ulrich R, Fröhling S, Benner A, Schlenk RF and Döhner H: Prognostic significance of partial tandem duplications of the MLL gene in adult patients 16 to 60 years old with acute myeloid leukemia and normal cytogenetics: A study of the acute myeloid leukemia study group Ulm. J Clin Oncol 20: 3254-3261, 2002.
15. Ufkin ML, Peterson S, Yang X, Driscoll H, Duarte C and Sathyanarayana P: miR-125a regulates cell cycle, proliferation, and apoptosis by targeting the ErbB pathway in acute myeloid leukemia. Leuk Res 38: 402-410, 2014.
16. Morris VA, Zhang A, Yang T, Stirewalt DL, Ramamurthy R, Meshinchi S and Oehler VG: MicroRNA-150 expression induces myeloid differentiation of human acute leukemia cells and normal hematopoietic progenitors. PLoS One 8: e75815, 2013.
17. Edgar R, Domrachev M and Lash AE: Gene expression omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 30: 207-210, 2002.
18. Diboun I, Wernisch L, Orengo CA and Koltzenburg M: Microarray analysis after RNA amplification can detect pronounced differences in gene expression using limma. BMC Genomics 7: 252, 2006.

19. Hsu SD, Lin FM, Wu WY, Liang C, Huang WC, Chan WL, Tsai WT, Chen GZ, Lee CJ, Chiu CM, *et al*: miRTarBase: A database curates experimentally validated microRNA-target interactions. Nucleic Acids Res 39 (Database Issue): D163-D169, 2011.
20. Hsu SD, Tseng YT, Shrestha S, Lin YL, Khaleel A, Chou CH, Chu CF, Huang HY, Lin CM, Ho SY, *et al*: miRTarBase update 2014: An information resource for experimentally validated miRNA-target interactions. Nucleic Acids Res 42 (Database Issue): D78-D85, 2014.
21. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T: Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res 13: 2498-2504, 2003.
22. Cheng L, Lin H, Hu Y, Wang J and Yang Z: Gene function prediction based on the Gene Ontology hierarchical structure. PLoS One 9: e107187, 2014.
23. Kanehisa M, Sato Y, Kawashima M, Furumichi M and Tanabe M: KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 44: D457-D462, 2016.
24. Zheng Q and Wang XJ: GOEAST: A web-based software toolkit for Gene Ontology enrichment analysis. Nucleic Acids Res 36: W358-W363, 2008.
25. Carmona-Saez P, Chagoyen M, Tirado F, Carazo JM and Pascual-Montano A: GENECODIS: A web-based tool for finding significant concurrent annotations in gene lists. Genome Biol 8: R3, 2007.
26. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
27. Cramer-Morales K, Nieborowska-Skorska M, Scheibner K, Padget M, Irvine DA, Sliwinski T, Haas K, Lee J, Geng H, Roy D, *et al*: Personalized synthetic lethality induced by targeting RAD52 in leukemias identified by gene mutation and expression profile. Blood 122: 1293-1304, 2013.
28. Barreyro L, Will B, Bartholdy B, Zhou L, Todorova TI, Stanley RF, Ben-Neriah S, Montagna C, Parekh S, Pellagatti A, *et al*: Overexpression of IL-1 receptor accessory protein in stem and progenitor cells and outcome correlation in AML and MDS. Blood 120: 1290-1298, 2012.
29. Kikushige Y, Shima T, Takayanagi S, Urata S, Miyamoto T, Iwasaki H, Takenaka K, Teshima T, Tanaka T, Inagaki Y and Akashi K: TIM-3 is a promising target to selectively kill acute myeloid leukemia stem cells. Cell Stem Cell 7: 708-717, 2010.
30. Majeti R, Becker MW, Tian Q, Lee TL, Yan X, Liu R, Chiang JH, Hood L, Clarke MF and Weissman IL: Dysregulated gene expression networks in human acute myelogenous leukemia stem cells. Proc Natl Acad Sci USA 106: 3396-3401, 2009.
31. Tan YS, Kim M, Kingsbury TJ, Civin CI and Cheng WC: Regulation of RAB5C is important for the growth inhibitory effects of MiR-509 in human precursor-B acute lymphoblastic leukemia. PLoS One 9: e111777, 2014.
32. Havelange V, Stauffer N, Heaphy CC, Volinia S, Andreeff M, Marcucci G, Croce CM and Garzon R: Functional implications of microRNAs in acute myeloid leukemia by integrating microRNA and messenger RNA expression profiling. Cancer 117: 4696-4706, 2011.
33. Forrest AR, Kanamori-Katayama M, Tomaru Y, Lassmann T, Ninomiya N, Takahashi Y, de Hoon MJ, Kubosaki A, Kaiho A, Suzuki M, *et al*: Induction of microRNAs, mir-155, mir-222, mir-424 and mir-503, promotes monocytic differentiation through combinatorial regulation. Leukemia 24: 460-466, 2010.
34. O'Connell RM, Rao DS, Chaudhuri AA, Boldin MP, Taganov KD, Nicoll J, Paquette RL and Baltimore D: Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder. J Exp Med 205: 585-594, 2008.
35. Marcucci G, Maharry KS, Metzeler KH, Volinia S, Wu YZ, Mrózek K, Nicolet D, Kohlschmidt J, Whitman SP, Mendler JH, *et al*: Clinical role of microRNAs in cytogenetically normal acute myeloid leukemia: miR-155 upregulation independently identifies high-risk patients. J Clin Oncol 31: 2086-2093, 2013.
36. Chuang MK, Chiu YC, Chou WC, Hou HA, Chuang EY and Tien HF: A 3-microRNA scoring system for prognostication in de novo acute myeloid leukemia patients. Leukemia 29: 1051-1059, 2015.
37. Zhi F, Cao X, Xie X, Wang B, Dong W, Gu W, Ling Y, Wang R, Yang Y and Liu Y: Identification of circulating microRNAs as potential biomarkers for detecting acute myeloid leukemia. PLoS One 8: e56718, 2013.
38. Qu YL, Wang HF, Sun ZQ, Tang Y, Han XN, Yu XB and Liu K: Up-regulated miR-155-5p promotes cell proliferation, invasion and metastasis in colorectal carcinoma. Int J Clin Exp Pathol 8: 6988-6994, 2015.
39. Baba O, Hasegawa S, Nagai H, Uchida F, Yamatoji M, Kanno NI, Yamagata K, Sakai S, Yanagawa T and Bukawa H: MicroRNA-155-5p is associated with oral squamous cell carcinoma metastasis and poor prognosis. J Oral Pathol Med 45: 248-255, 2016.
40. Merhautova J, Hezova R, Poprach A, Kovarikova A, Radova L, Svoboda M, Vyzula R, Demlova R and Slaby O: miR-155 and miR-484 are associated with time to progression in metastatic renal cell carcinoma treated with sunitinib. Biomed Res Int 2015: 941980, 2015.
41. Park MH, Cho SA, Yoo KH, Yang MH, Ahn JY, Lee HS, Lee KE, Mun YC, Cho DH, Seong CM and Park JH: Gene expression profile related to prognosis of acute myeloid leukemia. Oncol Rep 18: 1395-1402, 2007.
42. Conacci-Sorrell M, Zhurinsky J and Ben-Ze'ev A: The cadherin-catenin adhesion system in signaling and cancer. J Clin Invest 109: 987-991, 2002.
43. Giles RH, van Es JH and Clevers H: Caught up in a Wnt storm: Wnt signaling in cancer. Biochim Biophys Acta 1653: 1-24, 2003.
44. Kühnl A, Valk PJ, Sanders MA, Ivey A, Hills RK, Mills KI, Gale RE, Kaiser MF, Dillon R, Joannides M, *et al*: Downregulation of the Wnt inhibitor CXXC5 predicts a better prognosis in acute myeloid leukemia. Blood 125: 2985-2994, 2015.
45. Wang Y, Krivtsov AV, Sinha AU, North TE, Goessling W, Feng Z, Zon LI and Armstrong SA: The Wnt/beta-catenin pathway is required for the development of leukemia stem cells in AML. Science 327: 1650-1653, 2010.
46. Simon M, Grandage VL, Linch DC and Khwaja A: Constitutive activation of the Wnt/beta-catenin signalling pathway in acute myeloid leukaemia. Oncogene 24: 2410-2420, 2005.
47. Ye M, Zhang J, Zhang J, Miao Q, Yao L and Zhang J: Curcumin promotes apoptosis by activating the p53-miR-192-5p/215-XIAP pathway in non-small cell lung cancer. Cancer Lett 357: 196-205, 2015.
48. Jin H, Qiao F, Wang Y, Xu Y and Shang Y: Curcumin inhibits cell proliferation and induces apoptosis of human non-small cell lung cancer cells through the upregulation of miR-192-5p and suppression of PI3K/Akt signaling pathway. Oncol Rep 34: 2782-2789, 2015.
49. Kas K, Voz ML, Hensen K, Meyen E and Van de Ven WJ: Transcriptional activation capacity of the novel PLAG family of zinc finger proteins. J Biol Chem 273: 23026-23032, 1998.
50. Landrette SF, Kuo YH, Hensen K, Barjesteh van Waalwijk van Doorn-Khosrovani S, Perrat PN, Van de Ven WJ, Delwel R and Castilla LH: Plag1 and Plagl2 are oncogenes that induce acute myeloid leukemia in cooperation with Cbfb-MYH11. Blood 105: 2900-2907, 2005.