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Identification of miRNA-mRNA Network Associated with Acute Myeloid Leukemia Survival

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Acute myeloid leukemia (AML) is a common hematologic malignancy of adults. The pathophysiological mechanism of AML is not well understood. The purpose of this study was to examine the crucial miRNAs and mRNAs associated with AML survival.

Material/Methods: The full clinical dataset of miRNA and mRNA expression profiling of AML patients was downloaded from The Cancer Genome Atlas database. Univariate Cox regression analysis was performed to obtain those miRNAs and mRNAs associated with AML survival. A miRNA-mRNA interaction network was constructed. The underlying functions of mRNAs were predicted through Kyoto Encyclopedia of Genes and Genomes (KEEG) pathway enrichment. The expression levels of miRNAs and mRNAs were detected by quantitative real-time polymerase chain reaction (qRT-PCR).

Results: Fourteen miRNAs and 830 mRNAs associated with AML survival were identified. Of the 14 miRNAs, hsa-mir-425, hsa-mir-1201, and hsa-mir-1978 were identified as risk factors and the other 11 miRNAs were identified as protective factors of AML survival. For target-genes of miRNAs, GTSF1, RTN4R, and CD44 were the top risk factor target-genes associated with AML survival. An interaction network was constructed that including 607 miRNA-target gene pairs associated with AML survival. Target-genes associated with AML survival were significantly enriched in several pathways including pancreatic secretion, calcium signaling pathway, natural killer cell mediated cytotoxicity, and Alzheimer's disease. The qRT-PCR results were consistent with our bioinformatics analyses.

Conclusions: The miRNA hsa-mir-425 was identified as the top risk factor miRNA of AML survival and CD44 was identified as one of the top three risk factor target-genes associated with AML survival. Both hsa-mir-425 and CD44 may play key roles in progression and development of AML through calcium signaling pathway and natural killer cell mediated cytotoxicity.

MeSH Keywords: Database • Leukemia, Myeloid, Acute • Prognosis • Survival Analysis

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Background

Acute myeloid leukemia (AML) is a common hematological malignancy with myeloblast proliferation in the bone marrow of adults and children. AML patients have highly heterogeneous disease course and outcomes. According to the French-American-British (FAB) classification, AML is defined as having eight subtypes: M0, M1, M2, M3, M4, M5, M6, and M7 [1,2].

It has been estimated that 50%–60% of AML patients display cytogenetic abnormalities and significantly heterogeneous outcomes [3,4]. Cytogenetic abnormalities include chromosomes translocation, inversion, and deletion; some chromosome abnormalities, such as t(8;21), t(16;16), inv(16), and t(15;17), predict better prognosis of AML patients. Chromosomes abnormalities, including -5,-7, abn(3q), del(5q) and other complex karyotypes, predict poor prognosis [5]. AML patients with normal karyotypes, *FLT3* mutation, and negative mutation of *NPM1* possess intermediate prognosis. A number of groups have endeavored to come to an agreement on prognosis risk evaluation of AML chromosomes abnormalities [6–9], but discrepancy exists among different group standards. For example, del(7q) is considered to be an intermediate-risk abnormality based on CALGB standards but it is considered to be a poor-risk based on SWOG standards [10].

Although there has been progress in understanding the mechanisms of pathogenesis of AML, it is still difficult to predict clinical outcomes for AML patients. Over the past decades, the prognostic prediction of AML patients has largely depended on cytogenetic abnormalities. More recently, molecular genetic changes have been identified to predict prognosis and guide clinical treatment. Molecular genetic changes consist of mutations of *CEBPA, NPM1, FLT3,* and *c-KIT. CEBPA* mutation and *NPM1* mutation (absence of *FLT3* mutation) suggest a favorable prognosis for AML patients with longer complete remission duration and overall survival. However, *c-KIT* mutation suggests an intermediate prognosis, and *FLT3* mutation suggests an unfavorable prognosis [11].

In this study, we applied bioinformatics and univariate Cox regression analysis to identify the miRNAs and target-genes associated with overall survival of AML patients, and aimed to provide valuable information for further AML patient classification and therapy planning.

Material and Methods

Patients and samples

Two hundred study patients with AML were retrieved from The Cancer Genome Atlas (TCGA) data portal. The full clinical dataset was downloaded (up to March 16, 2015). The exclusion criteria for AML patients were as follows: 1) history of other malignancy; 2) history of AML treatment; and 3) samples with clinical data but without miRNA or mRNA sequence data. In the TCGA database, 49 AML patients had a history of treatment before collection of blood samples and all of them received treatment with hydroxyurea. Overall, 120 AML patients with available corresponding clinical data, including age, gender, race, vital status, and follow-up, were included in our study.

miRNA and mRNA expression data

The miRNA and mRNA expression data (level 3) of the AML patients were downloaded from the TCGA data portal (up to March 16, 2015). The miRNA and mRNA expression profiling was generated from the Illumina Genome Analyzer sequencing platforms (Illumina Inc., San Diego, CA, USA).

Survival analysis

The association between miRNA expression and overall survival was carried out using univariate Cox regression. A set of miRNAs that significantly correlated with survival was identified with the threshold of *p*-value less than 0.05. The association between mRNA expression and overall survival was sequentially performed using univariate Cox regression; the threshold was a *p*-value less than 0.05, which indicated that a set of mRNAs was significantly associated with survival. Hazard ratio (HR) >1 indicated a risk factor associated with AML survival and HR <1 indicated a protective factor associated with AML survival.

Identification of target-genes of miRNAs

To obtain the target-genes of miRNAs associated with AML survival, the miRNAs were integrated into the mirWalk database (*http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/*), in which the correlations between target-genes and miRNAs have been confirmed. Six algorithms including RNA22, miRanda, miRDB, miRWalk, PICTAR2, and Targetscan were conducted to predict target-genes of miRNAs. The genes, simultaneously predicted by more than four algorithms, were identified as the target-genes of the miRNAs.

miRNA-target gene network

miRNAs associated with AML survival and target-genes associated with AML survival were identified to construct the interaction network using Cytoscape software (*http://cytoscape.org*). In the miRNA-target gene network, a circular node represented mRNA and a rectangle node represented miRNA, and their association was represented by a solid line.

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KEGG pathway enrichment

The underlying functions of target-genes associated with AML survival were predicted by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using the GeneCodis software. FDR <0.05 was the cut-off for selecting significant KEGG pathway.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA of peripheral blood of AML patients was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The SuperScript III Reverse Transcription Kit (Invitrogen, Carlsbad, CA, USA) was used to synthesize the cDNA according to the manufacturer's instructions. Then qRT-PCR reactions were performed using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) on the Applied Biosystems 7500 (Applied Biosystems, Foster City, CA, USA). For detection of miRNA expression level, we used miRcute miRNA First-Strand cDNA Kit (Tiangen, Bejing, China) and miRcute miRNA qPCR Detection Kit (Tiangen, Bejing, China). U6 and β-actin were used as internal controls for miRNA and mRNA expression, respectively. The relative expression of target-genes was calculated using the ΔCT equation. The PCR primers were used as follows:
IL15RA forward-5'CAACAGCCAAGAACTGGGAAC3';
IL15RA reverse-5'AGTTTGCCTTGACTTGAGGTAGC3';
CD44 forward-5' ATCATCTTGGCATCCCTCTTG 3';
CD44 reverse-5'TGTCCTCCACAGCTCCATTG 3';
β-actin forward-5' CTGAAGTACCCCATCGAGCAC 3';
β-actin reverse- 5' ATAGCACAGCCTGGATAGCAAC 3';
hsa-miR-200c forward-5' CGTCTTACCCAGCAGTGTTTG3';
hsa-miR-425 forward-5' AATGACACGATCACTCCCGTTGA 3';
U6 forward-5' CTCGCTTCGGCAGCACA 3';
U6 reverse-5' AACGCTTCACGAATTTGCGT 3'.

Statistical analysis

At least three independent experiments were performed for statistical evaluation. The SPSS19.0 program was used for general statistical analysis. The qRT-PCR experimental data were expressed as means ± standard deviation. The statistical significance was evaluated using the Student's *t*-test; $p<0.05$ was considered a significant difference.

The peripheral blood samples for qRT-PCR examination were obtained from AML patients diagnosed at the Department of Hematology, Taian City Central Hospital. Our study was approved by the Ethics Committee of Taian City Central Hospital and complied with the Declaration of Helsinki. All participants provided written informed consent to participate in this study.

Results

miRNAs associated with survival time of AML patients

The miRNA expression and survival time of AML patients were obtained from the TCGA database and univariate Cox regression analysis was performed. We obtained 14 miRNAs associated with survival time; the threshold was $p<0.05$, as shown in Table 1. HR >1 indicated a risk factor and HR <1 indicated a protective factor. In our study, hsa-mir-1201, hsa-mir-1978, and hsa-mir-425 were identified as risk factors, and hsa-mir-10b, hsa-mir-193b, hsa-mir-194-1, hsa-mir-196a-1, hsa-mir-1976, hsa-mir-200c, hsa-mir-23b, hsa-mir-30a, hsa-mir-452, hsa-mir-509-1, and hsa-mir-589 were identified as protective factors for survival time of AML patients.

mRNA associated with survival time of AML patients

The mRNA expression and survival time of AML patients were obtained from the TCGA database and univariate Cox regression analysis was performed. We obtained 830 mRNAs associated with survival time; the threshold was $p<0.05$, as shown in Table 2. We identified 830 mRNAs associated with AML survival including 482 mRNAs identified as risk factors and 348 mRNAs identified as protective factors (data not shown). The top 20 risk factor mRNAs and top 20 protective factor mRNAs associated with survival time ($p<0.05$) are presented in Table 2; POLR3C, TULP2, CIRBP, WNT7A, and FARS2 were the top 5 risk factor mRNAs. SPIC, ZBTB2, VCAM1, AMPD1, and SOX9 were the top 5 protective factor mRNAs associated with survival time of AML patients.

The target-gene prediction of miRNA associated with survival time

The target genes of 14 miRNAs associated with survival time were predicted and target-genes of 12 miRNAs were available in the mirWalk database. A total of 607 miRNA-target gene pairs were obtained, as shown in the Supplementary Table 1. The predictive target-genes of miRNAs (Figure 1) and mRNAs associated with survival time overlapped; 109 predictive target-genes associated with survival time were obtained (underlined genes in Supplementary Table 1). GTSF1, RTN4R, and CD44 were the top risk factor target-genes associated with AML survival.

Regulatory network construction of miRNA and mRNA associated with survival time

To obtain insights into the regulatory relationship between the 12 miRNAs and 109 target-genes associated with survival time, a regulatory network was constructed. As shown in Figure 1, rectangle nodes represent miRNAs associated with

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Table 1. miRNAs associated with AML survival.

miRNA	Beta	HR	95 %CI (low)	95%CI (high)	*P*-value
hsa-mir-425	0.78098	2.1836	1.1866	4.0184	0.012081
hsa-mir-1978	0.77576	2.1722	1.1343	4.1601	0.019282
hsa-mir-1201	0.65208	1.9195	1.0231	3.6012	0.042227
hsa-mir-589	–0.64776	0.52322	0.27722	0.98751	0.045633
hsa-mir-23b	–0.65778	0.518	0.28334	0.94702	0.03261
hsa-mir-196a-1	–0.65789	0.51794	0.2715	0.98809	0.045895
hsa-mir-200c	–0.66085	0.51641	0.27139	0.98263	0.044075
hsa-mir-509-1	–0.6679	0.51278	0.27829	0.94487	0.032206
hsa-mir-1976	–0.69919	0.49699	0.25874	0.95462	0.035777
hsa-mir-194-1	–0.72271	0.48543	0.24412	0.9653	0.039331
hsa-mir-193b	–0.80362	0.4477	0.22526	0.88981	0.021841
hsa-mir-452	–0.82757	0.43711	0.2298	0.83143	0.011644
hsa-mir-10b	–0.84543	0.42937	0.22207	0.8302	0.011965
hsa-mir-30a	–0.85097	0.427	0.22278	0.81843	0.010358

HR – hazard ration; CI – confidence interval; Beta – regression coefficient; AML – acute myeloid leukemia.

survival time and circular nodes represent mRNAs associated with survival time. The miRNAs hsa-mir-30a, hsa-mir-589, hsa-mir-10b, hsa-mir-452, and hsa-mir-200c had high connectivity with the mRNAs associated with AML patient survival time, and regulated 27, 24, 22, 22, 21 mRNAs, respectively.

KEGG pathway enrichment

KEGG pathway analysis was used to identify biological functions of miRNA target-genes. In all, 105 of the 109 target-genes associated with survival time were enriched in the KEGG database. Four signaling pathways were significantly enriched, including pancreatic secretion, calcium signaling pathway, natural killer cell mediated cytotoxicity, and Alzheimer's disease, as shown in Table 3.

qRT-PCR validation of miRNA and mRNA associated with survival

To validate the expression status of miRNAs and mRNAs associated with survival, miRNAs (hsa-miR-200c and hsa-miR-425) and mRNA (IL15RA and CD44) were preliminarily quantified by qRT-PCR. Sixteen AML patients were divided into two groups, a low-moderate risk group and a high-risk group, based on karyotype and gene mutation detection according to the standards of the Medical Research Council. As Figure 2A, 2C, and 2D show, the expression levels of IL15RA (p=0.0003), CD44 (p=0.028) and hsa-miR-425 (p=0.053) were significant upregulated in the high-risk group compared with the low-moderate risk group. As Figure 2B shows, the expression level hsa-miR-200c (p=0.033) was significant downregulated in the high-risk group compared with the low-moderate risk group.

Discussion

In our study, hsa-mir-425 was the top risk factor associated with AML survival, as shown in Table 1. It has been reported that mir-425-5p is upregulated in human gastric cancer and contributes to gastric cancer cell proliferation, invasion and metastasis *in vitro* and *in vivo* [12–14]. Over-expression of miR-425 enhances cell proliferation, colony formation, and cell metastasis in esophageal squamous cell carcinoma by targeting SMAD2 [15]. Ge et al. reported that mir-425 was significantly associated with recurrence-free survival (RFS) and overall survival of chromophobe renal cell carcinoma, and miRNA expression signatures including mir-191, mir-19a, mir-210, and mir-425, were identified as predictors of clinical prognosis [16].

In our study, hsa-mir-30a was the top protective factor associated with AML survival (Table 1). Over-expression of BCR-ABL1 is associated with chronic myeloid leukemia; and downregulation of mir-30a enhances BCR-ABL expression and promotes chronic myeloid leukemia tumorigenesis [17]. The *MYBL2* gene encodes a transcription factor; and over-expression of *MYBL2* could be implicated in tumorigenesis of colorectal cancer and AML [18,19]. In AML, MYBL2 over-expression is associated with

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Table 2. mRNAs associated with AML survival.

Gene symbol	Beta	HR	95%CI (low)	95%CI (high)	*P*-value
Risky mRNAs (top 20)					
POLR3C	1.299177	3.666278842	1.822439	7.375612	0.00027
TULP2	1.271691	3.56687887	1.659339	7.667284	0.001126
CIRBP	1.212915	3.363273616	1.676527	6.747048	0.000638
WNT7A	1.13283	3.104429621	1.563505	6.164025	0.001207
FARS2	1.128418	3.090761914	1.589044	6.011672	0.000886
HSBP1L1	1.101514	3.008717605	1.49912	6.038464	0.001941
SPATA24	1.093419	2.984460963	1.507142	5.909864	0.001708
GTSF1	1.088786	2.97066512	1.516522	5.81914	0.001504
LOC150197	1.087166	2.965857274	1.512986	5.813873	0.001547
PRR4	1.085033	2.959538025	1.426825	6.138709	0.003558
ZSCAN16	1.059951	2.886230787	1.496316	5.567227	0.001565
tAKR	1.057348	2.878727159	1.41916	5.83942	0.003389
TMCO6	1.048799	2.854219977	1.445137	5.637231	0.002525
GLRA1	1.046813	2.848559639	1.431752	5.667387	0.002858
KCNQ5	1.040786	2.83144076	1.412616	5.675325	0.003349
CCDC61	1.030272	2.801828015	1.446379	5.427514	0.002258
METRN	1.014433	2.757800065	1.305514	5.825647	0.007844
HIST2H2BF	1.012048	2.751231083	1.452881	5.209838	0.001892
RTN4R	1.011228	2.748975915	1.384951	5.456416	0.003839
PKP3	1.008338	2.741042689	1.428346	5.26015	0.002429
Protective mRNAs (top 20)					
SPIC	–1.31893	0.2674205	0.129581	0.551884	0.00036
ZBTB2	–1.27059	0.2806665	0.142188	0.554012	0.00025
VCAM1	–1.26516	0.2821953	0.142927	0.557167	0.000267
AMPD1	–1.21566	0.2965143	0.147273	0.59699	0.000662
SOX9	–1.19687	0.3021391	0.15331	0.595449	0.000545
CXCL10	–1.18323	0.306289	0.146201	0.641673	0.001714
MOCS1	–1.17538	0.3087017	0.155751	0.611851	0.000759
CXCL12	–1.16587	0.3116504	0.159361	0.60947	0.000657
SDC3	–1.14539	0.3181006	0.16393	0.617262	0.000708
SDC1	–1.14055	0.3196427	0.161413	0.632981	0.001068
FER1L4	–1.12792	0.3237057	0.160942	0.651075	0.001558
TRIM25	–1.12791	0.32371	0.161198	0.650058	0.00152
EBF3	–1.1233	0.3252052	0.169878	0.622555	0.000698
CDH15	–1.11564	0.3277049	0.15995	0.671401	0.002299
CD163L1	–1.10857	0.3300322	0.16721	0.651404	0.001396
IGF1	–1.10036	0.3327518	0.171385	0.646052	0.001152
ADAMTS9	–1.07894	0.3399546	0.174127	0.663706	0.001573
C10orf81	–1.0656	0.34452	0.172913	0.686437	0.002448
ZCCHC24	–1.06124	0.3460263	0.172931	0.692381	0.00271
CCL18	–1.04882	0.3503516	0.180175	0.681262	0.001993

HR – hazard ration; CI – confidence interval; Beta – regression coefficient; AML – acute myeloid leukemia.

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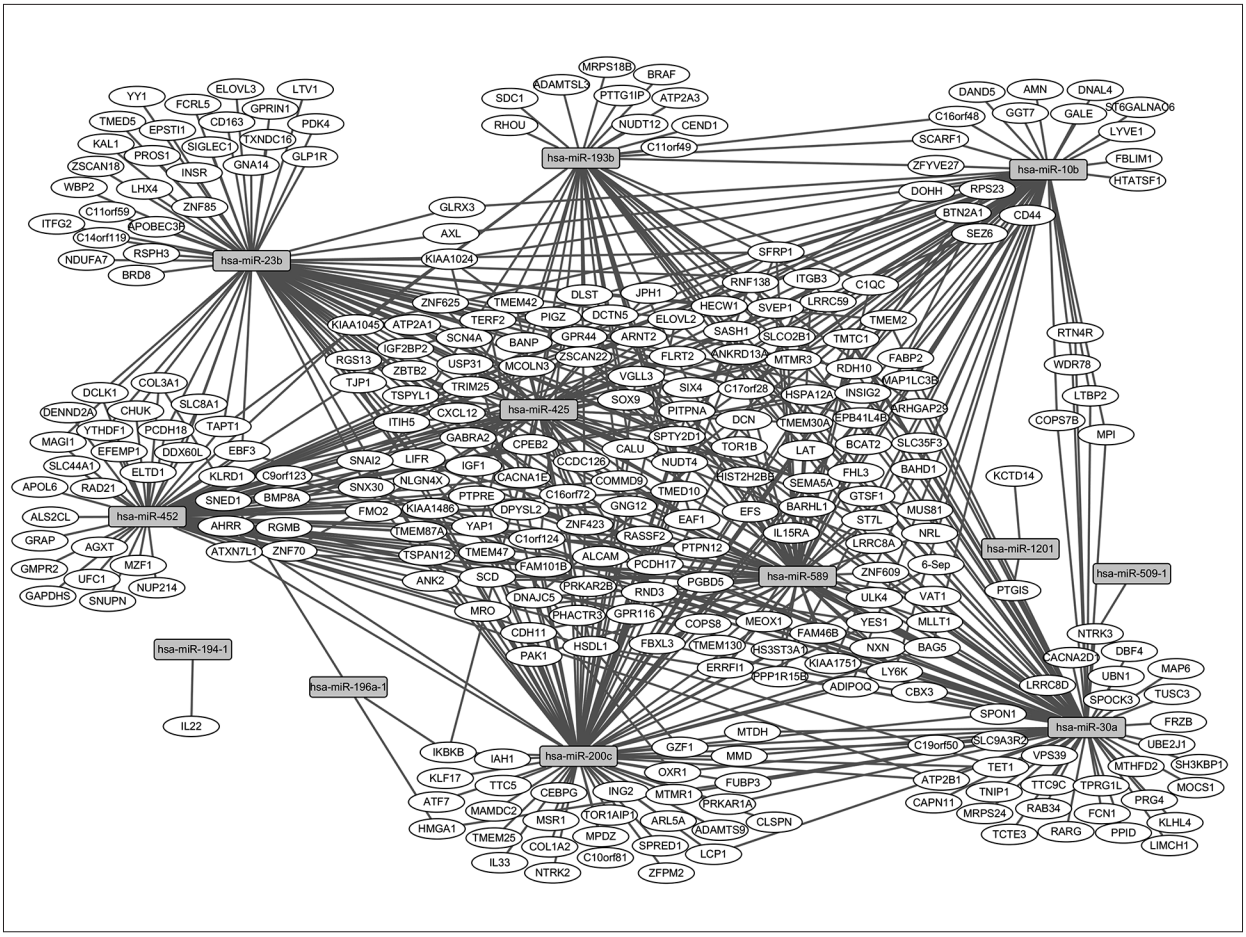

Figure 1. Interaction network of miRNAs-target-genes associated with AML. Circular nodes represent target-genes and rectangle nodes represented miRNAs. The solid line represents association between miRNA and target-gene.

Table 3. KEGG signaling pathway enrichment.

KEGG ID	KEGG term	No.	FDR	Genes
hsa 04972	Pancreatic secretion	3	0.000131	ATP2B1, ATP2A1, ATP2A3
hsa04020	Calcium signaling pathway	3	0.000131	ATP2B1, ATP2A1, ATP2A3
hsa04650	Natural killer cell mediated cytotoxicity	3	0.006809	BRAF, KLRD1, LAT
hsa05010	Alzheimer's disease	3	0.013968	ATP2A1, ATP2A3, NDUFA7

FDR – false discovery rate; No. – number of genes.

the miRNA-30 family (including mir-30a, mir-30b, mir-30c) and predicts unfavorable prognosis of AML patients [19,20].

CD44 was one of the top three risk factor target-genes associated with AML survival. A number of studies have reported that CD44 is associated with AML survival. In elderly patients with refractory AML, the overall survival of patients with PTEN-positive and CD44-negative expression is longer than patients with PTEN-negative and CD44-positive expression [21]. Knockdown of CD44 enhances chemo-sensitivity of AML cells to adriamycin (ADM) and cytosine arabinoside (Ara-C) [22]. CD44 activation enhances primary acute monoblastic leukemia blast survival and increases apoptosis resistance of THP-1 monoblastic leukemia cells. Moreover, CD44 activation upregulates the expression of anti-apoptotic Mcl-1 protein, which is essential for apoptosis resistance of THP-1 cells [23]. Calcium signaling pathway was one of the significantly enriched pathways in our study (Table 3). Calcium can act in signal transduction resulting from activation of ion channels or as a second messenger. Calcium signaling through ion channels is important

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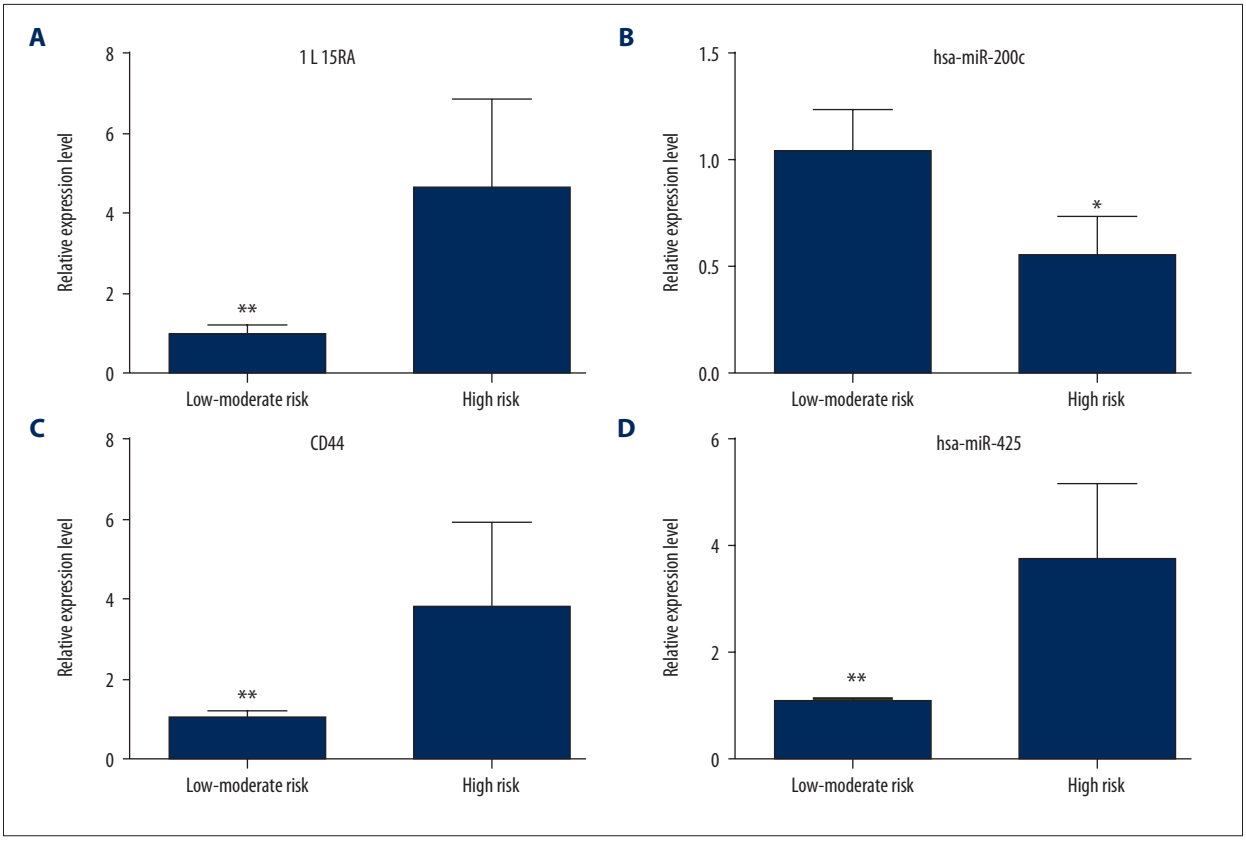

Figure 2. Verification of expression levels of miRNAs and mRNAs in the low-moderate risk group and the high risk group of AML patients by qRT-PCR. (**A**) IL15RA; (**B**) hsa-miR-200c; (**C**) CD44; (**D**) hsa-miR-425. * is $p<0.05$ and ** is $p<0.01$.

to maintain depolarization of the heart and neuronal synaptic transmission. Calcium ions play a vital role in muscle contraction, neuronal transmission, cell motility, cell growth or proliferation [24–27]. It has been reported that calcium signaling pathway is related to progression and development of various cancers including lung adenocarcinoma [28], colorectal cancer [29], glioblastoma [30], breast cancer [31], and Burkitt lymphoma [32]. Calcium signaling pathway may play a key role in the progression and development of AML.

Natural killer cell mediated cytotoxicity was one of the significant enrichment KEGG pathways in our study (Table 3). Natural killer cell mediated cytotoxicity contributes to the innate immune response against numerous malignancies. Progression and development of malignancy is promoted by tumor cells escaping from immune surveillance of immune effector cells, including natural killer cells [33]. The phenomenon of tumor cells escaping from immune surveillance is observed in numerous malignancies including breast cancer [34], head-and-neck squamous carcinoma [35], and leukemia. Leukemia stem cells play a central role in the relapse and refractory of AML. Leukemic stem-like cells from AML cell line KG1a cells are resistant to chemotherapy and natural killer cell-mediated cytotoxicity [36]. Acute lymphoblastic leukemia could resist natural killer cell-mediated cytotoxicity [37]. Calcium signaling pathway and natural killer cell mediated cytotoxicity correlate with the progression and development of numerous tumors. It has been suggested that these two pathways may play key roles in AML progression and contribute to AML survival.

We identified the miRNAs and target-genes associated with AML survival. In our study, IL15RA, TNIP1, CD44, and hsa-mir-425 were risk-factor miRNA/target-genes associated with AML survival. In addition, hsa-mir-10b and hsa-mir-30a were protective-factor miRNAs associated with AML survival. As Figure 2 shows, IL15RA, CD44, and hsa-miR-425 were obviously upregulated in the high-risk group, and hsa-miR-200c was significantly downregulated in the high-risk group compared with the low-moderate risk group of AML patients. The qRT-PCR results were in accordance with other study results.

There were limitations to our study. First, although the miRNAs and mRNAs associated with survival time of AML patients identified in our study may have implications in the understanding of AML tumorigenesis and development of targeted therapy of AML, the roles of identified miRNAs and mRNAs in AML need to be further explored in laboratory work. Second, although we obtained miRNAs and target-genes associated

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with AML survival, we did not construct a prognostic model of AML survival. In future work, we will use miRNAs and target-genes to construct a prognostic signature to predict survival time of AML patients, and then verify the prognostic signature through clinical observations.

Conclusions

A number of miRNAs and mRNAs that correlated with survival of AML patients were identified in our study, which might provide valuable information for identification of potentially prognostic biomarker for AML survival.

Conflict interest

All of the authors declare that they have no conflict interest.

Supplementary Table

Supplementary Table 1. The target-genes of miRNAs associated with AML survival.

miRNA	Count of targets	Count of target related to survival	Target mRNAs
hsa-mir-10b	66	22	JPH1, TRIM25, AXL, HTATSF1, VGLL3, SLCO2B1, RTN4R, C17orf28, CD44, IGF2BP2, ITGB3, NRL, SCARF1, HSPA12A, RDH10, SASH1, ANKRD13A, KIAA1024, MAP1LC3B, SFRP1, COPS7B, BTN2A1, NUDT4, FLRT2, SPTY2D1, ERRFI1, LTBP2, LRRC59, PPP1R15B, DNAJC5, C16orf48, GGT7, BAG5, WDR78, GPR44, FBLIM1, SEZ6, TMTC1, RPS23, CALU, DNAL4, GLRX3, DOHH, ZSCAN22, ST7L, ELOVL2, BAHD1, MPI, AMN, GALE, SIX4, KIAA1751, KIAA1045, INSIG2, TMEM2, ZFYVE27, MTMR3, ARNT2, FHL3, DAND5, FABP2, LYVE1, PITPNA, LY6K, ADIPOQ, ST6GALNAC6
hsa-mir-1201	2	0	KCTD14, PTGIS
hsa-mir-193b	44	15	SOX9, SDC1, TRIM25, C1QC, HECW1, EPB41L4B, ADAMTSL3, RHOU, ITGB3, SCARF1, SVEP1, TMEM30A, PTPRE, ANKRD13A, SLC35F3, CEND1, MRPS18B, NUDT4, TMED10, ZNF423, DNAJC5, C16orf48, ATP2A3, EAF1, LRRC8A, RNF138, CACNA1E, CALU, NUDT12, C11orf49, ST7L, BRAF, PTTG1IP, SNX30, ARHGAP29, COMMD9, SIX4, 6-Sep, KIAA1045, ZFYVE27, DLST, YAP1, DCTN5, TAPT1
hsa-mir-194-1	1	0	IL22
hsa-mir-196a-1	3	2	IKBKB, COL3A1, HMGA1
hsa-mir-200c	95	21	IGF1, ADAMTS9, PTPN12, C10orf81, VGLL3, COL1A2, OXR1, MRO, C1orf124, CEBPG, EPB41L4B, GZF1, CD44, NTRK2, SLC9A3R2, KLF17, ITGB3, TTC5, HSPA12A, CDH11, RDH10, C16orf72, SASH1, TMEM47, KIAA1486, KIAA1024, MAP1LC3B, SFRP1, YES1, BTN2A1, PHACTR3, RND3, RND3, NUDT4, TOR1AIP1, TJP1, SPTY2D1, GNG12, ERRFI1, NLGN4X, TMED10, ZNF423, DNAJC5, ATP2B1, BAG5, ATF7, SNAI2, SNAI2, LRRC8A, IKBKB, TMTC1, RNF138, RPS23, CALU, ATXN7L1, FUBP3, ALCAM, AHRR, ZFPM2, ZFPM2, ING2, TSPAN12, CLSPN, MPDZ, SCD, TET1, FBXL3, MAMDC2, GPR116, ELOVL2, MTDH, TMEM25, SNX30, ARL5A, MSR1, SPRED1, PRKAR2B, TERF2, MMD, 6-Sep, IAH1, MTMR1, SPON1, PAK1, HSDL1, LCP1, IL33, YAP1, SEMA5A, PRKAR1A, PITPNA, LY6K, ADIPOQ, HS3ST3A1, COPS8

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miRNA	Count of targets	Count of target related to survival	Target mRNAs
hsa-mir-23b	86	16	ZBTB2, CXCL12, CXCL12, EBF3, IGF1, RASSF2, AXL, VGLL3, ITFG2, HECW1, GNA14, OXR1, PCDH17, MAGI1, EPB41L4B, GZF1, C17orf28, PROS1, ITIH5, EPSTI1, ITGB3, HSPA12A, NDUFA7, RDH10, RSPH3, C16orf72, LHX4, KIAA1024, SFRP1, NUDT4, FLRT2, KAL1, ZSCAN18, TJP1, SPTY2D1, NLGN4X, LIFR, TMED10, ZNF423, SIGLEC1, EAF1, RGS13, DCLK1, CHUK, GPRIN1, CACNA1E, CALU, PCDH18, ALCAM, GLRX3, CPEB2, TSPAN12, TXNDC16, SCN4A, CD163, DDX60L, INSR, ZSCAN22, C11orf59, ST7L, PDK4, PDK4, ELOVL2, LTV1, WBP2, USP31, YTHDF1, SNX30, BRD8, YY1, SLC8A1, TERF2, SIX4, ELOVL3, TMED5, INSIG2, TMEM2, ZNF85, ARNT2, SEMA5A, FCRL5, APOBEC3F, PITPNA, GLP1R, C14orf119, TAPT1
hsa-mir-30a	102	27	SOX9, CXCL12, EBF3, MOCS1, SPOCK3, UBN1, VGLL3, NXN, RTN4R, FRZB, HECW1, OXR1, TUSC3, PCDH17, EPB41L4B, TTC9C, GZF1, TNIP1, MTHFD2, CACNA2D1, RARG, LRRC8D, SLC9A3R2, ITGB3, ITGB3, RAB34, TCTE3, RDH10, TMEM30A, NTRK3, TMEM47, ANKRD13A, VAT1, SLC35F3, KIAA1024, MAP1LC3B, YES1, COPS7B, DBF4, RND3, KLHL4, SPTY2D1, ERRFI1, LTBP2, LIMCH1, LIFR, TMED10, TMED10, PPP1R15B, FCN1, ATP2B1, WDR78, EAF1, PRG4, CBX3, LRRC8A, UBE2J1, UBE2J1, TMTC1, RNF138, CALU, MRPS24, MLLT1, MAP6, FUBP3, CPEB2, C19orf50, DPYSL2, VPS39, ZSCAN22, TET1, FBXL3, ELOVL2, MTDH, RGMB, PTGIS, BAHD1, MPI, SNX30, ARHGAP29, C9orf123, COMMD9, MMD, CAPN11, SIX4, PPID, 6-Sep, KIAA1751, INSIG2, TMEM2, TMEM87A, TMEM87A, MTMR1, SH3KBP1, SPON1, TPRG1L, HSDL1, LCP1, LCP1, PRKAR1A, HMGA1, LY6K
hsa-mir-425	57	20	SOX9, JPH1, IGF1, TRIM25, HIST2H2BE, VGLL3, DCN, C17orf28, ITIH5, CCDC126, ITGB3, TMEM42, TMEM30A, C16orf72, GABRA2, ANKRD13A, ZNF625, KIAA1486, SFRP1, PHACTR3, SNED1, RND3, NUDT4, FLRT2, SPTY2D1, GNG12, NLGN4X, LRRC59, TMED10, ZNF423, ZNF70, ATP2A1, EAF1, RGS13, LRRC8A, IKBKB, SEZ6, BMP8A, CALU, AHRR, CPEB2, SCN4A, DPYSL2, DOHH, PIGZ, BANP, ZSCAN22, MUS81, GPR116, USP31, C9orf123, TSPYL1, KLRD1, MTMR3, HSDL1, DLST, MCOLN3
hsa-mir-452	81	22	EBF3, IGF1, TRIM25, RASSF2, PTPN12, VGLL3, PGBD5, FMO2, MRO, PCDH17, C1orf124, MAGI1, IGF2BP2, ALS2CL, RAD21, HSPA12A, ANK2, APOL6, SASH1, TMEM47, PTPRE, KIAA1486, SNUPN, MAP1LC3B, SFRP1, YES1, SNED1, FLRT2, GNG12, NLGN4X, LIFR, TMED10, GRAP, ATP2B1, ZNF70, GPR44, FAM101B, SNAI2, DCLK1, CHUK, DENND2A, COL3A1, TMTC1, BMP8A, RNF138, CACNA1E, PCDH18, UFC1, ATXN7L1, EFEMP1, AHRR, CPEB2, DPYSL2, MZF1, DDX60L, GAPDHS, SCD, TET1, SLC44A1, FBXL3, ELOVL2, ELTD1, RGMB, YTHDF1, SNX30, SLC8A1, PRKAR2B, C9orf123, COMMD9, TSPYL1, KLRD1, KIAA1045, TMEM87A, AGXT, ARNT2, GMPR2, YAP1, NUP214, DCTN5, TAPT1, COPS8
hsa-mir-509-1	1	0	NTRK3
hsa-mir-589	69	24	ZBTB2, GTSF1, C1QC, RASSF2, HIST2H2BE, VGLL3, NXN, SLCO2B1, PGBD5, FMO2, MRO, PCDH17, DCN, EPB41L4B, CCDC126, ITGB3, NRL, CDH11, SVEP1, ANK2, TMEM130, EFS, C16orf72, SASH1, PTPRE, ULK4, IL15RA, VAT1, KIAA1486, SFRP1, YES1, ZNF609, GNG12, ERRFI1, LIFR, PPP1R15B, DNAJC5, BAG5, FAM101B, CBX3, TMTC1, BMP8A, RPS23, ATXN7L1, MLLT1, LAT, DPYSL2, SCD, ZSCAN22, TOR1B, FBXL3, ST7L, BAHD1, FAM46B, COMMD9, SIX4, KIAA1751, BARHL1, PAK1, FHL3, FABP2, YAP1, SEMA5A, PITPNA, MEOX1, DCTN5, HS3ST3A1, BCAT2, COPS8

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