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Comprehensive analysis the potential biomarkers for the high-risk of childhood acute myeloid leukemia based on a competing endogenous RNA network

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ABSTRACT

Acute myeloid leukemia (AML) is a common form of hematological malignancies, the discovery of non-coding RNA (ncRNA) plays an important role in diverse biological processes including hematopoietic differentiation and proliferation. However, the interaction mechanism of key RNAs and their regulatory network in childhood AML are still to be elucidated. RNA profiles were downloaded from the Therapeutically Applicable Research to Generate Effective Treatment (TARGET) database and identified specific lncRNAs, miRNAs, and mRNAs in high-risk group of childhood AML. A lncRNA-mRNA-miRNA ceRNA network in childhood AML was constructed. A total of 2064 mRNAs, 615 lncRNAs, and 60 miRNAs were identified as significantly differentially expressed, and 13 lncRNAs, 7 miRNAs, and 67 mRNAs were incorporated in the ceRNA network. Functional analysis showed that these DEmRNAs were significantly enriched in Ras signaling pathway, TGF-beta signaling pathway, and other tumor-related pathways. Among the network, 10 RNAs (*LINC00471*, *hsa-mir-100*, *hsa-mir-150*, *ANP32E*, *ERMP1*, *MYO1B*, *PAPD7*, *PTGIS*, *TERF1*, and *VEGFA*) was associated with high-risk group of childhood AML and functions were significant for prognosis. Then, these findings together provide a new insight into the pathogenesis of high-risk group of childhood AML that can assist clinicians clarify the function of lncRNA to guide the treatment and in-depth study.

1. Introduction

Acute myeloid leukemia (AML) is a common form of hematological malignancies characterized by abnormal proliferation of immature myeloid cells [1,2]. Chemotherapy and hematopoietic stem cell transplantation are the standard treatment for AML, and the 5-year overall survival rate for children is 67%, but it declines to 54%, 32%, and 7% when the patients aged 20 to 49, 50 to 64, 65 and older, respectively [3,4]. Although the diagnostic approaches and therapeutic efficacy of AML has gradually improved, but refractory acute leukemia still response and die after remission, and short survival period. Therefore, there is an urgent need for early prognostic markers and novel therapeutic targets.

Non-coding RNA (ncRNA) are RNA molecules that play critical roles in diverse biological processes including hematopoietic differentiation and proliferation [5,6]. Although ncRNAs are not involved in protein coding, includes micro RNAs (miRNAs), long noncoding (lncRNAs), and circular RNAs (circRNAs), but they directly regulate transcription, post-transcriptional protein coding in the form of RNA, and it opens up new prospects for treatment, diagnosis and prognosis of AML [7]. With the discovery of numerous RNAs and further research, the competing endogenous RNAs (ceRNAs) network has emerged in the development of AML which revealed a new mechanism for interactions between RNAs [8]. Studies has shown that miRNAs are involved in the initiation of cancers, the 5′ regions of miRNA can bind to sequences on the target mRNAs' 3′UTRs, known as microRNA recognition elements, which usually inhibit the expression of target genes [9]. The hypothesis of ceRNA described complex communication network, lncRNA can act as natural miRNA sponges to inhibit miRNA functions by binding to adsorbed miRNA [10,11]. These studies established the good foundation for understanding the lncRNA/miRNA/mRNA interaction mechanism.

At present, the ceRNA hypothesis has been shown to be associated with the development of different kinds of hematological tumor. In AML, lncRNA *SBF2-AS1* controlled cell proliferation via acting as a ceRNA against *miR-188-5p* [12]; LncRNA *NEAT1* repressed the expression of *miR-23a-3p* and therefore modulated cell proliferation and apoptosis by regulating *SMC1A* [13]; In chronic myeloid leukemia (CML), lncRNA *UCA1* contributes to imatinib resistance via acting as a

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ceRNA against *miR-16* [14]. In diffuse large B cell lymphoma (DLBCL), lncRNA *SMAD5-AS1* modulated cell proliferation via Wnt/Beta-catenin pathway by regulating *miR-135b-5p* to elevate expression of *APC* [15]. In multiple myeloma, lncRNA *CCAT1* promotes cancer progression by acting as a ceRNA against *miR-181a-5p* to modulate *HOXA1* expression [16]. Therefore, studies have shown that lncRNA-miRNA-mRNA ceRNA regulatory network is involved in the occurrence and development of hematopoietic malignancies. However, the research on childhood AML are still limited with recent developments in microarray technology, and there is a lack of comprehensive analysis of mRNAs, miRNAs and lncRNAs related to high-risk childhood AML by high-throughput sequencing and large-sample size.

In this study, we focused on high-risk group of childhood AML, the expression profiles of lncRNAs, miRNAs, and mRNAs between high-risk (n = 160) and low-risk (n = 404) group were obtained from TARGET database. After which, the aberrant lncRNA-miRNA-mRNA ceRNA network was constructed in high-risk group of childhood AML. Finally, we identified 13 lncRNAs, 7 miRNAs and 67 mRNAs to construct a lncRNA-miRNA-mRNA ceRNA network. Among the network, 10 RNAs (*LINC00471*, *hsa-mir-100*, *hsa-mir-150*, *ANP32E*, *ERMP1*, *MYO1B*, *PAPD7*, *PTGIS*, *TERF1*, and *VEGFA*) was identified in childhood AML and functions were significant for prognosis. The results of this study can assist clinicians clarify the function of lncRNA through lncRNA-miRNA-mRNA ceRNA network in AML and provide novel lncRNAs as potential diagnostic biomarkers.

2. Materials and methods

2.1. Data resource and pretreatment

The raw RNA and miRNA sequencing data of childhood AML patients were obtained from the TARGET database (https://ocg.cancer.gov/). Patient clinical data, including outcome and staging information, were also downloaded (up to April 29, 2019). Exclusion criteria were set as follows: (i) samples without clinical data, (ii) samples without complete information of risk stratification and overall survival period. Then, there are 564 samples used in this study, including 404 low risk samples and 160 high risk samples. GraphPad Prism (Version 8.0.2) was used to analyze the overall survival in high risk childhood AML. Finally, we obtained lncRNA, miRNA, and mRNA expression profiles of low risk and high risk of childhood AML patients. The RNA-seq data and clinical data are publicly available and available on open-access. Therefore, no further approval was required from the local ethics committee.

2.2. Differential expression analysis

We used Perl language (http://www.perl.org/,version 5.30.0) to extract the gene matrix file data and transform the ensembl IDs to gene names based on the Ensembl database (http://www.ensembl.org). The 'edgeR' package [17] in R 3.5.2 software(https://www.r-project.org/) was utilized to identify the differentially expressed RNAs in childhood AML, the downloaded data were calibrated, standardized and analyzed for differences to obtain differentially expressed lncRNA, miRNA, and mRNA molecules between the high risk group and low risk group in childhood AML. For all the P values, we used false discovery rate (FDR) to correct the statistical significance of multiple testing. The screening criteria of the three kinds of dysregulated RNAs were as follows: FDR < 0.01 and |log2fold change| > 1. Finally, hierarchical cluster analysis was used gplots and heatmap packages in the R platform.

2.3. Construction of the ceRNA (DElncRNA-DEmiRNA-DEmRNA) regulatory network

First, the names of DEmiRNAs were transformed into human mature miRNA names from starBase v2.0 (http://starbase.sysu.edu.cn/) [18]. In this study, miRcode(http://www.mircode.org/) database was used to predict lncRNA-miRNA interactions, so DElncRNAs and DEmiRNAs that could be compared successfully were obtained [19]. In addition, predicting the DEmiRNA target genes using the online websites miRDB (http://www.mirdb.org/), miRTarBase (http://mirtarbase.mbc.nctu.edu.tw), and TargetScan (http://www.targetscan.org) [20–22]. To further improve the reliability of bioinformatics analysis, a Venny diagram was used to obtain the portion of the target mRNA that overlaps with the differentially expressed mRNA in high risk childhood AML and was further analyzed as DEmRNA. Finally, we established matched DElncRNA–DEmiRNA and DEmiRNA–DEmRNA pairs.

Table 1
Clinical characteristics of 564 patients with childhood AML.

	Low risk (n = 404)	High risk (n = 160)	Total (n = 564)	P-value
Age				0.096
< 14	259(64.1%)	115(71.9%)	374(66.3%)	
≥14	145(35.9%)	45(28.1%)	190(33.7%)	
Gender				0.103
Male	210(52%)	96(60%)	306(54.3%)	
Female	194(48%)	64(40%)	258(45.7%)	
White blood cell				3.29E−04
< 50	236(58.4%)	66(41.3%)	302(53.5%)	
≥50	168(41.6%)	94(58.8%)	262(46.5%)	
FAB category				< 0.01
M0	2(0.5%)	8(5%)	10(1.8%)	
M1	50(12.4%)	34(21.3%)	84(14.9%)	
M2	150(37.1%)	23(14.4%)	173(30.7%)	
M3	1(0.2%)	0(0.0%)	1(0.2%)	
M4	127(31.4%)	37(23.1%)	164(29.1%)	
M5	18(4.5%)	23(14.4%)	41(7.3%)	
M7	0(0.0%)	3(1.9%)	3(0.5%)	
Not Classified	56(13.8%)	32(20.0%)	88(15.6%)	
Gene fusion				< 0.01
RUNX1-RUNX1T1	151(37.4%)	3(1.9%)	154(27.3%)	
CBFB-MYH11	128(31.7%)	3(1.9%)	131(23.2%)	
NUP98-NSD1	0(0.0%)	21(13.1%)	21(3.7%)	
DEK-NUP214	0(0.0%)	10(6.3%)	10(1.8%)	
CBFB-MYH11	0(0.0%)	3(1.9%)	3(0.5%)	
NA or other fusion	125(30.9%)	120(75%)	245(43.4%)	
FLT3/ITD positive				2.60E−65
Yes	34(8.4%)	130(81.3%)	164(29.1%)	
No	370(91.6%)	30(18.8%)	400(70.9%)	
NPM mutation				5.30E−01
Yes	69(17.1%)	23(14.4%)	92(16.3%)	
No	330(81.7%)	134(83.8%)	464(82.3%)	
Unknown	5(1.2%)	3(1.9%)	8(1.4%)	
CEBPA mutation				2.95E−05
Yes	59(14.6%)	3(1.9%)	62(11%)	
No	342(84.7%)	154(96.3%)	496(87.9%)	
Unknown	3(0.7%)	3(1.9%)	6(1.1%)	
WT1 mutation				4.36E−06
Yes	19(4.7%)	27(16.9%)	46(8.2%)	
No	380(94.1%)	131(81.9%)	511(90.6%)	
Unknown	5(1.2%)	2(1.3%)	7(1.2%)	

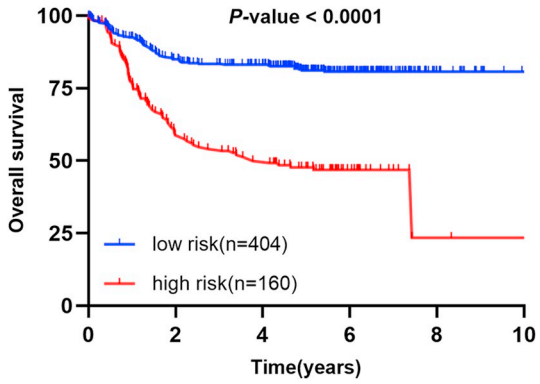

Fig. 1. Overall survival rate of high-risk group with childhood AML.

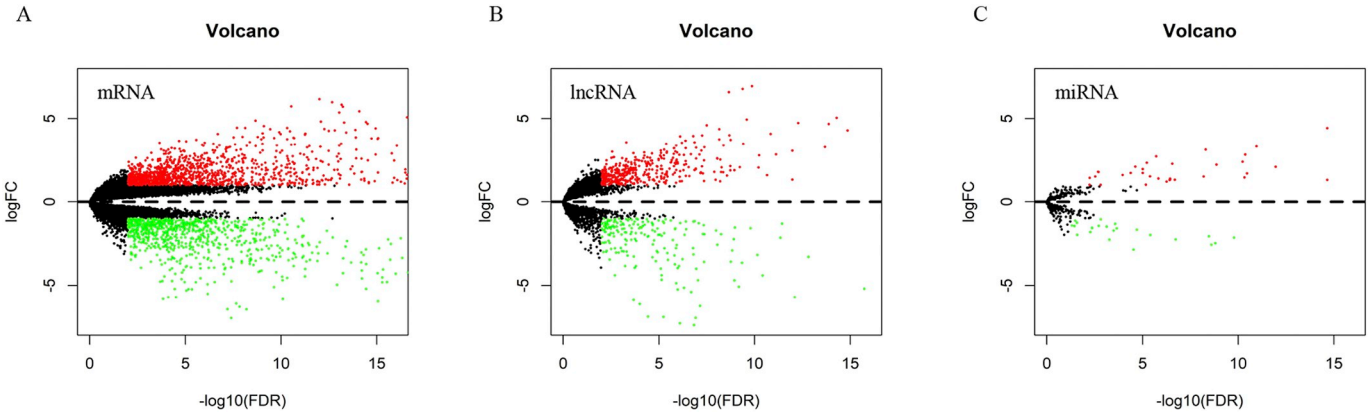

Fig. 2. Volcano plots reflecting number, significance and reliability of differentially expressed RNAs in high-risk childhood AML. The red dots indicate upregulation and green dots indicate downregulation of lncRNAs (A), miRNAs(B) and mRNAs(C). The x-axis represents an adjusted FDR and the y-axis represents the value of log2 FC. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Thirteen DElncRNAs interact with the seven DEmiRNAs retrieving miRcode database.

lncRNA	miRNA
SNHG14	*hsa-mir-106a hsa-mir-150 hsa-mir-155 hsa-mir-424 hsa-mir-497 hsa-mir-363*
SPATA13	*hsa-mir-106a hsa-mir-100 hsa-mir-150 hsa-mir-363*
C20orf203	*hsa-mir-150 hsa-mir-424 hsa-mir-497 hsa-mir-363*
C10orf95	*hsa-mir-150 hsa-mir-424 hsa-mir-497*
LINC00470	*hsa-mir-150 hsa-mir-424 hsa-mir-497*
CCDC13-AS1	*hsa-mir-150 hsa-mir-424 hsa-mir-497*
ADARB2-AS1	*hsa-mir-150 hsa-mir-424 hsa-mir-497*
C20orf166-AS1	*hsa-mir-106a hsa-mir-150*
LINC00501	*hsa-mir-150 hsa-mir-363*
C9orf139	*hsa-mir-150*
LINC00471	*hsa-mir-106a*
LINC00158	*hsa-mir-150*
FAM201A	*hsa-mir-150*

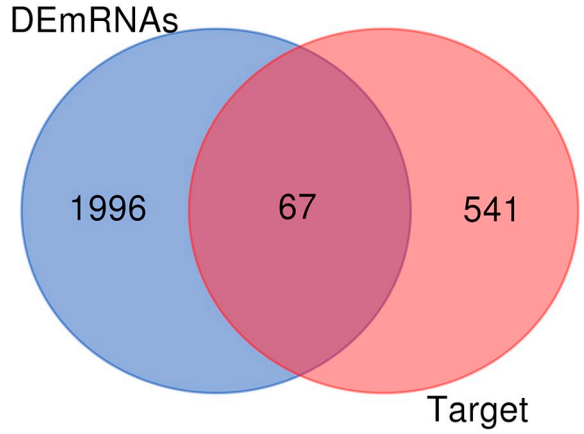

Fig. 3. Venn diagram of differentially expressed mRNAs involved in the competing endogenous RNA (ceRNA) regulation network. The mRNAs expressed in the blue area are all the DEmRNAs in high-risk childhood AML. The target number is in the red area, and the purple area represents the DEmRNAs that are located in both the differential expression and target groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The ceRNA network of DElncRNA-DEmiRNA-DEmRNA was constructed by Cytoscape(version 3.7.1) based on ceRNA hypothesis. The open source software platform of Cytoscape (https://cytoscape.org/) [23] was used to visualize the three types of RNAs and their interactions, which finally led to the ceRNA network graph.

2.4. Functional enrichment analysis and survival analysis

To explore the biological processes and pathways of aberrantly expressed DEmRNA in the ceRNA network, Gene Ontology (GO) Biological Process term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses [24] were conducted using the clusterProfiler package of R software [25]. The significant results of biological process (BP), cellular component (CC) and molecular function (MF) were identified by the GOplot package of R software [26], and p-value of < 0.05 was considered to be significantly enriched.

2.5. Associations of DElncRNAs, DEmiRNAs, and DEmRNAs and patient prognosis

Each sample in database was independent of each other, and it contained sample data, such as gene expression, prognosis, and survival time, the 'Survival' package in R software was used to analyze the specific DElncRNA, DEmiRNA and DEmRNA associated with survival. Survival curves were generated using the Kaplan-Meier method between DERNAs signature and childhood AML patient prognosis. $P < 0.05$ was considered as the threshold for significance in analyses.

3. Results

3.1. Patient characteristics

The detailed clinical and characteristics of study population were summarized in Table 1. All 564 childhood patients were diagnosed as AML, including 404 low-risk group and 160 high-risk group. The median age for all patients was 10 years, and no significant gender-related differences (male to female ratio: 1.18). According to the clinical risk classification characteristics, the overall survival rate of high-risk group was significantly lower than that of low-risk group (Fig. 1).

3.2. Differentially expressed lncRNA, miRNA, and mRNA

A total of 2064 mRNAs, 615 lncRNAs, and 60 miRNAs were found to be differentially expressed in high risk childhood AML ($|\log2FC| >$ and adjusted P value < 0.01 as the standards), of which 1197 mRNAs (57.9%), 413 lncRNAs (67.1%) and 35 miRNAs (58.3%) were upregulated while others were downregulated. Volcano plots, visually demonstrating the distribution of RNAs, are shown in Fig. 2.

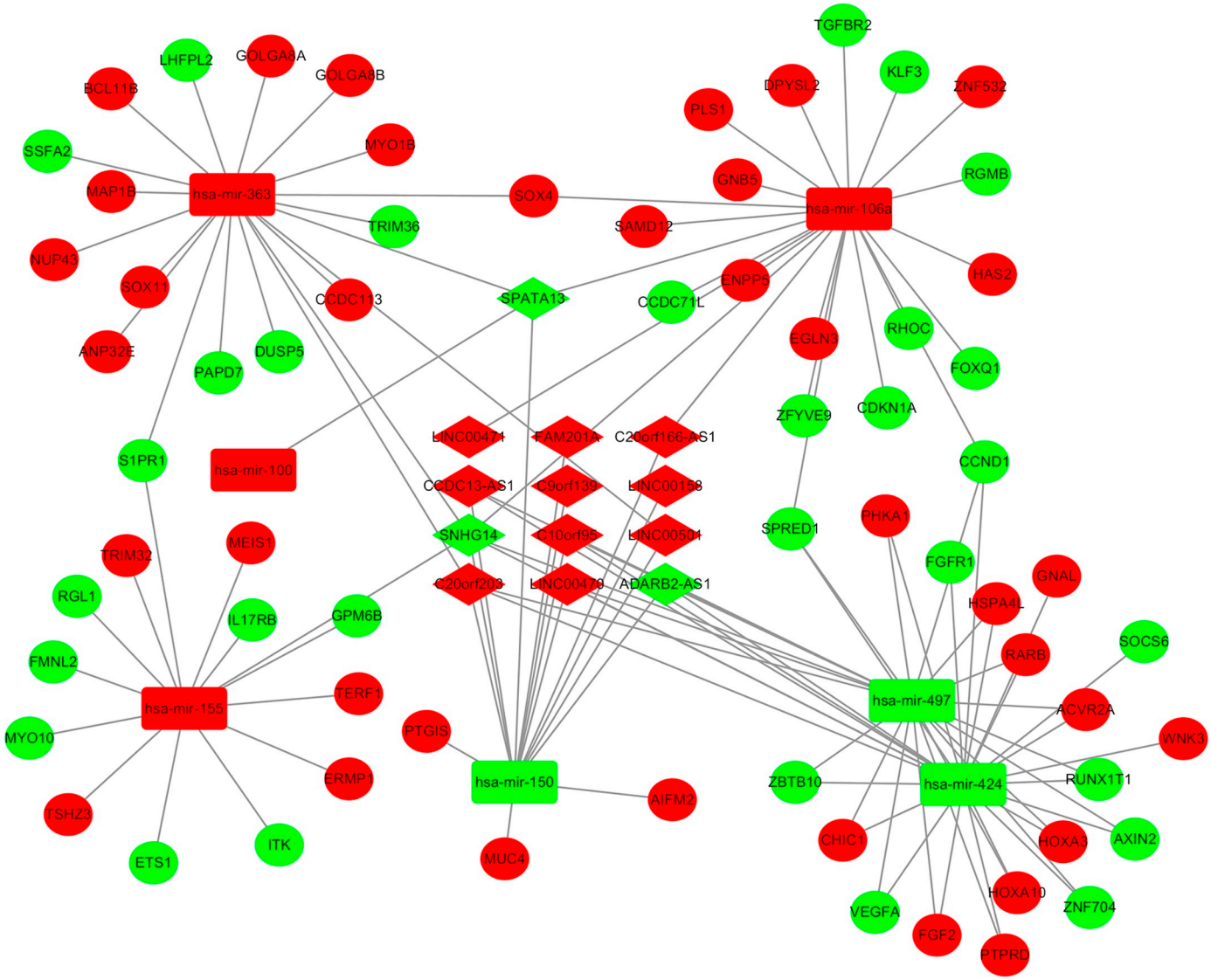

Fig. 4. DElncRNA mediated ceRNA regulatory network in high-risk childhood AML. Diamond denotes lncRNA, square represents miRNA, and round rectangle represents mRNA. All shapes in red and green colors stand for upregulation and downregulation, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Target prediction and construction of the ceRNA regulatory network

Our study used the 615 DElncRNAs retrieved from the miRcode database, the results revealed 876 pairs of interacting lncRNAs and miRNAs has been identified. From the 60 retrieved DEmiRNAs, we predicted that 34 DElncRNA-DEmiRNA interactions, with 13 DElncRNAs targeting 7 DEmiRNAs (Table 2). We next conducted target prediction for 7 modified DEmiRNAs, which were included in all 3 databases (miRDB, miRTarBase, and TargetScan), and miRNAs targeted mRNAs not included in DEmRNAs were discarded. Then we obtained 541 target genes, which intersected with 1996 DEmRNA, resulting in 67 DEmRNA (Fig. 3). In order to detect the significant relationships between DElncRNA, DEmiRNA, and DEmRNA in high-risk childhood AML, we constructed the ceRNA network by Cytoscape software(Fig. 4). The ceRNA network consisted of 13 lncRNAs (three downregulated and ten upregulated; Table 3), 7 miRNAs (three downregulated and four upregulated; Table 3), and 67 mRNAs (twenty-nine downregulated and thirty-eight upregulated).

3.4. Functional annotation of the ceRNA network

To understand the biological processes and pathways of aberrantly expressed mRNA in the ceRNA network, we conducted the GO and KEGG enrichent analysis. The enriched gene ontology (GO) networks are shown in Fig. 5. The biological processes (BP) included 'skeletal system development', 'heart morphogenesis', and 'positive regulation of endothelial cell chemotaxis'. In addition, the most enriched GO terms in cellular component (CC) were 'serine/threonine protein kinase complex', and that in molecular function (MF) was 'transcription factor activity, RNA polymerase II proximal promoter sequence-specific DNA binding'.

The KEGG results elucidated the potential biological functions(P-value < 0.05), a total of 20 significantly enriched pathways were obtained (Table 4). Among these pathways, 'Signaling pathways regulating pluripotency of stem cells', '*Ras* signaling pathway', 'TGF-beta signaling pathway', '*FoxO* signaling pathway', '*p53* signaling pathway', and '*PI3K-Akt* signaling pathway' are linked with the progression of AML [27–30]. Additionally, some other pathways such as 'Transcriptional misregulation in cancer', 'Gastric cancer', 'Renal cell carcinoma', 'Melanoma', 'Pancreatic cancer', and 'Breast cancer' were also tumor-

Table 3
Thirteen specific lncRNAs and seven specific miRNAs in ceRNA network construction.

	Expression change	LogFC	P-value	FDR
C10orf95	Up-regulation	2.08	3.71E−12	5.94E−10
LINC00470	Up-regulation	3.94	2.95E−09	1.61E−07
CCDC13-AS1	Up-regulation	1.82	1.17E−04	1.21E−03
C20orf166-AS1	Up-regulation	2.46	1.22E−06	2.57E−05
C9orf139	Up-regulation	1.78	5.38E−11	5.56E−09
LINC00471	Up-regulation	1.25	3.17E−05	4.16E−04
SPATA13	Down-regulation	−2.25	4.63E−05	5.57E−04
LINC00158	Up-regulation	1.72	1.30E−03	8.49E−03
C20orf203	Up-regulation	1.83	1.77E−08	7.73E−07
LINC00501	Up-regulation	1.62	8.26E−05	8.94E−04
FAM201A	Up-regulation	1.94	1.54E−06	3.15E−05
ADARB2-AS1	Down-regulation	−5.72	2.09E−15	7.98E−13
SNHG14	Down-regulation	−1.19	1.80E−06	3.60E−05
hsa-mir-150	Down-regulation	−1.05	1.71E−04	1.51E−03
hsa-mir-424	Down-regulation	−1.73	3.43E−05	3.22E−04
hsa-mir-497	Down-regulation	−1.44	2.33E−04	1.94E−03
hsa-mir-106a	Up-regulation	1.52	2.68E−10	6.42E−09
hsa-mir-100	Up-regulation	1.51	3.76E−04	2.95E−03
hsa-mir-363	Up-regulation	1.52	2.75E−07	4.09E−06
hsa-mir-155	Up-regulation	1.31	1.04E−17	2.24E−15

related pathways. The mentioned pathways are showed in Fig. 6.

3.5. Prognostic overall survival assessment of DElncRNAs, DEmiRNAs, and DEmRNAs

To identify the correlation between RNAs in the ceRNA network and the prognosis of childhood patients with high-risk AML, overall survival curves were generated using the Kaplan-Meier method between DERNAs ($P < 0.05$ was the cutoff threshold). Finally, following the topological analysis of the ceRNA network (Fig. 7), the expression levels of *LINC00471*($P = 1.04e-02$) was associated with poor OS, as well as *ANP32E*($P = 1.51e-02$), *ERMP1*($P = 3.52e-02$), *MYO1B*($P = 1.74e-02$), *PAPD7*($P = 3.86e-03$), *PTGIS*($P = 3.53e-02$), *TERF1*($P = 4.87e-02$), *VEGFA*($P = 9.96e-04$), *hsa-mir-100*($P = 1.41e-02$), and *hsa-mir-150*($P = 4.04e-02$). The results showed that the increased expression of *LINC00471*, *ANP32E*, *ERMP1*, *MYO1B*, *PTGIS* and *TERF1*, and the decreased period, which may be a procancer factor in childhood AML patients. However, the increased expression of *hsa-mir-100*, *hsa-mir-150*, *PAPD7*, and *VEGFA* demonstrated a good prognosis and potentially could be designated as a AML suppressor gene. Interestingly, we observed that *hsa-mir-150* and *PTGIS* had opposite prognostic effects, and *PTGIS* was the direct target of *hsa-mir-*

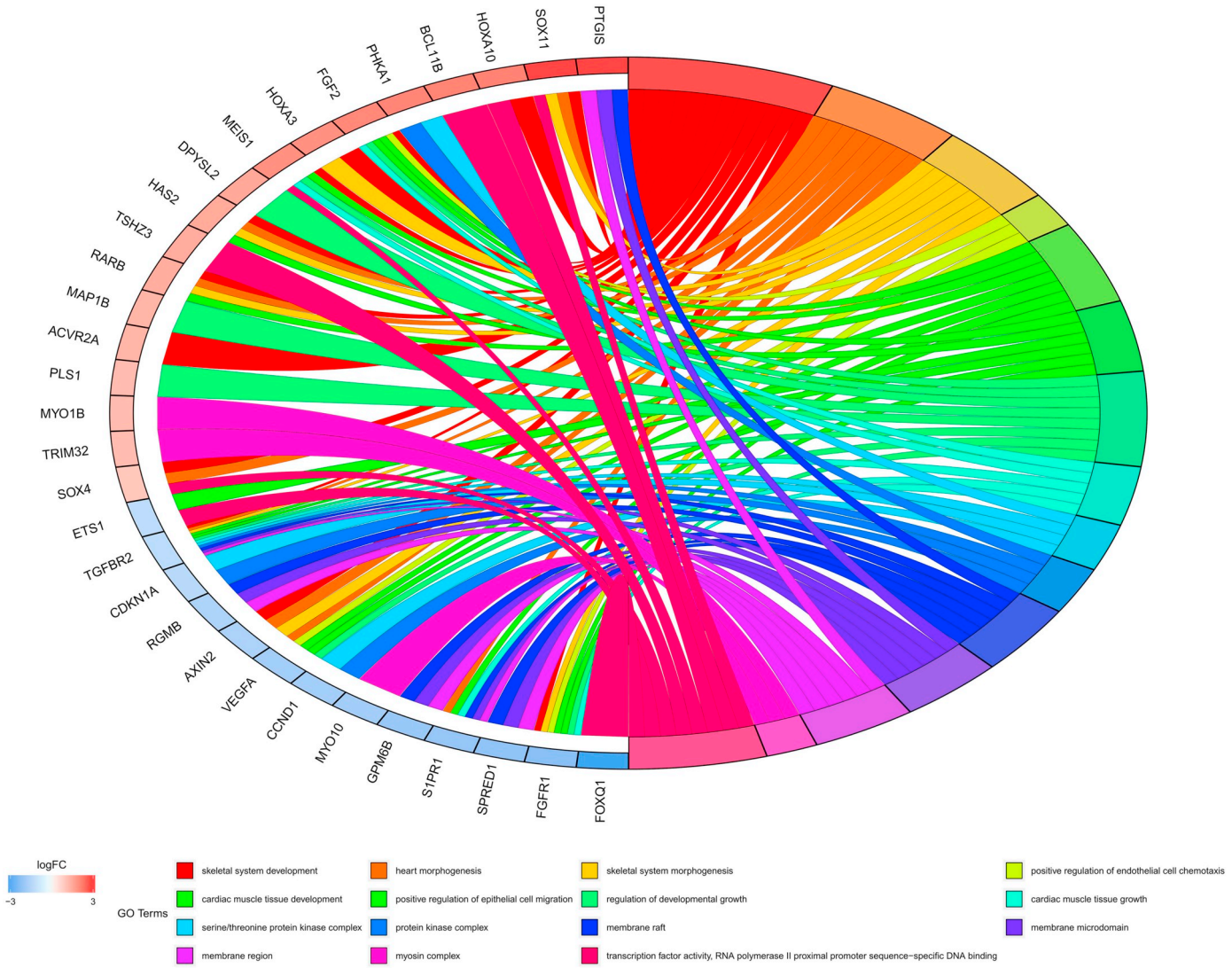

Fig. 5. The enriched GO terms of DEmRNAs involved in the ceRNA network of high-risk childhood AML.

Table 4
The top twenty of KEGG pathway enrichment analysis of genes involved in the ceRNA regulation network of high-risk childhood AML.

ID	Description	P-value	geneID	Count
hsa05202	Transcriptional misregulation in cancer	1.72E−05	*BCL11B,TGFBR2,RUNX1T1,KLF3,HOXA10,CDKN1A,MEIS1*	7
hsa05226	Gastric cancer	5.09E−05	*TGFBR2,FGF2,CDKN1A,AXIN2,CCND1,RARB*	6
hsa05211	Renal cell carcinoma	2.58E−04	*VEGFA,CDKN1A,ETS1,EGLN3*	4
hsa05218	Melanoma	3.04E−04	*FGFR1,FGF2,CDKN1A,CCND1*	4
hsa05212	Pancreatic cancer	3.56E−04	*TGFBR2,VEGFA,CDKN1A,CCND1*	4
hsa04550	Signaling pathways regulating pluripotency of stem cells	3.89E−04	*FGFR1,FGF2,AXIN2,MEIS1,ACVR2A*	5
hsa05224	Breast cancer	5.03E−04	*FGFR1,FGF2,CDKN1A,AXIN2,CCND1*	5
hsa04014	Ras signaling pathway	5.72E−04	*FGFR1,GNB5,VEGFA,RGL1,FGF2,ETS1*	6
hsa05210	Colorectal cancer	6.01E−04	*TGFBR2,CDKN1A,AXIN2,CCND1*	4
hsa05219	Bladder cancer	8.33E−04	*VEGFA,CDKN1A,CCND1*	3
hsa04350	TGF-beta signaling pathway	8.40E−04	*TGFBR2,RGMB,ACVR2A,ZFYVE9*	4
hsa05167	Kaposi sarcoma-associated herpesvirus infection	1.46E−03	*GNB5,VEGFA,FGF2,CDKN1A,CCND1*	5
hsa05205	Proteoglycans in cancer	2.14E−03	*FGFR1,VEGFA,FGF2,CDKN1A,CCND1*	5
hsa05213	Endometrial cancer	2.28E−03	*CDKN1A,AXIN2,CCND1*	3
hsa04068	FoxO signaling pathway	2.94E−03	*TGFBR2,S1PR1,CDKN1A,CCND1*	4
hsa05223	Non-small cell lung cancer	3.30E−03	*CDKN1A,CCND1,RARB*	3
hsa04115	p53 signaling pathway	4.23E−03	*AIFM2,CDKN1A,CCND1*	3
hsa05220	Chronic myeloid leukemia	4.92E−03	*TGFBR2,CDKN1A,CCND1*	3
hsa04151	PI3K-Akt signaling pathway	4.93E−03	*FGFR1,GNB5,VEGFA,FGF2,CDKN1A,CCND1*	6
hsa04218	Cellular senescence	5.85E−03	*TGFBR2,CDKN1A,ETS1,CCND1*	4

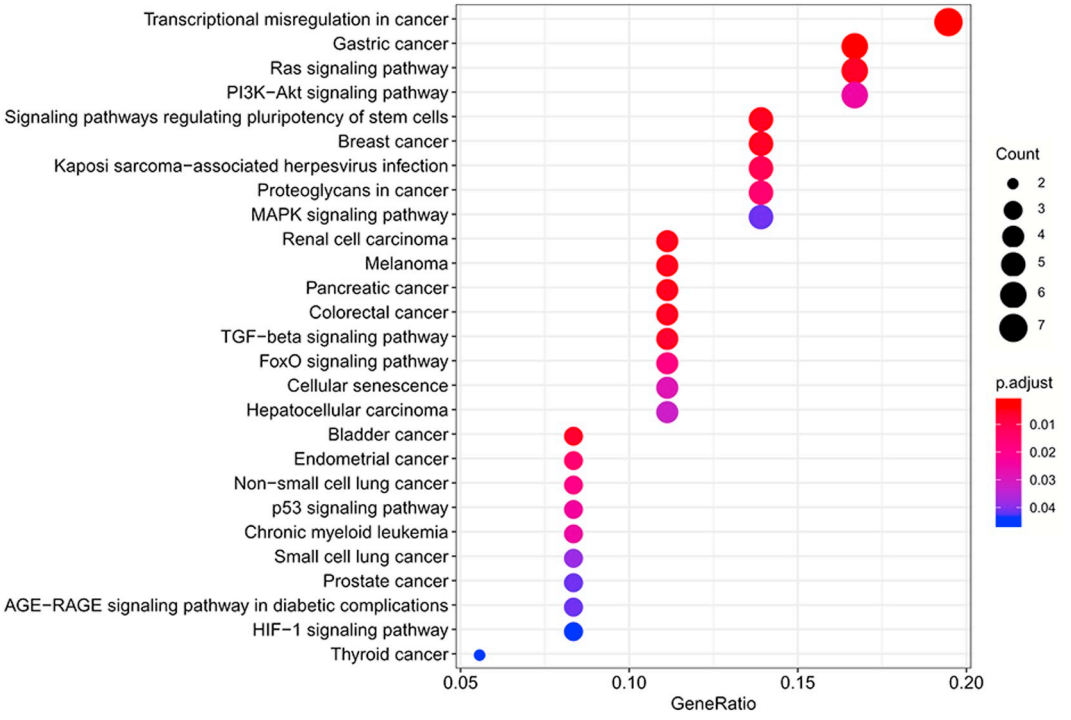

Fig. 6. The enriched KEGG pathway of DEmRNAs involved in the ceRNA network of high-risk childhood AML.

150, which are consistent with the results of previous studies.

Based on the ceRNA theory that lncRNAs can affect miRNA and act as miRNA sponges to further regulate mRNA [31], so there should be a positive correlation between mRNA and lncRNA regulated by the same miRNA. We constructed a correlation between the expression of DE-miRNA and DElncRNA (Fig. 8), and many molecules were highly correlated, which also verified the reliability of ceRNA network.

4. Discussion

Leukemia is the most common malignancies in children (< 14 years), with multiple types of molecular and cellular heterogeneity. AML accounts for 15–20% of the childhood leukemia [4]. With the continuous optimization of transplantation, chemotherapy and other treatment strategies, prognosis for AML improved remarkably during the past decades [32]. However, the therapeutic effects were poor in high-risk groups due to the high recurrence rate [33]. Recently, the ceRNA hypothesis has been reported to represent a group of RNAs with miRNA binding sites that competitively bind to miRNAs and inhibit their regulation of target genes [31]. Based on the ceRNA theory, miRNAs and their ceRNA targets (such as mRNA and lncRNA) can form complex ceRNA networks. Several studies has also revealed that dysregulation of lncRNAs contributes to pathogenesis and prognosis of cancer, and can serve as tumor-related predictors [34–36]. Therefore, elucidation of the molecular mechanisms is indispensable for diagnosing, and treating AML.

Our whole workflow is shown in Fig. 9. The present study analyzed the large-scale RNA-seq data from TARGET databases with high risk AML, we identified the differentially expressed RNAs, and further constructed a ceRNA network with AML-specific lncRNAs, miRNAs and

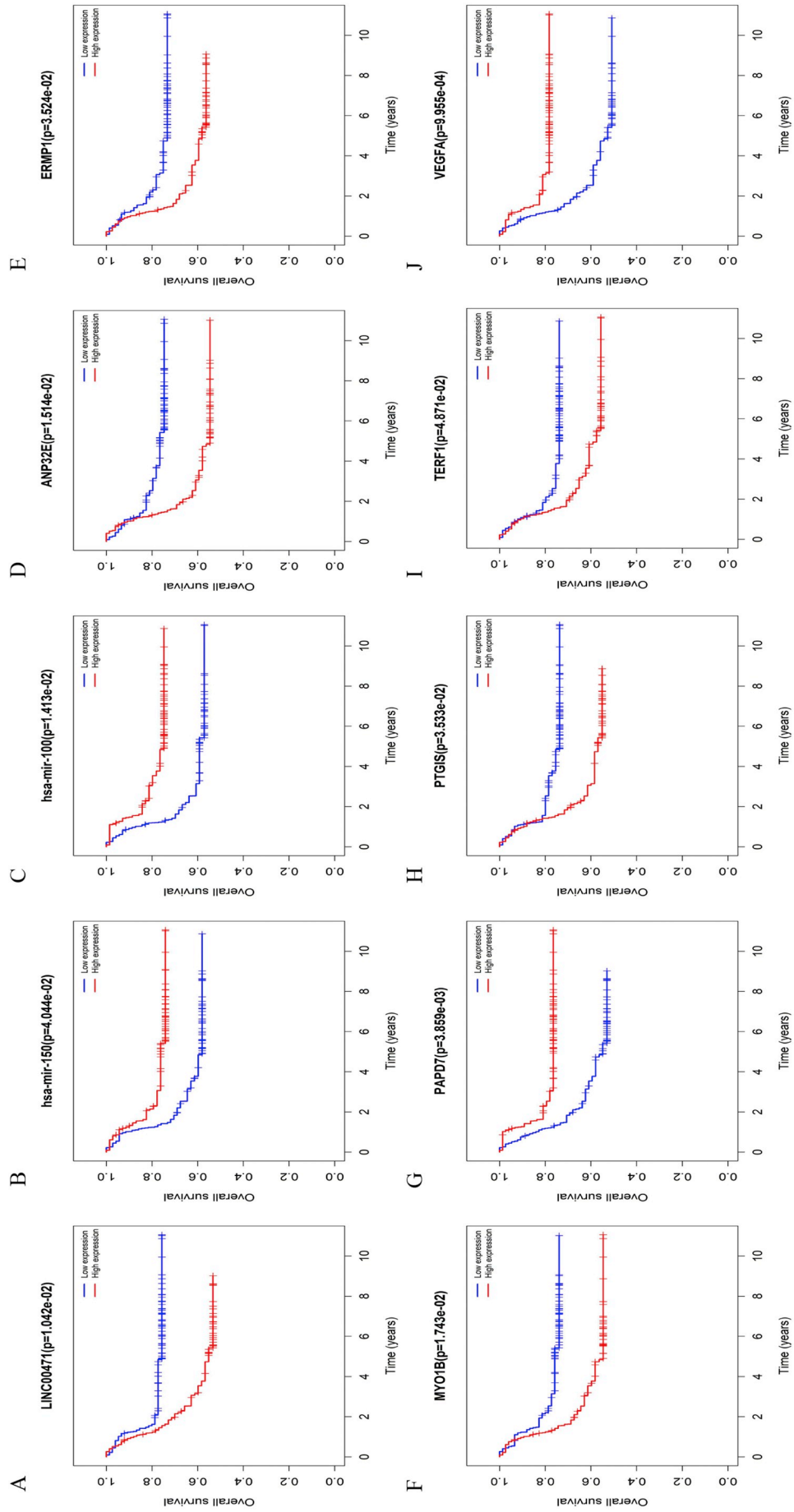

Fig. 7. Kaplan-Meier survival curves of (A) DE1ncRNAs, (B–C) DEmiRNAs, and (D–J) DEmRNAs most relevant to overall survival (OS) of high-risk AML patients in the ceRNA network.

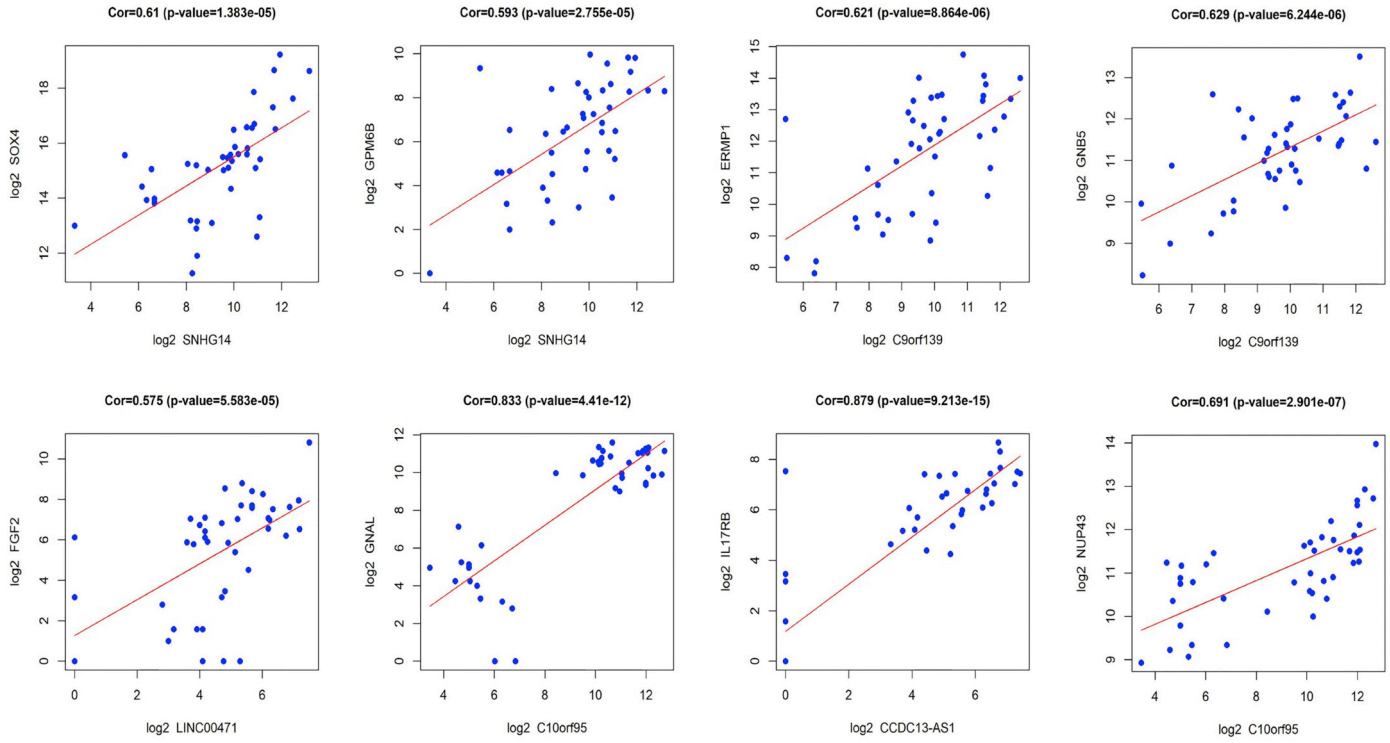

Fig. 8. Correlational analyses between the expression levels of DElncRNA and DEmRNAs in ceRNA network(Cor: correlation coefficient).

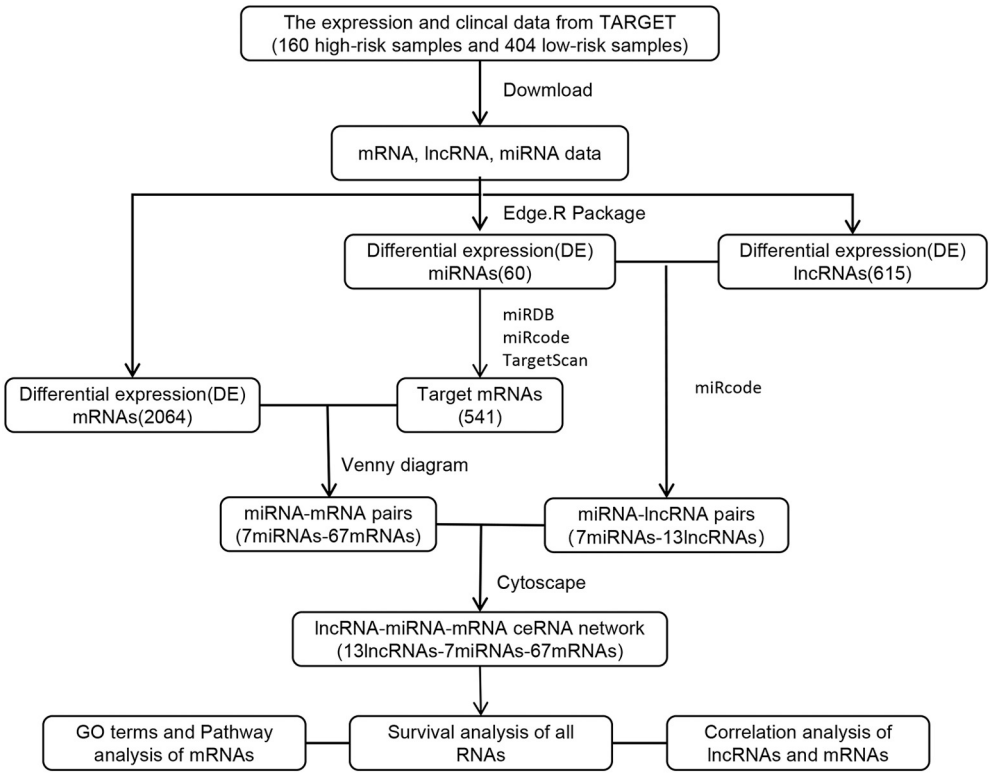

Fig. 9. The flow chart of construction of lncRNAs-miRNAs-mRNA regulation network in the high-risk of childhood acute myeloid leukemia.

mRNAs by bioinformatics. In the ceRNA network, we found 13 DElncRNAs and 67 DEmRNAs that were targeted by 7 common DE-miRNAs. To assess the specific biological roles of the RNAs, we further investigated the association between the aberrantly expressed RNAs and overall survival, 1 lncRNAs (*LINC00471*), 2 miRNAs (*hsa-mir-100* and *hsa-mir-150*), and 7 mRNAs (*ANP32E*, *ERMP1*, *MYO1B*, *PAPD7*, *PTGIS*, *TERF1*, and *VEGFA*) were found to be associated with overall survival in childhood AML with high risk patients. GO function and KEGG pathway analyses were performed to acquire an in-depth understanding of the ceRNA network. The functional enrichment analyses indicated that the DEmRNAs were mainly enriched in skeletal system development, positive regulation of endothelial cell chemotaxis,

transcription factor activity, RNA polymerase II proximal promoter sequence-specific DNA binding. The pathway analysis further revealed that 20 pathways were enriched, and primarily involved: *Ras*, *TGF-beta*, *FoxO*, *p53*, *PI3K-Akt* signaling pathway, and signaling pathways regulating pluripotency of stem cells, which are consistent with the findings of current AML research. Additionally, some other pathways such as transcriptional misregulation in cancer, Gastric cancer, Renal cell carcinoma, and Melanoma were also tumor-related pathways. Finally, the correlation between DEmiRNA and DElncRNA were constructed to verify the reliability of ceRNA network.

Among these key RNAs in the network, several studies has reported that *miR-150* was associated with tumor progression, and holds great potential as a therapeutic target in treating various types of hematopoietic malignancies [37]. *MiR-150* has been shown to play crucial roles in the pathogenesis of aggressive transformed, high-grade and refractory lymphomas [38]. *Mir-150* can directly target *FOXA1* to regulate the CD8 + T cell differentiation [39] and can affect the prognosis of various tumors [40]. Musilova K et al. suggested that the role of the *MYC/miR-150/FOXP1* axis in malignant B cells as a determinant of follicular lymphoma aggressiveness and its high-grade transformation [41]. Decreased expression and increased expression of miR-100 correlated with progression and poor prognosis of cancer [42]. Therefore, miRNAs are potentially useful biomarkers as a key factor in signal cascades. Yang G et al. suggested that the mechanism of *p53* protein ubiquitination mediated by *miR-100* was characterized in apoptosis [43]. *LINC00471* was identified to be associated with the progression of esophageal squamous cell carcinoma, and could be used as predictor of the survival rate of patients [44]. *ANP32* as a novel substrate of caspase-3 enhances the activation and apoptosis of myeloid leukemic cells [45]. Kumar R proved that *TERF2* as a biomarker involved in the disease progression of multiple myeloma [46]. It was reported that AML leukemogenesis involving a *VEGFA*-mediated non-cell-intrinsic mechanism [47], and *VEGFA* levels is related to vascular morphology within AML bone marrow. Based on these findings, these key RNAs play an important role in the development of AML in ceRNA networks, as previous reported.

Taking together, our study identified many RNAs of literature validated, and abnormalities in the ceRNA network may lead to tumorigenesis. A few novel RNAs as competing endogenous RNAs perform the important function for AML. However, despite the fact that our results provide many potential biomarkers for high risk AML, but still have certain limitations. More experimental validations are needed to confirm the contribution of our proposed RNAs in AML. In addition, whether this model is applicable to adult AML, all wait for the research and the practice confirmation.

In summary, our research results constructed a ceRNA network associated with high-risk group of childhood AML, and provide novel lncRNAs as potential diagnostic biomarkers. Based on the total survival analysis, we obtained a total of ten prognostic biomarkers associated with AML, and further demonstrate the contribution of RNAs interactions to the pathogenesis of AML. This discovery further investigated the molecular pathogenesis of AML that can assist clinicians clarify the function of lncRNA to guide the treatment and in-depth study.

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Declaration of competing interest

The author reports no conflicts of interest in this work.

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