Revealing key lncRNAs in cytogenetically normal acute myeloid leukemia by reconstruction of the lncRNA–miRNA–mRNA network based on the competitive endogenous RNA theory

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Abstract

Background: Cytogenetically normal acute myeloid leukemia(CN-AML) is a heterogeneous disease with different prognosis.Researches on prognostic indicators and therapy targets of CN-AML are still ongoing.Instead of protein-coding genes,more and more researches were focused on the non-coding RNAs especially long non-coding RNAs(lncRNAs) which may play an important role in the development and prognosis of AML.Although a large number of lncRNAs had been found, our knowledge of their function and pathological significance is still in its infancy.The purpose of this research is to identify the key lncRNAs and explore their function in CN-AML by reconstructing the lncRNA–miRNA–mRNA network based on the competitive endogenous RNA(ceRNA) theory.

Results: We reconstructed a global triple network based on the ceRNA theory using the data from National Center for Biotechnology Information Gene Expression Omnibus and published literature. According to the topological algorithm,we identified the key lncRNAs which had both the higher node degrees and the higher number of lncRNA–miRNA and miRNA–mRNA pairs in the ceRNA network. Meanwhile, Gene Ontology (GO) and pathway analysis were performed using databases such as DAVID,KOBAS and Cytoscape plug-in ClueGO respectively.The lncRNA–miRNA–mRNA network was composed of 90 lncRNAs,33mRNAs,26 miRNAs and 259 edges in the lncRNA upregulated group,and 18 lncRNAs,11 mRNAs,6 miRNAs and 45 edges in the lncRNA downregulated group.The functional assay showed that 53 pathways and 108 GO terms were enriched. Three lncRNAs(XIST,GABPB1-AS1,TUG1)could possibly be selected as key lncRNAs wihch may play an important role in the development of CN-AML.Particularly,GABPB1-AS1 was highly expressed in CN-AML by both bioinformatics analysis and experimental verification in AML cell line(THP-1) by quantitative real-time polymerase chain reaction.In addition, GABPB1-AS1 was also negatively correlated with overall survival of AML patients.

Conclusion: The lncRNA–miRNA–mRNA network revealed key lncRNAs and their functions in CN-AML.Particularly,lncRNA GABPB1-AS1 was firstly proposed in AML.We believe that GABPB1-AS1 is expected to become a candidate diagnostic biomarker or potential therapeutic target.

Background

Cytogenetically normal acute myeloid leukemia (CN-AML), a most common AML type,is characterized by the absence of microscopically detectable chromosome abnormalities.This is a heterogeneous disease with different prognosis.With the advent of high-throughput technologies,some prognostic gene expression signatures have been proposed in CN-AML. For example,patients with FLT3-ITD mutation always had a bad prognosis[1],while NPM1 or CEBPA mutations indicated a good prognosis[2].

However,the vast majority of transcripts that were detected by high-throughput technologies do not appear to be protein-coding genes. Recently,accumulating evidence has suggested that rather than being transcriptional noise,many non-coding RNAs,especially the long non-coding RNAs(lncRNAs),served as master regulators that affect the expression levels of dozens or even hundreds of target genes[3],and may play an important role in pathogenesis of AML[4]. For example, lncRNA NEAT1 repressed the expression

of miR-23a-3p and therefore modulated cell proliferation and apoptosis in AML by regulating SMC1A [5]. Furthermore, Garzon et al built a prognostic lncRNA score system for older patients (>60 years) with cytogenetically normal AML[6].But the functional mechanisms of lncRNAs in CN-AML were complicated and unclear.

Noteworthy, competing endogenous RNA(ceRNA) network is a promising module to facilitate lncRNAs function in complex pathologic conditions. CeRNA network is a complex post-transcriptional regulatory network using miRNA response elements (MREs) to compete for the binding of miRNAs thereby implementing mutual control between mRNAs, lncRNAs and miRNA[7].

In summary, in order to explore the detailed pathogenesis of lncRNAs in AML, we reconstructed a global triple network in CN-AML compared with healthy controls using the data from National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) based on the ceRNA theory.

Results

Differentially expressed lncRNA, miRNA, and mRNA in CN-AML

A total of 127 mRNAs,3380 lncRNAs, and 82 miRNAs were found to be differentially expressed in CN-AML group compared with normal controls(|log2fold change| >1 and adj.P value < 0.05 as the standards), of which 76 mRNAs (59.84%), 1751 lncRNAs (51.80%) and 19 miRNAs (23.17%) were upregulated while others were downregulated. Volcano plots, visually demonstrating the distribution of RNAs, were shown in Figure 1.

Reconstruction of the lncRNA–miRNA–mRNA network

To speculate on the functions of lncRNAs acting as miRNA targets, a network among lncRNAs, miRNAs, and mRNAs was reconstructed and then visualized. As shown in Figure 2, there were 90 lncRNAs, 33mRNAs, 26 miRNAs and 259 edges in the lncRNA upregulated group, and 18 lncRNAs, 11 mRNAs, 6 miRNAs and 45 edges in the lncRNA downregulated group.

Topological analysis of the CN-AML related lncRNA–miRNA–mRNA network

It is well known that hub nodes play critical roles in biological networks. Therefore, we calculated all node degrees of the lncRNA– miRNA–mRNA network. According to the previous study by Han et al.[8]in which they defined a hub as a node degree exceeding 5,we found that 42 nodes could be chosen as hub nodes, including 10 lncRNAs, 31 miRNAs, and 1 mRNA(CDK6). Moreover, the number of the first relationship pairs of lncRNA–miRNA and the secondary relationship pairs of miRNA–mRNA were calculated. The results were shown in Table 1. Interestingly, we found that three lncRNAs (XIST,GABPB1-AS1,TUG1)not only had higher node degrees, but also had a higher number of lncRNA–miRNA and miRNA–mRNA pairs. This suggested that the three lncRNAs may play crucial roles in the development of CN-AML, which could be selected as the key lncRNAs.

Table 1 The topological algorithm of differentially expressed lncRNAs (Top10)

Number	Gene name	Node degree	lnc-miRNA pairs	mi-mRNA pairs	Total
1	XIST	20	20	38	58
2	GABPB1-AS1	10	10	13	23
3	TUG1	10	10	23	33
4	MIRLET7BHG	7	7	11	18
5	AC092127.1	6	6	12	18
6	SNHG1	6	6	9	15
7	NEAT1	5	5	11	16
8	SNHG3	5	5	9	14
9	H19	5	5	9	14
10	AC074117.1	5	5	9	14

Functional annotation of the lncRNA–miRNA–mRNA network

To understand the biological processes and pathways of DEmRNAs in the lncRNA–miRNA–mRNA network, we conducted the KEGG and GO enrichment analysis.

The KEGG results elucidated the potential biological functions(p-value < 0.05), a total of 53 significantly enriched pathways were obtained. Among these pathways, 'PI3K-Akt signaling pathway'[9],'Ras signaling pathway'[10], 'MAPK signaling pathway'[11], 'FoxO signaling pathway'[12], were linked with the progression of AML. Additionally, some other pathways such as 'Pathways in cancer'[13], 'ErbB signaling pathway'[14]were also tumor related pathways.Genes RUNX1、FLT3、KIT、FASLG、AKT3、MAPK8、GADD45A and PIK3R1 were enriched in greater than or equal to three pathway terms. The mentioned pathways were showed in Figure 3.

The DEmRNAs were classified into three functional groups: molecular function group, biological process group, and cellular component group. As shown in Figure 4, in the biological process group, DEmRNAs mainly enriched in G1/S transition of mitotic cell cycle, cell cycle arrest and protein phosphorylation.In the molecular function group, DEmRNAs mainly enriched in protein binding, ATP binding and protein kinase activity.In the cellular component group, DEmRNAs mainly enriched in nucleus,nucleoplasm and cytosol.

Reconstruction of the key lncRNA–miRNA–mRNA sub-networks

The key three lncRNAs(XIST,GABPB1-AS1,TUG1) and their linked miRNAs/mRNAs were extracted and used to reconstruct the new subnetworks as follows(Figure 5).Pathway analysis showed that LncRNA XIST related mRNA were significantly enriched in 16 pathway terms including 'PI3K-Akt signaling pathway','FoxO signaling pathway', 'p53 signaling pathway'[15] and 'Ras signaling pathway', all of which had been shown to play important roles in AML.LncRNA GABPB1-AS1 related mRNA were significantly enriched in 6 pathway terms including 'PI3K-Akt signaling pathway' and 'Pathways in cancer'pathway terms.LncRNA TUG1 related mRNA were significantly enriched in 12 pathway terms including 'PI3K-Akt signaling pathway','FoxO signaling pathway',and 'Ras signaling pathway',all of which had been shown to play important roles in AML.

Survival analysis of the key RNAs

We conducted the survival analysis of the key lncRNAs(showing in Table 1) and mRNAs(showing in Figure 3b) involved in the lncRNA–miRNA–mRNA network by Kaplan–Meier curve with P value<0.05.Only three lncRNAs(GABPB1-AS1,SNHG3,SNHG1)and two mRNA(FLT3,AKT3) significantly related to overall survival(OS) of AML patients(Figure 6).

The expression of GABPB1-AS1 in AML by quantitative real-time polymerase chain reaction(qRT-PCR)

To validate the above bioinformatics analysis, we used qRT-PCR to detect lncRNA GABPB1-AS1 expression in AML cell line(THP-1). GABPB1-AS1 which belonged to the core of the lncRNA–miRNA–mRNA network was significantly high expressed in THP-1 cells compared with HS-5 cells(control cell line) (p□0.01)(Figure 7).

Discussion

Acute myeloid leukemia(AML) is the most common type of acute leukemia in adults. According to the WHO classification,patients with recurring cytogenetic abnormalities,such as t(8,21), inv(16)(p13q22) or t(15,17) often had a better prognosis[16].But for those with normal karyotype,there is an another story. Actually, CN-AML is a heterogeneous disease. Although with the advent of high-throughput sequencing and other methods, many genetic changes (such as mutations of gene FLT3,NPM1) which were closely related to the prognosis of CN-AML have been discovered,but the pathogenesis and prognostic markers of CN-AML were not yet fully understood.In recent years, more and more studies have focused on the epigenetic regulation of AML.Non-coding RNA (such as lncRNA or miRNA) is an important part of the epigenetic regulation. Recent studies have shown that lncRNAs are closely related to tumor cell proliferation, invasion, metastasis, apoptosis and tumor angiogenesis[17]. However, the specific regulatory mechanism of lncRNAs in CN-AML is still unclear. Since lncRNAs can regulate miRNAs abundance by binding and sequestering them which were knowed as "miRNA sponges", lncRNAs can regulate the expression of target mRNAs[18].Thus, it has been shown that an efficient way to infer the potential function of lncRNAs is by studying their relationship with miRNAs and/or mRNAs, whose

functions have been annotated.Therefore, this suggested that we can use the ceRNA network to explore the specific functional roles and prognostic significance of lncRNAs in CN-AML.

In this study, we conducted the difference analysis between CN-AML patients and normal controls to find differentially expressed RNAs by GEO data sets (GSE142699, GSE142698 and GSE103828) and document retrieval.Then the target lncRNAs and mRNAs of miRNAs were searched through the online databases. Afterwards,we took the intersection between the target RNAs with the above-mentioned differentially expressed RNAs to obtain DElncRNA–DEmiRNA and DEmiRNA–DEmRNA pairs, in which the expression of DEmiRNAs were negatively correlated with DElncRNA and DEmRNA.Finally ,a three-level network diagram of lncRNA–miRNA–mRNA was constructed.

We performed KEGG and GO analysis on the DEmRNAs in the lncRNA–miRNA–mRNA network.The pathway analysis showed that 29 pathways were significantly enriched(P-value 0.05).Pathways 'PI3K-Akt signaling pathway','MAPK signaling pathway' and 'Ras signaling pathway', which have been shown to play important roles in AML,were invovled. Furthermore,108 GO terms were significantly enriched with P-value 0.05.These significant GO terms involved G1/S transition of mitotic cell cycle, cell cycle arrest and protein phosphorylation for the biological process group;protein binding, ATP binding and protein kinase activity for the molecular function group;and nucleus,nucleoplasm and cytosol for the cellular component group.

In order to find the key lncRNAs, which can be used as potential novel biomarkers for clinical diagnosis and treatment targets of CN-AML, the hub nodes and the number of relationship pairs were used. In this study, three lncRNAs (XIST GABPB1-AS1 TUG1) were observed to be topological key nodes whose node degrees and the number of lncRNA–miRNA and miRNA–mRNA pairs were significantly higher compared to other lncRNAs. This indicated that these lncRNAs had profound implications for AML, which can be considered as key lncRNAs.

LncRNA XIST

The XIST locus produces a 17–20 kb RNA that coats the X chromosome in cis, and plays an essential role in X chromosome inactivation (XCI)[19].Much previous work has focused on the role of XIST in initiating X-inactivation in the early developing embryo. However,XIST has been reported to function as an oncogene or a tumor suppressor in different human malignancies, which is implicated in many aspects of carcinogenesis including tumor apoptosis, cell cycle, initiation, invasion, metastasis, stemness, autophagy and drug resistance[20].For example,XIST was highly expressed in breast cancer and was closely associated with a poor prognosis and the resistance to chemotherapy[21].Down-regulation of XIST has been reported to reduce chemoresistance in non-small cell lung cancer cells by inhibiting autophagy [22]. In AML,XIST was found to be up-regulated in patients'bone marrow cells. In addition, silencing of XIST could repress AML bone marrow cell proliferation while enhancing cell apoptosis and adriamycin sensitivity[23].

In this study,lncRNA XIST was highly expressed in CN-AML and had the highest node degrees and the highest number of lncRNA–miRNA and miRNA–mRNA pairs in the ceRNA network among all the lncRNAs.This means XIST may play an important role in CN-AML which is consistent with previous findings mentioned above.The pathway analysis of key lncRNA XIST–miRNA–mRNA sub-network showed that 16 pathways were significantly enriched.Pathways'PI3K-Akt signaling pathway','FoxO signaling pathway','p53 signaling pathway' and 'Ras signaling pathway', which had been shown to play important roles in AML,were involved.

LncRNA GABPB1-AS1

LncRNA GAbinding protein transcription factor subunit beta-1 antisense RNA 1 (GABPB1-AS1) is the antisense RNA of GABPB1 mRNA, which is located in the cytoplasm and has a total length of 4139nt[24].GABPB1-AS1 was identified for the first time in human-induced pluripotent stem cells (hiPSCs). The expression level of GABPB1-AS1 is increased in hiPSCs under the chemical stresses (cadmium, hydrogen peroxide, and cycloheximide)[25].LncRNA GABPB1-AS1 aslo played a role in several cancers.But the results were contradictory.As a tumor suppressor gene, Qi et al found that GAPBPB1-AS1 inhibited the antioxidant ability of hepatocellular carcinoma cancer cells and cell proliferation by inhibiting the expression of GABPB1 and peroxiredoxin 5 (PRDX5)[26].In addition,GABPB1-AS1 inhibited clear cell renal cell carcinoma growth and played a tumor suppressor role through an miR-1246/PCK1 axis[27].As a tumor activator gene, GABPB1-AS1 can bind to miR-519e-5p and destroied its tumor suppressive function in cervical cancer pathogenesis[28].Forthermore, findings suggested that the decrease in GABPB1-AS1 expression associated with decreased breast cancer risk[29].Alkhateeb et al also revealed that the aberrant expression of GABPB1-AS1 can be used as a potential prostate cancer biomarker[30].However, the role of GABPB1-AS1 in AML is still unclear.

In this study, for the first time, we came to a conclusion that lncRNA GABPB1-AS1 was highly expressed in CN-AML by both bioinformatics analysis and qRT-PCR verification in AML cell line(THP-1).The pathway analysis of key lncRNA GABPB1-AS1–miRNA–mRNA sub-network showed that 6 pathways were significantly enriched and primarily involved 'PI3K-Akt signaling pathway' and 'Pathways in cancer'pathway terms.In addition,the survival analysis told us that patients with lower expression of GABPB1-AS1 had better prognosis.In conclusion,GABPB1-AS1 would like to be a potential prognostic marker and therapeutic target of AML.

LncRNA TUG1

LncRNA Taurine-Upregulated Gene1 (TUG1), located on chromosome 22q12, a critical oncogenic lncRNAs of human, has been proved to take part in hematological cancers. Interestingly, Some scholars demonstrated that TUG1 was highly expressed in tissues and cell lines of AML patients, and the high expression of TUG1 was also closely related to poor prognosis of AML[31].TUG1 induced cell proliferation, and restrained cell apoptosis in AML by targeting aurora kinase A[32].TUG1 facilitates the cell viability and metastasis by targeting miR-370-3p/MAPK1/ERK in AML[33].In addition,TUG1 silencing

decreased the IC50 of adriamycin, and promotes adriamycin-induced apoptosis in AML cells by miR-34a/EZH2 axis[34],providing a potential therapeutic target for AML.

In our study,LncRNA TUG1 was highly expressed in CN-AML and had the higher topological parameters in the ceRNA network.This means TUG1 may play an important role in CN-AML which was consistent with previous findings mentioned above.The pathway analysis of key lncRNA TUG1–miRNA–mRNA sub-network showed that 16 pathways were enriched.Pathways 'PI3K-Akt signaling pathway','FoxO signaling pathway',and 'Ras signaling pathway', which have been shown to play important roles in AML,were involved.

In summary, our research results constructed a lncRNA–miRNA–mRNA network associated with CN-AML, and provided novel lncRNAs(especially GABPB1-AS1) as potential diagnostic and prognostic biomarkers.The specific functional mechanism of these key lncRNAs in AML needs further experimental exploration.

Conclusion

Based on the ceRNA theory, we reconstructed a lncRNA– miRNA–mRNA network for CN-AML compared with normal controls for the first time.According to the topological algorithm,our study further found that three lncRNAs(XIST,GABPB1-AS1,TUG1) could possibly be selected as key lncRNAs wich may play an important role in the development of CN-AML.Particularly,LncRNA GABPB1-AS1 was firstly proposed in AML.GABPB1-AS1 was highly expressed in CN-AML by both bioinformatics analysis and qRT-PCR verification in AML cell line(THP-1).In addition, it was also negatively correlated with OS of AML patients.So GABPB1-AS1 is expected to become a candidate diagnostic biomarker or potential therapeutic target.In addition,we conducted the functional analysis of the ceRNA network to advance our understanding of the pathogenesis of CN-AML from the perspective of lncRNAs.Further studies are needed to explore the biological functions and molecular mechanisms of these specific lncRNAs in CN-AML.

Methods

Raw data

GEO is a public functional genomics data repository supporting MIAME-compliant data submissions. Array- and sequence-based data were accepted.

In order to find the differentially expressed miRNAs,mRNAs and lncRNAs in CN-AML compared with normal controls,databases(GSE142699,GSE142698 and GSE103828)were downloaded respectively from NCBI GEO.

In addition,we also searched in pubmed[35] by keywords 'acute myeloid leukemia and lncRNA' to find lncRNAs which have been reported to have significantly differential expressions in CN-AML.

The RNA-seq data and clinical data are publicly available on open-access. Therefore, no further approval was required from the local ethics committee.

Screening differentially expressed lncRNAs, miRNAs and mRNAs

The 'edgeR' package[36] was utilized to identify the differentially expressed RNAs in CN-AML compared with normal controls, the downloaded data were calibrated, standardized and analyzed for differences to obtain differentially expressed lncRNA, miRNA, and mRNA molecules between the CN-AML group and control . The screening criteria of the three kinds of dysregulated RNAs were as follows: adj.p value< 0.05 and |log2fold change| > 1.

For lncRNAs, differentially expressed lncRNAs also includes the ones obtained from the published literature.

Prediction of target lncRNAs and mRNAs of differentially expressed miRNAs

In this study, starbase website[37]was used to predict lncRNA-miRNA interactions.In addition,the online websites miRDB[38], miRTarBase[39],TargetScan[40]were used to predict target genes.In TargetScan, predicted targets are ranked according to the predicted efficacy of targeting as calculated using cumulative weighted context++scores of the sites, and scores -0.1 were selected.Genes that appear in at least two databases or more are regarded as target genes.

Reconstruction of the lncRNA–miRNA–mRNA network

To further improve the reliability of bioinformatics analysis,we obtained the portion of the target mRNAs or lncRNAs that overlaped with the differentially expressed mRNAs or lncRNAs in CN-AML compared with normal controls,and overlapping RNAs were further analyzed as differentially expressed mRNAs(DEmRNAs) or differentially expressed lncRNAs(DElncRNAs). Finally, we established matched DElncRNA–DEmiRNA and DEmiRNA–DEmRNA pairs.

The lncRNA–miRNA–mRNA network was reconstructed based on ceRNA theory as follows: For a given co-expressed lncRNA– mRNA pair, both lncRNA and mRNA in this pair were targeted and co-expressed negatively with a certain common miRNA, and this lncRNA–miRNA–mRNA was identified as the co-expression competing triplet. The lncRNA–miRNA–mRNA network was reconstructed by assembling all co-expression competing triplets, which were identified above, and was visualized using Cytoscape software. Simultaneously, all node degrees of the lncRNA–miRNA–mRNA network were calculated.

Functional enrichment analysis

To assess functional enrichment, Gene Ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of mRNAs in the lncRNA–miRNA– mRNA network were performed using multiple online databases, including DAVID(Database for Annotation, Visualization, and Integration

Discovery)[41] and KOBAS[42] with $p < 0.05$ as the cut-off criterion. Then the KEGG pathway interaction network was reconstructed using Cytoscape plug-in ClueGO.

Reconstruction of the key lncRNA–miRNA–mRNA sub-networks

Every lncRNA, its linked miRNAs and mRNAs in the global triple network were extracted and used to reconstruct the new subnetwork using Cytoscape software. Meanwhile, the number of the first relationship pairs of lncRNA–miRNA and the secondary relationship pairs of miRNA–mRNA were calculated. Using the node degree of lncRNAs, the number of the first relationship pairs of lncRNA–miRNA plus the secondary relationship pairs of miRNA–mRNA, the key lncRNAs were collected. For further analysis, we performed pathway annotations for each of the key lncRNAs by using their first mRNA neighbors in the key lncRNA–miRNA–mRNA sub-networks.

Survival analysis

To explore the relationship between prognosis and the key lncRNAs, miRNAs and mRNAs involved in the ceRNA network, Kaplan–Meier curve were carried out at a P value<0.05 using the online websites GEPIA2(Gene Expression Profiling Interactive Analysis)[43] .

RNA extraction, reverse transcription(RT), and quantitative real-time polymerase chain reaction(qRT-PCR)

The total RNAs were extracted using TRIzol kits (Pufei,Shanghai,China) for qRT-PCR analyses.Reverse transcription was then conducted by applying the Promega RT reagent Kit(Promega M-MLV M1705,Madison,USA). qRT-PCR using SYBR Green Mix (TAKARA,Dalian,China) was carried out on Roche LightCycler 480II system in triplicate. Primers for GABPB1-AS1 and β-actin were synthesized by Genechem Co.,Ltd.(Shanghai, China). The mRNA expressions were normalized to β-actin.The expressing levels of lncRNAs were defined based on the threshold cycle (Ct), and calculated using the $2^{-\Delta\Delta CT}$ method.The primers were as follows:

GABPB1-AS1 forward primer:CAACTAGGCAGACTGGGACG,

GABPB1-AS1 reverse primer:AGGTGGCAGTAATCCAAGCA,

β-actin forward primer:GCGTGACATTAAGGAGAAGC,

β-actin reverse primer:CCACGTCACACTTCATGATGG.

Statistical analysis

All data collected from three independent experiments are presented as mean ± standard deviation. Survival curves were plotted using the Kaplan–Meier method and the log-rank test.Student's t test for comparison between two groups were performed for statistical analysis using SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA). A p value of less than 0.05 was identified as having statistical significance.

Abbreviations

CN-AML(Cytogenetically normal acute myeloid leukemia), lncRNAs(long non-coding RNAs), ceRNA(competing endogenous RNA), MREs(miRNA response elements), NCBI GEO(National Center for Biotechnology Information Gene Expression Omnibus), DEmRNAs (differentially expressed mRNAs), DEmiRNAs(differentially expressed miRNAs), DElncRNAs (differentially expressed lncRNAs), RT(reverse transcription), qRT-PCR(quantitative real-time polymerase chain reaction),CT(cycle threshold),OS(overall survival).

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: The datasets generated and/or analysed during the current study are available in the NCBI GEO repository, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE142699,[44] https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE142698,[45] https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE103828.[46]

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Authors' contributions: MZW and TS designed the work;TS,LD and YG collected data from the public datasets and literature; TS,MZW and HZ analyzed data;HZ,LD and YG performed qRT-PCR experiment in cell lines;TS drafted the work;MZW revised it.All authors read and approved the final manuscript.

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Figures

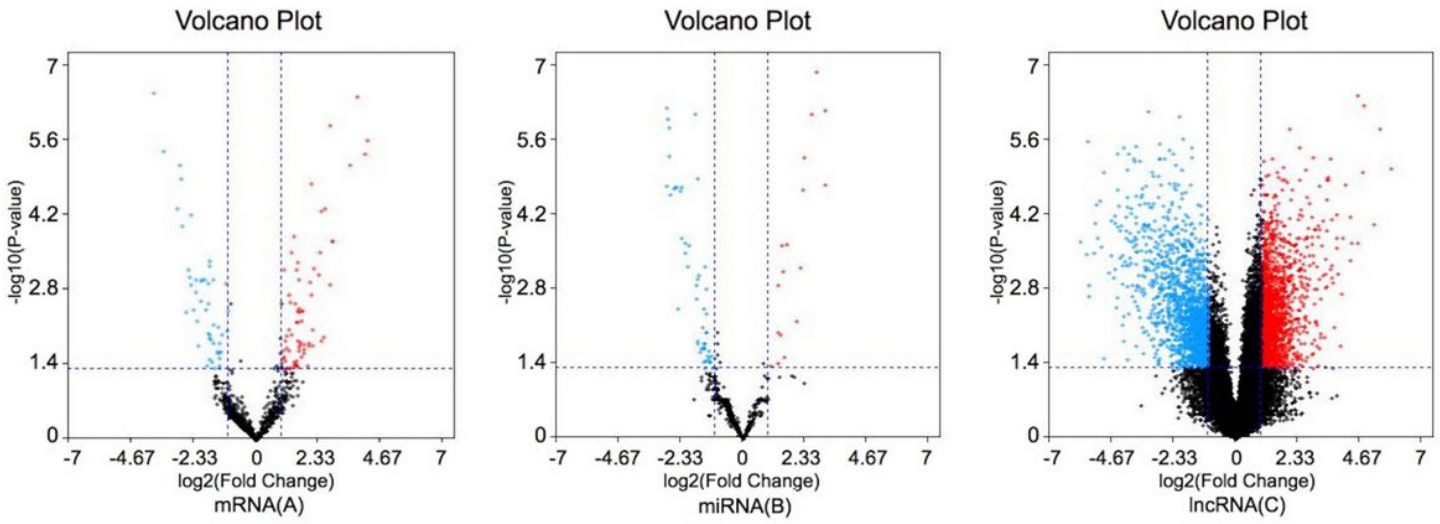
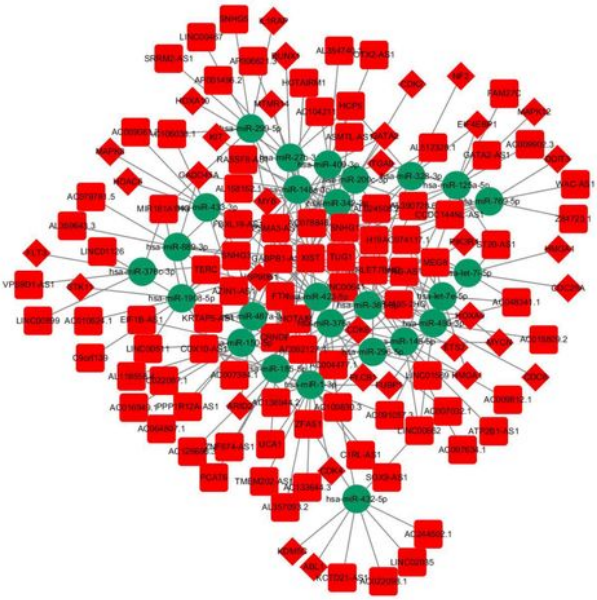
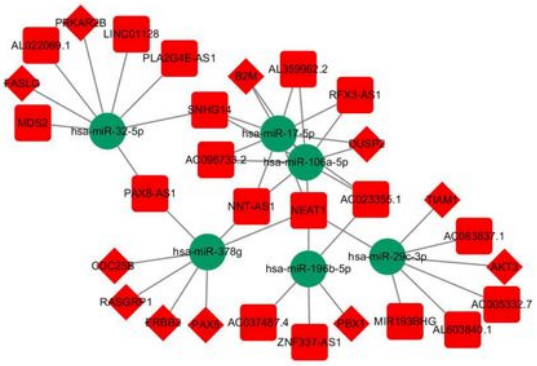


Figure 1

Volcano plots reflecting number, significance and reliability of differentially expressed RNAs in CN-AML compared with normal controls.The red dots indicate upregulation and blue dots indicate downregulation of mRNAs (A), miRNAs(B) and LncRNAs(C). The x-axis represents the value of log2(Fold Change) and the y-axis represents the value of -log10(p-value).



LncRNA upregulated group(A)



LncRNA downregulated group(B)

Figure 2

The view of the lncRNA–miRNA–mRNA network in CN-AML. The square represents lncRNAs, the rhombus represents mRNAs, and the circle represents miRNAs.There were 90 lncRNAs, 33mRNAs, 26 miRNAs and 259 edges in the lncRNA upregulated group(A), and 18 lncRNAs, 11 mRNAs, 6 miRNAs and 45 edges in the lncRNA downregulated group(B).

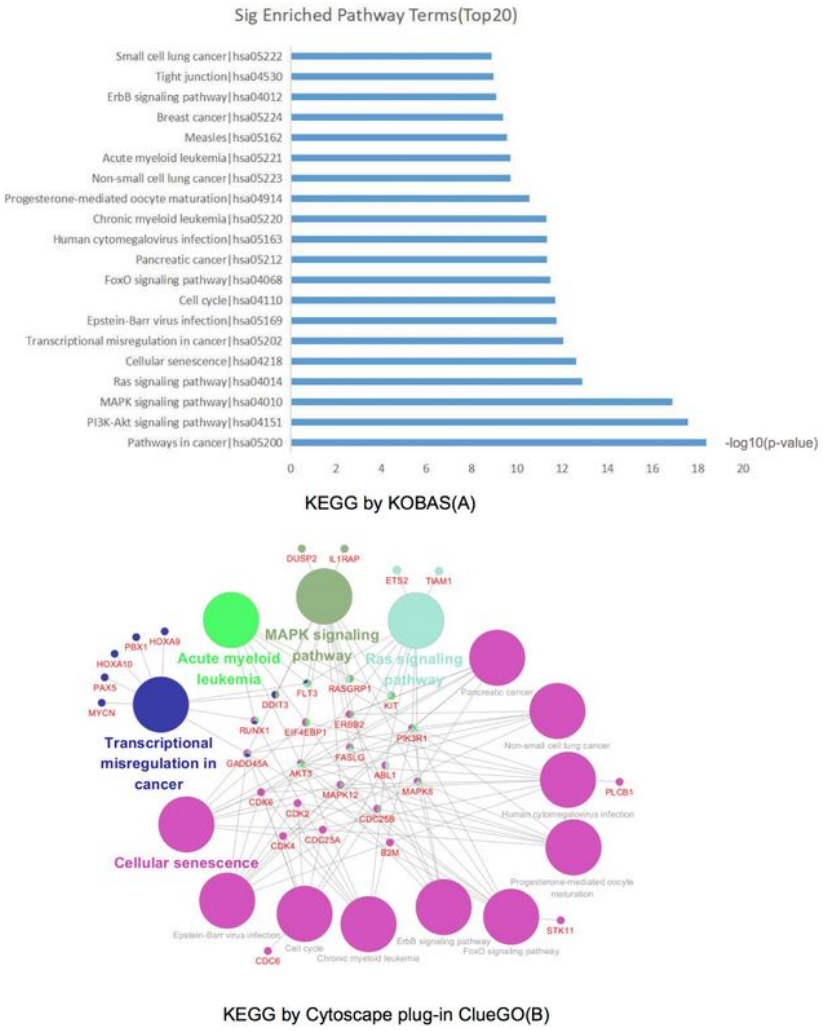


Figure 3

KEGG pathway analysis of DEmRNAs in the lncRNA–miRNA–mRNA network.(A)The top20 significantly enriched pathway terms by KOBAS.(B)The KEGG pathway interactions by Cytoscape plug-in ClueGO. Genes RUNX1,FLT3,KIT,FASLG,AKT3,MAPK8,GADD45A and PIK3R1 were enriched in pathways greater than two terms by Cytoscape plug-in ClueGO.

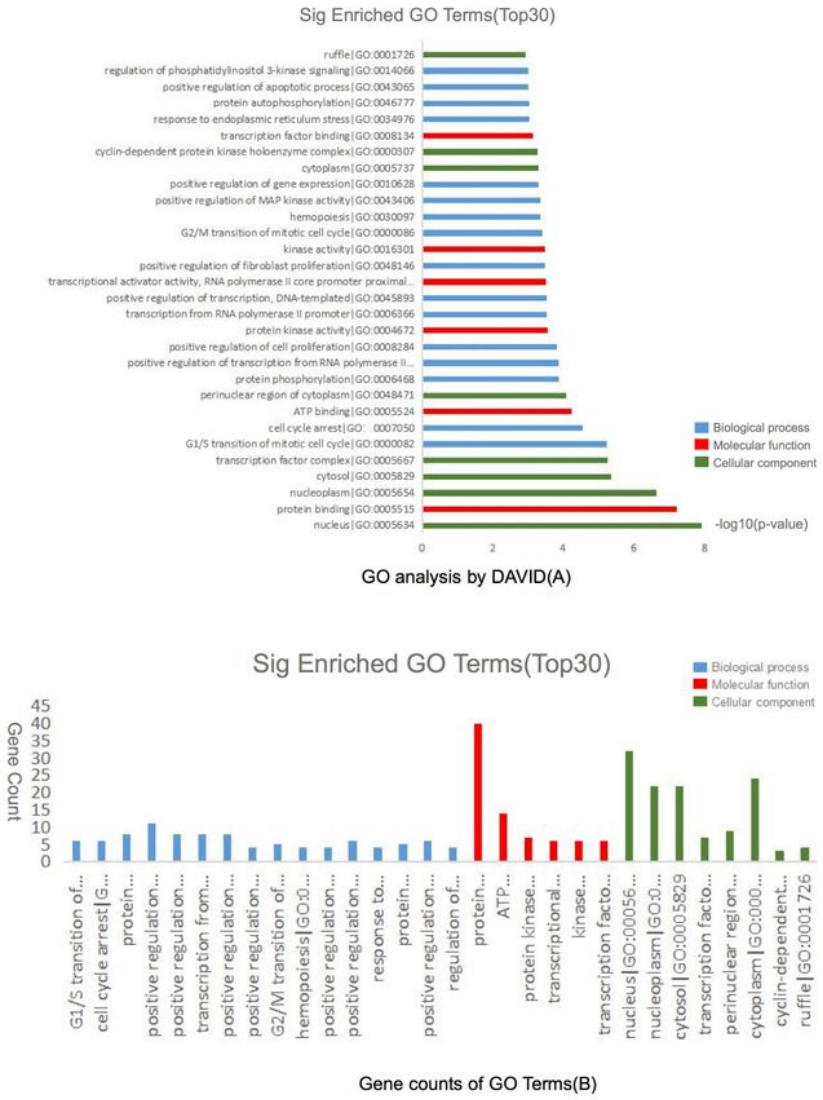
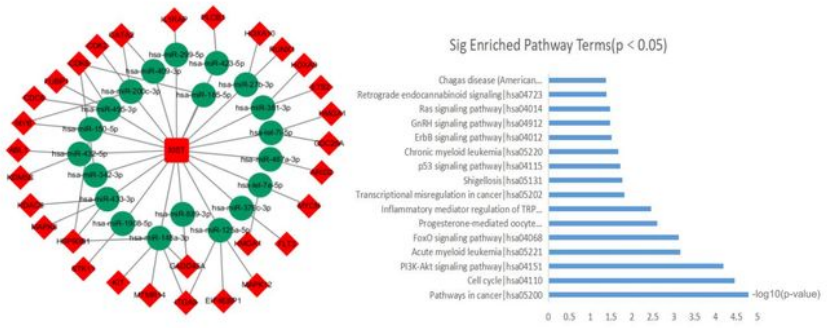
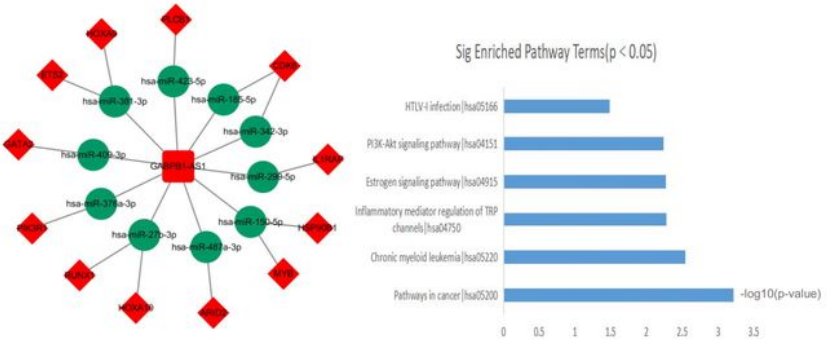


Figure 4

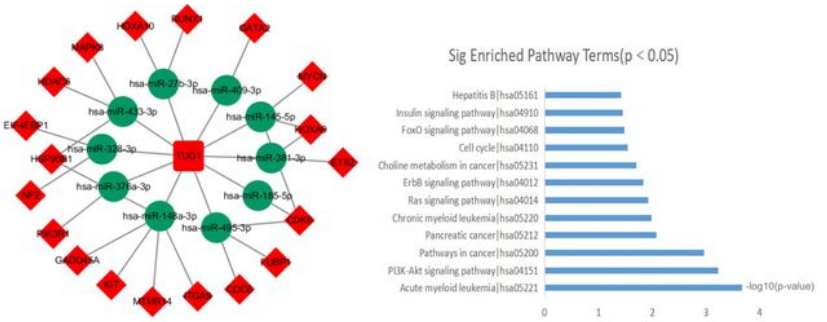
Gene Ontology analysis and significantly enriched GO terms of DEmRNAs in the lncRNA–miRNA–mRNA network.(A)Significantly Enriched GO Terms of DEmRNAs in the lncRNA–miRNA–mRNA network based on their functions.(B)GO analysis classified DEmRNAs in the lncRNA–miRNA–mRNA network into 3 groups(i.e.,molecular function,biological process and cellular component).



The sub-network and pathway analsis of lncRNA XIST(A)



The sub-network and pathway analsis of lncRNA GABPB1-AS1(B)



The sub-network and pathway analsis of lncRNA TUG1(C)

Figure 5

The sub-networks of three key lncRNAs and their KEGG pathway analysis by KOBAS.(A)The sub-network of lncRNA XIST and the significantly enriched pathway terms of its related mRNAs.(B)The sub-network of lncRNA GABPB1-AS1 and the significantly enriched pathway terms of its related mRNAs.(C)The sub-network of lncRNA TUG1 and the significantly enriched pathway terms of its related mRNAs.

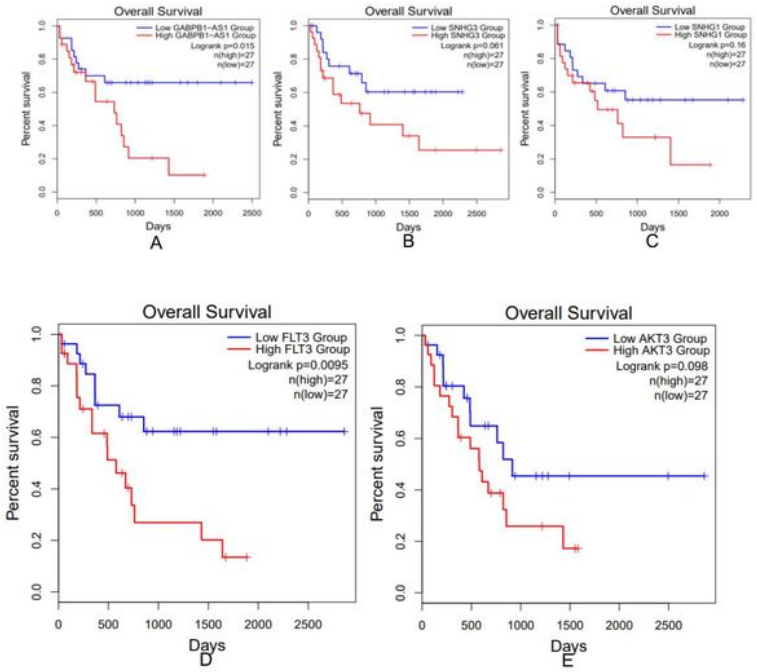


Figure 6

Kaplan–Meier curves of three lncRNAs(GABPB1-AS1(A),SNHG3(B),SNHG1(C))and two mRNA(FLT3(D),AKT3(E)) in AML with logrank p□0.05.

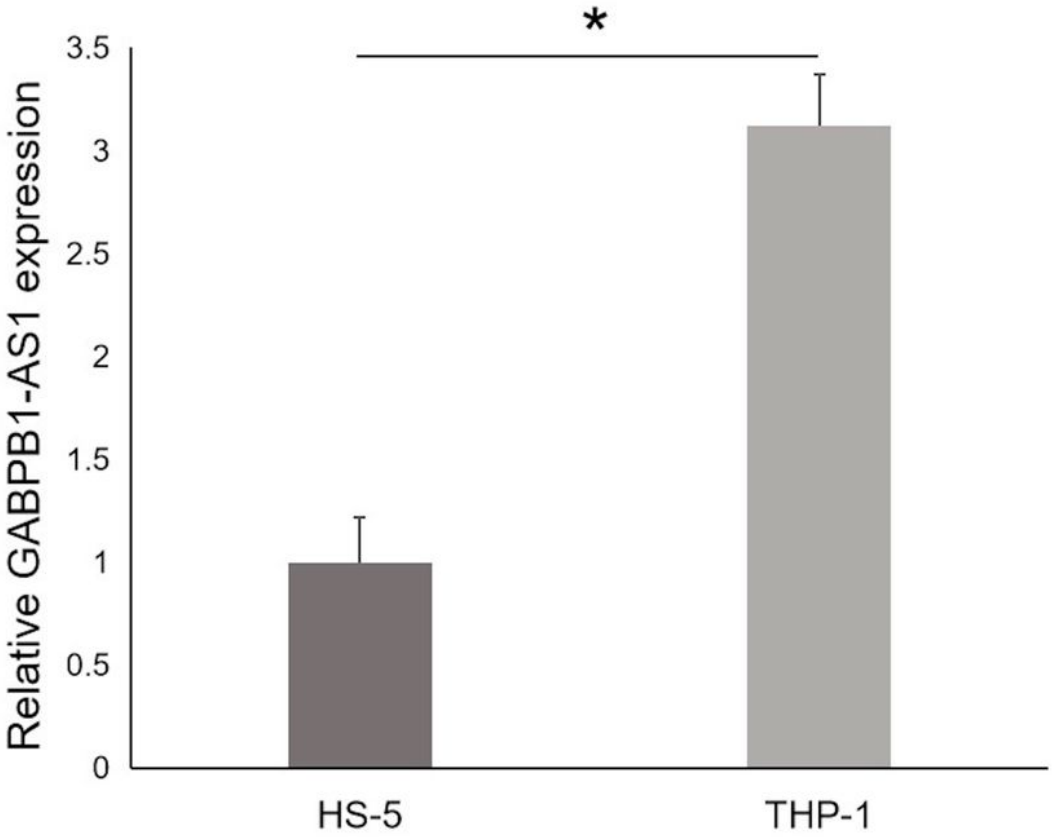


Figure 7

Comparing differences in the expression levels of GABPB1-AS1 between THP-1 cells and normal HS-5 cells. *p<0.01.