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RESEARCH ARTICLE

Identification The Hub Genes In HL-60 Leukemia Cells On Decitabine Through Bioinformatics Analysis

Fuxue Meng*, Lonakuan Li, Qin Zheng

Medical Experiment Center, the Third Affiliated Hospital of Guizhou Medical University, Duyun 558000, Guizhou Province, China.

Corresponding Author: Fuxue Meng

Abstract

Objective: Aims to identification the hub genes in HL-60 acute myeloid leukemia cells treated with decitabine, provide meaningful insights into the pathogenesis of AML. **Methods**: The gene expression profile of GSE24224 was obtained from GEO database, GO and pathway enrichment were performed though DAVID, established a PPI network from the STRING database and was displayed through the weight network diagram of omicShare tools. MiRNA targets and RBP targets prediction were carried out to further unravel the functions of hub genes identified. **Results**: 1558 differentially expressed genes were identified. GO and pathway analyses mainly involved citrulline metabolic process, positive regulation of neutrophil extravasation, positive regulation of cell adhesion molecule production, phospholipase A2 inhibitor activity, hematopoietic cell lineage, TGF-β signaling pathway, p53 signaling pathway. The miRNA and RBP targets prediction hinted that the 10 hub genes identified plays an important role in prognosis of AML. **Conclusion**: This study intimated that hub genes C3, C3AR1, FPR2, GNA11, PTAFR, ITGAM, ANXA1, LPAR1 CXCR4 and FPR1 identified predictively targeted hsa-miR-520d-5p, hsa-miR-362-5p, hsa-miR-224-5p, hsa-miR-1913, hsa-miR-196b-5, hsa-miR-188-5p, hsa-miR-130a-5p, hsa-miR-204-5p, hsa-miR-211-5p, hsa-miR-671-5p, hsa-miR-296-3p and hsa-miR-23b-3p and ADAR1, DGCR8, DKC1, ELAVL1, FBL, FUS, HNRNPC, IGF2BP2, NOP58, TAF15, U2AF2 and UPF1 proteins may regulate the occurrence and development of AML. This study provides meaningful insights and ideas for further understanding the pathogenesis of AML.

Key words: acute myeloid leukemia; hub genes; HL-60; bioinformatics analysis; decitabine

Introduction

Acute myeloid leukemia (AML) is a malignant clonal proliferative disease derived from hematopoietic stem cells, and its 5-year survival rate is very low. In the past few decades, the development of sequencing technology has resulted in the accumulation of a large number of omics data for various complex diseases. The subsequent development of bioinformatics revealed to us more rapid and intuitive multiple gene expression patterns[1;2].

Decitabine, as a specific inhibitor of DNA methyltransferase (DNMT), can be incorporated into DNA during the replication process, irreversibly or covalently bound to DNMT, depleting the storage of DNMT in the cell, and making DNA progressive demethylation. Decitabine can also enhance the body's anti-tumor immunity through non-methylated anti-tumor effects, stimulate the expression of tumor-associated antigens, or change the cellular immune status by regulating regulatory T cells; it can be mediated by P53 damage repair pathway promotes apoptosis of leukemia cells[3;4]. Study[5] have shown that compared with the

simulated control group, there are significant differences in gene expression in the HL-60 cellS group treated with decitabine, which plays a key role in the function of HL-60 cells, but the specific mechanism is still unclear.

Therefore, this study utilized bioinformatics analysis methods to analyze the HL-60 cell gene chip that was acted by decitabine, in order to discover biomarkers related to the disease, and provide a theoretical basis for revealing the molecular mechanism of the occurrence and development of AML.

Methods

Microarray data collection and DEG screening

The publicly accessible data GSE24224 was obtained from the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc =GSE24224) that deposited by Fabiani et al[5]. Containing six samples (three decitabine treated HL-60 cells and three mock treated HL-60 cells) were utilized in the present study. Gene expression profiling (GEP) was performed using the Affymetrix U133 Plus 2.0 expression array. All hybridization reactions were performed using GeneChip Fluidics Station 450, and GeneChips were scanned using the Affymetrix GeneChip Scanner 3000 (Affymetrix, USA). The raw data of GSE24224 was processed using the Affy package pair in R, using correction, normalization and log2 conversion[6]. The differentially expressed genes (DEGs) in decitabine treated HL-60 cells compared with mock treated HL-60 cells were determined using limma package[7;8]. DEGs were screened with a false discovery rate (FDR) corrected P<0.05 and |log fold-change (FC)|>1, then were confirmed using the GEO2R application from GEO.

Functional enrichment analysis

Investigation into the functions of enriched DEGs may improve understanding of their involvement in AML. In the present study, functional enrichment analysis of DEGs based on DAVID (https://david.ncifcrf.gov/tools.jsp), a widely used web-based genomic functional annotation tool. DEGs were subjected to molecular function and pathway studies by Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. $P < 0.05$ was set as cutoff values.

Protein-protein interaction (PPI) network construction and Hub genes identification

Genes are likely to function together rather than alone in complex diseases. Hub nodes in the network may represent key role. In the present study, protein-protein interaction (PPI) network analysis was performed to investigate the DEGs based on the STRING online database (https://string-db.org/cgi/input.pl). The minimum required interaction score was set as medium confidence > 0.8. In addition, the network was constructed through the weight network diagram of omicShare tools (https://www.omicshare.com/tools/index.php/) to confirm the hub genes and the genes with top-ten highest-weight nodes were defined as hub genes.

miRNA target prediction

In this study, the targets of the hub genes were predicted using four databases: miRWalk (http://mirwalk.umm.uni-heidelberg.de/), TargetScan (http://www. targetscan.org), miRMap (https://mirmap.ezlab.org) and starBase v2.0 (http://starbase.sysu.edu.cn/). The screening criterion was that the miRNA target exists in the four databases concurrently. The Venny 2.1 Online Tool (https://bioinfogp.cnb.csic.es/tools/venny/) was used to find overlapping genes between DEGs and predictive genes of DEMs. The miRNA-gene regulatory network was depicted and visualized using Cytoscape 3.7.1 (https://cytoscape.org/).

RNA binding protein (RBP) target prediction

As the core position of the post-transcriptional regulatory network, RNA binding protein (RBP) participates in multiple processes of RNA processing, including alternative splicing, RNA transport and stability maintenance, RNA localization, and mRNA translation [9]. In order to further understand the function of the hub gene, we used the RBP-mRNA module on the starBase v2.0 software to performed RBP prediction.

Results

Differentially expressed gene acquisition

By the cutoff of a 1-fold change and $P < 0.05$, 1592 differentially expressed were identified filtered out. After removing the genes without gene symbol, 1558 differentially expressed genes remain, of which 1368 were up-regulated genes and 190 were down-regulated genes. The heatmap with clustering of differentially

expressed gene were illustrated, and volcano plots were generated to demonstrate the distribution of the differentially expressed gene (Figure 1).

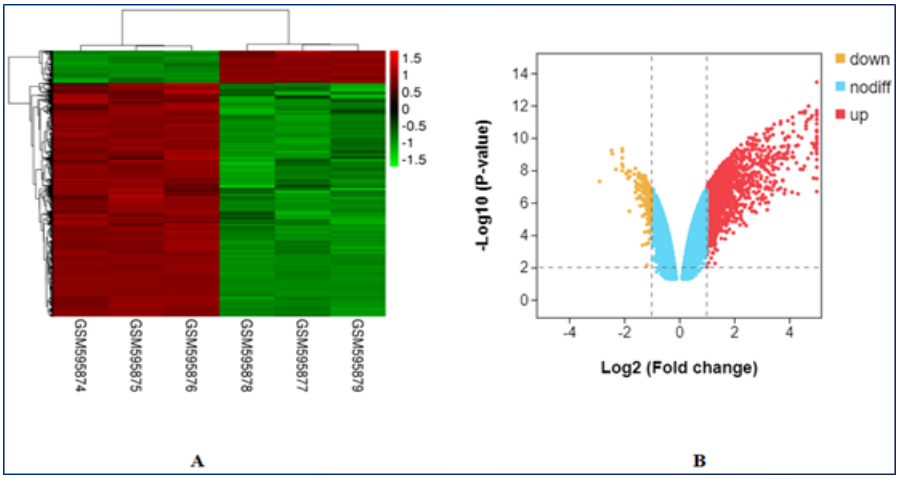

Figure 1 Heatmap and volcano plots showing the diffffferential expression genes between decitabine treated HL-60 cells and group. A: The Heatmap of diffffferential expression genes; B: the volcano plots diffffferential expression genes (|log 2 FoldChange| ≧ 1 and P value < 0.05).

GO and pathway analysis

The GO functional enrichment resulted in DEGs mapped to 198 GO terms. With *P*<0.05 as the significant enrichment criterion, top ten of significant enriched functional clusters were screened. Of them, the DEGs GO enrichment mainly involved citrulline metabolic process, positive regulation of neutrophil extravasation, positive regulation of cell adhesion molecule production, phospholipase A2 inhibitor activity, MRF binding, phagolysosome, synaptonemal complex. KEGG enrichment analysis with *P*<0.05 was used as an enrichment screening standard, involved such as staphylococcus aureus infection, hematopoietic cell lineage, TGF-beta signaling pathway, p53 signaling pathway (Figure 2) .

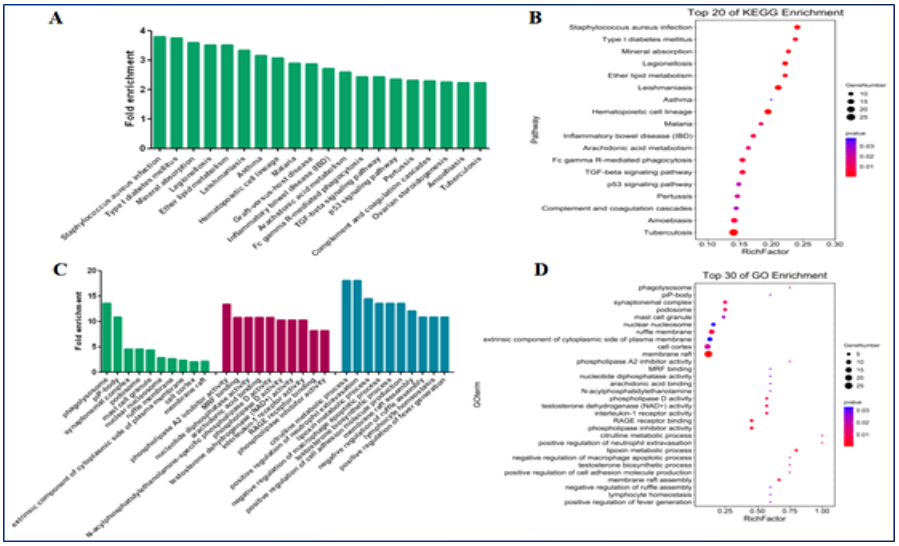

Figure 2 GO and KEGG analysis of DEGs. A: KEGG enrichment histogram analysis of DEGs ; B: KEGG enrichment bubble chart of DEGs; C: GO enrichment histogram analysis of DEGs; D: GO enrichment bubble chart of DEG (*P*<0.05).

PPI network analysis

To screen and identification the hub genes in HL-60 cells, we established a PPI network from the STRING database with scores of > 0.8. Then, the network was constructed through the weight

network diagram of omicShare tools to confirm the hub genes and the genes with top-ten highest-weight node of DEGs such as C3, C3AR1, FPR2, GNA11, PTAFR, ITGAM, ANXA1, LPAR1 CXCR4 and FPR1 were defined as hub genes (Figure 3).

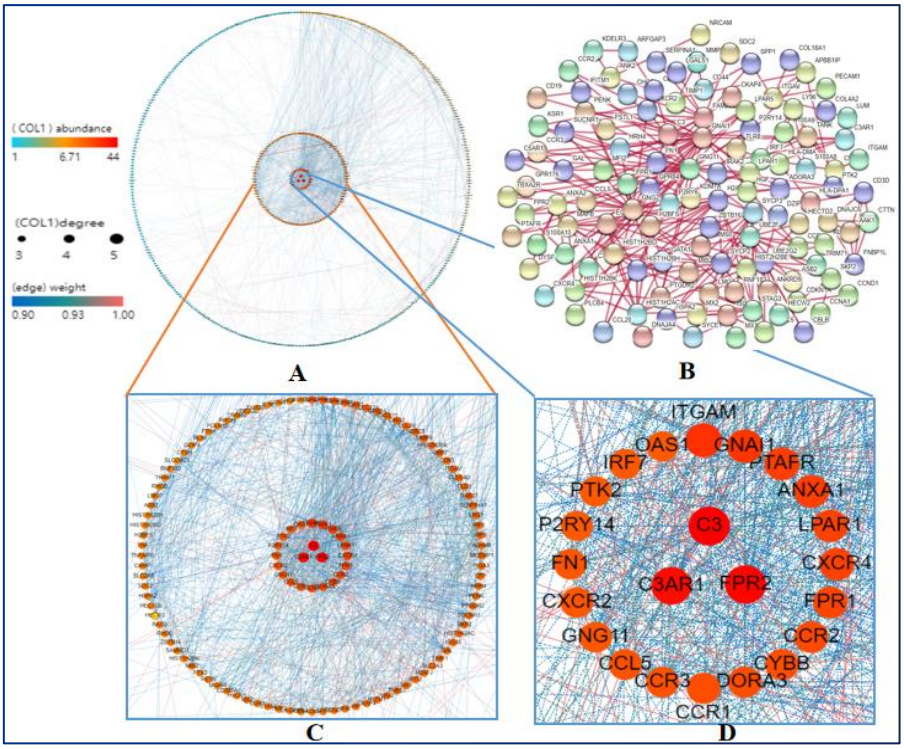

Figure 3 PPI network construction and hub genes determination. B: PPI network of DEGs; A,C,D: the weight network of DEGs, C3, C3AR1, FPR2, GNA11, PTAFR, ITGAM, ANXA1, LPAR1 CXCR4 and FPR1 identified as the hub genes (scores > 0.8).

MiRNA target prediction

For further understand the functions of the hub genes, the miRNA target prediction were carried out through four databases: miRWalk, TargetScan, miRMap and starBase v2.0. The C3 and FRP1 genes only have overlapping miRNA targets in three of the databases, and the remaining eight hub genes existed overlapping miRNA targets in the four databases, as the figure 4 shows.

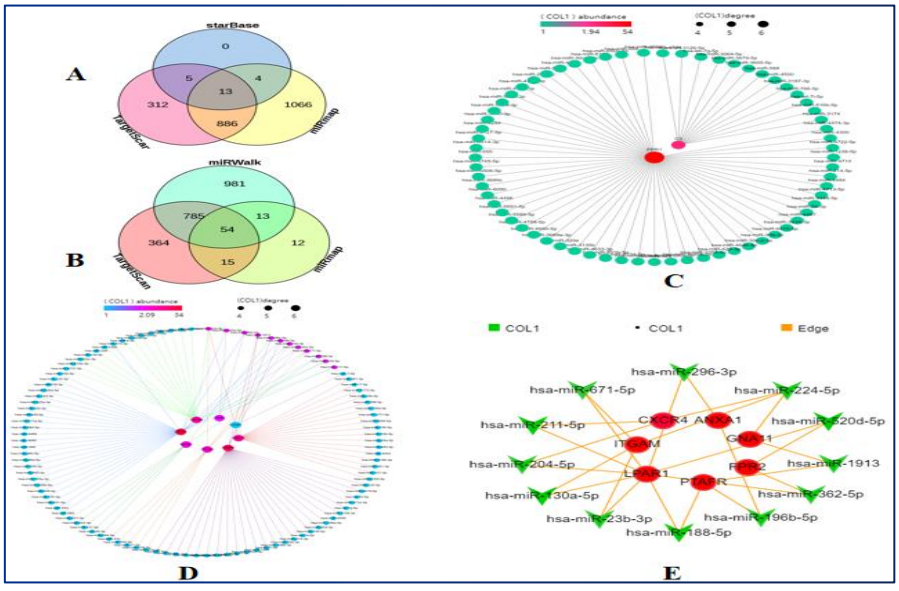

Figure 4 miRNA target prediction of hub genes. A, B: Predict miRNA targets the wenn map of C3 and FRP1 in three databases that predict miRNA targets; C: Eight hub genes for predicted miRNA targets network; D: Network of 7 hub genes with overlapping miRNA targets.

RNA binding protein (RBP) target prediction

RNA in the cell interacts with RBP to form a ribonucleoprotein (RNP) complex, which plays an important role in RNA synthesis, transport, stability, translation, and cellular localization. In order to further understand the function of hub genes, we carried out the RBP prediction. The results show that the top 10 RBP targets predicted by the 8 hub genes with the strongest weight were ELAVL1, FUS, DKC1, HNRNPC, ADAR, IGF2BP2, NOP58, TAF15, UPF1, U2AF2 (Figure 5).

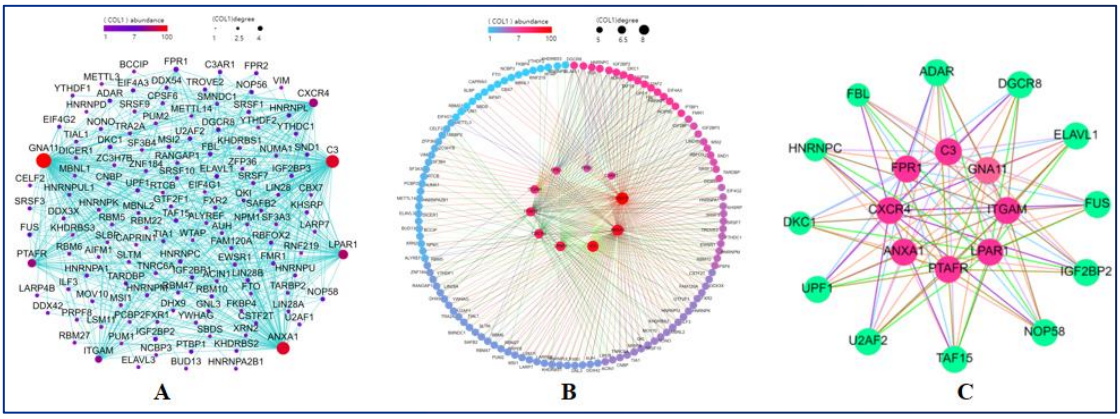

Figure 5 RBP targets prediction of hub genes. A, B: The networks of RBP targets. The larger point, darker the color, indicating the stronger the correlation and the greater the frequency of action; C: Screening of RBP targets with strong correlation.

Discussion

This study obtained gene expression profiles of HL-60 cells that decitabine induced and mock control group GSE24224 from GEO database and carried out DEGs screening, to understand the biological functions biological and the enrichment pathways involved through GO and KEGG analysis. Hereafter, PPI and weighted network analysis were conducted to identify the hub genes that play a key regulatory role in HL-60 cells. Further performed miRNA targets prediction and RBP targets prediction to clarify the possible mechanism of the hub gene for AML.

In this study, we screened 1558 DEGs, of which 1368 were up-regulated and 190 were down-regulated. Based on GO enrichment analyses, it was found that DEGs mainly involved molecular functions including hematopoietic cell lineage, TGF-β signaling pathway, p53 signaling pathway. The TGF-β signaling pathway plays a negative role in the regulation of cell proliferation and differentiation in the hematopoietic system. When ZFYVE16 is overexpressed, the negative regulation of the TGF-β signaling pathway is enhanced, which may inhibit the malignant proliferation activated by FLT3[10]. In addition, TGF-β stimulates the tumor pre-osteolytic and osteolytic factor production , Thereby stimulating further bone absorption [11;12]. This classifies TGF-β as as an important factor responsible for the feedforward vicious circle that drives tumor growth in the bones. In addition, abnormal reactivation of TGF-β usually leads to carcinogenic behavior [13;14]. Their role in tumorigenesis usually reflects their role in embryonic development, but also extends to other features often observed in cancer, such as cachexia and bone loss [15]. In the stage of prostate tumorigenesis, increased TGF-β production leads to the degradation of extracellular matrix, immunosuppression and angiogenesis, all of which lead to escape cell death and increase cell survival rate, which is conducive to the reproduction of cancer cells [16]. The regulation of p53 gene activity is mainly at the post-transcriptional level. After phosphorylation of p53 protein is activated, it becomes p-p53, which then

acts by inducing cell cycle arrest and inducing apoptosis to inhibit tumor cell proliferation [17;18].

After the above research and analysis, PPI networks were constructed by STRING, and from the intersection, 10 hub genes were obtained, which were verified and show with weighted network analysis. The analytic results of C3, C3AR1, FPR2, GNA11, PTAFR, ITGAM, ANXA1, LPAR1 CXCR4 and FPR1 were statistically significant, which suggested that these ten genes possibly play a key regulatory role in AML.

In order to further understand the functions of ten hub genes, we performed miRNA targets prediction and RBP targets prediction. miRNA is an endogenous single-stranded small RNA with a length of 21-25 bases, which can regulate the expression of target genes by specifically binding to mRNA to degrade or inhibit protein translation of mRNA. Studies have shown that miRNAs are often located in tumorigenesis-related regions or fragile sites, amplification regions, heterozygous loss regions or breakpoint regions. The specificity of miRNA expression in tumor cells is specific to tumor occurrence, development, invasion, metastasis, and metastasis. It plays an important role in the prognosis and other processes, providing potential therapeutic targets or new strategies[19;20]. In this study, we selected 7 hub genes whose miRNA prediction targets were hsa-miR-520d-5p, hsa-miR-362-5p, hsa-miR-224-5p, hsa-miR-1913, hsa-miR-196b-5, hsa-miR-188-5p, hsa-miR-130a-5p, hsa-miR-204-5p, hsa-miR-211-5p, hsa-miR-671-5p, hsa-miR-296-3p and hsa-miR-23b-3p. Li[21] showed that hsa-miR-362-3p was highly expressed as a marker in AML, and suggesting that it is related to the poor prognosis of AML patients. The prediction result of hsa-miR-1913 in AML is consistent with the result of Wang[22].

RBP refers to the general term for proteins that directly bind to RNA. RNA in cells interacts with RBP to form a ribonucleoprotein (RNP) complex, which plays an important role in RNA synthesis, transport, stability, translation, and cell positioning[23]. In the current study, the hub genes C3, GNA11, PTAFR, ITGAM, ANXA1, LPAR1 and CXCR4 were predicted to be strongly associated with proteins ADAR1, DGCR8, DKC1, ELAVL1, FBL, FUS, HNRNPC, IGF2BP2, NOP58, TAF15, U2AF2 and UPF1. Interestingly, Xiao[24] and Peng[25]reported that ADAR1 plays an important role in acute myeloid leukemia. Among them, Xiao's study demonstrate that ADAR1 may be involved in the regulation of the proliferation of AML cells partially via regulation of the Wnt signaling pathway[24]. He et al reported that IGF2BP2 overexpression indicates poor survival in patients with AML, IGF2BP2 may serve as a biomarker to predict the prognosis of AML and as a potential target in AML[26].

Conclusion

In conclusion, the present study identified a panel of 10 genes in AML HL-60 cells. Gene function and pathway investigation indicated that these genes were mainly engaged in citrulline metabolic process, positive regulation of neutrophil extravasation, positive regulation of cell adhesion molecule production, phospholipase A2 inhibitor activity, hematopoietic cell lineage, TGF-β signaling pathway, p53 signaling pathway. Moreover, the miRNA and RBP targets prediction hinted that the 10 hub genes identified plays an important role in prognosis of AML. Hub genes C3, C3AR1, FPR2, GNA11, PTAFR, ITGAM, ANXA1, LPAR1 CXCR4 and FPR1 predictively targeted hsa-miR-520d-5p, hsa-miR-362-5p, hsa-miR-224-5p, hsa-miR-1913, hsa-miR-196b-5, hsa-miR-188-5p, hsa-miR-130a-5p, hsa-miR-204-5p, hsa-miR-211-5p, hsa-miR-671-5p, hsa-miR-296-3p and hsa-miR-23b-3p and ADAR1, DGCR8, DKC1, ELAVL1, FBL, FUS, HNRNPC, IGF2BP2, NOP58, TAF15, U2AF2 and UPF1 proteins may regulate the occurrence and development of AML, but further verification is needed. In short, this study provides meaningful insights and ideas for further understanding the pathogenesis of AML.

Abbreviation

AML Acute myeloid leukemia

DNMT DNA methyltransferase

DEGs Differentially expressed genes

RBP RNA binding protein

C3AR1 Complement component 3a receptor 1

FPR2 Formyl peptide receptor 1

GNA11 G protein subunit alpha 11

PTAFR Platelet activating factor receptor

ITGAM Integrin subunit alpha M

ANXA1 Annexin A1

LPAR1 Lysophosphatidic acid receptor 1

CXCR4 C-X-C motif chemokine receptor 4

FPR1 Formyl peptide receptor 1

ELAVL1 ELAV like RNA binding protein 1

FUS FUS RNA binding protein

DKC1 Dyskerin pseudouridine synthase 1

HNRNPC Heterogeneous nuclear ribonucleoprotein C

ADAR Adenosine deaminase, RNA specific

IGF2BP2 Insulin like growth factor 2 mRNA binding protein 2

NOP58 NOP58 ribonucleoprotein

TAF15 TATA-box binding protein associated factor 15

UPF1 UPF1, RNA helicase and ATPase

U2AF2 U2 small nuclear RNA auxiliary factor 2

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Authors contributions

The design, study conduct, and financial support for this research were provided by Meng FX, Li LK and Zheng Q participated in the interpretation of data, review, and approval of the publication.

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Availability of data and materials

The original data of this research come from the third platform GEO database (GSE24224), and bioinformatics analysis was carried out through relevant analysis tools.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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