Am J Transl Res 2020;12(5):1683-1699
www.ajtr.org /ISSN:1943-8141/AJTR0104587

Original Article

Circular RNA profile of acute myeloid leukaemia indicates circular RNA annexin A2 as a potential biomarker and therapeutic target for acute myeloid leukaemia

Yi Ding*, Yan Dong*, Huina Lu, Xiu Luo, Jianfei Fu, Bing Xiu, Aibin Liang, Wenjun Zhang

Department of Hematology, Tongji Hospital, Tongji University School of Medicine, Shanghai 200065, P.R. China.
**Equal contributors.*

Received November 7, 2019; Accepted April 2, 2020; Epub May 15, 2020; Published May 30, 2020

Abstract: This study was to investigate the circular RNA (circRNA) expression profile and the potential circRNAs as biomarkers and therapeutic targets for acute myeloid leukaemia (AML). CircRNA expression profile in bone marrow mononuclear cells from 5 AML patients and 5 healthy donor controls (HCs) was evaluated by microarray. Then, 10 candidate circRNAs (top 5 upregulated and top 5 downregulated) from microarray were validated by RT-qPCR in 130 AML patients and 50 HCs. Finally, the effects of circRNA annexin A2 (circ-ANXA2) knockdown on cell proliferation, apoptosis, chemosensitivity to cytarabine, daunorbicin and potential target microRNAs were assessed in THP-1 and KG-1 cells. By microarray, 173 upregulated and 181 downregulated circRNAs were found in AML patients than HCs, and these circRNAs were found in AML patients compared with HCs, and these circRNAs were implicated in AML-related pathways such as ErbB and EGFR pathways. By RT-qPCR, 9 of 10 candidate circRNAs (including circ-RPS6KB1, circ-CSMD2, circ-PTK2, circ-ANXA2, circ-PWP2, circ-RBM5, circ-ZZEF1, circ-GSK3B and circ-FOXP1) were dysregulated in AML patients compared with HCs. Circ-ANXA2 correlated with higher disease risk, poor risk stratification, lower complete remission level, shorter event-free survival and overall survival in AML. In cellular experiments, circ-ANXA2 was upregulated in AML cell lines, and its knockdown suppressed proliferation, enhanced apoptosis of THP-1 and KG-1 cells and increased their chemosensitivity to cytarabine and daunorbicin. Additionally, circ-ANXA2 knockdown promoted microRNA (miR)-23a-5p and miR-503-3p expression in THP-1 and KG-1 cells. In conclusion, our findings provide a macroscopic view of the circRNA expression profile in AML, and demonstrate that circ-ANXA2 may be a potential biomarker and therapeutic target for AML.

Keywords: Acute myeloid leukaemia, circular RNA, expression profile, circular RNA annexin A2, reverse transcription quantitative polymerase chain reaction

Introduction

Acute myeloid leukaemia (AML), a molecularly and clinically heterogenous malignant disorder, is marked by abnormally proliferated and differentiated myeloid progenitor cells in the bone marrow, blood and other tissues and is the most common type of leukaemia in adults [1, 2]. Patients present with clinical symptoms, including anaemia, haemorrhage, infection, fever and abnormal metabolism, as the disease manifests [3]. Despite progress in intensive chemotherapy and allogeneic stem cell transplantation in the past five decades, the prognosis for most AML patients remains dismal, with a 5-year survival of only approximately 24% as a result of the challenges in treatment, such as refractoriness, relapse, treatment resistance or treatment-related mortality [4-6]. Therefore, potential biomarkers for aiding in the assessment and prognosis improvement of AML are worth investigating.

In recent years, the remarkable evolution of high-throughput sequencing and novel computation approaches has fuelled the discovery of novel RNA species and has provided critical insights into the pathology and pathogenesis of various diseases [7]. Circular RNAs (circRNAs), a novel class of non-coding RNAs, have a covalently closed loop configurations without either 5' end caps or 3' polyadenylated tails, which render them highly stable and abundant in cytoplasm, blood and other body fluids [8, 9].

Emerging evidence has revealed that a select number of circRNAs, such as circ-HIPK3 and circ-CBFB, participate in physiological and pathological processes such as tumorigenesis, uncontrolled growth, cell proliferation and migration, and the invasion of various cancers [8, 10]. Furthermore, the development of microarray approaches has also yielded a rapidly expanding understanding of circRNA expression profiles in multiple cancers. However, current cancer studies on the circRNA expression profile center on solid tumors [11-13]. For instance, among the 17,151 identified circRNAs, 67 circRNAs are upregulated and 78 circRNAs are downregulated in gastric cancer tissues, compared with their expression in noncancerous tissues [11]; and in high grade serous ovarian cancer, a total of 710 circRNAs are differentially expressed (354 upregulated and 356 downregulated) [13].

For haematological malignancies, one study illustrated that 361 differentially expressed circRNAs (201 upregulated and 160 downregulated) were present in chronic myeloid leukemia (CML) patients, compared to the circRNA expression in healthy individuals [14]. However, in AML, circRNA expression profiles have been rarely reported.

In the present study, we initially assessed the circRNA expression profile in 5 AML patients and 5 healthy donor controls (HCs) by microarray. Then, we selected 10 candidate circRNAs (top 5 upregulated and top 5 downregulated) from the microarray for validation by reverse transcription quantitative polymerase chain reaction (RT-qPCR) based on samples in 130 AML patients and 50 HCs. Of note, we found that circular RNA annexin A2 (circ-ANXA2) was upregulated and correlated with poor risk stratification, lower level of complete remission (CR) and shorter survival profiles of AML patients. Therefore, we subsequently conducted cellular experiments to discover the effect of circ-ANXA2 knockdown on the regulation of cell proliferation and apoptosis, cell chemosensitivity to cytarabine and daunorbicin and use of potential target microRNAs (miRNAs) in AML patients.

Materials and methods

Subjects

One hundred thirty adult patients with *de novo* AML admitted to Tongji Hospital, Tongji University School of Medicine, between January 2016 and June 2019 were consecutively enrolled in this study. The inclusion criteria were as follows: (i) diagnosed as de novo AML in accordance with the Health Organization (WHO) classification of AML (2008); (ii) age older than 18 years; (iii) no other malignancies; and (iv) available for regular followed up. Patients were excluded if they were (i) acute promyelocytic leukemia; (ii) secondary AML or recurrent AML; (iii) previously treated by radiotherapy, chemotherapy, or stem cell transplantation, (iv) infected with Human Immunodeficiency Virus, or (v) pregnant or lactating. Furthermore, a total of 50 bone marrow (BM) donors were recruited as HCs for the current study during the same period. The Institutional Review Board of Tongji Hospital, Tongji University School of Medicine approved this study prior to its initiation, and all subjects signed the informed consents forms.

Clinical data and bone marrow collection

Basic clinical data of the enrolled patients were documented, including age, gender, FAB (French-American-British) classification, karyotype/cytogenetics examinations results, molecular genetic examinations, risk stratification (according to NCCN Guideline 2013) and white blood cell (WBC) count at diagnosis. Prior to initial treatment, BM samples were collected from all patients. Additionally, the BM samples of the HCs were obtained by donation after participants signed informed consents forms. All collected BM samples were processed by density gradient centrifugation, and then BM mononuclear cells (BMMCs) were obtained and stored at -80°C until further examination.

Treatment and follow-up

After diagnosis was established, conventional induction therapy consisting of 3 days of anthracycline (e.g., daunorubicin at least 60 mg/m^2, idarubicin at 10-12 mg/m^2, or the anthracenedione or mitoxantrone at 10-12 mg/m^2) and 7 days of cytarabine (100-200 mg/m^2 cont. iv) or therapy of comparable intensity was administered to patients. The response evaluation was commonly performed between 21 and 28 days after the start of treatment. CR of patients was identified in accordance with the response criteria for AML proposed by an international expert panel [15]. In addition, surveillance continued every 1-3 months for the first two years and then every 6 months for the fol-

Table 1. Top 10 differentially expressed circRNAs (5 upregulated and 5 downregulated) by microarray

CircRNA	Probe	Type	Chromosome	Start	End	Log_2FC	*P* value	Adjusted *P* value	Gene Symbol	Trend
hsa_circ_0044907	ASCRP002470	exonic	chr17	58013575	58013902	4.258369	0.00017	0.007068	RPS6KB1	UP
hsa_circ_0011501	ASCRP000548	exonic	chr1	34254200	34276465	4.072882	2.72E-06	0.001382	CSMD2	UP
hsa_circ_0002483	ASCRP004960	exonic	chr8	141874410	141900868	3.50402	0.002921	0.030178	PTK2	UP
hsa_circ_0035559	ASCRP001891	exonic	chr15	60653139	60656722	3.082955	0.000183	0.007348	ANXA2	UP
hsa_circ_0000075	ASCRP000632	exonic	chr1	59787207	59812070	3.032165	0.001768	0.022465	FGGY	UP
hsa_circ_0092315	ASCRP005358	intronic	chr21	45545508	45545708	-3.85318	2.85E-05	0.003037	PWP2	Down
hsa_circ_0003795	ASCRP003676	exonic	chr3	50142509	50143142	-3.82337	5.35E-09	2.44E-05	RBM5	Down
hsa_circ_0041506	ASCRP002292	exonic	chr17	3984669	3989949	-3.14828	3.10E-06	0.00142	ZZEF1	Down
hsa_circ_0066887	ASCRP003739	exonic	chr3	119562101	119562200	-3.14408	4.12E-06	0.001514	GSK3B	Down
hsa_circ_0001320	ASCRP003712	exonic	chr3	71064699	71102924	-2.94792	2.60E-05	0.002959	FOXP1	Down

Top 5 upregulated and 5 downregulated circRNAs were selected by the rank of absolute value of Log_2FC. circRNA: circular RNA; FC: fold change.

lowing 2-3 years. Patients were consecutively followed through June 30, 2019. Event-free survival (EFS) was calculated from the date of the initial therapy to the date of treatment failure, relapse after CR, or death; patients not known to have any of these events were censored on the date that they were last examined. Overall survival (OS) was calculated from the date of initial therapy to the date of death; patients for whom life (or death) was unknown at the time of the last scheduled follow-up were censored on the date that they were last known to be alive.

Microarray and bioinformatics

Five BMMC samples from the AML patients and 5 BMMC samples from the HCs were randomly screened and subjected to microarray assay. Briefly, total RNA was extracted from each sample using TRIzol™ Reagent (Invitrogen, USA), RNA integrity was assessed by Agilent 2100 Bioanalyzer (Agilent, USA), and RNA was quantified with a NanoDrop ND-1000 spectrophotometer (Thermo, USA). After the RNA assays were completed, the liner RNAs were diminished using RNase R (Epicentre, USA). Subsequently, the circRNA expression profile of each sample was detected using an Agilent-069978 Arraystar Human CircRNA microarray V1 (Agilent, USA) by Genergy Bio (Shanghai, China) as previous study reported [16]. For data processing, quantile normalization and low-intensity filtering were carried out using the R software package (R version 3.1.2), and the circRNAs detected in more than 50% of the samples were marked as "Detected" and further analyzed. Bioinformatics analysis was performed using the R software package (R version 3.1.2). In brief, principal component analysis (PCA) of the circRNA expression profile was performed by Factoextra package; heatmap of circRNA expression profile was plotted by the Pheatmap package; differentially expressed circRNAs were analyzed by the Limma package, and circRNAs with a fold change (FC) ≥2.0 and an adjusted *P* value (BH multiple test correction) <0.05 was used to identify differentially expressed circRNAs, which were displayed using Volcano Plots; Gene Ontology (GO) and Kyoko Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of differentially expressed circRNAs based on located genes were performed, and the results were assessed by by Fisher exact test; GO, KEGG, human-phenotype-ontology (HP) and Disease Ontology (DOID) enrichment analyses of the differentially expressed circRNAs were also performed on the basis of target miRNAs and assessed by Fisher's exact test.

Screening and validation of 10 candidate circRNAs

Ten candidate circRNAs, namely, 5 upregulated and 5 downregulated circRNAs, were selected from differentially expressed circRNAs identified in the microarray analysis according to the rank of the absolute value of Log_2FC (shown in **Table 1**). Then, those 10 candidate circRNAs were further validated in samples from all the AML patients (N=130) and HCs (N=50) by Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR).

Cell culture

Human HL-60, THP-1, Kasumi-1 and KG-1 AML cell lines were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) (Braunschweig, Germany). All the

cells were cultured in 90% RPMI 1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) and maintained at 37°C in a humidified atmosphere of 5% CO_2. The relative expression of circ-ANXA2 was determined by RT-qPCR using BMMCs of HCs as control.

Transfection

The circ-ANXA2 Knock-down (KD) plasmid and control KD plasmid were constructed using pRNAT-U6.1/Neo by Guangzhou RiboBio Co., Ltd (Guangzhou, China). The plasmids were transfected into THP-1 and KG-1 cells with Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's manual. The cells transfected with the circ-ANXA2 KD plasmids were named KD-Circ cells; correspondingly, the cells transfected with control KD plasmids were named KD-NC cells. Cell viability was measured by Cell Counting Kit-8 (CCK-8) assay at 0 h, 24 h, 48 h and 72 h after transfection, and cell apoptosis was detected by Annexin V/Propidium Iodide (AV/PI) assay 48 h after transfection. Furthermore, the relative expression of circ-ANXA2 and potential target miRNAs, including hsa-miR-23a-5p, hsa-miR-139-3p and hsa-miR-503-3p were determined by RT-qPCR 24 h after transfection, and the potential target miRNAs were predicted using miRanda software (http://miranda.org.uk/).

Drug sensitivity

Twenty-four hours after transfection, 20,000 cells were seeded in each well of 96-well plates and then treated with different concentrations of cytarabine (Sigma-Aldrich, USA) and daunorubicin (Sigma-Aldrich, USA). After 48 h-treatment, cell viability was determined using CCK-8 assay. The drug concentration required to inhibit growth by 50% (IC_{50}) was calculated for all the treated cells using Probit regression analysis.

RT-qPCR

Total RNA was extracted using TRIzol™ Reagent (Invitrogen, USA) according to the manufacturer's instructions. Then, the delinearization of the isolated RNA was conducted for the detection of circRNAs using RNase R (Epicentre, USA) before the synthesis of complementary DNA. However, delinearization was not performed for the detection of miRNAs, GAPDH and U6. Subsequently, complementary DNA was synthesized using iScript™ cDNA Synthesis Kit (Bio-Rad, USA). Then, qPCR was carried out using SYBR® Advantage® qPCR Premix (Clontech, USA). The sequences of the primers used are listed in Table S1. GAPDH and U6 were set as the internal reference for circRNAs and miRNAs respectively. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression of circRNAs and miRNAs.

CCK-8 assay

According to the instructions, CCK-8 was used for cell viability (Sigma, USA). Then, cell proliferation was determined by optical density as measured by microplate reader (Bio Tek, USA).

AV/PI assay

Cells were collected, washed and then incubated with 5 μL of AV and 5 μL of PI staining solution (R&D, USA) for 15 minutes according to the manufacturer's instructions. Subsequently, cell apoptosis was assessed using CytoFLEX system (Beckman Coulter, USA), and the data were analyzed by Flowjo Software 7.6 (FlowJo-LLC, USA).

Statistical analysis

All statistical analyses were performed in GraphPad Prism software version 6.01 (GraphPad Software Inc., USA) and SPSS software version 22.0 (IBM Corporation, USA). Data were expressed as means and standard deviation (SD), median and interquartile range (IQR) or count (percentage). The significance of comparisons between two groups were determined by unpaired t test or Wilcoxon rank sum test. The significance of comparisons among groups were determined by Kruskal-Wallis H rank sum test. Multiple comparisons were subjected to Dunnett's t test. Correlations were identified by Spearman's rank correlation test. The ability of circRNAs to predict AML risk level was evaluated using receiver operating characteristic (ROC) curve and the area under the curve (AUC). EFS and OS were assessed by Kaplan-Meier method, and the results were compared with those from log-rank test. The IC_{50} was calculated using Probit regression analysis. $P<0.05$ indicated statistically significant.

Table 2. Clinical characteristics of AML patients

Items	AML patients (N=130)
Age (years), mean ± SD	52.2±14.9
Gender, No. (%)	
Female	50 (38.5)
Male	80 (61.5)
WBC (×10^9/L), median (IQR)	16.0 (9.2-28.9)
FAB classification, No. (%)	
M2	47 (36.2)
M4	36 (27.6)
M5	47 (36.2)
Cytogenetics, No. (%)	
NK	72 (55.4)
inv(16) or t(16;16)	13 (10.0)
CK	9 (6.9)
t(9;11)	5 (3.9)
t(8;21)	5 (3.8)
+8	4 (3.1)
-7 or 7q-	4 (3.1)
t(9;22)	3 (2.3)
11q23	2 (1.5)
-5 or 5q-	1 (0.8)
Others (not included in better or poor risk)	12 (9.2)
MK, No. (%)	6 (4.6)
FLT3-ITD mutation, No. (%)	30 (23.1)
Isolated biallelic CEBPA mutation, No. (%)	15 (11.5)
NPM1, No. (%)	52 (40.0)
Risk stratification, No. (%)	
Better	43 (33.1)
Intermediate	50 (38.4)
Poor	37 (28.5)

AML, acute myeloid leukemia; SD, standard deviation; WBC, white blood cell; IQR, interquartile range; FAB classification, French-American-Britain classification; NK, normal karyotype; CK, complex karyotype; MK, monosomal karyotype; FLT3-ITD, internal tandem duplications in the FMS-like tyrosine kinase 3; CEBPA, CCAAT/enhancer-binding protein α; NPM1, nucleophosmin 1.

Results

Clinical features of AML patients

There were 50 (38.5%) female and 80 (61.5%) male AML patients with a mean age of 52.2± 14.9 years. The number of AML patients with M2, M4 and M5 FAB classification was 47 (36.2%), 36 (27.6%) and 47 (36.2%) respectively, and the number of AML patients with better, intermediate and poor risk stratification was 43 (33.1%), 50 (38.4%) and 37 (28.5%) respectively. The detailed characteristics of WBC, cytogenetics and molecular genetics were shown in **Table 2**.

CircRNA expression profile, volcano plots and enrichment analyses

PCA plots revealed a clear distinction between the 5 AML patients and 5 HCs, which suggested that circRNA expression profile could distinguish AML patients from HCs (**Figure 1A**). The heatmap analysis exhibited that circRNA expression profile were relatively consistent and tended to be consistent in both AML patients and HCs (**Figure 1B**). Volcano plots were used to identify differentially expressed circRNAs in the AML patients and HCs, and they revealed that 173 circRNAs were upregulated and 181 circRNAs were downregulated in AML patients, compared with those in HCs (FC≥2.0, P_{adj}<0.05) (**Figure 1C**). GO enrichment analysis based on located genes showed that the differentially expressed circRNAs were enriched in biological process category (such as positive regulation of cell proliferation, cell migration and response to drug), cellular component category (such as cytosol, nucleoplasm and cytoplasm) and molecular function category (such as protein binding, ATP binding and RNA binding) (**Figure 1D**). In addition, KEGG enrichment analysis based on located genes showed that the differentially expressed circRNAs were enriched in AML related signaling pathways, such as the ErbB signaling, EGFR tyrosine kinase inhibitor resistance and mTOR signaling pathways (**Figure 1E**). For GO enrichment analysis based on target miRNAs, the differentially expressed circRNAs in the patients with AML were enriched in biological process category (e.g. VDJ recombination, double-strand break repair via nonhomologous end joining and DNA ligation), cellular components category (e.g. DNA dependent protein kinase DNA ligase, nonhomologous end joining complex and nuclear telomer cap complex) and molecular function category (e.g. deoxyribose 5 phosphate lyase activity,

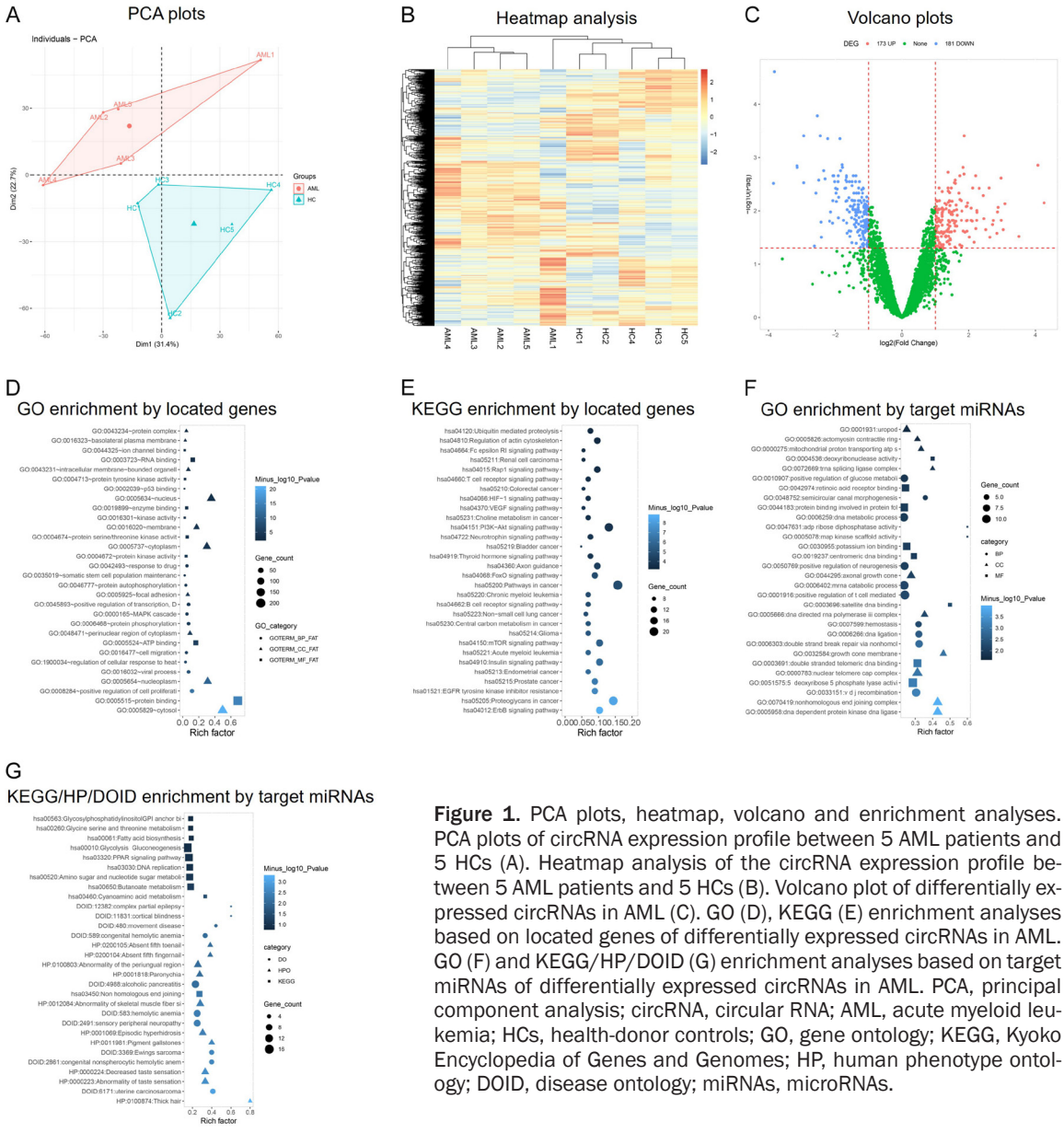

Figure 1. PCA plots, heatmap, volcano and enrichment analyses. PCA plots of circRNA expression profile between 5 AML patients and 5 HCs (A). Heatmap analysis of the circRNA expression profile between 5 AML patients and 5 HCs (B). Volcano plot of differentially expressed circRNAs in AML (C). GO (D), KEGG (E) enrichment analyses based on located genes of differentially expressed circRNAs in AML. GO (F) and KEGG/HP/DOID (G) enrichment analyses based on target miRNAs of differentially expressed circRNAs in AML. PCA, principal component analysis; circRNA, circular RNA; AML, acute myeloid leukemia; HCs, health-donor controls; GO, gene ontology; KEGG, Kyoko Encyclopedia of Genes and Genomes; HP, human phenotype ontology; DOID, disease ontology; miRNAs, microRNAs.

double stranded telomeric DNA binding and retinoic acid receptor binding) (**Figure 1F**). Regarding KEGG/HP/DOID enrichment analysis based on the target miRNAs, the differentially expressed circRNAs in patients with AML were enriched in disease ontology category (e.g. uterine carcinosarcoma, congenital nonspherocytic hemolytic anemia and haemolytic anemia), human phenotype ontology category (e.g. thick hair, abnormality of taste sensation and episodic hyperhidrosis) and cellular signaling pathways (e.g. nonhomologous end joining, cyanoamino acid metabolism and PPAR signaling pathway) (**Figure 1G**).

The value of candidate circRNAs in distinguishing AML patients from HCs

The top 5 upregulated and 5 downregulated circRNAs identified by bioinformatic analysis of AML patients data were compared to those selected from HC data based on the rank of absolute value of Log_2FC. The detailed information of these circRNAs is shown in **Table 1**. According to the RT-qPCR results, 9 candidate circRNAs were differentially expressed, including circ-RPS6KB1 ($P<0.001$) (**Figure 2A**), circ-CSMD2 ($P<0.001$) (**Figure 2B**), circ-PTK2 ($P=0.011$) (**Figure 2C**) and circ-ANXA2 ($P<0.001$)

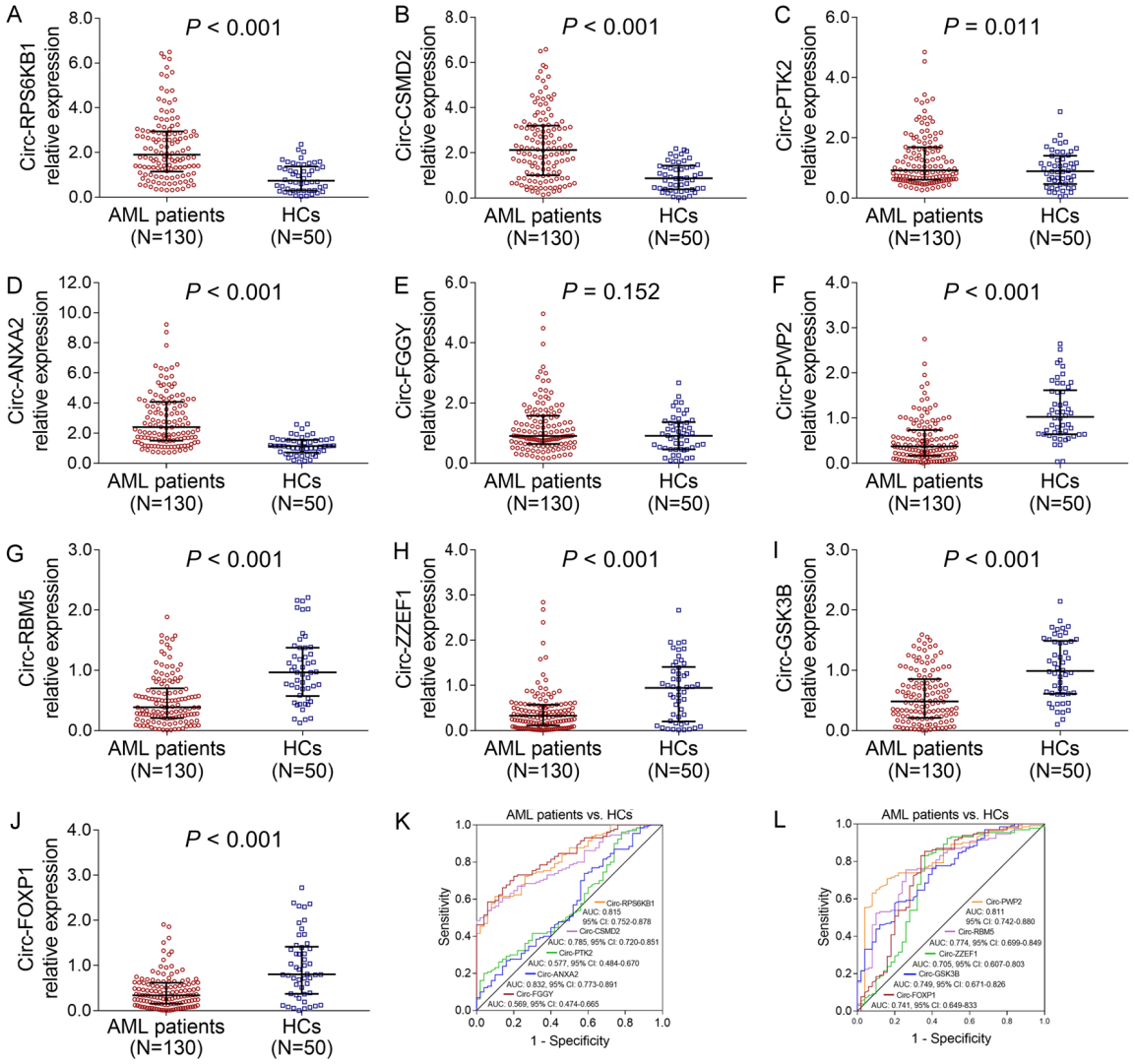

Figure 2. Candidate circRNAs in AML patients and HCs. Comparisons of circ-RPS6KB1 (A), circ-CSMD2 (B), circ-PTK2 (C), circ-ANXA2 (D), circ-FGGY (E), circ-PWP2 (F), circ-RBM5 (G), circ-ZZEF1 (H), circ-GSK3B (I) and circ-FOXP1 (J) relative expressions between AML patients and HCs. The performance of circ-RPS6KB1, circ-CSMD2, circ-PTK2, circ-ANXA2, circ-FGGY (K), circ-PWP2, circ-RBM5, circ-ZZEF1, circ-GSK3B and circ-FOXP1 (L) in differentiating AML patients from HCs. CircRNAs, circular RNAs; AML, acute myeloid leukemia; HCs, health-donor controls; circ, circular RNA; ROC, receiver operating characteristic.

(**Figure 2D**) for which the relative expressions was elevated; circ-PWP2 (*P*<0.001) (**Figure 2F**), circ-RBM5 (*P*<0.001) (**Figure 2G**), circ-ZZEF1 (*P*<0.001) (**Figure 2H**), circ-GSK3B (*P*<0.001) (**Figure 2I**) and circ-FOXP1 (P<0.001) (**Figure 2J**) for which the relative expression was decreased, and circ-FGGY (*P*=0.152) (**Figure 2E**) for which the relative expression was similar in AML patients and HCs. Subsequent ROC curve analyses revealed that circ-RPS6KB1 (AUC: 0.815, 95% CI: 0.752-0.878), circ-CSMD2 (AUC: 0.785, 95% CI: 0.720-0.851) and circ-ANXA2 (AUC: 0.832, 95% CI: 0.773-0.891) (**Figure 2K**), circ-PWP2 (AUC: 0.811, 95% CI: 0.742-0.880), circ-RBM5 (AUC: 0.774, 95% CI: 0.699-0.849), circ-ZZEF1 (AUC: 0.705, 95% CI: 0.607-0.803), circ-GSK3B (AUC: 0.749, 95% CI: 0.671-0.826) and circ-FOXP1 (AUC: 0.741, 95% CI: 0.649-0.833) distinguished AML patients from HCs (**Figure 2L**), whereas circ-PTK2 (AUC: 0.577, 95% CI: 0.484-0.670) and circ-FGGY (AUC: 0.569, 95% CI: 0.474-0.665) did not differentiate AML patients from HCs (**Figure 2K**).

Association of candidate circRNAs with FAB stratification and risk stratification of AML patients

No correlation of circ-RPS6KB1 (*P*=0.437), circ-CSMD2 (*P*=0.790), circ-PTK2 (*P*=0.345),

circ-ANXA2 (P=0.617), circ-FGGY (P=0.174), circ-PWP2 (P=0.716), circ-RBM5 (P=0.617), circ-ZZEF1 (P=0.853), circ-GSK3B (P=0.436) or circ-FOXP1 (P=0.369) with FAB classification was observed in AML patients (**Table 3**). In terms of risk stratification, circ-ANXA2 (P=0.001) was correlated with poor risk stratification in AML patients, while there was no correlation between circ-RPS6KB1 (P=0.425), circ-CSMD2 (P=0.550), circ-PTK2 (P=0.591), circ-FGGY (P=0.120), circ-PWP2 (P=0.782), circ-RBM5 (P=0.498), circ-ZZEF1 (P=0.834), circ-GSK3B (P=0.597) or circ-FOXP1 (P=0.440) with risk stratification in AML patients (**Table 3**).

Correlation of candidate circRNAs with treatment response in AML patients

AML patients who achieved CR had lower circ-ANXA2 expression (P=0.004) compared with that in AML patients who did not achieve CR, whereas no difference of circ-RPS6KB1 (P=0.128), circ-CSMD2 (P=0.537), circ-PTK2 (P=0.548), circ-FGGY (P=0.718), circ-PWP2 (P=0.460), circ-RBM5 (P=0.218), circ-ZZEF1 (P=0.546), circ-GSK3B (P=0.215) or circ-FOXP1 (P=0.667) expression was displayed in the CR patients or the patients without CR (**Table 4**). These results implied that circ-ANXA2 was correlated with lower CR in AML patients.

Correlation of candidate circRNAs with survival profiles in AML patients

In AML patients, circ-ANXA2 high expression was correlated with shorter EFS (P=0.013) (**Figure 3D**), whereas circ-RPS6KB1 (P=0.105) (**Figure 3A**), circ-CSMD2 (P=0.892) (**Figure 3B**), circ-PTK2 (P=0.340) (**Figure 3C**), circ-FGGY (P=0.451) (**Figure 3E**), circ-PWP2 (P=0.907) (**Figure 3F**), circ-RBM5 (P=0.496) (**Figure 3G**), circ-ZZEF1 (P=0.976) (**Figure 3H**), circ-GSK3B (P=0.496) (**Figure 3I**) or circ-FOXP1 (P=0.193) (**Figure 3J**) was not correlated with EFS. As for OS, circ-ANXA2 high expression was correlated with shorter OS (P=0.001) (**Figure 4D**), whereas circ-RPS6KB1 (P=0.091) (**Figure 4A**), circ-CSMD2 (P=0.554) (**Figure 4B**), circ-PTK2 (P=0.391) (**Figure 4C**), circ-FGGY (P=0.336) (**Figure 4E**), circ-PWP2 (P=0.711) (**Figure 4F**), circ-RBM5 (P=0.663) (**Figure 4G**), circ-ZZEF1 (P=0.523) (**Figure 4H**), circ-GSK3B (P=0.089) (**Figure 4I**) or circ-FOXP1 (P=0.152) (**Figure 4J**) was not correlated with OS in AML patients.

Comparison of circ-ANXA2 between BMMCs of HCs and human AML cell lines

Among the 10 selected circRNAs, circ-ANXA2 not only predicted AML risk, but also correlated with poor risk stratification, lower CR, shorter EFS and OS in AML. Thus, we conducted experiments to explore the function of circ-ANXA2 in the AML tumorigenesis. It was shown that circ-ANXA2 relative expression was upregulated in HL-60 cells (P<0.05), THP-1 cells (P<0.001), Kasumi-1 cells (P<0.01) and KG-1 cells (P<0.001) (human AML cell lines) compared with control cells (BMMCs of HCs) (Figure S1).

Effect of circ-ANXA2 knockdown on cell proliferation and apoptosis in THP-1 and KG-1 cells

In THP-1 cells, circ-ANXA2 relative expression was lower in KD-Circ group than that in KD-NC group at 24 h after transfection (P<0.001) (**Figure 5A**), which indicated that transfections were successful. Furthermore, cell proliferation was reduced in KD-Circ group compared with KD-NC group at 48 h (P<0.05) and 72 h (P<0.01) after transfection (**Figure 5B**). As for cell apoptosis rate, it was increased in KD-Circ group compared with KD-NC group at 48 h after transfection (P<0.001) (**Figure 5C**, **5D**). Regarding KG-1 cells, circ-ANXA2 relative expression was decreased in KD-Circ group compared with KD-NC group at 24 h after transfection (P<0.001) (**Figure 5E**), which suggested that transfections were successful. The cell proliferation was inhibited in KD-Circ group compared with KD-NC group at 48 h (P<0.05) and 72 h (P<0.05) after transfection (**Figure 5F**). And the cell apoptosis rate was promoted in KD-Circ group compared with KD-NC group at 48 h after transfection (P<0.01) (**Figure 5G**, **5H**).

Effect of circ-ANXA2 knockdown on chemosensitivity to cytarabine and daunorbicin in THP-1 and KG-1 cells

In THP-1 cells, the relative cell viability under 0.2 μM (P<0.05), 0.4 μM (P<0.05), 0.8 μM (P<0.01), 1.6 μM (P<0.05) cytarabine treatment and the IC_{50} value of cytarabine (P<0.001) were decreased in KD-Circ group compared with KD-NC group (**Figure 6A**, **6B**). The relative cell viability under 0.02 μM (P<0.05), 0.04 μM (P<0.01), 0.08 μM (P<0.05) daunorbicin treatment and IC_{50} value of daunorbicin (P<0.01)

Table 3. Correlation of candidate circRNAs with FAB and risk stratification in AML patients

Items	FAB classification				Risk stratification			
	M2 (n=47)	M4 (n=36)	M5 (n=47)	*P* value	Better (n=43)	Intermediate (n=50)	Poor (n=37)	*P* value
Circ-RPS6KB1, median (IQR)	1.960 (1.099-2.764)	1.890 (1.394-3.431)	1.790 (1.012-2.833)	0.437	1.812 (1.011-2.741)	1.793 (0.917-2.974)	1.960 (1.404-3.097)	0.425
Circ-CSMD2, median (IQR)	2.023 (0.851-3.083)	2.169 (0.890-3.347)	2.203 (1.122-3.272)	0.790	2.328 (1.134-3.479)	2.103 (0.983-3.073)	1.838 (0.821-3.568)	0.550
Circ-PTK2, median (IQR)	1.003 (0.638-1.702)	0.732 (0.502-1.680)	1.055 (0.670-1.684)	0.345	0.876 (0.561-1.613)	0.991 (0.698-1.687)	0.825 (0.582-2.030)	0.591
Circ-ANXA2, median (IQR)	1.916 (1.421-3.393)	2.402 (1.447-4.203)	2.650 (1.684-4.146)	0.617	2.014 (1.390-3.003)	2.060 (1.252-4.269)	2.871 (1.925-5.746)	0.001
Circ-FGGY, median (IQR)	1.230 (0.774-1.765)	0.877 (0.546-1.776)	0.902 (0.609-1.348)	0.174	1.028 (0.654-1.668)	0.832 (0.551-1.379)	1.099 (0.767-1.747)	0.120
Circ-PWP2, median (IQR)	0.390 (0.193-0.883)	0.388 (0.172-0.682)	0.340 (0.152-0.637)	0.716	0.312 (0.101-0.875)	0.395 (0.191-0.736)	0.340 (0.182-0.637)	0.782
Circ-RBM5, median (IQR)	0.402 (0.152-0.808)	0.507 (0.272-0.842)	0.340 (0.221-0.634)	0.617	0.340 (0.139-0.774)	0.384 (0.229-0.630)	0.499 (0.245-0.779)	0.498
Circ-ZZEF1, median (IQR)	0.294 (0.081-0.595)	0.310 (0.101-0.570)	0.333 (0.192-0.571)	0.853	0.239 (0.136-0.571)	0.374 (0.096-0.561)	0.339 (0.107-0.603)	0.834
Circ-GSK3B, median (IQR)	0.591 (0.236-1.059)	0.520 (0.232-0.749)	0.394 (0.148-0.787)	0.436	0.559 (0.205-1.004)	0.451 (0.278-0.797)	0.467 (0.140-0.834)	0.597
Circ-FOXP1, median (IQR)	0.332 (0.159-0.553)	0.278 (0.108-0.599)	0.431 (0.176-0.659)	0.369	0.364 (0.228-0.622)	0.354 (0.200-0.614)	0.244 (0.088-0.635)	0.440

Comparison of candidate circRNAs among different FAB classification patients was determined by Kruskal-Wallis H test. Correlation of candidate circRNAs with risk stratification was analyzed by Spearman's rank correlation test. FAB classification, French-American-Britain classification; AML, acute myeloid leukemia; IQR, interquartile range.

Table 4. Correlation of candidate circRNAs with CR in AML patients

Items	CR patients (n=101)	Non-CR patients (n=29)	*P* value
Circ-RPS6KB1, median (IQR)	1.812 (1.211-2.799)	2.579 (1.041-4.637)	0.128
Circ-CSMD2, median (IQR)	1.981 (0.872-3.225)	2.255 (1.476-3.134)	0.537
Circ-PTK2, median (IQR)	0.936 (0.614-1.677)	0.739 (0.585-1.845)	0.548
Circ-ANXA2, median (IQR)	2.078 (1.385-3.717)	3.779 (1.899-5.554)	0.004
Circ-FGGY, median (IQR)	0.909 (0.665-1.630)	0.895 (0.614-1.446)	0.718
Circ-PWP2, median (IQR)	0.362 (0.158-0.676)	0.385 (0.215-0.976)	0.460
Circ-RBM5, median (IQR)	0.383 (0.207-0.639)	0.499 (0.198-0.942)	0.218
Circ-ZZEF1, median (IQR)	0.304 (0.157-0.578)	0.339 (0.061-0.545)	0.546
Circ-GSK3B, median (IQR)	0.546 (0.236-0.881)	0.407 (0.117-0.834)	0.215
Circ-FOXP1, median (IQR)	0.350 (0.153-0.626)	0.334 (0.173-0.521)	0.667

Comparison of candidate circRNAs between CR and non-CR patients was determined by Wilcoxon rank sum test. CR, complete remission; AML, acute myeloid leukemia; IQR, interquartile range.

were lower in KD-Circ group than that in KD-NC group (**Figure 6C**, **6D**). In KG-1 cells, the relative cell viability under 0.02 μM ($P<0.05$), 0.04 μM ($P<0.05$), 0.08 μM ($P<0.05$), 0.16 μM ($P<0.01$), 0.32 μM ($P<0.05$) cytarabine treatment and IC_{50} value of cytarabine ($P<0.001$) were attenuated in KD-Circ group compared with KD-NC group (**Figure 6E**, **6F**). The relative cell viability under 0.04 μM ($P<0.01$), 0.08 μM ($P<0.05$) daunorbicin treatment and IC_{50} value of daunorbicin ($P<0.05$) were lower in KD-Circ group than that in KD-NC group (**Figure 6G**, **6H**).

Effect of circ-ANXA2 knockdown on regulating miR-23a-5p, miR-139-3p and miR-503-3p in THP-1 and KG-1 cells

Subsequently, we analyzed the effect of circ-ANXA2 on its potential target miRNAs (obtained by prediction using miRanda (http://miranda.org.uk/)). In THP-1 cells, miR-23a-5p ($P<0.05$) (**Figure 7A**) and miR-503-3p ($P<0.01$) (**Figure 7C**) relative expressions were upregulated, whereas miR-139-3p (**Figure 7B**) relative expression was similar in KD-Circ group compared with KD-NC group. In KG-1 cells, miR-23a-5p ($P<0.05$) (**Figure 7D**) and miR-503-3p ($P<0.001$) (**Figure 7F**) relative expressions were increased, whereas miR-139-3p (**Figure 7E**) relative expression was of no difference in KD-Circ group compared with KD-NC group.

Discussion

AML is a heterogenous malignancy with a high mortality rate in the majority of patients. In AML, the occurrence and progression of leukaemia are attributed to somatic gene mutations and non-random chromosomal translocations in leukemic stem cells [1, 17]. Due to the genetic and epigenetic heterogeneity of AML, challenges in the treatment of patients remain a major obstacle for obtaining overall optimal prognosis [18]. Therefore, the investigation of genetic biomarkers for determining the development and progression of AML is urgent. Circular RNAs, as proposed novel biomarkers, have gained wide attention due to their highly stable nature and specific expression patterns in blood and other bodily fluids [9]. In addition, the emergence and application of high-throughput biomedical technologies enable us to characterize the circRNA expression profile in several cancers [12, 14, 19, 20]. In non-small cell lung cancer, circRNAs microarray analysis showed that a total of 1377 circRNAs are differentially expressed (989 upregulated and 388 downregulated) in gefitinib effective NSCLC patients compared with the expression levels in the gefitinib ineffective NSCLC patients [12]. In esophageal squamous cell cancer, 1045 up-regulated and 1032 down-regulated circRNAs were found, compared with their expression in non-tumor tissues [19]. For haematological malignancies, one study revealed that 201 circRNAs were upregulated and 160 circRNAs were downregulated in CML patients compared to the levels in healthy individuals [14]; however, the relevant study about AML was limited. Therefore, the present study performed microarray analysis in 5 AML patients and 5 HCs, and reported that a total of 354 circRNAs were differentially expressed, among which 173 circRNAs were upregulated and 181 circRNAs were downregu-

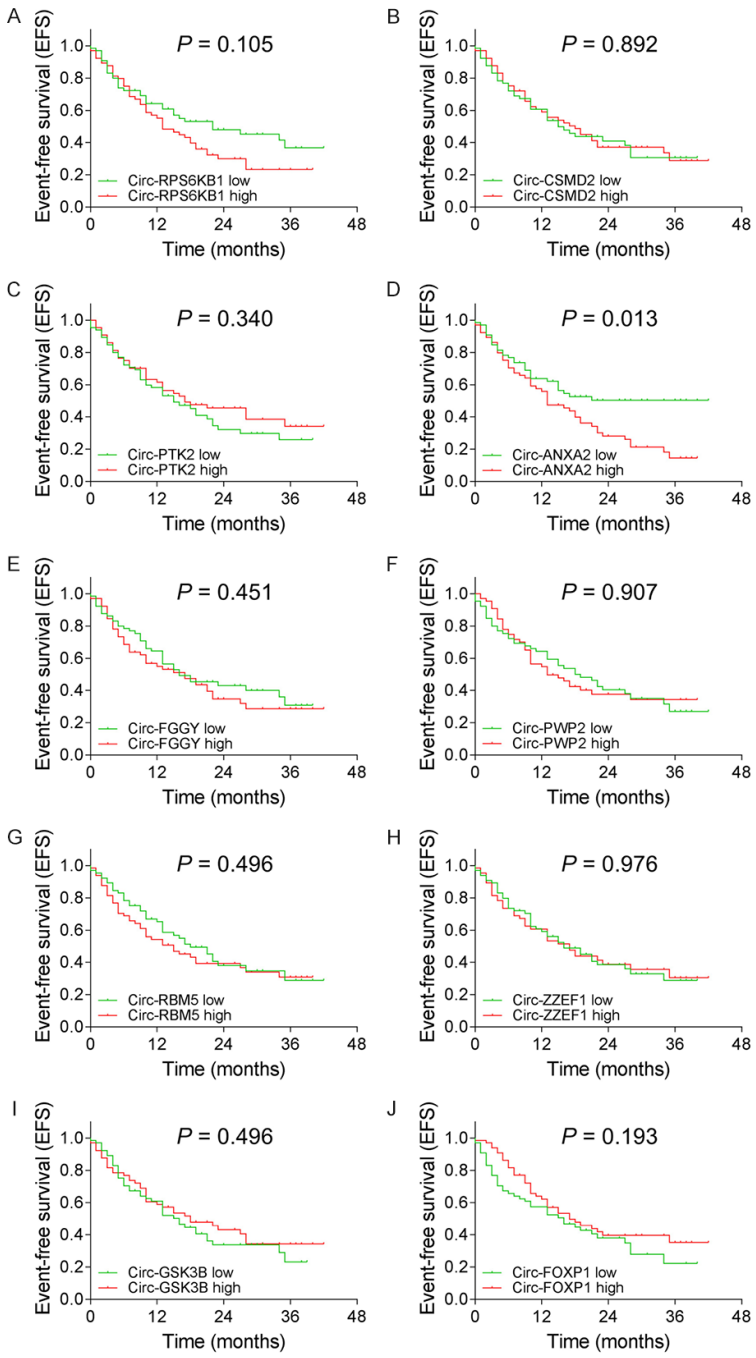

Figure 3. Association of candidate circRNAs with EFS in AML patients. The difference of EFS between circ-RPS6KB1 high expression vs circ-RPS6KB1 low expression (A), circ-CSMD2 high expression vs circ-CSMD2 low expression (B), circ-PTK2 high expression vs circ-PTK2 low expression (C), circ-ANXA2 high expression vs circ-ANXA2 low expression (D), circ-FGGY high expression vs circ-FGGY low expression (E), circ-PWP2 high expression vs circ-PWP2 low expression (F), circ-RBM5 high expression vs circ-RBM5 low expression (G), circ-ZZEF1 high expression vs circ-ZZEF1 low expression (H), circ-GSK3B high expression vs circ-GSK3B low expression (I), circ-FOXP1 high expression vs circ-FOXP1 low expression (J) AML patients. EFS, event-free survival; circRNAs, circular RNAs; AML, acute myeloid leukemia; circ, circular RNA.

lated in AML patients compared with the levels in HCs. Additionally, enrichment analyses showed that these differentially expressed circRNAs played vital roles in several biological processes (such as positive regulation of cell proliferation, cell migration and response to drug) and some AML-related signaling pathways, such as ErbB signaling pathway, EGFR tyrosine kinase inhibitor resistance and mTOR signaling pathway.

Although the advent of microarray has made the detection of circRNAs accessible in recent years, their accuracy re mains limited. In addition, the sample size for microarray (5 AML patients and 5 HCs) was relatively small. Hence, to verify the microarray data, the present study selected top 5 upregulated circRNAs and top 5 downregulated circRNAs derived from the microarray analysis to validate their clinical values by RT-PCR in 130 AML patients and 50 HCs. The present study revealed that 9 of 10 candidate circRNAs were verified to be differentially expressed, among which circRNA-RPS6KB1, circRNA-CSMD2, circRNA-PTK2 and circ-ANXA2 were increased, while circRNA-PWP2, circRNA-RBM5, circRNA-ZZEF1, circRNA-GSK38 and circRNA-FOXP1 were reduced in AML patients, compared with the levels in HCs. Subsequent ROC curve analysis disclosed that circRNA-RPS6KB1, circRNA-CSMD2, circ-ANXA2, circRNA-PWP2, circRNA-RBM5, circRNA-ZZEF1, circRNA-GSK38 and circRNA-FOXP1 could distinguish AML patients from HCs. The possible reasons could be as follows: (1) These differentially expressed

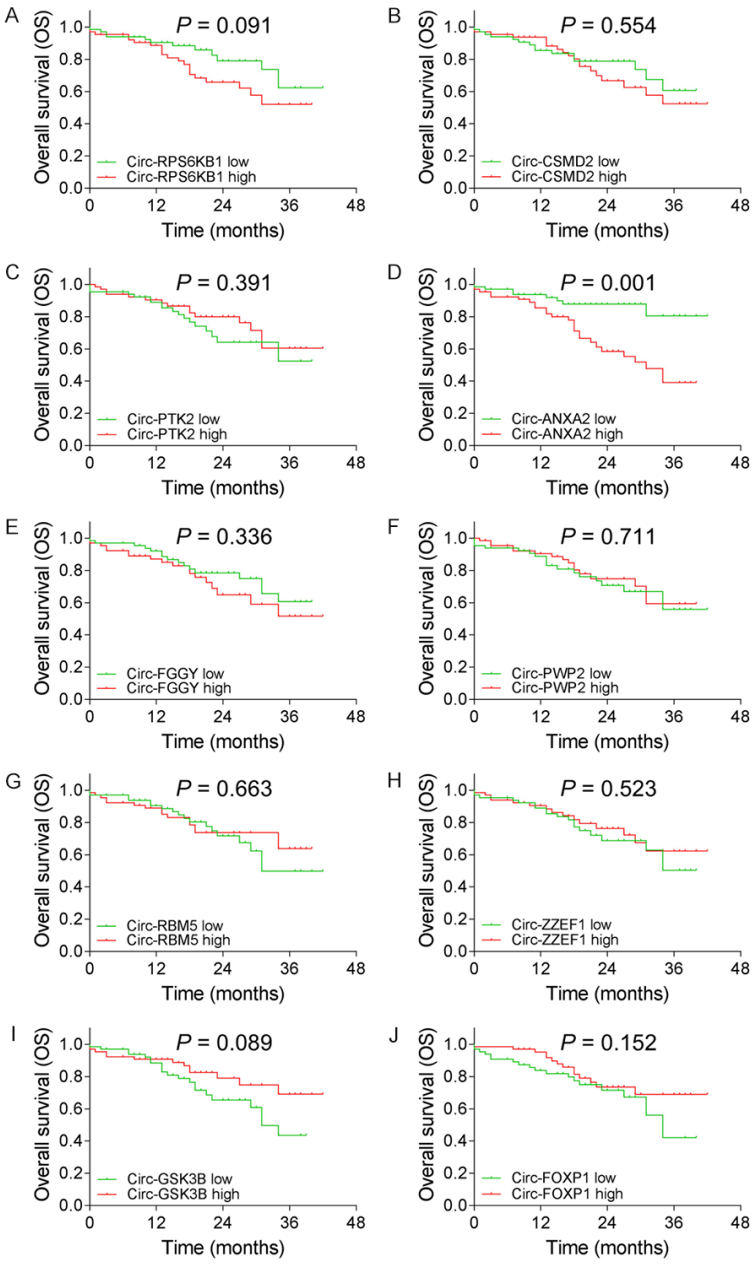

Figure 4. Association of candidate circRNAs with OS in AML patients. The difference of OS between circ-RPS6KB1 high expression vs circ-RPS6KB1 low expression (A), circ-CSMD2 high expression vs circ-CSMD2 low expression (B), circ-PTK2 high expression vs circ-PTK2 low expression (C), circ-ANXA2 high expression vs circ-ANXA2 low expression (D), circ-FGGY high expression vs circ-FGGY low expression (E), circ-PWP2 high expression vs circ-PWP2 low expression (F), circ-RBM5 high expression vs circ-RBM5 low expression (G), circ-ZZEF1 high expression vs circ-ZZEF1 low expression (H), circ-GSK3B high expression vs circ-GSK3B low expression (I), circ-FOXP1 high expression vs circ-FOXP1 low expression (J) AML patients. OS, overall survival; circRNAs, circular RNAs; AML, acute myeloid leukemia; circ, circular RNA.

circRNAs affected the transcription of their parental genes (such as oncogenes or tumor suppressor genes), which promoted/suppressed the abnormality of gene expression and the malignant transformation of haematopoietic cells, thus resulting in higher/lower AML risk [21]. For instance, circRNA-FOXP1 might promote the transcription of its parental gene FOXP1, which acts as a tumor suppressor, thus decreasing the AML risk [22, 23]. (2) These differentially expressed circRNAs regulated carcinogenesis by competitively binding to their target microRNAs [21]. For instance, circRNA-RPS6KB1 might completely bind to microRNA-127 and inhibit the anti-oncogenic function of microRNA-127 on abnormal proliferation of myeloid progenitor cells, thereby promoting neoplastic progression in AML (retrieved from tissue specific circRNA database: http://gb.whu.edu.cn/TSCD/) [24].

Specific circRNAs have been reported to exhibit prognostic potential in haematological malignancies [25, 26]. For instance, one study showed that circHIPK3 was elevated in peripheral blood mononuclear cells and serum samples from patients with chronic myeloid leukemia (CML) compared to the level in the samples from healthy individuals and its high expression could be used to predict shorter OS in CML patients [25]. Another study showed that chronic lymphocytic leukemia patients with circ-CBFB high expression had lower OS compared to chronic lymphocytic leukemia patients with low circ-CBFB expression [26]. However, the prognostic value of specific circRNAs in AML has been rarely reported. The present study revealed that, among these candidate cir-

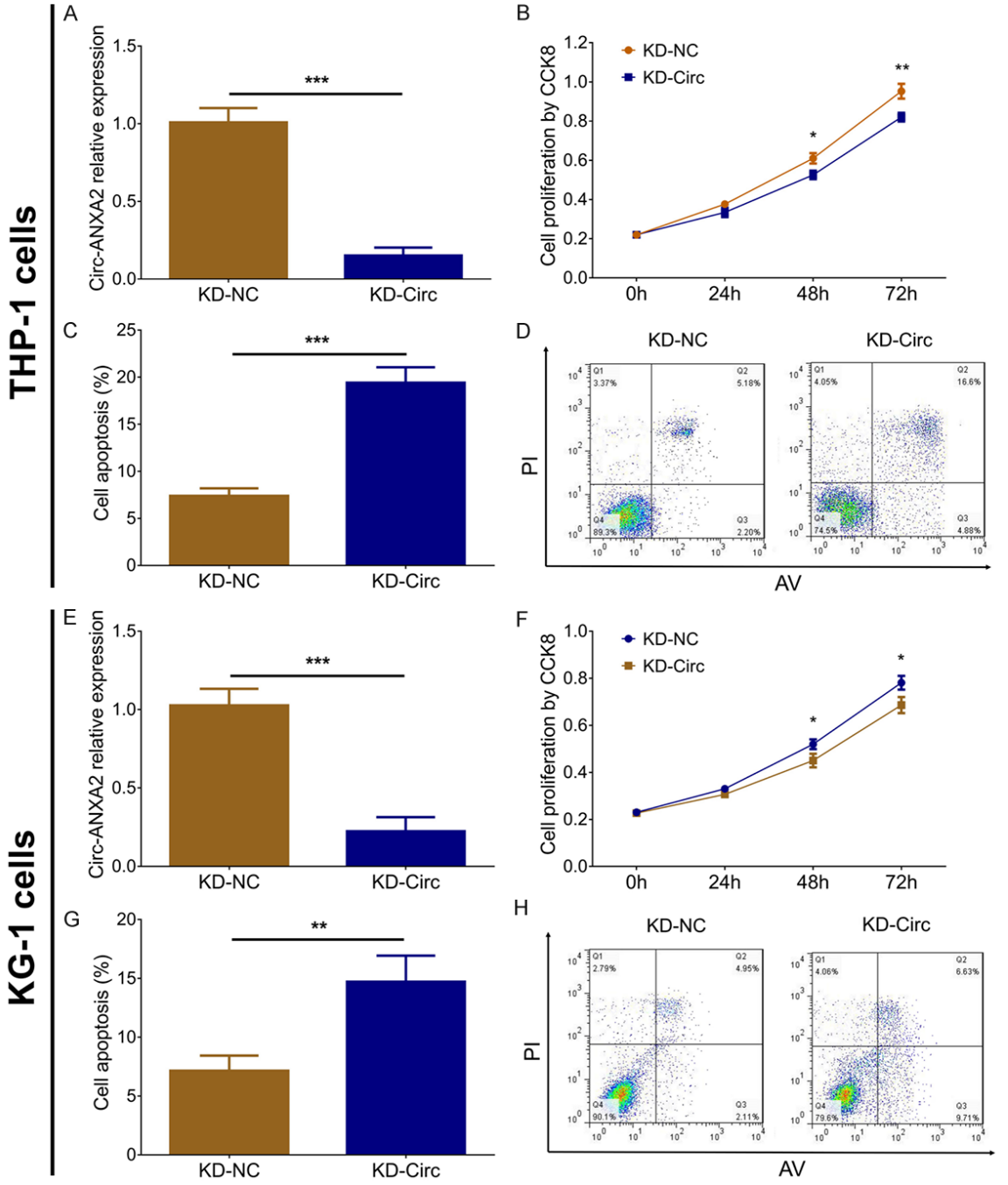

Figure 5. Circ-ANXA2 knockdown affected cell proliferation and apoptosis in THP-1 and KG-1 cells. The circ-ANXA2 relative expression after transfections (A), the effect of circ-ANXA2 knockdown on cell proliferation (B) and apoptosis (C, D) in THP-1 cells. The circ-ANXA2 relative expression after transfections (E), the effect of circ-ANXA2 knockdown on cell proliferation (F) and apoptosis (G, H) in KG-1 cells. Circ, circular RNA.

cRNAs, circ-ANXA2 was associated with poor risk stratification, decreased CR, shorter EFS and OS in AML patients. We proposed several explanations: (1) Circ-ANXA2 might enhance the transcription of its parental gene ANXA2, which stimulates fibrinolysis and tumor cell invasion and suppressed the sensitivity of tumor cells to chemotherapy, manifesting a disease state and attenuating survival in AML patients [27, 28]. (2) Our subsequent experi-

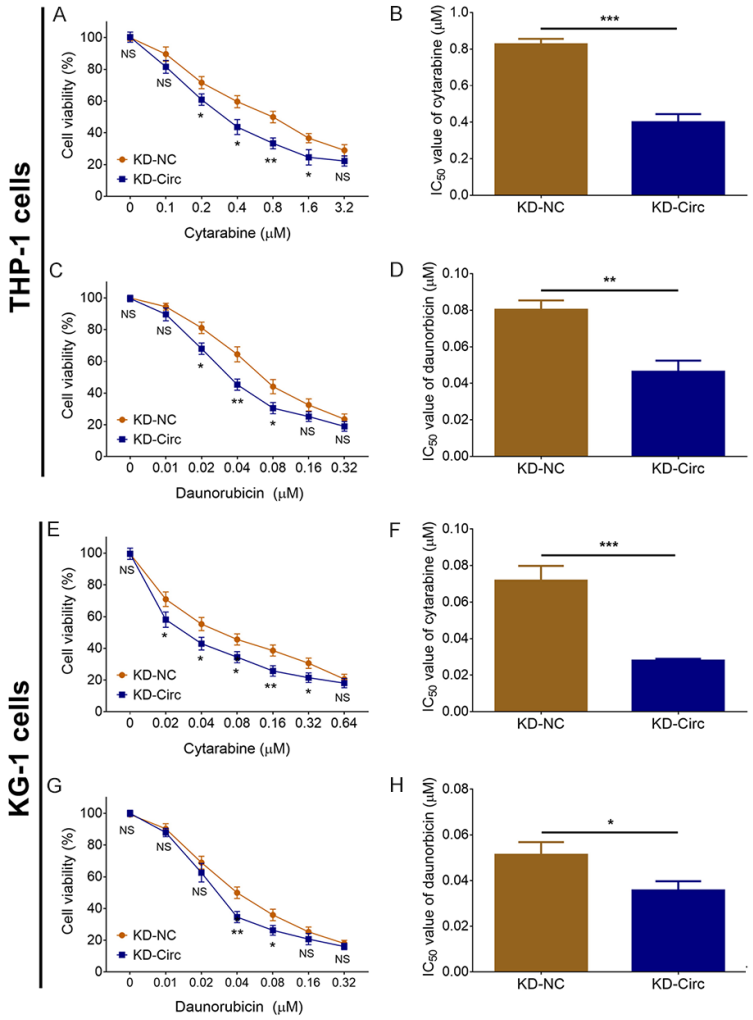

Figure 6. Circ-ANXA2 knockdown affected chemosensitivity to cytarabine and daunorubicin in THP-1 and KG-1 cells. The effect of circ-ANXA2 knockdown on relative cell viability under different concentration of cytarabine treatment (A) and IC_{50} value of cytarabine (B) in THP-1 cells. The effect of circ-ANXA2 knockdown on relative cell viability under different concentration of daunorubicin treatment (C) and IC_{50} value of daunorubicin (D) in THP-1 cells. The effect of circ-ANXA2 knockdown on relative cell viability under different concentration of cytarabine treatment (E) and IC_{50} value of cytarabine (F) in KG-1 cells. The effect of circ-ANXA2 knockdown on relative cell viability under different concentration of daunorubicin treatment (G) and IC_{50} value of daunorubicin (H) in KG-1 cells. Circ, circular RNA; IC_{50}, the half maximal inhibitory concentration.

ments observed that circ-ANXA2 negatively regulated miR-23a-5p and miR-503-3p, which are known to suppress the proliferation and invasion of tumor cells and induce the apoptosis of tumor cells; therefore, its high expression correlated with advanced disease stage and unsatisfactory prognosis in AML patients [29, 30]. (3) Our subsequent experiments illuminated that circ-ANXA2 facilitated the cell proliferation and inhibited the cell apoptosis and the chemosensitivity to cytarabine and daunorubicin; therefore, its high expression was associated with poor prognosis in AML patients.

Based on our precious clinical results, circ-ANXA2 not only exhibited a good predictive value for AML risk but also correlated with poor risk stratification, attenuated CR, reduced EFS and OS in AML patients. In addition, the identification of circ-ANXA2 and ANXA2 genes have indicated that they participate in the progression and drug resistance in leukaemia [28, 31, 32], Thus, the present study compared the circ-ANXA2 expression in the control cells and AML cells and detected the effect of circ-ANXA2 knockdown on regulating cellular functions of THP-1 and KG-1 cells, which illustrated that circ-ANXA2 was upregulated in AML cells compared with BMMCs of HCs, and circ-ANXA2 knockdown suppressed the proliferation and enhanced the apoptosis of the AML cells while increasing their chemosensitivity to cytarabine and daunorbincin and the relative miR-23a-5p and miR-503-3p expression. The possible explanation was as follows: circ-ANXA2 might function as a microRNA (miRNA) sponge to dampen the activity of miRNAs such as MiR-23a-5p and miR-503-3p, which are known to increase chemosensitivity and reduce tumor growth by inhibiting cancer stem cells proliferation and self-renewal [33, 34]. Thereby, circ-ANXA2 knockdown inhibited cell proliferation and promoted cell apoptosis and chemosensitivity in AML samples. These observations were supported by previously obtained evidence that the located gene of circ-ANXA2 (ANXA2) have implications in the leukaemogenesis of haematological malignancies [28, 35, 36]. For instance, one study observed that the upregulation of

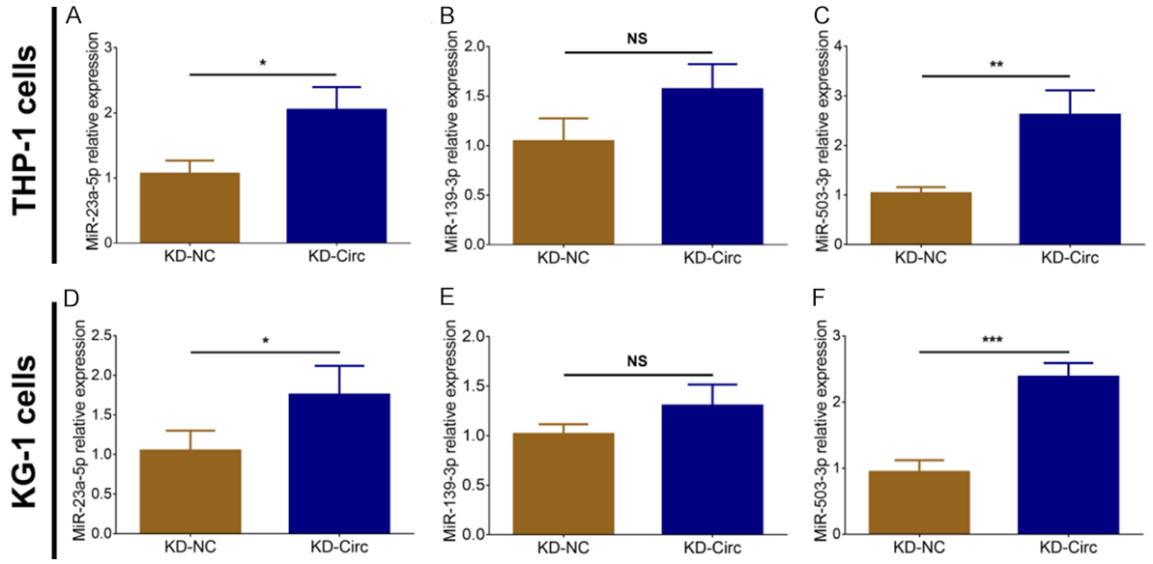

Figure 7. Circ-ANXA2 knockdown affected the potential target miRNAs expressions in THP-1 and KG-1 cells. The effect of circ-ANXA2 knockdown on regulating miR-23a-5p (A), miR-139-3p (B) and miR-503-3p (C) in THP-1 cells. The effect of circ-ANXA2 knockdown on regulating miR-23a-5p (D), miR-139-3p (E) and miR-503-3p (F) in KG-1 cells. Circ, circular RNA; miRNAs, micro RNAs; miR, micro RNA.

annexin A2-S100A10 heterotetramer stimulated fibrinolysis and cell invasion via mediating the overproduction of plasmin in AML [28]. Another study showed that downregulated ANXA2 impaired the generation of tissue plasminogen activator and subsequent hyperfibrinolysis in acute promyelocytic leukemia cells [35]. Additionally, ANXA2 inhibitor facilitated the sensitivity of ALL cells to chemotherapy, such as dexamethasone and vincristine, by disrupting the interaction between ALL cells and osteoblasts [36].

In conclusion, the present study provides a landscape of differentially expressed circRNAs in AML and validates that circ-ANXA2 holds clinical significance as a biomarker for predicting development, progression and prognosis of AML. In addition, cellular experiments illustrate that the inhibition of circ-ANXA2 suppresses cell proliferation, facilitates cell apoptosis, chemosensitivity to cytarabine and daunorubicin, miR-23a-5p and miR-503-3p expressions in AML. These findings might potentially help in the application of personalized treatment and prognosis to improve the outcomes for AML patients.

Disclosure of conflict of interest

None.

Address correspondence to: Aibin Liang and Wenjun Zhang, Department of Hematology, Tongji Hospital, Tongji University School of Medicine, 389 Xincun Road, Shanghai 200065, P.R. China. Tel: +86-18601670600; E-mail: lab7182@tongji.edu.cn (ABL); zhangwenjun@tongji.edu.cn (WJZ)

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Table S1. Primers list

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
Circ-RPS6KB1	GAAGAACTTCTGGCTCGAAAGGT	CTTGAACTTCTCCAGCGTCCC
Circ-CSMD2	GGCTCCTCTTCCAGACTGATG	GGTTGAGTGCTGTGATGATCCA
Circ-PTK2	AGAGGAGGTTCACTGGCTTCA	TGGTTGGCTCACTATTGCTTTCA
Circ-ANXA2	ACCTGCTCAGTATGACGCTTCT	CTGGTAGGCGAAGGCAATATCC
Circ-FGGY	ATCAATGAGACCAAGCACAGTGT	GCAGTTCCTCCACCTCTTTCAG
Circ-PWP2	GCCAGCATGTAGACCTCAGT	AAATGCCAAGGACACTTTCCC
Circ-RBM5	GCAGAACAGAGGCTTCGCATT	TCGTATCACAGTAGTAATCCACAGA
Circ-ZZEF1	TCACCGAGCAGGAGCACAA	CCTCAGAAGCAGCCAGTACATC
Circ-GSK3B	GCTCGGATTCAAGCAGCT	AGGAGGAATAAGGATGGTAGCC
Circ-FOXP1	ACATGCCTCTACCAATGGACAG	TGCTGCTGCTGGAGGAGAA
MiR-23a-5p	ACACTCCAGCTGGGGGTTCCTGGGGATGGG	TGTCGTGGAGTCGGCAATTC
MiR-139-3p	ACACTCCAGCTGGGTGGAGACGCGGCCCTG	TGTCGTGGAGTCGGCAATTC
MiR-503-3p	ACACTCCAGCTGGGGGGGTATTGTTTCCGC	TGTCGTGGAGTCGGCAATTC
U6	CGCTTCGGCAGCACATATACTA	ATGGAACGCTTCACGAATTTGC
GAPDH	GACCACAGTCCATGCCATCAC	ACGCCTGCTTCACCACCTT

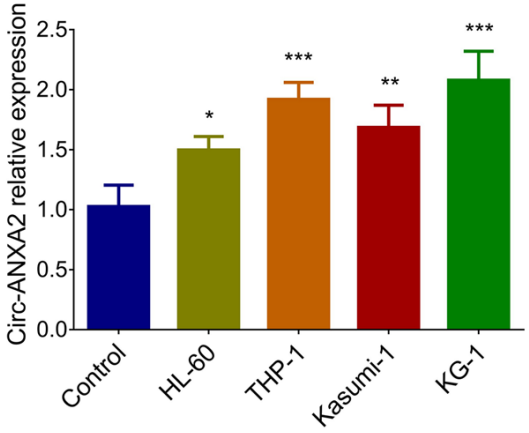

Figure S1. Circ-ANXA2 in control, HL-60, THP-1, Kasumi-1 and KG-1 cells. Comparison of circ-ANXA2 relative expression between BMMCs of HCs (control) and AML cells (HL-60, THP-1, Kasumi-1 and KG-1 cells). Circ, circular RNA; BMMCs, bone marrow mononuclear cells; HCs, health-donor controls; AML, acute myeloid leukemia.