Using circ-ANAPC7 as a novel type of biomarker in the monitoring of acute myeloid leukemia

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Research

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Abstract

Background

Circular RNAs (circRNAs) that occupy gene expression at the transcriptional or post-transcriptional level have great potential to be biomarker for types of cancers. We have screened one altered circRNA named circ-ANAPC7 in acute myeloid leukemia (AML) before. In this study, we aimed to validate its expression by enlarging sample size and illuminating the diagnostic and monitoring value of circ-ANAPC7 in AML.

Methods

Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was supposed to confirm the expression of circ-ANAPC7 of AML patients. We assessed the correlation of circ-ANAPC7 and clinical variables using Spearman correlation test. Receiver operating characteristic (ROC) curve was carried out to evaluate the diagnostic value.

Results

Circ-ANAPC7 was first found to be upregulated in AML, and its expression was correlated to WBC counts and blast percentage in bone marrow. ROC curve analysis revealed that circ-ANAPC7 has significant value of AML diagnosis (AUC = 0.915, $P < 0.001$). Furthermore, the expression level of circ-ANAPC7 was changed accompanied with disease condition transformation.

Conclusions

Circ-ANAPC7 could be used as a biomarker to monitor disease condition of AML.

Background

Acute myeloid leukemia (AML) is a heterogeneous disease characterized by the clonal proliferation of immature myeloid progenitor cells in the bone marrow, compressing normal blood cell production and leading to bone marrow failure ultimately(1, 2). Although the overall survival (OS) rate of patients with AML has improved due to mature chemotherapy and novel multimodality therapy, such as FLT3-specific tyrosine kinase inhibitors (3), Bcl-2 family inhibitors(4), as well as hematopoietic stem cell transplantation(5), a number of patients with AML are still showed no response (NR) to the present therapy, or relapsed from transient complete remission (CR).

According to National Comprehensive Cancer Network (NCCN) guideline, AML patients were divided to three risk status by monosomal karyotype, poor-risk cytogenetic and molecular abnormalities. High-risk AML responds poorly to available induction therapy and is likely to relapse despite consolidation treatment(6, 7), and most patients with AML die from progressive disease after relapse. Therefore, searching for ideal biomarkers is essential to predict early relapse of AML. Hundreds of somatic mutations were validated using

deep sequencing in relapsed AML several years ago(8). Recently, researchers have focused on searching for new prognostic biomarkers on noncoding RNA (ncRNA), including circular RNA.

Circular RNAs (circRNAs) are a class of RNAs shaping a covalently closed continuous loop which have no 5'-3' polarity or polyA tail, which regulate gene expression at the transcriptional or post-transcriptional level by interacting with microRNAs (miRNAs) or other molecules(9). Increasing researches indicate that circRNAs may participate in the development of many types of diseases (10–12), especially in cancers, including pancreatic ductal adenocarcinoma (13), gastric cancer (14), lung cancer (15) and AML (16, 17).

In previous study, we have conducted a microarray screening of circRNA changes in bone marrow mononuclear cells from 5 AML patients and 5 IDA controls, and confirmed the selected circRNA named circ-ANAPC7 (ID: hsa_circRNA_101141 in CircBase: <http://circbase.org/cgi-bin/simplesearch.cgi>) up-expression in 87 AML patients. Circ-ANAPC7 is located at chr12:110819556–110834257, and its associated-gene symbol is anaphase promoting complex subunit 7 (ANAPC7). Here, we analyze the relationship between expression level of circ-ANAPC7 and clinical features of AML by enlarging the sample size, trying to demonstrate its value in the pathogenesis and monitoring of AML.

Methods

Patients and clinical specimens

Approval for this study was obtained from the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University (Approval number: 2015186). After obtaining written informed consent from all patients, we collected bone marrow specimens from 144 newly diagnosed AML patients and 80 iron deficiency anemia (IDA) control patients at the Department of Hematology, Second Affiliated Hospital of Xian Jiaotong University, between 2015 and 2018. The diagnosis, stage and risk status of AML were made in accordance with the National Comprehensive Cancer Network (NCCN) (2018 version 1).

Total RNA extraction

Total RNA was collected from bone marrow samples of AML and IDA using TRIzol reagent (Invitrogen, Germany), according to manufacturer's instructions, and stored at -80°C until use. RNA purity and concentration were detected by NanoDrop ND-1000 (Thermo Fisher Scientific, Wilmington, DE).

Reverse transcription

RNA samples were reversely transcribed into cDNA using a Primescript RT master mix with random primers in accordance with manufacturer's protocols (Takara).

qRT-PCR detection of circ-ANAPC7

The real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed by SYBR Premix Ex Taq™ II (Tli RNaseH Plus) (Takara) and StepOne Software v2.1, according to manufacturer's instructions. The reaction conditions were as follows: 95 °C for 30 s, 40 cycles of 95 °C for 5 s and 60 °C for 30 s. Primers of detected circRNA was designed and synthesized by Invitrogen (Shanghai, China). The

sequences of β -actin and circ-ANAPC7 primers were as follows: 5'- GTGGCCGAGGACTTTGATTG – 3' (sense) and 5'-CCTGTAACAACGCATCTCATATT – 3'(antisense) for β -actin; 5'- GGGAGCAGCACTTAGGAACAT – 3' (sense) and 5'-AAAGCTGGTACTTCTGAGGTGG-3' (antisense) for circ-ANAPC7. β -actin was measured as an internal control for each sample. All of the quantitative PCR reactions were conducted in triplicate. $2^{-\Delta Ct}$ was utilized to reflect the expression level of circRNA. All results are expressed as the mean $\pm$ SD of three independent experiments.

Statistical analysis

Variable was compared between two groups using the Mann-Whitney Wilcoxon rank test. A receiver operating characteristic (ROC) curve was set up to evaluate the diagnostic value of circ-ANAPC7. Spearman rank correlation coefficient was used to analyze the correlation between clinical variables and circANAPC7 expression of AML patients. Overall survival rate and event-free survival rate were estimated by the Kplan-Meier analysis and compared using the log-rank test. Prognostic factors for survival were identified by Cox regression analysis. All tests were two-sided, and $P < 0.05$ was defined as a significant difference. All statistical analyses were performed with Statistical Product and Service Solutions (SPSS) Statistics for Windows, Version 18.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA).

Result

Circ-ANAPC7 expression was upregulated in AML

The expression level of circ-ANAPC7 was detected in 144 newly diagnosed AML, 123 complete remission (CR) AML and 80 IDA patients. Baseline characteristics of 144 newly diagnosed AML patients we detected are presented in Table 1. As shown in Fig. 1, its expression levels in newly diagnosed AML were significantly higher than those CR patients and IDA ($P < 0.001$). Furthermore, there was no significant difference in the expression of circ-ANAPC7 between CR and IDA patients.

Table 1
Clinical characters and laboratory measures of de nove AML patients.

Characteristics	Number (n = 144)
Gender, no (%)	71 (49.31)
Male	73 (50.69)
Female	
Age, mean (range)	50 (15–85)
FAB type, no (%)	
M1	3 (2.08)
M2	22 (15.23)
M3	9 (6.25)
M4	46 (31.94)
M5	58 (40.28)
M6	4 (2.28)
M7	2 (1.39)
Risk stage, no (%)	
Low	21 (14.58)
Intermediate	75 (52.08)
High	48 (33.34)
Refractory, no (%)	
Yes	61 (42.36)
No	83 (57.64)
Percentage of blasts in BM (%), mean (range)	66.45 (23.50-97.38)
WBC count ($\times 10^9/L$), mean (range)	40.65 (0.55-153.49)
SD, standard deviation; FAB, French–American–British classification system; BM, bone marrow; WBC, white blood cell.	

Potential diagnostic value of circ-ANAPC7 in AML

To evaluate the potential diagnostic value of circ-ANAPC7, we conducted a receiver operating characteristic (ROC) curve analysis (Fig. 2). The area under the curve was 0.915 (95% CI 0.865–0.966, $P < 0.001$), which means the expression level of circ-ANAPC7 in bone marrow of AML could separate the patients with AML from the healthy controls.

Spearman correlation analysis of clinical variables and circ-ANAPC7 expression in AML patients

We performed Spearman correlation analysis to evaluate the correlation between clinical characteristics of AML and the expression level of circ-ANAPC7, to estimate whether it's significant and differential expression in AML was relevant biomarkers for the diagnosis or monitor of AML.

As shown in Table 2, the expression level of circANAPC7 was correlated to white blood cell (WBC) count of AML patients ($r = 0.545$, $P < 0.01$) and percentage of blasts in bone marrow (($r = 0.470$, $P < 0.05$). Furthermore, WBC count was correlated with risk statues ($r = 0.242$, $P < 0.05$) and primitive cells in bone marrow of AML ($r = 0.338$, $P < 0.05$), while the percentage of blasts in bone marrow has a positive relationship with refractoriness ($r = 0.240$, $P < 0.05$).

Table 2

Spearman rank correlation coefficients of clinical variables and expression of circANAPC7 in AML patients.

	gender	age	FAB type	Risk statue	WBC	HB	PLT	Primitive cells in BM	Refracriness
Circ-ANAPC7	0.051	-0.023	0.016	-0.031	0.545**	-0.091	-0.191	0.470*	-0.092
gender	-	-0.014	0.101	0.020	-0.062	-0.133	0.176	-0.160	-0.137
age		-	0.052	0.060	0.041	0.019	0.151	0.170	0.032
FAB type			-	0.192*	-101	0.226	0.258*	-0.046	0.109
Risk statue				-	0.242*	-0.063	0.221*	0.102	0.173
WBC					-	-0.021	-0.141	0.338*	0.178
HB						-	0.062	-0.052	-0.097
PLT							-	-0.061	-0.134
Primitive cells in BM								-	0.240*
* $P < 0.05$; ** $P < 0.01$									

Potential ability of monitoring disease condition of AML

We chose 24 AML patients who undergo the condition of newly diagnosed, complete remission (CR) and relapse of dynamical monitor the expression of circ-ANAPC7. We discover that the expression level of circ-ANAPC7 changed accompanied with the disease condition transformation. It was overexpressed in newly diagnosed and relapsed AML patients. When patients got CR, the expression level of circ-ANAPC7 decreased ($P < 0.05$) (Fig. 3). In the continuous complete remission patients, the expression level of circ-ANAPC7 was at a minimal level always.

Long-term effect of the expression of circ-ANAPC7 in AML

By multivariate analysis, WBC count $\geq 10 \times 10^9/L$, blasts percentages in the bone marrow and refractory AML were significantly associated with inferior survival (Table 3).

Table 3
Prognostic factors for survival identified by Cox regression analysis in AML

Risk factors	Pvalue	HR	95% CI
WBC count	0.011	1.026	[1.006, 1.049]
Blast percentage in BM	0.019	1.341	[1.149, 1.570]
Refractory AML	0.015	5.289	[1.289, 11.065]
HR, hazard ratio; CI, confidence interval; WBC, white blood cell; BM, bone marrow			

To explore long-term effect of circ-ANAPC7 expression in AML patients, we conducted a survival analysis. As shown in Fig. 4, we found that the expression level of circ-ANAPC7 was not related to overall survival (OS) and disease-free survival (DFS) of AML patients ($P > 0.05$).

Discussion

Recently, circRNA, a large family of noncoding RNAs (ncRNAs), received extensive attention of researchers along with the development of transcriptome sequencing technologies. CircRNAs are alternative transcripts from exonic backsplicing of coding genes (exonic circRNAs) in mammalian cells, with abundant, stable, evolutionarily conserved and cell-type specific characteristics (18). Some circRNAs called miRNA sponges have miRNA response elements (MREs) and display important miRNA activities, enhancing the complexity of RNA regulatory networks and being involved in gene expression(19, 20).

A growing number of studies highlight the diagnostic and therapeutic potential of circRNAs in many types of cancers. CircRNA_100269 was downregulated in gastric cancer (GC) and suppresses tumor cell growth by targeting miR-630, which comprised a novel pathway that regulates proliferation of GC cells (21). CircRNA_100782 regulated the proliferation of pancreatic carcinoma by acting as miR-124 sponge through the IL6-STAT3 pathway (22). In non-small cell lung cancer, hsa_circ_0007385 knockdown resulted in significant suppression of the proliferation, migration and invasion of NSCLC cells(23). Microarray profiling and bioinformatics analyses were also performed in esophageal cancer (24) and cardiac fibroblasts(25).

Furthermore, the expression profile of circRNAs in AML patients has been done in one study, and a large number of circRNAs possibly expressed in a leukemia specific manner are identified, especially hsa_circ_0004277, which was down-expressed in AML patients compared with healthy controls (17). Another study showed that hsa_circ_0075001 expression correlates positively with total NPM1 expression in AML, but is independent of the NPM1 mutational status (16).

In our previous investigation, we also demonstrated a different circRNAs expression profiling in AML patients, and one circRNA named circ-ANAPC7 was significantly upregulated in AML patients in our preliminary validation(26). As for this study, we enlarge the number of clinical samples to verify the result before and illuminate the relationship between expression level of circ-ANAPC7 and the clinical features of AML patients.

The results showed circ-ANAPC7 was significantly upregulated in AML patients, which was in line with the result before. Furthermore, we found that the expression level of circ-ANAPC7 was related to the count of WBC, but not the FAB type, risk statue, percentage of blasts in bone marrow, indicating that the altered expression level of circ-ANAPC7 may be related to the pathogenesis of AML, but not the types or risk statues of AML. Survival analysis predicted the expression level of circ-ANAPC7 had no influence on the OS and DFS, that is, it could not be used as a prognostic factor in AML.

The count of WBC, blast percentage in the bone marrow and refractory AML was the independent factors for survival of AML patients, which were consistent with the previous study(27, 28). ROC analysis stated that circ-ANAPC7 have greatly potential diagnostic value for AML. Ambulatory monitoring of expression of circ-ANAPC7 in matched-pair AML samples showed it changed accompanied with the disease condition transformation. Therefore, we speculate that the expression level of circ-ANAPC7 might be used as a predictive index for supervising early recurrence, advising inchoate treatment to prolong OS.

According to bioinformatics analysis in our previous research, we have predicted that circ-ANAPC7 may function as a sponge to adsorb miR-181 family(26). MiR-181 has been proved upregulated in AML, which was related to longer OS of AML patients(29, 30). Furthermore, elevated miR-181 expression was associated with increased survival in 395 American patients, and reduced survival in 325 Chinese one, which indicated that miR-181 can be used as a promising prognostic biomarker in AML patients, depending on the origin of patient population.(31) In a subsequent study, we will devote ourselves to dig out the mechanism between circ-ANAPC7 and miR-181.

However, there is still some limitation in our study. First, the sample size of our study is still relatively small, and these data should be confirmed in large-scale studies and populations with different races and regions. Second, we do not have data in circ-ANAPC7 expression level of plasma or serum from AML patients, which is our next research plan.

Conclusions

In summary, we validated that circ-ANAPC7 was upregulated in AML patients. The clinical analysis revealed that circ-ANAPC7 may be a predictive index for diagnosing and supervising early recurrence of AML. What's more, additional molecular mechanisms and biological functions of circ-ANAPC7 merit deeper investigation.

Declarations

Ethics approval and consent to participate

Approval for this study was obtained from the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University (Approval number: 2015186).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Ying Shen performed the qRT-PCR examination and wrote the manuscript. Yachun Jia, Ru Zhang and Hongli Chen FC analyzed and interpreted the patient data of AML patients. Ting Wang performed the qRT-PCR examination. Aili He and Yun Yang contributed to the concept of the study. All authors read and approved the final manuscript.

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Figures

Figure 1

The expression of circ-ANAPC7 in newly diagnosed acute myeloid leukemia (AML), complete remission (CR) patients of AML and iron deficiency anemia (IDA) patients. *** $P < 0.001$

Figure 2

Receiver operating characteristic (ROC) curve of circ-ANAPC7.

Figure 3

The expression level of circ-ANAPC7 was measured in matched-pair samples acquired from 18 available follow-up AML patients at the time when they were at ND, CR and R stage. ND, newly diagnosed; CR, complete remission; R, relapsed.

Figure 4

Overall survival (OS) and Disease-free survival (DFS) of AML patients.