Blood Cells, Molecules and Diseases 75 (2019) 41–47

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

Silencing of circ_0009910 inhibits acute myeloid leukemia cell growth through increasing miR-20a-5p

Lei Ping, Chen Jian-Jun, Liao Chu-Shu, Liu Guang-Hua, Zhou Ming*

Laboratory of Hematology, Hunan Provincial People's Hospital, The First-Affiliated Hospital of Hunan Normal University, Changsha 410002, Hunan Province, China

ARTICLE INFO

Editor: Mohandas Narla

Keywords:
Acute myeloid leukemia
CircRNA
MiR-20a-5p
Therapeutic target
Prognosis

ABSTRACT

Acute myeloid leukemia (AML) is the most common acute leukemia in adults, which is aggressive cancer. CircRNAs are abundantly expressed in the hematologic malignancy cells. In this study, we aimed to investigate the expression profiling of circRNAs in AML. We performed circRNA-sequencing to identify differentially expressed circRNAs in bone marrow samples from AML patients and iron-deficiency anemia (control). Furthermore, we found that circ_0009910 was significantly upregulated in AML patients compared with iron-deficiency anemia patients. High circ_0009910 expression predicted a poor risk and outcome of AML patients. Further experiments in vitro and in vivo demonstrated that knockdown of circ_0009910 inhibited AML cell proliferation and induced apoptosis through sponging miR-20a-5p. Our findings firstly identify that circ_0009910 is significantly upregulated in AML bone marrow samples and might serve as a novel outcome biomarker for AML. Both circ_0009910 and miR-20a-5p may be potential therapeutic targets for future AML treatment.

1. Introduction

Acute myeloid leukemia (AML) is the most common acute leukemia in adults, which is aggressive cancer [1]. AML is characterized by high heterogeneity and rapid proliferation of immature myeloid cells, resulting interference of normal blood cells production [2]. Recent studies of AML on the genetic characterization has revealed that AML is often associated with dysregulated non-coding RNA [3,4]. Thousands of unannotated coding and noncoding transcripts, including microRNAs, long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs) by high-throughput sequencing technologies. Aberrant expression of miRNAs and lncRNAs has been shown to be associated with many human cancers, including AML [5]. More recently, circRNAs have been identified as biomarkers for acute myeloid leukemia patient prognosis [6].

CircRNAs are initially considered as by-products of mis-spliced RNAs and drew little attention in the past. However, recent investigations find that circRNAs play critical role in gene expression and participate in the pathogenesis of cancer [7]. By circRNA microarray, a large number of circRNAs have been identified possibly expressed in a leukemia specific manner [8]. CircRNAs are abundantly expressed in the hematologic malignancy cells [9]. For example, circRNA-DLEU2 is up-regulated in AML tissues and cells. It promotes AML tumor formation in vivo by suppressing miR-496 and promoting PRKACB expression [10].

Given the general deregulation in splicing mechanisms in AML, altered circRNAs could contribute to leukemogenesis. In this study, we aimed to investigate the expression profiling of circRNAs in AML. We performed circRNA-sequencing to identify differentially expressed circRNAs in bone marrow samples from 3 AML patients and 3 iron-deficiency anemia (IDA) control (control group). Furthermore, we found that circ_0009910 (hsa_circRNA_100053, circBase) was significantly upregulated in AML patients compared with IDA. High circ_0009910 expression predicted a poor outcome of AML patients. Further experiments in vitro and in vivo demonstrated that knockdown of circ_0009910 inhibited AML cell proliferation and induced apoptosis through increasing miR-20a-5p. Our findings provide a novel therapeutic target for AML treatment.

2. Materials and methods

2.1. Patients and samples

Seventy patients with AML and age matched 70 patients diagnosed with IDA as a control group were enrolled in this study from Hunan Provincial People's Hospital, Hunan Normal University. The medical records of AML patients with clinical staging and survival information

* Corresponding author at: Department of Hematology, Hunan Provincial People's Hospital, The First-Affiliated Hospital of Hunan Normal University, Jiefang West Road 61, Changsha 410002, Hunan Province, China.
E-mail address: hnchouming@163.com (Z. Ming).

https://doi.org/10.1016/j.bcmd.2018.12.006
Received 14 November 2018; Accepted 25 December 2018
Available online 28 December 2018
1079-9796/

Table 1
Clinical information of human samples used in microarray.

Samples	Gender	Age	Diagnosis of FAB subtypes	FLT-ITD3	NPM1	Karyotype	Risk status
1	M	53	M3	+	+	Normal	Poor-risk
2	M	46	M4	+	−	Normal	Poor-risk
3	F	32	M5	−	+	Normal	Poor-risk
4	F	41	IDA	N/A	N/A	N/A	N/A
5	M	58	IDA	N/A	N/A	N/A	N/A
6	M	29	IDA	N/A	N/A	N/A	N/A

M, male; F, female; FAB, French-American-British classification; FLT-ITD3, FMS-like tyrosine kinase-3; NPM1, Nucleophosmin 1; N/A, not applicable.

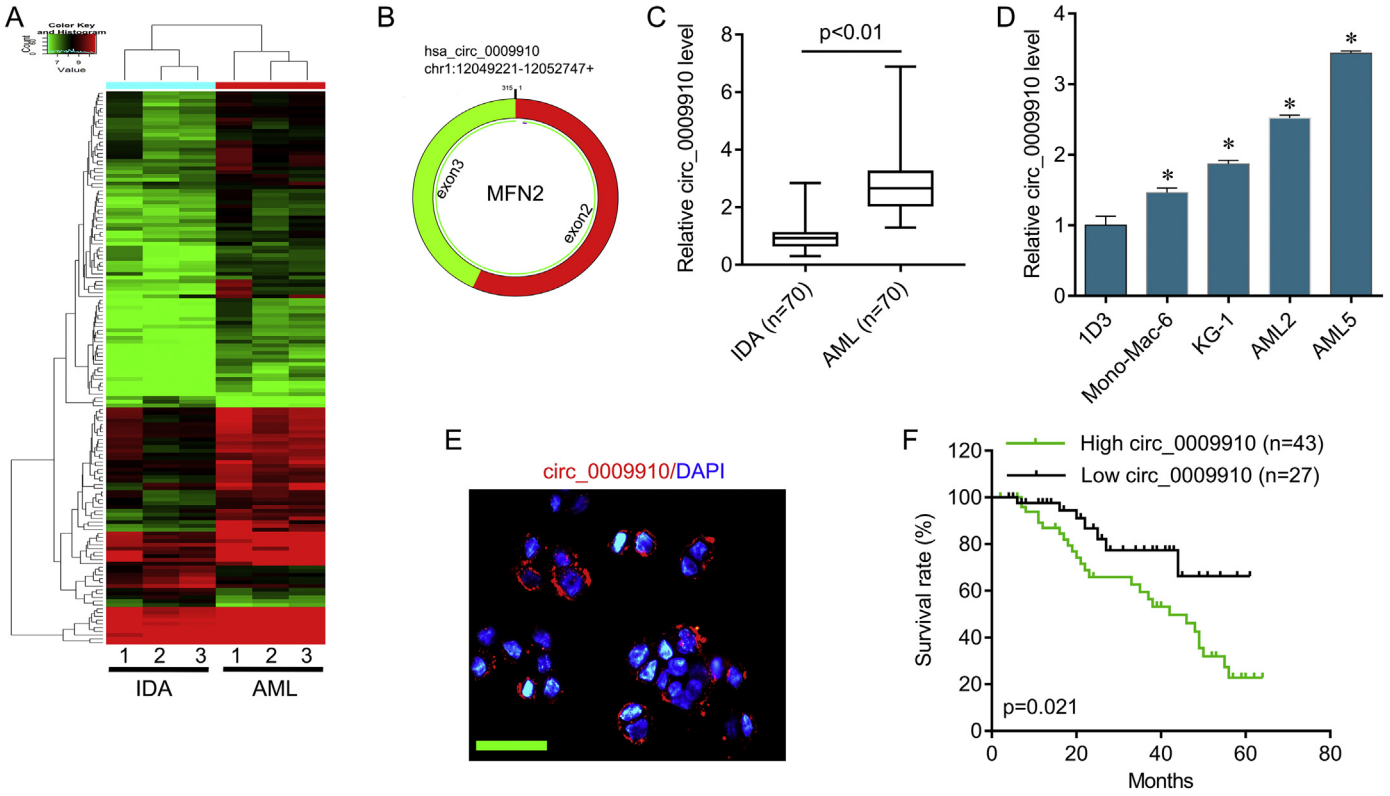

Fig. 1. The circRNAs expression in AML. (A) Heatmap for differentially expression circRNAs in bone marrow samples from AML patients and IDA controls (n = 3). (B) Schematic diagram of circ_0009910 that derived from exon2 and exon3 of MFN2. (C) QPCR was performed to measure the expression of circ_0009910 in bone marrow samples from AML patients (n = 70) and IDA controls (n = 70). (D) QPCR was performed to measure the expression of circ_0009910 in four leukemia cell lines and normal 1D3 cells. (E) ISH was performed to detect the expression of circ_0009910 (red) in AML5 cells. Blue color indicates nuclear. Bar = 100 μm. (F) The Kaplan-Meier survival cure showed the survival rate in patients with high circ_0009910 expression (n = 43) and those with low circ_0009910 expression (n = 27). AML, acute myeloid leukemia. *p < 0.05. IDA, iron-deficiency anemia. AML, acute myeloid leukemia. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

were collected. None of the patients had previously undergone chemotherapy. All specimens were obtained from bone marrow aspiration, and after removal were immediately preserved at −80 °C until required. This project was approved by the Ethic Committee of The Hunan Provincial People's Hospital, Hunan Normal University. Written informed consent were obtained from all subjects.

2.2. CircRNAs microarray analysis

Bone marrow samples were randomly selected from 3 AML patients and 3 IDA controls for circRNA microarray (the information of these samples was shown in Table 1). Total RNA was extracted with TRIzol® (Thermo Fisher Scientific, Inc.) from bone marrow samples. CircRNA microarray was analyzed using Arraystar Human circRNA Array V2 analysis (Arraystar) by Kangchen BioTech Inc. (Shanghai, China). Briefly, total RNAs were digested with Rnase R (Epicentre, Inc.) to remove linear RNAs and enrich circular RNAs. Then, the enriched circular RNAs were amplified and transcribed into fluorescent circRNA utilizing a random priming method (Arraystar Super RNA Labeling Kit; Arraystar). The labeled circRNAs were hybridized onto the Arraystar Human circRNA Array V2 (8x15K, Arraystar). After having washed the slides, the arrays were scanned by the Agilent Scanner G2505C. Agilent Feature Extraction software (version 11.0.1.1) was used to analyze acquired array images. Differentially expressed circRNAs were then identified by analyzing fold change (> 2), as well as the p-value (< 0.05).

2.3. Cell lines

The human cell line 1D3 and 4 leukemia cell lines (Mono-Mac-6, KG-1, AML2 and AML5) were obtained from Cellbank of Chinese Academy of Sciences. Cells were grown routinely in RPMI-1640 medium (Invitrogen, CA, USA) supplemented with 10% fetal bovine serum (Gibco, CA, USA) and cultured in a 37 °C humidified atmosphere

Table 2
Clinical association between circ_0009910 levels and clinicopathological variables of patients with AML.

Variable	Circ_0009910		χ^2 test p value
	High expression (n = 43)	Low expression (n = 27)	
Age			0.740
< 40	20	17	
≥40	23	10	
Gender			0.743
Male	19	14	
Female	24	13	
FAB subtypes			0.438
M1	8	4	
M2	7	5	
M3	17	10	
M4	6	5	
M5	5	3	
Risk status			0.029
Better-risk	8	10	
Intermediate	10	10	
Poor-risk	25	7	

of 5% CO_2.

2.4. Plasmids construction and transfection

MiR-20a-5p mimics, miR-20a-5p inhibitor and their control were purchased from RiboBio (Guangzhou, China). SiRNA aimed at circ_0009910 (siRNA-1, siRNA-2) was synthesized by Gene-Pharma (Shanghai, China). Lipofectamine 3000 (Life Technologies) was used for plasmid transfection following the manufacturer's instructions.

2.5. Quantitative PCR analysis

Trizol reagent (Invitrogen, CA, USA) was used to extract total RNA from cells or bone marrow samples. ABScript II cDNA First-Strand Synthesis Kit (ABclonal Biotechnology Co., Ltd., Wuhan, China) was used to reverse transcript cDNA from 500 ng of RNA according to the manufacturer's protocol. The expression of circRNAs was measured by SsoFast EvaGreen supermix (Bio-Rad Laboratories (Shanghai) Co., Ltd. Shanghai, China) according to manufacturers' instructions. Expression of β-actin was used as an endogenous control. MiR-20a-5p-specific stem-loop primer was used to perform reverse transcription and quantification of miR-20a-5p was performed using MiRcute miRNA qRT-PCR Kit (SYBR Green) (TIANGEN, catalog number FP304, and U6 was used as an endogenous reference). QPCR was performed at the condition: 95.0 °C for 3 min, and 39 circles of 95.0 °C for 10s and 60 °C for 30 s. The primers were used as following: divergent primers for

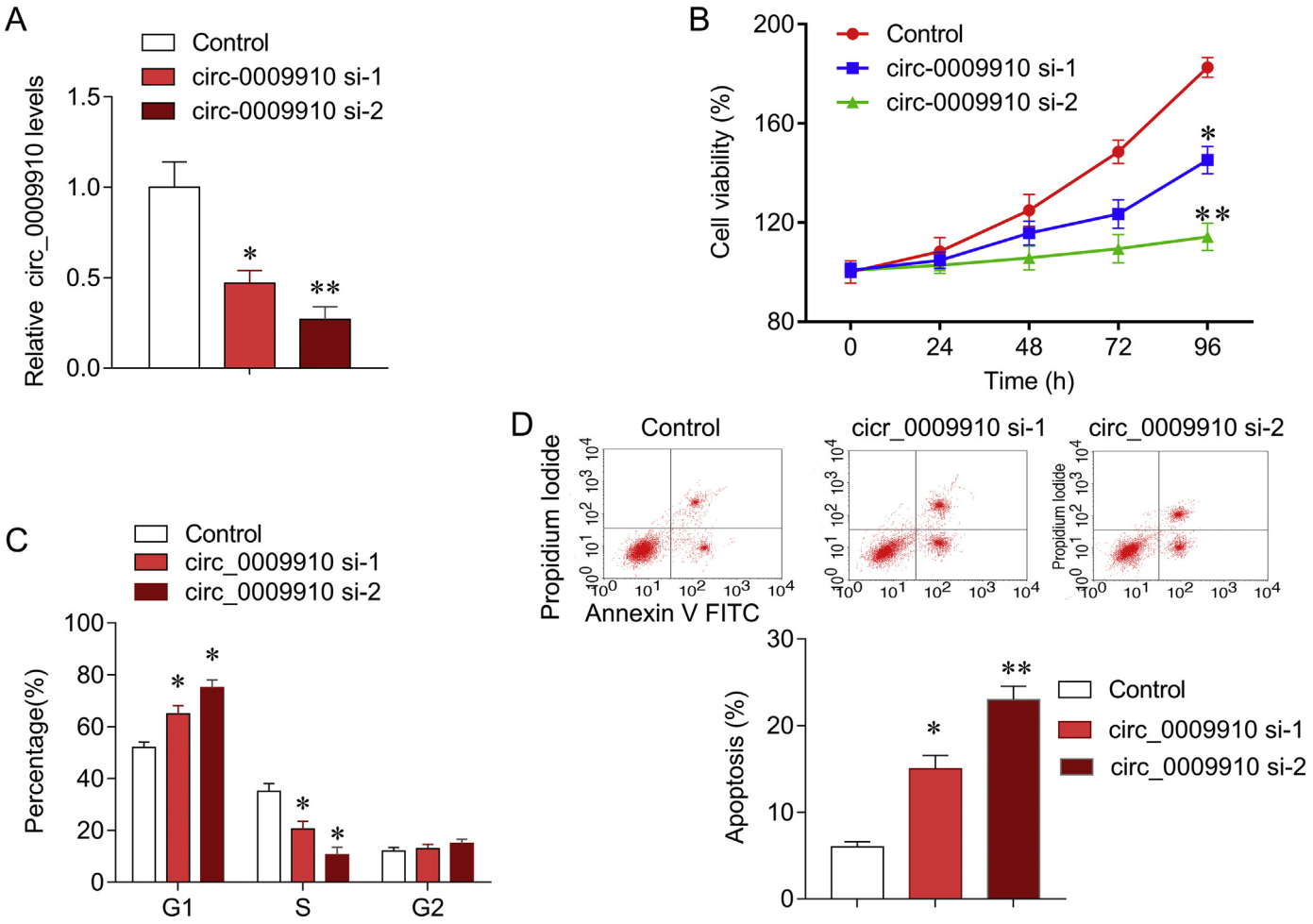

Fig. 2. Knockdown of circ_0009910 inhibits AML5 cell proliferation, arrests cell cycle and induces apoptosis. (A) QPCR was performed to measure the expression of circ_0009910 in AML5 cells after siRNA treatment. (B) AML5 cell proliferation rates were measured using CCK-8. (C) AML5 cell cycle was measured by flow cytometric analysis. (D) AML5 cell apoptosis was measured by flow cytometric analysis. *p < 0.05, **p < 0.01.

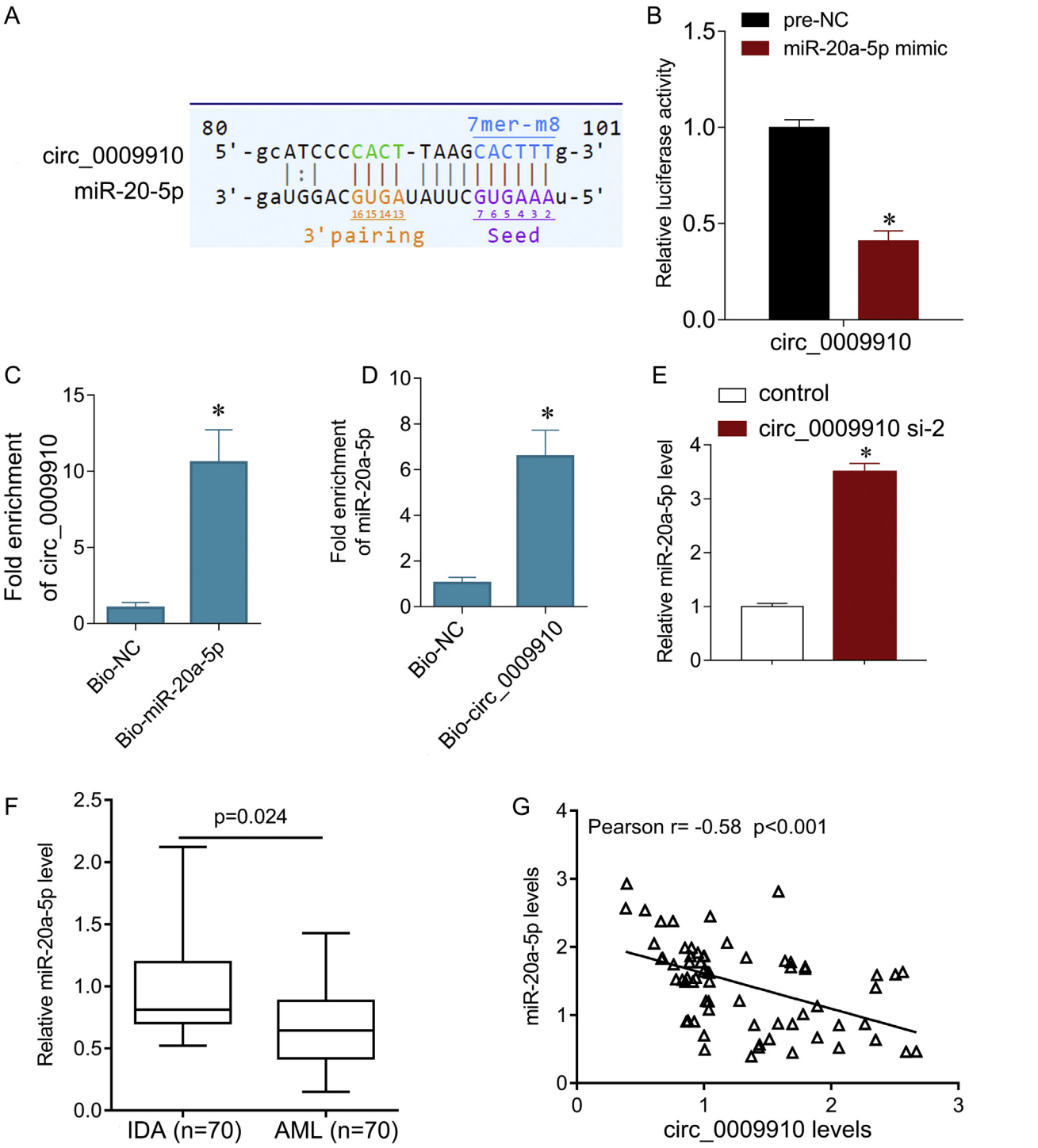

Fig. 3. Circ_0009910 interacts with miR-20a-5p. (A) The binding sites of miR-20a-5p and circ_0009910. (B) The relative luciferase activities were inhibited in the AML5 cells co-transfected with circ_0009910 and miR-20a-5p mimic. Firefly luciferase activity was normalized to *Renilla* luciferase. (C) Level of circ_0009910 in the sample pulled down by biotinylated miR-20a-5p was measured using real-time PCR. (D) Level of miR-20a-5p in the sample pulled down by biotinylated circ_0009910 was measured using real-time PCR. (E) The expression of miR-20a-5p in AML5 cells after circ_0009910 siRNA treatment. (F) QPCR was performed to measure the expression of miR-20a-5p in bone marrow samples from AML patients (n = 70) and IDA controls (n = 70). (G) Correlation of circ_0009910 and miR-20a-5p in AML patients. *p < 0.05. Error bars stand for the mean ± SD of at least triplicate experiments. IDA, iron-deficiency anemia. AML, acute myeloid leukemia.

circ_0009910, forward, 5′-CAGAACTGGACCCCGTTACC-3′, reverse, 5′-ATGCATTCACCTCAGCCATG-3′. β-actin, forward, 5′-TTGTTACAGG AAGTCCCTTGCC-3′, reverse, 5′-ATGCTATCACCTCCCCTGTGTG-3′. $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression of targets.

2.6. RNA–FISH

Cy3-labeled circ_0009910 probes were purchased from RiboBio (Guangzhou, China). The images were obtained using Fluorescent in Situ Hybridization kit (RiboBio) following the manufacturer's instructions. Briefly, following 3 h incubation with prehybridization solution at 37 °C, slides were incubated with Cy3-labeled circ_0009910 probe

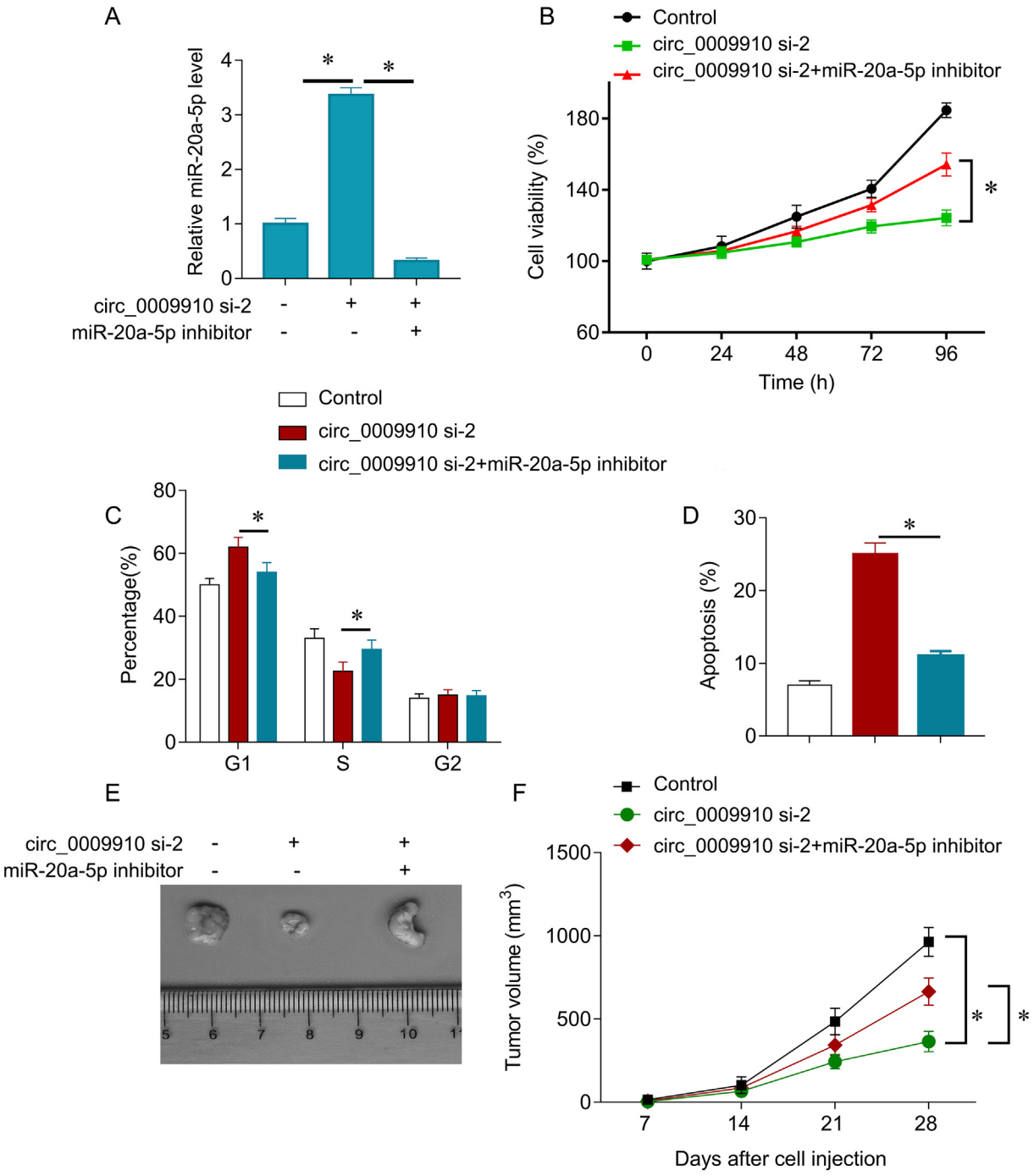

Fig. 4. Knockdown of circ_0009910 inhibits AML5 cell growth through miR-20a-5p. (A) QPCR was performed to measure the expression of miR-20a-5p in AML5 cells after circ_0009910 siRNA and miR-20a-5p inhibitor treatment. (B) CCK-8 assay was used to determine cell viability of AML5 cells. (C) AML5 cell cycle was measured by flow cytometric analysis. (D) AML5 cell apoptosis was measured by flow cytometric analysis. (E) Mice were sacrificed and the tumors were obtained from mice on day 28 post-injections. (F) The tumor volumes were measured every week. *$p < 0.05$.

overnight at 55 °C. The slices were washed with PBS for three times and counterstained with 4′,6-diamidino-2-phenylindole (DAPI) for 90 s. Images of slides were captured with an Olympus BX51 microscope (Tokyo, Japan).

2.7. Luciferase reporter assay

The wild-type miR-20a-5p binding site of circ_0009910 was designed and subcloned into pGL3 Basic vector (Promega, Madison, WI). MiR-20a-5p mimics were co-transfected with an internal control plasmid, pRL-TK (*Renilla* luciferase reporter plasmid, Promega), into AML5 cells, followed by transfection with circ_0009910. The luciferase activity was determined with the Dual Luciferase Reporter Assay Kit (Promega) according to the manufacturer's instruction.

2.8. RNA pull-down assay

Purified RNAs were labeled using biotin with Pierce RNA 3′End Desthiobiotinylation Kit (Thermo Fisher Scientific, Waltham, MA). Positive control (biotin-labeled miR-20a-5p, Bio-miR-20a-5p; biotin-labeled circ_0009910, Bio-circ_0009910) and biotinylated RNAs (NC-Bio) were indicated with cell lysates. Magnetic beads were used for

each binding reaction and the eluted RNA was measured by qRT-PCR.

2.9. CCK-8 cell proliferation assay

AML5 cell proliferation rates were measured using Cell Counting Kit-8 (CCK-8) (Beyotime, Hangzhou, China). 0.5×10^4 cells were seeded in each 96-well plate for 24 h. After treatment, the cells were further incubated for 24 h, 48 h, 72 h and 96 h, respectively. 10 μl CCK-8 reagents were added to each well at 1 h before the endpoint of incubation. OD 570 nm value in each well was determined by a microplate reader.

2.10. Cell cycle and apoptosis analysis

Annexin V apoptosis detection kit (Life technologies, Grand Island, NY) was used for analysis of apoptosis. After treatment, AML5 cells were trypsinized, collected, and resuspended. About 2×10^5 cells were harvested and washed twice with cold phosphate buffer saline (PBS), then resuspended in 500 μl binding buffer. 10 μl Annexin V-FITC and 10 μl Propidium Iodide were added to the solution and mixed well. For cell cycle analysis, the cells were only stained with Propidium Iodide. After 15 min incubation, the cells were analyzed using flow cytometric analysis (BD Biosciences, San Jose, CA).

2.11. Tumor xenograft in nude mice

Animal experiments were approved by the Ethical Committee for Animal Research of Hunan Provincial People's Hospital, Hunan Normal University. The AML5 cells were treated with circ_0009910 siRNA-2 with or without miR-20a-5p inhibitor. The cells untreated were used as control. After treatment, the cells (1×10^6 cells/100 μl) were subcutaneously injected into nude mice (n = 6 per group). The tumor sizes were measured regularly and calculated using the formula: $0.5 \times L \times W^2$ where L and W are the long and short diameter of the tumor, respectively.

2.12. Statistical analysis

All data from 3 independent experiments were expressed as mean ± SD and processed using SPSS17.0 statistical software. The overall survival rate estimates over time were calculated using the Kaplan-Meier method with log-rank test. The correlation between circ_0009910 expression and miR-20a-5p in AML patients was evaluated by Pearson correlation analysis. When the patients had circ_0009910 expression that was higher than the average, they were defined as high circ_0009910 group, otherwise they were low circ_0009910 group. The association of circ_0009910 expression with clinicopathological variables of patients with AML was analyzed by χ^2 test. The difference among the groups was estimated by Student's *t*-test or one-way ANOVA with Tukey post hoc test. $p < 0.05$ was statistically significant.

3. Results

3.1. Differentially expressed circRNAs in AML patients

CircRNA microarray was performed to screen differentially expressed circRNAs. Numerous differentially expressed circRNAs were analyzed in AML patients compared with the IDA controls (Fig. 1A). The results of the present study revealed that circ_0009910 expression was significantly upregulated in AML patients compared with in IDA controls. Circ_0009910 was derived from exon2 and exon 3 of Mitofusin-2 (MFN2) and located on chr1:12049221–12052747 + (Fig. 1B). We also confirmed circ_0009910 expression by qPCR in bone marrow from 70 AML patients and 70 IDA controls and found that circ_0009910 expression was increased in AML patients compared with in IDA controls (Fig. 1C). We also found that circ_0009910 was significantly increased in leukemia cell lines, especially in AML5 cells, compared with control cells (Fig. 1D). ISH results showed that circ_0009910 expressed in cytoplasm (Fig. 1D). In addition, we analyzed the clinical association between circ_0009910 levels and clinicopathological variables of patients with AML. There were no significant association between circ_0009910 levels and age, gender and FAB subtypes; whereas more AML patients with poor-risk were found high circ_0009910 group (Table 2). We further analyzed the relationship between circ_0009910 levels and survival time in AML patients. The Kaplan-Meier survival cure showed that the patients with high circ_0009910 expression had shorter overall survival rate than those with low circ_0009910 expression (Fig. 1F).

3.2. Knockdown of circ_0009910 inhibits AML5 cells growth

We investigated the biological role of circ_0009910 in AML5 cells. We treated AML5 cells with circ_0009910 siRNA (si-1 and si-2) and found that the expression of circ_0009910 was significantly decreased in AML5 cells (Fig. 2A). circ_0009910 downregulation significantly inhibited AML5 cell viability (Fig. 2B). In addition, circ_0009910 downregulation arrested cell cycle G1-S phase (Fig. 2C). The apoptosis rate in AML5 cells with reduced circ_0009910 was also significantly increased compared with in the control cells (Fig. 2D).

3.3. MiR-20a-5p was a target microRNA of circ_0009910

We screened the possible targets of circ_0009910 by using bioinformatic tools, and found that miR-20a-5p was a potential target of circ_0009910. Their target sequences were shown in Fig. 3A. We further confirmed their interaction in AML5 cells. As shown in Fig. 3B, co-transfection of miR-20a-5p mimics and the luciferase vector containing circ_0009910 resulted in a significant decrease of relative luciferase activity in AML5 cells. Subsequently, to test whether circ_0009910 can serve as a sponge RNA for miR-20a-5p, RNA pull-down assay with biotinylated circ_0009910 (Bio-circ_0009910) probe increased the level of miR-20a-5p than control (Bio-NC), and vice versa (Fig. 3C, D). In addition, we observed that knockdown of circ_0009910 significantly upregulated miR-20a-5p expression (Fig. 3E). Furthermore, we also confirmed that miR-20a-5p expression was reduced in AML patients compared with IDA controls (Fig. 3F), which was negatively associated with circ_0009910 expression (Fig. 3G). These results indicate that miR-20a-5p can serve as a direct microRNA target of circ_0009910.

3.4. Circ_0009910 control AML5 cells growth through miR-20a-5p

To investigate whether circ_0009910 regulated AML5 cells growth through miR-20a-5p, circ_0009910 siRNA (si-2) and miR-20a-5p inhibitor was transfected into AML5 cells. We observed that miR-20a-5p expression was increased by circ_0009910 siRNA and decreased by its inhibitor (Fig. 4A). We found that miR-20a-5p inhibitors reversed circ_0009910 siRNA-mediated cell proliferation inhibition, cell cycle arrest and cell apoptosis (Fig. 4B, C and D). Moreover, we confirmed these results in animal model. We observed that circ_0009910 siRNA inhibited tumor growth, which was restored by miR-20a-5p inhibitor (Fig. 4D). These results revealed that circ_0009910 promotes AML5 cells growth through inhibiting miR-20a-5p.

4. Discussion

In this study, we found that circ_0009910 was significantly increased in AML compared with IDA controls. Further analysis showed that higher circ_0009910 predicted a poor risk and worse prognosis for AML patients. CircRNAs have been proved to be ubiquitous and critical in regulating cellular processes and diseases, especially in cancers [11]. Emerging evidence reveals the function of circRNAs in cancer and may

potentially serve as a required novel biomarker and therapeutic target for cancer treatment [12,13]. For example, circRNA-101368 is significantly upregulated in hepatocellular carcinoma (HCC) tissue and is correlated with poorer prognosis in patients with HCC [14].

RNA-sequencing reveal thousands of circRNAs in leukemia [15]. A novel circular RNA (circRNA), circBA9.3, is derived from BCR-ABL1. CircBA9.3 can promote the proliferation and inhibit apoptosis of cancer cells by enhancing c-ABL1 and BCR-ABL1 oncoprotein expression, which is associated with TKI therapy resistance [16]. Circ-ANAPC7 is significantly upregulated in AML and might participate in AML pathogenesis by sponging miR-181 family [8]. Here, we identified that circ_0009910 was an exonic circRNA that located at chrome 1 (12049221 to 12052747), which was host in mitofusin 2. High circ_0009910 was associated with poor risk and outcome of AML patients.

Further investigation revealed that circ_0009910 knockdown inhibited AML cell proliferation, arrested cell cycle and induced cell apoptosis. We also explored the potential mechanism by which circ_0009910 knockdown inhibits AML cell growth. Our results demonstrated that circ_0009910 expression was expressed in cytoplasm, suggesting that it may exhibit its function by sponging miRNAs. Bioinformation screening shown that circ_0009910 interacted with miR-20a-5p. Dual-luciferase and RIP experiments confirmed that circ_0009910 interacted with miR-20a-5p. In vitro and in vivo experiments elucidated that circ_0009910 knockdown inhibited AML cell growth by increasing miR-20a-5p. MiR-20a-5p has been found to be a double faces gene. MiR-20a-5p is upregulated in triple-negative breast cancer, nasopharyngeal cancer, hepatocellular carcinoma, colorectal cancer. It can promote proliferation, invasion and metastasis as well as radio-resistance by targeting multiple genes, including runt-related transcription factor 3 (RUNX3), Rab27B and Smad4 [17–20]. However, miR-20a-5p is downregulated in osteosarcoma and neuroblastoma. MiR-20a-5p inhibits cell proliferation and promoted apoptosis through negative regulation of autophagy-related gene 7 (ATG7) and thus autophagy suppression in SH-SY5Y cells [21]. Forced expression of miR-20a-5p counteracts osteosarcoma cell chemoresistance in vitro and in vivo through targeting kinesin family member 26B (KIF26B) by modulating the activities of the MAPK/ERK and cAMP/PKA signaling pathways [22]. In recent study, we showed that miR-20a-5p was downregulated in AML and negatively associated with circ_0009910 expression. Circ_0009910 knockdown could induce miR-20a-5p expression to suppress cancer cell growth, suggesting that miR-20a-5p acts as a tumor suppressor in AML. These results clarify that circ_0009910 inhibits cell proliferation and promotes apoptosis through negative regulation of miR-20a-5p in AML cells.

5. Conclusion

Our study firstly identifies that circ_0009910 is significantly upregulated in AML bone marrow samples and might serve as a novel outcome biomarker for AML. Both circ_0009910 and miR-20a-5p may be potential therapeutic targets for future AML treatment.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

L P and Z M conceived the study and participated in the study design, performance, coordination and manuscript writing. C JJ, L CS and L GH performed the research. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

References

[1] I. Abou Dalle, C.D. DiNardo, The role of enasidenib in the treatment of mutant IDH2 acute myeloid leukemia, Ther. Adv. Hematol. 9 (2018) 163–173.
[2] C. Talati, K. Sweet, Recently approved therapies in acute myeloid leukemia: a complex treatment landscape, Leuk. Res. 73 (2018) 58–66.
[3] J.A. Wallace, R.M. O'Connell, MicroRNAs and acute myeloid leukemia: therapeutic implications and emerging concepts, Blood 130 (2017) 1290–1301.
[4] A. Zebisch, S. Hatzl, M. Pichler, A. Wolfler, H. Sill, Therapeutic resistance in acute myeloid leukemia: the role of non-coding RNAs, Int. J. Mol. Sci. 17 (2016).
[5] X. Wang, K. Chen, G. Guo, J.L. Chen, Noncoding RNAs and their functional involvement in regulation of chronic myeloid leukemia, Brief. Funct. Genomics 15 (2016) 239–248.
[6] W. Li, C. Zhong, J. Jiao, P. Li, B. Cui, C. Ji, et al., Characterization of hsa_circ_0004277 as a new biomarker for acute myeloid leukemia via circular RNA profile and bioinformatics analysis, Int. J. Mol. Sci. 18 (2017).
[7] A. Bonizzato, E. Gaffo, G. Te Kronnie, S. Bortoluzzi, CircRNAs in hematopoiesis and hematological malignancies, Blood Cancer J. 6 (2016) e483.
[8] H. Chen, T. Liu, J. Liu, Y. Feng, B. Wang, J. Wang, et al., Circ-ANAPC7 is upregulated in acute myeloid leukemia and appears to target the MiR-181 family, Cell. Physiol. Biochem. 47 (2018) 1998–2007.
[9] S. Hirsch, T.J. Blatte, S. Grasedieck, S. Cocciardi, A. Rouhi, M. Jongen-Lavrencic, et al., Circular RNAs of the nucleophosmin (NPM1) gene in acute myeloid leukemia, Haematologica 102 (2017) 2039–2047.
[10] D.M. Wu, X. Wen, X.R. Han, S. Wang, Y.J. Wang, M. Shen, et al., Role of circular RNA DLEU2 in human acute myeloid leukemia, Mol. Cell. Biol. 38 (2018).
[11] S. Lux, L. Bullinger, Circular RNAs in cancer, Adv. Exp. Med. Biol. 1087 (2018) 215–230.
[12] R. Ojha, R. Nandani, N. Chatterjee, V.K. Prajapati, Emerging role of circular RNAs as potential biomarkers for the diagnosis of human diseases, Adv. Exp. Med. Biol. 1087 (2018) 141–157.
[13] J. Guarnerio, M. Bezzi, J.C. Jeong, S.V. Paffenholz, K. Berry, M.M. Naldini, et al., Oncogenic role of fusion-circRNAs derived from cancer-associated chromosomal translocations, Cell 165 (2016) 289–302.
[14] S. Li, H. Gu, Y. Huang, Q. Peng, R. Zhou, P. Yi, et al., Circular RNA 101368/miR-200a axis modulates the migration of hepatocellular carcinoma through HMGB1/RAGE signaling, Cell Cycle 17 (19–20) (2018) 2349–2359.
[15] L. Song, Y. Xiao, Downregulation of hsa_circ_0007534 suppresses breast cancer cell proliferation and invasion by targeting miR-593/MUC19 signal pathway, Biochem. Biophys. Res. Commun. 503 (2018) 2603–2610.
[16] Y. Pan, J. Lou, H. Wang, N. An, H. Chen, Q. Zhang, et al., CircBA9.3 supports the survival of leukaemic cells by up-regulating c-ABL1 or BCR-ABL1 protein levels, Blood Cells Mol. Dis. 73 (2018) 38–44.
[17] D. Cheng, S. Zhao, H. Tang, D. Zhang, H. Sun, F. Yu, et al., MicroRNA-20a-5p promotes colorectal cancer invasion and metastasis by downregulating Smad4, Oncotarget 7 (2016) 45199–45213.
[18] Y. Chen, X. Wang, J. Cheng, Z. Wang, T. Jiang, N. Hou, et al., MicroRNA-20a-5p targets RUNX3 to regulate proliferation and migration of human hepatocellular cancer cells, Oncol. Rep. 36 (2016) 3379–3386.
[19] D. Huang, G. Bian, Y. Pan, X. Han, Y. Sun, Y. Wang, et al., MiR-20a-5p promotes radio-resistance by targeting Rab27B in nasopharyngeal cancer cells, Cancer Cell Int. 17 (2017) 32.
[20] X. Bai, G. Han, Y. Liu, H. Jiang, Q. He, MiRNA-20a-5p promotes the growth of triple-negative breast cancer cells through targeting RUNX3, Biomed. Pharmacother. 103 (2018) 1482–1489.
[21] Y. Yu, J. Zhang, Y. Jin, Y. Yang, J. Shi, F. Chen, et al., MiR-20a-5p suppresses tumor proliferation by targeting autophagy-related gene 7 in neuroblastoma, Cancer Cell Int. 18 (2018) 5.
[22] Y. Pu, Q. Yi, F. Zhao, H. Wang, W. Cai, S. Cai, MiR-20a-5p represses multi-drug resistance in osteosarcoma by targeting the KIF26B gene, Cancer Cell Int. 16 (2016) 64.