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MiR-592 functions as a tumor suppressor in acute myeloid leukemia by targeting ROCK1 and predicts patients' prognosis

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Abstract. – OBJECTIVE: Dysregulation of miR-592 has been reported in several tumors. However, its role in acute myeloid leukemia (AML) remains unknown. The present study aimed at investigating the expression pattern and biological function of miR-592 in AML and to elucidate the mechanism involved.

PATIENTS AND METHODS: qRT-PCR was used to analyze the expression of miR-592 in bone marrow and serum obtained from AML patients and healthy controls. The associations between serum miR-592 expression and clinical features and prognosis of AML patients were statistically analyzed. Then we detected the effect of miR-592 on proliferation, metastasis and apoptosis by CCK-8 assay, Transwell assays and flow cytometry, respectively. Dual-luciferase reporter assays were performed to validate the regulation of a putative target of miR-592. Rescue experiments were performed to confirm whether ROCK1 was a direct and functional target of miR-592 in AML.

RESULTS: We found that the expression level of miR-592 was significantly lower in AML patients and AML cell lines. Low expression of serum miR-592 was associated with advanced French-American-British classification, cytogenetics and poor prognosis. Multivariate analysis confirmed that serum miR-592 expression was an independent prognostic factor for AML patients. Functionally, overexpression of miR-592 suppressed AML cell growth and metastasis, and promoted apoptosis. Further mechanistic investigation showed ROCK1 was a direct target gene of miR-592. Finally, ROCK1 overexpression rescued the effect of miR-592-mediated AML cell proliferation and metastasis.

CONCLUSIONS: These findings suggest that miR-592 acted as a tumor suppressor by targeting ROCK1 and may serve as a potential biomarker in AML.

Key Words:
miR-592, Acute myeloid leukemia, ROCK1, Tumorigenesis, Prognosis.

Introduction

Leukemia is the most common of pediatric cancers accounting for about 30% of diagnoses[1]. Acute myeloid leukemia (AML), characterized by arrested differentiation and abnormal proliferation, is one of the two main subtypes and a heterogeneous malignant disease[2]. Risk factors include smoking, previous chemotherapy or radiation therapy and myelodysplastic syndrome[3]. Although several improvements have been made in diagnosis and therapy of patients with AML patients, only approximately 40 % of patients with AML achieved long-term survival[4,5]. Several important reasons for poor prognosis of AML patients include relapse which is common and associated diseases[6]. In clinical practice, precise prediction of prognosis can develop the chance of cure for a specific person. Thus, investigating the pathogenesis of AML is necessary. MicroRNAs (miRNAs) are recently identified noncoding small RNAs of 19-25 nucleotides and widely distributed in eukaryotes, which could play important roles in the regulation of gene expression at the post-transcriptional level[7,8]. It is estimated that more and one-third of genes could be modulated by miRNAs and each miRNA could target several mRNAs[9]. Growing evidences show that miRNAs exert biological functions in regulating important cellular functions such as proliferation, differentiation, invasion and apoptosis[10,11]. Of note, increasing studies have reported that miRNAs play essential roles in multiple biological processes of cancer, and thus promoted the development and progression of tumors by acting as tumor suppressors or tumors promoter according to the types of tumors[12-14]. Although the researches about the roles of miRNAs in common tumors such as lung cancer and liver cancer[15,16], the expression pattern and biological function of miRNAs in AML

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progression remain largely unclear. miR-592, located at 7q31.33, is a dysregulated miRNA in several tumors and plays an important functional role[17,18]. Interestingly, up-regulation of miR-592 was reported in gastric cancer[19], colorectal cancer[20], while down-regulation of miR-592 was reported in glioma[21], breast cancer[22] and hepatocellular carcinoma[23], which suggested that miR-592 may paly different roles according to the types of tumors. To our best knowledge, the expression pattern and biological function of miR-592 in AML patients have not been investigated. In this study, for the first time, we reported miR-592 as an overexpressed miRNA in AML and acted as a potential prognostic biomarker for AML patients and tumor promoter *in vitro*.

Patients and Methods

Clinical Specimens

AML and non-tumor specimens were obtained from 94 patients from April 2010 to September 2013 in the Jining No.1 People's Hospital. Liquid nitrogen was applied to frozen all the specimens and the tissues were stored at -80°C. The clinical information of the patients was listed in Table I. Before surgery, the patients had not received any cancer-related treatment. All the specimens were obtained based on the written informed consents from the patients. The Ethics Committee of Jining No.1 People's Hospital had approved the present study.

Cell Lines and Cell Transfection

One human normal stromal cell, HS-5, and three AML cell lines (HL-60, THP-1 and NB4) were obtained from Jennio Biotech Co., Ltd. (Guangzhou, Guangdong, China). Then, 10% fetal bovine serum containing Roswell Park Memorial Institute-1640 (RPMI-1640) medium was applied to culture the AML cells. For cell transfection, an EnoGeneFec transfection kit (EnoGene, Nanjing, Jiangsu, China) was used. The miRNA mimics (Control mimics and miR-592 mimics), and ROCK1 overexpressing plasmids (pcDNA3.1-ROCK1) were purchased from Western Biotechnology (Yuzhong, Chongqing, China).

Table I. Relationship between the expression of miR-592 and clinicopathological parameters in AML.

Clinical variables	No. of patients	Serum miR-592 expression		*p*-value
		Low	High	
Gender				NS
Male	42	20	22	
Female	52	26	26	
Age (year)				NS
< 6	39	21	18	
≥ 6	55	25	30	
Leukocyte (μL)				NS
< 10,000	60	32	28	
≥ 10,000	34	14	20	
French-American-British classification				0.008
M0	3	1	2	
M1/M2	39	15	24	
M3	31	13	18	
M4/M5	13	11	2	
M7	8	6	2	
Extramedullary disease				NS
Absent	61	31	33	
Present	30	15	15	
Cytogenetics				0.011
Favorable	36	11	25	
Intermediate	26	12	14	
Unfavorable	32	23	9	
Day 7 response to treatment				NS
Favorable	61	29	32	
Unfavorable	33	17	16	
Self injury	Rare (self-protection before fall)	Yes		

Real-time PCR Assay

Total RNA of HL-60, THP-1 and NB4 cells were extracted by a Total RNA Superfast Purification kit (HaoxinBio, Hangzhou, Zhejiang, China). A light cycler 480 Real-time PCR instrument (Roche, Basel, Switzerland) was then employed to conduct the qRT-PCR assays using TUREscript One Step qRT-PCR kit (YuduoBio, Pudong, Shanghai, China). For miR-592 detection, we applied a High-Specificity miRNA QRT-PCR Detection kit (Yeasen, Pudong, Shanghai, China). The primers for miR-592, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or ROCK1 were listed in Table II. GAPDH and U6 were used as an internal control of ROCK1 or miR-592, respectively.

Cell Counting Kit-8 (CCK-8) Assay

In short, HL-60, THP-1 and NB4 cells were firstly transfected with control mimics or miR-592 mimics. After 24 h, these cells (1×103 cells/well) were placed into 96-well plates. Subsequently, 10 μl CCK-8 reagents (Newgainbio, Wuxi, Jiangsu, China) were added into each well at 24 h, 48 h, 72 h and 96 h, and an AMR-100 microreader (Allsheng, Hangzhou, Zhejiang, China) was applied to read the 450 nm absorbance.

Western Blot Analysis

We used a HaoRanBio total protein extraction kit (HaoRanBio, Xuhui, Shanghai, China) to lyse the HL-60, THP-1 and NB4 cells. Afterwards, 20 μg of the protein lysates were subjected to electrophoresis and then transferred to polyvinylidene difluoride (PVDF) membranes. After the membranes were blocked with 5% Bovine Serum Albumin (BSA), they were sequentially immunoblotted with anti-human ROCK1 or GAPDH antibody (Sino Biological Inc., Yizhuang, Beijing, China). After the membranes were incubated with secondary antibodies, the ROCK1 or GAPDH protein bands were visualized by a NcmECL Ultra ECL kit (NcmBio, Suzhou, Jiangsu, China).

Flow Cytometry Analysis

Briefly, after HL-60, THP-1 and NB4 cells were transfected with miR-592 mimics or corresponding plasmids, the cell cycle and cell apoptosis were analyzed by flow cytometry using a FlowSight flow cytometer (Merck Millipore, Billerica, MA, USA). For cell cycle examination, an EZCellTM Cell Cycle Analysis kit (AmyJet Scientific, Wuhan, Hubei, China) was utilized. For cell apoptotic rates determination, we used a Servicebio Annexin V/PI cell apoptosis analysis kit (Servicebio, Wuhan, Hubei, China).

Table II. The sequences of primers for RT-PCR.

Name	Sequences (5′-3′)
miR-592: forward	TTGTGTCAATATGCGATGATGT
miR-592: reverse	GCGAGCACAGAATTAATAGCAC
ROCK1: forward	AACATGCTGCTGGATAAATCTGG
ROCK1: reverse	TGTATCACATCGTACCATGCCT
U6: forward	CTCGCTTCGGCAGCACA
U6: reverse	AACGCTTCACGAATTTGCGT

Dual-Luciferase Reporter Assay

The wild-type ROCK1 3'-untranslated region (3'-UTR) sequences (wt ROCK1) or the mutant ROCK1 3'-UTR sequences (mut ROCK1) were cloned into the pMIR-Report vectors by PackGene Biotechnology Co., Ltd. (Guangzhou, Guangdong, China). Subsequently, the HL-60, THP-1 or NB4 cells were co-transfected with wt ROCK1 vectors or mut ROCK1 vectors as well as miR-592 mimics or control mimics. Next, a Dual-Luciferase Reporter assays kit (Zikerbio, Shenzhen, Guangdong China) was employed to evaluate the luciferase activities in HL-60, THP-1 or NB4 cells.

Transwell Assays

The migration and invasion of the HL-60, THP-1 or NB4 cells were determined by transwell assays. Firstly, miR-592 mimics or ROCK1 overexpressing plasmids were transfected into HL-60, THP-1 or NB4 cells. Next, the treated AML cells were resuspended in the serum-free RPMI-1640 medium, and 70 μl of the AML cells (5×105 cells/ml) were placed into the upper chambers of the inserts with (for transwell invasion assays) or without (for transwell migration assays) Matrigel pre-treated. The lower chambers of the inserts were added with 15% fetal bovine serum (FBS) containing Roswell Park Memorial Institute-1640 (RPMI-1640) medium. After 24 h, 0.1% crystal violet (JissKang Biotech, Qingdao, Shandong, China) was utilized to stain the migratory or invaded cells, and the cell number was counted by the use of a DYS-810 microscope (DianYing, Pudong, Shanghai, China).

Statistical Analysis

The data of the experiments in this study were analyzed by SPSS (version 19.0; (IBM, Armonk, NY, USA). One-way ANOVA or t-tests were per-

formed for the comparisons. A multiple-range least significant difference (LSD) was used for intergroup comparisons. The expressing relationship between miR-592 and ROCK1 in AML specimens was assessed by the Pearson's correlation coefficient test. Survival curves were constructed with the Kaplan-Meier method and compared by the log-rank test. The significance of survival variables was analyzed using the Cox multivariate proportional hazards model. A p <0.05 was considered statistically significant.

Results

Downregulation of miR-592 in AML Patient Samples and Cell Lines

Firstly, we performed RT-PCR to explore whether miR-592 was abnormally expressed in AML patients. As shown in Figure 1A, it was observed that the expression level of bone marrow miR-592 was significantly down-regulated in AML patients compared to healthy controls (p < 0.01). In addition, we also showed that patients with AML had a higher serum miR-592 levels than healthy the controls (p < 0.01). On the other hand, the results of RT-PCR indicated that miR-592 expression was significantly down-regulated in three AML cell lines (HL-60, THP-1 and NB4) compared to HS-5 cell line. Thus, our data, for the first time, revealed that miR-592 acted as a down-regulated miRNA in AML and may play a functional role.

The Prognostic Value of miR-592 in AML Patients

To explore the potential clinical significance of miR-592 in AML patients, we divided the 94 AML

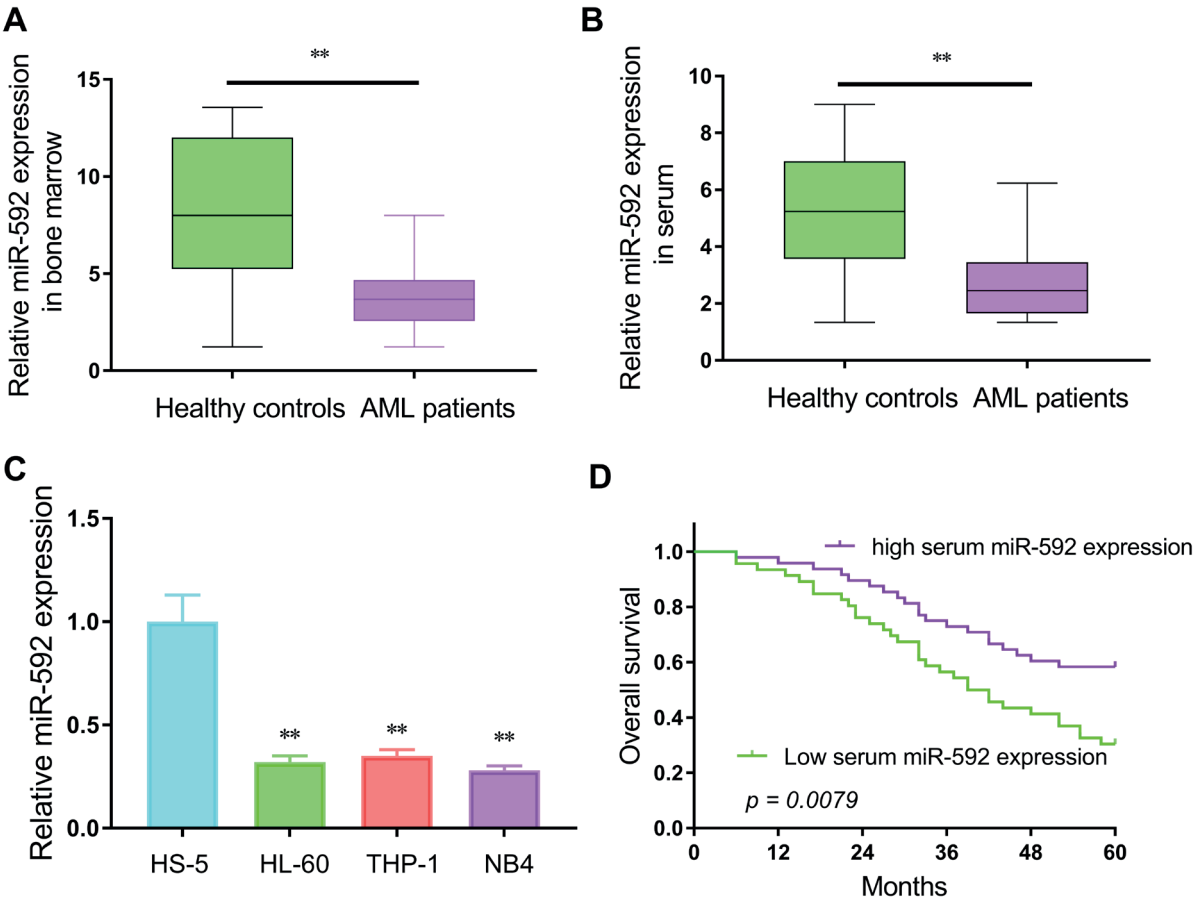

Figure 1. Down-regulated miR-592 expression was negatively correlated with prognosis of AML. ***(A)*** qRT-PCR analysis detected the miR-592 expression in bone marrow of AML patients and healthy controls. ***(B)*** qRT-PCR analysis detected the miR-592 expression in serum of AML patients and healthy controls. ***(C)*** The expression levels of miR-592 in AML cell lines (HL-60, THP-1 and NB4) and HS-5. ***(D)*** Kaplan-Meier curves for overall survival of 94 AML patients, divided according to miR-592 expression levels. *p < 0.05, **p < 0.01.

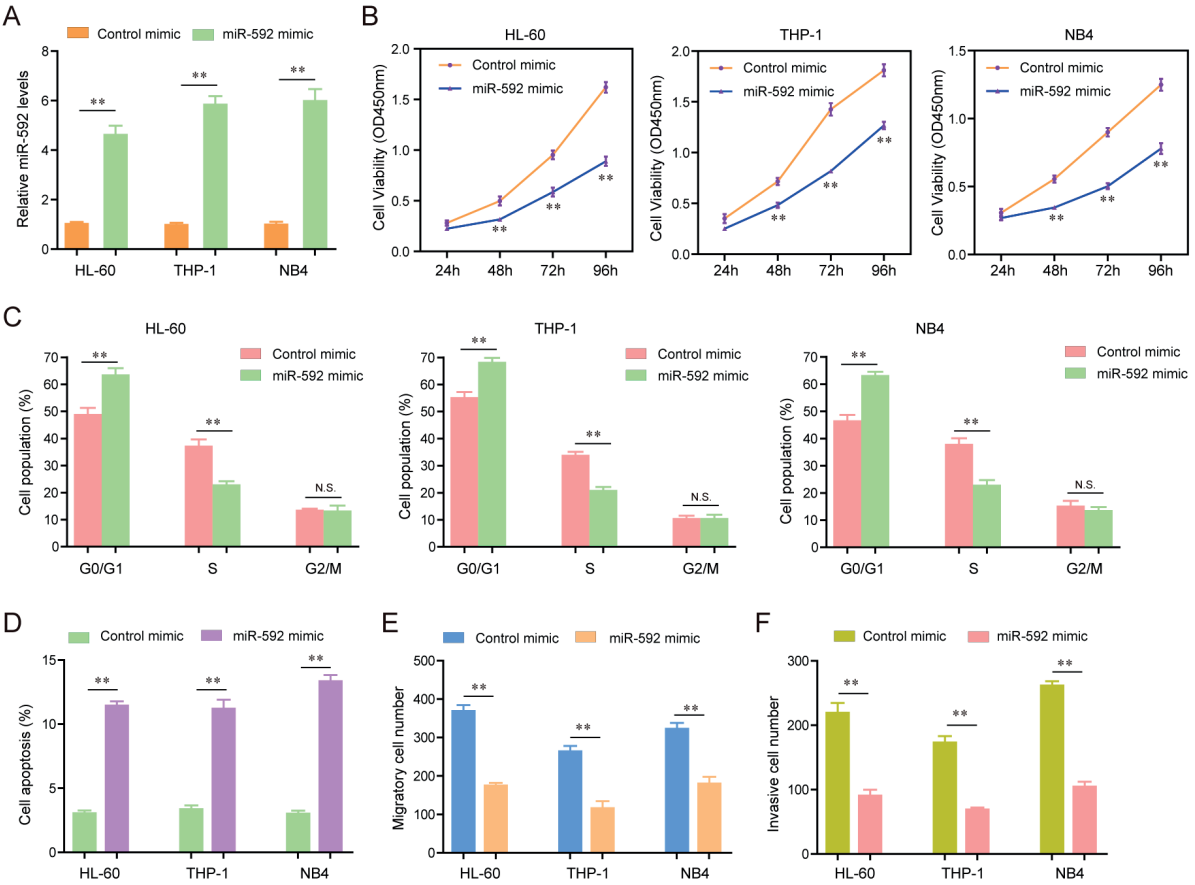

Figure 2. The influence of miR-592 on the proliferation, cell cycle, apoptosis, migration and invasion of HL-60, THP-1 and NB4 cells. ***(A)*** The qRT-PCR analysis determined the relative miR-592 expression in HL-60, THP-1 and NB4 cells. ***(B)*** CCK-8 assays detected the proliferation of HL-60, THP-1 and NB4 cells. ***(C)*** Cell cycle analysis of HL-60, THP-1 and NB4 cells transfected with control mimic or miR-592 mimic. ***(D)*** Flow cytometry examined the apoptosis of HL-60, THP-1 and NB4 cells. ***(E)*** Transwell migration assays evaluated the migratory abilities of HL-60, THP-1 and NB4 cells. ***(F)*** Transwell invasion assays assessed the changes of invasive abilities of HL-60, THP-1 and NB4 cells when they were transfected with miR-592 mimics. *$p < 0.05$, **$p < 0.01$.

patients into a high expression group (n = 46) and a low expression group (n =48), according to the median expression level of miR-592. The results of chi-square test indicated that low expression of serum miR-592 was significantly associated with advanced French-American-British classification ($p = 0.008$) and cytogenetics ($p = 0.011$) (Table I). However, there were no significant associations between serum miR-592 expression and other clinical features including gender, age, leukocyte, extramedullary disease and Day 7 response to treatment (all $p > 0.05$). Furthermore, we performed Kaplan-Meier method to further understand the prognostic value of serum miR-592 in AML patients and the results showed that patients with decreased serum miR-592 expression had shorter overall survival than those with elevated expression of serum miR-592 ($p = 0.0037$). More importantly, univariate and multivariate confirmed that serum miR-592 expression was an independent molecular biomarker for the predicting of overall survival of AML patients (Table III).

The Effects of miR-592 on the Proliferation, Cell Cycle, Apoptosis, Migration and Invasion of AML Cells

To dissect the functional roles of miR-592 in the development and progression of AML, we transfected negative control miRNA mimic (Control mimic) or miR-592 mimic into three AML cell lines, HL-60, THP-1 and NB4 cells. The results of qRT-PCR analysis suggested that enhancing expression of miR-592 resulted in remarkable increased miR-592 levels in HL-60, THP-1 and NB4 cells (Figure 2A). We next conducted CCK-8 assays to evaluate the effects of miR-592 on the proli-

Table III. Univariate and multivariate analysis of clinicopathological factors for overall survival in 94 patients with AML.

	Univariate analysis			Multivariate analysis		
Risk factors	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Cytogenetics Unfavorable *vs.* Favorable/Intermediate	3.552	1.423-4.532	0.008	2.965	1.216-4.016	0.013
French-American-British classification M7 *vs.* M1-M6	3.757	1.532-5.215	0.004	3.216	1.321-4.452	0.005
Serum miR-592 expression High *vs.* Low	3.469	1.328-4.867	0.009	3.127	1.156-4.137	0.014

feration of AML cells. The data revealed that transfection of miR-592 mimics notably suppressed the cell growth of HL-60, THP-1 and NB4 cells (Figure 2B). Furthermore, the cell cycle and apoptosis of AML cells transfected with miR-592 mimics were determined using flow cytometry. The data indicated that G0/G1 cell cycle arrest was induced when HL-60, THP-1 and NB4 cells were transfected with miR-592 mimics (Figure 2C). In addition, forcing the expression of miR-592 also accelerated the apoptotic rats of the AML cells (Figure 3D). Besides, the influence of miR-592 on metastasis of AML was also evaluated. The results of transwell migration assays confirmed that overexpression of miR-592 led to a significant decline of migratory cell number of HL-60, THP-1 as well as NB4 cells (Figure 3E). Similarly, transwell invasion assays demonstrated that transfection of miR-592 mimics dramatically reduced the invasive cell number of HL-60, THP-1 and NB4 cells (Figure 3F). Therefore, our data demonstrated that miR-592 played essential roles in regulating the development and progression of AML.

ROCK1 was the Direct Target of miR-592 in AML Cells

To facilitate the further elucidation of the underlying molecular mechanisms, we predicted the potential targets of miR-592 via searching "miRDB" website (http://www.mirdb.org/) and a putative target site of miR-592 in the 3'UTR of ROCK1, a well-studied oncogene, was identified (Figure 3A). Subsequently, we performed dual luciferase reporter assays to experimentally certify that miR-592 was directly interacted with ROCK1. The results of luciferase reporter analysis demonstrated that overexpression of miR-592 remarkably reduced the relative luciferase activities of HL-60, THP-1 as well as NB4 cells transfected with plasmids containing wild type 3'UTR of ROCK1 (wt ROCK1), while there was no influence on luciferase activities of AML cells when they were co-transfected with mutant 3'UTR of ROCK1 containing plasmids (mut ROCK1) and miR-592 mimics (Figure 3B). Additionally, ectopic expression of miR-592 obviously reduced the relative mRNA and protein levels of ROCK1 in HL-60, THP-1 and NB4 cells (Figure 3C and D). Furthermore, we performed Pearson's correlation analysis to evaluate the expressing correlation between miR-592 and ROCK1. The data revealed that a negative correlation was exhibited between ROCK1 expression and miR-592 expression in AML samples (Figure 3E). Taken together, these data validated that ROCK1 was a direct target of miR-592.

ROCK1 Reversed the Inhibitory Effects of miR-592 on the Proliferation, Apoptosis, Migration and Invasion of AML Cells

Considering the above findings, miR-592 was directly interacted with ROCK1, we speculated whether miR-592 modulated the development and progression of AML via ROCK1. Hence, rescue experiments were carried out in the following study using HL-60, THP-1 and NB4 cells. The qRT-PCR assays suggested that ectopic expression of miR-592 significantly reduced the mRNA levels of ROCK1, while transfection of the ROCK1 overexpressing plasmids notably increased the ROCK1 levels (Figure 4A). Moreover, CCK-8 assays demonstrated that enhancing the expression of ROCK1 abrogated the suppressive effects of miR-592 on AML cell growth (Figure 4B). Thereafter, we employed flow cytometry to detect the alteration of apoptotic rates in HL-60 as well as THP-1 cells after they were transfected with miR-592 mimics or corresponding plasmids. As the data shown in Figure 4C, overexpression of miR-592 promoted apoptosis of HL-60 and THP-1 cells, while co-transfection of miR-592 mimics and ROCK1 overexpressing plasmids reduced

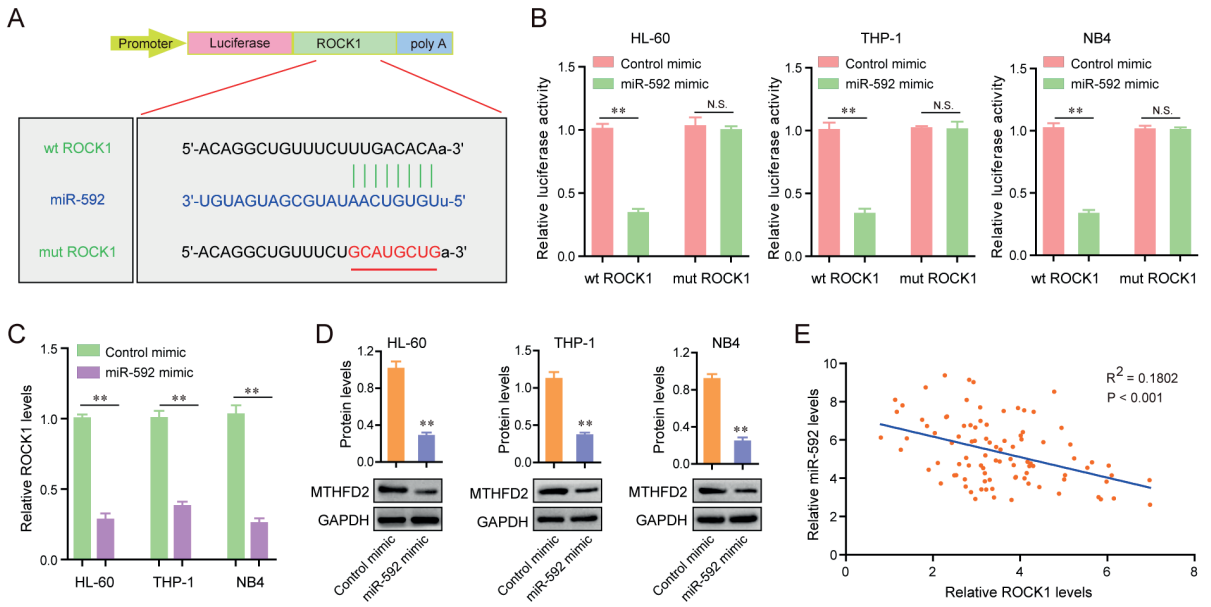

Figure 3. miR-592 directly targeted ROCK1 in AML cells. ***(A)*** "miRBD" predicted the binding sites of miR-592 in the 3'-untranslated region (3'-UTR) of ROCK1. (B) The luciferase activities of HL-60, THP-1 and NB4 cells transfected with related miRNA mimics or plasmids were detected by dual luciferase reporter assays. ***(C)*** The qRT-PCR analysis evaluated the relative ROCK1 expression. ***(D)*** Western blot assays detected the relative protein levels of ROCK1. ***(E)*** Pearson's correlation analysis evaluated the expressing relationship between miR-592 and ROCK1. *$p < 0.05$, **$p < 0.01$.

the apoptotic rates. Besides, by conducting the transwell migration assays, we found that ectopic expression of ROCK1 significantly increased the migratory abilities of HL-60 and THP-1 cells, which reversed the inhibitory effects of miR-592 on the cell migration (Figure 4D). Analogously, the invasive capabilities of HL-60 and THP-1 cells were remarkably reduced after transfecting with miR-592 mimics, while re-introduction of ROCK1 notably increased the invasive cell number of HL-60 and THP-1 cells (Figure 4E). Collectively, these data provided evidence that miR-592 modulated the development and progression of AML via ROCK1.

Discussion

Acute myeloid leukemia (AML) is a heterogeneous disease that is associated with a very poor prognosis[24]. With the change of life style and some other factors, the incidence rate of AML increased in recent years[25]. Identification of effective prognostic biomarkers were of vital importance to design the best therapeutic regimens for individualized treatment[26]. Increasing evidence revealed that deregulation of miRNAs is implicated in many tumors and the development of high throughput sequencing made it easy to detect dysregulated miRNAs[27,28]. In addition, more and more miRNAs were reported to act as important regulators whose expression levels were significantly associated with clinical prognosis of AML patients[29,30]. However, the studies about the potential of miRNAs as diagnostic and prognostic biomarkers in AML patients were limited. In this study, our attention focused on a tumor-related miRNA, miR-592, which has been reported to be abnormally expressed in several tumors and play important roles in regulation of tumor behaviors. By performing RT-PCR, we found that miR-592 expression was significantly down-regulated in both AML patients and cell lines. Furthermore, the association between serum miR-592 expression and clinical factors was statistically analyzed and the results showed that low miR-592 expression was significantly associated with advanced French-American-British classification and cytogenetics. Furthermore, survival assay indicated that low serum miR-592 expression was associated with shorter five-year overall survival. More importantly, univariate and multivariate analysis confirmed that miR-592 played a significant role of independent prognostic markers in overall sur-

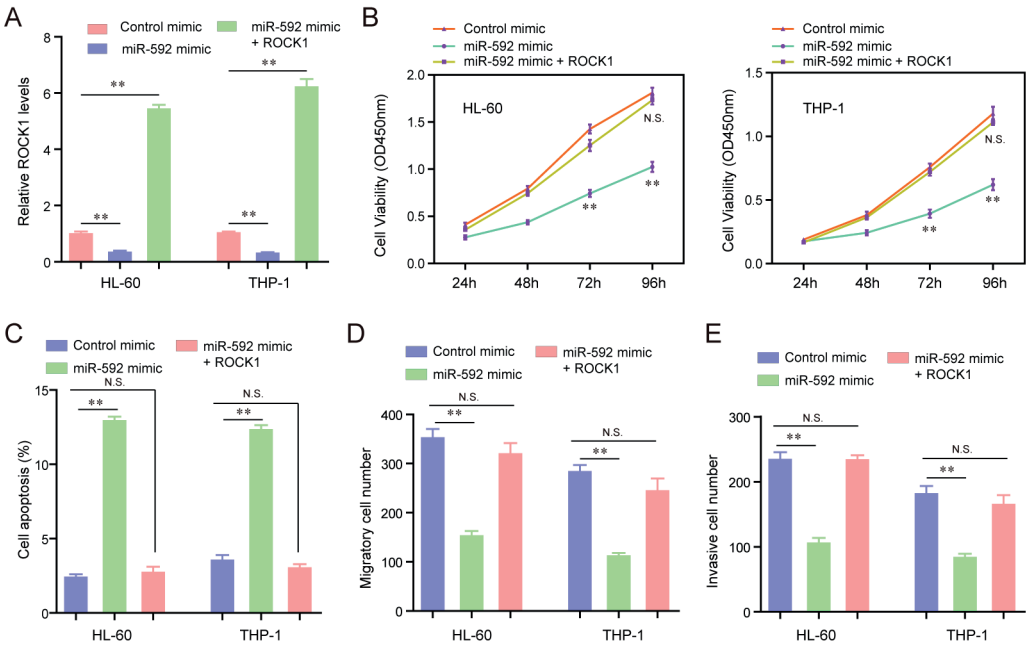

Figure 4. ROCK1 abrogated the suppressive effects of miR-592 on cell proliferation, apoptosis, migration as well as invasion. ***(A)*** The qRT-PCR analysis examined the relative ROCK1 expression in HL-60, THP-1 and NB4 cells. ***(B)*** Growth curves were determined by CCK-8 assays. ***(C)*** The apoptotic rates of HL-60, THP-1 and NB4 cells were assessed by flow cytometry. ***(D)*** The migratory cell number was detected by transwell migration assays. ***(E)*** The invasive cell number was determined by transwell invasion assays. $*p < 0.05$, $**p < 0.01$.

vival rates of AML patients. Thus, our studies provided first clinical evidence that miR-592 play a negative regulator in AML and could be used as a potential prognostic biomarker.

Recently, the expression pattern and biological function of miR-592 have been reported in several tumors. For instance, He et al[19] reported that miR-592 was highly expressed in gastric cancer and its forced expression could promoted tumor proliferation and metastasis by modulating MAPK/ERK signaling pathways. Fu et al[20] revealed that the expression levels of miR-592 was significantly up-regulated in human colorectal cancer and knockdown of miR-592 suppressed colorectal cancer cells proliferation and metastasis by targeting FoxO3A. Above findings indicated that miR-592 functioned as a tumor promoter in colorectal cancer and gastric cancer. On the other hand, interestingly, in non-small cell lung cancer, it was reported that miR-592 was lowly expressed and its lower levels in patients were associated with advanced clinical stages. *In vitro* and *in vivo* revealed that overexpression of miR-592 promoted cell proliferation, colony formation and metastasis by targeting SOX931.

Li et al[32] also suggested that miR-592 exhibited its tumor-suppressive roles in hepatocellular carcinoma by targeting DEK. Those findings revealed that miR-592 play different roles in development and progression of tumors according to the circumstance. However, the biological function of miR-592 in AML remains unknown. Based on our above findings, we assume that miR-592 may act as a tumor suppressor in AML and gain-of function assays were performed; we found that overexpression of miR-592 suppressed AML cells proliferation, migration and invasion, and promoted apoptosis. Thus, our findings revealed that miR-592 exhibited tumor-suppressive roles in AML just like its roles in hepatocellular carcinoma and non-small cell lung cancer.

Rho-associated protein kinase (ROCK) is a kinase belonging to the AGC family of serine-threonine kinases[33]. It includes ROCK1 and ROCK2. The ROCK1 genes contain 33 exons which lie the 18q11 chromosomal regions[34]. It has been confirmed that ROCK1 was involved in various biological progress and acted as critical regulator of actin-myosin contraction[35]. Recently, previous studies showed that ROCK1 provided a feedback mechanism and modulated the activity of upstream proteins Rac1 and RhoA, which played important roles in progression of tumors[36,37]. Thus, the roles of ROCK1 in tumor

progression also get attention. Indeed, increasing evidences had shown that ROCK1 was involved in the regulation of various tumors by modulating cell motility, metastasis, and angiogenesis[38,39]. In addition, it has been reported that the suppression of ROCK1 results in apoptosis in leukaemia cells, indicating that ROCK1 acted as a positive regulator in progression of AML[40]. Here, we identified ROCK1 as a direct target gene of miR-592 in AML cell by Dual-luciferase reporter assay and Western blot. Overexpression of miR-592 suppressed the levels of ROCK1 in AML cells and the expression of ROCK1 was negatively correlated with the expression level of miR-592 in the bone narrow of AML patients. Importantly, ROCK1 overexpression abolished the regulatory effect of up-regulation of miR-592 on three AML cells with decreased cell proliferation, migration and invasion, and increased apoptosis. Thus, our results implied that miR-592 played as tumor suppressor in the AML partly by inhibiting ROCK1 expression.

Conclusions

We provided first evidences that miR-592 was an independent prognostic factor for AML patients and that it suppressed the proliferation and metastasis of AML cells *in vitro* by targeting ROCK1. Our findings indicated that miR-592 may act as a novel prognostic biomarker and potential therapeutic agent for the treatment of AML.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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