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High expression of lnc-CRNDE presents as a biomarker for acute myeloid leukemia and promotes the malignant progression in acute myeloid leukemia cell line U937

Y. WANG, Q. ZHOU, J.-J. MA

Department of Hematology, Yantai Yuhuangding Hospital, Yantai, China

Yan Wang and *Qin Zhou* contributed equally to this work

Abstract. – OBJECTIVE: To detect the expression of long non-coding RNA-CRNDE in patients with acute myeloid leukemia and its effect on proliferation and apoptosis in acute myeloid leukemia cell line U937.

PATIENTS AND METHODS: 81 cases of newly diagnosed acute myeloid leukemia (AML) were enrolled, and 35 non-malignant hematological patients were selected as controls. Quantitative RT-PCR (qRT-PCR) was performed to detect the expression of lncRNA-CRNDE in the bone marrow specimens of the subjects, and the difference between the two groups was also compared. The correlation between the expression of lncRNA-CRNDE and the sex, age, classification and total survival of clinical patients was analyzed according to the clinical data. U937 cells and monocytes isolated from normal people were cultured, and the expression of lncRNA-CRNDE in acute myeloid leukemia cell line U937 and normal monocytes was compared. SiRNA-CRNDE and pcDNA-CRNDE were transfected into U937 cells, and cell counting kit-8 (CCK-8) assay was performed to detect proliferation of U937 cells, Annexin V/PI flow cytometry was carried out to detect cell apoptosis. Cell cycle was measured by flow cytometry.

RESULTS: The expression of lncRNA-CRNDE in patients with AML and U937 cells was significantly higher than that in non-malignant hematological controls. Results of clinical data showed that the expression of lncRNA-CRNDE was associated with the classification and total survival of myeloid leukemia in clinical patients. After transfection of siRNA-CRNDE, the proliferation and cloning ability of U937 cells decreased, while the apoptosis increased ($p < 0.01$) and cells were arrested in G0-G1 phase. Meanwhile, after transfection of pcDNA-CRNDE, the proliferation ability of U937 cells increased significantly, which indicated that the expression of lncRNA-CRNDE might play an essential role in promoting the proliferation of U937 cells.

CONCLUSIONS: LncRNA-CRNDE is highly expressed in the bone marrow tissues of AML patients, and the expression level is negatively correlated with the total survival of those clinical patients. Meanwhile, the expression is higher in FAB type M4 and M5 than that in M1, M2 and M3. LncRNA-CRNDE promotes the proliferation and cell cycle of U937 cells, and inhibits cell apoptosis, which is expected to become a molecular marker for predicting and treating AML.

Key Words:
Acute myeloid leukemia, LncRNA-CRNDE, LncRNA.

Introduction

Leukemia is one of the hematopoietic malignancies commonly seen in children and young adults. Its mortality rate ranks first among children and tumor deaths under 35 years old[1,2]. Acute myeloid leukemia (AML) is mainly caused by the malignant colony proliferation of the primitive myeloid cells in the hematopoietic system[3], which is characterized by abnormal proliferation of marrow blasts and the inhibition of the growth of normal hematopoietic cells[4]. Although hematopoietic stem cell transplantation and the emergence of some new drugs have shown some therapeutic effects on AML[5], there is still no curable treatment for other types of AML except for acute promyelocytic leukemia. Therefore, to find new diagnostic targets and further exploration of the pathogenesis is very important to improve the efficacy of AML[6].

Long non-coding RNA (lncRNA) is a kind of RNA with a length over 200 nt and has no function of protein coding[7]. Kapranov et al[8] have

Corresponding Author: Junjie Ma, MD; e-mail: majunjieyt@sina.com

found that only about 1/5 of human genome are protein encoding genes, which implies that the abundance of the non-coding RNA is at least four times than that of the coding RNA. Nowadays, around 520 thousand human lncRNAs have been identified[8,9]. Current studies have shown that non-coding RNA functions a lot in regulating the growth of organisms, cell differentiation, subcellular distribution and human diseases[10], which is specifically achieved by mechanisms of the recruitment of chromatin remodeling complex, regulation of transcription, promotion of translation and prevention of mRNA degradation[11,12]. In addition, various researchers have found that lncRNA is also involved in the development and progression of leukemia and other hematological diseases[13]. It participates in the proliferation, apoptosis, and invasion of tumor cells by acting the same role as oncogenes or tumor suppressor genes[14,15].

Based on the analysis of clinical data and functional experiments, we found that the expression of lncRNA-CRNDE was up-regulated in AML, and it was correlated with the total survival time and disease classification of patients. In the present study, we detected the expression of lncRNA-CRNDE in U937 cell line and normal mononuclear cells by qRT-PCR. Also, we observed the effects of lncRNA-CRNDE on proliferation and apoptosis of U937 cell line by RNA interference and overexpression.

Patients and Methods

Patients

A total of 116 patients treated in the Hematology Department of our hospital from June 2015 to March 2017 were enrolled. Their bone marrow specimens were collected and were approved by the medical Ethics Committee. Moreover, the informed consent of all patients was obtained. 81 AML patients were diagnosed as clarified AML by the standard of the French-America-British (FAB) and World Health Organization (WHO). All the patients were initially treated without any other malignancies and were not received other anti-tumor therapies. The median age was 35.7 years (18 to 64 years), including 47 males and 34 females.

Cell Culture

The acute myeloid leukemia cell line (U937) was provided by the American Type Collection Center (ATCC, Manassas, VA, USA) and cultured in completed Roswell Park Memorial Institute-1640 (RPMI-1640) (Gibco, Rockville, MD, USA), supplemented with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA). The cells were grown at 37°C with 5% CO_2. When the cell fusion reached 80%, the cell passage was performed, and the inoculation density was 1:2.

Transfection of siRNA and pcDNA

The cells were seeded into the 6-well plates, and Lipofectamine 2000 (Entranster-R4000 for pcDNA) was used for transfection when the cell fusion (Confluence) was around 60%. Firstly, 500 μL of serum-free suspension containing 10 μL of Lipofectamine 2000 and 10 μL of siRNA-CRNDE with the concentration of 20 nM (4 μL of Entranster-R4000 and 2.68 μg of pcDNA-CRNDE were used for pcDNA) were added. Next, 1.5 mL of RPMI-1640 medium (Gibco, Rockville, MD, USA) was added for cell culture. For the control group, an equal amount of Lipofectamine 2000 and siRNA-NC (Entranster-R4000 and pcDNA-NC for pcDNA) were added as the experimental group. The culture medium was replaced 6 h after transfection.

qRT-PCR

Total RNA was extracted according to the requirements of the TRIzol kit. 50 μL of reaction system were formulated according to the qRT-PCR instructions, and the reverse transcription reaction was performed under the following conditions: reverse transcription reaction at 50°C for 30 min, and denaturation of reverse transcriptase at 92°C for 3 min. The obtained cDNA was amplified by the following PCR amplification reaction conditions: denaturation at 92°C for 10 s, annealing at 55°C for 20 s, extension at 68°C for 20 s, for a total of 40 cycles. β-action was used as the internal reference, and the relative expression of CRNDE was represented by $2^{-\Delta\Delta Ct}$. Primers used in qRT-PCR were as follows: CRNDE: 5'-TGGATGCTGTCAGCTAAGTTCAC-3' (forward), 5'-TTCCAGTGGCATCCTCCTTATC-3' (reverse); β-action: 5'-CTCCATCCTGGCCTCGCT-GT-3' (forward), 5'-GCTGTCACCTTCACCGT-TCC-3' (reverse). Small interference sequences used in transfection were as follows: si-CRNDE:5'-UAUGGAAGCAUCACACUUAACACCU-3', si-Scramble: 5'-GGATGATCGAAGATGAGACTAGCTT-3'.

Cell Counting Kit-8 (CCK-8) Assay for Cell Proliferation

The transfection point was set as 0 h, the control cells and the treatment cells were seeded into the 96-well plates with 6 replicate wells for each and 5,000 cells in each well. After 6 h, the activity of cells after adherence was measured (0 h); then, at the following four time points of 24 h, 48 h, 72 h and 96 h, 20 µL of CCK-8 solution were added to each well and incubated at 37°C with 5% CO_2 for 2-3 h. The microplate reader was used to measure the absorbance (D) values at 450 wavelengths. Meanwhile, only the CCK-8 solution and the medium (with no cells) were added to the blank controls.

Colony Formation Assay

The transfection point was set as 0 h, the medium was replaced after 6 h, and 3,000 cells were reseeded into the medium plates after 24 h and incubated at 37°C with 5% CO_2 for 14 days; the culture medium was replaced every 2 days. The culture medium was discarded, phosphate-buffered saline (PBS) was used to wash twice, 5% paraformaldehyde was applied to fix the colonies for 30 min. Then, the waste liquid was discarded, 0.1% crystal violet solution was added to each plate and discarded after 30 min; phosphate-buffered saline (PBS) was used to wash. Finally, images of all the dishes were captured and counted.

Statistical Analysis

We used statistical product and service solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) for all the statistical analysis and GraphPad Prism 6.0 (Version X; La Jolla, CA, USA) for editing the images. Kaplan-Meier survival curve was used for survival analysis, and the statistical indicators in the survival analysis were included in the COX regression. The measurement data were represented as $\bar{x} \pm s$; the difference was compared by t-test, while x^2-test was performed for categorical variables. $p < 0.05$ was considered statistically significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.001$.

Results

Expression of CRNDE in Bone Marrow and Cells of AML Patients

We detected the expression of lncRNA-CRNDE in 81 AML patients and 35 non-malignant hematological patients. Results showed that the expression level of lncRNA-CRNDE was significantly higher in AML patients. The median expression level of lncRNA-CRNDE (5.274 ± 0.2518, n = 85) relative to β-actin in AML patients was 3.37 times higher than that of the controls (1.565 ± 0.1047, n = 31) (Figure 1A). Moreover, the expression level of lncRNA-CRNDE in the U937 cell line was higher than the normal cells (Figure 1B).

Relationship Between the Expression of CRNDE and Clinical Characteristics and Prognosis of AML

We investigated the relationship between the expression of CRNDE and pathological character-

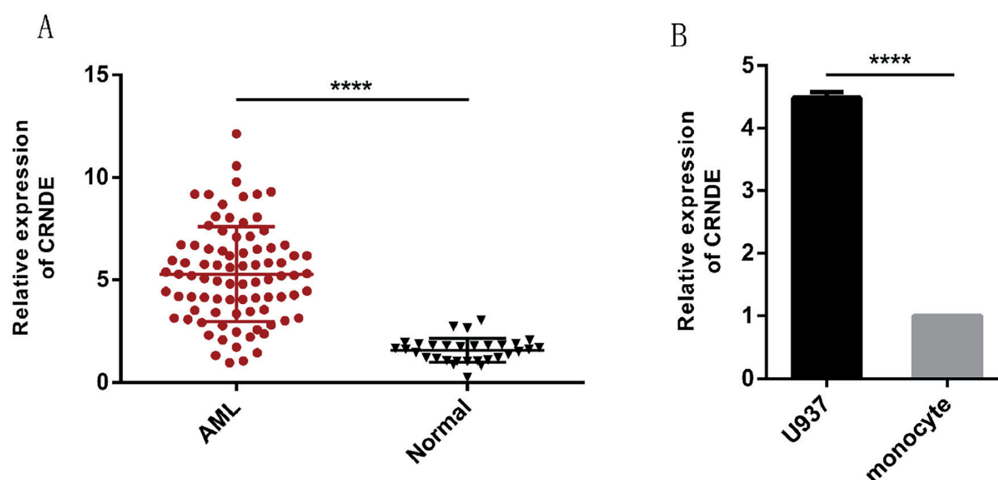

Figure 1. ***A,*** The expression of lncRNA-CRNDE in the bone marrow tissues of AML patients (n = 81) was higher than the patients with non-malignant hematological diseases (n = 35). ***B,*** The expression of lncRNA-CRNDE was higher in myeloid leukemia cell line U937 than that in normal monocytes.

Table I. Clinical data of patients.

Parameters	Relative lncRNA-CRNDE expression		*p*-value
	Fold change > 3.37	Fold change ≤ 3.37	
Age (years)			
< 35	28	20	0.38
≥ 35	16	17	
Gender			
Male	28	20	0.25
Female	14	17	
FAB			
M1	2	3	
M2	10	11	
M3	4	5	0.74
M4	10	6	
M5	18	12	

istics of the patients and the results indicated that there was no significant relationship between the expression level of lncRNA-CRNDE and age, sex and disease classification of AML patients (Table I). Through comparison between each group, we found the expression was higher in AML patients with FAB type M4 and M5 than that in M1, M2 and M3 (Figure 2A). In addition, analysis of the clinical data suggested that the total survival time of AML patients was negatively correlated with the expression of lncRNA-CRNDE (Figure 2B).

Down-regulation of CRNDE Inhibited the Proliferation and Colony Formation of the U937 Cell Line, Promoting Its Apoptosis

After transfection of siRNA-CRNDE, the expression of lncRNA-CRNDE was significantly decreased in the U937 cell line (Figure 3A). We also detected the proliferation ability of the experimental group and the control group by CCK-8 assay at 0 h, 24 h, 48 h, 72 h, and 96 h after inoculation. Results revealed that the proliferation ability of the U937 cell line was significantly suppressed at 48 h, 72 h and 96 h after transfection of siRNA-CRNDE ($p < 0.05$) (Figure 3B). The colony formation assay indicated that the number of clones formed in the normal control group (siRNA-NC) was much more than that of the experimental group (siRNA-CRNDE) (Figure 3C), suggesting that the ability of colony formation was weakened after the interference of lncRNA-CRNDE in the U937 cell line. Additionally, flow cytometry analysis demonstrated that apoptosis of the U937 cell line was increased (Figure 3D) and the cells were blocked at G0/G1 phase (Figure 3E) after the treatment of lncRNA-CRNDE.

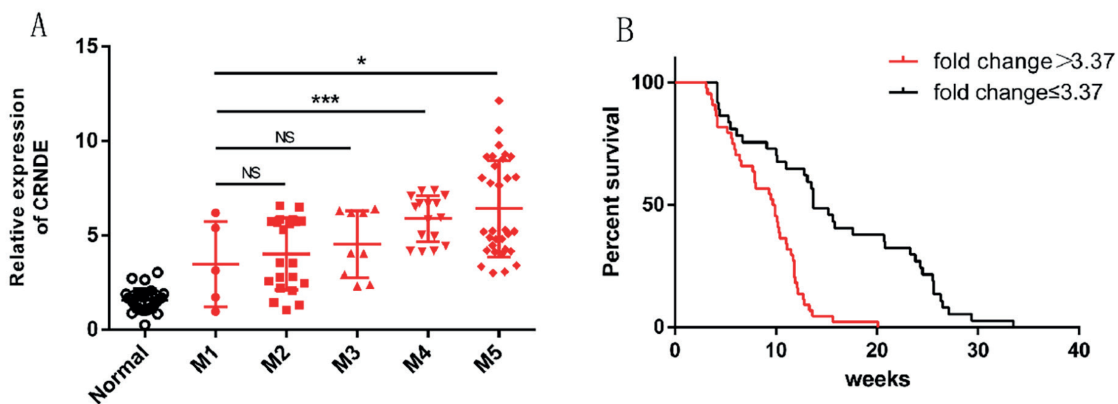

Figure 2. ***A,*** The expression of lncRNA-CRNDE in the bone marrow tissues of FAB type M4 and M5 was higher than that of type M1, M2 and M3. ***B,*** The total survival time of AML patients with higher expression of lncRNA in the bone marrow tissues (fold change > 3.37) was significantly lower than those with a relatively low expression (fold change ≤ 3.37).

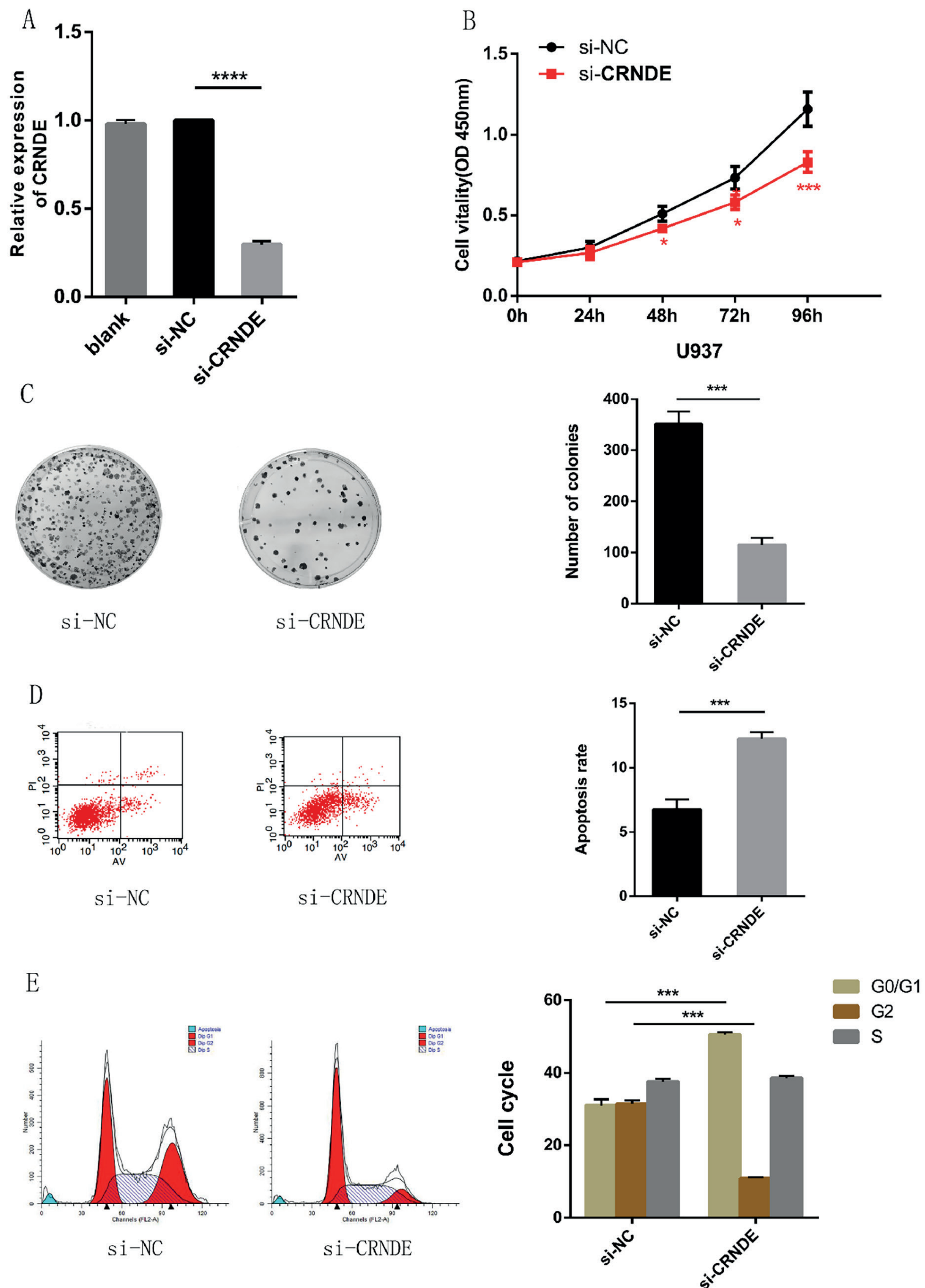

Figure 3. ***A,*** After transfection of si-CRNDE, the expression of lncRNA-CRNDE in U937 cells was significantly reduced. ***B,*** CCK-8 assay showed that the proliferation of U937 cells was significantly inhibited after transfection of si-CRNDE. ***C,*** Colony formation assay showed that the ability to form cloned cell groups was decreased significantly in U937 cells after transfection of si-CRNDE. ***D,*** Apoptosis analysis showed that the apoptosis of U937 cells was significantly increased after transfection of si-CRNDE. ***E,*** Cell cycle analysis showed that the cell cycle of U937 cells was blocked in G0/G1 phase after transfection of si-CRNDE.

Overexpression of CRNDE Promoted the Proliferation and Colony Formation of the U937 Cell Line

After the construction of the corresponding plasmids, pcDNA-NC and pcDNA-CRNDE were transferred to the U937 cell line for subsequent experiments (Figure 4A). The CCK-8 assay revealed that, compared with transfection of pcDNA-NC as a negative control, the D450 value of the U937 cell line transfected with pcDNA-CRNDE increased significantly, indicating that overexpression of lncRNA-CRNDE could promote the proliferation of the U937 cells (Figure 4B). The colony formation assay showed that the number of colonies formed in the control group (pcDNA-NC) was much lower than that of the overexpression (pcDNA-CRNDE) group (Figure 4C). The above experiments illustrated that lncRNA-CRNDE might promote the proliferation of the U937 cells.

Discussion

AML is a hematopoietic malignancy with large heterogeneity both in cell genetics and molecular genetics[16]. In recent years, with the rapid development of molecular biology, some abnor-

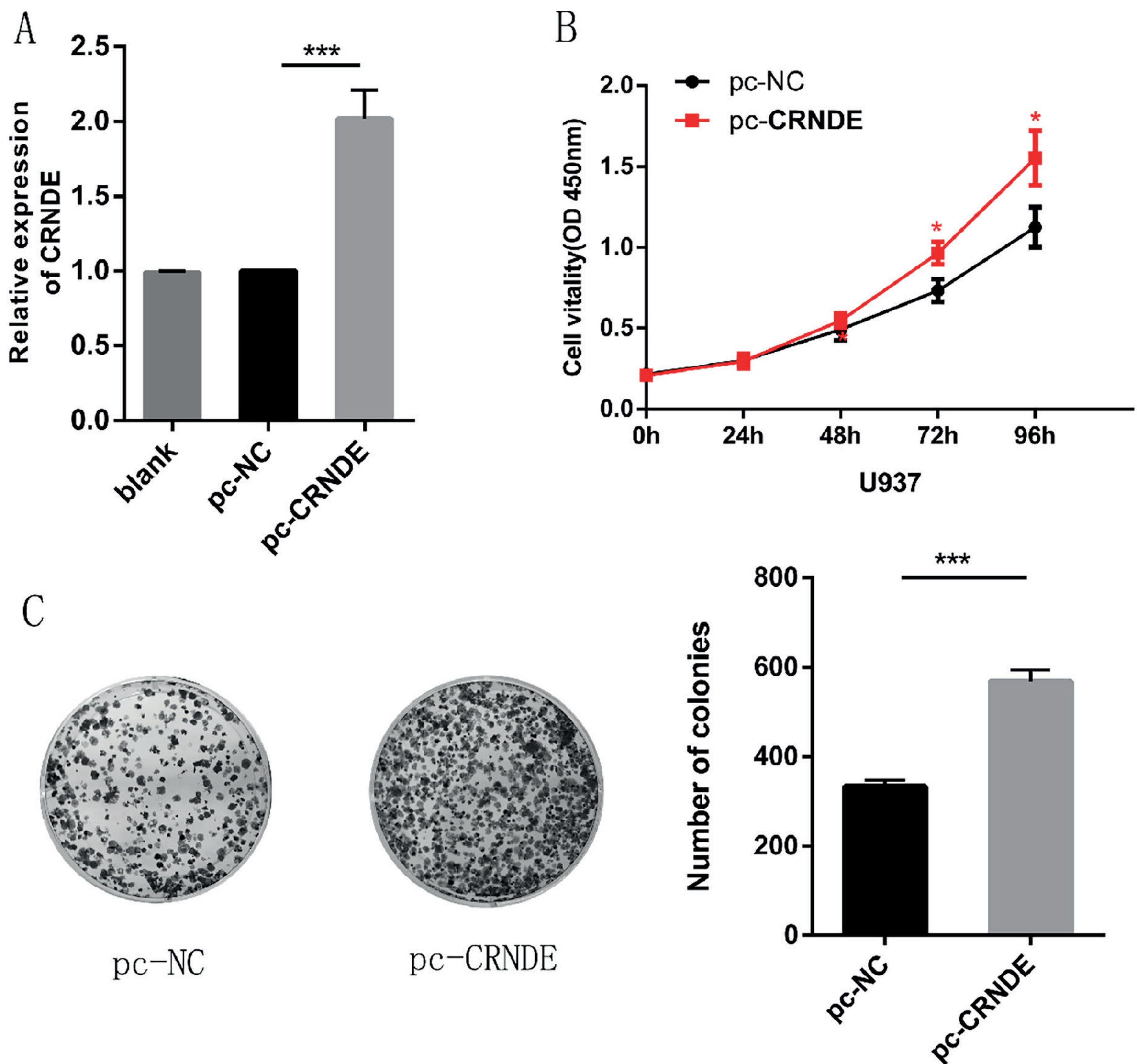

Figure 4. ***A,*** The expression of lncRNA-CRNDE in U937 cells was significantly increased after transfection of pc-CRNDE. ***B,*** CCK-8 assay showed that the proliferation of mice and human osteocytes was obviously active after transfection of pc-CRNDE. ***C,*** Colony formation assay showed that the ability to form cloned cell groups was increased significantly in U937 cells after transfection of pc-CRNDE.

mal changes have been detected at the molecular genetic level in AML patients. Chromosomal abnormalities, gene mutations and gene expression levels have become important prognostic indicators for AML[17,18], which is of great importance for clinical prognosis evaluation and treatment of AML. In clinical practice, we have found that the therapeutic effects of these patients vary markedly, suggesting that some patients may be accompanied by some abnormal changes in the molecular level. However, we have not yet discovered these changes, which implies that new detection methods and treatment should be used to achieve good clinical efficacy. Therefore, some markers may reflect clinical diagnosis; we considered prognosis as particularly necessary in choosing the proper and effective treatment.

Finding new molecular markers and evaluating their risk stratification for AML patients can not only guide the choice of treatment plan, but also provides a theoretical and experimental basis for the development of targeted therapeutic drugs. With the increasing understanding of the lncRNA function, the association between lncRNA and the occurrence of diseases has attracted more and more attention[19]. The regulation of gene expression by lncRNA is mainly reflected in three levels, including epigenetic modification regulation, transcriptional regulation and posttranscriptional regulation[20]. Multiple studies have shown that abnormal expression of lncRNA can lead to the development of tumor[21]. Though the understanding of the pathogenesis of lncRNA is not deep enough, it is commonly expressed in tumor cells as an ultra-conservative element in the process of species evolution[22]. This kind of ultra conservative element originally plays an essential function in the development of normal individuals; however, its abnormal expression may ultimately lead to tumors[23,24].

In the present study, qRT-PCR detection indicated that the expression of lncRNA-CRNDE in the bone marrow tissues of AML patients was high, which was also increased in tumor cell line U937. *In vitro* experiments demonstrated that the interference with lncRNA-CRNDE could suppress the growth of tumor cells, arrest cell cycle in the G0/G1 phase and promote cell apoptosis. Similarly, after overexpression of lncRNA-CRNDE, cell proliferation ability of the experimental group was significantly enhanced when compared with the negative control group and the blank control group. All the related experimental results suggested that lncRNA-CRNDE might significantly promote the proliferation of the U937 cell line and inhibit cell apoptosis.

The expression of lncRNA-CRNDE in AML patients was increased, which was especially higher in M4 and M5 than M1, M2 and M3, leading to a shorter total survival time. LncRNA-CRNDE could promote the proliferation and cell cycle of the U937 cell line, and inhibit apoptosis. It is suggested that more researches should be focused on the biological functions and the relevant mechanisms of lncRNA-CRNDE in AML in future.

Conclusions

l ncRNA-CRNDE is highly expressed in the bone marrow tissues of AML patients, and the expression level is negatively correlated with the total survival time. Meanwhile, in patients of FAB type M4 and M5, its expression is higher than patients of FAB type M1, M2, and M3. As lncRNA-CRNDE promotes proliferation and cell cycle of U937 and suppresses cell apoptosis, it is expected to become a molecular marker and potential therapeutic target for predicting AML and its prognosis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Grosso DA, Hess RC, Weiss MA. Immunotherapy in acute myeloid leukemia. Cancer Am Cancer Soc 2015; 121: 2689-2704.
2) Xia HL, Li CJ, Hou XF, Zhang H, Wu ZH, Wang J. Interferon-gamma affects leukemia cell apoptosis through regulating Fas/FasL signaling pathway. Eur Rev Med Pharmacol Sci 2017; 21: 2244-2248.
3) Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, Koboldt DC, Fulton RS, Delehaunty KD, McGrath SD, Fulton LA, Locke DP, Magrini VJ, Abbott RM, Vickery TL, Reed JS, Robinson JS, Wylie T, Smith SM, Carmichael L, Eldred JM, Harris CC, Walker J, Peck JB, Du F, Dukes AF, Sanderson GE, Brummett AM, Clark E, McMichael JF, Meyer RJ, Schindler JK, Pohl CS, Wallis JW, Shi X, Lin L, Schmidt H, Tang Y, Haipek C, Wiechert ME, Ivy JV, Kalicki J, Elliott G, Ries RE, Payton JE, Westervelt P, Tomasson MH, Watson MA, Baty J, Heath S, Shannon WD, Nagarajan R, Link DC, Walter MJ, Graubert TA, DiPersio JF, Wilson RK, Ley TJ. Recurring mutations found by sequencing an acute myeloid

leukemia genome. N Engl J Med 2009; 361: 1058-1066.

4) Montalban-Bravo G, Garcia-Manero G. Myelodysplastic syndromes: 2018 update on diagnosis, risk-stratification and management. Am J Hematol 2018; 93: 129-147.

5) Dixon SB, Lane A, O'Brien MM, Burns KC, Mangino JL, Breese EH, Absalon MJ, Perentesis JP, Phillips CL. Viral surveillance using PCR during treatment of AML and ALL. Pediatr Blood Cancer 2018; 65(1). doi: 10.1002/pbc.26752.

6) Ustun C, Marcucci G. Emerging diagnostic and therapeutic approaches in core binding factor acute myeloid leukaemia. Curr Opin Hematol 2015; 22: 85-91.

7) Wu B, Zhang XJ, Li XG, Jiang LS, He F. Long non-coding RNA Loc344887 is a potential prognostic biomarker in non-small cell lung cancer. Eur Rev Med Pharmacol Sci 2017; 21: 3808-3812.

8) Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, Stadler PF, Hertel J, Hackermuller J, Hofacker IL, Bell I, Cheung E, Drenkow J, Dumais E, Patel S, Helt G, Ganesh M, Ghosh S, Piccolboni A, Sementchenko V, Tammana H, Gingeras TR. RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science 2007; 316: 1484-1488.

9) Hon CC, Ramilowski JA, Harshbarger J, Bertin N, Rackham OJ, Gough J, Denisenko E, Schmeier S, Poulsen TM, Severin J, Lizio M, Kawaji H, Kasukawa T, Itoh M, Burroughs AM, Noma S, Djebali S, Alam T, Medvedeva YA, Testa AC, Lipovich L, Yip CW, Abugessaisa I, Mendez M, Hasegawa A, Tang D, Lassmann T, Heutink P, Babina M, Wells CA, Kojima S, Nakamura Y, Suzuki H, Daub CO, de Hoon MJ, Arner E, Hayashizaki Y, Carninci P, Forrest AR. An atlas of human long non-coding RNAs with accurate 5' ends. Nature 2017; 543: 199-204.

10) Chernyak N, Kushnir T. Giving preschoolers choice increases sharing behavior. Psychol Sci 2013; 24: 1971-1979.

11) Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E, Chang HY. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 2007; 129: 1311-1323.

12) Carrieri C, Cimatti L, Biagioli M, Beugnet A, Zucchelli S, Fedele S, Pesce E, Ferrer I, Collavin L, Santoro C, Forrest AR, Carninci P, Biffo S, Stupka E, Gustincich S. Long non-coding antisense RNA controls Uchl1 translation through an embedded SINEB2 repeat. Nature 2012; 491: 454-457.

13) Zebisch A, Hatzl S, Pichler M, Wolfler A, Sill H. Therapeutic resistance in acute myeloid leukemia: the role of non-coding RNAs. Int J Mol Sci 2016; 17(12). pii: E2080.

14) Fang C, Qiu S, Sun F, Li W, Wang Z, Yue B, Wu X, Yan D. Long non-coding RNA HNF1A-AS1 mediated repression of miR-34a/SIRT1/p53 feedback loop promotes the metastatic progression of colon cancer by functioning as a competing endogenous RNA. Cancer Lett 2017; 410: 50-62.

15) Ma X, Qi S, Duan Z, Liao H, Yang B, Wang W, Tan J, Li Q, Xia X. Long non-coding RNA LOC554202 modulates chordoma cell proliferation and invasion by recruiting EZH2 and regulating miR-31 expression. Cell Prolif 2017; 50(6). doi: 10.1111/cpr.12388.

16) Salerno L, Romeo G, Modica MN, Amata E, Sorrenti V, Barbagallo I, Pittala V. Heme oxygenase-1: a new druggable target in the management of chronic and acute myeloid leukemia. Eur J Med Chem 2017; 142: 163-178.

17) Mrozek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. Blood Rev 2004; 18: 115-136.

18) Byrd JC, Mrozek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, Pettenati MJ, Patil SR, Rao KW, Watson MS, Koduru PR, Moore JO, Stone RM, Mayer RJ, Feldman EJ, Davey FR, Schiffer CA, Larson RA, Bloomfield CD. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood 2002; 100: 4325-4336.

19) Sun T. Long noncoding RNAs act as regulators of autophagy in cancer. Pharmacol Res 2017 Nov 10. pii: S1043-6618(17)30988-X. doi: 10.1016/j.phrs.2017.11.009. [Epub ahead of print]

20) Tang Y, Zhou T, Yu X, Xue Z, Shen N. The role of long non-coding RNAs in rheumatic diseases. Nat Rev Rheumatol 2017; 13: 657-669.

21) Peng WX, Koirala P, Mo YY. LncRNA-mediated regulation of cell signaling in cancer. Oncogene 2017; 36: 5661-5667.

22) Calin GA, Liu CG, Ferracin M, Hyslop T, Spizzo R, Sevignani C, Fabbri M, Cimmino A, Lee EJ, Wojcik SE, Shimizu M, Tili E, Rossi S, Taccioli C, Pichiorri F, Liu X, Zupo S, Herlea V, Gramantieri L, Lanza G, Alder H, Rassenti L, Volinia S, Schmittgen TD, Kipps TJ, Negrini M, Croce CM. Ultraconserved regions encoding ncRNAs are altered in human leukemias and carcinomas. Cancer Cell 2007; 12: 215-229.

23) Qi X, Chen Z, Liu D, Cen J, Gu M. Expression of Dlk1 gene in myelodysplastic syndrome determined by microarray, and its effects on leukemia cells. Int J Mol Med 2008; 22: 61-68.

24) Sun L, Gao J, Yuan LX, Chen TT, Pan LL, Zhou CY, Zhu YP. [Expression of mitochondrial ferritin in K562 leukemic cell during TPA-induced cell differentiation]. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2007; 15: 272-277.