

Original Article

LncRNA LINC00265/miR-485-5p/IRF2-mediated autophagy suppresses apoptosis in acute myeloid leukemia cells

Feng Zhang^{1*}, Qianwen Li^{1*}, Kai Zhu¹, Junfeng Zhu¹, Jiajia Li¹, Yuan Yuan¹, Pingping Zhang¹, Lili Zhou², Lin Liu¹

Departments of ¹Hematology, ²Hematology Laboratory, The First Affiliated Hospital of Bengbu Medical College, Bengbu 233004, Anhui, PR China. *Equal contributors and co-first authors.

Received November 25, 2019; Accepted May 18, 2020; Epub June 15, 2020; Published June 30, 2020

Abstract: Background: Extensive studies have shown that long non-coding RNAs (lncRNAs) play important roles in multiple cancers. The present study aimed to investigate the role and mechanism of lncRNA LINC00265 in the regulation of apoptosis in acute myeloid leukemia (AML) cells. Methods: Gain- or loss-of-function experiments were conducted in AML cells to explore the effect of LINC00265 on AML. Autophagy was assessed by examining levels of Beclin-1, p62, and ratio of LC3-II/LC3-I. Cell proliferation and apoptosis of AML cells were evaluated by CCK-8 assay and flow cytometry, respectively. RNA pull-down was performed to enrich miR-485-5p interacted with LINC00265. The interaction between miR-485-5p and IRF2 3'UTR was analyzed by luciferase reporter assay. Results: LINC00265 expression was significantly up-regulated, whereas miR-485-5p was down-regulated in serum of AML patients and AML cell lines. LINC00265 promoted, whereas miR-485-5p suppressed autophagy in AML cells. Mechanistically, LINC00265 functioned as a ceRNA for miR-485-5p to facilitate IRF2 expression. More importantly, LINC00265 overexpression or miR-485-5p inhibitor reversed the 3-methyladenine (3-MA, an autophagy inhibitor)-mediated proliferation-inhibitory and pro-apoptotic effects, whereas LINC00265 silencing or miR-485-5p mimic overturned the proliferation-promoting and anti-apoptotic effects of autophagy activator rapamycin. Conclusion: LINC00265 attenuates AML cell apoptosis by inducing autophagy via miR-485/IRF2 axis.

Keywords: LINC00265, autophagy, apoptosis, acute myeloid leukemia

Introduction

Acute myeloid leukemia (AML) is an aggressive malignant disease defined by abnormal expansion of myeloid blasts [1]. Despite rapid progress in the treatment for AML, the long-term survival rate was still low [2]. Thus, elucidating the molecular mechanisms underlying the pathogenesis of AML is of great significance to explore novel therapeutic strategies for AML.

Autophagy is a vital, physiological catabolic process for cell survival by which cells clear damaged organelles and recycle nutrients to maintain homeostasis [3]. Increasing evidences reveal that activation of apoptosis and autophagy, two interdependent machineries, often occurs simultaneously [4]. Studies have suggested that understanding the crosstalk

between apoptosis and autophagy in AML is critical to improving treatment efficiency. Evidence has indicated that targeting autophagy might provide new therapeutic options for treatment of AML [5]. For instance, Liu et al reported that Atg5-dependent autophagy contributed to AML development [6].

Accumulating investigation demonstrates that long non-coding RNAs (lncRNAs) play crucial roles in diverse biological processes. Dozens of lncRNAs have been identified as regulators of normal and/or malignant hematopoiesis [7]. The latest study found that SBF2-AS1 [8], HOTAIR [9], KCNQ10T1 [10], LINC00265 [11], LINC00899 [12], etc, were significantly up-regulated in the serum of AML patients, which can be used as a target for the diagnosis, prognosis and treatment of AML. Among them,

LINC00265 induced autophagy in AML cells

LINC00265 may regulate the proliferation, migration and invasion function of AML cells by modulation of the PI3K/AKT signaling pathway [11], but the autophagy regulation of AML by LINC00265 has not been elucidated.

Our previous study showed that interferon-regulatory factor 2 (IRF2) induced autophagy and inhibited apoptosis in AML cells through binding to the inositol polyphosphate-4-phosphatase, type-II (INPP4B) promoter [13]. A large number of studies have reported that miR-485-5p was down-regulated in a variety of tumors and plays an anti-cancer role [14-17], but its expression and role in AML are still unknown. Through software predictive analysis, we found that miR-485-5p could bind to the 3'UTR of IRF2, and LINC00265 harbored a miR-485-5p binding site. Therefore, we hypothesized that LINC00265 promoted autophagy and inhibited apoptosis of AML cells by regulating the signal axis of miR-485/IRF2.

In this study, we examined the expression and functional roles of LINC00265 and miR-485-5p in AML cell lines. Furthermore, we investigated their effects on the interplay between autophagy and apoptosis. Our findings provide new insights into the role of LINC00265 and miR-485-5p in regulating AML progression.

Material and method

Blood samples

A total of 20 newly diagnosed patients with AML participants and 20 healthy controls were recruited by The First Affiliated Hospital of Bengbu Medical College. Peripheral venous blood samples were collected from each participant in a fasting state in the morning. A written informed consent was obtained by each participant and the research protocol was approved by the Ethics Committee of The First Affiliated Hospital of Bengbu Medical College.

qRT-PCR analysis

Total RNA was extracted from serum samples or AML cell lines using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed to cDNA using a PrimeScript RT Reagent Kit (TaKaRa, Dalian, China). The expression of matured mouse miR-485-5p was determined by a stem-loop real-time PCR system using

Maxima™ SYBR Green qPCR Master Mix and StepOne™ sequence detector. The relative quantification of LINC00265 expression and mRNA levels of IRF2, Beclin-1 and LC3 were determined using SYBR Premix Dimmer Eraser kit (TaKaRa) by the ABI7900 system (Applied Biosystem). Change in level of expression was compared with GAPDH or U6.

Cell transfection and treatment

The acute myeloid cells OCI/AML-2, acute monocyte leukemia cell line THP-1 and human normal stromal cells HS-5 were purchased from the ATCC. Specific small interfering RNAs (siRNA) against LINC00265, miR-485-5p mimic/inhibitor and their negative control siRNAs (NC) were synthesized by GenePharma (Shanghai, China). The LINC00265 overexpressed plasmid and an empty plasmid vector (as negative control) were also purchased from GenePharma. OCI/AML-2 or THP-1 cells were transfected with si-LINC00265, miR-485-5p mimic, LINC00265 plasmid or miR-485-5p inhibitor for 48 h using Lipofectamine2000 reagent (Invitrogen, CA, USA) according to the manufacturer's protocols. Following transfection, cells were treated with 3-methyladenine (3-MA; an autophagy inhibitor; 10 mmol/L; Sigma-Aldrich, USA), rapamycin (an autophagy activator; 10 µg/L; LC Laboratories, USA) or 0.1% DMSO (vehicle; Sigma-Aldrich) for another 24 h. After this, cells were harvested to determine the expression of autophagy-related proteins and cell proliferation and apoptosis.

Western blot analysis

Total proteins were extracted from OCI/AML-2 cells or THP-1 cells using RIPA lysis buffer (Beyotime, Shanghai, China). Equal volume of protein were subjected to SDS-PAGE and transferred onto PVDF. Subsequently, the membranes were blocked in PBS with 5% skim milk, and then incubated overnight at 4°C with primary antibodies, followed by another 2 h of incubation with HRP conjugated secondary antibodies at room temperature. The immunoreactive bands were detected using the ECL kit (Thermo Scientific, Waltham, MA, USA). The primary antibodies were as follows: IRF2 (1:1000 dilution, Cell Signaling Technology, USA), Beclin-1 (1:200, Santa Cruz, CA, USA), LC3-I (1:1000, Santa Cruz, CA, USA), LC3-II (1:1000,

LINC00265 induced autophagy in AML cells

Santa Cruz, CA, USA) and p62 (1:1000 dilution, Cell Signaling Technology, USA).

RNA pull-down

The AML cells were transfected LINC00265 and vector (ctrl). Biotinylated LINC00265 was used to carry out RNA pull-down. The lysates were collected and incubated with streptavidin magnetic bead (Invitrogen). The product was then treated with RNase-free DNase I (Roche, USA) and purified using RNeasy Mini Kit (Qiagen, USA). The miR-485-5p was detected by qRT-PCR from the pull-down samples.

Luciferase reporter assay

Our bioinformatics analysis (targetscan) revealed that IRF2 was a putative target of miR-485-5p. A dual-luciferase reporter assay was performed to evaluate the interaction between miR-485-5p and the 3'-UTR of IRF2 mRNA. The cells were co-transfected with IRF2-WT and IRF2-Mut and miR-485-5p mimic or mimic NC using Lipofectamine2000. The dual-luciferase assay kit (Promega, Madison, WI, USA) was used to determine the luciferase activity. Firefly luciferase activity was normalized to *Renilla* luciferase activity.

Cell proliferation assay

Cell proliferation was evaluated using the CCK-8 kit (Beyotime). Briefly, the treated cells were collected, and the culture supernatants were then changed to fresh medium with 10% CCK-8. The absorbance at 450 nm was measured by the Multi-Mode Microplate Reader.

Cell apoptosis assay

Cell apoptosis was analyzed using an Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) cell apoptosis kit (Invitrogen) according to the instructions. The cells were detected by flow cytometry and results were analyzed by FlowJo software.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 5.0, the data were represented as mean \pm standard deviation (SD). Statistical analyses were performed with a t-test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

LINC00265 was highly expressed in AML patients and AML cell lines

Compared with the healthy control, serum LINC00265 expression in AML patients was significantly up-regulated (**Figure 1A**), while the miR-485-5p expression was obvious down-regulated in AML patients (**Figure 1B**). And then, we detect the expression of LINC00265 and miR-485-5p in two AML cell lines. As shown in **Figure 1C**, we found that higher expression of LINC00265 was detected in two AML cell lines when compared with HS-5. Similarly, the expression level of miR-485-5p was significantly decreased in the two AML cell lines (**Figure 1D**). In addition, we also detect the expression of autophagy-related LC3 and Beclin-1 in two AML cell lines. As shown in **Figure 1E**, the expression of LC3 and Beclin-1 were both increased in two AML cell lines when compared with HS-5. Taken together, our findings suggested that LINC00265 was highly expressed in AML patients and AML cell lines, and on the contrary, miR-485-5p was lowly expressed in AML patients and AML cell lines.

The effect of LINC00265 and miR-485-5p on AML cell autophagy

To investigate the effect of LINC00265 on AML cell autophagy, the OCI/AML-2 cell was transfected with si-ctrl and si-LINC00265, and the THP-1 cell was transfected with vector and LINC00265 plasmid. The expression of LINC00265 was significantly decreased by the si-LINC00265 (**Figure 2A**). As shown in **Figure 2B**, we found that si-LINC00265 caused the decreased LC3-II/LC3-I ratio and Beclin-1 protein expression, and the increased p62 protein expression, suggested that knockdown of LINC00265 inhibited AML cell autophagy. The expression of LINC00265 was significantly increased by the LINC00265 plasmid (**Figure 2C**). As shown in **Figure 2D**, we found that LINC00265 plasmid caused the increased LC3-II/LC3-I ratio and Beclin-1 protein expression, and the reduced p62 protein expression, suggested that over-expression of LINC00265 promoted AML cell autophagy.

To investigate the effect of miR-485-5p on AML cell autophagy, the OCI/AML-2 cell was trans-

LINC00265 induced autophagy in AML cells

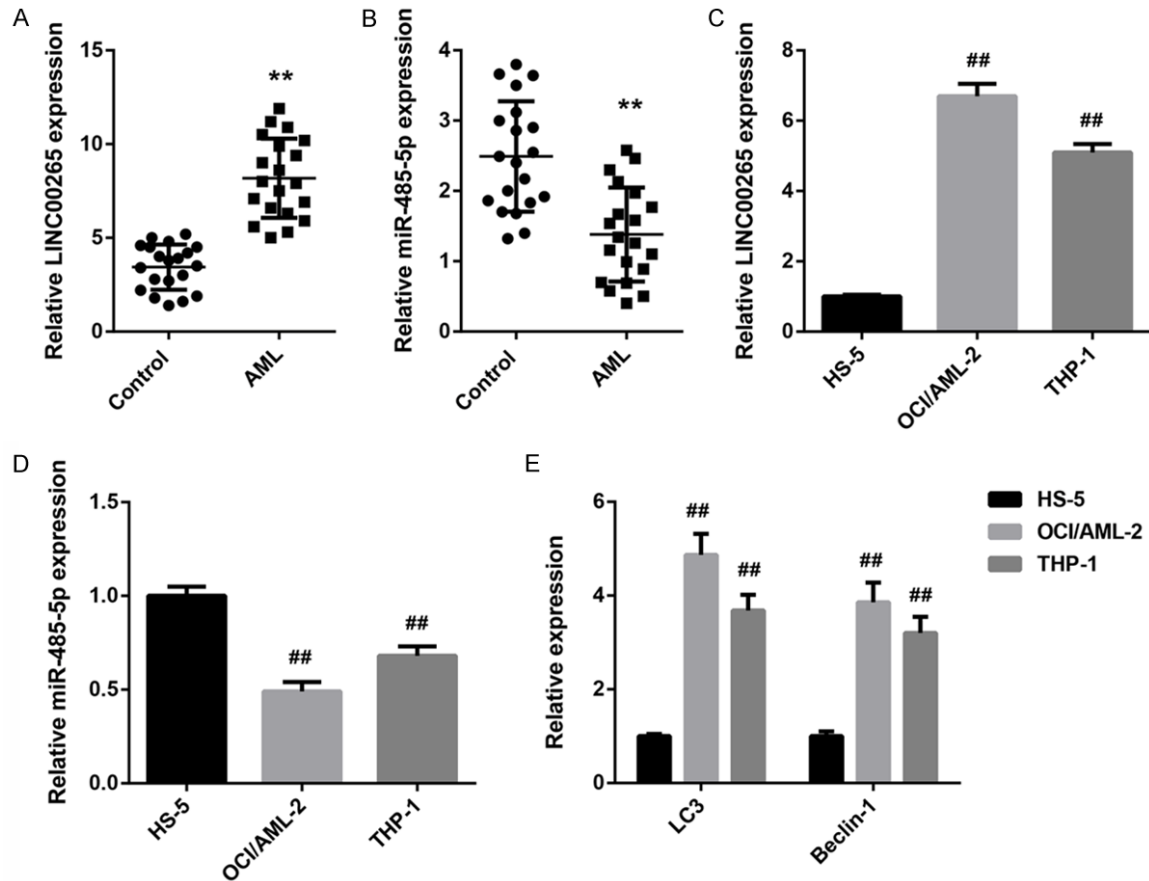


Figure 1. LINC00265 was highly expressed in peripheral blood from AML patients. A, B. Peripheral blood was collected from AML patients and healthy donors (control), the expression of LINC00265 and miR-485-5p were determined by RT-PCR. C, D. The expression of LINC00265 and miR-485-5p in HS-5, OCI/AML-2 and THP-1 cell were determined by RT-PCR. E. The expression of LC3 and Beclin-1 in HS-5, OCI/AML-2 and THP-1 cell were determined by RT-PCR. **P<0.01 vs control; ##P<0.01 vs HS-5.

ected with mimic NC and miR-485-5p mimic, and the THP-1 cell was transfected with inhibitor NC and miR-485-5p inhibitor. The expression of miR-485-5p was significantly up-regulated by the miR-485-5p mimic (**Figure 3A**). As shown in **Figure 3B**, we found that miR-485-5p mimic caused the decreased LC3-II/LC3-I ratio and Beclin-1 protein expression, and the increased p62 protein expression, suggested that over-expression of miR-485-5p inhibited AML cell autophagy. The expression of miR-485-5p was significantly down-regulated by the miR-485-5p inhibitor (**Figure 3C**). As shown in **Figure 3D**, we found that miR-485-5p inhibitor caused the increased LC3-II/LC3-I ratio and Beclin-1 protein expression, and the reduced p62 protein expression, suggested that inhibition of miR-485-5p promoted AML cell autophagy.

LINC00265 regulated IRF2 expression by competitively binding to miR-485-5p

The results from RNA pull-down showed that the abundance of miR-485-5p in biotin-labeled LINC00265 group was significantly higher than that in control group (**Figure 4A** and **4B** represented OCI/AML-2 and THP-1 cells, respectively). Moreover, the luciferase activity of HEK-293 cells after co-transfected with IRF2 3'UTR-WT and miR-485-5p mimic was significantly lower than that in the mimic NC group. However, miR-485-5p mimic had no significant effect on the luciferase activity in cells transfected with IRF2 3'UTR-Mut (**Figure 4C**). And then, to further determine the effect of LINC00265 on IRF2 expression, the THP-1 cell was transfected with vector and LINC00265 plasmid, and the OCI/AML-2 cell was transfected with si-ctrl and si-

LINC00265 induced autophagy in AML cells

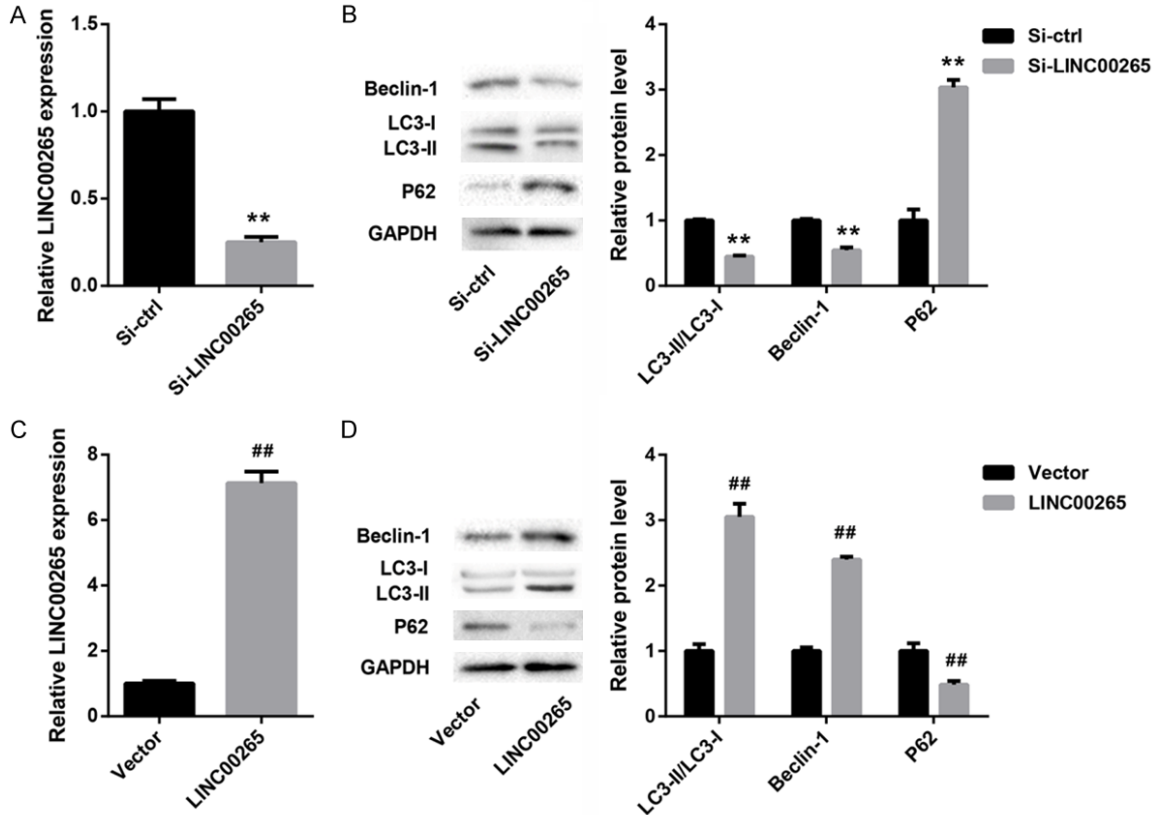


Figure 2. The LINC00265 could induce AML cell autophagy. A. The expression level of LINC00265 in OCI/AML-2 cell transfected with si-ctrl and si-LINC00265. B. The protein expression of Beclin-1, LC3-1, LC3-II and p62 in OCI/AML-2 cell transfected with si-ctrl and si-LINC00265. C. The expression level of LINC00265 in THP-1 cell transfected with vector and LINC00265 plasmid. D. The protein expression of Beclin-1, LC3-1, LC3-II and p62 in THP-1 cell transfected with vector and LINC00265 plasmid. ** $P < 0.01$ vs si-ctrl; ## $P < 0.01$ vs vector.

LINC00265. As shown in **Figure 4D** and **4E**, compared with transfected with vector, the IRF2 mRNA and protein expression were both increased in THP-1 cell-transfected with LINC00265. However, compare to transfected with si-ctrl, the IRF2 mRNA and protein expression were both decreased in OCI/AML-2 cell-transfected with si-LINC00265 (**Figure 4F** and **4G**). These results indicated that LINC00265 regulated IRF2 expression by competitively binding to miR-485-5p.

miR-485-5p/IRF2 mediated the effect of LINC00265 on AML cell autophagy

As we have known that the LINC00265 and miR-485-5p could regulate AML cells autophagy, and LINC00265 could regulate IRF2 expression by competitively binding to miR-485-5p, thus, we next mainly verified whether the regulation of autophagy by LINC00265 through miR-485-5p/IRF2 axis. The western blot ex-

periment described in **Figure 5A** showed that miR-485-5p inhibitor transfection up-regulated the OCI/AML-2 cell autophagy, showing increased LC3-II/LC3-I ratio and Beclin-1 protein expression, and the decreased p62 protein expression, while si-LINC00265 transfection down-regulated the OCI/AML-2 cell autophagy, showing reduced LC3-II/LC3-I ratio and Beclin-1 protein expression, and the increased p62 protein expression. Further, we found that miR-485-5p inhibitor could reverse the reduced autophagy by si-LINC00265. **Figure 5B** showed that IRF2 over-expression up-regulated the OCI/AML-2 cell autophagy, showing increased LC3-II/LC3-I ratio and Beclin-1 protein expression, and the decreased p62 protein expression, while si-LINC00265 transfection down-regulated the OCI/AML-2 cell autophagy, showing reduced LC3-II/LC3-I ratio and Beclin-1 protein expression, and the increased p62 protein expression. Further, we found that IRF2 over-expression could reverse the redu-

LINC00265 induced autophagy in AML cells

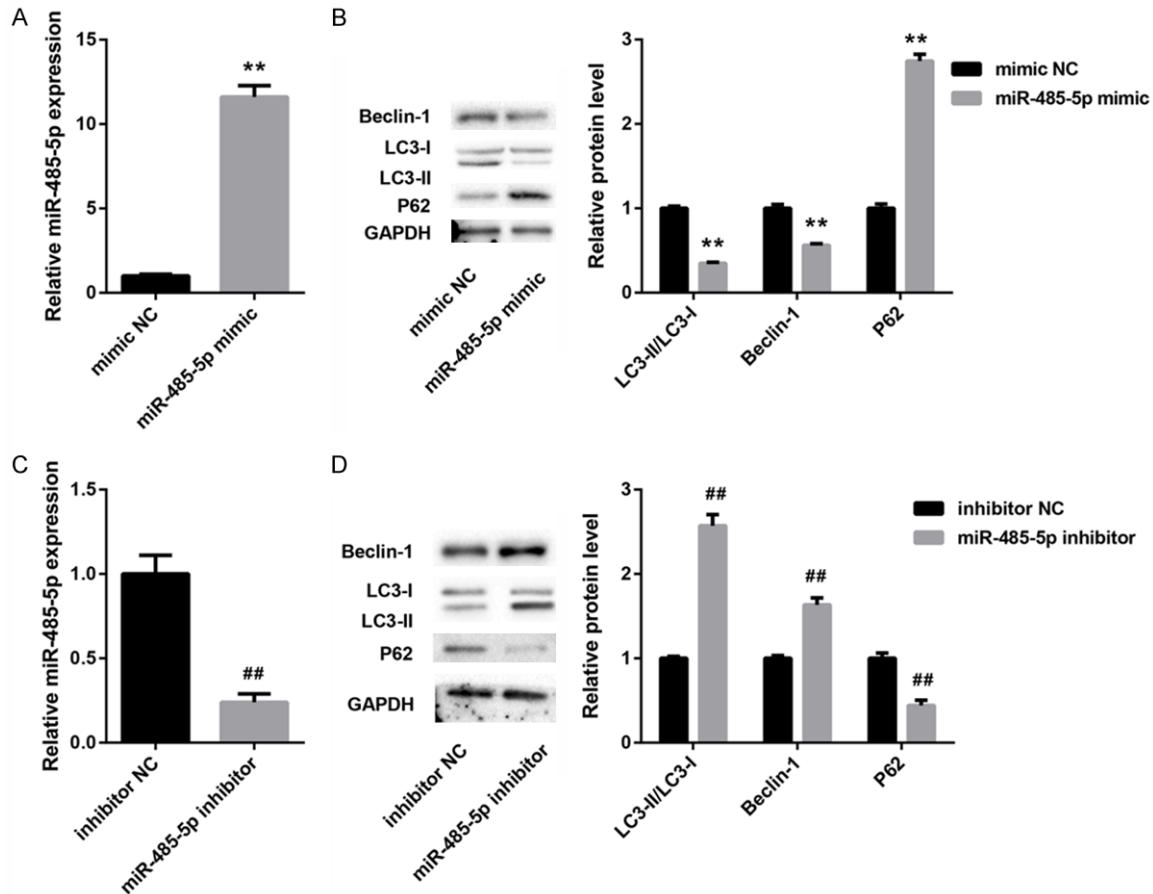


Figure 3. The miR-485-5p could inhibit AML cell autophagy. A. The expression level of miR-485-5p in OCI/AML-2 cell transfected with mimic NC and miR-485-5p mimic. B. The protein expression of Beclin-1, LC3-I, LC3-II and p62 in OCI/AML-2 cell transfected with mimic NC and miR-485-5p mimic. C. The expression level of miR-485-5p in THP-1 cell transfected with inhibitor NC and miR-485-5p inhibitor. D. The protein expression of Beclin-1, LC3-I, LC3-II and p62 in THP-1 cell transfected with inhibitor NC and miR-485-5p inhibitor. ** $P < 0.01$ vs mimic NC; ## $P < 0.01$ vs inhibitor NC.

ced autophagy by si-LINC00265. These results indicated that miR-485-5p/IRF2 mediated the effect of LINC00265 on AML cell autophagy.

The effect of LINC00265/miR-485-5p-mediated autophagy on proliferation and apoptosis of AML cells

Finally, we determined whether LINC00265 overexpression or miR-485-5p inhibitor suppressed AML cell apoptosis through autophagy activation. The CCK8 assay results showed that treatment with 3-MA, an autophagy inhibitor, inhibited but LINC00265 over-expression and miR-485-5p inhibitor suppressed AML cell proliferation (Figure 6A and 6C). Furthermore, 3-MA promoted but LINC00265 over-expression and miR-485-5p inhibitor suppressed AML cell apoptosis (Figure 6B and 6D). Moreover,

treatment with rapamycin, an autophagy activator, markedly facilitated the proliferation of AML cells (Figure 7A and 7C) and suppressed apoptosis (Figure 7B and 7D), which were reversed by LINC00265 knockdown or miR-485-5p mimic. Jointly, our findings manifested that LINC00265 or miR-485-5p affected apoptosis via regulating autophagy in AML cells.

Discussion

In the present study, we found that LINC00265 was significantly highly expressed in serum of AML patients and AML cell lines, and promoted the autophagy of AML cells, while miR-485-5p was significantly lowly expressed in serum of AML patients and AML cell lines, and suppressed the autophagy of AML cells. Next, we have proved that LINC00265 over-expression

LINC00265 induced autophagy in AML cells

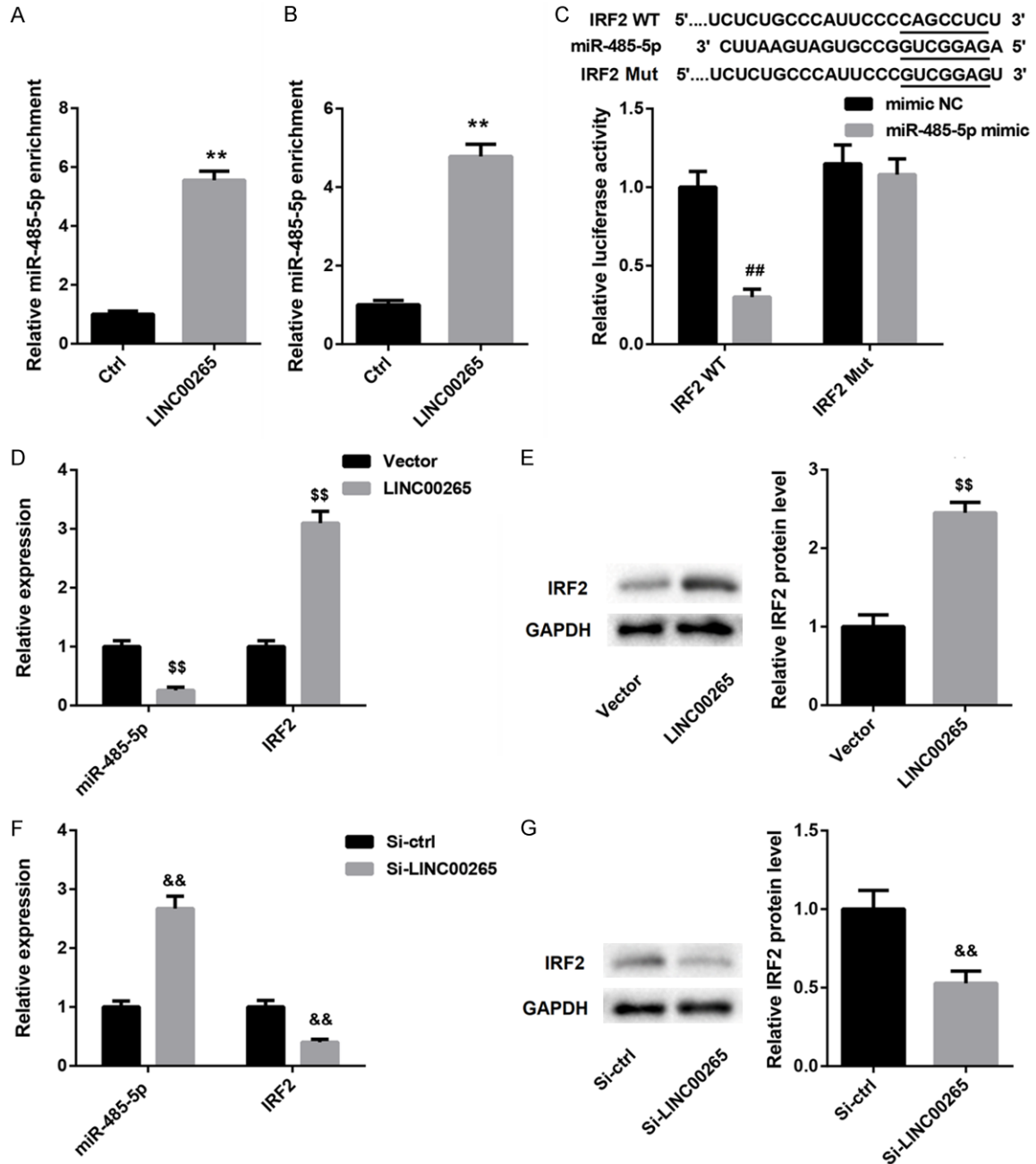


Figure 4. LINC00265 regulated IRF2 expression by competitively binding to miR-485-5p. A, B. RNA pull-down was performed to enrich miR-485-5p interacted with LINC00265 in OCI/AML-2 and THP-1 cells. C. A putative binding site of miR-485-5p in 3'UTR of IRF2 was predicted by miRanda. Luciferase activity was detected after co-transfected with miR-485-5p and IRF2-wt or IRF2-mut in HEK-293. D, E. The expression of miR-485-5p and IRF2 in THP-1 cell transfected with vector and LINC00265 plasmid. F, G. The expression of miR-485-5p and IRF2 in OCI/AML-2 cell transfected with si-ctrl and si-LINC00265. **P<0.01 vs ctrl; ##P<0.01 vs mimic NC; \$\$P<0.01 vs vector; &&P<0.01 vs si-ctrl.

or miR-485-5p inhibitor reversed 3-MA-mediated proliferation-inhibitory and pro-apoptotic effects, while LINC00265 silencing or miR-485-5p mimic overturned the proliferation-promoting and anti-apoptotic effects of rapamycin.

In addition, LINC00265 regulated IRF2 expression by competitively binding to miR-485-5p. So, we concluded that LINC00265 inhibited apoptosis through IRF2-induced autophagy by sponging miR-485-5p in AML cells.

LINC00265 induced autophagy in AML cells

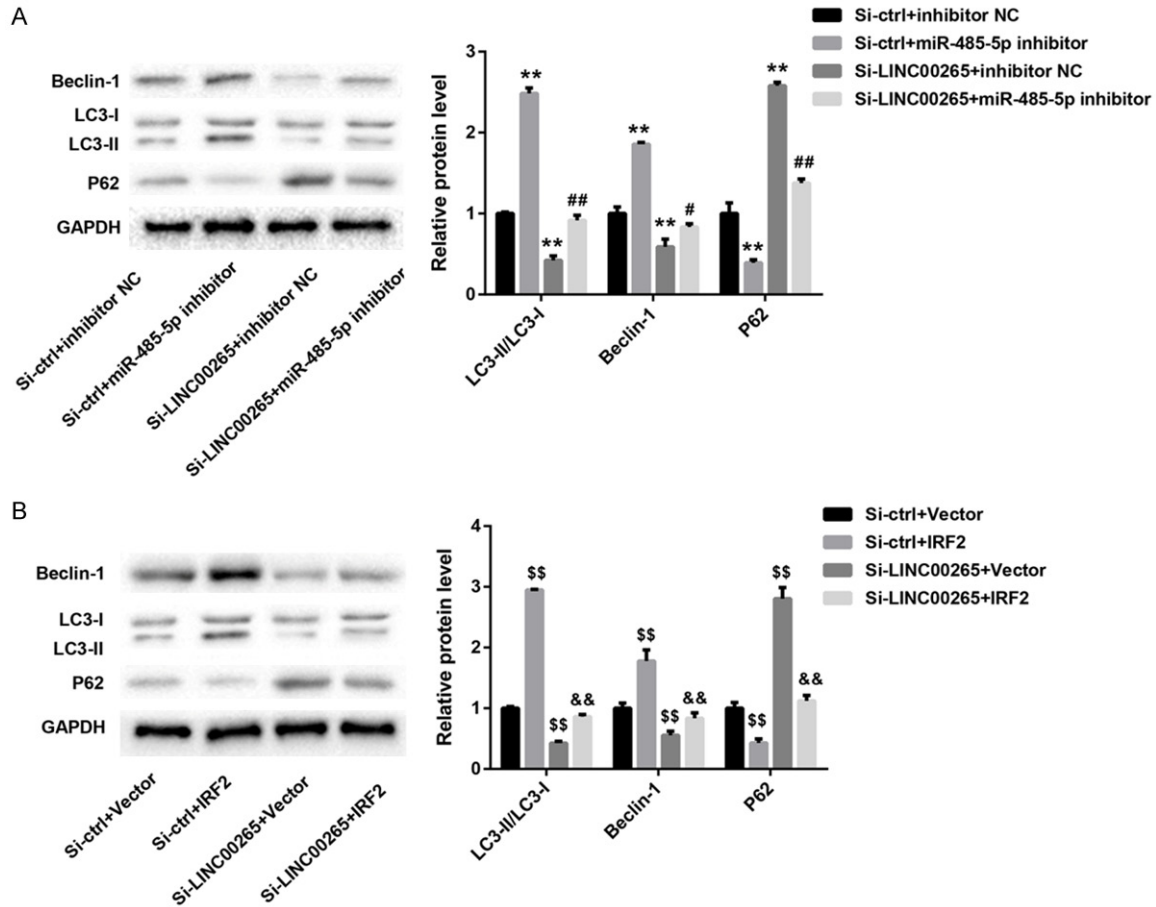


Figure 5. LINC00265 attenuates AML cell apoptosis by inducing autophagy via miR-485/IRF2 axis. A. The protein expression of Beclin-1, LC3-I, LC3-II and p62 in OCI/AML-2 cell transfected with si-LINC00265 and miR-485-5p inhibitor. B. The protein expression of Beclin-1, LC3-I, LC3-II and p62 in THP-1 cell transfected with si-LINC00265 and IRF2 plasmid. ** $P < 0.01$ vs si-ctrl+inhibitor NC; ## $P < 0.01$ vs si-LINC00265+inhibitor NC; \$\$ $P < 0.01$ vs si-ctrl+vector; && $P < 0.01$ vs si-LINC00265+IRF2.

LINC00265 is a new identified lncRNA in lung adenocarcinoma, and up-regulation of LINC00265 may act as a prognostic biomarker for this disease [18]. Subsequently, LINC00265 was demonstrated to be significantly highly expressed in the bone marrow and serum of AML patients, and ROC analyses showed that serum LINC00265 levels were reliable in distinguishing patients with AML from normal controls. Clinical assay indicated that AML patients with higher serum LINC00265 expression suffered poorer overall survival [11]. In this study, we found that LINC00265 was significantly highly expressed in the serum of AML patients, which was identical with those reported in literature. Functionally, it was reported that over-expression of LINC00265 suppressed the capability of proliferation, migration and invasion in AML cell lines [11]. And this study dem-

onstrated that over-expression of LINC00265 promoted AML cell autophagy, and knockdown of LINC00265 inhibited AML cell autophagy. Further, by using CCK8 assay and flow cytometry, we further illustrated that LINC00265 promoted cell proliferation and inhibited cell apoptosis via inducing autophagy in AML cells.

Previous studies have indicated that miR-485-5p exerted its antitumor effect by targeting a variety of genes, such as flotillin-2 (FLOT2) [16], paired box 3 (PAX3) [17], CD147 [19], KLK7 [20] and so on. This study reported for the first time that the expression of miR-485-5p was decreased in serum of AML patients and AML cell lines, which suggested that miR-485-5p may play anticancer role in AML. The next cell experiments confirmed that over-expression of miR-485-5p inhibited AML cell

LINC00265 induced autophagy in AML cells

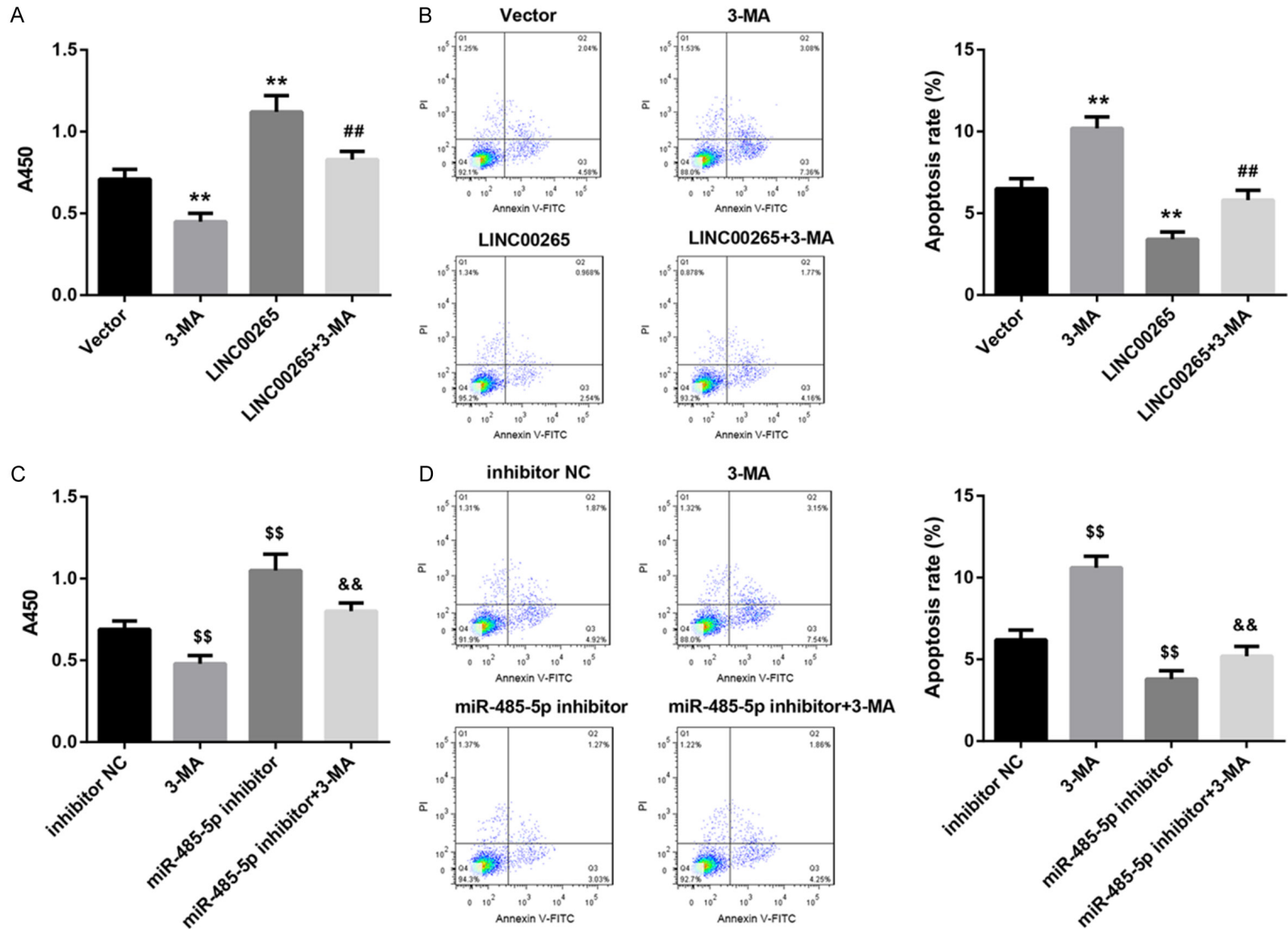


Figure 6. 3-MA reversed the effect of LINC00265 over-expression or miR-485-5p inhibitor on AML cell proliferation and apoptosis. A and C. The cell proliferation was detected by CCK8 assay. B and D. The cell apoptosis was detected by flow cytometry. **P<0.01 vs vector; ##P<0.01 vs LINC00265; \$\$P<0.01 vs inhibitor NC; &&P<0.01 vs miR-485-5p inhibitor.

LINC00265 induced autophagy in AML cells

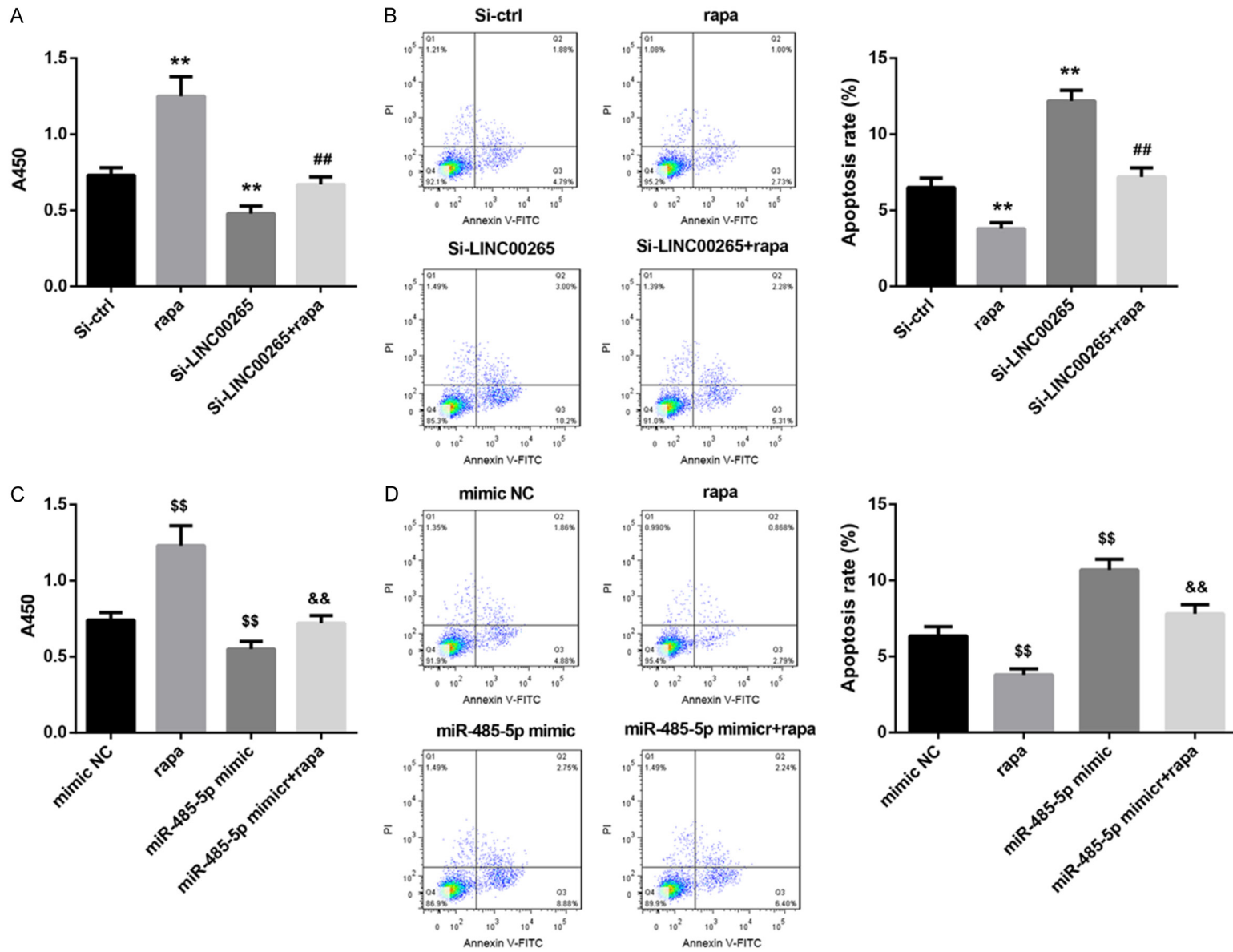


Figure 7. Rapamycin reversed the effect of si-LINC00265 or miR-485-5p mimic on AML cell proliferation and apoptosis. A and C. The cell proliferation was detected by CCK8 assay. B and D. The cell apoptosis was detected by flow cytometry. **P<0.01 vs si-ctrl; ##P<0.01 vs si-LINC00265; \$\$P<0.01 vs mimic NC; &&P<0.01 vs miR-485-5p mimic.

autophagy, and inhibition of miR-485-5p promoted AML cell autophagy. Further, using autophagy inhibitor (3-MA) or autophagy activator (rapamycin) to block or induce autophagy process, we found that miR-485-5p inhibited cell proliferation and promoted cell apoptosis via suppressing autophagy in AML cells.

LncRNA had a variety of regulatory modes, and currently one of the most widely studied mechanisms was an endogenous RNA, competing with RNA of coding genes for combining with miRNA and affects the function, which was known as endogenous competitive RNA (ceRNA) [21]. Here, by performing the RNA pull-down and qRT-PCR, we confirmed that LINC00265 can bind to miR-485-5p, and they are negative correlation. Another key node of ceRNA theory was the target genes of miRNA. Our previous research have proved that IRF2 induced autophagy and inhibited apoptosis in AML cells [13]. And here, IRF2 as the target gene of miR-485-5p by using bioinformatics prediction and the double luciferase reporter assay results showed that miR-485-5p could regulate IRF2 expression by combining its 3'UTR. Overexpression of LINC00265 could up-regulate IRF2 expression and knockdown of LINC00265 down-regulate IRF2 expression. In addition, knocking down miR-485-5p or overexpression of IRF2 could rescue the autophagy by si-LINC00265. Therefore, it was certain that LINC00265/miR-485-5p/IRF2 signaling axis suppressed AML cell apoptosis through autophagy induction.

Conclusions

In conclusion, we demonstrate for the first time that LINC00265 inhibited the apoptosis of AML cells by inducing autophagy via the miR-485-5p/IRF2 axis, and thus may be a new target for gene therapy in AML.

Acknowledgements

This study was funded by Anhui University Natural Science Research Project (grant no. KJ2018A0235) and Key Project of Natural Science Foundation of Bengbu Medical College (grant no. BYKY1743ZD).

Disclosure of conflict of interest

None.

Address correspondence to: Lin Liu, Department of Hematology, The First Affiliated Hospital of Bengbu Medical College, 287 Changhuai Road, Bengbu 233004, Anhui, PR China. Tel: +86-0552-3097071; E-mail: liulin7071@yeah.net

References

- [1] Kucukyurt S and Eskazan AE. New drugs approved for acute myeloid leukaemia in 2018. *Br J Clin Pharmacol* 2019; 85: 2689-2693.
- [2] Guerra VA, DiNardo C and Konopleva M. Venetoclax-based therapies for acute myeloid leukemia. *Best Pract Res Clin Haematol* 2019; 32: 145-153.
- [3] Robainas M, Otano R, Bueno S and Ait-Oudhia S. Understanding the role of PD-L1/PD1 pathway blockade and autophagy in cancer therapy. *Onco Targets Ther* 2017; 10: 1803-1807.
- [4] Nikolettou V, Markaki M, Palikaras K and Tavernarakis N. Crosstalk between apoptosis, necrosis and autophagy. *Biochim Biophys Acta* 2013; 1833: 3448-3459.
- [5] Folkerts H, Hilgendorf S, Wierenga ATJ, Jaques J, Mulder AB, Coffey PJ, Schuringa JJ and Velting E. Inhibition of autophagy as a treatment strategy for p53 wild-type acute myeloid leukemia. *Cell Death Dis* 2017; 8: e2927.
- [6] Liu Q, Chen L, Atkinson JM, Claxton DF and Wang HG. Atg5-dependent autophagy contributes to the development of acute myeloid leukemia in an MLL-AF9-driven mouse model. *Cell Death Dis* 2016; 7: e2361.
- [7] Ng M, Heckl D and Klusmann JH. The regulatory roles of long noncoding RNAs in acute myeloid leukemia. *Front Oncol* 2019; 9: 570.
- [8] Tian YJ, Wang YH, Xiao AJ, Li PL, Guo J, Wang TJ and Zhao DJ. Long noncoding RNA SBF2-AS1 act as a ceRNA to modulate cell proliferation via binding with miR-188-5p in acute myeloid leukemia. *Artif Cells Nanomed Biotechnol* 2019; 47: 1730-1737.
- [9] Wang SL, Huang Y, Su R and Yu YY. Silencing long non-coding RNA HOTAIR exerts anti-oncogenic effect on human acute myeloid leukemia via demethylation of HOXA5 by inhibiting Dnmt3b. *Cancer Cell Int* 2019; 19: 114.
- [10] Cheng P, Lu P, Guan J, Zhou Y, Zou L, Yi X and Cheng H. LncRNA KCNQ10T1 controls cell proliferation, differentiation and apoptosis by sponging miR-326 to regulate c-Myc expression in acute myeloid leukemia. *Neoplasma* 2020; 67: 238-248.
- [11] Ma L, Kuai WX, Sun XZ, Lu XC and Yuan YF. Long noncoding RNA LINC00265 predicts the prognosis of acute myeloid leukemia patients and functions as a promoter by activating PI3K-AKT pathway. *Eur Rev Med Pharmacol Sci* 2018; 22: 7867-7876.

LINC00265 induced autophagy in AML cells

- [12] Wang Y, Li Y, Song HQ and Sun GW. Long non-coding RNA LINC00899 as a novel serum biomarker for diagnosis and prognosis prediction of acute myeloid leukemia. *Eur Rev Med Pharmacol Sci* 2018; 22: 7364-7370.
- [13] Zhang F, Li J, Zhu J, Liu L, Zhu K, Cheng S, Lv R and Zhang P. IRF2-INPP4B-mediated autophagy suppresses apoptosis in acute myeloid leukemia cells. *Biol Res* 2019; 52: 11.
- [14] Bao W, Cao F, Ni S, Yang J, Li H, Su Z and Zhao B. lncRNA FLVCR1-AS1 regulates cell proliferation, migration and invasion by sponging miR-485-5p in human cholangiocarcinoma. *Oncol Lett* 2019; 18: 2240-2247.
- [15] Peng Y, Leng W, Duan S and Hong M. Long noncoding RNA BLACAT1 is overexpressed in hepatocellular carcinoma and its downregulation suppressed cancer cell development through endogenously competing against hsa-miR-485-5p. *Biomed Pharmacother* 2019; 116: 109027.
- [16] Gao F, Wu H, Wang R, Guo Y, Zhang Z, Wang T, Zhang G, Liu C and Liu J. MicroRNA-485-5p suppresses the proliferation, migration and invasion of small cell lung cancer cells by targeting flotillin-2. *Bioengineered* 2019; 10: 1-12.
- [17] Wang R, Zuo X, Wang K, Han Q, Zuo J, Ni H, Liu W, Bao H, Tu Y and Xie P. MicroRNA-485-5p attenuates cell proliferation in glioma by directly targeting paired box 3. *Am J Cancer Res* 2018; 8: 2507-2517.
- [18] Li DS, Ainiwaer JL, Sheyhiding I, Zhang Z and Zhang LW. Identification of key long non-coding RNAs as competing endogenous RNAs for miRNA-mRNA in lung adenocarcinoma. *Eur Rev Med Pharmacol Sci* 2016; 20: 2285-2295.
- [19] Hu XX, Xu XN, He BS, Sun HL, Xu T, Liu XX, Chen XX, Zeng KX, Wang SK and Pan YQ. miRNA-485-5p functions as a tumor suppressor in colorectal cancer cells by targeting CD147. *J Cancer* 2018; 9: 2603-2611.
- [20] Zhang Y, Hu J, Zhou W and Gao H. lncRNA FOXD2-AS1 accelerates the papillary thyroid cancer progression through regulating the miR-485-5p/KLK7 axis. *J Cell Biochem* 2018; [Epub ahead of print].
- [21] Thomson DW and Dinger ME. Endogenous microRNA sponges: evidence and controversy. *Nat Rev Genet* 2016; 17: 272-283.