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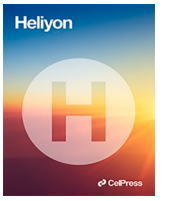

Research article

LncRNA LBX2-AS1 inhibits acute myeloid leukemia progression through miR-455-5p/MYLIP axis

Gongli Fu [a], Hao Wu [a], Xiaomiao Wu [a], Yang Yang [b], Cuihua Fan [a,*]

[a] Department of Hematology Ward, Shulan (Hangzhou)Hospital Affiliated to Zhejiang Shuren University Shulan International Medical College, Hangzhou, Zhejiang, China
[b] Department of Hematology, The Second Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China

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ABSTRACT

Acute myeloid leukemia (AML) is a common blood cancer primarily affecting the bone marrow and blood cells, which is prevalent among adults. Long non-coding RNAs (lncRNAs) have been shown to play a crucial role in the development and progression of AML. LBX2-AS1 is a recently discovered lncRNA that has been linked to the pathogenesis and progression of several types of cancer. This study aimed to investigate the role and possible mechanisms of LBX2-AS1 in AML. Expression levels of LBX2-AS1, miR-455-5p, and their target genes were detected in AML samples and cells by RT-qPCR. Cell proliferation and apoptosis were determined by Cell Counting Kit-8 and 5-ethynyl-2′-deoxyuridine assays, and flow cytometry, respectively. LBX2-AS1 was down-regulated in AML specimens and cells, and overexpression of LBX2-AS1 significantly inhibited cell proliferation and enhanced apoptosis *in vitro*. We also determined the effects of LBX2-AS1 overexpression in an AML mouse model by *in vivo* bioluminescence imaging. Mechanistically, LBX2-AS1 acts as a competitive endogenous RNA, which promotes myosin regulatory light chain interacting protein (MYLIP) expression by sponging miR-455-5p. Knockdown of *MYLIP* or up-regulation of miR-455-5p antagonized the effect of LBX2-AS1 overexpression on the progression of AML. LBX2-AS1 may thus be a valuable therapeutic target for AML.

1. Introduction

Acute myeloid leukemia (AML) is a highly aggressive hematological malignancy that arises from the abnormal proliferation and differentiation of myeloid progenitor cells [1]. The disease mostly affects older adults, with better cure rates in younger patients [2]. The heterogeneity of AML cytogenetics has led to the development of more specific and personalized therapeutic methods [3,4]; however, the cure of AML still presents a major challenge because of relapse and drug resistance. There is thus a crucial need to identify new diagnostic and therapeutic targets to improve AML treatment outcomes.

Long non-coding RNAs (lncRNAs) are transcripts >200 nucleotides in length, which play a role in regulating a wide range of various physiological and pathological processes, including the development of certain cancers, such as AML [5–7]. LBX2-AS1 is a newly discovered lncRNA that is transcribed from the intron of chromosome 2p13.1 [8], and which has been identified as a cancer-associated gene in several cancer types [9–11]. The expression, function, and molecular mechanism of LBX2-AS1 in AML,

* Corresponding author. Shulan Hospital Affiliated to Zhejiang Shuren University Shulan International Medical College, No. 848, Dongxin Road, Hangzhou, 310011, China.
E-mail address: popsyfanfan@126.com (C. Fan).

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however, are currently unclear. Analysis of the Cancer Genome Atlas (TCGA) databases showed that LBX2-AS1 expression was significantly decreased in AML patient samples, indicating its potential significant involvement in the onset and progression of AML; however, its biological function in AML remains unexplored.

MicroRNAs (miRNAs) are crucial modulators of gene expression, functioning to suppress or degrade their target mRNA molecules, thereby influencing a wide range of cellular processes, including tumor advancement and metastasis [12,13]. The upregulation and biological functions of miR-455-5p have been reported in many tumors [14–16]. LncRNAs can indirectly affect downstream gene expression and function by sponging miRNAs [17]. We predicted that LBX2-AS1 might be a potential target of miR-455-5p, and that miR-455-5p might also bind to myosin regulatory light chain interacting protein (MYLIP), based on the starBase online bioinformatics tools. MYLIP was found to be significantly downregulated in AML samples and cell lines, and has been shown to inhibit epithelial-mesenchymal transition in several cancers to regulate cellular activities. These findings suggest that a miR-455-5p/MYLIP axis downstream of LBX2-AS1 may regulate AML progression. Therefore, this study aimed to examine the expression and role of LBX2-AS1 in AML and to explore the potential underlying mechanism.

2. Materials and methods

2.1. Clinical specimens and cell lines

Blood samples were collected from healthy donors and patients with AML at diagnosis, from the Hematology Ward Department of Shulan (Hangzhou) Hospital. Patients and donors provided written informed consent, and the study was conducted in accordance with the ethical guidelines (Declaration of Helsinki) and approved by the local ethics committee of Zhejiang Cancer Hospital. Peripheral blood mononuclear cells were isolated from blood samples by Ficoll gradient density centrifugation using Ficoll-Paque PLUS (Merck, Germany). MOLM-13 and HL60 cells were purchased from the American Type Culture Collection (USA). Cells were cultured in RPMI 1640 medium supplemented with 10 % (v/v) fetal bovine serum and 5 % CO_2 at 37 °C. The media were changed every 3 days.

2.2. Cell transfection

We purchased LBX2-AS1 overexpression vector and negative control vector, miR-455-5p mimic and NC mimic, miR-455-5p inhibitor and NC inhibitor, si-MYLIP and NC siRNA from GenePharma (Shanghai, China). Subsequently, we seeded MOLM-13 and HL60 cells at a density of $1x10^6$ cells per well in a 6-well plate and performed cell transfection using Lipofectamine 2000 (Invitrogen, USA).

2.3. Quantitative real-time PCR (RT-qPCR)

According to the manufacturer's instructions, total RNA was extracted from AML tissues and cell samples using TRIzol (Invitrogen, USA). The total RNA was reverse transcribed using the PrimeScript RT Kit (Takara, Japan) and the miRNA First-Strand Synthesis Kit (Takara, Japan) as per the manufacturer's instructions. The relative expression levels of LBX2-AS1, miR-455-5p, and MYLIP were determined using the TB Green Kit (Takara, Japan) with GAPDH or U6 as internal controls. The qRT-PCR reaction conditions were as follows: 95 °C for 30 s for one cycle; 95 °C for 5 s, 60 °C for 30 s for 40 cycles, and calculation was performed using the $2^{-\Delta\Delta Ct}$ method. The primer sequences are as follows: LBX2-AS1 forward, 5′- CGTGGGGAATGGACCCATAG -3′, LBX2-AS1 reverse, 5′- GGACTTGCCCTTGGTGACTC-3′; miR-455-5p forward, 5′- GTGCCTTTGGACTACATC-3′, miR-455-5p reverse, 5′- GAACATGTCTGCGTATCTC-3'; MYLIP forward, 5′- ACGGTCACCAAGGAATCTGGGA -3′, MYLIP reverse, 5′- CCTTCAAGTCACGGCTATACTGC -3′.

2.4. CCK-8 assay

AML cells were seeded in 96-well plates at a density of 2×10^4. After seeding, the plates were incubated for 0, 24, 48, 72, and 96 h, respectively, after which 10 μL of CCK-8 solution (Dojindo, Japan) was added to each well. The plates were then incubated at 37 °C for an additional 2 h to allow the CCK-8 reagent to react with the cells. The absorbance of the samples was then measured at a wavelength of 450 nm using a microplate reader. This measurement provides information on the metabolic activity of the cells, which can be used as an indicator of cell viability and proliferation. To ensure the reliability of the results, the experiments were repeated independently three times to give triplicate measurements for each time point.

2.5. EdU assay

We assessed cell proliferation and DNA synthesis using a Cell-Light EdU Apollo488 *In Vitro* Imaging Kit (Ribobio). AML cells were treated with EdU at a concentration of 50 μM for 2 h, fixed using paraformaldehyde, and 0.5 % Triton-X-100 solution was then added to permeabilize the cells to allow better access for the staining reagents. Nucleic acids within the cells were visualized using Hoechst stain, and the fluorescently stained cells were visualized and captured using a fluorescence microscope(Olympus). This imaging technique allowed the detection and quantification of EdU-labeled cells, indicating active DNA synthesis and cell proliferation.

2.6. Cell apoptosis analysis

AML cells were transfected with OE-LBX2-AS1 plasmids or *MYLIP* siRNA for 48 h, harvested, washed twice with phosphate-buffered saline, and treated with an Annexin V-FITC Apoptosis Detection Kit (Dojindo), according to the manufacturer's instructions. Flow cytometry was used to detect stained cells and analyze the level of apoptosis.

2.7. Western blot analysis

Total protein was harvested in RIPA buffer supplemented with protease/phosphatase inhibitors (Beyotime, China), and the total protein concentration was then determined using the BCA assay kit (Beyotime, China). The proteins were separated by SDS-PAGE and transferred onto a PVDF membrane. After washing with TBS containing 0.1 % Tween-20, the membrane was blocked in Tris-buffered saline with 5 % non-fat milk for 2 h. Subsequently, the membrane was incubated with primary antibodies overnight, followed by a 2-h incubation with the secondary antibodies conjugated with horseradish peroxidase. The following antibodies were used: GAPDH (1:2000; CST, USA), N-cadherin (1:1000; CST), Snail (1:1000; CST), and MYLIP (1:1000; Sigma, USA).

2.8. AGO2-RIP assay

To investigate the interaction with RNA-binding proteins, we conducted RIP experiments using the Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, Germany) following the manufacturer's instructions. MOLM-13 and HL60 cells were lysed in RIP lysis buffer, and the lysates were then incubated overnight at 4 °C with magnetic beads conjugated with AGO2 or IgG antibodies. Following protein digestion with proteinase K, RNA was extracted using TRIzol reagent, and the expression of LBX2-AS1 was analyzed by RT-qPCR.

2.9. Luciferase reporter assay

We verified the regulatory relationship between LBX2-AS1 and miR-455-5p by dual-luciferase reporter assay. Briefly, the pmirGLO

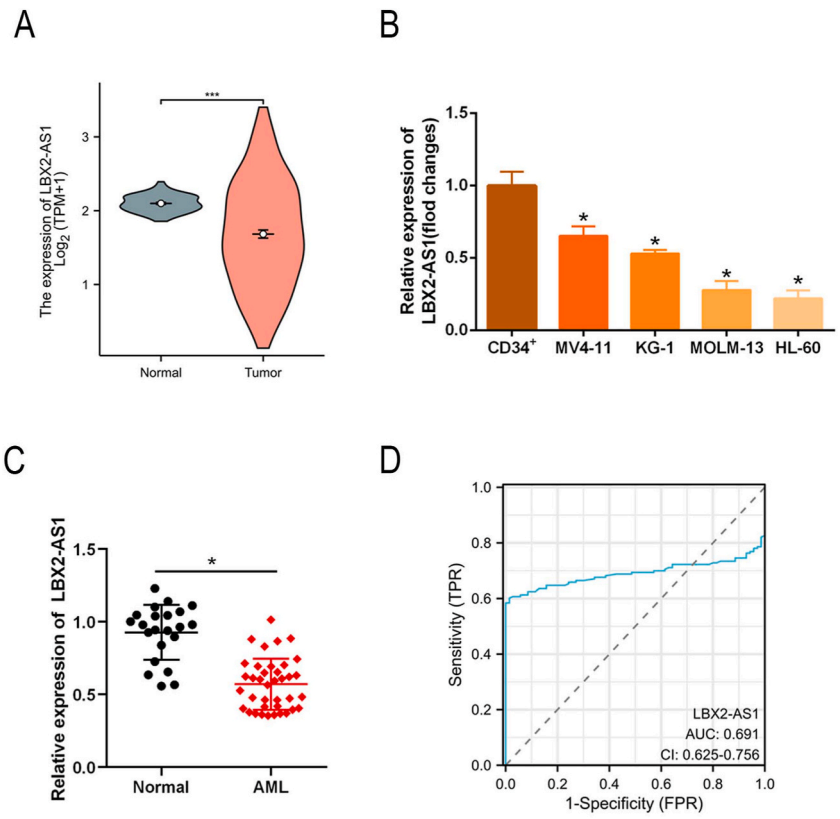

Fig. 1. LBX2-AS1 was overexpressed in AML tissues and cells.
(A) LBX2-AS1 expression was upregulated in tumor tissues (n = 173) compared with normal tissues (n = 70) in TCGA database ($P < 0.05$). (B, C) Expression levels of LBX2-AS1 in cell lines and clinical samples detected by RT-qPCR. (D) The ROC curves analysis showed that LBX2-AS1 can be a latent discriminator of AML. *$P < 0.05$ by student's t-test. The data were expressed as mean ± SD (n = 3).

vector containing wild-type or mutated LBX2-AS1 or miR-455-5p binding sites was co-transfected with into 293T cells with miR-455-5p mimic or negative control. At 48 h post-transfection, the luciferase activity of the cells was measured using a Transcription Factor Reporter Assay system. The findings were depicted as the ratio of firefly to *Renilla* luciferase activity across a minimum of three independent experiments.

2.10. Xenograft AML model

Female non-obese diabetic severe combined immunodeficiency gamma mice were purchased from Gempharmatech Co., Ltd. (Nanjing, China) and 6–8-week-old mice were housed under specific pathogen-free conditions in individually ventilated cages. A xenograft AML model was created by injection of vector and OE-LBX2-AS1 HL-60-Luc cells via the tail vein. Leukemia growth was measured by *in vivo* bioluminescence imaging. The mice were sacrificed 6 weeks after transplantation and femur bone marrow cells were harvested and analyzed by flow cytometry and using FlowJo software. All experiments and procedures were approved by Ethical Review Committee for Animals of Shulan Hospital.

2.11. Statistical analysis

All data were analyzed using GraphPad Prism 7.0 software and presented as mean ± standard deviation. Statistical significance was determined with *t*-tests or one-way analysis of variance (ANOVA). Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Expression levels of LBX2-AS1 were decreased in AML patients and cell lines

Based on an integrated analysis of TCGA databases, LBX2-AS1 expression was significantly decreased in patients with AML (Fig. 1A). In our study, we evaluated the relative expression levels of LBX2-AS1 in four AML cell lines and $CD34^+$ cells, and showed that LBX2-AS1 was significantly downregulated in AML cell lines compared with $CD34^+$ cells (Fig. 1B). We also detected the relative expression levels of LBX2-AS1 in patients with AML (n = 38) and healthy volunteers (n = 22) using RT-qPCR. The expression of LBX2-AS1 was significantly decreased in patients compared to healthy volunteers (Fig. 1C). To investigate the association between LBX2-AS1 expression and clinical pathological features in 38 AML patients, we used the median expression level of LBX2-AS1 as a cutoff point to divide the patients into two groups: high expression group and low expression group. As shown in Table 1, we found a significant correlation between LBX2-AS1 expression levels and BM blasts ($p = 0.022$) and Cytogenetics ($p = 0.013$). However, there was no significant correlation with other clinical features such as gender, age, FAB classification, and FLT3 mutation. The ROC curves analysis showed that LBX2-AS1expression a potential diagnostic biomarker for AML, with AUC values of 0.691 [95 % confidence interval (CI) 0.625–0.756]. This indicates that LBX2-AS1 can serve as a biomarker for AML.

3.2. Overexpression of LBX2-AS1 inhibited cell proliferation and enhanced apoptosis in AML cells

To investigate the impact of LBX2-AS1 on cell proliferation and apoptosis, we infected MOLM-13 and HL-60 AML cells with an

Table 1
Relationship between LBX2-AS1 expression and clinicopathologic features in AML.

Characteristics	Low expression of LBX2-AS1	High expression of LBX2-AS1	P value
n	19	19	
Gender			0.168
Female	7	10	
Male	12	9	
Age			0.139
<60	10	13	
≥60	9	6	
BM blasts			0.022*
<50 %	5	10	
≥50 %	14	9	
FAB classifications			0.436
M0-M2	12	11	
M3	2	1	
M4-M7	5	7	
Cytogenetics			0.013*
Favorable	3	7	
Intermediate	7	8	
Unfavorable	9	4	
FLT3 mutation			0.208
Negative	14	16	
Positive	5	3	

LBX2-AS1(OE-LBX2-AS1) overexpression plasmid(Fig. 2A). OE-LBX2-AS1 significantly repressed cell proliferation in AML cells compared with control cells, as shown by CCK-8 assay results (Fig. 2B). Similarly, the proliferation of OE-LBX2-AS1 cells was significantly lower compared with control cells according to EdU assay (Fig. 2C). Furthermore, LBX2-AS1 upregulation significantly increased apoptosis in AML cells as shown by flow cytometry (Fig. 2D and E). These results indicate that LBX2-AS1 can inhibit cell proliferation and promote apoptosis in AML.

3.3. LBX2-AS1 acted as a sponge for miR-455-5p in AML cells

LncRNAs primarily function as competing endogenous RNAs (ceRNAs) and regulate the expression of their target genes by binding and sequestering their target miRNAs. We screened potential target miRNAs of LBX2-AS1 using starBase online software and identified miR-455-5p. The predicted binding sequences between miR-455-5p and LBX2-AS1 are illustrated in Fig. 3A. Co-transfection of an miR-455-5p mimic and the 3′-untranslated region (UTR) of wild-type LBX2-AS1 significantly reduced the luciferase activity (Fig. 3B). Additionally, overexpression of LBX2-AS1 downregulated the expression of miR-455-5p in AML cells (Fig. 3C). The interaction between LBX2-AS1 and miR-455-5p in AML cells was confirmed by RNA immunoprecipitation (RIP)-qPCR assay using an anti-AGO2 antibody (Fig. 3D), which showed that LBX2-AS1 directly targeted miR-455-5p in MOLM-13 and HL-60 cells.

3.4. LBX2-AS1 inhibited AML progression by targeting miR-455-5p

We conducted rescue experiments to investigate the correlation between LBX2-AS1 and miR-455-5p in AML. We assessed how

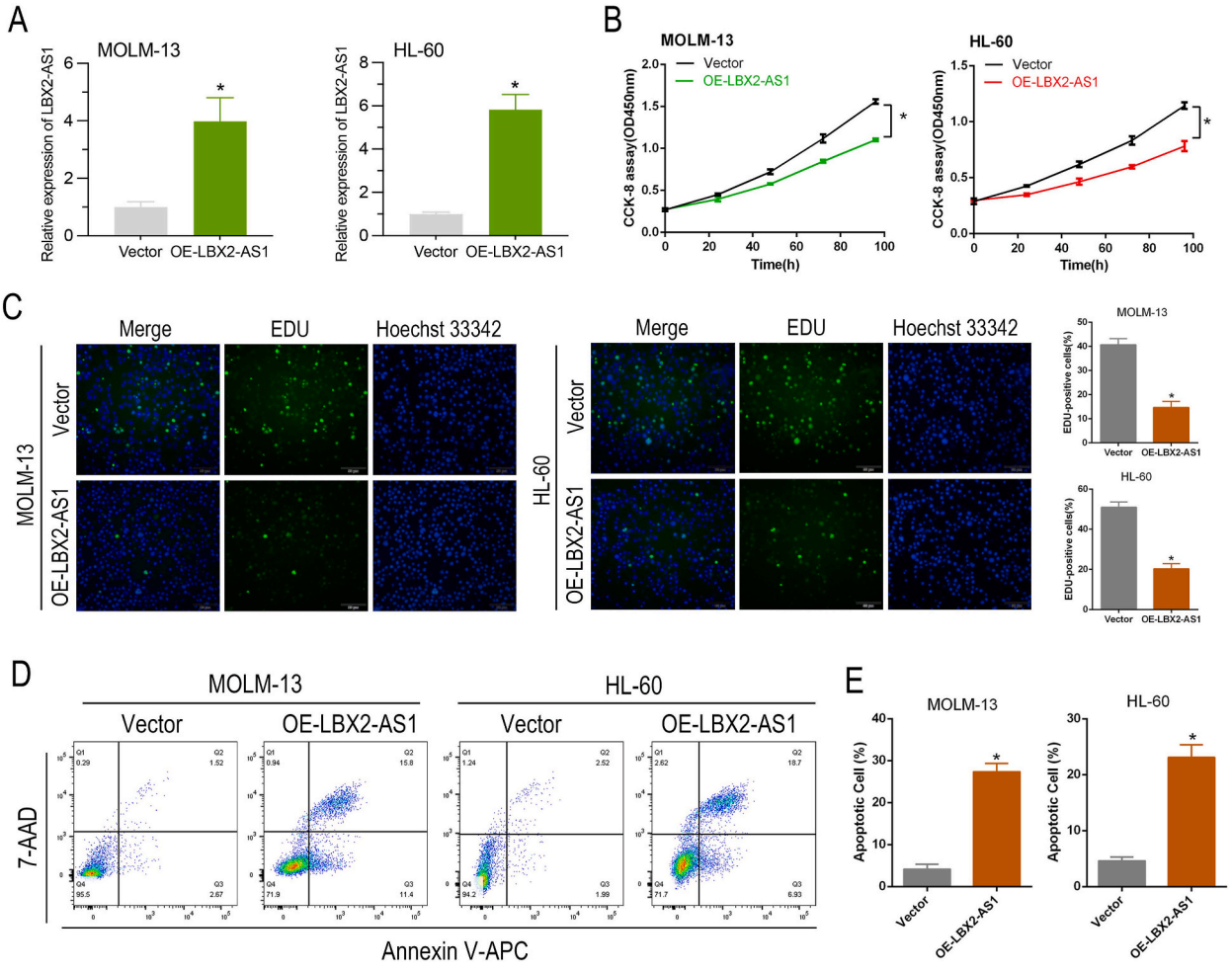

Fig. 2. LBX2-AS1 over-expression inhibited AML cell proliferation *in vitro*.
(A) Expression level of LBX2-AS1 in MOLM-13 and HL-60 cells after transfection detected by RT-qPCR. (B) The proliferation ability of AML cells transfected with OE-LBX2-AS1 was determined by CCK-8 assay ($P < 0.05$). (C) OE-LBX2-AS1 decreased cell proliferation in AML cells, as shown by EdU analysis, and EDU-positive cells were counted ($P < 0.05$). (D, E) OE-LBX2-AS1 accelerated apoptosis in AML cells, as shown by flow cytometry, and apoptotic cells were counted ($P < 0.05$). *$P < 0.05$ by student's t-test. The data were expressed as mean ± SD (n = 3).

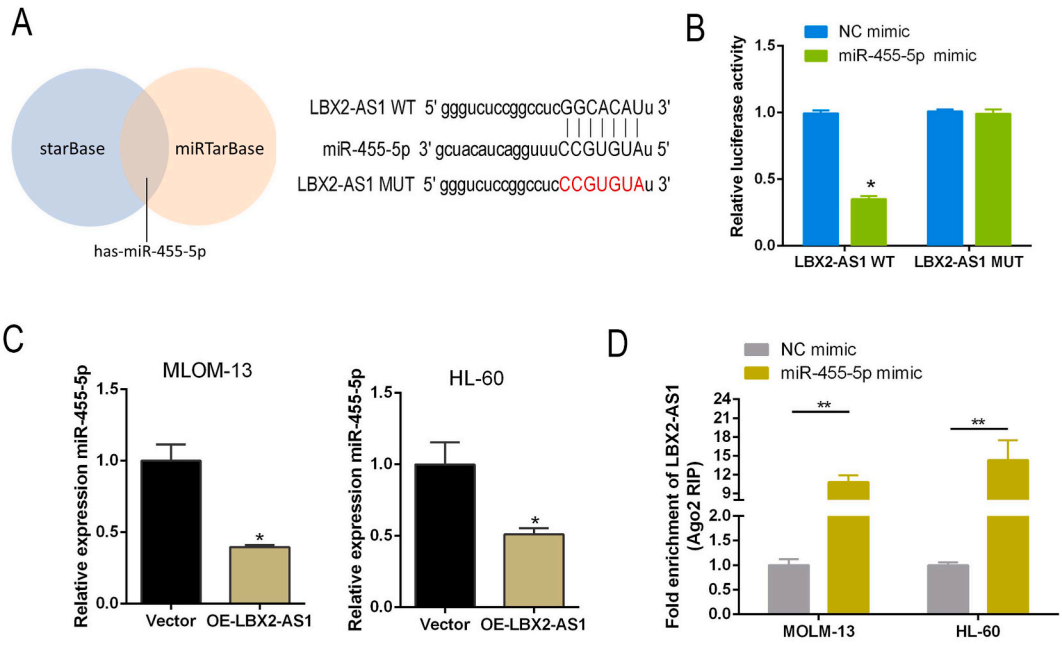

Fig. 3. LBX2-AS1 could bind to miR-455-5p.
(A) Venn diagram of starBase and miRTarBase online database predictions for target miRNA of LBX2-AS1. (B) Interaction between LBX2-AS1 and miR-455-5p was determined by luciferase reporter assay. (C) MiR-455-5p expression levels were lower in OE-LBX2-AS1-transfected compared with vector-transfected group. (D) Interaction between LBX2-AS1 and miR-455-5p was detected by RIP assay. $^{*}P < 0.05$, $^{**}P < 0.05$ by student's t-test. The data were expressed as mean ± SD (n = 3).

LBX2-AS1 relied on miR-455-5p to affect cell proliferation and apoptosis in AML cells by co-transfecting OE-LBX2-AS1 plasmids and miR-455-5p mimics into MOLM-13 and HL-60 cells. The results revealed that the miR-455-5p mimic was able to reverse the over-expression level of LBX2-AS1 by RT-qPCR (Fig. 4A). CCK-8 and EdU assays showed that miR-455-5p mimics partially reversed the inhibitory effects of OE-LBX2-AS1 on the proliferation of AML cells (Fig. 4B–C). Furthermore, LBX2-AS1 upregulation increased apoptosis in AML cells, as shown by flow cytometry analysis, and this was partly counteracted by the miR-455-5p mimics (Fig. 4D). In summary, these findings suggest that LBX2-AS1 inhibited inhibit cell proliferation and promote apoptosis in AML by targeting miR-455-5p.

3.5. *MYLIP as a direct target gene of miR-455-5p*

We utilized several online miRNA target-prediction databases, including starBase, TargetScan, miRTarBase, and miRDB, to identify the targets of miR-455-5p. Ten potential target genes were predicted by all databases (Fig. 5A), among which *MYLIP* displayed the most significant upregulation in AML cells (Fig. 5B). The predicted target regions between miR-455-5p and *MYLIP* are shown in Fig. 5C. In addition, luciferase activity was significantly decreased in cells co-transfected with miR-455-5p mimic and the 3′-UTR of wild-type *MYLIP* (Fig. 5D). Furthermore, endogenous *MYLIP* mRNA was pulled down from cell lysates in RIP-qPCR assay, which confirmed that miR-455-5p directly targeted *MYLIP* (Fig. 5E). RT-qPCR analysis demonstrated that the miR-455-5p mimic significantly inhibited *MYLIP* expression, whereas the miR-455-5p inhibitor significantly upregulated *MYLIP* expression (Fig. 5F), and these results were validated by Western blot analysis (Fig. 5G). In summary, these findings suggest that *MYLIP* acted as a direct target gene of miR-455-5p in AML cells.

3.6. *MYLIP knockdown reversed the effect of LBX2-AS1 on AML cell progression*

To investigate the potential correlation between LBX2-AS1 and *MYLIP* in regulating cell proliferation and apoptosis in AML, we co-transfected MOLM-13 and HL-60 cells with OE-LBX2-AS1 plasmids and *MYLIP* small interfering RNA (siRNA). The results revealed that the MYLIP siRNA was able to reverse the expression level of LBX2-AS1 and miR-455-5p by RT-qPCR (Fig. 6A–B). CCK-8 and EdU assays showed that *MYLIP* knockdown partially reversed the inhibitory effects of OE-LBX2-AS1 on cell proliferation in AML cells (Fig. 6C–E). In addition, flow cytometry analysis revealed LBX2-AS1 upregulation increased apoptosis in AML cells, which was partly counteracted by *MYLIP* knockdown (Fig. 6F). Western blot further demonstrated that LBX2-AS1 upregulation significantly increased expression of the epithelial marker protein N-cadherin and Snail. These effects were reversed in cells co-transfected with si-MYLIP (Fig. 6G). In conclusion, our results suggest that *MYLIP* knockdown reversed the effect of LBX2-AS1 on the progression of AML cells.

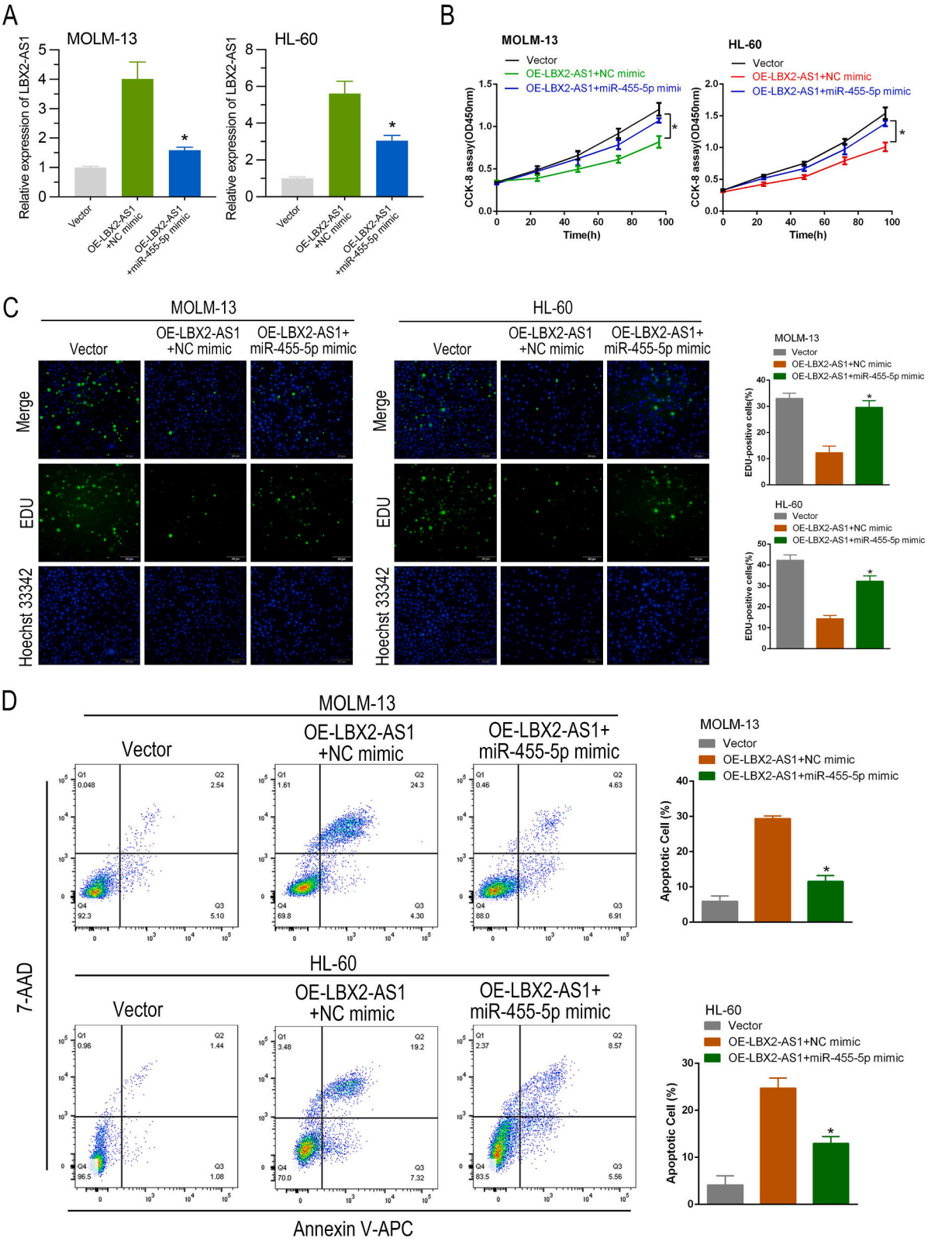

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Fig. 4. miR-455-5p reversed the effect of LBX2-AS1 overexpression on cell proliferation.
(A) Expression level of LBX2-AS1 in MOLM-13 and HL-60 cells after transfection detected by RT-qPCR. (B) The proliferation ability of AML cells co-transfected with OE-LBX2-AS1 and miR-455-5p mimics, respectively, were determined by CCK-8 assays. (C) OE-LBX2-AS1 decreased the proliferation of AML cells, as shown by EdU analysis, and miR-455-5p partially reversed that effect. (D) OE-LBX2-AS1 accelerated apoptosis in AML cells, as shown by flow cytometry, and miR-455-5p partially reversed that effect. *$P < 0.05$, **$P < 0.05$ by student's t-test. The data were expressed as mean ± SD (n = 3).

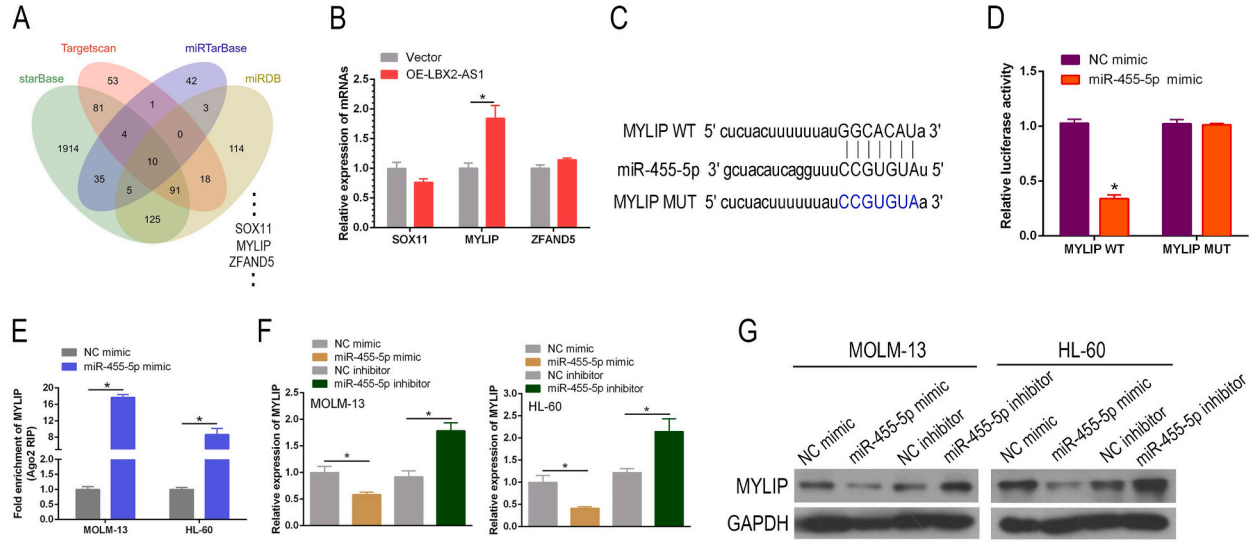

Fig. 5. miR-455-5p regulated the expression of *MYLIP* by targeting it.
(A) Venn diagram of four different online database predictions of target genes of miR-455-5p. (B) Three potential miR-455-5p target genes were detected by RT-qPCR in the OE-LBX2-AS1 group. Online prediction showed that miR-455-5p had potential binding sites with *MYLIP*. (C, D) Interaction between miR-455-5p and *MYLIP* was determined by luciferase reporter assay and RIP assay. (E) Western blot showed that the expression of MYLIP was regulated by miR-455-5p. *$P < 0.05$ by student's t-test. The data were expressed as mean ± SD (n = 3).

3.7. LBX2-AS1 overexpression inhibited AML progression in a mouse model

We further investigated the effect of LBX2-AS1 on AML progression *in vivo* by generating an AML mouse model via intravenous injection of OE-LBX2-AS1 HL-60-Luci cells. LBX2-AS1 overexpression significantly inhibited leukemia progression, as demonstrated by reduced levels of whole-body bioluminescence 4 weeks after tumor injection (Fig. 7A and B). Moreover, analysis of bone marrow samples revealed a significant decrease in the proportion of residual HL-60-Luci cells following LBX2-AS1 overexpression (Fig. 7C and D). Taken together, these findings suggest that LBX2-AS1 overexpression effectively suppressed the progression of AML cells *in vivo*.

4. Discussion

AML is a common, life-threatening hematological malignant tumor. Recent studies have shown that non-coding RNAs, such as lncRNAs and miRNAs, may be involved in the regulation of AML [18–20]. For instance, CD27-AS1 is a highly expressed lncRNA in AML and increased *PBX3* expression by sponging miR-224-5p, thus promoting AML cell growth and aggressiveness [21], and lncRNA HOTAIRM1 induced by mutant *NPM1* also promoted autophagy and proliferation in this distinct leukemia subtype [22]. Nevertheless, the specific functions of lncRNAs in AML are not fully understood. We therefore investigated the recently identified lncRNA LBX2-AS1 and showed that it was significantly downregulated in AML tissues and cell lines, and its overexpression inhibited cell proliferation and enhanced apoptosis in AML cells. Furthermore, LBX2-AS1 was found to regulate AML progression via the miR-455-5p/MYLIP axis. These results suggest that LBX2-AS1 may play a crucial role in AML development and could be a potential therapeutic target for AML treatment.

LBX2 antisense RNA 1 (LBX2-AS1) is a newly discovered lncRNA that was initially reported as a tumor promoter and predictor of poor prognosis in esophageal squamous cell carcinoma [8]. Recent research has shown that LBX2-AS1 plays an oncogenic role in various malignancies. For instance, LBX2-AS1 was upregulated in gastric cancer, and silencing it inhibited the proliferative, migratory, and invasive capacities of gastric cancer cells [23,24]. Similarly, high expression of LBX2-AS1 was observed in ovarian cancer and was associated with unfavorable survival outcomes [25]. In addition, LBX2-AS1 has been shown to promote the growth of hepatocellular carcinoma and gastric cancer through different mechanisms, and increased levels of LBX2-AS1 were linked to a poor prognosis and disease progression via Notch signaling in patients with non-small cell lung cancer [26]. These findings indicate that LBX2-AS1 may be significantly upregulated in most tumor tissues, and its expression levels are negatively correlated with prognosis and survival.

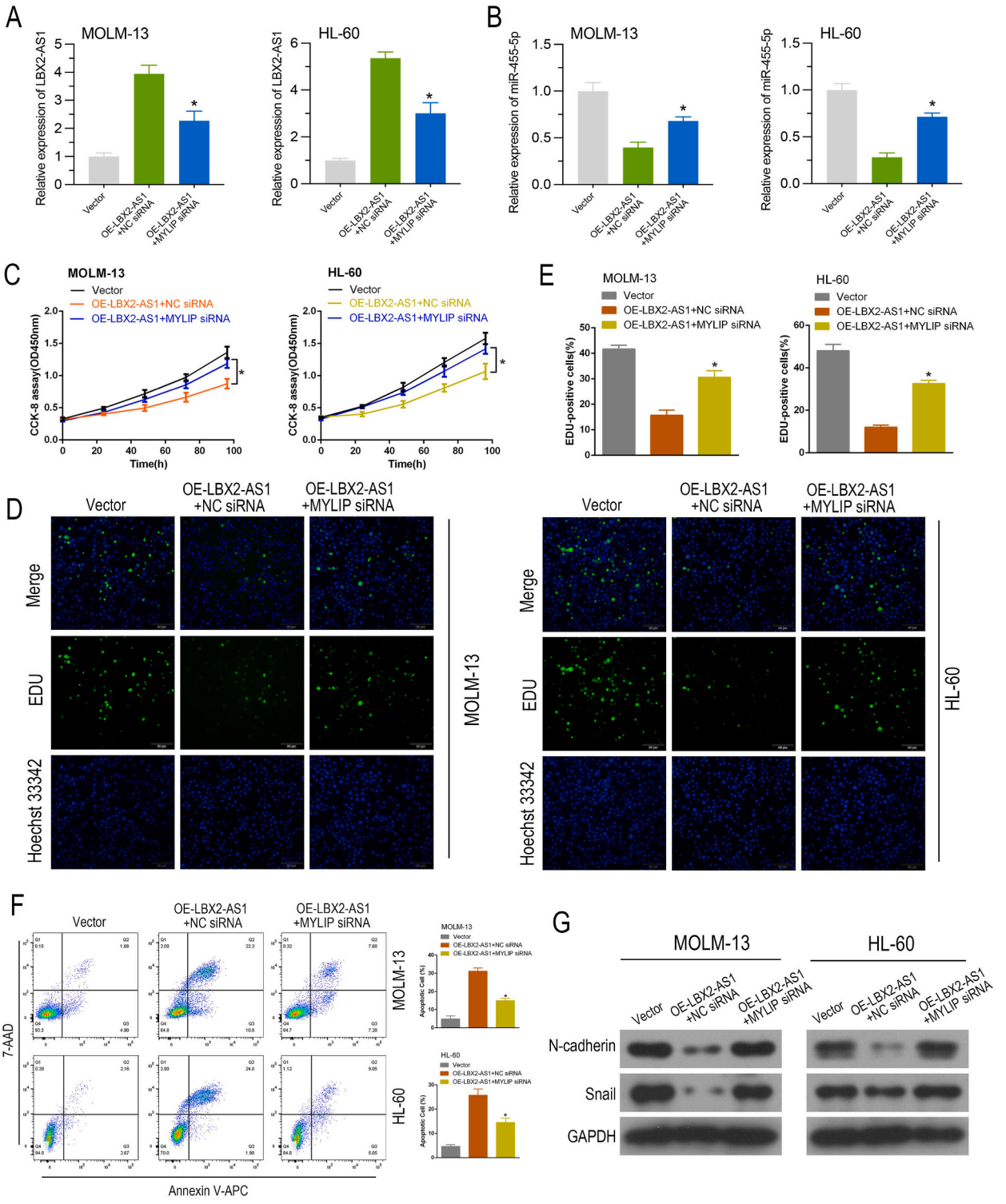

Fig. 6. *MYLIP* knockdown reversed the effect of LBX2-AS1 on AML cell progression.
(A) Expression level of LBX2-AS1 and miR-455-5p in MOLM-13 and HL-60 cells after transfection detected by RT-qPCR. (B) The proliferation abilities of AML cells co-transfected with OE-LBX2-AS1 and *MYLIP* siRNA, respectively, were determined by CCK-8 assays. (C, D, E) OE-LBX2-AS1 decreased the proliferation of AML cells, as shown by EdU analysis, and *MYLIP* siRNA partially reversed that effect. (F) OE-LBX2-AS1 accelerated apoptosis in AML cells, as shown by flow cytometry, and *MYLIP* siRNA partially reversed that effect. (G) Western blot showed that the expression of the epithelial marker protein was rescued by *MYLIP* siRNA. $^{*}P < 0.05$ by student's t-test. The data were expressed as mean ± SD (n = 3).

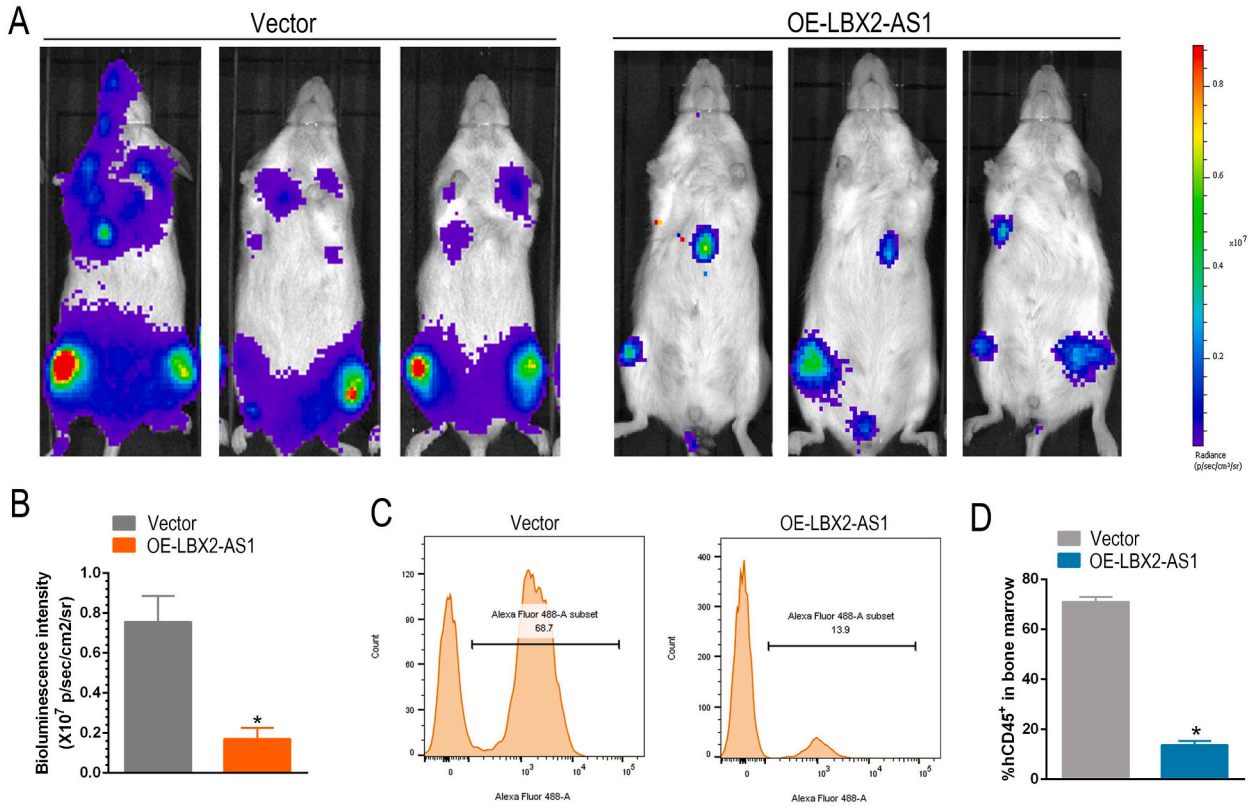

Fig. 7. LBX2-AS1 overexpression reduced leukemia progression in an AML mouse model.
(A, B) LBX2-AS1 overexpression significantly reduced levels of whole-body bioluminescence compared with the vector group. (C, D) LBX2-AS1 overexpression significantly reduced the proportion of residual HL-60-Luci cells from bone marrow, as shown by flow cytometry. $^{*}P < 0.05$ by student's t-test. The data were expressed as mean ± SD (n = 3).

Despite evidence suggesting that LBX2-AS1 is involved in promoting tumor development in various cancer types, the molecular mechanisms underlying its oncogenic functions and regulatory networks remain unclear. The current study showed that LBX2-AS1 was significantly downregulated in AML, and functional experiments confirmed that LBX2-AS1 acted as a tumor suppressor in AML, consistent with analysis of TCGA database. Meanwhile, in a study on abdominal aortic aneurysm, Li et al. found that downregulation of LBX2-AS1 significantly increased cell proliferation and inhibited apoptosis, while overexpressing LBX2-AS1 had the opposite effect [27]. LBX2-AS1 has also demonstrated the ability to suppress cellular proliferation in certain types of cells. In summary, these results suggest that LBX2-AS1 may play unknown regulatory roles in the development of different tumors, but further in-depth research is needed.

Previous studies suggested that the lncRNA LBX2-AS1 could act as a ceRNA to sponge miRNAs, such as miR-597-3p, miR-491-5p, miR-422a, and miR-4685-5p [25,28,29]. In the present study, we showed that miR-455-5p acted as a tumor promoter in AML cells. Furthermore, LBX2-AS1 functioned as a ceRNA to regulate miR-455-5p expression levels and modulate the expression of miR-455-5p target genes, such as *MYLIP*. MYLIP (also known as IDOL) is a cytoskeletal protein involved in regulating cell migration and movement [29–31]. By interacting with cell membrane proteins and the myosin cytoskeleton, MYLIP plays a crucial role in maintaining cellular morphology, remodeling cytoskeletal proteins, modulating cell motility, and promoting cell adhesion with the extracellular matrix. Recent studies also showed that MYLIP significantly inhibited the proliferation, migration, and invasion of lung cancer cells, indicating that it might function as a tumor suppressor gene in lung cancer [32]. *MYLIP* has also been identified as a target gene of miR-19b/miR-190b-5p in breast cancer and is involved in inhibiting tumor growth and metastasis [33]. The current research revealed that *MYLIP* interacted directly with miR-455-5p, and that downregulation of *MYLIP* could effectively reverse the tumor-suppressive effect of LBX2-AS1 overexpression.

5. Conclusion

In conclusion, the results of this study demonstrate that LBX2-AS1 is downregulated in AML and functions as a tumor suppressor by regulating the miR-455-5p/MYLIP axis. These findings provide promising new therapeutic targets and strategies for the treatment of AML.

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Ethics approval

The study protocol was approved by the Medical Ethics Committee of Zhejiang Chinese Medical University (No. 2020249).

Data availability statement

The authors declare that all data supporting the findings of this study are available within the article.

CRediT authorship contribution statement

Gongli Fu: Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Hao Wu:** Formal analysis, Data curation. **Xiaomiao Wu:** Resources, Data curation. **Yang Yang:** Formal analysis, Data curation. **Cuihua Fan:** Writing – review & editing, Visualization, Project administration, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24812.

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