Neoplasma 2025; 72(6): 405–414

doi:10.4149/neo_2025_250619N270

LINC00324 suppresses abnormal proliferation and promotes apoptosis of leukemia cells through the miR-10b-5p/PTEN pathway

Yiyu LI[1,2,#], Bo WANG[1,2,#], Faquan LIN[3], Yangyang ZHANG[1,2], Yue LAN[4], Yi LIU[1,2], Wei BAO[1,2], Binbin XIE[1,2], Qingniao ZHOU[1,2], Jicheng ZHOU[4,*], Qiyan ZENG[1,2,*]

[1]Department of Biochemistry and Molecular Biology, School of Basic Medicine, Guangxi Medical University, Nanning, China; [2]Education Department of Guangxi Zhuang Autonomous Region, Key Laboratory of Biological Molecular Medicine Research, Guangxi Medical University, Nanning, China; [3]Clinical Laboratory, First Affiliated Hospital of Guangxi Medical University, Nanning, China; [4]Department of Hematology, First Affiliated Hospital of Guangxi Medical University, Nanning, China

**Correspondence: zengnn6@hotmail.com; zhoujicheng@gxmu.edu.cn*
[#]Contributed equally to this work.

Received June 19, 2025 / Accepted November 27, 2025

Long non-coding RNAs (lncRNAs) are known to participate in leukemia development, but the molecular mechanisms of many remain unclear. The expression of LINC00324 was examined in peripheral blood samples from leukemia patients and in leukemia cell lines by RT-qPCR. Functional studies were performed to evaluate how LINC00324 overexpression or knockdown affected cell proliferation and apoptosis. Flow cytometry was used to detect apoptosis. Western blotting was applied to measure Ki-67, BAX, Bcl-2, and PTEN protein levels. Dual-luciferase reporter assays were used to confirm the interaction among LINC00324, miR-10b-5p, and PTEN. LINC00324 expression was markedly reduced in leukemia samples and cell lines. Upregulation of LINC00324 inhibited the proliferation of Jurkat and HL-60 cells and increased apoptosis, while its silencing produced the opposite trend. Western blotting showed that LINC00324 overexpression decreased Ki-67 and Bcl-2 but increased BAX expression. Rescue experiments demonstrated that miR-10b-5p mimics reversed, whereas inhibitors restored, the effects of LINC00324. Bioinformatics prediction and luciferase validation identified PTEN as a direct target of miR-10b-5p, and LINC00324 enhanced PTEN expression by sponging miR-10b-5p. LINC00324 regulates the proliferation and apoptosis of leukemia cells through the miR-10b-5p/PTEN axis. These findings add to the understanding of lncRNA-mediated regulatory mechanisms in leukemia.

Key words: LINC00324; leukemia; diagnosis; miR-10b-5p; PTEN

Leukemia, a highly heterogeneous and malignant disease, is characterized by the uncontrolled proliferation of abnormal white blood cells (WBCs) in the bone marrow and other hematopoietic tissues, causing function loss of normal hematopoietic cells and severe disorder symptoms, including thrombocytopenia, anemia, and immunodeficiency [1]. Although chemotherapy and hematopoietic stem cell transplantation have improved the prognosis, more than two-thirds of adult leukemia patients fail to achieve complete remission, highlighting the necessity of the study on the pathology of leukemia and the development of effective drugs.

Long non-coding RNAs (lncRNAs) are defined as RNA molecules with more than 200 nucleotides, without protein-coding capacity [2]. LncRNAs are involved in multi-cellular processes at both transcriptional and post-transcriptional levels [3, 4]. The expression patterns of lncRNAs are highly cell-type specific, and emerging evidence suggests that they could act as potential biomarkers for diagnosis and prognosis in cancers [5–8]. Recent findings have also shown that metabolism-related lncRNA signatures can predict tumor prognosis and therapeutic response in various cancers [9, 10], further emphasizing their translational potential.

In leukemia, lncRNAs have been implicated in critical pathophysiological processes, including modulation of gene expression networks, regulation of apoptotic pathways, and development of chemoresistance [11–13]. For instance, ANRIL was recently reported to influence the proliferation and apoptosis of acute myeloid leukemia (AML) cells through GRK2-mediated signaling [14], supporting the essential roles of lncRNAs in leukemic progression. It was reported that highly expressed lncRNA PVT1 affected the proliferation of NB4 cells, showing a potential therapeutic target in acute promyelocytic leukemia [15]. Moreover,

lncRNA HOTAIR was proven to indicate a poor outcome in leukemia patients [16, 17].

Long intergenic non-protein coding RNA 324 (LINC00324), located at 17p13.1, has been reported to regulate tumor behavior through several miRNA-related pathways, such as the miR-139-5p/IGF1R and miR-195-5p/TRIM29 axes [18, 19]. Our previous work indicated that LINC00324 might act as a tumor suppressor in breast cancer, although its regulatory mechanism remained unclear [20]. In this study, we examined LINC00324 expression in leukemia patients and cell lines and further investigated its effects on cell proliferation and apoptosis. Based on bioinformatic prediction and experimental validation, including rescue assays and protein-level detection, we identified the LINC00324/miR-10b-5p/PTEN pathway as a key regulatory axis in leukemia cells. These findings may provide a basis for understanding the biological function of LINC00324 and its potential therapeutic value in leukemia.

Patients and methods

Patient characteristics and sample collection. This study enrolled 47 AML patients diagnosed and treated at the Affiliated Hospital of Guangxi Medical University (Nanning, China) from September 2020 to January 2021. The cohort comprised 9 *de novo* AML cases, 27 patients in complete remission (CR), and 11 patients in partial remission (PR). Additionally, 39 age-matched healthy individuals were recruited as controls. All participants provided written informed consent prior to biological sample collection. The study protocol was approved by the Medical Ethics Committee of Guangxi Medical University (Approval Number: 20150306-1).

Quantitative real-time PCR (qRT-PCR) assay. Total RNA was isolated from peripheral WBCs (PWBCs) or cell lines using TRIzol reagent. Peripheral blood samples were used because they are easily accessible and have been widely applied in AML biomarker studies. The RNA concentration was determined by ultraviolet spectrophotometry. Subsequent reverse transcription reactions were carried out with the PrimeScript™ RT Reagent Kit (Takara Bio, Dalian, China) following the manufacturer's protocols. PCR assay was performed in triplicate using the Eppendorf PCR Detection System. Primer sequences of LINC00324 were as follows: forward 5'-GACGAGCCCTCCTTTACCTT-3' and reverse 5'-CTGGGATTGAGATGCTTTCT-3'. The primer sequences of GAPDH were as follows: forward 5'-CATGAGAAGTATGACAACAGCCT-3' and reverse 5'-AGTCCTTCCACGATACCAAAGT-3'. The reaction system contained SYBR Green Premix Ex Taq™ (Takara Bio) as a double-stranded DNA-specific fluorescent dye. Relative expression level of LINC00324 was calculated using the $2^{-\Delta\Delta Ct}$ method.

Cell culture. The human leukemia cell lines HL60, K562, Jurkat, and normal PWBC cells were obtained from the Shanghai Institute of Biosciences Cell Resource Center, Chinese Academy of Sciences (Shanghai, China). All cells were maintained in RPMI-1640 medium (Hyclone, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) at 37 °C in an atmosphere humidified with 5% CO_2.

siRNA transfection. For loss-of-function analysis, specific small interfering RNA (siRNA) targeting LINC00324 (si-LINC00324: 5'-UUGGUUACCGACUUGGUGCTT-3') along with scrambled negative control siRNA (si-NC: 5'-UUCUCCGAACGUGUCACGUTT-3') were commercially synthesized by Wuhan GeneCreate Biological Engineering Co., Ltd. (China). siRNA transfection was performed according to the manufacturer's instructions with the RNAiMAX Transfection Reagent (Invitrogen, USA). Following a 72 h post-transfection incubation period under standard culture conditions (37 °C, 5% CO_2), transfected cells were harvested for subsequent functional analyses.

miRNA mimic and inhibitor transfection. For functional rescue experiments, miR-10b-5p mimics, inhibitors, and their corresponding negative controls were purchased from RiboBio (Guangzhou, China). HL-60 and Jurkat cells were transfected using Lipofectamine RNAiMAX (Invitrogen, USA) following the manufacturer's protocol. The final concentration of each oligonucleotide was 50 nM. After 48 h of incubation under standard culture conditions (37 °C, 5% CO_2), cells were collected for subsequent proliferation, apoptosis, and protein expression assays.

Cell proliferation assay. Cell proliferation assay was conducted using a Cell Counting Kit-8 (CCK-8; Dojindo, Shanghai, China). Briefly, 200 µl of cell suspension (5×10^4/well) was added to 96-well plates (6 wells/sample) and incubated at 37 °C in an atmosphere of 5% CO_2 for 24, 48, or 72 h. Next, cells were further incubated for 4 h with 10 µl of the CCK-8 reagent. Finally, the optical density (OD) values were quantified using a microplate reader with absorbance measurement at 450 nm.

Apoptosis analysis. Cell apoptosis was quantitatively analyzed using the Annexin V Apoptosis Assay Kit (Beyotime, Shanghai, China). Briefly, cells (1×10^5/sample) were harvested and subsequently washed twice with ice-cold phosphate-buffered saline (PBS, pH 7.4), stained with 5 µl Annexin V-FITC according to the manufacturer's instructions, and incubated for 30 min at room temperature in the dark. After incubation, flow cytometry was performed using the BD FACS Calibur flow cytometer.

Plasmid construction. For lentivirus-mediated overexpression of LINC00324, the human full-length DNA of LINC00324 was cloned into the pCDH-CMV-MCS-EF1-GFP lentiviral expression vector. Lentiviral particles were generated through transient transfection of 293T cells using Lipofectamine-mediated delivery of the recombinant plasmid along with packaging vectors psPAX2 and pMD2.G. Viral supernatant was harvested 48 h post-transfection. Then, Jurkat cells and HL-60 cells were seeded in 6-well or 12-well plates at 50–60% confluency and infected with lentiviral particles before subsequent experiments.

Western blot analysis. Total proteins were extracted from leukemia cells using RIPA lysis buffer containing protease inhibitors (Beyotime, Shanghai, China). The protein concentration of each sample was quantified by the BCA method. Equal amounts of protein were resolved on 10% SDS-polyacrylamide gels and then transferred to PVDF membranes (Millipore, USA). The membranes were blocked with 5% skimmed milk for 1 h and subsequently incubated overnight at 4 °C with primary antibodies against Ki-67(ab16667, Abcam, UK; 1:1000), BAX(5023T, Cell Signaling Technology, USA; 1:1000), Bcl-2(3498T, Cell Signaling Technology, USA; 1:1000), PTEN(9559T, Cell Signaling Technology, USA; 1:1000), and GAPDH (2118T, Cell Signaling Technology, USA; 1:1000). After washing with TBST buffer, the membranes were exposed to HRP-conjugated secondary antibodies for 1 h at room temperature. Protein signals were developed using an enhanced chemiluminescence reagent (Thermo Fisher, USA) and analyzed by ImageJ software for relative intensity measurement.

Bioinformatic prediction. The potential binding sites between miR-10b-5p and its target genes were predicted using TargetScan, miRDB, and miRTarBase databases. Candidate genes with consistent predictions across all databases were selected for experimental validation. PTEN, E2F7, and NFAT were identified as potential targets, and their expression levels were subsequently evaluated by qRT-PCR and western blotting after miR-10b-5p mimic transfection.

Dual-luciferase reporter assay. Wild-type (WT) and mutant (MUT) fragments of LINC00324 or the PTEN 3'-UTR containing the predicted miR-10b-5p binding sites were cloned into the pmirGLO reporter vector (Promega, USA). 293T cells were co-transfected with the reporter plasmid and miR-10b-5p mimics or negative control using Lipofectamine 3000 (Invitrogen, USA). After 48 h, luciferase activity was determined using the Dual-Luciferase Reporter Assay System (Promega, USA). Firefly luciferase activity was normalized to Renilla luciferase activity.

Statistical analyses. Statistical analysis was performed with SPSS version 17.0 (SPSS Inc., USA). The differences between the two groups were analyzed using Student's t-test, while one-way ANOVA was used for comparing more than two groups. Correlation analysis was performed using the Spearman coefficient. Receiver operating characteristic curves (ROC curves) were drawn for evaluating diagnostic performance. Data are expressed as mean ± standard deviation (SD). A p-value <0.05 was considered statistically significant.

Results

Decreased levels of LINC00324 in leukemia. We first detected the expression levels of LINC00324 in leukemia and found a significant decrease in PWBCs from both AML and ALL patients (Figures 1A, 1B; p<0.0001 and p<0.001). Comparative analysis across leukemia subtypes (M2, M3, M4, M5) showed that the expression profile of LINC00324 was also significantly reduced (Figure 1C, Table 2; p<0.001). To evaluate the potential of LINC00324 in therapeutic efficacy and diagnosis, we analyzed the difference in LINC00324 levels between *de novo*, CR, and PR patients. As shown in Figure 1D and Table 2, *de novo* AML patients (p<0.0001) and those with PR (p<0.001) exhibited persistently suppressed LINC00324 levels compared to healthy participants. Diagnostic performance evaluation through ROC curve analysis yielded an area under the curve (AUC) of 0.8456 (p<0.0001; 95% CI: 0.7472–0.9439), with optimal diagnostic accuracy achieved at a cutoff value of 0.65 (sensitivity: 87.2%; specificity: 61.7%) (Figure 1E). We then further analyzed the clinical relevance of LINC00324 in relation to several factors. As shown in Table 1, the expression level of LINC00324 was positively correlated with platelet count (p<0.05), but no correlation with other characteristics, including gender, age, AML subtype, hemoglobin, and bone marrow blast count. In

Table 1. Relationship between LINC00324 and clinical characteristics of AML patients.

Characteristics	X ± s	p-value
Age (years)		0.612
<40	0.65 ± 0.49	
≥40	0.56 ± 0.5	
Gender		0.110
Man	0.70 ± 0.56	
Female	0.45 ± 0.27	
AML subtype		0.980
M2	0.41 ± 0.28	
M3	0.41 ± 0.32	
M4	0.47 ± 0.36	
M5	0.41 ± 0.29	
Hemoglobin (g/l)		0.467
<60	0.43 ± 0.31	
≥60	0.47 ± 0.35	
Platelets ($\times 10^9$/l)		0.023
<30	0.39 ± 0.28	
≥30	0.87 ± 0.52	
Bone marrow blasts (%)		0.427
<60	0.42 ± 0.29	
≥60	0.48 ± 0.31	

Table 2. Clinical pathological parameters of patients with AML.

Feature	n	%
FAB classification		
M2	5	10.6
M3	13	27.7
M4	19	40.3
M5	10	21.3
Response Criteria		
de novo	9	19.1
CR	27	57.4
PR	11	23.4

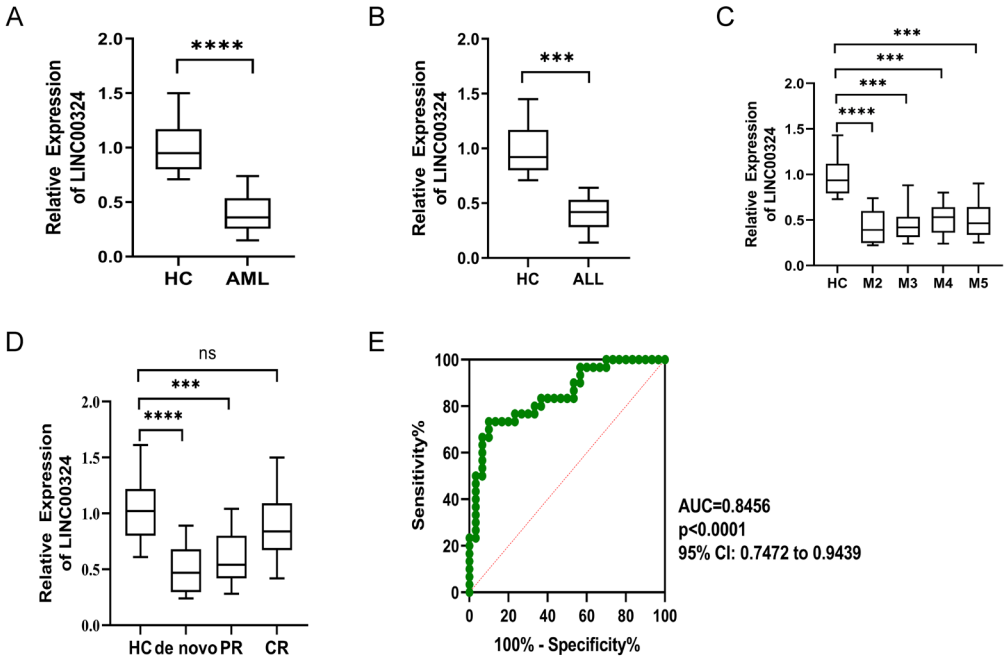

Figure 1. Decreased expression of LINC00324 in leukemia patients. A) Comparative analysis of LINC00324 expression levels in WBCs from AML patients. B) Differential expression of LINC00324 in WBCs from ALL patients. C) Subtype-specific expression profiling of LINC00324 in AML patients. D) LINC00324 levels in samples from *de novo*, CR, and PR patients. (E) Diagnostic performance evaluation of LINC00324 using ROC curve analysis. Data are expressed as mean ± SD; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

summary, these data suggest that LINC00324 may serve as a promising biomarker for therapeutic efficacy and diagnosis in AML.

LINC00324 inhibits the proliferation of leukemia cells. Although the role of LINC00324 in several solid tumors has been studied, its function in leukemia cells remains unclear. We first examined LINC00324 expression in leukemia cell lines (HL-60, K562, and Jurkat) and PWBCs. As shown in Figure 2A, LINC00324 was markedly downregulated in leukemia cells compared with PWBCs. To explore its biological role, we conducted knockdown experiments on Jurkat cells and overexpression experiments on HL-60 cells. The efficiency of siRNA-mediated silencing and lentiviral overexpression was confirmed (Figures 2B, 2C). CCK-8 assays showed that LINC00324 overexpression suppressed cell proliferation, whereas LINC00324 knockdown enhanced it (Figures 2D, 2E). Flow-cytometric analysis further revealed that the apoptotic rate increased in LINC00324-overexpressing cells but decreased after LINC00324 silencing (Figure 2F). Consistent with these findings, western blot analysis demonstrated reduced Ki-67 and Bcl-2 levels and elevated BAX expression after LINC00324 overexpression, while the opposite trend was observed in LINC00324-silenced cells (Figure 2G). These results indicate that LINC00324 suppresses abnormal proliferation and promotes apoptosis in leukemia cells.

LINC00324 regulates the proliferation of leukemia cells by acting as the sponge of miR-10b-5p. Our previous study suggested that LINC00324 can function as a molecular sponge for miR-10b-5p [20]. To determine whether this mechanism also exists in leukemia cells, we conducted a dual-luciferase reporter assay. As shown in Figure 3A, miR-10b-5p mimics significantly reduced the luciferase activity of the LINC00324 wild-type vector, but not the mutant construct, confirming the direct interaction between LINC00324 and miR-10b-5p. We next examined whether miR-10b-5p affects the biological function of LINC00324 in leukemia cells. Overexpression of LINC00324 in HL-60 cells inhibited cell proliferation, while co-transfection with miR-10b-5p mimics reversed this effect (Figure 3B). In contrast, the enhanced proliferation caused by LINC00324 silencing in Jurkat cells was attenuated by miR-10b-5p inhibitors (Figure 3C). Flow-cytometric analysis showed that LINC00324 overexpression increased the apoptotic rate, whereas this effect was weakened by miR-10b-5p mimics; conversely, miR-10b-5p inhibitors restored apoptosis in LINC00324-silenced cells (Figure 3D). Western blot analysis revealed that LINC00324 overexpression decreased Ki-67 and Bcl-2 and increased BAX levels, while miR-10b-5p mimics reversed these protein changes. Similarly, miR-10b-5p inhibitors counteracted the effects of LINC00324 knockdown (Figure 3E). These results indicate that LINC00324 suppresses abnormal proliferation

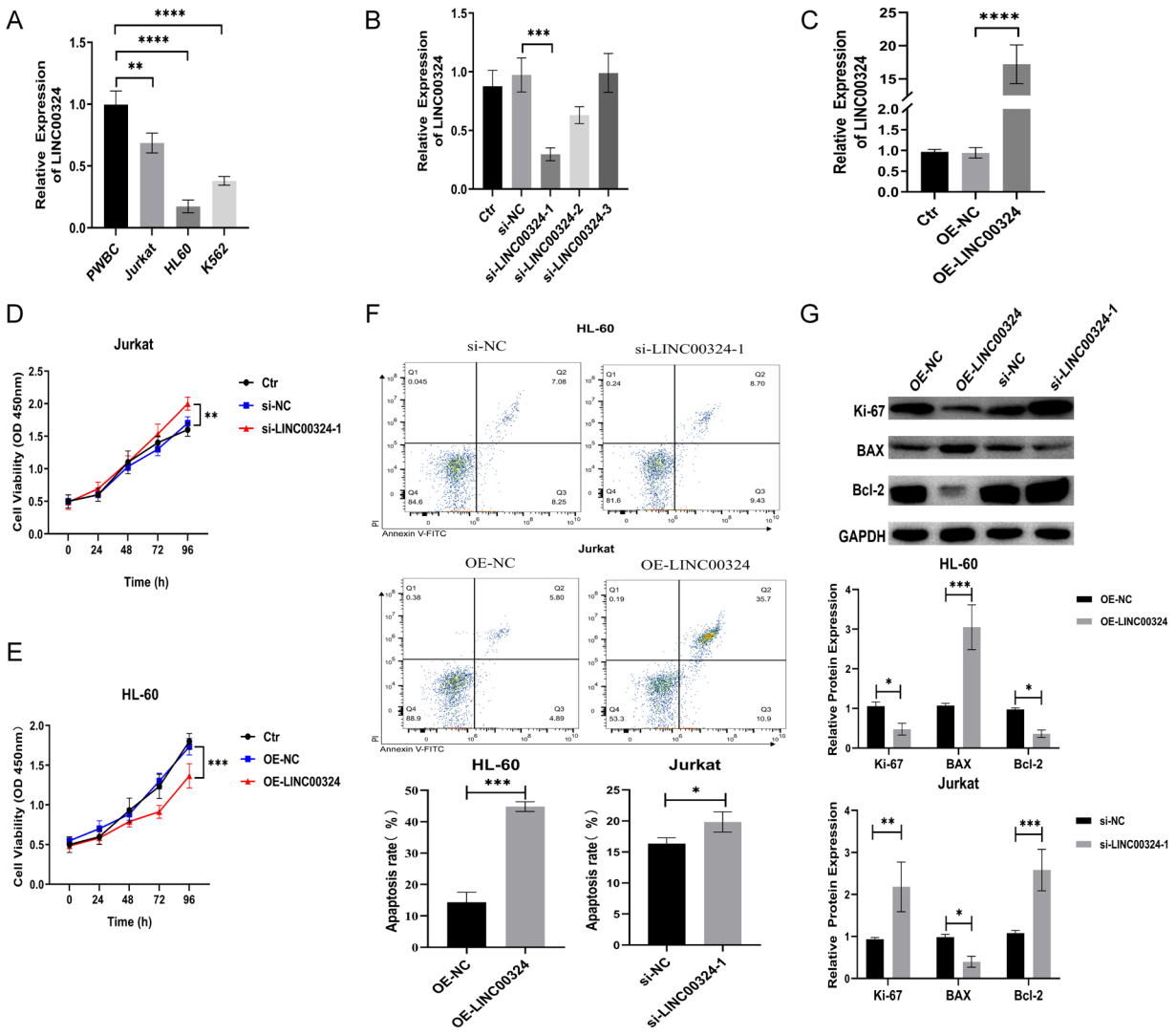

Figure 2. LINC00324 inhibits the proliferation and promotes apoptosis of leukemia cells. A) Relative LINC00324 expression in PWBCs and leukemia cell lines (HL-60, K562, and Jurkat). B) Knockdown efficiency of si-LINC00324 in Jurkat cells. C) Overexpression efficiency of LINC00324 in HL-60 cells. D, E) The CCK-8 assay results showed that after knocking down the LINC00324 gene in Jurkat cells or overexpressing LINC00324 in HL-60 cells, the proliferation of the cells was affected. F) Apoptosis analysis by Annexin V/PI staining showing an increased apoptotic rate in LINC00324-overexpressing cells and a decreased rate after LINC00324 silencing. G) Western blot detection of Ki-67, BAX, and Bcl-2 expression after LINC00324 overexpression or knockdown. Data are presented as mean ± SD; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

and promotes apoptosis in leukemia cells partly by sponging miR-10b-5p.

PTEN is the direct target of miR-10b-5p. To identify downstream targets of the LINC00324/miR-10b-5p axis, bioinformatic prediction was first carried out. PTEN, E2F7, and NFAT were predicted as potential targets (Figure 4A). After miR-10b-5p mimic transfection, PTEN mRNA and protein levels were markedly reduced, while E2F7 and NFAT showed no obvious change (Figures 4B, 4C). Luciferase reporter assays confirmed that miR-10b-5p directly binds to the 3'-UTR of PTEN (Figure 4D). In addition, PTEN expression was increased by LINC00324 overexpression and decreased after LINC00324 silencing (Figures 4E, 4F). These results indicate that PTEN is a direct target of miR-10b-5p and that LINC00324 upregulates PTEN by sequestering miR-10b-5p in leukemia cells.

Discussion

An increasing number of molecular biomarkers have been tried to make a prognosis of leukemia, including non-coding RNAs, proteins, and metabolites [1, 12, 21]. Among them, lncRNAs have demonstrated critical regulatory roles in

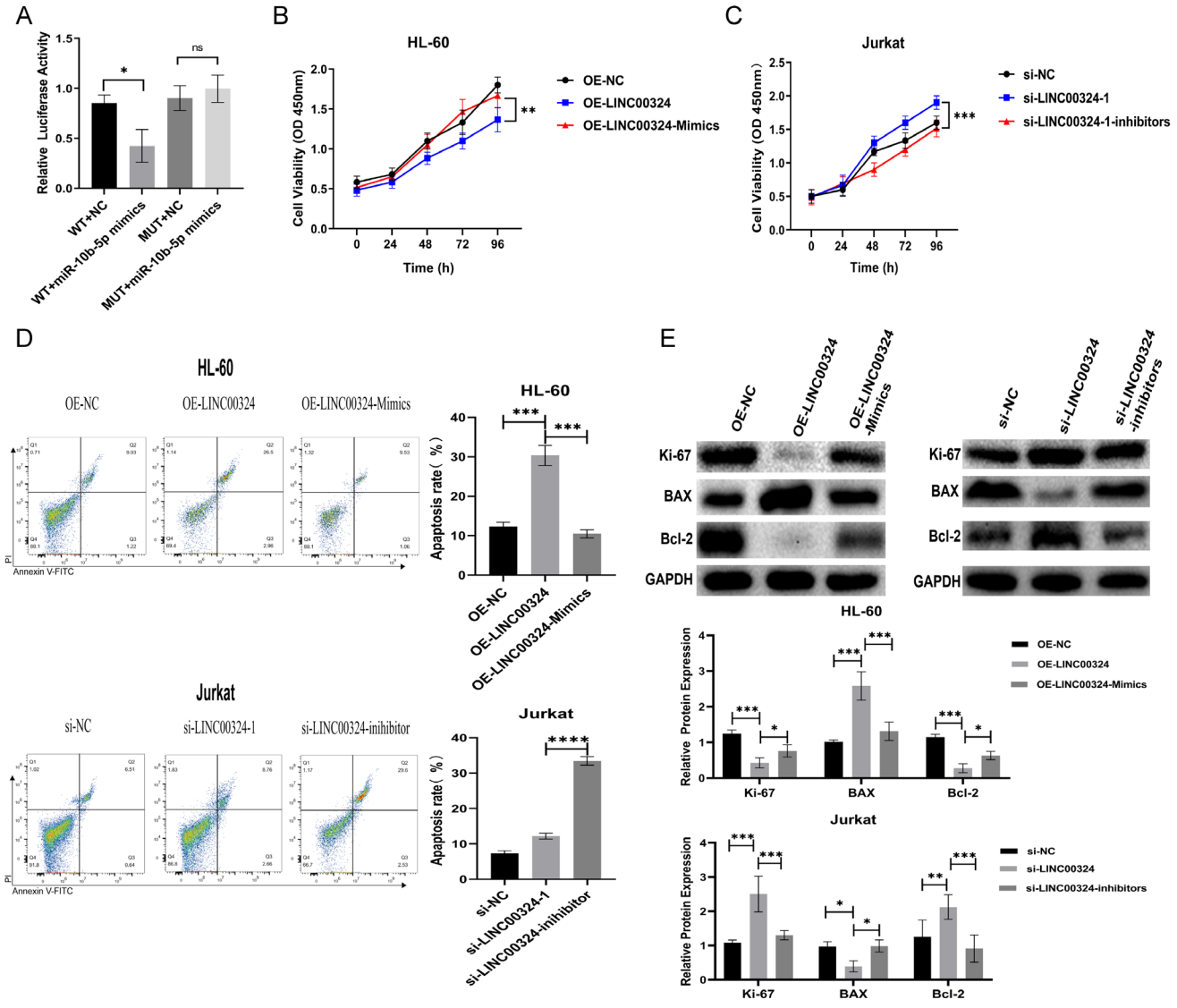

Figure 3. LINC00324 regulates leukemia-cell proliferation and apoptosis through miR-10b-5p. A) Dual-luciferase reporter assay confirming the binding of miR-10b-5p to LINC00324. B, C) The CCK-8 assay results showed that the miR-10b-5p mimics were able to reverse the cell proliferation effect mediated by the overexpression of LINC00324 in HL-60 cells, while the miR-10b-5p inhibitors were able to rescue the proliferation effect mediated by the knockdown of LINC00324 in Jurkat cells. D) Annexin V/PI flow-cytometric analysis showing changes in apoptosis under different treatment conditions. E) Western blot analysis of Ki-67, BAX, and Bcl-2 expression after miR-10b-5p mimic or inhibitor treatment in LINC00324-modified cells. Data are presented as mean ± SD; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

regulating leukemia cell proliferation and apoptosis by modulating cellular processes, including survival, differentiation, proliferation, and metastatic potential [11, 13, 22, 23]. Notably, accumulating clinical evidence correlates lncRNA dysregulation with leukemia characteristics and outcomes [11, 24]. HOTAIRM1 and NEAT1 are reported to act as vital regulators in the differentiation of leukemia cells [25–27]. In addition, high levels of TUG1, MALAT-1, HOTAIR, and lnc-SOX6-1 have been associated with poor prognosis in leukemia [28–31].

Although LINC00324 has been implicated in the development of tumors, including gastric cancer, glioma, and colorectal cancer [32–35], it demonstrates tumor-suppressive functions in the tumorigenesis of breast cancer [20]. An elevated level of LINC00324 is associated with the long-term survival of breast cancer patients [36]. Our preliminary investigation further identified a potential tumor-suppressive role of LINC00324 in leukemia, despite its unclear molecular mechanism [27]. These conflicting observations underscore the need to systematically investigate the context-depen-

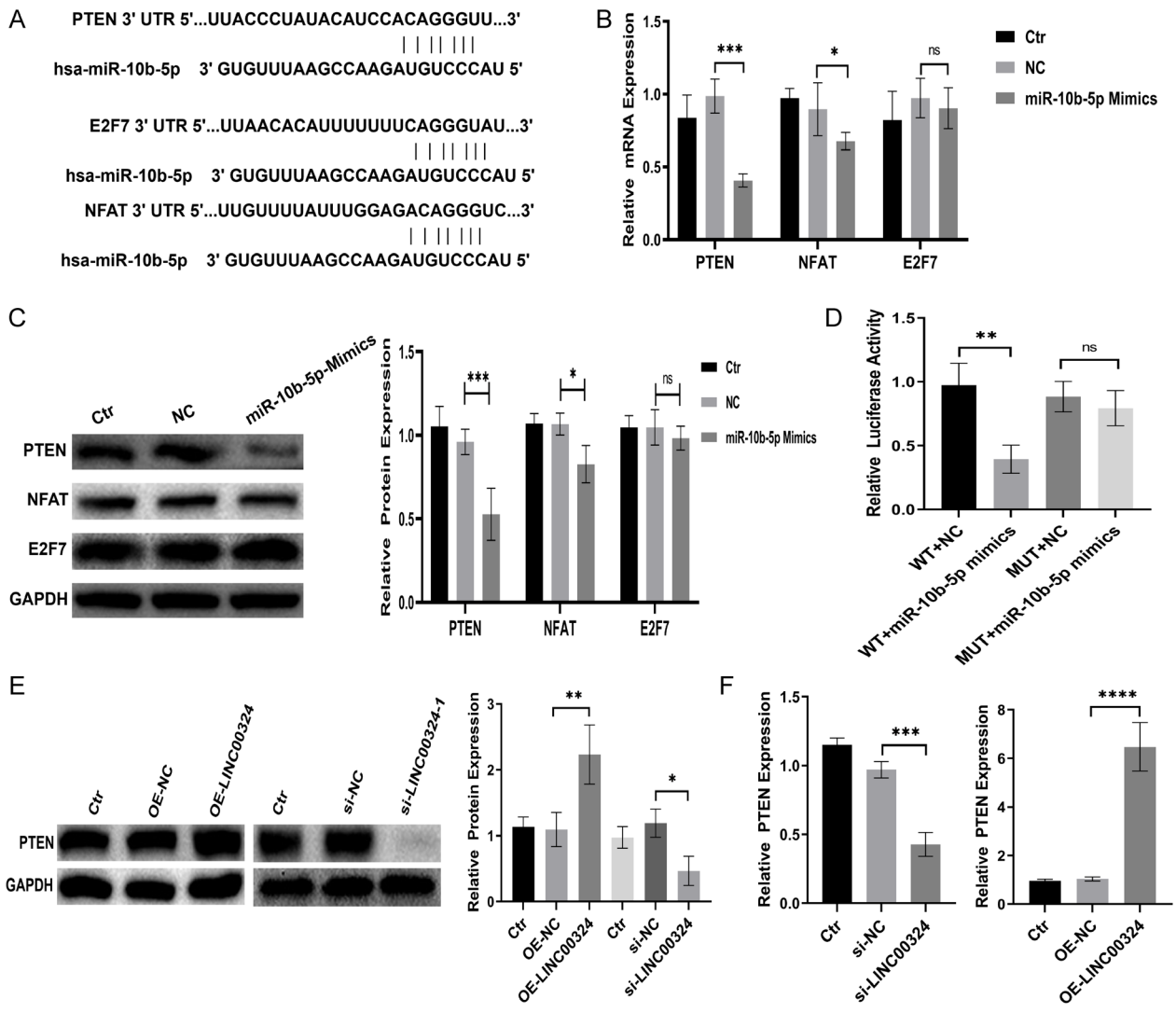

Figure 4. LINC00324 regulates PTEN expression through miR-10b-5p. A) Bioinformatic prediction showing the potential binding of miR-10b-5p to PTEN, E2F7, and NFAT. B) qRT-PCR analysis of PTEN, E2F7, and NFAT expression after miR-10b-5p mimic transfection. C) Western blot validation of PTEN, E2F7, and NFAT protein levels following miR-10b-5p mimic treatment. D) Dual-luciferase assay confirming direct interaction between miR-10b-5p and the 3'-UTR of PTEN. E) Western blot was used to detect the expression of PTEN protein after overexpression or knockdown of LINC00324. F) qRT-PCR results showing PTEN expression changes after LINC00324 overexpression or knockdown. Data are shown as mean ± SD; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

dent functionality of LINC00324 in hematologic malignancies. In this study, we identified significant downregulation of LINC00324 in leukemia patients, especially in *de novo* and PR subgroups. This expression pattern was recapitulated in leukemia cell lines, suggesting a potential association between LINC00324 deficiency and abnormal proliferation of leukemia cells. The experimental data suggest that decreased LINC00324 expression levels may correlate with leukemia pathogenesis.

Functional studies employing siRNA-mediated knockdown demonstrated an enhanced proliferative capacity in Jurkat cells, whereas overexpression of LINC00324 significantly attenuated HL-60 cell proliferation. This tumor-suppressive activity contrasts with previously reported oncogenic functions of LINC00324 in cancers [35, 37, 38]. The reason for this contradiction might arise from the fact that LINC00324 exhibits different expression patterns and functions in different tissues or cancer types [32], as well as the tumor microenvironment and cellular context [33]. These mechanistic hypotheses warrant systematic validation through subsequent investigations.

We acknowledge that the cellular composition of peripheral blood differs between AML patients and healthy controls, which may partly influence gene expression patterns. Never-

theless, peripheral blood-based analyses have been widely used in AML research because of their accessibility and reproducibility. Similar approaches have been reported in previous biomarker studies [39, 40]. Future studies using $CD34^+$ bone marrow cells will help further validate the current findings.

Emerging evidence has elucidated the pivotal regulatory roles of lncRNAs through acting as molecular sponges for miRNAs [41]. Several miRNAs, including miR-3164 [42], miR-769-5p [43], and miR-615-5p [44], were identified as the downstream factors of LINC00324 in cancers. Our previous investigations have also confirmed that LINC00324 binds to cytoplasmic miR-10b-5p and suppresses its activity in breast cancer [20]. Therefore, we sought to investigate whether this miRNA sponging mechanism extends to hematopoietic malignancies, and it was validated with a dual-luciferase reporter assay system in leukemia cell models.

To elucidate the molecular target of miR-10b-5p, we conducted bioinformatics-based screening of potential candidate genes and subsequently profiled their expression levels in miR-10b-5p-overexpressing cell models. Among the predicted targets, PTEN exhibited the most significant downregulation. This inverse correlation was further validated through dual-luciferase reporter assays, confirming direct targeting of PTEN by miR-10b-5p. PTEN is a critical tumor suppressor gene that plays a central role in regulating tumor cell apoptosis by modulating the PI3K/AKT/mTOR signaling pathway, activating pro-apoptotic proteins such as BAD and caspase-9, and inhibiting anti-apoptotic factors like Bcl-2[40]. The observed miR-10b-5p-mediated PTEN suppression suggests a novel epigenetic mechanism underlying tumor cell survival and apoptosis resistance. Consistent with this, LINC00324 overexpression upregulated PTEN and shifted the balance of BAX, Bcl-2, and Ki-67 expression, supporting its regulatory role in leukemia cell proliferation and apoptosis.

Collectively, our findings demonstrate that LINC00324 exhibits significant downregulation in leukemic malignancies. Through functional characterization, we established that enforced LINC00324 expression substantially suppresses leukemia cell proliferation via modulating the miR-10b-5p/PTEN signaling axis. Mechanistic investigations revealed that this tumor-suppressive effect is mediated through competitive binding of miR-10b-5p, thereby restoring PTEN expression. The identification of the LINC00324-mediated pathway provides both diagnostic biomarkers and potential targeted therapeutic approaches for leukemia management, advancing our understanding of non-coding RNA biology in hematological malignancies. Although these findings were based on cellular models, they provide mechanistic insight into the LINC00324/miR-10b-5p/PTEN axis in leukemia. Further studies using patient-derived bone marrow cells and animal models will help to clarify its biological and clinical relevance.

In conclusion, LINC00324 may act as a regulator of leukemia cell proliferation and apoptosis through the miR-10b-5p/PTEN signaling axis, providing a basis for further functional and *in vivo* investigations.

Acknowledgments: Thank all the authors for their support for this research. This work was supported by grants from the the National Natural Science Foundation of China (grant number 81560434 and 32260164) and Natural Science Foundation of Guangxi Zhuang Autonomous Region (grant numbers: 2017GXNSFAA198151, 2018GXNSFAA281240 and 2023GXNSFAA026080).

References

[1] BHAT AA, YOUNES SN, RAZA SS, ZARIF L, NISAR S et al. Role of non-coding RNA networks in leukemia progression, metastasis and drug resistance. Mol Cancer 2020; 19: 57. https://doi.org/10.1186/s12943-020-01175-9

[2] YUAN Y, TANG Y, FANG Z, WEN J, WICHA MS et al. Long Non-Coding RNAs: Key Regulators of Tumor Epithelial/Mesenchymal Plasticity and Cancer Stemness. Cells 2025; 14: 227. https://doi.org/10.3390/cells14030227

[3] BAGHERI-MOHAMMADI S, KARAMIVANDISHI A, MAHDAVI SA, SIAHPOSHT-KHACHAKI A. New sights on long non-coding RNAs in glioblastoma: A review of molecular mechanism. Heliyon 2024; 10: e39744. https://doi.org/10.1016/j.heliyon.2024.e39744

[4] KUMAR A, YAP KC, BHARATHWAJ CHETTY B, LYU J, HEGDE M et al. Regulating the regulators: long non-coding RNAs as autophagic controllers in chronic disease management. J Biomed Sci 2024; 31: 105. https://doi.org/10.1186/s12929-024-01092-9

[5] CHEN Y, YE X, HU M, HU Y, DING J. Long non-coding RNAs in pancreatic cancer. Clin Chim Acta 2025; 566: 120040. https://doi.org/10.1016/j.cca.2024.120040

[6] HENDRI AZ, SURYAWATI S, HERIYANTO DS, HARDIANTI MS, PIKATAN NW et al. Long non-coding RNAs as prognostic biomarkers in non-muscle invasive bladder cancer: A systematic review. Narra J 2024; 4: e1233. https://doi.org/10.52225/narra.v4i3.1233

[7] KADIAN LK, VERMA D, LOHANI N, YADAV R, RANGA S et al. Long non-coding RNAs in cancer: multifaceted roles and potential targets for immunotherapy. Mol Cell Biochem 2024; 479: 3229–3254. https://doi.org/10.1007/s11010-024-04933-1

[8] YANG C, SHU J, LI Y, ZHAO N, LIU X et al. Long non-coding RNAs are involved in the crosstalk between cancer-associated fibroblasts and tumor cells. Front Immunol 2024; 15: 1469918. https://doi.org/10.3389/fimmu.2024.1469918

[9] LEI H, XIANG T, ZHU H, HU X. A Novel Cholesterol Metabolism-Related lncRNA Signature Predicts the Prognosis of Patients with Hepatocellular Carcinoma and Their Response to Immunotherapy. Front Biosci (Landmark Ed) 2024; 29: 129. https://doi.org/10.31083/j.fbl2903129

[10] LIN YH. Crosstalk of lncRNA and Cellular Metabolism and Their Regulatory Mechanism in Cancer. Int J Mol Sci 2020; 21: 2947. https://doi.org/10.3390/ijms21082947

[11] ALIMOHAMMADI M, ABOLGHASEMI H, CHO WC, REITER RJ, MAFI A et al. Interplay between LncRNAs and autophagy-related pathways in leukemia: mechanisms and clinical implications. Med Oncol 2025; 42: 154. https://doi.org/10.1007/s12032-025-02710-8

[12] ALLURI A, SAXENA P, MISHRA A, GUTTI RK. Association of long non-coding RNA in lipid metabolism: Implications in leukemia. Int J Biochem Cell Biol 2025; 184: 106785. https://doi.org/10.1016/j.biocel.2025.106785

[13] FARAJZADEH M, FATHI M, JALALI P, MAHMOUD-SALEHI KHESHTI A, KHODAYARI S et al. Long noncoding RNAs in acute myeloid leukemia: biomarkers, prognostic indicators, and treatment potential. Cancer Cell Int 2025; 25: 131. https://doi.org/10.1186/s12935-025-03763-5

[14] WANG S, ZHANG C, HU H, XU J, ZHANG J et al. GRK2 Protein Mediates the ANRIL, a lncRNA, to Affect the Proliferation and Apoptosis of Kasumi-1 Cells. Front Biosci (Landmark Ed) 2024; 29: 362. https://doi.org/10.31083/j.fbl2910362

[15] ZENG C, YU X, LAI J, YANG L, CHEN S et al. Overexpression of the long non-coding RNA PVT1 is correlated with leukemic cell proliferation in acute promyelocytic leukemia. J Hematol Oncol 2015; 8: 126. https://doi.org/10.1186/s13045-015-0223-4

[16] DASHTI NR, FADAVI D, REZAEI R, RAHGOZAR S, MOAFI A. Circulating lncRNA HOTAIR is a biomarker for pediatric acute lymphoblastic leukemia and mediator of miR-326 exosomal export. Sci Rep 2025; 15: 4901. https://doi.org/10.1038/s41598-025-87857-0

[17] LI ML, WANG Y, XU YN, LU QY. Overexpression of LncRNA-HOTAIR promotes chemoresistance in acute leukemia cells. Int J Clin Exp Pathol 2020; 13: 3044–3051.

[18] ZHANG M, LIN B, LIU Y, HUANG T, CHEN M et al. LINC00324 affects non-small cell lung cancer cell proliferation and invasion through regulation of the miR-139-5p/IGF1R axis. Mol Cell Biochem 2020; 473: 193–202. https://doi.org/10.1007/s11010-020-03819-2

[19] XU J, LI Z, SU Q, ZHAO J, MA J. Suppression of long noncoding RNA LINC00324 restricts cell proliferation and invasion of papillary thyroid carcinoma through downregulation of TRIM29 via upregulating microRNA-195-5p. Aging (Albany NY) 2020; 12: 26000–26011. https://doi.org/10.18632/aging.202219

[20] WANG B, ZHANG Y, ZHANG H, LIN F, TAN Q et al. Long intergenic non-protein coding RNA 324 prevents breast cancer progression by modulating miR-10b-5p. Aging (Albany NY) 2020; 12: 6680–6699. https://doi.org/10.18632/aging.103021

[21] CHATTERJEE M, GUPTA S, KUMAR U, PARASHAR D, MAITRA A et al. Extracellular vesicles in acute myeloid leukemia: The role in disease pathogenesis, potential biomarker, and application in clinical settings. Crit Rev Oncol Hematol 2025; 211: 104743. https://doi.org/10.1016/j.critrevonc.2025.104743

[22] Li S, Gao R, Han X, Wang K, Kang B et al. MALAT1/miR-582-5p/GALNT1/MUC1 axis modulates progression of AML leukemia stem cells by regulating JAK2/STAT3 pathway. Ann Hematol 2024; 103: 5273–5283. https://doi.org/10.1007/s00277-024-06043-w

[23] WILSON C, SWAROOP P, KUMAR S, CHOPRA A, SHARAWAT SK. Molecular leveraging of HOX-embedded noncoding RNAs in the progression of acute myeloid leukemia. Hum Cell 2024; 38: 24. https://doi.org/10.1007/s13577-024-01149-9

[24] PRIYA, GARG M, TALWAR R, BHARADWAJ M, RUWALI M et al. Clinical relevance of long non-coding RNA in acute myeloid leukemia: A systematic review with meta-analysis. Leuk Res 2024; 147: 107595. https://doi.org/10.1016/j.leukres.2024.107595

[25] GASIC V, KARAN-DJURASEVIC T, PAVLOVIC D, ZUKIC B, PAVLOVIC S et al. Diagnostic and Therapeutic Implications of Long Non-Coding RNAs in Leukemia. Life (Basel) 2022; 12: 1770. https://doi.org/10.3390/life12111770

[26] GOURVEST M, BROUSSET P, BOUSQUET M. Long Noncoding RNAs in Acute Myeloid Leukemia: Functional Characterization and Clinical Relevance. Cancers (Basel) 2019; 11: 1638. https://doi.org/10.3390/cancers11111638

[27] GAO J, WANG F, WU P, CHEN Y, JIA Y. Aberrant LncRNA Expression in Leukemia. J Cancer 2020; 11: 4284–4296. J Cancer 2020; 11: 4284–4296. https://doi.org/10.7150/jca.42093

[28] LI Q, SONG W, WANG J. TUG1 confers Adriamycin resistance in acute myeloid leukemia by epigenetically suppressing miR-34a expression via EZH2. Biomed Pharmacother 2019; 109: 1793–1801. https://doi.org/10.1016/j.biopha.2018.11.003

[29] HUANG J, FANG J, CHEN Q, CHEN J, SHEN J. Epigenetic silencing of E-cadherin gene induced by lncRNA MALAT-1 in acute myeloid leukaemia. J Clin Lab Anal 2022; 36: e24556. https://doi.org/10.1002/jcla.24556

[30] ZHANG YY, HUANG SH, ZHOU HR, CHEN CJ, TIAN LH et al. Role of HOTAIR in the diagnosis and prognosis of acute leukemia. Oncol Rep 2016; 36: 3113–3122. https://doi.org/10.3892/or.2016.5147

[31] GUAN X, WEN X, XIAO J, AN X, YU J et al. Lnc-SOX6-1 upregulation correlates with poor risk stratification and worse treatment outcomes, and promotes cell proliferation while inhibits apoptosis in pediatric acute myeloid leukemia. Int J Lab Hematol 2019; 41: 234–241. https://doi.org/10.1111/ijlh.12952

[32] GHAFOURI-FARD S, SAFARZADEH A, HUSSEN BM, TAHERI M, RASHNOO F. A concise review on the role of LINC00324 in different cancers. Pathol Res Pract 2022; 240: 154192. https://doi.org/10.1016/j.prp.2022.154192

[33] JIN X, ZHU J, YU H, SHI S, SHEN K et al. Dysregulation of LINC00324 promotes poor prognosis in patients with glioma. PLoS One 2024; 19: e0298055. https://doi.org/10.1371/journal.pone.0298055

[34] WANG S, CHENG Y, YANG P, QIN G. Silencing of Long Noncoding RNA LINC00324 Interacts with MicroRNA-3200-5p to Attenuate the Tumorigenesis of Gastric Cancer via Regulating BCAT1. Gastroenterol Res Pract 2020; 2020: 4159298. https://doi.org/10.1155/2020/4159298

[35] XIA Q, SHEN J, WANG Q, KE Y, YAN Q et al. LINC00324 in cancer: Regulatory and therapeutic implications. Front Oncol 2022; 12: 1039366. https://doi.org/10.3389/fonc.2022.1039366

[36] ZHAO H, FENG K, LEI J, SHU Y, BO L et al. Identification of somatic mutation-driven enhancers and their clinical utility in breast cancer. iScience 2024; 27: 108780. https://doi.org/10.1016/j.isci.2024.108780

[37] NI S, WEI Z, LI D. Effect of lncRNA LINC00324 on cervical cancer progression through down-regulation of miR-195-5p. J Obstet Gynaecol 2023; 43: 2285384. https://doi.org/10.1080/01443615.2023.2285384

[38] SUN GK, XU ZJ, NAN FY, TANG LJ, YAO DM. Dysregulation of LINC00324 associated with methylation facilitates leukemogenesis in de novo acute myeloid leukemia. Int J Lab Hematol 2022; 44: 567–575. https://doi.org/10.1111/ijlh.13809

[39] GUO L, KOU R, SONG Y, LI G, JIA X et al. Serum hsa_circ_0079480 is a novel prognostic marker for acute myeloid leukemia. J Clin Lab Anal 2022; 36: e24337. https://doi.org/10.1002/jcla.24337

[40] POGOSOVA-AGADJANYAN EL, HUA X, OTHUS M, APPELBAUM FR, CHAUNCEY TR et al. Verification of prognostic expression biomarkers is improved by examining enriched leukemic blasts rather than mononuclear cells from acute myeloid leukemia patients. Biomark Res 2023; 11: 31. https://doi.org/10.1186/s40364-023-00461-0

[41] GHAFOURI-FARD S, GLASSY MC, ABAK A, HUSSEN BM, NIAZI V et al. The interaction between miRNAs/lncRNAs and Notch pathway in human disorders. Biomed Pharmacother 2021; 138: 111496. https://doi.org/10.1016/j.biopha.2021.111496

[42] CHEN H, WEI L, LUO M, WANG X, ZHU C et al. LINC00324 suppresses apoptosis and autophagy in nasopharyngeal carcinoma through upregulation of PAD4 and activation of the PI3K/AKT signaling pathway. Cell Biol Toxicol 2022; 38: 995–1011. https://doi.org/10.1007/s10565-021-09632-x

[43] DONG Y, WAN G, YAN P, QIAN C, LI F et al. Long noncoding RNA LINC00324 promotes retinoblastoma progression by acting as a competing endogenous RNA for microRNA-769-5p, thereby increasing STAT3 expression. Aging (Albany NY) 2020; 12: 7729–7746. https://doi.org/10.18632/aging.103075

[44] PAN ZH, GUO XQ, SHAN J, LUO SX. LINC00324 exerts tumor-promoting functions in lung adenocarcinoma via targeting miR-615-5p/AKT1 axis. Eur Rev Med Pharmacol Sci 2018; 22: 8333–8342. https://doi.org/10.26355/eurrev_201812_16531