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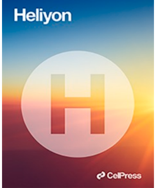

Research article

The AC010247.2/miR-125b-5p axis triggers the malignant progression of acute myelocytic leukemia by IL-6R

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A R T I C L E I N F O

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A B S T R A C T

AML is a malignant tumor derived from the hematopoietic system, which has a poor prognosis and its incidence is increasing recent years. LncRNAs bind to miRNAs as competitive endogenous RNAs to regulate the occurrence and progression of AML , with IL-6R playing a crucial role in hematological malignancies. However, the mechanism by which noncoding RNAs regulate IL6R expression in AML remains unclear. This study found that the AC010247.2/miR-125b-5p axis promotes AML progression by regulating IL-6R expression. Specifically, knocking down or inhibiting AC010247.2 and miR-125b-5p affected IL6R and its downstream genes. Mechanistically, AC010247.2 acts as a ceRNA for miR-125b-5p, influencing IL-6R expression. Additionally, AC010247.2's regulation of AML progression partially depends on miR-125b-5p. Notably, the AC010247.2/miR-125b-5p/IL6R axis serves as a better polygenic diagnostic marker for AML. Our study identifies a key ceRNA regulatory axis that modulates IL6R expression in AML, providing a reliable multigene diagnostic method and potential therapeutic target.

1. Introduction

Acute myeloid leukemia (AML) is a common invasive hematopoietic malignancy, which is characterized by heterogeneity and rapid progression [1,2]. AML is highly destructive and affects a wide population, with an average survival rate of 5%–15 % and a high recurrence rate, especially in older patients [2]. AML is diagnosed by bone marrow biopsy and peripheral blood samples [3]. Bone marrow biopsies are invasive, and to obtain successful results with the testing of peripheral blood samples, the samples must have leukemic blasts in circulation, which are usually present at an advanced stage of the disease with a high disease burden [4]. The current standard treatments for AML include induction chemotherapy, intensive consolidation therapy, and autologous or allogeneic hematopoietic stem cell transplantation [5]. Although most patients respond to standard therapy and show complete remission, relapses are frequent after treatment. Patients undergoing intensive therapy and allogeneic stem cell transplantation have a very poor

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prognosis, with a relatively high treatment-related morbidity and mortality [6]. Thus, new biomarkers must be explored urgently for the effective early diagnosis of AML, and new treatment methods with less toxicity and better efficiency are needed.

Non-coding RNAs (ncRNAs), which are transcribed from most of the non-protein-coding parts of the genome, do not encode proteins but can regulate gene expression and protein function. These molecules are key in physiology and pathology [7]. MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are included in this category [8,9]. miRNAs are single-stranded RNA molecules, typically 19–24 nucleotides long, derived from longer RNA precursors [10]. The primary function of miRNAs is to bind to specific target mRNA through a part of the complementary sequence of the 3′ UTR. This causes mRNA instability, translation inhibition, and mRNA cleavage, thus silencing mRNA and inhibiting expression [11,12]. During tumorigenesis and development, miRNA can perform both tumor-suppressor and tumor-promoting functions by targeting different mRNAs. For example, miR-21 is expressed at high levels in breast cancer cells, and anti-miR-21 can inhibit tumor growth [13]. The expression of let-7e is also enhanced in esophageal squamous cell carcinoma, which promotes tumor migration and invasion [14]. However, miR-146a-5p plays different roles in different tumors [15]. Many studies have explored miRNAs in the blood, and their adverse effects in the blood primarily include promoting tumor progression and increasing drug resistance in hematological tumors. Abnormally high expression of miR-155 or miR-29a could promote the progression of AML [16,17]. miR-125b also contributes to leukemia development, while miR-125b-5p can be a biomarker for lung cancer diagnosis and prognosis [18,19].

Long noncoding RNAs (lncRNAs) are non-protein-coding RNAs over 200 nt in length, which are transcribed by RNA polymerase II [20,21]. LncRNAs are involved in various biological functions, and their mechanisms are diverse. For example, they can interact with signaling proteins, regulate the translation of specific mRNAs, and act as miRNA sponges [20–22]. In MiRNA sponges, competitive endogenous RNAs (ceRNAs) such as lncRNAs have miRNA action sites and regulate mRNA expression by competitively binding to miRNAs [23,24]. Studies increasingly show that lncRNAs play a significant role in various tumors. LINC00673 promoted lung cancer progression by targeting miR-150-5p [25]. LncRNA REG1CP is upregulated in colorectal cancer and promoted the growth of colorectal cancer xenografts [26]. In addition, findings from numerous studies have shown that lncRNAs can promote the progression of hematological tumors. LncRNA UCA1 could promote the proliferation of AML cells by inhibiting cell cycle regulators and binding to miR-126, thereby enhancing the proliferation, migration and invasion of AML cells [27,28]. As a ceRNA, LINC01679 regulates the signaling axis of miR-3150a-3p/SLC17A9, which helps to inhibit the induction and progression of prostate cancer [29].

IL-6R is a binding receptor for IL-6 and comprises an IL-6Rα subunit and a gp130 subunit [30]. As a proinflammatory cytokine, IL-6 can participate in the immune response, hematopoietic system, and also play a role in tumors [31]. The IL-6 signaling pathway regulates inflammation and other immune responses by binding to IL-6R to form a complex that causes gp130 to activate JAK1/2 and Tyk2, which in turn activates STAT1 and STAT3 as well as the MAPK cascade [32]. As a key proinflammatory factor, IL-6 has been linked to tumor progression in various studies. The expression levels of IL-6 and IL-6R are significantly increased in prostate carcinogenesis and tumor progression [33]. MiRNA can regulate the IL-6/IL-6R signaling pathway in different tumors, affecting tumor growth and metastasis. For example, in colorectal cancer, p53 activation interferes with IL-6-induced tumor migration and invasion by downregulating miR-34a-dependent IL-6R expression [34]. The role of IL-6/IL-6R in hematological tumors is also significant. IL-6 can act as a growth factor for myeloma cells [35], and IL-6R is expressed at high levels in several multiple myeloma subtypes [36]. Meanwhile, IL-6R expression is elevated in patients with high-risk myelodysplastic syndromes. Targeting IL-6 signaling can improve the progression of MDS to AML [37]. The IL-6/IL-6R system can regulate the proliferation of blast cells in AML [38]. However, the mechanism by which IL-6R expression is regulated by ncRNAs in AML has not been elucidated.

We found that AC010247.2 could act as a ceRNA of miR-125b-5p to regulate the expression of IL6R, which played an important role. First, we observed a significant correlation between the expressions of AC010247.2, miR-125b-5p, and IL6R with AML progression. Next, we predicted and confirmed that AC010247.2 binds to miR-125b-5p, functioning as a molecular sponge to regulate IL6R expression. In the end, the AC010247.2/miR-125b-5p/IL6R axis could be confirmed as a joint molecular marker.

2. Materials & methods

2.1. Data acquisition

The expression data of mRNAs, miRNAs, and lncRNAs were obtained from the same patients with AML from the TCGA database. Besides, the overall survival and status of patients were also obtained.

2.2. Univariate regression analysis

Obtain survival data (overall survival and survival status) for AML patients, along with the expression data of associated mRNA, miRNA, and lncRNA. Conduct univariate Cox regression analysis to calculate the hazard ratio (HR), 95 % confidence interval (CI), and p-value for each RNA, in order to assess its impact on survival.

2.3. Construction of the ceRNA axes

Based on the ceRNA theory, miRNA can target mRNA to promote its degradation and downregulate its expression. However, lncRNAs can indirectly affect mRNA expression by competitively binding to miRNAs. Therefore, we first identified the lncRNA-miRNA relationships by utilizing the miRcode database (http://www.mircode.org/) in accordance with the findings from the univariate regression analysis of lncRNAs and miRNAs. Next, we used the miRNAs in the lncRNA-miRNA relationships to screen potential mRNAs.

The screening criteria were targeted mRNA numbers in datasets $\geqq 3$, which included TargetScan, miRDB, miRTarbase, and ENCORI. Then, we derived the miRNA-mRNA relationships. Additionally, the relationships between lncRNA-miRNA and miRNA-mRNA were refined to build the ceRNA network. The potential axes were HR value > 1 for lncRNAs and mRNAs, HR value < -1 for miRNAs, HR value < -1 for lncRNAs and mRNAs, and HR value > 1 for miRNAs. The software Cytoscape (version 3.9.1) was used to visualize the regulatory relationships. The sankey diagram was prepared using the R package ggalluvial and ggplot2.

2.4. DEG analysis

Differential gene expression analysis in AML patients from the TCGA database was conducted using the R package. The "limma-voom" was chosen to analyze the count data from the patients. Finally, the results with log2FC ≥ 1 and adjusted $P < 0.05$ were considered significant.

2.5. GSEA

The overlapping genes of lncRNA-DEG and mRNA-DEG, miRNA-DEG and mRNA-DEG, and the three groups were acquired to perform GSEA. The R package clusterProfiler was used.

2.6. Development and validation of a prognostic model

In constructing the predictive model using LASSO Cox regression analysis, the gene expression data of AML patients from the TCGA database was split equally into a training set and a validation set. The training set was used to build and train the model, resulting in the following risk score model: risk score = AC010247.2 $\times$ 0.217770873061909 + miR-125b-5p $\times$ −0.106136612443171 + IL6R $\times$ 0.33300227489702. The validation set was then used to test the predictive capability of the model. According to the median risk score, the test set was divided into low-risk group and high-risk group. Finally, ROC curve was drawn to verify the effectiveness of the model.

2.7. Cell culture

We cultured human AML THP-1 and Kasumi cells for the study. THP-1 cells were grown in RPMI-1640 medium enriched with 10 % bovine serum albumin (FBS), 0.05 mM 2-mercaptoethanol, and antibiotics (100 μg/mL penicillin and streptomycin) at a temperature of 37 °C in a 5 % CO_2. In contrast, Kasumi cells were maintained in the same RPMI-1640 medium but with 20 % FBS, along with the same concentrations of penicillin and streptomycin, also at 37 °C in 5 % CO_2.

2.8. Transfection

The miRNA mimics and inhibitors were provided from Biomics (Nantong, China). shRNA targeting IL6R was constructed, with the following sequence: shIL6R 5′-CCGGTATCGGGCTGAACGGTCAAAGCTCGAGCTTTGACCGTTCAGCCCGATATTTTTG-3'. The ASO for lncRNA AC010247.2 was purchased from RiboBio (Guangzhou, China).

2.9. Western blot

Cellular lysis was performed using RIPA lysis buffer, and protein quantification was conducted using a BCA kit (Thermo Scientific, MA, USA). Samples were prepared according to protein concentration for SDS-PAGE, then transferred to NC membranes. After blocking, the membranes were incubated with the appropriate primary and secondary antibodies. Finally, the blots were developed and photographed. The antibody ratios are as follows: IL6R (ab271042; Abcam; 1:1000), STAT3 (ab68153; Abcam; 1:1000), p-STAT3 (ab76315; Abcam; 1:20000), ACTB (AC004; Abclonal; 1:3000), anti-mouse HRP (31430; Thermo; 1:3000), and anti-rabbit HRP (31460; Thermo; 1:3000).

2.10. qPCR assay

First, total RNA was extracted and cDNA was generated by reverse transcription. Specific primers were designed based on the target gene sequence, followed by PCR cycling, where fluorescent signals were monitored in real time. The data was then analyzed to calculate the expression level of the target gene. The primer sequences are as follows:

IL6R forward: 5′- CCGAGATCTGGCTTTTACTTAAACCG -3′
IL6R reverse: 5′- CAGGAATTCACTTGCTCTGTCACCC -3′
has-miR-125b-5p : 5′- TCCCTGAGACCCTAACTTGTGA'
AC010247.2 forward : 5′- TCTGCTTCCGAGGCATTCA-3′
AC010247.2 reverse : 5′- CCTTCCCTTCTGTCCTTCCA.
ACTB forward : 5′- ATGTGGCCGAGGACTTTGATT-3′
ACTB reverse : 5′- AGTGGGGTGGCTTTTAGGATG-3'.

2.11. CCK8 assay

Cells with different treatments were seeded in a 96-well plate and incubated. After 48 h, CCK-8 reagent was added. After 2 h, absorbance was measured at 450 nm using a microplate reader.

2.12. Luciferase reporter assay

We used PGL4.15 to construct plasmids containing the IL6R-wild type and mutant type. Dual-Luciferase Reporter Assay System Kit (E1910; Promega, Madison, WI, USA) was used according to the manufacturer's instructions.

2.13. Statistical analysis

The KM curve illustrates the survival analysis differences between the two groups. The ROC curve analysis was used to evaluate the predictive ability of the model. The experimental results were analyzed using GraphPad Prism 8. An unpaired *t*-test was employed for intergroup comparisons, with $P < 0.05$ defined as statistically significant.

3. Results

3.1. Identification of lncRNAs, miRNAs, and mRNAs linked to the progression of AML

The expression of tumor biomarkers often appear apparent changes in the progression of cancers. Some of these genes can play a tumor-promoting or tumor-suppressing role in regulating tumor progression. However, made up of these genes ceRNA shaft in the AML regulation not yet not clear. To gain deeper insights into its potential mechanisms, we obtained gene expression data from AML patients via the TCGA database. We conducted univariate COX regression analysis on these genes, identifying 2446 mRNAs, 91 miRNAs, and 848 lncRNAs. Fig. 1A–H and S1 show the top five results of these mRNAs, miRNAs, and lncRNAs.

3.2. AC010247.2/miRNA-125b-5p/IL6R may be an essential ceRNA axis regulating AML tumors

On the basis of the above results, we boiled the regulation ceRNA network AML malignant cancer progression. The network contains 20 lncRNAs, 4 miRNAs, 29 mRNAs, and 7 groups of interaction axes (Fig. 2A and S2). Among the mRNAs, IL6R can bind IL6 for signal transmission. It contributes to the promotion of AML occurrence and progression. Therefore, we focused on the ceRNA axis that regulates IL6R expression. Fifteen lncRNAs might regulate IL6R through miRNA-125b-5p (Fig. S2A). AC133919.2, AC010247.2, LINC01637, LINC02145, and LINC01679 have a relatively high abundance in AML tumors and are more likely to be functional. Hence, we selected the AC133919.2/miRNA-125b-5p, AC010247.2/miRNA-125b-5p, LINC01637/miRNA-125b-5p, LINC02145/miRNA-125b-5p, and LINC01679/miRNA-125b-5p axes for further experiments(Fig. 2B).

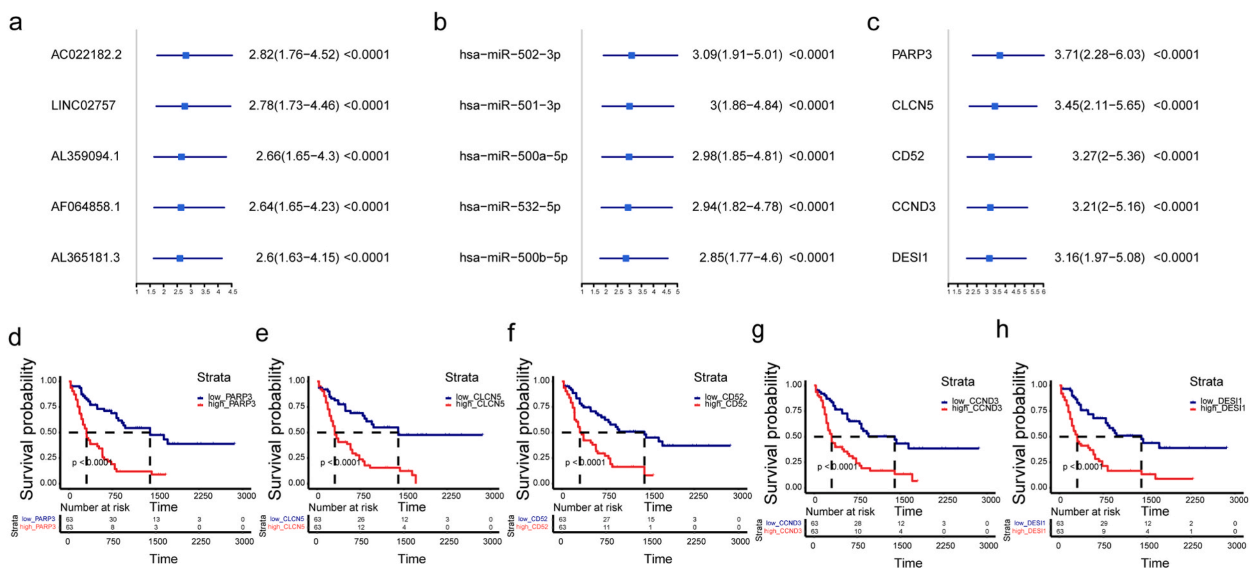

Fig. 1. The Univariate cox regression results of lncRNAs, miRNAs, and mRNAs in TCGA AML patients. (a–c) The top 5 results of Univariate cox regression results in lncRNAs, miRNAs, and mRNAs. (d–h) The overall survival curves of the top 5 mRNAs in AML patients. Blue lines indicate low expression of mRNA and red lines indicate high expression of mRNA. The groups between low and high expression of mRNA were stratified according to the median expression level of mRNA. Data were analyzed by log-rank test.

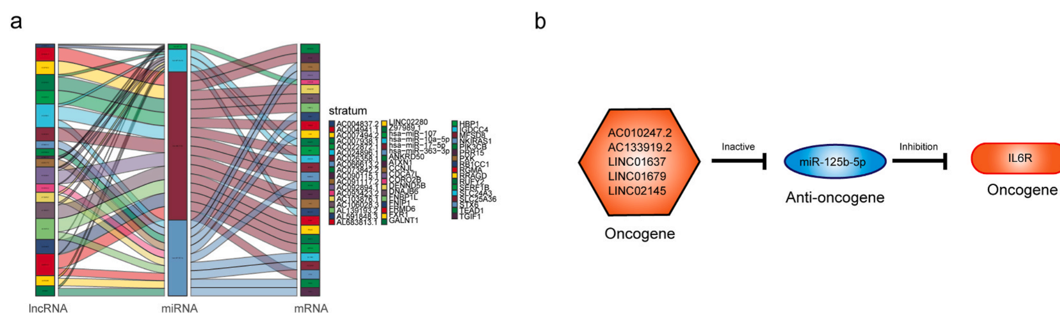

Fig. 2. Construction of AML related ceRNA network by integrated analysis. (a) lncRNA-miRNA-mRNA regulatory axes extracted from this ceRNA network. (b) The pontential lncRNA/miR-125b-5p/IL6R axis.

3.3. AC010247.2 may regulate the mRNA and protein expression of IL6R

To illustrate the potential functions of these targeted genes, we performed differentially expressed gene analysis and survival analysis for these five axes. LINC01637 and AC010247.2 showed relatively high expression levels compared with normal samples in patients with AML (Fig. 3A), which shows that these lncRNAs may serve as oncogenes in AML. Furthermore, the survival analysis and correlation between lncRNAs and IL6R indicated that AC010247.2 is critical among the five lncRNAs (Fig. 3B–C). The AUC curves also indicated similar result (Fig. S3). To further investigate their influences, we knocked down the five lncRNAs in AML using the antisense oligonucleotides (ASOs) to determine IL6R protein and mRNA expression. IL6R expression is regulated predominantly by AC010247.2, whereas the other molecules showed no effect (Fig. 3D–E). Therefore, AC010247.2 may be the primary lncRNA that regulates IL6R expression.

3.4. AC010247.2, miR-125b-5p, and IL6R proteins modulated malignant progression in AML

The above results indicate that AC010247.2-miR-125b-5p-IL6R axis may function in AML to influence its malignant progression. Next, based on the median expression levels of AC010247.2, miR-125b-5p, and IL6R obtained from TCGA for AML patients, we divided the patients into high-expression and low-expression groups. Moreover, GSEA enrichment analysis revealed that the expression of these three genes was primarily regulated in immune response and signal transduction pathways (Fig. 4A–C). To evaluate the effects of AC010247.2, miR-125b-5p, and IL6R on cell proliferation, we performed CCK8 assays after individually knocking out each of these genes. The findings indicated that the genes were involved in the regulation of the growth of AML cells (Fig. 4D). In conclusion, AC010247.2, miR-125b-5p and IL6R co-regulate the malignant progression of AML.

3.5. lncRNAs AC010247.2 and miR-125b-5p can regulate IL-6R protein expression in AML tumors

We performed Pearson correlation analysis on the three genes to further explore the relationship among the expression of AC010247.2, miR-125b-5p, and IL6R proteins (Fig. 5A). We knocked down AC010247.2 with ASO and detected the changes in miR-125b-5p and IL6R protein expression and the expression of their downstream genes. IL6R and its downstream genes were down-regulated, whereas miR-125b-5p exhibited an upregulation (Fig. 5B). Next, we overexpressed and inhibited miR-125b-5p using mimics and inhibitors, respectively, to examine the changes in the AC010247.2 and IL6R protein pathways. The miR-125b-5p negatively regulates the AC010247.2 and IL6R pathways (Fig. 5C–D). These findings demonstrated that lncRNA AC010247.2 and miR-125-b-5p can regulate the expression of IL6R protein in AML, which was consistent with the ceRNA shaft observed trends.

3.6. AC010247.2, as a ceRNA of miR-125b-5p, regulates IL6R protein expression and affects the malignant progression of AML tumors

In order to clarify whether AC010247.2 serves as the ceRNA of miR-125b-5p, complementary mutations of AC010247.2 and miR-125b-5p predicted binding sites were constructed (Fig. 6A).We performed the reporter gene assay to detect the mutation changes in luciferase activity before and after changeing the miR-125b-5p levels. Wild-type AC010247.2 luciferase activity was affected by miR-125b-5p, nevertheless the mutant type showed no significant expression changes (Fig. 6B). Next, we predicted the enrichment of genes co-regulated by two or three genes in the AC010247.2/miR-125b-5p/IL6R axis in the AML tumor malignant progression pathway. The results indicated that the enrichment was especially pronounced in the proliferative pathway (Fig. 6C–D and S4). To test this finding, we examined proliferation after the knockdown of AC010247.2 in control and AML cells treated with miR-125b-5p inhibitors. A reduction in tumor cell proliferation was observed after AC010247.2 knockdown in the control group, while no significant difference was observed in the inhibitor group (Fig. 6E–F). These findings demonstrated that AC010247.2 as miR-125-b-5p ceRNA, adjusted the IL6R proteins, and affected the malignant progress of AML.

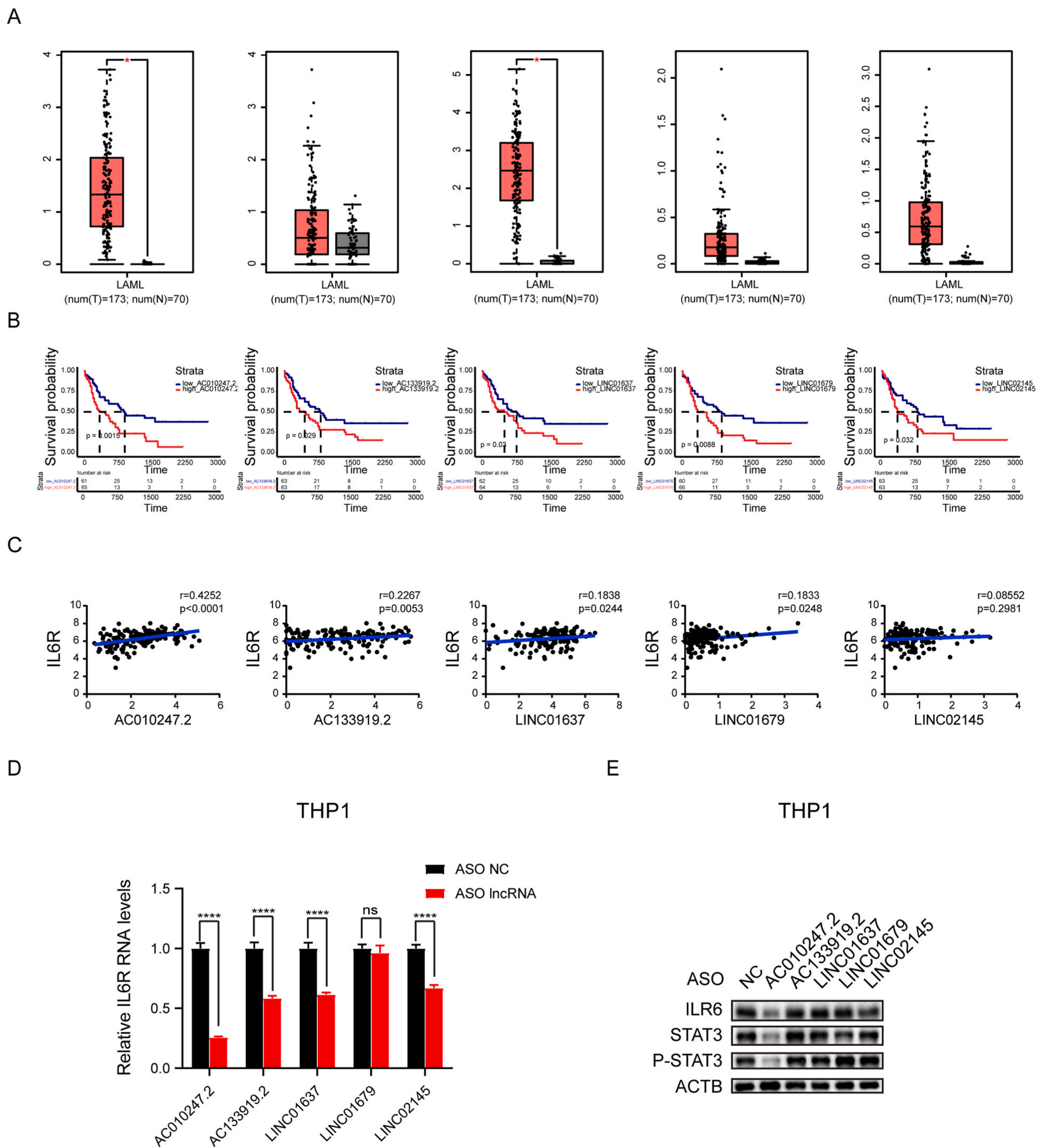

Fig. 3. AC010247.2 may regulate the mRNA and protein levels of IL6R. (a) The expression of pontential lncRNAs between tumor and normal tissues in AML patients. The red box indicates the tumor tissue and the black box indicates the normal tissue. (b) The survival curves of pontential lncRNAs in AML patients. Blue lines indicate low expression of lncRNA and red lines indicate high expression of lncRNA. The groups between low and high expression of lncRNA were stratified according to the median expression level of lncRNA. Data were analyzed by log-rank test. (c) Correlation analysis between IL6R and 5 lncRNAs (AC010247.2, AC133919.2, LINC01637, LINC01639, and LINC02145). (d) IL6R mRNA levels in THP1 cells with ASO knockdown 5 pontential lncRNAs was detected. (e) The protein levels of IL6R and its doenstream genes in THP1 cells with ASO knockdown 5 pontential lncRNAs were detected. The original blot images were added in the supplementary western files. *$P < 0.05$; ****$P < 0.0001$; ns means no significant.

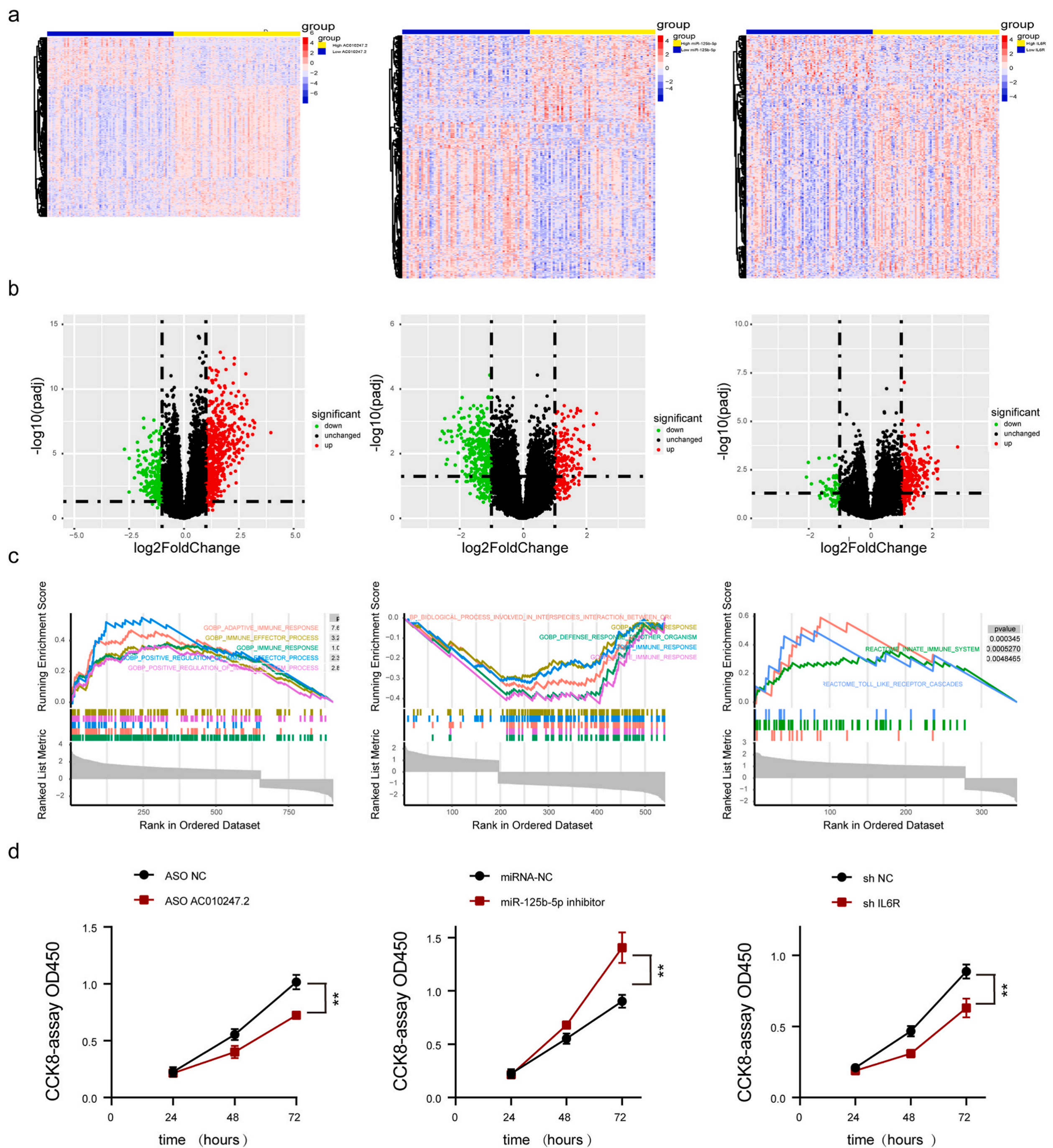

Fig. 4. AC010247.2, miR-125b-5p, and IL6R proteins regulate AML malignant progression. (a) The heatmap (up pannel) and (b) volcano plots (down pannel) of the differentially expressed genes in low expression group and high expression group among AC010247.2, miR-125b-5p, and IL6R. The groups between low and high expression of the 3 genes were stratified according to the median expression level of them. Red dots, significantly upregulated genes. Green dots, significantly downregulated genes. Black dots, nondifferentially expressed genes. (c) The GO functional analysis of the related biological processes of differentially expressed genes in low expression group and high expression group among AC010247.2, miR-125b-5p, and IL6R. (d) The CCK8 results in THP1 cells with knockdown or inhibition of AC010247.2, miR-125b-5p, and IL6R. $^{**}P < 0.01$.

3.7. AC010247.2/miR-125b-5p/IL6R axis could effectively predict the prognosis of AML

Previous results revealed that all three genes are related to the malignant progression of AML tumors (Fig. S5A). However, the malignant progression of AML tumors results from the joint regulation of a multi-gene network. Therefore, single genes have certain

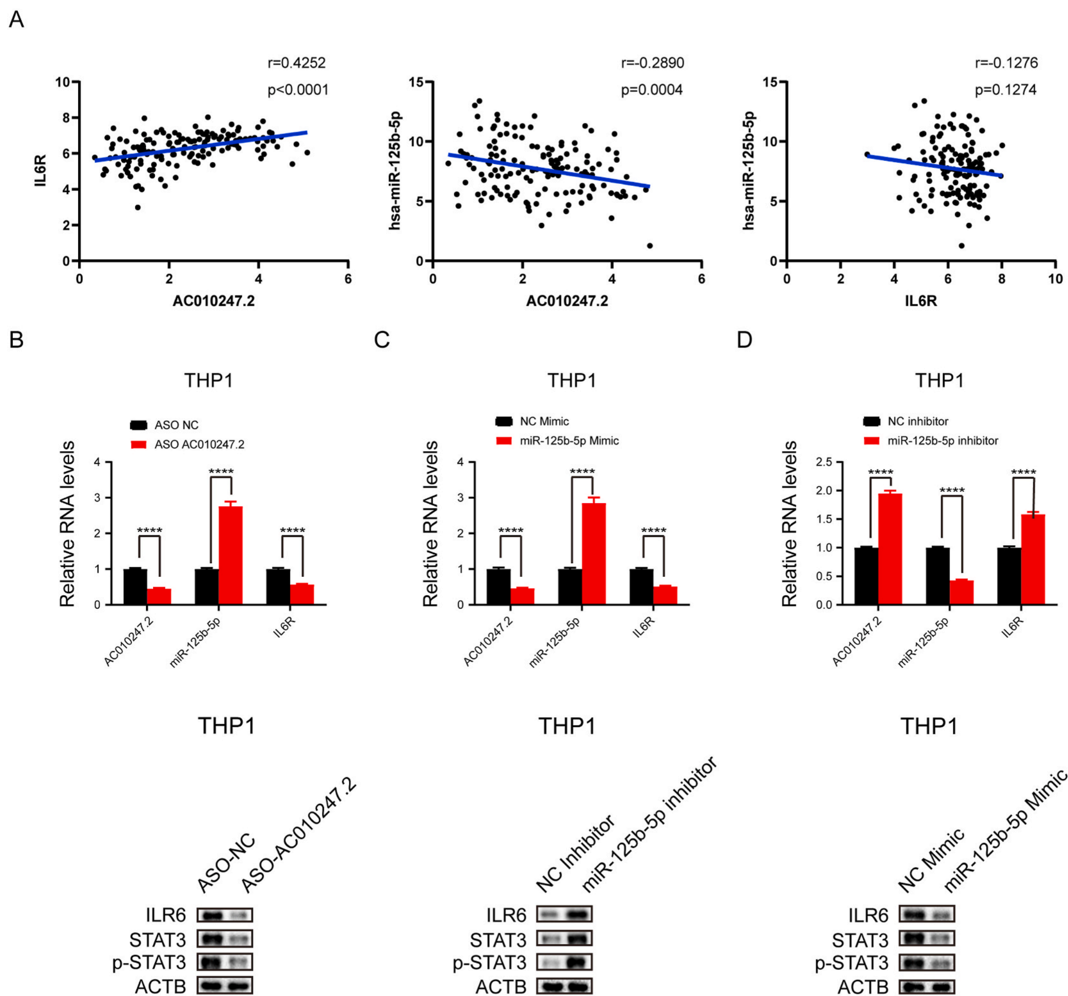

Fig. 5. lncRNA AC010247.2 and miR-125b-5p can regulate IL6R protein in AML tumors. (a) Correlation analysis between IL6R, AC010247.2, and miR-125b-5p. (b) The protein and RNA levels of IL6R, AC010247.2, and miR-125b-5p in THP1 with ASO knockdown AC010247.2 was detected. (c) The protein and RNA levels of IL6R, AC010247.2, and miR-125b-5p in THP1 treated with miR-125b-5p mimic was detected. (d) The protein and RNA levels of IL6R, AC010247.2, and miR-125b-5p in THP1 treated with miR-125b-5p inhibitor was detected. The original blot images were added in the supplementary western files. ****$P < 0.0001$.

limitations as biomarkers for the diagnosis of AML. A prognostic model based on AC010247.2/miR-125b-5p/IL6R expression was established by LASSO regression analysis (Fig. 7A–D, risk score = AC010247.2 × 0.217770873061909 + miR-125b-5p × −0.106136612443171 + IL6R × 0.33300227489702). Higher risk scores were associated with shorter survival, and AUC values significantly surpassed those of the single-gene model in both training and validation sets (Fig. 7E–G and S5B-D). In conclusion, the predictive model using the three genes could more accurately diagnose AML tumors.

4. Discussion

AML has a poor prognosis and high malignancy, making early diagnosis and effective treatment challenging. Thus, new diagnostic and treatment methods are extremely necessary. As the receptor of IL-6, IL-6R participates in the regulation of the signaling pathway and immune infiltration of IL-6. IL-6 can induce the infiltration of T cells and immunosuppressive MDSCs [39]. IL-6R also plays a crucial role in hematological malignancies and can regulate the growth of AML cells. With the advancement of bioinformatics, biomarkers have shown significant advantages in the diagnosis and prognosis of tumors. Nevertheless, gene regulation network in tumor development and progression is a complex one. Single gene diagnostic biomarkers are usually not adequately comprehensive for the accurate assessment of the prognosis [40]. Therefore, exploring the regulatory network of IL-6R in AML and developing a multigene diagnostic model is crucial. NcRNAs are key players in AML progression. MiRNA primarily promotes the progression of AML by targeting and binding mRNAs to inhibit mRNA expression. LncRNAs can act as a molecular sponge to bind miRNAs, regulate mRNAs, and further promote AML tumor progression. Findings from previous studies have shown that miRNAs can target IL-6R to

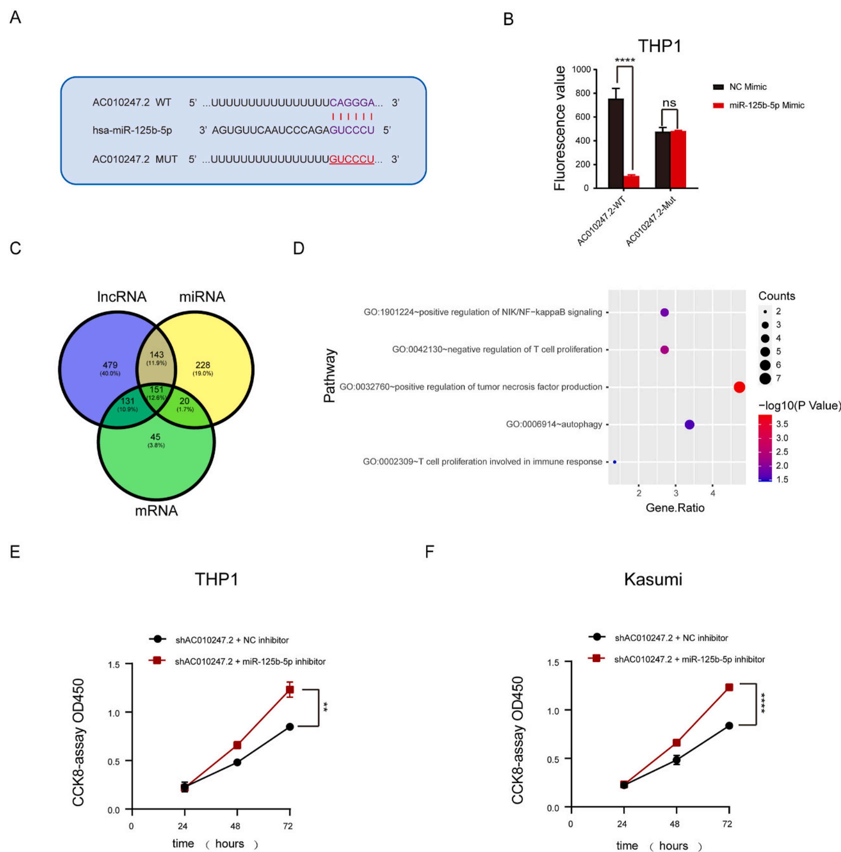

Fig. 6. AC010247.2 regulates IL6R protein and affects the malignant progression of AML tumors. (a) Predicted binding site between miR-125b-5p and IL6R. (b) Luciferase reporter gene test was used to test the relationship between AC010247.2 and miR-125b-5p treated with miR-125b-5p mimic in THP1 cell. (c) Venn diagrams represent the intersections of differentially expressed mRNAs in in low expression group and high expression group among AC010247.2, miR-125b-5p, and IL6R. The groups between low and high expression of the 3 genes were stratified according to the median expression level of them. (d) GSEA analysis are performed by the overlap genes. (e) The CCK8 results in sh AC010247.2 THP1 cells treated with NC inhibitor or miR-125b-5p inhibitor. (f) The CCK8 results in sh AC010247.2 Kasumi cells treated with NC inhibitor or miR-125b-5p inhibitor. $^{**}P < 0.01$; $^{****}P < 0.0001$; ns means no significant.

downregulate its expression in various tumors [41]. LncRNAs expressed at high levels can upregulate IL-6R by targeting miRNAs. For example, LINC01116 can target miR-520a-3p, leading to IL-6R upregulation, thereby activating the JAK-STAT pathway and promoting osteosarcoma development [42]. Despite the important role of IL-6R in the blood system, the ceRNA network of IL-6R in AML has not been reported. Here, we show that AC010247.2 acts as a ceRNA of mir-125b-5p in AML to regulate IL-6R expression. The findings of these studies established a synergistic regulatory network between IL-6R and ncRNAs in AML, offering a theoretical foundation for the diagnosis and treatment of IL-6R. Besides, AML is a hematological tumor with different gene alteration types. Our study may not be meaningful in all AML patients due to the limited samples. Therefore, we will collect more AML samples and investigate their specific ceRNA networks in different types in the future.

As a receptor of IL-6, IL-6R participates in the signal transduction of IL-6. IL-6 can regulate tumor initiation and progression, affecting functions such as proliferation, invasion, and migration [43]. Both IL-6 and IL-6R are highly expressed in AML and the secretion of IL-6 can act synergistically with other cytokines to regulate the proliferation of pluripotent stem cells, leading to tumorigenesis and affecting the prognosis of patients with AML [44,45]. Mechanistically, the growth support of leukemic cells by IL-6 may be related to paracrine stimulation, which induces the activation of STAT3 [46]. The activation of IL-6 signaling involves ligand-dependent tyrosine phosphorylation of the protein tyrosine kinases Jak1, Jak2, Tyk2, and STAT3. Among them, STAT3, as a signaling transcription factor, has been shown to promote the initiation and progression of various solid tumors. Its activation occurs in various tumor processes, including cell proliferation, differentiation, apoptosis, and drug sensitivity [47]. Meanwhile, the activation of the IL-6/STAT3 pathway has been noted in AML patients, which increases the chemoresistance of AML [43,48]. Therefore, the role of Il-6, an important component of IL-6 signaling, in the initiation and progression of AML cannot be ignored. Here, we defined the role of IL-6R in the regulation of AML malignant progression and determined that AC010247.2 and miR-125b-5p could further regulate AML through IL-6R *in vitro*. However, the *in vivo* validation of the ceRNA axis regulating the malignant progression warrants further studies.

Recently, many single-gene biomarkers have been used for the diagnosis and prognosis of tumors, but there remain several limitations in their wide clinical use. The process of tumor initiation and progression is complex, involving the regulation of multiple

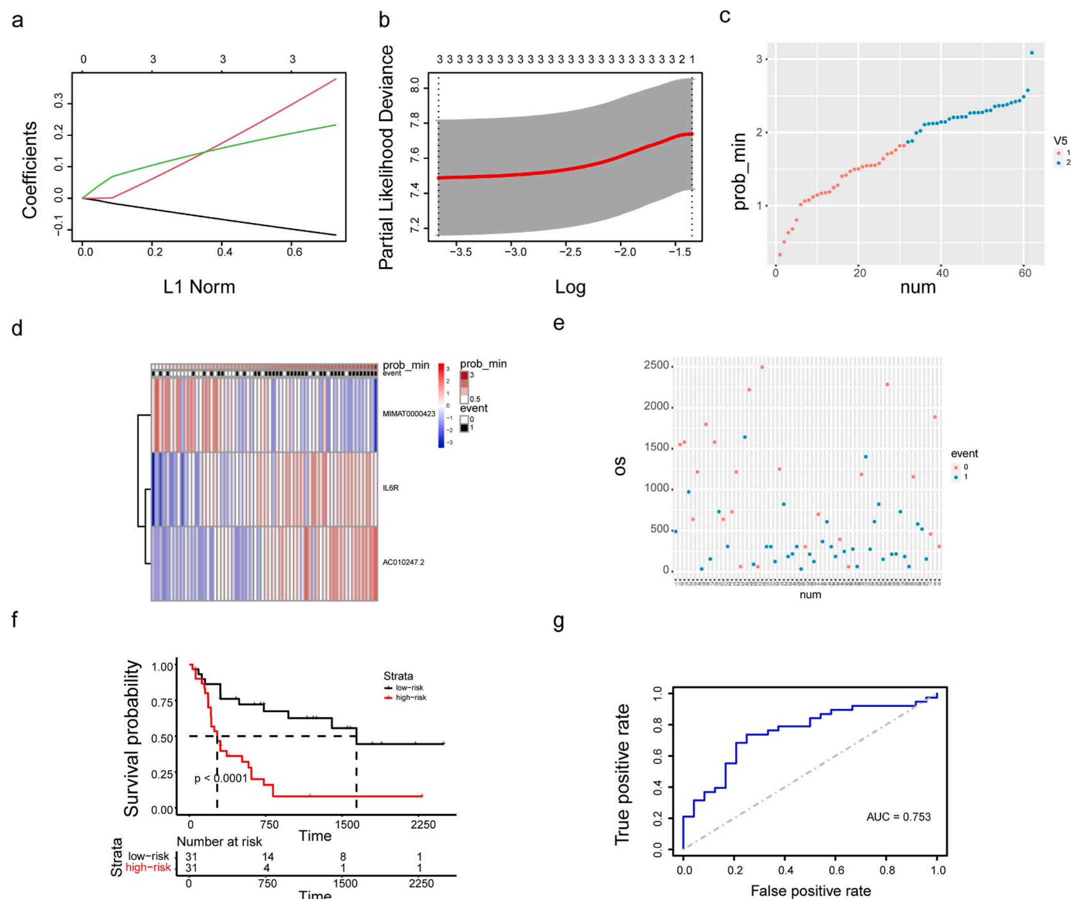

Fig. 7. Construction of a risk-prediction model of the training set and analyses of model performance. (a) LASSO regression coefficient profile of the 3 genes. (b) LASSO deviance profile of the 3 genes. (c) The risk scores of AML in TCGA database. (d) The heatmap of the riskscore and survival status in AML patients. (e) The distribution of survival status and risk scores in AML patients. (f) Kaplan-Meier curves of patients in low riskscore group and high riskscore group for overall survival. The groups between low and high riskscore were stratified according to the median value of riskscore. (g) ROC curve of the risk score model.

factors and environmental influence. A single gene biomarker cannot accurately assess the prognosis of the disease [40,49]. Moreover, a single gene's expression is also influenced by other factors [40]. Thus, common single-gene markers often lack sensitivity and specificity in clinical practice. A single marker such as CEA has various influencing factors and cannot be used for the diagnosis of specific tumors alone, but only as a reference for correlation with clinical findings [50]. CA199 has limited use as a marker because it is difficult to detect in early-stage tumors [51]. Therefore, various methods have been used to establish a polygenic prognostic model, but their accuracy and sensitivity remain insufficient, which make the models inapplicable to clinical practice. The emergence of AI has considerably improved the accuracy of prognostic models. The LASSO regression model is a common machine learning algorithm that handles complex collinear data by compressing some coefficients and setting others to zero, thus maintaining the benefits of subset shrinkage [52]. It can estimate parameters and select variables concurrently and realize the analysis of multiple collinear problems. The LASSO regression model can prevent over-fitting in data processing and create a simpler and more practical model [53]. It can also establish multiple classifiers to predict the prognosis of multiple genes [54]. Evidence from studies shows that LASSO optimizes the selection of genetic markers and increases the accuracy and stability of prognosis prediction [55,56]. The feasibility of the AC010247.2/miR-125b-5p/IL6R axis as a combined molecular marker was predicted and validated using a LASSO regression model. The multigene predictive model, comprised of these three genes, demonstrated greater accuracy and sensitivity than the single-gene predictive model.

5. Conclusion

In conclusion, our study established a prognosis-related ceRNA network in AML. Specifically, AC010247.2 and miR-125b-5p regulate IL6R expression within this network. Mechanistically, they interact to competitively modulate IL6R expression. Ultimately, this axis can serve as a molecular biomarker for predicting AML prognosis.

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Data availability

The datasets used in this study are available on request.

CRediT authorship contribution statement

Fang Xie: Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jialu Xu:** Software, Methodology, Formal analysis, Data curation. **Lina Yan:** Visualization, Validation, Software, Resources, Data curation. **Xia Xiao:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Liang Liu:** Writing – review & editing, Writing – original draft, Supervision, 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37715.

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