

IGF2BP3 Promoted the Overproliferation of AML Cells via Stability of *NRXN2* mRNA

IGF2BP3'ün *NRXN2* mRNA Stabilitesi Üzerinden AML Hücrelerinde Aşırı Proliferasyonu Teşvik Etmesi

Wenyue Ma, Letian Sun, Siling Liu, Ning Hao

Nanjing University of Technology, School of Biological and Pharmaceutical Engineering, Nanjing, P.R. China

Abstract

Objective: Acute myeloid leukemia (AML) is a malignant tumor with high incidence and mortality. This study aimed to investigate whether insulin-like growth factor 2 mRNA-binding protein 3 (IGF2BP3) regulates the stability of *neurexin 2* (*NRXN2*) mRNA in an N6-methyladenosine (m⁶A)-dependent manner and to explore its role in AML progression.

Materials and Methods: Differentially expressed genes of AML were identified using the DESeq2 R package and verified by the TCGA-AML database. *NRXN2* silencing, *IGF2BP3* downregulation, and *IGF2BP3* overexpression were performed in the human HL-60 AML cell line and proliferation and apoptosis were investigated. The potential m⁶A site of *NRXN2* mRNA was predicted with the SRAMP website. *IGF2BP3* enrichment and m⁶A modification of *NRXN2* mRNA were detected by real-time polymerase chain reaction using the Magna MeRIP m⁶A Kit and by RNA immunoprecipitation real-time polymerase chain reaction, respectively. An *NRXN2* mutant lacking the predicted m⁶A site (*NRXN2*-mut [c.1770A>T]) was constructed and transfected into HL-60 cells.

Results: *IGF2BP3* and *NRXN2* were upregulated in AML and their expression was negatively correlated with overall survival. A specific m⁶A modification site was identified on *NRXN2* mRNA. *NRXN2*-mut (c.1770A>T) decreased the m⁶A modification level and reduced the stability of *NRXN2* mRNA, as well as its enrichment by *IGF2BP3*. *NRXN2* silencing and *IGF2BP3* knockdown suppressed proliferation and promoted apoptosis in HL-60 cells, accompanied by decreased Bcl-2 and PCNA protein levels and increased cleaved caspase 3. *IGF2BP3* overexpression promoted proliferation and inhibited apoptosis, effects that were reversed by the co-expression of the *NRXN2* mutant.

Conclusion: *IGF2BP3* promoted the stability of *NRXN2* mRNA in an m⁶A-dependent manner, thereby promoting the overproliferation of AML cells.

Keywords: Acute myeloid leukemia, *Neurexin 2*, Insulin-like growth factor 2 mRNA-binding protein 3, N6-methyladenosine

Öz

Amaç: Akut miyeloid lösemi (AML), yüksek insidans ve mortalite oranına sahip malign bir tümördür. Bu çalışma, insülin benzeri büyüme faktörü 2 mRNA bağlayıcı protein 3'ün (IGF2BP3) *nörexin 2* (*NRXN2*) mRNA stabilitesini N6-metiladenosin (m⁶A) bağımlı bir şekilde düzenleyip düzenlemediğini araştırmayı ve AML progresyonundaki rolünü incelemeyi amaçlamıştır.

Gereç ve Yöntemler: AML'nin farklı şekilde eksprese olan genleri DESeq2 R paketi kullanılarak tanımlanmış ve TCGA-AML veritabanı ile doğrulanmıştır. İnsan HL-60 AML hücre hattında *NRXN2* susturulması, *IGF2BP3* azaltılması ve *IGF2BP3* aşırı ekspresyonu gerçekleştirilmiş ve proliferasyon ile apoptoz araştırılmıştır. *NRXN2* mRNA'sının potansiyel m⁶A bölgesi SRAMP web sitesi ile tahmin edilmiştir. *IGF2BP3* zenginleşmesi ve *NRXN2* mRNA'sının m⁶A modifikasyonu, sırasıyla Magna MeRIP m⁶A Kiti kullanılarak gerçek zamanlı polimeraz zincir reaksiyonu ve RNA immünopresipitasyon gerçek zamanlı polimeraz zincir reaksiyonu ile tespit edilmiştir. Tahmin edilen m⁶A bölgesinden yoksun bir *NRXN2* mutan (NRXN2-mut [c.1770A>T]) oluşturulmuş ve HL-60 hücrelerine transfeke edilmiştir.

Bulgular: *IGF2BP3* ve *NRXN2*, AML'de yukarı regüle edilmiştir ve ekspresyonları genel sağkalım ile negatif korelasyon göstermiştir. *NRXN2* mRNA'sı üzerinde spesifik bir m⁶A modifikasyon bölgesi tanımlanmıştır. *NRXN2*-mut (c.1770A>T), m⁶A modifikasyon seviyesini azaltmış ve *NRXN2* mRNA stabilitesini ve *IGF2BP3* tarafından zenginleşmesini düşürmüştür. *NRXN2* susturulması ve *IGF2BP3* baskılanması, HL-60 hücrelerinde proliferasyonu baskılamış ve apoptozu desteklemiş, bununla birlikte Bcl-2 ve PCNA protein seviyeleri azalmış ve kaspaz 3 parçalanması artmıştır. *IGF2BP3* aşırı ekspresyonu proliferasyonu desteklemiş ve apoptozu inhibe etmiştir; bu etkiler *NRXN2* mutanın birlikte ekspresyonu ile tersine çevrilmiştir.

Sonuç: *IGF2BP3*, m⁶A bağımlı bir şekilde *NRXN2* mRNA stabilitesini destekleyerek AML hücrelerinin aşırı çoğalmasını teşvik etmiştir.

Anahtar Sözcükler: Akut miyeloid lösemi, *Nörexin 2*, insülin benzeri büyüme faktörü 2 mRNA bağlayıcı protein 3, N6-metiladenosin



Address for Correspondence/Yazışma Adresi: Ning Hao, Ph.D., Nanjing University of Technology, School of Biological and Pharmaceutical Engineering, Nanjing, P.R. China
E-mail: haoning29@163.com ORCID: orcid.org/0009-0004-8514-3058

Received/Geliş tarihi: April 28, 2025
Accepted/Kabul tarihi: November 19, 2025
Epub: November 20, 2025



©Copyright 2026 by Turkish Society of Hematology Turkish Journal of Hematology, Published by Galenos Publishing House.
Licensed under a Creative Commons Attribution-NonCommercial (CC BY-NC-ND) 4.0 International License.

Introduction

Acute myeloid leukemia (AML) is a hematological malignancy characterized by the uncontrolled proliferation of hematopoietic cells [1,2,3]. In AML, the incidence of new cases is approximately 4.3 per 100,000 [4]. Over the past two decades, the global incidence of AML has gradually increased [5]. Thus, the burden of AML has become heavier, and the 5-year survival rate for AML is 29%, which poses a major challenge in clinical practice [6]. Insights into the genetic landscape and molecular mechanisms of AML are critical for advances in AML prognosis and therapy. It is necessary to investigate differentially expressed genes (DEGs) in AML and elucidate their specific mechanisms.

Neurexin 2 (*NRXN2*) is a member of the neurexin family that mediates cell adhesion and intracellular signaling [7]. The role of *NRXN2* in neurodevelopmental disorders is frequently mentioned, including autism [8], schizophrenia [9], and spontaneous seizures [10]. Intriguingly, increasing studies report that changes in *NRXN2* contribute to the occurrence and development of cancer. For example, *NRXN2* was found to be differentially expressed in endometrial cancer, with a potential effect on the PI3K/Akt pathway [11]. Furthermore, *NRXN2* was identified as a biomarker of medulloblastoma due to the correlation between *NRXN2* upregulation and decreased overall survival in cases of medulloblastoma [12]. The regulatory mechanism of *NRXN2* in AML has only recently been documented. One report revealed that *NRXN2* was correlated with the survival of patients with AML with high risk of poor prognosis [13], indicating the potential relationship between *NRXN2* and AML progression. However, the regulatory role of *NRXN2* in AML remains unclear.

N⁶-methyladenosine (m⁶A) is one of the most common modifications of RNA, characterized by the addition of a methyl group to the nitrogen atom (N⁶) at the sixth carbon position of adenosine (A) [14]. The m⁶A modification involves a series of proteins, divided into “writer,” “eraser,” and “reader” categories. Central to the functional significance of m⁶A is the reader, which can recognize and bind to the target RNA via the m⁶A site. Insulin-like growth factor 2 mRNA-binding protein 3 (IGF2BP3), the most prevalent m⁶A reader, enhances mRNA stability by recognizing and binding to the m⁶A site [15,16]. It plays a critical role in tumorigenesis and cancer progression. For example, the upregulation of IGF2BP3 promotes malignant behaviors such as proliferation and migration in bladder cancer cells, thereby driving bladder cancer advancement [17]. In triple-negative breast cancer, IGF2BP3 regulates glycolysis and lactate secretion in tumor-associated macrophages [18]. These findings suggest the significant association of IGF2BP3 with aggressive malignancies. The role of IGF2BP3 in AML is increasingly gaining attention. Overexpression of IGF2BP3 facilitates the malignant proliferation of hematopoietic progenitor cells, inducing oncogenic transformation and

accelerating leukemogenesis [19]. Notably, elevated IGF2BP3 expression in AML correlates strongly with poor survival outcomes [20]. However, the precise molecular mechanisms underlying the biofunction of IGF2BP3 in AML remain to be fully clarified.

In the present study, *NRXN2* is identified to be a DEG of AML based on bioinformatic analysis, and it can promote the proliferation of AML cells. Furthermore, IGF2BP3 is capable of stabilizing *NRXN2* mRNA in AML cells in an m⁶A-dependent manner. Collectively, our findings indicate the oncogenic role of *NRXN2* in AML and we provide novel insight for the targeted therapy of AML.

Materials and Methods

Experimental Design

The human KG-1, THP-1, and HL-60 AML cell lines and human peripheral blood mononuclear cells (PBMCs) were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (FBS) at 37 °C in a 5% CO₂ atmosphere. To determine the function of *NRXN2* in AML cells, HL-60 cells were allocated into two experimental groups: the si-NC group, transfected with small interfering RNA (siRNA) as a negative control, and the si-*NRXN2* group, transfected with *NRXN2*-specific siRNA. To assess the impact of IGF2BP3, HL-60 cells were divided into the si-NC group and the si-*IGF2BP3* group, transfected with siRNA as a negative control and *IGF2BP3*-specific siRNA, respectively. To investigate the IGF2BP3/*NRXN2* signaling axis, HL-60 cells were divided into three distinct groups: the si-NC+oe-NC group, transfected with negative-control siRNA and an empty overexpression vector; the si-*IGF2BP3*+oe-NC group, transfected with *IGF2BP3* siRNA and an empty vector; and the si-*IGF2BP3*+oe-*NRXN2* group, transfected with *IGF2BP3* siRNA and an *NRXN2* overexpression vector. To further investigate the role of IGF2BP3-mediated m⁶A modification in regulating *NRXN2*, HL-60 cells were divided into the following groups: the oe-NC group, transfected with an empty overexpression vector; the oe-*IGF2BP3* group, co-transfected with an *IGF2BP3* overexpression vector; and the oe-*IGF2BP3*+*NRXN2*-mut group, co-transfected with an *IGF2BP3* overexpression vector and an *NRXN2* mutant construct (hereafter referred to as *NRXN2*-mut [c.1770A>T]) lacking the predicted m⁶A site. All siRNAs, overexpression vectors, and the *NRXN2* mutant were designed and synthesized by Sangon Biotech (Shanghai, P.R. China). Transfections were conducted using the Lipofectamine 3000 kit (Thermo Fisher Scientific, Waltham, MA, USA).

Bioinformatic Analysis

RNA sequencing data described in a previous study (<https://doi.org/10.5061/dryad.h9w0vt4t2>) were used to screen and identify the DEGs of AML using the DESeq2 R package with log₂ fold

change|>1.5 and $p<0.05$. The TCGA-AML database was used to verify the expressions of DEGs and their association with the survival of patients with AML using the GEPIA website. Kaplan-Meier curves were used to analyze the survival rates of patients with AML.

Real-Time Polymerase Chain Reaction

NRXN2 and *IGF2BP3* were quantified by real-time polymerase chain reaction (qPCR). After the measurement of RNA purity with a spectrophotometer at 260 nm and 280 nm, RNA was extracted using TRIzol reagent, reverse-transcribed (RT) into cDNA, and quantified using the FastKing One Step RT-qPCR Kit (SYBR Green) (#4993103, TIANGEN, Beijing, P.R. China). The RT-qPCR reaction was performed using the CFX Connect Real-Time System (Bio-Rad, Hercules, CA, USA). *GAPDH* was used as the internal reference gene. The primer sequences were as follows: *GAPDH*-F, 5'- GTC TCC TCT GAC TTC AAC AGC G-3'; *GAPDH*-R, 5'- ACC ACC CTG TTG CTG TAG CCA A-3'; *NRXN2*-F, 5'- GAC AAT GAG TGG CACA CGG TGA-3'; *NRXN2*-R, 5'- CCG TCT CAA TGT TGT GGA ACT CC-3'; *IGF2BP3*-F, 5'-TCG TGA CCA GAC ACC TGA TGA G-3'; *IGF2BP3*-R, 5'- GGT GCT GCT TTA CCT GAG TCA G-3'.

Western Blotting

HL-60 cells were lysed using RIPA buffer containing protease inhibitors, and then cell lysates were centrifuged at 10,000 x g for 10 min at 4 °C. The supernatant was collected, followed by the measurement of protein concentration using the BCA assay (Beyotime, Shanghai, P.R. China). Proteins were then separated by SDS-PAGE electrophoresis (Bio-Rad, USA) and transferred to PVDF membranes (Millipore, Burlington, MA, USA) using a semi-dry transfer system (Bio-Rad, USA). The membranes were blocked with 5% non-fat milk in TBST buffer for 1 h at room temperature. They were then incubated overnight at 4 °C with primary antibodies, including anti-Bcl-2 (ab182858, 1/2000, Abcam, Waltham, MA, USA), anti-PCNA (ab92552, 1/10000, Abcam, USA), anti-cleaved caspase 3 (ab32042, 1/500, Abcam, USA), and anti-GAPDH (ab9485, 1/2500, Abcam, USA), followed by HRP-conjugated secondary antibody (ab6721, 1/10000, Abcam, USA) for 1 h. Protein blots were visualized using an ECL substrate (Beyotime, P.R. China) and imaged with a chemiluminescence detection system (Bio-Rad, USA). *GAPDH* was used as a loading control.

MeRIP-qPCR

The m⁶A modification of *NRXN2* mRNA was detected using the Magna MeRIP m⁶A Kit (17-10499, Sigma-Aldrich, St. Louis, MO, USA). The total RNA extracted using the TRIzol reagent was verified and fragmented using RNA fragmentation buffer (pH 7.0) at 94 °C for 15 min. Fragments were then incubated overnight at 4 °C with anti-m⁶A antibody conjugated to protein A/G magnetic beads in IP buffer. IgG and the input RNA sample were included as controls. After washing, the bound RNA was eluted using proteinase K (Sigma-Aldrich, USA) and purified. The

enrichment of *NRXN2* mRNA was quantified by qPCR with gene-specific primers.

RIP-qPCR

Cell lysates were centrifuged at 12,000 x g for 10 min at 4 °C. The cleared lysates were then incubated overnight at 4 °C with anti-IGF2BP3 antibody or IgG-bound magnetic beads. Input RNA was used as a reference. Beads were washed and bound RNA-protein complexes were digested with proteinase K. The RNA was collected for qPCR analysis. *NRXN2* mRNA enrichment was quantified by RNA immunoprecipitation-qPCR.

Detection of RNA Stability

RNA stability was assessed using the actinomycin D assay. Following grouping treatments, cells were incubated with 10 µg/mL actinomycin D (MedChemExpress, Monmouth Junction, NJ, USA). Cells were collected immediately before actinomycin D treatment (0 h) and at 1, 3, 6, and 9 h after treatment. *NRXN2* mRNA levels at each time point were quantified by qPCR.

Cell Proliferation Detected by CCK-8 Assay

HL-60 cells were seeded onto 96-well plates at a density of 5x10³ cells/well using RPMI-1640 medium supplemented with 10% FBS, followed by overnight incubation at 37 °C with 5% CO₂. The cells were treated according to their designated groups and were then incubated with CCK-8 reagent (Beyotime, P.R. China) at a dose of 10 µL per 100 µL of medium, followed by 1 h of incubation at 37 °C. Optical density (OD) was measured at 450 nm using a microplate reader (Thermo Fisher Scientific, USA). Subsequently, the proliferation rate was calculated by OD normalized to control group.

Cell Apoptosis Detected by Flow Cytometry

HL-60 cells were harvested and washed twice with phosphate-buffered saline, followed by centrifugation at 300 x g for 5 min. Cells resuspended in binding buffer were then incubated with 5 µL of annexin V-FITC and 5 µL of propidium iodide for 15 min at room temperature, protected from light. The stained cells were diluted with binding buffer and analyzed immediately using a flow cytometer (Beckman, Krefeld, Germany). The apoptosis rate was calculated using FlowJo software.

Statistical Analysis

In this work, all experiments were repeated 3 times. Independent t-tests and one-way analysis of variance were used for statistical comparisons between groups, and post-hoc comparisons were performed using Tukey's test. Values of $p<0.05$ indicated significant differences. Kaplan-Meier curves were used to analyze the survival rates of patients with AML using the Kaplan-Meier Plotter website (<https://kmplot.com/analysis/index.php?p=home>).

Results

NRXN2 Was Upregulated in AML

The DEG analysis of AML was performed based on the transcript sequence dataset described in a previous study and a total of 13,962 DEGs were identified (Figure 1A). The relationship between DEGs and overall survival in AML was investigated using

the GEPIA 2.0 database. Importantly, upregulated *NRXN2* was positively related to lower overall survival in AML (Figure 1B). Furthermore, we verified that *NRXN2* was significantly elevated in AML according to the TCGA and GTEx databases (Figure 1C). We observed that *NRXN2* showed upregulation in AML cells, including HL-60, KG-1 and THP-1, as compared to PBMCs ($p < 0.05$) (Figures 1D and 1E).

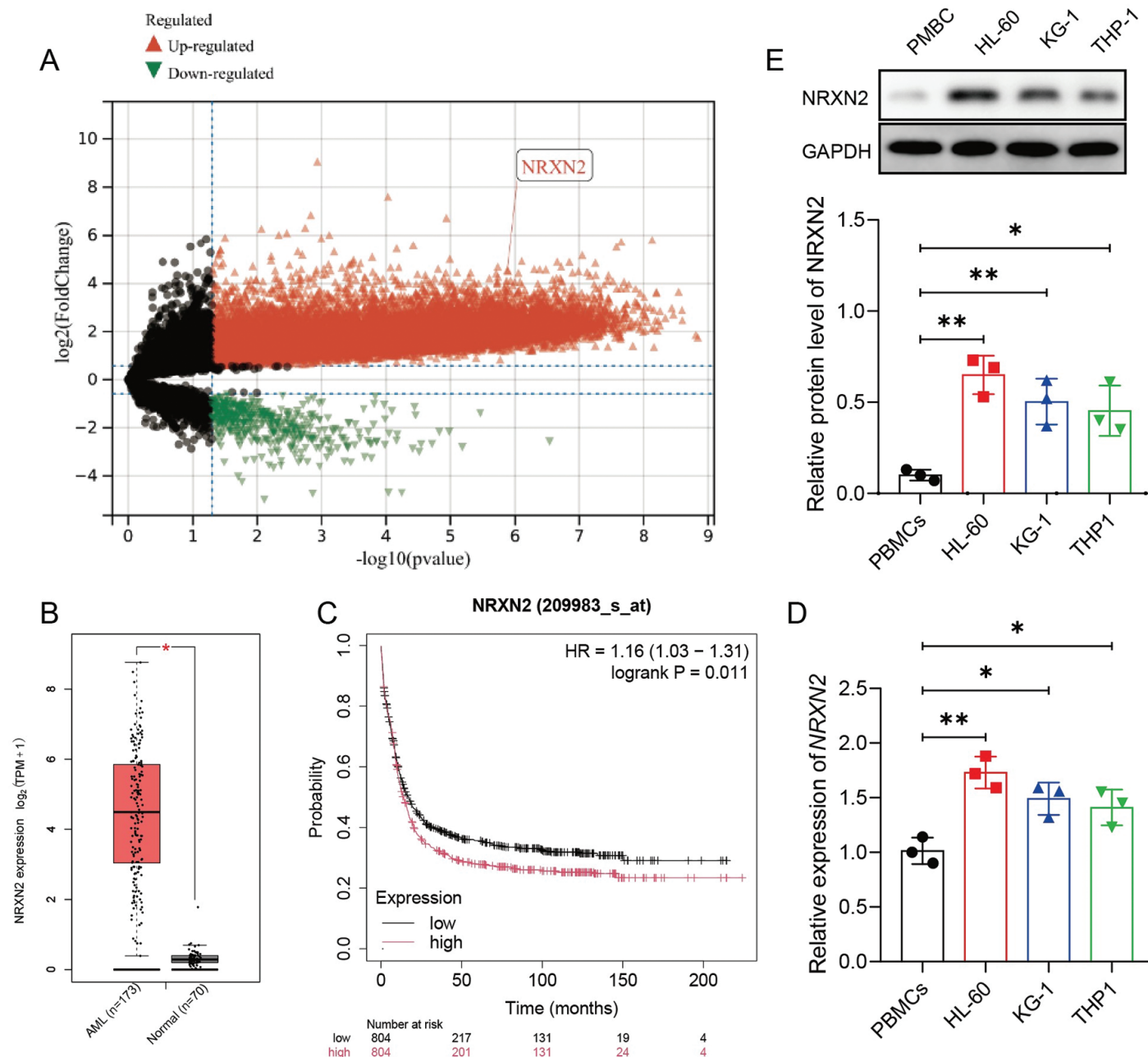


Figure 1. *NRXN2* was upregulated in acute myeloid leukemia (AML). A) Analysis of differentially expressed genes for AML. B) Normalized expression of *NRXN2* based on the TCGA-AML database: transcriptomic data of AML samples (tumor group, n=173) and normal samples (normal group, n=70) were extracted from the TCGA-AML database and normalized using the transcripts per million (TPM) method. C) Overall survival of patients with high and low expression of *NRXN2* based on the TCGA-AML database. D) *NRXN2* in peripheral blood mononuclear cells and in HL-60, KG-1, and THP-1 cells as quantified by real-time polymerase chain reaction. E) *NRXN2* in peripheral blood mononuclear cells and in HL-60, KG-1, and THP-1 cells as detected by western blotting.

*: $p < 0.05$, **: $p < 0.01$; HR: hazard ratio; PBMCs: peripheral blood mononuclear cells.

NRXN2 Enhanced the Proliferation of AML Cells

To investigate the effects of *NRXN2* on AML, *NRXN2* was downregulated by its specific siRNA ($p < 0.05$) (Figures 2A and 2B). Intriguingly, the si-*NRXN2* group showed a decrease in cell proliferation and an increase in cell apoptosis in AML cells compared to the si-NC group ($p < 0.05$) (Figures 2C and 2D). The expression levels of proliferation- and apoptosis-related factors, including Bcl-2, PCNA, and cleaved caspase 3, were compared between the si-NC and si-*NRXN2* groups. Significantly lower levels of Bcl-2 and PCNA were observed in the si-*NRXN2* group compared to the si-NC group ($p < 0.05$), while cleaved caspase 3 was significantly higher ($p < 0.05$) (Figure 2E).

NRXN2 mRNA Was Regulated by IGF2BP3 via an m⁶A-Dependent Pathway

To explore the mechanisms responsible for the aberrant expression of *NRXN2* in AML, the potential involvement of m⁶A RNA methylation was examined. The m⁶A modification sites on *NRXN2* were predicted utilizing the online SRAMP tool (Figure 3A). To disrupt the predicted m⁶A site, an m⁶A methylation-deficient mutant of *NRXN2* (*NRXN2*-mut [c.1770A>T]) was generated. As shown in Figures 3B and 3C,

the *NRXN2*-mut (c.1770A>T) group experienced a decrease in *NRXN2* expression compared to the *NRXN2*-wt group ($p < 0.05$). *NRXN2*-mut (c.1770A>T) resulted in a decrease in the m⁶A level of *NRXN2* mRNA ($p < 0.05$) (Figure 3D). There was a significant reduction in the RNA stability of *NRXN2* mRNA due to the *NRXN2* mutant (c.1770A>T), suggesting an m⁶A site contributing to the RNA stability of *NRXN2* mRNA (Figure 3E). According to the prediction of the Starbase website, IGF2BP3, an m⁶A reader, might bind to *NRXN2* mRNA. Intriguingly, the *NRXN2*-mut (c.1770A>T) group showed lower interaction between IGF2BP3 and *NRXN2* mRNA, whereas the *NRXN2*-wt group exhibited higher interaction between IGF2BP3 and *NRXN2* mRNA (Figure 3F). Next, *IGF2BP3* silencing led to the downregulation of *NRXN2* ($p < 0.05$) (Figures 3G and 3H); further studies showed that IGF2BP3 overexpression caused the upregulation of *NRXN2* as well as the RNA stability of *NRXN2* mRNA, but the *NRXN2* mutant (c.1770A>T) reversed the effects of IGF2BP3 on *NRXN2* mRNA (Figures 3I-3K). The level of m⁶A-modified *NRXN2* mRNA in the oe-IGF2BP3+*NRXN2*-mut (c.1770A>T) group was lower than that in the oe-IGF2BP3 group ($p < 0.05$) (Figure 3L). Collectively, IGF2BP3 promotes the stability of *NRXN2* mRNA via an m⁶A-dependent pathway.

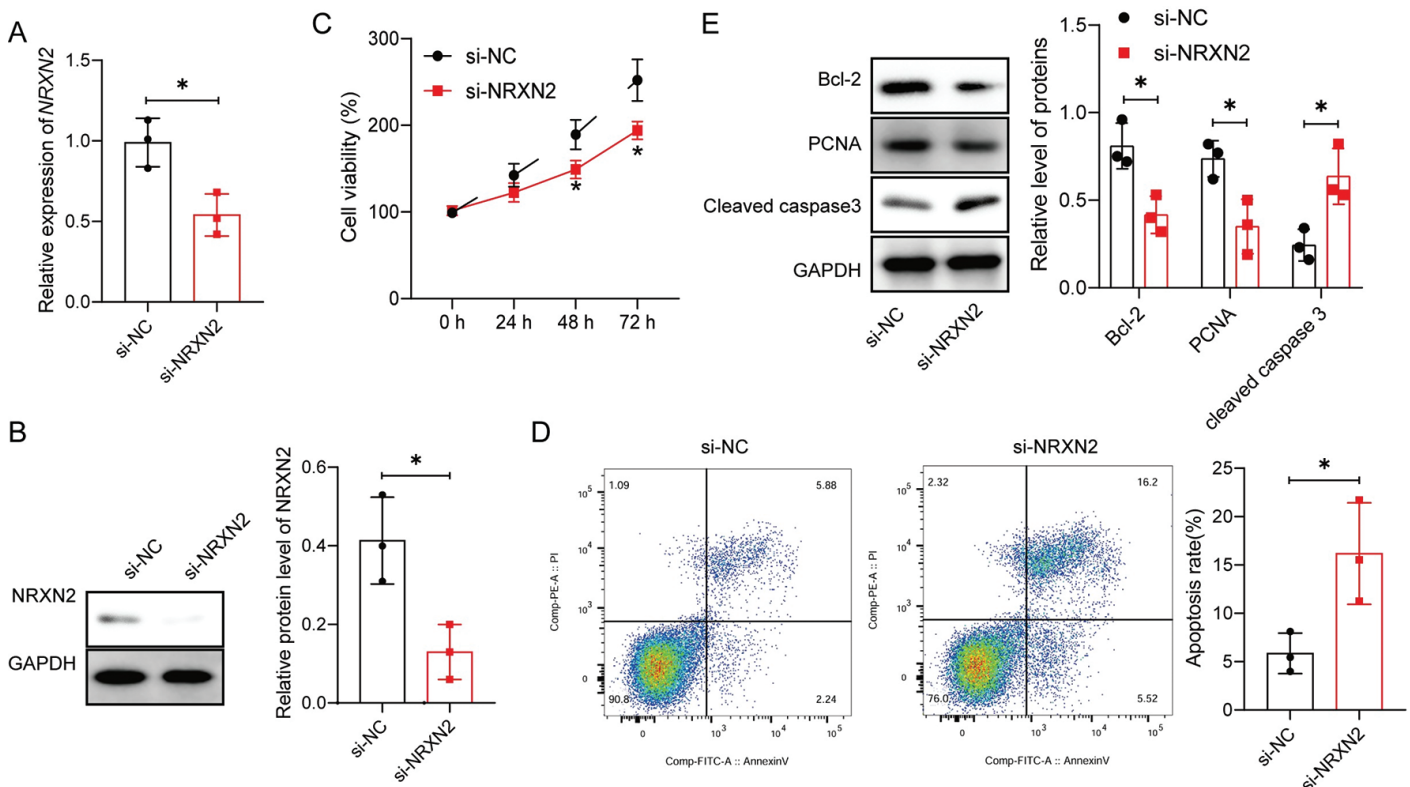


Figure 2. NRXN2 enhanced the proliferation of acute myeloid leukemia cells. HL-60 cells were transfected with control siRNA (si-NC) or NRXN2-specific siRNA (si-*NRXN2*). A, B) *NRXN2* in the si-NC and si-*NRXN2* groups was detected by real-time polymerase chain reaction (A) and western blotting (B). C) Cell proliferation was studied by CCK-8 assay. D) Cell apoptosis was detected by flow cytometry. E) Apoptosis-related proteins were detected by western blotting.

*: $p < 0.05$.

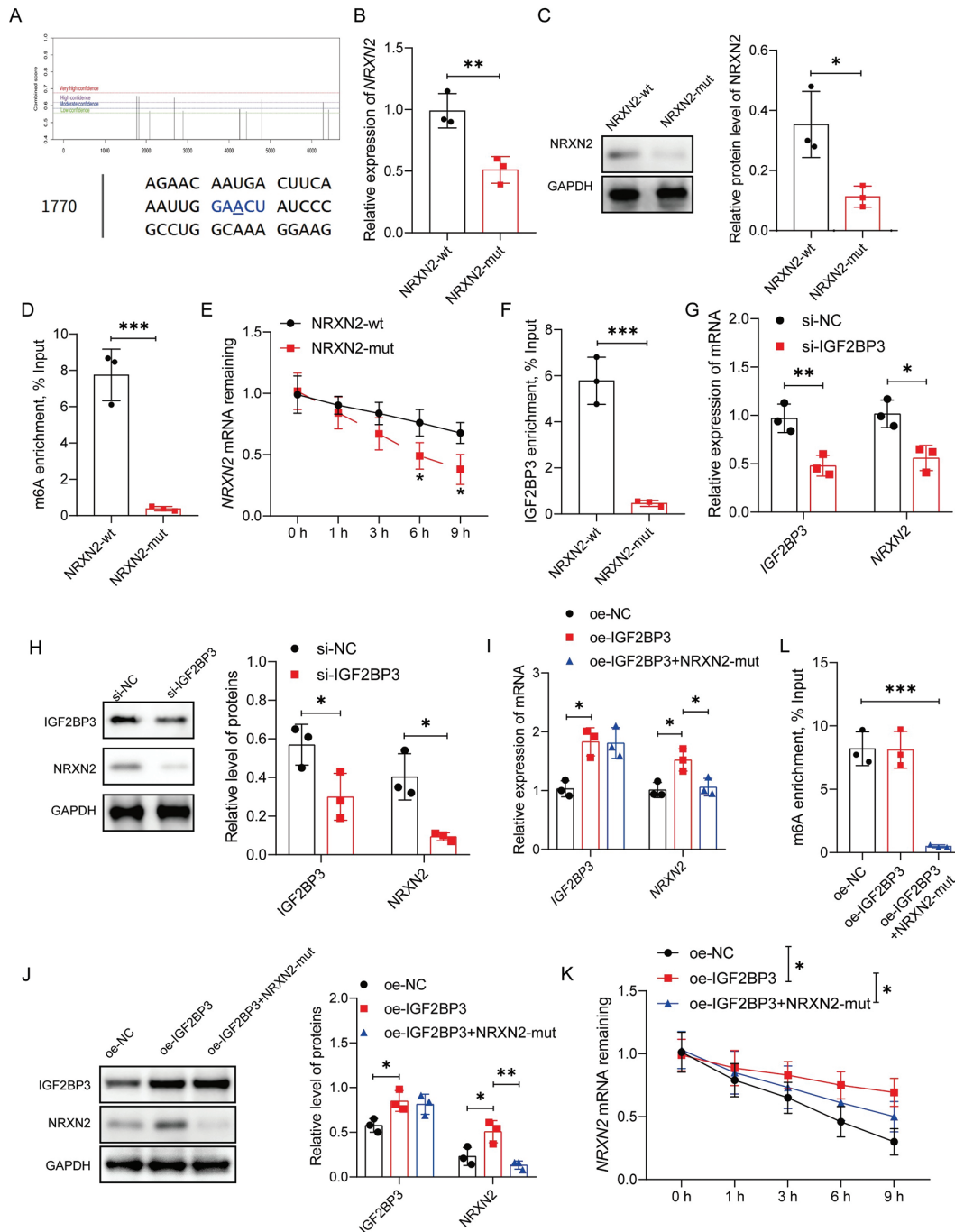


Figure 3. *NRXN2* mRNA was regulated by IGF2BP3 through an m⁶A-dependent pathway. A) Prediction of m⁶A modification sites on *NRXN2* mRNA using the online SRAMP tool. B, C) *NRXN2* expression in cells transfected with wild-type *NRXN2* (*NRXN2*-wt) or an m⁶A site mutant (*NRXN2*-mut [c.1770A>T]) was detected by real-time polymerase chain reaction (B) and western blotting (C). D) The m⁶A modification level of *NRXN2* mRNA in the *NRXN2*-wt and *NRXN2*-mut (c.1770A>T) groups was detected by real-time polymerase chain reaction using the Magna MerIP m⁶A Kit. E) RNA stability of *NRXN2* mRNA in the *NRXN2*-wt and *NRXN2*-mut (c.1770A>T) groups was assessed by actinomycin D assay. F) The interaction between *IGF2BP3* and *NRXN2* mRNA in the *NRXN2*-wt and *NRXN2*-mut (c.1770A>T) groups was determined by RNA immunoprecipitation-real-time polymerase chain reaction. G, H) *IGF2BP3* and *NRXN2* expression levels in HL-60 cells transfected with control siRNA (si-NC) or *IGF2BP3*-specific siRNA (si-*IGF2BP3*) were detected by real-time polymerase chain reaction (G) and western blotting (H). I, J) *IGF2BP3* and *NRXN2* expression levels in HL-60 cells from the oe-NC, oe-*IGF2BP3*, and oe-*IGF2BP3*+*NRXN2*-mut (c.1770A>T) groups were detected by real-time polymerase chain reaction (I) and western blotting (J). K) RNA stability of *NRXN2* mRNA in the oe-NC, oe-*IGF2BP3*, and oe-*IGF2BP3*+*NRXN2*-mut (c.1770A>T) groups. L) m⁶A modification level of *NRXN2* mRNA in the oe-NC, oe-*IGF2BP3*, and oe-*IGF2BP3*+*NRXN2*-mut (c.1770A>T) groups was detected by real-time polymerase chain reaction using the Magna MerIP m⁶A Kit.

*: p<0.05, **: p<0.01, ***: p<0.001.

IGF2BP3 Promoted the Malignant Behaviors of AML Cells

The mRNA and protein expression levels of IGF2BP3 in AML cell lines, including HL-60, KG-1, and THP-1, were significantly elevated compared to those in PBMCs ($p < 0.05$), with the highest expression observed in HL-60 cells (Figures 4A and 4B). According to the TCGA-AML database, *IGF2BP3* expression was negatively correlated with the overall survival of patients with AML ($p < 0.05$) (Figure 4C). As shown in Figures 4D and 4E, IGF2BP3 silencing caused a reduction in cell apoptosis and an increase in cell proliferation in AML cells ($p < 0.05$). At the molecular level, IGF2BP3 silencing contributed to reduced expression levels of Bcl-2 and PCNA and the higher expression of cleaved caspase 3 ($p < 0.05$) (Figure 4F).

IGF2BP3 Promoted the Proliferation of AML Cells via Upregulation of *NRXN2*

To determine whether IGF2BP3 modulates AML cells through its target *NRXN2* mRNA, a co-transfection of si-*IGF2BP3* and oe-*NRXN2* was performed in AML cells. Significantly, oe-*NRXN2* increased the si-*IGF2BP3*-decreased *NRXN2* levels of AML cells

($p < 0.05$) (Figures 5A and 5B). Additionally, *NRXN2* overexpression reversed the changes caused by si-*IGF2BP3* in proliferation and apoptosis, characterized by increased proliferation and decreased apoptosis ($p < 0.05$) (Figures 5C and 5D). *NRXN2* overexpression offset the role of si-*IGF2BP3* for Bcl-2, PCNA, and cleaved caspase 3, characterized by upregulation of Bcl-2 and PCNA and downregulation of cleaved caspase 3 ($p < 0.05$) (Figure 5E).

Discussion

The pathogenesis of AML is complex, with suboptimal outcomes of therapy. This study identified an IGF2BP3/*NRXN2* regulatory axis that played a critical role in the proliferation of AML cells. *NRXN2* mRNA carried m⁶A modifications, and IGF2BP3 could bind to *NRXN2* mRNA through the m⁶A-modified site, thereby enhancing its post-transcriptional stability and ultimately promoting leukemia cell proliferation while inhibiting apoptosis.

As a member of the neurexin family, *NRXN2* has been primarily investigated in neurological disorders. However, recent studies have begun to report its involvement in cancer progression, including medulloblastoma, endometrial cancer, and thyroid

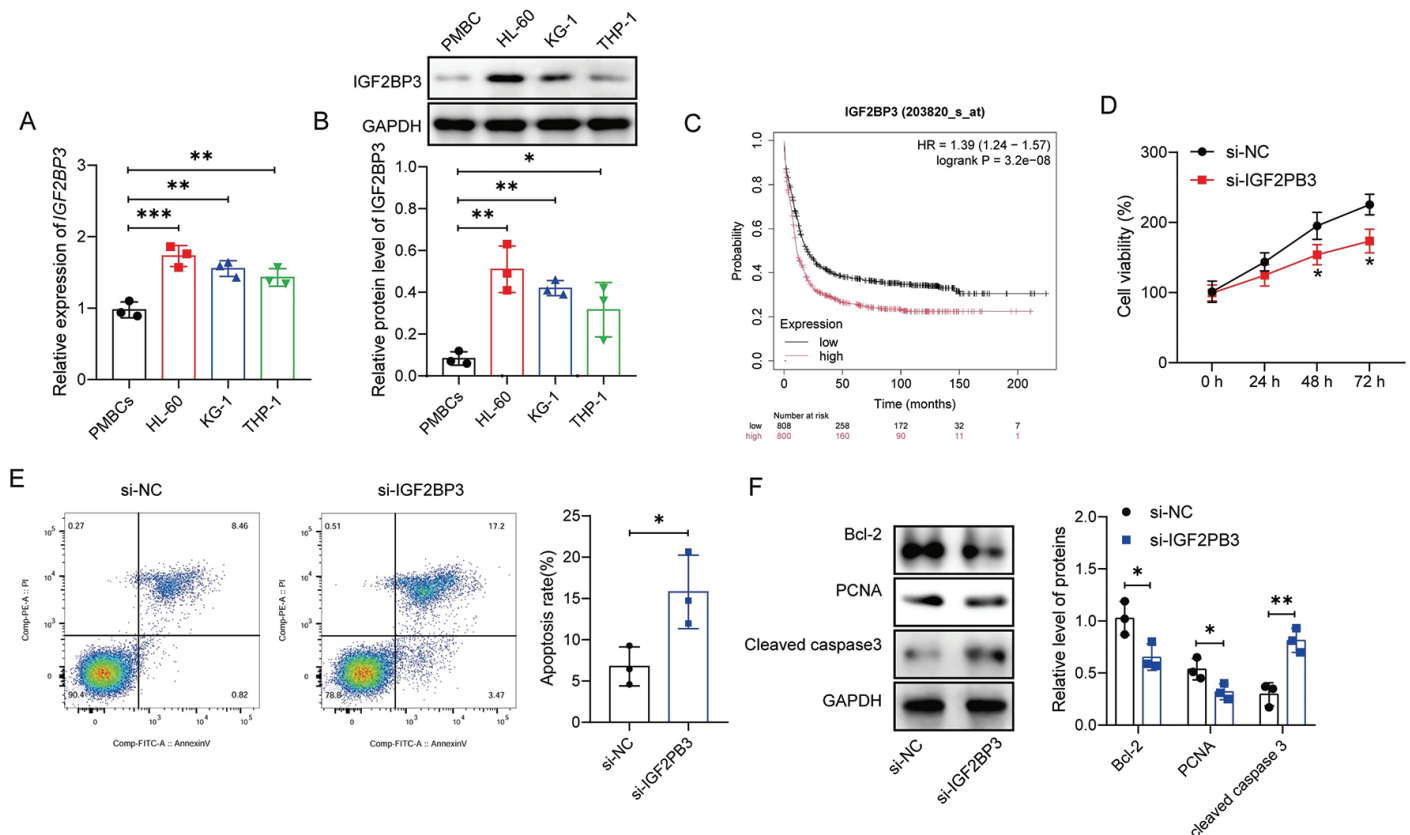


Figure 4. IGF2BP3 promoted the malignant behavior of acute myeloid leukemia cells. A, B) IGF2BP3 in peripheral blood mononuclear cells and HL-60, KG-1, and THP-1 cells was detected by real-time polymerase chain reaction (A) and western blotting (B). C) Overall survival of patients with high and low expression of *IGF2BP3*. D, E) HL-60 cells were transfected with control siRNA (si-NC) or *IGF2BP3*-specific siRNA (si-*IGF2BP3*), and then cell proliferation (D) and apoptosis (E) were assessed by CCK-8 assay and flow cytometry, respectively. F) Apoptosis-related proteins were detected by western blotting.

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$; HR: hazard ratio; PBMCs: peripheral blood mononuclear cells.

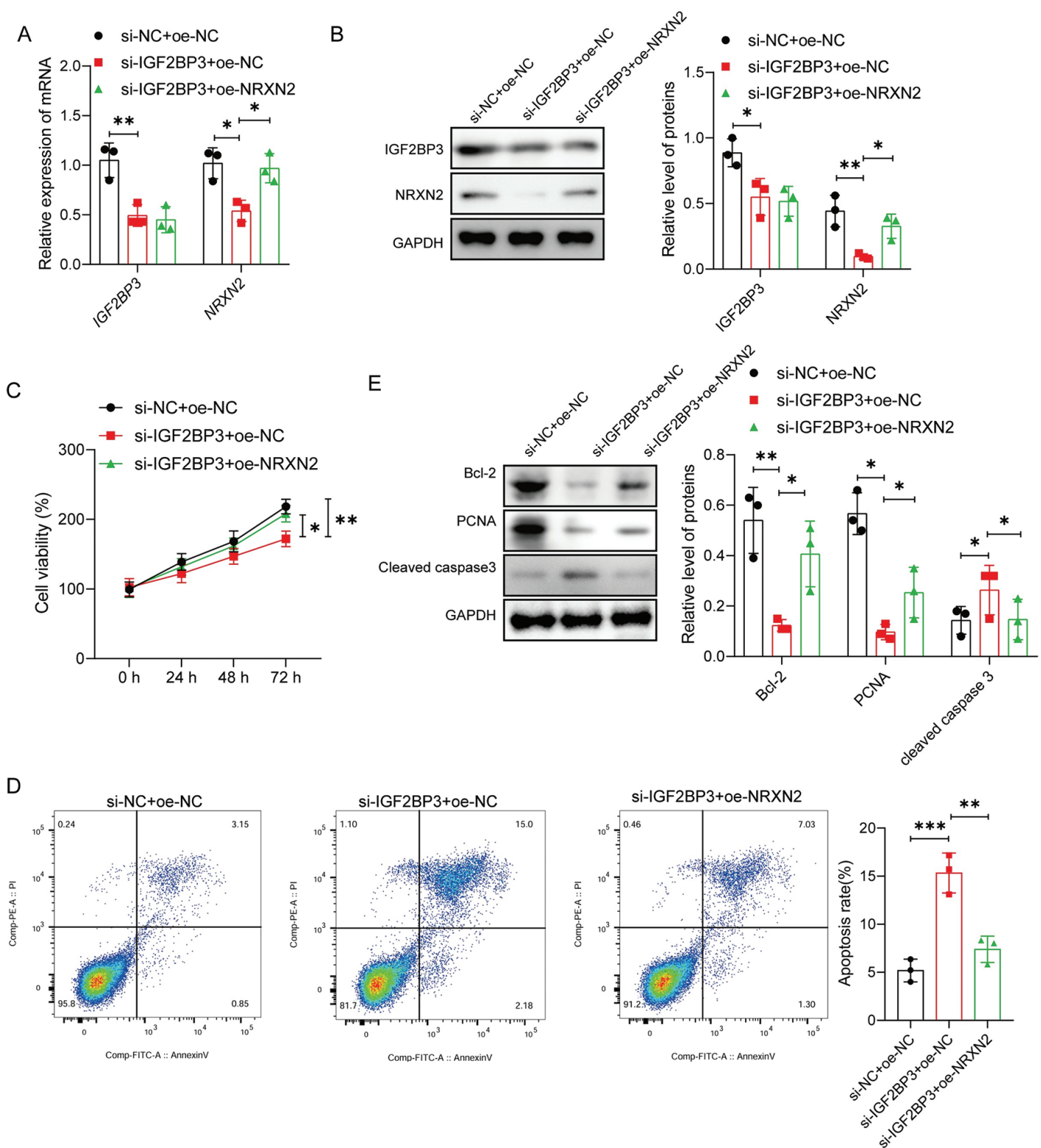


Figure 5. IGF2BP3 promoted the proliferation of acute myeloid leukemia cells by upregulating *NRXN2*. HL-60 cells were subjected to the following treatments as described in the main text: si-NC+oe-NC, si-IGF2BP3+oe-NC, and si-IGF2BP3+oe-NRXN2. A, B) *IGF2BP3* and *NRXN2* were detected by real-time polymerase chain reaction (A) and western blotting (B). C) Cell proliferation was studied by CCK-8 assay. D) Cell apoptosis was detected by flow cytometry. E) Apoptosis-related proteins were detected by western blotting.

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

carcinoma [21,22]. An analysis of the GSE dataset demonstrated the upregulation of *NRXN2* in leukemia. Our finding was subsequently validated through the TCGA-AML cohort and in vitro experiments, which confirmed a significant correlation between elevated *NRXN2* expression and reduced survival rates in AML patients. These results suggest a potential oncogenic role for *NRXN2* in leukemia. Abnormal *NRXN2* expression can induce cellular alterations. Cuttler et al. [21] demonstrated that *NRXN2* mutations reduce viability and promote apoptosis/oxidative stress in SH-SY5Y neuroblastoma cells. Ma and Zhang [22] showed that aberrant *NRXN2* expression enhances proliferation and migration in thyroid cancer. Our experiments revealed that *NRXN2* silencing in AML HL-60 cells markedly reduced proliferation while increasing apoptosis, accompanied by elevated cleaved caspase 3 and suppressed Bcl-2/PCNA expression. These findings inversely indicate that *NRXN2* upregulation can facilitate the overproliferation of AML cells.

The m⁶A modification, the most prevalent RNA epigenetic alteration, is associated with AML. A series of dysregulated m⁶A-related genes in AML have been shown to correlate with tumor immunity [23]. One function of m⁶A modification is to enhance the stability of target RNAs, thereby increasing their intracellular abundance. This facilitation ultimately promotes translation and leads to elevated levels of the corresponding protein. Using bioinformatics tools, the m⁶A modification site on *NRXN2* mRNA was predicted. Site-directed mutagenesis was then performed at this predicted site to generate *NRXN2*-mut (c.1770A>T). qPCR and western blotting analyses revealed decreased levels of both *NRXN2* mRNA and protein, suggesting the potential involvement of this site in maintaining the stability of *NRXN2* mRNA. The *NRXN2*-mut (c.1770A>T) construct reduced the m⁶A level of *NRXN2* and accelerated mRNA decay. Thus, an m⁶A modification site was identified on *NRXN2* mRNA. The m⁶A modification at this site enhances the stability of *NRXN2* mRNA, thereby upregulating *NRXN2*. As an m⁶A reader, IGF2BP3 contains a hydrophobic groove in its KH domain that recognizes m⁶A motifs, enabling target RNA stabilization [14]. In this work, our findings demonstrated that mutation of the m⁶A modification sites on *NRXN2* mRNA significantly reduced the recruitment of IGF2BP3 to *NRXN2* mRNA, indicating that m⁶A sites on *NRXN2* mRNA are essential for IGF2BP3 binding. Functioning in an m⁶A-dependent manner, IGF2BP3 stabilizes target RNAs. For instance, IGF2BP3 could promote the stability of *HMGB1* mRNA in bladder cancer to induce tumorigenesis [17]. Our findings indicate that the knockdown of IGF2BP3 accelerates the decay of *NRXN2* mRNA, whereas its overexpression enhances the stability of *NRXN2* mRNA. Recent studies have linked elevated IGF2BP3 expression in leukemia to chemotherapy resistance, immune dysregulation, and poor prognosis [24,25]. Thus, the upregulation of IGF2BP3 is a key cause of the overexpression of *NRXN2* in AML. Subsequently, IGF2BP3 was found to promote

the proliferation of HL-60 cells and to inhibit apoptosis via *NRXN2* upregulation, suggesting an m⁶A-dependent mechanism by which IGF2BP3 elevated *NRXN2* through recognition of the m⁶A site of *NRXN2* mRNA, thereby fueling the proliferation of HL-60 cells. Importantly, our findings indicate that IGF2BP3 and *NRXN2* are promising anticancer targets for AML. The silencing of IGF2BP3 and the downregulation of *NRXN2* were both observed to inhibit the proliferation of HL-60 cells and induce apoptosis.

Conclusion

Although the m⁶A-mediated mechanism underlying *NRXN2* upregulation in AML has been elucidated and the IGF2BP3/*NRXN2* axis has been established, the upstream drivers of IGF2BP3 dysregulation in AML require further investigation. Further studies should explore how this axis influences AML drug resistance. In addition, future prospective studies could explore the potential utility of IGF2BP3 and *NRXN2* in the early diagnosis and prognosis of AML, with parallel efforts to develop AML-specific early diagnostic tools and risk prediction models.

IGF2BP3 can recognize the m⁶A-modified site of *NRXN2* mRNA, contributing to the stabilization of *NRXN2* at the post-transcriptional level in AML cells, thereby promoting the overproliferation of AML cells. Our findings provide novel insight into the oncogenic mechanism of AML progression and indicate new targets in AML therapy.

Ethics

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

Footnotes

Authorship Contributions

Concept: N.H.; Design: N.H.; Data Collection or Processing: W.M., L.S., S.L.; Analysis or Interpretation: W.M., L.S., S.L.; Literature Search: W.M., L.S.; Writing: W.M., L.S.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Shimony S, Stahl M, Stone RM. Acute myeloid leukemia: 2025 update on diagnosis, risk-stratification, and management. *Am J Hematol*. 2025;100:860-891.
2. Wysota M, Konopleva M, Mitchell S. Novel therapeutic targets in acute myeloid leukemia (AML). *Curr Oncol Rep*. 2024;26:409-420.
3. Mishra SK, Millman SE, Zhang L. Metabolism in acute myeloid leukemia: mechanistic insights and therapeutic targets. *Blood*. 2023;141:1119-1135.

4. Vakiti A, Reynolds SB, Mewawalla P. Acute myeloid leukemia. Treasure Island, StatPearls, 2024. Available online at <https://www.ncbi.nlm.nih.gov/books/NBK507875/>
5. Yi M, Li A, Zhou L, Chu Q, Song Y, Wu K. The global burden and attributable risk factor analysis of acute myeloid leukemia in 195 countries and territories from 1990 to 2017: estimates based on the global burden of disease study 2017. *J Hematol Oncol.* 2020;13:72.
6. Forsberg M, Konopleva M. AML treatment: conventional chemotherapy and emerging novel agents. *Trends Pharmacol Sci.* 2024;45:430-448.
7. Khoja S, Haile MT, Chen LY. Advances in neurexin studies and the emerging role of neurexin-2 in autism spectrum disorder. *Front Mol Neurosci.* 2023;16:1125087.
8. Lu J, Zhu Y, Williams S, Watts M, Tonta MA, Coleman HA, Parkington HC, Claudianos C. Autism-associated miR-873 regulates *ARID1B*, *SHANK3* and *NRXN2* involved in neurodevelopment. *Transl Psychiatry.* 2020;10:418.
9. Khoja S, Chen LY. Conditional deletion of neurexin-2 impaired behavioral flexibility to alterations in action-outcome contingency. *Sci Rep.* 2024;14:10187.
10. Haile MT, Khoja S, de Carvalho G, Hunt RF, Chen LY. Conditional deletion of neurexin-2 alters neuronal network activity in hippocampal circuitries and leads to spontaneous seizures. *Transl Psychiatry.* 2023;13:97.
11. Shen Y, Tian Y, Ding J, Chen Z, Zhao R, Lu Y, Li L, Zhang H, Wu H, Li X, Zhang Y. Unravelling the molecular landscape of endometrial cancer subtypes: insights from multiomics analysis. *Int J Surg.* 2024;110:5385-5395.
12. Zhu S, Lin F, Chen Z, Jiang X, Zhang J, Yang Q, Chen Y, Wang J. Identification of a twelve-gene signature and establishment of a prognostic nomogram predicting overall survival for medulloblastoma. *Front Genet.* 2020;11:563882.
13. Huang W, Zheng S, Wang Q, Zhao N, Long Z. Identification and validation of a prognostic risk-scoring model based on the level of TIM-3 expression in acute myeloid leukemia. *Sci Rep.* 2023;13:15658.
14. Wei G. RNA m⁶A modification, signals for degradation or stabilisation? *Biochem Soc Trans.* 2024;52:707-717.
15. Liu X, Chen J, Chen W, Xu Y, Shen Y, Xu X. Targeting IGF2BP3 in cancer. *Int J Mol Sci.* 2023;24:9423.
16. Mancarella C, Scotlandi K. IGF2BP3 from physiology to cancer: novel discoveries, unsolved issues, and future perspectives. *Front Cell Dev Biol.* 2019;7:363.
17. Lv L, Wei Q, Zhang J, Dong Y, Shan Z, Chang N, Zhao Y, Bian P, Yi Q. IGF2BP3 prevent HMGB1 mRNA decay in bladder cancer and development. *Cell Mol Biol Lett.* 2024;29:39.
18. Liang Y, Ye F, Luo D, Long L, Wang Y, Jin Y, Wang L, Li Y, Han D, Chen B, Zhao W, Wang L, Yang Q. Exosomal circSIPA1L3-mediated intercellular communication contributes to glucose metabolic reprogramming and progression of triple negative breast cancer. *Mol Cancer.* 2024;23:125.
19. Palanichamy JK, Tran TM, Howard JM, Contreras JR, Fernando TR, Sterne-Weiler T, Katzman S, Toloue M, Yan W, Basso G, Pigazzi M, Sanford JR, Rao DS. RNA-binding protein IGF2BP3 targeting of oncogenic transcripts promotes hematopoietic progenitor proliferation. *J Clin Invest.* 2016;126:1495-1511.
20. Zhang N, Shen Y, Li H, Chen Y, Zhang P, Lou S, Deng J. The m⁶A reader IGF2BP3 promotes acute myeloid leukemia progression by enhancing RCC2 stability. *Exp Mol Med.* 2022;54:194-205.
21. Cuttler K, de Swardt D, Engelbrecht L, Kriel J, Cloete R, Bardien S. Neurexin 2 p.G849D variant, implicated in Parkinson's disease, increases reactive oxygen species, and reduces cell viability and mitochondrial membrane potential in SH-SY5Y cells. *J Neural Transm (Vienna).* 2022;129:1435-1446.
22. Ma C, Zhang Y. NRXN2 possesses a tumor suppressor potential via inhibiting the growth of thyroid cancer cells. *Comput Math Methods Med.* 2021;2021:7993622.
23. Wang Y, Bin T, Tang J, Xu XJ, Lin C, Lu B, Sun TT. Construction of an acute myeloid leukemia prognostic model based on m⁶A-related efferocytosis-related genes. *Front Immunol.* 2023;14:1268090.
24. Li Z, Liu X, Wang L, Zhao H, Wang S, Yu G, Wu D, Chu J, Han J. Integrated analysis of single-cell RNA-seq and bulk RNA-seq reveals RNA N6-methyladenosine modification associated with prognosis and drug resistance in acute myeloid leukemia. *Front Immunol.* 2023;14:1281687.
25. LE N, Yang JJ, Liu YC, Zhang XW, Wang H, Wen YN, Jiao YF, Wang LL, Dou LP. Prognostic value of IGF2BP3 gene expression levels in patients with acute myeloid leukemia. *Zhongguo Shi Yan Xue Ye Xue Za Zhi.* 2024;32:355-364.