Journal of http://www.satursonpublishing.com/
Research in Multidisciplinary Methods and Applications
ISSN: 2957-3920

Volume 3, Issue 7, July 2024

hnRNPR Regulates MYB Expression Via Interacting with Enhancer lncRNA MY34UE-AS in Human Leukemia Cells

Mengjie Shi[1,2], Yucheng Wang[1,2,3], Chao Liu[1,2], Siyu Shen[1,2], Junfang Zhang[1,2,3*], Bingshe Han[1,2,3*]

[1] Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai Ocean University, Shanghai 201306, China; [2] National Demonstration Center for Experimental Fisheries Science Education, Shanghai Ocean University, Shanghai 201306, China; [3] Marine Biomedical Science and Technology Innovation Platform of Lin-gang Special Area, Shanghai 201306, China

[*]Corresponding to: Junfang Zhang, Bingshe Han

Abstract: The heterogeneous nuclear ribonucleoprotein (hnRNP) family significantly influences the development and progression of various cancers, yet the specific role of its major member hnRNPR in leukemia is not yet understood. Our study discovered a long non-coding RNA (lncRNA) enhancer, known as MY34UE-AS, transcribed from the -34kb enhancer of the MYB gene. It has the ability to increase MYB expression in human leukemia cells. This study demonstrated that hnRNPR can bind to MY34UE-AS through its RRM2 domain. Overexpression of hnRNPR increased MYB expression and enhanced K562 cell proliferation and migration, while hnRNPR knockdown had the opposite effects. Further investigation revealed that MY34UE-AS is crucial for the aforementioned functions of hnRNPR. In summary, these findings suggest that hnRNPR plays a role in MYB regulation and the development of leukemia by interacting with MY34UE-AS.

Keywords: hnRNPR; Enhancer RNA; MYB; Leukemia

1 INTRODUCTION

The transcription factor MYB plays a crucial role in regulating the proliferation and differentiation of hematopoietic cells (Pattabiraman and Gonda, 2013). MYB plays vital role in various stages during thymocyte development (Allen et al.; Pearson and Weston), myelopoiesis (Sumner et al.), erythropoiesis (Vegiopoulos et al.), and B-cell development (Thomas et al.). Dysregulation of MYB is often associated with blood disorders, mainly leukemia. MYB overexpression is found across all AML and acute lymphoid leukemia (ALL) subtypes, and is linked to a poor prognosis (Zuber et al.). Collectively, dysregulation of MYB significantly contributes to the development of human leukemia. Consequently, MYB can be as a potential target for leukemia therapy.

Precisely regulated MYB expression is required under physiological conditions. The transcription of MYB is mainly blocked by an attenuation site located within the first intron (Drabsch et al.; Hugo et al., 2006). Recent studies have found that MYB is controlled by distal enhancer elements located 84 kb upstream, and 140 kb downstream of the MYB gene (Stadhouders R Fau - Aktuna et al.). Enhancers can significantly promote the expression of target genes with notable cell type specificity (Bulger and Groudine; Su et al.). Many active enhancers undergo bidirectional transcription to produce eRNAs in a tissue-specific manner (Andersson et al.; Wu et al.). These eRNAs can maintain enhancer activity and regulate gene transcription through several mechanisms (Fang et al.; Hsieh et al.; Lam et al.; Li et al.; Melo et al.; Mousavi et al.). For instance, the efficient enhancement of p53 transcription depends on the production of eRNAs from p53-bound enhancer regions (Melo et al.). Furthermore, eRNAs play a role in the development and advancement of leukemia (Wan et al.). Specifically, the eRNA SEELA drives leukemia progression by modulating the transcription of SERINC2 (Fang et al.). According to our recent study, in human leukemia cells, an enhancer, termed as MY34UE-AS, has been discovered at the -34 kb region of MYB, leading to the upregulation of MYB expression. However, the underlying mechanisms by which MY34UE-AS regulates MYB expression have not been thoroughly explored.

The hnRNPs are large RNA-binding proteins that can regulate many biological processes, such as tumor initiation, tumor development, and drug resistance (Chatterji and Rustgi; Klingenberg et al.; Kudinov et al.). A series of hnRNPs have been reported to be involved in cancers, including hnRNPK in lung cancer (Li et al.) and acute promyelocytic leukemia

Journal of http://www.satursonpublishing.com/
Research in Multidisciplinary Methods and Applications
ISSN: 2957-3920

Volume 3, Issue 7, July 2024

(Padovani et al.), hnRNPC in pancreatic cancer (Yang et al.) and hnRNPA1 in acute myeloid leukemia (AML) (Song et al.). hnRNPR is involved in pathophysiological processes (Yang et al.) and various cancers (Hu et al.; Li et al.; Liu et al.; Chen et al., 2019). hnRNPR modulates transcription via interaction with ncRNA 7SK (Ji et al., 2022), activates the c-fos promoter (Fukuda et al.). hnRNPR can also mediate target gene mRNA splicing (Duijkers et al., 2019). hnRNPR mediates UPF3B mRNA splicing to drive hepatocellular carcinoma metastasis (Wang et al.; Chen et al., 2019), and mediates the splicing of exon 7 in SMN1/2 pre-mRNAs with spinal muscular atrophy (Jiang et al., 2023). Despite numerous studies have indicated that hnRNPR is involved in biological processes and cancer, its specific role and molecular mechanisms in the progression of leukemia remain unclear.

In conclusion, through its RRM2 domain, hnRNPR is capable of binding to MY34UE-AS, which is transcribed from the -34 kb enhancer in MYB, to promote MYB expression and to an increase in proliferation and migration of human leukemia K562 cells.

2 Materials And Methods

2.1 Cell Culture

RPMI-1640 medium (11875093, Gibco) and DMEM medium (10569044, Gibco) containing 10% fetal bovine serum (10099141, Gibco) and 1% penicillin-streptomycin-glutamine solution (SV30082.01, Hyclone) were respectively used to culture human erythroleukemic K562 cells (CCL-243, ATCC) and HEK 293T cells (CRL-3216, ATCC). Cells were maintained in a wild environment at 37°C with 5% CO2.

2.2 Quantitative Real-Time PCR Analysis(RT-QPCR)

Total RNA from cell samples was isolated using TRIzol reagent (15596018, Invitrogen). Then, after RNA extraction, 1 μg of total RNA was converted to cDNA using PrimeScriptTM RT reagent Kit with DNA Eraser (RR047A, Takara) and RT-qPCR was used in LightCycler® 480 Instrument II. GAPDH was used for normalization as an internal reference in the results. The expression data was analyzed using the relative quantification method, calculated by 2-ΔΔCt. Primer sequences are included in the supporting information: Supplementary Table 1.

2.3 Western Blot Analysis

In accordance with manufacturer's instructions, proteins were extracted from cells and concentrations were determined by the Modified Bradford Protein Assay Kit (C503041, Sangon Biotech). SDS-PAGE was used to separate the protein (30 μg) and transfer the membrane to the primary antibody after blocking it with 5% skimmed milk in TBST and incubated with the primary antibody overnight at 4 °C, followed by 1 h at 25 °C with secondary antibody conjugated to horseradish peroxidase. The ClarityTM Western ECL Substrate (1705060, Bio-Rad) was used to visualize antibody binding.

2.4 Plasmid Construction

The ORFs encoding the full-length and truncated forms of hnRNPR were PCR-amplified using PrimeStar Max DNA Polymerase (R040A, Takara). Then the PCR products were cloned into pcDNA3.1(-) and pLVX-IRES-neo, and the FLAG-coding sequence was inserted to the C-terminal of above hnRNPR proteins. In order to knock down hnRNPR permanently, shRNA-encoding oligos were inserted into pLKO.1-puro (8453, Addgene). The sequences for sh RNA of hnRNPR, hnRNPR truncation, and pcDNA3.1-hnRNPR, pLVX-hnRNPR are separately shown in supplementary Table 2, 3 and 4.

2.5 Lentivirus Production And Infection

HEK 293T cells were co-transfected with pCMV-VSVG, pCMV-dR8.91, and pLKO.1-hnRNPR-shRNA or pLVX-hnRNPR using TurboFect Transfection Reagent (R0531, Thermo Scientific) with 1:10:10 mass ratio. Viral media were collected 48 h later, and used to infect K562 cells in the presence of 8 μg/mL polybrene (H9268, Sigma-Aldrich).

2.6 Biotin-Labeled RNA Pull-Down And Mass Spectrometry Analysis

Biotin-labeled RNA, MY34UE-AS or MY34UE-S, was transcribed in vitro using the MEGAscript T7 Transcription Kit (AM1333, Invitrogen). Then, the proteins of incubation attached to the RNA were extracted and analyzed for composition using mass spectrometry at Novogene (Beijing, China).

2.7 RNA Immunoprecipitation(RIP)

Cells were lysed in immunoprecipitation lysis buffer according to previously reported instructions (Gagliardi and Matarazzo). Magnetic beads were coupled with either anti-FLAG antibody or control anti-lgG at room temperature for 1 h, followed by incubation with the cell extract for overnight at 4°C. The immunoprecipitated RNA was isolated and subjected to RTq-PCR to examine the expression of candidate genes.

2.8 Cell Proliferation Assay

Cell proliferation was assessed utilizing the Enhanced Cell Counting Kit-8 (C0042, Beyotime). Cells were plated at a density of 2,000 cells per well, with each well containing 100 μL of complete medium, in 96-well plates. The optical density at 450 nm was subsequently recorded to evaluate cell growth.

2.9 Cell Migration Assay

Cell migration ability was evaluated using a Transwell assay conducted in 24-well Transwell chambers with 8 μm pore-sized membranes (CLS3422, Corning, New York). Cells were seeded at a concentration of 5×105 cells/mL in 100 μL of serum-free medium in the upper chamber, while the lower chamber was filled with 600 μL of medium containing 10% FBS. Following incubation, the membranes were fixed with 3.7% paraformaldehyde and stained with Giemsa (E607314, Sangon Biotech). Cell migration was observed under a microscope, and photographs were taken of five randomly selected fields.

Journal of http://www.satursonpublishing.com/
Research in Multidisciplinary Methods and Applications
ISSN: 2957-3920

Volume 3, Issue 7, July 2024

2.10 STATISTICAL ANALYSES

Data were expressed as the means ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software) and Microsoft Excel. A Student's two-tailed t-test was used for comparisons between two groups. One-way ANOVA was conducted to compare multiple groups. All data were presented in triplicate. Significance levels were indicated as follows: (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$.

3 RESULTS

3.1 HNRNPR INTERACTS WITH MY34UE-AS

We have recently discovered MY34UE-AS, an enhancer lncRNA, transcribed from the antisense strand of the -34kb enhancer of the MYB locus (supplementary Figure 1), and this lncRNA can positively regulate MYB expression. Since accumulating evidence indicates that lncRNAs often function through RNA-protein interaction, we conducted RNA pull-down assay to capture potential interacting proteins of MY34UE-AS. The RNA pull-down assay is shown as the flow diagram (Figure 1A). Through mass spectrometry analysis, hnRNPR was identified as a protein interacting with MY34UE-AS (Figure 1B). To further verify the interaction between hnRNPR and MY34UE-AS, HEK 293T cells successfully expressed hnRNPR or TARDBP with a C-terminal FLAG tag when MY34UE-AS was present. RNA immunoprecipitation (RIP) assay (Figure 1C) showed that MY34UE-AS was significantly enriched by hnRNPR, meanwhile there was no association detected between MY34UE-AS and TARDBP (Figure 1D).

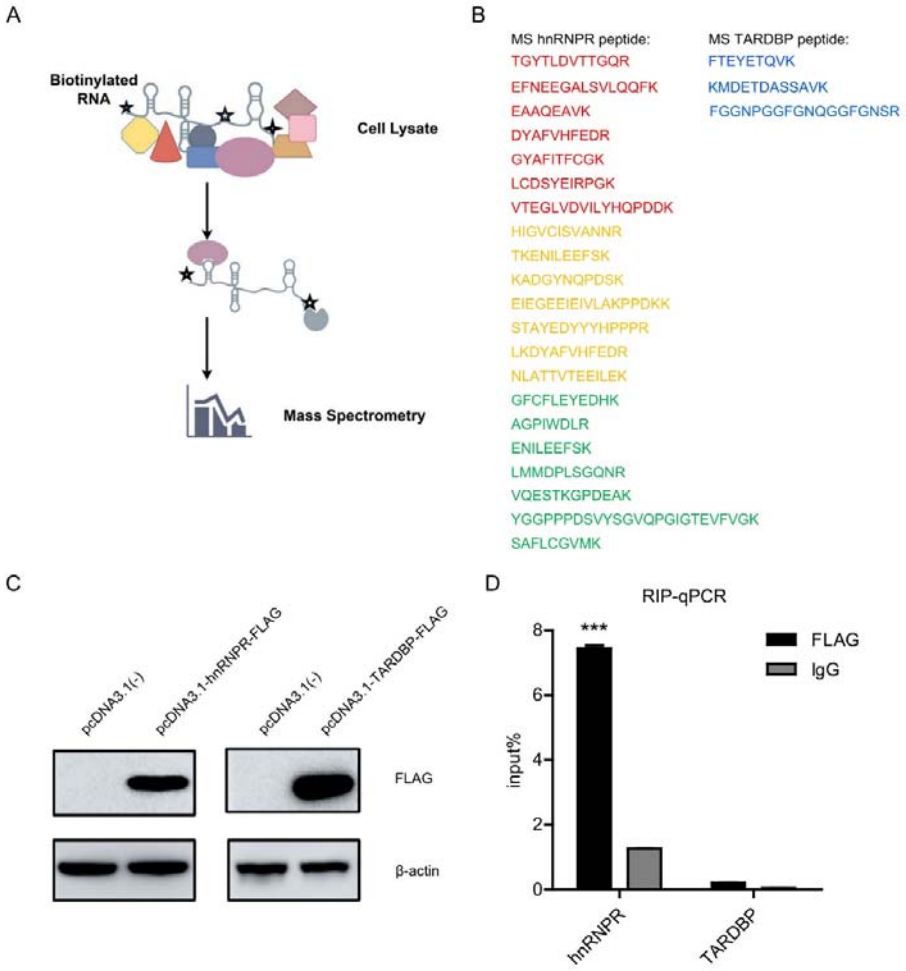

FIGURE 1. HNRNPR INTERACTS WITH MY34UE-AS. (A) SCHEMATIC DIAGRAM ILLUSTRATING THE RNA PULL-DOWN EXPERIMENT FOR IDENTIFICATION OF PROTEINS ASSOCIATED WITH MY34UE-AS BY FIGDRAW. (B) THE PEPTIDES OF HNRNPR AND TARDBP WERE REVEALED WITH MASS SPECTROMETRY (MS) ASSAY. (C, D) CO-TRANSFECTION OF HEK 293T CELLS WITH OVEREXPRESSION OF MY34UE-AS AND SPECIFIC FLAG-TAGGED PROTEINS, WESTERN BLOT WAS CONFIRMED THEIR EXPRESSION (C), AND RIP-QPCR PERFORMED THAT HNRNPR COULD SPECIFICALLY BIND TO MY34UE-AS (D).

Journal of http://www.satursonpublishing.com/
Research in Multidisciplinary Methods and Applications
ISSN: 2957-3920

Volume 3, Issue 7, July 2024

3.2 HNRNPR'S RRM2 DOMAIN INTERACTS WITH MY34UE-AS

To delve deeper into the interaction between MY34UE-AS and hnRNPR, we constructed four truncated forms of hnRNPR (165-636, 245-636, 332-636, 419-636 amino acid) based on its domains predicted by SMART web (as shown in Figure 2A). All these truncated forms of hnRNPR were effectively co-transfected with the MY34UE-AS in HEK 293T cells. Subsequently, RIP-qPCR analysis using anti-FLAG antibody revealed that the removal of RRM2 region significantly hindered the interaction with MY34UE-AS, suggesting that RRM2 plays a crucial role in the interaction with MY34UE-AS (Figure 2B). Western blot showed the expression of full-length and truncated forms of hnRNPR (Figure 2C). These findings collectively demonstrated that hnRNPR through its RRM2 domain can specifically binds to MY34UE-AS, forming an RNA/protein complex.

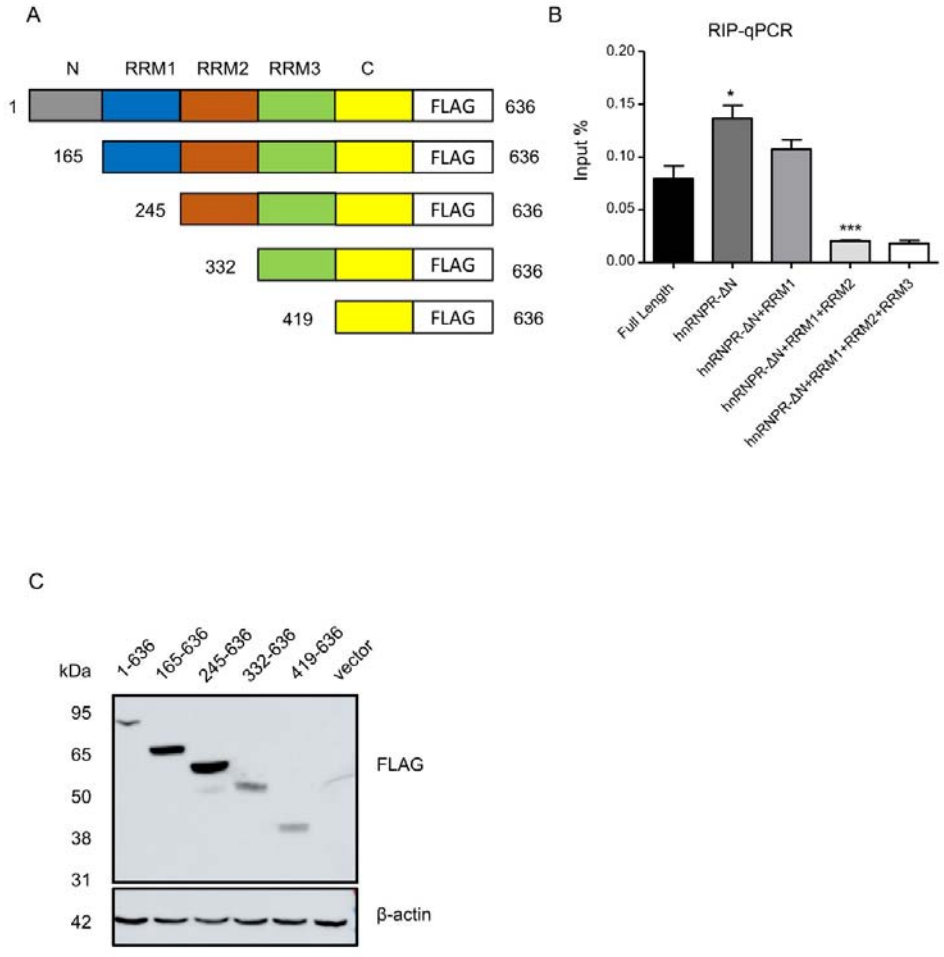

FIGURE 2. HNRNPR RRM2 DOMAIN INTERACTS WITH MY34UE-AS. (A) A SCHEMATIC DIAGRAM SHOWS HNRNPR AND ITS TRUNCATED MUTANTS. (B, C) IN VITRO BINDING ASSAY DEPICTING MY34UE-AS LEVELS WERE INVESTIGATED BY RIP (B) AFTER INCUBATION WITH FULL-LENGTH (1-636 AMINO ACIDS), △N (165-636 AMINO ACIDS), △N+△RRM1 (245-636 AMINO ACIDS), △N+△RRM1+△RRM2 (332-636 AMINO ACIDS), AND △N+△RRM1+△RRM2+△RRM3 (419-636 AMINO ACIDS) TRUNCATIONS. THE FULL-LENGTH AND TRUNCAT-IONS OF FLAG-TAGGED RECOMBINANT HNRNPR PROTEIN WERE VALIDATED BY WESTERN BLOT ASSAY (C) (*P < 0.05, ***P < 0.001).

3.3 HNRNPR ENHANCES MYB EXPRESSION THROUGH ITS INTERACTION WITH MY34UE-AS.

To probe hnRNPR's impact on MYB expression, we examined MYB levels by constructing pcDNA3.1(-)-hnRNPR expressing vector to transfect K562 cells. RT-qPCR analysis performed an increase in both hnRNPR (Figure 3A) and MYB (Figure 3B) mRNA levels. Conversely, using shRNA to knockdown hnRNPR resulted in a reduction in both hnRNPR (Figure 3C) and MYB (Figure 3D) levels. These findings affirmed the

Journal of http://www.satursonpublishing.com/
Research in Multidisciplinary Methods and Applications
ISSN: 2957-3920

Volume 3, Issue 7, July 2024

positive regulatory role of hnRNPR in MYB expression in human erythroleukemic K562 cells.

To elucidate the regulatory mechanisms involving hnRNPR and MY34UE-AS on MYB, knockdown of hnRNPR and overexpression of MY34UE-AS were co-transfected in K562 cells. The findings revealed that MYB was upregulated by overexpressing MY34UE-AS, while hnRNPR knockdown downregulated MYB. Interestingly, hnRNPR knockdown partially reversed the enhanced MYB expression induced by MY34UE-AS overexpression, suggesting collaborative upregulation of MYB expression by hnRNPR and MY34UE-AS (Figure 3E-F).

Collectively, these findings strongly indicated that the interaction between MY34UE-AS and hnRNPR activates MYB expression.

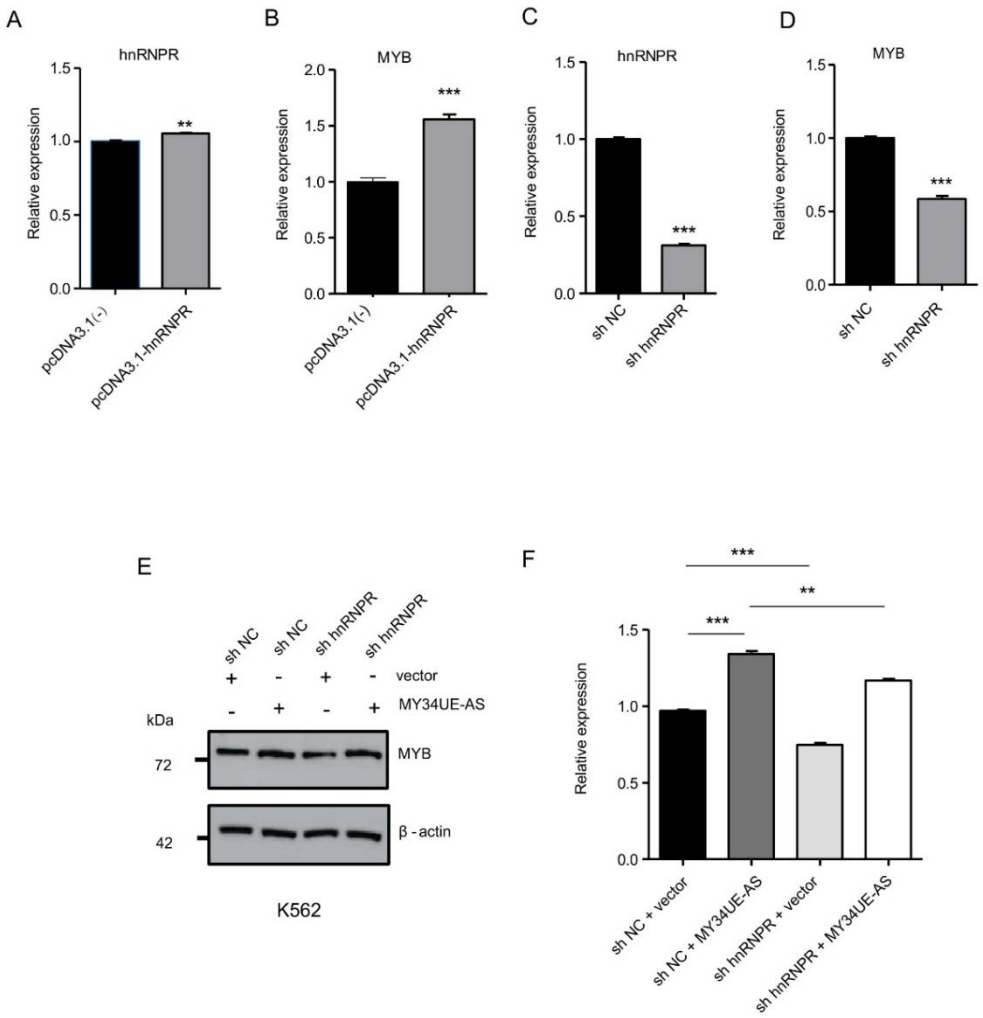

FIGURE 3. HNRNPR REGULATES MYB EXPRESSION THROUGH INTERACTION WITH MY34UE-AS. (A-D) RT- PCR ANALYSIS SHOWED THE EXPRESSION OF HNRNPR AND MYB IN K562 CELLS. (E, F) WESTERN BLOT ASSAY (E) AND RIP-QPCR ANALYSIS (F)WERE SHOWED THE LEVELS OF MYB IN K562 CELLS CO-TRANSFECTED WITH SH NC OR SH HNRNPR, AND PCDNA3.1(-) (VECTOR) OR PCDNA3.1(-)-MY34UE-AS (MY34UE-AS) (P < 0.01, ***P < 0.001, ****P < 0.0001).**

3.4 *HNRNPR IS ESSENTIAL FOR PROMOTING K562 CELL GROWTH*

To elucidate the potential fuction of hnRNPR in leukemia progression, its effect on K562 cell proliferation and migration was examined. Using lentivirus, hnRNPR was either knockdown or overexpressed in K562 cells which were subjected to CCK8 and Transwell assays (Figure 4A-D). The findings demonstrated that hnRNPR overexpression markedly increased K562 cell proliferation (Figure 4A) and migration

Journal of http://www.satursonpublishing.com/
Research in Multidisciplinary Methods and Applications
ISSN: 2957-3920

Volume 3, Issue 7, July 2024

(Figure 4C), while hnRNPR knockdown significantly inhibited cell proliferation (Figure 4B) and migration (Figure 4D).

Additionally, to investigate the association between hnRNPR and MY34UE-AS in K562 cell migration, there was an overexpression of MY34UE-AS in K562 cells, following the knockdown of hnRNPR. These findings indicated that overexpression of MY34UE-AS enhanced K562 cell migration, and knockdown of hnRNPR reduced cell migration. Moreover, hnRNPR shRNA partially reversed the increased migration induced by MY34UE-AS overexpression (Figure 4E).

Collectively, our findings suggested that hnRNPR facilitates K562 cell growth through its interaction with MY34UE-AS.

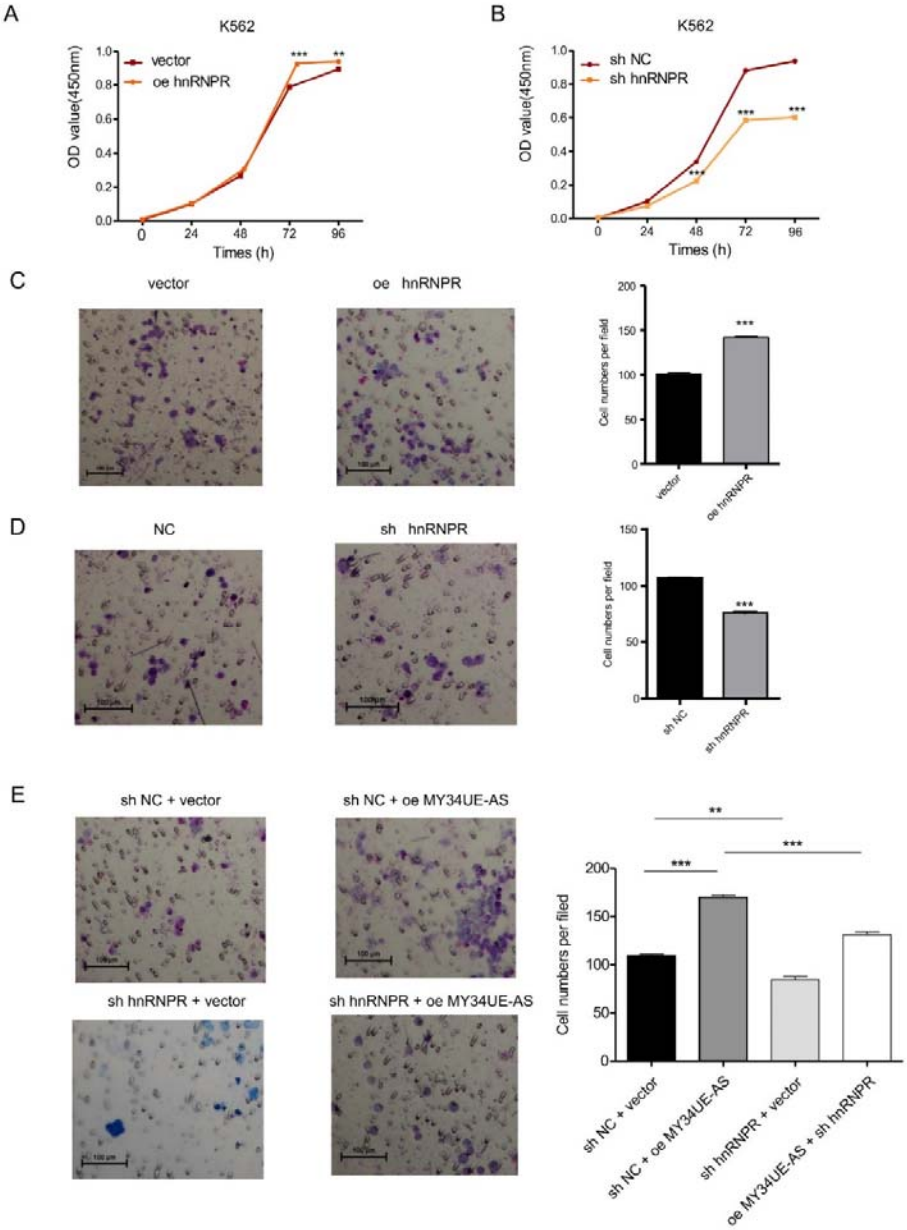

FIGURE 4. HNRNPR AND MY34UE-AS INTERACTION PROMOTES GROWTH OF K562 CELLS. (A-B) CCK-8 ASSAYS WAS USED TO DETERMINE THE PROLIFERATION ABILITY OF K562 CELLS BY KNOCKING DOWN OR OVEREXPRESSING HNRNPR. (C-D) TRANSWELL ASSAYS SHOWED THAT HNRNPR KNOCKDOWN OR OVEREXPRESSION COULD INHIBIT OR PROMOTE K562 CELLS MIGRATION. (E) REPREHENSIVE IMAGES (LEFT PANEL) OR QUANTIFICATION (RIGHT PANEL) OF TRANSWELL ASSAY INDICATING THE MIGRATION CAPABILITY OF K562 CELLS WAS CO-TRANSFECTED WITH SH NC OR SH HNRNPR AND PCDNA3.1(-) (VECTOR) OR PCDNA3.1(-)-MY34UE-AS (OE MY34UE-AS) (*P<0.05; **P<0.01; ***P<0.001).

Journal of http://www.satursonpublishing.com/
Research in Multidisciplinary Methods and Applications
ISSN: 2957-3920

Volume 3, Issue 7, July 2024

4 DISCUSSION

MYB plays a crucial role in hematopoiesis and is associated with critical cellular functions including proliferation, differentiation, and apoptosis (Fry and Inoue, 2019). Ectopic expression of MYB can block proliferation of myeloid cells in primary murine cells (Gonda et al.), and MYB has high levels of its expression in human myeloid leukemia (Westin Eh Fau - Gallo et al.). MYB dysregulation is a prevalent feature in leukemia, yet comprehensive understanding of its transcriptional regulation mechanism remains unclear. Increasing evidence suggests that distal enhancers regulate the MYB gene. The enhancers located at -84 kb and -71 kb regulate MYB expression. (Stadhouders R Fau - Aktuna et al.). Additionally, the -28kb enhancer of c-myb is involved in c-myb regulation during differentiation of myeloid progenitor M1 cells (Zhang et al.). Our previous study demonstrated enhancer lncRNA from 34kb upstream of MYB can promote MYB expression, cell growth in human erythroleukemic K562 cells. Our research indicated that MYB is influenced by several distant enhancers and their RNA enhancers. This suggested that these lncRNAs can be potential targets for diagnosing and treating leukemia.

Enhancer RNAs (eRNAs) are synthesized from enhancers located upstream or downstream of their target genes. They function to regulate their target genes, contributing to the progression of various diseases. Specifically, an eRNA transcribes from the -81kb murine Myb enhancer, which becomes the therapeutic target in β-globin hemoglobinopathies (Kim et al.). ARIEL eRNA originated from ARID5B can upregulate ARID5B expression to maintain T-ALL cell growth and survival (Tan et al.). Our study demonstrated that MY34UE-AS transcibed from the -34kb enhancer of MYB can enhance MYB expression in human erythroleukemic K562 cells, subsequently stimulating the growth of leukemia cells.

LncRNAs can interact with binding partners to perform crucial functions in gene regulation. For example, lncRNA CTHCC can bind to hnRNPK to promote hepatocellular carcinogenesis (Xia et al.), while lncRNA SNHG1 interacts with hnRNPL to promote the metastasis of prostate cancer (Tan et al., 2021). Furthermore, hnRNPR can interact with lncRNA LDLR-AS to enhance triglyceride accumulation in fish (Cao et al.). Our research revealed hnRNPR as a binding partner for MY34UE-AS, promoting MYB expression in human leukemia cells. These results indicated that members of the hnRNP family may play a significant role in the regulatory function of lncRNAs. Moreover, hnRNPR consists of three RNA recognition motif (RRM) domains and one RGG box (Han et al.). Notably, previous research has demonstrated that the RRM2 domain of the protein exhibits a high RNA-binding affinity and specific recognition (Maris et al.). Our study also found that the primary binding site for MY34UE-AS is the RRM2 domain of hnRNPR.

hnRNPs have been identified as potential biomarkers for cancer (Li et al.; Xu et al.). However, there are limited reports on hnRNPR in human tumors. The initial report on hnRNPR in tumors performed its role in promoting gastric cancer development (Chen et al., 2019). Recent studies have observed increased expression of hnRNPR in various cancers, linked to poor prognosis (Yang et al.). For instance, overexpression of hnRNPR has been connected with increased 18F - FDG absorption in ESCA and with anticipated poor survival (Liu et al.). Additionally, hnRNPR has been found to promote neuroblastoma progression by stabilizing ASCL1 (Hu et al.). However, the function and regulatory mechanisms of hnRNPR in leukemia remains unknown. Our findings, for the first time, showed that hnRNPR boosts the growth of human leukemia cells by increasing MYB expression.

In conclusion, our results suggest that hnRNPR enhances MYB expression by binding to MY34UE-AS via its RRM2 domain, promoting leukemia cell proliferation and migration in vitro, thereby driving tumorigenesis in leukemia.

Consequently, these results show that targeting the hnRNPR/MY34UE-AS axis may hold potential as a therapeutic approach for leukemia.

SUPPLEMENTARY TABLES AND FIGURES

Supplementary Figure 1

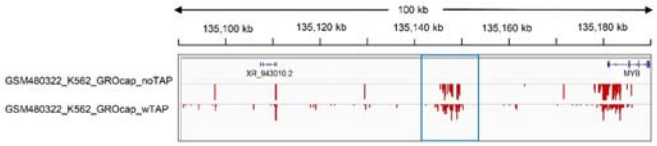

FIGURE S1. GRO-SEQ DATA SHOWS THE TRANSCRIPTION STATES OF THE -34KB REGION OF MYB IN K562 CELLS.

Journal of http://www.satursonpublishing.com/
Research in Multidisciplinary Methods and Applications
ISSN: 2957-3920

Volume 3, Issue 7, July 2024

Supplementary Table 1

Primer name	Primer sequence (5'-3')
hnRNPR-qPCR-F	CCGCTCTGCCCTGCATAATA
hnRNPR-qPCR-R	GGCCTGCCTCTATCAGTGTC
GAPDH-F	GGAAGGTGAAGGTCGGAGTCA
GAPDH-R	GTCATTGATGGCAACAATATCCACT
MYB_F	AAGGTCGAACAGGAAGGTTATC
MYB_R	ACTGTTCTTCTGGAAGCTTGT
-34k-lncRNA-qPCR-F	GTTCTGGGGAACAGGAGAGC
-34k-lncRNA-qPCR-R	ACTGCCAGTATAAGGAATCCAGT

Supplementary Table 2

Primer name	Primer sequence (5'-3')
sh-hnRNPR-F	CCGGGCACTGCGTATGAAGATTATTCTCGAGAATAATCT TCATACGCAGTGCTTTTTG
sh-hnRNPR-R	AATTCAAAAAGCACTGCGTATGAAGATTATTCTCGAGAAT AATCTTCATACGCAGTGC

Supplementary Table 3

Primer name	Primer sequence (5'-3')
hnRNPR-ΔN-F	CCGCTCGAGATGACGGAGGTATTTGTAGGC
hnRNPR-ΔN-R	GGGGTACCCTACTTGTCATCGTCGTCCTTG
hnRNPR-ΔN+RRM1-F	CCGCTCGAGATGAACAACAGACTTTTTGTTGGA
hnRNPR-ΔN+RRM1-R	GGGGTACCCTACTTGTCATCGTCGTCCTTG
hnRNPR-ΔN+RRM1+RRM2-F	CCGCTCGAGATGGTGGAAGAACCAGATCC
hnRNPR-ΔN+RRM1+RRM2-R	GGGGTACCCTACTTGTCATCGTCGTCCTTG
hnRNPR-ΔN+RRM1+RRM2+RRM3-F	CCGCTCGAGATGAGGAAAGAGCGCCAAG
hnRNPR-ΔN+RRM1+RRM2+RRM3-R	GGGGTACCCTACTTGTCATCGTCGTCCTTG

Supplementary Table 4

Primer name	Primer sequence (5'-3')
pcDNA3.1-hnRNPR-flag-F	CCGCTCGAGATGGCTAATCAGGTGAATGGTAA
pcDNA3.1-hnRNPR-flag-R	GGGGTACCCTACTTGTCATCGTCGTCCTTG
-34k-lncRNA-F	GGAATTCATGGGAGAGAAACTCAGAACATTTC
-34k-lncRNA-R	GACTAGTTTAAATTGTTTATTGTTCTCTGTTACATTTAAAA TG
pLVX-hnRNPR-flag-F	CCCTCGAGATGGCTAATCAGGTGAATGGTAA
pLVX-hnRNPR-flag-R	GCTCTAGA CTACTTGTCATCGTCGTCCTTG

TABLE S1-S4. VARIOUS PRIMER SEQUENCES WERE SHOWN IN SUPPLEMENTARY TABLE.

FUNDING

This study was funded by the National Natural Science Foundation of China (81770165 to B.H., and 31372516 to J.Z.). The work was also supported by the First-Class Discipline Program for Fishery from the Shanghai municipal government.

REFERENCES

[1]Pattabiraman, D.R. and Gonda, T.J. (2013). Role and potential for therapeutic targeting of MYB in leukemia. Leukemia. 2013, 27(2):269-77.

[2]Allen, R.D., 3rd, Bender Tp Fau - Siu, G. and Siu, G. c-Myb is essential for early T cell development. Genes Dev. 1999, 13(9):1073-8.

[3]Pearson, R. and Weston, K. c-Myb regulates the proliferation of immature thymocytes following beta-selection. EMBO J. 2000, 19(22):6112-20.

[4]Sumner, R., Crawford A Fau - Mucenski, M., Mucenski M Fau - Frampton, J. and Frampton, J. Initiation of adult myelopoiesis can occur in the absence of c-Myb whereas subsequent development is strictly dependent on the transcription factor. Oncogene. 2000, 19(30):3335-42.

[5]Vegiopoulos, A., García P Fau - Emambokus, N., Emambokus N Fau - Frampton, J. and Frampton, J. Coordination of erythropoiesis by the transcription factor c-Myb. Blood. 2006, 107(12):4703-10.

[6]Thomas, M.D., Kremer Cs Fau - Ravichandran, K.S., Ravichandran Ks Fau - Rajewsky, K., Rajewsky K Fau - Bender, T.P. and Bender, T.P. c-Myb is critical for B cell development and maintenance of follicular B cells. Immunity. 2005, 23(3):275-86.

[7]Zuber, J. et al. An integrated approach to dissecting oncogene addiction implicates a Myb-coordinated self-renewal program as essential for leukemia maintenance. Genes Dev. 2011, 25(15):1628-40.

[8]Drabsch, Y. et al. Mechanism of and requirement for estrogen-regulated MYB expression in estrogen-receptor-positive breast cancer cells. Proc Natl Acad Sci U S A. 2007, 104(34):13762-7.

[9]Hugo, H. et al. (2006). Mutations in the MYB intron I regulatory sequence increase transcription in colon cancers. Genes Chromosomes Cancer. 2006, 45(12):1143-54.

[10]Stadhouders R Fau - Aktuna, S. et al. HBS1L-MYB intergenic variants modulate fetal hemoglobin via long-range MYB enhancers. J Clin Invest. 2014, 124(4):1699-710.

[11]Su, G. et al. Enhancer architecture-dependent multilayered transcriptional regulation orchestrates RA signaling-induced early lineage differentiation of ESCs. Nucleic Acids Res. 2021, 49(20):11575-11595.

[12]Bulger, M. and Groudine, M. Functional and mechanistic diversity of distal transcription enhancers. Cell. 2011, 144(3):327-39.

[13]Wu, H., Nord, A.S., Akiyama, J.A., Shoukry, M., Afzal, V., Rubin, E.M., Pennacchio, L.A. and Visel, A. Tissue-specific RNA expression marks distant-acting developmental enhancers. PLoS Genet. 2014, 10(9):e1004610.

[14]Andersson, R. et al. An atlas of active enhancers across human cell types and tissues. Nature. 2014, 507(7493):455-461.

[15]Lam, M.T. et al. Rev-Erbs repress macrophage gene expression by inhibiting enhancer-directed transcription. Nature. 2013, 498(7455):511-5.

[16]Melo, C.A. et al. eRNAs are required for p53-dependent enhancer activity and gene transcription. Mol Cell. 2013, 49(3):524-35.

[17]Li, W. et al. Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. Nature. 2013, 498(7455):516-20.

[18]Mousavi, K., Zare H Fau - Dell'orso, S., Dell'orso S Fau - Grontved, L., Grontved L Fau - Gutierrez-Cruz, G., Gutierrez-Cruz G Fau - Derfoul, A., Derfoul A Fau - Hager, G.L., Hager Gl Fau - Sartorelli, V. and Sartorelli, V. eRNAs promote transcription by establishing chromatin accessibility at defined genomic loci. Mol Cell. 2013, 51(5):606-17.

[19]Fang, B. et al. Circadian enhancers coordinate multiple phases of rhythmic gene transcription in vivo. Cell. 2014, 159(5):1140-1152.

[20]Hsieh, C.L. et al. Enhancer RNAs participate in androgen receptor-driven looping that selectively enhances gene activation. Proc Natl Acad Sci U S A. 2014, 111(20):7319-24.

[21]Wan, L., Li, W., Meng, Y., Hou, Y., Chen, M. and Xu, B. Inflammatory Immune-Associated eRNA: Mechanisms, Functions and Therapeutic Prospects. Front Immunol. 2022, 13:849451.

[22]Fang, K. et al. Cis-acting lnc-eRNA SEELA directly binds histone H4 to promote histone recognition and leukemia progression. Genome Biol. 2020, 21(1):269.

[23]Kudinov, A.E., Karanicolas, J., Golemis, E.A. and Boumber, Y. Musashi RNA-Binding Proteins as Cancer Drivers and Novel Therapeutic Targets. Clin Cancer Res. 2017, 23(9):2143-2153.

[24]Chatterji, P. and Rustgi, A.K. RNA Binding Proteins in Intestinal Epithelial Biology and Colorectal Cancer. Trends Mol Med. 2018, 24(5):490-506.

[25]Klingenberg, M.A.-O. et al. The Long Noncoding RNA Cancer Susceptibility 9 and RNA Binding Protein Heterogeneous Nuclear Ribonucleoprotein L Form a Complex and Coregulate Genes Linked to AKT Signaling. Hepatology. 2018, 68(5):1817-1832.

[26]Li, M. et al. Downregulation of HNRNPK in human cancer cells inhibits lung metastasis. Animal Model Exp Med. 2019, 2(4):291-296.

[27]Padovani, K.S. et al. Crosstalk between hnRNP K and SET in ATRA-induced differentiation in acute promyelocytic leukemia. FEBS Open Bio. 2021, 11(7):2019-2032.

[28]Yang, N.A.-O., Liu, L.A.-O., Liu, X.A.-O., Chen, Y.A.-O., Lu, J.A.-O. and Wang, Z.A.-O. hnRNPC Promotes Malignancy in Pancreatic Cancer through Stabilization of IQGAP3. Biomed Res Int. 2022, 2022:6319685.

[29]Song, L. et al. microRNA-451-modulated hnRNP A1 takes a part in granulocytic differentiation regulation and acute myeloid leukemia. Oncotarget. 2017, 8(33):55453-55466.

[30]Yang, Y., Sun, J.D. and Xiang, Z.A.-O.X. Pan-cancer analysis of the oncogenic role of HNRNPR in human tumors. Cell Biol Int. 2023, 47(8):1406-1426.

[31]Chen, E.B. et al. (2019). HnRNPR-CCNB1/CENPF axis contributes to gastric cancer proliferation and metastasis. Aging (Albany NY) 2019, 11(18):7473-7491.

[32]Hu, T., Zeng, C., Song, Z., Liu, Q., Chen, S., Huang, W., Ma, Q. and Li, H. HNRNPA2B1 and HNRNPR stabilize ASCL1 in an m6A-dependent manner to promote neuroblastoma progression. Biochim Biophys Acta Mol Basis Dis. 2024, 1870(3):167050.

[33]Liu, X.Y., Gao, Y., Kui, X.Y., Liu, X.S., Zhang, Y.H., Zhang, Y., Ke, C.B. and Pei, Z.A.-O. High expression of HNRNPR in ESCA combined with (18)F-FDG PET/CT metabolic parameters are novel biomarkers for preoperative diagnosis of ESCA. J Transl Med. 2022, 20(1):450.

[34]Li, Y., Wang, H., Wan, J., Ma, Q., Qi, Y. and Gu, Z. The hnRNPK/A1/R/U Complex Regulates Gene Transcription and Translation and is a Favorable Prognostic Biomarker for Human Colorectal Adenocarcinoma. Front Oncol. 2022, 12:845931.

[35]Ji, C., Deng, C., Antor, K., Bischler, T., Schneider, C., Fischer, U., Sendtner, M. and Briese, M. (2022). hnRNP R negatively regulates transcription by modulating the association of P-TEFb with 7SK and BRD4. EMBO Rep. 2022, 23(9):e55432.

[36]Fukuda, A., Nakadai T Fau - Shimada, M., Shimada M Fau - Hisatake, K. and Hisatake, K. Heterogeneous nuclear ribonucleoprotein R enhances transcription from the naturally configured c-fos promoter in vitro. J Biol Chem. 2009, 284(35):23472-80.

[37]Duijkers, F.A. et al. (2019). HNRNPR Variants that Impair Homeobox Gene Expression Drive Developmental Disorders in Humans. Am J Hum Genet. 2019, 104(6), 1040-1059.

[38]Wang, H. et al. HnRNPR-mediated UPF3B mRNA splicing drives hepatocellular carcinoma metastasis. J Adv Res. 2024:S2090-1232(24)00072-9.

[39]Jiang, T., Qu, R., Liu, X., Hou, Y., Wang, L. and Hua, Y. (2023). HnRNPR strongly represses splicing of a critical exon associated with spinal muscular atrophy through binding to an exonic AU-rich element. J Med Genet. 2023, 60(11), 1105-1115.

[40]Gagliardi, M. and Matarazzo, M.R. RIP: RNA Immunoprecipitation. Methods Mol Biol. 2016;1480:73-86.

[41]Fry, E.A. and Inoue, K. (2019). c-MYB and DMTF1 in Cancer. Cancer Invest. 2019, 37(1):46-65.

[42]Gonda, T.J., Buckmaster C Fau - Ramsay, R.G. and Ramsay, R.G. Activation of c-myb by carboxy-terminal truncation: relationship to transformation of murine haemopoietic cells in vitro. EMBO J. 1989, 8(6):1777-83.

[43]Westin Eh Fau - Gallo, R.C., Gallo Rc Fau - Arya, S.K., Arya Sk Fau - Eva, A., Eva A Fau - Souza, L.M., Souza Lm Fau - Baluda, M.A., Baluda Ma Fau - Aaronson, S.A., Aaronson Sa Fau - Wong-Staal, F. and Wong-Staal, F. Differential expression of the amv gene in human hematopoietic cells. Proc Natl Acad Sci U S A. 1982, 79(7):2194-8.

[44]Zhang, J., Han, B., Li, X., Bies, J., Jiang, P., Koller, R.P. and Wolff, L. Distal regulation of c-myb expression during IL-6-induced differentiation in murine myeloid progenitor M1 cells. Cell Death Dis. 2016, 7(9):e2364.

[45]Kim, J., Diaz, L.F., Miller, M.J., Leadem, B., Krivega, I. and Dean, A. An enhancer RNA recruits MLL1 to regulate transcription of Myb. bioRxiv. 2023, 12:2023.09.26.559528.

[46]Tan, S.A.-O. et al. The enhancer RNA ARIEL activates the oncogenic transcriptional program in T-cell acute lymphoblastic leukemia. Blood. 2019, 134(3):239-251.

[47]Xia, A. et al. The cancer-testis lncRNA lnc-CTHCC promotes hepatocellular carcinogenesis by binding hnRNP K and activating YAP1 transcription. Nat Cancer. 2022, 3(2):203-218.

[48]Tan, X. et al. (2021). LncRNA SNHG1 and RNA binding protein hnRNPL form a complex and coregulate CDH1 to boost the growth and metastasis of prostate cancer. Cell Death Dis. 2021, 12(2):138.

[49]Cao, X., Fang, W., Li, X., Wang, X., Mai, K. and Ai, Q. Increased LDL receptor by SREBP2 or SREBP2-induced lncRNA LDLR-AS promotes triglyceride accumulation in fish. iScience. 2022, 25(7):104670.

[50]Han, S.P., Tang Yh Fau - Smith, R. and Smith, R. Functional diversity of the hnRNPs: past, present and perspectives. Biochem J. 2010, 430(3):379-92.

[51]Maris, C., Dominguez C Fau - Allain, F.H.T. and Allain, F.H. The RNA recognition motif, a plastic RNA-binding platform to regulate post-transcriptional gene expression. FEBS J. 2005, 272(9):2118-31.

[52]Li, Y. et al. HNRNPL Circularizes ARHGAP35 to Produce an Oncogenic Protein. Adv Sci (Weinh). 2021, 8(13):2001701.

[53]Xu, H., Li, P., Wang, X., Zhuang, H. and Hua, Z.C. Emerging roles of hnRNP A2B1 in cancer and inflammation. Int J Biol Macromol. 2022, 221:1077-1092.