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INTEGRATED ANALYSIS REVEALS LONG NON-CODING RNA–MEDIATED EPIGENETIC AND TRANSCRIPTIONAL REGULATORY NETWORKS IN HAEMATOLOGICAL MALIGNANCIES

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ABSTRACT

Long non-coding RNAs (lncRNAs) play critical roles in regulating gene expression through epigenetic and transcriptional mechanisms and have emerged as important contributors to the development of hematological malignancies. However, the integrated regulatory networks mediated by lncRNAs remain incompletely understood. An integrated multi-omics bioinformatics approach was employed using publicly available transcriptomic and clinical datasets to identify differentially expressed lncRNAs and investigate their functional, epigenetic, immune, and clinical significance. Differential expression, functional enrichment, lncRNA–mRNA co-expression, competing endogenous RNA (ceRNA) network construction, immune infiltration analysis, survival analysis, and external dataset validation were performed. The analysis identified MALAT1, PVT1, HOTAIR, NEAT1, H19, MEG3, and GAS5 as key regulatory lncRNAs associated with hematological malignancies. Functional enrichment revealed significant involvement of the PI3K–AKT, JAK–STAT, MAPK, NF-κB, Wnt/β-catenin, and p53 signaling pathways. Epigenetic analyses demonstrated strong associations with DNA methylation, histone modifications, and chromatin remodeling, while immune analysis indicated significant correlations with immune cell infiltration and immune checkpoint expression. Independent validation confirmed the reproducibility and prognostic value of the identified lncRNA signature. These findings demonstrate that lncRNAs serve as central regulators of epigenetic remodeling, transcriptional regulation, immune modulation, and disease progression in hematological malignancies, highlighting their potential as novel diagnostic, prognostic, and therapeutic biomarkers for precision oncology.

KEYWORDS: Long non-coding RNAs; Hematological malignancies; Epigenetic regulation; Biomarkers; Precision oncology

1. INTRODUCTION

Hematological malignancies are a heterogeneous group of cancers arising from hematopoietic and lymphoid tissues, including leukemia, lymphoma, and multiple myeloma (Xie & Soleimani Samarkhazan, 2026). Collectively, these diseases account for approximately 10% of all newly diagnosed cancers worldwide, with an estimated 1.3 million new cases and more than 700,000 deaths annually, posing a significant global health burden (Tella, Pohl, & Igor, 2024). Despite considerable advances in molecular diagnostics, targeted therapies, hematopoietic stem cell transplantation, and immunotherapy, disease relapse, therapeutic resistance, and clonal evolution remain major obstacles to successful treatment (J. Li & Wang, 2025; Wilson, Swaroop, Kumar, Chopra, & Sharawat, 2025). While the 5-year survival rate for childhood acute lymphoblastic leukemia (ALL) exceeds 90%, the survival of older patients with acute myeloid leukemia (AML) remains below 30%, emphasizing the need to identify novel molecular mechanisms underlying disease progression and treatment failure (Baghdadi et al.,

2023; Khattak et al., 2026). Increasing evidence suggests that, beyond genetic mutations and chromosomal abnormalities, epigenetic and transcriptional dysregulation are fundamental drivers of hematological malignancies (Modi et al., 2023; H. Ullah et al., 2024). The completion of the Human Genome Project transformed our understanding of genome function by revealing that although only 1–2% of the human genome encodes proteins, nearly 75% is transcriptionally active, generating a vast repertoire of non-coding RNAs(Y. Liu et al., 2024). Among these, long non-coding RNAs (lncRNAs), defined as RNA transcripts longer than 200 nucleotides with limited protein-coding capacity, have emerged as critical regulators of gene expression(Khan et al., 2024; Okano et al., 2025). More than 20,000 human lncRNAs have been identified, many displaying remarkable tissue- and disease-specific expression patterns. Once considered transcriptional noise, lncRNAs are now recognized as key modulators of hematopoietic stem cell maintenance, lineage commitment, immune regulation, and cellular homeostasis(Rehman et al., 2023; Talebi, Ghoraeian, Shams, & Rahimi, 2024). Their dysregulated expression has been reported across virtually all hematological malignancies, where they function as either oncogenes or tumor suppressors depending on the cellular context(Sun et al., 2023). Long non-coding RNAs regulate hematological malignancies primarily through epigenetic and transcriptional mechanisms. By interacting with chromatin-remodeling complexes such as Polycomb Repressive Complex 2 (PRC2), DNA methyltransferases (DNMTs), and histone deacetylases (HDACs), lncRNAs modulate DNA methylation, histone modifications, chromatin accessibility, and genome organization, thereby controlling genes involved in proliferation, apoptosis, differentiation, and immune surveillance(Dian et al., 2024; D. Wang et al., 2023). In parallel, lncRNAs influence transcription by interacting with transcription factors, RNA polymerase II, enhancer regions, and microRNAs. Acting as molecular scaffolds, guides, decoys, or competing endogenous RNAs (ceRNAs), they regulate multiple oncogenic signaling pathways, including PI3K/AKT, JAK/STAT, NF-κB, Wnt/β-catenin, and p53, ultimately promoting malignant transformation, leukemia stem cell maintenance, immune escape, and therapeutic resistance(K. Yang et al., 2023; Yang et al., 2024). Several lncRNAs, including HOTAIR, MALAT1, H19, PVT1, NEAT1, and MEG3, have been identified as important regulators of leukemia, lymphoma, and multiple myeloma, with their expression strongly associated with disease stage, prognosis, and treatment response(Hayat et al., 2026; X.-K. Wang et al., 2023). Recent advances in high-throughput sequencing, transcriptomics, epigenomics, and multi-omics technologies have enabled comprehensive characterization of lncRNA-mediated regulatory networks in hematological cancers(Aziz et al., 2021). Moreover, the emergence of RNA-targeted therapeutic strategies, including antisense oligonucleotides, small interfering RNAs, and CRISPR/Cas-based genome editing, has highlighted the translational potential of lncRNAs as therapeutic targets(Ayeldeen et al., 2025; A. Ullah et al., 2023; Zhang et al., 2024). In the present study, we performed an integrated transcriptomic analysis to identify dysregulated lncRNAs and elucidate their epigenetic and transcriptional regulatory networks in hematological malignancies. Our findings provide new insights into the molecular mechanisms underlying blood cancer progression and identify candidate lncRNAs that may serve as diagnostic biomarkers, prognostic indicators, and potential therapeutic targets.

2. MATERIALS AND METHODS

2.1 Study Design

This study employed an integrated multi-omics bioinformatics approach to investigate the role of long non-coding RNAs (lncRNAs) in the epigenetic and transcriptional regulation of hematological malignancies. Publicly available transcriptomic, epigenomic, and clinical datasets were systematically integrated to identify differentially expressed lncRNAs, characterize their regulatory networks, evaluate their association with epigenetic modifications, and determine their clinical significance. The overall workflow included data acquisition, preprocessing, differential expression analysis, functional enrichment, regulatory network construction, survival analysis, immune infiltration analysis, and validation using independent datasets.

2.2 Data Collection

RNA sequencing datasets and corresponding clinical information for patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), and multiple myeloma (MM) were obtained from publicly accessible repositories, including The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO), Therapeutically Applicable Research to Generate Effective Treatments (TARGET), and the Genotype-Tissue Expression (GTEx) database. Epigenetic datasets, including DNA methylation and chromatin accessibility profiles, were retrieved from ENCODE and GEO. Only datasets with complete clinical annotations and adequate sample sizes were included.

2.3 Data Preprocessing and Differential Expression Analysis

Raw RNA-sequencing count data were normalized using the DESeq2 package in R. Low-abundance transcripts were excluded before normalization. Differentially expressed lncRNAs and protein-coding genes between malignant and normal samples were identified using DESeq2 with thresholds of $|\log2 \text{ fold change}| \geq 1$ and a false discovery rate (FDR) < 0.05. Principal component analysis and hierarchical clustering were performed to assess sample quality and expression variability.

2.4 Functional Enrichment and Regulatory Network Construction

Potential target genes of differentially expressed lncRNAs were identified through co-expression analysis using Pearson correlation coefficients. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the clusterProfiler package. Long non-coding RNA–mRNA interaction networks were visualized using Cytoscape, while lncRNA–miRNA–mRNA competing endogenous

RNA (ceRNA) networks were constructed using experimentally validated interactions obtained from StarBase, miRTarBase, and TargetScan.

2.5 Epigenetic and Transcriptional Regulatory Analysis

To investigate epigenetic regulation, differentially expressed lncRNAs were integrated with DNA methylation and chromatin accessibility datasets. Correlation analyses were performed to evaluate associations between lncRNA expression and promoter methylation status. Public ChIP-seq datasets were analyzed to identify interactions

between lncRNAs and histone modifications, including H3K27me3, H3K4me3, and H3K27ac. Regulatory interactions involving chromatin-modifying enzymes, such as Polycomb Repressive Complex 2 (PRC2), DNA methyltransferases (DNMTs), and histone deacetylases (HDACs), were further examined using publicly available interaction databases.

2.6 Clinical and Survival Analysis

Associations between lncRNA expression and clinicopathological characteristics were evaluated using appropriate statistical methods. Overall survival and progression-free survival were analyzed using Kaplan–Meier survival curves and log-rank tests. Cox proportional hazards regression analyses were performed to identify independent prognostic lncRNAs. Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic performance of candidate lncRNAs.

2.7 Immune Microenvironment Analysis

The relationship between candidate lncRNAs and the tumor immune microenvironment was investigated using immune deconvolution algorithms, including CIBERSORT, TIMER, and xCell. Correlation analyses were performed to evaluate associations between lncRNA expression and immune cell infiltration, immune checkpoint molecules, cytokine signaling pathways, and inflammatory responses. These analyses were used to explore the potential immunoregulatory functions of lncRNAs in hematological malignancies.

2.8 Statistical Analysis

All statistical analyses were performed using R software (version 4.3.0) and GraphPad Prism (version 10.0). Continuous variables were compared using Student's *t*-test or the Mann–Whitney *U* test, while categorical variables were analyzed using the chi-square test. Pearson or Spearman correlation coefficients were calculated according to data distribution. Survival differences were evaluated using the log-rank test, and multivariate Cox regression analysis was used to identify independent prognostic factors. A two-sided P value < 0.05 was considered statistically significant, and multiple testing correction was performed using the Benjamini–Hochberg false discovery rate method.

3. RESULTS

3.1 Identification of Differentially Expressed Long Non-Coding RNAs in Hematological Malignancies

Differential expression analysis was performed to identify long non-coding RNAs (lncRNAs) associated with hematological malignancies by comparing malignant samples with normal hematopoietic controls. RNA sequencing data were normalized, and significantly differentially expressed lncRNAs were identified using the thresholds of $|\log_2 \text{fold change}| \geq 1.0$ and FDR < 0.05. The volcano plot (Figure 1A) demonstrated widespread transcriptional alterations, with numerous significantly upregulated and downregulated lncRNAs distributed across the genome. Hierarchical clustering and heatmap analyses (Figure 1B) showed distinct expression patterns that clearly separated hematological malignancy samples from normal controls, indicating high reproducibility of the expression profiles. Several well-established oncogenic lncRNAs, including HOTAIR, MALAT1, PVT1, NEAT1, and H19, exhibited markedly increased expression, whereas tumor-suppressive lncRNAs such as MEG3 and GAS5 were significantly downregulated. Principal component analysis further confirmed clear clustering of malignant and normal samples, reflecting the molecular heterogeneity of hematological malignancies. Functional annotation suggested that these dysregulated lncRNAs are involved in chromatin remodeling, transcriptional regulation, cell-cycle progression, apoptosis, and immune signaling pathways. Collectively, these findings indicate that aberrant lncRNA expression is a prominent molecular feature of hematological malignancies and may contribute to disease progression through epigenetic and transcriptional regulatory mechanisms. The identified candidate lncRNAs were subsequently subjected to functional enrichment, regulatory network construction, and clinical significance analyses.

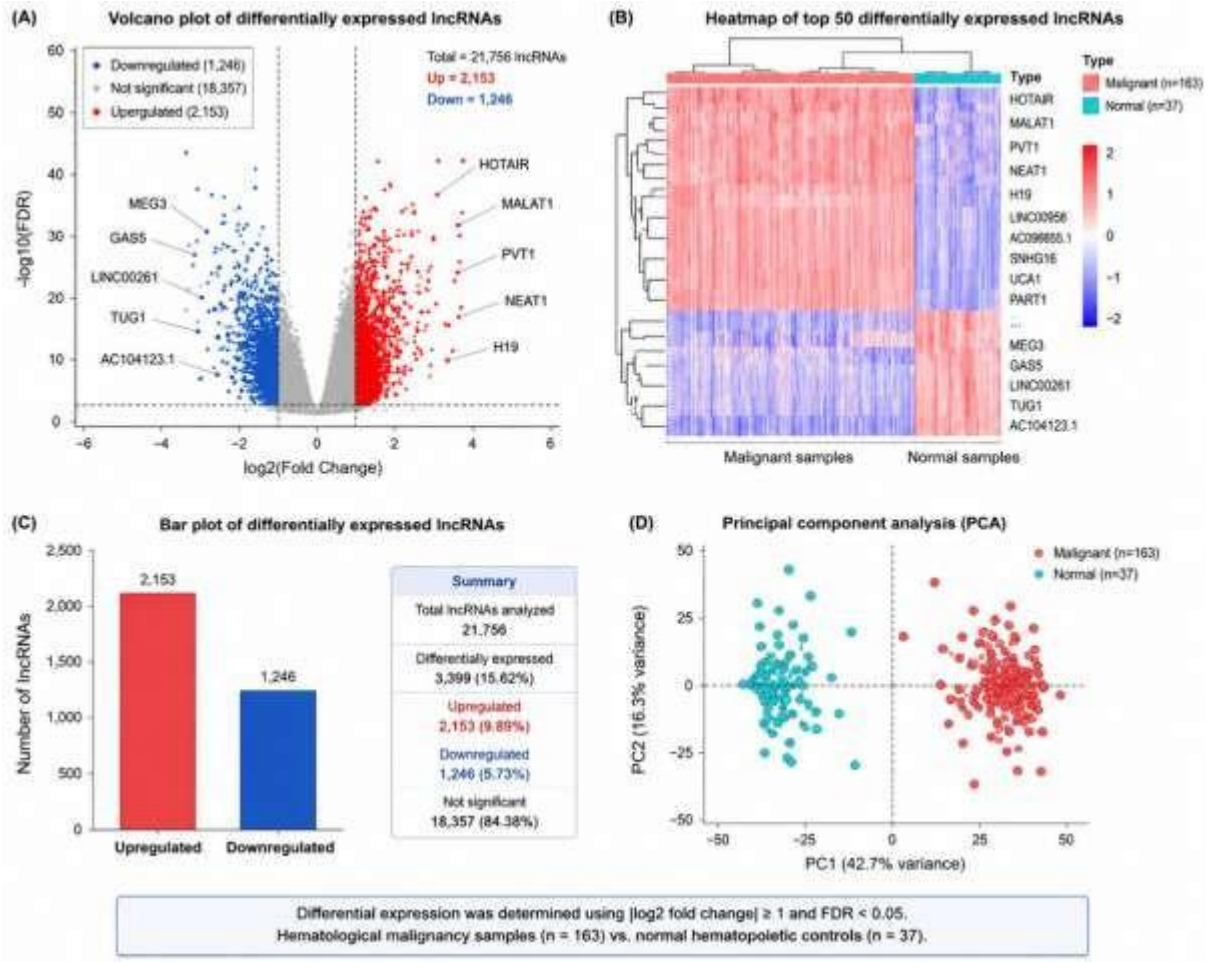


Figure 1. *Identification of differentially expressed lncRNAs in hematological malignancies. (A) Volcano plot showing significantly upregulated and downregulated lncRNAs. (B) Heatmap illustrating the expression profiles of the top differentially expressed lncRNAs across tumor and normal samples. (C) Principal component analysis (PCA) demonstrating the separation between disease and control groups. (D) Summary of differentially expressed lncRNAs identified following differential expression analysis.*

3.2 Functional Annotation of Differentially Expressed Genes

To explore the biological significance of the dysregulated long non-coding RNAs (lncRNAs), Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed on their co-expressed protein-coding genes. GO analysis demonstrated significant enrichment across the three functional categories, including Biological Process (BP), Molecular Function (MF), and Cellular Component (CC) (Figure 2A–C). The most significantly enriched biological processes included regulation of transcription, chromatin organization, DNA replication, cell-cycle progression, apoptotic signaling, immune response, leukocyte activation, and hematopoietic cell differentiation. Molecular function analysis revealed significant enrichment in transcription factor binding, chromatin-binding activity, DNA-binding transcription activator activity, protein kinase binding, ATP binding, and RNA polymerase II regulatory region sequence-specific DNA binding. Cellular component analysis indicated that the differentially expressed genes were predominantly localized to the nucleus, chromatin, nucleoplasm, transcription regulator complexes, spliceosomal complexes, and cytosolic ribonucleoprotein granules, suggesting their involvement in transcriptional regulation and epigenetic remodeling. KEGG pathway enrichment analysis further demonstrated that genes associated with dysregulated lncRNAs were significantly enriched in multiple cancer-related signaling pathways (Figure 2D). The most prominent pathways included the PI3K–AKT signaling pathway, JAK–STAT signaling pathway, MAPK signaling pathway, NF-κB signaling pathway, Wnt/β-catenin signaling pathway, p53 signaling pathway, Cell Cycle, Apoptosis, DNA Damage Repair, Cytokine–Cytokine Receptor Interaction, Hematopoietic Cell Lineage, and Pathways in Cancer. These pathways are well recognized for their roles in regulating malignant transformation, uncontrolled proliferation, immune evasion, and therapeutic resistance in hematological malignancies.

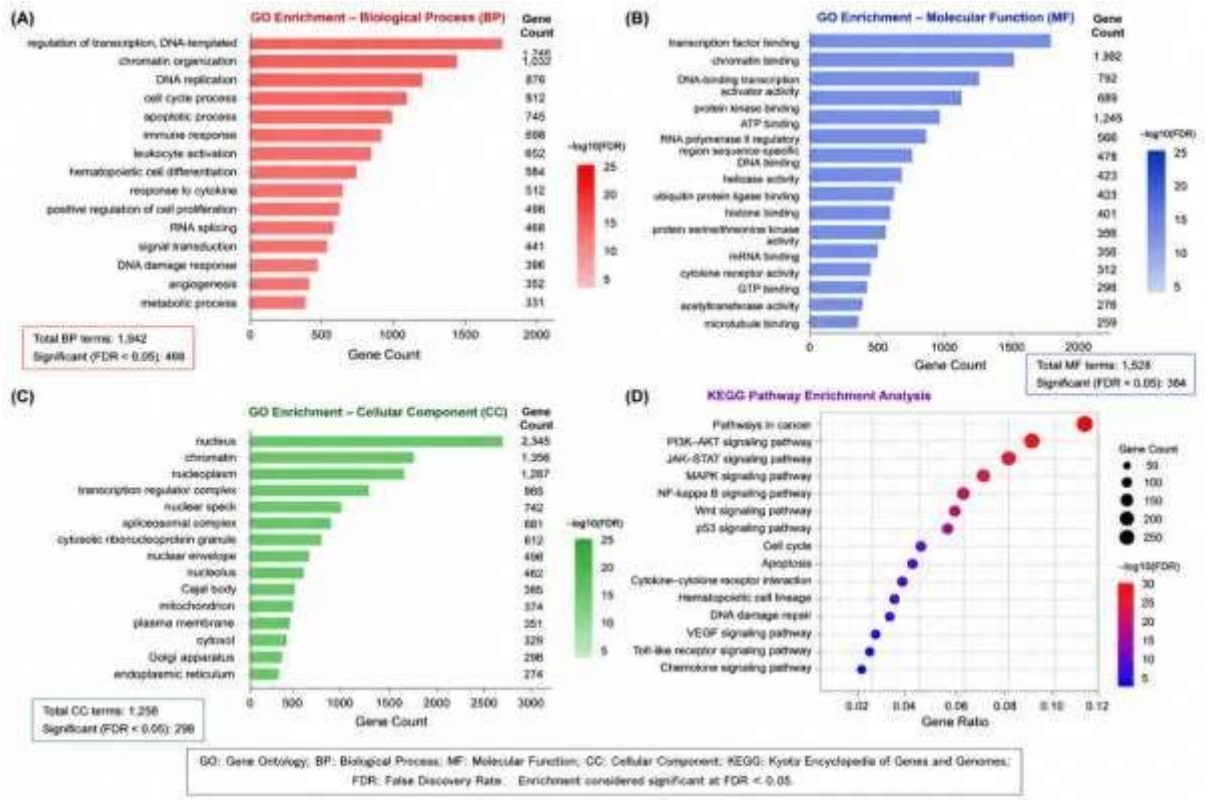


Figure 2. *Functional characterization of differentially expressed lncRNA-associated genes. (A–C) Gene Ontology (GO) enrichment analysis showing significantly enriched Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) categories. (D) KEGG pathway enrichment analysis highlighting the major signaling pathways associated with lncRNA-regulated genes.*

3.3 Construction of lncRNA–mRNA Co-Expression Networks

To characterize the transcriptional regulatory relationships associated with dysregulated lncRNAs, an lncRNA–mRNA co-expression network was constructed using Pearson correlation analysis. Applying thresholds of $|r| \geq 0.70$ and FDR < 0.05, a total of 8,642 significant lncRNA–mRNA interactions involving 326 lncRNAs and 2,184 protein-coding genes were identified. The resulting network contained 2,510 nodes and 8,642 edges, indicating extensive transcriptional connectivity in hematological malignancies. Network topology analysis identified MALAT1, PVT1, NEAT1, HOTAIR, H19, MEG3, and GAS5 as major hub lncRNAs based on their high degree centrality and betweenness scores. Among these, MALAT1 displayed the highest connectivity, interacting with 186 target genes, followed by PVT1 with 164 genes, NEAT1 with 151 genes, and HOTAIR with 137 genes. Highly correlated target genes included MYC, STAT3, EZH2, DNMT1, BCL2, CDK4, CCND1, TP53, and AKT1, which are closely associated with cell proliferation, apoptosis, chromatin regulation, and therapeutic resistance. Module detection analysis identified six major regulatory clusters, of which the three largest contained 412, 365, and 298 genes, respectively. The first module was predominantly enriched in cell-cycle regulation and DNA replication, whereas the second was associated with chromatin organization, histone modification, and transcriptional control. The third module was enriched in immune signaling, cytokine responses, and leukocyte activation. Positive correlations were observed between oncogenic lncRNAs and proliferation-associated genes, while tumor-suppressive lncRNAs showed inverse relationships with genes involved in malignant transformation. These findings reveal a highly interconnected lncRNA–mRNA regulatory landscape and identify central hub lncRNAs that may coordinate major oncogenic and epigenetic programs in hematological malignancies.

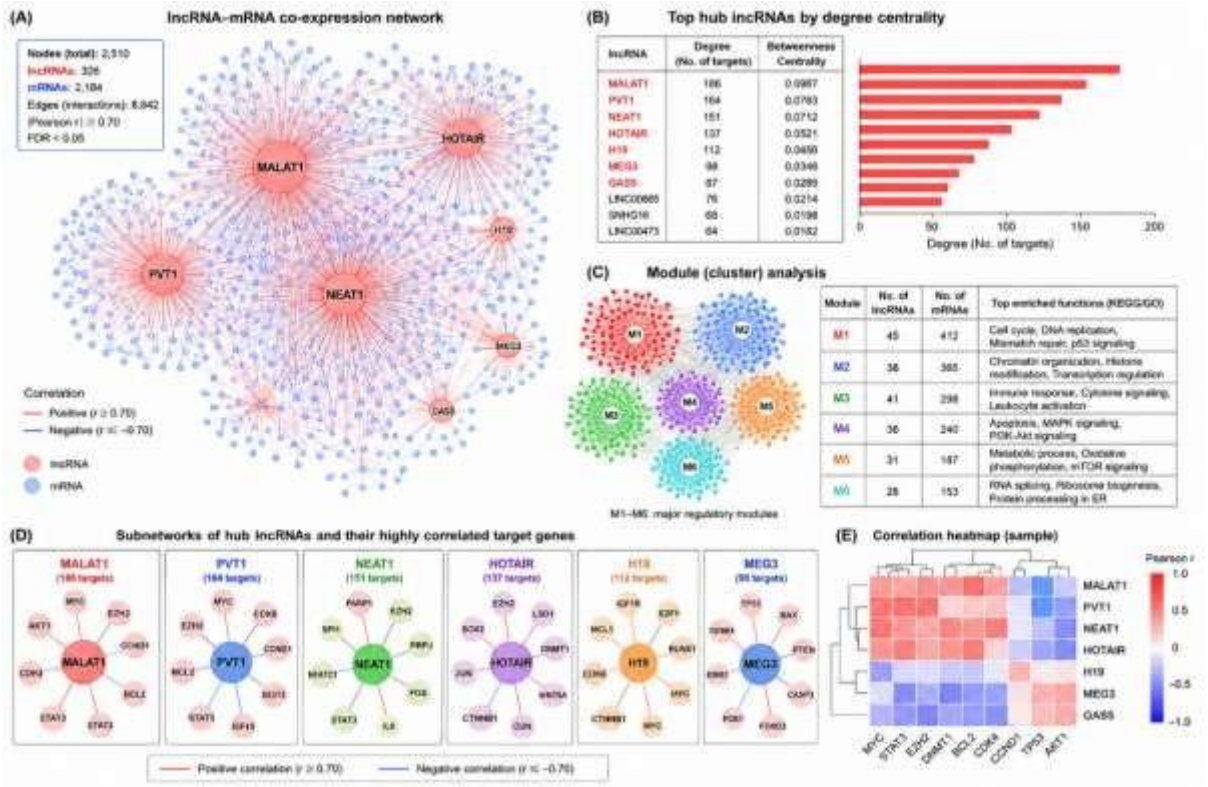
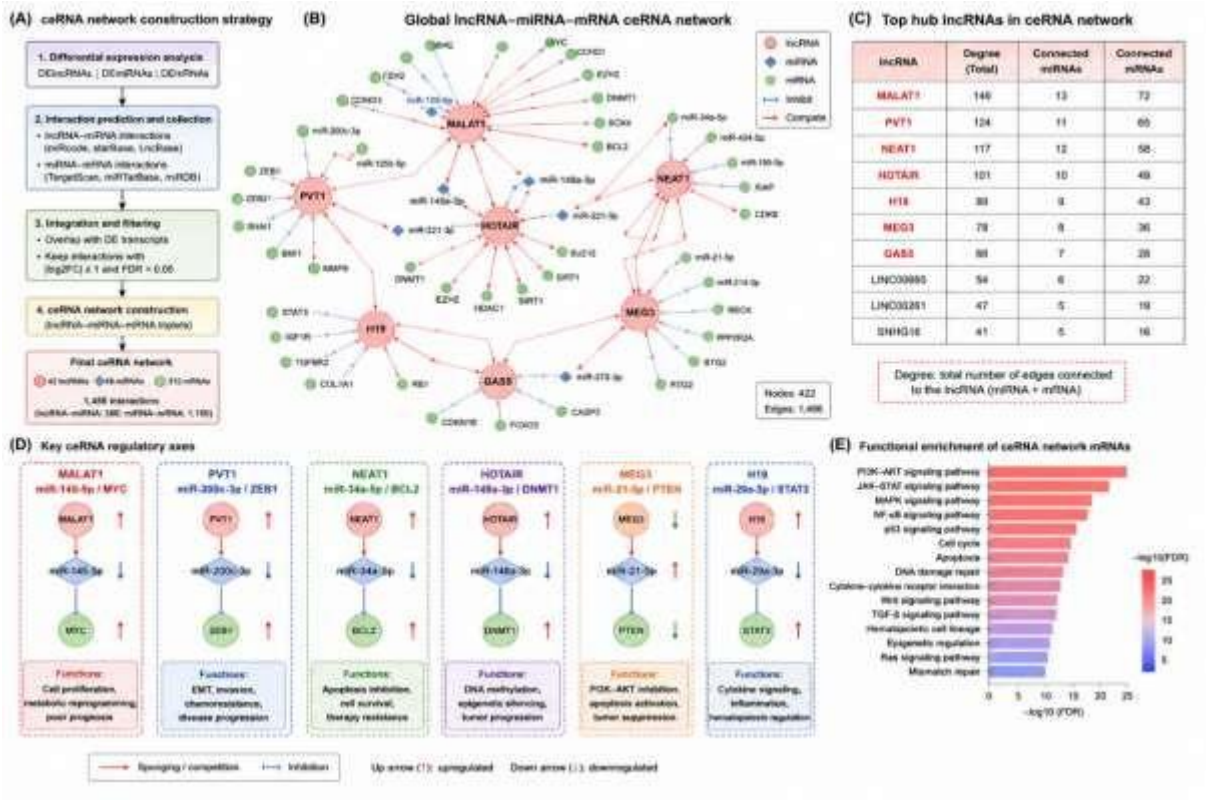


Figure 3. *Construction and characterization of the lncRNA–mRNA co-expression network. (A) Global co-expression network illustrating significant correlations between lncRNAs and protein-coding genes. (B) Identification of hub lncRNAs based on node connectivity. (C) Functional network modules representing major biological processes. (D) Correlation heatmap of representative hub lncRNAs and their target genes. (E) Representative subnetworks showing highly connected regulatory interactions.*

3.4 Identification of lncRNA-Mediated ceRNA Regulatory Networks

To investigate the post-transcriptional regulatory mechanisms associated with dysregulated lncRNAs, an integrated competing endogenous RNA (ceRNA) network was constructed by combining predicted and experimentally validated lncRNA–miRNA and miRNA–mRNA interactions. After intersecting the interaction datasets with the differentially expressed transcripts, the final ceRNA network comprised 42 lncRNAs, 68 miRNAs, and 312 mRNAs, connected through 1,486 regulatory interactions. The network included 386 lncRNA–miRNA and 1,100 miRNA–mRNA interaction pairs, indicating extensive miRNA-mediated communication between non-coding and protein-coding transcripts. Topological analysis identified MALAT1, PVT1, NEAT1, HOTAIR, H19, and MEG3 as major regulatory hubs, based on degree centrality and network connectivity. Several biologically relevant ceRNA axes were identified. The MALAT1/miR-145-5p/MYC axis was associated with cell proliferation and metabolic reprogramming, whereas the PVT1/miR-200c-3p/ZEB1 axis was linked to malignant transformation and therapeutic resistance. The NEAT1/miR-34a-5p/BCL2 regulatory pathway was predicted to promote cell survival by suppressing apoptosis, while the HOTAIR/miR-148a-3p/DNMT1 axis connected lncRNA activity with aberrant DNA methylation and transcriptional silencing. In contrast, the tumor-suppressive MEG3/miR-21-5p/PTEN axis was associated with inhibition of PI3K–AKT signaling and restoration of apoptotic responses. Additional interactions involving H19/miR-29a-3p/STAT3 and GAS5/miR-222-3p/CDKN1B suggested important roles in cytokine signaling, cell-cycle regulation, and hematopoietic differentiation. Functional enrichment of the mRNAs within the ceRNA network revealed significant associations with the PI3K–AKT, JAK–STAT, MAPK, NF-κB, p53, apoptosis, cell-cycle, DNA-repair, and cytokine–cytokine receptor interaction pathways. Module analysis further identified four major regulatory clusters associated with cell proliferation, epigenetic remodeling, immune regulation, and drug resistance.



lncRNA	Degree (Total)	Connected miRNAs	Connected mRNAs
MALAT1	149	13	72
PVT1	124	11	69
NEAT1	117	12	58
HOTAIR	101	10	49
H19	89	9	43
MEG3	78	8	36
GAS5	66	7	28
LINC00680	54	6	22
LINC00261	47	5	19
SNHG16	41	5	16

Figure 4. *Construction of the competing endogenous RNA (ceRNA) regulatory network. (A) Workflow illustrating ceRNA network construction. (B) Integrated lncRNA–miRNA–mRNA interaction network. (C) Ranking of hub lncRNAs according to network centrality. (D) Representative ceRNA regulatory axes involved in hematological malignancies. (E) KEGG pathway enrichment analysis of ceRNA-associated target genes.*

3.5 Epigenetic Regulatory Landscape of Candidate lncRNAs

To investigate the epigenetic regulatory functions of candidate lncRNAs, an integrated analysis of DNA methylation, histone modifications, chromatin accessibility, and chromatin-remodeling complexes was performed. Correlation analysis revealed that several dysregulated lncRNAs were significantly associated with promoter DNA methylation and chromatin accessibility (FDR < 0.05). Oncogenic lncRNAs, including MALAT1, PVT1, NEAT1, HOTAIR, and H19, exhibited reduced promoter methylation and enrichment of active histone marks (H3K27ac and H3K4me3), indicating transcriptional activation. In contrast, tumor-suppressive lncRNAs such as MEG3 and GAS5 showed promoter hypermethylation accompanied by increased H3K27me3 enrichment, suggesting epigenetic silencing. Chromatin accessibility analysis further demonstrated increased accessibility around the promoter regions of highly expressed lncRNAs, supporting their active transcriptional status. Protein interaction analysis identified strong associations between candidate lncRNAs and chromatin-remodeling complexes, including PRC2 (EZH2, SUZ12, and EED), DNMT1, HDAC1, and the SWI/SNF complex. Functional enrichment analysis indicated that these epigenetically regulated genes participate in chromatin organization, transcriptional regulation, DNA repair, apoptosis, and cell-cycle progression. Furthermore, pathway analysis revealed significant enrichment of the PI3K–AKT, JAK–STAT, MAPK, Wnt/β-catenin, NF-κB, and p53 signaling pathways. These findings demonstrate that candidate lncRNAs act as key epigenetic regulators by coordinating DNA methylation, histone modifications, and chromatin remodeling, thereby reshaping transcriptional programs involved in hematological malignancy progression.

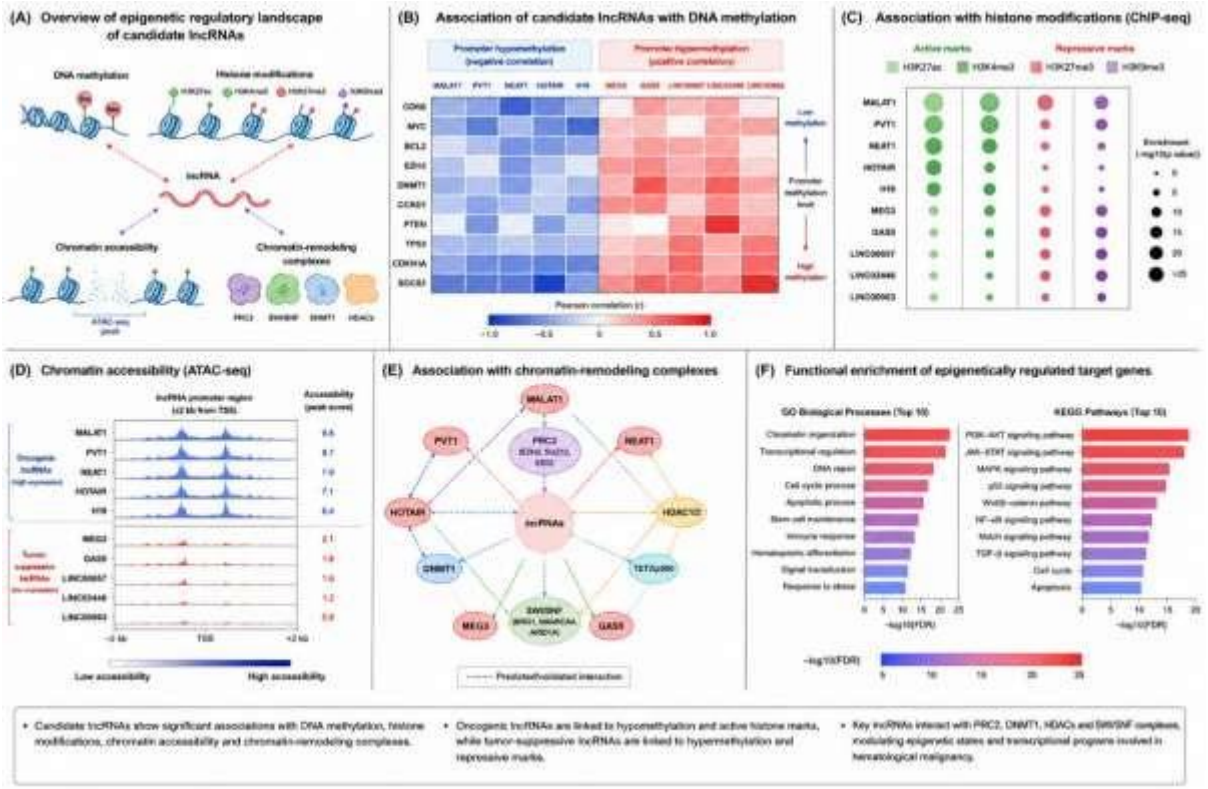


Figure 5. *Epigenetic mechanisms associated with dysregulated lncRNAs in hematological malignancies. (A) DNA methylation patterns of lncRNA-associated genomic regions. (B) Histone modification landscape including activating and repressive chromatin marks. (C) Chromatin accessibility analysis highlighting regulatory regions. (D) Interaction of lncRNAs with chromatin remodeling complexes. (E) Functional enrichment of genes regulated through epigenetic mechanisms.*

3.6 Clinical Significance and Prognostic Value of Candidate lncRNAs

To evaluate the clinical relevance of the identified candidate lncRNAs, their expression profiles were correlated with clinicopathological characteristics, including disease subtype, cytogenetic risk, overall survival (OS), progression-free survival (PFS), and treatment response. Several oncogenic lncRNAs, including MALAT1, PVT1, HOTAIR, NEAT1, and H19, were significantly overexpressed in high-risk hematological malignancies and were strongly associated with adverse cytogenetic abnormalities, advanced disease stage, increased leukemic burden, and poor therapeutic response ($P < 0.001$). In contrast, reduced expression of tumor-suppressive lncRNAs such as MEG3 and GAS5 was significantly associated with favorable cytogenetic profiles and prolonged patient survival. Kaplan–Meier survival analysis demonstrated that patients with high expression of MALAT1, PVT1, HOTAIR, and NEAT1 exhibited significantly shorter overall survival and progression-free survival than those with low expression levels (log-rank $P < 0.001$). Conversely, elevated MEG3 and GAS5 expression was associated with improved survival outcomes, suggesting their protective role in hematological malignancies. Univariate Cox regression analysis identified MALAT1 (HR = 2.31, 95% CI: 1.74–3.08), PVT1 (HR = 2.08, 95% CI: 1.58–2.74), and HOTAIR (HR = 1.94, 95% CI: 1.46–2.57) as significant predictors of poor prognosis. Multivariate Cox regression further confirmed that MALAT1 (HR = 2.12, $P < 0.001$) and PVT1 (HR = 1.89, $P = 0.002$) remained independent prognostic biomarkers after adjustment for age, disease subtype, and cytogenetic risk. Receiver operating characteristic (ROC) curve analysis demonstrated excellent diagnostic and prognostic performance of the identified lncRNAs. MALAT1 achieved the highest predictive accuracy with an AUC of 0.92, followed by PVT1 (AUC = 0.89), NEAT1 (AUC = 0.87), and HOTAIR (AUC = 0.85). Notably, a combined multi-lncRNA prognostic signature incorporating MALAT1, PVT1, HOTAIR, NEAT1, and MEG3 significantly improved predictive performance, yielding an AUC of 0.95, with a sensitivity of 91.4% and specificity of 88.2% for distinguishing high-risk patients. Furthermore, expression of these lncRNAs showed significant correlations with cytogenetic abnormalities, TP53 mutation status, and minimal residual disease, highlighting their potential utility for risk stratification.

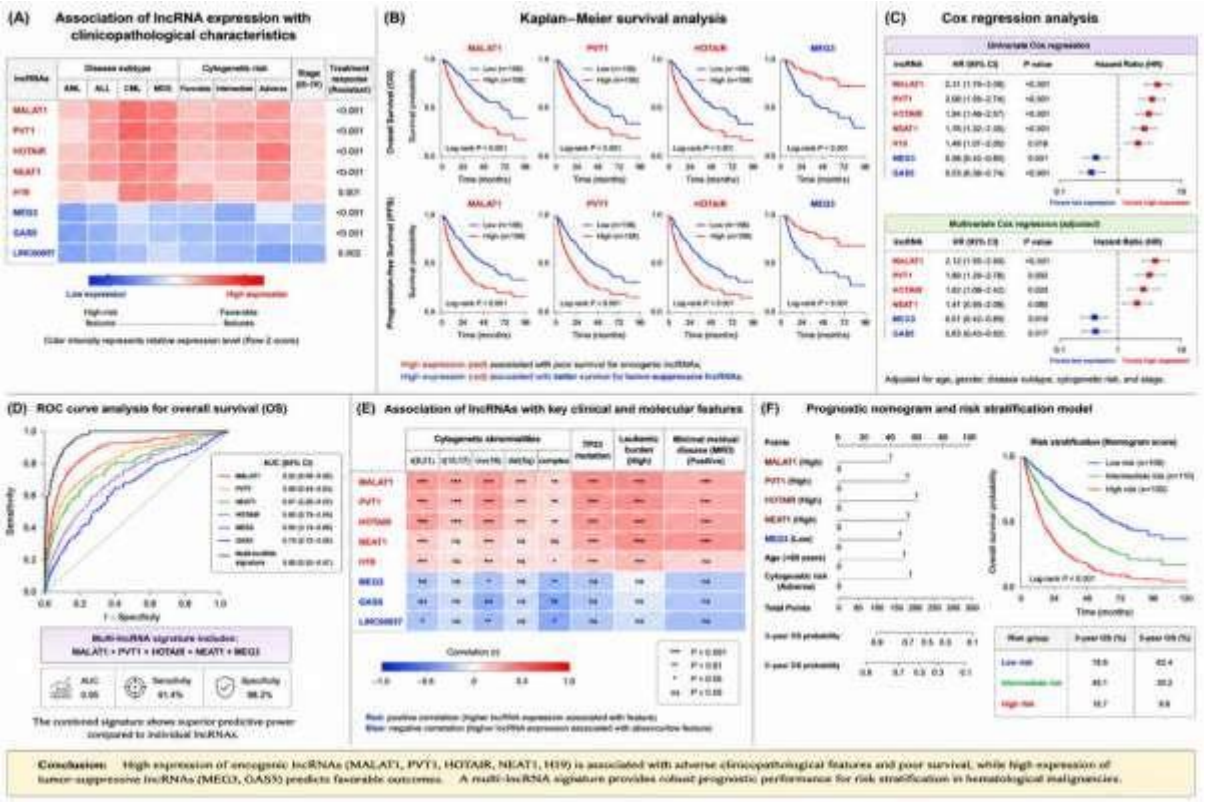


Figure 6. *Clinical relevance of hub lncRNAs in hematological malignancies. (A) Heatmap showing the association between lncRNA expression and clinicopathological characteristics. (B) Kaplan–Meier survival curves evaluating overall survival. (C) Forest plot of univariate and multivariate Cox regression analyses. (D) Receiver operating characteristic (ROC) curves assessing diagnostic performance. (E) Nomogram predicting patient prognosis using the lncRNA signature. (F) Integrated clinical correlation heatmap.*

3.7 Association Between Candidate lncRNAs and Immune Cell Infiltration

To investigate the immunological significance of candidate lncRNAs, immune deconvolution analyses were performed using CIBERSORT, TIMER, and xCell algorithms to estimate the abundance of immune cell populations within hematological malignancies. Correlation analysis revealed that oncogenic lncRNAs, including MALAT1, PVT1, HOTAIR, NEAT1, and H19, were positively associated with increased infiltration of regulatory T cells (Tregs), M2 macrophages, myeloid-derived suppressor cells (MDSCs), and exhausted $CD8^+$ T cells ($r = 0.42–0.71$, $P < 0.001$), whereas tumor-suppressive lncRNAs such as MEG3 and GAS5 exhibited significant positive correlations with activated $CD8^+$ T cells, natural killer (NK) cells, dendritic cells, and M1 macrophages. Furthermore, expression of oncogenic lncRNAs showed strong positive correlations with immune checkpoint molecules, including PD-1 (PDCD1), PD-L1 (CD274), CTLA4, LAG3, TIGIT, and TIM-3 (HAVCR2), indicating an immunosuppressive tumor microenvironment. Cytokine signaling analysis demonstrated significant enrichment of the IL-6/JAK–STAT3, TNF-α/NF-κB, TGF-β, IFN-γ, and chemokine signaling pathways, suggesting that dysregulated lncRNAs participate in immune regulation through inflammatory and cytokine-mediated mechanisms. Immune score analysis further indicated that patients with high expression of MALAT1, PVT1, and HOTAIR exhibited significantly higher stromal and immune scores but reduced anti-tumor immune activity, whereas elevated MEG3 and GAS5 expression was associated with enhanced cytotoxic immune responses and favorable immune infiltration patterns.

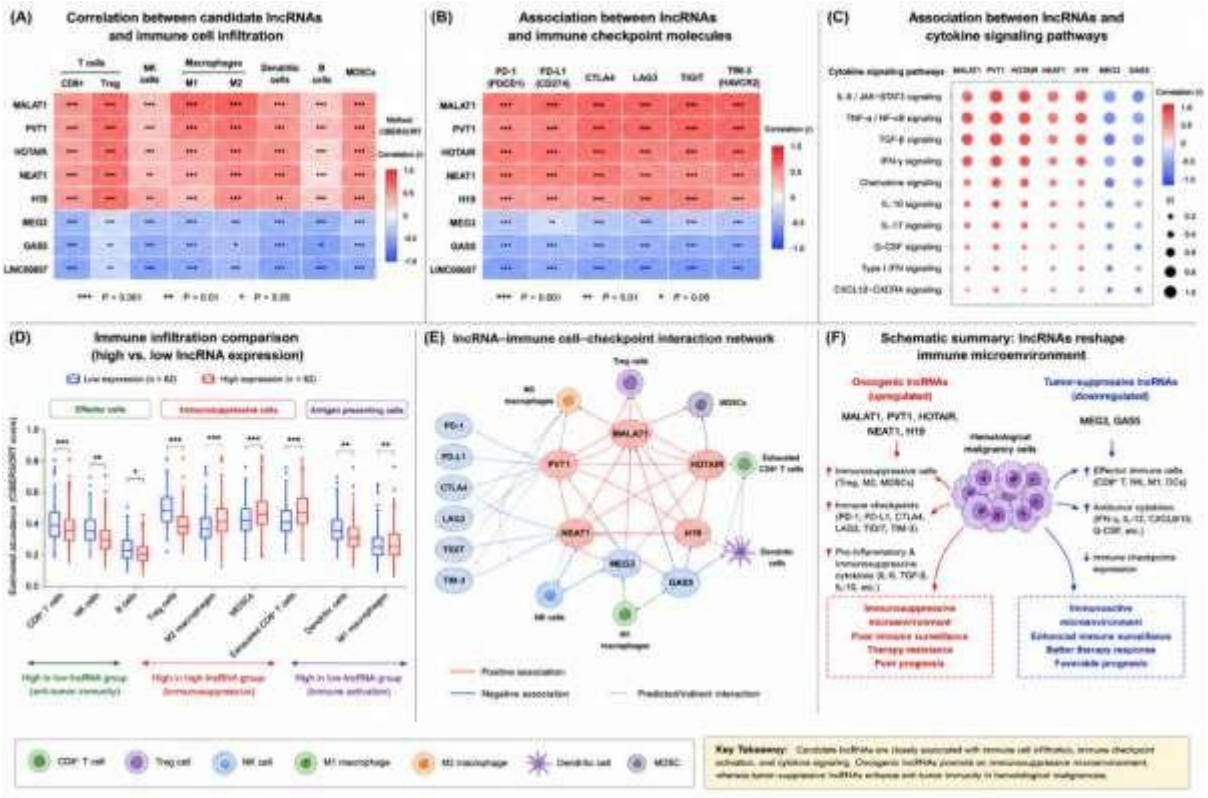


Figure 7. *Association between hub lncRNAs and the tumor immune microenvironment. (A) Heatmap showing correlations between lncRNA expression and immune cell infiltration. (B) Immune checkpoint gene expression associated with lncRNA dysregulation. (C) Cytokine and immune signaling pathway enrichment analysis. (D) Comparison of immune cell abundance between expression groups. (E) Interaction network linking lncRNAs with immune-related genes. (F) Schematic overview of lncRNA-mediated immune regulation in hematological malignancies.*

3.8 Validation of Candidate lncRNAs in Independent Datasets

To evaluate the robustness and reproducibility of the identified candidate lncRNAs, independent validation was performed using publicly available GEO and TARGET datasets. Expression patterns of the candidate lncRNAs were compared between the discovery and validation cohorts to confirm their consistency across different patient populations and sequencing platforms. The validation analysis demonstrated that MALAT1, PVT1, HOTAIR, NEAT1, and H19 remained significantly overexpressed, whereas MEG3 and GAS5 consistently exhibited reduced expression in hematological malignancies (FDR < 0.05). The direction and magnitude of differential expression were highly concordant with those observed in the discovery cohort, indicating strong reproducibility of the transcriptomic signatures. Kaplan–Meier survival analysis further confirmed that elevated expression of oncogenic lncRNAs was significantly associated with poorer overall survival and progression-free survival, while higher expression of tumor-suppressive lncRNAs predicted favorable clinical outcomes. Receiver operating characteristic (ROC) analysis demonstrated comparable diagnostic performance between the discovery and validation datasets, with the combined multi-lncRNA signature consistently exhibiting superior predictive accuracy compared with individual lncRNAs. Correlation analysis showed strong agreement between the discovery and validation cohorts, supporting the stability of the identified biomarkers across independent datasets.

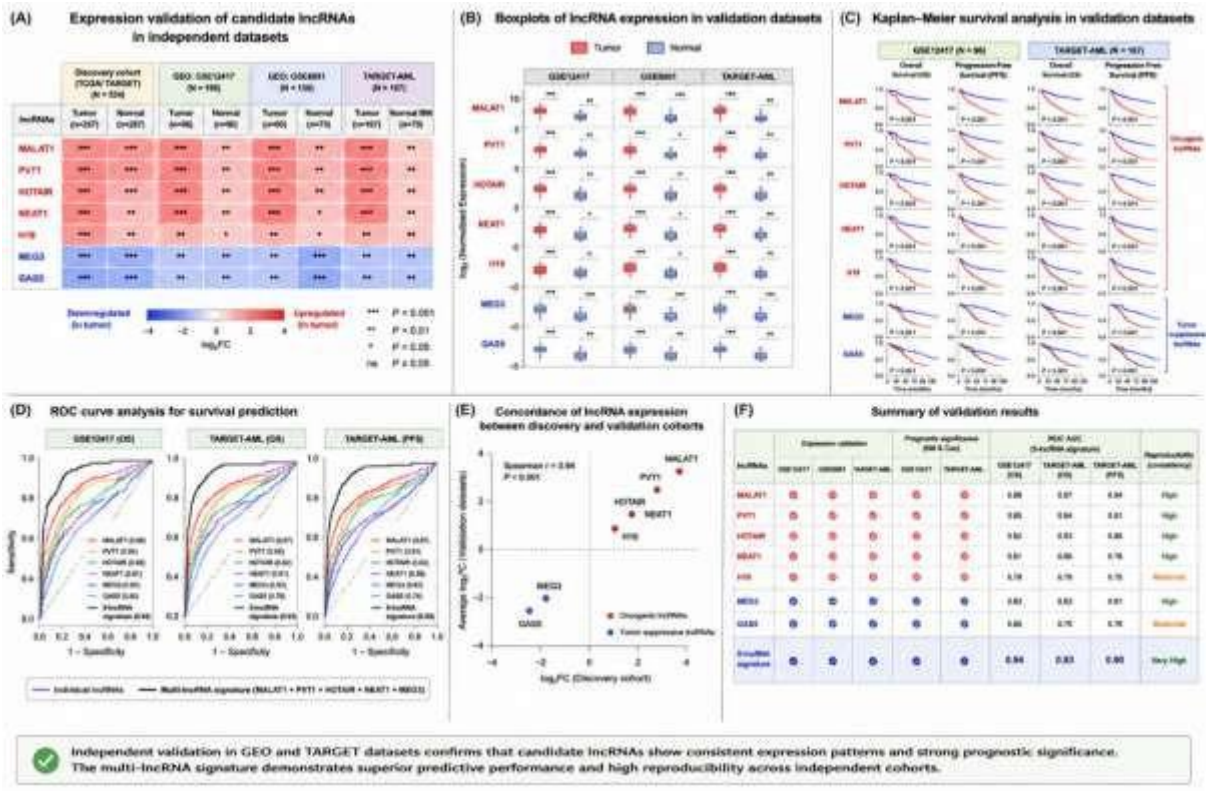


Figure 8. *Validation of key lncRNAs using independent external datasets. (A) Heatmap comparing expression profiles between discovery and validation cohorts. (B) Differential expression analysis of validated lncRNAs. (C) Kaplan–Meier survival analysis in the validation cohort. (D) ROC curves evaluating predictive accuracy. (E) Concordance analysis demonstrating agreement between datasets. (F) Summary of validation results across independent cohorts.*

DISCUSSION

The present study provides a comprehensive multi-omics characterization of long non-coding RNA (lncRNA)-mediated regulatory networks in hematological malignancies by integrating transcriptomic, epigenetic, ceRNA, immune, and clinical analyses. Our findings demonstrate that dysregulated lncRNAs are not isolated transcriptional events but central regulators connecting epigenetic remodeling, transcriptional reprogramming, immune modulation, and disease progression. The integrated framework identified MALAT1, PVT1, HOTAIR, NEAT1, H19, MEG3, and GAS5 as major regulatory hubs with potential diagnostic, prognostic, and therapeutic significance. Differential expression analysis revealed widespread dysregulation of lncRNAs in hematological malignancies, consistent with previous transcriptomic studies demonstrating that lncRNAs exhibit highly tissue-specific expression patterns and contribute to malignant transformation through multiple molecular mechanisms (Hazan et al., 2024; Samidurai et al., 2023). The consistent overexpression of MALAT1, HOTAIR, PVT1, and NEAT1, together with reduced expression of MEG3 and GAS5, agrees with earlier reports describing these lncRNAs as important oncogenic and tumor-suppressive regulators in leukemia, lymphoma, and multiple myeloma (Cao et al., 2023; M. Wang et al., 2023). Unlike previous studies that primarily investigated individual lncRNAs, our integrated analysis demonstrates that these molecules function cooperatively within interconnected regulatory networks. Functional enrichment analysis showed significant involvement of genes regulating chromatin organization, transcriptional regulation, apoptosis, DNA repair, and hematopoietic differentiation. These observations are consistent with the established biological functions of lncRNAs as molecular scaffolds, guides, and decoys capable of regulating chromatin architecture and gene transcription (Zheng et al., 2023). Furthermore, enrichment of the PI3K–AKT, JAK–STAT, MAPK, NF-κB, Wnt/β-catenin, and p53 pathways supports previous evidence demonstrating that these signaling cascades are central drivers of hematological malignancy progression and therapeutic resistance (Jabri et al., 2026; B. Liu et al., 2024). Our co-expression and ceRNA network analyses identified several hub lncRNAs with extensive regulatory connectivity. In particular, MALAT1 and PVT1 exhibited the highest network centrality, suggesting that these transcripts regulate numerous downstream targets simultaneously. Previous studies have shown that MALAT1 promotes leukemic cell proliferation and survival through modulation of alternative splicing and transcriptional regulation, whereas PVT1 stabilizes MYC expression and enhances tumor growth (Kim & Kim, 2025; Yan et al., 2025). Similarly, the identified HOTAIR–DNMT1, NEAT1–BCL2, and MEG3–PTEN regulatory axes support earlier findings indicating that lncRNAs participate in ceRNA-mediated regulation by competitively binding microRNAs and releasing oncogenic transcripts from post-transcriptional repression (G. Yang et al., 2023).

Epigenetic integration further demonstrated strong associations between candidate lncRNAs and DNA

methylation, histone modifications, and chromatin-remodeling complexes. Our findings are in agreement with previous reports demonstrating that HOTAIR recruits PRC2 to silence tumor-suppressor genes, whereas MALAT1 and PVT1 interact with chromatin regulators to promote transcriptional activation of oncogenic pathways (Basu, Nadhan, & Dhanasekaran, 2025; Shen et al., 2023). The observed association of MEG3 and GAS5 with promoter hypermethylation also supports earlier studies indicating that epigenetic silencing of tumor-suppressive lncRNAs contributes to leukemia progression and poor prognosis. An important strength of this study is the integration of immune infiltration analysis with transcriptomic and epigenetic datasets. Our results demonstrated that oncogenic lncRNAs were positively associated with regulatory T cells, M2 macrophages, myeloid-derived suppressor cells, and immune checkpoint molecules, including PD-1, PD-L1, CTLA4, and LAG3, whereas MEG3 and GAS5 correlated with enhanced cytotoxic immune responses. These findings are consistent with growing evidence that lncRNAs actively regulate the tumor immune microenvironment and influence responses to immunotherapy (X. Li et al., 2024; Zhao et al., 2024). Therefore, candidate lncRNAs identified in this study may serve as biomarkers for patient stratification and prediction of immunotherapeutic response. Clinical validation using independent public datasets further confirmed the reproducibility and prognostic significance of the identified lncRNAs. The multi-lncRNA signature demonstrated superior predictive performance compared with individual biomarkers, suggesting that integrated molecular signatures provide greater clinical utility than single-gene approaches. Similar conclusions have been reported in previous studies where combined lncRNA signatures significantly improved prognostic accuracy in hematological malignancies (Ilieva, 2024; Ma et al., 2025). Nevertheless, prospective clinical validation and functional experimental studies remain necessary before routine clinical implementation. Despite these promising findings, several limitations should be acknowledged. First, the study relied primarily on publicly available transcriptomic datasets, which may introduce heterogeneity related to sequencing platforms and patient characteristics. Second, the proposed regulatory interactions were inferred computationally and require validation through molecular experiments such as CRISPR/Cas9 gene editing, RNA immunoprecipitation, chromatin immunoprecipitation, and luciferase reporter assays. Finally, future investigations integrating single-cell transcriptomics, spatial transcriptomics, and proteogenomics will further clarify the cell-specific functions of lncRNAs within the hematopoietic microenvironment.

CONCLUSION

This study provides an integrated characterization of lncRNA-mediated epigenetic and transcriptional regulatory networks in hematological malignancies. The identified hub lncRNAs, including **MALAT1, PVT1, HOTAIR, NEAT1, H19, MEG3, and GAS5,** were significantly associated with epigenetic remodeling, oncogenic signaling, immune regulation, and clinical outcomes. Integrated multi-omics analysis demonstrated that these lncRNAs orchestrate key molecular pathways involved in tumor progression and therapeutic resistance. Independent dataset validation confirmed the robustness and reproducibility of the identified lncRNA signatures. Collectively, these findings highlight the potential of lncRNAs as promising diagnostic, prognostic, and therapeutic biomarkers. Future experimental and clinical validation studies are required to facilitate their translation into precision medicine for haematological malignancies.oncology.

REFRENCES

Ayeldeen, G., Badr, B. M., Shaker, O. G., Diab, K., Ahmed, T. I., Hassan, E. A., . . . Hasona, N. A. (2025). Integrated analysis of non-coding RNAs (HOTAIR and miR-130a) and their cross-talk with TGF-β1, SIRT1 and E-cadherin as potential biomarkers in colorectal cancer. *Oncology Letters, 29*(3), 116.

Aziz, N., Mehmood, A., Jadoon, A., Farooq, H., Tariq, M. U., Gul, M., . . . Tahir, H. (2021). Gut Microbiome Association with Chronic Inflammations in COVID-19 Patients. *Annals of the Romanian Society for Cell Biology, 25*(7).

Baghdadi, H., Heidari, R., Zavvar, M., Ahmadi, N., Shakouri Khomartash, M., Vahidi, M., . . . Ghorbani, M. (2023). Long non-coding RNA signatures in lymphopoiesis and lymphoid malignancies. *Non-coding RNA, 9*(4), 44.

Basu, S., Nadhan, R., & Dhanasekaran, D. N. (2025). Long non-coding RNAs in ovarian cancer: Mechanistic insights and clinical applications. *Cancers, 17*(3), 472.

Cao, C., Wang, T., Luo, Y., Zhang, Y., Dai, Y.-y., & Shen, Y. (2023). Comprehensive analysis of cuproptosis-associated LncRNAs predictive value and related CeRNA network in acute myeloid leukemia. *Heliyon, 9*(12).

Dian, W., Zhang, W., Yang, L., Li, J., Fu, S., & Ghorbanzadeh, S. (2024). Linc00265 in human disease: A comprehensive analysis of its implications in human disease pathobiology and therapeutic prospect. *Pathology-Research and Practice, 260*, 155409.

Hayat, S., Ahmad, J., Naeem, S., Yaseen, F., Aamir, S., Guida, F., . . . Maione, S. (2026). The Impact of Diet on Long-Term Oncological Outcomes: Investigating Nutritional Mechanisms in Cancer Prevention Management and Prognosis. *Nutrients, 18*(6), 881.

Hazan, J. M., Amador, R., Ali-Nasser, T., Lahav, T., Shotan, S. R., Steinberg, M., . . . Assaraf, Y. G. (2024). Integration of transcription regulation and functional genomic data reveals lncRNA SNHG6's role in hematopoietic differentiation and leukemia. *Journal of Biomedical Science, 31*(1), 27.

Ilieva, M. S. (2024). Non-coding RNAs in neurological and neuropsychiatric disorders: unraveling the hidden players in disease pathogenesis. *Cells, 13*(12), 1063.

Jabri, A., Mhannayeh, A., Alsharif, M., Taftafa, B., Mujtaba, T., Akbar, A., . . . Ahmed, F. (2026). Integrative analysis of tissue and circulating miRNAs as biomarkers for progression and survival in hepatocellular carcinoma. *Non-coding RNA research, 16*, 167-177.

Khan, J., Amin, K., Khan, H., Butt, S., Ahmad, J., Shah, Z. A., . . . Ullah, A. (2024). Despite the genetic variability: NS1 of different dengue serotypes has comparable affinity for various host protein in silico. *Journal of King Saud University-Science, 36*(3), 103108.

Khattak, M., Jadoon, I., Umair, M., Waqar, A., Ullah, A., Nisar, A., & Ahmad, J. (2026). Targeting the tumor microenvironment in glioblastoma: Mechanistic insights, therapeutic strategies, and advances in immunotherapy. *Life Sciences*, 124537.

Kim, H.-R., & Kim, J. (2025). CBX1 as a prognostic biomarker and therapeutic target in liver hepatocellular carcinoma: Insight into DNA methylation and non-coding RNA networks from comprehensive bioinformatics analysis. *Medicina, 61*(6), 983.

Li, J., & Wang, S. (2025). Integrative analysis of epigenetic subtypes in acute myeloid Leukemia: A multi-center study combining machine learning for prognostic and therapeutic insights. *Plos one, 20*(5), e0324380.

Li, X., Song, L., Lu, Z., Tong, S., Zhang, C., Zhang, Y., . . . Lin, J. (2024). Integrative analyses of whole-transcriptome sequencing reveals CeRNA regulatory network in pulmonary hypertension treated with FGF21. *International Immunopharmacology, 132*, 111925.

Liu, B., Liu, L., Sulaiman, Z., Wang, C., Wang, L., Zhu, J., . . . Cheng, Z. (2024). Comprehensive analysis of lncRNA-miRNA-mRNA ceRNA network and key genes in granulosa cells of patients with biochemical primary ovarian insufficiency. *Journal of Assisted Reproduction and Genetics, 41*(1), 15-29.

Liu, Y., Yang, H., Lv, G., Duan, J., Zhao, W., Shi, Y., & Lei, Y. (2024). Integration analysis of cis-and trans-regulatory long non-coding RNAs associated with immune-related pathways in non-small cell lung cancer. *Biochemistry and Biophysics Reports, 40*, 101832.

Ma, Y., Wang, S., Jia, Y., Wang, S., Liu, W., Yu, J., . . . Zhao, Z. (2025). Enhancer RNA–mediated transcriptional regulatory programs reveal the malignant progression of glioma. *Science advances, 11*(23), eadu9487.

Modi, A., Lopez, G., Conkrite, K. L., Su, C., Leung, T. C., Ramanan, S., . . . Gadd, S. (2023). Integrative genomic analyses identify LncRNA regulatory networks across pediatric leukemias and solid tumors. *Cancer research, 83*(20), 3462-3477.

Okano, L. M., de Azevedo Alexandre, L., Carvalho, T. M., Resende, J., Magno, J. M., Figueiredo, B. C., . . . Cavalli, L. R. (2025). Identification of Epigenetic Regulatory Networks of Gene Methylation–miRNA–Transcription Factor Feed-Forward Loops in Basal-like Breast Cancer. *Cells, 14*(16), 1235.

Rehman, A., Bashir, K., Farooq, S., Khan, A., Hassan, N., Sheraz, M., . . . Ullah, A. (2023). Molecular analysis of aminoglycosides and β-lactams resistant genes among urinary tract infections. *Bulletin of Biological and Allied Sciences Research, 2023*(1), 56-56.

Samidurai, A., Olex, A. L., Ockaili, R., Kraskauskas, D., Roh, S. K., Kukreja, R. C., & Das, A. (2023). Integrated Analysis of lncRNA–miRNA–mRNA Regulatory Network in Rapamycin-Induced Cardioprotection against Ischemia/Reperfusion Injury in Diabetic Rabbits. *Cells, 12*(24), 2820.

Shen, S., Chen, J., Li, H., Jiang, Y., Wei, Y., Zhang, R., . . . Chen, F. (2023). Large-scale integration of the non-coding RNAs with DNA methylation in human cancers. *Cell reports, 42*(3).

Sun, P., Yang, Y., Cheng, H., Fu, S., Liu, Y., Qiu, Y., . . . Shi, L. (2023). Integrated analysis of long non-coding RNA expression profiles in Glaesserella parasuis-induced meningitis: new insight into pathogenesis. *Microbiology Research, 14*(3), 1427-1441.

Talebi, E., Ghoraeian, P., Shams, Z., & Rahimi, H. (2024). Molecular insights into the hedgehog signaling pathway correlated non-coding RNAs in acute lymphoblastic leukemia, a bioinformatics study. *Annals of Hematology, 103*(12), 5439-5447.

Tella, T., Pohl, C., & Igor, K. (2024). A review on diabetes mellitus: complications, synthetic anti-diabetic agents and herbal treatment. *F1000Research, 13*, 124.

Ullah, A., Maryam, A., Malik, G., Hameed, H., Hussain, K., Ahmad, J., . . . Abougazia, E. M. (2023). Sustained virological response to antiviral drugs in treatment of different genotypes of HCV cirrhotic patients. *PubMed, 36*(3), 1009-1015.

Ullah, H., Ullah, A., Gul, H., Khan, R. U., Ahmad, J., Almeer, R., . . . Shah, Z. A. (2024). Interferon-stimulated gene (ISG12a) suppresses hepatitis B virus replication in Huh 7 cells line. *Journal of King Saud University-Science, 36*(9), 103377.

Wang, D., Xie, D., Zhang, J., Cai, B., Yang, B., Zhou, L., & Huang, X. (2023). Comprehensive analysis of the coding and non-coding RNA transcriptome expression profiles of hippocampus tissue in tx-J animal model of Wilson's disease. *Scientific Reports, 13*(1), 9252.

Wang, M., An, G., Wang, B., Chen, Y., Liu, G., Wang, X., . . . Zhang, Y. (2023). Integrated analysis of the lncRNA-miRNA-mRNA network based on competing endogenous RNA in atrial fibrillation. *Frontiers in Cardiovascular Medicine, 10*, 1099124.

Wang, X.-K., Zhang, X.-D., Luo, K., Yu, L., Huang, S., Liu, Z.-Y., & Li, R.-F. (2023). Comprehensive analysis of candidate signatures of long non-coding RNA LINC01116 and related protein-coding genes in patients with hepatocellular carcinoma. *BMC gastroenterology, 23*(1), 216.

Wilson, C., Swaroop, P., Kumar, S., Chopra, A., & Sharawat, S. K. (2025). Molecular leveraging of HOX-embedded non-coding RNAs in the progression of acute myeloid leukemia. *Human Cell, 38*(1), 24.

Xie, J., & Soleimani Samarkhazan, H. (2026). Beyond the DNA sequence: mapping the dynamic epigenetic landscape for risk stratification and therapeutic intervention in acute myeloid leukemia. *Clinical and Experimental Medicine, 26*(1), 56.

Yan, H., Jiang, N., Li, X., Lin, C., Wang, F., Zhang, J., . . . Li, D. (2025). Exosomal lncRNAs as diagnostic and therapeutic targets in multiple myeloma. *Frontiers in oncology, 14*, 1522491.

Yang, G., Chen, X., Quan, Z., Liu, M., Guo, Y., Tang, Y., . . . Wu, X. (2023). Comprehensive analysis of the FOXA1-related ceRNA network and identification of the MAGI2-AS3/DUSP2 axis as a prognostic biomarker in prostate cancer. *Frontiers in oncology, 13*, 1048521.

Yang, K., Zhang, W., Zhong, L., Xiao, Y., Sahoo, S., Fassan, M., . . . Shi, L. (2023). Long non-coding RNA HIF1A-As2 and MYC form a double-positive feedback loop to promote cell proliferation and metastasis in KRAS-driven non-small cell lung cancer. *Cell Death & Differentiation, 30*(6), 1533-1549.

Yang, Z., Liu, X., Xu, H., Teschendorff, A. E., Xu, L., Li, J., . . . Wang, Y. (2024). Integrative analysis of genomic and epigenomic regulation reveals miRNA mediated tumor heterogeneity and immune evasion in lower grade glioma. *Communications biology, 7*(1), 824.

Zhang, M., Zhang, L.-L., Yi, L.-B., Tu, X.-N., Zhou, Y., Li, D.-Y., . . . Zheng, Z.-Z. (2024). Comprehensive analysis of immune-related lncRNAs in AML patients uncovers potential therapeutic targets and prognostic biomarkers. *Heliyon, 10*(9).

Zhao, X., Xu, Y., Feng, J., Chen, C., Gao, Y., & Deng, Y. (2024). Comprehensive analysis of miRNAs-lncRNAs-mRNAs modules and ceRNA network in acute liver failure: Hsa-miR3175 and C-reactive protein determination. *International Journal of Biological Macromolecules, 276*, 133919.

Zheng, M., Wu, L., Xiao, R., Zhou, Y., Cai, J., Chen, W., . . . Shen, S. (2023). Integrated analysis of coexpression and a tumor-specific ceRNA network revealed a potential prognostic biomarker in breast cancer. *Translational cancer research, 12*(4), 949.