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# m6A-Associated Long Non-Coding RNA as a Potential Diagnostic Biomarker for Systemic Lupus Erythematosus

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(Received 7 October 2023; revised 13 October 2023; accepted 8 November 2023; first published online 31 December 2023)

## Abstract

**Objective:** To identify novel diagnostic biomarkers for Systemic Lupus Erythematosus (SLE) by selecting m6A-associated long non-coding RNAs (lncRNAs) through bioinformatics analysis. **Methods:** Multiple SLE datasets were obtained from the Gene Expression Omnibus (GEO). The datasets were analyzed for m6A-related genes and lncRNAs. Differentially expressed m6A-associated lncRNAs were further investigated, and mRNAs co-expressed with these lncRNAs were subjected to KEGG pathway analysis, followed by ROC curve plotting. **Results:** A correlation was observed between six lncRNAs (ANKRD10-IT1, C2orf27A, EGOT, HCG18, POLR2J4, TCL6) and six m6A methyltransferases (RBM15, HNRNPA2B1, ZC3H13, YTHDF2, RBM15B, LRPPRC). The differential analysis indicated that lncRNA POLR2J4 and lncRNA ANKRD10-IT1 were significantly downregulated in SLE patients. ROC curve showed that lncRNA POLR2J4 had an AUC of 0.799 and lncRNA ANKRD10-IT1 had an AUC of 0.516. KEGG analysis revealed that proteins co-expressed with lncRNA POLR2J4 were enriched in T-cell receptor signaling pathways, Toll-like receptor signaling pathways, mTOR signaling pathways, and other SLE-related pathways. Those co-expressed with lncRNA ANKRD10-IT1 were involved in PI3K-Akt, mTOR, and cell apoptosis pathways.

**Keywords:** Systemic Lupus Erythematosus; Bioinformatics analysis; m6A; lncRNA; Biomarker

Systemic Lupus Erythematosus (SLE) is an autoimmune diffuse connective tissue disease that poses serious threats to human health. Given the current uncertainties around the cause and pathogenesis of SLE, clinical diagnosis is often delayed, with

varying degrees of internal organ damage observed when symptoms finally manifest. Therefore, identifying more sensitive and specific diagnostic biomarkers is crucial.

The pathogenesis of SLE involves various factors, including genetics, hormones, environment, and epigenetic changes. Among these, RNA modifications, especially methylation at the 6th position of adenosine (N<sup>6</sup>-methyladenosine, m<sup>6</sup>A), act as post-transcriptional gene expression regulators. Recent studies have explored the role of m<sup>6</sup>A modification in SLE, with Li et al. analyzing potential associations between m<sup>6</sup>A modifications and SLE, and Luo et al. observing a declining trend of the m<sup>6</sup>A-binding protein YTHDF2 mRNA level in SLE patients compared to healthy controls. The role of m<sup>6</sup>A-regulated lncRNA could be crucial in immune cell proliferation and migration.

lncRNAs are non-coding RNAs longer than 200 nucleotides. They play vital roles in various biological events, such as epigenetic regulation, gene dosage compensation, cell cycle regulation, and cellular differentiation. Several studies report that many lncRNAs are associated with the pathogenesis of autoimmune inflammatory diseases like SLE and RA. Some lncRNAs participate in the progression of SLE through epigenetic regulation mechanisms, affecting SLE therapeutic drug action.

These findings underline the involvement of m<sup>6</sup>A and lncRNA in SLE pathogenesis. Recent research has emphasized the interaction between m<sup>6</sup>A and lncRNA, especially regarding lncRNA stability and degradation. Therefore, uncovering the mechanisms of m<sup>6</sup>A-associated lncRNAs in the progression of SLE might have significant implications for diagnostic and prognostic targets. This study uses bioinformatics to integrate network platform data to explore m<sup>6</sup>A-associated lncRNAs, laying a foundation for the development of potential diagnostic biomarkers for SLE.

## **1. Materials and Methods**

### **1.1 Data Retrieval**

Data were retrieved from the GEO database ([www.ncbi.nlm.nih.gov/geo](http://www.ncbi.nlm.nih.gov/geo)) using “Systemic lupus erythematosus” as the keyword, obtaining two datasets, GSE46907 and GSE50772. The samples were Peripheral Blood Mononuclear Cells (PBMCs). Specifically, GSE46907 utilized platforms GPL96 and GPL97, each containing 5 normal and 5 SLE samples, while GSE50772 used platform GPL570 with 20 normal and 61 SLE samples.

### **1.2 Data Merging and Re-annotation**

R software (version 4.02) was employed, utilizing the SVA and Limma packages, to merge samples from different platforms within the GSE46907 dataset and correct for batch effects. Principal Component Analysis (PCA) was subsequently performed to confirm correction efficacy. Perl software (version 5.30.2) was used for gene annotation of both datasets, distinguishing between mRNA and lncRNA.

### 1.3 Extraction of m6A Methylation Genes and lncRNA

Based on current research, 21 m6A related genes were identified, including readers (YTHDC1, YTHDC2, YTHDF1, YTHDF2, YTHDF3, IGF2BP1, IGF2BP2, IGF2BP3, RBMX, HNRNPA2B1, HNRNPC), writers (METTL3, METTL14, METTL16, RBM15, RBM15B, WTAP, VIRMA (KIA1499), and ZC3H13) and erasers (FTO and ALKBH5). The Limma package in R software was used to extract expression levels of m6A genes and lncRNA from the GSE46907 dataset.

### 1.4 Correlation Between m6A Methylation Genes and lncRNA Expression

Using the Limma package in R, with a set correlation threshold greater than 0.5 and  $P < 0.05$ , correlations between m6A methylation genes and lncRNA in the GSE46907 dataset were calculated.

### 1.5 Differential Expression Analysis of lncRNA

SPSS Statistics 20 was used to analyze the differential expression of lncRNA from step 1.4 in the GSE46907 dataset.

### 1.6 Diagnostic Analysis of m6A-related lncRNA and Co-expression with mRNA

Expression levels of m6A-related lncRNA from step 1.5 were obtained from the GSE46907 dataset. The survROC package in R was used to plot the ROC curve, considering  $P < 0.01$  as statistically significant to validate the diagnostic value of m6A-related lncRNA. Based on the ROC curve, positively correlated lncRNA were selected. Using the Limma package in R, with an absolute correlation value greater than 0.5 and  $P < 0.05$ , co-expressed mRNA in the GSE50772 dataset with lncRNA were retrieved.

### 1.7 KEGG Pathway Analysis

The co-expressed mRNA from step 1.6 was subjected to KEGG pathway analysis via the KOBAS online website (<http://kobas.cbi.pku.edu.cn/kobas3>), considering  $P < 0.05$  as statistically significant.

## 2. Results

After merging and batch correction of the 20 data points from different platforms in the GSE46907 dataset, PCA indicated a random distribution of the data, showing evident batch correction effects (Figure 1).

Correlation analysis between m6A methylation genes and lncRNA expression revealed correlations for 6 lncRNAs (ANKRD10-IT1, C2orf27A, EGOT, HCG18, POLR2J4, TCL6) and 6 m6A methylation enzymes (RBM15, HNRNPA2B1, ZC3H13, YTHDF2, RBM15B, LRPPRC) (Figure 2).

Differential analysis showed that lncRNA POLR2J4 and lncRNA ANKRD10-IT1 were significantly downregulated in SLE patients ( $P < 0.05$ ) (Figure 3).

Verification in the GSE46907 dataset with ROC diagnostic curves indicated that the Area Under the Curve (AUC) for lncRNA ANKRD10-IT1 was 0.516 ( $P = 0.238$ )

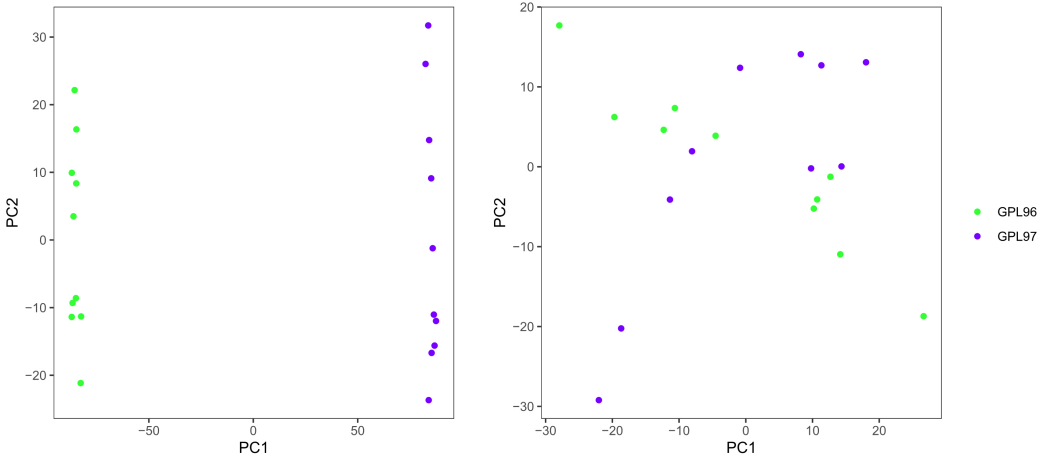


**Figure 1.** Batch correction results (left: before correction; right: after correction).

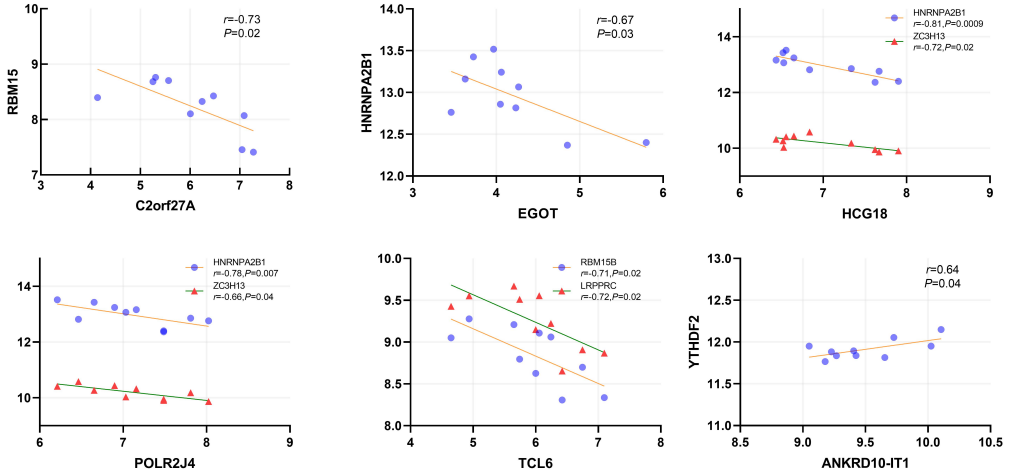


**Figure 2.** Correlation between m6A methylation genes and lncRNA expression.

and for lncRNA POLR2J4 was 0.799 ( $P < 0.0001$ ) (Figure 4). Therefore, lncRNA POLR2J4 was selected for further analysis.

lncRNA POLR2J4 co-expressed with 448 mRNAs ( $|r| > 0.5$ ,  $P < 0.05$ ). KEGG results showed that proteins co-expressed with lncRNA POLR2J4 were enriched in T-cell receptor signaling pathways, Toll-like receptor signaling pathways, mTOR signaling pathway, and Human T-lymphotropic virus type 1 infection among other SLE-related signaling pathways (Figure 5).

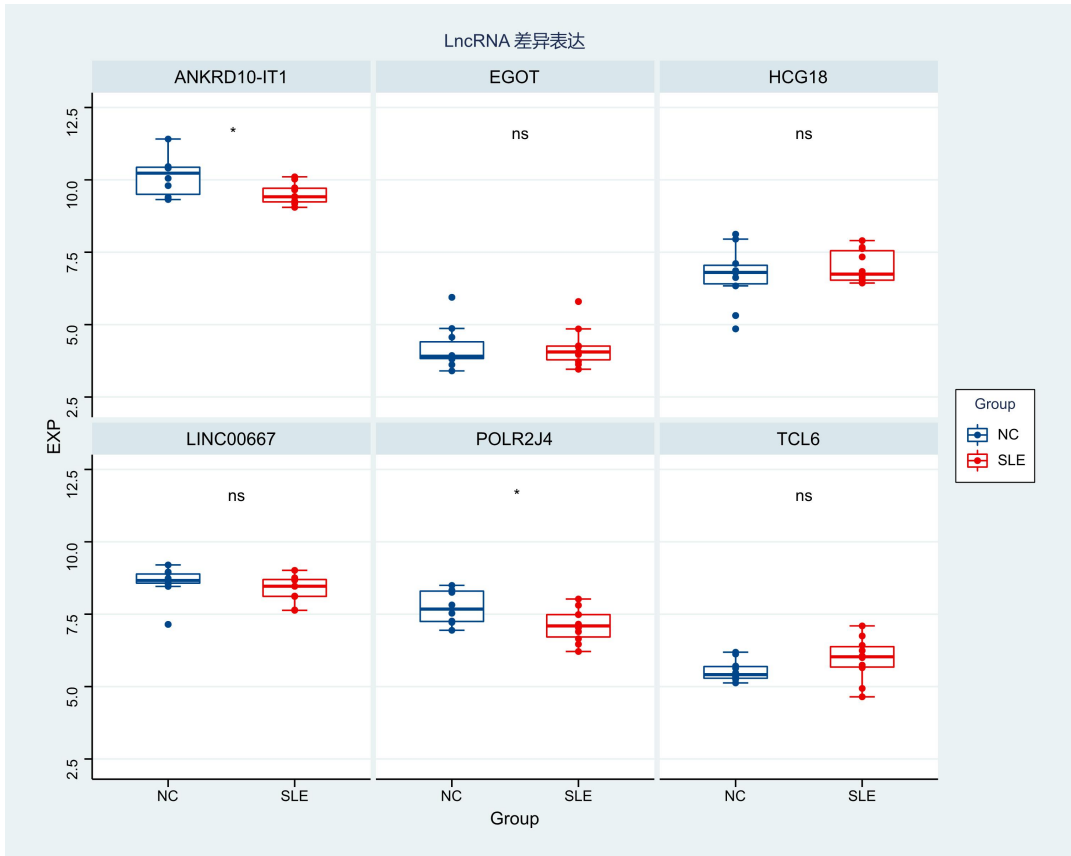


**Figure 3.** Differential expression of lncRNA in normal individuals vs. SLE patients' PBMCs.

### 3. Discussion

The etiology and pathogenesis of SLE remain unclear. Currently, its diagnosis largely relies on clinical symptoms and laboratory indicators such as serological and immunological tests. Earlier diagnosis facilitates earlier treatment, which in turn prevents organ damage and improves prevention and treatment outcomes. m6A methylation is the most common form of mRNA modification and plays a crucial role in post-transcriptional gene expression regulation. Many connective tissue diseases, including SLE, osteoporosis, osteoarthritis, rheumatoid arthritis, and spinal diseases, are believed to be associated with m6A and its related enzymes[11,12]. A deeper understanding of the role of m6A RNA methylation in various connective tissue diseases will aid in developing drugs that selectively target m6A and related proteins. Current research has shown that lncRNA can regulate gene expression at epigenetic, transcriptional, and post-transcriptional levels[13], and it is closely linked with the onset, progression, and prevention of human tumors, neurological disorders, and autoimmune diseases[14]. Abnormal expression of multiple lncRNAs, including NEAT1, Gas5, may be involved in the onset and progression of SLE[15]. Modulators of signaling pathways play a role in disease onset and progression and offer a new direction for

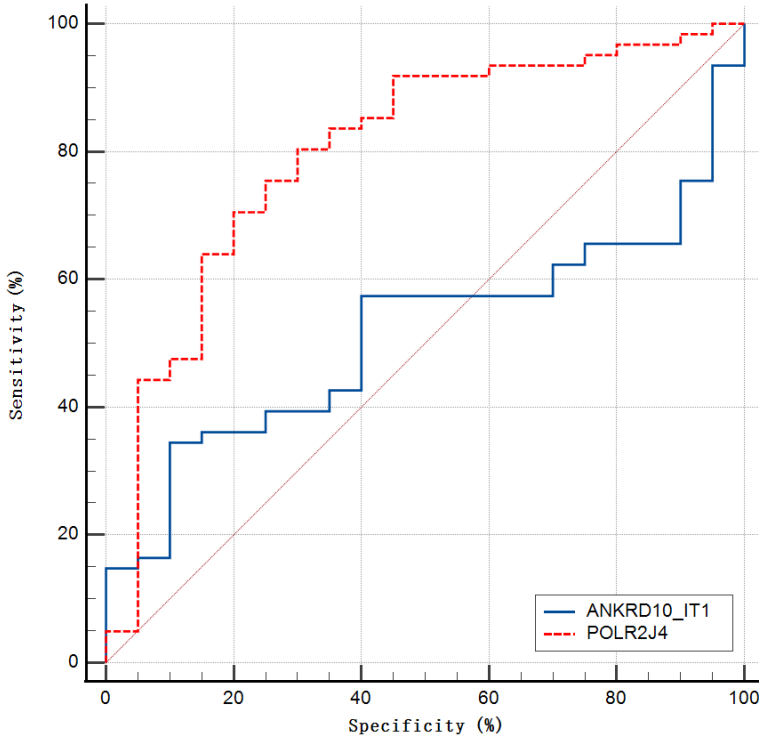


**Figure 4.** ROC diagnostic curves.

studying new therapeutic targets and biomarkers for SLE diagnosis. While efforts have been made to elucidate the downstream mechanisms of differentially expressed lncRNAs in SLE, the upstream regulatory mechanisms, especially those involving m6A modifications in lncRNAs, have not received much attention. Hence, focusing on the interaction between m6A modifications and lncRNAs will provide insights into their roles in SLE, aiding in the identification of potential diagnostic and prognostic markers or therapeutic targets for SLE.

With the proliferation and widespread application of gene chip technology, researchers aiming to develop biomarkers for SLE diagnosis and prognosis now have significant convenience. To explore new diagnostic and prognostic lncRNAs for SLE patients, this study examined lncRNA expression profiles and clinical data from the GEO database. Initially, co-expression networks were constructed to identify lncRNAs associated with m6A. In the GSE46907 dataset, six lncRNAs (ANKRD10-IT1, C2orf27A, EGOT, HCG18, POLR2J4, TCL6) and six m6A methyltransferases (RBM15, HNRNPA2B1, ZC3H13, YTHDF2, RBM15B, LRPPRC) were identified to have correlations. Differential analysis revealed that lncRNA POLR2J4 was significantly downregulated in SLE patients. The ROC diagnostic curve indicated statistical significance for POLR2J4, suggesting it as a potential diagnostic biomarker for

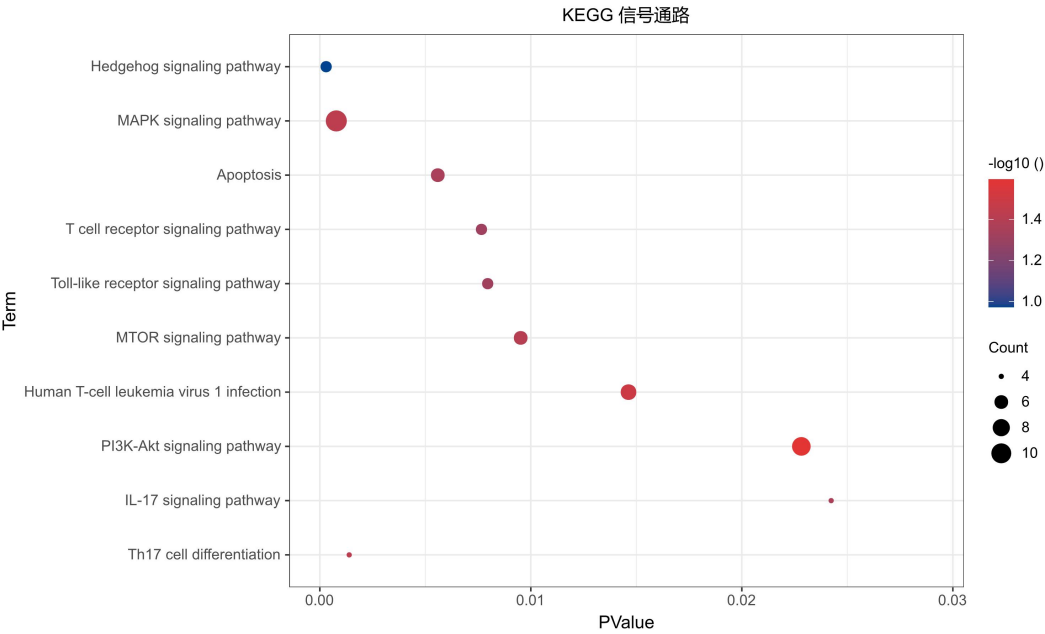


**Figure 5.** KEGG pathway analysis of m6A methylation-related lncRNA co-expressed mRNAs.

SLE.

The POLR2J family of RNA polymerase II subunits includes four members: POLR2J1, POLR2J2, POLR2J3, and POLR2J4[16,17]. There are no published studies detailing their specific biological functions to date. However, research suggests that POLR2J4 may be a potential predictor for the onset and prognosis of hepatocellular carcinoma[18,19], colorectal cancer[20], and breast cancer[21]. Notably, the expression of POLR2JA and estrogen receptors has been significantly correlated with bone metastasis in breast cancer. ANKRD10-IT1 has been identified as a prognostic marker for triple-negative breast cancer and liver cancer[22-23]. To date, there is no report on POLR2J in SLE. By analyzing co-expressed proteins, we can infer its potential function. The results indicate enrichment in Toll-like receptor signaling pathways, the mTOR signaling pathway, and several other pathways related to the pathogenesis of SLE. This suggests that POLR2J may be involved in the onset and progression of SLE by modulating these signaling pathways.

Research has shown that the Immunoreceptor Tyrosine-based Activation Motif (ITAMs) are crucial intracellular domains of the T cell activation receptor (TCR/CD3). Its functionality relies on the normal expression of CD3 and FcR. In lupus patients, T cells synthesize reduced CD3 and increased FcR, causing the Syk signaling pathway to dominate over the ZAP-70 signaling pathway. This results in overexpression of genes such as CD40L, leading to abnormal B cell proliferation and differentiation, excessive secretion of pathogenic autoantibodies and cytokines, and abnormal antibody isotype switching, all of which contribute to the onset and progression of SLE[24]. Treatments targeting T cell signaling, such as tacrolimus and cyclosporine

A, have shown efficacy in reducing proteinuria and maintaining renal function in lupus nephritis[25]. This study found that POLR2J4 may regulate the expression of CD3D and ZAP70 in SLE, suggesting that the POLR2J4-mediated abnormal activation of T cells in SLE may be linked to these two key molecules. The underlying mechanism warrants further investigation.

HNRNPA2B1, ZC3H13, and YTHDF2 are proteins involved in m6A regulation, including m6A methyltransferases, m6A demethylases, and methylation reader proteins. ZC3H13 is an m6A methyltransferase that can promote the nuclear localization of complexes modified by RNA m6A [30]. HNRNPA2B1 can directly bind to m6A RNA, regulating RNA splicing and processing [31]. There have been no studies on these two in SLE. YTHDF2 acts as an RNA-binding protein, can recognize m6A modifications, and ultimately translate them into various functional signals [32]. Studies have shown that in SLE, the expression of YTHDF2 decreases and is directly proportional to complement C3 [33]. This study found that lncRNA POLR2J4 is associated with HNRNPA2B1 and ZC3H13, suggesting that m6A methylation regulation of both might take place, possibly by altering local structures and affecting the binding of lncRNA with proteins, contributing to the onset and progression of SLE.

#### 4. Conclusion

In this study, we revealed the correlation between m6A-related lncRNA expression and SLE. By analyzing lncRNA expression profiles from the GEO database, we identified lncRNA POLR2J4 as a potential diagnostic biomarker for SLE. The study also suggests that POLR2J4 may play a role in SLE pathogenesis by modulating specific signaling pathways. This finding provides a new direction for understanding SLE at the molecular level and offers potential for the development of novel therapeutic strategies.

#### Acknowledgments

We sincerely thank the contributors of the GEO database for making their data publicly available, which has facilitated this research. Additionally, we express our gratitude to the entire research team and everyone who provided support during the course of this study.

#### Conflicts of Interest

The authors declare no conflicts of interest.

#### FUNDING

The present study was funded by the Chinese Medicine Research Project of Traditional Chinese Medicine Bureau Of Guangdong Province (20221342), and the Shenzhen Futian District Health and Public Welfare Research Project (grant no. FTWS2022021).

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