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LINC00641 Expression and a Predicted LINC00641-Associated RNA Network in Multiple Sclerosis

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Research Article

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Abstract

Background

Multiple sclerosis (MS) is a chronic neuroinflammatory disease characterized by demyelination and neurodegeneration. Long non-coding RNAs may contribute to gene-regulatory changes in MS, but the involvement of LINC00641 has not been investigated.

Methods and Results

LINC00641 expression was measured by quantitative real-time PCR in peripheral blood samples from 48 patients with MS and 48 healthy controls. LINC00641-associated mRNAs were retrieved from ENCORI and integrated with differential-expression analysis of the GSE137143 RNA-seq dataset. Protein-interaction analysis, functional enrichment, miRNA-target prediction, and receiver operating characteristic analyses were then performed. LINC00641 was significantly upregulated in MS and showed case-control discriminatory performance (AUC = 0.74). Six LINC00641-associated genes—DDX5, HNRNPC, THRAP3, YY1, SUPT16H, and HP1BP3—were significantly upregulated in the RNA-seq dataset, with individual AUC values of 0.64–0.71. A logistic-regression model combining the six genes showed an AUC of 0.806. Eleven predicted miRNAs were shared among the six genes, and enrichment analysis linked the gene set mainly to RNA splicing, mRNA processing, and chromatin organization.

Conclusions

LINC00641 is upregulated in MS, and integration of experimental expression data with transcriptomic and network analyses identifies a LINC00641-associated RNA signature linked to RNA-processing pathways. These findings support independent validation and functional investigation of LINC00641 and the associated network as candidate molecular biomarkers and regulators in MS.

1 Introduction

Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disorder of the central nervous system in which altered immune regulation, demyelination, and neuronal injury are accompanied by broad changes in gene expression. Non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), contribute to gene regulation through effects on transcription, RNA stability, and post-transcriptional control [1–3]. Dysregulation of these RNA species has therefore become an important area of investigation in MS and other neurological disorders.

Several lncRNAs have already been associated with MS-related immune and inflammatory processes. HOTAIR, for example, has been reported to show altered expression in MS and has been investigated in experimental models of demyelination and immune regulation [4–8]. These observations support the

broader possibility that disease-associated lncRNAs participate in RNA-regulatory networks relevant to MS.

LINC00641 has been studied mainly in cancer biology. In glioma, LINC00641 has been reported to modulate the miR-4262/NRGN axis and to influence cell proliferation and apoptosis [9]. Transcriptomic studies have also included LINC00641 in lncRNA-based signatures associated with glioblastoma outcome and pathways related to cell adhesion and signaling [10]. However, its expression and potential molecular associations in MS have not been characterized.

In this study, we evaluated LINC00641 expression in peripheral blood samples from patients with MS and healthy controls. We then integrated the experimental findings with public transcriptomic data and interaction resources to identify LINC00641-associated genes, predicted miRNA interactions, enriched biological processes, and their potential case-control discriminatory value in MS.

2 Materials and Methods

2.1 Study population

The study included peripheral blood samples from 48 patients with MS, encompassing relapsing–remitting MS (RRMS) and secondary progressive MS (SPMS), and 48 healthy control participants. Patients with MS were 31–61 years of age and had Expanded Disability Status Scale (EDSS) scores ranging from 1 to 6. Control participants were 50–61 years of age and had no personal or family history of MS. Older controls were selected to reduce the likelihood of including individuals with undiagnosed or subclinical MS [11, 12]. The study was approved by the Ethics Committee of the University of Isfahan (IR.UI.REC.1402.016), and written informed consent was obtained from all participants before enrollment.

2.2 RNA extraction and quantitative real-time PCR

Total RNA was extracted from peripheral blood cells using RNX_PLUS reagent (CinnaGen, Tehran, Iran) according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized using a ROJE Technologies RT-PCR kit (Tehran, Iran). Quantitative real-time PCR (qRT-PCR) was performed on a Bio-Rad CFX system (Hercules, CA, USA) using a SYBR Green PCR kit (Yekta Tajhiz Azma, Tehran, Iran). Relative LINC00641 expression was calculated using the 2^−ΔΔCt method, with GAPDH as the internal reference gene. Primers were designed using Oligo 7 software (version 7) on the basis of melting temperature, GC content, and expected amplicon size. Amplification specificity was assessed by visualization of PCR products on a 1.5% agarose gel relative to a 100-bp DNA ladder. Primer sequences are provided in Table 1.

Table 1
Primer sequence of LINC00641 and the housekeeping gene (GAPDH).

gene	RNA type	Primer (5' – 3')	Forward/Reverse	Tm	Product length
LINC00641	lncRNA	TTGAGCAGTGCCTGGCTAAC	Forward	60.61	112
		CACGTTGTGGGTGGGAGAAG	Reverse	60.88	
GAPDH	Housekeeping	ACAGGGTGGTGGACCTCAT	Forward	60.15	175
		AGGGGTCTACATGGCAACTG	Reverse	59.38	

2.3 Prediction of the LINC00641-associated mRNA interaction network

ENCORI (starBase v3.0) [13] was used to retrieve mRNAs predicted or experimentally supported to interact with LINC00641. Proteins encoded by these LINC00641-associated mRNAs were subsequently analyzed using STRING [14] to construct a protein–protein interaction (PPI) network. Proteins with a network degree of at least 2 were retained to prioritize more highly connected nodes for downstream analyses.

2.4 Differential-expression analysis of LINC00641-associated genes in MS

Publicly available RNA-seq data from GEO accession GSE137143 (GPL24676; Illumina NovaSeq 6000; Homo sapiens) were analyzed to assess expression changes in LINC00641-associated genes [15]. The dataset contains transcriptomic profiles from sorted CD4 + T cells, CD8 + T cells, and CD14 + monocytes obtained from treatment-naïve patients with MS and healthy controls. Raw count data were analyzed in R using DESeq2 [16]. Genes with an adjusted P value (padj) < 0.05 were considered differentially expressed; positive and negative log2 fold-change values were used to describe higher and lower expression, respectively. Expression distributions were visualized using boxplots, and group differences were additionally evaluated with the Wilcoxon rank-sum test. LINC00641-associated mRNAs were cross-referenced with the differentially expressed genes to identify overlapping candidates for downstream analysis. Visualizations were generated using ggplot2 [17].

2.5 Discriminatory-performance analysis

Receiver operating characteristic (ROC) analysis was used to assess the ability of the selected LINC00641-associated genes to distinguish MS samples from healthy controls on the basis of normalized expression values. The area under the ROC curve (AUC) was calculated for each gene. The six selected genes were also combined in a logistic-regression model, and the resulting model was evaluated within the same RNA-seq dataset. These analyses were treated as exploratory assessments of case-control discrimination rather than as independent clinical validation.

2.6 miRNA interaction analysis

miRWalk [18] was used to identify predicted miRNAs targeting the selected LINC00641-associated genes. Only predicted interactions involving the 3' untranslated region and seed region were retained. The resulting miRNA–mRNA network was visualized in Cytoscape [20]. Overlap among miRNAs predicted to target the six genes was summarized using an UpSet plot generated with UpSetR [19].

2.7 Statistical analysis of qRT-PCR data

qRT-PCR data were analyzed and visualized in GraphPad Prism version 8. Normality was assessed using the Shapiro–Wilk test. Differences between the independent MS and control groups were evaluated using an unpaired t-test for normally distributed data or a Wilcoxon rank-sum test for non-normally distributed data, as appropriate. A two-sided P value < 0.05 was considered statistically significant. ROC analysis was also performed in GraphPad Prism to evaluate the discriminatory performance of LINC00641 expression.

3 Results

3.1 LINC00641 is upregulated in MS

LINC00641 expression was significantly higher in peripheral blood samples from patients with MS than in healthy controls (Fig. 1a). ROC analysis showed an AUC of 0.7396, indicating measurable case-control discrimination by LINC00641 expression in the study cohort (Fig. 1b).

3.2 Identification of LINC00641-associated genes with increased expression in MS

ENCORI-derived LINC00641-associated mRNAs were mapped to their encoded proteins and evaluated in STRING. The complete PPI network contained several interaction clusters (Fig. 2a). Restricting the network to proteins with degree ≥ 2 generated a more highly connected subset (Fig. 2b). Cross-referencing these candidates with the GSE137143 differential-expression results identified six genes—DDX5, HNRNPC, THRAP3, YY1, SUPT16H, and HP1BP3—with significantly higher expression in MS samples (Fig. 2c). Their positions in the overall differential-expression analysis and their expression distributions are shown in Fig. 3a and Fig. 3b, respectively.

3.3 Discriminatory performance of individual LINC00641-associated genes

ROC analysis of variance-stabilized expression values showed above-chance discrimination for all six genes (Fig. 4). THRAP3 had the highest AUC (0.713), followed by HP1BP3 (0.698), HNRNPC (0.683), YY1 (0.653), DDX5 (0.651), and SUPT16H (0.644). These results indicate that each gene carries measurable case-control signal within the analyzed dataset.

3.4 Exploratory performance of the combined six-gene model

A logistic-regression model integrating expression of all six genes showed an AUC of 0.806 (Fig. 5a). Within GSE137143, this value was higher than the AUC of any individual gene (Fig. 5b). Because model fitting and evaluation were performed in the same dataset, this analysis represents exploratory discrimination and requires independent validation before clinical interpretation.

3.5 Predicted miRNA interactions and construction of the LINC00641-associated RNA network

miRWalk analysis identified predicted miRNAs targeting the six selected genes (Fig. 6a). An UpSet analysis showed that 11 predicted miRNAs were shared across all six gene sets (Fig. 6b). Integration of LINC00641, the six associated genes, and these shared predicted miRNAs generated a candidate LINC00641-associated RNA interaction network (Fig. 6c). This network is intended as a hypothesis-generating representation of potential RNA-regulatory relationships rather than evidence of direct regulatory interactions. The RNAs included in the final predicted network are listed in Supplementary Table S1.

3.6 Functional enrichment of the six LINC00641-associated genes

KEGG and Gene Ontology enrichment analyses were performed for DDX5, HNRNPC, THRAP3, YY1, SUPT16H, and HP1BP3. The spliceosome pathway was significantly enriched (adjusted P = 0.0024), with DDX5 and HNRNPC contributing to this term. Additional enriched pathways included transcriptional misregulation in cancer and proteoglycans in cancer. Gene Ontology Biological Process analysis identified chromatin organization (adjusted P = 1.07 × 10^−4), mRNA catabolic process, and regulation of alternative mRNA splicing via the spliceosome among the most significantly enriched terms. Collectively, the enrichment results link the six-gene set mainly to RNA processing, splicing regulation, mRNA metabolism, and chromatin organization. Detailed enrichment results are provided in Table 2.

Table 2
Functional enrichment analysis of 6 diagnostic mRNAs.

KEGG			
Term	**Adjusted P-value**	**Combined Score**	**Genes**
Spliceosome	0.002465225	476.3096764	DDX5;HNRNPC
Transcriptional misregulation in cancer	0.059952482	59.68054719	DDX5
Proteoglycans in cancer	0.059952482	54.60105617	DDX5
Gene Ontology - Biological Process			
Term	**Adjusted P-value**	**Combined Score**	**Genes**
Chromatin Organization (GO:0006325)	1.07E-04	1707.149654	YY1;SUPT16H;HP1BP3;HNRNPC
mRNA Catabolic Process (GO:0006402)	0.003267717	1982.427537	DDX5;THRAP3
Regulation of Alternative mRNA Splicing, via Spliceosome (GO:0000381)	0.003267717	1713.735028	DDX5;THRAP3
mRNA Splicing, via Spliceosome (GO:0000398)	0.016026261	304.2469676	DDX5;HNRNPC
mRNA Processing (GO:0006397)	0.016026261	277.671411	DDX5;HNRNPC

4 Discussion

This study combines experimental measurement of LINC00641 in peripheral blood with transcriptomic and network analyses to investigate its potential relevance to MS. LINC00641 was significantly upregulated in the study cohort, and six LINC00641-associated genes identified through interaction and differential-expression analyses also showed increased expression in the public RNA-seq dataset. The selected genes were enriched for functions related to RNA processing, splicing, and chromatin organization, and predicted miRNA interactions were used to construct a candidate LINC00641-associated RNA network.

Altered RNA processing is increasingly recognized as an important component of MS biology. Alternative splicing can influence immune function and disease susceptibility, as illustrated by the well-characterized effects of IL7R exon 6 splicing and PLP1/DM20 isoform regulation [27–30]. The enrichment of the present six-gene set for spliceosomal and mRNA-processing functions is therefore consistent with a broader connection between RNA-regulatory abnormalities and MS.

DDX5 is an RNA helicase with established roles in RNA structure, transcriptional regulation, pre-mRNA splicing, and non-coding RNA processing. Previous work has reported increased DDX5 expression in treatment-naïve RRMS compared with healthy controls and associations between DDX5, RMRP, and RORC expression [31]. Its inclusion in the present gene set provides an independent line of support for an association between altered RNA-processing machinery and MS.

HNRNPC is a heterogeneous nuclear ribonucleoprotein involved in pre-mRNA processing and other aspects of mRNA metabolism. Although the specific contribution of HNRNPC to MS requires further study, dysregulation of the related hnRNP A1 protein has been linked experimentally to abnormal RNA splicing, neurite loss, and neurodegenerative features relevant to MS [32]. Together with the enrichment results, these observations support further investigation of RNA-binding and splicing factors represented in the identified LINC00641-associated gene set.

The predicted miRNA analysis identified 11 miRNAs shared among all six selected genes. These relationships are computational predictions and do not establish that LINC00641 directly regulates the six genes through a competing-endogenous-RNA or other post-transcriptional mechanism. Instead, the integrated network provides a focused set of candidate interactions that can be tested experimentally in future studies. Similarly, the ROC analyses indicate case-control signal in the studied datasets but do not by themselves establish clinical diagnostic utility.

4.1 Limitations

Several limitations should be considered. First, although LINC00641 expression was measured experimentally in an independent clinical cohort, the downstream mRNA and miRNA relationships were derived from public transcriptomic data and interaction-prediction resources and therefore require functional validation. Second, the discriminatory performance of the six-gene logistic-regression model was evaluated within the same RNA-seq dataset used to derive the candidates; independent validation and internal resampling approaches will be needed to establish model generalizability. Third, the age distributions of the MS and control groups differed, which may influence peripheral-blood gene-expression measurements. Finally, the present study does not establish direct regulatory interactions among LINC00641, the predicted miRNAs, and the six associated genes.

5 Conclusion

This study identifies increased peripheral-blood expression of LINC00641 in MS and describes a LINC00641-associated molecular signature comprising DDX5, HNRNPC, THRAP3, YY1, SUPT16H, and HP1BP3. Integration with predicted miRNA interactions and functional enrichment links this gene set primarily to RNA splicing, mRNA processing, and chromatin organization. The expression and ROC analyses further indicate potential case-control discriminatory value of LINC00641 and the six-gene signature in the studied cohorts. These findings provide a basis for independent validation and functional investigation of the proposed LINC00641-associated RNA network in MS.

Abbreviations

AS
alternative splicing
AUC
area under the curve
CNS
central nervous system
DEG
differentially expressed gene
EDSS
Expanded Disability Status Scale
GEO
Gene Expression Omnibus
GO
Gene Ontology
KEGG
Kyoto Encyclopedia of Genes and Genomes
lncRNA
long non-coding RNA
miRNA
microRNA
MS
multiple sclerosis
PPI
protein–protein interaction
qRT-PCR
quantitative real-time PCR
ROC
receiver operating characteristic
RRMS
relapsing–remitting multiple sclerosis
SPMS
secondary progressive multiple sclerosis
VST
variance-stabilizing transformation.

Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

The study protocol was approved by the Ethics Committee of the University of Isfahan (approval code: IR.UI.REC.1402.016). The study was conducted in accordance with the ethical standards of the institutional ethics committee and the Declaration of Helsinki and its subsequent amendments.

Consent to participate:

Informed consent was obtained from all individual participants included in the study.

Clinical trial number:

not applicable.

Declarations

of Generative AI use

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Author Contribution

N.S. performed the experiments and data analysis and wrote the manuscript. Z.S. and Z.H. supervised the study and critically revised the manuscript. Z.S. is the corresponding author and is responsible for the manuscript and communication with the journal. All authors reviewed and approved the final manuscript.

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Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request

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Figures

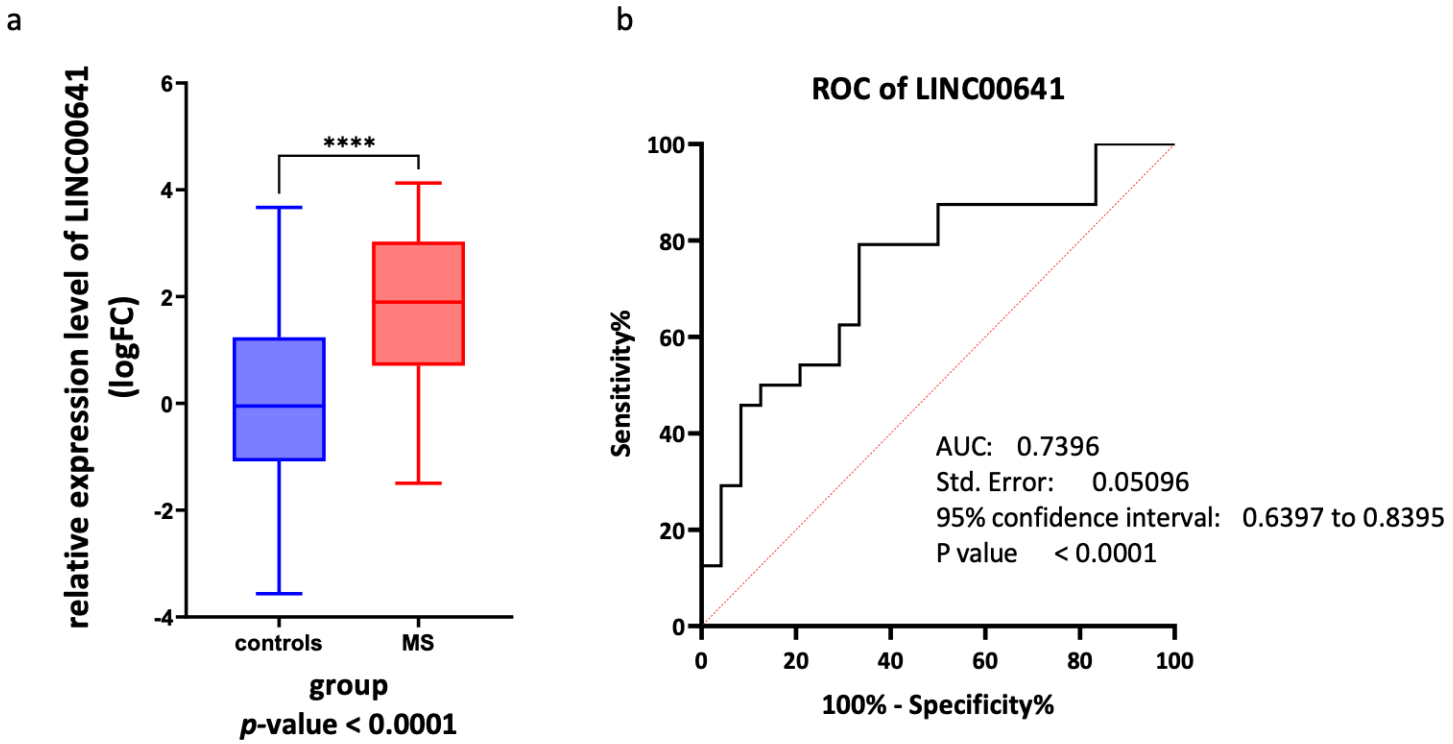


Figure 1

Expression and case-control discriminatory performance of LINC00641 in MS. (a) Boxplot showing significantly higher LINC00641 expression in patients with MS than in healthy controls. (b) ROC curve for discrimination between MS and control samples based on LINC00641 expression (AUC = 0.7396).

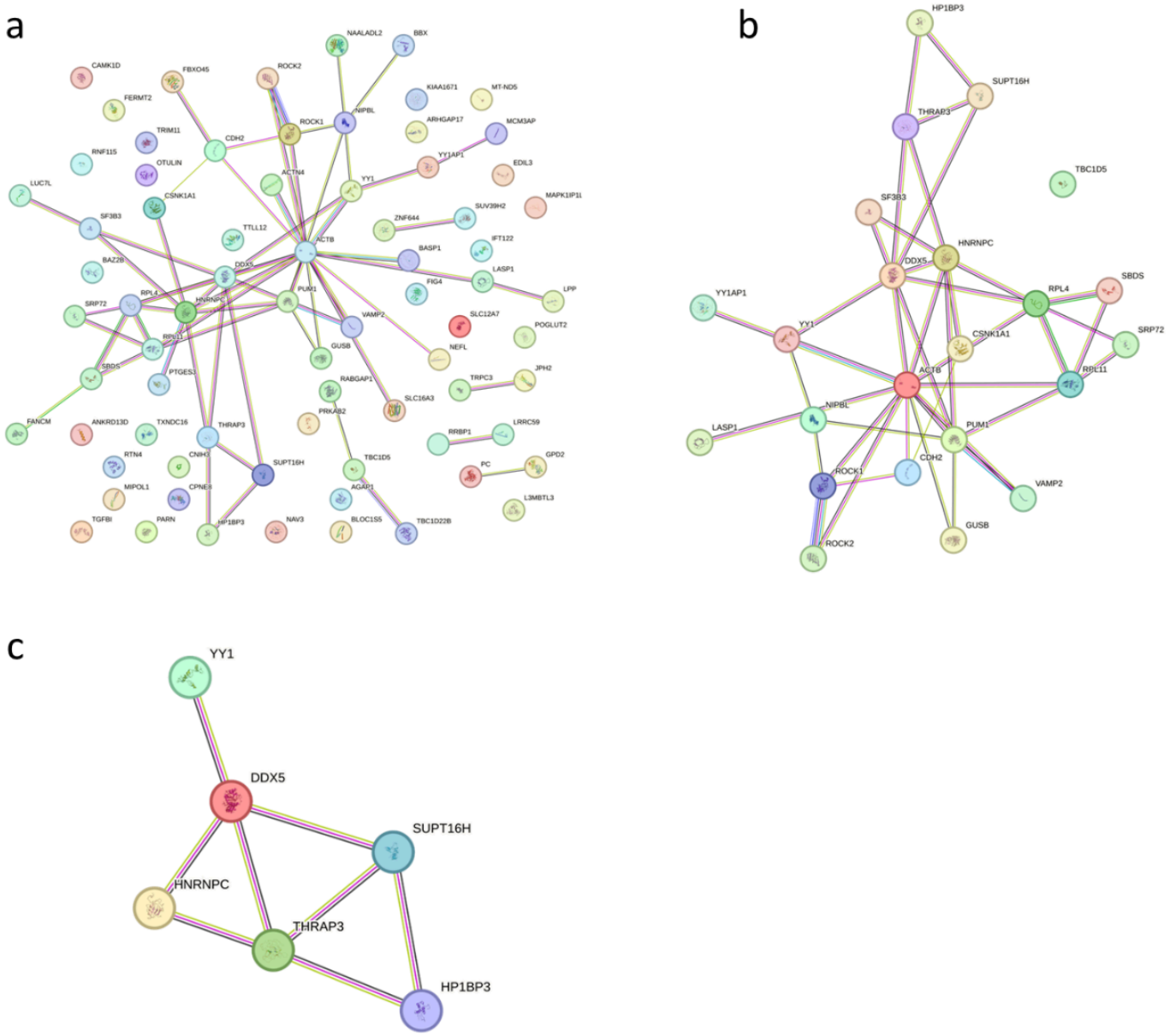


Figure 2

Construction and filtering of the LINC00641-associated interaction network. (a) Full PPI network generated from proteins encoded by LINC00641-associated mRNAs retrieved from ENCORI and analyzed using STRING. (b) Refined interaction network containing proteins with degree ≥2. (c) Six LINC00641-associated genes—DDX5, HNRNPC, THRAP3, YY1, SUPT16H, and HP1BP3—showed significantly higher expression in MS samples and were retained for downstream analyses.

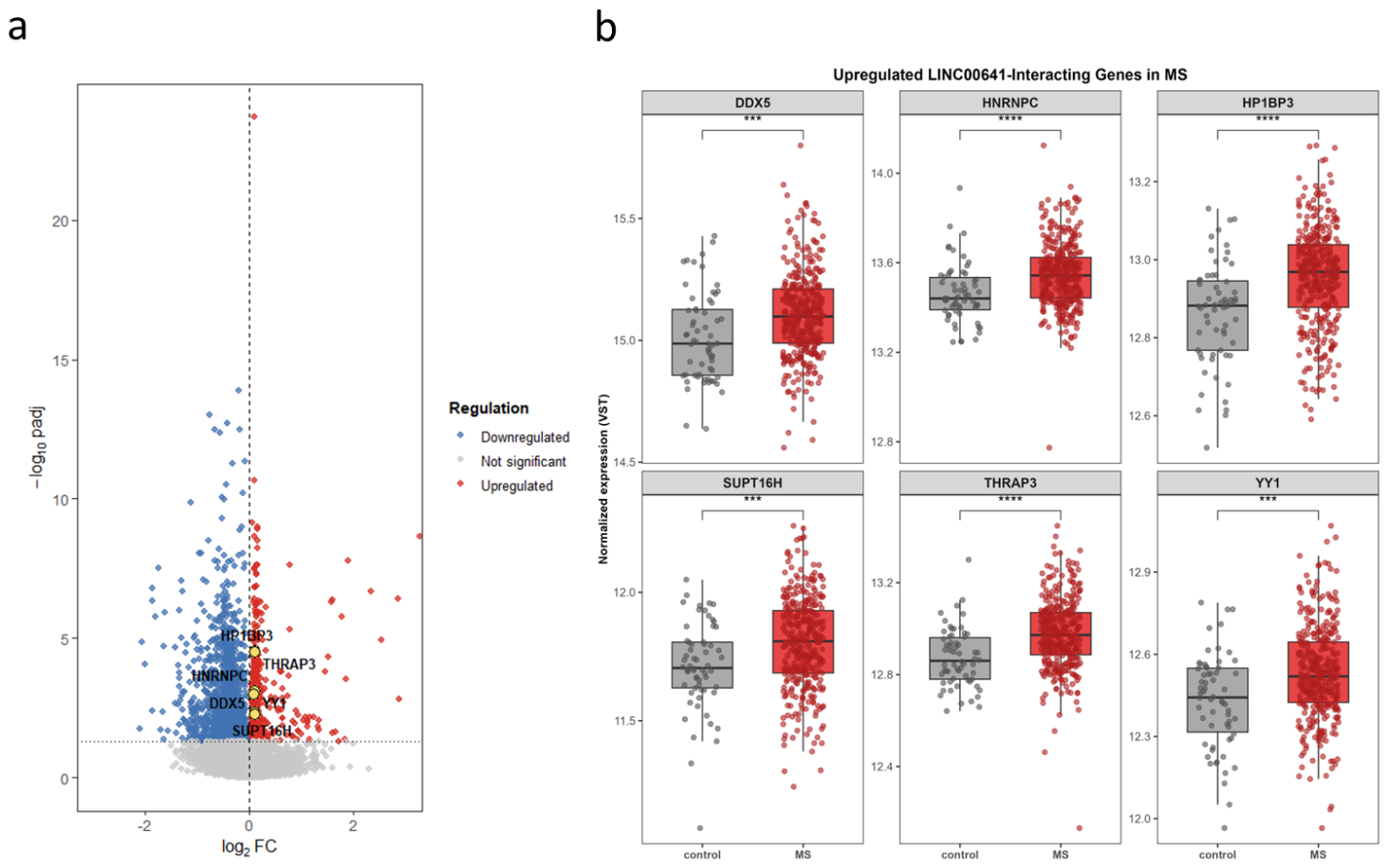


Figure 3

Differential expression of LINC00641-associated genes in MS. (a) Volcano plot showing the distribution of differentially expressed genes in MS versus healthy controls. The six selected LINC00641-associated genes are highlighted. (b) Expression distributions of DDX5, HNRNPC, THRAP3, YY1, SUPT16H, and HP1BP3 in MS and control samples. All six genes showed significantly higher expression in MS (adjusted $P < 0.05$).

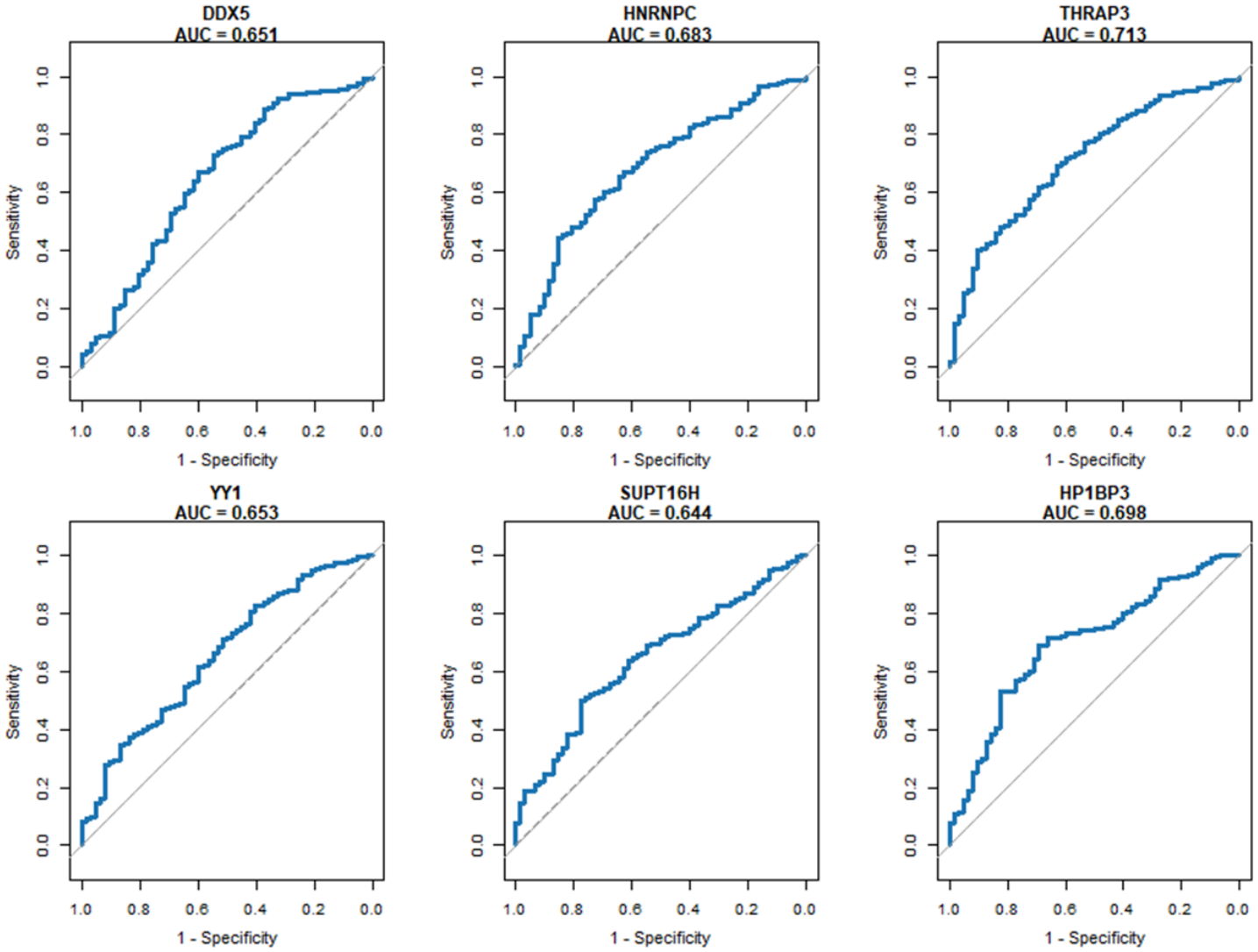


Figure 4

ROC analysis of the six LINC00641-associated genes. ROC curves and AUC values are shown for DDX5, HNRNPC, THRAP3, YY1, SUPT16H, and HP1BP3 using variance-stabilized expression values from GSE137143. Individual AUC values ranged from 0.644 to 0.713.

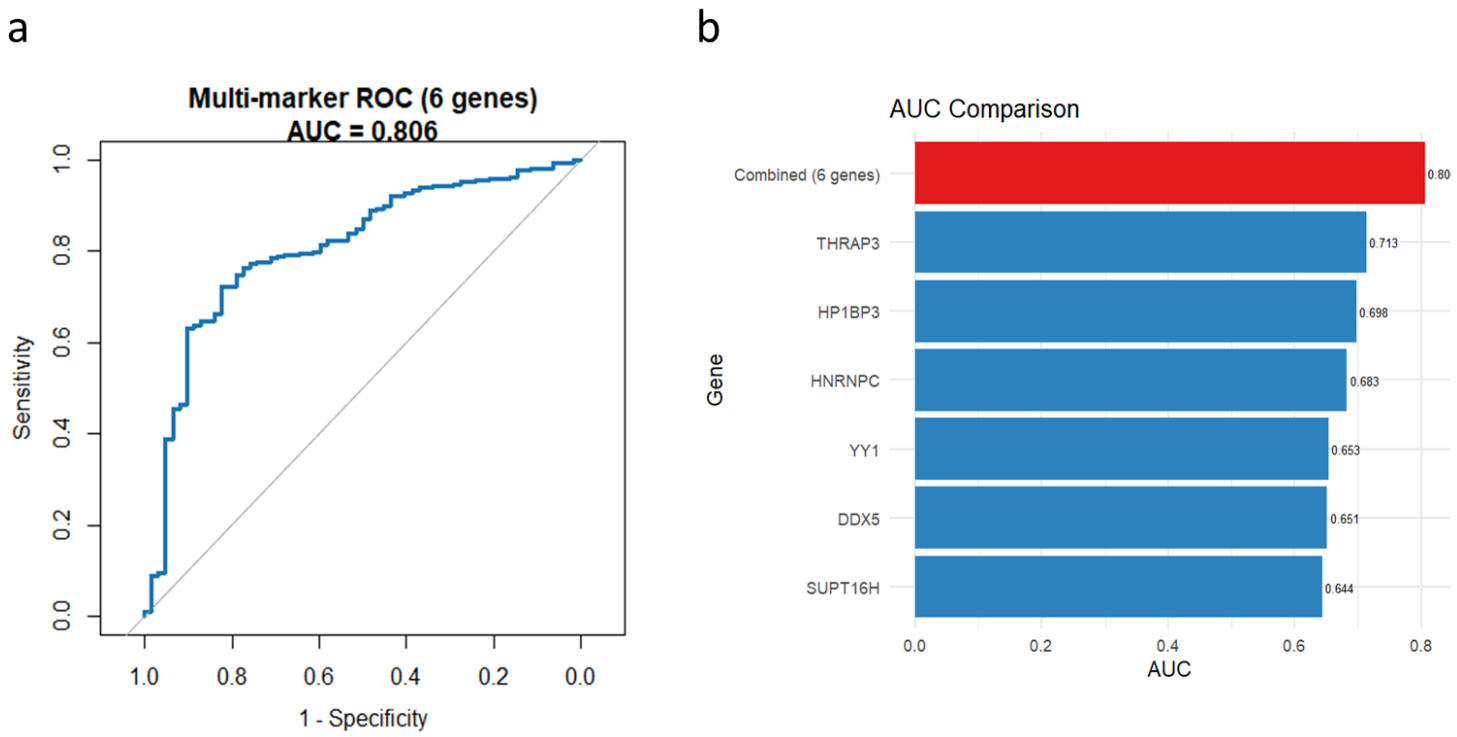


Figure 5

Exploratory discriminatory performance of the combined six-gene model. (a) ROC curve for the logistic-regression model combining DDX5, HNRNPC, THRAP3, YY1, SUPT16H, and HP1BP3 (AUC = 0.806). (b) Comparison of the AUC of the combined model with those of the individual genes. Performance was evaluated within GSE137143 and therefore requires independent validation.

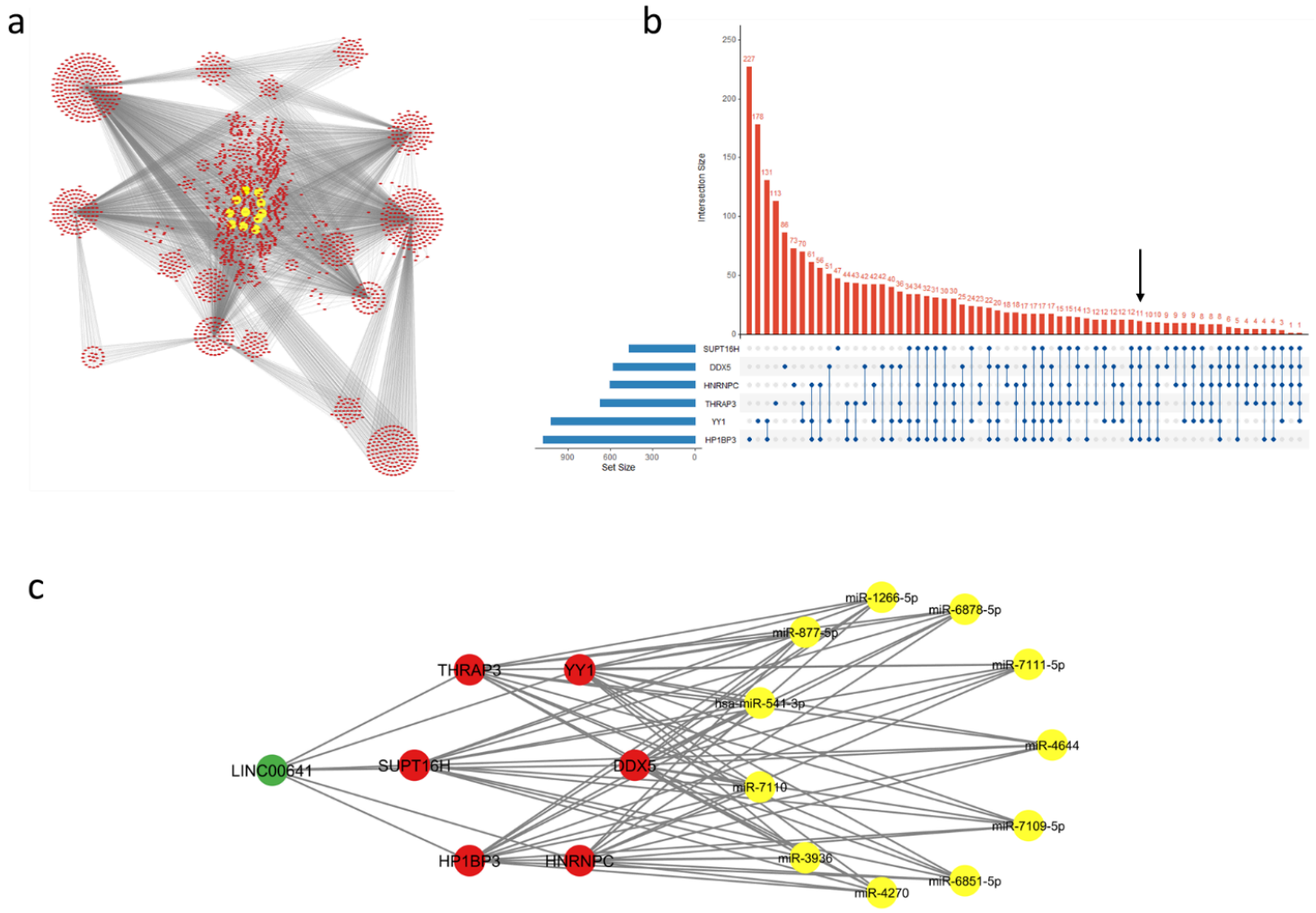


Figure 6

Predicted miRNA interactions and construction of the LINC00641-associated RNA network. (a) Predicted miRNA–mRNA interaction network for the six selected genes; yellow nodes indicate miRNAs shared by all six gene sets. (b) UpSet plot showing overlap among predicted miRNAs, including 11 miRNAs shared across all six genes. (c) Candidate LINC00641-associated RNA network integrating LINC00641, the six selected genes, and the 11 shared predicted miRNAs. The network represents predicted associations and is intended as a hypothesis-generating model.