

Integrated Analysis of lncRNA-miRNA-mRNA ceRNA Network and Identification of Hub lncRNAs in Molecular Subtypes of Gastric Cancer as Potential Prognostic Indicators

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Abstract

Background: Gastric cancer (GC) is the fourth most common cause of death among cancers in the world, and the most prevalent type of GC is adenocarcinoma. The Cancer Genome Atlas (TCGA) project introduced 4 molecular subtypes of GC adenocarcinomas: Epstein-Barr virus (EBV), microsatellite instability (MSI), genomically stable (GS), and chromosomal instability (CIN). However, the function of long noncoding RNAs (lncRNAs) in these subtypes still remains unknown. Here, we aimed to construct a competing endogenous RNA (ceRNA) network to clarify the role of lncRNAs in each GC subtype and predict patients' overall relapse-free survival (RFS) time.

Methods: The RNA-seq data of miRNAs, lncRNAs, and mRNAs related to different GC subtypes and their corresponding normal samples from TCGA were analyzed, and a combination of signed and unsigned weighted gene co-expression network analysis (WGCNA) (csuWGCNA) was recruited to determine co-expressing gene modules. Then, lncRNA-miRNA and miRNA-mRNA interactions were predicted, and the ceRNA regulatory network was established, followed by the identification of hub lncRNAs for any GC subtype. Gene set enrichment analyses were applied to protein-coding genes of each module to investigate their functions. Finally, survival analysis was performed for identified hub lncRNAs.

Results: Differentially expressed lncRNAs, miRNAs, and mRNAs related to each GC subtype were identified, ceRNA networks were constructed, and in each subtype, the top 5 lncRNAs with the most MCC scores were chosen as hub lncRNAs. Survival analysis revealed that ACI38356.1, LINC01270, and ALI18506.1 lncRNAs were associated with the RFS of CIN subtype, LINC02099, ALI57871.2, AC068580.3, and AC004264.1 lncRNAs with EBV subtype, ALI17335.1 and AC011416.3 lncRNAs with MSI subtype, and MIR210HG and LINC00565 lncRNAs with GS subtype patients.

Conclusion: The identified hub lncRNAs provide insights for understanding molecular mechanisms underlying GC subtype transformation and may be useful to predict the RFS of corresponding patients; more research is required to confirm the results.

Keywords

Gastric cancer molecular subtypes, ceRNA, csuWGCNA, hub lncRNA, prognosis factor

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Introduction

Gastric cancer (GC) is a major global health challenge. The GLOBOCAN 2022 report indicates that GC is the fifth most common cancer and the fourth leading cause of cancer-related mortality worldwide, with over 1 million new cases annually and approximately 1 in every 12 cancer-related deaths.¹ Adenocarcinomas account for over 95% of GC cases.² Men face a 2-fold higher risk of developing and dying from GC compared to women.³ Despite declining global incidence and mortality rates, GC remains prevalent in Asia, contributing to nearly 75% of new cases and deaths.

With a 5-year survival rate of around 20%, GC is among the deadliest cancers.⁴ Extensive research has explored gene expression profiles in GC, identifying numerous

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Table 1. Number of different subtype samples and corresponding normal samples in both RNA-seq and miRNA-seq data.

Sample subtype	RNA-seq tumor (n)	miRNA-seq tumor (n)	RNA-seq corresponding normal (n)	miRNA-seq corresponding normal (n)
CIN	155	158	13	15
MSI	69	77	9	15
EBV	33	34	1	1
GS	90	104	8	9

differentially expressed sequences potentially driving its onset and progression.⁵⁻⁷ Recent bioinformatics studies, utilizing single-cell RNA sequencing and competing endogenous RNA (ceRNA) network analyses, have further unraveled the molecular complexity of GC, highlighting potential diagnostic and therapeutic targets.⁸⁻¹⁰ The Cancer Genome Atlas (TCGA) project has characterized 4 molecular subtypes of GC: Epstein-Barr virus-positive (EBV), microsatellite instable (MSI), genomically stable (GS), and chromosomally unstable (CIN) tumors.¹¹ While these subtypes offer valuable insights, the specific contributions of long noncoding RNAs (lncRNAs) to their molecular profiles remain largely unexplored, necessitating further investigation to inform personalized therapeutic approaches.

Long noncoding RNAs are RNA molecules exceeding 200 nucleotides in length, typically lacking protein-coding capacity.¹² Their high tissue specificity positions them as promising biomarkers for various diseases, including cancer.^{13,14} Growing evidence underscores the pivotal role of lncRNAs in GC progression, including cell proliferation, invasion, metastasis, and angiogenesis.¹⁵⁻¹⁸ The lncRNAs regulate diverse biological processes through multiple mechanisms, notably as competing endogenous RNAs (ceRNAs).¹⁹⁻²¹ The ceRNA framework elucidates a sophisticated post-transcriptional regulatory network involving lncRNA-miRNA-mRNA interactions, which significantly influence cancer development.²²⁻²⁴ Recent studies have highlighted lncRNA-mediated ceRNA networks as critical regulators in several cancers such as breast, colorectal, lung, and GC, identifying them as potential prognostic indicators and therapeutic targets.²⁵⁻³⁰

To date, comprehensive analyses of lncRNAs within GC molecular subtypes, particularly their clinical significance and mechanistic roles, remain scarce. In this study, we utilized a bioinformatics approach to analyze high-throughput RNA sequencing data from TCGA (<http://cancergenome.nih.gov/>), focusing on the Stomach Adenocarcinoma (STAD) dataset. Our objective was to construct an lncRNA-miRNA-mRNA ceRNA network to identify key lncRNAs, assess their clinicopathological relevance, and elucidate their mechanistic contributions to the progression of GC molecular subtypes.

Materials and Methods

Data collection

The TCGA database (<http://cancergenome.nih.gov/>) is a repository created by the US National Cancer Institute,

with data on 33 different cancer types. The RNA-seq and miRNA-seq data for GC patients were downloaded from the TCGA database (Table 1); the gene expression profile has been measured experimentally using the Illumina HiSeq2000 RNA Sequencing platform by the TCGA genome characterization center of the University of North Carolina. Clinical data such as survival-time, TNM staging, age, and gender were also retrieved. Hierarchical clustering was performed to detect sample outliers.

Data processing of differentially expressed genes

The mRNA, lncRNA, and miRNA differential expression analysis was performed separately in the R software (version 4.4.3; <https://cran.r-project.org/>) along with “DESeq2” package for each subtype.³¹ Raw *P*-values were adjusted according to the Benjamini and Hochberg method. Normal gastric tissue samples were limited across molecular subtypes (Table 1). Given that all normal samples share identical tissue origin (gastric mucosa), we aggregated all normal samples into a common reference set to maximize statistical power and ensure robustness. Subtype-specific differential expression was then assessed by comparing tumor samples from each molecular subtype against this pooled normal cohort, and differentially expressed genes (DEGs) defined as genes, with a corrected *P*-value < .05.

Finally, volcano plots were visualized using the “EnhancedVolcano” package to illustrate the distribution of each lncRNA according to the log₂ fold change (log₂FC) and false discovery rate (FDR) (Figure 1).

Weighted co-expression network analysis of lncRNAs and mRNAs

Weighted gene co-expression network analysis (WGCNA) is able to categorize co-expressing genes into multiple clusters and further investigate the relationship between co-expression modules and clinical phenotypes.³² The WGCNA may be calculated with signed or unsigned methods, but both methods fail to capture weak and moderate negative correlations, which may be important in gene regulation exerted by lncRNAs and miRNAs. Thus, we used a combination of signed and unsigned weighted gene co-expression network analysis (csuWGCNA), which combines the signed and unsigned methods and improves the detection of negative correlations, as well as reducing bias toward highly expressed genes, which is particularly useful for noncoding RNA analysis such as miRNAs and lncRNAs.³³

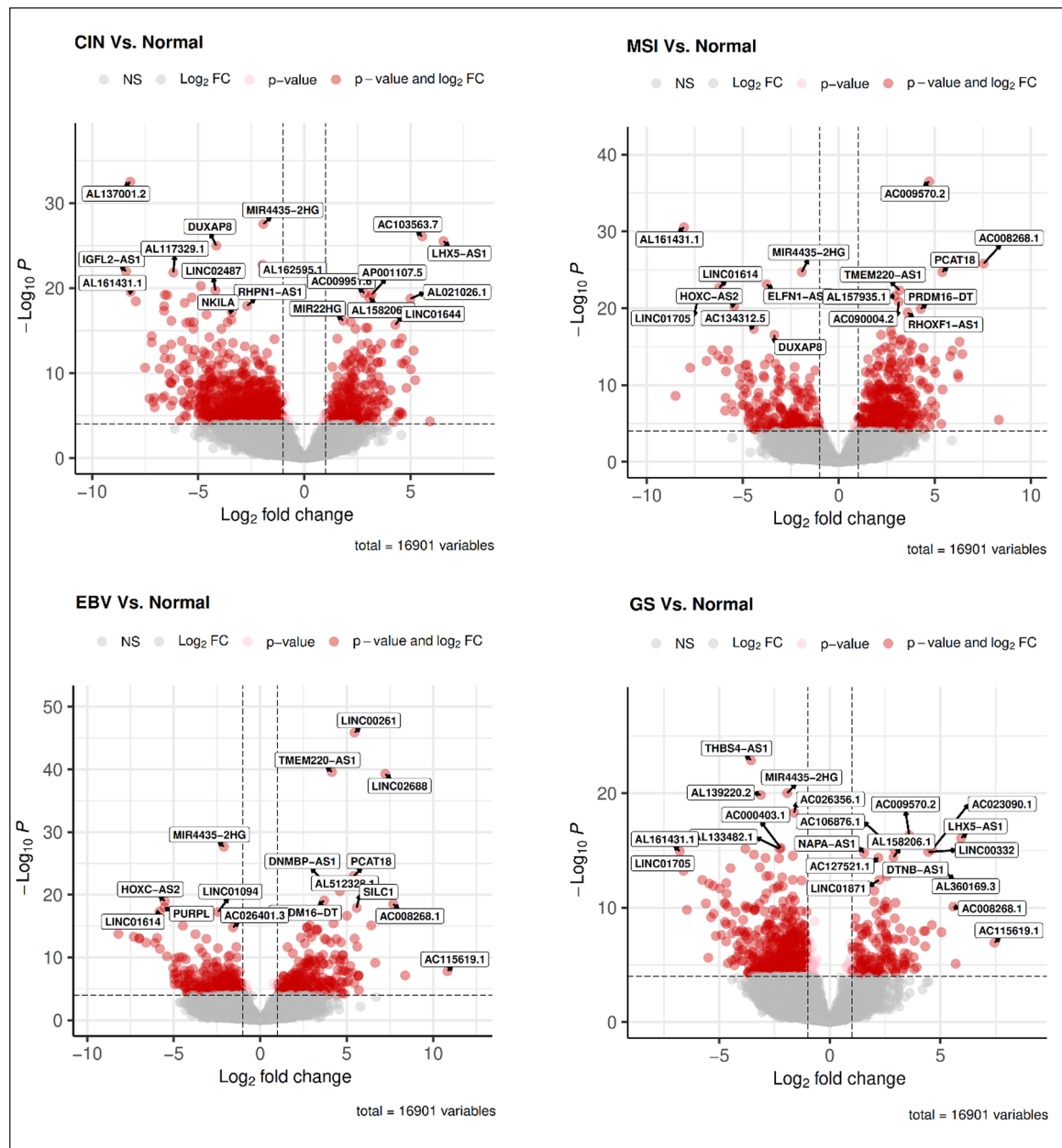


Figure 1. Volcano plots representing differentially expressed lncRNAs in each GC subtype. The horizontal line at false discovery rate (FDR) = 0.05.

First, the variance stabilizing transformation (VST) value was calculated via the DESeq2 package for each read count, and the input lncRNAs and mRNAs were filtered by removing those with less than 5 VST values in at least 75% of samples. Next, the Pearson correlation coefficient between all gene pairs in the selected samples was calculated to construct an adjacency matrix, then the adjacency function was defined, and module segmentation was performed based on the threshold of minimal module size of 30 genes. In addition, the correlation between modules and GC subtypes was calculated, and subtype-related modules were identified.

Gene set enrichment analysis

In order to investigate molecular function and corresponding pathways in which mRNAs of selected modules participate,

gene set enrichment analysis (GSEA) was performed via web-based gene set analysis toolkit (WebGestalt)³⁴ using Reactome terms. The FDR-value < 0.05 was considered statistically significant.

Construction of lncRNA-miRNA-mRNA competing endogenous RNA network

Differentially expressed miRNAs corresponding to each subtype that could target the lncRNAs and mRNAs in selected modules were predicted from RNAInter v4.0 (Kang, Tang et al. 2022) and StarBase database (<http://starbase.sysu.edu.cn/starbase2/>), and pairs with a combined score > 0.4 were extracted. The lncRNA-mRNA pairs regulated by the same miRNAs were integrated into lncRNA-miRNA-mRNA network and visualized by Cytoscape. By

Table 2. Number of differentially expressed genes corresponding to each subtype.

Subtype	lncRNA		mRNA		miRNA	
	Upregulated (n)	Downregulated (n)	Upregulated (n)	Downregulated (n)	Upregulated (n)	Downregulated (n)
CIN	583	2504	5963	5849	356	152
EBV	570	982	4405	6205	243	167
GS	412	2397	3968	5382	183	169
MSI	814	634	4987	6405	302	107

performing topology analysis on the network, hub lncRNAs were identified using the Maximal Clique Centrality (MCC) method and ranked according to MCC score. The top 5 hub lncRNAs were chosen for subsequent analysis.

Immune cell infiltration analysis

To characterize the tumor immune microenvironment across GC molecular subtypes, we performed immune cell deconvolution using the xCell algorithm.³⁵ xCell calculates enrichment scores for immune and stromal cell types based on gene expression signatures. The normalized gene expression matrix transcripts per million (TPM; values) of TCGA-STAD samples was used as input. Differences in immune cell infiltration between tumor subtypes and normal gastric tissue were assessed using the Dunnett test, which controls for multiple comparisons against a common control group (normal tissue). Associations between hub lncRNA expression levels and immune cell infiltration scores in tumor samples were evaluated using the Spearman rank correlation. *P*-values were adjusted for multiple testing using the Benjamini-Hochberg method.

Survival analysis of hub lncRNAs

The Kaplan-Meier (KM) plotter tool³⁶ was recruited to evaluate the prognostic value of hub lncRNAs by determining their relapse-free survival (RFS) time. Patients were divided into 2 groups, high- and low-expression patients, according to the ratio of expression level of lncRNA to the median expression level of that lncRNA in the subtype patients, and KM survival curves were calculated. A *P*-value less than 0.05 was regarded as the cut-off criterion.

Results

Identification of differentially expressed lncRNAs, miRNAs, and mRNAs

Differentially expressed lncRNAs, miRNAs, and mRNAs were identified distinctively between tumor and respective normal tissue pairs of different subtypes of GC. We detected 3087 differentially expressed lncRNAs, including 583 upregulated and 2504 downregulated in CIN subtype, 1552 differentially expressed lncRNAs, including 570 upregulated and 982 downregulated in EBV subtype, 2809 differentially expressed lncRNAs, including 412 upregulated and 2397 downregulated in GS subtype, and 1448 differentially

expressed lncRNAs, including 814 upregulated and 634 downregulated in MSI subtype. Figure 1 represents corresponding key differentially expressed lncRNAs for each subtype. Similarly, differentially expressed miRNAs and mRNAs corresponding to each subtype were identified (Table 2 and Supplementary Figures 1 and 2).

Construction of weighted gene co-expression network and modules significance calculation

By filtering input lncRNAs and mRNAs with VST value less than 5 in at least 75% of samples, 12 583 mRNAs and 2655 lncRNAs remained for the WGCNA analysis. Obvious outlier samples were removed (Supplementary Figure 3), and a soft threshold=12 was selected to guarantee high-scale independence and low mean connectivity (Figure 2A). A scale-free network was constructed, and as shown in Figure 2B, the first set of modules was identified, then correlated modules were merged, and in total 39 modules were obtained. The correlations of each module eigengene with GC subtypes were calculated. We found that the saddle brown module ($R=0.74$, $P=1e-30$) was significantly associated with the CIN subtype, the bisque4 module ($R=0.71$, $P=7e-28$) with the GS subtype, thistle1 ($R=0.57$, $P=3e-16$) and violet ($R=0.4$, $P=8e-8$) with the EBV subtype, and coral1 ($R=0.48$, $P=2e-11$) and plum ($R=0.43$, $P=4e-9$) with the MSI subtype (Figure 3 and Supplementary Figure 4), and these modules were selected for further analysis (Table 3).

Construction of lncRNA-miRNA-mRNA competing endogenous RNA network

Differentially expressed miRNAs corresponding to each subtype and the lncRNAs and mRNAs in selected modules were used, and miRNA-lncRNA interactions and miRNA-mRNA interactions were combined and visualized using the Cytoscape version 3.10.4 to construct a complete lncRNA-miRNA-mRNA ceRNA network related to each subtype. In each subtype, the top 5 lncRNAs with the most MCC scores were chosen as hub lncRNAs, including lncRNAs AC138356.1, LINC01270, AP003469.2, AL139384.1, and AL118506.1 for CIN subtype, lncRNAs LINC02099, AL157871.2, AC068580.3, MIR3945HG, and AC004264.1 for EBV subtype, lncRNAs AL117335.1, AC100861.1, AC016065.1, AC139019.1, and AC011416.3 for MSI subtype, and lncRNAs MIR210HG, AC010931.1, AL136090.2, AC005618.1, and LINC00565 for GS subtype. A selected

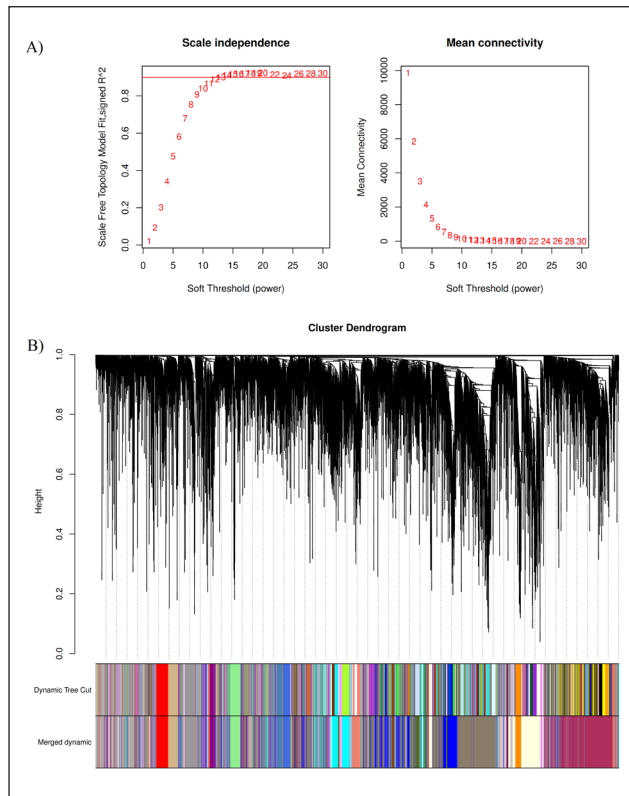


Figure 2. WGCNA analysis of lncRNAs and mRNAs. (A) Indication of the scale-free fitting index of the network topology obtained by the soft-threshold power analysis method. (B) Hierarchical clustering dendrogram of identified co-expressed genes. Each colored row represents a color-coded module, which contains a group of highly connected genes.

part of the ceRNA network related to hub lncRNAs for each subtype is shown in Figure 4.

Gene set enrichment analysis

In order to investigate dysregulated pathways corresponding to each subtype, the upregulated and downregulated mRNAs of selected modules were analyzed. The main pathways for the upregulated genes in the CIN subtype were related to the neural system, and the downregulated genes corresponded to membrane trafficking and vesicle transport pathways. Upregulated and downregulated genes in the GS subtype were mostly related to signal transduction and immune system, respectively. Dysregulated pathways in EBV subtype for upregulated genes were most related to immunity, while downregulated ones participated in different subjects such as neural system, metabolism, and signaling. In the case of MSI subtype, upregulated genes were mostly connected to membrane affairs, whereas downregulated ones were in association with protein modification, signaling, and innate immunity (Figure 5).

Immune cell infiltration analysis

Analysis of immune cell infiltration revealed significant heterogeneity in the tumor immune microenvironment

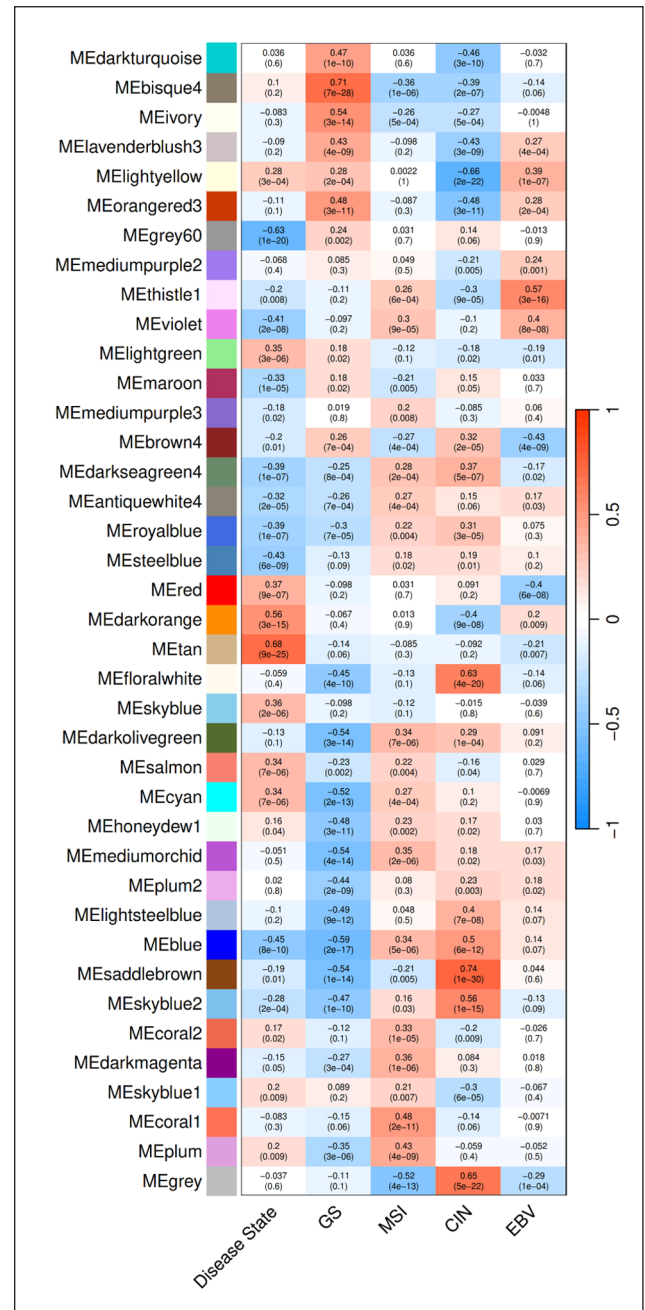


Figure 3. Heatmap of module-phenotype relationships depicting correlations between module eigengenes and GC molecular subtypes. Numbers in the table correspond to the correlation and the P-value in parentheses. The degree of correlation is illustrated with the color legend.

across GC molecular subtypes (Figures 6 and 7). Using the xCell deconvolution algorithm, we quantified enrichment scores for 21 immune and stromal cell types in TCGA-STAD samples, including both tumor and adjacent normal tissues. Correlation analysis between hub lncRNA expression and immune cell infiltration levels in the CIN and EBV molecular subtypes revealed a striking inverse pattern. A broad panel of immune cells—including CD4⁺ and CD8⁺ T cells, regulatory T cells, effector memory T cells, TEMRA cells, B cells, plasma cells, myeloid cells (monocytes, neutrophils, granulocytes, macrophages, conventional dendritic

Table 3. Most significant modules correlated to each GC subtype and their specifications.

Module color	No. genes	No. lncRNAs	No. mRNAs	Correlated subtype	Correlation	P-value
Saddlebrown	222	20	202	CIN	0.74	1e-30
Bisque4	123	13	110	GS	0.71	7e-28
Thistle1	118	17	101	EBV	0.57	3e-16
Violet	204	23	181	EBV	0.4	8e-8
Coral1	92	8	84	MSI	0.48	2e-11
Plum	71	7	64	MSI	0.43	4e-9

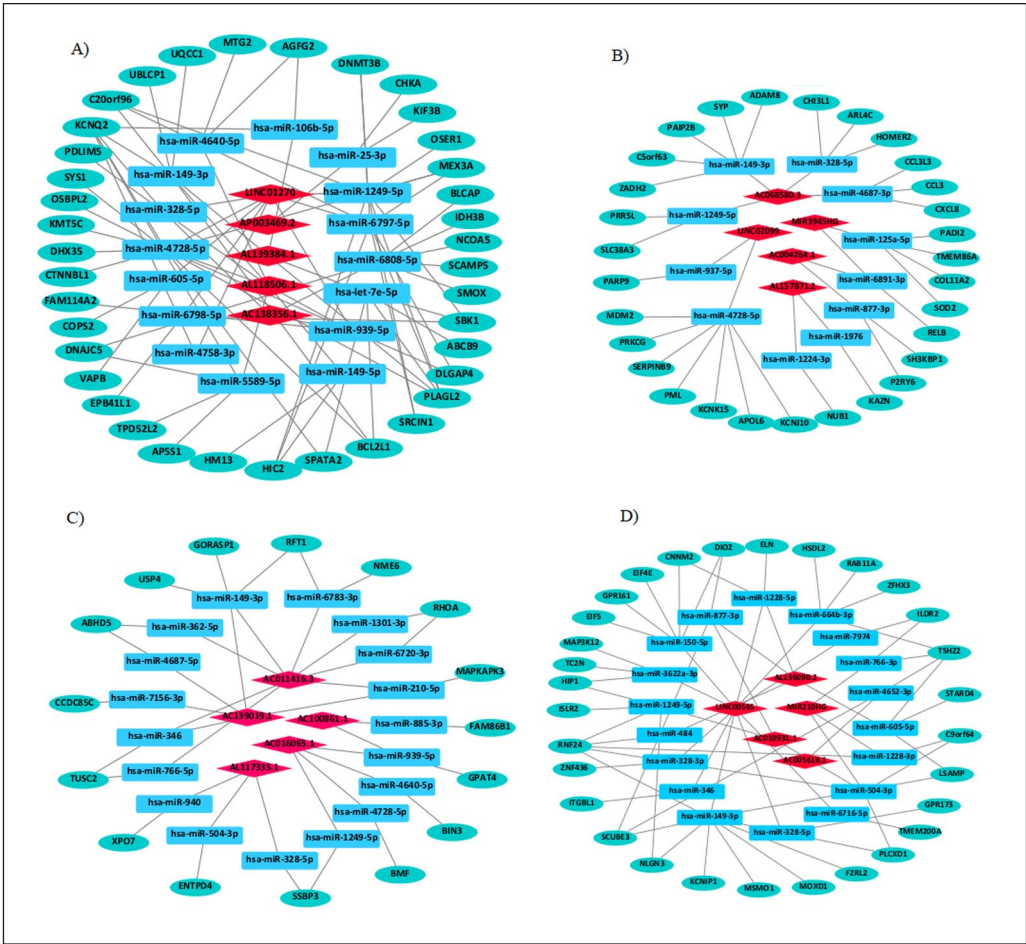


Figure 4. The lncRNA-miRNA-mRNA ceRNA subnetwork corresponding to (A) CIN identified hub lncRNAs, (B) EBV identified hub lncRNAs, (C) MSI identified hub lncRNAs, and (D) GS identified hub lncRNAs. Diamonds, rectangles, and ellipses represent lncRNAs, miRNAs, and mRNAs, respectively. Gray lines indicate the interactions between miRNA-lncRNA and miRNA-mRNA.

cells), NK cells (cytotoxic and regulatory subsets), eosinophils, basophils, and fibroblasts—showed significant negative correlations with CIN-specific hub lncRNAs. In contrast, the same immune cell populations exhibited strong positive correlations with EBV-specific hub lncRNAs. These findings suggest that hub lncRNAs in the CIN subtype may be associated with immunosuppressive or immune-excluded microenvironments, whereas in the EBV subtype, they likely contribute to an immunologically active and inflamed tumor microenvironment (Figure 6B).

Survival analysis and potential prognostic factor identification

To identify the lncRNAs with potential prognostic characteristics, univariate Cox regression analysis was performed for hub lncRNAs corresponding to each subtype. The results show that 3 lncRNAs (AC138356.1, LINC01270, and AL118506.1) were significantly associated with RFS of the CIN subtype, 4 lncRNAs (LINC02099, AL157871.2, AC068580.3, and AC004264.1) with the EBV subtype, 2

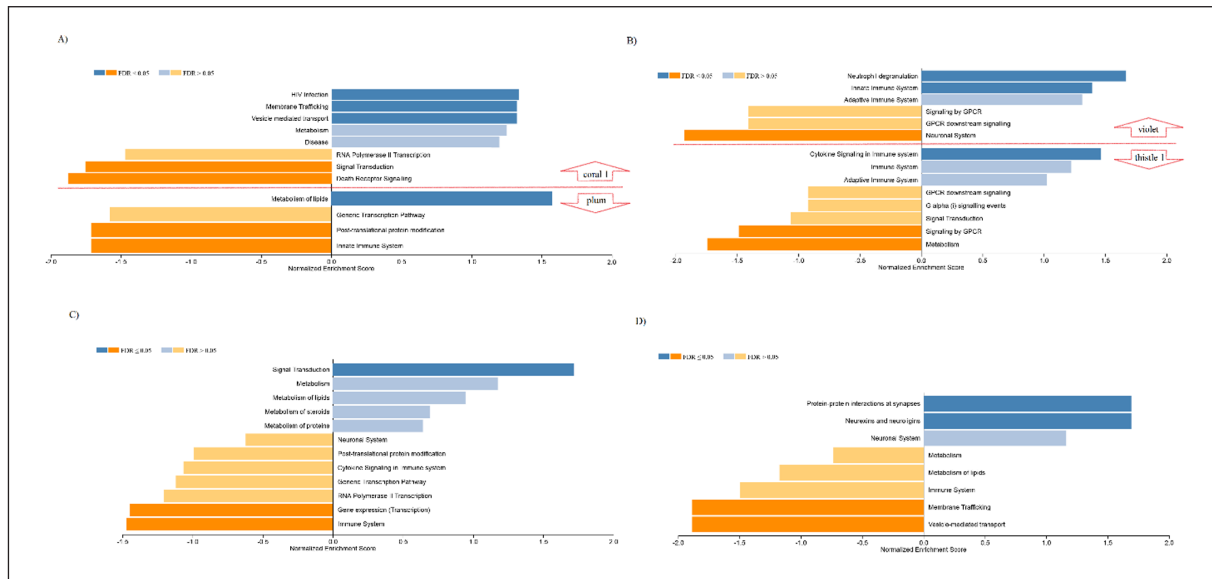


Figure 5. Gene set enrichment analysis for modules correlated to (A) MSI subtype, (B) EBV subtype, (C) GS subtype, and (D) CIN subtype, using the Reactome pathway database.

Higher positive (blue bars) or negative (orange bars) enrichment scores (x-axis) represent up or downregulation, respectively. The color legend on the top left corner represents FDR values.

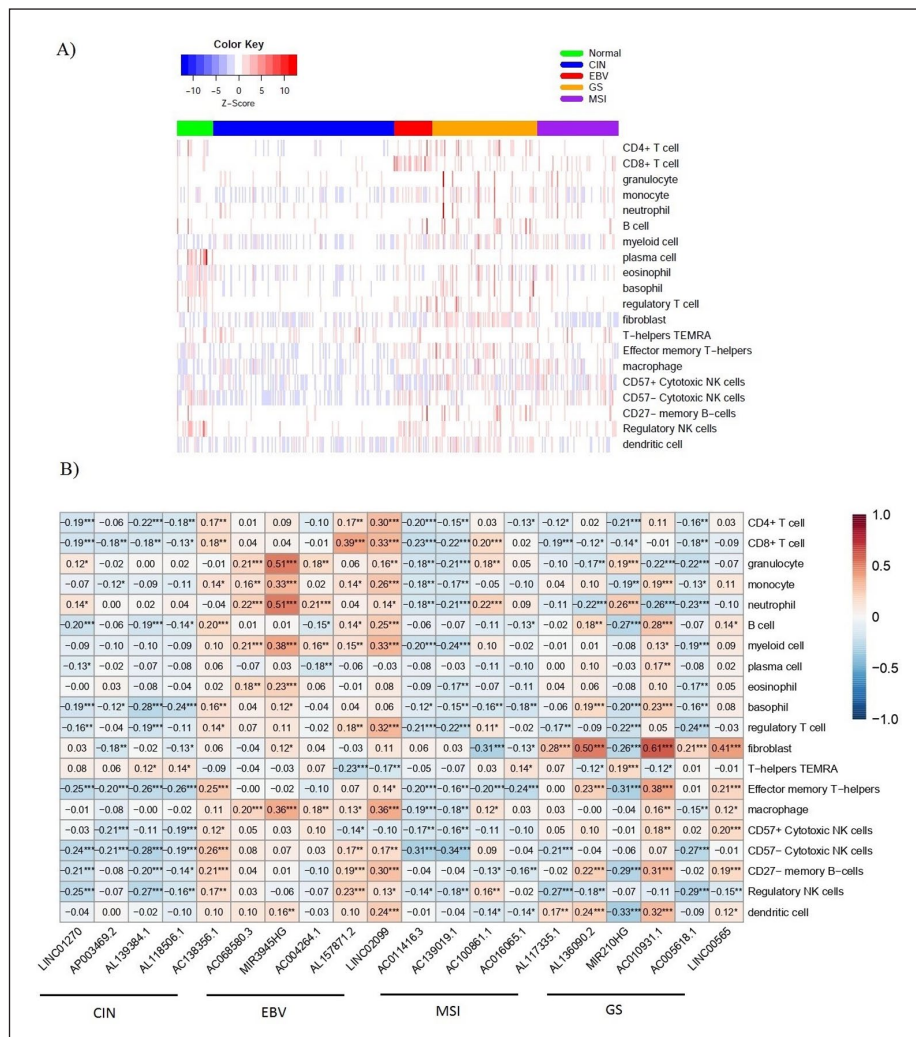


Figure 6. (A) Heatmap of immune cell infiltration scores across GC molecular subtypes and normal tissues. Rows represent the immune cell types, and columns represent the individual samples grouped by subtype (CIN, EBV, MSI, GS, Normal). Color intensity indicates the z-score normalized enrichment scores. (B) Heatmap of Spearman correlation coefficients between hub lncRNA expression and immune cell infiltration scores. The color scale indicates the correlation strength (red: positive, blue: negative). Asterisks denote significance levels: *P.adj < 0.05, **P.adj < 0.01, ***P.adj < 0.001.

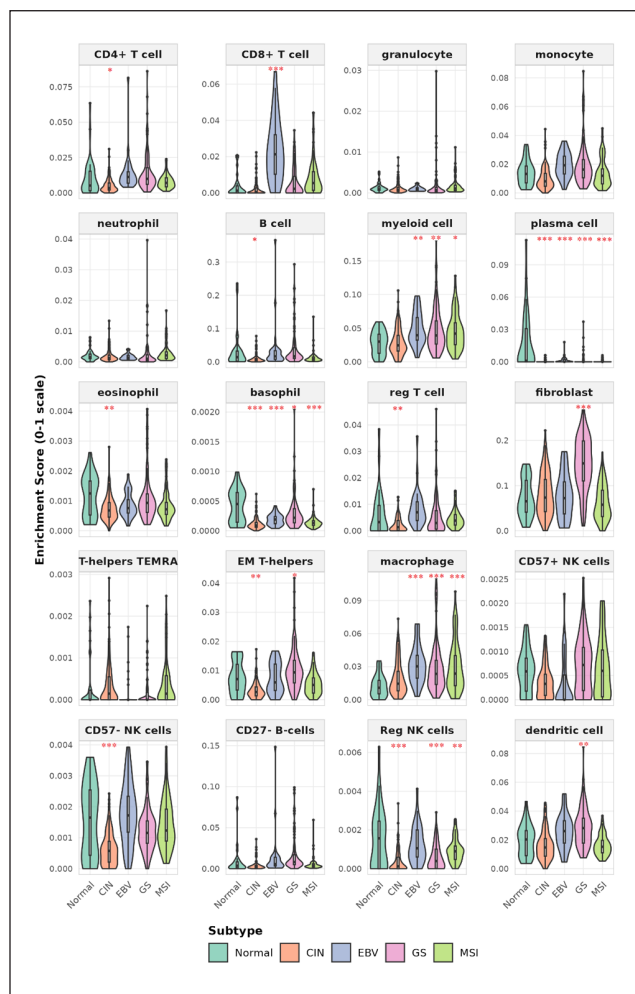


Figure 7. Violin plots showing distribution of selected immune cell infiltration scores across GC subtypes. Each panel represents 1 immune cell type.

Red asterisks indicate statistical significance from the Dunnett test comparing each tumor subtype to normal tissue: * $P_{adj} < 0.05$, ** $P_{adj} < 0.01$, *** $P_{adj} < 0.001$.

lncRNAs (AL117335.1 and AC011416.3) with the MSI subtype, and 2 lncRNAs (MIR210HG and LINC00565) with the GS subtype patients ($P < .05$) (Figure 8). The hazard ratio (HR) of these lncRNAs was more than 1, indicating that these lncRNAs may be a potential prognostic factor for their corresponding GC subtype. Cox analysis results of the rest of hub lncRNAs indicated P -values higher than .05; thus, these lncRNAs were not associated with patients' RFS time.

Differential expression analysis of hub lncRNAs

As the survival analysis based on the hub lncRNAs was meaningful, we investigated whether there is a difference in the expression levels of hub lncRNAs in the tumor and normal tissues. The results confirmed that except 1 lncRNA (AC005618.1), all others indicated significant differential expression in tumor tissues in comparison with corresponding normal samples (Figure 9). It is interesting that lncRNA

AC005618.1 did not indicate association with patients' RFS time.

Discussion

The comprehensive integrative analysis of the genome and proteome of GC tissues from TCGA uncovered 4 molecularly distinct subtypes.¹¹ They analyzed protein-coding and noncoding gene expression data of GC samples and identified potential prognostic miRNA biomarkers for predicting the survival of patients; however, the exact role of lncRNAs in the genomic context of these subtypes still remained to be elucidated in order to provide a complete insight into this kind of malignancy. It has been indicated that lncRNAs are great biomarkers for different cancers, such as ovarian cancer, colorectal cancer, thyroid cancer, and so on.³⁷⁻³⁹ Moreover, lncRNAs have been implicated in GC development.^{40,41} A large body of evidence indicated lncRNAs as players in the proliferation, invasion, metastasis, and angiogenesis of GC.^{15,17,18} Emerging evidence shows that lncRNAs and miRNAs are simultaneously implicated in a series of molecular processes, including transcriptional regulation, proliferation, differentiation, and metastasis.^{42,43} In the present study, the expression profile of lncRNAs along with miRNAs and mRNAs was investigated in 4 molecular subtypes of GC. We discovered 2827, 1405, 2403, and 1330 differentially expressed lncRNAs in CIN, EBV, GS, and MSI subtypes, respectively. Considering that the WGCNA has great clustering efficiency for genomic materials with the correlated expression pattern, the ceRNA network and WGCNA were combined to discover more reliable subtype-related hub lncRNAs. Since conventional WGCNA fails to capture weak and moderate negative correlations, which might be important in gene regulation exerted by lncRNAs and miRNAs, we used csuWGCNA, which combines the signed and unsigned methods and improves the detection of negative correlations and is especially useful for noncoding RNAs such as miRNAs and lncRNAs. The purpose of finding hub lncRNAs, which can regulate the biological behavior of different GC subtypes, is to provide new ideas for understanding the molecular mechanisms of the initiation and progression of different cancer subtypes and new targets for the treatment of GC.

In the present study, we systematically analyzed GC subtype-related genes, constructed ceRNA network, and identified 5 hub lncRNAs related to each subtype. Gene set enrichment analysis on significantly correlated modules was also performed in order to discover the differences in cell pathway activity variation related to lncRNA function in any subtype. Finally, prognostic risk factors were evaluated for predicting RFS.

First, 5 hub lncRNAs were identified by integrating RNA-RNA connections for any GC subtype, which were lncRNAs AC138356.1, LINC01270, AP003469.2, AL139384.1, and AL118506.1 for CIN subtype, lncRNAs LINC02099, AL157871.2, AC068580.3, MIR3945HG, and AC004264.1 for EBV subtype, lncRNAs AL117335.1, AC100861.1, AC016065.1, AC139019.1, and AC011416.3

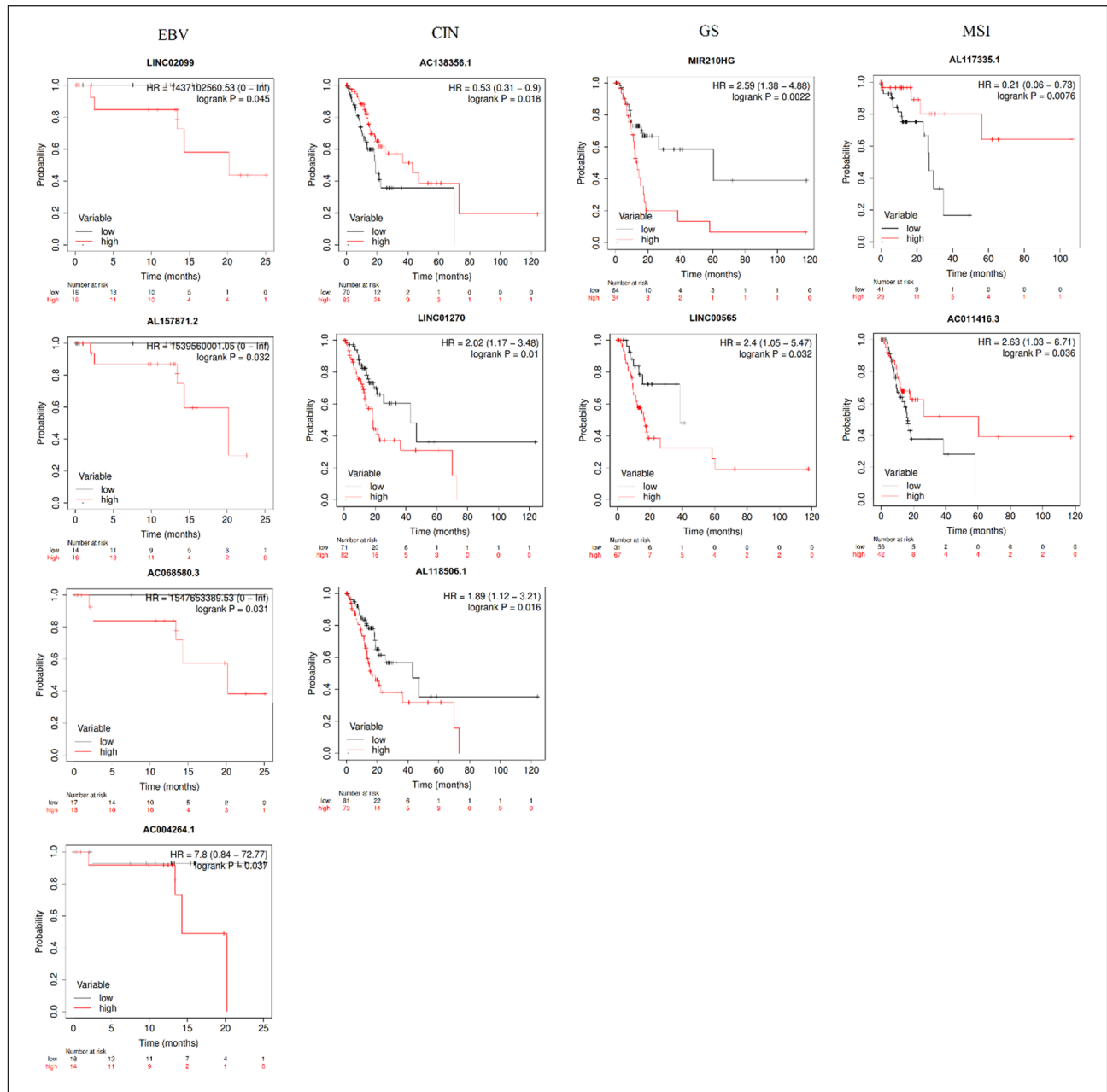


Figure 8. Kaplan-Meier survival curves of identified hub lncRNAs associated with relapse-free survival of their corresponding GC subtype patients.

for MSI subtype, and lncRNAs MIR210HG, AC010931.1, AL136090.2, AC005618.1, and LINC00565 for GS subtype. A significant expression difference could be observed between tumor subtypes and normal tissues for any selected lncRNA except AC005618.1 for the GS subtype; however, key gene regulators may not show very huge ectopic gene expression in different cancerous states, as their small variations can affect a lot of downstream genes.⁴⁴

In the case of CIN subtype, a major gene regulated by identified hub lncRNAs such as AC138356.1, LINC01270, and AL118506.1 is BCL2L1, a BCL-2 family member that acts as an apoptosis regulator and is involved in a wide

variety of cellular activities. There is evidence that BCL2L1 is related to tumorigenesis and invasion and contributes to treatment and drug sensitivity in GC.⁴⁵⁻⁴⁷ In GS subtype, *HIP1* gene is also an apoptosis regulator gene, which is regulated by hub lncRNAs LINC00565 and AC010931.1 and is associated with GC migration, invasion, and prognosis.⁴⁸ MDM2 is a protein altered in EBV subtype regulated by hub lncRNA LINC02099, which is related to invasion and apoptosis in GC.^{49,50} In MSI subtype, the MAPKAPK3 gene is one of those regulated by hub lncRNA AC011416.3, which is proven to affect tumor cell proliferation in GC.⁵¹

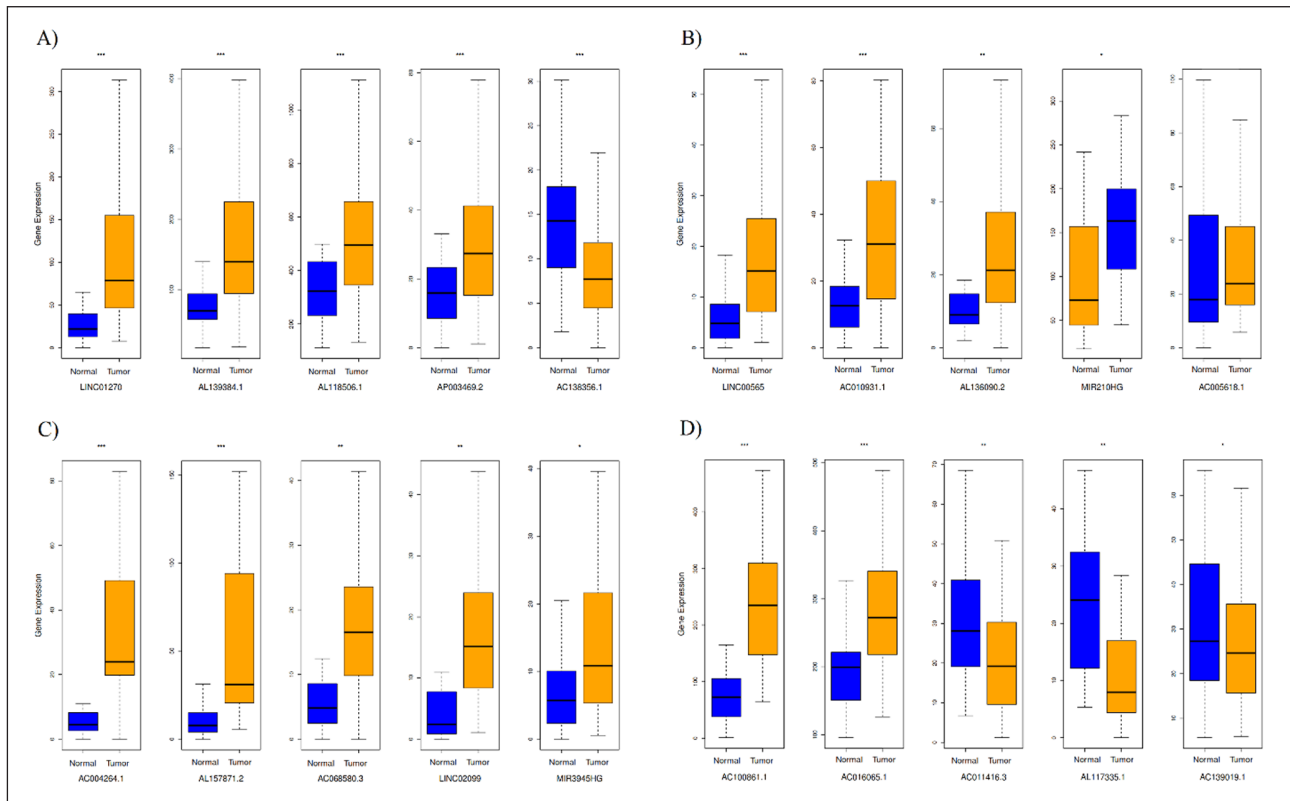


Figure 9. Expression of hub lncRNAs in (A) CIN GC subtype, (B) GS GC subtype, (C) EBV GC subtype, and (D) MSI GC subtype, compared with corresponding normal tissues.

* $P < .05$, ** $P < .01$, *** $P < .001$.

Compared with protein-encoding mRNAs, lncRNAs show greater tissue specificity, so they are preferential biomarkers for many diseases;^{13,14} nevertheless, there is less studies on lncRNAs corresponding to GC. Univariate Cox regression analysis for hub lncRNAs indicated an association of lncRNAs AC138356.1, LINC01270, and AL118506.1 with the CIN subtype, LINC02099, AL157871.2, AC068580.3, and AC004264.1 with the EBV subtype, AL117335.1 and AC011416.3 with the MSI subtype, and MIR210HG and LINC00565 with the GS subtype prognosis.

MIR210HG is shown to promote the metastasis of GC, and in line with our finding, it is introduced as a potential therapeutic target⁵² and implicated to the prognosis of colorectal carcinoma.^{53,54} There is also evidence that LINC01270 aggravates the progression of GC⁵⁵ and LINC00565 promotes proliferation and inhibits apoptosis of GC.⁵⁶ As there is no study to investigate the exact role of lncRNAs in different molecular subtypes of GC; hence, the lncRNAs introduced in this study may be used as potential biomarkers or therapeutic targets of GC subtypes for subsequent studies.

The robust positive correlation observed between EBV-associated hub lncRNAs and diverse immune cell populations aligns with the immunostimulatory and pro-inflammatory nature of the EBV subtype. This finding is consistent with our GSEA results, which demonstrated significant enrichment of immune activation pathways among

genes positively correlated with these hub lncRNAs. The concordance between immune cell infiltration patterns and pathway-level enrichment underscores the functional relevance of these lncRNAs in promoting an immunologically active tumor microenvironment. In contrast, the negative correlations seen in the CIN subtype suggest a role in immune suppression or exclusion, further highlighting the context-dependent regulatory functions of subtype-specific lncRNAs. These results reinforce the biological significance of the identified hub lncRNAs and support their potential relevance in immunotherapy. The EBV-associated lncRNAs may serve as biomarkers for immune-responsive tumors or as modulators to enhance therapeutic efficacy, whereas CIN-associated lncRNAs represent candidate targets for overcoming immune resistance. Functional validation is warranted to elucidate their precise mechanistic roles in tumor immune crosstalk.

There were several limitations in the present study. Even though there are a lot of studies on GC, but there are a few data about GC subtypes, which imitated bioinformatics analysis of these subtypes, as well as their comparison and validation. In addition, regulation of ceRNA network is very intricate, and ceRNA interactions are influenced by multiple factors, such as the subcellular localization of ceRNA components, miRNA-lncRNA-mRNA affinity, RNA editing, and RNA-binding proteins.²³ This study only investigated the miRNAs, lncRNAs, and mRNAs putative interactions, which requires further validation.

Conclusion

In conclusion, we constructed a ceRNA network by bioinformatics analysis of the lncRNA, miRNA, and mRNA expression profiles of 4 different molecular subtypes of GC from the TCGA database. We identified hub lncRNAs that may be part of key regulators of differential tumorigenesis factors for each subtype and may be useful to predict the RFS of corresponding patients and also used as therapeutic targets. The exact mechanism of action of these lncRNAs in GC subtypes should be investigated to determine their feasibility as diagnostic or therapeutic biomarkers.

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Ethical considerations

This study utilized only publicly available data and did not involve any animals or human subjects.

Author Contributions

Seyed Navid Goftari: Data curation; Software; Investigation; Writing – original draft; Conceptualization; Formal analysis.

Ahmad Reza Bahrami: Data curation; Writing – review & editing; Validation; Methodology.

Maryam M Matin: Supervision; Writing – review & editing; Conceptualization; Funding acquisition; Project administration; Validation.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Supplemental Material

Supplemental material for this article is available online.

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