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Up-Regulation of *MYB* Gene Constructs a Prognosis Biomarker for Survival and Prognostic in Liver Hepatocellular Carcinoma

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Abstract

Background & Aims: MYB proteins are highly conserved DNA-binding domain proteins and aberrant gene regulation plays a role in the development of multiple cancer types. Prior studies have identified *MYB* gene expression as a prognostic tool. However, the role of the *MYB* gene in liver hepatocellular carcinoma (LIHC) is uncertain.

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Approach & Results: The RNA expression profile, variance analysis, copy number alteration, and prognostic value of *MYB* gene in LIHC patients was evaluated using TCGA data. Pathway enrichment analysis of *MYB* was conducted using the R package cluster profiler. In addition, we deeply explored the interaction between *MYB* gene and immunosuppressive tumor microenvironment (iTME) in LIHC patients. *MYB* gene expression was substantially higher in LIHC patients than normal. High *MYB* gene expression predicted worse long-term survival in LIHC patients. We also identified *MYB* gene expression was positively correlated with the expression of immune checkpoint genes, DNA damage repair (DDR) genes, and CD8+ T cell effector genes.

Conclusion: Our results revealed expression level alterations of *MYB* in LIHC patients significantly impacts overall patient survival and warrants further study to implement *MYB* expression monitoring as a prognostic tool for patients diagnosed with LIHC.

Keywords *TCGA, MYB, prognostic biomarker, liver hepatocellular carcinoma (LIHC), immunosuppressive tumor microenvironment (iTME)*

1. Introduction

Primary liver cancer is characterized by the formation of tumor cells originating from the liver and is one of the most common cancers in the world. Hepatocellular carcinoma (LIHC) is the most common subtype of primary liver cancer, accounting for more than 70% of all primary liver cancers [1, 2]. There are many risk factors for LIHC including but not limited to long-term alcohol abuse, liver cirrhosis, aflatoxin intake, and chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection [3]. Currently, the primary methods for treating LIHC patients are surgical treatment and radiotherapy. However, the curative effect of LIHC remains unsatisfactory and the recurrence rate is high [4]. Therefore, further investigation into the genes driving LIHC progression is necessary to identify novel targets for prognostic testing and therapeutic treatment of LIHC patients.

The transcription factor, *MYB*, is highly conserved from plants to vertebrates [5]. It was first identified in the chicken leukemia virus [6]. Research shows that the *MYB* gene is highly expressed in hematopoietic progenitor cells (HPC) and performs a crucial role in the development of hematopoietic system [7]. *MYB* is also a known regulator of cellular proliferation, differentiation, and apoptosis pathways. As previously reported, *MYB* is aberrantly expressed in human leukemia [8], breast cancer [9], and pancreatic cancer [10] and negatively impacts overall patient survival and prognosis. *MYB* also has known functions in LIHC. In LIHC cells, *MYB* acts as a transcriptional regulator of Yes-associated protein (YAP) to promote cell growth both *in vivo* and *in vitro* [11, 12].

In this study, we defined the RNA expression characteristics and the diagnostic and prognostic values of the *MYB* gene in LIHC. We also analyzed the correlation between *MYB* gene expression and

immune cell infiltration, immune checkpoint genes, and signaling pathways. In our study, we aimed to establish a MYBcluster method to predict the clinical outcome of LIHC patients. By investigating the role of *MYB* gene in LIHC patients, we further illustrate the unique function of *MYB* in LIHC patients.

2. Materials and Methods

2.1. Data Collection

The RNA expression profiles and clinical information of LIHC was obtained from the UCSC Xena (Link 1) database. The gene alteration profiles of MYB were downloaded from the cBioPortal database (Link 2).

2.2. Prognostic Analysis of *MYB* Hene

Kaplan-Meier analyses were performed to analyze the influence of the *MYB* gene on the survival of patients in LIHC using the R package survival.

2.3. Gene Enrichment Analysis

To explore the function of the *MYB* gene and its biological effects in LIHC, GSVA (gene set variation analysis) enrichment analysis was performed using R package “GSVA” to evaluate the correlation between the *MYB* gene and pathway based on the MsigDB database (Link 3). We also conducted the GSEA (gene set enrichment analysis) of *MYB* in LIHC using R package “clusterprofiler” based on KEGG (Kyoto Encyclopedia of Genes and Genomes), and Reactome database. Gene correlation analysis results are presented in the form of heatmaps using the pheatmap package.

2.4. Immunosuppressive Tumor Microenvironment (iTME) Analysis

The R package ESTIMATE was used to calculate the stromal score, immune score, and tumor purity score of each patient in TCGA cohort. The association between MYB and these scores were analyzed.

2.4. Correlation Analysis between MYB and Tumor Infiltrating Cells

To further explore the role of *MYB* in iTME, we conducted the correlation analysis between *MYB* and immune infiltration cells in LIHC. The infiltration data were downloaded from TIMER2 database (Link 4). To study the specific role of *MYB* in iTME, we further analyzed the association between *MYB* and immunosuppressive signature, CD8+ T cell effector genes, and immune checkpoint genes at the LIHC level. The visualization of all boxplots in this step was conducted with the ggplot2 package.

2.5. Statistical Analysis

In this article, Student’s t-test and Kruskal-Wallis were performed for the gene expression difference. Log-Rank test was used to evaluate the survival of the patients. All analyses were performed using R software (version 4.1.1). The results of $P < 0.05$ were considered statistically significant.

3. Results

3.1. High MYB Expression Negatively Impacts the Prognosis of LIHC Patients

First, we evaluated the differential RNA expression of *MYB* in LIHC patients compared to normal liver tissue controls. We found the expression of *MYB* in tumor cells was significantly higher than that in normal liver

tissue (p< 0.001) (Figure 1A). RNA array analysis showed that the *MYB* gene expression in tumor tissues higher than hepatitis and hepatocirrhosis (control group) tissues (Figure 1B). Interestingly, after we dig into the different grade of liver cancer patients. We found that the expression of *MYB* is positively corrected with the disease stage (Figure 1C). A survival curve of patients with high or low *MYB* gene expression was then examined, and showed high expression of *MYB* had a significant negative impact on LIHC patient survival ($p < 0.05$) (Figure 1D). These results identify *MYB* gene expression as a novel prognostic marker in LIHC patients.

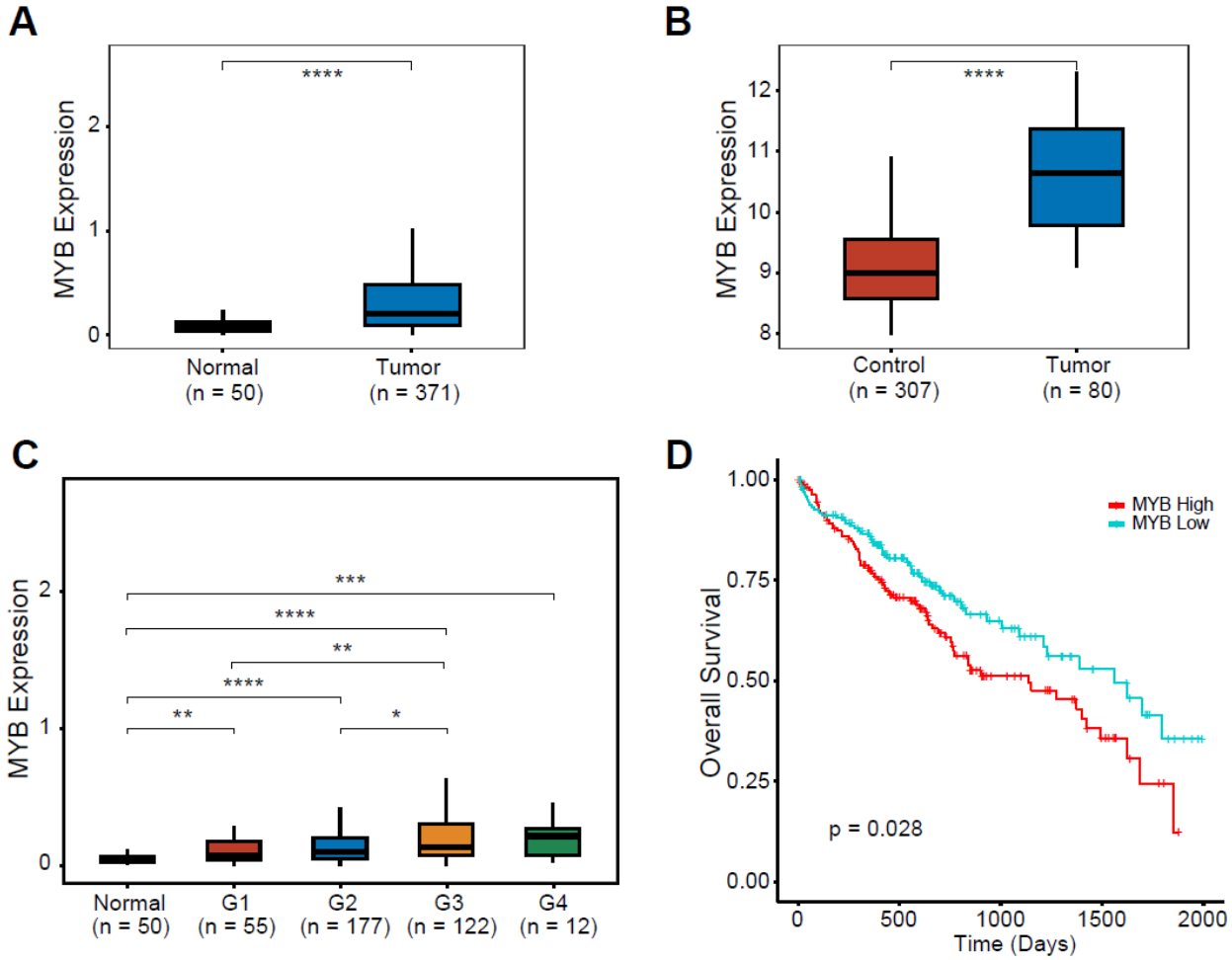

FIGURE 1: The role of *MYB* gene in LIHC compare with normal.

A) RNA expression levels of the *MYB* gene in tumor (blue) and normal liver tissues (red) based on the TCGA database. Statistical significance was determined using the Wilcoxon test. Asterisks indicate statistical significance. **B)** RNA expression levels of the *MYB* gene in tumor (blue) and normal liver tissues (red) based on the GEO dataset GSE10143. Statistical significance was determined using the Wilcoxon test. Asterisks indicate statistical significance. **C)** RNA expression levels of the *MYB* gene across different tumor grades in the TCGA database. Statistical significance was assessed using the Wilcoxon test. Asterisks indicate statistical significance. **D)** Overall survival analysis of LIHC patients with high and low MYB gene expression based on the TCGA database.

*P < 0.05; **P < 0.01; ***P < 0.001.

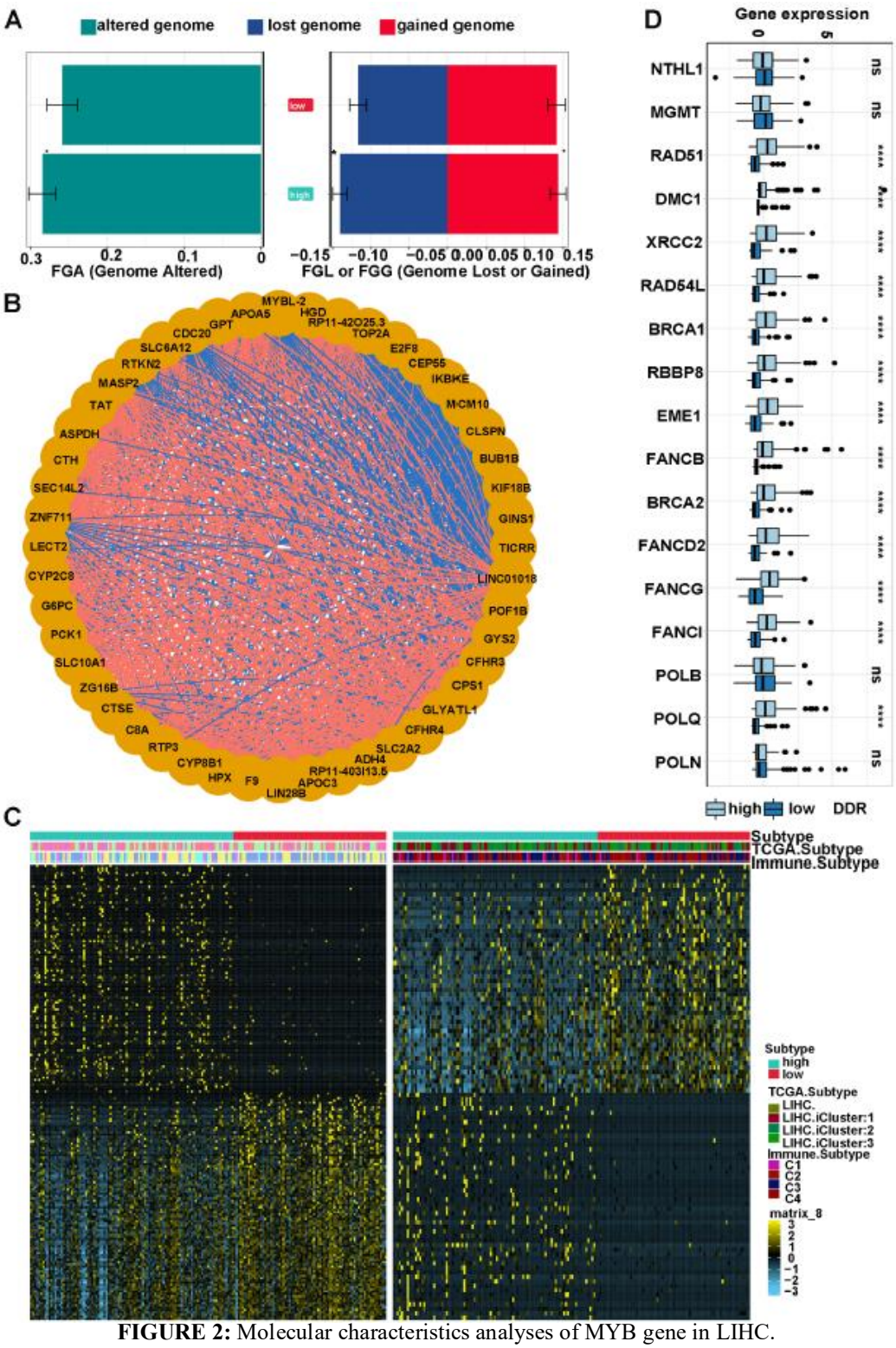

FIGURE 2: Molecular characteristics analyses of MYB gene in LIHC.

A) Bar plot showing the fraction of the genome altered between two MYBcluster subtypes in the TCGA-LIHC cohort. **B)** Network activity analysis based on 48 differentially expressed genes (DEGs) related to COX in the TCGA-LIHC cohort. **C)** Heatmap illustrating subtype-specific upregulated and downregulated biomarkers in the three identified subtypes using limma analysis in the TCGA-LIHC cohort. **D)** Expression levels of DDR-related genes in high and low MYBcluster groups in LIHC.

3.2. Molecular Characteristics of High or Low *MYB* Gene Expression Groups in LIHC

Given the differential *MYB* gene expression in two MYBcluster groups, we sought to explore the signatures of tumor microenvironment (*TME*) genes associated with MYBcluster. Compared with the MYBcluster-low, MYBcluster-high had a high *TMB* gene expression and higher levels of overall CNVs (Figure 2A). The R/limma package software was used to find 48 DEGs associated with the two-cluster subtypes. The network activity of differentially expressed genes (DEGs) was investigated in (Figure 2B). Subtype-specific upregulated or downregulated biomarkers were identified by starting with differential expression analysis (DEA). The most DEGs sorted by log2Fold are chosen as the biomarkers for each MYBcluster subtype. These biomarkers must pass the R/limma package analysis to identify subtype-specific downregulated genes in (Figure 2C) in left and upregulated genes in right. With a consideration of DNA damage repair (DDR) related genes on TMB, we also explored 17 RNA levels and found 13 genes with significant expression alterations between the two MYBcluster groups (Figure 2D). In conclusion, we identified a collection of differentially expressed *TME* gene signatures between the two MYBcluster groups, which potentially indicated that aberrant MYB gene expression in LIHC cell affects the expression of TME gene signatures.

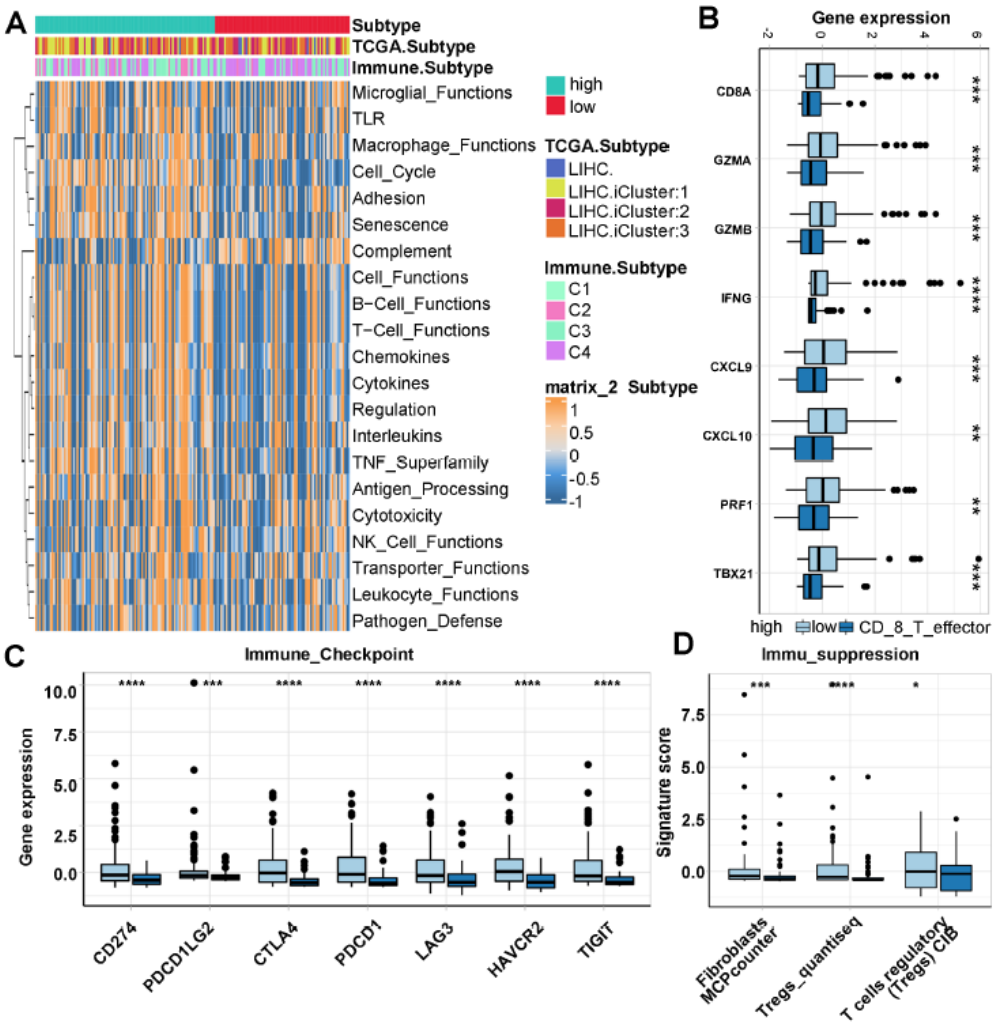

FIGURE 3: Immunosuppressive tumor microenvironment (TME) analysis of MYB gene in LIHC.

A) Heatmap of the enrichment scores of immune cell sets of interest for the two MYBcluster subtypes in LIHC. **B)** RNA expression levels of eight CD8+ T cell effector genes in high and low MYBcluster groups. **C)** Expression levels of immune checkpoint genes in high and low MYBcluster groups. **D)** Signature scores of immune suppression in high and low MYBcluster groups.

3.3. Immune Infiltrating Patterns of MYBcluster group

Further exploration of the diverse alteration patterns associated with differential *MYB* expression was necessary to gain a deeper understanding of role of MYB in LIHC. The functions based on the expression of IM regulators in the *MYB* three subtypes were investigated in (Figure 3A). CD8+ T cell effector genes include several such as *CD8A*, *GZMA*, *GZMB*, *IFNG*, *CXCL9*, *CXCL10*, *PRF1* and *TBX21*. All CD8+ effector genes were significantly up-regulated ($p < 0.05$) in the high MYBcluster group. This result indicates that T cells in this group are more efficient at recognizing antigens and initiating inflammation and antitumor immunity. As a novel biomarker, CD8+ T cell effector gene signature has high sensitivity in predicting the effect of immunotherapy, and after the above analysis, we also concluded that it is positively correlated with MYBcluster. Boxplot analysis shows the key 8 genes for CD8+ T cell effectors in (Figure 3B).

As a regulator of immune tolerance, immunological checkpoints can play an important role in protecting normal cells from indiscriminate attacks, thereby restoring the body's normal immune function. Activation of inhibitory checkpoint molecules prevents cancer cells from being damaged and attacked. Conversely, inhibition of inhibitory checkpoint molecules can result in immunological attack of tumor tissue, making these checkpoints potential promising targets for cancer immunotherapy. In LIHC samples, we investigated aspects related to immune checkpoint gene expression and found that *CD274* (*PD-L1*), *PDCD1*, *PDCD1LG2*, *CTLA4*, *HAVCR2*, *LAG3*, and *TIGIT* were significantly overexpressed in the high MYBcluster group (Figure 3C). Immune suppressive signature scores for LIHC samples were inferred using the ESTIMATE package. As shown in (Figure 3D), an increase in the feature score and a decrease in LIHC purity corresponded to a high MYBcluster. These results indicate increased levels of stromal and immune cells in the iTME of these LIHC specimens. Tumors with high MYBcluster were also positively associated with increased levels of multiple immune infiltrates. From these data, it can be inferred that LIHC has a complex iTME, where a mixture of tumor cells and anti-tumor cells, and immune activation and suppression molecules coexist.

3.4. Functional Annotations and Pathway Enrichment Analyses of MYBcluster Groups

To comprehensively characterize the transcriptional differences associated with MYBcluster classification, we first performed principal component analysis (PCA), which revealed a distinct separation between the low MYBcluster group (positioned on the left) and the high MYBcluster group (positioned on the right) along the Dim1 axis (Figure 4A), indicating substantial global gene expression differences between the two subtypes. Differential gene expression analysis using *EdgeR*, *DESeq2*, and *limma* identified 333 overlapping differentially expressed genes (DEGs) (Figure 4B). Subsequently, the ssGSEA algorithm was applied to calculate HALLMARK and KEGG pathway signature scores for each sample. The

analysis demonstrated that cell cycle-related pathways were predominantly enriched in the low MYBcluster group, whereas pathways associated with metabolic regulation were significantly enriched in the high MYBcluster group (Figure 4C). Pathway significance ranking was performed using the limma algorithm, and the top 10 enriched pathways were visualized (Figure 4D). Notably, the high MYBcluster LIHC group exhibited significant enrichment in multiple immune activation pathways, including TNFα signaling via NF-κB, reactive oxygen species pathway, peroxisome, interferon-α response, coagulation, bile acid metabolism, lipogenesis, fatty acid metabolism, xenobiotic metabolism, and oxidative phosphorylation.

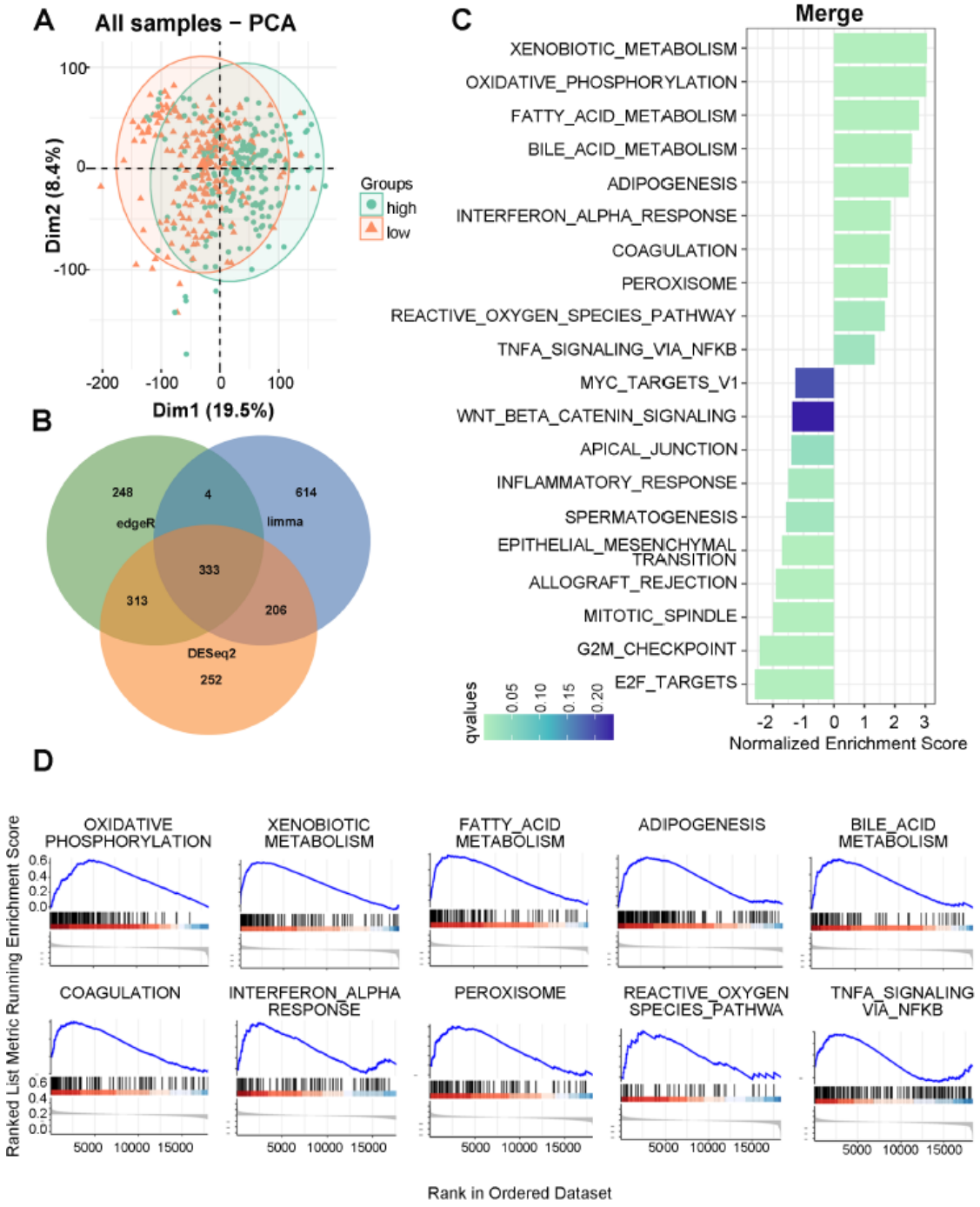

FIGURE 4: Functional annotation and pathway enrichment analysis of MYBcluster groups.

A & B) RNASeqstat2 pipeline used for transcriptome analysis. **A)** Principal component analysis (PCA) and **B)** a Venn plot showing overlapping differentially expressed genes (DEGs) identified by limma, edgeR, and DESeq2. **C)** GSEA HALLMARK pathway analysis of signaling pathways in high and low MYBcluster groups. **D)** Enrichment analysis of the top 10 HALLMARK pathways in high and low MYBcluster groups.

4. Discussion

As a transcription factor family, the *MYB* family is widely present in the eukaryotic genome and has function to regulate cell proliferation and cell differentiation pathways by regulating the transcription of target genes [13, 14]. Previous studies show that *MYB* is an important hematopoietic system transcription factor and is upregulated in most human acute leukemias [15, 16]. In recent years, a growing number of studies have shown that the *MYB* gene family plays a key role in tumor development [17, 18]. For example, aberrant *MYB* gene expression was detected in ovarian cancer (OC) cases but not in normal ovarian tissue [19], and *B-MYB* overexpression was found to promote tumor growth by regulation the cell cycle, thereby affecting tumor prognosis [20, 21]. However, there has been no studies on the role of *MYB* gene expression on LIHC patient prognosis. In our study, we identified the RNA expression of *MYB* was not only significantly upregulated in LIHC samples but also positively correlated with disease grades. The survival analysis demonstrated that patients with higher expression of *MYB* have a poorer prognostic value. It suggests that *MYB* gene expression can be used as a novel prognostic biomarker for LIHC patients.

In recent years, TME, particularly iTME, has become a major focus of cancer immunity research [22, 23]. Our findings reveal a significant correlation between *MYB* gene expression and CD8+ T cell effector genes, one of the key biomarkers of effective tumor immunotherapy, in LIHC samples. Therefore, *MYB* gene overexpression in LIHC may limit T cell infiltration and lead to immune escape of tumor cells. We also identified a positive correlation between *MYB* gene expression and immune checkpoint genes, which may also contribute to immunological escape of tumor cells. In summary, our results provide supportive evidence that *MYB* gene overexpression is associated with the immunosuppressive tumor microenvironment in LIHC.

In conclusion, our study demonstrates that *MYB* gene expression has high prognostic value and correlates significantly with an immunosuppressive TME. These data suggest *MYB* could be implemented as an immune-related biomarker for LIHC clinical prognosis, as well as a potential therapeutic target for LIHC immunotherapy.

Disclosure

TCGA and UCSC Xena belong to public databases. Our study is based on open-source data, so there are no ethical issues.

Conflicts of Interests

None.

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Author Contributions

Cai, Fan and Li performed data analyses and wrote the manuscript, Gan helped with data analyses and prepare for the manuscript. Kong and Lu conceived of the research, designed the methods and analyzed data. Cai, Fan and Li contributed equally to this work. All authors read and approved the final version of this manuscript.

Competing Interests

None.

Abbreviation

DDR: DNA Damage Repair
DEA: Differential Expression Analysis
DEGs: Differentially Expressed Genes
GSEA: Gene Set Enrichment Analysis
GSVA: Gene Set Variation Analysis
HBV: Hepatitis B Virus
HCV: Hepatitis C Virus
HPC: hematopoietic Progenitor Cells
KEGG: Kyoto Encyclopedia of Genes and Genomes
LIHC: Liver Hepatocellular Carcinoma
OC: Ovarian Cancer
TME: Tumor Microenvironment
iTME: Immunosuppressive Tumor Microenvironment
uniCox: Univariate Cox Regression
YAP: Yes-Associated Protein

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