

Upregulation of *MYBL2* independently predicts a poorer prognosis in patients with clear cell renal cell carcinoma

SHAN-SHAN SUN¹, YANG FU² and JIAN-YANG LIN¹

Departments of ¹Pharmacy and ²Urology, The First Hospital of China Medical University, Shenyang, Liaoning 110001, P.R. China

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Abstract. MYB protooncogene-like 2 (*MYBL2*) is a transcription factor that is upregulated and significantly associated with various human cancer types. However, the potential role of *MYBL2* in clear cell renal cell carcinoma (ccRCC) is yet to be elucidated. Therefore, the expression and biological functions of *MYBL2* in ccRCC were assessed in the current study using The Cancer Genome Atlas (TCGA). A Wilcoxon signed-rank test was performed to compare *MYBL2* expression between ccRCC and normal tissues. Moreover, the association between *MYBL2* expression and various clinicopathological factors was estimated using both the Wilcoxon signed-rank test and logistic regression. The differences in prognosis between patients with high- and low-*MYBL2* expression were analyzed via the Kaplan-Meier method and Cox regression analysis. Finally, gene set enrichment analysis (GSEA) was performed to investigate the biofunctions of *MYBL2* in ccRCC. It was revealed that *MYBL2* was upregulated in ccRCC, and that the *MYBL2* high-expression phenotype was significantly associated with sex, a high histological grade, an advanced clinical stage, tumor stage, lymph node metastasis, distant metastasis and poor overall survival (OS). It was also revealed, via the Cox regression analysis, that the upregulation of *MYBL2* expression was able to independently predict a poor prognosis in patients with ccRCC. GSEA indicated that the intestinal immune network for IgA production, primary immunodeficiency, the janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway, the

cytosolic DNA-sensing pathway, the p53 signaling pathway and the chemokine signaling pathway were all enriched in the high-*MYBL2* expression datasets. In conclusion, the present findings indicate that *MYBL2* may be used as an independent prognostic factor in patients with ccRCC.

Introduction

Renal cell carcinoma (RCC) is the most common renal malignancy, causing approximately 99200 new cases and almost 39100 deaths in Europe in 2018 (1). Clear cell renal cell carcinoma (ccRCC) is the most common pathological type of RCC, accounting for 80-90% of total cases globally in 2016 (2). Although surgical resection is an effective treatment option for early ccRCC, the prognosis for patients with metastatic ccRCC is poor (3). Targeted therapy is a promising anticancer strategy (4) and the identification of novel hub genes specific to ccRCC may improve targeted treatment and confer significant benefit on patients.

MYB protooncogene-like 2 (*MYBL2*), a member of the myeloblastosis family of transcription factors, influences numerous factors affecting cell cycle progression (5). Recently, an increasing number of studies have demonstrated that *MYBL2* expression is significantly associated with various carcinomas. For example, upregulated *MYBL2* expression was identified in gastric cancer and predicted a poor prognosis, indicating that *MYBL2* may serve as a promising biomarker (6). Overexpressed *MYBL2* promotes proliferation and inhibits apoptosis of breast cancer cells targeted by miR-143-3p (7). Moreover, *MYBL2* knockdown suppressed the growth of esophageal squamous cell and colorectal carcinoma cells via regulation of the cell cycle (8). In other cancer types, including hepatocellular carcinoma (HCC) (9), non-small cell lung cancer (NSCLC) (10) and pancreatic ductal adenocarcinoma (11), high *MYBL2* expression also promoted the progression of tumors and resulted in a poorer prognosis. However, the influence of *MYBL2* expression on ccRCC is yet to be elucidated.

Therefore, *MYBL2* expression was analyzed in ccRCC, and the association between *MYBL2* and the prognosis of patients with ccRCC was determined in the current study. Moreover, gene set enrichment analysis (GSEA) was performed to investigate the biological functions of *MYBL2* in ccRCC. According to previous studies, it was hypothesized that *MYBL2* may

Correspondence to: Professor Jian-Yang Lin, Department of Pharmacy, The First Hospital of China Medical University, 155 Nanjing North Street, Heping, Shenyang, Liaoning 110001, P.R. China
E-mail: linjianyangdy@126.com

Abbreviations: RCC, renal cell carcinoma; ccRCC, clear cell renal cell carcinoma; TCGA, The Cancer Genome Atlas; GSEA, gene set enrichment analysis; NES, normalized enrichment score; FDR, false discovery rate; OS, overall survival; HCC, hepatocellular carcinoma

Key words: *MYBL2*, clear cell renal cell carcinoma, prognosis, The Cancer Genome Atlas, gene set enrichment analysis

independently predict the prognosis of ccRCC patients and may represent a novel therapeutic target.

Materials and methods

Patient samples. The mRNA expression data and corresponding clinical data from patients with ccRCC were downloaded from the official website of The Cancer Genome Atlas database (National Institutes of Health), dataset TCGA-Kidney Renal Clear Cell Carcinoma. A total of 611 gene expression data profiles and 537 clinical data profiles were retrieved from TCGA ccRCC database in July 2019. The 611 gene expression data profiles were used to evaluate the differential expression of *MYBL2* between tumor (539) and healthy tissues (72). Subsequently, patients lacking gene expression data or all clinicopathological characteristics were excluded, 530 ccRCC profiles with both gene expression and clinical data were included for further analysis. Some patients had incomplete clinicopathological characteristics data, such as T stage (12), therefore these patients were included in analysis of clinical information they possessed, but excluded in the analysis of clinicopathological characteristics they lacked. Hence, some variables in Table I do not total 530.

GSEA. GSEA was used to investigate the potential functions of *MYBL2* in ccRCC. The pretense of GSEA is to use predefined gene sets (usually from functional annotation or results of previous studies) to rank genes according to the degree of differential expression in two types of samples, and then test whether the preselected gene sets are enriched at the top or bottom of this ranking table. In the present study, the 'c2.cp.kegg.v6.2.symbols.gmt' gene sets from the Molecular Signatures Database (software.broadinstitute.org/gsea/msigdb/index.jsp) were analyzed using GSEA 3.0 software (BROAD Institute). To obtain normalized enrichment scores (NESs), the nominal P-value and false discovery rate (FDR) q-value were determined. The number of gene set permutations for each analysis was set at 1,000, and the phenotype label was the expression level of *MYBL2*. Gene sets with a nominal P-value of <0.05 and an FDR q-value of <0.25 were considered significantly enriched (13).

Statistical analysis. The Mann-Whitney U test was used to compare *MYBL2* expression in tumor and healthy tissues. The Wilcoxon signed-rank test was used to compare *MYBL2* expression in paired tissues. To examine the association between clinicopathological factors and *MYBL2* expression (after excluding healthy tissue sample data) the Mann-Whitney U test was performed for comparison between two groups (sex, lymph node metastasis and distant metastasis), and the Kruskal-Wallis test and Bonferroni's post-hoc test was performed for multi-group comparison [tumor (T) stage, clinical stage and histological grade] (12), then logistic regression was conducted for further analysis. The median value of *MYBL2* expression was selected as the cutoff value, and patients with ccRCC were subsequently divided into high- and low-expression groups. Kaplan-Meier survival curves were constructed and log rank tests performed to evaluate the association between *MYBL2* expression and overall survival (OS). A univariate Cox regression analysis was

Table I. Clinicopathological characteristics of patients with clear cell renal cell carcinoma, retrieved from The Cancer Genome Atlas database.

Clinical characteristics	Value
Median age at diagnosis, years (range)	61 (21-90)
Sex, %	
Female	35.1
Male	64.9
Histological grade ^a , %	
I	2.7
II	43.5
III	39.5
IV	14.3
Clinical stage ^a , %	
I	50.3
II	10.8
III	23.3
IV	15.6
T stage ^a , %	
T1	51.1
T2	13.1
T3	33.8
T4	0.02
Lymph node metastasis N stage ^a , %	
N0	93.7
N1	6.3
Distant metastasis M stage ^a , %	
M0	84.3
M1	15.7

^a(ref. 12). T, tumor; N, node; M, metastasis.

performed to explore the association of clinicopathological factors and *MYBL2* expression with OS, and a multivariate Cox regression analysis was performed to investigate whether *MYBL2* can independently influence prognosis. All statistical analyses were performed using R version 3.5.3 software (14), Statistical Product and Service Solutions (SPSS) 26.0 software (IBM Corp.) and GraphPad Prism 8.0.2 software (GraphPad Software).

Results

Patient characteristics. Of the 530 included patients, the median patient age was 61 years (range, 26-90), and there were 14, 227, 206 and 75 patients with histological grade I, II, III and IV, respectively. A total of 265, 57, 123 and 82 patients presented with clinical stage I, II, III and IV, respectively. There were also 271, 69, 179 and 11 patients with T1, T2, T3 and T4, respectively. Notably, 239 and 16 patients presented with node (N)0 and N1, respectively, and there were 420 and 78 patients with metastasis (M)0 and M1, respectively. The characteristics of the included patients with ccRCC are summarized in Table I.

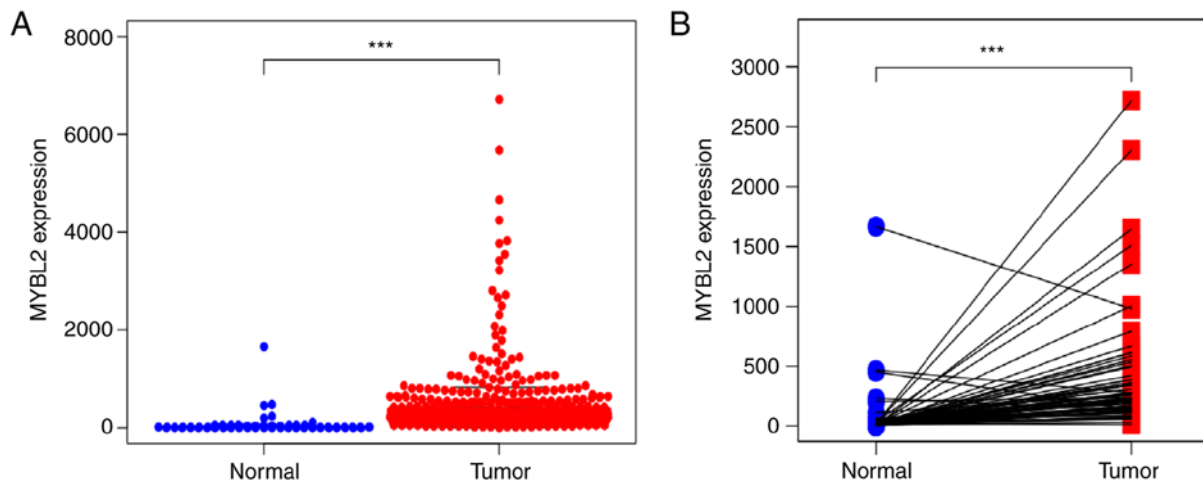


Figure 1. Comparison of *MYBL2* expression between ccRCC and healthy tissues using data from The Cancer Genome Atlas. A Mann-Whitney U test was used to compare *MYBL2* expression between tumor and healthy tissues. A Wilcoxon signed-rank test was used to compare *MYBL2* expression in paired tumor and non-tumorous tissues. (A) The expression of *MYBL2* in ccRCC tissues (n=537) was significantly higher compared with normal tissues (n=72). (B) Compared with 72 paired normal tissues, ccRCC tissues exhibited significant upregulation of *MYBL2*. ***P<0.001. ccRCC, clear cell renal cell carcinoma.

A number of samples retrieved from TCGA had incomplete clinicopathological characteristics, therefore, samples lacking specific clinicopathological characteristics will be excluded when these clinicopathological characteristics were counted.

Upregulation of *MYBL2*. It was revealed that *MYBL2* was significantly upregulated in ccRCC tissues (P<0.001; Fig. 1A). In addition, *MYBL2* was also significantly upregulated in ccRCC tissues compared with paired healthy tissues (P<0.001; Fig. 1B). The data from Fig. 1A and B were derived from TCGA.

Association of *MYBL2* expression and clinicopathological factors. The median value of *MYBL2* expression was selected as the cut-off value and patients with ccRCC were divided into high- and low-expression groups. The median follow-up times for the high- and low-*MYBL2* expression groups were 967 and 951 months, respectively. As indicated in Fig. 2A-F, the upregulation of *MYBL2* was significantly associated with sex, an advanced T stage, lymph node metastasis, distant metastasis, clinical stage and histological grade. Logistic regression demonstrated that increased *MYBL2* expression was significantly associated with age, sex, histologic grade, clinical stage, T stage, lymph node metastasis and distant metastasis (Table II). The data from Fig. 2A-F was derived from TCGA.

***MYBL2* can be considered a prognostic factor for ccRCC.** Kaplan-Meier analysis revealed that patients with ccRCC with high *MYBL2* expression exhibited a poorer OS rate compared with the low-expression group (Fig. 2G). Moreover, the univariate Cox regression analysis indicated that high *MYBL2* expression was strongly associated with a poor prognosis. Other clinical variables, such as age, grade, stage, T stage, lymph node metastasis and distant metastasis were all associated with OS. Moreover, multivariate analysis revealed that high *MYBL2* expression, age and distant metastasis were independently associated with a poor prognosis (Table III). Therefore, *MYBL2* may be an independent prognostic biomarker for patients with ccRCC.

Biological pathogenesis of *MYBL2* in ccRCC. GSEA revealed that the ‘intestinal immune network for IgA production’, ‘primary immunodeficiency’, ‘the janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway’, ‘the cytosolic DNA-sensing pathway’, ‘the p53 signaling pathway’ and ‘the chemokine signaling pathway’ (Fig. 3A-F) were enriched in the high *MYBL2* expression datasets (Table IV).

Discussion

In the present study, analyzing the data from TCGA provided a direction for further research and generated genomic and statistical evidence to support novel candidate cancer targets for therapeutic and diagnostic development. In the current study, it was revealed that the upregulation of *MYBL2* was significantly associated with sex, high histological grade, advanced clinical stage, T stage, lymph node metastasis, distant metastasis and poor OS rate, and may therefore be used as an independent prognostic factor for patients with ccRCC, according to data from TCGA. Furthermore, the results of GSEA demonstrated that *MYBL2* upregulation was significantly associated with classical tumor-associated pathways and immune-associated pathways.

Previous findings have demonstrated that *MYBL2* regulates the progression of malignant tumors by inhibiting the cell cycle and activating tumor-associated genes and pathways via transcription factor activity (15). The upregulation of *MYBL2* has been reported in various cancer types, suggesting that *MYBL2* may promote tumorigenesis. For example, the upregulation of *MYBL2* was revealed to promote progression of the G1-S phase transition by suppressing insulin-like growth factor-binding protein 3; thus, *MYBL2* may represent a novel therapeutic biomarker in NSCLC (16). Other findings indicated that *MYBL2* regulated breast cancer cell proliferation and apoptosis via the targeting of microRNA-143-3p (7). Therefore, *MYBL2* may serve as an effective therapeutic target. Notably, the high expression and carcinogenic effects of *MYBL2* in ccRCC were described in detail in the present study.

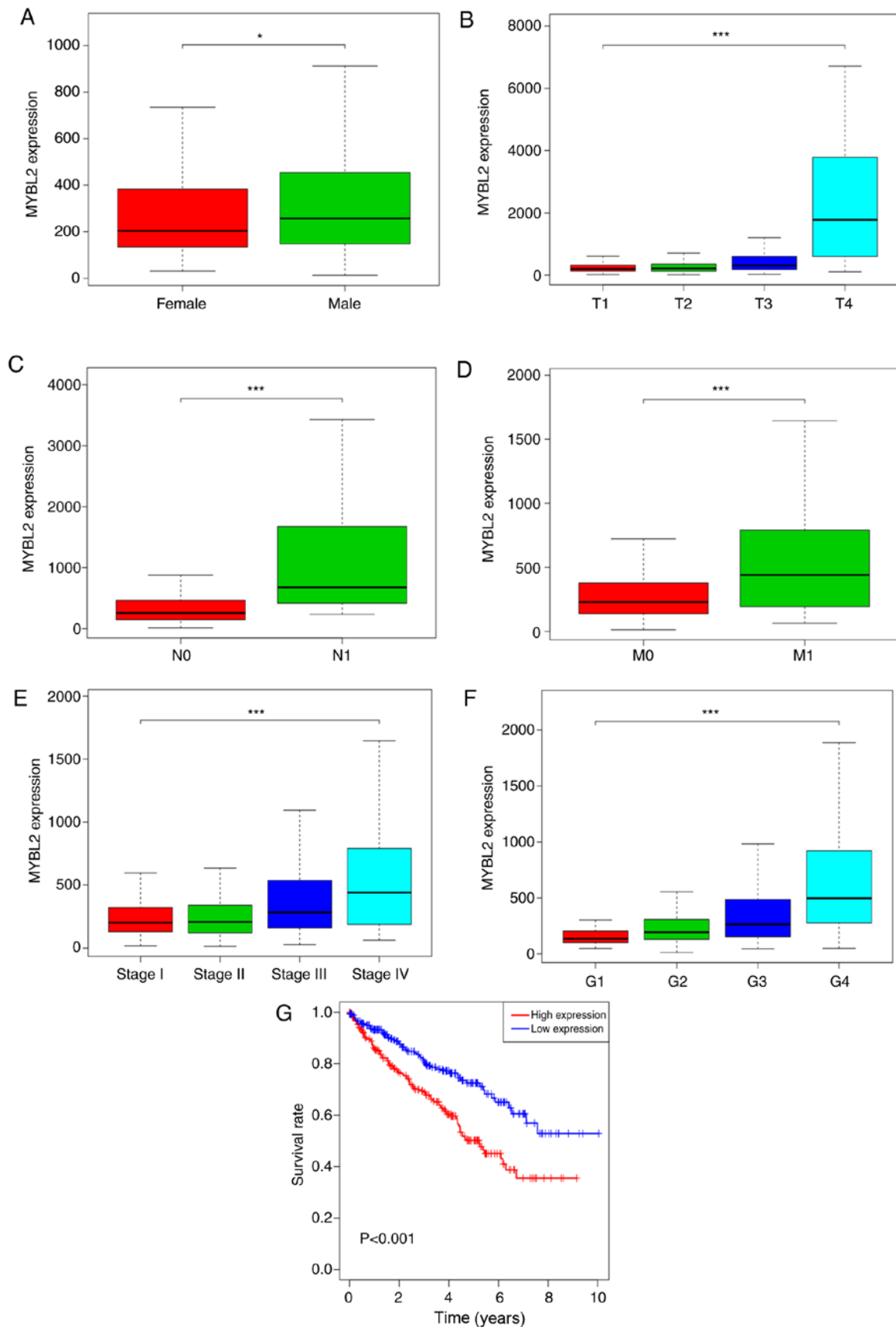


Figure 2. Analysis of TCGA datasets was conducted to determine expression of *MYBL2* and its association with clinicopathological variables. The median value of *MYBL2* expression was considered the cutoff value. To examine the association between clinicopathological factors and *MYBL2* expression after excluding normal sample data, the Mann-Whitney U test was performed for comparison between two groups [sex, N stage and M stage (12)], the Kruskal-Wallis test was used for multigroup comparison [T stage, clinical stage and histological grade (12)]. The association between *MYBL2* expression and OS was estimated using Kaplan-Meier survival curves. (A) Sex; (B) T stage; (C) N stage; (D) M stage; (E) histological grade; and (F) clinical stage. (G) Kaplan-Meier curves revealed that the prognosis of patients with ccRCC with high *MYBL2* expression was poorer than that of patients with ccRCC with low *MYBL2* expression. *P<0.05, ***P<0.001 TCGA, The Cancer Genome Atlas; ccRCC, clear cell renal cell carcinoma; *MYBL2*, MYB protooncogene-like 2; T, tumor; N, node; M, metastasis.

Table II. Association of MYB protooncogene-like 2 expression levels with the clinicopathological factors of patients using logistic regression analysis.

Clinical characteristics	Total, n	Odds ratio for MYBL2 expression (Confidence Interval)	P-value
Age, years	530	0.98 (0.96-0.99)	0.003 ^b
Sex, male vs. female	530	1.54 (1.08-2.21)	0.018 ^a
Histological grade ^d , IV vs. I	89	22.15 (5.36-151.97)	<0.001 ^c
Clinical stage ^d , IV vs. I	347	2.99 (1.78-5.11)	<0.001 ^c
T stage ^d , T4 vs. T1	530	6.19 (1.56-41.15)	0.021 ^a
N stage ^d , N1 vs. N0	255	4.91 (1.54-21.83)	0.015 ^a
M stage ^d , M1 vs. M0	498	3.85 (1.39-2.29)	0.001 ^b

^aP<0.05, ^bP<0.01, ^cP<0.001. ^d(ref. 12). Patients were stratified into high- and low-expression groups, with the median expression level selected as the cut-off value. T, tumor; N, node; M, metastasis.

Table III. Association of OS and clinicopathological characteristics in patients from TCGA database using Cox regression.

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age, years	1.02	1.00-1.04	0.012 ^a	1.03	1.01-1.05	0.001 ^b
Sex, male vs. female	1.01	0.67-1.54	0.951	1.11	0.71-1.73	0.657
MYB protooncogene-like 2 expression, high vs. low	1.52	1.29-1.78	<0.001 ^c	1.29	1.08-1.55	0.004 ^b
Histological grade ^d	2.24	1.68-2.99	<0.001 ^c	1.27	0.91-1.79	0.164
Clinical stage ^d	1.86	1.54-2.25	<0.001 ^c	1.11	0.71-1.74	0.645
T stage ^d	3.31	2.17-5.06	<0.001 ^c	1.61	0.77-3.38	0.208
N stage ^d	2.93	1.52-5.67	0.001 ^b	1.31	0.64-2.68	0.456
M stage ^d	4.07	2.63-6.30	<0.001 ^c	2.25	1.03-4.79	0.041 ^a

^aP<0.05, ^bP<0.01, ^cP<0.001. ^d(ref. 12). HR, hazard ratio; CI, confidence interval; T, tumor; N, node; M, metastasis.

Table IV. Gene sets enriched in the high-MYB protooncogene-like 2 expression phenotypes.

Gene set name	NES	NOM P-value	FDR q-value
KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	2.202	<0.001 ^b	<0.001
KEGG_PRIMARY_IMMUNODEFICIENCY	2.146	<0.001 ^b	<0.001
KEGG_JAK_STAT_SIGNALING_PATHWAY	1.999	<0.001 ^b	0.005
KEGG_CYTOSOLIC_DNA_SENSING_PATHWAY	1.993	<0.001 ^b	0.004
KEGG_CHEMOKINE_SIGNALING_PATHWAY	1.953	0.002 ^a	0.006
KEGG_P53_SIGNALING_PATHWAY	1.948	0.002 ^a	0.006

^aP<0.01, ^bP<0.001. Gene sets with NOM P-value <0.05 and FDR q-value <0.25 were considered significant. NES, normalized enrichment score; NOM, nominal; FDR, false discovery rate; JAK-STAT, janus kinase-signal transducer and activator of transcription; KEGG, Kyoto Encyclopedia of Genes and Genomes.

GSEA was then performed; it is able to detect the expression changes in gene sets rather than in individual genes and is considered more flexible and reliable than traditional methods, such as Gene Ontology and Kyoto Encyclopedia of Genes and Genomes, making it one of the most commonly used pathway analysis methods to study the biological

function of tumors (17). However, for genes with complex interactions and insufficient annotation information, the sensitivity of GSEA is reduced due to functional class scoring, the approach of GSEA (18). GSEA demonstrated that *MYBL2* is involved in various pathways associated with tumor progression, such as the intestinal immune

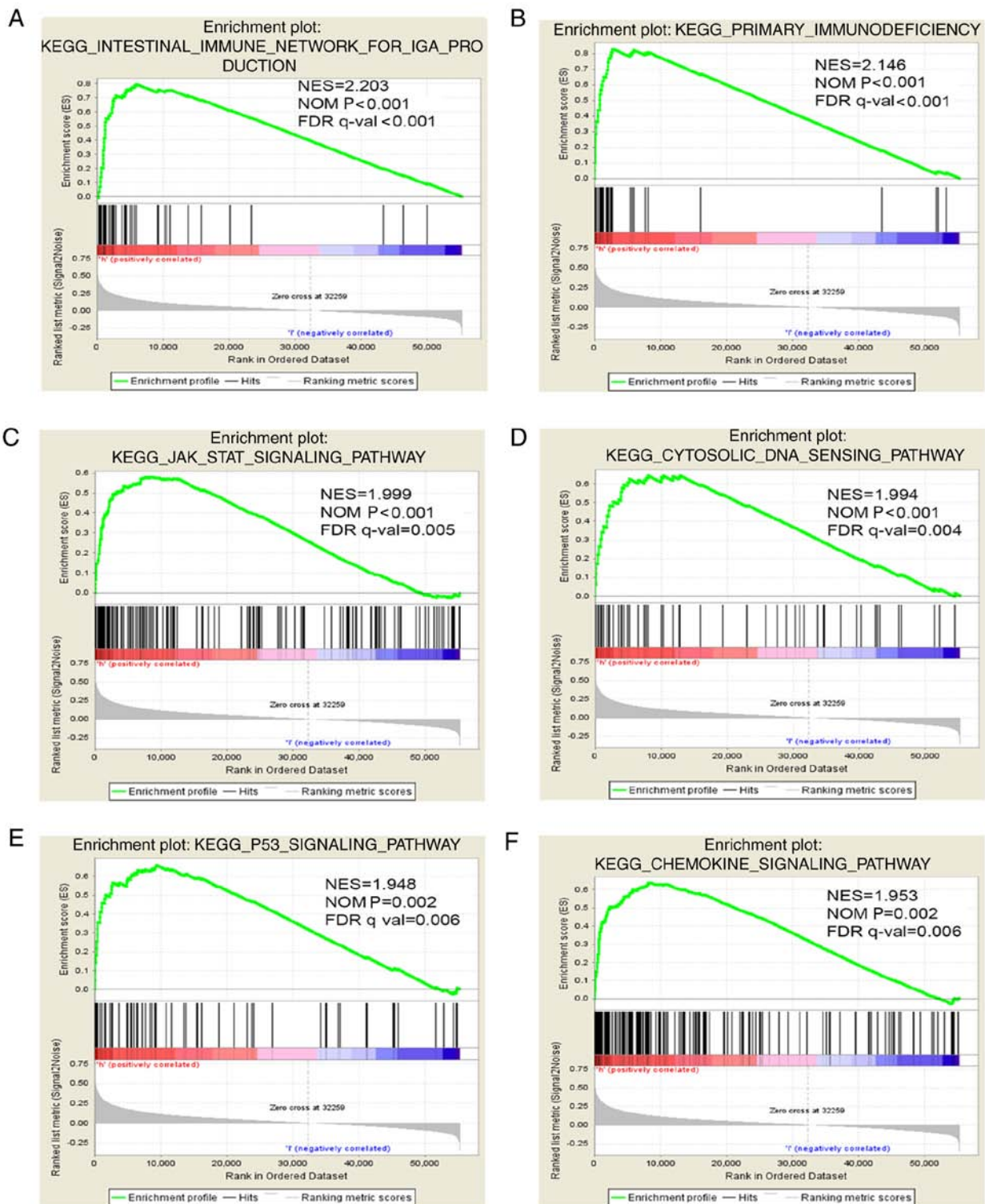


Figure 3. Enrichment plots from GSEA. GSEA was conducted to identify the differentially activated signaling pathways in the high *MYBL2* expression ccRCC datasets. GSEA revealed that (A) 'the intestinal immune network for IgA production', (B) 'primary immunodeficiency', (C) 'the janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway', (D) 'the cytosolic DNA-sensing pathway', (E) 'the p53 signaling pathway' and (F) 'the chemokine signaling pathway' were significantly enriched in patients with upregulated *MYBL2* expression. The data from (A-F) were derived from GSEA. ccRCC, clear cell renal cell carcinoma; *MYBL2*, MYB protooncogene-like 2; GSEA, gene set enrichment analysis; NES, normalized enrichment score; NOM, nominal; FDR, false discovery rate; q-val, q-value.

network for IgA production, primary immunodeficiency, the JAK/STAT signaling pathway, the cytosolic DNA-sensing pathway, the p53 signaling pathway and the chemokine signaling pathway. Some of these pathways have been reported to be associated with other cancer types. In HCC

cells, increased apolipoprotein B mRNA editing enzyme catalytic subunit 3F expression promoted cell proliferation and migration, which were mediated by immune-associated pathways (19). Moreover, a high incidence of cancer, especially lymphoma, has been reported in subjects with primary

immunodeficiency (20). Numerous studies have reported the association between JAK/STAT and various malignancies and inflammatory pathologies, suggesting that JAK-targeted drugs may be successful for the treatment of cancer and immune-mediated diseases (21-27). A previous study indicated that the downregulation of *MYBL2* caused cell cycle arrest at the G₂/M phase via the p53-p21-DREAM-CDE/CHR pathway (28).

However, there were still certain limitations to the current study. Notably, the number of normal tissues was significantly lower than the number of tumorous tissues retrieved from TCGA database. The factors associated with patient prognosis, such as the use of drugs, surgical treatment and surgical details, were lacking. Moreover, the protein levels or direct mechanisms underlying the role of *MYBL2* in ccRCC could not be assessed using TCGA database. Therefore, accounting for various confounding factors to more accurately assess the association between *MYBL2* and OS, and exploring the specific molecular mechanisms involved represent a promising focus for future research.

In conclusion, it was revealed that the upregulation of *MYBL2* in ccRCC was associated with certain advanced clinical factors and was able to independently predict a poor prognosis, indicating that *MYBL2* expression may represent a promising biomarker and potential therapeutic target for the treatment of patients with ccRCC. However, the protein expression levels of *MYBL2* and the specific molecular mechanisms underlying poor prognosis of ccRCC need to be further explored in future research.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in The Cancer Genome Atlas repository, (portal.gdc.cancer.gov/).

Authors' contributions

JYL and YF designed this research project. SSS and YF contributed to data collection, analysis and interpretation. All authors participated in writing of the manuscript for the relevant sections. All authors read and approved the final manuscript, and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work is appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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