

Clinical Significance of MYBL2 Expression in Hepatocellular Carcinoma: Impact on Overall Survival

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Purpose: Transcription factor MYB proto-oncogene like 2 (MYBL2) is involved in cell cycle regulation and proliferation, and it has been implicated in various cancers. However, its expression pattern and clinical significance in hepatocellular carcinoma (HCC) are unclear. Therefore, the aim of this study was to evaluate the tissue expression of MYBL2 in patients with HCC and evaluate associations with clinicopathological factors and prognosis.

Patients and Methods: We enrolled 88 patients with HCC who underwent hepatectomy from October 2023 to January 2025 and analyzed MYBL2 expressions in non-tumorous and tumorous tissue samples using immunohistochemistry. The patients were then categorized into high and low expression groups, and clinicopathological factors were compared. Cox proportional hazard regression and Kaplan-Meier survival analyses were used to explore the prognostic significance of MYBL2 expression.

Results: MYBL2 immunohistochemistry score was significantly associated with survival (p for trend = 0.038). Kaplan-Meier analysis demonstrated that those with a high MYBL2 expression were associated with lower overall survival compared to those with a low MYBL2 expression ($p = 0.029$). An independent association was found between high MYBL2 expression in tumorous tissues and poor overall survival ($p = 0.047$). However, multivariate analysis indicated that MYBL2 expression was not an independent risk factor for overall survival.

Conclusion: The results indicated an association between MYBL2 and HCC prognosis, suggesting that it could be used as a prognostic biomarker and potentially be a therapeutic target. Further research is required to clarify the molecular mechanisms by which MYBL2 drives tumor progression and to explore its potential in targeted therapy for HCC.

Keywords: hepatocellular carcinoma, MYBL2, overall survival, immunohistochemistry

Introduction

Hepatocellular carcinoma (HCC) is a significant health issue and the third leading cause of cancer-related mortality worldwide.¹ It is the most common type of liver cancer, accounting for 75%–85% of all cases.^{2,3} The incidence of HCC continues to rise, especially in patients with chronic liver diseases such as hepatitis B and C virus (HBV/HCV) infections,⁴ non-alcoholic fatty liver disease,^{5,6} and liver cirrhosis.^{6,7} Despite advances in diagnostic techniques and

therapeutic strategies such as liver transplantation, surgical resection, and targeted therapies,^{8,9} the prognosis of HCC remains poor due to diagnosis at a late stage, frequent tumor recurrence, and resistance to treatment.¹⁰ Identifying novel biomarkers and molecular targets involved in HCC progression is essential for improving early detection, risk stratification, and therapeutic outcomes.

Transcription factor MYB proto-oncogene like 2 (MYBL2), also known as B-Myb, has been shown to play a critical role in regulating both differentiation and proliferation across various cell types.^{11,12} A previous study reported high expressions of MYBL2 in rapidly proliferating cells, and that this played a crucial role in regulating genes essential for the G1-S and G2-M phases of the cell cycle.¹³ The researchers concluded that MYBL2 expression and activation are governed by genetic mechanisms. Furthermore, they showed that MYBL2 upregulation accelerated the growth and progression of both premalignant and malignant liver tissues by disrupting cell cycle regulation and activating key genes and pathways involved in tumor development.¹³ MYBL2 has also been shown to be overexpressed in breast,¹⁴ lung,¹⁵ and colorectal cancers,¹⁶ and it has been associated with tumor progression and poor prognosis.^{16,17} However, the association between MYBL2 expression and clinicopathological factors in patients with HCC is unclear.

Several studies have suggested that MYBL2 may contribute to tumorigenesis by promoting cell proliferation,¹² inhibiting apoptosis,¹⁸ and enhancing genomic instability.¹⁹ In HCC, dysregulation of cell cycle control is a hallmark of malignant transformation, and factors that drive aberrant cell cycle progression are critical for tumor development.²⁰ An elevated MYBL2 expression has been linked to aggressive tumor behavior in other cancers,^{21,22} raising the possibility that MYBL2 may also serve as a prognostic biomarker in HCC.

The aims of this study were therefore to evaluate the expression of MYBL2 in tumorous and non-tumorous tissue samples from HCC patients using immunohistochemical (IHC) analysis, explore the associations between MYBL2 expression and clinicopathological factors, and whether MYBL2 can serve as a prognostic biomarker in these patients.

Material and Methods

Study Participants

A total of 88 patients who underwent resection for HCC from October 2023 to January 2025 were enrolled (22 females and 66 males; mean age, 61 years; range, 38–88 years). Information on the patients was obtained from the hospital's medical records. The inclusion criteria were patients who: (1) had a pathologically confirmed diagnosis of primary HCC; (2) underwent curative-intent hepatectomy at our institution from October 2023 to January 2025; (3) had available tumor and adjacent non-tumor liver tissue samples for histopathological evaluation; and (4) had complete clinicopathologic and follow-up data. The exclusion criteria were patients who: (1) presented with recurrent HCC at the time of surgery; (2) had a history of other primary malignancies; (3) received preoperative anti-cancer therapy; or (4) had insufficient tissue or incomplete clinical data. Tumors were staged following the American Joint Committee on Cancer guidelines. The enrolled patients attended regular outpatient follow-up visits when they were assessed for recurrence. Immediately after resection, tumor and matched adjacent non-tumor tissue samples were divided into two portions. One portion was fixed in 10% neutral buffered formalin, embedded in paraffin, and used for histopathological evaluation and IHC. The other portion was snap-frozen at -80°C to preserve molecular integrity for potential future molecular analysis. Only the formalin-fixed, paraffin-embedded tissues were analyzed in the present study. This study was approved by the Institutional Review Board of E-Da Hospital (no. EMRP-112-138), and signed informed consent forms were obtained from each participant.

IHC Staining and Analysis of MYBL2 Expression

For the IHC analysis, sections of the tissue specimens were obtained from formalin-fixed, paraffin-embedded tissue blocks, mounted on slides, and dried at 60°C for 1 hour. Xylene was used to deparaffinize the sections, followed by rehydration with graded alcohol. Heat-induced epitope retrieval was conducted using ethylenediaminetetraacetic acid for 30 minutes at 100°C . The slides were blocked with peroxide for 10 minutes at room temperature, and then incubated with rabbit polyclonal B-Myb antibody (Abcam, ab114055) at a dilution of 1:100 for 60 minutes at 37°C . Color was developed using 3, 3'-diaminobenzidine tetrahydrochloride at room temperature for 10 minutes. Finally, the slides

were counterstained with hematoxylin for 5 minutes, and then mounted and observed under a light microscope. The MYBL2 IHC results were scored as follows: score 1, <25% positively stained cells; score 2, 25–50% positively stained cells; score 3, 51–75% positively stained cells; and score 4, >75% positively stained cells. All scores were evaluated by two independent qualified pathologists, with cases of disagreement resolved by consensus.

Statistical Analysis

Categorical data are shown as frequency (%), and Fisher's exact test was used to explore differences. Survival and trends among the MYBL2 IHC score groups were explored with Fisher's exact test and Cochran-Armitage trend test, respectively. The Kaplan-Meier method was used to assess survival, and between-group differences were examined with the Log rank test. Univariate and multivariate Cox proportional hazard analyses were performed to identify factors independently associated with survival and calculate hazard ratios (HRs) with corresponding 95% confidence intervals (CIs). A p-value < 0.05 was considered statistically significant. The statistical analysis was performed using JMP v10.0 (SAS Institute, Cary, NC).

Results

High MYBL2 Expression Was Correlated with Poor Differentiation and Advanced Stage

MYBL2 immunoreactivity was analyzed using a 4-tier grading system from 1 to 4, as shown in [Figure 1A to D](#). MYBL2 was found to be expressed in the cytoplasm and/or nuclear areas of the tumor tissue specimens. As shown in [Figure 1E](#), a lower expression of MYBL2 was observed in the non-tumor tissue specimens.

Analysis of the patients' clinicopathological characteristics based on MYBL2 grade in the tumor tissue specimens showed that compared to the patients with MYBL2 grade 1 or 2, those with MYBL2 grade 3 or 4 had a significantly higher prevalence of alcohol consumption, TNM stage II, and poorer overall survival (all $p < 0.05$, [Table 1](#)). However, no significant differences between the two groups were observed regarding early recurrence, TNM stage I or IIIa-IVb, pathological differentiation, etiology, tumor size, age or sex ([Table 1](#)). Survival analysis showed that survival was significantly associated with MYBL2 IHC score (p for trend = 0.038, [Figure 2](#)). Specifically, the proportions of surviving patients with MYBL2 grades 1, 2, 3, and 4 were 69.8%, 85.0%, 60.0%, and 30.0%, respectively, while the corresponding mortality rates were 30.2%, 15.0%, 40.0%, and 70.0%. These results demonstrate a clear trend of decreasing survival with increasing MYBL2 expression, consistent with a dose-response relationship. Patients with the highest MYBL2 score (grade 4) had markedly reduced survival compared to those with a lower expression. This finding suggests that MYBL2 overexpression may reflect more aggressive tumor biology and worse clinical outcomes.

High MYBL2 Expression Was Associated with Lower Overall Survival

We then assessed overall survival in the patients with a high (grade 3 or 4) or low (grade 1 or 2) MYBL2 expression. The results showed that a high MYBL2 expression was significantly associated with lower overall survival ([Figure 3](#)). In addition, poor differentiation, age ≥ 65 years and high MYBL2 expression were significantly associated with poor overall survival in univariate analysis ([Table 2](#)). However, multivariate analysis revealed that only poor differentiation was an independent prognostic factor, but not age, sex, HBV/HCV infection, tumor size, stage, or MYBL2 expression ([Table 3](#)). These results showed that poor tumor differentiation was the strongest independent predictor of overall survival in our cohort. Although MYBL2 expression was correlated with adverse outcomes in univariate analysis, this correlation did not persist after adjustment, suggesting that MYBL2 expression may reflect underlying histologic aggressiveness rather than serve as an independent prognostic factor.

Discussion

In this study, we found that a higher MYBL2 expression in patients with HCC was associated with adverse clinicopathologic features and poorer overall survival, although MYBL2 was not an independent prognostic factor after multivariable adjustment. These findings support a potential role for MYBL2 as a biomarker of aggressive tumor

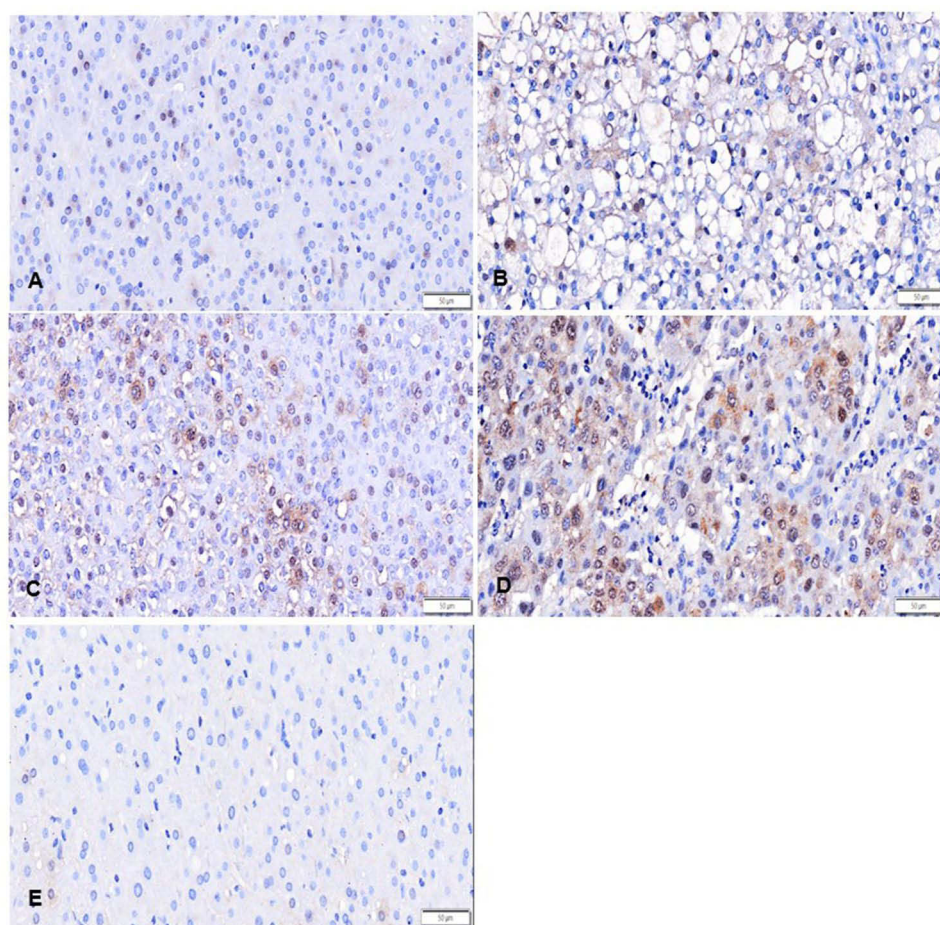


Figure 1 Cytoplasmic MYB proto-oncogene like 2 (MYBL2) immunoreactivity in hepatocellular carcinoma tissue specimens. Immunoreactivity was analyzed using a 4-tier grading system: grade 1 (A), grade 2 (B), grade 3 (C), grade 4 (D), and non-tumor tissue (E). All specimens were formalin-fixed, paraffin-embedded and stained using standardized protocols. Representative images were selected and validated by two experienced pathologists blinded to clinical outcomes.

biology in HCC. While previous experimental studies in other tumor types have linked MYBL2 to cell cycle regulation, genomic instability, and chemoresistance,^{11,12,14–16,19} and one genetic study suggested that MYBL2 may contribute to susceptibility to HCC,¹³ our findings are limited to IHC correlations. There were three main findings in this study. First, the patients with a high MYBL2 expression were associated with poorer survival compared to those with a low expression. Second, poor differentiation, age ≥ 65 years, and a high expression of MYBL2 were significantly associated with poorer overall survival in univariate analysis. Third, a low MYBL2 expression was significantly associated with better overall survival, highlighting the potential role of MYBL2 as a prognostic biomarker in HCC.

The finding of significantly poorer overall survival in the patients with a high MYBL2 expression is consistent with previous studies.^{12,17,22,23} Guan et al reported that patients with primary HCC and a high MYBL2 expression were associated with inferior overall survival outcomes.²³ Similarly, Musa et al demonstrated that MYBL2 was overexpressed and associated with poor outcomes in patients with various cancer types.¹² Frau et al also reported that MYBL2 plays a role in human cancers, that it is overexpressed in HCC, and that its expression and activation are regulated by genetic factors.¹³ In addition, they reported that the upregulation of MYBL2 could promote the rapid growth and progression of both premalignant and malignant liver lesions by disrupting cell cycle control and activating genes and pathways associated with tumor development.¹³

MYBL2 has been associated with promoting epithelial-mesenchymal transition (EMT), a process that enables cancer cells to acquire invasive and metastatic properties.²⁴ EMT is characterized by the loss of epithelial cell polarity and adhesion followed by the acquisition of a mesenchymal phenotype, which then enhances cellular motility and

Table 1 Correlation Between MYBL2 Expression and Clinicopathological Characteristics

Clinical Parameter	ALL Cases	MYBL2 Grade 1, 2, N (%)	MYBL2 Grade 3, 4, N (%)	p-Value
No	88	63	25	
Sex				
Male	66(75.0)	46(73.0)	20(80.0)	0.592
Female	22(25.0)	17(27.0)	5(20.0)	
Age (yr)				
<65	58(65.9)	44(69.8)	14(56.0)	0.225
≥65	30(34.1)	19(30.2)	11(44.0)	
Alcohol drinking				
Yes	29(33.0)	14(22.2)	15(60.0)	0.001
No	59(67.0)	49(77.8)	10(40.0)	
Tumor size (cm)				
<5	58(65.9)	43(68.2)	15(60.0)	0.619
≥5	30(34.1)	20(31.8)	10(40.0)	
Etiology				
HCC	20(22.7)	13(20.6)	7(28.0)	0.574
HCC/HBV	41(46.6)	30(47.6)	11(44.0)	0.816
HCC/HCV	25(28.4)	19(30.2)	6(24.0)	0.612
HCC/HBV/HCV	2(2.3)	1(1.6)	1(4.0)	0.490
Pathological differentiation				
Well	8(9.1)	6(9.5)	2(8.0)	0.992
Moderately	72(81.8)	52(82.6)	20(80.0)	0.774
Poorly	8(9.1)	5(7.9)	3(12.0)	0.377
TNM stage				
I	63(71.6)	49(77.8)	14(56.0)	0.051
II	15(17.0)	7(11.1)	8(32.0)	0.023
IIIa-IVb	10(11.4)	7(11.1)	3(12.0)	0.945
Early recurrence				
Absent	36(40.9)	26(41.3)	10(40.0)	0.913
Occurs	52(59.1)	37(58.7)	15(60.0)	
Survival status				
Survival	59(67.0)	47(74.6)	12(48.0)	0.024
Death	29(33.0)	16(25.4)	13(52.0)	

Abbreviations: MYBL2, MYB proto-oncogene like 2; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus.

invasiveness.²⁵ MYBL2 has been demonstrated to promote EMT by regulating the expressions of key EMT markers including E-cadherin, N-cadherin, vimentin, and Smad/SNAI1.^{16,24,26} Specifically, MYBL2 has been shown to down-regulate the epithelial marker E-cadherin while upregulating mesenchymal markers such as vimentin and N-cadherin, thereby promoting cellular detachment, invasion, and metastasis.^{12,16} Moreover, MYBL2 has been associated with activating the transcription of Snail, a key EMT regulator, further enhancing the EMT process and the metastatic potential of HCC cells.^{24,27} This suggests that MYBL2 not only facilitates primary tumor growth but also contributes to metastasis, thereby exacerbating disease severity.^{12,13,23}

The oncogenic activity of MYBL2 in HCC can be attributed to its complex regulatory network, which involves multiple downstream effectors and signaling pathways.²⁸ At the molecular level, MYBL2 drives cell cycle progression by regulating the transcription of genes critical for the G1/S and G2/M transitions.¹³ Specifically, MYBL2 enhances the expressions of CDK1, CCNB1, and CCNA2 by forming a complex with the MuvB core, which consists of LIN9, LIN37, LIN52, LIN54, and RBBP4.^{29,30} During the late G1 and early S phases, this MYBL2-MuvB complex transactivates G2/M genes, ensuring proper cell cycle progression and rapid division of HCC cells.¹² In the late S phase, the MuvB-MYBL2 complex recruits FOXM1, forming a ternary complex that drives the transcription of late cell cycle genes essential for mitotic progression.^{31,32} This regulatory mechanism highlights the pivotal role of MYBL2 in modulating

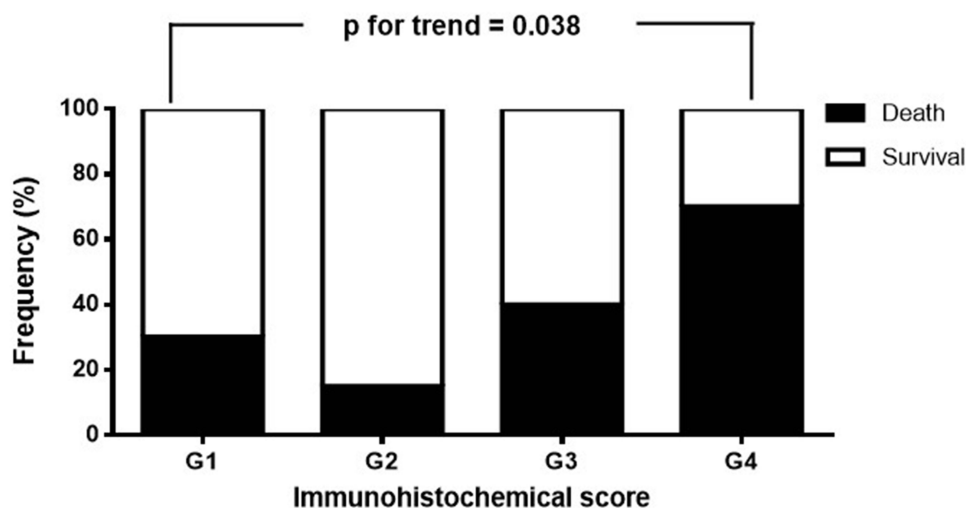


Figure 2 The frequencies of death and survival of the patients with hepatocellular carcinoma stratified by MYB proto-oncogene like 2 (MYBL2) immunohistochemical (IHC) score. MYBL2 staining scores were assigned based on formalin-fixed paraffin-embedded sections by two qualified pathologists blinded to clinical outcomes; representative images reflect the spectrum of expression observed. Survival status was significantly associated with MYBL2 IHC score.

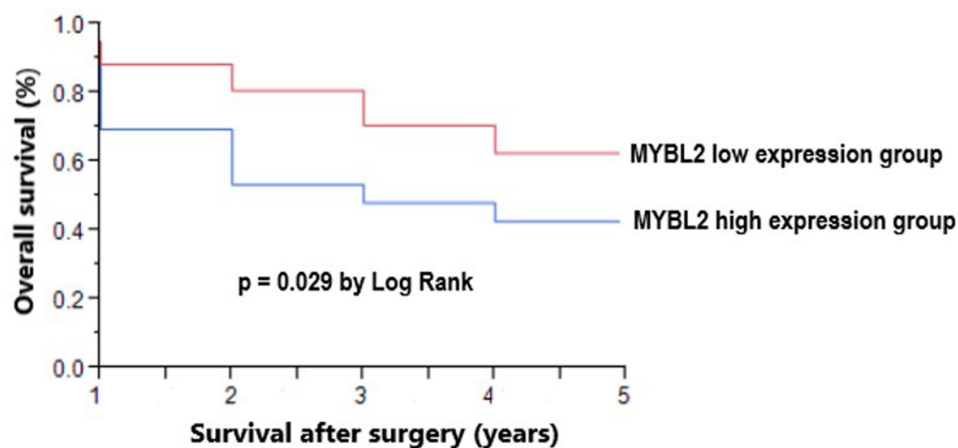


Figure 3 Overall survival in patients with low and high expressions of MYB proto-oncogene like 2 (MYBL2) in tumor tissue. The overall survival rate was significantly higher in the group with a low MYBL2 expression.

cell cycle transition, thereby sustaining the high proliferative capacity of HCC cells.¹² In addition, MYBL2 has been shown to be a key regulator of purine synthesis and to promote HCC progression by transcriptionally activating IMPDH1.³³ However, further investigations are required to clarify the association between the expression of MYBL2 in HCC tissues with tumor progression.

MYBL2 has been associated with p53-mutant HCC, where it drives aggressive tumor growth by bypassing p53-dependent cell cycle checkpoints.¹² Chen et al proposed that an elevated MYBL2 expression could promote gastric cancer cell proliferation and suppress apoptosis by modulating the PI3K/AKT and BCL2/BAX/Cleaved-caspase-3 signaling pathways, and consequently that MYBL2 could serve as a potential therapeutic target and prognostic biomarker for gastric cancer.³⁴ In addition, MYBL2 has been shown to regulate three key oncogenic pathways: (1) Wnt/ β -catenin signaling, which enhances tumor cell survival and self-renewal;^{35,36} (2) the PI3K/AKT/mTOR pathway, which promotes cell proliferation and resistance to therapy;^{34,37} and (3) TGF- β signaling, reinforcing EMT and metastatic potential.^{12,38} The molecular and cellular mechanisms underlying the role of MYBL2 in HCC tumorigenesis were not analyzed in the present study, and further investigations are needed to explore this issue.

Table 2 Univariate Analysis of Factors Affecting Overall Survival in the Patients with Hepatocellular Carcinoma

Variable	Hazard Ratio	95% CI	p-Value
Age: ≥65 years versus <65 years	2.715	1.301–5.848	0.008
Sex: Male versus female	1.564	0.647–4.647	0.341
Differentiation status:			
Poorly versus well and moderately	4.557	1.646–10.865	0.005
Hepatitis B Virus: Positive versus negative	0.525	0.234–1.108	0.092
Hepatitis C Virus: Positive versus negative	1.400	0.625–2.952	0.399
Tumor size: >5 cm versus ≤5 cm	1.274	0.593–2.649	0.525
Stage: 3, 4 versus 1, 2	2.272	0.837–5.259	0.101
MYBL2 in tumor: High versus low	2.145	1.012–4.462	0.047

Abbreviation: MYBL2, MYB proto-oncogene like 2.

Table 3 Multivariate Analysis of Factors Affecting Overall Survival in the Patients with Hepatocellular Carcinoma Using a Cox's Proportional Hazard Model

Variable	Hazard Ratio	95% CI	p-Value
Age: ≥65 years versus <65 years	2.488	0.999–6.412	0.050
Sex: Male versus female	1.071	0.353–3.726	0.907
Differentiation status:			
Poorly versus well and moderately	4.995	1.531–15.038	0.009
Hepatitis B Virus: Positive versus negative	0.622	0.237–1.633	0.330
Hepatitis C Virus: Positive versus negative	0.810	0.306–2.129	0.666
Tumor size: >5 cm versus ≤5 cm	1.176	0.449–3.012	0.737
Stage: 3, 4 versus 1, 2	2.575	0.842–7.156	0.094
MYBL2 in tumor: High versus low	1.944	0.860–4.341	0.108

Notes: In multivariate Cox regression analysis, values included for analysis were age, sex, differentiation status, hepatitis B Virus, hepatitis C Virus, tumor size, stage, and MYBL2 in tumor.

Abbreviation: MYBL2, MYB proto-oncogene like 2.

Infection with HBV/HCV has been shown to induce genetic instability and be a major risk factor for HCC.^{39,40} Currently, there is no clear evidence directly linking MYBL2 with HCV or HBV infection. However, HCV infection can activate multiple cellular signaling pathways including STAT3, ERK and JNK, which can in turn promote the expression of genes such as MMP-2 and Bcl-2, which play crucial roles in cell proliferation and survival.⁴¹ In addition, MYBL2 has been identified as an oncogene in various cancers, with its overexpression strongly associated with poor prognosis.^{12,17,22,23} Therefore, while no direct evidence has linked MYBL2 to HCV or HBV infection, the changes in cellular signaling pathways induced by these viral infections may indirectly influence MYBL2 expression or function. Our results (Table 2) did not show a significant difference in MYBL2 expression between the patients with HBV or HCV infection, possibly due to the nature of MYBL2 regulation in HCC, which is influenced by tumor biology rather than viral infection status.

We also found a significant association between high MYBL2 expression with poor overall survival in univariate analysis, indicating a potential role in HCC progression. However, this association was not found in multivariate analysis, suggesting that MYBL2 may not be an independent prognostic factor. The prognostic effect of MYBL2 may be influenced by other clinical variables such as tumor stage, size, or differentiation status. In addition, no significant association was found between MYBL2 expression and disease-free survival, implying that its relevance may lie more in the general aggressiveness of the tumor rather than recurrence after treatment. These findings suggest that while MYBL2 may contribute to tumor biology, its prognostic utility is limited compared to established clinical factors.

This study has several limitations. First, it was conducted at a single center with a relatively small sample size (88 HCC patients), which may limit the generalizability of our findings. Larger, multicenter cohorts are needed to validate the clinical significance of MYBL2 in HCC prognosis. Second, we primarily relied on IHC staining to assess the expression of MYBL2 in tissue samples. While this method can provide valuable insights into protein localization and expression levels, quantitative techniques such as Western blotting or RT-qPCR could further confirm the expression of MYBL2 and its correlation with clinical outcomes. Third, although we observed that MYBL2 expression was associated with adverse clinicopathologic features and overall survival, we did not perform *in vitro* experiments or *in vivo* animal studies to validate the mechanistic role of MYBL2 in HCC progression. Therefore, the proposed mechanisms should be considered as contextual information rather than direct evidence from our data. These limitations highlight that our findings should be interpreted as an initial clinicopathologic correlation, and further functional studies such as *in vitro* and *in vivo* experiments are warranted to explore the role of MYBL2 in tumor growth, metastasis, and therapeutic resistance. Fourth, although we assessed the association between MYBL2 expression and clinicopathological characteristics, we did not explore potential interactions with key oncogenic pathways or viral infections (HBV/HCV) that may influence MYBL2 expression in HCC. Future research should focus on these factors to gain a more comprehensive understanding. Finally, the follow-up period for survival analysis may not have been long enough to fully capture long-term outcomes in HCC patients. A longer follow-up period could provide a more robust evaluation of MYBL2 as a prognostic biomarker.

In brief, the practical significance of our findings is that MYBL2 expression, which can be measured in routine formalin-fixed paraffin-embedded sections, was correlated with aggressive tumor features and poor survival. Although the association was not independent after adjustment, this finding is also clinically informative, as it suggests that MYBL2 reflects underlying histologic aggressiveness rather than serving as a standalone biomarker. This study is novel in that it directly evaluates the expression of MYBL2 in resected human HCC tissues with survival follow-up, thus bridging prior molecular research on MYBL2 with clinicopathologic and prognostic data. These insights may guide both risk stratification and the design of future mechanistic studies.

Conclusion

In summary, a high MYBL2 expression in patients with HCC was associated with poor differentiation, advanced stage, and poorer overall survival, indicating that MYBL2 may serve as a marker of aggressive tumor biology. However, MYBL2 did not remain an independent prognostic factor after adjusting for established clinicopathologic features. These findings suggest that MYBL2 has potential value as a supportive biomarker, but that findings should be interpreted with caution. Further studies including mechanistic investigations and validation in larger cohorts are required to clarify the role of MYBL2 in hepatocarcinogenesis and its utility in clinical prognostication.

Abbreviations

MYBL2, MYB proto-oncogene like 2; HCC, Hepatocellular carcinoma; HBV/HCV, Hepatitis B and hepatitis C virus; IHC, Immunohistochemical; CI, Confidence interval; EMT, Epithelial-mesenchymal transition.

Data Sharing Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Ethics Approval and Consent to Participate

The present study was approved by the Human Research Ethics Committee of E-Da Hospital (EDAH IRB No. EMRP-112-138) and written informed consent was provided by all patients prior to the study start. All procedures were performed in accordance with the ethical standards of the Institutional Review Board and The Declaration of Helsinki, and its later amendments or comparable ethical standards.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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