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Immunohistochemical Expression of EZH2 and INI-1 in Hepatocellular Carcinoma: Prognostic Significance in a Retrospective Cohort Study

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ABSTRACT

Objective: Enhancer of zeste homologue 2 (EZH2) serves as the enzymatic component of polycomb repressive complex 2, while SMARCB1/INI-1 is an essential subunit of the SWI/SNF chromatin remodeling complex; both function as epigenetic regulators with proposed roles in hepatocarcinogenesis. This study aimed to evaluate the expression of EZH2 and INI-1 by immunohistochemistry (IHC) in hepatocellular carcinoma (HCC) and to determine their association with clinicopathological features and survival outcomes.

Material and Methods: We retrospectively studied 103 patients with histologically proven HCC who were treated at a single center between 2011 and 2019. EZH2 and INI-1 were evaluated by IHC in archival, paraffin-embedded specimens, with a case considered positive when at least 1% of tumor nuclei showed distinct staining. Relationships with clinicopathological variables were tested using the chi-square test or Fisher's exact test, and overall survival (OS) and progression-free survival (PFS) were assessed using Kaplan-Meier estimation and Cox proportional hazards modeling.

Results: EZH2 positivity occurred in 45 patients (43.7%), whereas INI-1 loss occurred in 11 (10.7%). Over a median follow-up of 42.0 months, 71 deaths and 86 progression events were observed. EZH2-positive cases exhibited significantly shorter median OS (8.0 vs. 27.0 months; p=0.007) and PFS (5.0 vs. 9.0 months; p=0.012). In univariate Cox regression analysis, EZH2 positivity was a significant risk factor for both mortality [hazard ratio (HR): 1.88; 95% confidence interval (CI): 1.17-3.02; p=0.009] and disease progression (HR: 1.69; 95% CI: 1.10-2.60; p=0.018). However, EZH2 did not retain independent prognostic significance in multivariate analysis adjusted for Barcelona Clinic Liver Cancer (BCLC) stage for either OS (HR: 1.55; 95% CI: 0.95-2.53; p=0.078) or PFS (HR: 1.39; 95% CI: 0.89-2.17; p=0.146). INI-1 loss showed a trend toward longer PFS (22.0 vs. 6.0 months; p=0.051) and OS (30.0 vs. 15.0 months; p=0.097), but these differences did not reach statistical significance.

Conclusion: EZH2 positivity is associated with inferior survival in HCC; however, this association does not remain independent of BCLC stage, indicating a stage-dependent rather than independent prognostic effect. INI-1 loss showed only borderline exploratory associations with survival that require validation in larger series. Together, these observations support the biological relevance of epigenetic regulators in HCC.

Keywords: Hepatocellular carcinoma; EZH2; INI-1; SMARCB1; prognosis; immunohistochemistry; epigenetics; biomarker

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for a large share of malignancies and is among the leading contributors to cancer mortality worldwide. In 2020, approximately 906,000 individuals worldwide were newly diagnosed with liver cancer—of which HCC was the dominant histological type—and the disease ranked third in cancer-related deaths. Given a five-year relative survival of approximately 18% and approximately 830,000 deaths recorded that same year, the small margin between new diagnoses and fatalities underscores the aggressive behavior of this tumor.[1-3]

Efforts to deliver precision medicine in HCC are constrained by the disease's pronounced heterogeneity, both across different tumors and within a single lesion; the limited

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number of biomarkers validated for prognostic and treatment-selection purposes; and the drawbacks inherent to invasive tissue sampling. Consequently, identifying accessible and dependable molecular markers remains essential for improving risk stratification and tailoring therapy to the individual patient.[4]

HCC develops through a multistep sequence in which genetic and epigenetic alterations progressively accumulate. Among these, epigenetic dysregulation has been recognized as a central driver of tumor development, acting chiefly through disturbances in deoxyribonucleic acid (DNA) methylation, histone modifications, and non-coding ribonucleic acids (RNAs).[5,6] Two histone-modifying systems exert opposing effects on chromatin: polycomb repressive complex 2 (PRC2) silences transcription, while the SWI/SNF remodeling complex renders chromatin accessible for transcriptional activation.[7,8]

As the catalytic subunit of PRC2, enhancer of zeste homologue 2 (EZH2) represses transcription by trimethylating histone H3 at lysine 27 (H3K27me3)—a modification that condenses chromatin and inactivates genes.[9] Because many of the genes silenced are tumor suppressors, this process constitutes an important oncogenic driver in hepatocarcinogenesis.[10,11] Accumulating evidence implicates EZH2 as a regulator of cell proliferation and disease progression in HCC, primarily through the silencing of tumor suppressor genes such as chromodomain helicase DNA binding protein 5 (CHD5), a confirmed suppressor that favors senescence and restrains proliferation. By trimethylating H3K27 at the CHD5 promoter, *EZH2* silences this protective gene and thereby supports tumor growth.[12,13]

Opposing PRC2, the SWI/SNF complex restores an open, transcription-permissive chromatin configuration. A central component of this complex, SMARCB1/INI-1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1) behaves as a tumor suppressor across a range of cancers.[8] Although the loss of INI-1 has been thoroughly described in rhabdoid and other tumors, little is known about INI-1 expression in HCC or whether it has prognostic relevance.[14]

Therefore, this study aimed to evaluate the expression of EZH2 and INI-1 by immunohistochemistry (IHC) in a single-institution cohort of HCC patients and to determine their association with clinicopathological features and survival outcomes, and to assess their value as prognostic biomarkers.

MATERIAL AND METHODS

Study Design and Population

This single-institution, retrospective cohort study was conducted following approval from the Çukurova University Faculty of Medicine Clinical Research Ethics Committee (approval number: 38; date: 13.04.2018) and in accordance with the Declaration of Helsinki. Reporting adheres to the STROBE statement. This study was funded by the Çukurova University Scientific Research Projects Unit (project number: TTU-2018-10738).

The study cohort comprised 103 patients with pathologically confirmed HCC who were managed at our institution between 2011 and 2019. Because the analysis was retrospective and relied on fully de-identified records, the ethics committee waived the need for individual informed consent. Clinicopathological information—age at diagnosis, sex, etiology, Barcelona Clinic Liver Cancer (BCLC) stage, and Child-Pugh class—was retrieved from patient charts. Treatment modalities administered during the disease course, including surgical resection, liver transplantation, locoregional therapies (transarterial chemoembolization, transarterial radioembolization, and percutaneous thermal ablation), systemic therapy (sorafenib), cytotoxic chemotherapy, and best supportive care were recorded where available.

Immunohistochemical Analysis

IHC analysis was performed on archival formalin-fixed, paraffin-embedded tissue blocks from all 103 cases. Five-micron-thick sections were processed on a Ventana BenchMark XT automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA) using the ultraView Universal DAB Detection Kit.

To assess EZH2 protein expression, sections were incubated with a primary mouse monoclonal antibody against EZH2 (Clone 11; Cat. No. 415M-15; Cell Marque, Rocklin, CA, USA). For INI-1 assessment, a primary mouse monoclonal antibody targeting SMARCB1/INI-1 was used (Clone: MRQ-27, Cat. No. 272M-15; Cell Marque, Rocklin, CA, USA). All stained slides were evaluated by an experienced pathologist who was blinded to the clinical data. For both markers, a case was considered positive if distinct nuclear staining was observed in ≥1% of the tumor cells. Loss of INI-1 expression was defined as the absence of nuclear staining in tumor cells, with retained expression in surrounding non-neoplastic cells serving as an internal positive control. A ≥1% nuclear threshold was applied because EZH2 nuclear staining in HCC is frequently heterogeneous and focal, and a low cut-off maximizes sensitivity for detecting any tumoral EZH2 expression in a routine diagnostic setting. Because staining intensity and H-score were not systematically recorded, we use the term "EZH2 positivity" rather than "overexpression" throughout, to reflect a binary, presence-based scoring approach.

Statistical Analysis

All statistical analyses were conducted using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Associations between biomarker expression status and clinicopathological parameters were assessed using the chi-square test or Fisher's exact test. Overall survival (OS) and progression-free survival (PFS) were estimated by the Kaplan-Meier method and compared using the log-rank test. The prognostic value of EZH2 was further evaluated by univariate and multivariate Cox proportional hazards regression. OS was measured from the date of diagnosis until death from any cause, whereas PFS was measured from the start of treatment until radiological progression, recurrence, or death, whichever occurred first. A two-sided p-value of <0.05 was considered statistically significant for all analyses.

The multivariate Cox model included EZH2 status and BCLC stage, with 71 OS events and 86 PFS events for four estimated parameters (approximately 17.8 and 21.5 events per variable, respectively), exceeding the recommended minimum of ten. Complete-record analysis was used without imputation. The proportional hazards assumption was assessed using scaled Schoenfeld residuals and was satisfied for all covariates in both the OS and PFS models (all p>0.05). Child-Pugh class was not entered into the multivariate model because liver function and performance status are already incorporated within BCLC staging, thereby avoiding collinearity. The median follow-up duration was estimated using the reverse Kaplan-Meier method.

RESULTS

Patient Characteristics

A total of 103 patients with pathologically confirmed HCC were included in this retrospective study. The baseline clinicopathological characteristics of the cohort are summarized in Table 1. The cohort was predominantly male (86.4%; n=89), with a median age at diagnosis of 68 years (range, 17-89). The most common underlying etiology was hepatitis B (57.3%), followed by non-viral causes (29.1%) and hepatitis C (13.6%). At diagnosis, the majority of patients were classified as Child-Pugh class A (58.3%), and early-stage disease (BCLC stages 0 and A) comprised 43.7% of the cohort (Table 1). With respect to treatment, 43 patients (41.7%) received locoregional therapy with or without sorafenib; the remaining patients received surgical, systemic, or supportive approaches, alone or in combination, according to BCLC stage and liver function. Detailed patient-level treatment allocation could not be uniformly retrieved for all cases owing to the retrospective design.

EZH2 and INI-1 Expression

IHC analysis was performed on all 103 tumor specimens. Representative IHC images are shown in Figure 1A-D. Using the predefined cut-off (≥1% distinct nuclear staining), EZH2 positivity was detected in 45 patients (43.7%), while 58 patients (56.3%) were classified as EZH2-negative. INI-1 nuclear expression was retained in 92 patients (89.3%), while loss of expression was observed in 11 patients (10.7%).

Association of EZH2 Expression with Clinicopathological Features

The association between EZH2 positivity and clinicopathological parameters is presented in Table 2. No statistically significant association was observed between EZH2 status and underlying etiology (p=0.789). Although a higher proportion of EZH2 positivity was observed in advanced BCLC stages (65.2% in stage C vs. 30.0% in stage

TABLE 1: Baseline clinicopathological characteristics of the study cohort (n=103).

Characteristic	Values[a]
Age (years), median (range)	68 (17-89)
Sex, n (%)	
Male	89 (86.4)
Female	14 (13.6)
Etiology, n (%)	
Hepatitis B	59 (57.3)
Hepatitis C	14 (13.6)
Non-viral	30 (29.1)
Child-Pugh score, n (%)	
Class A	60 (58.3)
Class B	30 (29.1)
Class C	13 (12.6)
BCLC stage, n (%)	
Stage 0	5 (4.9)
Stage A	40 (38.8)
Stage B	14 (13.6)
Stage C	23 (22.3)
Stage D	21 (20.4)
EZH2 expression, n (%)	
Positive	45 (43.7)
Negative	58 (56.3)
INI-1 (SMARCB1) expression, n (%)	
Retained	92 (89.3)
Loss	11 (10.7)

[a] Data are presented as number (percentage) unless otherwise indicated. INI-1/SMARCB1, SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1.
BCLC: Barcelona Clinic Liver Cancer; EZH2: Enhancer of zeste homologue 2.

A), this trend did not reach statistical significance (p=0.064) (Table 2).

Survival Analysis

At the time of analysis, 71 of 103 patients (68.9%) had died, and 86 (83.5%) had experienced disease progression, resulting in 71 OS events and 86 PFS events. The median follow-up duration, estimated by the reverse Kaplan-Meier method, was 42.0 months. Median OS and PFS for the entire cohort were 16.0 months [95% confidence interval (CI): 9.8-22.2] and 6.0 months (95% CI: 3.7-8.3), respectively. Kaplan-Meier survival analysis demonstrated that EZH2 positivity was significantly associated with inferior survival outcomes. EZH2-positive patients had a significantly shorter median OS than the EZH2-negative group (8.0 vs. 27.0 months; log-rank p=0.007) (Figure 2). A similar association was observed for PFS, with a median of 5.0 months in the EZH2-positive group compared with 9.0 months in the EZH2-negative group (log-rank p=0.012) (Figure 3). As expected, worsening Child-Pugh class and advanced BCLC stage were also significantly associated with reduced median OS and PFS (Table 3).

In an exploratory analysis of INI-1 expression, the INI-1-loss group demonstrated numerically longer median PFS compared with the INI-1-retained group (22.0 vs. 6.0 months); however, this difference did not reach statistical significance (log-rank p=0.051). Similarly, median OS was numerically longer in the INI-1-loss group (30.0 vs. 15.0 months; log-rank p=0.097). Among the 11 patients with INI-1 loss, 6 died and 8 progressed; the median OS and PFS were 30.0 and 22.0 months, respectively. Notably, this subgroup was enriched for early-stage disease: 7 of 11 patients were classified as BCLC stage 0 or A (stage 0, n=2; stage A, n=5), with only 2 patients in stage B and 1 patient each in stages C and D. This stage imbalance may largely account for the numerically longer survival observed in the INI-1-loss group. Given the small subgroup size and this distributional skew, these findings are strictly exploratory and hypothesis-generating. In a further exploratory analysis restricted to the 43 patients who received locoregional therapy with or without sorafenib, EZH2 positivity remained associated with significantly shorter median PFS (4.0 vs. 14.0 months; log-rank p=0.014) and numerically shorter OS (16.0 vs. 41.0 months; p=0.077), supporting the relevance of EZH2 within a treated subgroup.

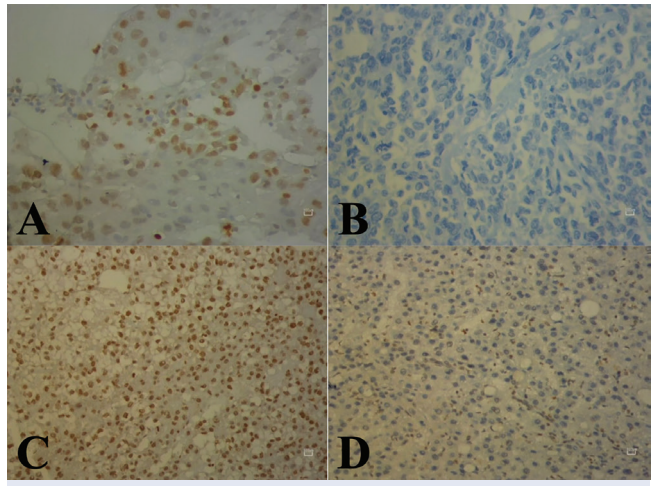

FIGURE 1: Representative immunohistochemical staining patterns for EZH2 and INI-1 in hepatocellular carcinoma. **(A)** EZH2-positive tumor demonstrating distinct nuclear staining in tumor cells (scale bar =100 μm). **(B)** EZH2-negative tumor with absent nuclear immunoreactivity (scale bar =100 μm). **(C)** Retained INI-1 expression showing diffuse nuclear staining in tumor cells (scale bar =100 μm). **(D)** Loss of INI-1 expression in tumor cells with retained nuclear staining in adjacent non-neoplastic cells serving as an internal positive control (scale bar =100 μm).

EZH2: Enhancer of zeste homologue 2

TABLE 2: Association between EZH2 positivity and clinicopathological parameters.

Characteristic	Total (n=103)	EZH2 positive (n=45)	EZH2 negative (n=58)	p-value[a]
Etiology, n (%)				
Hepatitis B	59	26 (44.1)	33 (55.9)	0.789
Hepatitis C	14	5 (35.7)	9 (64.3)	
Non-viral	30	14 (46.7)	16 (53.3)	
BCLC stage, n (%)				
Stage 0	5	1 (20.0)	4 (80.0)	0.064
Stage A	40	12 (30.0)	28 (70.0)	
Stage B	14	7 (50.0)	7 (50.0)	
Stage C	23	15 (65.2)	8 (34.8)	
Stage D	21	10 (47.6)	11 (52.4)	

[a] Chi-square test or Fisher's exact test. EZH2: Enhancer of zeste homologue 2.

Cox Regression Analysis

Univariate and multivariate Cox proportional hazards regression analyses were performed to identify prognostic factors for both OS and PFS (Table 3). In univariate analysis, EZH2 positivity was a significant risk factor for both shorter OS [hazard ratio (HR): 1.88; 95% CI: 1.17-3.02; p=0.009] and shorter PFS (HR: 1.69; 95% CI: 1.10-2.60; p=0.018). Advanced BCLC stage was also significantly associated with poorer outcomes in a stage-dependent manner: compared with stage A, stage C was associated with substantially increased risks of death (HR: 3.79; 95% CI: 1.96-7.32; p<0.001) and of progression (HR: 2.59; 95% CI: 1.44-4.67; p=0.002), while stage D conferred the highest risk for both OS (HR: 23.44; 95% CI: 10.41-52.76; p<0.001) and PFS (HR: 8.50; 95% CI: 4.44-16.28; p<0.001). Worsening Child-Pugh class was significantly associated with inferior OS (class B vs. A: HR: 2.11; 95% CI: 1.23-3.61; p=0.007; class C vs. A: HR: 11.94; 95% CI: 5.61-25.42; p<0.001) and PFS (class C vs. A: HR: 6.50; 95% CI: 3.30-12.81; p<0.001).

In multivariate analysis adjusted for BCLC stage, EZH2 positivity did not retain independent prognostic significance for either OS (HR: 1.55; 95% CI: 0.95-2.53; p=0.078) or PFS (HR: 1.39; 95% CI: 0.89-2.17; p=0.146); BCLC stage remained the dominant independent predictor of survival (Table 3).

In a sensitivity analysis that combined BCLC stages 0 and A into a single early-stage reference category (thereby retaining all 103 patients), the direction and magnitude of the EZH2 effect were similar. EZH2 reached nominal significance for OS (HR: 1.71; 95% CI: 1.05-2.79; p=0.030) but not for PFS (HR: 1.51; 95% CI: 0.97-2.35; p=0.067); BCLC stage remained the dominant predictor. Given that the point estimate hovered near the significance threshold across model specifications, we interpret EZH2 as indicating a borderline stage-dependent prognostic signal rather than a robust independent effect. To examine whether EZH2 retained discriminatory value within a single stage, we additionally analyzed the BCLC stage C subgroup (n=23), the largest advanced-stage group. EZH2 positivity was associated with significantly shorter median OS (7.0 vs. 23.0 months; log-rank p=0.002) and a non-significant trend for PFS (4.0 vs. 6.0 months; p=0.058), indicating that EZH2 may carry prognostic information even within advanced-stage disease.

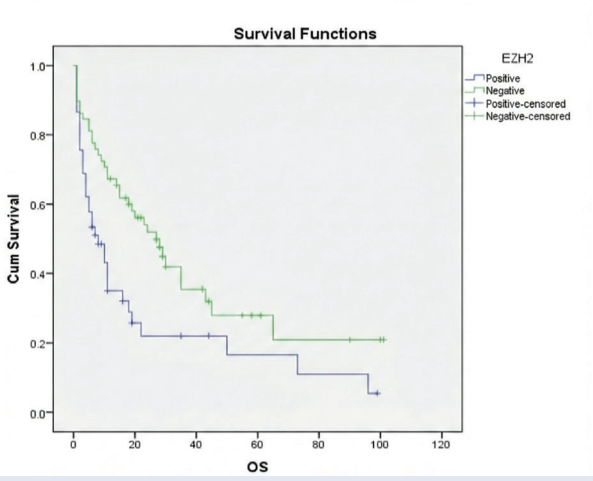

FIGURE 2: Kaplan-Meier curves for OS according to EZH2 expression status. EZH2-positive patients (n=45) demonstrated significantly shorter median OS compared with EZH2-negative patients (n=58) (8.0 vs. 27.0 months; log-rank p=0.007).

EZH2: Enhancer of zeste homologue 2; OS: Overall survival

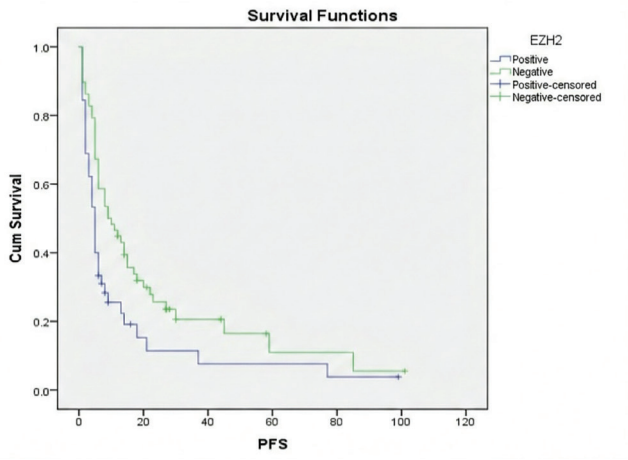

FIGURE 3: Kaplan-Meier curves for progression-free survival according to EZH2 expression status. Median PFS was 5.0 months in the EZH2-positive group vs. 9.0 months in the EZH2-negative group (log-rank p=0.012).

EZH2: Enhancer of zeste homologue 2; PFS: Progression-free survival

TABLE 3: Survival analysis and Cox proportional hazards regression for overall survival and progression-free survival.

Panel A. Kaplan-Meier survival estimates.

Variable	n	Median PFS (months)	p-value	Median OS (months)	p-value
EZH2 expression					
Positive	45	5.0	0.012	8.0	**0.007**
Negative	58	9.0		27.0	
INI-1 expression					
Retained	92	6.0	0.051	15.0	**0.097**
Loss	11	22.0		30.0	

TABLE 3: Continued.

Panel A. Kaplan-Meier survival estimates.

Variable	n	Median PFS (months)	p-value	Median OS (months)	p-value
Child-Pugh class					
A	60	9.0	<0.001	29.0	**<0.001**
B	30	6.0		9.0	
C	13	1.0		1.0	
BCLC stage					
A	40	15.0	<0.001	45.0	**<0.001**
B	14	5.0		15.0	
C	23	5.0		11.0	
D	21	1.0		1.0	

Panel B. Cox proportional hazards regression analysis.

Variable	PFS HR (95% CI)	p-value	OS HR (95% CI)	p-value
Univariate analysis				
EZH2 (positive vs. negative)	1.69 (1.10-2.60)	0.018	1.88 (1.17-3.02)	0.009
Age (per year)	1.01 (0.99-1.02)	0.532	1.01 (0.99-1.03)	0.284
Sex (female vs. male)	1.05 (0.57-1.94)	0.880	0.91 (0.45-1.83)	0.785
Etiology (overall)	—	>0.16	—	>0.16
INI-1 loss vs. retained	0.49 (0.23-1.02)	0.056	0.50 (0.21-1.16)	0.104
BCLC B vs. A	1.88 (0.97-3.62)	0.060	2.20 (1.03-4.72)	0.043
BCLC C vs. A	2.59 (1.44-4.67)	0.002	3.79 (1.96-7.32)	<0.001
BCLC D vs. A	8.50 (4.44-16.28)	<0.001	23.44 (10.41-52.76)	<0.001
Child-Pugh B vs. A	1.37 (0.85-2.23)	0.199	2.11 (1.23-3.61)	0.007
Child-Pugh C vs. A	6.50 (3.30-12.81)	<0.001	11.94 (5.61-25.42)	<0.001
Multivariate analysis (EZH2 + BCLC)				
EZH2 (positive vs. negative)	1.39 (0.89-2.17)	0.146	1.55 (0.95-2.53)	0.078
BCLC B vs. A	1.75 (0.90-3.39)	0.100	2.03 (0.94-4.38)	0.071
BCLC C vs. A	2.49 (1.37-4.51)	0.003	3.69 (1.90-7.18)	<0.001
BCLC D vs. A	8.28 (4.31-15.92)	<0.001	23.55 (10.34-53.61)	<0.001
Sensitivity analysis (BCLC 0 + A combined reference)				
EZH2 (positive vs. negative)	1.51 (0.97-2.35)	0.067	1.71 (1.05-2.79)	0.030

P-values were determined by the log-rank test (Panel A) and Cox proportional hazards regression (Panel B). Bold p-values indicate statistical significance (p<0.05). BCLC stage (n=5; 1 death, 1 progression event) was excluded from the primary Cox regression owing to limited events; a sensitivity analysis combining BCLC 0 and A is shown. The proportional hazards assumption was verified (scaled Schoenfeld residuals, all p>0.05). Child-Pugh class was not included in the multivariate model as it is incorporated within the BCLC staging system.
BCLC: Barcelona Clinic Liver Cancer; CI: Confidence interval; HR: Hazard ratio; OS: Overall survival; PFS: Progression-free survival; EZH2: Enhancer of zeste homologue 2.

DISCUSSION

In this single-institution retrospective study of 103 HCC patients, we found that EZH2 positivity, detected by IHC in 43.7% of cases, was significantly associated with poorer survival outcomes. Specifically, EZH2 positivity was associated with markedly reduced median OS (8.0 vs. 27.0 months; p=0.007) and median PFS (5.0 vs. 9.0 months; p=0.012). Although in univariate analysis EZH2 positivity emerged as a significant prognostic factor for both OS (HR: 1.88; 95% CI: 1.17-3.02; p=0.009) and PFS (HR: 1.69; 95% CI: 1.10-2.60; p=0.018), it did not retain independent prognostic value in multivariate analysis adjusted for BCLC stage (OS: HR: 1.55; 95% CI: 0.95-2.53; p=0.078; PFS: HR: 1.39; 95% CI: 0.89-2.17; p=0.146), suggesting that its prognostic impact may be mediated, at least in part, through its association with tumor burden and disease stage. Accordingly, EZH2 positivity should be regarded as a marker associated with more aggressive, advanced-stage disease rather than as a stage-independent prognostic factor.

The observed EZH2 positivity rate of 43.7% aligns with previously reported frequencies in HCC. A recent study analyzing mitotic cell cycle genes in HCC identified EZH2 as one of the key diagnostic biomarkers with individual diagnostic performance (area under the curve >0.81) across multiple datasets.[15] Similarly, Wang et al.[16] reported that EZH2 was overexpressed in HCC and significantly associated with advanced tumor stage and poor prognosis. Our findings are consistent with and extend this body of evidence. Bae et al.[17] demonstrated that EZH2 overexpression was an independent biomarker for poor outcomes in HCC, with high expression being negatively correlated with patient age and positively associated with alpha-fetoprotein (AFP) levels >20 ng/mL; in keeping with this, EZH2-positive tumors in our cohort also showed higher AFP levels (median 43 vs. 13 ng/mL). Chien et al.[18] revealed that EZH2-mediated stage-dependent methylation patterns correlate with immune polarization, metabolic suppression, and unfavorable outcomes, providing biological insights into EZH2-driven disease progression.

The biological basis for these findings is well supported. EZH2 mediates transcriptional repression through H3K27me3, leading to silencing of critical genes involved in cell cycle control, apoptosis, and differentiation. Multiple pathways have been implicated, including EZH2-mediated *SOCS3* silencing through the CTCF-vigilin complex, activating STAT3 signaling,[19] and epigenetic modulation of *miR-1224* forming a positive feedback circuit with CREB to activate YAP signaling.[20] Wu et al.[21] further demonstrated that *SIRT7* super-enhancer-driven reprogramming involves EZH2-dependent H3K27me3 at tumor suppressor promoters. Beyond direct tumor cell regulation, emerging single-cell RNA-seq data indicate that EZH2 modulates the MIF-CD74 axis, affecting T-cell activation and exhaustion,[22] while glutamate-NMDAR signaling influences tumor-associated macrophage infiltration through EZH2 regulation.[23] These findings suggest that EZH2 may simultaneously drive tumor cell proliferation and modulate anti-tumor immune responses.

Importantly, our multivariate analysis revealed that EZH2 did not function as an independent prognostic factor for either OS (HR: 1.55; 95% CI: 0.95-2.53; p=0.078) or PFS (HR: 1.39; 95% CI: 0.89-2.17; p=0.146) when BCLC stage was included in the model. This finding suggests that the prognostic value of EZH2 may be confounded by or closely associated with tumor stage and overall disease burden. One plausible interpretation is that EZH2 positivity is a molecular feature enriched in more advanced tumors, and thus its prognostic signal is captured by established staging systems. This interpretation is supported by the observed trend toward higher EZH2 positivity in advanced BCLC stages (65.2% in stage C vs. 30.0% in stage A; p=0.064), which was not statistically significant, likely due to limited sample size. Li et al.[24] similarly identified EZH2 as a prognostically relevant gene in HBV-related HCC with enhanced stratification when combined with clinical parameters, and Liu et al.[25] demonstrated improved prognostic accuracy when integrating EZH2 with clinicopathological features in an oxidative stress risk model.

As an exploratory analysis, we also assessed the expression of SMARCB1/INI-1, a core subunit of the SWI/SNF chromatin remodeling complex, which functionally antagonizes PRC2-mediated gene silencing. Loss of INI-1 expression was observed in only 10.7% of cases. Although a numerical trend toward longer survival was noted in the INI-1-loss group for both PFS (p=0.051) and OS (p=0.097), these differences did not reach statistical significance. With only 11 INI-1-loss cases, the analysis is underpowered and the CIs are wide; moreover, 7 of these 11 patients had early-stage disease, suggesting that the paradoxical direction of the association most likely reflects stage imbalance rather than a protective biological effect, and these observations should be regarded strictly as hypothesis-generating. The high rate of retained INI-1 expression (89.3%) in our cohort contrasts with the findings of Mochizuki et al.[26], who reported that only 1 of 30 HCC cases demonstrated nuclear SMARCB1 staining in their diagnostic study; this discrepancy likely reflects several factors. First, the two cohorts were assembled in fundamentally different clinical contexts: Mochizuki et al.[26] examined SMARCB1 specifically as a diagnostic discriminator between alpha-fetoprotein-producing gastric carcinoma and HCC, whereas our cohort comprised unselected, pathologically confirmed cases of HCC. Second, differences in antibody clones, staining platforms, and—most importantly—the threshold used to define retained versus lost expression may substantially alter the reported frequency of INI-1 loss. Such methodological heterogeneity in SMARCB1/INI-1 interpretation is well recognized and underscores the need for standardized scoring before cross-study comparisons can be made reliably. To our knowledge, this is the first report to examine the prognostic implications of INI-1 expression in HCC, and future studies with larger sample sizes are warranted to clarify its potential role.

Emerging evidence supports the potential therapeutic relevance of targeting EZH2 in HCC. Salani et al.[27] demonstrated that circulating H3K27me3/H3K36me3 ratios, reflecting EZH2 activity, predicted response to sorafenib in advanced HCC. Additionally, disruption of EZH2-dependent regulatory networks, such as the lncRNA-AC079061.1/VIPR1 axis, has been shown to suppress HCC development.[28] These findings suggest that pharmacological inhibition of EZH2, potentially in combination with immunotherapy or targeted agents, merits further clinical investigation.

Study Limitations

Our study has several strengths, including complete clinicopathological annotation, consistent survival follow-up, and standardized IHC methodology with blinded pathological evaluation. Furthermore, the simultaneous assessment of two functionally antagonistic epigenetic regulators (EZH2 and INI-1) provides a broader perspective on the role of chromatin remodeling in HCC. However, several limitations warrant acknowledgment. The retrospective design and modest sample size (n=103) may limit statistical power, particularly for the INI-1-loss subgroup (n=11), as evidenced by the observed borderline associations. The binary ≥1% cut-off, while clinically pragmatic, does not capture intensity or H-score information; although the proportion of positive nuclei was recorded (median 30%, range 1-80% among positive cases), intensity-based scoring was not available. The limited number of events also constrained the number of covariates that could be modeled simultaneously. Although AFP values were available for most patients and were discussed descriptively, AFP was not incorporated into the formal survival analyses because it lies outside the primary EZH2-INI-1 focus of this study and because the limited number of events constrained the multivariate model. In addition, several other standard prognostic variables—including ECOG performance status, tumor size and number, vascular invasion, extrahepatic spread, and histological grade—were not consistently documented and could not be reliably analyzed. As BCLC staging already incorporates performance status, tumor burden, and vascular/extrahepatic involvement, it captures much of the information carried by these individual variables. Detailed treatment-stratified modeling was not feasible, limiting our ability to assess the predictive value for therapeutic response. Finally, our cohort was treated between 2011 and 2019, predating the routine use of immune checkpoint inhibitor-based regimens such as atezolizumab plus bevacizumab, now a first-line standard for advanced HCC. Whether EZH2 expression influences outcomes or responses in the contemporary immunotherapy era—particularly given evidence that EZH2 modulates anti-tumor immunity—remains an open and clinically relevant question. Prospective multicenter studies with larger cohorts, refined cut-off values, integration of complementary biomarkers, and evaluation of EZH2-targeted therapies are needed to validate and extend these findings.

CONCLUSION

EZH2 positivity, detected in 43.7% of HCC patients, was significantly associated with inferior OS and PFS. Although EZH2 demonstrated prognostic value in univariate analysis, it did not retain independent significance after adjustment for BCLC stage, indicating that its prognostic value is stage-dependent and does not constitute an independent prognostic effect. INI-1 loss, observed in 10.7% of cases, showed only borderline, exploratory associations with survival that did not reach statistical significance. These findings, together with consistent evidence from the broader literature, support the biological relevance of epigenetic regulators in HCC and warrant further investigation in larger prospective studies to clarify the roles of EZH2 and INI-1 as prognostic biomarkers and potential therapeutic targets.

Ethics

Ethics Committee Approval: This single-institution, retrospective cohort study was conducted following approval from the Çukurova University Faculty of Medicine Clinical Research Ethics Committee (approval number: 38; date: 13.04.2018).

Informed Consent: Retrospective study.

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Footnotes

Authorship Contributions

Concept: M.M.K., B.Ş., Design: M.M.K., F.D., Data Collection or Processing: M.M.K., F.D., Analysis or Interpretation: M.M.K., B.Ş., Literature Search: M.M.K., Writing: M.M.K., B.Ş.

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REFERENCES

1. Vogel A, Meyer T, Sapisochin G, Salem R, Saborowski A. Hepatocellular carcinoma. Lancet. 2022;400(10360):1345-1362. [Crossref] [PubMed]
2. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-249. [Crossref] [PubMed]
3. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022;72(1):7-33. [Crossref] [PubMed]
4. Akabane M, Imaoka Y, Kawashima J, Pawlik TM. Advancing precision medicine in hepatocellular carcinoma: current challenges and future directions in liquid biopsy, immune microenvironment, single nucleotide polymorphisms, and conversion therapy. Hepat Oncol. 2025;12(1):2493457. [Crossref] [PubMed] [PMC]
5. Goodall GJ, Wickramasinghe VO. RNA in cancer. Nat Rev Cancer. 2021;21(1):22-36. [Crossref] [PubMed]
6. Sandoval J, Esteller M. Cancer epigenomics: beyond genomics. Curr Opin Genet Dev. 2012;22(1):50-55. [Crossref] [PubMed]
7. Kadoch C, Copeland RA, Keilhack H. PRC2 and SWI/SNF Chromatin Remodeling Complexes in Health and Disease. Biochemistry. 2016;55(11):1600-1614. [Crossref] [PubMed]

8. Wilson BG, Roberts CW. SWI/SNF nucleosome remodellers and cancer. Nat Rev Cancer. 2011;11(7):481-492. [Crossref] [PubMed]
9. Gao SB, Zheng QF, Xu B, et al. EZH2 represses target genes through H3K27-dependent and H3K27-independent mechanisms in hepatocellular carcinoma. Mol Cancer Res. 2014;12(10):1388-1397. [Crossref] [PubMed]
10. Wang J, Wang GG. No easy way out for EZH2: its pleiotropic, noncanonical effects on gene regulation and cellular function. Int J Mol Sci. 2020;21(24):9501. [Crossref] [PubMed] [PMC]
11. Timp W, Feinberg AP. Cancer as a dysregulated epigenome allowing cellular growth advantage at the expense of the host. Nat Rev Cancer. 2013;13(7):497-510. [Crossref] [PubMed] [PMC]
12. Zhao R, Wang N, Huang H, Ma W, Yan Q. CHD5 a tumour suppressor is epigenetically silenced in hepatocellular carcinoma. Liver Int. 2014;34(6):e151-e160. [Crossref] [PubMed]
13. Xie CR, Li Z, Sun HG, et al. Mutual regulation between CHD5 and EZH2 in hepatocellular carcinoma. Oncotarget. 2015;6(38):40940-40952. [Crossref] [PubMed] [PMC]
14. Kohashi K, Oda Y. Oncogenic roles of SMARCB1/INI1 and its deficient tumors. Cancer Sci. 2017;108(4):547-552. [Crossref] [PubMed] [PMC]
15. Sucularli C. Machine learning-based identification of diagnostic and prognostic mitotic cell cycle genes in hepatocellular carcinoma. PLoS One. 2025;20(8):e0331118. [Crossref] [PubMed] [PMC]
16. Wang X, He M, Zhou L, Chen W. EZH2 expression in hepatocellular carcinoma and its relationship with circadian rhythm-related genes. Sci Rep. 2025;15(1):42177. [Crossref] [PubMed] [PMC]
17. Bae AN, Jung SJ, Lee JH, Lee H, Park SG. Clinical value of EZH2 in hepatocellular carcinoma and its potential for target therapy. Medicina (Kaunas). 2022;58(2):155. [Crossref] [PubMed] [PMC]
18. Chien YC, Wu GW, Wu JY, Liu LC, Hsieh YH, Yu YL. Stage-dependent EZH2 methylation correlates with immune polarization, metabolic suppression, and unfavorable outcomes in hepatocellular carcinoma. Int J Med Sci. 2025;22(16):4201-4213. [Crossref] [PubMed] [PMC]
19. Wei L, Liu Q, Huang Y, et al. Knockdown of CTCF reduces the binding of EZH2 and affects the methylation of the SOCS3 promoter in hepatocellular carcinoma. Int J Biochem Cell Biol. 2020;120:105685. [Crossref] [PubMed]
20. Yang S, Jiang W, Yang W, et al. Epigenetically modulated miR-1224 suppresses the proliferation of HCC through CREB-mediated activation of YAP signaling pathway. Mol Ther Nucleic Acids. 2021;23:944-958. [Crossref] [PubMed] [PMC]
21. Wu F, Xu L, Tu Y, et al. Sirtuin 7 super-enhancer drives epigenomic reprogramming in hepatocarcinogenesis. Cancer Lett. 2022;525:115-130. [Crossref] [PubMed]
22. Zhou Y, Xu Y, Ye M, et al. Integrated single-cell RNA-seq analysis reveals that EZH2 regulates the MIF-CD74 axis to modulate T cell activation and exhaustion in hepatocellular carcinoma. J Transl Med. 2025;23(1):1040. [Crossref] [PubMed] [PMC]
23. Chen J, Sun HW, Wang RZ, et al. Glutamate promotes CCL2 expression to recruit tumor-associated macrophages by restraining EZH2-mediated histone methylation in hepatocellular carcinoma. Oncoimmunology. 2025;14(1):2497172. [Crossref] [PubMed] [PMC]
24. Li Y, Liu SS, Jing HR, Qian HW, Li RC. Identifying key genes involved in HBV-related hepatocellular carcinoma: diagnose, prognosis, interaction and immune analysis. Discov Oncol. 2025;16(1):1510. [Crossref] [PubMed] [PMC]
25. Liu Y, Li Y, Chen L, et al. Construction of an oxidative stress risk model to analyze the correlation between liver cancer and tumor immunity. Curr Cancer Drug Targets. 2025;25(1):49-63. [Crossref] [PubMed]
26. Mochizuki K, Kawai M, Odate T, et al. SMARCB1/INI1 is diagnostically useful in distinguishing α-fetoprotein-producing gastric carcinoma from hepatocellular carcinoma. Anticancer Res. 2018;38(12):6865-6868. [Crossref] [PubMed]
27. Salani F, Latarani M, Casadei-Gardini A, et al. Predictive significance of circulating histones in hepatocellular carcinoma patients treated with sorafenib. Epigenomics. 2022;14(9):507-517. [Crossref] [PubMed]
28. Lin XH, Zhang DY, Liu ZY, et al. lncRNA-AC079061.1/VIPR1 axis may suppress the development of hepatocellular carcinoma: a bioinformatics analysis and experimental validation. J Transl Med. 2022;20(1):379. [Crossref] [PubMed] [PMC]