

RESEARCH

Open Access



EZH2 expression in colorectal carcinoma: an evaluation of clinicopathological and prognostic value

Diren Vuslat Cagatay^{1*} , Mecdi Gurhan Balci^{2,4} , Gizem Issin^{2,4} and Fatih Demir³

*Correspondence:

Diren Vuslat Cagatay
direncagatay@gmail.com

¹Department of Pathology,
Gumushane State Hospital,
Eskibaglar, 29000 Gumushane,
Turkey

²Department of Pathology, Faculty
of Medicine, Erzincan Binali Yildirim
University, 24100 Erzincan, Turkey

³Department of Pathology, Faculty
of Medicine, Duzce University,
81620 Duzce, Turkey

⁴Department of Pathology,
Mengucek Gazi Training and
Research Hospital, Erzincan Binali
Yildirim University, Erzincan, Turkey

Abstract

Background Enhancer of zeste homolog 2 (EZH2), the catalytic subunit of Polycomb Repressive Complex 2, catalyzes trimethylation of histone H3 lysine 27 and has been implicated in tumor progression.

Aim Our study aims to assess the relationship between EZH2 expression and clinicopathologic data, and survival in colorectal carcinomas (CRC).

Materials and methods EZH2 immunohistochemistry was performed on tumor blocks from 124 CRC patients. Based on H-scores, cases were stratified into low- and high-expression groups. EZH2 status was correlated with demographic, clinical, and pathologic features, and overall survival was analyzed with the Kaplan–Meier method and log-rank test.

Results A total of 124 cases of CRC; 12 (9.7%) cases were classified as low EZH2 expression, and 112 (90.3%) cases were classified as high EZH2 expression. A significant association was found between EZH2 expression and microsatellite stability. High EZH2 expression was significantly enriched in microsatellite-stable tumors ($p=0.007$) and in left-sided lesions ($p=0.049$). No significant associations were detected with sex, stage, grade, lymph-node status, or vascular invasion. Kaplan–Meier analysis revealed no difference in overall survival between low- and high-EZH2 groups (log-rank $p=0.47$; hazard ratio = 1.13, 95% CI 0.45–2.85). EZH2 staining was uniformly strong in normal mucosa and adenomas, mirroring the high expression observed in most CRCs.

Conclusions EZH2 is highly expressed across the normal-adenoma-carcinoma sequence and, in our cohort, was not linked to overall survival or to most clinicopathologic parameters in CRC. The lower expression observed in a subset of MSI-H tumors warrants further investigation; present evidence remains insufficient to establish EZH2 as an independent prognostic biomarker.

Keywords Colorectal carcinoma, EZH2, Histone Methyl transferase, Prognosis, Microsatellite



1 Introduction

Colorectal carcinomas (CRCs) are the third most common cancer worldwide and a leading cause of cancer-related deaths [1]. Disease progression and response to treatment in CRCs are highly variable. Several factors contribute to this variability, including tumor stage, location and molecular characteristics of the tumor [2]. In recent years, genetic and epigenetic alterations have been shown to have important effects on tumor development, prognosis and therapeutic response [3, 4]. Among these molecular factors, enhancer of zeste homolog 2 (EZH2) has emerged as a potential prognostic marker and therapeutic target in several cancers [5].

EZH2 is a core component of the Polycomb Repressive Complex 2 (PRC2), which regulates histone methylation and chromatin modification. By silencing tumor-suppressor genes, EZH2 can promote tumor progression [6]. Numerous studies have reported that elevated EZH2 expression is associated with poor prognosis and aggressive clinicopathologic features in various malignancies, including prostate, breast, and ovarian cancers [7]. Beyond its prognostic value, EZH2 inhibitors is being actively explored as an anti-cancer strategy [8].

Investigations of EZH2 expression in CRC have yielded conflicting results. Some studies suggest that high EZH2 levels correlate with worse clinical outcomes and more aggressive disease [9, 10], whereas others find no association with patient prognosis [11]. These divergent findings highlight the need for a more comprehensive evaluation of EZH2 in CRC.

Therefore, in this study, we aimed to evaluate EZH2 expression comprehensively throughout the entire tumor area and to investigate its relationship with key prognostic factors in CRCs. Elucidation of the relationship between EZH2 and CRCs may help clarify the clinical significance of EZH2 expression in this cancer.

2 Materials and methods

2.1 Case selection

This study received ethical approval from the Ethics Committee of Erzincan Binali Yildirim University on 31 March 2023, under decision number 01/05. We analyzed the electronic records of the Department of Pathology at Erzincan Binali Yildirim University and identified cases of CRC that underwent surgery between January 2015 and January 2023. To ensure the relevance and quality of the data, we excluded cases that underwent chemotherapy or radiation therapy prior to surgery, those with fewer than two tumor blocks, those with synchronous second primary tumors, and those with incomplete clinical data. After applying these inclusion and exclusion criteria, a total of 124 cases were deemed eligible for the study.

In addition to CRC tissues, we extended our analysis to investigate the expression of EZH2 in different stages of tumorigenesis. For this, we included normal colon tissue, low-grade adenomas, and high-grade adenomas. We reviewed the documentation of patients who underwent polypectomy between January 2015 and January 2023. The corresponding slides were evaluated blindly by two experienced pathologists, who assessed the degree of dysplasia. In cases where there was disagreement between the two pathologists, consensus was reached through joint review. A total of 15 cases of low-grade tubular adenoma, 15 cases of high-grade tubulovillous adenoma, and 15 cases of normal colon tissue were selected. During the selection process, care was taken to exclude cases

with a history of CRC-related surgery or concurrent CRC. In total, 45 polypectomy cases were included for evaluation, in addition to the 124 CRC cases. The case selection process is summarized in Fig. 1.

2.2 Data collection

Clinicopathological data were collected from pathology reports and electronic health records. The variables collected included patient age, gender, tumor size, depth of invasion, lymph node involvement, tumor differentiation, tumor localization, presence of tumor deposits, lymphocytic response (both intratumoral and peritumoral), lymphovascular invasion, perineural invasion, microsatellite instability status, and survival data.

2.3 Immunohistochemical analysis and evaluation

All H&E-stained sections of the cases were re-evaluated by light microscopy. Blocks that best represented the tumor morphology and had a tumor rate greater than 80% were identified. Sections with a thickness of 4 microns were taken from these blocks. Sections were deparaffinized by incubation at 40 °C for 1 h. After deparaffinization, slides were stained with monoclonal anti-EZH2 antibody (clone 6A10, Leica) in a fully automated immunohistochemistry instrument (Leica BondMax, Melbourne, Australia). A reactive lymph node with germinal center, reported as a positive control in the Human Protein Atlas, was used as a staining control.

The stained slides were evaluated by a pathologist who was blinded to the clinical details of the cases. Cells showing nuclear staining with EZH2 were classified as EZH2-positive. For evaluation, both the intensity of nuclear staining and the percentage of positively stained cells were assessed. These evaluations were performed based on the criteria outlined in Table 1. The immunoreactivity score for each sample was calculated by multiplying the percentage of positively stained cells by the degree of nuclear staining intensity [12]. This 3-tier intensity scale (0–3) follows the immunoreactive score originally described by Remmele and Stegner and adopted by Abou Gabal et al. [9, 10] In cases where cells exhibited varying levels of nuclear staining intensity within the same preparation, the arithmetic mean of the immunoreactivity scores was taken. Cases with

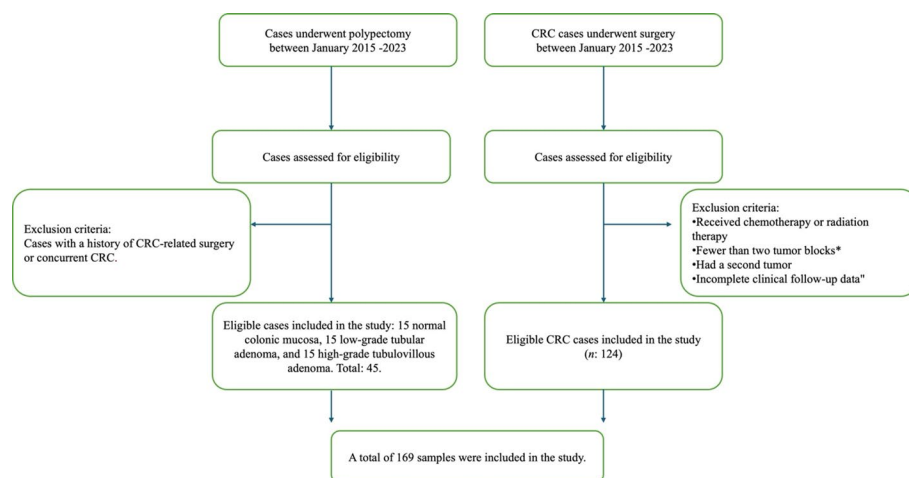


Fig. 1 Flowchart of Case Selection for EZH2 Expression Analysis. *Cases with fewer than two tumor blocks were excluded according to our laboratory's standard operating procedure to ensure sufficient tissue for reliable molecular analysis and patient safety

Table 1 Immunoreactivity scoring criteria for EZH2 expression

Percentage of Positive Cells	Stained Cell Score	Staining Intensity	Staining Intensity Score
None of cells	0	None	0
1–10% of cells	1	Weak staining	1
11–50% of cells	2	Moderate staining	2
Over 50% of cells	3	Strong staining	3
Immunoreactivity score (IRS) = Stained Cell Score X Staining Intensity Score			
IRS = 0 - negative	IRS = 1–4 - low expression	IRS = 5–9 - high expression	

Explanation of Staining Intensity: Weak Staining: Staining is faint and barely visible, Moderate Staining: Staining is clearly visible but not overly intense, Strong Staining: Intense and easily visible staining

Adapted from Cizkova et al. [13]

an immunoreactivity score of 1–3 were classified into the low-expression group, while those with a score of 4–9 were categorized into the high-expression group.

2.4 Statistical evaluation

Data were analyzed using IBM SPSS Statistics version 23. Two groups were defined based on EZH2 expression levels: the low EZH2 expression group and the high EZH2 expression group.

The normality of data distribution was assessed using the Kolmogorov-Smirnov test and the Shapiro-Wilk test. For normally distributed quantitative variables, the independent samples t-test was used, while the Mann-Whitney U test was applied for non-normally distributed data. The comparison of categorical variables (e.g., sex, tumor stage) between the low and high EZH2 expression groups was performed using the Pearson Chi-Square test, Fisher’s Exact test, and the Yates continuity correction when appropriate. To assess the robustness of the association between MSI status and EZH2 expression, a power analysis was conducted using R software (TTestIndPower function). The analysis revealed a statistical power of 52.56% ($p = 0.05$). The data included in the power analysis were the proportions of MSI cases in the low and high EZH2 expression groups.

Overall survival was defined as the interval from the date of surgery to death from any cause; patients alive on the census date (01 February 2024) were censored. Survival curves were generated by the Kaplan–Meier method, and differences were assessed with the log-rank test.

Results are presented as frequencies and percentages for categorical variables and as mean ± standard deviation for normally distributed data or median (range) for non-normally distributed data. A significance level of $p < 0.05$ was considered statistically significant.

3 Results

A total of 124 CRC, along with 15 normal colonic mucosa, 15 low-grade tubular adenoma, and 15 high-grade tubulovillous adenoma samples, were analysed. Fifty-four patients (43.5%) were female and 70 (56.5%) were male. The ages of the CRC cases ranged from 26 to 98 years, with a mean of 69.46 ± 14.14 years.

Regarding staining scores, 7 (5.6%) CRC cases had a score of 1, 32 (25.8%) had a score of 2, and 85 (68.6%) had a score of 3. In 7 cases, 1–10% of cells were stained (score 1); in 31 cases, 11–50% of cells were stained (score 2); and in 86 cases, more than 50% of cells were stained (score 3). Based on immunoreactivity scores, 12 (9.7%) CRC cases had low EZH2 expression, and 112 (90.3%) had high EZH2 expression. Representative images are

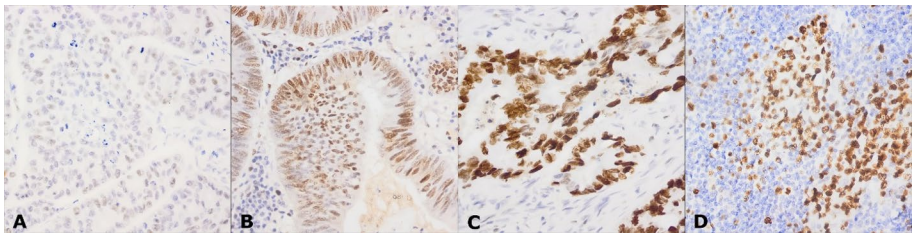


Fig. 2 Representative microscopic views of EZH2 staining: **A** score 1/weak staining in tumor cells, **B** score 2/moderate staining in tumor cells, **C** score 3/strong staining in tumor cells, and **D** positive and negative control in a reactive lymph node. Magnification: X400

Table 2 EZH2 stained cell ratio and nuclear staining intensity distribution in colorectal cancer specimens

	Number of Cases; n (%)
EZH2 Stained Cell Ratio	
Score 1	7 (5.6)
Score 2	31 (25)
Score 3	86 (69.4)
Nuclear Staining Intensity	
Score 1	7 (5.6)
Score 2	32 (25.8)
Score 3	85 (68.6)
Group according to the immunoreactivity score	
Low expression group	12 (9.7)
High expression group	112 (90.3)

The data regarding EZH2 Stained Cell Ratio and Nuclear Staining Intensity distribution were adapted from Abou Gabal et al. Evaluation of EZH2 and ERRA in colorectal carcinoma: an immunohistochemical study [9]

shown in Fig. 2, and the distribution of cases by staining intensity and stained cell ratio is in Table 2.

The mean age of patients with low EZH2 expression was 67.4 years, while those with high EZH2 expression had a mean age of 69.6 years, showing no significant difference ($p = 0.600$). EZH2 expression did not show a significant difference between men and women ($p = 0.164$). In terms of tumor size, the mean size was 4.75 cm for cases with low EZH2 expression and 4.5 cm for those with high expression. This difference was not statistically significant ($p = 0.324$).

EZH2 expression was not significantly associated with tumor invasion depth, lymph node metastasis, tumor grade, stage, lymphovascular invasion, perineural invasion, tumor budding, tumor deposits, or lymphocytic response ($p > 0.05$). EZH2 expression was significantly correlated with tumor location ($p = 0.049$), with high expression cases predominantly located in the left colon. The clinicopathological and demographic characteristics of the low and high EZH2 expression groups are summarized in Table 3.

In our CRC cases, 29 (23.4%) were MSI, and 95 (76.6%) were microsatellite stable (MSS). Among the low EZH2 expression group, 7 (58.3%) cases were MSI and 5 (41.7%) were MSS, while in the high EZH2 expression group, 22 (19.6%) cases were MSI and 90 (80.4%) were MSS (Table 4). A statistically significant association was found between microsatellite status and EZH2 expression ($p = 0.007$), with the majority of high EZH2 expression cases being MSS. A power analysis was conducted, revealing a statistical power of 52.56% at a significance level of $p = 0.05$.

Table 3 Distribution of cases according to clinicopathological features and EZH2 expression

Clinicopathological features	Groups according to the EZH2 Expression		P value
	Low EZH2 Expression group	High EZH2 Expression group	
Age	67.417 ± 14.829	69.685 ± 14.119	0.600 ^x
Gender			
Male	4 (33.3)	66 (58.9)	0.164 ^x
Female	8 (66.7)	46 (41.1)	
Histologic Grade			
Well differentiated	4 (33.3)	40 (35.7)	0.476 ^x
Moderately differentiated	0 (0)	11 (9.8)	
Poorly differentiated	8 (66.7)	61 (54.5)	
T Stage			
Tis-T1	0 (0)	23 (2.7)	0.913 ^x
T2	1 (8.3)	17 (15.2)	
T3	8 (66.7)	72 (64.3)	
T4	3 (25)	20 (17.9)	
N Lymph node metastasis			
0	7 (58.3)	77 (68.8)	0.552 ^x
1	2 (16.7)	20 (17.9)	
2	3 (25)	15 (13.3)	
Lymphovascular invasion			
Negative	6 (50)	64 (57.1)	0.867 ^x
Positive	6 (50)	48 (42.9)	
Perineural invasion			
Negative	7 (58.3)	68 (60.7)	1.000 ^y
Positive	5 (41.7)	44 (39.3)	
Intratumoral lymphocytic infiltration			
Absent	0 (0)	7 (6.3)	0.441 ^x
Mild	9 (75)	68 (60.7)	
Moderate	2 (16.7)	11 (9.8)	
Severe	1 (8.3)	26 (23.2)	
Tumor deposit			
Present	3 (25)	9 (8)	0.093 ^y
Absent	9 (75)	103 (92)	
Tumor budding			
Mild	10 (83.3)	94 (83.9)	0.816 ^x
Moderate	2 (16.7)	15 (13.4)	
Severe	0 (0)	3 (2.7)	
Peritumoral lymphocytic infiltration			
Mild	5 (41.7)	63 (56.3)	0.275 ^x
Moderate	4 (33.3)	17 (15.2)	
Severe	3 (25)	32 (28.5)	
Survival			
Alive	7 (58.3)	62 (55.4)	1.000 ^x
Ex	5 (41.7)	50 (44.6)	

Yates' continuity correction, Fisher's Exact test, and Pearson's Chi-Square test were used to compare categorical data

Bonferroni-corrected Z-test was used to examine multiple comparisons

The significance level was taken as $p < 0.05$

Table 4 Relationship between EZH2 expression and microsatellite distribution in cases

MSS (Microsatellite Stability)	Low EZH2 Expression group	High EZH2 Expression group	P value
MSS	5 (41.7)	90 (80.4)	0.007 ^y
MSI	7 (58.3)	22 (19.6)	

In addition to the CRC cases, 15 normal colonic mucosa, 15 low-grade tubular adenoma, and 15 high-grade tubulovillous adenoma samples were evaluated, and a strong and uniform EZH2 staining pattern was observed in all these tissues, with more than 90% of the cells exhibiting intense staining (Fig. 3). This strong staining was present regardless of the degree of dysplasia, with no significant difference between low-grade and high-grade adenomas. Moreover, no heterogeneity in staining intensity was observed in any of the tissue types, including both normal and adenomatous tissues. During the evaluation of the EZH2 staining pattern in the tumor tissue, heterogeneity in expression was observed between the tumor center and the most invasive area. Lower levels of EZH2 expression were found at the site of invasion compared to other regions (Fig. 4).

The follow-up periods ranged from 2 to 121 months (mean 40.6 ± 24.3 months) in the high EZH group and 16 to 100 months (mean 47.8 ± 26.9 months) in the low EZH group. Mortality rates were recorded as 50 cases (44.6%) in the high EZH group and 5 cases (41.7%) in the low EZH group.

The median overall survival was 36 months (95% CI: 30–42) in the high-EZH2 group compared to 38.5 months (95% CI: 26–60) in the low-EZH2 group. Kaplan–Meier analysis with log-rank testing demonstrated no statistically significant survival difference between groups ($p = 0.47$; Fig. 5). The hazard ratio of 1.13 (95% CI: 0.45–2.85) further confirmed the absence of clinically significant prognostic impact from EZH2 expression levels. However, the limited sample size of the low-EZH2 cohort ($n = 12$) resulted in wide confidence intervals, precluding definitive conclusions regarding EZH2's potential prognostic value.

4 Discussion

CRCs are the third most common cancer worldwide and the second leading cause of cancer-related deaths, with approximately 500,000 fatalities annually [1]. Advances in molecular detection have significantly enhanced our understanding of tumorigenesis, leading to the identification of novel biomarkers and the development of targeted therapeutic strategies.

One such biomarker, EZH2, has gained increasing attention in recent years due to its role in tumorigenesis. As an epigenetic regulator, EZH2 contributes to cancer progression and immune regulation by silencing tumor suppressor genes [6]. High EZH2 expression has been associated with poor prognosis in various cancers, including lung, breast, ovarian, and prostate cancers, suggesting its potential as a prognostic marker [7].

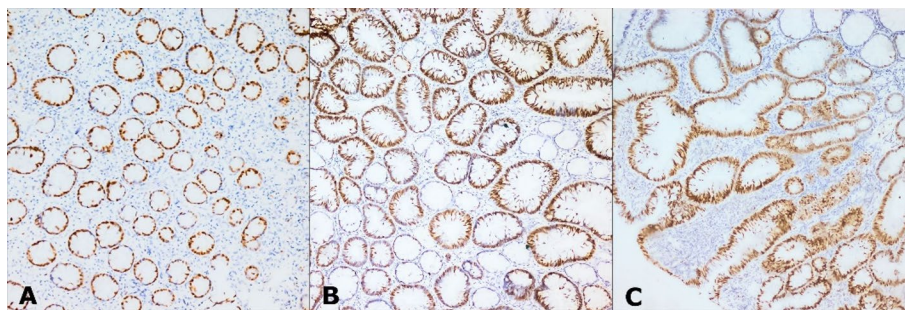


Fig. 3 Representative microscopic views of EZH2 expression: **A** normal colonic mucosa, **B** tubular adenoma, and **C** tubulovillous adenoma. Magnification: X100

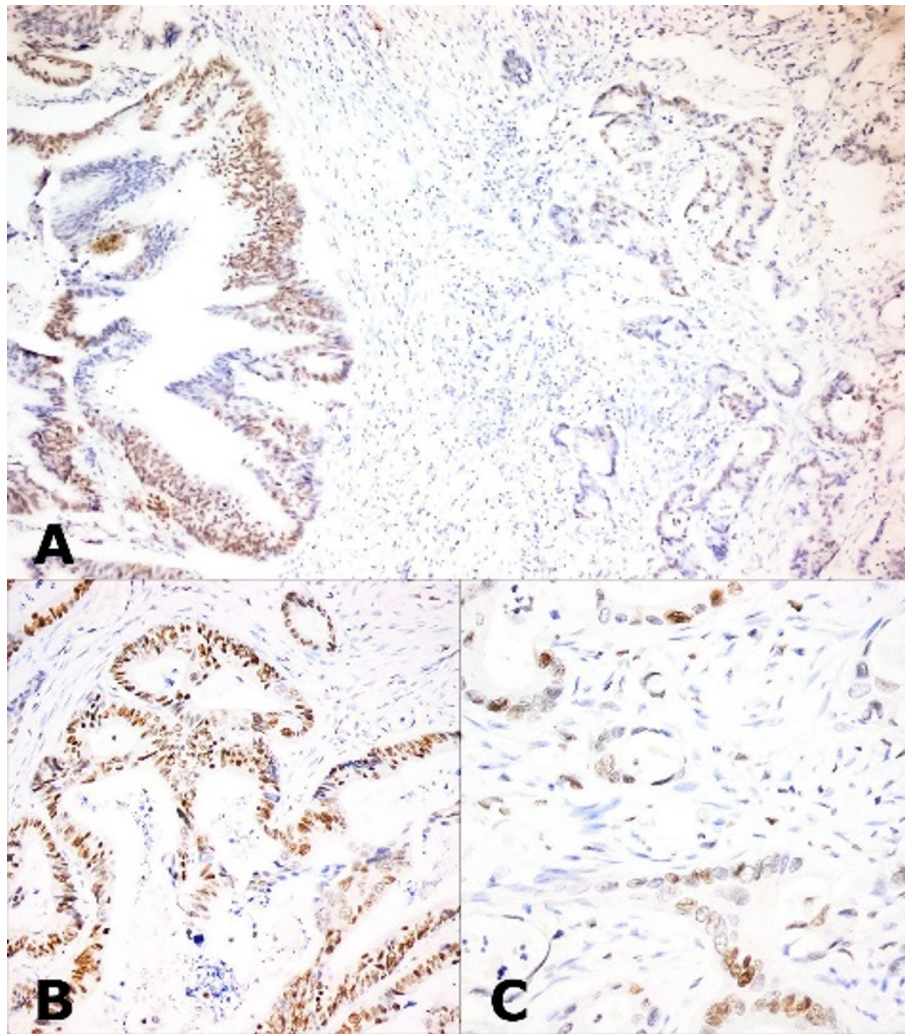


Fig. 4 Representative microscopic views of EZH2 expression: **A** central (left) and invasive (right) regions of the tumor, **B** central regions of the tumor, and **C** invasive regions of the tumor. Magnification: X100, X400

Beyond its prognostic relevance, EZH2 inhibition is being explored therapeutically; the FDA-approved inhibitor tazemetostat is now in basket trials that include CRC cohorts [11, 12].

EZH2 overexpression has been linked to poor prognosis, metastasis, and tumor aggressiveness in several cancers [7]. However, data on the relationship between EZH2 expression and prognosis in CRCs are inconsistent. Gabal H. et al. reported that EZH2 overexpression was associated with high tumor grade, advanced stage, lymph node metastasis, and worse survival rates [9]. Chen et al. found that high EZH2 expression in colorectal cancers correlated with tumor progression, stage, size, differentiation, and lymph node metastasis, all of which contributed to poor prognosis and worse survival [14]. Liu et al. also reported faster disease progression and significantly decreased survival in CRCs related with high EZH2 expression [15]. Furthermore, Mimori et al. found that high EZH2 expression could serve as both an oncogene and a prognostic marker, given its association with poor prognosis [16]. In contrast, Takawa et al. reported that high EZH2 expression was associated with a good prognosis in colorectal cancer [17]. Fluge et al. observed that patients with high EZH2 expression had longer disease-free

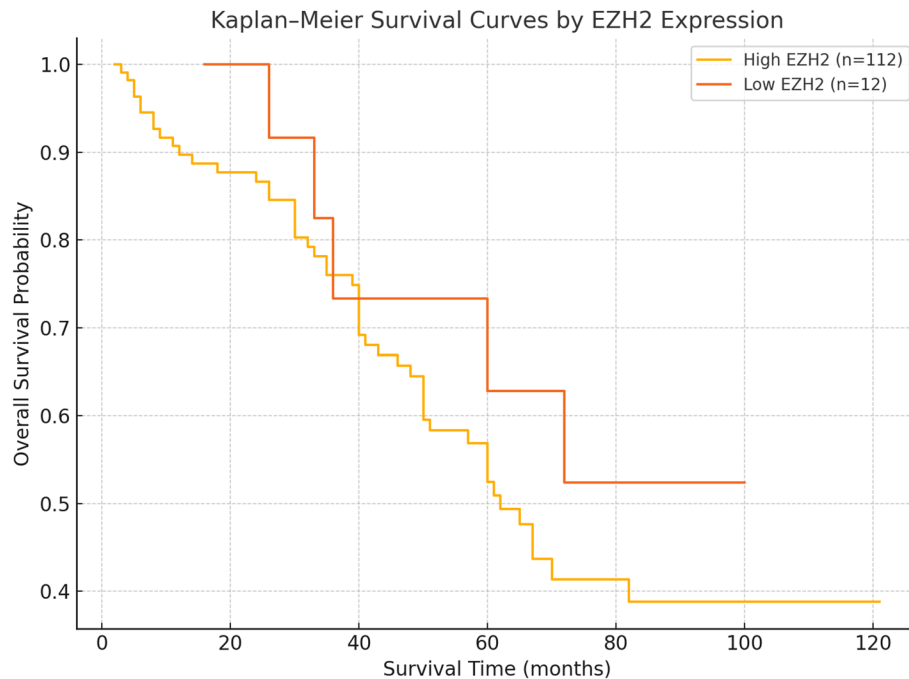


Fig. 5 Kaplan-Meier Survival Analysis by EZH2 Expression Status Kaplan-Meier curves demonstrate comparable overall survival between high-EZH2 ($n = 112$, red line) and low-EZH2 ($n = 12$, blue line) patient groups. Median survival was 36 months (95% CI: 30–42) for high-EZH2 versus 38.5 months (95% CI 26–60) for low-EZH2 expression (log-rank $p = 0.47$). Vertical ticks indicate censored observations

survival [18]. Laura-Viloria et al. performed a meta-analysis and suggested that high EZH2 expression has a protective role and prolongs survival in colorectal cancer, in contrast to findings in other solid tumors [19]. A comprehensive overview of studies evaluating EZH2 expression in colorectal cancers is presented in Table 5.

Considering the results of numerous studies and meta-analyses, conflicting data exist regarding the impact of EZH2 expression on patient prognosis, survival, and tumor aggressiveness. Our findings indicate that EZH2 expression was not significantly associated with tumor size, age, gender, tumor grade, stage, invasion depth, lymph node metastasis, tumor budding, intratumoral and peritumoral lymphocytic infiltration, lymphovascular invasion, perineural invasion, extratumoral large vessel invasion disease progression, or survival. Similarly, Wang CG et al. reported that high EZH2 expression was not associated with gender, tumor location, lymph node metastasis, or distant metastasis [20]. Kodach LI. et al. also found no significant correlation between EZH2 expression and survival [21].

The inconsistencies in the findings from previous studies regarding the relationship between EZH2 expression and CRCs may be attributed to several factors, such as the heterogeneity of the study populations, the size of the tumor areas examined, and the methods used to select study regions. Most previous studies utilized microarray methods to analyze small tumor areas, typically in the range of 1–6 mm, which may limit the ability to capture the full scope of EZH2 expression across the entire tumor. In contrast, we analyzed the entire tumor area, providing a broader assessment that may explain some of the divergent results.

We observed that in a subset of cases, EZH2 staining intensity was lower at the invasive front compared to the tumor center (Fig. 2). This pattern suggests a region-specific

Table 5 Summary of studies evaluating EZH2 expression in colorectal carcinoma

Author	Method	Number of Cases	Tumor Area	Important Findings
Wang et al. [20]	IHC	119	2 mm	High EZH2 expression is associated with poor prognosis.
Chen et al. [14]	PCR, Western Blot	95	Not specified	A significant correlation between high EZH2 expression and tumor stage, tumor size, and lymph node metastasis. Low EZH2 expression has been associated with increased survival.
Fluge et al. [18]	IHC	425	1 mm	High EZH2 expression is associated with increased survival.
Kodach et al. [21]	PCR, IHC	72	TMA	No significant correlation between EZH2 expression and survival.
Abdel Raouf et al. [22]	IHC	50	Whole slide	High EZH2 expression is associated with advanced tumor stage and lymph node metastasis.
Julian et al. [23]	IHC, WesternBlot	105	6 mm	EZH2 staining intensity decreased from the tumor center to the invasion area.
Abou Gabal et al. [9]	IHC	120	Whole slide	High EZH2 expression has been reported to be associated with high tumor stage, grade, lymph node metastasis, and decreased survival.
Sanches et al. [24]	IHC, Western blot, Immunofluorescence	75	TMA	High expression of EZH2 is associated with advanced tumor stages
Takawa et al. [17]	IHC	172	Not specified	High EZH2 expression associated with a good prognosis
Yamamoto et al. [25]	IHC, PCR	109	3–4 mm	Low EZH2 expression is associated with decreased survival.
Lauro Vilorio-Marques et al. [19]	Meta-analysis	1662	–	High EZH2 expression has been reported to have a protective role and is associated with increased survival.

modulation of EZH2 expression rather than a uniform staining artifact. The invasive margin of tumors is a dynamic microenvironment where cancer cells often undergo epithelial–mesenchymal transition (EMT). During EMT, transcriptional repressors like SNAIL and SLUG recruit chromatin modifiers that reduce PRC2 activity, leading to the downregulation of EZH2 and its associated histone mark, H3K27me3 [26, 27]. This facilitates the activation of gene programs involved in migration and invasion. Julian et al. similarly reported reduced EZH2 and H3K27me3 levels at the invasive margin, which contributed to a more aggressive cellular phenotype [23]. Our findings are consistent with this model and highlight that EZH2 expression can vary within the tumour.

Gabal H. and Samar M. et al. reported minimal EZH2 staining in normal colonic mucosa [9, 22]. By contrast, we found uniformly strong staining in normal epithelium and in both grades of adenoma. Since reagent sensitivity can significantly affect nuclear staining, these differences may have resulted from clone 6A10, ready-to-use dilution and stringent antigen recovery. Since down-regulation of EZH2 is linked to EMT and tumour progression [28], its persistent high expression in normal and dysplastic tissues may be related to the absence of EMT in these compartments.

The relationship between MSI status and EZH2 expression is likewise inconsistent across studies [23, 29]. A recent pan-cancer analysis revealed positive correlations between EZH2 and several MMR genes, suggesting crosstalk with the mismatch-repair system [30]. In our cohort, MSI-H tumors were over-represented in the low-EZH2 group (58.3% vs. 19.6%, $p = 0.007$); however, statistical power was limited (52.6%), and

this finding should be interpreted with caution. Larger studies are needed to clarify whether EZH2 truly modulates MSI biology or reflects underlying molecular subtype.

EZH2 inhibitors are emerging as a promising therapeutic strategy in cancer treatment [31]. As an epigenetic regulator, EZH2 mediates silencing of tumor suppressor genes through histone methylation, promoting tumor progression and metastasis. Inhibition of EZH2 restores the expression of these silenced genes, leading to tumor growth suppression, increased apoptosis, and reduced metastatic potential [32]. Furthermore, EZH2 inhibition can remodel the tumor microenvironment, enhancing immune responses. By increasing immune cell infiltration and upregulating antigen presentation, EZH2 inhibitors may sensitize tumors to immune checkpoint inhibitors, providing a complementary approach to immunotherapy, especially in immune-cold tumors like CRC [33].

EZH2 inhibition appears to be particularly effective in MSI-high CRC tumors, where ARID1A mutations are common [30]. These tumors tend to be more dependent on EZH2 for silencing immune-stimulatory genes and may exhibit greater sensitivity to EZH2-targeted therapies [34, 35]. Preclinical studies show that EZH2 inhibition can suppress CRC tumor growth and overcome chemotherapy resistance [31]. Clinical trials are investigating EZH2 inhibitors in combination with immunotherapy and chemotherapy, particularly in MSS CRC, with promising early-phase data [36]. While no EZH2 inhibitor is yet approved for CRC, ongoing research continues to explore their potential in treating both chemoresistant and advanced tumors, positioning EZH2 as an actionable target in CRC therapy.

Taken together, while preclinical and early clinical data highlight EZH2 as a promising therapeutic target, the complexity of its biological behavior in CRC—as reflected by our findings—underscores the need for further validation in larger, mechanistic studies. Importantly, although EZH2 inhibition shows therapeutic promise, the relationship between EZH2 expression levels and treatment efficacy in CRC remains to be clearly established.

Although this study provides valuable insights into the relationship between EZH2 expression and various clinical and pathological parameters in CRCs, several limitations must be acknowledged. First, the sample size, although adequate, may not fully capture the heterogeneity of CRCs, potentially limiting the generalizability of our findings. Secondly, the retrospective nature of the study may introduce selection bias and limit the ability to establish causality. In addition, although we used a comprehensive whole slide approach, the inherent variability in tumor regions may still influence the interpretation of EZH2 expression patterns. Finally, our study did not investigate the functional mechanisms underlying the observed associations, and further research is needed to elucidate the biological pathways by which EZH2 influences microsatellite stability and other tumor characteristics.

5 Conclusion

The role of EZH2 in colorectal carcinogenesis has received considerable attention due to its involvement in cancer initiation, progression and prognosis. In this study, we evaluated the association between EZH2 expression and various clinical and pathological parameters in CRC. Our results showed that there was no significant association between EZH2 expression and patient age, sex, tumour size, stage, depth of invasion,

lymph node metastasis, tumour budding, lymphocytic infiltration, lymphovascular invasion, perineural invasion, extratumoral large vessel invasion, or survival.

In our cohort, EZH2 expression was markedly lower in MSI colon cancers. Future research using next generation sequencing genomic profiling and larger studies investigating the relationship between microsatellite status, clinicopathological parameters and EZH2 in CRC will likely provide more insight.

Author contributions

Design of work: D.V.C., M.G.B., G.I, F.D. Data collection: D.V.C., M.G.B., G.I, F.D. Data analysis and interpretation: D.V.C., M.G.B. Drafting the article: D.V.C., M.G.B. Critical revision of the article: D.V.C., M.G.B., G.I, F.D. Final approval of the manuscript: D.V.C., M.G.B., G.I, F.D.

Funding

This study was supported by Erzincan University Scientific Research and Project Office (Project ID:910).

Data availability

The authors confirm that the data supporting the findings of this study are available within the article. Raw data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Erzincan Binali Yildirim University Ethics Committee (approval number 2023-01/05), and written informed consent was obtained from all participants or their legal guardians.

Consent for publication

Informed Consent was obtained from all the participants involved in the study.

Competing interests

The authors declare no competing interests.

Received: 5 March 2025 / Accepted: 13 June 2025

Published online: 19 June 2025

References

1. Baidoun F, Elshawy K, Elkeriaie Y, Merjaneh Z, Khoudari G, Sarmini MT, et al. Colorectal cancer epidemiology: recent trends and impact on outcomes. *Curr Drug Targets*. 2021;22(9):998–1009.
2. Chen K, Collins G, Wang H, Toh JWT. Pathological features and prognostication in colorectal cancer. *Curr Oncol*. 2021;28(6):5356–83.
3. Hong SN. Genetic and epigenetic alterations of CRC. *Intest Res*. 2018;16(3):327–37.
4. Issin G, Sayar I, Demir F, Bakkaloğlu IG, Gamsizkan M, Yildiz Z, et al. Clinicopathologic features and prognostic significance of immunohistochemistry and in situ hybridization based molecular classification in gastric carcinoma. *J Environ Pathol Toxicol Oncol*. 2023;42(4):1–14.
5. Duan R, Du W, Guo W. EZH2: a novel target for cancer treatment. *J Hematol Oncol*. 2020;13(1):104.
6. Chang CJ, Hung MC. The role of EZH2 in tumour progression. *Br J Cancer*. 2011;106(2):243–7.
7. Jiang T, Wang Y, Zhou F, Gao G, Ren S, Zhou C. Prognostic value of high EZH2 expression in patients with different types of cancer: A systematic review with meta-analysis. *Oncotarget*. 2016;7(4):4584–97.
8. Kim KH, Roberts CWM. Targeting EZH2 in cancer. *Nat Med*. 2016;22(2):128–34.
9. Abou Gabal HH, Ahmed NS, Meckawy GR, Yassin RAE, Hakim SA. Evaluation of EZH2 and ERα in colorectal carcinoma: an immunohistochemical study. *Pol J Pathol*. 2021;72(3):200–10.
10. Thomas B, Wolfgang W, Carsten B, Kunhard P, Paul-Georg K. Immunohistochemical detection of hormone receptors in breast carcinomas (ER-ICA, PgR-ICA): prognostic usefulness and comparison with the biochemical Radioactive-Ligand-Binding Assay(DCC). *Gynecol Oncol*. 1994;53(2):220–7.
11. Julie CM. Phase II study of Tazemetostat in solid tumors harboring an ARID1A mutation. 2024. <https://clinicaltrials.gov/study/NCT05023655>
12. Patrick Joseph Loehrer Sr. Cabozantinib and erlotinib for patients with EGFR and c-Met Co-expressing Metastatic Pancreatic Adenocarcinoma. 2020. <https://clinicaltrials.gov/study/NCT03213626>
13. Cizkova K, Foltynkova T, Gachechiladze M, Tauber Z. Comparative analysis of immunohistochemical staining intensity determined by light microscopy, ImageJ and QuPath in placental Hofbauer cells. *Acta Histochem Cytochem*. 2021;54(1):21–9.
14. Chen Z, Yang P, Li W, He F, Wei J, Zhang T, et al. Expression of EZH2 is associated with poor outcome in colorectal cancer. *Oncol Lett*. 2018;15(3):2953–61.
15. Liu XY, Liu H, Gu L, Zheng HL. EZH2 Expression and its correlation with clinicopathological features in patients with colorectal carcinoma. 2016;11(1):287–92.
16. Mimori K, Ogawa K, Okamoto M, Sudo T, Inoue H, Mori M. Clinical significance of enhancer of zeste homolog 2 expression in colorectal cancer cases. *Eur J Surg Oncol*. 2005;31(4):376–80

17. Takawa M, Masuda K, Kunizaki M, Daigo Y, Takagi K, Iwai Y, et al. Validation of the histone methyltransferase EZH2 as a therapeutic target for various types of human cancer and as a prognostic marker. *Cancer Sci*. 2011;102(7):1298–305.
18. Fluge O, Gravdal K, Carlsen E, Vonen B, Kjellekvold K, Refsum S, et al. Expression of EZH2 and Ki-67 in colorectal cancer and associations with treatment response and prognosis. *Br J Cancer*. 2009;101(8):1282–9.
19. Vilorio-Marqués L, Martín V, Díez-Tascón C, González-Sevilla MF, Fernández-Villa T, Honrado E, et al. The role of EZH2 in overall survival of colorectal cancer: A meta-Analysis. *Sci Rep*. 2017;7(1):1–8.
20. Wang CG, Ye YJ, Yuan J, Liu FF, Zhang H, Wang S. EZH2 and STAT6 expression profiles are correlated with colorectal cancer stage and prognosis. *World J Gastroenterol*. 2010;16(19):2421.
21. Kodach LL, Jacobs RJ, Heijmans J, van Noesel CJM, Langers AMJ, Verspaget HW, et al. The role of EZH2 and DNA methylation in the silencing of the tumour suppressor RUNX3 in colorectal cancer. *Carcinogenesis*. 2010;31(9):1567–75.
22. Abdel Raouf SM, Ibrahim TR, Abdelaziz LA, Farid MI, Mohamed SY. Prognostic value of TWIST1 and EZH2 expression in colon cancer. *J Gastrointest Cancer*. 2021;52(1):90–8.
23. Böhm J, Muenzner JK, Caliskan A, Ndreshkjana B, Erlenbach-Wünsch K, Merkel S, et al. Loss of enhancer of zeste homologue 2 (EZH2) at tumor invasion front is correlated with higher aggressiveness in colorectal cancer cells. *J Cancer Res Clin Oncol*. 2019;145(9):2227–40.
24. Sanches JGP, Song B, Zhang Q, Cui X, Yabasin IB, Ntim M, et al. The Role of KDM2B and EZH2 in regulating the stemness in colorectal cancer through the PI3K/AKT pathway. *Front Oncol*. 2021;11:637298.
25. Yamamoto I, Noshio K, Kanno S, Igarashi H, Kurihara H, Ishigami K, et al. EZH2 expression is a prognostic biomarker in patients with colorectal cancer treated with anti-EGFR therapeutics. *Oncotarget*. 2017;8(11):17810–8.
26. Li L, Liu J, Xue H, Li C, Liu Q, Zhou Y, et al. A TGF- β -MTA1-SOX4-EZH2 signaling axis drives epithelial-mesenchymal transition in tumor metastasis. *Oncogene*. 2020;39(10):2125–39.
27. Liu Y, Yang Q. The roles of EZH2 in cancer and its inhibitors. *Med Oncol*. 2023;40(6):167.
28. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. *Nature*. 2011;469(7330):343–9.
29. Joensuu EI, Nieminen TT, Lotsari JE, Pavicic W, Abdel-Rahman WM, Peltomäki P. Methyltransferase expression and tumor suppressor gene methylation in sporadic and familial colorectal cancer. *Genes Chromosomes Cancer*. 2015;54(12):776–87.
30. Luo L, Wang Z, Hu T, Feng Z, Zeng Q, Shu X, et al. Multiomics characteristics and immunotherapeutic potential of EZH2 in pan-cancer. *Biosci Rep*. 2023 ;43(1):BSR20222230
31. Eich ML, Athar M, Ferguson JE 3rd, Varambally S. EZH2-Targeted Therapies in Cancer: Hype or a Reality. *Cancer Res*. 2020;80(24):5449–58.
32. Xu M, Xu C, Wang R, Tang Q, Zhou Q, Wu W, et al. Treating human cancer by targeting EZH2. *Genes Dis*. 2024;12(3):101313.
33. Shin DS, Park K, Garon E, Dubinett S. Targeting EZH2 to overcome the resistance to immunotherapy in lung cancer. *Semin Oncol*. 2022;49(3–4):306–18.
34. Dang T, Guan X, Cui L, Ruan Y, Chen Z, Zou H, et al. Epigenetics and immunotherapy in colorectal cancer: progress and promise. *Clin Epigenetics*. 2024;16(1):123.
35. Huang J, Yin Q, Wang Y, Zhou X, Guo Y, Tang Y, et al. EZH2 inhibition enhances PD-L1 protein stability through USP22-mediated deubiquitination in colorectal cancer. *Adv Sci (Weinh)*. 2024;11(23):e2308045
36. Palmieri LJ, Cousin S, Spalato M, Guegan JP, Bessede A, Pernot S, et al. Targeting EZH2 to overcome the resistance to immunotherapy in microsatellite stable colorectal cancer: Results from the CAIRE study. *J Clin Oncol*. 2023;41(16):3599.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.