Original paper

Evaluation of EZH2 and ERRα in colorectal carcinoma: an immunohistochemical study

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The combined immunohistochemical evaluation of EZH2 (enhancer of zeste homolog 2) and ERRα (estrogen-related receptor α), in relation to clinicopathological prognostic factors and patients' outcome, has not been performed yet in colorectal carcinoma (CRC). In order to achieve this aim, 120 samples were extracted; 60 cases of CRC; and 60 samples from normal colonic tissue.
Our study showed that 63.3% and 38.3% of CRC cases reveal high EZH2 and high ERRα nuclear expression, respectively. 6.6% and 8.3% of normal colonic mucosa samples express low EZH2 and low ERRα nuclear expression, respectively. High EZH2 and high ERRα expression correlate with late tumor stages ($p = 0.001$ each), high grade ($p = 0.001$, $p = 0.009$ respectively), positive lymph node involvement ($p = 0.001$, $p = 0.002$ respectively) and larger tumor size ($p = 0.001$ each). There is a moderate highly statistically significant agreement ($\kappa = 0.467$, $p = 0.001$) between EZH2 and ERRα immunohistochemical expression. By Kaplan Meier analysis, high EZH2 and high ERRα show statistically significant shorter overall survival, and progression free survival than cases with low EZH2 and low ERRα immunohistochemical expression, respectively. Thus, EZH2 and ERRα might serve as potential promising prognostic markers in CRC.

Key words: EZH2, ERRα, colorectal carcinoma, normal colonic tissue, immunohistochemistry, prognostic.

Introduction

Colorectal carcinoma (CRC) is one of the most common malignancies worldwide, and is known to be associated with high mortality rate [1]. Thus, finding efficient prognostic biomarkers and new treatment modalities that aim at improving prognosis is a compelling demand. Enhancer of zeste homolog 2 (EZH2) constitutes one of the most investigated core subunits of polycomb repressive complex 2 (PRC2) that has a crucial role in transcriptional regulation and cellular proliferation. EZH2 functions in various biological processes with intricate associations between it and cancer initiation, progression, metastasis, drug resistance, and immunity regulation. This is why it is related to many diseases, including cancer [2]. It has been associated with poor prognostic factors in several malignancies including breast, endometrial, prostatic and renal cell carcinoma as well as malignant melanoma [3, 4, 5]. While some studies conducted on CRC correlate EZH2 to poor prognostic indicators and worse survival [6, 7], few failed to correlate it to prognostic indicators [8], and other studies correlate its high expression with better survival [9, 10]. Estrogen-related receptor α (ERRα) is one of estrogen orphan receptors involved

in energy homeostasis regulation. In the very few papers conducted on CRC, ERRα has been correlated with poor clinicopathological parameters and worse survival [11, 12, 13].

In breast cancer, EZH2 and ERRα have shown several interactions [14]. However, the exact interaction between them in colonic cells has not been fully elucidated. In colonic carcinoma, it has been suggested that the transcription factor "nuclear factor κ-light-chain enhancer of activated B cells" (NF-κB) induces EZH2 which in turn represses ERRα transcription by methylation of histone 3 residue 27 (H3K27) [15, 16, 17]. Interestingly, NF-κB, on the other hand, helps the recruitment of ERRα to stimulate its DNA transcriptional activity. Moreover, EZH2 may also function independently of methylation of H3K27 in colon. Thus, the overall effect of EZH2 and ERRα on each other and on CRC remain perplexing. Furthermore, EZH2 and ERRα might provide targets for therapeutic intervention in various types of cancer [2, 18]. However, the combined immunohistochemical analysis of both markers has not been yet tested in CRC. Thus, the aim of the current study is to evaluate the immunohistochemical expression of EZH2 and ERRα in CRC, to correlate their expression with clinicopathological prognostic parameters of this tumor, and to find any possible relationship between the two markers and tumor progression.

Material and methods

Tissue and patient data

The current study was conducted on 120 samples divided into two groups; 60 cases of primary colorectal carcinoma (CRC); and 60 samples from normal colonic tissue. Cases of both groups were obtained from the Archives of the Pathology Lab., Ain Shams University Hospitals. Such cases were diagnosed during the period from 2014 to 2017. CRC cases were obtained by surgical resection and fixed in phosphate buffered formalin. The surgical and histopathological reports were examined to determine clinicopathological data of the patients: age of the patients, tumor size and tumor site. In addition, imaging reports on lymph nodal involvement and metastatic workout were reviewed. Accordingly, the 60 CRC cases showed the following data; 2 cases were TNM stage I, 14 were stage II, 31 were stage III and 13 were stage IV; lymph nodal involvement was detected in 44 cases; 25 cases had a tumor size ≥ 5 cm; the tumor was colonic in 44 samples and rectal in 16 samples. Haematoxylin and eosin-stained slides (HE) were examined to evaluate and verify the histopathologic diagnosis and the tumor grade (CRC cases included 6 cases that were well differentiated, 43 moderately differentiated cases and 11 poorly differentiated cases).

Follow-up data were extracted from the archives of Clinical Oncology Department to determine:

- overall survival time (OS); which was calculated based on the date of diagnosis and the date of last follow-up or death,
- progression free survival (PFS); which was calculated based on the date of diagnosis and the date of progression (local recurrence or distant metastasis).

Only samples of cases of CRC patients who did not receive prior neoadjuvant therapy, that had enough tissue and available information on all covariates were included in the study.

Ethics statement

All patients who participated in this study signed a written, informed consent. The study was approved by the Research Ethical Committee at Faculty of Medicine, Ain Shams University.

Immunohistochemical staining

Four micrometer sections of formalin-fixed and paraffin-embedded samples of CRC and normal colonic tissue were prepared. The prepared tissue sections were fixed on poly-L- lysine coated slides overnight at 37°C. They were deparaffinized and rehydrated. Then the sections were heated in a microwave oven in 10mM citrate buffer (pH 6.0) for 20 min. After the blocking of endogenous peroxidase and incubation in Protein Block Serum-Free Solution (Dako Cytomation, Glostrup, Denmark) for 20 min, the sections were incubated at 4°C with primary antibodies. Biotinylated anti-mouse immunoglobulin and streptavidin conjugated to horseradish peroxidase were then added. Finally, 3,3`-diaminobenzidine as the substrate or chromogen was used to form an insoluble brown product. Finally, the sections were counterstained with hematoxylin, and mounted. With each run, sections of invasive breast duct carcinoma and human endocervix were used as a positive control for EZH2 and ERRα respectively [19, 20]. Negative control sections were incubated with normal mouse serum instead of the two primary antibodies.

Automated immunohistochemical staining was performed using two primary antibodies; mouse monoclonal anti-EZH2 (11) (Clone: 415M-18; Cell Marque, Sigma-Aldrich Co.,CA, USA ; 1:100 dilution) and ERRα mouse monoclonal antibody (Clone: ERRα (1ERR87): sc-65715; Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:100 dilution).Avidin-Biotin immunoperoxidase complex technique was used according to Hsu *et al.* [21] by applying the super sensitive detection kit (Biogenex, CA, USA).

Table I. Clinicopathologic characteristics among studied cases of colorectal carcinoma

	N	%
Pathologic Grade		
Well differentiated	6	10.0
Moderately differentiated	43	71.7
Poorly differentiated	11	18.3
TNM Staging		
I	2	3.3
II	14	23.3
III	31	51.7
IV	13	21.7
Lymph Node Involvement		
No	16	26.7
Yes	44	73.3
Size		
< 5	35	58.3
≥ 5	25	41.7
Site		
Colonic	44	73.3
Rectal	16	26.7

Interpretation of immunohistochemical staining

Immunohistochemical analysis of EZH2 and ERRα was blindly performed by two pathologists without any prior knowledge of the clinicopathological data. Any discrepancy between the two pathologists was assessed by a third senior pathologist to reach the consensus.

Immunoreactivity for EZH2 was divided into two groups; low expression (proportion of cells < 50%), or high expression (proportion of cells of ≥ 50%) [22, 23]. For estimation of nuclear EZH2 immunoreactivity, the proportion of positive cells was assessed as follows: 0, 0%; 1, 1 – 10%; 2, 10 – 50%; and 3, > 50%. In addition, EZH2 staining intensity was recorded as: 0, no staining; 1, weak; 2, moderate; and 3, strong staining. A score was obtained by multiplying the proportion of positive cells by the intensity score. A score of 0 to 3 was considered as low EZH2 expression, while a score of 4 to 9 was considered as high EZH2 expression [24].

For estimation of nuclear ERRα immunoreactivity, five to ten separate high-power fields (× 400) were examined for each CRC case, and the mean number of positively stained nuclei was estimated. The proportion of positively stained cells was accordingly assessed as follows: (0, < 5%; 1, 6 to 25%; 2, 26 to 50%; 3, 51 to 75%; 4, > 75%). The staining intensity was evaluated as follows: (0, no signal; 1, weak; 2, moderate; 3, strong). The score was calculated by multiplying proportion score by intensity score, such that a score = 0 was denoted as low ERRα expression, and a score > 0 represented high ERRα expression [25].

Data management and analysis

Continuous variables are expressed as mean and Standard Deviation. Categorical variables are expressed as frequencies and percents. Student t test was used to assess the statistical significance of the difference between two study group mean. Chi square and Fisher's exact test were used to examine the relationship between Categorical variables. Kappa statistics was used to examine the agreement between EZH2 and ERRα with values < 0 as indicating no agreement and 0-0.20 as slight, 0.21-0.40 as fair, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81-1 as almost perfect agreement. Kaplan Meier curves were used to describe overall and progression free survival (OS and PFS), while log rank test was used to compare the overall and PF survival. A significance level of $p < 0.05$ was used in all tests. All statistical procedures were carried out using SPSS version 20 for Windows (SPSS Inc, Chicago, IL, USA).

Results

A

Patients

A total of 60 cases of CRC are included in the current study, 38 of which are males (63.3%), and 22 are females (36.7%). The mean age is 47.70 years (SD, ±14.28) (range 17-73 years). Detailed clinicopathologic characteristics are presented in Table I.

Immunohistochemical analysis

Thirty-eight cases of CRC (63.3%) reveal high EZH2 nuclear expression. Meanwhile, only 23 CRC cases (38.3%) show high ERRα nuclear expression (Fig. 1). On the other hand, low EZH2 nuclear expression is focally detected in 4 out of 60 (6.6%) normal colonic mucosa samples, while low ERRα nuclear expression is detected in 5 out of 60 (8.3%) normal colonic mucosa samples included in the current study (Fig. 2).

Correlation between EZH2, ERRα and clinicopathological parameters

Both age and gender do not show significant relationships with either EZH2 or ERRα IHC expression. Both EZH2 and ERRα show statistically significant associations with TNM tumor stage, tumor grade, lymph node involvement and tumor size such that

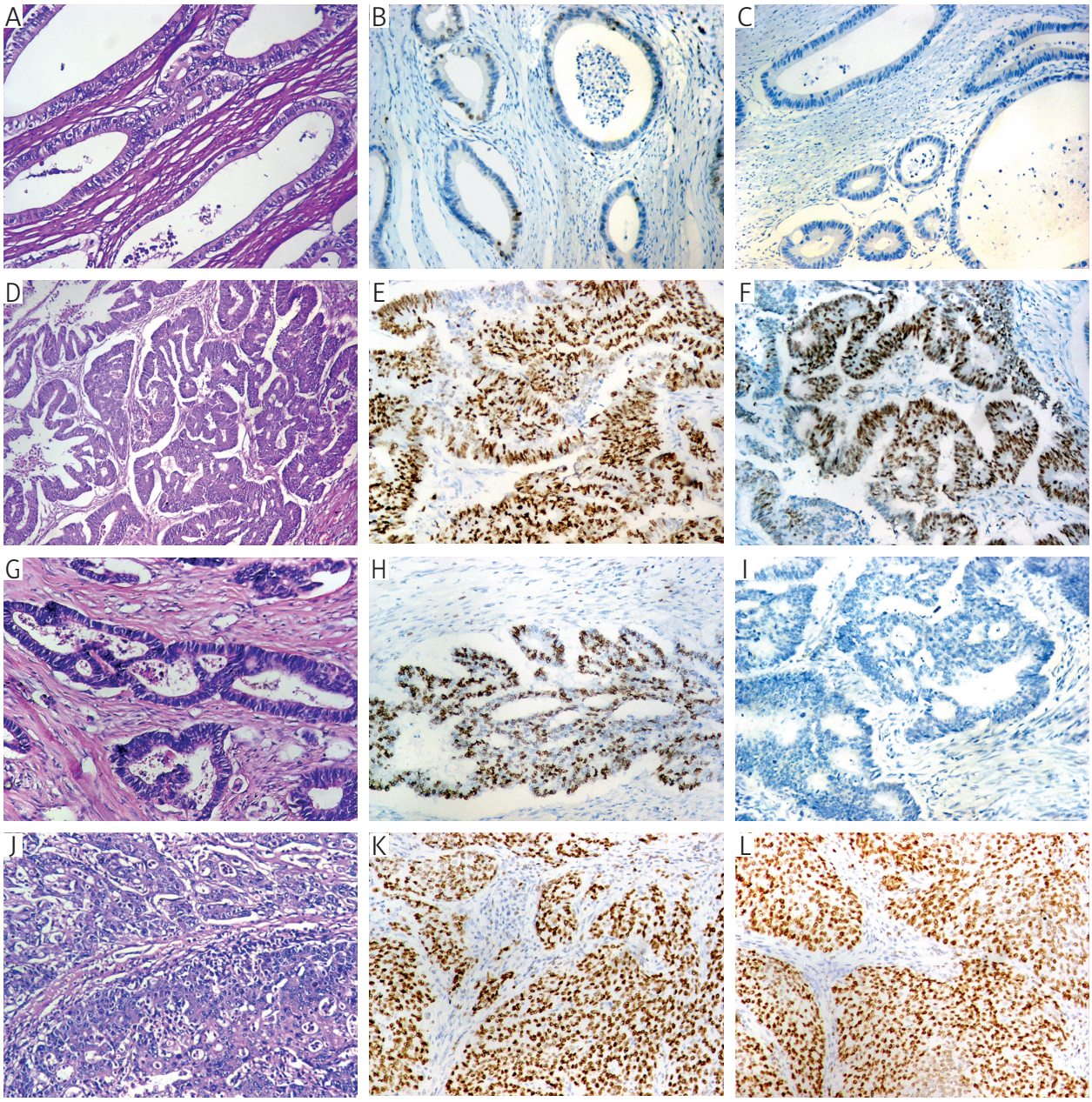

Fig. 1. A) A case of well-differentiated CRC (HE, 200×). B) Low EZH2 nuclear expression in tumor cells (IHC, 200×). C) Negative ERRα IHC expression in tumor cells (IHC, 200×). D) A case of moderately-differentiated CRC (HE, 100×). E) High EZH2 nuclear expression in tumor cells (IHC, 200×). F) High ERRα nuclear expression in tumor cells (IHC, 200×). G) Another case of moderately-differentiated CRC (HE, 200×). H) High EZH2 nuclear expression in tumor cells (IHC, 200×). I) Negative ERRα IHC expression in tumor cells (IHC, 200×). J) A case of poorly-differentiated CRC (HE, 200×). K) High EZH2 nuclear expression in tumor cells (IHC, 200×). L) High ERRα nuclear expression in tumor cells (IHC, 200×)

high EZH2 and high ERRα expression correlate with late tumor stage ($p = 0.001$ each), high grade ($p = 0.001$, $p = 0.009$ respectively), positive lymph node involvement ($p = 0.001$, $p = 0.002$ respectively) and larger tumor size ($p = 0.001$ each). However, the association with tumor site is insignificant for EZH2 IHC expression ($p = 0.258$), but shows statistical significance with ERRα expression ($p = 0.013$) (Tables II and III).

There is a moderate highly statistically significant agreement ($\kappa = 0.467$, $p = 0.001$) between EZH2 and ERRα IHC expression, such that 57.9% of high EZH2 cases show high ERRα expression and 95.5% of low EZH2 cases show low ERRα expression (Table IV).

Survival analysis

The OS, and PFS among CRC cases included in this study are 50.2% at 92 months, and 57.7% at

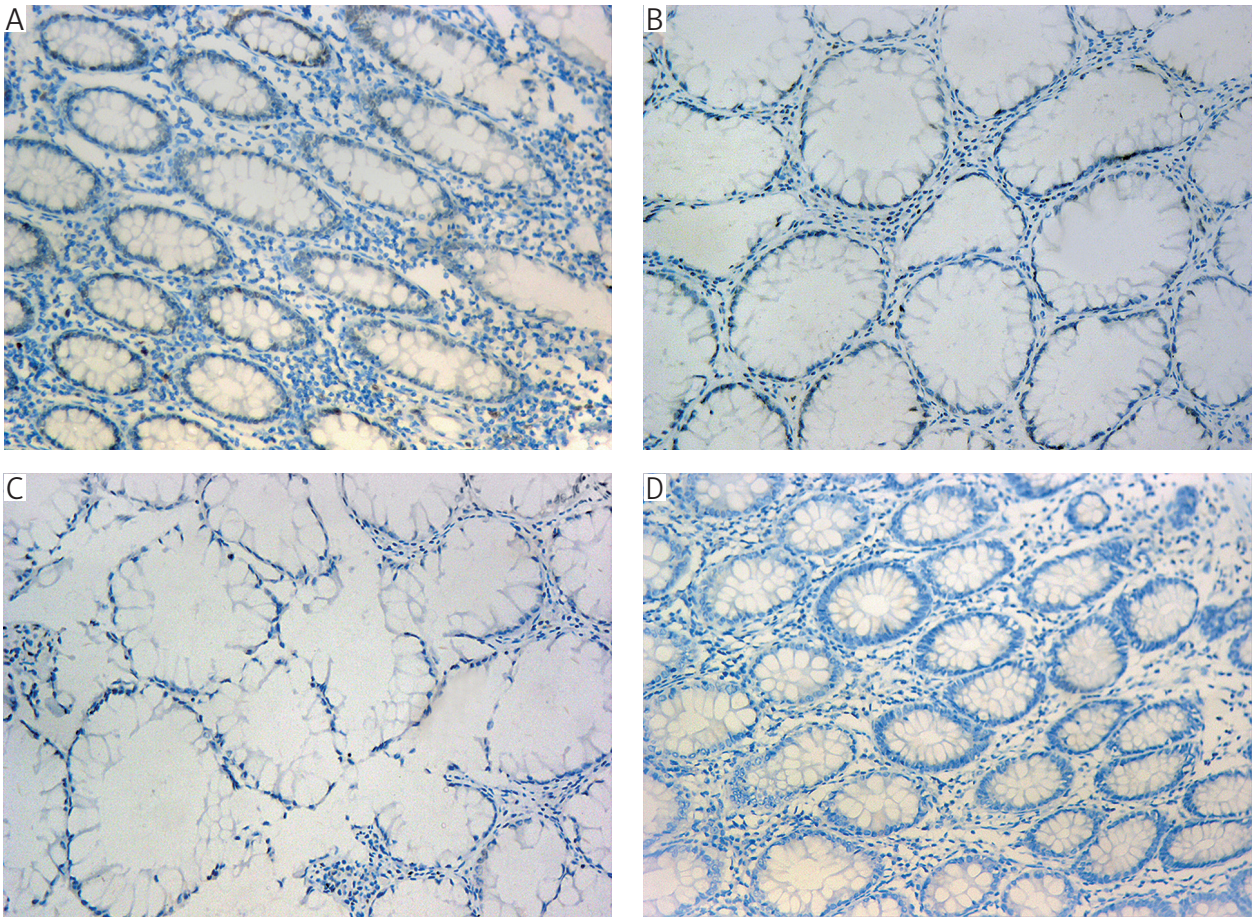

Fig. 2. Normal Colonic Tissue Samples. A) Low EZH2 nuclear expression in few epithelial cells (IHC, 200×). B) Low ERRα nuclear expression in few epithelial cells (IHC, 200×). C) Negative EZH2 IHC expression (IHC, 200×). D) Negative ERRα IHC expression (IHC, 200×)

92 months, respectively (Figs. 3A, 4A). Cases with high EZH2 IHC expression show statistically significant shorter OS, and PFS than cases with low EZH2 expression, such that OS was 29.8% at 76 months vs. 89.5% at 92 months, respectively ($p = 0.0001$); and PFS is 37.5% at 76 months vs. 94.7% at 92 months, respectively ($p = 0.001$) (Figs. 3B, 4B).

In the same sense, cases with high ERRα IHC expression reveal statistically significant shorter OS, and PFS than cases with low ERRα expression, such that OS is 13% at 70 months vs. 75.8% at 92 months, respectively ($p = 0.001$); and PFS is 8.7% at 67 months vs. 90.8% at 92 months, respectively ($p = 0.001$) (Figs. 3C, 4C).

Meanwhile, cases with combined high EZH2 and high ERRα IHC expressions also reveal statistically significant shorter OS, and PFS than cases showing combined low EZH2 and low ERRα expressions, such that OS is 9.1% at 70 months vs. 88.9% at 92 months, respectively, while low EZH2/high ERRα or high ERRα/low EZH2 show an OS of 62.5% at 76 months ($p = 0.001$); and PFS for both high vs. both low is 4.5% at 24 months vs. 94.4% at 92 months, respectively, while low EZH2/high ERRα or high ERRα/low EZH2 show a PFS of 87.5% at 76 months ($p = 0.001$) (Figs. 3D, 4D).

After adjustment of tumor stage, tumor grade, lymph node involvement, EZH2 and ERRα IHC expression, it is shown that EZH2 IHC Expression, ERRα IHC expression and tumor grade are independent factors affecting the OS of CRC cases (Table V).

After adjustment of tumor stage, tumor grade, lymph node involvement, EZH2 and ERRα IHC expression, it is shown that ERRα IHC expression and tumor grade are independent factors affecting the PFS of CRC cases (Table VI).

Discussion

In spite of advanced treatment, CRC accounts for 0.6 million deaths per year worldwide [26]. Thus, introducing new prognostic biomarkers and more personalized treatment modalities are greatly crucial especially in resistant cases. EZH2 was identified as a relevant coregulator of ERRα in breast cancer with a recommendation of more research to elucidate the underlying mechanisms [27]. However, the

Table II. Relationship between EZH2 IHC Expression and clinicopathological characteristics in the evaluated colorectal carcinoma cases

	High				P
	Low		High		
	Mean	±SD	Mean	±SD	
Age	45.77	15.20	48.82	13.80	0.431‡
Gender					
Male	15	39.5%	23	60.5%	0.553*
Female	7	31.8%	15	68.2%	
Pathologic Grade					
Well differentiated	6	100.0%	0	0.0%	0.001**
Moderately differentiated	15	34.9%	28	65.1%	
Poorly differentiated	1	9.1%	10	90.9%	
TNM Staging					
I	2	100.0%	0	0.0%	0.001**
II	11	78.6%	3	21.4%	
III	8	25.8%	23	74.2%	
IV	1	7.7%	12	92.3%	
Lymph Node Involvement					
No	13	81.3%	3	18.8%	0.001*
Yes	9	20.5%	35	79.5%	
Size					
< 5	21	60.0%	14	40.0%	0.001*
≥ 5	1	4.0%	24	96.0%	
Site					
Colonic	18	40.9%	26	59.1%	0.258*
Rectal	4	25.0%	12	75.0%	

‡Student t test

**Chi-Square Tests*

***Fisher exact test*

combined IHC expression of EZH2 and ERRα has never been evaluated in CRC.

The current study showed that low EZH2 nuclear expression was detected in 4 out of 60 (6.6%) normal colonic tissue samples. This was slightly higher than Abdel Raouf *et al.* [19] who showed 5% EZH2 nuclear expression in their samples of normal colonic tissue, but was similar to Liu *et al*. [6] who detected EZH2 expression in 6.67% of normal colonic tissue. Moreover, the current study showed low ERRα nuclear expression in 5 out of 60 (8.3%) normal colonic tissue samples. This was slightly lower than the percentage of expression shown by Liang *et al.* [13] where 10.9% of the included adjacent normal colonic tissues expressed low ERRα nuclear staining. These slight discrepancies might be attributed to different sample sizes in all of these studies as compared to ours.

There was a rise in EZH2 expression from normal colonic tissue to CRC in the current study as was also reported by Ohuchi *et al*. [27]; such that high nuclear EZH2 expression was detected in 63.3% of the current CRC cases which was in accordance with Liu *et al*. [6] CRC cases that showed 62.12% EZH2 expression. It was slightly lower than Abdel Raouf *et al.* [19] who showed high EZH2 expression in 73.3% in their colon cancer cases. This might be attributed to different sites of the specimens included in the latter study that only included colonic carcinoma without rectal carcinoma cases. Meanwhile, only 23 CRC cases (38.3%) showed high ERRα nuclear expression. This goes well with Liang *et al.* [13], whose CRC cases showed 39.1% ERRα nuclear expression.

Conflicting results exist among different studies concerning the possible role of EZH2 in CRC progression. Several studies demonstrated that EZH2

Table III. Relation between ERRα IHC Expression and clinicopathological characteristics in the evaluated colorectal carcinoma cases

	ERRα IHC Expression				P
	Low		High		
	Mean	±SD	Mean	±SD	
Age	49.16	14.56	45.35	13.80	0.318‡
Gender					
Male	23	60.5%	15	39.5%	0.811*
Female	14	63.6%	8	36.4%	
Pathologic Grade					
Well differentiated	6	100.0%	0	0.0%	0.009**
Moderately differentiated	28	65.1%	15	34.9%	
Poorly differentiated	3	27.3%	8	72.7%	
TNM Staging					
I	2	100.0%	0	0.0%	0.001**
II	13	92.9%	1	7.1%	
III	19	61.3%	12	38.7%	
IV	3	23.1%	10	76.9%	
Lymph Node Involvement					
No	15	93.8%	1	6.3%	0.002*
Yes	22	50.0%	22	50.0%	
Size					
< 5	33	94.3%	2	5.7%	0.001*
≥ 5	4	16.0%	21	84.0%	
Site					
Colonic	23	52.3%	21	47.7%	0.013*
Rectal	14	87.5%	2	12.5%	

‡Student t test

**Chi-Square Tests*

***Fisher exact test*

Table IV. Agreement between EZH2 and ERRα in the evaluated colorectal carcinoma cases

	EZH2 IHC Expression				κ	P
	Low		High			
	N	%	N	%		
ERRα IHC Expression						
Low	21	95.5%	16	42.1%	0.467	0.001
High	1	4.5%	22	57.9%		

Table V. Stepwise Backward Cox Regression of important factors affecting OS of the evaluated colorectal carcinoma cases

	Hazard ratio (HR)	P	95.0% CI for HR	
			Lower	Upper
High EZH2 IHC Expression*	4.920	0.042	1.062	22.791
High ERRα IHC Expression*	5.183	0.001	1.966	13.663
Poorly differentiated grade**	8.302	0.0001	3.028	22.763

**reference low expression*

***reference moderate/well differentiated grade*

Table VI. Stepwise Backward Cox Regression of important factors affecting PFS of the evaluated colorectal carcinoma cases

	Hazard ratio (HR)	P	95.0% CI for HR	
			Lower	Upper
High EZH2 IHC Expression*	10.948	0.052	0.979	122.416
High ERRα IHC Expression*	44.892	0.001	7.848	256.795
Poorly differentiated grade**	47.224	0.0001	7.810	285.540

**reference low expression*

***reference moderate/well differentiated grade*

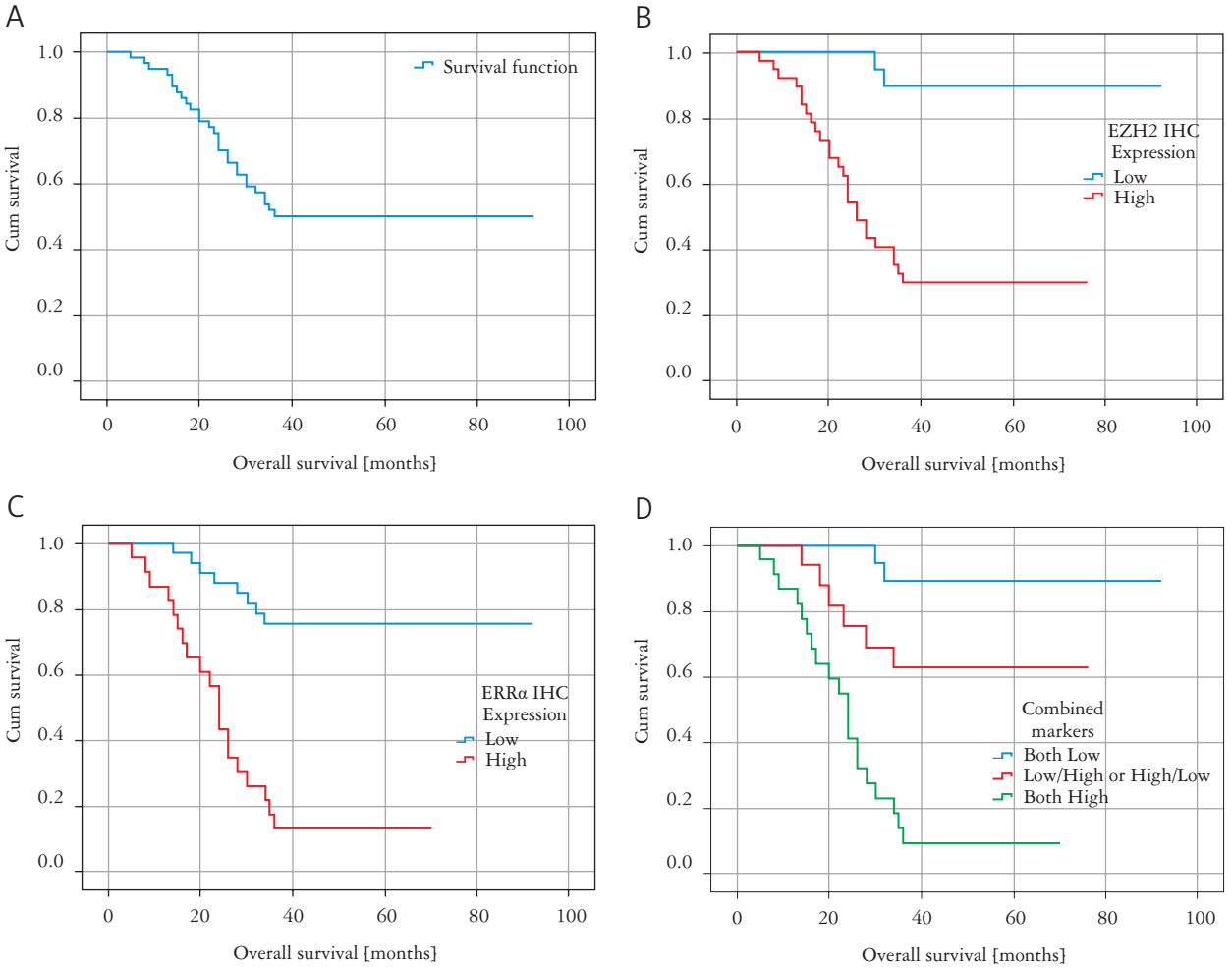

Fig. 3. Kaplan Meier analysis of overall survival (OS): A) OS among all CRC cases; B) correlation between high EZH2 IHC expression and shorter OS (p = 0.0001); C) correlation between high ERRα IHC expression and shorter OS (p = 0.001); D) correlation between combined EZH2 and ERRα IHC expression and OS (p = 0.001)

correlated with poorer prognosis [6, 7, 19, 28, 29, 30, 31, 32, 33, 34]. In contrast, other publications supported its association with better prognosis in CRC [9, 10, 35, 36, 37]. Moreover, few failed to correlate it to prognostic indicators [8]. The results of the current work were in accordance with the first group of studies where high EZH2 IHC expression was associated with high grade, late tumor stages, positive lymph node involvement and larger tumor sizes (p = 0.001 for each). Moreover, tumors expressing high EZH2 showed statistically significant shorter OS and PFS (p = 0.001 for each). These conflicting results might be attributed to the fact that the precise molecular pathways of EZH2 in CRC has not been fully elucidated; where some reports suggested both oncogenic and tumor-suppressing roles of EZH2 without knowing the controlling factors in each case, and some publications attributed this discrepancy to polymorphism [38, 39, 40, 41]. Other suggested explanations included using different

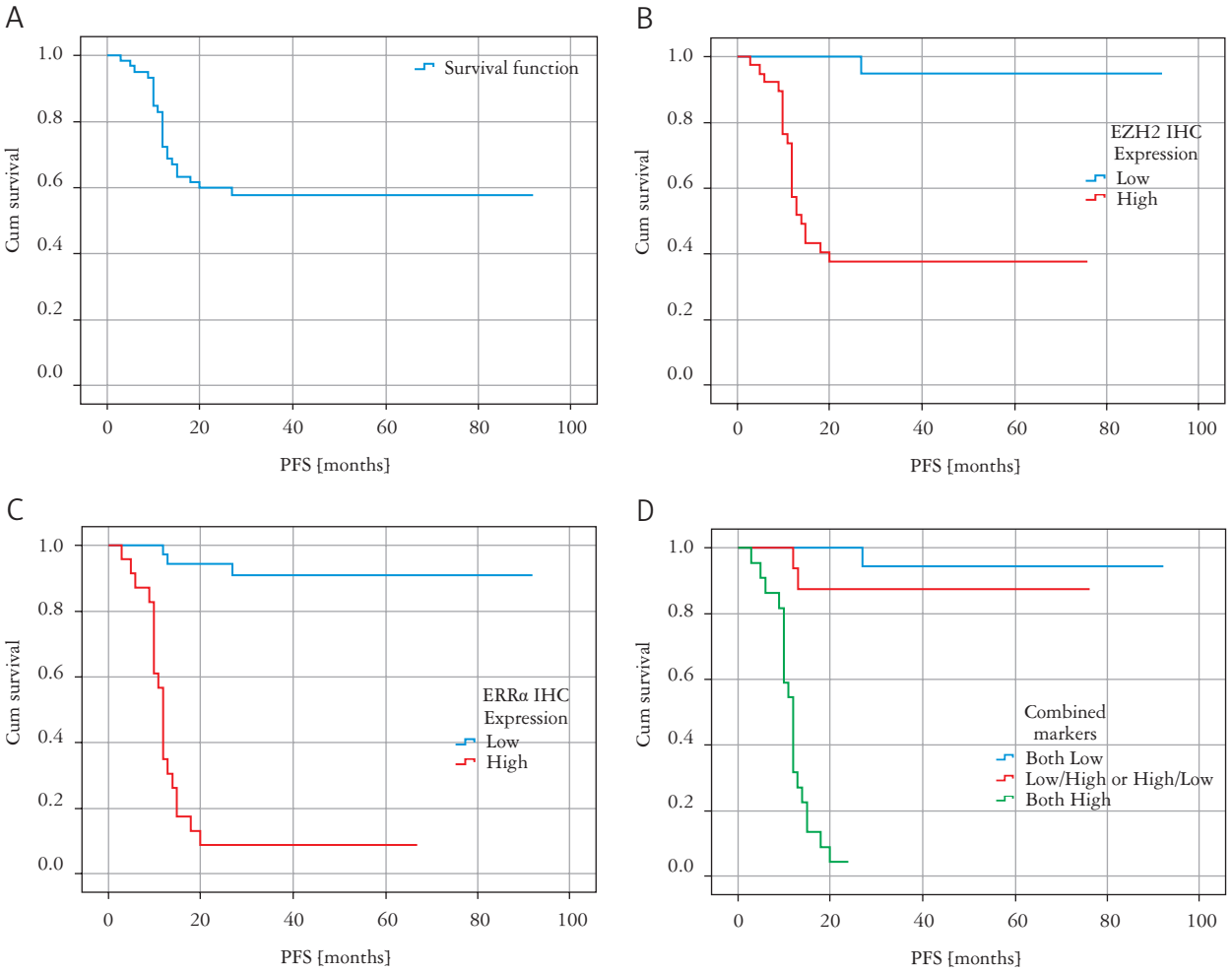

Fig. 4. Kaplan Meier analysis of progression free survival (PFS): A) PFS among all CRC cases; B) correlation between high EZH2 IHC expression and shorter PFS (p = 0.001); C) correlation between high ERRα IHC expression and shorter OS (p = 0.001); D) correlation between combined EZH2 and ERRα IHC expression and PFS (p = 0.001)

EZH2 scoring techniques, as well as differences in prognosis between rectal and colonic carcinomas, and even differences of prognostic impact of EZH2 IHC expression within the same tumor between its central part and its invasive front [42].

In the very few papers conducted on colonic cancer, ERRα has been correlated with poor clinicopathological parameters [11, 12, 13]. This was similar to the findings of the current study where high ERRα correlated with higher grade, late stage, lymph node involvement and large tumor sizes (p = 0.009, p = 0.001, p = 0.002, p = 0.001 respectively). In addition, high ERRα was associated with shorter OS and PFS by Kaplan Meier analysis (p = 0.001 for each). This was in conformity with the study conducted by Liang *et al*. [13] which suggested that high ERRα expression might contribute to cancer progression, and their CRC cases expressing high ERRα correlated with lower OS and local recurrence. Similarly, Nussler *et al*. [43] and Caiazza *et al.* [44] reported that ERRα was involved in CRC development and progression.

After adjustment of the other factors by backward stepwise Cox regression, both EZH2 and ERRα IHC expression, as well as tumor grade were independent factors affecting OS of CRC cases in the current work. Furthermore, backward stepwise Cox regression showed that only ERRα IHC expression and tumor grade affected PFS. Similarly, Liang *et al*. [13] and Ye *et al*. [25] also identified ERRα as an independent prognostic factor for patients with CRC.

The relationship between EZH2 and ERRα IHC expression in CRC has not been previously addressed. To the best of our knowledge, this is the first study to evaluate the combined IHC expression of both markers in CRC. There was a highly statistically significant moderate agreement between both markers (p = 0.001, κ = 0.467), where the majority of cases showed either high expression of both markers or low expression of both markers. Future studies providing in-depth understanding of genetic and signaling pathways of both markers are mandatory for tailoring personalized treatment of CRC, especially in case of therapy resistance. In this context, it should be

highlighted that each of these two markers could provide a target of therapy in CRC. Fussbroich *et al*. [29] reported that EZH2 participated in the proliferation of colonic cancer cells, and thus could serve as a potential therapeutic target. However, several general considerations should be kept in mind when dealing with EZH2 targeted therapy; which include the fact that EZH2 is expressed to a certain extent in normal tissues including normal colonic tissue. Furthermore, EZH2 is controlling differentiation of tissue-specific stem cells. It may also act as a tumor suppressor in certain disorders. Thus, to avoid unwanted side effects, more studies in this field become of paramount importance. Moreover, Bernatchez *et al.* [12] demonstrated that ERRα enhanced the proliferation of colonic cancer cells by incorporating nutrients into the cells and suggested that these biological roles could be the cornerstone for developing targeted therapeutic agents.

A limitation to this study is the relatively small sample size, but this could be attributed to the strict abidance to inclusion criteria. Further studies with larger cohorts are mandatory to validate the current results, and to investigate thoroughly any underlying genetic pathways combining both markers in CRC.

Overall, this is the first study to assess the combined IHC expression of EZH2 and ERRα in CRC. Concomitant high or low IHC expression of EZH2 and ERRα, or the single expression of any of them might comprehensively evaluate CRC progression and predict the overall survival of such patients. Moreover, they could serve as future targets of specific therapy provided that thorough research of their underlying genetic pathways would be conducted.

The authors declare no conflict of interest.

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