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Original article

Prognostic impact of changes in base excision repair machinery in sporadic colorectal cancer

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ABSTRACT

Objective: to evaluate the prognostic value of base excision repair proteins in sporadic colorectal cancer.
Methods: Pre-treatment tumor samples from 72 patients with sporadic colorectal adenocarcinoma were assessed for APC, MPG, Polβ, XRCC1 and Fen1 expression by immunohistochemistry. The associations of molecular data were analyzed in relation to clinical features and TNM staging as a prognosis predictor and disease-free survival.
Results: Higher levels of MPG, Polβ and XRCC1, but not Fen1, were associated with unfavorable pathological outcomes, such as poor cellular differentiation, advanced TNM stages, presence of lymphatic and perineural invasions and metastatic lymph nodes. MPG and Polβ overexpression were associated with right-sided CRC. However, only MPG high expression is associated with shorter disease-free survival in CRC patients.
Conclusions: Our results suggest that increased expression of MPG, Polβ and XRCC1 are more likely to evolve to poor pathological outcomes, but only the elevated expression of MPG protein predicts recurrence. The BER proteins appear to be suitable candidates to refine the TNM current staging of colorectal cancer.

1. Introduction

Colorectal cancer (CRC) is one of the most frequently neoplasia in Western countries (10–15% of all forms of cancer) and ranks second in cancer related deaths [1,2]. While only 6% of all cases present a hereditary genetic etiology, the sporadic CRC (~80% of all cases), which is the most prevalent form, still has a lack of knowledge about the etiological factors that triggers this disease [1,2]. Despite survival rates have increased in the past few years, at least a third of patients who undergo curative resection experience local tumor recurrence or metastasis [3,4]. Pathological staging is the only prognostic classification used in clinical practice to select patients for adjuvant chemotherapy. Furthermore, drug resistance is also a critical problem in CRC patients with comprehensive treatment, which is directly associated to the absence of predictive markers [5,6].

Among the earliest events leading to the development of sporadic CRC are the mutations in the central area of the *adenomatous polyposis coli* (*APC*) gene, which are strongly associated with familial predisposition to CRC and with the sporadic CRC [7,8]. Appropriate levels of functional APC are essential to many cellular and tissue integrities [9,10]. The major role of APC is to regulate β-catenin and Wnt signaling, interfering in processes such as apoptosis, cell adhesion, chromosomal instability, cell cycle and DNA repair [11].

The DNA repair system has evolved to deal with the modification or loss of DNA bases, as a sophisticated manner to fight against the mutations. However, changes in its normal functions respond as a major cause of human diseases, including cancer. Among these mechanisms, base excision repair (BER) is the most prevalent pathway for the

Abbreviations: CRC, colorectal cancer; TNM, tumor-node-metastasis; APC, *adenomatous polyposis coli*; BER, base excision repair; dRP, deoxyrbose phosphate; AP site, apurinic/abasic site; SN-BER, single-nucleotide base excision repair; LP-BER, long-patch base excision repair; MPG, N-methylpurine DNA glycosylase; Fen1, flap endonuclease; Polβ, DNA polymerase beta; XRCC1, X-ray repair complementing defective repair in Chinese hamster cells 1; QS, multiplicative quick score method; MMR, Mismatch Repair; LCRC, Left-sided colorectal cancer; RCRC, right-sided colorectal cancer

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removal of damaged bases generated by alkylation, oxidation or reduction [12] and proceeds through a sequence of reactions requiring several different enzymes. The first step involves excision of the damaged base by a DNA glycosylase enzyme, which leads to the formation of a potentially cytotoxic abasic site intermediate (AP site). Subsequently, the AP site is processed by an AP endonuclease (APE1), generating a strand break and a flap. At this point, DNA polymerase fills the gap, and DNA ligase seals the remaining nick, thus completing the BER process [13].

It has been reported that APC protein has a DNA repair inhibitory domain located towards the N-terminus, which interacts with the BER proteins Polβ and Fen1 [14]. APC has the ability to block Polβ −directed strand displacement synthesis in long patch-BER (LP-BER) or it can inhibit its lyase activity, thus blocking single nucleotide-patch BER (SN-BER) [15,16]. The consequence of the blocked LP- and SN-BER on cellular fate is not clear, but specially because both Fen1 and APC are considered tumor suppressors [16,17] and their levels are critical for the repair of the BER pathways in colon cancer [18] this combination of factors can differently drive the tumor cells in terms of aggressiveness.

DNA repair imbalance is related to malignant transformation by allowing greater vulnerability to the accumulation of DNA damage [19]. The elaboration of an expression profile that could produce reliable biomarkers is a priority need to guide colorectal cancer treatment and monitor therapeutic response, as well as for surveillance to detect recurrence. Thus, bearing in mind the importance of DNA repair in the disease development and therapy response, it seems quite reasonable to consider a categorization of colorectal tumors based on DNA repair characteristics.

2. Patients and methods

2.1. Study patients and collection of samples

In this study (case-series design), we retrospectively selected resection specimens of 72 individuals diagnosed with adenocarcinoma of the colon and rectum and who were admitted to colorectal surgery with curative intent by the same surgical team between 2010 and 2012 in South Brazil. Patients were excluded if at least one of the follow criteria was identified: suspicion of hereditary colorectal cancer (familial adenomatous polyposis and hereditary non-polypoid colorectal cancer); presence of colorectal cancer associated with inflammatory bowel disease; realization of neoadjuvant chemoradiation therapy. This study was approved by the Ethics Committee in Human Research of the participating institutions (No. 321.069). Written informed consent was obtained from all patients before their enrolment in the study.

Epidemiological, clinical and pathological data were obtained from the hospital medical records. Histopathological data (such as tumor subtype, depth of invasion, lymph node and/or metastasis distance and staging) were also extracted from the pathological reports. TNM system was used as the staging scale for prognosis. Colon tumors was classified into left-sided colorectal cancer (LCRC) and right-sided colorectal cancer (RCRC)

2.2. Immunohistochemistry

Formalin-fixed and paraffin-embedded samples were cut into 4 μm sections. After deparaffinization and rehydration, the sections were quenched with 3% H_2O_2 in methanol to block endogenous peroxidase. 5% bovine serum albumin was then applied to prevent non-specific binding. The sections were incubated with APC (dilution 1:100, AB15270, Abcam), Polβ (dilution 1:500, AB26343, Abcam), XRCC1 (dilution 1:50, AB1838, Abcam), Fen1 (dilution 1:800, AB17993, Abcam), MPG (dilution 1:100, EPR10959 (B), Abcam), then treated with the rabbit conjugated to horseradish peroxidase (DAKO) antibodies. Diaminobenzidine was used as chromogen and the sections were counterstained with haematoxylin. Tissues used as positive controls were recommended by the antibodies manufacturer as it follows: human testis tissue (anti-MPG, anti-Fen, anti-XRCC1); human small cell lung cancer tissue (anti-Polβ); and normal human colon tissue (anti-APC). Omission of the primary antibody was used as a negative control. Each immunohistochemical stain was performed in a group to prevent potentially staining irregularities encountered with separate immunohistochemistry runs.

Histological sections used for diagnostic and experimental purposes were obtained from the same tumoral area to minimize intratumoral heterogeneity bias. The quality (number, intensity, and pattern) of every staining procedure has been comparatively evaluated using consecutive control sections an independent experienced pathologist blinded to the objectives of this study. After the immunostaining, two observers assessed all cases independently. The few cases with discrepant scoring were re-evaluated jointly on a second occasion, and agreement was reached in all cases. Non-representative samples or samples with only a few tumor cells (< 100) were excluded from the data analysis.

2.3. Immunohistochemistry results evaluation

Positive staining for APC and BER proteins was defined as the observation of shades of brown nuclear staining the microscope ($\times 400$). Five hot spot fields containing at least 200 cells were captured and the positive cells were manually counted using the NIH-ImageJ software. To assess the immunohistochemical expression we used the multiplicative quick score method (QS) [20]. In order to minimize intratumoral heterogeneity bias, based on the distribution and intensity of staining, we used a semiquantitatively score (corresponding to staining intensity and percentage of reactive nuclei). According to the number of positive staining cell, the staining density was expressed semi-quantitatively as follows: 0, less than 5%; 1, 5–25%; 2, 25–50%; 3, 50% to 75%; or 4, more than 75%. We also evaluated the staining intensity was scored as follows: 0-negative staining; 1-weak staining; 2-moderate staining and 3-strong staining. Both values were multiplied together, and the staining score was stratified into two groups of immune reactivity: weak (score range, 0–4) or strong (score range, 5–12) (Supplementary Fig. 1).

2.4. Statistical analysis

Statistical analysis was performed using SPSS software version 22.0. Immunohistochemistry and clinical features correlations were analyzed through contingency tables, chi-square ($\chi 2$) test and Fisher's exact test. For tumor protein expression and associations with the disease-free interval, the Kaplan-Meier survival table method was used. To test the significance of the differences between the curves of the disease-free interval and protein expression levels, the Log-rank (Mantel-Cox) test was used. All statistical tests were two sided and $P \leq 0.05$ was considered significant.

2.5. Availability of data and materials

Any supplementary supporting data relating details of clinical and pathological analysis are available upon request from the corresponding author and can be found in the electronic medical record system of the Irmandade Santa casa de Misericórdia de Porto Alegre hospital.

3. Results

3.1. Clinicopathological findings

Table 1 summarizes the clinicopathological characteristics of the 72 included patients. The patients' ages varied from 29 to 88 years. Considering the anatomic location, 59 (80%) of the tumors were located in

Table 1
Clinicopathological profile of patients with colorectal adenocarcinoma (n = 72). T1: invasion through muscularis mucosa into submucosa, T2: invasion through submucosa into the muscularis propria, T3: invasion through the muscularis propria into subserosa but not to adjacent organs or tissues, T4: invasion of surrounding structures or with tumour cells on the free external surface of the bowel.

Characteristics		Number of cases (%)
Gender	Male	38 (53)
	Female	34 (47)
Average Surgical Age (years)		66.7 ± 12.4
Age, y	< 65	31 (43)
	> 65	41 (57)
Tumor Site	RCRC	19 (27)
	LCRC	40 (55)
	Rectum	13 (18)
Grade	Well to Moderately-differentiated	45 (63)
	Poorly-differentiated	27 (37)
Tumour ivasive depth	T1, T2	44 (61)
	T3, T4	28 (39)
TNM Stage	I-II	44 (61)
	III-IV	28 (39)
Lymphatic invasion	No	40 (55)
	Yes	32 (45)
Perineural invasion	No	60 (83)
	Yes	12 (17)
Lymph node metastasis	No	40 (55)
	Yes	32 (45)

the colon (19 right-sided colon and 40 left sided-colon), and 13 (20%) were located in the rectum. Regarding the tumor stage, 44 (61%) of the cases were considered in initial stages (TNM I or II), while 28 (39%) of the cases presented a more advanced stage. 45 tumors (63%) were considered well to moderately differentiated. Considering the presence of lymphatic and perineural invasions, 32 (46%) were positive for the first one and 12 to the second. Finally, 32 (45%) patients presented lymph node metastasis at the moment of the surgical procedure.

3.2. Changes in BER proteins MPG, Polβ and XRCC1 expression have prognostic value in colorectal cancer

The distribution of intensities of BER proteins staining and its correlation with clinical features are shown in Table 2 and, in Fig. 1, we show the representative images of immunohistochemistry positive staining for MPG (Fig. 1A), Polβ (Fig. 1B), Fen1 (Fig. 1C) and XRCC1 (Fig. 1D). MPG overexpression presented very consistent associations with clinical features of tumor aggressiveness, such as tumor invasive depth ($p < 0.001$), presence of lymphatic and perineural invasions ($p < 0.001$ and $p = 0.011$) and presence of metastatic lymph nodes ($p < 0.001$). In consonance, Polβ and XRCC1 overexpression were also associated with characteristics of poor prognosis. High expression of Polβ was associated with tumor invasive depth ($p < 0.001$), advanced TNM stages ($p < 0.001$) and presence of lymphatic invasion ($p = 0.004$) and lymph node metastasis ($p < 0.001$). Overexpression of XRCC1 was also associated with tumors with advanced TNM stages ($p < 0.001$) and with lymphatic invasion ($p < 0.001$) and lymph node metastasis ($p < 0.001$). Finally, regarding APC protein expression, we found that 57 tumors were considered as presenting low expression of APC (78%) and 15 that presented high expression (22%), however we did not find any association between the APC immunohistochemical expression and the clinicopathological features (Table 2). Representative images of the scores used to classify the tumors are shown in Fig. S1. Aside from Fen1, all the other base excision repair proteins correlated with the pathological and clinical features.

3.3. Polβ and APC are inversely expressed in colorectal tumors

To assess whether BER protein expression of MPG, Polβ, Fen1 and

Table 2
Increase of MPG, Polβ and XRCC1, but not Fen1 protein expression, in colorectal tumors, is associated with several unfavorable pathological outcomes. Associations were analyzed with the chi-square ($\chi 2$) test and Fisher's exact test. $P < 0.05$. Significant p values are highlighted in bold font. Number of cases (%).

Variable		APC			MPG			Polβ			XRCC1			Fen1		
		Low (n = 57)	High (n = 15)	*p value*	Low (n = 37)	High (n = 35)	*p value*	Low (n = 43)	High (n = 29)	*p value*	Low (n = 28)	High (n = 44)	*p value*	Low (n = 34)	High (n = 38)	*p value*
Age	< 65 years	27 (37)	4 (5)	0,241	16 (22)	15 (21)	0,974	17 (24)	14 (19)	0,479	11 (15)	20 (28)	0,634	15 (21)	16 (22)	0,863
	> 65 years	30 (42)	11 (16)		21 (29)	20 (28)		26 (36)	15 (21)		17 (24)	24 (33)		19 (26)	22 (31)	
Tumor Site	Colon	45 (62)	14 (20)	0,276	30 (42)	29 (40)	0,845	37 (51)	22 (31)	0,352	35 (49)	24 (33)	0,754	30 (42)	29 (40)	0,231
	Rectum	12 (17)	1 (1)		7 (8)	7 (10)		6 (8)	7 (10)		9 (12)	4 (6)		4 (6)	9 (12)	
Grade	Well to Moderately-differentiated	36 (50)	9 (12)	0,822	19 (26)	26 (36)	0,054	24 (33)	21 (30)	0,215	18 (25)	27 (37)	0,803	18 (25)	27 (38)	0,146
	Poorly- differentiated	21 (29)	6 (9)		18(25)	9 (12)		19 (26)	8 (11)		10 (14)	17 (24)		16 (22)	11 (15)	
Tumor invasive depth	T1-T2	34 (47)	10 (14)	0,890	35 (49)	9 (12)	**< 0.001**	35 (49)	9 (12)	**< 0.001**	24 (33)	20 (28)	0,391	21 (29)	23 (32)	0,594
	T3-T4	23 (32)	5 (7)		2 (3)	26 (36)		8 (11)	20 (28)		4 (6)	24 (33)		13 (18)	15 (21)	
TNM Stage	I-II	34 (47)	10 (14)	0,769	11 (15)	33 (46)	**< 0.001**	35 (49)	9 (12)	**< 0.001**	26 (36)	18 (25)	**0,001**	20 (28)	24 (33)	0,914
	III-IV	23 (32)	5 (7)		26 (36)	2 (3)		8 (11)	20 (28)		2 (3)	26 (36)		14 (19)	14 (20)	
Lymphatic invasion	No	30 (42)	10 (14)	0,392	31 (43)	9 (12)	**< 0.001**	30 (42)	10 (14)	**0,004**	23 (32)	17 (24)	**0,001**	21 (29)	19 (26)	0,350
	Yes	27 (37)	5 (7)		6 (9)	26 (36)		13 (18)	19 (26)		5 (7)	27 (37)		13 (18)	19 (26)	
Perineural invasion	No	48 (67)	12 (17)	0,705	35 (49)	25 (35)	**0,011**	38 (52)	22 (31)	0,204	25 (35)	35 (49)	0,346	27 (37)	33 (46)	0,530
	Yes	9 (12)	3 (4)		2 (3)	10 (13)		5 (7)	7 (10)		3 (4)	9 (12)		7 (10)	5 (7)	
Lymph node metastasis	No	32 (44)	9 (12)	0,788	31 (43)	10 (14)	**< 0.001**	32 (44)	9 (12)	**0,001**	25 (35)	15 (22)	**< 0.001**	22 (31)	19 (26)	0,240
	Yes	25 (35)	6 (9)		6 (9)	25 (34)		11 (15)	20 (29)		2 (3)	29 (40)		12 (17)	19 (26)	

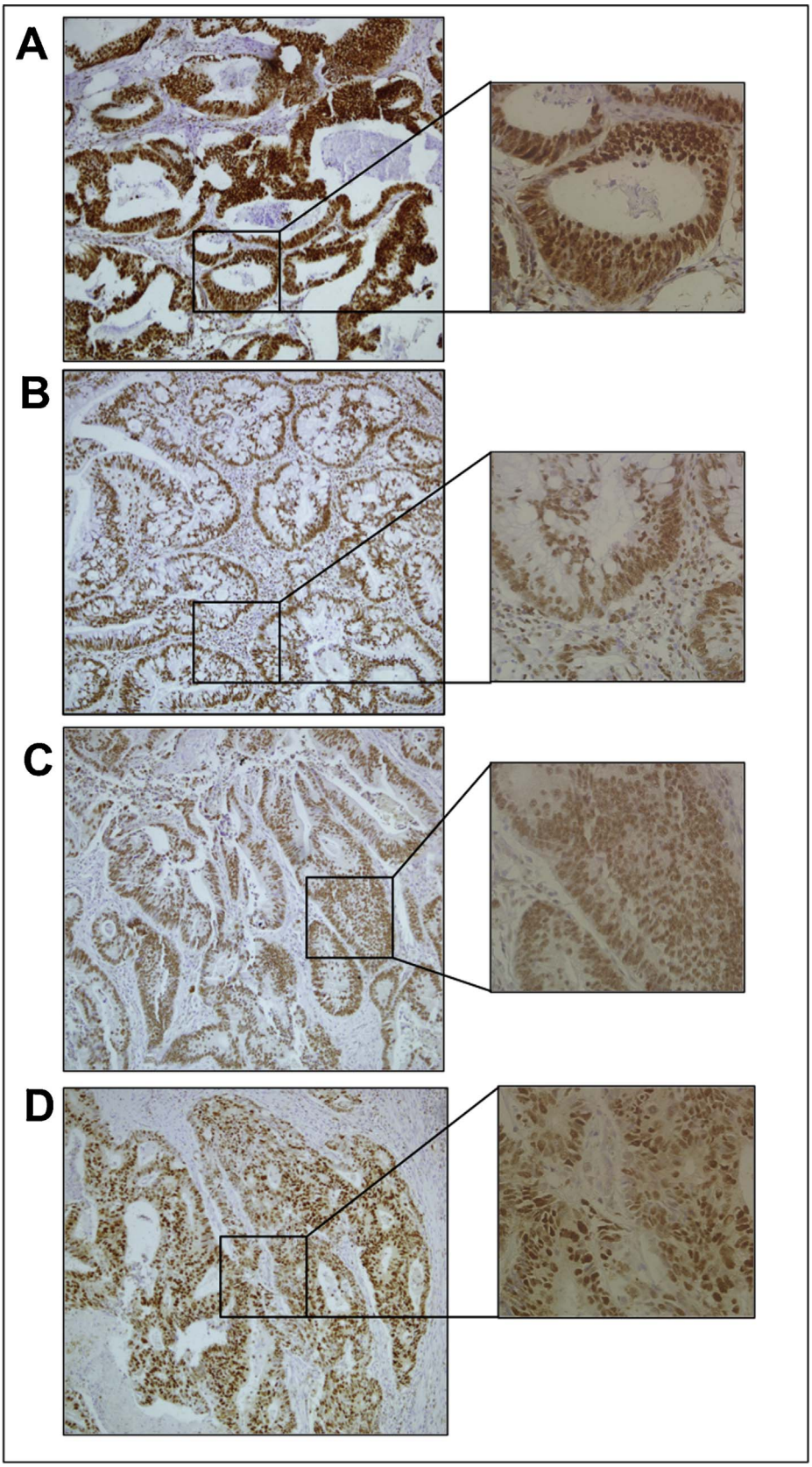
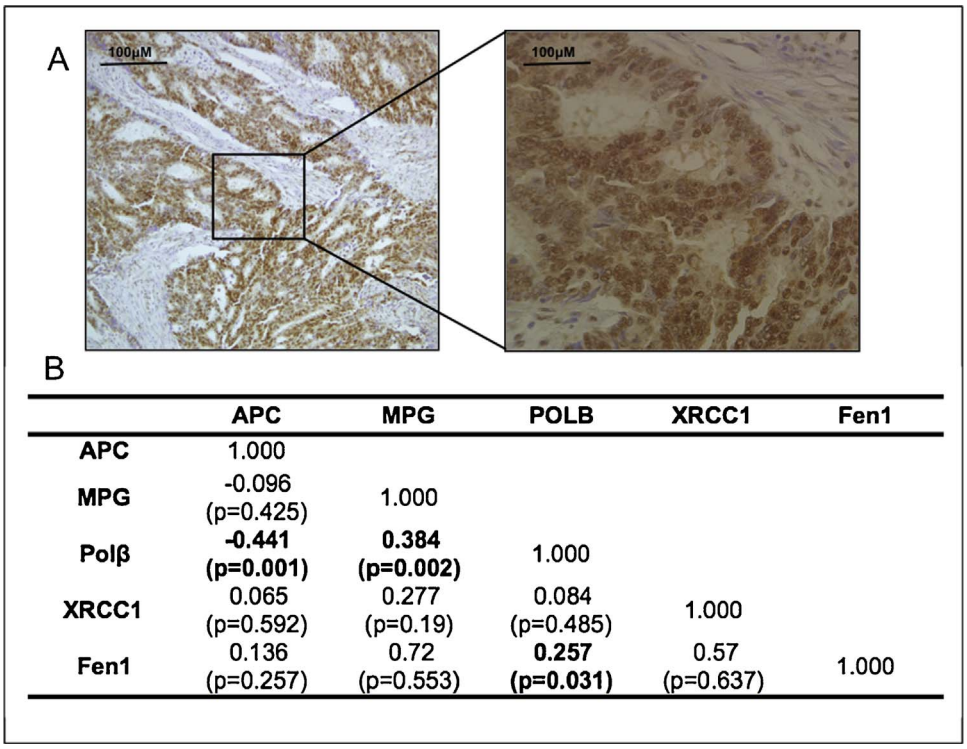


Fig. 1. Positive immunohistochemical staining of BER proteins in colorectal adenocarcinoma tissue samples. Representative immunohistochemical staining of BER proteins in colorectal cancer tissues. A: MPG; B: Polβ; C: XRCC1; D: Fen1. Original magnification: 100× (left), 200× (middle) and 400× (right).

XRCC1 were associated with each other or with APC, the Spearman's rank correlation coefficient (ρ; rho) was determined (Fig. 2A). We found that the higher is the expression of APC the lower is the expression of Polβ (-0.441; p = 0.001). On the other hand, we identified that a co-expression MPG and Polβ (0.384; p = 0.001) and of Polβ and Fen1 (0.257; p = 0.03) are present.



	APC	MPG	POLB	XRCC1	Fen1
APC	1.000				
MPG	-0.096 (p=0.425)	1.000			
Polβ	**-0.441 (p=0.001)**	**0.384 (p=0.002)**	1.000		
XRCC1	0.065 (p=0.592)	0.277 (p=0.19)	0.084 (p=0.485)	1.000	
Fen1	0.136 (p=0.257)	0.72 (p=0.553)	**0.257 (p=0.031)**	0.57 (p=0.637)	1.000

Fig. 2. Immunohistochemical staining of APC in colorectal adenocarcinoma tissue samples and co-expression analysis.
A: Representative immunohistochemical staining of APC in colorectal cancer tissues. Original magnification: 100× (left), 200× (middle) and 400× (right). B: Spearman rank correlation matrix of APC and BER protein expression. P < 0.05. Significant p values are highlighted in bold font.

3.4. MPG and Polβ overexpression are associated with right-sided CRC

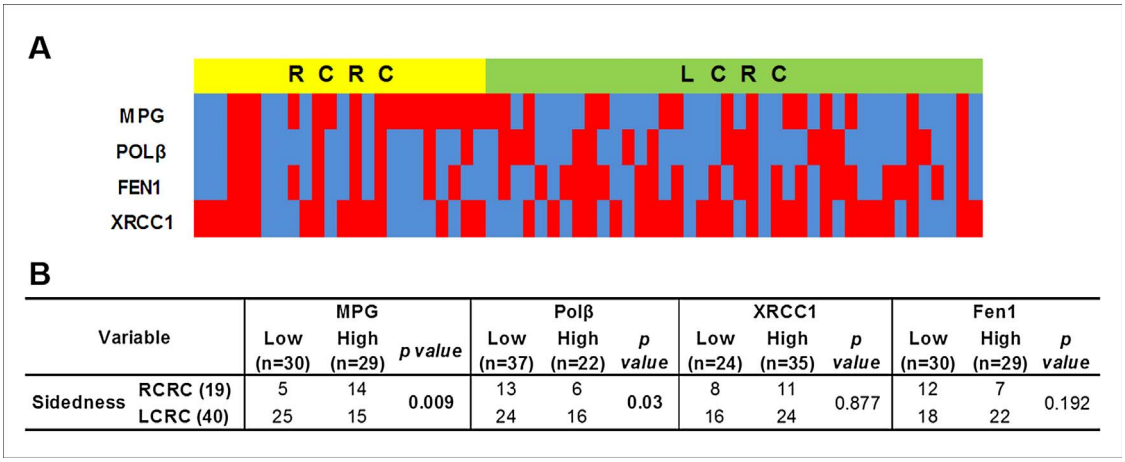
Since the distinction between right- and left-sided CRC (RCRC and LCRC, respectively) has been receiving growing attention, we performed an analysis to characterize the profile of BER signature between these two categories of colon cancer (Fig. 4). Surprisingly, we found that MPG and Polβ were overexpressed in RCRC (p = 0.009 and p = 0.03, respectively. Fig. 3B). In addition, BER pathway is imbalanced and has a heterogeneous expression pattern in both RCRC and LCRC (Fig. 3A).

3.5. MPG high expression is associated with poor disease-free survival in CRC patients

We investigated the prognostic implications of the BER high and low individual protein expression in CRC patients. The high expression of MPG was significantly correlated with shorter disease-free survival, compared to the low expression (p = 0.004; Fig. 3A). Despite the alteration in Polβ and XRCC1 protein levels are associated with several pathological outcomes, these proteins have no influence in disease-free survival of CRC patients (p = 0.674 and p = 0.641 respectively; Fig. 3B and D). Finally, Fen1 protein expression, also do not present prognostic in this analysis (p = 0.781; Fig. 3C)

4. Discussion

In human cells, while coordinated BER pathway avoids unnecessary toxic intermediate formation, such as AP sites and single strand breaks [21], imbalances are responsible for a wide range of cellular fates [22]. For example, when a DNA glycosylase (such as MPG) is inhibited, it may block BER initiation, creating an environment prone to the accumulation of both cytotoxic [23] and mutagenic base lesions [24],



Variable		MPG Low (n=30)	MPG High (n=29)	MPG *p value*	Polβ Low (n=37)	Polβ High (n=22)	Polβ *p value*	XRCC1 Low (n=24)	XRCC1 High (n=35)	XRCC1 *p value*	Fen1 Low (n=30)	Fen1 High (n=29)	Fen1 *p value*
Sidedness	RCRC (19)	5	14	**0.009**	13	6	**0.03**	8	11	0.877	12	7	0.192
	LCRC (40)	25	15		24	16		16	24		18	22	

Fig. 3. Right-sided colorectal tumors present overexpression on MPG and Polβ. A: Heat map presenting the differences in BER components protein expression in right- and left-sided CRC (RCRC and LCRC, respectively). Blue: low expression; Red: high expression. B: Associations between BER components protein expression and sidedness. Chi-square ($\chi 2$) test and Fisher's exact test. P < 0.05. Significant p values are highlighted in bold font. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

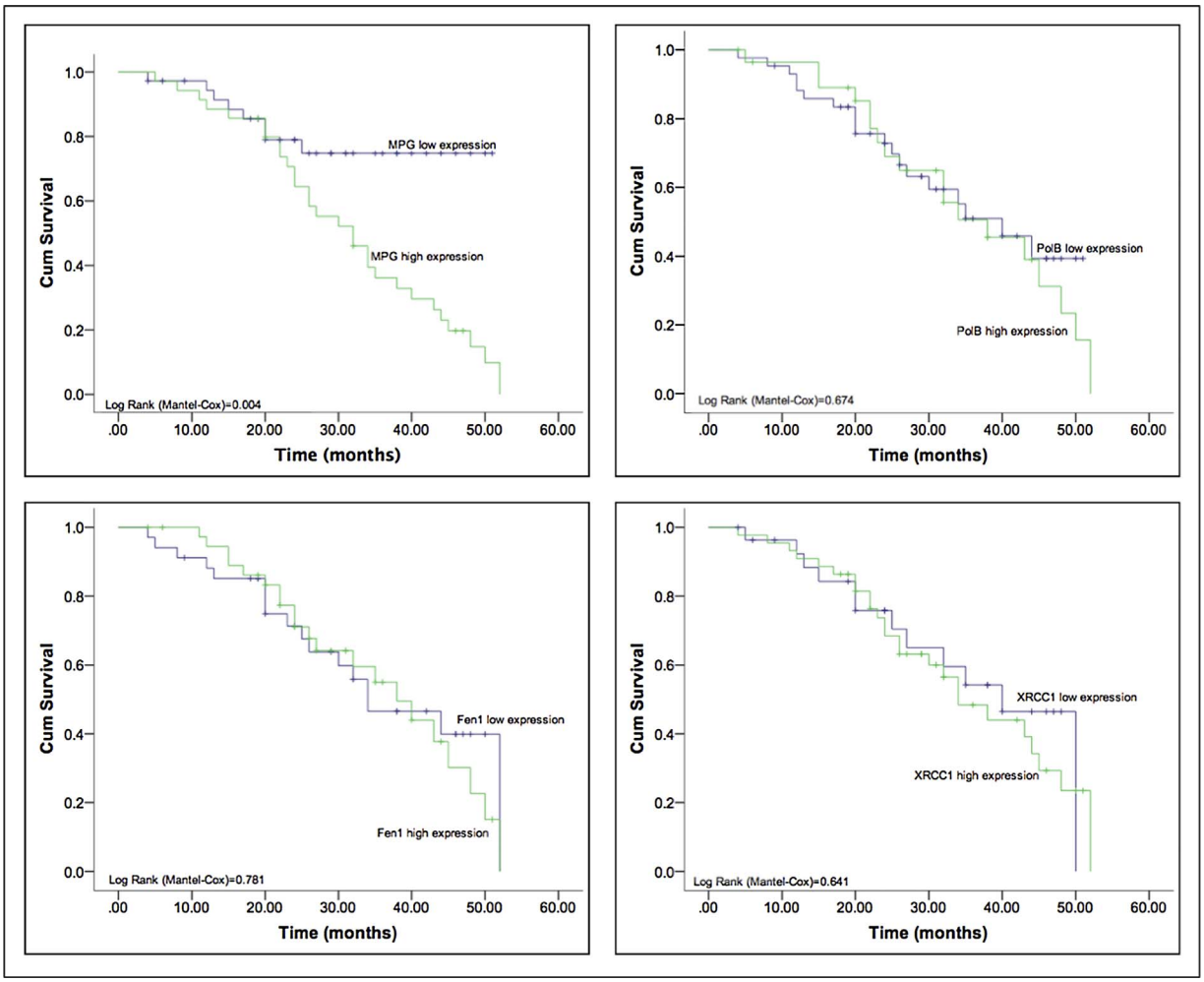


Fig. 4. Disease-free survival in colorectal cancer (CRC) patients according to BER expression profiles. Kaplan-Meier survival analyses of disease-free survival in CRC patients. A: High/low MPG; B: High/low Fen1; C: High/low Polβ; D: High/low XRCC1.

leading to a considerable level of cellular dysfunction. In contrast, once BER has initiated, overexpression of Polβ increases spontaneous mutagenesis [25] and the deficiency of XRCC1 impedes the single strand breaks repair, due to inefficient DNA termini clean up and nick ligation [26].

We found that increased immunohistochemical expression of MPG was associated with poor histopathological features. Additionally, the patients with high expression of MPG were the ones with shorter disease-free survival. BER can be initiated by MPG DNA glycosylase, which recognizes and removes a broad spectrum of alkylated bases [27]. Overexpression of MPG together with an imbalance of the other BER enzymes causes accumulation of AP sites [28], which are lethal to the cell if the subsequent proteins are not sufficient. On the other hand, the loss or down-regulation of MPG also results in an accumulation of unpaired N^3-methyladenine residues, resulting in stalled DNA replication and cell death [29]. Interestingly, we also found that MPG and Polβ were co-expressed, which points out to a greater BER pathway disruption due to an accumulation toxic intermediates and increased spontaneous mutagenesis.

Polβ is the main DNA polymerase involved in BER and has been shown to be overexpressed in a variety of human tumors [30]. In our study, we found that the higher is the expression of Polβ, the poorer is the pathological prognostic. Low activity of Polβ induces genomic instability and cellular transformation [31] and is one of the main factors for BER failure [32] and a potential driver for cell death via a non-apoptotic pathway [33]. In addition, we also found overexpression of XRCC1 and it was associated with poor clinical outcomes. XRCC1 complexes with Polβ to facilitate BER, and the disruption of this complex may inhibit the Polβ-mediated BER [34], converging to an accumulation of base lesions and non-processed abasic sites, which are lethally toxic.

To the best of our knowledge, we reported here for the first time the association between the differences in BER proteins expression in relation to CRC sidedness. Patients with right-sided CRCs are more likely to be female, older, and have mucinous, undifferentiated histology and higher microsatellite instability compared with those with left-sided CRCs [35]. In our study, we found that MPG and Polβ were overexpressed in RCRC, fact associated with features of tumor aggressiveness, as mentioned above. Currently, regarding DNA repair pathways and sidedness, mismatch repair (MMR) status proficiency in RCRC is an indicative of shorter survival [36,37]. Previously results of our group showed that CRC patients with MMR deficiency presented higher levels of MPG gene expression [38]. However, further investigation is warranted to depict why this association occurs and if it really has prognostic value.

We did not find any association of Fen1 expression and clinical features. A tumor suppressor function for Fen1 has been shown in preclinical models, indicating its involvement on carcinogenesis [39], since the Fen1 over expression may promote cancer progression and survival [40]. However, the high expression of Fen1 has been mainly observed in terms of therapeutic response in several cancer types, essentially because the efficacy of these therapeutic agents such as platinum drugs and alkylating agents [41] can be significantly reduced by the ability of cells to repair their DNA. It is possible that the use of Fen1

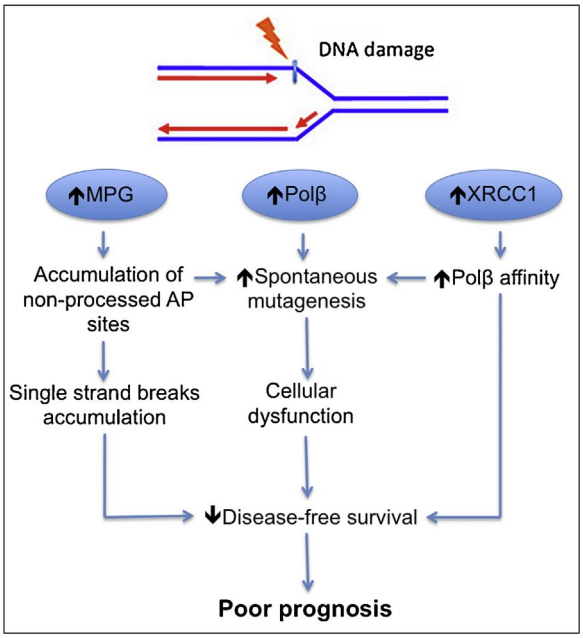


Fig. 5. Model depicting the possible mechanism by which the high expression of BER proteins in colorectal adenocarcinoma is associated with unfavorable clinicopathological outcome.

as a biomarker may be more helpful on predicting chemotherapy success than disease prognosis.

Recent findings point out to the involvement of nuclear APC in the regulation of DNA repair [42,43], which happens through the blockage of BER pathway. It has been reported that APC is capable of a direct interaction with Polβ and Fen1 [44]. Because of these interactions, APC blocks the entire LP-BER.

In our study, APC expression occurred in only 22% of primary tumors. Similarly, another study reported loss of APC expression in 83% of colon cancers [45]. Polβ and APC expression presented an inversed relation, corroborating the *in vitro* data of Polβ blockage by APC. The importance of this result might be applied in the therapeutic field of CCR. Since alkylating agents can increase de APC gene expression and, therefore, fortify the BER blockage, the neoplastic cells can be driven to an apoptotic pathway.

Finally, in Fig. 5, we present a model to depict the possible mechanism by which the elevated expression of BER proteins MPG, Polβ and XRCC1 in colorectal adenocarcinoma is associated with an unfavorable clinicopathological outcome. We believe that an overexpression of MPG and Polβ leads to an incompetence of BER to repair accumulated abasic sites and single strand breaks. A consequent persistence of these toxic intermediates at the site of damage together with an unbearable level of spontaneous mutagenesis will drive the cell to a carcinogenic process. Since BER is an error free DNA repair pathway, the acquired mutations might be preserved and, by evading apoptosis, tumor cells with more aggressive profile will lead to a poor prognosis to the patient, clinically represented by the reduction of disease free survival. These facts allow us to believe that the new therapies should target the inhibition of BER based on its gene and protein expression profile on colorectal tumors.

5. Conclusions

Our study revealed that the presence of alteration on BER proteins expression is associated with clinical and pathological features in colorectal cancer. More specifically, we demonstrated the prognostic significance of high expression of MPG, Polβ and XRCC1, and, for the first time, the association of MPG high expression with shorter disease-free survival in these patients, pointing out to the use of these proteins on the refinement of the current TNM staging. However, further large-scale clinical studies are needed to precisely determine the associations between candidate biomarkers, taking in account the tumor molecular heterogeneity and response to chemotherapy.

Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author's contribution

DBA: study design and conception, material and clinical data collection (operated on all of the patients), interpretation of the data and writing the manuscript. NML: study design and conception, IHQ and systematic review execution and analysis, writing the manuscript. HCG: IHQ and systematic execution and analysis. ANK, ER and GAL: clinical data collection and analysis. JS: supervision, interpretation of data and writing the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.prp.2017.11.012.

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