Received: 24 September 2019 | Accepted: 8 October 2019

DOI: 10.1002/jso.25737

Journal of SURGICAL ONCOLOGY WILEY

RESEARCH ARTICLE

The role of double-strand break repair, translesion synthesis, and interstrand crosslinks in colorectal cancer progression—clinicopathological data and survival

Gustavo A. Laporte MD, PhD[1,2] | Natália M. Leguisamo MD, PhD[2,3] | Helena de Castro e Gloria BS[2] | Daniel B. Azambuja MD[4] | Antonio N. Kalil MD, PhD[1] | Jenifer Saffi MD, PhD[2]

[1]Division of Surgical Oncology, Santa Rita Hospital/ISCMPA, Porto Alegre, Brazil

[2]Laboratory of Genetic Toxicology, Federal University of Health Sciences of Porto Alegre/ UFCSPA, Porto Alegre, Rio Grande do Sul, Brazil

[3]Institute of Cardiology of Rio Grande do Sul, University Foundation of Cardiology, Porto Alegre, Brazil

[4]Division of Coloproctology, ISCMPA, Porto Alegre, Brazil

Correspondence
Gustavo A. Laporte, Division of Surgical Oncology, Santa Rita Hospital, ISCMPA, Porto Alegre, RS 92990000, Brazil.
Email: laportegustavo@gmail.com

Funding information
Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior – CAPES, Conselho Nacional de Pesquisa – CNPq; Fundação de Amparo à Pesquisa do Rio Grande do Sul - FAPERGS

Abstract

Background and Objectives: DNA repair is a new and important pathway that explains colorectal carcinogenesis. This study will evaluate the prognostic value of molecular modulation of double-strand break repair (*XRCC2* and *XRCC5*); DNA damage tolerance/translesion synthesis (*POLH*, *POLK*, and *POLQ*), and interstrand crosslink repair (*DCLRE1A*) in sporadic colorectal cancer (CRC).

Methods: Tumor specimens and matched healthy mucosal tissues from 47 patients with CRC who underwent surgery were assessed for gene expression of *XRCC2*, *XRCC5*, *POLH*, *POLK*, *POLQ*, and *DCLRE1A*; protein expression of Polk, Ku80, p53, Ki67, and mismatch repair MLH1 and MSH2 components; CpG island promoter methylation of *XRCC5*, *POLH*, *POLK*, *POLQ*, and *DCLRE1A* was performed.

Results: Neoplastic tissues exhibited induction of *POLK* ($P < .001$) and *DCLRE1A* ($P < .001$) expression and low expression of *POLH* ($P < .001$) and *POLQ* ($P < .001$) in comparison to healthy paired mucosa. Low expression of *POLH* was associated with mucinous histology and T1-T2 tumors ($P = .038$); low tumor expression of *POLK* was associated with distant metastases ($P = .042$). CRC harboring *POLK* promoter methylation exhibited better disease-free survival (DFS) ($P = .005$).

Conclusions: This study demonstrated that low expression or unmethylated POLH and POLK were related to worse biological behavior tumors. However, POLK methylation was associated with better DFS. POLK and POLH are potential prognostic biomarkers in CRC.

KEYWORDS
colorectal cancer, DNA damage response, prognostic biomarkers

1 | INTRODUCTION

Colorectal cancer (CRC) is considered the third major cause of cancer-related deaths worldwide.[1-3] Survival rates and therapeutic decisions for CRC patients depend on pathology-related staging following the tumor-node-metastasis (TNM) classification.[4] However, despite modifications to improve prognostic staging, this algorithm still fails to predict recurrence and survival after resection for stage II and III CRC patients, resulting in heterogeneous and controversial oncological outcomes.[5]

Gustavo A. Laporte and Natália M. Leguisamo are co-first authors and have contributed equally to this study.

In the pursuit of eliminating TNM inconsistencies, CRC molecular complexity and its heterogeneous clinical presentations have been leading to the research of novel prognostic and predictive biomarkers, including DNA repair components. For example, 15% of sporadic CRC patients who harbor DNA mismatch repair (MMR) system defects and, consequently, microsatellite instability (MSI),[6] have better stage-adjusted survival and reduced likelihood of metastasis when compared with microsatellite stable tumors.[7,8] However, MSI has several limitations that restrict its use as a practical prognostic factor across all stages of CRC, as its clinical value is restricted to stage II CRC, where adjuvant chemotherapy is not recommended.[9]

Nevertheless, associations of DNA damage and imbalances in other pathways engaged in their repair with CRC risk, progression, response to therapy and prognosis have been widely reported. We and others recently reported that disturbances in gene and/or protein expression of DNA damage response sensors and effectors—including double-strand break repair (DSBR), DNA damage tolerance/translesion synthesis (DDT/TLS) and interstrand crosslink repair (ICLR) pathways—have minimal association with clinicopathological features and response to therapy in CRC.[10,11] Despite the lack of definitive evidence so far, a plethora of reports have been suggesting an intersection between CRC and DNA repair systems, which may be mediated by MMR defects (by inducing other somatic mutations that disrupt DNA repair mechanisms) or not.[12]

Double-strand breaks (DSBs) are the most critical type of genotoxic stress and their repair is a central cellular mechanism to preserve genomic stability.[13] DSBs are processed by homologous recombination or classical nonhomologous end-joining DNA repair pathways, and disruptions of these pathways favor the accumulation of damage in rapidly dividing cells, leading to mutagenesis or apoptosis.[14] Since DSBs result in the loss of integrity of both complementary strands, proficiency of error-prone repair is required. However, loss of genetic information and genomic instability is an immediate consequence to guarantee cell survival. DDT mechanisms are mediated by Y-family translesion DNA polymerases (such as pol κ, pol η, and pol θ), which bypass DNA adducts, imbalanced dNTP pools, and unusual template structures. As a consequence, to impede fork collapse and apoptosis due to unrepaired DSB, translesion DNA polymerases induce mutation.[15,16] So far, although a number of investigations have focused on the role of MMR, NER, and BER genes in CRC, fewer studies have evaluated DSBR, DDT/TLS, and ICLR roles from the perspective of expression characteristics and prognostic roles in CRC.[10,17-20]

Thus, since tumor heterogeneity and genomic instability are hallmarks of CRC, to pinpoint a role for DSBR, DDT/TLS and ICLR may offer a better understanding of these features. Finally, alterations in gene/protein expression within DSBR, DDT/TLS, and ICL components could affect the response to chemotherapy and, ultimately, the overall survival (OS) of these patients. Thus, we aimed to evaluate the prognostic role of molecular modulation of key DSBR, DDT/TLS, and ICLR components in sporadic CRC patients.

2 | MATERIAL AND METHODS

2.1 | Patients

A total of 47 CRC patients who underwent surgical treatment between 2013 and 2015 at Irmandade Santa Casa de Misericórdia de Porto Alegre Hospital were included in this

TABLE 1 Clinicopathological features of patients with CRC included in this study (n = 47)

Variable	n (%)
Total cases	47
Age (mean ± SD)	67.77 ± 11.49
Age, y	
≤65	19 (40.4)
>65	28 (59.6)
Gender	
Female	28 (59.6)
Male	19 (40.4)
Preoperative CEA, ng/mL	
≤5	25 (53.2)
>5	22 (46.8)
Tumor location	
Right side	17 (36.1)
Left side	30 (63.9)
Histology	
Well or moderately differentiated	19 (40.4)
Poorly differentiated	28 (59.6)
Mucinous	
No	43 (91.5)
Yes	4 (8.5)
Tumor invasive depth	
1-2	12 (25.5)
3-4	35 (74.5)
Lymph node status	
N−	24 (51.1)
N+	23 (48.9)
Vascular metastasis	
No	40 (85.1)
Yes	7 (14.9)
Lymph vascular invasion	
No	23 (48.9)
Yes	24 (51.1)
Perineural invasion	
No	20 (42.6)
Yes	27 (54.4)
Chemotherapy	
No	21(45.7)
Yes	25(54.3)
TNM stage	
I-II	23 (48.9)
III-IV	24 (51.1)
Relapse	
No	32^{80}
Yes	6^{20}

Abbreviations: CEA, carcinoembryonic antigen; CRC, colorectal cancer; TNM, tumor-node-metastasis.

study. Patients who had received neoadjuvant treatment and with a family history of hereditary CRC were excluded. Clinical data for each patient comprised age, sex, preoperative carcinoembryonic antigen (CEA) levels and the chemotherapy regimen completed. The pathological data comprised tumor site, histology, tumor grade, presence of lymph vascular, and perineural invasion and staging (according to 8th edition of AJCC/UICC).[21]

2.2 | Tumor samples

Fresh tissue specimens comprising tumor tissues (with at least 70% of neoplastic cells) and adjacent normal tumor-free regions (>10 cm distance from the tumor) of primary sporadic CRC were collected and assessed for gene expression, gene promoter methylation, and $BRAF^{V600}$ mutation status. Formalin-fixed paraffin-embedded CRC samples were used for protein expression.

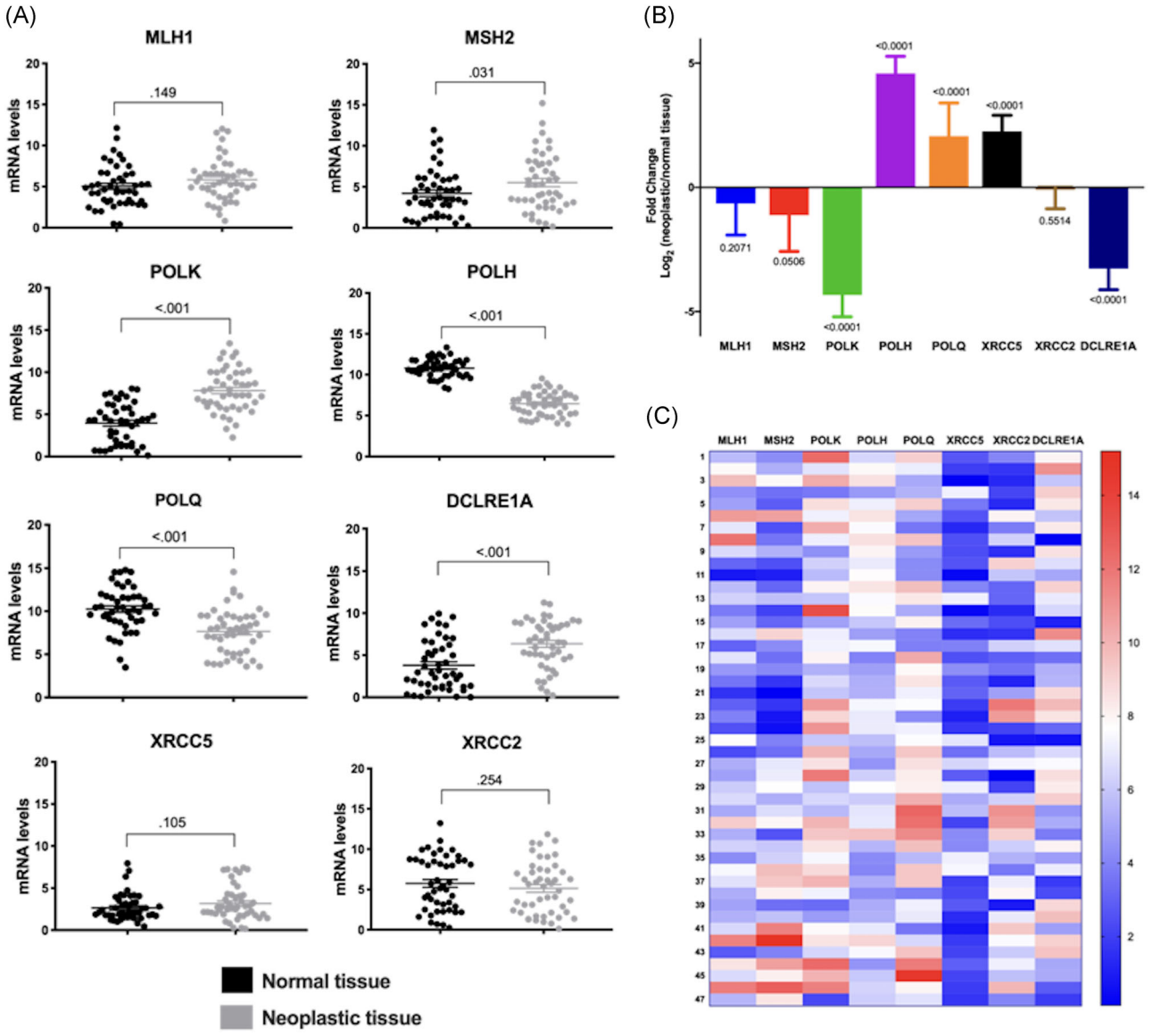

FIGURE 1 Molecular changes in DSBR, ICLR, and DDT/TLS compared colonic normal tissue and CRC tumors. A, Gene expression was quantified for a panel of genes by real-time qPCR analysis in neoplastic and normal mucosal tissues from 47 patients with sporadic colorectal cancer. The following genes were examined: MLH1, MSH2, POLK, POLH, POLQ, XRCC2, XRCC2, and DCLRE1A. Gene expression data are shown as scatter diagrams. B, Fold change between neoplastic and normal tissue quantified real-time qPCR analysis. C, A heat map of individual gene expression changes in sporadic colorectal cancer. Fold changes were calculated for neoplastic tissue vs adjacent normal tissue. Blue indicates decreased relative gene expression, red indicates increased relative gene expression and white indicates no change in gene expression. Gene expression means between normal and neoplastic tissue were compared using independent sample *t* Student or Mann-Whitney tests after Kolmogorov-Smirnov tests. CRC, colorectal cancer; DDT/TLS, DNA damage tolerance/translesion synthesis; DSBR, double-strand break repair; qPCR, quantitative polymerase chain reaction [Color figure can be viewed at wileyonlinelibrary.com]

2.3 | Quantitative reverse transcription-polymerase chain reaction

Gene expression of *XRCC2* and *XRCC5* (DSBR), *POLH*, *POLK*, and *POLQ* (DDT/TLS), *DCLRE1A* (ICLR), and *MLH1* and *MSH2* (MMR) were carried out in colorectal tumors and healthy paired tissues by RT2 Profiler PCR Array (SABiosciences, Qiagen). RNA extraction and cDNA synthesis were performed using RNeasy Mini Kit and RT2 PCR Array First Strand Kit (SABiosciences, Qiagen), respectively. Cataloged polymerase chain reaction (PCR) primers were used. Reaction was prepared using RT2 SYBR Green/Rox PCR Master Mix (SABiosciences, Qiagen). Data analysis was based on the $2^{-\Delta\Delta C_q}$ method (Livak et al, 2001) with normalization of raw data to two housekeeping genes (*EIF2B* and *PPIA*). Median fold change ($\log_2$(neoplastic tissue/normal tissue)) for each gene was used to categorize tumors into high or low expressors.

2.4 | Methylation PCR analysis

The methylation status of CpG islands of five genes (*XRCC5*, *POLH*, *POLK*, and *DCLRE1A*) was performed by methylation-sensitive restriction qPCR analysis using EpiTect Methyl II PCR assay (SABiosciences, Qiagen). Digested DNA was obtained with EpiTect Methyl II DNA restriction kit (#335452; SABiosciences, Qiagen) and used as a template for qPCR Assay using RT2 SYBR Green qPCR Mastermix (SABiosciences, Qiagen) under standard amplification conditions. Cataloged Epitect II Methyl PCR primers used were as follows: *POLH* (EPHS5112501-1A); *POLK* (EPHS511608-1A); *XRCC5* (EPHS108851-1A); and *DCLRE1A* (EPHS101928-1A) which were all purchased from Qiagen. Gene promoter methylation status was classified into unmethylated (<5%) and methylated (>5%).

2.5 | Immunohistochemistry

Immunohistochemistry for MLH1, MSH2, XRCC5 (Ku80), Polκ, p53, and ki67 was carried out according to MacDonald et al.[22] The sections were incubated with the following primary antibodies, all purchased from Abcam: anti-MLH1 (1:100), anti-MSH2 (1:200), anti-XRCC5 (1:200), anti-DNA Polymerase Kappa (1:300), anti-p53 (1:250), and anti-Ki67 (1:100) and then incubated with appropriate secondary antibodies (Spring). Diaminobenzidine was used as chromogen and the sections were counterstained with hematoxylin. Five hot spot fields containing at least 200 cells were captured and the positive cells were counted using the ImageJ software (National Institutes of Health, Bethesda, MD). Protein expression was evaluated using QuickScore and two observers scored all samples independently and blinded.[22]

2.6 | $BRAF^{V600E}$ mutation analysis

The exon 15 of the *BRAF* gene was amplified by polymerase chain reaction through Platinum Taq DNA Polymerase Kit (Invitrogen by Life Technologies) and appropriate primer pair: forward 5′-CTTC ATAATGCTTGCTCTGATAGGA-3' and reverse 5'-CAGGGCCAAAAA TTTAATCAGTGGA-3'. Sanger sequencing reaction was performed with the BigDye Terminator V3.1 Cycle Sequencing Kit (Life Technologies).

2.7 | Statistical analysis

Gene expression means between normal and neoplastic tissue were compared using independent sample *t* Student or Mann-Whitney tests after Kolmogorov-Smirnov tests. For correlation and survival analyses, continuous variables were dichotomized as previously stated. The association between molecular and clinical features was assessed by χ^2 test and Fisher's exact test. Kaplan-Meier analysis, with logrank test, was used to determine the OS and disease-free survival (DFS). Cox regression analysis for independent correlation of individual parameters with patients' OS and DFS. Statistical analysis was performed using SPSS software version 22.0.0. A two-sided test with $P < 0.05$ was considered statistically significant.

2.8 | Availability of data and materials

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

3 | RESULTS

3.1 | Characteristics of CRC patients

The main patient characteristics are shown in Table 1. A total of 47 patients were included in the final statistical analysis.

TABLE 2 Protein levels (Polκ, Ku80, Mlh1, Msh2, Ki67, and p53), methylation (*POLH*, *POLK*, *XRCC5*, and *DCLRE1A*) and BRAF mutation in neoplastic tissue

Variable Methylation	n (%) Unmethylated	n (%) Methylated Methylated
POLH	20 (57.1)	15 (42.9)
POLK	19 (52.8)	17 (47.2)
XRRC5	25 (67.5)	12 (32.5)
DCLRE1A	17 (58.6)	12 (41.4)
IHC	**Low**	**High**
XRCC5/Ku80	22 (46.8)	25 (53.2)
Polκ	21 (44.6)	26 (55.4)
MLH1	7 (14.9)	40 (85.1)
MSH2	6 (12.7)	41 (87.3)
p53	32 (68.1)	15 (31.9)
Ki67	7 (14.9)	40 (85.1)
	Wild	**Mutated**
BRAF	44 (93.6)	3 (6.4)

TABLE 3 Correlations between DNA gene repair expression of *POLH*, *POLK*, *POLQ*, *XRCC*, and *XRCC5*, methylation of *POLH*, *POLK*, *XRCC5*, and *DCLRE1A* and IHC of XRCC5, Pol ϰ, MLH1, MHS2, p53, and Ki67 scores with clinical parameters

	Gene expression						Methylation				IHC					
Variable	*POLH*	*POLK*	*POLQ*	*XRCC2*	*XRCC5*	*DCLRE1A*	*POLH* (n = 36)	POLK (n = 37)	XRCC5 (n = 37)	DCLRE1A (n = 32)	XRCC5	Pol k	MLH1	MSH2	p53	Ki67
Age, y	0.548	0.095	0.452	0.452	0.143	0.318	0.347	0.419	0.176	0.261	0.408	0.115	0.285	0.169	0.363	0.6
Gender	0.318	0.143	0.452	0.095	0.452	0.238	0.224	0.603	0.228	0.314	0.169	0.117	0.6	0.535	0.16	0.285
CEA, ng/mL	0.075	0.340	0.340	0.580	0.580	0.207	0.127	0.175	0.165	0.602	0.503	0.413	0.447	0.092	0.393	**0.042**
Tumor location	0.544	0.092	0.310	0.237	0.310	0.544	0.205	0.627	0.102	0.398	0.391	0.123	0.499	0.372	0.241	0.499
Histology	0.452	0.548	0.548	0.318	0.548	0.548	**0.023**	0.341	0.051	0.630	0.169	0.117	0.133	0.209	0.363	0.4
Mucinous	**0.050**	0.288	0.679	0.288	0.679	0.679	0.457	0.562	0.704	0.452	0.257	0.61	0.512	0.568	0.381	0.488
T	**0.038**	0.402	0.402	0.337	0.598	0.337	0.168	**0.011**	0.311	0.579	0.228	0.104	0.417	0.151	0.415	0.243
N	0.557	0.443	0.234	0.095	0.443	0.557	0.500	0.209	0.243	0.149	0.562	0.448	0.525	0.646	0.46	0.525
M	0.525	**0.042**	0.190	0.190	0.475	0.525	0.251	0.072	0.350	0.650	0.426	0.377	0.057	**0.035**	0.054	0.296
Lymph vascular invasion	0.557	0.443	0.443	0.095	0.443	0.557	0.253	0.324	0.121	0.252	0.438	0.237	0.525	0.646	0.234	0.525
Perineural invasion	0.433	0.337	0.567	0.337	0.337	0.433	0.485	0.286	0.401	0.615	0.467	0.064	0.648	0.201	0.532	0.352
Chemotherapy	0.194	0.18	0.374	0.607	0.374	0.5	0.163	0.132	0.502	0.37	0.165	0.48	0.601	0.422	0.198	0.399
TNM stage	0.230	0.562	0.155	0.334	0.155	0.562	0.363	0.121	0.407	0.252	0.241	0.241	0.574	0.397	0.381	0.574

Note: Associations of DNA repair gene expression, methylation, and IHC with clinical parameters were evaluated using χ^2 test and Fisher's exact test. Statistically significant are highlighted ($P < .05$).
Abbreviations: CEA, carcinoembryonic antigen; IHC, immunohistochemistry; M, metastase; N, lymph node; T, tumor; TNM, tumor-node-metastasis.

TABLE 4 Correlations between DNA repair gene expression, methylation, and IHC with BRAF mutation and IHC for MLH1, MSH2, p53 and Ki67.

	Gene expression						Methylation				IHC	
Variable	POLH	POLK	POLQ	XRCC2	XRCC5	DCLRE1A	PolH (n = 36)	PolK (n = 37)	XRCC5 (n = 37)	DCLRE1A (n = 32)	XRCC5	Pol k
BRAF	0.484	0.19	0.484	0.109	0.125	0.125	0.271	0.438	0.704	0.726	0.082	0.549
MLH1	0.226	**0.042**	0.475	0.226	0.525	0.226	0.386	0.13	0.47	0.65	0.129	0.265
MSH2	0.646	0.085	0.312	0.646	0.646	0.354	0.543	0.58	0.609	0.212	0.235	0.603
p53	0.124	0.3	0.54	0.54	0.234	0.46	0.564	0.627	0.609	0.267	0.451	0.177
Ki67	0.19	0.525	0.226	0.475	0.19	**0.042**	0.655	**0.036**	0.609	0.452	0.623	0.574

Note: The data were evaluated using χ^2 test and Fisher's exact test. Statistically significant are highlighted ($P < .05$).
Abbreviation: IHC, immunohistochemistry.

3.2 | Molecular changes in DSBR, ICLR, and DDT/TLS in CRC tumors

MSH2 ($P = .031$), *POLK* ($P < .001$), and *DCLRE1A* ($P < .001$) were overexpressed, while mean gene expression of *POLH* ($P < .001$) and *POLQ* ($P < .001$) were found reduced in neoplastic tissues in comparison to healthy paired mucosa (Figures 1A and 1C). Only *XRCC2* and MMR repair genes were considered normally expressed. *POLH*, *POLQ*, and *XRCC5* presented a mean 4.34-, 2.61-, and 1.74-fold expression induction, respectively. Conversely, *POLK* and *DCLRE1A* exhibited a 3.72- and 3.2-fold expression reduction, respectively (Figure 1B,C).

In neoplastic tissue, nearly 85% of patients presented high protein levels of MLH1 and/or MSH2. Yet, 15% showed absent or low levels of MLH1 or MSH2 proteins. Polκ and Ku80 levels were high in 55% of patients. Regarding proliferation markers expression, 68% of CRC patients presented low p53 levels and 85% of those same patients revealed high Ki67 expression (Table 2).

Low *XRCC5* gene expression was associated with promoter methylation ($P = .015$) and low XRCC5 (Ku80) protein expression ($P = .0001$). *POLK* overexpression was associated with high corresponding protein contents ($P = .0001$), but not with the absence of promoter methylation ($P = .581$) (Table S1). Promoter methylation and gene expression of *POLH* and *DCLRE1A* were not associated (data not shown).

3.3 | Associations of DSBR, ICLR, and DDT/TLS key components with clinicopathological and molecular features of CRC patients

Tumors with low expression of *POLH* exhibited mucinous histology ($P = .05$), but smaller invasive depth ($P = 0.038$). Low tumor expression of *POLK* was associated with the presence of distant metastases ($P = 0.042$). Promoter methylation of *POLK* was associated with smaller invasive depth ($P = .011$) and methylation of *POLH* to well-differentiated tumors (.023). In addition, *POLK* promoter methylation was associated with tumors with high Ki67 contents ($P = .036$) and low expression of DCLRE1A was associated with tumors with low Ki67 contents ($P = .042$) (Table 3). Overexpression of *POLK* was associated with tumors expressing MLH1 ($P = .042$) (Tables S2, S3, and S4). High tumor protein expression of MSH2 was associated with the absence of distant metastases ($P = .035$), while overexpression of Ki67 with lower preoperative CEA levels ($P = .042$) (Table 4). More detailed associations between clinicopathological features and molecular data are provided in Tables S5, S6, and S7.

3.4 | Prognostic value of DNA repair component modulation in patients with CRC

Kaplan-Meier's survival analyses indicated that patients whose tumors harbored *POLK* promoter methylation had better DFS ($P = .005$). Statistical tendencies were found for *POLK* promoter methylation and better OS ($P = .053$); overexpression of *POLQ* and better OS ($P = .076$) and DFS ($P = .068$); overexpression of XRCC5 expression and better survival ($P = .057$) (Figure 2). Other survival analyses are provided in Figures S1, S2, S3, S4, S5, and S6.

Univariate Cox regression analysis showed the prognostic significance of N+, M+, lymph vascular invasion, perineural invasion, stages III and IV, low tumor *POLQ* gene expression, tumor-unmethylated *POLK* gene promoter, and high XRCC5/Ku80 protein expression on OS. Unfortunately, these associations were not confirmed in our multivariate analysis (Table 5). For DFS, univariate analysis showed that male, preoperative CEA >5 ng/mL, N+, lymph vascular invasion, perineural invasion, chemotherapy realized stages III, low expression of *POLQ*, unmethylated *POLK* promoter and low or absent MSH2 protein expression were predictors of poor DFS, but not confirmed in multivariate analysis (Table 6).

4 | DISCUSSION

A growing body of evidence has been strengthening the need for more accurate tools to minimize the inconsistencies of the TNM staging system as prognostic and therapeutic guidance for CRC patients. Contribution of aberrant DNA repair and DNA damage response in carcinogenesis and its response to treatments has been well established. Furthermore, the study of DNA repair components as oncological molecular markers has already reached clinical practice, including *MGMT* promoter methylation status

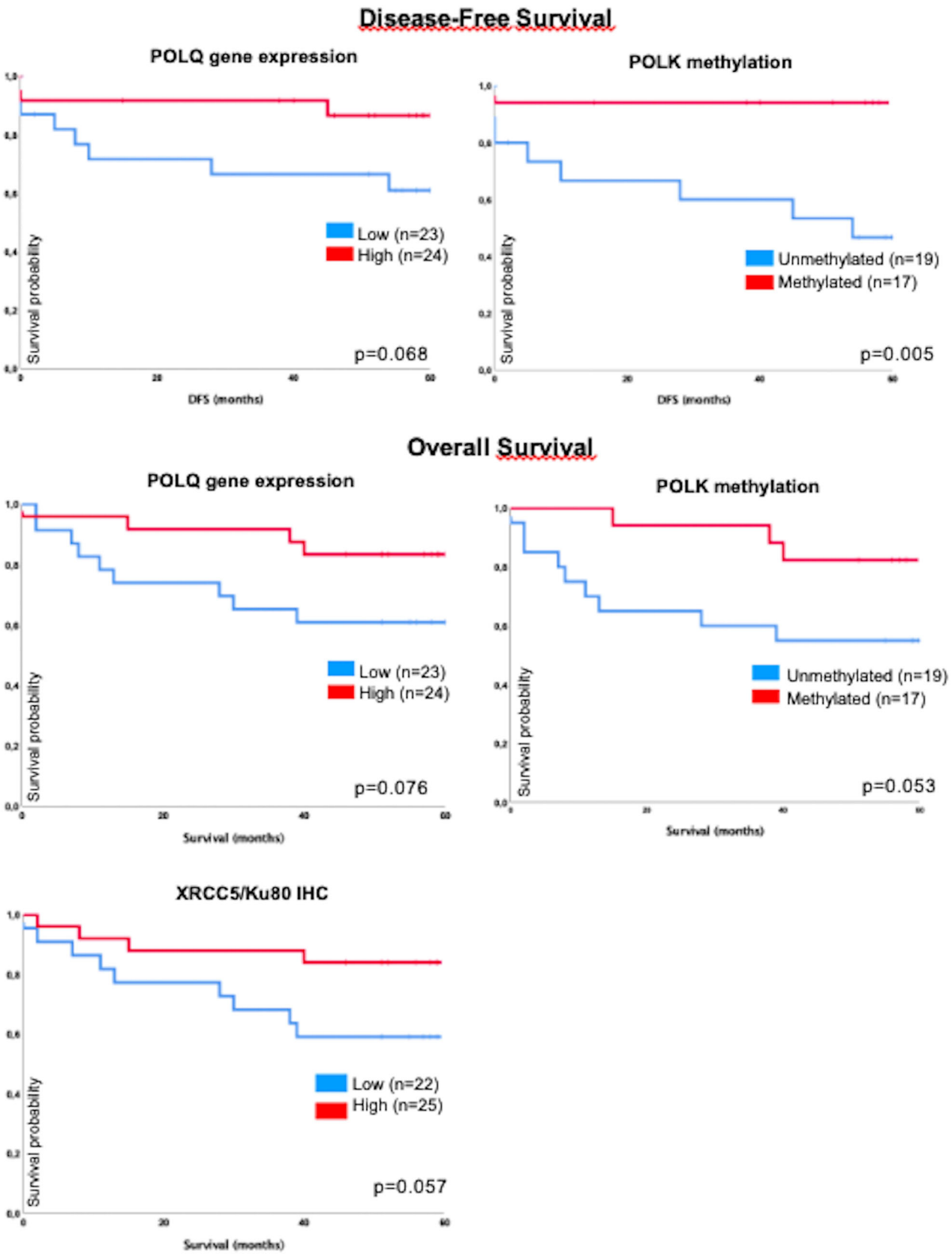

FIGURE 2 Overall and disease-free survival for POLQ gene expression, POLK methylation and IHC for XRCC5. The data were evaluated with the Kaplan-Meier test. IHC, immunohistochemistry [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 5 Overall survival calculated with univariate and multivariate cox regression tests

	Univariate analysis			Multivariate analysis		
Variable	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
Age, >65 y	1.708	(0.526-5.547)	.373			
Sex, male	1.917	(0.644-5.71)	.242			
CEA, >5	1.234	(0.404-3.774)	.712			
Left side	1.309	(0.403-4.253)	.654			
Poor differenciated	1.534	(0.472-4.982)	.477			
Mucinous	2.081	(0.461-9.394)	.341			
T3-T4	5.034	(0.654-38.764)	.121			
N+	4.021	(1.103-14.654)	.035	1.983	(0.176-22.359)	.58
M+	3.059	(0.938-9.976)	.064	1.63	(0.443-6.004)	.462
Lymph vascular invasion	4.021	(1.103-14.654)	.035	1.394	(0.194-10.006)	.741
Perineural invasion	3.582	(1.099-11.673)	.034	2.54	(0.643-10.038)	.184
Chemotherapy	2.911	(0.799-10.598)	.105			
Stage III-IV	4.14	(1.136-15.087)	.031	1.096	(0.144-8.353)	.929
Low Exp *POLH*	1.839	(0.602-5.624)	.285			
Low Exp *POLK*	1.213	(0.407-3.609)	.729			
Low Exp *POLQ*	2.782	(0.855-9.055)	.089	1.254	(0.215-7.33)	.801
High Exp *XRCC2*	1.131	(0.38-3.368)	.825			
Low Exp *XRCC5*	1.738	(0.568-5.32)	.332			
Low Exp *DCRLE1A*	1.616	(0.528-4.944)	.401			
Unmetilated *POLH*	1.134	(0.346-3.718)	.835			
Unmetilated *POLK*	3.363	(0.908-12.46)	.07	1.756	(0.306-10.062)	.451
Unmetilated *XRCC5*	1.533	(0.406-5.786)	.529			
Unmetilated *DCLRE1A*	2.778	(0.717-10.766)	.139			
Pol k IHC Low	1.54	(0.517-4.586)	.438			
XRCC5 IHC Low	2.968	(0.912-9.654)	.071	1.802	(0.376-8.646)	.461
BRAF mutated	1.363	(0.177-10.497)	.766			
MLH1 IHC Low	2.06	(0.566-7.491)	.273			
MSH2 IHC Low	1.266	(0.281-5.715)	.759			
p53 IHC High	1.352	(0.442-4.135)	.597			
Ki67 IHC Low	2.312	(0.301-17.785)	.421			

Abbreviations: CEA, carcinoembryonic antigen; CI, confidence interval; Exp, expression; HR, hazard ratio; IHC, immunohistochemistry; M, metastase; N, lymph node; T, tumor.

(glioblastoma),[23] *BRCA1/2* mutations (breast and ovarian cancer),[24-26] and MMR deficiency (colorectal, endometrial, ovarian, and other cancer types).[27-32]

POLK and *POLH* encode members of DNA polymerase type-Y-family of proteins, Pol κ, and Pol η, respectively. Variations in expression or activity of Y-family DNA polymerases could possibly produce TLS pathway imbalance and, therefore, mutagenesis.[33] However, the magnitude to which these alterations are oncogenic drivers or whether it impacts clinical outcomes is still unknown.

In our study, we found upregulation of *POLK* and downregulation of *POLH* in neoplastic tissues in comparison to paired normal tissues. The oncological relevance of pol κ and pol η in cancer is most firmly established concerning response to treatment. Upregulation of pol κ confers resistance to temozolomide in glioblastoma,[34,35] and upregulation of pol η to platinum drugs in HNSCC, lung, gastric adenocarcinomas, and ovarian cancers.[36-38] Contrary to our results, low levels of *POLK* were previously observed in CRC.[39,40] Conversely, others reported an increase of pol κ expression in brain and lung cancers.[41,42]

Low expression of *POLH* and *POLK* were found in tumors with mucinous histology and vascular metastasis, although in the early stages of development. *POLK* promoter methylation was strongly associated with better DFS. Conversely, unmethylated *POLH* and *POLK* promoters were associated with more advanced and poorly differentiated tumors.

Despite finding more aggressive colorectal tumors harboring high *POLK* levels, this fact was not a predictor of DFS and OS. On the other hand, *POLK* promoter methylation was associated with better

TABLE 6 Disease-free survival calculated with univariate and multivariate cox regression tests

	Univariate analysis			Multivariate analysis		
Variable	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
Age, <65 y	1.121	(0.342-3.677)	.851			
Sex, male	2.878	(0.841-9.851)	.092	2.287	(0.063-82.97)	.652
CEA, >5	5.845	(1.538-22.068)	.009	25.432	(0.258-2510.47)	.258
Right side	1.425	(0.435-4.676)	.559			
Poor differenciated	3.414	(0.736-15.838)	.117			
Mucinous	1.139	(0.145-8.934)	.901			
T3	36.142	(0.157-8313.8)	.196			
N+	6.049	(1.295-28.245)	.022	46.388	(0.083-25970.4)	.235
Lymph vascular invasion	5.587	(1.201-25.998)	.028	15.922	(0.026-9799.2)	.398
Perineural invasion	4.323	(1.141-16.372)	.021	16.76	(0.467-601.995)	.123
Chemotherapy	4.678	(1.003-21.807)	.049	6.629	(0.159-276.673)	.32
Stage III	3.687	(0.971-14.005)	.055	42.077	(0.201-8821.94)	.17
High Exp *POLH*	1.621	(0.474-5.546)	.442			
Low Exp *POLK*	2.05	(0.599-7.014)	.253			
Low Exp *POLQ*	3.151	(0.834-11.906)	.091	1.63	(0.244-10.867)	.629
Low Exp *XRCC2*	1.955	(0.572-6.682)	.285			
Low Exp *XRCC5*	1.936	(0.566-6.625)	.293			
High Exp *DCRLE1A*	1.155	(0.351-3.797)	.812			
Unmetilated *POLH*	1.23	(0.33-4.593)	.758			
Unmetilated *POLK*	10.263	(1.292-81.531)	.028	51.874	(0.221-12164.7)	.156
Unmetilated *XRCC5*	4.438	(0.554-35.552)	.16			
Metilated *DCLRE1A*	2.239	(0.409-12.26)	.353			
Pol K IHC Low	1.581	(0.482-5.183)	.45			
XRCC5 IHC Low	1.532	(0.467-5.031)	.482			
BRAF wild	22.171	(0-1117704.6)	.575			
MLH1 IHC Low	2.966	(0.776-11.334)	.112			
MSH2 IHC Low	3.253	(0.857-12.35)	.083	1.837	(0.343-9.842)	.478
p53 IHC Low	4.888	(0.625-38.213)	.13			
Ki67 IHC Low	26.615	(0.024-29262.2)	.358			

Abbreviations: CEA, carcinoembryonic antigen; CI, confidence interval; Exp, expression; HR, hazard ratio; IHC, immunohistochemistry; M, metastase; N, lymph node; T, tumor.

DFS, but we could not confirm it as an independent prognostic factor. Surprisingly, despite *POLK* gene and protein expression is associated ($P = .001$), such connection was not found between *POLK* expression and promoter methylation. It may indicate that promoter methylation is not the main mechanism regulating *POLK* transcription.

On its turn, *POLQ* (A-family) encodes pol θ DNA polymerase and is a component of an end-joining pathway for DSB. Defects in *POLQ* lead to DSB-mediated genomic instability.[43] Differently from previous reports,[44,45] our patients presented downregulation of *POLQ*, but no association with clinicopathological parameters was detected. Overexpression of pol θ has been implicated as an indicator of poor prognosis and decreased survival in breast, colorectal and NSCLC.[45-47] Nevertheless, to date, *POLQ* overexpression presented a weak association for better OS ($P = .076$) and DFS ($P = .068$).

DSBR (represented in our study by *XRCC2* and *XRCC5*) did not present alterations in gene expression between neoplastic and normal tissues nor associations with clinicopathological variables in CRC patients. To date, low XRCC5/Ku80 expression suggested poor OS in CRC patients included in our study ($P = .057$). XRCC5/Ku80 is associated with the risk of development of several tumors[48,49] and its activity may inhibit or promote the carcinogenic process, depending on the tumor type.[50] In CRC, downregulation of *XRCC5* and/or its protein product (Ku80) was associated with poor prognosis and better response to radiotherapy.[10,51-53] Regarding ICLR, despite *DCLRE1A* being upregulated in neoplastic tissues, it did not present associations with clinical features or survival in this study. *DCLRE1A* encodes SNM1A nuclease, and it has been linked to an important function in human ICLR.[54]

Finally, despite its sample size limitation, to the best of our knowledge, our study is one of the few to report associations between *POLK*, *POLH* modulation and clinical features and prognosis of CRC patients. Furthermore, we believe that this is the first study to evaluate *DCLRE1A* gene expression and promoter methylation in colorectal tumors.

5 | CONCLUSION

Components of the pathways involved in DSBR, DDT/TLS, and ICLR are a new horizon in the DNA repair pathway discussion. There are few reports about these and the influence on clinicopathological features and survival is still a big question. This study revealed that low expression or unmethylated *POLH* and *POLK* were related to worse tumors. In this context, *POLK* methylated was strongly associated with better DFS with a propensity for a better OS. On the other hand, another interesting finding is the high score of XRCC5/Ku80 in IHQ suggests a better survival. Finally, even with little information about these pathways in relation to their clinicopathological influence and survival, this knowledge may help to clarify the utility of specific adjuvant treatments based on the individual's genotype in the future.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ORCID

Gustavo A. Laporte http://orcid.org/0000-0002-5363-1055

REFERENCES

1. Frederiksen BL, Jørgensen T, Brasso K, Holten I, Osler M. Socio-economic position and participation in colorectal cancer screening. *Br J Cancer*. 2010;103(10):1496-1501.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424.
3. Siegel RL, Miller KD, Fedewa SA, et al. Colorectal cancer statistics. *CA Cancer J Clin*. 2017;67(3):177-193. 2017.
4. Amin MB, Greene FL, Edge SB, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA Cancer J Clin*. 2017;67(2):93-99.
5. Huang Y, Kim BYS, Chan CK, Hahn SM, Weissman IL, Jiang W. Improving immune-vascular crosstalk for cancer immunotherapy. *Nat Rev Immunol*. 2018;18(3):195-203.
6. Jang E, Chung DC. Hereditary colon cancer: lynch syndrome. *Gut Liver*. 2010;4(2):151-160.
7. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol*. 2005;23(3):609-618.
8. Malesci A, Laghi L, Bianchi P, et al. Reduced Likelihood of Metastases in Patients with Microsatellite-Unstable Colorectal Cancer. *Clin Cancer Res*. 2007;13(13):3831-3839.
9. NCCN - Colon Cancer. Colon Cancer. NCCN Guidel [Internet]. 2018;1–832. Available from: www.nccn.org
10. Laporte GA, Leguisamo NM, Kalil AN, Saffi J. Clinical importance of DNA repair in sporadic colorectal cancer. *Crit Rev Oncol Hematol*. 2018;126:168-185.
11. Mirza-Aghazadeh-Attari M, Darband SG, Kaviani M, et al. DNA damage response and repair in colorectal cancer: Defects, regulation and therapeutic implications. *DNA Repair*. 2018;69:34-52.
12. Bodor JN, Handorf EA, Feldman R, Hall MJ. Pathogenic somatic mutation (SM) of mismatch repair (MMR) genes and associations with microsatellite instability (MSI), tumor mutational burden (TMB) and SM in other DNA repair pathways in 24,223 tumor genomic profiles. *J Clin Oncol*. 2018;36(15_suppl):1505.
13. Shibata A, Jeggo PA. DNA double-strand break repair in a cellular context. *Clin Oncol*. 2014;26(5):243-249.
14. Jekimovs C, Bolderson E, Suraweera A, Adams M, O'Byrne KJ, Richard DJ. Chemotherapeutic compounds targeting the DNA double-strand break repair pathways: the good, the bad, and the promising. *Front Oncol*. 2014;4(86).
15. Sale JE. Competition, collaboration and coordination - determining how cells bypass DNA damage. *J Cell Sci*. 2012;125(7):1633-1643.
16. Zafar MK, Eoff RL. Translesion DNA synthesis in cancer: molecular mechanisms and therapeutic opportunities. *Chem Res Toxicol*. 2017;30(11):1942-1955.
17. Moreno V. Polymorphisms in genes of nucleotide and base excision repair: risk and prognosis of colorectal cancer. *Clin Cancer Res*. 2006;12(7 Pt 1):2101-2108.
18. Xu K, Song X, Chen Z, Qin C, He Y, Zhan W. XRCC2 promotes colorectal cancer cell growth, regulates cell cycle progression, and apoptosis. *Medicine*. 2014;93(28):e294.
19. Zhang Y, An JH, Liu YX, et al. XRCC2-deficient cells are highly sensitive to 5-fluorouracil in colorectal cancer. *Cell Physiol Biochem*. 2017;43(3):1207-1219.
20. Qin C-J, Song X-M, Chen Z-H, et al. XRCC2 as a predictive biomarker for radioresistance in locally advanced rectal cancer patients undergoing preoperative radiotherapy. *Oncotarget*. 2015;6(31):32193-32204.
21. Cooper C AJCC 8 th edition update *: Colorectal cancer Chapter 20.
22. McDonald JW, Pilgram TK. Nuclear expression of p53, p21 and cyclin D1 is increased in bronchioloalveolar carcinoma. *Histopathology*. 1999;34(5):439-446.
23. Binabaj MM, Bahrami A, ShahidSales S, et al. The prognostic value of MGMT promoter methylation in glioblastoma: A meta-analysis of clinical trials. *J Cell Physiol*. 2018;233(1):378-386.
24. Lee CK, Scott C, Lindeman GJ, et al. Phase 1 trial of olaparib and oral cyclophosphamide in BRCA breast cancer, recurrent BRCA ovarian cancer, non-BRCA triple-negative breast cancer, and non-BRCA ovarian cancer. *Br J Cancer*. 2019;120(3):279-285.
25. Hoskins PJ, Gotlieb WH. Missed therapeutic and prevention opportunities in women with BRCA-mutated epithelial ovarian cancer and their families due to low referral rates for genetic counseling and BRCA testing: a review of the literature. *CA Cancer J Clin*. 2017;67(6):493-506.
26. Baretta Z, Mocellin S, Goldin E, Olopade OI, Huo D. Effect of BRCA germline mutations on breast cancer prognosis. *Medicine*. 2016;95(40):e4975.
27. Kim GP, Colangelo LH, Wieand HS, et al. Prognostic and predictive roles of high-degree microsatellite instability in colon cancer: A National Cancer Institute-national surgical adjuvant breast and bowel project collaborative study. *J Clin Oncol*. 2007;25(7):767-772.
28. Markowitz SD, Bertagnolli MM. Molecular basis of colorectal cancer. *N Engl J Med*. 2009;361:2449-2460.

29. Kim MJ, Jeong S-Y, Choi S, et al. Survival paradox between stage IIB/C (T4N0) and stage IIIA (T1-2N1) colon cancer. *Ann Surg Oncol*. 2015;22(2):505-512.
30. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372(26):2509-2520.
31. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*. 2017;357(6349):409-413.
32. Halvarsson B, Anderson H, Domanska K, Lindmark G, Nilbert M. Clinicopathologic factors identify sporadic mismatch repair–defective colon cancers. *Am J Clin Pathol*. 2008;129(2):238-244.
33. Yang Y, Wang D, Jin L, et al. Prognostic value of the combination of microsatellite instability and BRAF mutation in colorectal cancer. *Cancer Manag Res*. 2018;10:3911-3929.
34. Bostian ACL, Maddukuri L, Reed MR, et al. Kynurenine signaling increases DNA polymerase kappa expression and promotes genomic instability in glioblastoma cells. *Chem Res Toxicol*. 2016;29(1):101-108.
35. Peng C, Chen Z, Wang S, et al. The error-prone DNA polymerase κ promotes temozolomide resistance in glioblastoma through Rad17-dependent activation of ATR-Chk1 signaling. *Cancer Res*. 2016;76(8):2340-2353.
36. Zhou W, Chen Y, Liu X, et al. Expression of DNA translesion synthesis polymerase η in head and neck squamous cell cancer predicts resistance to gemcitabine and cisplatin-based chemotherapy. *PLoS One*. 2013;8(12):e83978.
37. Teng K, Qiu M, Li Z, et al. DNA polymerase η protein expression predicts treatment response and survival of metastatic gastric adenocarcinoma patients treated with oxaliplatin-based chemotherapy. *J Transl Med*. 2010;8:126.
38. Ceppi P, Novello S, Cambieri A, et al. Polymerase mRNA expression predicts survival of non-small cell lung cancer patients treated with platinum-based chemotherapy. *Clin Cancer Res*. 2009;15(3):1039-1045.
39. Lemée F, Bavoux C, Pillaire MJ, et al. Characterization of promoter regulatory elements involved in downexpression of the DNA polymerase κ in colorectal cancer. *Oncogene*. 2007;26(23):3387-3394.
40. Pillaire MJ, Bétous R, Hoffmann JS. Role of DNA polymerase κ in the maintenance of genomic stability. *Mol Cell Oncol*. 2014;1(1):e29902.
41. Wang Y, Seimiya M, Kawamura K, et al. Elevated expression of DNA polymerase κ in human lung cancer is associated with p53 inactivation: Negative regulation of POLK promoter activity by p53. *Int J Oncol*. 2004;25(17):161-165.
42. O-Wang J, Kawamura K, Tada Y, et al. DNA polymerase kappa, implicated in spontaneous and DNA damage-induced mutagenesis, is overexpressed in lung cancer. *Cancer Res*. 2001;61(14):5366-5369.
43. Wood RD, Doublié S. DNA polymerase θ (POLQ), double-strand break repair, and cancer. *DNA Repair*. 2016;44:22-32.
44. Kawamura K, Bahar R, Seimiya M, et al. DNA polymerase theta is preferentially expressed in lymphoid tissues and upregulated in human cancers. *Int J Cancer*. 2004;109(1):9-16.
45. Pillaire M-J, Selves J, Gordien K, et al. A 'DNA replication' signature of progression and negative outcome in colorectal cancer. *Oncogene*. 2010;29(6):876-887.
46. Lemee F, Bergoglio V, Fernandez-Vidal A, et al. DNA polymerase up-regulation is associated with poor survival in breast cancer, perturbs DNA replication, and promotes genetic instability. *Proc Natl Acad Sci U S A*. 2010;107(30):13390-13395.
47. Allera-Moreau C, Rouquette I, Lepage B, et al. DNA replication stress response involving PLK1, CDC6, POLQ, RAD51 and CLASPIN upregulation prognoses the outcome of early/mid-stage non-small cell lung cancer patients. *Oncogenesis*. 2012;1:e30.
48. Pucci S, Polidoro C, Joubert A, et al. Ku70, Ku80, and sClusterin: A cluster of predicting factors for response to neoadjuvant chemoradiation therapy in patients with locally advanced rectal cancer. *Int J Radiat Oncol Biol Phys*. 2017;97(2):381-388.
49. Wang S, Wang M, Yin S, et al. A novel variable number of tandem repeats (VNTR) polymorphism containing Sp1 binding elements in the promoter of XRCC5 is a risk factor for human bladder cancer. *Mutat Res*. 2008;638(1-2):26-36.
50. Xiao Y, Wang J, Qin Y, et al. Ku80 cooperates with CBP to promote COX-2 expression and tumor growth. *Oncotarget*. 2015;6(10):8046-8061.
51. Komuro Y, Watanabe T, Hosoi Y, et al. The expression pattern of Ku correlates with tumor radiosensitivity and disease free survival in patients with rectal carcinoma. *Cancer*. 2002;95(6):1199-1205.
52. Han L, Wu Z, Zhao Q. Revealing the molecular mechanism of colorectal cancer by establishing LGALS3-related protein-protein interaction network and identifying signaling pathways. *Int J Mol Med*. 2014;33(3):581-588.
53. Lu Y, Gao J, Lu Y. Down-expression pattern of Ku70 and p53 coexisted in colorectal cancer. *Med Oncol*. 2015;32(4):98.
54. Buzon B, Grainger R, Huang S, Rzadki C, Junop MS. Structure-specific endonuclease activity of SNM1A enables processing of a DNA interstrand crosslink. *Nucleic Acids Res*. 2018;46(17):9057-9066.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Laporte GA, Leguisamo NM, Gloria HdCe, Azambuja DB, Kalil AN, Saffi J. The role of double-strand break repair, translesion synthesis, and interstrand crosslinks in colorectal cancer progression—clinicopathological data and survival. *J Surg Oncol*. 2020;121:906–916. https://doi.org/10.1002/jso.25737