Original Article

Role of ERCC1 expression in colorectal adenoma-carcinoma sequence and relation to other mismatch repair proteins expression, clinicopathological features and prognosis in mucinous and non-mucinous colorectal carcinoma

Abd AlRahman Mohammad Foda[1], Andrea Palicelli[2,3], Abdelhadi Shebl[1], Renzo Boldorini[2,4], Khaled Elnaghi[5,6], Amira K. ElHawary[1]

[1]Department of Pathology, Faculty of Medicine, Mansoura University, [5]Department of Internal Medicine, Medical Oncology Unit, Faculty of Medicine, Mansoura University, [6]Medical Oncology Unit, Oncology Center, Mansoura University, Mansoura, Egypt, [2]Department of Health Science, School of Medicine, University of Eastern Piedmont "Amedeo Avogadro", [4]Pathology Unit, "Maggiore della Carità" Hospital, Novara, [3]Unit of Pathology, "Arcispedale Santa Maria Nuova-IRCCS", Reggio Emilia, Italy

Address for correspondence:
Dr. Abd AlRahman Mohammad Foda, Department of Pathology, Faculty of Medicine, Mansoura University, Mansoura - 35516, Egypt.
E-mail: abdofoda@mans.edu.eg

ABSTRACT

Background: There are several DNA repair pathways that protect cellular DNA from injury, such as nucleotide excision repair (NER) and mismatch repair (MMR). The protein product of the excision repair cross-complementation group 1 (ERCC1) gene plays a pivotal role in NER**.** The exact relationship between MMR proteins and ERCC1 is not well known in colorectal carcinoma (CRC). **Aim of the Study:** To investigate expression of ERCC1 and MMR proteins in colorectal mucinous carcinoma (MA) and non-mucinous carcinoma (NMA) using tissue microarray technique. **Material and Methods:** We studied tumor tissue specimens from 150 patients with colorectal mucinous (MA) and non-mucinous adenocarcinoma (NMA). Tissue microarrays were constructed using modified mechanical pencil tips technique and immunohistochemistry for ERCC1, MLH1, MSH2, MSH6, and PMS2. **Results:** NMA showed a significantly more frequent aberrant cytoplasmic expression than MA while MA showed a more frequent intact nuclear expression than NMA. There were no significant differences between the NMA and MA groups in the expression of MMR proteins. In NMA cases, ERCC1 expression was significantly related to MMR status while was not significantly related in MA cases. ERCC1 expression was not significantly related to overall and disease-free survival in both NMA and MA groups. **Conclusion:** this study is the first to investigate the relation between MMR status and ERCC1 expression in colorectal MA and NMA. ERCC1 expression was significantly related to MMR status only in NMA cases. Hence, the current study emphasizes that further research about the relation between various DNA repair pathways is needed.

KEY WORDS: Colorectal, ERCC1, MMR, mucinous

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INTRODUCTION

Colorectal carcinoma (CRC) is one of the most prevalent cancers worldwide and it represents the fourth most common cause of cancer-related mortality. CRC is so heterogenous regarding its pathogenesis with many pathways.[1] One of the major pathways in colorectal carcinogenesis is the defect in the global

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"DNA damage response" that senses different types of damage and coordinates response. There are several recognized DNA repair pathways that protect cellular DNA from injury, such as nucleotide excision repair (NER), mismatch repair (MMR), double-strand break repair, base excision repair, and direct repair.[2]

MMR proteins (namely MLH1, MSH2, MSH6, and PMS2) are nuclear enzymes, which participate in repair of base-base mismatch that occur during DNA replication in proliferating cells. Loss of MMR proteins leads to an accumulation of DNA replication errors, particularly in areas of the genome with short repetitive nucleotide sequences, a phenomenon known as microsatellite instability (MSI). Colorectal MMR profile provides useful prognostic information, as patients with microsatellite unstable neoplasms have a better overall survival rate and a modified response to conventional chemotherapy. MSI also helps in predicting the treatment response of CRC, and could modify the chemotherapy protocols.[3]

On the other hand, nucleotide excision repair (NER) is perhaps the most flexible of the DNA repair pathways considering the diversity of DNA lesions it acts upon. The protein product of the excision repair cross-complementation group 1 (ERCC1) gene plays a pivotal role in NER. Low expression levels or loss of ERCC1 observed in cancer patients could be a cause of disease or a consequence of the same.[4]

There is conflicting data about role of ERCC1 in oncogenesis. In patients with squamous cell carcinoma of the head and neck it was found that the levels of ERCC1 were significantly lower than in healthy controls and that this low expression of ERCC1 was associated with statistically significant increased risk for this tumor type.[5] In contrast, no association between ERCC1 mRNA expression and risk of subsequent development of lung cancer was found.[6]

ERCC1 has also a major impact on response to multimodality treatment of various tumors. A prognostic association between high ERCC1 expression level and low response rates to platinum-based chemotherapy and overall survival has been established in patients with advanced NSCLC, bladder, biliary tract, pancreatic, colorectal, and ovarian cancers.[7]

In CRC, research in this area is highly desirable as the role of ERCC1 in CRC patient-tailored multimodality treatment is far from being firmly established for routine use. Moreover, the exact relationship between MMR proteins and ERCC1 is not yet well known in CRC. Most previous studies emphasized at metastatic CRCs, not primaries, and didn't clearly investigate the role of ERCC1 expression in various CRC subtypes. So, we aimed at this work to investigate the role of ERCC1 in colorectal adenoma-carcinoma sequence, expression of ERCC1 in CRC, its relation to clinicopathological, histological parameters, survival, and to MMR proteins expression in a large number of CRC cases, including various subtypes.

MATERIALS AND METHODS

Samples

A total of 150 cases of CRC specimens were obtained from patients who underwent surgery for histologically confirmed CRC between January 2007 and January 2012. Files of all resected CRC cases during this period were revised.

Seventy-five cases satisfied 2010 WHO selection criteria for "mucinous adenocarcinoma"(MA) (extracellular mucin-containing malignant cells >50% of the tumor; 56 cases) or "signet ring cell carcinoma" (SRCC) (signet ring cells >50% of the tumor cells, with prominent intra-cytoplasmic mucin; 19 cases) and were all included in the mucinous group.

Seventy-five cases of non-mucinous adenocarcinoma (NMA) were randomly chosen for comparison from the same period, including 47 cases of "conventional adenocarcinoma not otherwise specified"(CA) and 28 cases of "adenocarcinoma with mucinous component" (AMC) (<50% of the tumor).

Hematoxylin and Eosin (H and E) stained sections were examined to evaluate histopathological parameters and choose representative areas for tissue microarray (TMA) construction. Grading and TNM staging were performed according to established criteria.[8]

Exclusion criteria included: preoperative (neoadjuvant) chemotherapy; incomplete clinicopathological and follow-up information; insufficient tissue for immunostaining.

Thirty-five samples of normal colic mucosa and 45 adenomas were also included in the study.

The local scientific ethical committee approved the study and REMARK criteria were applied.[9]

Clinical parameters and histopathological evaluation

Clinical data of all cases were revised and all histological slides were re-examined, including the following parameters: age; gender; localization; size; shape; multiple tumors; histological type; grade; depth of invasion (T); tumor edges (pushing and/or infiltrative at microscopic examination); lymphovascular invasion; perineural invasion; peri- and intra-tumoral lymphocytic infiltration; extent of neutrophilic infiltrate; status of nearby and distant mucosa; whether the cancer was on the top of adenoma or not; number of lymph node metastases (N); distant metastases (M); TNM stage; status of surgical margins; associated schistosomiasis. Data regarding survival of the patients were obtained. Disease-free survival (DFS) was defined as the time from the date of surgery to the date of relapse or death, and overall survival (OS) was defined as the time from the date of surgery to the date of death. The median follow up of the patients was 30.7 months (Range from 1.3 to 111.1 months).

Tissue Microarray (TMA) construction

Three manual TMA blocks were constructed using modified mechanical pencil tip method as previously described.[10] Three

representative cores of 0.8 mm diameter were punched out from each case of CRC in addition to cores from normal and adenomatous tissues. Cores of various other normal tissues were included to serve as positive and negative controls. Four-μm thick sections from the TMA blocks were cut on ordinary slides for routine H and E evaluation and on charged slides for immunohistochemical studies.

Immunohistochemistry (IHC)

Immunohistochemistry was performed using an automated immunostainer (BenchMark, Roche, Tucson, USA). The slides were stained with the following antibodies: anti-ERCC1 (mouse monoclonal, Clone 8F1, 0.2 mg/ml conc.) from Neomarkers (Freemont, California, USA); anti-MLH1 (mouse monoclonal, Clone M1, pre-diluted), anti-MSH2 (mouse monoclonal, Clone G219-1129, pre-diluted), anti-MSH6 (mouse monoclonal, Clone 44, pre-diluted) and anti-PMS2 (rabbit monoclonal Clone EPR3947, pre-diluted) from Roche (Ventana Medical Systems, Arizona, USA).

Positive external controls included sections of pulmonary squamous cell carcinoma for ERCC1 and sections of normal colic mucosa for other stains. As a negative control, phosphate buffered saline was used to replace the monoclonal antibody whereas normal goat serum was used to replace polyclonal antibody.

Examination of the slides was done on an Olympus CX31 light microscope. Pictures were obtained by a PC-driven digital camera (Olympus E-620).

Evaluation of IHC

MLH1, MSH2, MSH6, and PMS2expressions were assessed for each case according to College of American Pathologists (CAP) guidelines (CAP Technology Assessment Committee, 2011).[11] Any positive reaction in the nuclei of tumor cells was considered as intact expression (normal), even if patchy or in only one core of the case. An interpretation of expression loss in tumor cells was made only if a positive reaction was seen in internal control cells, such as the nuclei of stromal, inflammatory, or non-neoplastic epithelial cells. For ERCC1, any staining either nuclear or cytoplasmic were reported as previously described.[12,13] Similarly, a positive internal control was a must in each core to be interpreted. Whenever tissue cores of any case were lost, it was not included in the results, which led to different number of cases in each analysis.

Statistical analysis

Data were analyzed, applying SPSS, version 16.0 for Windows (SPSS Inc, IBM, Chicago, Illinois). $\chi 2$ (Chi-square) test was used to test significant differences in categorical variables between various groups. Survival data were analyzed using Kaplan-Meier test. A comparison of survival curves was carried out using the log-rank test. For multivariate analysis, Cox proportional hazard models were performed. A 2-tailed $P \leq 0.05$ was considered significant in all tests.

RESULTS

Clinicopathological and histological features of CRC cases

The age range of the 150 analyzed cases was 20–80 years (mean age: 52.7 years). The patients were 93 men and 57 women. The clinicopathological and histological features of all cases were previously reported.[14] In brief, MA was significantly associated with younger age ($P = 0.017$), deeper invasion ($P = 0.008$), more frequent lymph node metastases ($P = 0.008$), and fewer peritumoral and intratumoral neutrophils ($P < 0.001$) than NMA. For the remaining factors, there were no significant differences between MA and NMA groups.

ERCC1 expression in colorectal normal, adenomatous, and carcinomatous tissues

ERCC1 showed loss of nuclear expression in 25.7% of normal colic mucosae, 33.3% of adenomas and 29.7% of carcinomas. ERCC1 expression was not significantly different between normal colic mucosae, adenomas and CRCs ($P = 0.529$). Aberrant cytoplasmic expression was also detected in 27 cases (18.2%) of CRC [Table 1 and Figure 1].

ERCC1 and other MMR proteins expression in CRC

ERCC1 showed complete loss of expression in 17 cases (11.5%), intact nuclear expression in 104 cases (70.3%) and aberrant cytoplasmic expression in 27 cases (18.2%). NMA showed a significantly more frequent aberrant cytoplasmic expression (74.1%) than MA (25.9%), while MA showed a more frequent intact nuclear expression (54.8%) than NMA (45.2%) ($P = 0.027$) [Table 2 and Figure 1]. Loss of nuclear expression was detected in 7 cases (4.7%) for MLH1, 23 cases (15.3%) for MSH2, 28 cases (18.7%) for MSH6 and 31 cases (21.5%) for PMS2. There were no significant differences between the NMA and MA groups in the expression of MMR proteins [Table 2 and Figure 2].

Relation of ERCC1 expression to CRC subtypes

Within NMA group, ERCC1 showed intact nuclear staining in 31 cases (66%) of CA, and 16 cases (57.1%) of AMC ($P = 0.082$). Similarly, ERCC1 showed intact nuclear staining in 41 cases (75.9%) of MA, and 16 cases (84.2%) of SRCC ($P = 0.242$). ERCC1 expression was not significantly associated with any of the CRC subtypes (data not shown) [Figure 1].

Relation of ERCC1 expression to other MMR proteins expression in CRC

In NMA cases, ERCC1 expression was significantly related to MMR status ($P = 0.045$). 74.5% of cases with preserved nuclear ERCC1 expression were MMR proficient (preserved nuclear MLH1, MSH2, MSH6, and PMS2 expressions), as well as 60% of cases with aberrant cytoplasmic ERCC1 expression. In addition, 71.4% of cases with negative ERCC1 expression were MMR deficient. ERCC1 expression either nuclear or cytoplasmic was significantly related to nuclear MSH6 ($P = 0.011$) and PMS2 ($P = 0.048$) expressions [Table 3].

Table 1: ERCC1 expression in colorectal normal, adenoma and carcinoma tissues

	Normal (n=35) *No. (%)*	*Adenoma (n=45)* *No. (%)*	*Carcinoma (n=148)* *No. (%)*	*Chi-square (χ^2)*	*P*
Nuclear ERCC1 expression					
Loss of expression	9 (25.7%)	15 (33.3%)	44 (29.7%)	0.548	0.760
Preserved expression	26 (74.3%)	30 (66.7%)	104 (70.3%)		

* $P \leq 0.05$ is significant

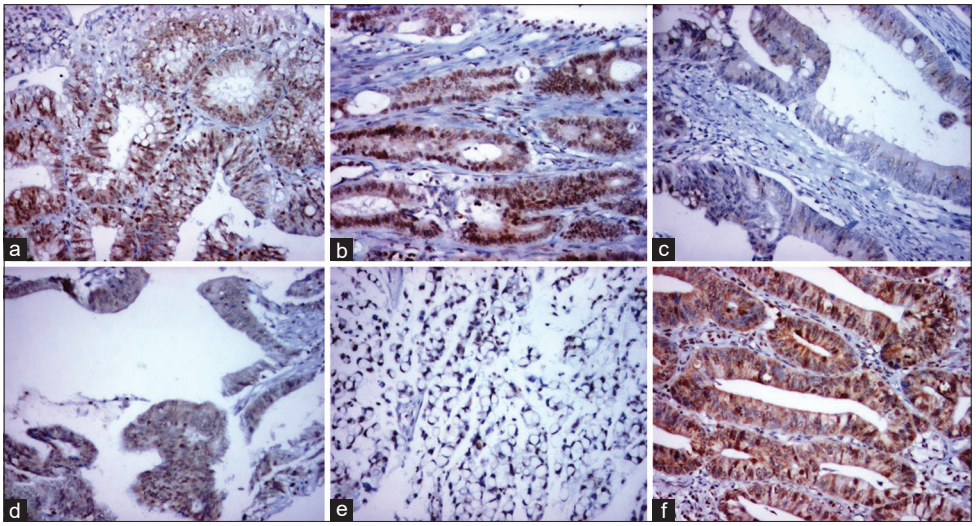

Figure 1: ERCC1 expression in colorectal adenoma and carcinoma subtypes (×200). (a) In adenoma (nuclear), (b) In conventional adenocarcinoma (nuclear), (c) In adenocarcinoma with mucinous component (nuclear, focal and faint), (d) In mucinous adenocarcinoma (nuclear), (e) In signet ring cell carcinoma (nuclear), (f) In conventional adenocarcinoma (cytoplasmic with some nuclear staining)

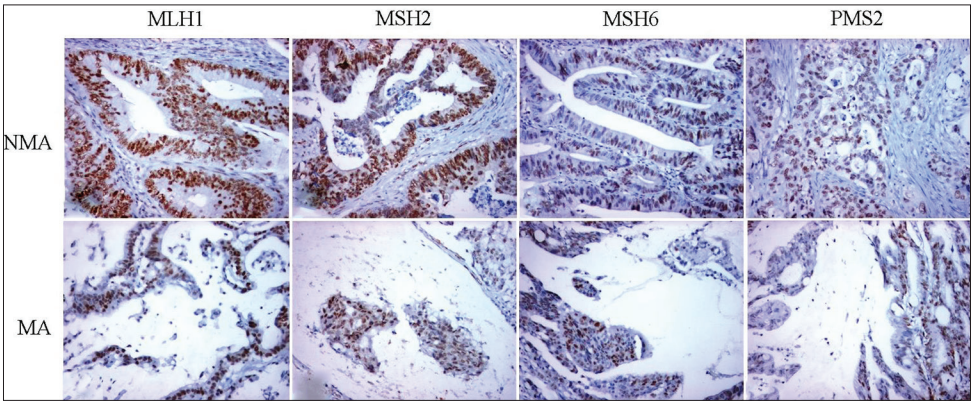

Figure 2: Nuclear MMR proteins expression in mucinous and non-mucinous colorectal carcinoma (×200)

In contrast, ERCC1 expression in MA cases was not significantly related to either MMR status or individual MMR proteins expression [Table 4].

Relation of ERCC1 expression to clinicopathological and histological parameters in CRC

ERCC1 expression was not significantly associated with any of the tested clinicopathological and histological parameters (data not shown) except with the presence of lymphovascular emboli within the tumor, which were found in about 70% of cases with preserved nuclear ERCC1 expression ($\chi 2 = 6.423$, $P = 0.040$).

Relation of ERCC1 expression to survival of CRC cases

DFS and OS of all NMA and MA cases were previously reported.[15] MA cases were significantly associated with worse DFS and OS than NMA cases ($P < 0.001$). To clarify the prognostic impact of ERCC1expression on survival of CRC cases, univariate and multivariate analyses were carried out for each group separately. Relation of ERCC1 expression to DFS and OS in NMA and MA cases was summarized at Table 5. In a univariate analysis, ERCC1 expression was not significantly related to DFS and OS in both NMA and MA groups [Table 5].

Table 2: ERCC1 and other MMR proteins expression in CRC

	NMA (n=75)	*MA (n=75)*	*Chi-square (χ^2)*	*P*
	No. (%)	*No. (%)*		
ERCC1 expression	(*n*=75)	(*n*=73)		
Negative	8 (47.1%)	9 (52.9%)	7.254	0.027*
Nuclear	47 (45.2%)	57 (54.8%)		
Cytoplasmic	20 (74.1%)	7 (25.9%)		
MLH1 expression	(*n*=75)	(*n*=75)		
Negative	2 (28.6%)	5 (71.4%)	1.349	0.246*
Positive	73 (51.0%)	70 (49.0%)		
MSH2 expression	(*n*=75)	(*n*=75)		
Negative	13 (56.5%)	10 (43.5%)	0.462	0.497
Positive	62 (48.8%)	65 (51.2%)		
MSH6 expression	(*n*=75)	(*n*=75)		
Negative	16 (57.1%)	12 (42.9%)	0.703	0.402
Positive	59 (48.4%)	63 (51.6%)		
PMS2 expression	(*n*=71)	(*n*=73)		
Negative	14 (45.2%)	17 (54.8%)	0.271	0.602
Positive	57 (50.4%)	56 (49.6%)		

*$P \leq 0.05$ is significant NMA: Non-mucinous adenocarcinoma, MA: Mucinous adenocarcinoma

Table 3: Relation of ERCC1 expression to other MMR proteins expression in NMA

	ERCC1 expression			*Chi-square (χ^2)*	*P*
	Negative	*Nuclear*	*Cytoplasmic*		
MMR status	(*n*=7)	(*n*=47)	(*n*=20)	6.211	0.045*
Proficient	2 (28.6%)	35 (74.5%)	12 (60.0%)		
Deficient	5 (71.4%)	12 (25.5%)	8 (40.0%)		
MLH1	(*n*=8)	(*n*=47)	(*n*=20)		
expression	1 (12.5%)	1 (2.1%)	0 (0.0%)	3.581	0.167
Negative	7 (87.5%)	46 (97.9%)	20 (100.0%)		
Positive					
MSH2	(*n*=8)	(*n*=47)	(*n*=20)	3.054	0.217
expression	3 (37.5%)	6 (12.8%)	4 (20.0%)		
Negative	5 (62.5%)	41 (87.2%)	16 (80.0%)		
Positive					
MSH6	(*n*=8)	(*n*=47)	(*n*=20)		
expression	5 (62.5%)	8 (17.0%)	3 (15.0%)	9.077	0.011*
Negative	3 (37.5%)	39 (83.0%)	17 (85.0%)		
Positive					
PMS2	(*n*=8)	(*n*=46)	(*n*=17)	6.085	0.048*
expression	4 (50.0%)	6 (13.0%)	4 (23.5%)		
Negative	4 (50.0%)	40 (87.0%)	13 (76.5%)		
Positive					

*$P \leq 0.05$ is significant, MMR: Mismatch repair

DISCUSSION

In clinical practice, oncologists have started to require information on ERCC1 expression on CRC tumors since its overexpression strongly suggests resistance to platinum chemotherapy but also has a favorable prognosis.[16] In addition, defining tumor subtypes of CRC based on pathway driven alterations has the potential to improve prognostication and guide targeted therapy.[17,18]

The current study presents a large dataset exploring the role of ERCC1 in colorectal adenoma-carcinoma sequence, expression of ERCC1 in CRC, its relation to clinicopathological, histological parameters, survival and to MMR proteins expression in of CRC cases, including various subtypes.

Table 4: Relation of ERCC1 expression to other MMR proteins expression in MA

	ERCC1 expression			*Chi-square (χ^2)*	*P*
	Negative	*Nuclear*	*Cytoplasmic*		
MMR status	(*n*=8)	(*n*=57)	(*n*=7)	2.127	0.345
Proficient	4 (50.0%)	36 (63.2%)	6 (85.7%)		
Deficient	4 (50.0%)	21 (36.8%)	1 (14.3%)		
MLH1 expression	(*n*=9)	(*n*=57)	(*n*=7)		
Negative	2 (22.2%)	2 (3.5%)	1 (14.3%)	4.937	0.085
Positive	7 (77.8%)	55 (96.5%)	6 (85.7%)		
MSH2 expression	(*n*=9)	(*n*=57)	(*n*=7)	1.800	0.407
Negative	2 (22.2%)	7 (12.3%)	0 (0.0%)		
Positive	7 (77.8%)	50 (87.7%)	7 (100.0%)		
MSH6 expression	(*n*=9)	(*n*=57)	(*n*=7)		
Negative	3 (33.3%)	9 (15.8%)	0 (0.0%)	3.265	0.195
Positive	6 (66.7%)	48 (84.2%)	7 (100.0%)		
PMS2 expression	(*n*=8)	(*n*=57)	(*n*=6)	1.907	0.385
Negative	2 (25.0%)	14 (24.6%)	0 (0.0%)		
Positive	6 (75.0%)	43 (75.4%)	6 (100.0%)		

* $P \leq 0.05$ is significant, MMR: Mismatch repair

Table 5: Univariate analysis of the relation of ERCC1 expression to survival in NMA and MA cases

	ERCC1 expression	*Median DFS (months)*	*P*	*Median OS (months)*	*P*
NMA	Negative	32	0.217	44	0.377
	Nuclear	61		61	
	Cytoplasmic	37		46	
MA	Negative	22	0.266	27	0.302
	Nuclear	15		22	
	Cytoplasmic	31		38	

* $P \leq 0.05$ is significant, NMA: Non-mucinous adenocarcinoma, MA: Mucinous adenocarcinoma, DFS: Disease-free survival, OS: Overall survival

In this study, it was found that there was positive ERCC1 protein expression in 70.3% of CRC cases. Likewise, Gajjar *et al.*[19] reported 72% positive ERCC1 immunoreactivity in patients with CRC. In another two studies, ERCC1 positivity was observed in 45% and 55% of Chinese patients with colorectal and stage III disease, respectively[20,21] [Supplementary Table 1]. This heterogeneity may be due to several factors including antibody used, scoring technique, and preparation of the paraffin embedded tissue blocks. Another important explanation for this discrepancy is that the frequencies of ERCC1 alleles were substantially different among patient populations with different ethnicity.[22]

Most colorectal carcinomas are considered to arise from conventional adenoma based on the concept of the adenoma-carcinoma sequence. Investigating biological markers expression in colorectal adenomas and carcinomas help in better understanding of these pathogenic pathways involved in colorectal tumorigenesis.[23] In the current study, ERCC1 expression was not significantly different between normal colonic mucosae, adenomas, and CRCs. In contrast, Sæbø *et al.*[24] found that mRNA levels of ERCC1 were up-regulated in both colorectal adenomas and carcinomas compared to corresponding normal colonic mucosa, indicating that increased expression of defense genes is an early event in the progression of colorectal adenomas

to carcinomas. As the current study, they also reported that the level of ERCC1 was not different between adenomas and CRC cases. The controversy from our results may be due to several factors including different methods of assessments and also the number of cases in their study was relatively small.

To the best of our knowledge, this study was the first to report the significant difference in ERCC1 immunohistochemical subcellular localization between NMA and MA subtypes. There was a wide discrepancy in the subcellular localization of ERCC1 in the literature. One study found that expression of ERCC1 protein was localized in the cytoplasm of epithelial cells of colon and rectum in 72% of cases.[19] The primary antibody used was mouse monoclonal anti-ERCC1 (clone 4F9). Another study by Li *et al.*[25] reported positive nuclear staining in 90.7% of CRC cases. The antibody used was mouse anti-human ERCC1 (clone OTI1A3). Also, Wang *et al.*[26] observed nuclear ERCC1 expression in gastric cancer cells, but as in our study, they also detected its expression in the cytoplasm of some tumor cells. In non-small cell carcinoma cases, Olaussen *et al.*[12] observed frequent cytoplasmic staining with FL297 and nuclear staining with 8F1 (the antibody used in the current study). Consequently, they believed that the 8F1 antibody is an acceptable tool to determine nuclear ERCC1 protein expression in tissues of solid tumors of epithelial origin, whereas FL297 leads to a puzzling cytoplasmic staining. Interestingly, we found cytoplasmic staining of ERCC1 as well using 8F1 antibody in about 18% of CRC cases, mainly of NMA histological type.

Universal assessment of immunohistochemical MMR staining is increasingly applied in colorectal cancer diagnostics in order to identify cases suspected of Lynch syndrome for further molecular diagnostics.[27] In the current study, loss of nuclear expression for MMR protein was 34.6% of cases which is slightly higher than published incidence rate detected by Hall *et al.*, (30.2%).[28] Sylvester *et al.*[29] and Kumar *et al.*[30] reported MSI incidence rate of 27% and 19.8% respectively in a large samples of African-American colorectal cancer patients. Li *et al.*[31] explained the low incidence of MMR mutation in his study (7.5%) as a significant proportion of CRC in China may follow tumorigenesis pathways distinct from the deficient MMR CRC progression sequence. The same explanation could be applied for the discrepancy in the incidence rates between different studies. Clearly, this possible heterogeneity could also have implications for CRC prognosis and the clinical management of disease.

In this study, no significant differences were found between the NMA and MA groups in the expression of MMR proteins. Our study showed that loss of nuclear expression was present in 36.1% of MA group. Similarly, Andrici *et al.*[32] reported that loss of nuclear expression was present in 36.0% of mucinous colorectal cancer keeping with previous studies reporting ranges from 29 to 42%.[33,34] In addition, Kaur *et al.*[35] found a significant association between abnormal MMR proteins expression and mucinous, signet ring and poorly differentiated CRC in contrast to CA cases which showed normal MMR proteins expression. In contrast, in this study, 33.7% of NMC group showed loss of nuclear expression compared with only 14.1% on non-mucinous tumors reported by Andrici *et al.*[32] and 18.5% reported by Vergouwe *et al.*[36] This difference may be explained by multi-factorial etiology of CRC involving hereditary and racial causes, environmental factors, and somatogenetic changes during tumor progression.[37]

Scarce studies had assessed ERCC1 expression in different pathological types of CRC by immunohistochemistry. We found that ERCC1 showed intact nuclear staining in 66% of CA, 57.1% of AMC, 75.9% of MA, and 84.2% of SRCC without significant association with any of the CRC subtypes. Similarly, Li *et al.*[25] found that most of cases of CA (90.7%), MA (88.4%) and SRCC (85.7%) showed positive ERCC1 nuclear staining but without statistical difference. However, they did not differentiate between CA and AMC as many of the studies did. To the best of our knowledge, this study was the first to assess ERCC1 expression in AMC. In contrast to our results, Shimamoto *et al.*[38] found that the lowest levels of expression of ERCC1 were observed in patients with mucinous adenocarcinoma, while the highest level of expression was observed in patients with poorly differentiated adenocarcinoma.

Li *et al.*[25] large dataset showed that CRC patients with retained expression of MMR tended to have positive ERCC1 expression, suggesting a collaboration of these two DNA repair pathways in maintaining cell integrity and normalcy. MMR proteins are responsible for correcting mismatched nucleotides and insertion-deletion loops in DNA caused by polymerase errors, chemical modifications, and recombination between heterologous DNA sequences,[39] while ERCC1 is a key molecule in the NER pathway, which is responsible for repairing DNA adducts induced by platinum drugs.[40,41] The underlying mechanisms of these potential interactions between these DNA repair proteins still needs to be elucidated to gain a better understanding of CRC pathogenesis and its prognosis. Similarly, Tóth *et al.*[42] reported that loss of MLH1 and MSH2 was associated with lower expression or loss of ERCC1 in colorectal liver metastasis. In the study of Zhang *et al.*,[43] they have examined MSI level and ERCC1 polymorphisms of CRC patients before receiving adjuvant chemotherapy and they found no significant correlation was found between MSI status and ERCC1 polymorphisms. However, we believe that it is not feasible to investigate the relationship between both DNA repair pathways in a large group of CRC cases involving multiple subgroups, especially MA which is well known to have specific pathogenic pathways and prognosis. To the best of our knowledge, the current study is the first to assess the relation of ERCC1 expression to MMR proteins expression in NMA and MA cases. This specification can explain the discrepancy found in previous studies. Only in NMA cases, ERCC1 expression was significantly related to MMR status, as reported by Li *et al.*[25] In contrast to Tóth *et al.*,[42] we found that ERCC1 expression either nuclear or cytoplasmic was significantly related to nuclear MSH6 and PMS2 expressions. This can be explained by the known genetic dissimilarity between primary tumors and their metastases.[44] In addition, ERCC1 expression in MA cases in our study was not significantly related to either MMR status or individual MMR proteins expression.

Similar to the study of Li, *et al.*,[25] ERCC1 expression in this study was not significantly associated with any of the clinicopathological and histological parameters. However, we could demonstrate significant relation between the presence of lymphovascular emboli within the tumor and preserved nuclear ERCC1 expression. In contrast, Kim *et al.*[45] and Shimamoto *et al.*[38] reported no significant difference between lymphovascular emboli and ERCC1 expression.

Some studies reported that patients with low levels of ERCC1 expression have an improved response and a longer OS in gastrointestinal tumors treated with FOLFOX.[46] One of the suggested explanations of this relation is the identification of several common and putatively functional single nucleotide polymorphisms (SNPs) of ERCC1.[47] The rs11615 T allele of ERCC1 polymorphism was found to be associated with high mRNA expression of the corresponding gene. Patients carrying the ERCC1 rs11615 T may have higher DNA repair capacity that could effectively reduce the anticancer effect of oxaliplatin, leading to poor prognosis of these patients. Thus, inter-individual difference in the NER capacity may influence clinical outcomes of the treated cancer patients.[22-48] Notably, Yin *et al.*[22] confirmed the existence of ethnical difference in the estimates for the ERCC1 allele. Although the underlying mechanisms are not clear, numerous factors may have played a role, such as gene-gene interaction from different genetic background, and gene environmental interaction from different lifestyles. These points can explain the lack of association between enzyme expression and survival in the present study. One more point is that the current study is the first to investigate OS and DFS in CRC subtypes (NMA and MA). Nevertheless, multivariate analysis of Kwon *et al.*[49] revealed that ERCC1 expression is significantly related to OS which indicates that immunohistochemical staining for ERCC1 may be useful for predicting the clinical outcomes of advanced gastric cancer patients treated with 5-FU and oxaliplatin. However, it is well known that immunohistochemical staining has many limitations attributable to its semi-quantitative nature, the staining technique, the enzyme antibody used, and inter-observer variation.

In conclusion, this study is the first to investigate the relation between MMR status and ERCC1 expression in mucinous and non-mucinous colorectal carcinoma. ERCC1 expression was significantly related to MMR status only in NMA cases. In contrast, ERCC1 expression in MA cases was not significantly related to MMR status or individual MMR proteins expression. Hence, the current study emphasizes that further research about the relation between various DNA repair pathways is needed. Owing to the well-known genetic differences between MA and NMA CRC, we emphasize that these relations should be investigated in each subgroup separately.

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Conflicts of interest
There are no conflicts of interest.

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