European Review for Medical and Pharmacological Sciences 2020; 24: 2994-3003

Influence of KRAS mutations on clinical outcome in patients with curatively resected stage III colon cancer treated with adjuvant chemotherapy

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Abstract. – OBJECTIVE: To profile and correlate KRAS mutations with outcome in stage III colon cancer (CC) patients who underwent adjuvant chemotherapy following curative resection surgery.

PATIENTS AND METHODS: In this retrospective study, eligible patients were those with resected stage III CC who underwent 6-months adjuvant chemotherapy, either with fluoropyrimidine monotherapy (FP) or with oxaliplatin-based regimens (O-FP). Disease-free survival (DFS) and overall survival (OS) were analyzed and computed using the Kaplan-Meier method and the log-rank test.

RESULTS: The study population included 148 patients (n=65 FP and n=83 O-FP). We identified KRAS mutations in 41/148 (27%) patients, of which 18 (44%) received FP and 23 (56%) O-FP. Five-year DFS and OS were significantly higher in patients with KRAS wild-type *vs.* mutant [DFS: 78 *vs.* 56%, HR: 0.47 (95% CI: 0.25; 0.87), *p*=0.01; OS: 73 *vs.* 68%, HR: 0.44 (95% CI: 0.21; 0.88), *p*=0.01]. In patients treated with FP, the 5-year DFS and OS was significantly improved in the KRAS wild-type *vs.* mutant group, respectively [DFS: 80 *vs.* 43%, HR: 2.88 (95% CI: 0.67; 3.76), *p*=0.014; OS: 85 *vs.* 68%, HR: 0.27 (95% CI: 0.10; 0.73), *p*=0.005]. Conversely, 5-year DFS and OS were not statistically different for patients with KRAS wild-type *vs.* mutations treated with O-FP, respectively [DFS: 78 *vs.* 65%, HR: 1.59 (95% CI: 0.67; 3.76), *p*=0.281; OS: 80 *vs.* 75%, HR: 0.73 (95% CI: 0.55; 2.12), *p*=0.57)].

CONCLUSIONS: Our results suggest that curatively resected stage III CC patients exhibiting wild-type KRAS status might benefit from FP alone. Conversely, an oxaliplatin-containing regimen should be recommended in KRAS mutated patients.

Key Words:
KRAS, Oxaliplatin, Chemotherapy, Colon cancer, Adjuvant.

Introduction

Despite undergoing curative surgical resection, up to 50% of patients with stage III colon cancer (CC) may experience recurrence[1]. In patients with curatively resected stage III CC, adjuvant chemotherapy has been shown to reduce the risk of disease relapse[2]. By treating stage III CC patients with 5-fluorouracil (5FU)-based adjuvant chemotherapy, the disease-free survival (DFS) and overall survival (OS) improve by approximately 17% and 15%, respectively[3]. Moreover, the addition of platinum-based oxaliplatin to 5-FU-based regimens has demonstrated superiority to 5-FU monotherapy in terms of DFS and OS, and has thus become the universally recommended adjuvant treatment for stage III CC[2-5]. Nevertheless, the majority of patients receive adjuvant treatment unnecessarily, either because they are cured with surgery alone or because they will

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relapse despite treatment. In the era of personalized medicine, and because oxaliplatin is associated with cumulative neurotoxicity, incorporating biomarkers into current clinical decision-making could potentially predict the efficacy of adjuvant chemotherapy and avoid unnecessary toxicity in patients who are unlikely to benefit, thus decreasing health expenditure[6].

Conventional tumor-node-metastasis (TNM) pathologic staging alone cannot predict which CC patients would benefit from adjuvant treatment and considerable effort has already been directed towards finding molecular biomarkers that can accurately predict tumor response in stage III CC patients at high risk of recurrence[7]. Preclinical data have suggested that sensitivity to 5-FU or to oxaliplatin may be predicted by the KRAS mutational status[8,9], although other studies[10,11] failed to confirm this assumption. At the clinical level, one study[12] reported the correlation between KRAS mutations and a shorter time to recurrence in stage III CC patients treated with oxaliplatin-based chemotherapy, thus supporting the influence of KRAS mutations as a negative prognostic factor. However, these results are in contrast with the majority of previous studies[13-17], which reported no association between KRAS mutations and survival in stage III CC in the adjuvant setting. Also, the correlation between KRAS mutational status and efficacy of fluoropyrimidines in stage III CC is controversial. The SWOG trial showed that KRAS mutated CC patients gained no benefit from receiving 5FU/folinic acid compared with observation or folinic acid alone, while KRAS wild-type patients significantly benefited from 5-FU/folinic acid (76 vs. 44%; HR, 0.4; 95% CI, 0.2-0.8)[18]. The CALGB 89803 trial also reported no influence of KRAS mutational status on DFS or OS in stage III disease[19].

KRAS mutations may occur in many tumor types together with the downregulation of ERCC1 (excision repair cross-complementation group 1), a protein involved in the mechanism of DNA damaged recognition and repair[20]. Indeed, it has been proposed that KRAS mutations in CC cells can predict oxaliplatin sensitivity by ERCC1 downregulation[10].

Notably, and to the best of our knowledge, only one study[21] has investigated KRAS mutational status and ERCC1 protein expression in the same cohort and compared the molecular profiles to clinical data. The authors of the study, by analyzing metastatic CC cases, found that in ERCC1 overexpressing patients' response rate and PFS were higher in mutated KRAS patients but not in those without ERCC1 overexpression.

On the basis of all these findings, we decided to analyze the KRAS mutational status as well as ERCC1 alterations (polymorphisms, mRNA and protein expression) in the same retrospective cohort of curatively resected stage III CC patients, treated either with fluoropyrimidines alone or in combination with oxaliplatin, and then, to compare the molecular patterns to clinical outcome.

Patients and Methods

Patient Population

This is a retrospective analysis of patients with histologically confirmed pathological stage III CC who underwent a curative resection and treated with adjuvant chemotherapy between December 1996 and October 2010, in different recruiting centres in Switzerland (Oncology Institute of Southern Switzerland, Ticino), Argentina (Hospital Udaondo, Instituto Fleming, Hospital Alemán, Sanatorio Municipal, Hospital Privado Comunidad, Sanatorio Británico, Clinica Oncomed, Clinica ISIS, Hospital Penna, Hospital Italiano de CBA, Hospital zonal Lopes de Lanus, Hospital Lucero, Hospital General San Martin, Hospital Churruca, Hospital zonal Evita Pueblo and COIR, Buenos Aires) and Italy (Istituto Ospedaliero MultiMedica, Castellanza). The staging was performed according to the Union for International Cancer Control (UICC) TNM classification of malignant tumors (8th edition) (Brierley 2016). A chest X-ray and abdominopelvic CT scan (CT A/P) were routinely performed before surgery. Additional examinations were considered only in case of clinical suspicion of distant metastases. In all cases, the macroscopic disease was cleared by surgery, and resection margins were free of tumor at histopathological examination.

Treatment and Follow-Up

After surgical resection, patients were treated with 6-months adjuvant chemotherapy, either with single-agent fluoropyrimidine (FP: modulated fluorouracil (5-FU) or capecitabine) and/or with oxaliplatin-based regimens (FOLFOX (folinic acid, 5-FU, oxaliplatin) or XELOX (oxaliplatin, capecitabine; also called CAPOX). Chemotherapy was initiated in all patients within 8 weeks of surgery. Patients in the FP monotherapy group received the standard de Gramont regimen[22] of leucovorin (LV) plus 5FU (LV5FU) for 6 months or

capecitabine (2,500 mg/m^2 po, days 1-14 q3w for 8 cycles). The combined treatment group received modified FOLFOX6 (mFOLFOX6: oxaliplatin 85 mg/m^2, calcium folinate 200 mg/m^2, 5FU 400 mg/m^2, and then, continuous infusion of 2,400 mg/m^2 for 48 h, every 14 days for 12 cycles), or CAPOX (oxaliplatin 130 mg/m^2 plus capecitabine 2000 mg/m^2 po, days 1-14, q3w for 8 cycles). Dose modifications were allowed according to the original schedules.

Follow-up included history taking, physical examination, and serum carcinoembryonic antigen (CEA) assay, every 3 months for the first year, every 6 months for the following 2 years, and thereafter annually, for a total follow-up period of 5 years or until disease recurrence. Abdominal and thoracic imaging was performed every 6 months for the first 3 years and then annually. Clinical outcome was monitored for each study participant from surgery to death or to the last follow-up date.

Molecular Analyses

Genomic DNA and total RNA were extracted from formalin-fixed paraffin-embedded (FFPE) tissue sections. A single representative FFPE tumor tissue block (containing ≥70% of neoplastic cells)[23] and a paired healthy mucosa tissue block (for RNA analysis) were selected for each sample. The DNA extraction was performed using the QIAamp Mini kit (Qiagen, Chatsworth, CA, USA) while the RNeasy FFPE Kit (Qiagen, Chatsworth, CA, USA) was used for RNA extraction, according to the manufacturer's instructions.

KRAS and ERCC1 Polymorphism Analyses

For *KRAS* gene analyses, we investigated exon 2, including codons 12 and 13, corresponding to the two hot-spot sites where more than 90% of KRAS mutations occur. Regarding *ERCC1*, the polymorphism at nucleotide 19007 (*AAT/AAC*, Asn118Asn) (exon 4) was analyzed. *KRAS* and *ERCC1* gene sequences were evaluated by Sanger sequencing using an ABI Prism 3130 automated sequencer (Applied Biosystems, Foster City, CA, USA), as previously described[24]. The files obtained were aligned and examined for mutations of the KRAS gene by Sequencing Analysis software v6.0 (Applied Biosystems, Foster City, CA, USA). Each sequence reaction was carried out at least twice, starting from independent PCR reactions. In each case, the detected mutation was confirmed in the sequence as sense and antisense strands.

Microsatellite Instability (MSI)

The status of MSI was assessed by the analysis of the microsatellite loci included in the panel of Bethesda (BAT25, BAT26, D2S123, D5S346, D17S250), as reported previously[24]. Instability in each locus was confirmed by the presence of an additional peak in the tumor sample in comparison with the normal paired tissue. Microsatellite stability (MSS) and low frequency of MSI (MSI-L) status were defined as instability at the 0 and 1 marker, respectively. High frequency of MSI (MSI-H) status was characterized by the presence of instability in 2 or more markers[25].

ERCC1 Reverse Transcription-PCR

To analyze *ERCC1* gene expression, a fluorescence-based real-time procedure (CFX96, Bio-Rad, Hercules, CA, USA) was adopted, using the protocol previously described[26]. *POLR2A* was chosen as the internal reference gene.

Total RNA was transcribed into cDNA using the High Capacity RNA-to-cDNA™ Master Mix protocol (Applied Biosystems, Foster City, CA, USA). Real Time-PCR was performed in the CFX96™ Real Time System (Bio-Rad, Hercules, CA, USA). Taqman® Gene Expression Master Mix and Taqman® assays probes (*POLR2A* probe: Hs00172187_m1 and *ERCC1* probe: Hs01012161_m1) were purchased from Applied Biosystems (Foster City, CA, USA). The sequences of primers and probes used for ERCC1 and POLR2A evaluation by RT-PCR were: ERCC1 Primer Fw: 5`-GGGAATTTGGCGACGTAATTC-3`, ERCC1 Primer Rev: 5'-GCGGAGGCTGAGGAACAG-3', TaqMan ERCC1 probe: 6FAM (carboxyfluorescein) 5'-CACAGGTGCTCTGGCCCAGCACATA-3'; POLR2A Primer Fw: 5'-ATGGAGATCCCCACCAATATCC-3', POLR2A Primer Rev: 5'-CATGGGACTGGGTGCTGAAC-3', TaqMan POLR2A probe: 5'-FAM-TGCTGGACCCACCGGCATGTTC TAMRA-3'.

Each sample was analyzed in triplicate. Final results[27,28] were determined by the formula $2^{-\Delta\Delta Ct}$, which standardizes the target with the reference gene in both tumor and normal tissue[29]. The median value of all scores was used as the threshold separating ERCC1 overexpressing (higher values) from ERCC1 normally expressing (lower values) cases.

ERCC1 Immunohistochemistry

The ERCC1 immunohistochemistry (IHC) analysis was performed on 3-µm thick tissue sections by using an anti-ERCC1 (clone 8F1, dilution 1:50; Thermo Fisher Scientific, Erembodegem, Belgium)

monoclonal antibody. The analysis was performed on the Ventana BENCHMARK® XT instrument using UltraView DAB kit (Ventana Medical Systems, Tucson, AZ, USA). Briefly, for epitope retrieval, slides were exposed on heat to EDTA, then, endogenous peroxidase activity was blocked by incubation with H_2O_2 3% (30 min EDTA and 4 min H_2O_2). Primary antibody incubation was carried out for 32 minutes at 37°C. Immunoreaction was revealed by secondary antibody incubation for 8 minutes with 3'-3'-diaminobenzidine as the chromogen, and Mayers hematoxylin as the counterstain. Endothelial cells of normal tonsil tissues and proliferating germinal centre lymphocytes were included as positive controls for ERCC1, as previously suggested[24].

Immunostaining was evaluated under a light microscope by an expert pathologist (RB). A positive staining was assigned when tumor cells showed nuclear reactivity. As to date there are no standardized guidelines for ERCC1 staining evaluation on colon tumors, an H-score, usually utilized in the evaluation of ERCC1 in non-small cell lung cancer, was applied[30]. The intensity of staining was scored on a scale of 0 to 3; 3 indicating the higher intensity using normal tonsil tissue as positive control. The percentage of positive tumor cells was scored as follows: 0 if 0%; 0.1 if 1% to 9%; 0.5 if 10% to 49%; 1 if 50% or more. Semi-quantitative H-scores were obtained from intensity multiplied with positive cells, with values ranging from 0 to 3. The median value of all H-scores was chosen as the cut-off point to determine positive or negative tissues according to the literature and to our previous work on gastric cancer[24,30].

Statistical Analysis

A two-tailed Fisher's exact test was used to calculate the *p*-values for the association among variables. The level of significance was set at $p<0.05$. The disease-free survival (DFS) and overall survival (OS) analyses were performed according to the Kaplan-Meier method, and survival curves were compared using the log-rank test. Data were analyzed using the IBM Statistical Package for Social Sciences (SPSS) Statistics 20 (IBM Corp., Armonk, NY, USA).

Results

Patient Characteristics

The study population consisted of 148 patients with a median age at diagnosis of 68 years (range 35-82). The median follow-up at the time of this analysis was 55 months (range 2-156). At the end of follow-up, 59 patients (40%) had relapsed. The disease relapse accounted for 37 of 46 deaths (80%). In the entire cohort, 65 patients (43%) received FP monotherapy and 83 (57%) received the combination.

Expression of ERCC1, KRAS, and MSI

All CC samples were assessed for ERCC1 expression (IHC and RT-PCR) and KRAS mutational status. ERCC1 was measured quantitatively in the tumor tissue, and the ratio of ERCC1 to the reference gene ranged between 0.2 and 16.8; the median value was calculated as 1.4 (95% CI 109.4-134.4). Seventy-two (48%) patients had an ERCC1 level greater than the median.

ERCC1 expression using IHC was categorised as score 0 in 53 of 148 cases (35%), 1+ in 56 cases (38%), 2+ and 3+ in 39 cases (27%). By using the H-score, the median value was 0.5 (range 0-5.3, 95% CI 127.9-162.3). Positive cases were considered those exhibiting an H-score higher than the median value (56 cases, 37%).

We identified KRAS mutations in 41 participants (27% of cases), of which 18 received FP monotherapy, and 23 received combination therapy with O-FP.

Overall, 14 cases (16%) with MSI-H tumors were detected in the combined treatment group compared to 12 cases (18%) in the monotherapy group.

Molecular Analysis and Clinical Outcome

Tables I and II summarise the DFS and OS data, respectively, according to molecular markers analyzed. The univariate analysis revealed no significant association between ERCC1 expression and survival. In the entire cohort, 5-year DFS and OS were significantly higher in KRAS wt. patients compared to KRAS mutant patients: 5-year DFS: 78 *vs.* 56%, $p=0.01$, HR: 0.47 (95% CI: 0.25, 0.87); 5-year OS: 73 *vs.* 68% $p=0.01$, HR: 0.44 (95% CI: 0.21, 0.88).

Also, in patients treated with FP, a better 5-year DFS and OS were observed in KRAS wt. patients: 5-year DFS: wt. *vs.* mutant 80 *vs.* 43%, $p=0.014$, HR: 2.88 (95% CI: 0.67, 3.76); 5-year OS: 85 *vs.* 68%, $p=0.005$, HR: 0.27 (95% CI: 0.10, 0.73). On the contrary, 5-year DFS and OS were not statistically different for wt. and mutated KRAS patients treated with O-FP: 5-year DFS: 78 *vs.* 65%, $p=0.281$, HR: 1.59 (95% CI: 0.67; 3.76); 5-year-OS: 80 *vs.* 75%, $p=0.57$, HR: 0.73 (95% CI: 0.55; 2.12). The 5-year DFS and OS showed no difference in both treatment groups between MSS/MSI-L and MSI-H.

Table I. Five-Year Disease-Free Survival (DFS) according to molecular classification.

Variable	Hazard Ratio (95% CI)	*p*-value
KRAS: wt. *vs.* mt:		
Overall	0.47 (0.25-0.87)	0.016
FP	2.88 (0.67-3.76)	0.014
O-FP	1.59 (0.67-3.76)	0.281
ERCC1 IHC: 0 *vs.* 1+, 2+, 3+:		
Overall	1.20 (0.59-2.42)	0.597
FP	1.01 (0.67-3.76)	0.441
O-FP	1.75 (0.53-4.23)	0.975
ERCC1 IHC: 0, 1+ *vs.* 2+, 3+:		
Overall	0.90 (0.44-1.86)	0.796
FP	1.12 (0.32-3.83)	0.353
O-FP	0.88 (0.35-2.18)	0.789
ERCC1 IHC: < Median *vs.* > Median:		
Overall	0.64 (0.32-1.28)	0.214
FP	0.45 (0.13-1.53)	0.207
O-FP	0.81 (0.34-1.94)	0.658
ERCC1 RT-PCR: <1 *vs.* >1:		
Overall	1.23 (0.63-2.40)	0.522
FP	1.57 (0.57-4.33)	0.378
O-FP	1.20 (0.42-2.54)	0.921
ERCC1 RT-PCR: <2 *vs.* >2:		
Overall	1.59 (0.47-2.73)	0.124
FP	1.42 (0.59-3.44)	0.425
O-FP	1.83 (0.79-4.25)	0.433
ERCC1 RT-PCR: <3 *vs.* >3:		
Overall	1.45 (0.71-2.94)	0.319
FP	1.96 (0.75-5.13)	0.187
O-FP	1.10 (0.37-3.27)	0.854
ERCC1 IHC: < Median *vs.* > Median:		
Overall	1.49 (0.81-2.75)	0.189
FP	1.44 (0.57-3.63)	0.422
O-FP	1.56 (0.69-3.53)	0.203
MSI-H *vs.* MSS, MSI-L:		
Overall	1.98 (0.61-6.14)	0.206
FP	1.67 (0.27-4.97)	0.830
O-FP	3.9 (0.55-29.19)	0.100

ERCC1: excision repair cross-complementing group 1; wt: wild-type; mt: mutant; IHC: immunohistochemistry; MSI-H: high frequency microsatellite sequence; RT-PCR: reverse transcription polymerase chain reaction; MSS: Microsatellite instability; MSI-L: low frequency microsatellite sequence; FP: fluoropyrimidine monotherapy; O-FP: oxaliplatin-FP combination regimen.

Discussion

The aim of this retrospective cohort study was to analyze the impact of KRAS or ERCC1 alterations on clinical outcomes in curatively resected stage III CC patients receiving either fluoropyrimidines alone or in combination with oxaliplatin. We found that stage III CC patients with wild-type KRAS showed better 5-year survival outcomes than patients with KRAS mutations. In addition, we observed that KRAS wild-type CC patients treated with FP exhibit better 5-year DFS and OS than KRAS mutant patients. On the contrary, KRAS mutational status made no difference to 5-year survival for patients receiving the adjuvant chemotherapy combination O-FP. To our knowledge, this is the second study to evaluate the role of KRAS mutations stratified by MSI/MSS status and ERCC1 protein expression in the same cohort of stage III colon cancer patients treated with adjuvant chemotherapy.

KRAS is an intracellular effector located downstream of the epidermal growth factor receptor (EGFR). In the metastatic setting, KRAS mutations confer resistance to anti-EGFR treatments. Post-hoc analysis of two large prospective adjuvant trials, PETACC-8 and N0147, confirmed the detrimental role of KRAS mutations on stage

Table II . Five-Year Overall Survival (OS) according to molecular classification.

Variable	Hazard Ratio (95% CI)	*p*-value
KRAS: wt. *vs.* mt:		
Overall	0.44 (0.21-0.88)	0.017
FP	0.27 (0.10-0.73)	0.005
O-FP	0.73 (0.55-2.12)	0.572
ERCC1 IHC: 0 *vs.* 1+, 2+, 3+:		
Overall	1.13 (0.59-2.90)	0.488
FP	2.07 (0.65-6.61)	0.195
O-FP	0.82 (0.28-2.42)	0.732
ERCC1 IHC: 0, 1+ *vs.* 2+, 3+:		
Overall	0.84 (0.36-1.97)	0.694
FP	1.37 (0.38-4.89)	0.634
O-FP	0.65 (0.20-2.07)	0.463
ERCC1 IHC: < Median *vs.* > Median:		
Overall	0.62 (0.27-1.41)	0.244
FP	0.74 (0.20-2.65)	0.639
O-FP	0.58 (0.19-1.73)	0.325
ERCC1 RT-PCR: <1 *vs.* >1:		
Overall	1.31 (0.59-2.92)	0.491
FP	1.46 (0.47-4.50)	0.482
O-FP	1.20 (0.38-3.76)	0.741
ERCC1 RT-PCR: <2 *vs.* >2:		
Overall	1.97 (0.89-3.51)	0.103
FP	1.35 (0.52-3.50)	0.537
O-FP	2.48 (0.93-6.62)	0.073
ERCC1 RT-PCR: <3 *vs.* >3:		
Overall	1.60 (0.74-3.46)	0.244
FP	1.71 (0.59-4.90)	0.334
O-FP	1.66 (0.53-5.18)	0.398
ERCC1 IHC: < Median *vs.* > Median:		
Overall	1.55 (0.76-3.17)	0.215
FP	1.19 (0.44-3.23)	0.725
O-FP	2.03 (0.73-5.56)	0.163
MSI-H *vs.* MSS, MSI-L:		
Overall	2.13 (0.51-8.92)	0.244
FP	2.36 (0.15-8.70)	1.695
O-FP	0.70 (0.15-3.11)	0.659

ERCC1: excision repair cross-complementing group 1; wt: wild-type; mt: mutant; IHC: immunohistochemistry; MSI-H: high frequency microsatellite sequence; RT-PCR: reverse transcription polymerase chain reaction; MSS: Microsatellite instability; MSI-L: low frequency microsatellite sequence; FP: fluoropyrimidine monotherapy; O-FP: oxaliplatin-FP combination regimen.

III colon cancer clinical outcomes[31,32]. Similar to our study, the pooled analysis of both PETACC-8 and N0147 trials confirmed a 1.5-fold higher risk of relapse and death in the KRAS mutated than in the KRAS wild-type population[33].

Several studies have shown that ERCC1 mRNA overexpression can be a negative prognostic factor for platinum-based chemotherapy in patients with a variety of other cancer types, including non-small-cell lung cancer[30], ovarian cancer[34], and gastric cancer[35]. Various outcomes regarding the prognostic correlation between ERCC1 expression and platinum-based chemotherapy in an adjuvant setting have been reported in colorectal patients. Shirota et al[36] reported a significant negative correlation between the mRNA levels of ERCC1 and the survival of patients with unresectable advanced colorectal cancer, who received FOLFOX chemotherapy after failure of 5-FU and irinotecan chemotherapy. Another study[37] investigated the expression of ERCC1 using immunohistochemistry in colorectal cancer patients with unresectable metastases that had been treated with the FOLFOX regimen. The median OS reported in the study was significantly longer in patients without ERCC1 expression (p=0.0474).

In stage III CC, patients treated with oxaliplatin-based chemotherapy with ERCC1 protein

overexpression had a lower DFS (54%) and OS (60%) than those with negative ERCC1 tumors (72% *vs.* 78%, respectively; p=0.009 for DFS values and p=0.02 for OS values)[20]. By contrast, ERCC1 status did not affect DFS (p=0.62) or OS (p=0.63) in the 5FU group.

We analyzed the association of mRNA expression levels of ERCC1 with OS and DFS in stage III colon cancer patients in this study. Our results indicate no significant association between survival and the mRNA expression levels of ERCC1. This finding suggests that the expression of ERCC1 is not applicable as a predictive factor for this cohort of stage III resected colon cancer patients receiving 5-FU and oxaliplatin-based adjuvant chemotherapy. Similarly, Kim et al[38] in 2015 found that ERCC1 expression was not significantly associated with the 5-year DFS and ERCC1 expression in patients with high-risk stages II and III colon cancer treated with FOLFOX adjuvant chemotherapy, not even in a subgroup analysis of stage III colon cancer patients. The fact that different studies have reported conflicting results may be partly attributable to the fact that mRNA expression levels of ERCC1 may vary at different cancer stages of cancer and between ethnic groups. Indeed, ethnic differences in ERCC1 polymorphic variants associated with altered nucleotide excision repair (NER) function have been observed between Caucasian, African, and Asian populations[39,40].

Multiple retrospective and population-based studies[41-43] have shown that patients with colorectal tumors displaying high loss of DNA mismatch repair (MSI-H) have a more favorable stage-adjusted prognosis than those with MSI-L/MSS tumors. However, the relationship between MSI status and chemotherapy outcome in colon cancer patients remains controversial[44]. In our study, the 5-year DFS and OS showed no statistically significant difference in either treatment group between MSS and MSI-H; however, an approximate 2-fold improved survival trend was observed in stage III colon cancer patients with MSI-H than MSH-L/MSS subgroups irrespective of the treatment regimen. In the PETACC3 trial, 600 stage II and III patients receiving 5-FU treatment displayed a significant difference in 5-year DFS between MSI and MSS tumors (p=0.0077), suggesting that MSI-improved prognosis can be maintained under adjuvant 5-FU[45,46]. By contrast, a systematic literature review of the predictive effect of MSI status in colorectal cancer in patients undergoing 5-FU based chemotherapy showed no significant survival differences between MSI and MSS[47]. An analysis of the Multicentre International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC) evaluated MMR status in 986 of the 2,240 patients enrolled[48]. In a modest number of patients with MSI-H colon cancers, a DFS benefit from FOLFOX compared with 5-FU alone was observed[49]. Hence, as reported in the literature, this putative association requires further investigation[44].

This study has some notable limitations: first, this study has a retrospective design, so selection bias and potential confounders may influence the results, and second, the sample size was too small to reliably determine the association between ERCC1 expression and DFS. Thus, more prospective studies with larger sample sizes are required.

Conclusions

The above results suggest that curatively resected stage III CC patients exhibiting wild-type KRAS status might benefit from FP alone. If confirmed in prospective studies, this finding could lead to a better patient selection, avoiding oxaliplatin and unnecessary toxicities. On the contrary, an oxaliplatin-containing regimen should be recommended in all KRAS mutated patients.

Funding Acknowledgments

This study was funded by Foundation Nelia and Amedeo Barletta.

Conflict of Interests

The authors declare that they have no conflict of interest.

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