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ERCC1, as Prognostic and Predictive Marker in Metastatic Colorectal Cancer Patients Treated with Oxaliplatin based Chemotherapy

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Abstract

Background and objectives: Excision repair cross-complementation group 1 gene (ERCC1) encodes an enzyme that is essential for the efficient repair of DNA damage induced by platinum compounds such as Oxaliplatin agent. Our study investigated the association between ERCC1 protein expression and clinical outcomes of metastatic colorectal cancer (CRC) patients administrated with combination Oxaliplatin (OX) and 5-fluorouracil (5-FU) treatment.

Patients and methods: Immunohistochemistry (IHC) analysis of the expression of ERCC1 protein was performed on tumor tissue of 30 patients with metastatic CRC, who received first line Oxaliplatin /5-fluorouracil treatment. Tumor response, progression free survival (PFS) and overall survival (OS) were evaluated.

Results: there was no significant association between ERCC1 expression and response to chemotherapy (p=0.880) or PFS (p=0.133) was observed, however patients negative for ERCC1 expression had a better OS than those positive for ERCC1 (p=0.043).

Conclusion: ERCC-1 expression is a good prognostic marker in patients with metastatic CRC who were treated with Oxaliplatin/5-fluorouracil, but may not be useful for the prediction of chemotherapeutic response in these patients.

Keywords: ERCC1, metastatic CRC, Oxaliplatin, survival, tumor response, prognostic marker, predictive marker, IHC.

Background

Colorectal cancer (CRC) is a major cause of cancer related death worldwide. CRC is the third most commonly diagnosed cancer in males and the second in females, with 1.4 million new cases and almost 694,000 deaths estimated to have occurred in 2012 [1]. The introduction of new cytotoxic and targeted agents for patients with metastatic CRC (mCRC) has improved overall survival(OS) rates, with expected median survival now in excess of 20 months with many patients surviving beyond two years[2]. The current treatment paradigm consists of 5-Fluorouracil based regimens in combination with either Oxaliplatin (FOLFOX) or irinotecan (FOLFIRI), potentially combined with therapy targeting either EGFR or VEGFR[3]. Prior large, prospective clinical trials have shown that when used as first line therapy options, response rates (RR) for either FOLFOX or FOLFIRI are around 55% [4]. Currently, oncologists have limited diagnostic tools to predict which first line chemotherapy option is best for an individual patient. Choice of first line therapy is of great importance in mCRC as it has been shown that patients who respond to first line therapy have longer OS[5].The cytotoxic effects of Oxaliplatin are principally attributable to the formation of bulky platinum-DNA adducts, and these adducts are recognized and repaired by the nucleotide excision repair (NER) pathway, which is a major cellular defense mechanism against the cytotoxic effects of platinum-based chemotherapeutic agent. The ERCC1 protein is major component of the NER complex, acting as the rate-limiting enzyme in the NER pathway[6]. ERCC1 expression levels have been previously described as potentially promising biomarkers in metastatic CRC [7]. Patients with low levels ofERCC1 expression have been reported to have an improved response and a longer OS in gastrointestinal tumors treated with FOLFOX [8].

We conducted this study to evaluate the potential prognostic and predictive role of ERCC1 protein expression as detected by immunohistochemistry (IHC)on tumor tissue of the metastatic colorectal cancer

patients treated with first line Oxaliplatin and 5-fluorouracil chemotherapy

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Patients and Methods

Patient population:

Between June 2013 and May 2015, a total of 30 CRC patients treated at the South Egypt Cancer Institute were recruited to participate in this study after approval of the local ethics committee and patients consent.

Inclusion criteria:

Patients of both gender, aged ≥18 years with histologically confirmed colorectal adenocarcinoma; metastatic disease at initial diagnosis according to American Joint Committee on Cancer and the Union for International Cancer Control (AJCC-UICC); 7th Edition. ECOG performance state ≤ 2, adequate hematological, renal and hepatic functions were included in the study.

Exclusion criteria:

Patients have been treated with prior palliative chemotherapy. Also, patients with inadequate organ functions or serious uncontrolled concomitant disease that would contraindicate the use of any of the chemotherapy drugs or interfere with cycle's regularity were excluded from the study.

Work-up:

The routine diagnostic work-up included clinical examination, CT scans of the abdomen, pelvis within 3 weeks before starting treatment, Chest image, blood sampling for complete blood count, renal and hepatic functions .Serum level of tumor marker CEA.

Treatment Schedule:

All patients received FOLFOX-4 (Oxaliplatin 85 mg/m^2 infused for 2 hours, on day 1.Leucovorin 200 mg IV over 2 hours, on days 1-2, before 5-FU.5-FU 400 mg/m^2 IV bolus, then continuous infusion of 600 mg/m^2 for 22 hours, on day 1–2, in a 2-week cycle).Or XELOX (Oxaliplatin 130 mg/m^2 infused for 2 hours, day 1.Xeloda 850 – 1000 mg/m^2, po, twice daily for 14 days followed by a7-day treatment-free interval in a 3-week cycle)regimen treatment. Treatment continued until disease progression, unacceptable toxicity or maximum response. The toxic effects were evaluated using the national cancer institute common toxicity criteria [9].According to the standard practice of our institution, in case of any grade III toxicity; treatment was interrupted until recovery and then restarted with a 20% dose reduction. Treatment was permanently stopped in case of any grade IV toxicity. Tumor response measured by the same method of assessment and same technique used to characterize each identified and reported lesion at baseline. Assessment was done every two cycles, accordance to the Response Evaluation Criteria in Solid Tumors (RECIST).

Immunohistochemical (IHC) staining

Briefly, four µm thick sections of each patient's sample were deparaffinised and rehydrated. The antigen retrieval for ERCC1 was performed with 0.01 mol/l citrate buffer (PH 6.0) in an 800 W microwave for 12 minutes. After blocking of endogenous peroxidase and non-specific reactions, sections were incubated for an hour at room temperature with diluted primary antibody (ERCC1 1/200; Primary mouse monoclonal ERCC-1 antibody (Clone 8F1) Novus biologicals). Ultra Vision Detection System Anti-Polyvalent, HRP/DAB (Ready-To-Use) [LAB VISION Corporation, catalogue #TP-015-HD, Fremont, California 94539-6406, USA] was used as visualization system following manufacturer's instructions. Immunohistochemical staining was developed using diaminobenzidine (DAB) as the substrate for 5 minutes. Sections then counterstained in Mayer's hematoxyline, dehydrated and mounted with DPX. Sections from Human tonsils used as positive control as recommended by the manufacturer datasheet. ERCC-1 positivity was identified as brownish nuclear staining [10].

Evaluation of Immunohistochemistry

The immunoreactivity was evaluated by a single pathologist without prior knowledge of the clinicopathological features and outcome data. The proportion of cells with ERCC1 expression was rated as follows:0 points for <5% positive tumor cells; 1 point for 5–25% positive cells; 2 points for 26–50%positive cells; 3 points for 51–75% positive cells; and 4 points for >75%positive cells, and the staining intensity graded as 0 for no staining, 1 for weak staining, 2 for moderate staining, and 3 for strong staining. The specimens were categorized into two groups according the overall immunoreactivity score (IRS) score into: ERCC1 negative (0-1point), and ERCC1 positive (≥2points) as previously described [11], (Figure 1).

Statistical analysis

All statistical analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, IL). Data were described as frequencies (percentages). Differences in distributions between the variables examined were analyzed by chi-square test. PFS was defined as the time from the start of treatment to the time of the first record of progression or to the date of death. OS was assessed as the time from the initiation of first-line chemotherapy to death from any cause or last follow-up. Survival curve was estimated with the Kaplan-Meier method and compared using the log-rank test. A P-value <0.05 was considered statistically significant.

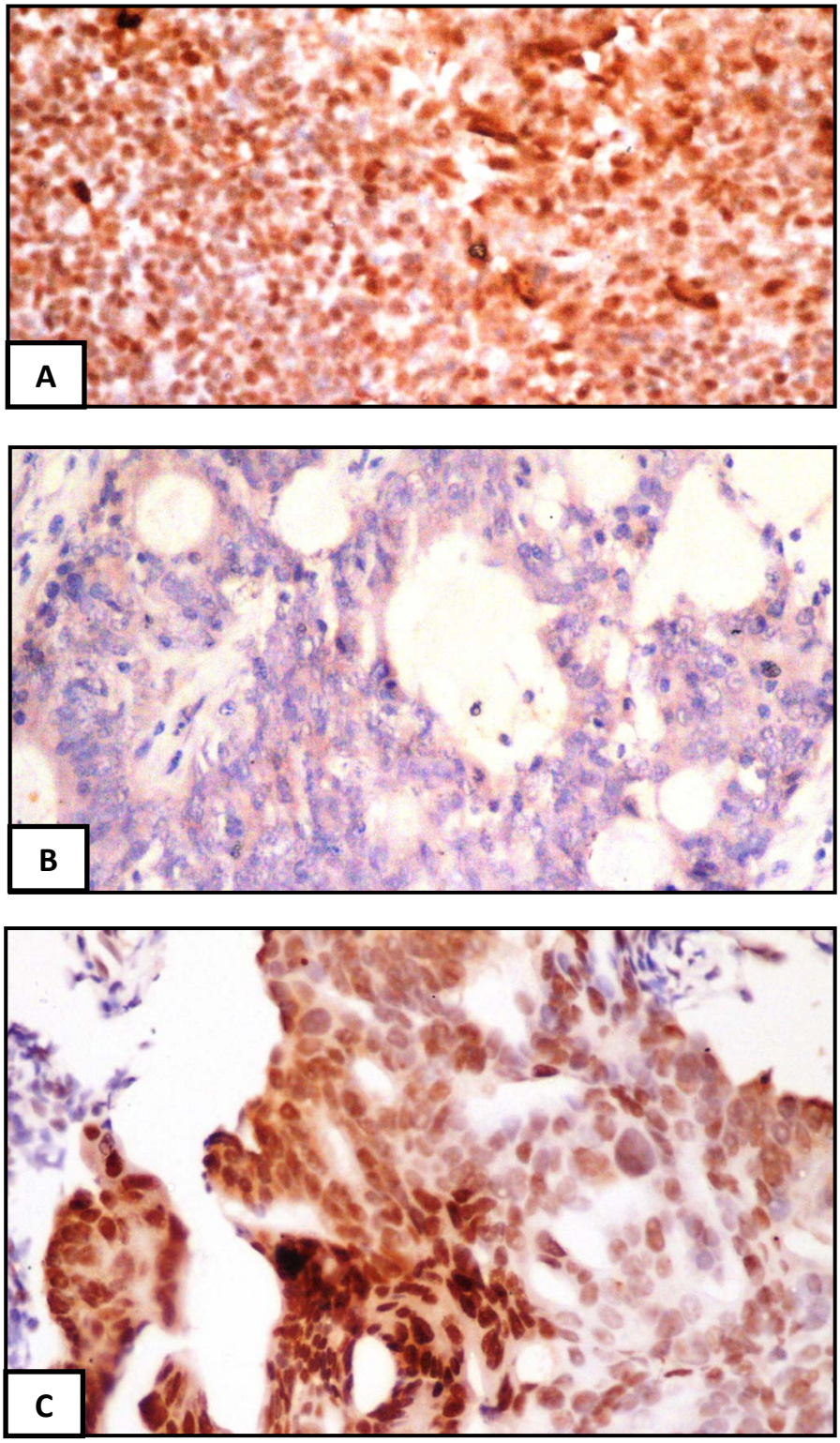

Figure 1: Immunohistochemical staining of ERCC1in colorectal tumor tissue. ERCC1 expressed as a brown nuclear staining. (A) human tonsil, as a positive control ; (B) Cancer colon tissue show negative expression; (C) cancer colon tissue show positive nuclear staining of ERCC1 expression

Results

The demographic data of the 30 patients with metastatic CRC are shown in (Table 1).The median age at diagnosis was 40 years (range, 19-69 years) with mean 41.2±12.3. We followed the patients until May 2015 with a median follow-up period of 19 months (range, 3– 24 months). ERCC1 expression was positive in10 cases (33.3%) while 20 cases (66.7%) had negative expression.

Table (1): The demographic characteristics of the 30 colorectal cancer patients in the study

Variable	No. (%)
Age	
- <40	16 (53.3%)
- ≥40	14 (46.7%)
Performance	
- ≤1	21 (70%)
- >1	9 (30%)
Gender	
- Male	15 (50%)
- Female	15 (50%)
Tumor site	
- Colon	13 (43.3%)
- Rectum	17 (56.7%)
Tumor histopathology	
- Adenocarcinoma	21 (70%)
- G1	2 (6.7%)
- G2	12(40%)
- G3	7 (23.3%)
- Other variants	9 (30%)
CEA (post-operative)	
- Normal	14 (57.6%)
- Abnormal	16 (42.4%)
ERCC1 expression	
- Positive	10 (33.3%)
- Negative	20 (66.7%)
Treatment	
- FOLFOX-4	29 (96.7%)
- XELOX	1 (3.3%)

Pattern of treatment related toxicity

Concerning the main treatment side effects; neurotoxicity, GIT toxicity (vomiting / diarrhea), myelo-suppression and hepatic toxicity are shown in (Table2).most cases were grades I or II. Three patients were irregularly on treatment due to grades II-III toxicity. Oxaliplatin and 5-FU doses were reduced by 20% in two patients due to grade III toxicity.

Tumor Response according to ERCC1 expression

The overall response rate for palliative first-line Oxaliplatin-based chemotherapy was 30% (9 of 30 cases). (Table 3) outlines the overall response by the levels of ERCC1 expression. There were no significant association between response to treatment and the ERCC1 expression (p=0.880). The only one patient who achieved CR was negative for ERCC1 expression in tumor tissues. There was a slight trend to better disease control rate (CR+PR+SD) among patients with negative ERCC1 (55% vs. 50 %.).

Survival analysis according to ERCC1 expression

Although the Median PFS was (5.5 vs. 9.5 months) in positive and negative ERCC1 respectively, there was no significant association between ERCC1 expression and PFS in this study (p=0.133; Figure 2).The OS was significantly higher in patients with negative ERCC1 tumors (75%) than in patients with positive ERCC1 tumors (25%, HR: 9.4; 95% CI: 7.3–12.7; p=0.043). The Median OS was (5.5 vs. 7 months) in positive and negative ERCC1 respectively (Figure 3).

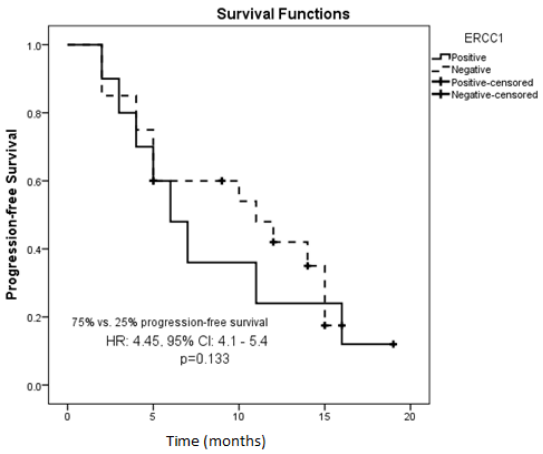

Figure 2: Relation between ERCC1 expression and PFS

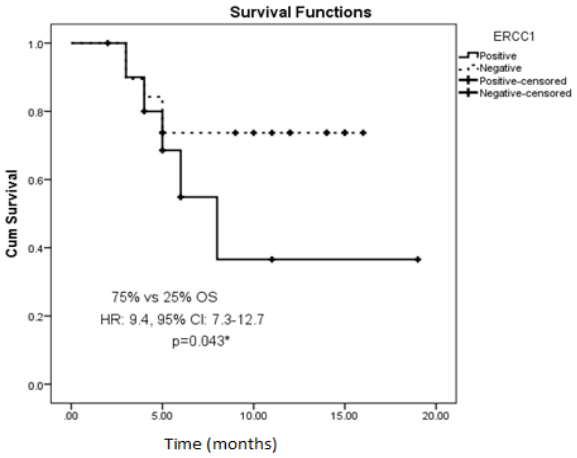

Figure 3: Relation between ERCC1 expression and OS

Table 2: The main side effects of chemotherapy

Side- effects	Anemia	Neutropenia	Thrombocytopenia	GIT	Neurotoxicity	Hepatic
GI	**13**	**5**	**2**	**5**	**6**	**2**
GII	**5**	**2**	**1**	**2**	**1**	**1**
GIII	**2**	**2**	**1**	**1**	**0**	**0**
GIV	**0**	**0**	**0**	**0**	**0**	**0**
Percent (%)	**66.7**	**30%**	**13.3%**	**26.7%**	**23.3%**	**10%**

Table 3: Tumor response according to ERCC1 expression

Response	ERCC1				P. value
	Positive (n=10)		Negative (n=20)		
	No.	%	No.	%	
CR	0	0.0	1	5.0	0.880
PR	3	30.0	5	25.0	
SD	2	20.0	5	25.0	
PD	5	50.0	9	45.0	

* Significant difference ($p<0.01$)

Discussion

FOLFOX and FOLFIRI, the two major backbone chemotherapy regimens used to treat metastatic CRC have been shown to have equivalent response rates in prior large, prospective clinical trials[4]. However, large proportion of patients with metastatic disease display varying levels of treatment resistance, indicating that the therapeutic efficacy has a remarkable inter-individual variability. Hence, it is important to find biomarkers that might enable the selection of which chemotherapy regimen offers the greatest chance for response in an individual patient. Several studies have investigated the influence of ERCC1 in resistance to platinum compound in CRC patients. The majority of which revealed that patients with low levels of ERCC1 protein expression were associated with favorable clinical outcomes of platinum based anti-cancer chemotherapy [12]. This finding is consistent with the known function of ERCC1 in DNA repair following platinum therapy. Cancer cells with ERCC1 overexpression may have higher DNA repair capacity that could effectively reduce the anticancer effect of Oxaliplatin, leading to poor prognosis of these patients. Moreover, ERCC1 expression often possesses a high DNA-repair capability, and so, upon exposure to Oxaliplatin, will undergo relatively less apoptosis. Apoptosis is also one of the main mechanisms through which platinum compounds exert their antineoplastic activity, so less apoptosis is related to poor therapy efficacy and leading to treatment failure[13].Shirota et al,2001[7]found that low mRNA ERCC1 expression is related to better survival in irinotecan-resistant CRC patients treated with OX. Lenz study found that the advanced CRC with high expression of ERCC1 mRNA should not accept OX-based chemotherapy [14].In addition, Opus study found that Patients in the FOLFOX4 arm in the high ERCC1 expression group had shorter PFS, overall survival and a lower response rate compared with those in the low ERCC1 expression group[15].Similarly, our study showed that patients with highERCC1 expression had poor OS. However, our results showed no significant difference in response rates or PFS according to ERCC1expression, this may have been due to the small sample size in this study. Nevertheless, several studies have reported various outcomes regarding the predictive correlation between ERCC1 and FOLFOX chemotherapy in metastatic CRC patients. Ishibashi et al, 2010[16] and Jaeet al, 2014 [17] also found no significant association between ERCC1 expression and response to chemotherapy.

In summary, our findings showed that ERCC-1 expression is a good prognostic marker in patients with metastatic CRC who were treated with Oxaliplatin/5-fluorouracil, but may not be useful for the prediction of chemotherapeutic response in these patients.

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