African Journal of Pharmacy and Pharmacology Vol. 6(32), pp. 2429-2432, 29 August, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP12.862
ISSN 1996-0816 ©2012 Academic Journals

*Full Length Research Paper*

# ERCC1 and ERCC2 polymorphisms predict the clinical outcomes of oxaliplatin-based adjuvant chemotherapy in colorectal cancer

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Accepted 17 August, 2012

**Platinum agents have shown to be effective in the treatment of colorectal cancer. We conducted a prospective study to investigate the effect of excision repair cross-complementation group 1 (*ERCC1 rs11615C>T*) and Excision repair cross-complementation group 2 (*ERCC2 rs13181T>G*) on the efficacy of oxaliplatin-based adjuvant chemotherapy in colorectal cancer patients. A total of 335 cases newly diagnosed by histologically procedure as primary advanced colorectal cancer were collected. The genotypes of *ERCC1 rs11615C>T* and *ERCC2 rs13181T>G* genotyping was conducted with TaqMan Gene Expression assays using the ABI PRISM®7900HT Sequence Detection System. All the patients were followed up until death or the end of November, 2011. The median follow-up period was 34.6 months, and 195 patients died during the follow-up. Compared with carrying *ERCC1 T/T* genotype, patients with a homozygous *ERCC1 C/C* genotype had a longer survival time. Similarly, the *ERCC2 G/G* genotype carriers had a lower risk of death from colorectal cancer compared with T/T genotype carriers. Our study suggested that the *ERCC1 rs11615C>T* and *ERCC2 rs13181T>G* single-nucleotide polymorphisms (SNPs) could be a predictive marker for the prognosis of colorectal cancer. Further studies are needed to validate the results of our study in Chinese population.**

**Key words:** Excision repair cross-complementation group 1 (*ERCC1 rs11615C>T*), excision repair cross-complementation group 2 (*ERCC2 rs13181T>G*), colorectal cancer, chemotherapy.

## INTRODUCTION

Colorectal cancer is the fifth leading cause of death in Chinese population, and there is an increasing trend of colorectal cancer morbidity in recent years (Steward et al., 2003). Despite the development of treatment in terms of colorectal cancer, the prognosis of colorectal cancer is poor. In recent years, chemotherapy either in the adjuvant or palliative setting is the standard treatment of choice for advanced colorectal cancer. However, interindividual variation was shown in colorectal cancer patients with similar clinical characteristics. Cisplatin or oxalipatin is commonly used with 5-fluorouracil (5-FU) as chemotherapy doublets in the treatment of colorectal cancer. The major feature of oxaliplatin-DNA adducts is to block the DNA replication of cancer cell and thus, to induce death of cell (Faivre et al., 2003; Reed, 2005) and the DNA damage, and repair was modified by the nucleotide excision repair (NER) pathways. Excision repair cross-complementation group 1 (*ERCC1 rs11615C>T*) and Excision repair cross-complementation group 2 (*ERCC2 rs13181T>G*) are the two important proteins in NER pathway. Previous studies showed that the polymorphisms of the two genes have an important role in influencing the response to chemotherapy and chemotherapeutic sensitivity (Ishibashi et al., 2011;

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Kuwabara et al., 2011; Noda et al., 2012). Therefore, the *ERCC1 rs11615C>T* and *ERCC2 rs13181T>G* might be two effective prognostic markers in colorectal cancer patients with chemotherapy treatment.

Previous reports on the association between the two gene polymorphisms and chemotherapy are inconsistent (Ishibashi et al., 2011; Kuwabara et al., 2011; Noda et al., 2012). We therefore, conducted a prospective study to investigate the effect of *ERCC1 rs11615C>T* and *ERCC2 rs13181T>G* on the efficacy of oxaliplatin-based adjuvant chemotherapy in colorectal cancer patients.

## MATERIALS AND METHODS

### Patients

A total of 335 cases newly diagnosed by histologically procedure as primary advanced colorectal cancer were collected. All these patients were treated by 5-FU/oxaliplatin regimen (FOLFOX6) chemotherapy. The clinical data of all patients were obtained from medical record. Exclusion criteria included a history of other malignancies and not a metastatic colorectal cancer.

All patients were treated with six cycles of chemotherapy. The regimen included intravenous leucovorin (400 mg/m$^2$) on the first day, intravenous 5-FU (400 mg/m$^2$) on the first day, followed by an intravenous dose of 2,400 mg/m$^2$ over 46 h, and intravenous oxaliplatin (85 mg/m$^2$) on the first day.

The potential lifestyle factors were included by a uniform questionnaire including sociodemographic characteristics, alcohol, smoking and family history of colorectal cancer.

### DNA extraction and genotyping

The peripheral blood samples were collected from all participants and they were stored at -20°C until DNA extraction. Genomic DNA was extracted by using a Qiagen Blood Kit (Qiagen, Chastworth, CA) according to the manufacturer's protocol.

For each single-nucleotide polymorphism (SNP), a pair of amplification primers and an extension primer was designed by using Assay design 3.1 software. The forward and reverse primers of *ERCC1 rs11615C>T* were 5'-AAGCTGGAAAAGACCCTGCC-3' and 5'-CTCACCTGAGGAACAGGGCA-3', respectively. The forward and reverse primers of *ERCC2 rs13181T>G* were 5'-CCCCCTCTCCCTTTCCTCTG-3' and 5'-AACCAGGGCCAGGCAAGAC-3', respectively. The genotypes of *ERCC1* rs11*615C>T* and *ERCC2 rs13181T>G* genotyping was conducted with TaqMan Gene Expression assays using the ABI PRISM®7900HT Sequence Detection System (Applied Biosystems, Poster City, CA). 20% of all the samples were genotyped again for quality control.

### Statistical analysis

All analyses were performed with SPSS Version 16.0 software (SPSS Inc., Chicago, IL, USA). The overall survival was the time from study entry until death regardless of cause. All statistical tests are two-sided. The Kaplan-Meier method was adopted to estimate survival curves, and the log-rank test was used to compare patients' survival time between genotype groups. Cox's proportional hazards model was used to estimate hazard ratios (HRs) and their 95% confidence intervals (CIs), representing the overall relative risk of relapse or death associated with polymorphisms of *ERCC1 rs11615C>T* and *ERCC2 rs13181T>G*. Primary death from ovarian cancer was defined as the failure event, and the time of survival as the time between diagnosis and death. All surviving patients were censored at the date of last follow-up. Statistical significance was defined as a two-sided P-value of less than 0.05.

## RESULTS

Among 335 patients, 307 enrolled, with a participation rate of 91.6%. All the patients were followed up until death or the end of November, 2011. The median follow-up period was 34.6 months, and 195 patients died during the follow-up. The mean age was 61.5 ± 6.9 years when diagnosed. About 64.7% of the patients were males. Almost all of the patients were adenocarcinoma. People who had a family history of colorectal cancer has short survival rate, and the HR (95% CI) was 1.65 (0.86 - 3.18) (Table 1).

Table 2 showed the genotype distributions for *ERCC1 rs11615C>T* and *ERCC2 rs13181T>G* among patients with colorectal cancer. Compared with carrying *ERCC1 T/T* genotype, patients with a homozygous *ERCC1 C/C* genotype had a longer survival time, and the median survival time was 43.2 months. The adjusted HR ((95% CI) of *ERCC1 C/C* genotype was 0.20 (0.10 - 0.79) compared with T/T genotype. Similarly, the *ERCC2 G/G* genotype carriers had a lower risk of death from colorectal cancer compared with T/T genotype carriers, and the median overall survival was 41.4 months. The adjusted HR (95% CI) of *ERCC2 G/G* genotype was 0.48 (0.19 - 0.97).

## DISCUSSION

In this present study, we provided evidence of an association between *ERCC1 rs11615C>T* and *ERCC2 rs13181T>G* SNPs and clinical outcomes of colorectal cancer in Chinese population. Our study showed that *ERCC1 rs11615C>T* and *ERCC2 rs13181T>G* SNPs could play an important role in the efficacy of chemotherapy for colorectal cancer, and thus to be associated with the survival of patients.

The oxaliplatin-based adjuvant chemotherapy is a very common chemotherapy for the treatment of patients with advanced colorectal cancer. However, there are few studies on the predictive factors of patients' response to the chemotherapy. Previous studies suggested that *ERCC1 rs11615C>T* and *ERCC2 rs13181T>G*, the two key enzymes of the NER pathway, play an important role in the prognosis of various cancers, such as bladder cancer, lung cancer, esophageal cancer and breast cancer as well as colorectal cancer (Kawashima et al., 2012; Das et al., 2012; Leichman et al., 2011; Goyal et al., 2010; Kuwabara et al., 2011). The proteins of NER pathway are thought to be repair DNA damage caused by platinum agents, and the polymorphisms of the enzyme in NER pathway could influence the DNA repair capability

**Table 1.** Survival of colorectal patients according to the demographic and clinical characteristics.

| Variable | Cases (%) N = 307 | Patient deaths (%) N = 195 | Five-year survival rate (%) | HR (95% CI) |
|---|---|---|---|---|
| Age (mean ± SD, years) | 61.5 ± 6.9 | | | |
| **Gender (%)** | | | | |
| Female | 108 (35.3) | 67 (34.5) | 36.7 | - |
| Male | 199 (64.7) | 128 (65.5) | 34.4 | 0.97 (0.67 - 1.41) |
| **Smoking** | | | | |
| No | 142 (46.4) | 85 (43.4) | 40.6 | - |
| Yes | 165 (53.6) | 110 (56.6) | 32.9 | 1.11 (0.76 - 1.63) |
| **Drinking** | | | | |
| No | 207 (67.3) | 126 (64.8) | 38.8 | - |
| Yes | 100 (32.7) | 69 (35.2) | 31.6 | 1.13 (0.76 - 1.68) |
| **Family history of colorectal cancer** | | | | |
| No | 284 (92.4) | 172 (45.7) | 39.4 | - |
| Yes | 23 (7.6) | 23 (11.9) | 0.5 | 1.65 (0.86 - 3.18) |
| **TNM stage** | | | | |
| III | 165 (53.6) | 89 (45.7) | 44.8 | - |
| IV | 142 (46.4) | 106 (54.3) | 24.2 | 1.38 (0.97 - 1.98) |
| **Histological subtype** | | | | |
| Other | 140 (45.7) | 80 (41.2) | 41.6 | - |
| Adenocarcinoma | 167 (54.3) | 115 (58.8) | 29.9 | 1.21 (0.84 - 1.73) |
| **Location** | | | | |
| Colon | 161 (52.5) | 88 (45.3) | 44.1 | - |
| Rectal | 146 (47.5) | 107 (54.7) | 25.4 | 1.34 (0.94 - 1.92) |

**Table 2.** Cox proportional regression analysis for the overall survival of *ERCC1 rs11615C>T* and *ERCC2 rs13181T>G* gene polymorphisms among colorectal cancer patients.

| Genotype | No. of patients (%) N = 307 | Patient deaths N = 195 | Median overall survival (Months) | HR (95% CI) | HR (95% CI)[1] |
|---|---|---|---|---|---|
| ***ERCC1 rs11615C>T*** | | | | | |
| T/T | 146 (47.6) | 104 (53.2) | 29.3 | - | - |
| C/T | 128 (41.7) | 79 (40.5) | 31.7 | 0.86 (0.58 - 1.28) | 0.82(0.49 - 1.07) |
| C/C | 33 (10.7) | 12 (6.3) | 36.8 | 0.51 (0.22 - 1.07) | 0.43(0.18 - 0.96) |
| ***ERCC2 rs13181T>G*** | | | | | |
| T/T | 143 (46.6) | 102 (52.4) | 28.9 | - | - |
| T/G | 130 (42.5) | 81 (41.5) | 30.8 | 0.87 (0.59 - 1.29) | 0.75(0.49 - 1.13) |
| G/G | 33 (10.9) | 12 (6.1) | 35.1 | 0.51 (0.22 - 1.07) | 0.43(0.15 - 0.94) |

[1], Adjusted for sex, age, TNM stage, histological subtype and location.

and efficacy of chemotherapy. The inactive of *ERCC1 rs11615C>T* and *ERCC2 rs13181T>G* had low activity of DNA repair and could induce the better efficacy of chemotherapy and longer survival of patients. Our study showed that the inactive genotypes of the two genes had longer survival of colorectal cancer. However, the results on the polymorphisms of two genotypes in colorectal cancer are inconsistent. Two studies conducted in Japan stated that *ERCC1* was not related to unresectable colorectal cancer (Ishibashi et al., 2010; Kuwabara et al., 2011). However, a meta-analysis showed that *ERCC1 rs11615C>T* polymorphisms are useful prognostic factors in oxaliplatin-based treatment of colorectal cancer. The inconsistency of these studies may be explained by differences in population background, source of subjects, sample size and by chance.

This study has several major limitations. Firstly, there was a certain risk of selection bias since they were not a random sample of the general population and may not fully represent the underlying base population. Secondly, due to selecting patients in one hospital, we only included 307 patients in our study. The sample size could limit the statistic power to find the difference, especially for the slightly rate of variant alleles. Further large sample study is still needed. Finally, we could only find few evidence for the role of the two gene polymorphisms in Chinese population. Future studies on the two gene polymorphisms are still needed.

Therefore, our study suggested that the *ERCC1 rs11615C>T* and *ERCC2 rs13181T>G* SNPs could be a predictive marker for the prognosis of colorectal cancer. Further studies are needed to validate the results of our study in Chinese population.

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