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Short Communication

Polymorphisms of folate metabolism-related genes and survival of patients with colorectal cancer in the Korean population

Moon Ju Jang [a,1], Jong Woo Kim [b,1], Young Joo Jeon [c], So Young Chong [a], Sung Pyo Hong [a], Seong Gyu Hwang [a], Doyeun Oh [a], Yun Kyung Cho [d], Young Geon Ji [e], Nam Keun Kim [b,*]

[a] Department of Internal Medicine, School of Medicine, CHA University, Seongnam, South Korea
[b] Department of Surgery, School of Medicine, CHA University, Seongnam, South Korea
[c] Institute for Clinical Research, School of Medicine, CHA University, Seongnam, South Korea
[d] Department of Internal Medicine, CHA Gangnam Medical Center, School of Medicine, CHA University, Seoul, South Korea
[e] Department of Preventive Medicine, CHA University, Seongnam, South Korea

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ABSTRACT

Background: 5-Fluorouracil (5-FU) is a cornerstone of chemotherapy for colorectal cancer (CRC), and the major targets of 5-FU are thymidylate synthase (TS), methylenetetrahydrofolate reductase (MTHFR), and reduced folate carrier 1 (RFC1). We hypothesized that polymorphisms in the genes encoding these proteins would be associated with CRC patient survival.
Patients and methods: We genotyped the following polymorphisms in 372 CRC patients: *TS* enhancer region (TSER), *TS* 1494del6, *MTHFR* 677C>T and 1298A>C, and *RFC1* −43T>C, 80G>A, and 696C>T. Using Kaplan–Meier curves, log-rank tests, and Cox proportional hazard models, we evaluated associations between these polymorphisms and overall survival (OS).
Results: The combined *TS* 1494 0bp6bp + 6bp6bp genotype was associated with reduced OS compared to the *TS* 1494 0bp0bp genotype. Among rectal cancer patients, the *RFC1* −43CC and 80AA genotypes were associated with favorable OS.
Conclusions: Our data suggest that *TS* and *RFC1* polymorphisms are associated with CRC prognosis in Korean patients. Further studies are needed to verify these findings.

1. Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer-related death in Western countries (Jemal et al., 2009). Although incidence rates are lower in Asian than Caucasian populations, a recent study has demonstrated that the incidence of CRC has been increasing every year in Korea (Jung et al., 2011). 5-Fluorouracil (5-FU)-based regimens have been used as the standard treatment in adjuvant and metastatic chemotherapy. Previous studies have reported that adjuvant therapy with a 5-FU-based regimen results in a 7% improvement in the 8-year overall survival (OS) rate, and palliative chemotherapy with a 5-FU-based regimen also increases the median survival time, to approximately 6 months (Sargent et al., 2009; Simmonds, 2000). Thus, 5-FU has become a cornerstone of chemotherapy for CRC patients.

Thymidylate synthase (TS), which catalyzes the methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), is the primary target of 5-FU. The generation of dTMP provides thymidine, a rate-limiting nucleotide essential for DNA synthesis and repair. The active metabolite of 5-FU, fluorodeoxyuridine monophosphate, binds to the nucleotide-binding site of TS, forming a stable complex with TS, for which folate acts as a co-factor; fluorodeoxyuridine monophosphate blocks binding of the complex's normal substrate, dUMP, and inhibits dTMP synthesis (Longley et al., 2003). Several studies have demonstrated an association between TS levels and the response to 5-FU (Leichman et al., 1997; Salonga et al., 2000). Previous studies have also reported that polymorphisms in the *TS* gene are associated with TS protein levels in CRC (Mauritz et al., 2009; Morganti et al., 2005; Yu et al., 2008). Thus, germline polymorphisms in this gene may affect its regulation.

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism and DNA synthesis. It catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate; the latter is the methyl donor for the conversion of homocysteine to methionine, whereas the former, and its derivatives, are essential cofactors for both

Abbreviations: 5-FU, 5-fluorouracil; CRC, colorectal cancer; TS, thymidylate synthase; TSER, thymidylate synthase enhancer region; MTHFR, methylenetetrahydrofolate reductase; RFC1, reduced folate carrier 1; OS, overall survival; RFS, relapse-free survival; HR, hazard ratio; CI, confidence interval.

* Corresponding author at: Institute for Clinical Research, School of Medicine, CHA University, 351, Yatap-dong, Bundang-gu, Seongnam, 463–712, South Korea. Tel.: +82 31 780 5762; fax: +82 31 780 5766.
E-mail addresses: nkkim@cha.ac.kr, namkkim@naver.com (N.K. Kim).

[1] Contributed equally to this work.

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thymidylate and de novo purine synthesis (Slattery et al., 1999). Decreased MTHFR enzymatic activity may lead to accumulation of 5,10-methylenetetrahydrofolate, thereby improving the efficacy of 5-FU treatment through stabilization of the ternary complex (Kawakami et al., 2003). Additionally, because reduced folate carrier 1 (RFC1) is involved in the metabolism of folate co-factors, it may modulate 5-FU clinical efficacy through TS inhibition.

Polymorphisms in genes encoding folate metabolism-related enzymes, including *TS*, *MTHFR*, and *RFC1*, may alter gene activity, influence clinical responsiveness to 5-FU therapy, and, consequently, affect CRC patient outcomes. However, previous epidemiological studies conflict regarding the effects of *TS*, *MTHFR*, and *RFC1* polymorphisms on the survival of CRC patients, and past studies have been conducted predominantly in Caucasian populations. In the present study, we investigated whether a polymorphism in the enhancer region of the *TS* gene (TSER, rs34743033), *TS* 1494del6 (rs16430), *MTHFR* [677C>T (rs1801133) and 1298A>C (rs1801131)], and *RFC1* [−43T>C (rs1131596), 80G>A (rs1051266), and 696C>T (rs12659)] influenced the outcomes of patients with surgically-resected CRC in Korea.

2. Materials and methods

2.1. Study population

Blood samples were collected from 372 Korean patients who underwent surgical resection at CHA Bundang Medical Center (Seongnam, Korea) between June 1996 and January 2009. The study included only patients who underwent surgical resection with curative intent and had histologically-proven adenocarcinomas. We retrospectively obtained information concerning the date of diagnosis, pathological stage, relapse, and death. The American Joint Committee on Cancer: Classification and Stage Groupings, 6th edition, was used for tumor staging. All study subjects were ethnic Koreans and provided written informed consent. The study protocol was approved by the Institutional Review Board of CHA Bundang Medical Center, Seongnam, South Korea.

2.2. Genetic analyses

DNA was extracted from leukocytes using a G-DEX™ II Genomic DNA Extraction kit (Intron Biotechnology, Seongnam, Korea), according to the manufacturer's instructions. Nucleotide polymorphisms were characterized using polymerase chain reaction–restriction fragment length polymorphism analyses. Primers and PCR conditions for each polymorphism analysis were as described previously (Chung et al., 2012; Kim et al., 2011; Rah et al., 2012; Yim et al., 2010). The accuracy of these genotyping methods was confirmed by sequencing 20% of the samples, chosen randomly. The concordance rate was 100%.

2.3. Statistical analysis

The genotypes for each single nucleotide polymorphism were analyzed as a three-group categorical variable (reference model) and were also grouped according to dominant and recessive models. Haplotype frequencies were estimated using the PHASE program. The Pearson's chi-squared test was used to calculate the significance of the differences in frequencies between two or more groups. OS was defined as the time from surgery until death or the last follow-up; relapse-free survival (RFS) was defined as the time from surgery until relapse or the last follow-up. Survival curves were created according to the Kaplan–Meier method, and the log-rank test was used to assess the significance of differences between groups. Cox regression models were used to analyze the independent prognostic importance of various markers, and results were adjusted for age, gender, tumor differentiation, tumor site, chemotherapy, and cancer stage. Hazard ratios (HRs) are presented with a 95% confidence interval (CI). All tests were two-tailed, and a *P*-value less than 0.05 was deemed to indicate a statistically significant difference. All statistical calculations were performed using SPSS (ver. 17; SPSS, Chicago, IL, USA).

3. Results

Baseline characteristics of the study population are described in Table 1. The 372 patients were comprised of 215 men and 157 women, and the mean age was 62.1 ± 12.1 years. Of the 372 patients, 221 (59.4%) had colon cancer and 151 (40.6%) had rectal cancer. Regarding histological differentiation, 318 patients (85.5%) had moderately differentiated cancer, 35 patients (9.4%) had well differentiated cancer, and 19 patients (5.1%) had poorly differentiated cancer. Pathological staging after curative resection was as follows: 34 patients (9.1%) had stage I cancer, 166 (44.6%) had stage II, 141 (37.9%) had stage III, and 31 (8.3%) had stage IV. Three hundred thirty-one (89.0%) patients received adjuvant chemotherapy, whereas 41 (11.0%) patients had non-adjuvant chemotherapy. Participants were followed for a median of 34 months (range, 4–173 months). The estimated 3-year OS and RFS rates for all patients were 79.0% and 79.3%, respectively (Table 1).

Seven folate metabolism-related gene polymorphisms were amplified. The genotype frequencies of these polymorphisms conformed to Hardy–Weinberg equilibrium ($P > 0.05$). The associations between these polymorphisms and patient clinicopathological factors are shown in Table 2. Tumor, node, metastasis stage III + IV, and lymph node invasion were associated with the *MTHFR* 677CT + TT, *TSER* 2R2R, *RFC1* −43TC + CC, *RFC1* 80AA, and *RFC1* 696TT genotypes. Additionally, compared to rectal cancer patients, colon cancer patients harbored a higher proportion of the *RFC1* −43CC genotype.

The associations between these polymorphisms and CRC patient survival are shown in Table 3. Multivariate Cox proportional analysis showed that the *TS* 1494 0bp6bp genotype had a significant effect on survival in the dominant model. Compared with the *TS* 1494 0 bp/0 bp genotype, the combined *TS* 1494 0bp6bp+6bp6bp genotype was associated with poor OS (HR = 1.91; 95% CI, 1.21–3.01; $P = 0.006$; Fig. 1A) and RFS (HR = 1.84; 95% CI, 1.16–2.90; $P = 0.010$; Fig. 1B). However, no associations between the other polymorphisms in folate metabolism-related genes and survival outcomes were found.

Next, we performed stratified analyses according to tumor size, site, and stage (Table 4). In the stratified analyses, we found that the

Table 1
Patient characteristics.

Characteristics	n = 372 (%)
Age, mean ± SD	62.11 ± 12.08
Gender, male	215 (57.8)
Primary tumor site	
Colon	221 (59.4)
Rectum	151 (40.6)
Histological differentiation	
Well	35 (9.4)
Moderate	318 (85.5)
Poor	19 (5.1)
Tumor size	
<5 cm	158 (42.5)
≥5 cm	214 (57.5)
Lymph node invasion	
No	202 (54.3)
Yes	170 (45.7)
TNM stage	
I	34 (9.1)
II	166 (44.6)
III	141 (37.9)
IV	31 (8.3)
Adjuvant chemotherapy	
No	41 (11.0)
Yes	331 (89.0)
Recurrence-free survival rate (3-year)	79.30%
Overall survival rate (3-year)	79.00%

SD, standard deviation; TNM stage, tumor, node, metastasis stage.