Original Research Article

Methylenetetrahydrofolate reductase C677T polymorphism and toxicity to 5-FU-based chemotherapy in colorectal cancer

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Abstract

Purpose: To investigate the toxicity of methylenetetrahydrofolate reductase (MTHFR) polymorphism in colorectal cancer patients treated with 5-fluorouracil (5-FU).
Methods: A total of 105 patients with colorectal cancer who underwent 5-FU therapy were included in this study. MTHFR C677T polymorphisms were determined using direct sequencing. Physical examination and the results of blood and urine tests were used to evaluate the toxicities, including gastrointestinal toxicity, hematopoietic toxicity, hair-skin toxicity and hand-foot syndrome.
Results: In 90.5 % of all patients, 5-FU toxicity was observed. With regard to MTHFR C677T mutation, 45.7 % heterozygote mutants and 19.0 % homozygote mutants were observed. MTHFR C677T polymorphism was statistically related to 5-FU toxicity (p = 0.000). In addition, MTHFR C677T mutation was closely related to hematopoietic toxicity (p = 0.005).
Conclusion: MTHFR C677T can be used for the prediction of 5-FU toxicity, and can also predict hematopoietic toxicity in patients with colorectal cancer.

Keywords: MTHFR genes, Polymorphism, Colorectal cancer, Biomarker, Toxicity

INTRODUCTION

5-fluorouracil (5-FU) has been used in the treatment of various cancers, especially in colorectal cancer (CRC) [1]. 5-FU has no anti-tumor effect, however, it can be involved in reducing DNA synthesis [2].

Methylenetetrahydrofolate reductase (MTHFR) is the most critical enzyme in the metabolism of folate and 5-FU [3]. Therefore, the MTHFR activity may predict the clinical responses and toxicity to 5-FU. The grade and type of toxicities primarily depend on demographic factors including gender, age, dose of 5-FU and different methods of administration [4]. Individual differences in fluoropyrimidine-related toxicity are partly explained by genetic factors.

The *MTHFR* gene is highly polymorphic [5]. One of the most common functional mutations in the *MTHFR*, C677T (rs1801133, A222V) has been identified the main variants in reducing the activity of this enzyme [6]. Therefore, *MTHFR*

C677T polymorphisms are considered a potential predictor of clinical responses and 5-FU toxicity. However, the evidence of genetic association is relatively weak and published results from previous studies are not consistent [7-10].

Therefore, the purpose of this study was to investigate the relationship between *MTHFR* C677T mutations and the clinical responses and toxicity of patients with CRC who underwent 5-FU therapy.

METHODS

Study population

One hundred and five CRC patients who were treated with 5-FU between 2016 and 2018 at the Hubei Hospital were enrolled in this study. Patients were eligible if they were adults, with biopsy-proven clinical T3/T4. Patients were excluded if they underwent pretreatment with any chemotherapeutic regimen, previous pelvic radiotherapy, and were allergic to 5-FU [11]. This study was approved by the ethics committee of the Hubei Hospital (approval no. 20160102) and followed the guidelines of the Helsinki Declaration [12].

Clinical data

Cohort characteristics of the 105 CRC patients are shown in Table 1. Among the 105 patients were 64 males (60.95 %) and 41 females (39.05 %), and the mean age of the patients was 58 ± 19 years (range 25-83). The proportion of tumor location in the colon and rectum was 62.9 % and 37.1 %, respectively. The histopathological types included adenocarcinoma (84.8 %), squamous cell carcinoma (9.5 %) and adenosquamous cell carcinoma (5.7 %). The stage of the tumors

Table 1: Characteristics of patients in the study cohort

Patient profile	Characteristic	Patients (%)
Gender n (%)	Male	60.95
	Female	39.05
Age at diagnosis	≤60	48.6
	>60	51.4
Tumor site	Colon	62.9
	Rectum	37.1
Histopathological type	Adenocarcinoma	84.8
	Others	15.2
Stage	Ⅲ	39.0
	Ⅳ	61.0
Intestinal perforation	Present	11.4
	Not present	88.6
Bowel obstruction	Present	15.2
	Not present	84.8

included stage Ⅲ (39 %), and stage IV (61 %), respectively.

Evaluation of toxicity

All adverse drug reactions and toxicities were recorded. Gastrointestinal toxicity, hematopoietic toxicity, hair and skin toxicity and hand-foot syndrome were defined as previously published. Adverse reactions were classified as grade 1, 2, 3 and 4 as defined as previously published [13].

Genotyping

DNA was extracted from peripheral blood. The DNA was diluted to 10 ng per well, and Polymerase Chain Reaction (PCR) was carried out and the PCR products were genotyped using direct sequencing (ABI 3100 DNA sequencer, Shanghai, China).

Statistical analysis

Data were analyzed using SPSS 19.0 (Chicago, IL, USA) and expressed as the mean ± SEM, or percentage (%). The relationship between genotypes and toxicity, and the Hardy-Weinberg equilibrium (HWE) were determined by Chi-square test. Statistical significance was defined as $p < 0.05$.

RESULTS

The types and grades of patients' toxicity

All patients were followed up in the first four cycles of treatment with 5-FU. Based on the evaluation criteria for adverse events, 90.5 % (n = 95) of patients suffered from toxicities, and the proportions of grades 1, 2, 3 and 4 were 31.4, 25.7, 35.3 and 10.4%, respectively. Details of toxicity classifications are presented in Table 2.

Patient genotypes

As shown in Table 3, no significant differences were observed in the Hardy–Weinberg equilibrium between groups. The genotype frequencies of CC, CT and TT were 35.2, 45.7, and 19.0 %, respectively, while allele frequencies of C and T were 58.1 and 41.9 %.

Gene polymorphism was associated with 5-FU toxicity

At least one of four types of toxicity was observed in patients with MTHFR C677T polymorphism ($p = 0.000$) (Table 4). The MTHFR C677T mutation was related to hematopoietic toxicity ($p = 0.005$, Table 5). However, the

Table 2: Types and grades of toxicity

Toxicity type	Grade of toxicities(%)			
	Grade 1	*Grade 2*	*Grade 3*	*Grade 4*
Gastrointestinal toxicity	5.7	6.7	2.9	0.9
Hematopoietic toxicity	15.2	10.5	23.8	7.6
Hair and skin toxicity	1.9	0.9	2.9	-
Hand-foot syndrome	8.6	7.6	5.7	1.9

MTHFR C677T mutation was not related to other toxicity groups.

DISCUSSION

MTHFR is a key enzyme in the metabolism of 5-FU.and the MTHFR C677T polymorphism can reduce MTHFR enzyme activity [14]. Therefore, the MTHFR C677T polymorphism may be closely related to the efficacy of 5-FU treatment. In this study, 90.5 % patients had adverse reactions when treated with 5-FU, and the MTHFR C677T frequency (45.7 % heterozygote mutants, 19.0 % homozygote mutants) was significantly higher than presented in previous reports [13,15].

The most important finding is that the MTHFR C677T mutation was related to the 5-FU toxicities when 5-FU was used alone, especially for the hematopoietic toxicity. Afzal *et al* reported that the MTHFR C677T polymorphism was closely related to fluoropyrimidine-related toxicity in CRC patients, however, no specific types of toxicity were mentioned [16]. In addition, Noor *et al* found that MTHFR C677T polymorphism increased the tumor response to 5-FU and increased gastrointestinal toxicity in CRC patients [17]. Loganayagam *et al* found that the MTHFR C677T polymorphism was closely related to hand-foot syndrome in CRC patients [18].

Moreover, Lu *et al* showed that the MTHFR C677T polymorphism increased gastrointestinal toxicity in gastric cancer patients [19]. However, Capitain *et al* found that the MTHFR C677T polymorphism was not associated with the toxicity of 5-FU in advanced CRC patients [20]. Based on the above-mentioned results, the relationship between the MTHFR C677T mutation and 5-FU toxicity in CRC patients remains controversial, and the reasons may be as follows.

Table 3: Distribution of genotype and allelic frequencies among all patients

Polymorphism	Amino acid change	Genotype (n)			Allelic frequency	
		CC	*CT*	*TT*	*C*	*T*
MTHFR C677T	A222V	37	48	20	58.1 %	41.9 %

Table 4: Relationship between MTHFR 677C>T mutation and all 5-FU-related toxicities

MTHFR 677C>T	All toxicities (n)		Risk ratio	*P*-value
	-	+		
CC	9	28	15.120	0.000
CT+TT	0	68		

Table 5: Relationship between MTHFR 677C>T mutation and the hematopoietic toxicity of 5-FU

MTHFR 677C>T	Hematopoietic toxicity (n)		Risk ratio	*P*-value
	-	+		
CC	32	35	7.754	0.005
CT+TT	0	12		

First, differences in sample size may lead to differences in results. Second, differences in study population and ethnic differences may have affected the frequency of gene mutations. Third, not only used 5-FU, but also combined with other drugs.

Genetic markers can predict either drug toxicity or efficacy, which is critical for patients who are undergoing treatment with cytotoxic agents. However, there are significant regional and ethnic diversities in genetic polymorphisms in MTHFR C677T, and the frequency of mutations varies widely worldwide. In addition, the results of the study are quite different, therefore, it is important in requiring a degree of homogeneity in the selection of patients and treatments.

Limitations of the study

There is a limitation of the present study that should be considered. The relationship between MTHFR C677T polymorphism and 5-FU-related toxicities in CRC patients needs to be confirmed in a larger sample size.

CONCLUSION

MTHFR C677T can be used for the prediction of both 5-FU toxicity and also hematopoietic toxicity in patients with CRC.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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