# Effect of *HtrA1* Polymorphism on Sensitivity to Chemotherapy in Patients with Colon Cancer

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
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Literature Search F
Funds Collection G

ABC 1,2    Yi Yao
DEG 1    Nan Li

1 Medical School of Chinese PLA, Chinese People's Liberation Army (PLA) General Hospital, Beijing, P.R. China
2 Department of Gastroenterology, Eighth Medical Center of People's Liberation Army (PLA) General Hospital, Beijing, P.R. China

Corresponding Author:    Nan Li, e-mail: sddseeww@126.com
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**Background:**    This study was performed to estimate the genetic effects of *HtrA1* polymorphisms rs1049331 and rs11200638 on treatment response in stage III colon cancer patients receiving 5-FU-based chemotherapy.

**Material/Methods:**    A total of 105 stage III colon cancer patients who received postoperative 5-FU based adjuvant chemotherapy were included in our study. Chemotherapy was performed in 3 cycles for the patients. *HtrA1* rs1049331 and rs11200638 polymorphisms were genotyped via polymerase chain reaction with sequencing method. The treatment response was estimated according to the RECIST guidelines.

**Results:**    The response rate of the eligible patients was 53.33%. For rs1049331, the presences of TT genotype and T allele indicted reduced chemotherapy sensitivity (adjusted TT: OR=1.736, 95%CI: 1.001-3.011, *P*=0.049; T: OR=1.801, 95%CI: 1.054-2.932, *P*=0.039). The rs11200638 polymorphism had no significant association with chemotherapy sensitivity in the study population (*P*>0.05 for all).

**Conclusions:**    *HtrA1* rs1049331 polymorphism, but not rs11200638 polymorphism, can influence individual sensitivity to 5-FU-based treatment in stage III colon cancer patients.

**MeSH Keywords:**    Antineoplastic Agents • Disease Resistance • Heat-Shock Proteins • Polymorphism, Genetic • Sigmoid Neoplasms

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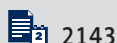
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## Background

Colon cancer is a common digestive tract malignancy and is a leading cause of cancer-related in men and women worldwide [1,2]. The high morbidity rate of colon cancer is closely correlated with a variety of risk factors, such as age, diet, smoking, alcohol, lack of physical activity, inflammatory bowel diseases, family history, exposure to radiation, and toxic factors [3–7]. In China, due to the changes in lifestyle and diet, the incidence and mortality rates of colon cancer have been increasing in recent years [8]. Effective treatment strategies are urgently needed to improve the clinical outcomes of colon cancer patients.

Currently, surgical resection, chemotherapy, radiotherapy, and immunotherapy are the main therapeutic strategies for colon cancer. Surgical treatment is preferred for patients diagnosed at early stages, but not all patients can be cured by surgery, especially those diagnosed at advanced stages. Chemotherapy is an effective strategy for advanced cancer patients. Capecitabine, an oral prodrug of 5-fluorouracil (5-FU), is accepted as the first-line chemotherapy drug for stage III colon cancer patients [9]. However, the clinical benefits of 5-FU-based chemotherapy vary according to individual sensitivity [10]. Chemotherapy sensitivity is correlated with multiple factors, such as cancer heterogeneity and individual genetic patterns. Exploring the individual genetic profile may be an effective way to improve chemotherapy sensitivity in clinical practice.

High-temperature requirement A-1 (*HtrA1*) is a member of a highly conserved family of heat shock proteins and serine proteases. *HtrA1* is involved in a variety of physiological and pathological processes, such as cell proliferation, apoptosis, arthritis, and embryogenesis [11]. *HtrA1* is also correlated with drug resistance [12]. Xiong et al. reported that decreased *HtrA1* expression can induce cisplatin resistance in colon cancer patients [13]. The expression of the *HtrA1* gene can be regulated by its genetic variants, such as single-nucleotide polymorphism (SNP). The genetic effects of *HtrA1* SNP on the response to 5-FU-based therapy in patients with colon cancer remains largely unclear.

In the present study, we investigated the roles of *HtrA1* gene polymorphisms rs1049331 and rs11200638 in chemotherapy sensitivity in unrelated Han Chinese colon cancer patients.

## Material and Methods

### Patients

This study was carried out at the Chinese PLA General Hospital. The experimental procedures were approved by the Ethics Committee of the hospital. All the patients provided signed informed consent before enrollment. A total of 105 stage III colon cancer patients who were treated by surgical resection followed by 5-FU-based adjuvant chemotherapy were included in our study. All the patients were pathologically diagnosed with stage III colon cancer, and none of them had received any preoperative chemotherapy. All the patients received 5-FU-based adjuvant chemotherapy with at least 3 cycles of treatment. The clinical information of patients was collected from their medical records.

### Sample collection

For DNA extraction, a 3-mL fasting peripheral blood sample was collected from each eligible patient before chemotherapy. Genomic DNA samples were isolated from the collected blood specimens using a DNA extraction kit (Tiangen Biotech, Beijing), and then stored at −20°C.

### *HtrA1* polymorphism analysis

*HtrA1* genetic polymorphisms rs1049331 and rs11200638 were genotyped via polymerase chain reaction (PCR) with direct sequencing method. The specific primers were designed using Primer Premier 5.0 software, and the sequences were as follows:
rs 1049331 forward: 5'-AGAGTCGCCATGCAGATCC-3';
reverse: 5'-CACAGGTTGGCGTAGGTGTT-3' [14];
rs11200638 forward: 5'-ATGCCACCCACAACAACTTT-3';
reverse: 5'-CGCGTCCTTCAAACTAATGG-3'.
The PCR reaction was carried out in a total of 20 µl mixture, containing 10 µL PCR Master Mix (2×), 1 µL genome DNA sample, 0.5 µL forward and reverse primers, and 8 µL deionized water. The amplification procedure was designed as follows: an initial denaturation at 94°C for 5 min, 35 cycles with denaturation at 94°C for 1 min, annealing at 54°C for 40 s, extension at 72°C for 45 s, and an additional extension at 72°C for 10 min. PCR products were detected using 1% agarose gel electrophoresis (AGE). Finally, the PCR products were sequenced by Shanghai Sangon Biotech Co. to confirm the genetic distribution.

### Chemotherapy outcomes estimation

The chemotherapy efficacy was estimated after 3 cycles of chemotherapy based on the revised RECIST (Response Evaluation Criteria in Solid Tumors) guidelines, version 1.0 [15]. The evaluation indexes included complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). CR refers to the disappearance of all lesions, PR is defined as a more than 10% reduction of the long diameter of a tumor or more than 15% reduction of tumor density. PD refers to more than 10% enlargement of tumor diameter, or the presence of new lesions. Conditions that could not meet the criteria of CR,

PR, or PD were defined as SD. The chemotherapy sensitivity was estimated by CR+PR, while chemotherapy resistance was evaluated by SD+PD.

## Statistical analysis

All data analyses were performed using SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA). The chi-square test was performed to compare the genotype and allele distribution of *HtrA1* SNPs among the study patients based on their chemotherapy response. The genetic influences of *HtrA1* polymorphisms on chemotherapy sensitivity were evaluated using odds ratio (OR) with 95% confidence interval (CI). The rough results were adjusted using logistic regression analysis. *P* values less than 0.05 indicated the statistical significance of the analysis results.

## Results

### Baseline characteristics of the eligible patients

We included 105 patients diagnosed with stage III colon cancer. There were 69 (65.71%) males and 36 (34.29%) females, with an average age of 54.16±10.79 years. The cancer lesions of 50 (47.62%) patients were located on the right side, while 55 (52.38%) cases had their lesions on the left side. Among the eligible patients, 50 (47.62%) were smokers. Histological examinations demonstrated that 64 (60.95%) cases had moderate differentiation, and the remaining 41 (39.05%) cases showed poor differentiation. According to T staging system, 42 (40%) patients were diagnosed at T1–T2 stages, and 63 (60%) cases were diagnosed at T3–T4 stages. All the patients were treated with 5-FU-based chemotherapy after surgical treatments: 41 (39.05%) patients received 5-FU+levamisol therapy, 52 (49.52%) patients received 5-FU+leucovorin chemotherapy, and 12 (11.43%) patients received Capecitabine treatment. According to the RECIST guidelines, 18 (17.14%) patients achieved CR, 38 (36.19%) reached PR, and 39 (37.14%) exhibited SD. In addition, 9 (8.57%) patients showed PD. The response rate (CR+PR) of the included patients was 53.33%. The demographic and clinical characteristics of the eligible patients are summarized in Table 1.

### Relationship between *HtrA1* polymorphisms and chemotherapy sensitivity

The genotype and allele distributions of *HtrA1* rs1049331 and rs11200638 polymorphisms were assessed. For rs1049331 SNP, the response rate of patients carrying the CC genotype was higher than in those carrying CT and TT genotypes (63.33% *vs.* 55.17% *vs.* 29.41%). Chi-square testing demonstrated that TT was a risk factor for chemotherapy sensitivity in comparison

**Table 1.** Clinical characteristics of eligible patients.

| Characteristics | Case (n=105, %) | |
|---|---|---|
| Mean age (years) | 54.16±10.79 | |
| Sex | | |
|   Male | 69 | (65.71%) |
|   Female | 36 | (34.29%) |
| Tumor location | | |
|   Right side | 50 | (47.62%) |
|   Left side | 55 | (52.38%) |
| Differentiation | | |
|   Moderate | 64 | (60.95%) |
|   Poor | 41 | (39.05%) |
| Smoker | | |
|   No | 55 | (52.38%) |
|   Yes | 50 | (47.62%) |
| T stages | | |
|   T1–T2 | 42 | (40%) |
|   T3–T4 | 63 | (60%) |
| Chemotherapy strategies | | |
|   5-FU+levamisol | 41 | (39.05%) |
|   5-FU+leucovorin | 52 | (49.52%) |
|   Capecitabine | 12 | (11.43%) |
| Chemotherapy response | | |
|   CR | 18 | (17.14%) |
|   PR | 38 | (36.19%) |
|   SD | 39 | (37.14%) |
|   PD | 9 | (8.57%) |
|   CR+PR | 56 | (53.33%) |

with CC genotype (OR=4.145, 95%CI: 1.152–14.918, *P*=0.025). Additionally, 59.32% individuals carrying the C allele showed sensitivity to 5-Fu chemotherapy and 45.65% of those carrying the T allele were sensitive. Presence of the T allele was associated with reduced chemotherapy sensitivity among colon cancer patients (OR=1.736, 95%CI: 1.001–3.011, *P*=0.049). After adjusting for confounding demographic and clinical variables, the TT genotype and T allele of *HtrA1* rs1049331 polymorphism were still significantly correlated with reduced chemotherapy sensitivity (TT: OR=1.736, 95%CI: 1.001–3.011, *P*=0.049; T: OR=1.801, 95%CI: 1.054-2.932, *P*=0.039) (Table 2).

For rs11200638, the response rates of the carriers of GG, GA, and AA genotypes were 62.07%, 50.85%, and 47.06%, respectively. We found that 56.41% of individuals carrying the G allele

**Table 2.** Genetic influences of *HtrA1* polymorphisms on treatment response among stage III colon cancer cases receiving 5-FU-based chemotherapy.

| SNPs | Chemotherapy efficacy | | | Rough results | | Adjusted results | |
|---|---|---|---|---|---|---|---|
| | CR+PR (n, %) | SD+PD (n, %) | Response rate (%) | OR (95% CI) | *P* | OR (95% CI) | *P* |
| rs1049331 | | | | | | | |
| CC | 19 (33.93%) | 11 (22.45%) | 63.33% | Reference | – | Reference | – |
| CT | 32 (64.29%) | 26 (53.06%) | 55.17% | 1.403 (0.568–3.469) | 0.462 | 1.247 (0.508–3.061) | 0.629 |
| TT | 5 (8.93%) | 12 (24.49%) | 29.41% | 4.145 (1.152–14.918) | 0.025 | 3.758 (1.265–11.343) | 0.021 |
| C | 70 (62.50%) | 48 (48.98%) | 59.32% | Reference | – | Reference | – |
| T | 42 (37.50%) | 50 (51.02%) | 45.65% | 1.736 (1.001–3.011) | 0.049 | 1.801 (1.054–2.932) | 0.039 |
| rs11200638 | | | | | | | |
| GG | 18 (32.14%) | 11 (22.45%) | 62.07% | Reference | – | Reference | – |
| GA | 30 (53.57%) | 29 (59.18%) | 50.85% | 1.528 (0.638–3.919) | 0.320 | 1.609 (0.700–4.154) | 0.296 |
| AA | 8 (14.29%) | 9 (18.37%) | 47.06% | 1.841 (0.548–6.188) | 0.322 | 1.912 (0.602–7.001) | 0.317 |
| G | 66 (58.93%) | 51 (52.04%) | 56.41% | Reference | – | Reference | – |
| A | 46 (41.07%) | 47 (47.96%) | 49.46% | 1.322 (0.765–2.284) | 0.316 | 1.451 (0.802–2.147) | 0.285 |

showed sensitivity to 5-FU chemotherapy, while 49.46% of A allele carriers showed sensitivity to 5-FU chemotherapy. Chi-square testing demonstrated that the genotypes and alleles of rs11200638 polymorphism had no significant association with chemotherapy sensitivity among stage III colon cancer patients ($P>0.05$ for all) (Table 2).

## Discussion

Colon cancer has a high mortality rate, especially for those diagnosed at advanced stages [16]. Chemotherapy can provide clinical benefits for colon cancer patients diagnosed with advanced stages. For patients diagnosed with stage III colon cancer, 5-FU-based treatments are considered to be the standard treatment strategy [17]. However, the survival advantages of patients who receive 5-FU treatment may be limited by chemotherapy resistance, thus leading to chemotherapy failure [18]. The genetic variants that can alter the drug metabolism pathways may influence individual sensitivity to chemotherapy [19]. In the present study, we investigated the genetic association of *HtrA1* rs1049331 and rs11200638 polymorphisms with chemotherapy sensitivity among stage III colon cancer patients who received 5-FU-based treatments. Analysis results revealed that

*HtrA1* rs1049331 SNP can reduce chemotherapy sensitivity, but rs11200638 cannot. These results might be of great help for individual chemotherapy in colon cancer patients.

*HtrA1* belongs to the serine protease family, and its deficient expression has been reported in several human cancers, such as pancreatic cancer [20], breast cancer [21], and hepatocellular carcinoma [22]. *HtrA1* can suppress malignant development and progression of cancer [23], and it also has been reported to be involved in chemotherapy resistance. For example, Xu et al. reported that loss of *HtrA1* expression can induce cancer stem cell-like properties of lung adenocarcinoma cells, leading to resistance to cisplatin treatment [24]. In colon cancer, it was reported that the expression of *HtrA1* was significantly reduced, which might be useful as a hallmark for early screening of colorectal cancer [25]. The study conducted by Xiong et al. demonstrated that downregulation of *HtrA1* enhanced the expression of X-linked inhibitor of apoptosis protein (XIAP) and activated PI3K/Akt pathway, thus contributing to cisplatin resistance of colon cancer [13]. The reduced expression of *HtrA1* in colon cancer is caused by multiple factors, and genetic polymorphisms may be an important endogenic cause. In view of this, the present study was performed to investigate the association of *HtrA1* polymorphisms with chemotherapy response in colon cancer patients.

In our study, *HtrA1* polymorphisms rs1049331 and rs11200638 were selected. Rs1049331 is located at the coding region of the *HtrA1* gene, which causes a synonymous change. In this study, we found that rs1049331 polymorphism was significantly correlated with chemotherapy sensitivity of colon cancer patients. The colon cancer patients carrying TT genotype and T allele were more likely to show resistance to 5-FU chemotherapy. Our results agree with the published literature. Feehan et al. reported that presence of the T allele can elevate the risk of age-related macular degeneration (AMD) by influencing neovascular subtype, and individualized treatments might be performed according to rs1049331 in AMD patients [26]. It was reported that rs1049331 does not influence the protein level of *HtrA1*, but it can alter the secondary structure of *HtrA1* mRNA, thus causing changes in structure and function of the protein [14]. Friedrich et al. reported that rs1049331 SNP alters the interaction between *HtrA1* and TGF-β1, thus influencing activity of the TGF-β signal pathway [27]. Excessive activation of the TGF-β pathway can contribute to aggressive tumor development and progression, as well as chemoresistance [28]. The *HtrA1* rs1049331 polymorphism can alter chemotherapy sensitivity through multiple targets or signaling pathways, and further investigations are required to explain the underlying mechanisms.

Rs11200638 is located at the promoter region of the *HtrA1* gene, and published studies have reported that variants in this locus can influence the protein and mRNA levels of *HtrA1* [29]. In view of this, we investigated the genetic association of rs11200638 polymorphism with chemotherapy sensitivity in colon cancer. The results demonstrated that there were no significant relationships between rs11200638 SNP and therapeutic response of colon cancer. Rs11200638 did not affect individual susceptibility to 5-FU-based chemotherapy among advanced colon cancer patients. However, a study by Mohamad et al.

reported that the rs11200638 polymorphism can influence the response to intravitreal ranibizumab therapy in AMD patients [30]. These conflicting results may be due to the different chemotherapy strategies, different study populations, and relatively small sample sizes of studies. Therefore, further investigations are required to confirm our results.

Despite of the encouraging results, several limitations in our study should be stated. Firstly, the sample size was relatively small, which could have reduced the statistical power of our results. Secondly, all the patients were of Chinese Han ethnicity, which limits extrapolation of our results to different populations. In addition, chemoresistance is regulated by multiple factors and genes, but the interactions between *HtrA1* and other factors were not explored in our study. Therefore, further well-designed studies with large sample size are required.

## Conclusions

*HtrA1* rs1049331 polymorphism can influence individual response to 5-FU-based chemotherapy in stage III colon cancer patients, but rs11200638 cannot. To the best of our knowledge, this is the first study to explore the genetic influences of *HtrA1* polymorphisms on response to 5-FU chemotherapy in colon cancer. The results obtained in our study may help guide 5-FU-based treatment among colon cancer patients. In everyday clinical practice, detection of the *HtrA1* rs1049331 polymorphism may improve 5-FU-based chemotherapy and contribute to the design of individualized chemotherapy for patients with colon cancer. Due to relatively small sample size and single Han population in this study, the results need to be verified in further studies, and the implications of our study for clinical practice need to be cautiously interpreted.

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