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*Original Article*

# Correlation analysis of peripheral DPYD gene polymorphism with 5-fluorouracil susceptibility and side effects in colon cancer patients

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**Abstract:** Objective: To investigate the correlation of peripheral DPYD gene polymorphism with 5-fluorouracil (5-FU) susceptibility and side effect in patients with colon cancer. Methods: The total DNA of peripheral mononuclear cells was extracted in 100 cases of colon cancer patients. Quantitative PCR was conducted to measure DPYD gene 14G1A, A1627G, T85C 3 loci polymorphism, and analyze the correlation of 5-FU susceptibility, side effects with DPYD gene polymorphism. Results: Mutations were detected at position 14G1A (mutation rate 14%), A1627G (mutation rate 11%), and T85C (mutation rate 17%). The effective rate of 3 loci of wild type was significantly higher than that of mutant type ($P < 0.05$). With respect to side effects such as myelosuppression, hand-foot syndrome, diarrhea, and gastrointestinal reactions, the incidence in mutation type was significantly higher than that in the wild type ($P < 0.05$). Conclusion: DPYD gene polymorphism plays a guiding role in predicting efficacy and toxicity of 5-FU, which can be used as an important reference index of 5-FU individualized administration scheme.

**Keywords:** Colon cancer, dihydropyrimidine dehydrogenase, gene polymorphism, 5-fluorouracil

## Introduction

Although 5-fluorouracil (5-FU) has been used for over 40 years in cancer chemotherapy field, it is still the most common drug in treating many solid tumors. Currently, the main chemotherapy schemes for advanced colon cancer include FOLFOX scheme (oxaliplatin, 5-FU) and FOLFIRI scheme (irinotecan, 5-FU) [1]. 5-FU was metabolized by dihydropyrimidine dehydrogenase (DPD), of which the activity is closely correlated with the sensitivity of 5-FU and side effects [2]. The DPD activity in human population shows Gaussian distribution and varies a lot among individuals, which is dependent on mutations of different loci of its coding DNA, DPYD gene [3]. Therefore, DPYD mutations are the molecular foundations of low DPD activity and the 5-FU toxicity. The aim of the study was to investigate the correlations of main three loci mutations of DPYD with 5-FU chemotherapy sensitivity and side effect in colon cancer patients, so as to define the dosage of 5-FU accurately for the reference of efficacy improvement.

## Materials and methods

### *General information*

A total of 100 patients (male 57; female 43) with colon cancer enrolled from March 2012 to December 2013 in Oncology Department were included in our study. The mean age of the population is 51.7±8.1 y, which ranged from 31 to 71 years old. All the included patients were diagnosed colon cancer by pathology, with 67 cases of poor differentiation and 33 cases of moderate or high differentiation. Dukes' classification: B stage 37, C stage 49, D stage 14. Inclusion criteria: 1) performance status (Eastern Cooperative Oncology Group, ECOG) 0-2 points; 2) expected survival ≥ 3 months; 3) all patients have measurable focus. Exclusion criteria: severe cardiopulmonary or cerebral disease; 2) severe hepatic or renal dysfunction; 3) white blood cells counts (WBC) < $4.0\times10^9$/L, platelet counts (PLT) < $100\times10^9$/L, hemoglobin (Hb) < 100 g/L. All the included patients received chemotherapy scheme FOLFOX4, with 3 cycles of chemotherapy courses.

**Table 1.** DPD polymorphism distributions in colon cancer patients [n (%)]

| Sites | Cases | Gene polymorphism | | |
|---|---|---|---|---|
| | | Wild type | Mutant type | Hybrid type |
| 14G1A | 100 | 86 (86.0) | 14 (14.0) | 0 |
| A1627G | 100 | 89 (89.0) | 11 (11.0) | 0 |
| T85C | 100 | 83 (83.0) | 17 (17.0) | 0 |

**Table 2.** Correlations of DPD polymorphism with 5-FU sensitivity in colon cancer patients

| Sites | | CR+PR | PD+SD | $\chi^2$ value | *P* value | OR | 95% CI |
|---|---|---|---|---|---|---|---|
| 14G1A | Wild type | 34 | 52 | 7.735 | 0.005 | 1.846 | 7.681-81.731 |
| | Mutant type | 3 | 11 | | | | |
| A1627G | Wild type | 35 | 54 | 10.867 | 0.001 | 2.159 | 8.978-53.227 |
| | Mutant type | 2 | 9 | | | | |
| T85C | Wild type | 33 | 48 | 6.141 | 0.013 | 1.694 | 5.097-36.154 |
| | Mutant type | 4 | 10 | | | | |

**Table 3.** Correlations of DPD gene 14G1A polymorphism with 5-FU side effect [n (%)]

| Side effect | Wild type (n=86) | Mutant type (n=14) | $\chi^2$ value | *P* value |
|---|---|---|---|---|
| Myelosuppression | 27 (31.4) | 8 (57.1) | 13.387 | 0.000 |
| Hand-foot syndrome | 24 (27.9) | 9 (64.3) | 26.661 | 0.000 |
| Diarrhea | 31 (36.1) | 11 (78.6) | 36.923 | 0.000 |
| Gastrointestinal reactions | 18 (20.9) | 9 (64.3) | 38.515 | 0.000 |
| Mucositis | 21 (24.4) | 7 (50.0) | 14.026 | 0.000 |

*Sample collection*

3 mL peripheral blood was extracted from each included patient before chemotherapy. We kept the serum after high-speed centrifugation of blood samples, and used the novel high-resolution melting (HRM) technique to analyze the DPD polymorphism. Afterwards, all participants received FOLFOX4 scheme.

*DPD polymorphism analysis*

DNA extraction kit (QIAGEN Company) was used to extract the total DNA of mononuclear leucocytes in peripheral blood. Software Primer Express 2.0 was used to design the primer of the three loci of DPYD (14G1A, A1627G, T85C). 14G1A: upstream primer 5'-TCCTCTGCAAAAATGTGAGAAGG-3', downstream primer 5'-GCTTTTCTTTGTCAAAAGGAGACTCA-3'. A1627: upstream primer 5'-GAACAAACTGCATAGCAACAATTCTC-3', downstream primer 5'-TCTCTGTTCTGTTTTGTTTTAGATGGA-3'. T85C: upstream primer 5'-CCTGGCTTTAAATCCTCGAACA-3', downstream primer 5'-GCAGTTCTTATCAGGATTTCTTTTCC-3'.

Reaction system: deionized water 30 μL, 10× buffer 4 μL, Taq enzyme 0.5 μL, dNTP 1.5 μL, $MgCl_2$ 10 μL, upstream primer 1 μL and downstream primer 1 μL, templates 2 μL, total reaction system 50 μL. Polymerase chain reaction (PCR) condition: 94°C 4 min; 94°C 30 sec, 56°C 30 sec, 72°C 30 sec, 40 cycles. Afterwards, DNA samples underwent HRM analysis in Rotor Gene Q (QIAGEN Company). PCR amplified products were recollected and purified, and analyzed the sequences.

*Efficacy assessment*

According to the WHO criteria [4], the assessment of chemotherapy efficacy is presented in the following. Complete remission (CR): all focus vanished without new focus occurrence. Partial remission (PR): 1) computed tomography (CT) shows that long diameter of tumor shrinks by ≥ 10%, and (or) tumor density reduces ≥ 15%; 2) no new focus; 3) no development of unpredictable focus. Progress development (PD): 1) long diameter of tumor enlarges by ≥ 10% and the density change doesn't meet the criterion of PR; 2) new focus. Stable development (SD): 1) do not meet the criteria of CR, PR or PD; 2) no symptom deteriorations induced by the cancer. The sensitivity of 5-FU was defined as CR+PR after chemotherapy. The tolerance of 5-PU was defined as PD+SD after chemotherapy.

*Statistical analysis*

Software SPSS 19.0 was used to analyze the data. Enumeration data was presented with rate, and rate was compared by $\chi^2$ test. Logistic equation was used to calculate OR, and $P < 0.05$ was defined as statistical significant.

**Table 4.** Correlations of DPD gene A1627G polymorphism with 5-FU side effect [n (%)]

| Myelosuppression | Wild type (n=89) | Mutant type (n=11) | $\chi^2$ value | *P* value |
|---|---|---|---|---|
| Myelosuppression | 37 (41.6) | 7 (63.6) | 9.706 | 0.002 |
| Hand-foot syndrome | 35 (39.3) | 6 (54.5) | 4.639 | 0.031 |
| Diarrhea | 29 (32.6) | 6 (54.5) | 9.755 | 0.002 |
| Gastrointestinal reactions | 25 (28.1) | 8 (72.7) | 39.786 | 0.000 |
| Mucositis | 41 (46.1) | 9 (81.8) | 27.641 | 0.000 |

**Table 5.** Correlations of DPD gene T85C polymorphism with 5-FU side effect [n (%)]

| Myelosuppression | Wild type (n=83) | Mutant type (n=17) | $\chi^2$ value | *P* value |
|---|---|---|---|---|
| Myelosuppression | 36 (43.4) | 11 (64.7) | 9.134 | 0.003 |
| Hand-foot syndrome | 31 (37.3) | 9 (52.9) | 4.914 | 0.027 |
| Diarrhea | 39 (47.0) | 13 (76.5) | 18.422 | 0.000 |
| Gastrointestinal reactions | 33 (39.8) | 10 (58.8) | 7.221 | 0.007 |
| Mucositis | 45 (54.2) | 12 (70.6) | 5.732 | 0.017 |

## Results

*Chemotherapy outcomes*

Among all the 100 patients after 3 cycles of chemotherapy courses, there were 2 cases of CR (2.0%), 35 cases of PR (35%), 38 cases of SD (38.0%), 25 cases of PD (25.0%), and the total efficacy rate was 37.0%.

*DPD gene polymorphism*

Mutations were detected at all target position, namely 14G1A (mutation rate 14%), A1627G (mutation rate 11%), and T85C (mutation rate 17%) (**Table 1**).

*DPD gene polymorphism and 5-FU sensitivity*

14G1A: the efficacy rate of 5-FU for wild type was 39.5%, which was significantly higher than that of mutant type (21.4%) ($P < 0.05$); 14G1A: the efficacy rate of wild type was 39.3%, which was significantly higher than that of mutant type (18.2%) ($P < 0.05$); T85C: the efficacy rate for wild type was 39.8%, which was significantly higher than that for mutant type (23.5%) ($P < 0.05$). The details are presented in **Table 2**.

*DPD gene polymorphism and 5-FU toxicity*

According to WHO, the chemotherapy side effect was classified as grade I-IV, and the presented study calculate moderate or severe side effect (III-IV) events. With respect to side effects such as myelosuppression, hand-foot syndrome, diarrhea, and gastrointestinal reactions, the incidence of side effects in mutation type was significantly higher than that in wild type ($P < 0.05$) (**Tables 3**-**5**).

## Discussions

Colon cancer is one of the most common malignant tumors in clinic, and chemotherapy plays an important role in treating colon cancer. In current practice, the main chemotherapy schemes for advanced colon cancer include FOLFOX scheme (oxaliplatin, 5-FU) and FOLFIRI scheme (irinotecan, 5-FU). Usually, the administration dosage of chemotherapy drugs depends on the weight and body surface area, but the chemotherapy efficacy and toxicity vary a lot among individuals. Although 5-FU has been used for over 40 years in cancer chemotherapy field, it is still the most common drug in treating many solid tumors. Other pyrimidine fluoride drugs include tegafur, camo fur, floxuridine, doxifluridine, and capecitabine, but they still need to be transformed into 5-FU to work.

DPD, encoded by DPYD, is the rate-limiting enzyme in the metabolic pathway of 5-FU [5], which transforms 80% 5-FU in the body into inactive products. Therefore, DPD is correlated with the metabolic velocity of 5-FU, which further affects the efficacy and toxicity of pyrimidine fluoride drugs. DPD rapidly degrades 85% of administered 5-FU, and as such, limits the amount of drug available for conversion into active metabolites [6]. Many other studies on gastric carcinoma, colon cancer, and breast cancer showed that the lower the DPD activity in tumor tissue predicted the stronger 5-FU chemotherapy sensitivity [7, 8]. That is to say, high DPD activity is correlated with tolerance and unfavorable efficacy. Thus, assessing DPD activity in tumor tissue plays an important role in avoiding severe side effect and predicting 5-FU chemotherapy sensitivity. The DPD activity in human population shows Gaussian distribution [9] and varies a lot among individuals, which is

dependent on mutations of different loci of its coding DNA, DPYD gene. Therefore, DPYD mutations are the molecular foundations of low DPD activity and the 5-FU toxicity. Although DPD activity is not totally dependent on DPYD mutations, but it is considered that DPYD mutations account for at least 57% low 5-FU activity. They also thought that it was feasible to predict DPD activity by analyze DPYD mutations [10].

DPYD, including 23 exons and 22 introns, is located in chromosome 1p22 with a full length of 950 kb. The initiation site is ATG of exon 1, and the pause site is TAA of exon 23. Intron 5 is the longest, and intron 17 is the shortest. DPYD coding region is unevenly distributed in 23 exons, among which over 40 mutant sites are identified currently and some will induce low DPD activity [11]. 28% cancer patients with grade III/IV side effect after receiving 5-FU were found IVS14+1G→A (site *2A) [12], so it is suggested site *2A polymorphism should be screened prior to 5-FU chemotherapy for cancer patients [13]. However, DPYD polymorphism has racial difference, and no DPYD polymorphism is detected in Chinese and Korean population. Thus, other researchers thought that polymorphism screen was not suitable for Chinese population. Very few domestic studies investigated the correlation of DPYD polymorphism with 5-FU toxicity [14]. It is deemed that mutant type in DPYD*5 population was more likely to have severe nausea, vomiting, and declined WBC than that of hybrid type, which was then followed by wild type [15]. They also thought that hybrid type was more likely to have severe nausea and vomiting than that of wild type in DPYD*9A populations.

The presented study investigated the three key mutant sites of DPYD in peripheral blood of colon cancer patients. Mutations were detected at position 14G1A (mutation rate 14%), A1627G (mutation rate 11%), and T85C (mutation rate 17%). These results indicated that DPYD polymorphism might be the main cause of side effect and toxicity differences. The further analysis indicated that significant differences of 5-FU sensitivity and toxicity were found among wild type and mutant type. 5-FU sensitivity of wild type was significantly higher than that of mutant type. Side effect events of wild type were significantly lower than that of mutant type, which was consistent with the report by Iwahashi et al. [16] That is to say, 5-FU tolerance and toxicity in colon cancer patients were closely correlated with DPYD polymorphism mutations.

In conclusion, DPYD gene polymorphism plays a guiding role in predicting efficacy and toxicity of 5-FU, which can be used as an important reference index of 5-FU individualized administration scheme.

## Disclosure of conflict of interest

None.

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