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The role of IVS14+1 G > A genotype detection in the dihydropyrimidine dehydrogenase gene and pharmacokinetic monitoring of 5-fluorouracil in the individualized adjustment of 5-fluorouracil for patients with local advanced and metastatic colorectal cancer: a preliminary report

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Abstract. – AIM: We retrospectively investigated the relationship between IVS14+1 G > A genotype of the dihydropyrimidine dehydrogenase (DPD) gene with plasma concentration of 5-fluorouracil (5-FU) as well as adverse reactions in 80 patients with locally advanced or metastatic colorectal cancer.

PATIENTS AND METHODS: Eighty patients with un-resectable locally advanced or metastatic colorectal cancer were treated with Folfox-6 regimen, which repeated every two weeks for at least three cycles. Single nucleotide polymorphisms for DPD gene were analyzed before chemotherapy by high-resolution melting (HRM) analysis. The plasma concentration of fluorouracil was measured by high performance liquid chromatography (HPLC) after continuous infusion of fluorouracil over 12 h in each cycle. The average values of plasma concentrations in each cycle were calculated, and the factors related to plasma concentration of 5-FU were screened by stepwise regression.

RESULTS: All patients were divided into three groups according to the predictive confidence interval of plasma concentration of 5-FU, and the average plasma concentrations of fluorouracil in each cycle of these three groups were less than or equal to 26.83 mg/L, 26.83-40.62 mg/L, and more than 40.62 mg/L, respectively. Stepwise regression analysis showed that the plasma concentration of fluorouracil was associated with myelosuppression, hand-foot syndrome, diarrhea, overall survival (OS) and DPD genotype. In efficacy, the median progression-free survival PFS (mPFS) and OS (mOS) of group 2 and group 3 were both significantly higher than those of group 1.

CONCLUSIONS: Among the advanced colorectal cancer patients receiving fluorouracil-based chemotherapy, those with plasma concentration of 5-FU above 26.83 mg/L can obtain better survival; for patients with heterozygous DPD IVS14+1 mutation, 5-FU dose should be appropriately reduced according to last plasma concentration to reduce adverse reactions, while the homozygous ones should avoid application of 5-FU and its derivatives.

Key Words:
Colonic neoplasms, Plasma concentration, 5-FU, Drug monitoring, Chemotherapy side effects, Kaplan-Meiers estimate, High resolution melting analysis.

Introduction

Colorectal cancer (CRC) ranks the fifth most common cancer in China, the incidence rate has been showing a rising trend in recent years along with diet, lifestyle changes, etc. and is close to that in some cities of the western developed countries. Approximately 50%-60% of CRC cases have distant metastases at diagnosis, and chemotherapy remains a major mean of the comprehensive treatment for patients with newly diagnosed metastasis and postoperative recurrence or metastasis. 5-FU is the most commonly prescribed anticancer drug with approximately 2 million patients receiving it each year to treat a

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wide variety of malignancies including CRC[1]. Although CRC chemotherapy has entered a new period from 5-FU monotherapy to combination chemotherapy with new drugs such as oxaliplatin and irinotecan, or molecular targeted drugs including bevacizumab and cetuximab etc., the position of 5-FU as a basis in CRC chemotherapy has not been shaken. Because of individual differences, the dose of 5-FU is generally calculated according to body surface area (BSA). However, an equal dose may result in suboptimal effectiveness or intolerable, even life-threatening toxicity which are correlated to 5-FU plasma steady concentration (or quantified by area under the plasma concentration-time curve (AUC)). A randomized, multi-center study of 5-FU monotherapy for advanced CRC found that the objective response rate (ORR) was 18.3% in conventional treatment group, in which dosage was calculated based on BSA. When dosage was individually determined using pharmacokinetically guided adjustments, the ORR was 33.7% ($p = 0.004$), the mOS between the two groups were 16 and 22 months ($p = 0.08$)[2]. In addition, combination 5-FU with oxaliplatin or irinotecan only increased the ORR by about 10%, to 40%-50%[3,4], indicating that 5-FU was still essential in the treatment of CRC currently.

DPD is the initial and rate-limiting enzyme in the metabolism of 5-FU, and approximately 80%-85% of 5-FU is metabolized by DPD (dihydropyrimidine dehydrogenase) in the liver to dihydro 5-FU (DHFU), an inactive product[5]. A number of studies have reported that patients with deficiency in DPD activity may suffer from serious toxicity after the administration of 5-FU[6-9]. Up to know, more than 30 SNPs (single-nucleotide polymorphisms) and deletion mutation of DPD gene have been found, and the most prevailing one is G to A mutation in the splicing-recognition sequence of intron 14 (known as 14G > A, DPYD*2A) contributing about 50% of the total mutation incidence, this mutation leads to absence of exon 14, which results in partial or complete deficiency of DPD activity[9]. Some studies have found that 27%~57% of cancer patients with IVS14+1G > A mutation suffered from severe 5-FU-associated toxicity[10]. Although some previous works investigated the relationship between DPD activity and 5-FU plasma concentration in cancer patients[11-14], there have been not comprehensive research on the association between 5-FU plasma concentration and IVS14+1G > A mutation of DPD gene in CRC patients. Herein, we retrospectively investigate the relationship between IVS14+1 G > A genotype in DPD gene with plasma concentration of 5-FU after chemotherapy and 5-FU-associated therapeutic efficacy as well as adverse reactions. Ultimately, we want to achieve a goal of improving efficacy and reducing toxicity by 5-FU dosage individual adjustment.

Patients and Methods

Patients

A total of 102 hospitalized patients with unresectable local advanced and metastatic CRC were received IVS14+1 G > A genotype detection before chemotherapy. 5-FU plasma concentration was detected after treatment which were confirmed with cytological, pathological and imaging data in Shanghai First People's Hospital (China) from June 2008 to October 2010, 22 cases were excluded due to certain factors such as having received targeted therapy, lost follow-up, 5-FU plasma concentration having not been detected in time, and so on. The eligible 80 patients, those only had received chemotherapy, composed of 48 males and 32 females ranging from age 32 to 76 with a median age of 58 years. For pathological types, 28 cases were with poorly differentiated adenocarcinoma, 16 cases were with moderately differentiated adenocarcinoma, 12 were cases with well differentiated adenocarcinoma, 8 were cases with mucinous adenocarcinoma and 16 cases were with tubular adenocarcinoma. For tumor sites, 58 cases were with colon cancer and 22 were cases with rectum cancer. 6 cases had stage III B disease and 74 cases had stage IV disease according to the AJCC (American Joint Committee on Cancer) Cancer Staging standard (6th edition). All of the cases, with ECOG (Eastern Cooperative Oncology Group) physical status scores from 0 to 2 and having measurable diseases as well as life expectancy of ≥ 3 months, were assigned to receive modified Folfox-6 (mFolfox-6) for 3 cycles as first-line palliative combination chemotherapy, the mean of 5-FU plasma concentrations of the three cycles for each patient was calculated after chemotherapy. Then, all patients were divided into three groups according to the predictive confidence interval of plasma concentration of 5-FU for retrospective analysis, which were less than or equal to 26.83 mg/L, 26.83-40.62 mg/L, and more than 40.62 mg/L, respectively.

Methods

5 ml of peripheral blood sample was obtained from each patient before chemotherapy (at about 4-6 AM) and centrifuged at the speed of 2,000 rpm for 15 min. Serum was reserved for DPD 14G1A SNPs detection by high-resolution melting (HRM) curve analysis (time for detection: 4-6 AM)[15], then the patients were received chemotherapy with mFolfox-6: a 2 hours infusion of oxaliplatin (100 mg/m^2) and leucovorin (200 mg/m^2), followed by 5-FU bolus (400 mg/m^2) on day 1 and a 46 hour continuous infusion (3.000 mg/m^2) for each cycle, repeated every 2 weeks. The plasma concentration of 5-FU was detected by high performance liquid chromatography (HPLC) (2 ml of peripheral blood sample, time for detection at about 4-6 AM) after continuous infusion of 5-FU[16].

Detection of DPD 14G1A SNPs

The purification of genomic DNA from blood was used serum DNA extraction kit (Qiagen, Hilden, Germany). 40 μl proteinase K was added in 2 ml centrifuge tube (packed with spin column) with 400 μl serum sample, kept in 56°C water bath for 10 min after full oscillation, then mixed with 400 μl ethanol, the mixture was centrifuged through the spin column for two times (620 μl per time): after centrifuging at 6.000 g for 1 min, put the spin column into another 2 ml centrifuge tube, washed with 500 μl buffer AW1 and buffer AW2 later, spun at 6.000 g for 1 min and 20,000 g for 3 min, respectively. Moved the spin column into another centrifuge tube, added 50 μl buffer AE (Adams-Evans), stood at room temperature for 5 min, spun at 6.000 g for 1 min again for nucleic acid quantification, and was stored at –20°C. Primer design refers to the sequence of malate dehydrogenase gene[17], and primers were synthesized by Invitrogen Corporation, Carlsbad, CA, USA. Primer sequences were as follows:

- **Upstream**: 5'- ACTAAAGGCTGACTTTCCA-GACAAC-3'
- **Downstream:** 5'-AACATTCACCAACT-TATGCCAA-3'

The PCR reaction mix (20 μl), containing 2 μl of 10×PCR Buffer, 0.4 μl of $MgCl_2$ (25 mM), 0.5 μl of dNTP (deoxyribonucleotide triphosphate: 10 mM), 1 μl of 20 × Eva-green saturated dye, 1 μl of Primers (10 μm), 1 μl of DNA templates, 0.2 μl of DNA Taq polymerase (5 U/μl) and 13.9 μl of ddH_2O, was amplified by real time quantitative PCR (polymerase chain reaction) with LightCycler480 II PCR instrument (Roche, Motel de Rennaz, Switzerland), the amplification condition and procedure were shown in Figure 1. After PCR, the melting curve was performed from 65°C to 90°C with a slope of 0.3°C/s followed by a finally cooling step to 40°C, and analyzed by the software of Light-Scanner Call IT. The PCR products were purified and recovered, and the sequence was analyzed by 3730 DNA analyzer.

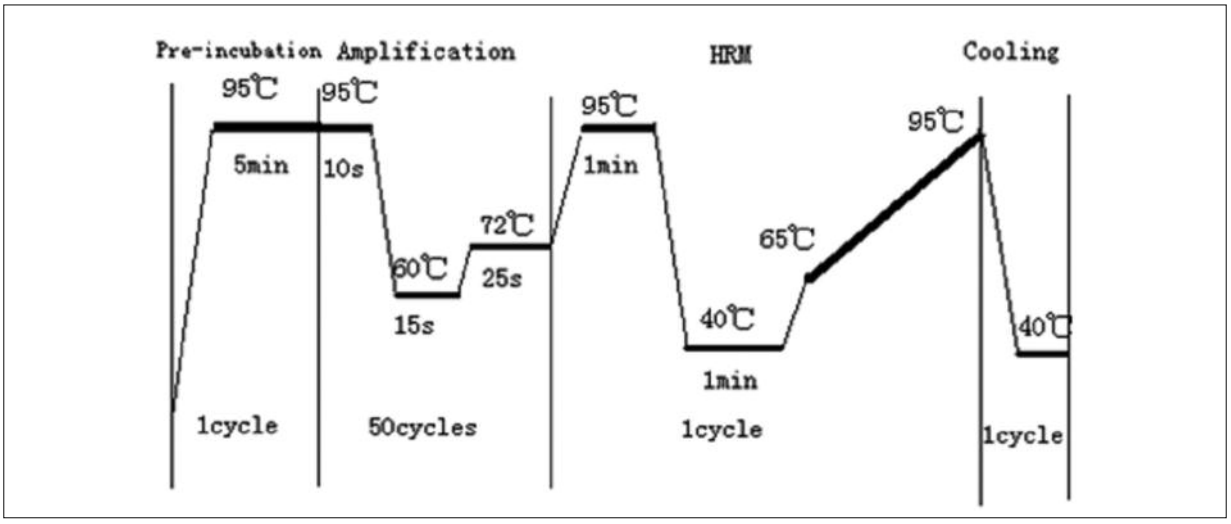

Figure 1. RT-PCR and HRM analysis for 14G1A sites in the dihydropyrimidine dehydrogenase gene included 4 parts: ***A,*** A denaturation step of 5 min at 95°C; ***B,*** Followed by 50 cycles of PCR, with denaturation (10 s at 95°C), annealing (15 s at 60°C), and extension (25 s at 72°C); ***C,*** Followed by a melting program of 95°C for 60 s (denaturation), 40°C for 60 s (annealing), and then 65 to 95°C at a transition rate of 45° with continuing monitoring of fluorescence; ***D,*** Cooling at 40°C for 1 cycle.

Detection of 5-FU Plasma Concentration

The 200 ng/ml of 5-FU standard stock solution and 5-bromouracil (5-Bru) internal standard solution were prepared with 5-FU and 5-Bru standard (Sigma Aldrich Co, St Louis, MO, USA). 5-FU standard stock solution was added to blank plasma samples which made them into plasma standard solution at different final concentration of 0.5, 1, 2.5, 5, 10, 25 and 50 ng/ml, then mixed with 25 μl of 5-Bru internal standard solution and 20 μl of 10% perchloric acid, after being centrifuged at the speed of 5,000 rpm for 10 min, the supernanant was filtered, 20 μl of which was tested. The HPLC system consisted of Millipore 510 HPLC pump, 490 ultraviolet detector (Waters Technology, Chicago, IL, USA), Diamonsil C_{18} colum n (250×4.6 mm i.d., 5 μm of particle diameter, Dikma Technologies Inc, Anaheim, CA, USA). A mobile phase of methanol: water (45:55, V/V) was used for all analyses at a flow-rate of 1.3 ml · min^{-1}, and plasma concentration was detected by UV absorption at 260 nm. The data collected for each amount for all seven curves were averaged and replotted to yield a composite standard curve for 5-FU. For each test sample, the peak area detected was used to calculate the amount of 5-FU present in the plasma sample by applying the linear equation obtained from the composite standard curve.

Evaluation Criteria and Follow-up

First efficacy follow-up was performed by imaging scans after 3 cycles for all patients, and all responses were confirmed at least 4 weeks after initial assessment and re-evaluated every 3 cycles with disease progression. Patients will be followed-up for overall survival every 3 months. The median follow-up was 16 months. The tumor assessments for response and progression were evaluated using the Response Evaluation Criteria in Solid Tumours (RECIST). Toxicity was graded according to National Cancer Institute common terminology criteria for adverse events (CTCAE version 3.0).

Statistical Analysis

Measurement data were expressed as mean ± standard deviation. The study was designed to have 90% power to accept the hypothesis and 5% significance to reject the hypothesis. Associations between response rate and polymorphism were assessed by a Chi-square test. Median PFS and OS according to prognostic factors were calculated using the Kaplan-Meier method, and differences were analyzed by log rank test. All values were two sided and statistical significance was accepted at the $p < 0.05$ level. SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

5-Fu Standard Curve Equation

A good linear relationship exists between 5-FU concentration and peak area, the regression equation was $y = 0.065 \times +0.178$ and the minimum detection limit of 5-Fu was 0.01 mg · L^{-1}.

Stepwise Regression Analysis of the Influencing Factors to 5-FU Plasma Concentration

In stepwise regression analysis, 5-FU plasma concentration was conducted as dependent variable, and independent variables include adverse reactions, short-term effect, PFS and OS. Stepwise regression analysis revealed that 5-FU plasma concentration was associated with bone marrow suppression, hand-foot syndrome, diarrhea, OS and DPD genotype (regression coefficients were seen in Table I). The 5-FU standardized predictive values were of linear distribution (the scatter plots of predictive values and regression line were shown in Figure 2B) and the residual frequency of them was of normal distribution (see Figure 2A).

The Relationship Between 5-FU Plasma Concentration and Efficacy

All patients were divided into three groups according to the predictive confidence interval of plasma concentration of 5-FU, which were less than or equal to 26.83 mg/L, 26.83-40.62 mg/L, and more than 40.62 mg/L, respectively. The clinical characteristics of the three groups were comparable (see Table II). The average plasma concentrations of 5-FU in group 1, group 2 and group 3 were 23.88±1.80 mg/L, 34.27±3.66 mg/L and 45.70±3.97 mg/L, respectively ($\chi^2 = 225.99$, $p < 0.001$). The disease control rate (DCR) of the three groups were 58.33%, 73.68% and 72.22%, which were significantly increased in group 2 and 3 than that of group 1 by 15.35% and 13.89%. Compared with group1, mPFS (median duration of progression-free survival) increased by 3.5 months from 5.0 to 8.5 months in group 2 and 3 ($\chi^2 = 21.09$, $p < 0.001$), and mOS (median overall survival) increased from 14.0 to 19.0 and 22.0 months in

Table I. Coefficient of the regression equation.

Model	Unstandardized coefficients		Standardized coefficient β	*t* value	*p*-value	95% confidence interval for B	
	B	Standard error				Lower	Upper
Constant	111.52	17.81		6.26	< 0.001	76.42	146.62
Bone marrow suppression	28.94	4.35	0.37	6.66	< 0.001	20.37	37.50
Diarrhea	-16.40	6.05	-0.15	-2.71	0.007	-28.32	-4.49
Hand-foot syndrome	17.56	4.99	0.18	3.52	0.001	7.74	27.38
DPD genotype	64.28	10.48	0.31	6.13	< 0.001	43.63	84.93
Overall survival	5.68	0.89	0.30	6.38	< 0.001	3.92	7.43

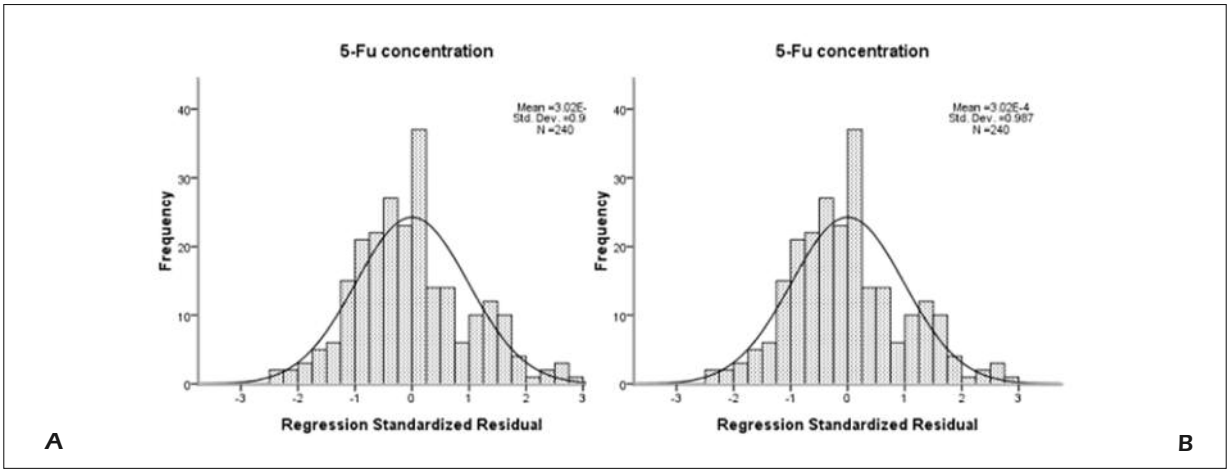

Figure 2. Distribution of standardized 5-FU predictive value: ***A,*** Figure 1 distribution histogram for residual frequency of 5-FU standardized predictive value. ***B,*** Scatter plot of 5-FU predictive value, regression line and linear regression model.

Table II. Comparison of clinical characteristics of the three groups (n).

Characteristic	Group			χ^2	*p* value
	1 (n=24)	2 (n=38)	3 (n=18)		
Gender				1.60	0.28
Male	15	22	11		
Female	9	16	7		
Age/yr				0.99	0.38
Median	61	58	57		
Range	52~76	32~72	49~74		
ECOG Score	0.75 ± 0.61	0.89 ± 0.73	0.83 ± 0.71	0.33	0.72
TNM stage					
III B	2	3	1	0.21	0.82
IV	22	37	17		

80 patients were divided into three groups according to confidence interval of plasma concentration of 5-FU, and there is no significant difference in the member number of each group.

group 2 and 3, respectively ($\chi^2 = 32.37$, $p < 0.001$, see Table III and Figure 4).

DPD Genotype

Among the 80 patients, 67 without DPD 14G1A mutation (G/G) were defined as wild type group, 11 with heterozygous mutation (G/A) and 2 with homozygous mutation (A/A) were defined as mutation group. The mutation rate was 16.25%.

The Relationship Between DPD Genotype and adverse reactions, survival

It can be shown from table IV that no significant differences were found among the groups, which were divided according to effective prediction for interval of 5-FU plasma concentration, for adverse reactions associated with 5-FU (χ^2 value of 1.29 to 4.03, p value of 0.12 to 0.32). While the patients of group 2 and 3 were divided into wild-type group and mutation group according to genotype for comparison, it was found that the incidence of grade III/IV myelosuppression, hand-foot syndrome and diarrhea in mutation group was significantly higher than that in wild type group (p = 0.04, p = 0.03 and p = 0.04), but no significant association was observed for mPFS and mOS (mPFS: 7.50±0.44 months vs. 8.50±0.40 months, p = 0.69; mOS: 21.00±1.12 months vs. 20.00±1.16 months, p = 0.72, see Figure 5).

Discussion

As a pyrimidine anti-metabolite, 5-FU has been one of the most frequently used anti-cancer drugs for more than 40 years, and is considered the backbone in treatments of malignancies in the gastrointestinal tract. For advanced CRC, the response rate of 5-FU alone in first-line setting was 10%-15% with a mOS of about 10 months, while in combination therapy setting was 40%-50% with a mOS of 16-20 months[18,19]. An ideal treatment of improving the outcome in patients with cancer is increasing safety without reducing the efficacy of chemotherapy. Individualizing doses and application schedules should be added to the efficacy and safety of chemotherapeutic agents, because of their narrow therapeutic index. The deficiency of improving the efficacy by increasing the dose of oxaliplatin or irinotecan lies in two aspects: on one hand the regulation range is relatively narrow; on the other hand the appearance of associated adverse reactions (such as neurotoxicity and chemotherapy-related diarrhea) may be significantly increased, which affect the conduct of chemotherapy and life quality for patients. A randomized, multi-center study of advanced colorectal also showed that patients administrated with individualized 5-FU adjusted by pharmacokinetics had a mOS equivalent to that of combination therapy[2]. In addition, the half-life of 5-FU by continuously intravenous infusion is about 10-20 min, and be able to reach steady plasma concentration after 5 half-lives in theory. Blood samples collecting was controlled about 4-6 AM in the early morning, which could avoid biases of plasma concentration detection and adverse reactions investigation caused by 5-FU circadian fluctuations, because peak concentration reached at 1-5 AM and positively correlated with

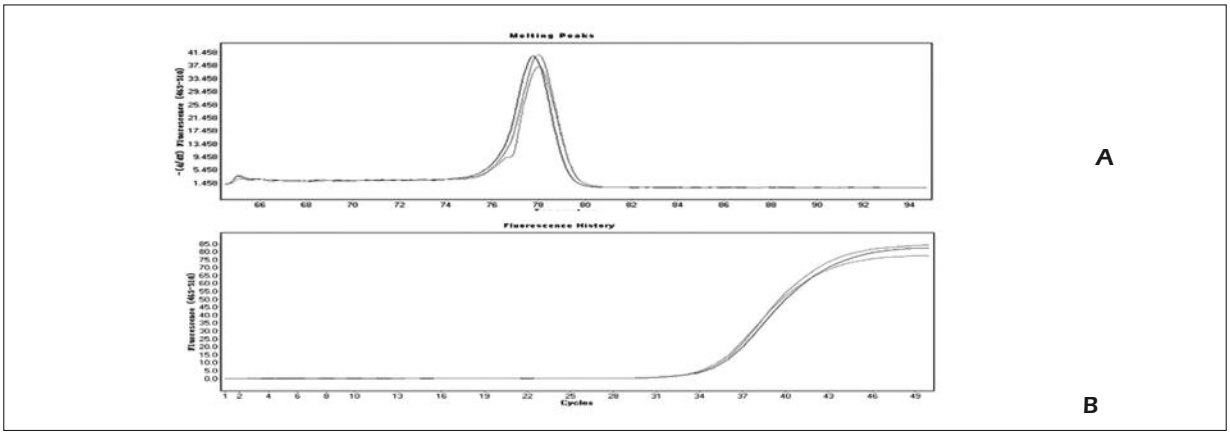

Figure 3. Mutation results for 14G1A in the dihydropyrimidine dehydrogenase gene by high-resolution melting analysis. ***A,*** Melting curves of different single nucleotide polymorphisms for 14G1A sites in the dihydropyrimidine dehydrogenase gene. ***B,*** Expansion plans of different single nucleotide polymorphisms for 14G1A sites in the dihydropyrimidine dehydrogenase gene.

Table III. Comparison of efficacy among the three groups (n, %).

Efficacy	Group based on 5-Fu concentration			χ^2	p	DPD gene phenotype		χ^2	p
	1 (n=24)	2 (n=38)	3 (n=18)			mt (n=13)	wt (n=43)		
Partial remission/n	2	7	4	3.96	0.08	1	9	0.83	0.52
Stable disease/n	12	21	9			6	25		
Progression disease/n	10	10	5			6	9		
DCR/%58.33%	73.68%	72.22%			53.85%	79.07%			
mPFS t/month	5.00 ± 0.56	8.50 ± 0.61	8.50 ± 2.00	21.09	<0.001	7.50 ± 0.44	8.50 ± 0.40	0.16	0.69
95% CI t/month	3.91-6.09	7.30-9.70	4.59-12.41			6.64-8.36	7.72-9.29		
mOS t/month	14.00 ± 0.43	19.00 ± 0.96	22.00 ± 2.06	32.37	<0.001	21.00 ± 1.12	20.00 ± 1.16	0.13	0.72
95% CI t/month	13.16-14.84	17.12-20.89	17.96-26.04			18.81-23.19	17.76-21.40		

80 patients were classified into three groups based on 5-FU plasma concentration. The patients in Group 2 and 3 were subdivided into two groups according to the DPD gene phenotype. The short-term and long-term clinic efficacy between groups each group were valuated. DCR: Discease contral rate; mPFS: Median progession free survival; mOS: Median overall survival; CI: confidence interval.

adverse reactions[20]. The monitoring of 5-FU plasma concentration seems principally useful for individual a posteriori dose adjustment, the following dose can be increased to improve the efficacy for patients with lower plasma concentrations, or decreased to reduce associated adverse reactions for patients with higher plasma concentrations. Previous studies have preliminary shown relationship between 5-FU pharmacokinetics and efficacy, patients with 5-FU plasma concentrations above a certain level could have a higher probability of objective response[21,22].

In our study, taking 5-FU plasma concentration as dependent variable, and adverse reactions, short-term effect, PFS, OS as independent variables, stepwise regression analysis revealed that 5-FU plasma concentration was associated with bone marrow suppression, hand-foot syndrome, diarrhea, OS and DPD genotype, which suggested that the higher plasma concentration patients got the longer mOS, However, the higher prevalence of adverse reactions such as myelosuppression, hand-foot syndrome and diarrhea, which may be related with DPD genotype. Then according to the predictive confidence interval of plasma concentration of 5-FU, all patients with an average plasma concentrations of 5-FU less than or equal to 26.83 mg/L, 26.83-40.62 mg/L, and more than 40.62 mg/L respectively in each cycle were divided into three groups (group 1, group 2 and group 3) to analyze the difference between efficacy and adverse reactions of the groups with different plasma concentrations. The results showed that the DCR, mPFS and mOS were significantly increased in group 2 and 3 than those in group 1, mPFS of group 2 and 3 were increased by 3.5 months than that of group 1 ($p < 0.001$). The increases of mOS were 5 months in group 2 and 8 months in Group 3 (both $p < 0.001$). All of these suggested that patients with plasma concentration above 26.83 mg $\cdot$ L^{-1} could achieve better outcome, while the others with plasma concentration below 26.83 mg $\cdot$ L^{-1} could improve the efficacy by enhancing the following dose intensity.

The activity of DPD may be an important determinant for predicting the efficacy and toxicity of 5-FU, because 80%-85% of 5-FU is metabolized by DPD. SNPs in genes encoding drug metabolizing enzymes such as DPD might be clinically important in the condition that they have a reliable effect on enzyme activity. Alleles variation (one or two variant alleles) results in partial or complete lack of DPD activity[23], and more

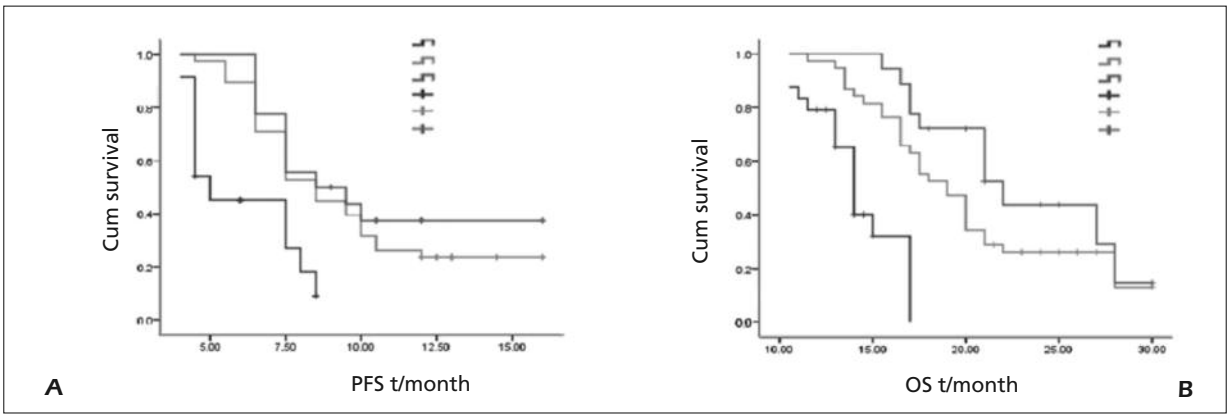

Figure 4. Comparison of progression-free survival and overall survival among the three groups. ***A,*** Progression-free survival among the three groups. ***B,*** Overall survival among the three groups.

than 40 known allelic variants or deletion mutation have been described associated with DPD activity[24-29], the most common is IVS14+1G > A, which accounts for about 50% of all. The G to A mutation changing an invariant splice donor site GT into AT which is no longer recognized by the splicing site, and this mutation leads to skipping of exon 14 immediately upstream of the mutated splice donor site in the process of DPD pre-mRNA splicing. As a result, the mature DPD mRNA lacks a 165 bp fragment encoding amino acid residues 581-635[30]. It was reported that approximately 3% of patients may be geno-typically heterozygous for a mutant DPD allele in the general population[10], but additional studies have shown significant variability among different ethnic subpopulations, for instance, IVS14+1G > A hadn't been found in a small Japanese or Taiwanese population[31]. In our study, we found 13 cases with IVS14+1G > A mutation, including 11 cases with heterozygous mutation (G/A) and 2 cases with homozygous mutation (A/A), a total mutation rate of 16.25%, was higher than that had been reported, the reason may be as follows: (1) sample size wasn't enough, which may result in publication bias; (2) as mentioned earlier, variation mat exist among different ethnic subpopulations; (3) HRM analysis used in this study, with high sensitivity and specificity, variants as low as 2% can be detected, compared with 20% by conventional PCR sequencing.

Patients in group 2 and 3 were further divided into wild type and mutation group according to DPD genotype, it was found that the frequency of grade III/IV myelosuppression, hand-foot syndrome and diarrhea in mutation group was significantly higher than those in wild type group. In addition, grade IV myelosuppression occurred in both 2 cases with homozygous mutation, which was consistent with previous studies[32,33].

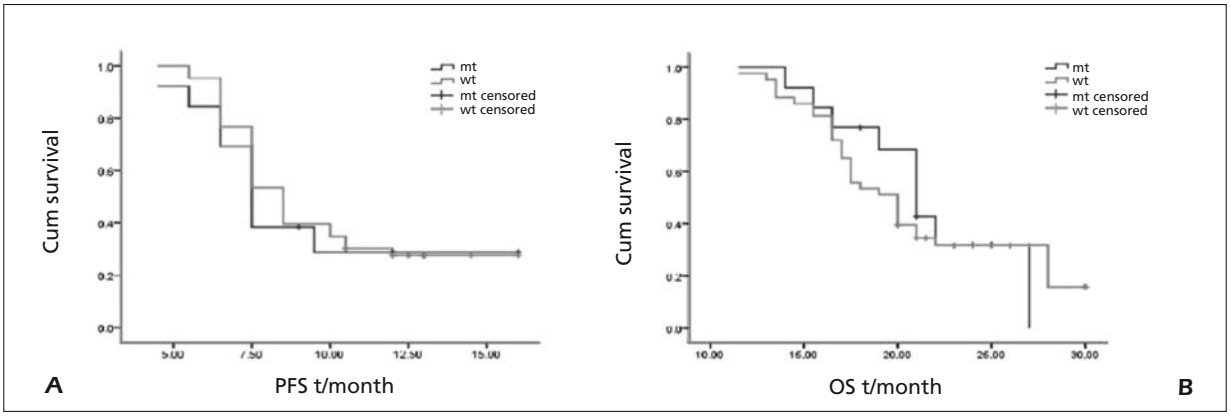

Figure 5. Comparison of progression-free survival and overall survival between the wild type and mutant groups. ***A,*** Pprogression-free survival between the wild type and mutant groups. ***B,*** Overall survival between the wild type and mutant groups.

Table IV. Comparison of adverse reactions among the three groups (person-time, %).

Adverse reaction	Group based on 5-Fu concentration			χ^2	p	DPD gene phenotype		χ^2	p
	1* (n=72)	2* (n=114)	3* (n=58)			Mt** (n=39)	Wt** (n=129)		
Marrow suppression									
I-II	25 (34.72)	52 (45.61)	13 (24.07)			15 (38.46)	50 (38.76)		
III-IV	3 (4.17)	14 (12.28)	38 (70.37)	1.58	0.28	24 (61.54)	28 (21.71)	8.54	0.04
Mucositis									
I-II	8 (11.11)	45 (39.47)	24 (44.44)			17 (43.59)	52 (40.31)		
III-IV	1 (1.39)	6 (5.26)	15 (61.11)	1.37	0.31	11 (28.21)	10 (7.75)	3.05	0.16
Gastrointestinal reaction									
I-II	27 (37.50)	42 (36.84)	24 (44.44)			17 (43.59)	49 (37.98)		
III-IV	3 (4.17)	7 (6.14)	22 (40.74)	1.29	0.32	16 (41.03)	13 (10.08)	3.41	0.14
Hand-foot syndrome									
I-II	16 (22.22)	48 (22.11)	14 (25.93)			10 (25.64)	52 (40.31)		
III-IV	4 (5.56)	13 (11.40)	28 (51.85)	2.17	0.21	16 (41.03)	25 (19.38)	10.71	0.03
Diarrhea									
I-II	15 (20.83)	52 (45.61)	16 (29.63)			15 (38.46)	53 (41.09)		
III-IV	6 (8.33)	9 (16.67)	21 (38.89)	4.03	0.12	16 (41.03)	24 (18.60)	9.31	0.04

80 patients were divided into different groups according to 5-FU concentration. The patients of group 2 and 3 were subclassified into two groups based on DPD gene phenotype. After 3 cycles of 5-FU chemotherapy treatment, adverse reactions were assessed. Grade III / IV adverse reaction of different groups was tested by CHI-square test. Person-time stands for the sum of patients' times of getting adverse reaction after chemotherapy. *1: ≤ 26.83 mg□L^{-1}; 2: 26.83~40.62 mg□L^{-1}; 3: > 40.62 mg□L^{-1}. **mt: DPD mutant; wt: DPD wide type.

No significant association was observed for mPFS and mOS, although DPD plays a key role in 5-FU plasma concentration according to previous report[34], reasons for the results may be: (1) the prevalence of mutation with IVS14+1G > A is relatively low; (2) DPD genotype may be inconsistent with phenotype[9], other types of mutations affecting DPD activity or compensation mutations may exist[35-38]. And these need to be verified by determination of DPD activity.

Conclusions

Our present work comprehensively investigate the effect of IVS14+1 G > A genotype of DPD gene on the clinical outcome of patients with specifically locally advanced or metastatic colorectal cancer. To our acknowledgment, there had been such works being reported. By retrospective analysis of 5-FU plasma concentration of patients with local advance or metastatic CRC after chemotherapy, we found that patients with 5-FU plasma concentration above 26.83 mg/L can obtain better survival, so those with 5-FU plasma concentration below 26.83 mg/L should further enhance dose intensity of 5-FU. Patients who are to receive 5-FU treatment should be required for genetic screening for DPD IVS14+1 mutation, 5-FU dose should be appropriately reduced for the next scheduled treatment according to last plasma concentration to reduce the risk of toxicities for heterozygous ones, while the homozygous ones should avoid application of 5-FU and its derivatives.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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