Pharmacogenomics and Personalized Medicine

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ORIGINAL RESEARCH

Rs11479 in *Thymidine Phosphorylase* Associated with Prognosis of Patients with Colorectal Cancer Who Received Capecitabine-Based Adjuvant Chemotherapy

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Objective: *Thymidine Phosphorylase (TYMP)* gene was of potential significance in the process of colorectal cancer (CRC) development and played an important role in capecitabine metabolism. This study was to identify the association between *TYMP* polymorphism and prognosis of postoperative patients with CRC who received capecitabine-based adjuvant chemotherapy.

Methods: A total of 218 patients with CRC who were treated with surgical resection and capecitabine-based adjuvant chemotherapy were included in this study retrospectively. Peripheral blood and peripheral blood mononuclear cell (PBMC) specimen of the patients were collected for the genotyping of *TYMP* polymorphism and *TYMP* mRNA expression, respectively. Univariate analysis of genotypes and prognosis was carried out by Kaplan–Meier survival analysis, Cox regression analysis was adopted in multivariate analysis. The mRNA expression of *TYMP* according to genotype status was analyzed using non-parameter test.

Results: Prevalence of rs11479 in *TYMP* among the 218 patients exhibited that minor allele frequency of rs11479 was 0.20 (GG 141 cases, GA 68 cases and AA 9 cases), which was in accordance with Hardy-Weinberg equilibrium (P=0.825). Association analysis suggested that the median disease-free survival (DFS) of patients with GG genotype and GA/AA genotype was 3.1 and 6.1 years, respectively (P=0.004). Furthermore, the median overall survival of patients with GG genotype and GA/AA genotype was 5.0 and 7.0 years, respectively (P=0.033). Multivariate Cox regression analysis exhibited that rs11479 polymorphism was an independent factor for DFS (HR = 1.64, P=0.009). Additionally, of the 65 PBMC specimens, mRNA expression results indicated that patients with GA/AA genotypes conferred significantly higher mRNA expression of *TYMP* than that of patients with GG genotype (P<0.001).

Conclusion: Polymorphism rs11479 in *TYMP* gene might predict the prognosis of patients with CRC who received capecitabine-based adjuvant chemotherapy through mediation of the mRNA expression of *TYMP*. The conclusion of this study should be validated in prospective clinical trials subsequently.

Keywords: colorectal cancer, adjuvant chemotherapy, capecitabine, prognosis, polymorphism

Introduction

Colorectal cancer (CRC) is one of the most common gastrointestinal tumors worldwide. It is estimated that there are approximately 56,000 new cases and 29,000 new deaths of CRC in China currently.[1] To our knowledge, surgical resection was established to be curable for patients with CRC over the past decades. Unfortunately, approximately 30% of the patients were diagnosed of distant metastasis, thus missing the opportunity for surgical resection.[2] Consequently, almost half of the patients with CRC were able to receive surgical resection clinically. Recent years had witnessed in-depth progress of molecular status in colon cancer or rectal cancer with advanced stage, more and more targeted drugs

Received: 12 November 2022
Accepted: 17 February 2023
Published: 31 March 2023

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with different targets of action were developed to provide considerable treatment options, thus bringing survival benefits for the patients consecutively.[3] Nevertheless, meaningful research progress in early-stage and postoperative patients with CRC was still limited comparatively. It was known that IDEA trial was proved to be the significant breakthrough with clinical significance recently, which suggested that 3 months rather than 6 months of adjuvant chemotherapy was recommended for low-risk stage III colon cancer to receive 5-FU related regimens.[4] Conclusions of IDEA trial helped to attenuate the unnecessary side reactions of chemotherapy to some extent in view of the fact that 6 months adjuvant chemotherapy regimens were established to be the standard of care for stage III patients with CRC for decades.[5] The IDEA trial also highlighted more work should be performed to refine the adjuvant chemotherapy for patients with CRC, which was in line with the aim of our study. Besides, with the adequate development of the more sensitive detection techniques, ctDNA had established to be the potential predictive value in the recurrence risk assessment for patients with CRC after surgical resection.[6] However, the predictive role regarding ctDNA for the intensity or duration of adjuvant chemotherapy among patients with CRC was still controversial and no standardized criteria were available for the detection of ctDNA currently.[7]

Capecitabine-based adjuvant chemotherapy was the standard of care for patients with CRC after surgical resection for decades, which improved 5-year survival rates of approximately 10%.[8] However, the benefits of capecitabine–based adjuvant chemotherapy for patients with stage II CRC to reduce recurrence and improve survival remained controversial currently.[9] Furthermore, large individual differences were observed in the clinical application of capecitabine-based adjuvant chemotherapy, indicating that considerable factors might compromise the efficacy of this regimen. Capecitabine could be transformed into 5-FU only through the key metabolism enzyme of *thymidine phosphorylase (TYMP)*, thus playing the cytotoxic effects to kill the tumor.[10] Noteworthily, previous study indicated that *TYMP* gene was similar with the structure of platelet-derived endothelial cell growth factor in tumor cells, which played an important role in promoting tumor angiogenesis and metastasis.[11] Therefore, considerable studies confirmed that *TYMP* was highly expressed in various kinds of tumors.

TYMP gene was located at chromosome 22q13.33 and contained 10 exons. As a member of the endothelial vascular growth factor family, *TYMP* gene was not conserved in Chinese population, which suggested that protein expression of *TYMP* also showed great individual differences among different individuals.[12] Relatively limited polymorphism studies of this gene in Chinese population were initiated. Especially in the field of colorectal cancer, rare research had been performed on this gene polymorphism currently.[13] Previous work initiated by Du et al performed the exploration of *TYMP* genetic variation on clinical outcome and safety of CRC.[14] They identified that polymorphism of *TYMP* might be of potential significance clinically. Besides, another study initiated by Huang et al investigated the clinical significance of *TYMP* gene polymorphism among patients with advanced gastrointestinal tumors.[15] Conclusion suggested that the expression level of *TYMP* gene was higher among patients with T allele in the advanced gastrointestinal tumors. Nevertheless, the relevance of *TYMP* polymorphism to the prognosis of patients with CRC who received capecitabine-based adjuvant chemotherapy after surgical resection remained unknown.

Consequently, the objective of this study was to identify the association between *TYMP* polymorphism and prognosis of patients with CRC who received surgical resection and capecitabine-based adjuvant chemotherapy retrospectively.

Methods

Study Design

Given that capecitabine-based adjuvant chemotherapy was licensed in China over ten years, considerable patients with CRC were treated with capecitabine-based adjuvant chemotherapy clinically. Therefore, this study was designed as real-world retrospective research. Appropriate patients with CRC underwent surgical resection were collected in the department of Gastrointestinal surgery of Affiliated Hospital of Hebei University from January 2010 to March 2021. Baseline characteristics of the patients were obtained from the hospitals electronic medical record system. Patients who fulfilled the eligibility criteria were included in the study retrospectively. Inclusion criteria were as the following: (1) aged ≥18 years; (2) Eastern Cooperative Oncology Group (ECOG) performance status of 0–2 score; (3) adequate cardiac function, renal function and bone marrow function to receive adjuvant chemotherapy appropriately; (4) pathological diagnosis of

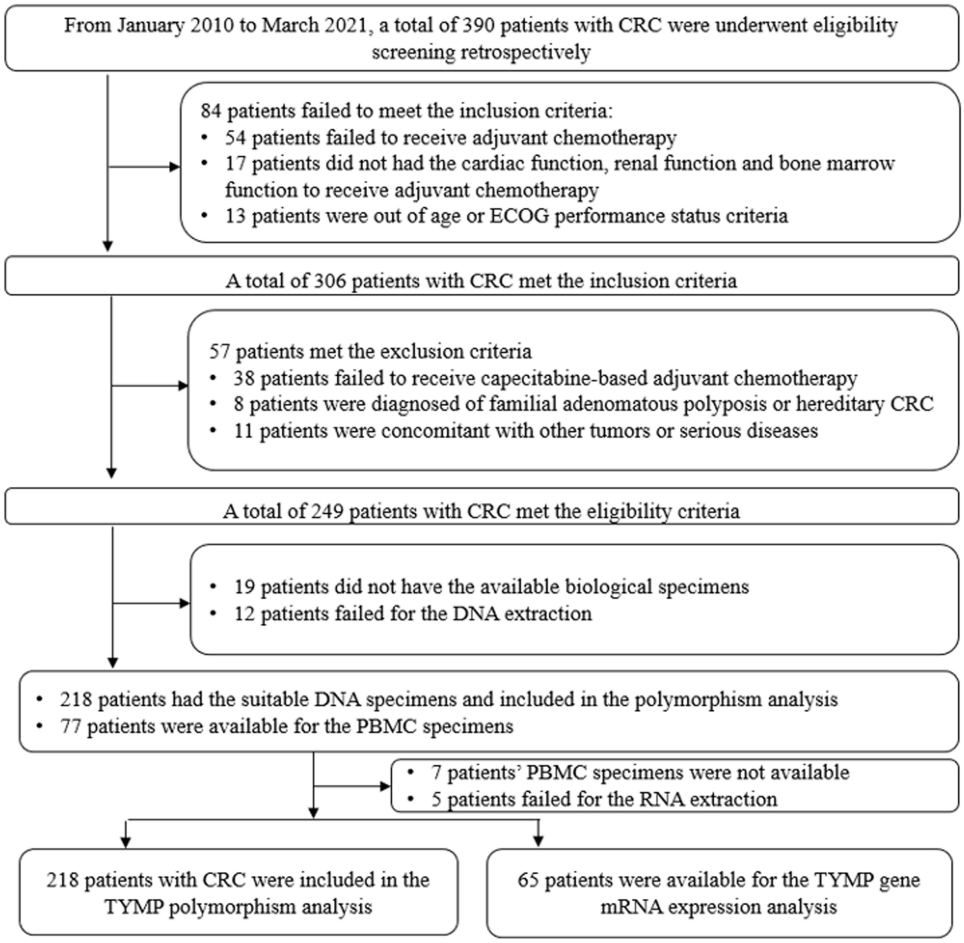

Figure 1 Flow chart of this retrospective study of the implication of *TYMP* polymorphism on the prognosis of patients with colorectal cancer who were treated with capecitabine-based adjuvant chemotherapy.

colon cancer or rectal cancer; (5) received surgical resection and postoperative adjuvant chemotherapy; (6) pathological staging of II or III. The exclusion criteria included: (1) failed to receive capecitabine-based adjuvant chemotherapy (capecitabine monotherapy or capecitabine-related regimens) after surgical resection; (2) peripheral blood specimen was not available for DNA extraction; (3) diagnosis of familial adenomatous polyposis or hereditary CRC; (4) concomitant with another tumor or serious diseases that might compromise the survival of the patients. The flow chart of this study is illustrated in Figure 1. Finally, a total of 218 patients with CRC patients were enrolled in this study. The primary endpoint of this study was the association between prognosis and polymorphism genotype status. The study was approved by the ethics committee of the Affiliated Hospital of Hebei University (approval no. 2021 MEC035). Informed consent was signed by each enrolled patient in accordance with the recommendation of the declaration of Helsinki.

Data Collection

All the patients with CRC included in this study were treated with capecitabine-based adjuvant chemotherapy. The chemotherapy regimens included capecitabine monotherapy and CAPEOX combination therapy. And the usage and dosage of capecitabine monotherapy was as follows: 3–4 weeks after surgical treatment, capecitabine, 1000–1250 mg/m^2, twice daily, day 1–14, every 21 days as one cycle. Additionally, the usage and dosage of CAPEOX regimen was the following: capecitabine 1000mg/m^2, twice daily, day 1−14, every 21 days as one cycle, combined with oxaliplatin, 80–130 mg/m^2, iv. infusion, day 1. The adjuvant chemotherapy period was administered for 8 cycles or depended on the actual situation of the patients.

Each patient was followed up at the onset of adjuvant chemotherapy. Initial follow-up was implemented when the patient received adjuvant chemotherapy in the hospital, where the baseline characteristics and the date of disease recurrence could be obtained through the electronic medical record system specifically. Subsequent follow-up was performed mainly by telephone. Patients were followed up every three months for the date of recurrence and treatment after recurrence, and the death status was mainly inquired. Furthermore, when a patient has recurrence or death, it must be confirmed by at least two research colleagues before it could be confirmed as a recurrent events or death events in survival analysis.

Procedure of Polymorphism Analysis

Peripheral blood specimen from each enrolled patient were collected when it was available. Genomic DNA was extracted using the traditional phenol chloroform method. Unfortunately, 19 patients failed to obtain the available peripheral blood specimen and 12 patients failed for the genomic DNA extraction. Consequently, a total of 218 patients with adjuvant chemotherapy had the suitable DNA specimens and included in the polymorphism analysis finally. The single nucleotide polymorphism included in this study was collected from the National Center for Biotechnology Information (NCBI) database with the minor allele frequency >10% among Chinese population or the previous study that identified the clinical significance of the polymorphisms. Consequently, polymorphisms included were rs11479, rs131804 and rs470119. In the preliminary analysis between the polymorphism status and prognosis, only rs11479 was significantly associated with prognosis. Therefore, the subsequent analysis of this study was focused on rs11479 polymorphism accordingly.

The rs11479 polymorphism was genotyped using the method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR product of rs11479 was amplified initially, the PCR product including this polymorphism was amplified, forward primer was 5'- TCTAACAGCCCCTCGCTCT-3', reverse primer was 5'-GGGTCACGTGTTCATCGAG-3'. And the size of PCR product was 266bp. A total of 2 μL PCR products were digested using the restriction enzyme *NdeI* (Thermo Fisher Scientific, USA). Genotyping results of rs11479 were distinguished through the size of PCR bands according to the previous study.[16] Additionally, genotyping results for rs11479 were confirmed in some randomly selected samples using ionization-time-of-flight mass spectrometry (Sequenom, SanDiego, CA) method, and the detailed methods were adopted from the previous study.[17] The two genotyping approaches reached a 100% consistency.

Peripheral Blood Mononuclear Cell (PBMC) Specimen Collection and *TYMP* Gene mRNA Expression Analysis

Fresh peripheral blood specimens stored in EDTA anticoagulated tubes were obtained from 77 randomly selected patients from the 218 patients. Fresh peripheral blood samples were first centrifuged to separate plasma, and the remainder was diluted and washed with an equal volume of phosphate-buffered saline (PBS). These samples were then added to an equal volume of lymphocyte separation solution Histopaque-1077, after which the intermediate layer of leukocytes was aspirated through centrifugation. Then the specimens were washed with an appropriate amount of PBS to obtain the fresh PBMC specimen. However, 7 patients' PBMC specimens were not available and 5 patients failed for RNA extraction experiment subsequently. Ultimately, a total of 65 mRNA specimens were available for the subsequent analysis and preserved in liquid nitrogen. Total RNA samples were extracted using TRizol reagents (Takara Biotechnology, China) according to the instruction and stored at −80°C for mRNA expression analysis. A total of 500ng RNA extracted from the PBMC specimens were used as the templates for reverse-transcription polymerase chain reaction. Relative quantitative analysis of *TYMP* gene mRNA expression was implemented using the LightCycler® 480 (Roche, Shanghai, China) with SYBR Premix EX Taq system. The forward primer of *TYMP* was 5'-ATGGCAGCCTTGATGACC-3', the reverse primer was 5'- TTATTGCTGCGGCGGCAG-3'. Amplified system was comprised of 10 μL SYBR Premix EX Taq, 0.2 μL of each primer (20μM), 7.6 μL double distilled water (ddH2O) and 2 μL cDNA. *TYMP* gene mRNA expression was determined by the method of comparative Ct ($2^{-\Delta\Delta Ct}$) with *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* mRNA expression being used as an endogenous control according to the previous research.[18]

Statistical Analysis

Correlation analysis was performed using SPSS version 25.0 (IBM, USA). Hardy-Weinberg equilibrium test was performed for the rs11479 genotype status using χ^2 test method. The significance of proportion variables and continuous variables according to rs11479 genotype status was analyzed using χ^2 test and the Mann–Whitney U nonparametric test (between the two groups), respectively. The primary analysis of this study was the association between disease-free survival (DFS) or overall survival (OS) and rs11479 genotype status. Survival curves were produced using Stata 14.0 to present DFS and OS data according to rs11479 genotype status using Log rank test to estimate the significant difference. DFS was defined as the date from the onset of surgical resection to the date of patients' recurrence of disease or death from any cause. OS was defined as the date from the onset of surgical resection to the date of patient's death from any cause. Those without death event by the end of the study follow-up, survival end points were censored at the date of data cut-off. Additionally, Cox multivariable analysis was introduced for DFS. $P<0.05$ was accepted as statistical significance.

Results

Baseline Characteristics of the 218 Patients with CRC According to *TYMP* rs11479 Genotype Status

Baseline characteristics of the 218 patients with CRC included in this study are presented in Table 1. All the 218 patients were treated with capecitabine-based adjuvant chemotherapy, capecitabine monotherapy was administered in 53 patients and CAPEOX (capecitabine combined with oxaliplatin) regimen was implemented in 165 patients. Interestingly, it should be noted that the actual median completion cycle of capecitabine-monotherapy regimen was 5 cycles (range: 3–8 cycles). And the actual median completion cycle of CAPEOX regimen was 4 cycles (range: 2–8 cycles).

As described in the method part, only *TYMP* rs11479 was of clinical significance preliminarily. The prevalence of rs11479 polymorphism among the 218 patients with CRC was: GG genotype 141 cases (64.7%), GA genotype 68 cases (31.2%), AA genotype 9 cases (4.1%), minor allele frequency (MAF) of rs11479 was 0.20. The distribution of the three genotypes was in accordance with Hardy-Weinberg equilibrium ($P=0.825$). Given that the frequency of AA genotype was relatively rare, patients with AA and GA genotypes were merged in the subsequent analysis. As presented in Table 1, patients with GG and GA/AA genotype were well balanced with similar baseline characteristics and no statistically significant difference was observed ($P>0.05$).

Table 1 Baseline Characteristics of the 218 Patients with CRC According to *TYMP* rs11479 Genotype Status

Baseline Characteristics	Total (N=218, %)	*TYMP* rs11479 Genotype Status		χ^2	*P*
		GG (N=141)	GA/AA (N=77)		
Age Median (range)	59 (28–83)	59 (31–83)	59 (28–81)	NA	0.519
Gender					
Male	127 (58.3)	84 (59.6)	43 (55.8)	0.285	0.593
Female	91 (41.7)	57 (40.4)	34 (44.2)		
ECOG PS score					
0	148 (67.9)	92 (65.2)	56 (72.7)	1.278	0.258
1–2	70 (32.1)	49 (34.8)	21 (27.3)		
Tumor location					
Colon cancer	153 (70.2)	99 (70.2)	54 (70.1)	0.000	0.990
Rectum cancer	65 (29.8)	42 (29.8)	23 (29.9)		
Pathological staging					
II	48 (22.0)	28 (19.8)	20 (26.0)	1.085	0.298
III	170 (78.0)	113 (80.2)	57 (74.0)		

(*Continued*)

Table 1 (Continued).

Baseline Characteristics	Total (N=218, %)	*TYMP* rs11479 Genotype Status		χ^2	*P*
		GG (N=141)	GA/AA (N=77)		
Histological type					
Adenocarcinoma	196 (89.9)	125 (88.7)	71 (92.2)	0.694	0.405
Other type	22 (10.1)	16 (11.3)	6 (7.8)		
MMR status					
dMMR	15 (6.9)	9 (6.4)	6 (7.8)	0.156	0.925
pMMR	109 (50.0)	71 (50.4)	38 (49.4)		
NA	94 (43.1)	61 (43.3)	33 (42.8)		
Adjuvant radiotherapy					
Yes	47 (21.6)	28 (19.9)	19 (24.7)	0.683	0.408
No	171 (78.4)	113 (80.1)	58 (75.3)		
Adjuvant chemotherapy regimen					
Capecitabine monotherapy	53 (24.3)	36 (25.5)	17 (22.1)	0.323	0.570
CAPEOX	165 (75.7)	105 (74.5)	60 (77.9)		
Completion of scheduled chemotherapy					
Capecitabine completion cycle Median (range)	5 (3–8)	5 (3–8)	5 (3–8)	NA	0.512
CAPEOX completion cycle Median (range)	4 (2–8)	4 (2–8)	4 (3–8)	NA	0.437

Abbreviations: CRC, colorectal cancer; *TYMP, thymidine phosphorylase*; ECOG, Eastern Cooperative Oncology Group; PS, performance status; MMR, mismatch repair; dMMR, deficiency of mismatch repair; pMMR, proficiency of mismatch repair; CAPEOX, capecitabine plus oxaliplatin; NA, not available.

Implications of *TYMP* rs11479 Polymorphism on the Prognosis of the 218 Patients with CRC

All the 218 patients with CRC included in this study were available for prognostic assessment. The data cut-off date of this study was September 2021. The median follow-up duration from the date of patients included in this study to the date of data cut-off was 5.5 years (follow-up range: 0.25–10 years). At the date of data cut-off, a total of 121 disease recurrence events were observed and a total of 110 death events were observed. Collectively, the prognostic data of the 218 patients with CRC was presented in Figures 2 and 3. As shown in Figure 2, the median DFS of the 218 patients was 4.6 years [95% confidence interval (CI): 3.80–5.40], 3-year DFS rate was 59.04% (95% CI: 52.13–65.29%) and 5-year

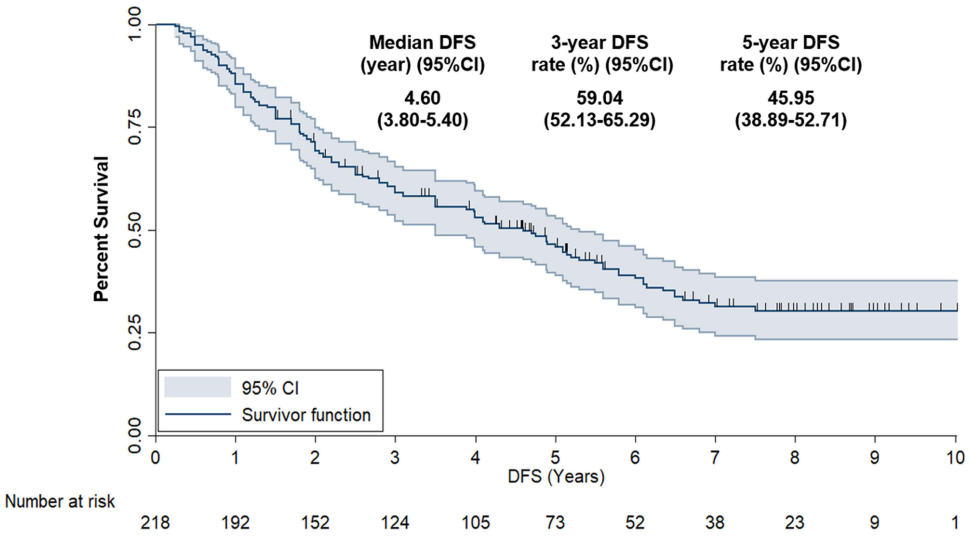

Figure 2 Disease-free survival of the 218 patients with colorectal cancer who received capecitabine-based adjuvant chemotherapy.

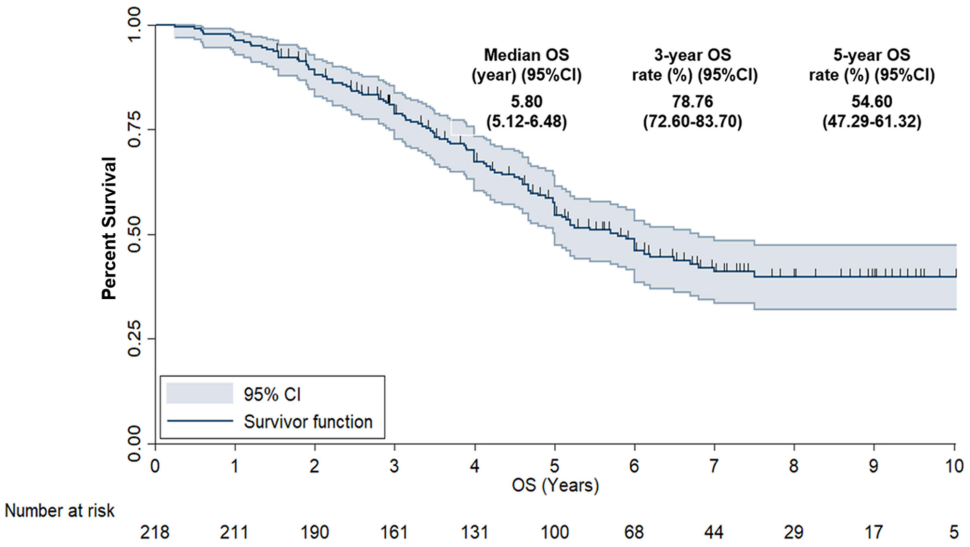

Figure 3 Overall survival of the 218 patients with colorectal cancer who received capecitabine-based adjuvant chemotherapy.

DFS rate was 45.95% (95% CI: 38.89–52.71%), respectively. Furthermore, as illustrated in Figure 3, the median OS of the 218-patient cohort was 5.8 years (95% CI: 5.12–6.48), the 3-year OS rate was 78.76% (95% CI: 72.60–83.70%) and 5-year OS rate was 54.60% (95% CI: 47.29–61.32%).

In order to identify the clinical significance of *TYMP* rs11479 polymorphism, association analysis between genotyping status of rs11479 and DFS was performed firstly. As exhibited in Figure 4, the median DFS of the patients with GG and GA/AA genotype of rs11479 polymorphism was 3.1 years (95% CI: 2.08–4.12) and 6.1 (95% CI: 4.65–7.55) years, respectively. And the 3-year DFS rate was 50.38% (95% CI: 41.78–58.36%) and 74.97% (95% CI: 63.58–83.25%), which was statistically significant (χ^2=8.164, *P*=0.004). Furthermore, the association between rs11479 genotype status and OS is illustrated in Figure 5, the median OS of the patients with GG and GA/AA genotype of rs11479 polymorphism was 5.0 (95% CI: 4.05–5.95) years and 7.0 (95% CI: NA-NA) years, respectively. The 5-year OS rate of patients with GG and GA/AA genotype of rs11479 polymorphism was 49.65% (95% CI: 40.52–58.11%) and 63.31% (95% CI: 50.83–73.44%). And the difference was statistically significant (χ^2=4.562, *P*=0.033).

Furthermore, the median DFS according to different baseline characteristic subgroups in univariate analysis was performed simultaneously. As exhibited in Table 2, only ECOG performance status score and pathological staging were

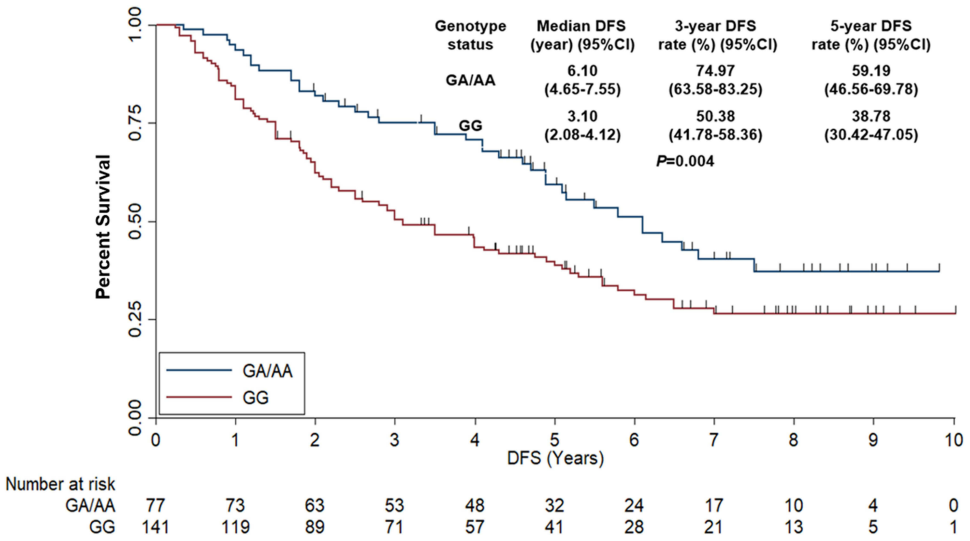

Figure 4 Disease-free survival of the 218 patients with colorectal cancer according to *TYMP* rs11479 genotype status (log-rank P value in the KM plot).

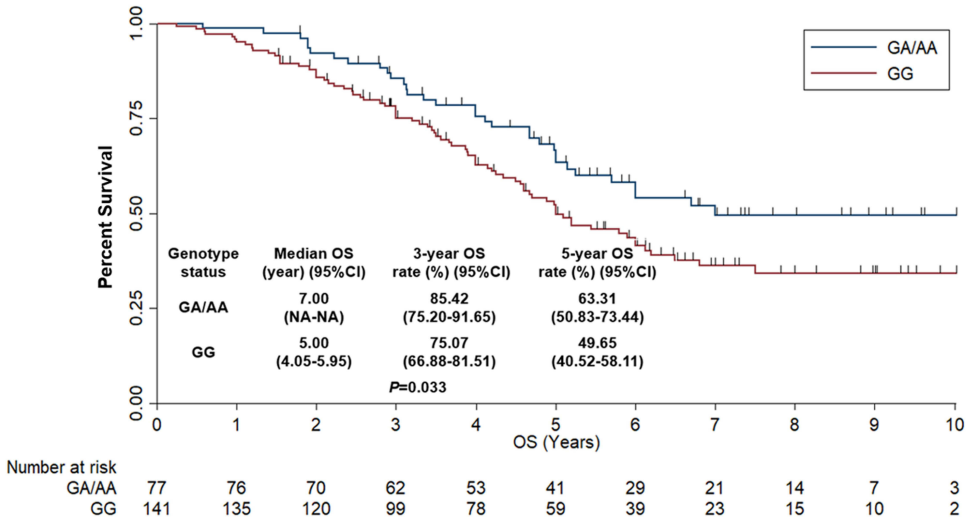

Figure 5 Overall survival of the 218 patients with colorectal cancer according to *TYMP* rs11479 genotype status (log-rank P value in the KM plot).

significantly associated with DFS in univariate analysis according to baseline characteristics, which suggested that the median DFS of patients with ECOG 0 score was dramatically longer than that of patients with the 1–2 score (median DFS: 5.80 vs 3.89 years, *P*=0.009), the median DFS of patients with pathological staging of II was significant longer than that of patients with III (median DFS: 6.15 vs 3.89 years, *P*=0.002). Furthermore, a multivariate Cox regression model was introduced for DFS adjustment including the baseline characteristics which was significant in the univariate analysis as illustrated in Table 2. The multivariate analysis results are illustrated in Table 2 as well, after the multivariate adjustment, the significant statistical difference was still observed between *TYMP* rs11479 polymorphism and DFS, which suggested that rs11479 polymorphism was an independent factor for DFS [hazard ratio (HR)=1.64, *P*=0.009]. Besides, as presented in Table 2, after adjusted in the Cox regression analysis, both ECOG score (HR = 0.65, *P*=0.015) and pathological staging (HR = 0.57, *P*=0.007) were independent factors for DFS.

Relevance of rs11479 Polymorphism to *TYMP* Gene mRNA Expression

As mentioned in the methods part, a total of 65 PBMC specimens were suitable for *TYMP* gene mRNA expression analysis finally. The expression of *TYMP* gene mRNA was detected by extracting RNA from the 65 PBMC specimens and the relevance analysis between the genotype status of rs11479 polymorphism and *TYMP* mRNA expression was analyzed accordingly. Firstly,

Table 2 Univariate and Multivariate Analysis of DFS Among the 218 Patients with Colorectal Cancer

Baseline characteristics	Median DFS (95% CI)	*P* (univariate analysis)	Multivariate analysis	
			HR (95% CI)	*P*
Age				
<59	5.30 (4.50–6.10)	0.313		
≥59	4.50 (3.65–5.35)			
Gender				
Female	5.50 (4.59–6.41)	0.435		
Male	4.55 (3.72–5.38)			
ECOG PS score				
0	5.80 (4.93–6.67)	0.009	0.65 (0.46–0.88)	0.015
1–2	3.89 (3.11–4.67)			
Tumor location				
Colon cancer	5.10 (4.28–5.92)	0.531		
Rectum cancer	4.10 (5.25–4.95)			

(*Continued*)

Table 2 (Continued).

Baseline characteristics	Median DFS (95% CI)	*P* (univariate analysis)	Multivariate analysis	
			HR (95% CI)	*P*
Pathological staging				
II	6.15 (5.09–7.21)	0.002	0.57 (0.33–0.81)	0.007
III	3.89 (3.02–4.76)			
Histological type				
Adenocarcinoma	5.10 (4.08–6.12)	0.461		
Other type	4.30 (3.55–5.05)			
MMR status				
dMMR	4.10 (3.24–4.96)	0.614		
Other status	4.89 (4.02–5.76)			
Adjuvant radiotherapy				
Yes	5.00 (4.09–5.91)	0.619		
No	4.30 (3.51–5.09)			
Adjuvant chemotherapy regimen				
Capecitabine monotherapy	4.12 (3.31–4.93)	0.315		
CAPEOX	4.89 (4.01–5.77)			
TYMP rs11479 genotype status				
GG	3.10 (2.08–4.12)	0.004	1.64 (1.25–2.70)	0.009
GA/AA	6.10 (4.65–7.55)			

Abbreviations: CRC, colorectal cancer; DFS, disease free survival; *TYMP, thymidine phosphorylase*; ECOG, Eastern Cooperative Oncology Group; PS, performance status; MMR, mismatch repair; dMMR, deficiency of mismatch repair; CAPEOX, capecitabine plus oxaliplatin; CI, confidence interval; HR, hazard ratio.

the genotyping status of rs11479 polymorphism in the 65 PBMC specimens was in the following: GG genotype 42 cases (64.6%), GA genotype 20 cases (30.8%) and AA genotype 3 cases (4.6%). The MAF was 0.20 and the frequency of three genotypes was in accordance with the Hardy-Weinberg equilibrium (P=0.756) as well, which was comparable to the genotype distribution among the 218 patients with CRC. Similarly, GA and AA genotypes were merged in the subsequent analysis. As illustrated in Figure 6, the relative expression of *TYMP* mRNA in PBMC of patients with GA/AA genotype was significantly

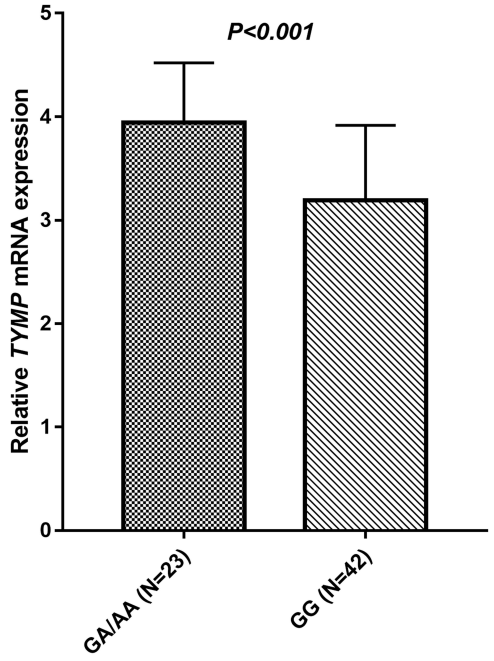

Figure 6 The relative expression level of *TYMP* mRNA according to *TYMP* rs11479 genotype status.

higher than that of patients with GG genotype of rs11479 polymorphism (3.945±0.575 vs 3.191±0.726), which was statistically significant (t = 4.294, $P<0.001$).

Discussion

CRC was established to be a highly heterogeneous digestive system malignancy.[19] Currently, traditional chemotherapy still played an important role in the treatment for CRC. However, it should be noticed that ORR of chemotherapy was relatively limited, which exhibited the urgent exploration of potential biomarkers that might predict the efficacy of traditional chemotherapy was an important research direction.[20] At present, biomarkers that might predict the effectiveness to traditional chemotherapy were mainly focused on genetic polymorphisms, ctDNA and somatic gene mutations.[21] Specifically, a recently reported polymorphism study initiated by Su et al indicated that *programmed cell death-ligand 1 (PD-L1)* gene polymorphism 901T>C could be used as a potential biomarker to involve in the prognosis of patients with CRC receiving capecitabine-based adjuvant chemotherapy through mediation of the mRNA expression of *PD-L1*.[22] Furthermore, another study initiated by Tie et al investigated the clinical significance of ctDNA for guiding the prognosis of patients with high-risk stage III colon cancer, which indicated that the monitoring of ctDNA might be useful to predict the prognosis of patients with high-risk stage III colon cancer.[23] Additionally, Li et al performed a study to explore the predictive association between *PIK3CA* and *TP53* somatic mutations status and OS for patients with stage II and III CRC.[24] The results suggested that patients with *PIK3CA* and *TP53* double mutations were correlated with worse OS. Collectively, all the above findings demonstrated that genome DNA status of colon cancer or rectal cancer might predict the prognosis of patients with CRC who were treated with conventional chemotherapy to some extent.[25]

Although this study was designed as a retrospective analysis, we still carried out prognostic analysis among the 218 patients with CRC who received capecitabine-based adjuvant chemotherapy. And the results exhibited that the median DFS of the 218 patients was 4.6 years (95% CI: 3.80–5.40) and the median OS of the 218-patient cohort was 5.8 years (95% CI: 5.12–6.48). The DFS and OS data in our study seemed to be lower than that in NO16968 clinical trial which was implemented to identify the feasibility of oxaliplatin combined with capecitabine as adjuvant therapy for stage III CRC.[26] The discrepancy between the two studies might be attributed to the following explanation: our study included more patients with an ECOG performance status of 2 score than that in the NO16968 study and the results of the Cox analysis in our study indicated that patients with ECOG of 2 score conferred a worse prognosis, which was consistent with the previous study.[27] Besides, it should be noted that this study was designed as a retrospective analysis. Management of the patients in retrospective study was not sufficient and normative compared with well-designed phase III clinical trial. Furthermore, the actual completion of capecitabine monotherapy regimen in this study was only 5 cycles, and the CAPEOX regimen was only 4 cycles. The insufficient adjuvant chemotherapy might stand a good chance to compromise the survival of the patients to some extent.[28]

Considerable previous studies suggested that genetic variation of drug metabolism gene might contribute to the effectiveness of a variety of drugs.[29] To our knowledge, our study might be the first exploration disclosed that the carriers of A allele in rs11479 of *TYMP* gene might be sensitive to capecitabine administration and benefit from capecitabine adjuvant chemotherapy in Chinese patients with CRC who received capecitabine-based adjuvant chemotherapy by influencing the mRNA expression of *TYMP*. Interestingly, a previous study initiated by Du et al was reported.[14] A total of 235 patients with CRC underwent surgical treatment were included in their study retrospectively and they investigated the influence of *TYMP* genetic variation on clinical outcomes of patients with CRC. The conclusion in their study suggested that 5633C>T of *TYMP* might impact the prognosis of the patients. The idea of the study design was consistent with that of our study. However, the heterogeneous adjuvant chemotherapy regimens in their study might compromise the clinical significance of 5633C>T of *TYMP* in the analysis. Another recent study initiated by Chen et al performed the implication of *TYMP* genetic variation on the clinical outcome of gastric cancer patients who received capecitabine-based adjuvant chemotherapy.[16] A total of 198 patients with gastric cancer participated in the study and the clinical significance of *TYMP* genetic variation was implemented. Furthermore, the conclusion exhibited that rs11479 of *TYMP* had favorable influence on the clinical outcomes of gastric cancer patients received capecitabine-based adjuvant chemotherapy, which was in concert with our study even the tumors in the two studies were different. Additionally, another study initiated by Liu et al investigated the relevance of *TYMP* genetic variation to the survival of gastrointestinal

cancer patients treated with fluoropyrimidines.[15] A total of 141 metastatic gastrointestinal cancer patients who received 5-FU-based first-line chemotherapy were included and the results suggested that A allele gene carriers were associated with higher *TYMP* gene expression, which was consistent with the mRNA expression results in our study. However, they failed to identify the positive association of the polymorphism with OS mainly own to the relatively limited sample size. Furthermore, a previous study initiated by Jennings et al found that T allele gene carriers of rs11479 were correlated with higher incidence of adverse reaction among patients with CRC receiving capecitabine therapy, which was partly in line with the results of our study, highlighting the possibility that A allele carriers of rs11479 might be more sensitive to capecitabine administration.[30]

Additionally, we had searched and read the relevant papers regarding how the genetic changes could cause post-translational modification to the level and function of *TYMP* protein carefully. Fortunately, we found a previous study initiated by Zhang et al investigated the clinical significance of *deoxycytidine kinase (DCK)* polymorphism in acute myeloid leukemia.[31] They also identified that rs67437265 which was located at coding region (missense polymorphism similar as rs11479 in our study) affected the *DCK* mRNA expression significantly and the potential explanation was that rs67437265 was in strong linkage disequilibrium with rs4643786 that was located at 3'-untranslated region, which might explain the *DCK* mRNA expression and clinical significance of rs67437265. As a result, we speculated that rs11479 in our study might be in linkage disequilibrium with another polymorphism in *TYMP*, thus contributing to the *TYMP* mRNA expression level and function of *TYMP* protein to some extent.

Additionally, it should be noted that another two polymorphisms were also included in this study. Unfortunately, both rs131804 and rs470119 were negative of the association with DFS in the preliminary analysis, which was consistent with the previous study that not all polymorphisms in *TYMP* were of clinical significance.[32] We speculated that possible explanation was that not all polymorphisms in one gene might change the function of that gene and confer potential clinical significance as the previous study indicated.[33]

Interestingly, the relationship between the expression level of *TYMP* gene and the prognosis of CRC remained controversial in recent study.[34] Our study preliminarily suggested that patients with higher mRNA expression of *TYMP* might be more likely to benefit from capecitabine administration, thus conferring a superior prognosis, which was consistent with the results of previous study initiated by Lu et al.[35] A total of 57 patients with advanced gastric cancer who received capecitabine-related regimens were included Lu' study and they found that the mRNA expression of *TYMP* was positively associated with overall response and OS. Nevertheless, on the other hand, considerable studies found that *TYMP* gene was an important factor to promote tumor angiogenesis, which demonstrated that higher expression of *TYMP* gene was associated with a higher tumor burden, resulting in a tendency that the tumor was easy to recurrence and metastasis and the worse prognosis.[36] Collectively, *TYMP* gene played a dual role in vivo. On the one hand, it stimulated angiogenesis of tumor cells to motivate tumor growth. On the other hand, *TYMP* was an important metabolic gene of capecitabine and a key gene for 5-FU chemotherapy drugs to play a cytotoxic role and kill tumor cells.[37] Collectively, the conclusion in our study was needed to be confirmed in large-scale clinical trials subsequently.

The limitations of this study were as follows: firstly, the sample size of the study was relatively small, clinical significance of *TYMP* polymorphism in patient with CRC was still needed to be evaluated in a larger population. Secondly, this study was designed as a retrospective analysis and some bias might not be avoided. However, the clinical significance of *TYMP* rs11479 was fully evaluated, we thought our study was of clinical significance for the prognostic evaluation of patients with CRC who received surgical resection and capecitabine-based adjuvant chemotherapy.

Conclusions

Collectively, this study provided real-world evidence regarding the prognostic data of patients with CRC who were treated with surgical resection and capecitabine-based adjuvant chemotherapy retrospectively. Simultaneously, the prognostic association analysis suggested that GA/AA genotype of rs11479 in *TYMP* gene might be involved in the superior prognosis of patients with CRC who received capecitabine-based adjuvant chemotherapy through mediation of mRNA expression of *TYMP*. The present study was of clinical implication for the prognostic evaluation of patients with CRC who received capecitabine-based adjuvant chemotherapy in clinical practice, which suggested that when patients were genotyped of the adverse genotype status of rs11479, longer duration of capecitabine-based adjuvant chemotherapy

or replacement of other adjuvant chemotherapy regimens was needed for the patients to reduce the recurrence risk in the future.

Disclosure

The authors declare no conflicts of interest in this work.

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