

Influence of thymidylate synthase gene polymorphisms on the survival of colorectal cancer patients receiving adjuvant 5-fluorouracil

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The present study aimed to prospectively investigate the influence of thymidylate synthase (TS) polymorphisms (5'-TSER, 3'-TSUTR) on the disease-free survival (DFS) and overall survival (OS) of patients with colorectal cancer (CRC) who were treated with adjuvant 5-fluorouracil (5-FU) therapy. Patients were followed up for 19 ± 14 months (median \pm SD). TS genotypes were determined from the peripheral blood mononuclear cells of 166 patients by polymerase chain reaction–polyacrylamide gel electrophoresis and restriction fragment length polymorphism methods. 5'-TSER 3R homozygotes showed significantly longer DFS ($P=0.048$) and OS ($P=0.009$). The 5'-TSER and 3'-TSUTR genotype combination groups showed a significant difference for DFS ($P=0.039$) and OS ($P=0.029$). Significantly better DFS ($P=0.049$) and OS ($P=0.043$) were observed for 6 bp/6 bp genotypes in 5'-TSER heterozygotes ($n=80$). Based on this, and on hazard ratios obtained by Cox regression analysis of the DFS of genotype–combinations, the patients were classified as belonging to prognostic groups A and B. The DFS and OS of these two groups showed a highly significant difference ($P=0.002$ and 0.001). In the multivariate Cox regression model, beside tumour location, the prognostic classification (groups A and B) proved to be an independent prognostic factor. Our data suggest that those TS genotypes and their combinations

(group A: 3R/3R with any 3'-TSUTR genotype and 2R/3R with 6 bp/6 bp), which have been reported earlier as having high TS expression, predict significantly longer DFS and OS. We found that a combination of germline TS polymorphisms is an independent prognostic marker in selecting CRC patients with worse prognosis, and it may be worthwhile to examine whether these patients would benefit from an alternative therapy. *Pharmacogenetics and Genomics* 15:723–730 © 2005 Lippincott Williams & Wilkins.

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Introduction

5-fluorouracil (5-FU) plays a central role in the treatment of colorectal cancer (CRC). Although adjuvant chemotherapy has been shown to significantly improve survival, especially in Dukes' C disease, many patients are treated without any proven clinical benefit [1,2].

Recent work suggests that tumour site, gender, p53 alterations and microsatellite instability may be important determinants of any benefit obtained from this treatment, but the need remains to identify further predictive and prognostic factors for the patients' response, to avoid unnecessary and sometimes toxic treatments [3].

One of the most important molecular targets of 5-FU is thymidylate synthase (TS), which catalyses the transformation of 2'-deoxyuridine-5'-monophosphate (dUMP) to

2'-deoxythymidine-5'-monophosphate (dTMP), an essential precursor for DNA replication. An active metabolite of 5-fluorouracil, 5-fluorodeoxyuridine-5'-monophosphate (FdUMP), prevents DNA synthesis by forming a stable complex with TS and 5,10-methylenetetrahydrofolate (MTHF) as cofactors, thus blocking the conversion of dUMP to dTMP [4]. Studies performed *in vitro* have demonstrated a relationship between TS levels and response to 5-FU [5]. In clinical studies, high TS levels (mRNA, protein, enzyme activity) are generally associated with a poor response, especially in advanced CRCs [6,7]. In several studies, a high TS level is associated with poor postoperative outcome independently of the Dukes' stage [2,8]. However, several reports indicate that patients with high TS expression may benefit from 5-FU-based chemotherapy in the adjuvant setting [9,10]. The identification of various factors that are significant in

the regulation of TS expression would be of considerable value for understanding the mechanism of 5-FU sensitivity and resistance. The promoter enhancer region of TS gene (*TYMS*) is polymorphic, containing a double (2R) or triple (3R) tandem repeat of a 28-bp sequence, which is present on the transcript, and known to be involved in the translational autoregulation mechanism of TS expression [11]. The 3R/3R homozygote genotype was shown to be related with higher TS mRNA expression [12]. The second, equally common polymorphism is a 6 bp ins/del type polymorphism at the TS 1494del6 locus on the 3'-UTR of the TS mRNA. This polymorphism influences message instability. 0 bp homozygous individuals had significantly lower TS mRNA levels compared to those of heterozygotes [13]. Most studies have determined the TS polymorphisms from the tumour tissue, although it would be a major advantage to analyse the genotype of the host, which offers a quick and non-invasive approach.

The present study was designed to prospectively investigate whether the 5'-TSER and 3'-TSUTR polymorphisms determined from the peripheral blood mononuclear cells and their combined analysis have any predictive value for disease-free survival (DFS) and overall survival (OS) of CRC patients treated with adjuvant 5-FU-based chemotherapy.

Patients and methods

Patients

From May 1995 to June 2004, 166 patients with Dukes' B2 and C CRC, who underwent radical resection at the Department of Surgery of our Institute and received 5-fluoropyrimidine-based adjuvant chemotherapy, entered this study. Their median age was 58 years (range 23–82 years). Each patient provided their written informed consent and the study was approved by the Ethics Committee of the Institute. The demographic and clinicopathological characteristics of patients are summarized in Table 1.

Treatment

All patients received 5-FU-based chemotherapy in the adjuvant setting. Patients with rectal cancer also received preoperative radiotherapy. Chemotherapy was bolus FUFA according to the Mayo regimen (FA 20 mg/m², 5-FU 425 mg/m²/day, days 1–5, every 28 days, six cycles) or continuous infusional 5-FU plus folinic acid (deGramont protocol: FA 200 mg/m², 5-FU bolus 400 mg/m², continuous infusional 5-FU 600 mg/m²/day, days 1–2, every 14 days, 12 cycles), or continuous infusional 5-FU (400 mg/m²/day, days 1–5, every 28 days, six cycles). In stage II (Dukes' B) patients, chemotherapy was indicated only for patients at higher risk (obstruction, perforation, grade 3 tumour, young age, lymphovascular or perineural tumour cell invasion, etc.).

Molecular genetic methods

Peripheral blood mononuclear cells (PBMC) were extracted from anticoagulated (10 ml EDTA 15%) blood by Ficoll gradient. DNA from the PBMCs of the patients was isolated according to the manufacturer's instructions using the MasterPure DNA purification kit (Epicentre Technologies, Madison, Wisconsin, USA). The standard protocol and reaction conditions of the polymerase chain reaction (PCR) analyses have been reported in detail previously [14].

5'-TSER polymorphism genotypes were determined by using primers (forward) 5'-GTGGCTCCTGCGTTTCCCCC-3' and (reverse) 5'-TCCGAGCCGGCCACAGGCAT-3', as described by Kawakami *et al.* [15]. Reaction products were analysed by non-denaturing polyacrylamide gel electrophoresis, on a 10% gel, and the 28-bp difference in allele size (220 bp for the 3R allele and 192 bp for the 2R allele) could discriminate between alleles.

3'-TSUTR polymorphism genotypes were examined by PCR amplification, followed by restriction fragment length polymorphism (RFLP) analysis as described by Ulrich *et al.* [16]. The primers used for PCR amplification were: 5'-CAAATCTGAGGGAGCTGAGT-3' (forward) and 5'-CAGATAAGTGGCAGTACAGA-3' (reverse). PCR products were digested with *DraI* endonuclease. After 1 h of digestion at 37°C, products were separated on a 10% non-denaturing polyacrylamide gel. The RFLP analysis discriminated between alleles by the presence of a *DraI* recognition site. In both PCR analysis methods, sequencing, beside the fragment size, was used to validate the amplified products.

Statistical analysis

For the analysis of the frequency distribution of 5'-TSER and 3'-TSUTR polymorphism, patients were subdivided into groups on the basis of different clinicopathological parameters. The differences were tested with contingency table exact tests. The studied parameters as potential prognostic factors of survival were analysed by univariate technique. Survival was estimated by Kaplan–Meier method, and the log-rank test was used for comparisons. Univariate and multivariate Cox's regression analyses employing NCSS statistical software (NCSS, Kaysville, Utah, USA) were used to calculate the hazard ratios (HR) and 95% confidence intervals. For multivariate Cox analysis a 'hierarchical forward with switching' model was also applied.

Results

The clinicopathological characteristics of 166 patients with colorectal cancer and the genotype frequencies of the two TS polymorphisms are presented in Table 1. Among the patients, the distribution of 5'-TSER polymorphism was 18% 2R/2R, 48% 2R/3R and 34% 3R/3R

Table 1 Distribution of clinicopathological parameters and cross tabulations with 5'-TSER and 3'-TSUTR genotypes

Characteristics	n	%	5'-TSER			P ^a	3'-TSUTR			P
			2R/2R	2R/3R	3R/3R		0 bp/0 bp	0 bp/6 bp	6 bp/6 bp	
All patients (%)	166	100	29 (18)	80 (48)	57 (34)		17 (10)	79 (48)	70 (42)	
Sex										
Male	91	55	18	40	33	0.477	14	38	39	0.035
Female	75	45	11	40	24		3	41	31	
Age (years)										
<58	84	51	15	42	27	0.837	8	41	35	0.924
≥ 58	82	49	14	38	30		9	38	35	
Dukes' stage										
B2	46	28	9	21	16	0.847	4	20	22	0.699
C	120	72	20	59	41		13	59	48	
Grade										
1/2	145	87	27	67	51	0.425	15	65	62	0.622
3	21	13	2	13	6		1	12	8	
Tumour location										
Rectum	82	49	15	40	27	0.958	6	41	35	0.074
Sigmoid	35	21	6	18	11		5	21	9	
Colon	49	30	8	22	19		6	17	26	
Treatment type										
Bolus	70	42	12	35	23	0.934	11	31	28	0.152
Continuous	96	58	17	45	34		6	48	42	
3'-TSUTR										
0 bp/0 bp	17	10	0	8	9	0.038				
0 bp/6 bp	79	48	11	38	30					
6 bp/6 bp	70	42	18	34	18					

^aExact test; n, number of patients.

and that of 3'-TSUTR genotypes 0 bp/0 bp, 0 bp/6 bp and 6 bp/6 bp was 10%, 48% and 42%, respectively. No significant differences in the frequencies of 5'-TSER and 3'-TSUTR genotypes could be demonstrated according to age, Dukes' stage, grade, tumour location and type of chemotherapy. However, the genotype distributions between males and females showed a significant difference for 3'-TSUTR ($P < 0.035$); the frequency of the 0 bp/0 bp homozygotes was 15% among males and only 4% among females. No similar difference could be demonstrated in the case of 5'-TSER genotypes. A significant association was found between the distribution of 5'-TSER and 3'-TSUTR polymorphisms ($P = 0.038$).

During the follow-up period of 19 ± 14 months (median \pm SD), 58 patients (34.9%) relapsed (11 local recurrences, 47 distant metastases). Based on the results of log-rank test and univariate Cox regression model (Table 2), it appears that patients treated with continuous 5-FU-based chemotherapy had significantly better outcome ($P = 0.036$) than those treated by bolus regimen. Considering the clinicopathological similarities between the colon and sigmoid colon tumours, survival analysis was performed by comparing the group of patients with colon and sigmoid tumours with that of rectal cancer patients. Patients with colon and sigmoid tumours showed a more favourable DFS than patients with rectal tumours ($P = 0.065$), although the difference was not statistically significant.

Evaluating the relationship between the survival and TS genotypes, it was observed that patients containing at

least one 5'-TSER 2R allele showed significantly shorter DFS and OS than 3R homozygous patients ($P = 0.048$). At the same time, neither the 3'-TSUTR genotypes, nor the other investigated clinicopathological parameters were significantly associated with DFS or OS.

Although no significant influence of 3'-TSUTR polymorphism on the survival was identified, the 0 bp/0 bp genotype group had higher hazard ratio compared to that of patients containing at least one 6 bp allele.

Combining the two polymorphisms, the patients could be divided into eight groups (it is noteworthy that, 2R/2R and 0 bp/0 bp genotype combination was lacking among our patients). Kaplan-Meier survival curves showed a significant difference between genotype combinations (DFS: log-rank test, $P = 0.039$ and, for trend, $P = 0.027$; OS: log-rank test, $P = 0.029$ and, for trend, $P = 0.016$) (Figs 1 and 2).

In the case of 5'-TSER homozygotes (3R/3R and 2R/2R), the combination of two polymorphisms did not result in significantly different survival curves. By contrast, analysing the survival curves of 5'-TSER heterozygous (2R/3R) patients ($n = 80$) grouped according to the 3'-TSUTR polymorphism resulted in significantly better DFS and OS ($P = 0.049$ and 0.043 , respectively) being observed in the case of 6 bp/6 bp genotypes compared to that of patients carrying at least one 0 bp allele.

To demonstrate that the combined TS polymorphism might be an independent prognostic factor for survival,

Table 2 Univariate analysis of survival

	<i>n</i>	Disease-free survival				Overall survival			
		ms	<i>P</i> ^a	HR ^b	95% CI	ms	<i>P</i>	HR	95% CI
Sex									
Male	91	20	0.055	1		26	0.718	1	
Female	75	22		0.59	0.35–1.02	25		1.15	0.54–2.45
Age (years)									
< 58	84	21	0.877	1		27	0.267	1	
≥ 58	82	21		0.94	0.56–1.57	24		1.48	0.69–3.16
Dukes' stage									
B2	46	24	0.481	1		29	0.119	1	
C	120	20		1.23	0.69–2.19	24		2.12	0.80–5.63
Grade									
1/2	145	21	0.559	1		26	0.817	1	
3	21	20		0.80	0.38–1.70	25		0.87	0.26–2.89
Tumour location									
Rectum	82	18	0.182	1		23	0.214	1	
Sigmoid	35	23	1 vs. 2, 0.120	0.59	0.29–1.21	28	1 vs. 2, 0.094	0.35	0.10–1.20
Colon	49	24	1 vs. 3, 0.137	0.64	0.35–1.16	28	1 vs. 3, 0.353	0.80	0.35–1.82
			1 vs. 2+3, 0.065	0.62	0.37–1.04		1 vs. 2+3, 0.184	0.60	0.28–1.29
Treatment type									
Bolus	70	20	0.036	1		27	0.615	1	
Continuous	96	22		0.58	0.35–0.97	25		0.82	0.39–1.75
5'-TSER									
3R/3R	57	24	0.134	1		29	0.027	1	
2R/3R	80	20	1 vs. 2, 0.093	1.73	0.94–3.21	24	1 vs. 2, 0.012	3.99	1.35–11.84
2R/2R	29	18	1 vs. 3, 0.080	1.95	0.92–4.14	23	1 vs. 3, 0.081	3.19	0.85–11.90
			1 vs. 2+3, 0.048	1.79	1.00–3.22		1 vs. 2+3, 0.009	3.79	1.31–10.98
3'-TSUTR									
0 bp/0 bp	17	20	0.552	1		26	0.659	1	
0 bp/6 bp	79	22	1 vs. 2, 0.325	0.69	0.32–1.52	27	1 vs. 2, 0.696	0.75	0.25–2.27
6 bp/6 bp	70	20	1 vs. 3, 0.307	0.65	0.29–1.47	24	1 vs. 3, 0.406	0.59	0.18–1.91
			1 vs. 2+3, 0.285	0.67	0.32–1.42		1 vs. 2+3, 0.467	0.68	0.23–1.95

^aLog-rank test;^bHR estimated by Cox proportional hazard regression model. HR, hazard ratio; CI, confidence interval; ms, mean survival time (months); *n*, number of patients.

multivariate Cox regression analysis was performed. To avoid the interpretation difficulties by applying multicategorical variables in COX regression analysis, it appeared to be useful to also generate binary variable for TS polymorphisms (instead of the eight categories).

Considering the significantly better survival of 6 bp homozygotes of 5'-TSEr heterozygotes (2R/3R 6 bp/6 bp), it was reasonable to couple this genotype group to the 3R homozygotes of which DFS and OS proved to be significantly longer (Table 2). Furthermore, the hazard ratios of relapse obtained by univariate Cox regression analysis of the eight combined genotype groups were also relevant to define two major prognostic groups. In group A, the patients belonging to the 3R homozygous group, regardless of their 3'-TSUTR polymorphism, together with the 3'-TSUTR 6 bp homozygotes from the 2R/3R heterozygous group are at a lower risk of relapse (HR ≤ 1). By contrast, in group B, the 2R homozygotes, regardless of their 3'-TSUTR genotype, together with those with the 2R/3R genotype carrying at least one 0 bp allele are at a higher risk for relapse (HR > 1) (Table 3).

Further survival analysis of the patients classified into groups A and B demonstrated a highly significant difference between the two prognostic groups for both

DFS and OS ($P = 0.002$ and $P = 0.001$, respectively) (Figs 3 and 4).

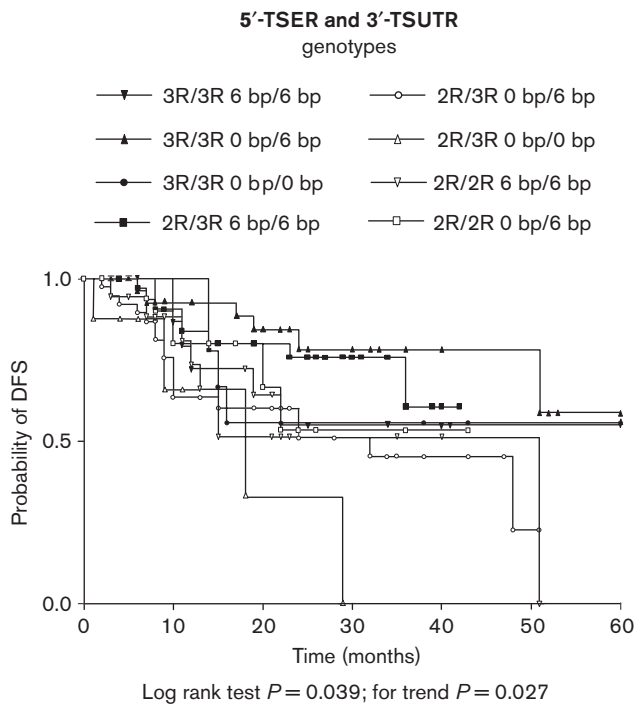
In the multivariate Cox regression model, beside Dukes' stage, tumour location and treatment type, the TS prognostic classification into groups A and B proved to be an independent prognostic factor for DFS. Tumour location and TS prognostic classification were found also to be independent prognostic factors for OS (Table 4).

Using a 'hierarchical forward with switching' multivariate Cox regression model for DFS, tumour location ($P = 0.003$) treatment type (0.001) and TS polymorphism (0.004), and for OS only, the TS polymorphisms (0.004) were selected as independent variable subsets (data not shown).

Discussion

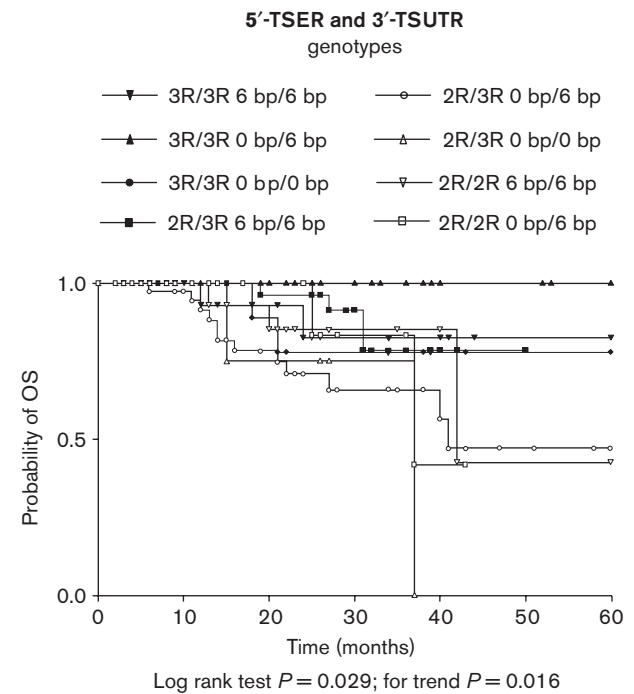
Recently, the possibility to determine the TS status from normal tissue or even from PBMC, has received great attention, because the latter would represent an easily accessible tissue sample for characterizing the TS status of the host [17]. However, the question remains to be answered of whether there is a correlation between the TS status/polymorphisms of the host and the patients' prognosis (DFS and OS).

Fig. 1



Kaplan-Meier analysis of the disease-free survival of patients, stratified according to different 5'-TSER and 3'-TSUTR genotype combinations.

Fig. 2



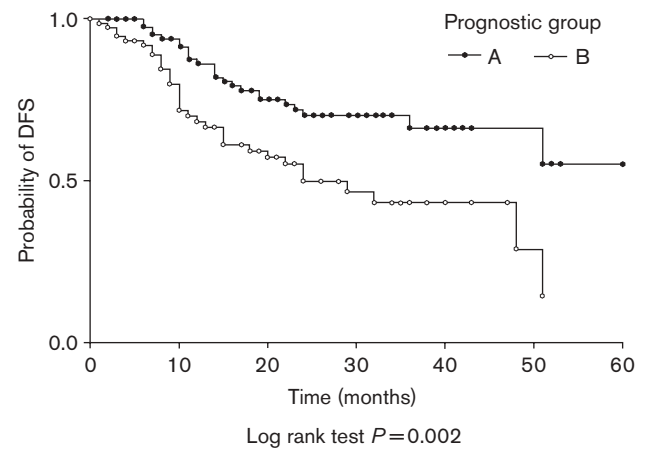
Kaplan-Meier analysis of the overall survival of patients, stratified according to different 5'-TSER and 3'-TSUTR genotype combinations.

Table 3 Determination of the prognostic groups according to different genotype-combinations by applying univariate Cox regression analysis of DFS

Genotype groups	<i>n</i>	HR	95% CI	Prognostic group ^a
3R/3R 6 bp/6 bp	18	1.0		A
3R/3R 0 bp/6 bp	30	0.5	0.15–1.49	
3R/3R 0 bp/0 bp	9	0.9	0.27–3.44	
2R/3R 6 bp/6 bp	34	0.7	0.23–1.96	
2R/3R 0 bp/6 bp	38	1.6	0.62–3.97	B
2R/3R 0 bp/0 bp	8	3.0	0.84–10.75	
2R/2R 6 bp/6 bp	18	1.6	0.55–4.56	
2R/2R 0 bp/6 bp	11	1.1	0.31–3.88	

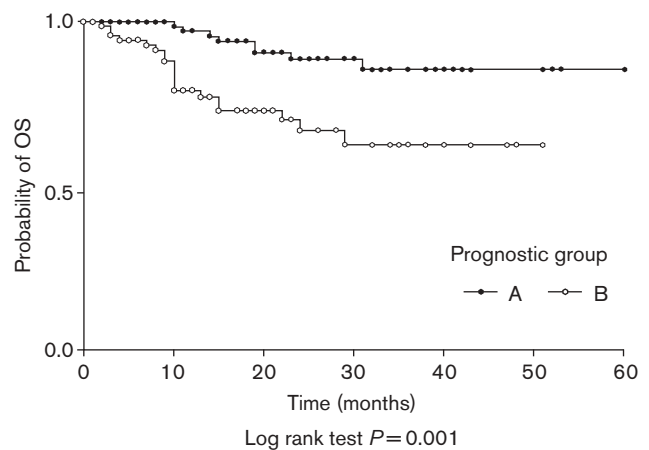
^aGenotypes with HR > 1 and with HR ≤ 1 were classified as prognostic groups A (lower relapse risk) and B (higher relapse risk). *n*, number of patients; HR, hazard ratio; CI, confidence interval.

Fig. 3



Disease-free survival of patients belonging to prognostic groups A and B. See the text, for the specification of prognostic groups A and B.

Fig. 4



Overall survival of patients belonging to prognostic groups A and B. See the text, for the specification of prognostic groups A and B.

Table 4 Multivariate analysis of survival

	n	Disease-free survival			Overall survival		
		HR ^a	95% CI	P	HR	95% CI	P
Sex							
Male	91	1			1		
Female	75	0.60	0.34–1.07	0.086	1.25	0.54–2.90	0.600
Age (years)							
< 58	84	1			1		
≥ 58	82	1.03	0.61–1.75	0.904	1.83	0.83–4.06	0.137
Dukes' stage							
B2	46	1			1		
C	120	2.04	1.03–4.06	0.042	2.68	0.91–7.87	0.074
Grade							
1/2	145	1			1		
3	21	1.33	0.62–2.85	0.466	0.81	0.24–2.78	0.742
Tumour location							
Rectum	82	1			1		
Sigmoid/colon	84	0.44	0.23–0.83	0.011	0.36	0.14–0.95	0.039
Treatment type							
Bolus	70	1			1		
Continuous	96	0.35	0.19–0.65	0.001	0.46	0.19–1.10	0.082
TS prognostic group							
B	57	1			1		
A	80	0.46	0.27–0.79	0.005	0.30	0.13–0.69	0.005

^aHR estimated by Cox proportional hazard regression model. n, number of patients; HR, hazard ratio; CI, confidence interval.

The present study is the first to demonstrate that the combination of 3'-TSUTR and 5'-TSER polymorphisms measured from the PBMC of CRC patients receiving adjuvant 5-FU-based chemotherapy is an independent prognostic factor of survival. These results suggest that those germline TS genotypes, which have been reported to involve high TS expression [12,13,18–20] can predict significantly better DFS and OS.

Based on previous reports demonstrating an association between TS 3R/3R or 6bp/6bp genotypes and higher protein or mRNA expression [13,18,19], it might be presumed that patients with 3R/3R combined with any 3'-TSUTR genotype, and those with 2R/3R together with 6bp/6bp genotype belong to the high TS expressing group (group A). By contrast, all other TS genotype combinations are associated with medium or low TS expression (group B).

On surveying earlier reports on the value of tumoural TS as a prognostic factor of the survival after adjuvant chemotherapy, the published results are controversial. Several studies indicate that high TS expression of tumour is a significant predictor of poorer DFS and/or OS in the adjuvant setting [1,8,21–26] whereas other publications demonstrate significantly improved survival rate of patients with high intratumoural TS level [2,9,27–29].

A recent study demonstrated that metastatic CRC patients with germline 3R/3R genotype responded better to 5-FU treatment, with a higher response rate and longer time to progression [30]. However, it should be noted that the results of palliative chemotherapy cannot be transferred to adjuvant setting and vice versa.

The aim of adjuvant chemotherapy is to eradicate circulating tumour cells and micrometastases [2]. There might be also TS independent mechanisms (i.e. RNA directed action of 5-FU or induction of apoptosis responsible for the favourable prognosis of patients with TS polymorphisms relating high TS expression). By studying the Fas (CD95/Apo1), a 5-FU-inducible mediator of apoptosis, Longley *et al.* [31] demonstrated that certain tumours despite expressing high levels of TS may still be sensitized to Fas mediated apoptosis by 5-FU.

The survival of patients with CRC may also be influenced by the folate-deficient state, which can be related to the TS polymorphisms. Odin *et al.* [32] showed that, in CRC patients, high folypolyglutamate synthase (FPGS) gene expression in the normal mucosa was associated with better tumour-specific survival. The mucosa samples with high FPGS levels also expressed high TS and elevated total folate levels. A relationship between folate level and TS polymorphisms has been reported by Chen *et al.* [33]. Compared with the mean folate level among individuals with the 3R/3R genotype, those with the 2R/3R and 2R/2R ($P = 0.03$) genotypes had lower levels of plasma folate ($P = 0.03$ for trend). No effects of 3'-TSUTR polymorphism on plasma folate levels were observed. By contrast, Trinh *et al.* [34] demonstrated an association of 3R/3R genotype with low plasma folate levels among Chinese individuals. Moreover, Kealey *et al.* [35] found that the median red blood cell folate concentration was much higher for 3-TSUTR 0 bp/0 bp subjects compared to either 6 bp/6 bp or 0 bp/6 bp individuals. 3R homozygotes showed a nonsignificant trend for association with lower folate intermediate levels (tetrahydrofolate and 5,10-methylenetetrahydrofolate).

determined in the tumour tissue of CRC patients [36]. Similarly, the 5'-TSER polymorphism was not associated with serum folate concentrations in healthy young Caucasians [37]. Further investigations are needed to reveal the real relationship, if any, between the folate level and TS polymorphisms. Moreover, this presumed relationship may be different in the case of healthy individuals, in colorectal carcinogenesis or during anti-folate (5-FU, methotrexate, etc.) chemotherapy.

On the other hand, the serious toxicity, which was observed in patients with low TS expression or TS genotypes involving low TS expression in the normal tissues, might also contribute to the worse prognosis of these patients [38,39]. Our patients belonging to group B (low TS) also showed more frequent toxic side-effects (30% versus 22%, $P=0.034$) compared to patients in group A (high TS). It is well known that 5-FU-caused toxicity among others manifests in the form of diarrhea, nausea, anorexia, vomiting and thus low energy intake. Dray *et al.* [40] presented evidence indicating that low energy intake is associated with significantly shorter survival of CRC patients; therefore, it is presumable that patients with a low TS expression involving serious side-effects could have worse prognosis after adjuvant 5-FU therapy.

The inhibition of the cellular TS activity and the incorporation of the fluorinated nucleotides into RNA and DNA may cause a slower renewal of the normal mucosa and of other tissues, and thus may also result in a worse outcome. Patients with low tumoural TS mRNA levels who received adjuvant 5-FU chemotherapy developed tumour recurrence more frequently than those with high tumoural TS levels [29].

Although no precise explanation of our results can yet be given, our data suggest that 5'-TSER and 3'-TSUTR polymorphisms of the host have prognostic value on the survival of CRC patients who are treated with adjuvant 5-FU therapy. Further clinical trials are ongoing aiming to determine whether 5-FU-based adjuvant chemotherapy should be combined with other available drugs, such as oxaliplatin or irinotecan, in patients with an unfavourable prognosis.

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