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ORIGINAL ARTICLE

Thymidylate synthase gene polymorphisms as important contributors affecting hepatocellular carcinoma prognosis

X. Wang[a,b,1], X. Sun[a,1], X. Du[a], F. Zhou[a], F. Yang[a], J. Xing[a], G. Dong[c,*], X. Guo[a,*]

[a] State Key Laboratory of Cancer Biology, Experimental Teaching Center of Basic Medicine, Fourth Military Medical University, 169, Changle West Road, Xi'an 710032, China
[b] Department of Breast Cancer Center, Shaanxi Cancer Hospital, Xi'an 710061, China
[c] Department of General Surgery, The General Hospital of PLA, 28, Fuxing Road, Beijing 100853, China

Summary
Background: Thymidylate synthase (TYMS), a key rate-limiting enzyme in the folate metabolism, plays essential roles in the development of several malignancies including hepatocellular carcinoma (HCC). Nonetheless, the association of the single nucleotide polymorphisms (SNPs) in *TYMS* gene with the prognosis of Chinese HCC patients remains unknown.
Methods: A total of 492 HCC patients who underwent surgery treatment were included in this study. Five functional SNPs (rs2847153, rs2853533, rs502396, rs523230, and rs9967368) in *TYMS* gene were genotyped using the iPLEX genotyping system. Multivariate Cox proportional hazards regression model and Kaplan–Meier curve were used to analyze the association of SNPs with survival and recurrence of HCC patients.
Results: Two SNPs (rs523230 and rs9967368) in *TYMS* gene were significantly associated with the overall survival of HCC patients. Patients carrying homozygous variant genotype (VV) of rs523230 had significantly decreased risk of death (hazard ratio [HR], 0.68; 95% confidence interval [CI], 0.46–1.00; $P = 0.048$) when compared with those carrying homozygous wild-type (WW) or heterozygous (WV) genotypes, while patients carrying WV + VV genotype of rs9967368 had significantly increased risk of death (HR, 1.46; 95% CI, 1.05–2.04; $P = 0.026$) when compared with those carrying WW genotypes. Cumulative effect analysis showed a significant dose-dependent effect of unfavorable SNPs on OS.
Conclusions: Our study for the first time demonstrates the association of SNPs in *TYMS* gene and clinical outcome of HCC, suggesting that rs523230 and rs9967368 in *TYMS* gene might be used to predict clinical outcome of Chinese HCC patients.

Abbreviations: HCC, hepatocellular carcinoma; SNPs, single nucleotide polymorphisms; HR, Hazard ratio; CI, Confidence interval.

* Corresponding authors.
E-mail addresses: guanglongdong@126.com (G. Dong), fmmuxguo@163.com (X. Guo).

[1] Xiaoyan Wang and Xiacheng Sun contribute equally to this work.

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Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide.[1,2]. Even under huge improvements in surveillance and clinical treatment strategies, the prognosis of HCC patients remains poor. In order to reduce medical cost and iatrogenic injury caused by suboptimal management in HCC patients, individualized treatment greatly varies according to disease status. In fact, according to clinical practice guideline, even TNM staging of HCC, the most widely used staging method, could not identify the disease status precisely and accurately. In accordance with these, it seems to be urgent to explore novel biomarkers to distinguish HCC patients with different disease status and clinical outcomes, which would lead to an appropriate individual treatment.

Thymidylate synthase (TYMS), a key rate-limiting enzyme in the folate metabolism, catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). This conversion essentially influences DNA repair, methylation and syntheses through the production of nucleotide [3]. The genetic mutations in *TYMS* gene have been demonstrated to influence the activities of enzyme and the prognosis of many malignant tumors [3—7]. The *TYMS* gene contains polymorphic 28-bp tandem repeat sequence in the 5′-untranslated region enhancer region (TSER) affecting the translational efficiency [8]. And the triple repeat has showed greater gene expression than the double repeat, which mediates more effective binding between ribosome and *TYMS* mRNA [8,9]. Additionally, mutation with 6-bp deletion of the sequence TTAAAG at nt1494 has been found to be associated with *TYMS* gene expression [10,11]. Moreover, recent studies have shown that expression levels of *TYMS* mRNA and protein are prognostic indicators for colorectal and non-small-celllung cancer patients [12]. But there are limited data from the association study of the *TYMS* genetic polymorphisms and death risk of HCC.

Furthermore, single nucleotide polymorphisms (SNPs) may account for much diversity of genetic traits in human beings, such as cancer susceptibility, prognosis and therapeutic responses [13,14]. The SNPs in *TYMS* gene are considered as an essential role in the development of malignancies in several studies. The relationship between SNPs of *TYMS* gene and several malignant tumors, such ascolorectal, lung, pancreatic and gastric cancers, has been investigated in previous studies [3,5,6]. Among women but not men, a decreased risk has been observed in colorectal cancer patients with the variant TSER 2rpt/2rpt genotype [15]. Further studies have demonstrated that susceptibility of cancers is associated with SNPs of *TYMS* gene. Among male patients with advanced colorectal cancer, the significant effect of *TYMS* polymorphisms on the clinical evolution has been observed [16]. Analogously, it is suggested that three SNPs (rs16430 6 bp del/ins, rs2790 A > G and rs1059394 C > T) in the miRNA binding sites of *TYMS* might be associated with developing risk and survival of gastric cancer patients [1]. In a population-based case-control study, SNPs of *TYMS* gene (rs10502289, rs2298583, and rs2298581) have been demonstrated to be associated with a marginally significant decrease in risk of endometrial cancer under the dominant model [17]. In another study among NSCLC patients in our lab, SNP rs2847153 in *TYMS* gene has been demonstrated as a potential biomarker for predicting clinical outcome [18]. In a clinical research among Chinese people, the enhanced TYMS activity has been demonstrated to be an essential element in minimizing uracil misincorporation into DNA, which protect against the development of HCC [19]. However, whether SNPs in *TYMS* gene are associated with the patient prognosis in HCC remains to be unknown.

In this study, association of five functional SNPs in *TYMS* gene with the overall survival (OS) or recurrence-free survival (RFS) of HCC patients have been investigated. And for the first time, based on large number clinical data analysis, we systematically investigated the comprehensive effects of SNPs of *TYMS*on the clinical outcomes of HCC patients.

Materials and methods

Study population

A total of 518 Chinese Han patients who had resectable HCC were recruited at Xijing Hospital, The Fourth Military Medical University (Xi'an, China) and Eastern Hepatobiliary Surgery Hospital, Secondary Military Medical University (Shanghai, China) between January 2009 and January 2012. HCC was diagnosed based on clinical manifestation, medical history, imaging and serum examination according to the National Comprehensive Cancer Network clinical practice guidelines. All cases had no previous history of other cancers or did not undergo anticancer treatments before surgery. After recruitment, all patients received surgery as the first-line treatment within a week after diagnosis. We finally analyzed 492 patients after excluding 26 patients, which including 3 patients with incomplete clinical data and 23 patients died within 1 month postsurgery. Written informed consent was obtained from each participant before enrollment. This study was approved by Hospital's Ethics Committees both in Shanghai and Xi'an.

Demographic and clinical data

Demographic data were collected by well-trained interviewers. Detailed clinical information was collected through medical chart review or consulting with treating physicians. The follow-up information was updated every 3-month interval through onsite interview, direct calling, or medical chart review by a trained clinical specialist. The latest follow-up data in this analysis were obtained in January 2013. For each patient, 5-mL of venous blood was collected for genomic DNA extraction using the E.Z.N.A. Blood DNA Midi Kit (Omega Bio-Tek, Norcross, GA, USA) in the laboratory.

SNP selection and genotyping

The candidate functional SNPs in *TYMS* gene were selected using a set of web-based SNP selection tools (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm). The criteria are as follows:

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