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RAPID COMMUNICATION

Thymidylate synthase and thymidine phosphorylase gene expression as predictive parameters for the efficacy of 5-fluorouraci-based adjuvant chemotherapy for gastric cancer

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Abstract

AIM: To investigate the prognostic role of thymidylate synthase (TS) and thymidine phosphorylase (TP) mRNA levels in T3 or T4 gastric cancer treated with 5-fluorouraci-based adjuvant chemotherapy.

METHODS: Fifty-one patients with T3 or T4 gastric cancer received systemic 5-fluorouraci-based adjuvant chemotherapy, and intratumoral expression of TS and TP in 51 gastric cancer tissue samples was tested by real-time quantitative PCR.

RESULTS: The median disease-free survival (DFS) time was 10.2 mo in the patients. There were no significant differences in DFS between the groups with high and low levels of TP. However, the group with low level of TS had a longer DFS (14.4 mo *vs* 8.3 mo, $P = 0.017$). The median overall survival (OS) time was 18.5 mo, and there were significant differences in OS between the groups with high and low levels of TS or TP (for TS, 17.0 mo *vs* 21.3 mo, $P = 0.010$; for TP, 16.6 mo *vs* 22.5 mo, $P = 0.009$). Moreover, the coupled low expression of these two genes was strongly associated with a longer survival time of patients as compared with that of a single gene.

CONCLUSION: Expression of TS and TP mRNA is a useful predictive parameter for the survival of postoperative gastric cancer patients after 5-fluorouracil-based adjuvant chemotherapy.

Key words: 5-fluorouracil; Gastric cancer; Thymidine phosphorylase; Thymidylate synthase

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INTRODUCTION

Since originally synthesized in 1957, 5-fluorouracil (5-FU) has been widely applied in clinical practice. Although 5-FU is considered one of the most effective agents against gastrointestinal carcinoma, its efficacy rate is only 10%-15% for gastric and colon cancer, and differs greatly among patients[1,2]. As a pyrimidine analog, 5-FU exerts its antitumor effects through anabolism, which is determined by the rate of catabolism. Thus, the expression level of genes coding for key enzymes in metabolism may play a pivotal role in the sensitivity and efficacy of 5-FU[3].

Thymidylate synthase (TS) is the target enzyme for 5-FU and catalyzes methylation of fluorodeoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), which is an important process of DNA biosynthesis[4,5]. Several reports indicate that TS expression in clinical tumor samples is significantly related to the response to 5-FU in gastric cancer patients[6]. Recent studies demonstrate that TS mRNA level may be an important independent prognosticator of survival in patients with gastric cancer[7].

Thymidine phosphorylase (TP) is an enzyme involved in pyrimidine nucleoside metabolism. TP is expressed in a wide variety of solid tumors (carcinomas of the breast, stomach, colon, pancreas and lung), and its content is higher in tumor tissues than in adjacent normal tissues[8]. TP levels might affect the sensitivity of 5-FU, and transfection of TP cDNA into cancer cells increases their sensitivity to 5-FU[9]. It was also reported that high expression of TP in tumors is indicative of a poor prognosis [10].

In this study, we examined the mRNA level of TS/TP in a panel of 51 patients with T3 or T4 gastric adenocarcinoma to probe its predictive value for the efficacy of 5-FU-based adjuvant chemotherapy. In addition, we tested if combination of expression levels of the analyzed genes has an increased effect on the prediction of patient survival.

MATERIALS AND METHODS

Patients and tissue samples

A total of 51 patients with T3 or T4 gastric adenocarcinoma who underwent gastrectomy at the 4th Affiliated Hospital of Suzhou University from August 2003 to April 2005 were enrolled in this study. Tumor tissue samples were obtained during surgery and stored in liquid nitrogen until preparation of RNA extracts. Inclusion criteria for patients included: (1) patients with histologically proved gastric cancer, (2) patients without early recurrence or non-curable resection, (3) patients receiving no other adjuvant treatments, such as radiotherapy or immunotherapy, (4) patients with their performance status score of 0-1 and a life expectancy over 6 mo. All patients gave their informed consent, and the study was approved by the Institutional Ethics Review Board.

Chemotherapy schedule

After surgery, all patients underwent 6 courses of 5-FU-based adjuvant chemotherapy. The chemotherapy protocol included intravenous infusion of 5-FU (375-500 mg/m^2 per day, d 1-5) and leucovorin (LV) (100-200 mg/m^2 per day, d 1-5) at three-week interval between two courses. The therapy was terminated or shifted to second-line chemotherapy when disease progression was observed or unacceptable toxicity appeared. All patients were treated in our hospital from 2003 to 2005.

Total RNA isolation and cDNA synthesis

RNA was extracted by using Trizol (Invitrogen Corporation, CA) according to the manufacturer's instructions. The amount of total RNA was estimated by ultraviolet absorbance at 260 nm, and the quality was determined by agarose gel electrophoresis in the presence of formaldehyde. cDNA strand synthesis was performed using a reverse transcription system (Promega Corporation, US).

Real-time quantitative PCR assay for TS and TP

Quantitative PCR was performed with the LightCycler TS/TP mRNA quantification kit^{PLUS} (Roche Diagnostics, Germany) as follows: denaturation of cDNA at 95℃ for 5 min; 40 cycles of amplication of cDNA at 95℃ for 10 s, at 62℃ for 10 s, at 72℃ for 10 s; cooling the rotor and thermal cycler at 40℃ for 30 s. LightCycler TS/TP calibrator RNA was also used to compensate for the constant differences between detection of the target (TS/TP) and reference gene (glucose- 6-phosphate dehydrogenase, G6PDH) and to provide a constant calibration point between PCR runs. The amount of mRNA encoding for TS/TP was calculated using the LightCycler relative quantification software (Roche). All gene expression analyses were performed in a blinded fashion by the laboratory investigators.

Statistical analysis

To evaluate the association of TS and TP with disease-free survival (DFS) and overall survival (OS), TS and TP expression levels were categorized into a low and high value, using the median concentration as a cut point. The maximal χ^2 method was used to determine which expression value best segregated patients into poor and good prognosis subgroups. The survival rate was calculated by the Kaplan-Meier method and statistical analysis was performed using the log-rank test. Cox's proportional hazard model was used to assess the prognostic importance of gene expressions adjusted for the following clinicopathologic features: age, gender, histological type, lymphatic metastasis. $P < 0.05$ was considered statistically significant. All analyses were performed using SPSS software (version 10.0.5; SPSS Inc. Chicago, IL).

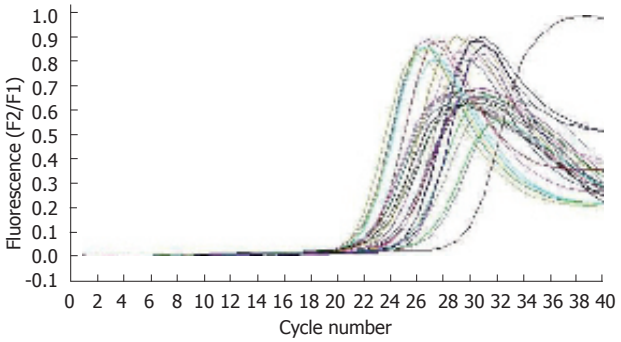

Figure 1 Amplication curve of RT-PCR on the target (TS/TP) and reference gene (G6PDH). TS: thymidylate synthase; TP: thymidine phosphorylase; G6PDH: glucose- 6-phosphate dehydrogenase.

RESULTS

Expression of 5-fluorouracil-related genes TS and TP in gastric tumor

A total of 51 eligible patients were enrolled in the study between August 2003 and April 2005. The quantitation of TS and TP mRNA levels in gastric cancer tissues was successfully performed in all specimens (Figure 1). Intratumoral expression of TS and TP genes ranged from 0.09 to 4.60 (median: 0.94) and 3.99 to 196.28 (median: 21.20), respectively.

No significant differences were observed between the mRNA levels of TS/TP and clinicopathological features such as gender, age and stage. The relationship between TS and TP expression levels and clinicopathologic factors are summarized in Table 1.

DFS in relation to expression of TS and TP

The median DFS of the 51 eligible patients was 10.2 mo. When the median value (21.20) of TP was assigned as the cut-off value, there was no significant difference in DFS between the high and low level groups (9.5 mo *vs* 14.0 mo, $P = 0.068$). Patients with a low expression level of TS showed a trend to correlate with prolonged DFS (14.4 mo *vs* 8.3 mo, $P = 0.017$).

OS in relation to expression of TS and TP

The median DFS was 16.2 mo in patients with low TS and TP expression, but only 8.30 mo in patients with high TS and TP expression. The median OS was 18.5 mo. When the median value of 5-fluorouracil-related TS and TP genes was designated as a cut-off value, there were significant differences in OS between the groups with high TS (TP) expression and low TS (TP) expression (17.0 mo *vs* 21.3 mo,

Table 1 Relationship between TS/TP mRNA levels and clinicopathologic factors

Factor	*n*	TS		*P*	TP		*P*
		High value	Low value	value	High value	Low value	value
Age (yr)							
≥ 60	22	9	13	0.313	12	10	0.492
< 60	29	16	13		13	16	
Gender							
Male	39	19	20	0.938	18	21	0.460
Female	12	6	6		7	5	
Histological Type							
Ⅱ	25	15	10	0.124	11	14	0.482
Ⅲ	26	10	16		14	12	
Lymphatic metastasis							
Positive	41	22	19	0.323	21	20	0.777
Negative	10	3	7		4	6	

TS: thymidylate synthase; TP: thymidine phosphorylase.

Table 2 Coupled expression of TS + TP and patients survival

	TS + TP		*P*-value
	High value	Low value	
n	16	17	
DFS	8.3 (3.7-23.7)	16.2 (5.8-30.7)	0.013
OS	16.2 (5.3-29.7)	25.0 (9.2-32.1)	0.003

DFS: disease-free survival; OS: overall survival; TS: thymidylate synthase; TP: thymidine phosphorylase.

$P = 0.010$; 16.6 mo *vs* 22.5 mo, $P = 0.009$), (Figure 2A-B). Tumors tended to be associated with a low TS/TP mRNA expression level and a prolonged patient survival.

Coupled expression of TS and TP and patients survival

The relationship between the expression levels of TS and TP was determined for the survival of patients (Table 2). As Figure 2C shows, the survival of 16 patients with high expression of TS and TP was significantly shorter than that of 17 patients with low expression of TS and TP (for OS, 16.2 mo *vs* 25.0 mo, $P = 0.003$; for DFS, 8.3 mo *vs* 16.2 mo, $P = 0.013$). Thus, the coupled low expression level of the two genes resulted in longer OS as well as DFS in gastric adenocarcinoma patients in this study.

Analysis of clinicopathological features and prognosis

No significant differences were observed in OS and clinicopathological features. However, there existed a significant negative correction between OS and TS or TP expression. Histological type, lymphatic metastasis and TS gene expression were analyzed by multivariate regression analysis, showing that TS expression level could be identified as a potential predictive marker for OS with a 2.52 hazard ratio for high TS expression (95% confidence interval = 1.30-4.862, $P = 0.006$).

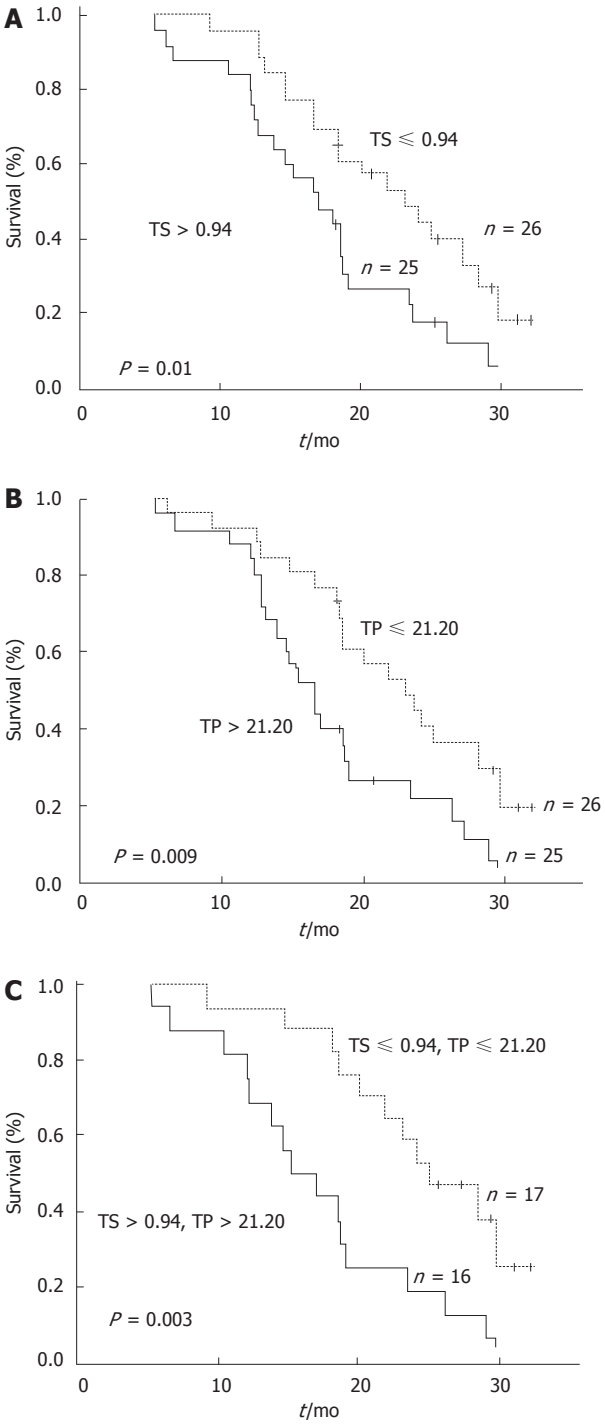

Figure 2 Kaplan-Meier survival curve for patients with intratumoral TS (**A**), TP (**B**), TS + TP mRNA levels above and below the median level of TS/TP (**C**). TS: thymidylate synthase; TP: thymidine phosphorylase.

DISCUSSION

Since the response rate of gastrointestinal carcinoma to 5-FU as a single agent, is less than 30% and differs greatly among patients[3], it is imperative to identify some indexes, which can be used to predict the efficacy of 5-FU in clinical settings. In the present study, we analyzed the mRNA levels of two genes in 51 patients with T3 or T4 gastric adenocarcinoma involved in 5-FU metabolism before 5-FU-based adjuvant chemotherapy to see if it is associated with survival. Our results showed that there was a significant correlation between TS/TP expression levels and survival of gastric cancer patients receiving postoperative FU-based adjuvant chemotherapy. The

identification of predicative markers for chemosensitivity by pharmacogenomics means could clarify which subset of patients might gain benefit, and enable clinicians to design individualized chemotherapy regimens[11,12].

The primary biochemical mechanism responsible for cytotoxicity of 5-FU is the formation of 5-fluorouridine monophosphate (FdUMP), which can bind tightly to and inhibit thymidylate synthase in the presence of 5, 10-methylene tetrahydrofolate. TS catalyzes the reductive methylation of deoxyuridine-5'-monophosphate (dUMP) to deoxythymidine-5'-monophosphate (dTMP), which is the only pathway for de novo synthesis of dTMP, so inhibition of TS by FdUMP disrupts intracellular nucleotidepools necessary for DNA synthesis[13,14]. As the main target of fluoropyrimidines, the expression level of TS is assumed to influence the efficacy of chemotherapy, although the amount of TS is not unanimously recognized as a determinant factor for 5-FU sensitivity[7,15-18]. For example, Lenz *et al*[7] showed that TS mRNA level influences response to 5-FU-based chemotherapy and survival of patients with primary gastric cancer. However, in two other studies[16,17], TS mRNA levels did not reach statistically significant association with survival. In this work, patients with a lower TS expression level showed a trend to correlate with a longer DFS ($P = 0.017$) and a longer OS ($P = 0.010$) than those with a high TS expression.

TP is known to be higher in tumor tissue than in surrounding normal tissue[19]. When 5-FU is administered, it is anabolized to FdUMP by TP in tumors[20,21]. It was reported that the expression of TP can predict the efficacy of fluoropyrimidine chemotherapy and survival of patients[21,22]. In the present study, although there was no significant difference in DFS in relation to TP expression, there existed significant differences in OS between the patients with high and low TP expression. Additionally, there would have an increased effect on prediction of survival in gastric cancer patients when TS and TP expressions were analyzed. These results indicate that measurement of TS and TP expression can identify a better survival prognosis of patients.

In summary, mRNA expression in TS and TP is a potential indicator in predicting the survival of patients with T3 or T4 gastric adenocarcinoma treated with 5-FU-based adjuvant chemotherapy. Further study is required to confirm our results and to establish the advantages of pre-treatment tumor biopsy for TS/TP screening, which permits a more rational decision on whether to precede a FU-based therapy.

COMMENTS

Background

5-fluorouracil (5-FU) is one of the most effective agents against gastrointestinal cancer. Its efficacy differs greatly among patients. As a pyrimidine analog, 5-FU exerts its antitumor effects through anabolism, which is determined by the rate of catabolism. Thus, the expression level of genes coding for key enzymes (such as TS and TP) in metabolism may play a pivotal role in the sensitivity and efficacy of 5-FU.

Research frontiers

As the key enzymes of fluoropyrimidines, the expression levels of TS and TP are assumed to influence the efficacy of chemotherapy, although TS or TP is not unanimously recognized as a determinant factor for 5-FU sensitivity. Some studies showed that TS or TP mRNA level may serve as an important independent prognostic factor of survival in patients with solid tumor, including gastric cancer. However, there are no related reports about the relationship between TS/TP mRNA expressions and the clinical outcome of gastric cancer patients treated with 5-FU-based adjuvant chemotherapy.

Innovations and breakthroughs

The mRNA expression in TS and TP is a potential indicator in predicting the survival of patients with gastric adenocarcinoma. To our knowledge, it is the first report on the effect of TS/TP mRNA expression on the prognosis of Chinese patients with gastric cancer treated with 5-FU-based adjuvant chemotherapy.

Applications

Prospective controlled clinical trials are required to confirm our results and to establish the advantages of pre-treatment tumor biopsy for TS/TP screening, which permits a more rational decision on whether to precede a fluoropyrimidine-based therapy.

Terminology

5-fluorouracil (5-FU) is a widely used medicine in treatment of solid tumor. Thymidylate synthase (TS) is the target enzyme of 5-FU and catalyzes methylation of fluorodeoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), which is an important process of DNA biosynthesis. Thymidine phosphorylase (TP) is an enzyme involved in pyrimidine nucleoside metabolism.

Peer review

The authors investigated the level of thymidylate synthase (TS) and thymidine phosphorylase (TP) mRNA in T3 or T4 gastric cancer patients treated with 5-fluorouraci-based adjuvant chemotherapy, and found that they were associated with overall survival and disease-free survival of the patients. They concluded that mRNA level of TS and TP may be a useful predictive parameter for the survival of postoperative gastric cancer patients after 5-fluorouracil-based adjuvant chemotherapy.

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