Review article

Prediction of clinical outcome of S-1-based chemotherapy for gastric cancer patients

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Abstract

S-1, an oral fluoropyrimidine has been considered to be a key drug in the treatment of advanced gastric cancer in Japan as a standard chemotherapy option. Individual variations in the enzyme activity of the 5-FU metabolic pathway can affect the extent of 5-FU metabolism and affect the efficacy of S-1 based chemotherapy. In this review, the role of genetic factors in affecting the therapeutic efficacy of S-1 is discussed, with special emphasis on enzymes involved in the 5-FU metabolic pathway. The gene expressions of thymidylate synthase, thymidine phosphorylase, and orotate phosphoribosyltransferase, in particular, are discussed in relation to the efficacy of S-1 monotherapy. The predictive values of these candidate genes may, however, be overcome when other drugs are combined with S-1. In gastric cancer patients, pharmacogenetic studies, based on either the pathway approach or a global approach, are promising for identifying both survival benefit and clinical benefit more accurately than studies based on a candidate approach, especially for the new era of S-1 combination therapy as the standard regimen. However, large controlled studies are needed to justify changes in chemotherapeutic strategies; from a “one-size fits all” to a “tailor-made” approach.

Key words Thymidylate synthase · Dihydropyrimidine dehydrogenase · Thymidine phosphorylase · Orotate phosphoribosyltransferase · Pharmacogenetics

Introduction

A growing body of evidence suggests that individual tumor variations in drug-metabolizing enzymes, DNA repair enzymes, or angiogenic enzymes may affect anti-cancer drug efficacy [1, 2]. However, whether the pharmacogenetic variations are useful in predicting drug response to and survival with specific chemotherapy

regimens remains unknown. Determination of significant associations between gene expressions and defined clinical endpoints (e.g., survival and response) may improve the prediction of treatment success and thereby the tailoring of chemotherapy.

S-1 (Taiho Pharmaceutical, Tokyo Japan) is an oral anticancer agent consisting of tegafur (FT), 5-chloro-2, 4-dihydropyridine (CDHP), and potassium oxonate (Oxo) in a molar ratio of 1:0.4:1 [3]. A randomized phase III study of 5-fluorouracil (5-FU) alone versus a combination of irinotecan and cisplatin versus S-1 alone in advanced gastric cancer showed a significant noninferiority of the S-1-alone to the 5-FU alone regimen (Japan Clinical Oncology Group 9912) [4]. Another randomized phase III study, of S-1 alone versus S-1 plus cisplatin in the treatment of advanced gastric cancer, demonstrated a significant survival benefit in the patients treated with S-1 plus cisplatin [5]. Based on the results obtained from these randomized phase III trials, S-1 has been considered to be a key drug in the treatment of advanced gastric cancer in Japan as a standard chemotherapy option.

In this article, we address some of the latest advances in the area of pharmacogenetics as it applies to S-1 based chemotherapy for patients with gastric cancer. We focus on the factors that affect the therapeutic efficacy of S-1-based chemotherapy, with special emphasis on enzymes involved in the 5-FU metabolic pathway.

Metabolic pathway of fluoropyrimidines

Tegafur is converted into 5-FU mainly by cytochrome P450 (CYP450) enzymes in the liver (Fig. 1). CYP2A6 is the main CYP450 enzyme involved in tegafur activation, but CYP1A2 and CYP2C8 also play a significant role [6].

In humans, 80%–90% of the administered 5-FU is catabolized rapidly to the inactive metabolite α -fluoro-

Fig. 1. Metabolic pathway of 5-fluorouracil (5-FU) and 5-FU analogues including tegafur. For explanation of abbreviations and metabolic pathways, see text. Modified from reference 1, with permission. 5'-dFUR, 5'-deoxy-5-fluoro-uridine; FUDP, 5-fluorouridine-5'-diphosphate; FdUDP, 5-fluoro-2'-deoxyuridine-5'-diphosphate; CD, cytidine deaminase; CE, carboxylesterase; TK, thymidine kinase; UK, uridine kinase

β -alanine via dihydrofluorouracil (FUH₂) by the first and rate-limiting enzyme, dihydropyrimidine dehydrogenase (DPD) [7] (Fig. 1). 5-FU degradation occurs in all tissues, including tumor tissues, but is highest in the liver [8]. The main mode of action of 5-FU is considered to be through its active metabolite: 5-fluoro-uridine-5'-triphosphate (FUTP) or 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP). FUTP can be incorporated into RNA, while FdUMP suppresses thymidylate synthase (TS), an essential DNA de-novo synthetic enzyme that catalyzes the methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) [9, 10]. FdUMP and TS form covalent ternary complexes with 5, 10-methylenetetrahydrofolate (5, 10-CH₂-FH₄) that subsequently inhibit DNA synthesis (Fig. 1).

The anabolic conversion of 5-FU into nucleotides such as FUTP or FdUMP is essential for its action, as illustrated in Fig. 1, where pathway 1 shows the direct conversion of 5-FU to FUMP by orotate phosphoribosyltransferase (OPRT) in the presence of 5-phosphoribosyl-1-pyrophosphate (PRPP). Pathway 2 shows the indirect conversion of 5-FU to FUMP in a sequence of reactions, with the conversion of 5-FU to 5-fluorouridine (FUR) catalyzed by uridine phosphorylase (UP) in the presence of ribose-1-phosphate (Rib-1-P). Pathway 3 shows the indirect conversion of 5-FU to FdUMP by 2'-deoxy-5-fluorouridine (FdUR) catalyzed by thymidine phosphorylase (TP) in the presence of deoxyribose-1-phosphate (dRib-1-P) [11]. In humans, the preferential use of the OPRT pathway (pathway 1) was revealed to correlate with a higher sensitivity to 5-FU [11].

The relative expression of these enzymes participating in the 5-FU metabolic pathway may be related to treatment efficacy and toxicity. In general, high expression of DPD, high expression of TS, or low expression of OPRT in gastrointestinal cancers would be expected

to predict a poor response to 5-FU-based chemotherapy and uracil/tegafur (UFT) [11–13].

Prediction of clinical outcome with S-1 monotherapy

Prediction in terms of enzymes involved in the 5-FU metabolic pathway

Thymidylate synthase (TS). We previously demonstrated that the median value of TS gene expression in responding tumors was significantly lower than that in nonresponding tumors in 27 patients with metastatic gastric cancer treated with S-1 monotherapy [14]. Another study including 59 patients confirmed the predictive values for response and survival of TS gene expression in S-1 treatment for metastatic gastric cancer [15]. Patients with tumors with low TS gene expression survived longer than those with tumors with high TS gene expression, with the difference showing statistical significance. Miyamoto et al. [16] reported that S-1 showed antitumor effects, in terms of tumor shrinkage and survival, regardless of the expression status of TS, when TS expression was evaluated by an immunohistochemical method using an anti-recombinant human TS polyclonal antibody. This finding did not agree with our results; a possible explanation for the different findings may be the differences in methods used for measuring TS expression, because the detection method for gene expression is a more quantitative and sensitive evaluation method than the immunohistochemical method [1].

Dihydropyrimidine dehydrogenase (DPD). CDHP, a constituent of S-1, reversibly inhibits the activity of DPD in the liver, producing higher plasma 5-FU concentrations, followed by an increased antitumor effect [3, 12]. Takechi et al. [17] demonstrated that CDHP

inhibited 5-FU degradation, through the inhibition of intratumoral DPD activity, and that CDHP enhanced 5-FU cytotoxicity in human cancer cells both in vitro and in vivo. Actually, as indicated in three independent studies [15, 18, 19], the antitumor effect of S-1 for gastric cancer was not influenced by intratumoral *DPD* gene expression. Miyamoto et al. [16] reported that patients with positive DPD expression showed a slightly higher response rate and longer survival than those with negative DPD expression when DPD expression was evaluated by the immunohistochemical method, but the differences were not statistically significant. In 61 patients with gastric scirrhous carcinoma, the response rate was significantly higher in patients with DPD-positive tumors than in those with DPD-negative ones in the S-1 group, as compared with the 5-FU group [20]. These results, taken together, suggest that S-1 has anti-tumor activity even in tumors with high DPD expression, which are essentially resistant to 5-FU.

We have demonstrated that *TS* and *DPD* gene expression in gastric cancer tissue differed according to the histological differentiation of the tumor; *TS* gene expression was higher in differentiated adenocarcinoma, while *DPD* gene expression was higher in undifferentiated adenocarcinoma [18]. In a phase II study of S-1 for metastatic gastric cancer, the response rate of patients with undifferentiated type was higher than that of patients with differentiated type [21]. The higher response rate of S-1 for the undifferentiated type may be, at least in part, explained by both low *TS* expression and the inhibition of high *DPD* expression by CDHP.

Orotate phosphoribosyltransferase (OPRT). S-1 contains Oxo, which decreases the levels of FUMP and 5-FU incorporated into RNA (F-RNA) by about 70% in the small intestine, through the inhibition of OPRT activity [22]. Oxo is distributed at high levels in the digestive tract after oral administration, and thus reduces 5-FU-induced gastrointestinal toxicity such as diarrhea [23]. If Oxo in plasma inhibits intratumoral OPRT, the antitumor effect of S-1 might be diminished

and the predictive value of OPRT expression might be overcome. We have reported that *OPRT* gene expression in gastric tumors was related to a higher response rate and longer survival in 59 gastric cancer patients treated with S-1 [1], as well as in colorectal cancer patients treated with UFT (which does not include Oxo) [11]. These findings were concordant with an in vivo study that demonstrated that the decrease in the levels of FUMP and F-RNA was limited to 0–20% in tumor regions, without affecting the antitumor effect of 5-FU [22].

“Polygenic” approach

The expression of each gene (e.g., *TS* and *OPRT*) involved in the 5-FU metabolic pathway, as discussed above, may be useful for predicting the clinical outcome of S-1 monotherapy. However, these genes involved in the 5-FU pathway do not act in isolation. The use of the expression of more than a single gene, such as a combination of *TS*, *DPD*, and *TP*, or a combination of *DPD* and *OPRT*, has been reported to permit the identification of a high percentage of responding patients with colorectal cancer [11–13].

We evaluated whether the response to S-1 treatment in patients with gastric cancer could be predicted in terms of the expression of five genes involved in the 5-FU pathway (*TS*, *DPD*, *OPRT*, *TP*, and *UP*) [15]. On univariate analyses, low *TS*, high *OPRT*, and low *TP* expressions were significantly associated with tumor shrinkage and long survival, whereas *DPD* and *UP* gene expressions did not correlate with response or survival (Table 1). Multivariate analyses of the response to S-1 treatment, in terms of the expression of these five genes, revealed that *OPRT* and *TS* were independent variables for response (Table 1) and *TS* and *TP* were independent variables for survival (Table 2). When *OPRT* and *TS* were combined, a significantly increased accuracy rate, of 91.5%, was seen for response, as compared with 74.5% obtained from the information on *TS* gene

Table 1. Logistic regression analysis for response

Variable	Univariate			Multivariate		
	OR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>
DPD	1.20	0.96–1.52	0.122	—	—	—
TS	0.005	0.00–0.08	0.002	0.003	0.00–0.25	0.0433
OPRT	63.5	9.64–1137.7	0.0005	46.3	6.51–1316.3	0.0036
TP	0.98	0.96–0.99	0.022	—	—	—
UP	1.16	0.98–1.39	0.086	—	—	—

In the multivariate analysis, stepwise regression was used to select variables. Probabilities to enter and remove variables were settled at less than 0.05 and greater than 0.1, respectively
OR, odds ratio; CI, confidence interval

Table 2. Cox proportional hazard analysis for survival

Variable	Univariate			Multivariate		
	HR	95% CI	P	HR	95% CI	P
DPD	1.08	0.97–1.21	0.1811	—	—	
TS	5.96	2.74–12.93	<0.0001	4.75	2.17–10.34	<0.0001
OPRT	0.58	0.39–0.85	0.0056	—	—	
TP	1.009	1.005–1.013	<0.0001	1.008	1.004–1.012	0.0002
UP	0.94	0.87–1.02	0.1461	—	—	

In the multivariate analysis, stepwise regression was used to select variables. Probabilities to enter and remove variables were settled at less than 0.05 and greater than 0.1, respectively
HR, hazard ratio; CI, confidence interval

expression (Fig. 2). Similarly, an increased hazard ratio, of 10.29, was observed for death in patients with high *TS* and/or high *TP* expression, compared with those with low *TS* and low *TP* (Fig. 2). Thus, simple combinations of two genes, *OPRT* and *TS* for response and *TS* and *TP* for survival, may allow the identification of gastric cancer patients who will benefit from S-1 chemotherapy.

Prediction from other markers not involved in the 5-FU metabolic pathway

Although the enzymes involved in the 5-FU metabolic pathway appear to be the most promising for predicting response or resistance to fluoropyrimidines, several other markers, including apoptosis-related factors such as p53 and bcl-2, and the angiogenic factor, vascular endothelial growth factor (VEGF), have been investigated in terms of prediction of clinical outcome in fluoropyrimidine-based chemotherapy [1]. However, there is little information about the predictive values of other markers not involved in the 5-FU metabolic pathway in treatment with S-1 chemotherapy.

Boku et al. [24] investigated the relationship between VEGF expression and antitumor effect in 44 patients treated with S-1 chemotherapy. The response rates of patients with VEGF-positive and VEGF-negative tumors were 40% and 54%, respectively (the difference was not statistically significant), when VEGF expression was evaluated by the immunohistochemical method [24].

Prediction of clinical outcome of S-1 combination chemotherapy

Irinotecan combination

We reported that when S-1 was combined with irinotecan, the predictive value of *TS* gene expression for clinical

outcome was overcome; there was no statistically significant difference in response rate or survival between patients with low *TS* tumors and those with high *TS* tumors in 26 patients treated with S-1 combined with irinotecan [14]. These results were confirmed by Takiuchi et al. [19]. There was no relationship between *TS* gene expression and tumor response in the S-1 and irinotecan treatment group ($n = 11$), with responses being observed in some patients having tumors with high *TS* gene expression [19]. In vivo studies indicated that *TS* activity was downregulated by irinotecan in a dose-dependent manner in xenografts with high levels of *TS* expression, suggesting that the use of irinotecan results in an environment in which S-1 is more likely to exert its antitumor effect [19].

Cisplatin combination

In the neoadjuvant setting using S-1 combined with cisplatin, high *TS* protein expression in preoperative biopsy samples was observed in 8% of 13 responders and 31% of 25 nonresponders, without a statistically significant difference, whereas high expression of p53 was observed in 8% of the responders, but in 56% of the nonresponders [25]. Boku et al. [26] failed to demonstrate a significant relationship between *TS* protein expression and the effect of 5-FU combined with cisplatin. These results suggest that the effect of a cisplatin-containing regimen for gastric cancer treatment may not be predicted by *TS* protein expression.

It seems that patients with VEGF-positive tumors may receive a greater benefit from cisplatin-containing chemotherapy (such as cisplatin plus irinotecan and cisplatin plus 5-FU) than those with VEGF-negative tumors [26–28]. These results have been recapitulated in a cohort treated with S-1 plus cisplatin [24]. Patients with VEGF-positive tumors survived remarkably longer than those with VEGF-negative tumors. In addition, in patients treated with S-1, docetaxel, and cisplatin combination chemotherapy, positive immunohistochemical staining for VEGF was observed in 4/4 of the

Fig. 2. **A** Orotate phosphoribosyltransferase (*OPRT*) and thymidylate synthase (*TS*) gene expressions according to non-response (*open circles*) and response (*filled circles*). Median values for the expressions of both genes are shown by dotted lines. **B** Combination of *TS* and thymidine phosphorylase (*TP*) gene expressions and survival. Kaplan-Meier curves of overall survival from start of chemotherapy. Modified from reference 15, with permission

downstaged patients, whereas positive immunohistochemical staining for VEGF was observed in only 5/10 of the nondownstaged patients [29].

The predictive values of nucleotide excision repair-related factors such as *ERCC1* and *GADD45A* have

been reported in cisplatin and 5-FU combination chemotherapy [30, 31]. However, to our best knowledge, there is little information about the association between nucleotide excision repair-related factors and clinical outcome in patients treated with S-1 combined with cisplatin.

Taxane combination

The combination of S-1 and docetaxel exhibited greater growth-inhibitory effects in human tumor xenograft models than treatment with S-1 alone or docetaxel alone [32]. This synergistic antitumor effect was greater for the combination of S-1 and docetaxel than for a combination of 5-FU and docetaxel. The expressions of *TS* and *DPD* at the protein level were decreased to 50% and 73% of control levels, respectively, and that of *OPRT* was increased by 3.9-fold, by treatment with docetaxel in combination with 5-FU [32]. These findings suggested that biochemical modulation of these two drugs had occurred. Thus, the predictive values of *TS* and *OPRT* gene expressions for clinical outcome may be overcome when S-1 is combined with docetaxel, similar to findings that the predictive value of *TS* is overcome when S-1 is combined with irinotecan [14].

Future perspectives

To date, only relatively small studies have identified *TS*, *OPRT*, and *TP* as potential candidates to predict the clinical outcome of S-1 monotherapy for gastric cancer. Well-controlled prospective clinical trials with adequate sample size and statistical power are necessary to demonstrate the predictive values of these candidates for clinical outcome. In addition, further studies are warranted to resolve methodological issues in the measurement of biomarkers; i.e., how to measure them and what tissues are to be measured, as mentioned elsewhere [1].

Pharmacogenetic studies to predict clinical outcome are categorized into the following three approaches: (1) the candidate approach: evaluating several genes, to be narrowed down from the information previously reported in *in vivo* and *in vitro* studies, by reverse-transcriptase polymerase chain reaction (RT-PCR) or real-time RT-PCR; (2) the pathway approach: evaluating a few dozen genes involved in pathways such as the 5-FU metabolic pathway, the apoptosis pathway, or the cell-cycle pathway, by low-density array [33]; and (3) the global approach: identifying potential chemoresistance-related genes within a panel of hundreds to thousands of genes, by DNA microarray [2] (Fig. 3). Potential candidates such as *TS*, *OPRT*, *TP*, and *VEGF* have been identified by the candidate approach.

	Candidate	Pathway	Global

No. of Genes	1-2	$\sim 10^{1-2}$	$\sim 10^{3-4}$
Examples	DPD TS OPRT	5-FU metabolism Apoptosis Cell cycle	
Method	RT-PCR Real-time PCR	Low density array	Micro array

Fig. 3. Three approaches for pharmacogenetic study. Pharmacogenetic studies are categorized into three approaches, the candidate approach (*leaf*), the pathway approach (*tree*), and the global approach (*forest*), in terms of the number of genes evaluated and the methods applied. The relationship between these three approaches is compared to that between leaves, trees, and a forest. *DPD*, Dihydropyrimidine dehydrogenase; *RT-PCR*, reverse-transcriptase polymerase chain reaction

Most enzymes function in complex networks with several regulatory mechanisms. It is unlikely that any one candidate with modest effects on enzyme function will affect treatment outcome, whereas a combination of several candidates within the same pathway might result in significant disturbance. The “polygenetic approach,” examining two-gene combinations in the 5-FU metabolic pathway is a good example of the pathway approach. The next step will be the real 5-FU pathway approach that includes all 29 genes reported to be involved in the 5-FU metabolic pathway by Marsh and McLeod [34]. As 5-FU mimics uracil, 5-FU is catabolized and anabolized by the same biological pathway as that for the naturally occurring pyrimidines. Not surprisingly, these 5-FU pathway genes correlate with one other [11, 15, 33]. These complicated correlations in the 5-FU pathway may diminish any additional benefit to be gained for the prediction of clinical outcome from measuring the expression of all 29 genes.

When S-1 combination chemotherapy is used, the predictive values of candidate genes for the outcome of S-1 monotherapy might be overcome. The global approach has great potential to identify large numbers of potential candidates to predict the clinical outcome of S-1 combination therapy for gastric cancer. However, examining thousands of genes can also be anticipated to generate many false-positive results [2]. The possible candidate genes hypothesized through the global approach should be prospectively validated in other cohorts of patients treated with the same regimens.

Conclusions

The final goal of the individualization of treatment is to select the right patient and the right drug, and to determine the right dose of the drug. Studies examining the expression of enzymes involved in the metabolism of S-1 in tumor tissues can serve as surrogate markers predictive of tumor shrinkage and survival. Research on the prediction of adverse events and determination of the optimal dosing of S-1 by genotyping is also underway [35, 36]. It is anticipated that integration of these studies will realize the aim of the individualization of S-1 based chemotherapy. Further work may determine whether candidate markers can be employed in the stratification of patients for different treatment regimens — S-1 or non-S-1 regimens, S-1 monotherapy or S-1 based combination therapy, or various drugs combined with S-1.

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