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ORIGINAL ARTICLE

Thymidylate synthase and thymidine phosphorylase as predictive markers of capecitabine monotherapy in patients with anthracycline- and taxane-pretreated metastatic breast cancer

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Abstract
Purpose The primary purpose of this study was to evaluate the role of thymidylate synthase (TS) and thymidine phosphorylase (TP) as biomarkers to predict clinical outcomes of capecitabine monotherapy in patients with anthracycline- and taxane-pretreated metastatic breast cancer (MBC).
Methods Of the patients who were previously treated with anthracycline and taxane regimens, 90 patients who had available tissue block for immunohistochemistry with measurable lesions were included. All patients received capecitabine (2,500 mg/m^2/day) for 14 days every 3 weeks.
Results High TS expression was more common among patients with triple-negative (TN) subtype than among patients with other subtypes (33% for hormone receptor+, 8% for HER2+, and 58% for TN, $P = 0.023$). The median PFS was significantly lower in patients with high TS (6.6 vs. 3.0 months; $P = 0.017$) and low TP expressions (6.0 vs. 3.3 months; $P = 0.013$). A high TS and a low TP expressions were identified as unfavorable independent risk factors for PFS to capecitabine monotherapy in multivariate analysis (hazard ratio [HR], 1.7, $P = 0.037$ for high TS score; HR, 1.8, $P = 0.014$ for low TP score).
Conclusions Our data suggest that high TS and low TP scores correlate with a shorter PFS for capecitabine monotherapy in patients with anthracycline- and taxane-pretreated MBC.

Keywords Thymidylate synthase · Thymidine phosphorylase · Immunohistochemistry · Capecitabine · Metastatic breast cancer

Introduction

Capecitabine, one of the most widely used cytotoxic agents for the treatment of breast cancer, achieves a high tumor control rate with low toxicity, even in heavily pretreated patients with metastatic breast cancer (MBC). Capecitabine with or without ixabepilone is approved by the Food and Drug Administration for the treatment of metastatic breast cancer (MBC) after the use of an anthracycline and taxane regimen, or in patients for whom anthracycline therapy is contraindicated [1–5]. Capecitabine, an oral fluoropyrimidine prodrug, is metabolized to 5-fluorouracil (FU) through several steps, the last of which is conversion of 5′-deoxy-5-fluorouridine to 5-FU by thymidine phosphorylase (TP). TP expression has been observed in many normal human tissues [6]. Because TP is overexpressed in several tumor tissues, capecitabine can be selectively activated in tumor tissues and affect tumor cells [7–10]. TP is the rate-limiting enzyme in the activation of capecitabine, suggesting that TP might contribute to the sensitivity of tumors to this agent [11]. Puglisi et al. suggested that TP may have a role in predicting the response to capecitabine [12]. They also published retrospective data regarding TP expression and the clinical response in patients with MBC treated with capecitabine and provided further evidence of TP

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expression in patients with MBC as a biomarker of sensitivity to capecitabine treatment. However, the predictive role of TP in patients with breast cancer is still needed to be proven.

Thymidylate synthase (TS) catalyzes the conversion of deoxyuridine monophosphate (dTUP) to deoxythymidine monophosphate (dTMP)—the critical source of thymidine nucleotides for DNA synthesis and repair. TS is the target enzyme for 5-FU; the activity of TS is inhibited by the action of the 5-FU metabolite and 5-fluoro-deoxyuridine-monophosphate (FdUMP). Several studies have demonstrated that high TS levels correlate with 5-FU resistance in various malignancies, especially in colorectal cancer [13–17]. However, the predictive role of TS expression in response to capecitabine in patients with MBC has not been clearly documented [18].

On the basis of the mechanism of action of capecitabine and previous data of TP and TS, we hypothesized that tumors with high TP expression are more likely to respond to capecitabine, and tumors with high TS expression are more resistant to capecitabine. Because prior chemotherapy before the use of capecitabine can affect TP expression [19], we confined the study population to those patients who were pretreated with an anthracycline- and taxane-based regimen. The purpose of the present study was to evaluate the predictive role of TS and TP in capecitabine monotherapy for anthracycline- and taxane-pretreated patients with MBC.

Patients and methods

Patients

Patients with MBC were treated with capecitabine after anthracycline- and taxane-containing regimens at the Samsung Medical Center from 2000 to 2008 were analyzed for this study. All pathologic specimens were reviewed by two experienced pathologists who determined the primary tumor characteristics (histologic and nuclear grade), and the receptor status of ER, PgR, and HER2 using IHC staining. ER- and PgR-positivity was defined as having an Allred score from 3 to 8 by IHC using antibodies to ER (Immunotech, France) and PgR (Novocastra, UK). HER2 status was evaluated using antibody (DAKO, USA) and/or fluorescence in situ hybridization (FISH). Grades 0 and 1 for HER2 by IHC were defined as a negative result, and grade 3 as a positive result. Amplification of HER2 was confirmed by FISH if HER2 was rated 2+ by IHC. The patients were divided into 3 groups according to their ER, PgR, and HER2 status: hormone receptor (HR)-positive group, HER2-positive group, triple-negative group. The hormone receptor (HR)-positive group was defined as ER and/or PgR-positive irrespective of HER2 status. The HER2 group was defined as HR-negative and HER2-positive patients. The triple-negative breast cancer (TNBC) group was defined as ER-negative, PR-negative, and HER2-negative. Our study protocol was approved by the Institutional Review Board of Samsung Medical Center.

Treatment

All patients received capecitabine (2,500 mg/m^2/day) for 2 weeks with 1 week rest every 3 weeks till progression or unacceptable toxicity or patient's refusal. When grade 2 or more hematologic or non-hematologic toxicities including hand-foot syndrome were developed, doses were reduced to 2,000 mg/m^2/day of capecitabine. When these toxicities were repeated twice, treatment was delayed for 1 week.

Response and survival assessment

Patients were evaluated at baseline and after every two cycles of treatment with capecitabine. The response was evaluated according to RECIST. The response rate (RR) reflects the proportion of complete response (CR) and partial response (PR), and disease control rate (DCR) reflects the proportion of CR, PR, and stable disease (SD). The progression-free survival (PFS) was defined as the time from the start of capecitabine monotherapy to the time of first documentation of progressive disease (PD) or death from any cause, and overall survival (OS) as the time from the start of capecitabine monotherapy to death or last follow-up.

Immunohistochemical staining and scoring

Tumor specimens from patients who were diagnosed based on histologic samples were evaluated for biomarker analyses. Full specimen sections (4 μm thick) were cut from paraffin blocks and mounted on adhesive-coated or charged-glass slides. An IHC method was used to determine the level of protein expression for TS and TP as well as ER, PgR, and HER2.

IHC staining was performed using the streptavidin–biotin complex method and the TechMate^{TM} 1,000 automated staining system (DakoChemmate, Glostrup, Denmark). Primary antibodies and the working dilutions used were as follows: TS mouse monoclonal antibody (mAb; clone TS106/4H4B1, 1:50 dilution in Tris/EDTA buffer [pH 9.0]; Zymed, San Francisco, CA, USA) and TP mouse mAb (PGF.44C, 1:100 dilution; NeoMarkers, Fremont, CA, USA). Deparaffinized sections were treated with 3% hydrogen peroxide in methanol for 10 min to inhibit endogenous peroxidase. Sections were immersed in 0.01 M citrate buffer (pH 6.0) and heated in a pressure cooker for

30 min. Sections were then incubated with primary antibody for 50 min at room temperature. Each section was treated sequentially with biotinylated secondary antibody (anti-mouse immunoglobulin) and streptavidin-peroxidase complex (DakoChemmate). 3,3′-diaminobenzidine tetrahydrochloride was used as a chromogen, and Mayer's hematoxylin counterstain was applied. Negative controls (isotype-matched irrelevant antibody) were run simultaneously.

The slides were assessed without knowledge of the clinical outcome by two experienced pathologists. TS and TP protein expression was detected in both cytoplasm and nucleus.

The intensity and extent of the IHC staining was graded on a semi-quantitative scale from 0 to 3, as follows: 0 = no staining; 1 = weak staining; 2 = strong staining; and 3 = very strong staining. The area of most intense staining was graded on a scale from 0 to 4, in which 0 = no staining, 1 = 0–10% staining of tumor cells, 2 = 10–25% staining of tumor cells, 3 = 25–50% staining of tumor cells, and 4 = >50% staining of tumor cells. A TS score was calculated by multiplying the intensity and extent grades. The intensity and percentage grades were multiplied, and these products were named "score." The TS level of expression was defined as positive if the score was >100, and the TP level of expression if the score was >50. These numbers were approximate values of their means.

Statistical analysis

The X^2 test or Fisher's exact test was used to study the association between the expression of TP or TS and other variables. The cutoff values of TP and TS scores were searched though receiver operating characteristic (ROC) curves analysis. The PFS and OS were estimated by the Kaplan–Meier product limit method. The log-rank test was used to compare survival rates. A P value <0.05 was considered significant. A Cox proportional hazards regression model was used to assess the effect of each potential predictive variable on the PFS. All potential prognostic variables were included in the model, and the variables were then removed from this model one at a time in a backward selection process using the likelihood ratio test and a significance level of 0.05.

Results

Patient cohort (Fig. 1)

We identified 243 patients who received a capecitabine monotherapy after anthracycline- and taxane-containing regimens for the treatment of MBC at Samsung Medical Center from 2000 to 2008. After excluding 110 patients who were not evaluable for response or did not have any measurable lesion, 133 patients were remained. Of these 133 patients, 90 patients who had available tissues for IHC were identified as our final patients' cohort (Fig. 1).

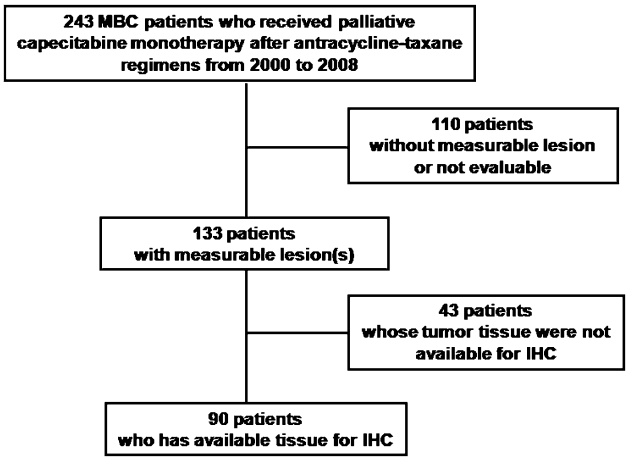

Fig. 1 Patients' cohort

Patient characteristics (Table 1)

The median age at diagnosis was 49 years (range, 26–76 years), and the median duration of capecitabine treatment was 3.6 months (range, 1.0–33.5 months). Of the

Table 1 Patients' characteristics

	No. of patients	%
Median age (years)	48.5 (range: 26-76)	
Median duration of Tx (months)	3.6 (range: 1.0-33.5)	
Subtype		
HR+	49	54.4
HER2+	9	10.0
TN	32	35.6
TS score ≥100	24	26.7
TP score <50	49	54.4
Chemotherapy line		
1st	20	22.2
2nd	31	34.4
≥3rd	39	43.3
Metastatic organs		
Liver	13	14.4
Lung	25	27.8
Bone	28	31.1
Bone marrow	5	5.6
Brain	3	3.3
Lymph nodes	24	26.7
Skin	8	8.9
Soft tissue	2	2.2

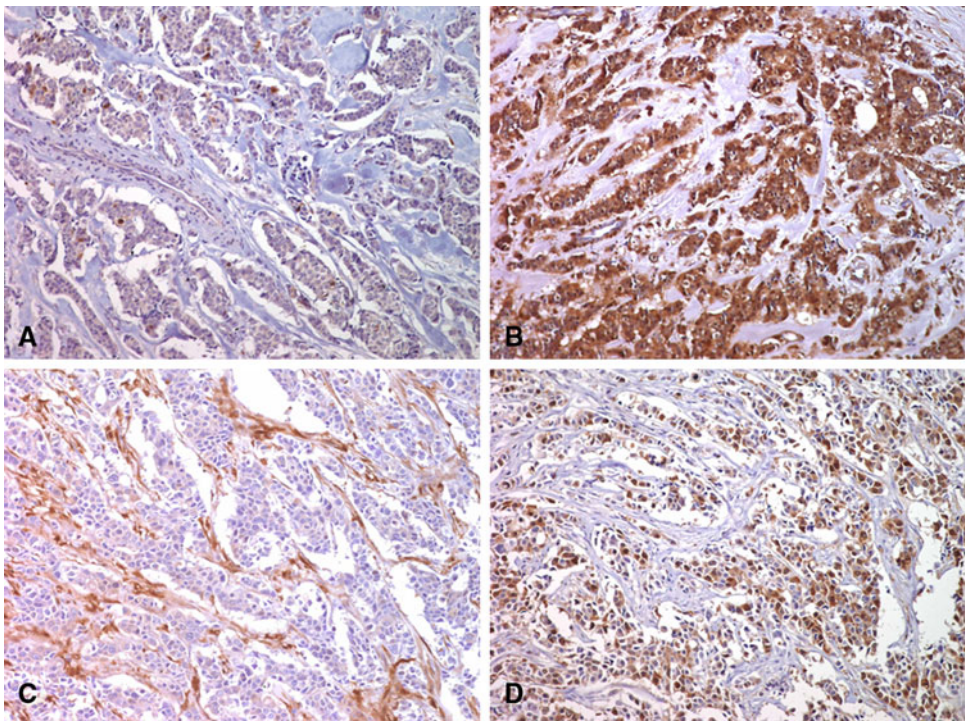

Fig. 2 Immunohistochemistry of breast cancer samples; Sample for TS, negative (**a**) and positive (**b**). Sample for TP negative (**c**) and positive (**d**) (original magnification: ×200)

90 patients, 54.4% were HR-positive group, 10% were HER2-positive and HR-negative group, and 35.6% were both HR-negative and HER2-negative (triple negative) group. Of the 90 patients, 22.2% received capecitabine as first-line chemotherapy in metastatic setting, 34.4% as second-line, and 43.3% as third-line chemotherapy. The most frequent site of metastasis was bone (31.1%), followed by lung (27.8%), lymph nodes (26.7%), and liver (14.4%).

Level of TS and TP expression, and the clinical characteristics (Fig. 2; Table 2)

The cutoff values of TS and TP scores using ROC curves were 100 and 50, respectively (AUC; 0.643 for TS score, 0.544 for TP score).

Among the 90 patients, high TS expression was found in 26.7%, while low TP expression was found in 54.4%. Neither age nor line of chemotherapy after metastasis was different between the high and low TS expression groups. Notably, high TS expression (TS score ≥100) was more common in patients with the triple-negative (TN) subtype than in those with other subtypes (16% for HR+, 22% for HER2+, and 44% for TN, $P = 0.023$). All metastatic sites were not different between the high and low TS expression groups, except the brain, in which all three of the patients had high TS expression.

The level of TP expression had no correlation with age, line of chemotherapy after metastasis, tumor subtypes, and metastatic sites.

Clinical response to capecitabine (Table 3)

The duration of response to capecitabine treatment was 2.1 months in the high TS expression group, and 4.0 months in the low TS expression group ($P = 0.007$). The overall response rate (ORR) was 8.3% in the high TS expression group, and 31.8% in the low TS expression group ($P = 0.024$). The disease control rate was lower in the high TS expression group than the low TS expression group (45.8% vs. 72.7%, $P = 0.018$). The ORR and disease control rate (DCR) were not significantly different between the high and low TP expression groups.

Progression-free survival analysis (PFS; Fig. 3)

The median PFS was 3.0 months (range, 1.8–4.2 months) in the high TS expression group and 6.6 months (3.5–9.7 months) in the low TS expression group (Fig. 3a). The difference between the two groups was statistically significant ($P = 0.017$). The median PFS was significantly shorter in the low TP expression group compared with the high TP expression group (4.4 vs. 6.0 months, $P = 0.013$, Fig. 3B).

Overall survival analysis (OS; Fig. 4)

The median OS was 14.1 months (range, 9.8–18.4 months) in the high TS expression group and 29.9 months (21.8–38.0 months) in the low TS expression group (Fig. 4a). The difference between the two groups was

Table 2 Level of TS, TP expressions, and clinical characteristics

	Thymidylate synthase (TS) expression			Thymidine phosphorylase (TP) expression			Total ($N = 90$)
	TS < 100 ($N = 66$)	TS ≥ 100 ($N = 24$)	*P* value	TP < 50 ($N = 49$)	TP ≥ 50 ($N = 41$)	*P* value	
Age	48.0 (33–76)	49 (26–66)	0.609	46.0 (26–67)	49.0 (33–76)	0.386	48.5 (26–76)
ChemoTx line			0.261			0.447	
1st	12 (18.2%)	8 (33.3%)		9 (18.4%)	11 (26.8%)		20 (22.2%)
2nd	25 (37.9%)	6 (25.0%)		16 (32.7%)	15 (36.6%)		31 (34.4%)
≥3rd	29 (43.9%)	10 (41.7%)		24 (49.0%)	15 (36.6%)		39 (43.3%)
Subtype			0.023			0.182	
HR+	41(62.1%)	8 (33.3%)		23 (46.9%)	26 (63.4%)		49 (54.4%)
HER2+	7 (10.6%)	2 (8.3%)		7 (14.3%)	2 (4.9%)		9 (10.0%)
TN	8 (27.3%)	14 (58.3%)		19 (39.8%)	13 (31.7%)		32 (35.0%)
Metastasis							
Liver	10 (15.2%)	3 (12.5%)	0.897	8 (16.3%)	5 (12.2%)	0.579	13 (14.4%)
Lung	16 (24.2%)	9 (37.5%)	0.214	17 (34.7%)	8 (19.5%)	0.109	25 (27.8%)
Bone	23 (34.8%)	5 (20.8%)	0.204	16 (32.7%)	12 (29.3%)	0.730	28 (31.1%)
BM	4 (6.1%)	1 (4.2%)	0.834	2 (4.1%)	3 (7.3%)	0.656	5 (5.6%)
Brain	0 (0%)	3 (12.5%)	0.017	0 (0%)	3 (7.3%)	0.091	3 (3.3%)
LN	17 (25.8%)	7 (29.2%)	0.746	15 (30.6%)	9 (22.0%)	0.355	24 (26.7%)
Skin	5 (7.6%)	3 (12.5%)	0.435	5 (10.2%)	3 (7.3%)	0.723	8 (8.9%)
Soft tissue	1 (1.5%)	1 (4.2%)	0.464	1 (2.0%)	1 (2.4%)	0.978	2 (2.2%)

HR+ ER and/or PgR-positive irrespective of HER2 status, *HER2+* HR-negative and HER2-positive patients, *TN* ER-negative, PR-negative, and HER2-negative

Table 3 TS, TP expressions and response to capecitabine therapy

	Total ($N = 90$)	TS < 100 ($N = 66$)	TS ≥ 100 ($N = 24$)	*P* value	TP < 50 ($N = 49$)	TP ≥ 50 ($N = 41$)	*P* value
Response				0.060			0.212
CR	3 (3.3%)	3 (4.5%)	0 (0%)		0 (0%)	3 (7.3%)	
PR	19 (21.1%)	17 (25.8%)	2 (8.3%)		9 (18.4%)	10 (24.4%)	
SD	37 (41.1%)	28 (42.4%)	9 (37.5%)		22 (44.9%)	15 (36.6%)	
PD	31 (34.4%)	18 (27.3%)	13 (54.2%)		18 (36.7%)	13 (31.7%)	
ORR	23 (25.6%)	21 (31.8%)	2 (8.3%)	0.024	10 (20.4%)	13 (31.7%)	0.221
DCR	59 (65.6%)	48 (72.7%)	11 (45.8%)	0.018	31 (63.3%)	28 (68.3%)	0.617

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *DCR* disease control rate

statistically significant ($P = 0.008$). The median OS was shorter in the low TP expression group compared with the high TP expression group with marginal significance (20.4 vs. 29.9 months, $P = 0.043$, Fig. 4b).

Relationship between therapeutic outcomes and TP and TS expression (Tables 4, 5)

The clinical factors predicting PFS after capecitabine treatment were detected during univariate and multivariate analysis are shown in Table 4. Tumor subtype, level of TS expression, and level of TP expression significantly correlated with the PFS. We performed multivariate analysis and included the factors that were statistically significant based on univariate analysis for the PFS. High TS expression (HR, 1.7; 95% CI, 1.0–2.9; $P = 0.04$) and low TP expression (HR, 1.8; 95% CI, 1.1–2.9; $P = 0.014$) retained statistical significance at the multivariate level to predict the PFS.

The patients were classified into four groups according to the levels (high vs. low) of TS and TP; high TS/high TP group, high TS/low TP group, low TS/high TP group, and low TS/low TP group (Fig. 3c). Among four groups, high TS and low TP group particularly showed shorter PFS than other groups. So, we compared PFS of high TS and low TP group with other groups that were got together into one group. The high TS and low TP group had a shorter PFS than

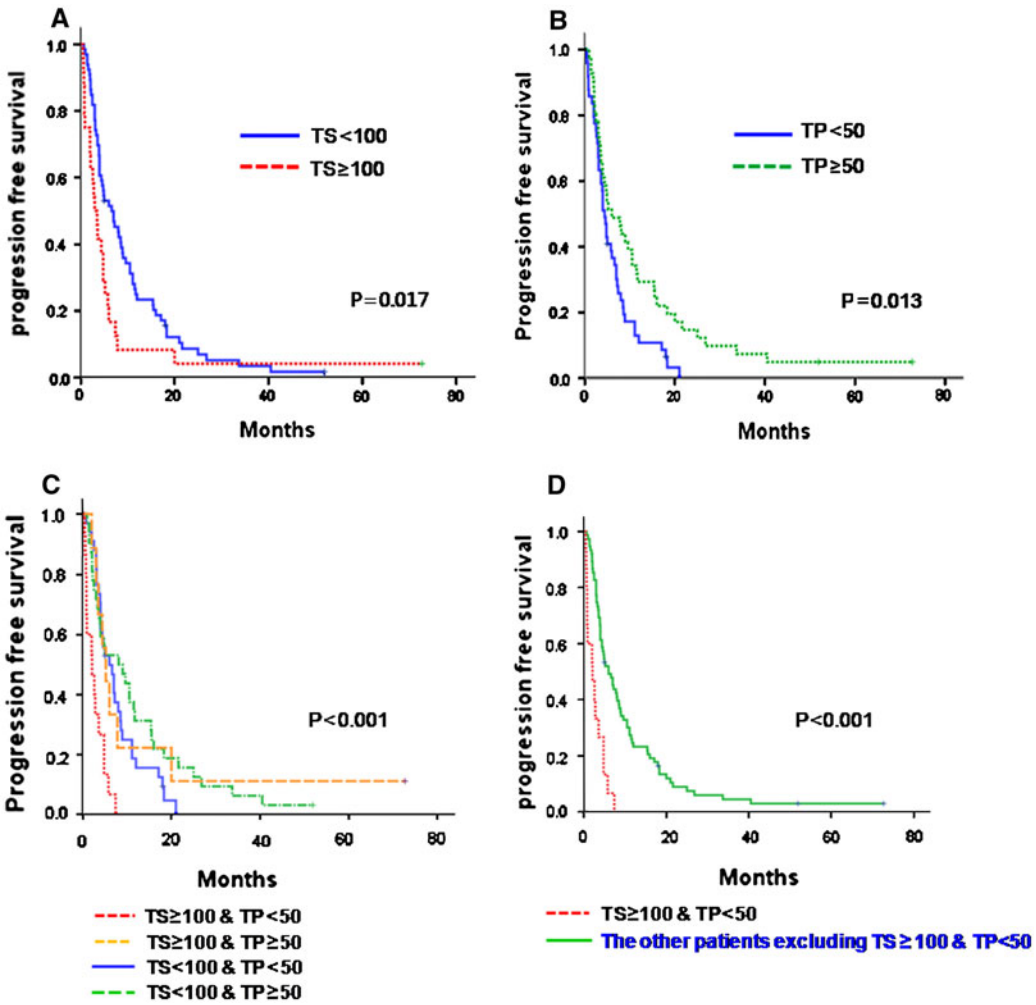

Fig. 3 Progression-free survival (PFS) curves according to TS and TP expressions. **a** *Upper solid line* indicates PFS of the patients with TS < 100, and *lower dotted line* indicates PFS of the patients with TS ≥ 100 (median PFS 6.6 vs. 3.0 months, $P = 0.017$). **b** *Upper dotted line* indicates PFS of the patients with TP < 50, and *lower solid line* indicates PFS of the patients with TP ≥ 50 (median PFS 6.0 vs. 3.3 months, $P = 0.013$). **c** *Upper most semi-dotted line* indicates PFS of the patients with TS < 100 and TP ≥ 50, *Solid line* indicates PFS of the patients with TS < 100 and TP < 50, *long dotted line* indicates PSF of the patients with TS ≥ 100 and TP ≥ 50, and *lower most short dotted line* indicates PFS of the patients with TS ≥ 100 and TP < 50 (median PFS 8.1 vs. 6.0 vs. 4.8 vs. 2.1 months). **d** *Upper solid line* indicates PFS of the patients excluding the patients with TS ≥ 100 and TP < 50, and *lower dotted line* indicates PFS of the patients with TS ≥ 100 and TP < 50 (median PFS 6.0 vs. 2.1 months, $P < 0.001$)

the other 3 groups (Fig. 3d; median PFS, 2.1 months [range, 1.0–4.2 months] vs. 6.0 months [range, 3.8–8.2 months]; $P < 0.001$). Based on multivariate analysis with tumor subtype, high TS with low TP expression was the most potent predictor for a short PFS (HR, 3.8; 95% CI, 2.1–6.9).

We also performed OS analysis like PFS analysis as before. Table 5 showed the univariate and multivariate analysis of clinical factors for OS. Among them, high TS (HR, 2.1; 95% CI, 1.1–3.0; $P = 0.018$) expression and low TP expression (HR, 1.8; 95% CI, 1.1–3.2; $P = 0.032$) were statistically significant at multivariate level to predict OS. When the patients were divided into four groups according to TS and TP expression, the high TS and low TP group had shorter OS than the other three groups (Fig. 4d; median OS, 10.4 months [range, 3.6–17.2] vs. 28.5 [range, 21.7–35.3]; $P < 0.001$). Like PFS analysis, high TS with low TP expression was the most potent predictor for a short OS at multivariate analysis with tumor subtype (HR, 2.9; 95% CI, 1.4–6.0).

Discussion

Our study showed that high TS expression correlates with a worse response and a shorter PFS than low TS expression, and high TP expression correlates with a longer PFS than

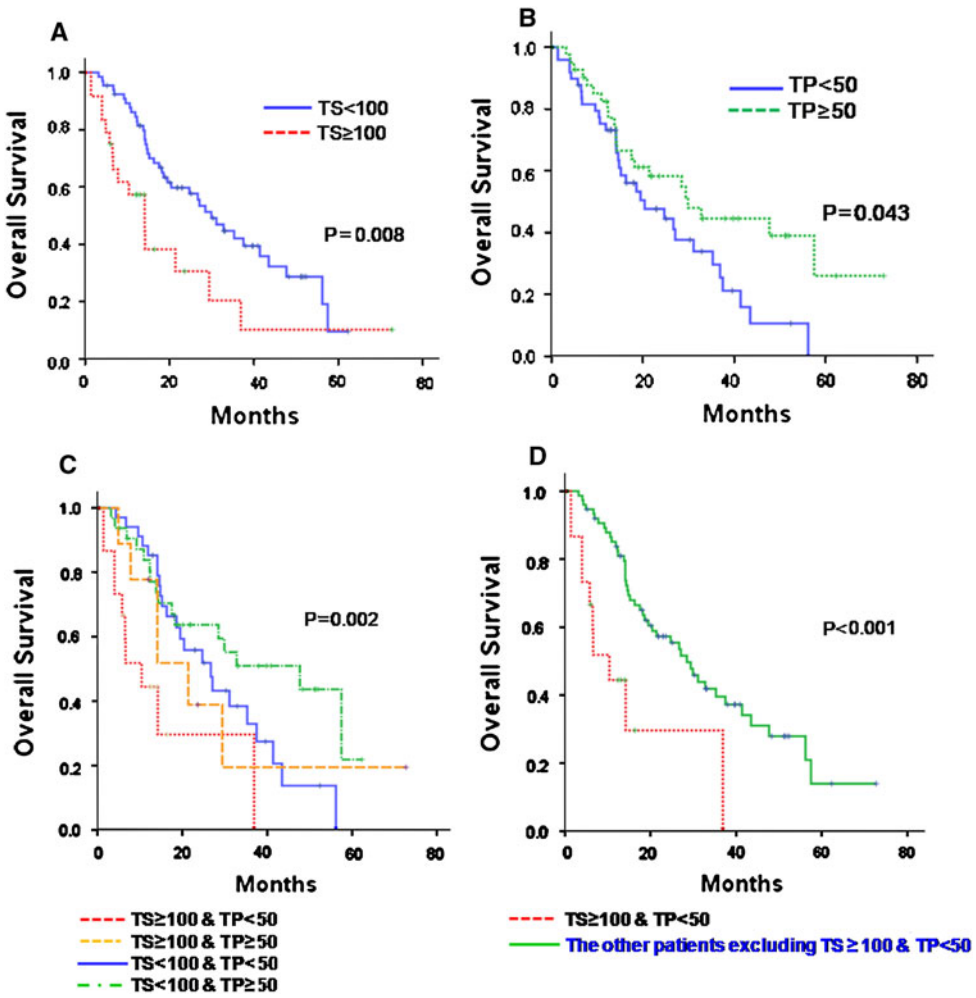

Fig. 4 Overall survival (OS) curves according to TS and TP expressions. **a** *Upper solid line* indicates OS of the patients with TS $<$ 100, and *lower dotted line* indicates OS of the patients with TS $\geq$ 100 (median OS 29.9 vs. 14.1 months, $P = 0.008$). **b** *Upper dotted line* indicates OS of the patients with TP $\geq$ 50, and *lower solid line* indicates OS of the patients with TP $<$ 50 (median OS 20.4 vs. 29.9 months, $P = 0.043$). **c** *Upper most semi-dotted line* indicates OS of the patients with TS $<$ 100 and TP $\geq$ 50, *solid line indicates* OS of the patients with TS $<$ 100 and TP $<$ 50, *long dotted line* indicates OS of the patients with TS $\geq$ 100 and TP $\geq$ 50, and *lower most short dotted line* indicates OS of the patients with TS $\geq$ 100 and TP $<$ 50 (median OS 47.7 vs. 26.7 vs. 21.4 vs. 10.4 months, $P = 0.002$). **d** *Upper solid line* indicates OS of the patients excluding the patients with TS $\geq$ 100 and TP $<$ 50, and *lower dotted line* indicates OS of the patients with TS $\geq$ 100 and TP $<$ 50 (median OS 10.4 vs. 28.5 months, $P < 0.001$)

low TP expression to capecitabine monotherapy in patients with anthracycline- and taxane-pretreated MBC. TS and TP play a key role in 5-FU resistance [8, 10, 13–18, 20].

Our results suggest that a low TS level appears to be related with a better response rate and a longer PFS than a high TS level. TS might be associated with a resistant mechanism of 5-FU. Interestingly, the present study demonstrated that the patients with TNBC had higher levels of TS than other subtypes (58.3% for TNBC vs. 33.3% for HER2+ vs. 8.3% for HR+ve, $P = 0.023$). This finding should be evaluated whether or not TS expression is related with biologic aggressiveness of the tumor. In addition, it would be possible that poor response to capecitabine therapy in TNBC might be related with higher levels of TS. In other words, TS expression could be a mechanism of capecitabine resistance in TNBC. However, it implies that TS might have prognostic role regardless of treatment. These findings also support the notion that HR status could have a potential role in predicting the benefit of capecitabine treatment. Siva et al. examined the predictive factors of capecitabine treatment in patients with MBC and reported that only ER-positivity correlated with the response to capecitabine [21]. Muss et al. compared standard chemotherapy and capecitabine in early-stage BC patients $\geq$65 years of age and concluded that capecitabine was inferior to standard chemotherapy, particularly in those with HR-negative tumors [22]. In addition, it could partially explain resistance of TNBC to many chemotherapeutic agents as well as 5-FU derivatives.

In contrast, TP is the marker of a more aggressive and malignant tumor phenotype that has increased resistance to cytotoxic agents due to the loss of apoptotic potential [18, 19]. Our study demonstrated a significant association between TP expression and clinical outcome to capecitabine chemotherapy. TP expression was shown to have a trend as a positive predictive factor for the FU response. These results are consistent with previous reports and can be explained by the knowledge that high TP levels are most commonly associated with responsiveness to 5-FU [18, 23–27].

Table 4 Univariate and multivariate analysis for PFS

Univariate analysis			
	No. of patients	Median PFS (95% CI)	*P* value
Subtype			0.007
HR+	49	8.0 (4.3–11.7)	
HER2+	9	3.6 (3.2–4.0)	
TN	32	3.5 (1.8–5.2)	
TS			0.017
<100	66	6.6 (3.5–9.7)	
≥100	24	3.0 (1.8–4.2)	
TP			0.013
<50	49	4.4 (3.5–5.3)	
≥50	41	6.0 (1.9–10.1)	

Multivariate analysis			
	P value	HR	95% CI for exp (B)
TS ≥ 100	0.037	1.743	1.034–2.938
TP < 50	0.014	1.790	1.122–2.854
Subtype	0.153	1.123	0.958–1.316

HR hazard ratio

Table 5 Univariate and multivariate analysis for OS

Univariate analysis			
	No. of patients	Median OS (95% CI)	*P* value
Subtype			0.039
HR+	49	29.9 (23.7–36.1)	
HER2+	9	14.1 (3.9–24.3)	
TN	32	16.3 (9.2–23.4)	
TS			0.008
<100	66	29.9 (22.5–37.3)	
≥100	24	14.1 (7.7–20.5)	
TP			0.043
<50	49	20.4 (9.4–31.4)	
≥50	41	29.9 (16.0–43.8)	

Multivariate analysis			
	P value	HR	95% CI for exp (B)
TS ≥ 100	0.018	2.130	1.137–3.991
TP < 50	0.032	1.848	1.053–3.245
Subtype	0.351	1.106	0.895–1.368

HR hazard ratio

Taken together, the patients with high TS and low TP showed the worst clinical outcomes in univariate and multivariate analyses (Tables 4, 5).

Several studies have reported a TP-inducing effect of anthracyclines and taxanes [19, 28, 29]. Andreetta et al. reported that patients with tumor with high TP expressions and previously treated with a taxane-based therapy achieved a better clinical benefit and a significantly longer time-to-progression than patients who did not receive these treatments before capecitabine [30]. In order to avoid the effect of these confounding variables, this study included the patients who were previously treated with both anthracycline and taxanes.

The ability to identify the subpopulation of patients who are most likely to respond to a particular therapy in advance would not only increase the likelihood of response, but would also avoid unnecessary treatment of those patients who have little probability of benefiting from the treatment [31]. With respect to the chemotherapy of BC, our study has clinical importance in that TS and TP expression correlates with PFS to capecitabine, one of the most commonly used agents in daily practice.

In conclusion, this work provides further evidence that high TS and low TP expression in BC tissues could represent predictive markers of capecitabine treatment. Furthermore, high TS expression may have prognostic role in BC. Following prospective studies are needed.

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