OncoTargets and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Clinical significance of the thymidylate synthase, dihydropyrimidine dehydrogenase, and thymidine phosphorylase mRNA expressions in hepatocellular carcinoma patients receiving 5-fluorouracil-based transarterial chemoembolization treatment

This article was published in the following Dove Press journal:
OncoTargets and Therapy
2 July 2013
Number of times this article has been viewed

Hongyun Zhao[1,*]
Yuanyuan Zhao[2,*]
Ying Guo[1]
Yan Huang[2]
Suxia Lin[3]
Cong Xue[2]
Fei Xu[2]
Yang Zhang[1]
Liping Zhao[2]
Zhihuang Hu[2]
Li Zhang[1,2]

[1]State Key Laboratory of Oncology in South China and National Anti-Cancer Drug Clinical Research Centre, [2]State Key Laboratory of Oncology in South China and Department of Medical Oncology, [3]Department of Pathological Oncology, Sun Yat-sen University Cancer Center, Guangzhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Li Zhang
Department of Medical Oncology, Sun Yat-sen University Cancer Center, 651 Dong Feng Road East, Guangzhou 510060, People's Republic of China
Tel +86 20 8734 3458
Fax +86 20 8734 3392
Email zhangli6@mail.sysu.edu.cn

Purpose: To determine whether 5-fluorouracil (5-FU) sensitivity is associated with the mRNA expressions of thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD), and thymidine phosphorylase (TP) in patients with hepatocellular carcinoma (HCC) treated with 5-FU-based transarterial chemoembolization (TACE).

Methods: Formalin-fixed, paraffin-embedded tumor specimens from 40 patients treated with 5-FU-based TACE were selected for the examination of TS, DPD, and TP expression level by a quantitative real-time reverse transcription- polymerase chain reaction (PCR) technique. Patients were categorized into high and low expression groups according to the median expression level of each enzyme. Associations between the mRNA expression levels of TS, DPD, and TP and clinical parameters including treatment efficacies, clinicopathological factors, and prognosis were assessed.

Results: High DPD expression was associated with worse treatment outcome, including intrahepatic disease progression rate (hazard ratio [HR] for high DPD versus low DPD, 2.212; 95% confidence interval [CI], 1.030–4.753; $P = 0.042$), extrahepatic disease progression rate (HR for high versus low DPD, 3.171; 95% CI, 1.003–10.023; $P = 0.049$), and progression-free survival (HR for high versus low DPD, 2.308; 95% CI, 1.102–4.836; $P = 0.027$). No correlation was found between the mRNA expression of TS/TP and treatment outcome.

Conclusion: DPD mRNA expression level was negatively correlated with the clinical outcomes of HCC patients treated with 5-FU-based TACE. These results provide indirect evidence that high DPD mRNA expression is a predictive marker of treatment resistance for 5-FU.

Keywords: dihydropyrimidine dehydrogenase, 5-fluorouracil, hepatocellular carcinoma, thymidylate synthase, thymidine phosphorylase, transarterial chemoembolization

Introduction

Hepatocellular carcinoma (HCC) is the most common type of liver cancer; occurrence rate ranks sixth among all types of cancers, and it is the third most deadly cancer worldwide.[1] The People's Republic of China is one of the high-risk areas for HCC due to the high prevalence of chronic hepatitis B virus infection;[2,3] Chinese HCC patients represent more than half of the cases in the entire world.[4] Despite various diagnostic techniques, most HCC patients are not diagnosed until the cancer reaches

an advanced stage,[5] and most of them eventually succumb to the disease. The overall 5-year survival rate for patients with HCC is lower than 10%; the recurrence rate after surgery is approximately 40% to 50%.[6]

Numerous randomized controlled trials and meta-analyses have demonstrated that transarterial chemoembolization (TACE) is the only HCC treatment that increases survival rates.[7,8] The pyrimidine antimetabolic agent 5-fluorouracil (5-FU) has been widely used in the treatment of gastrointestinal cancer for nearly 50 years,[9] and it was the first chemotherapeutic agent evaluated for the treatment of HCC.[10] It is used alone or in combination with other chemotherapeutic drugs and administered by hepatic arterial infusion therapy and TACE as chemotherapy for HCC.[11,12]

Previous reports demonstrated that the tumor response to 5-FU is at least partially correlated with the expression of enzymes such as dihydropyrimidine dehydrogenase (DPD), thymidylate synthase (TS), and thymidine phosphorylase (TP) in gastric or colon cancers.[13,14] Due to their involvement in nucleotide and fluoropyrimidine metabolism/function, the expression and activity levels of TS, DPD, and TP are therefore potentially important not only as predictive markers for response to 5-FU but also as prognostic factors.[15] For HCC, some reports suggest the expression levels of DPD, TS, and TP in HCC are different from those of healthy individuals;[16,17] and these differences in DPD or TS expression may be related to clinicopathological factors.[16,18] However, few studies have investigated the relationship between the expression of 5-FU-related enzymes and 5-FU treatment effectiveness in HCC, and the significance of these biomarkers remains undetermined or controversial in HCC. Since TACE is the most widely used treatment method for HCC and 5-FU is one of the drugs commonly used in TACE, we hypothesize that expression of TS/DPD/TP may contribute to the treatment efficacy of 5-FU-based TACE. Here, we report our discovery of the associations between TS/DPD/TP gene expression and their clinicopathological/predictive significance in HCC patients who have received 5-FU-based TACE.

Materials and methods

Patient information

A total of 40 HCC patients who first underwent hepatectomy and then were treated with TACE by regular 5-FU injection after recurrence between January 2004 and May 2008 at the Cancer Center, Sun Yat-sen University were selected; patients with a history of malignant diseases were excluded. This study was approved by the Medical Ethics Committee of Sun Yat-sen University. Tumor differentiation grade was defined according to the criteria of the World Health Organization. Median patient age was 44 years (26–68 years); patients included 33 men and seven women. The necessity for TACE treatment was due to the following factors: ineligible for radical surgery at diagnosis (nine patients; 22.5%), incomplete surgery (nine patients; 22.5%), and relapse after surgery (22 patients, 55%). All patients received TACE therapy by the Seldinger technique. Medical chemotherapy was then implemented via super-selective cannulation to the target artery, injection of iodized oil mixture, and gelatin sponge embolism if necessary. The main chemotherapeutics adopted were combinations of 5-FU, epirubicin, pirarubicin, mitomycin, and carboplatin. The dose of anticancer agent lipiodol emulsion and fine pieces of the embolic material was determined based on the tumor size and lesion extension. All patients received a median of two treatments (range, 1–6 treatments) throughout the follow-up period.

Microdissection of the primary tumors

Formalin-fixed, paraffin-embedded tumor specimens collected during the hepatectomy before TACE were examined by a pathologist (SXL) after hematoxylin and eosin staining. Total mRNA was extracted using FFPE tissue kit (Catalog No. 74404, Qiagen, Hilden, Germany) according to manufacturer instructions, followed by cDNA reverse transcription as previously described.

cDNA preparation and quantitative measurement of mRNA

cDNA was prepared by a 20 μL reaction system composed of 10 μm reverse transcription primers, 200 nmoL of dNTPs, 200 units (U) Taq enzyme, 0.25 moL $MgCl_2$, and Buffer A (pH 8.0). The reaction program was 42°C for 1 hour, 95°C for 5 minutes, and then cooling down on ice. The expression levels of TS, TP, DPD, and the internal control of β-actin were quantified using the MX3000P (Stratgene, La Jolla, CA, USA) with 25 μL polymerase chain reaction (PCR) reaction system containing 10 μm for each primers and probes, 1 U Taq enzyme, 200 nmol dNTPs, 0.25 mol $MgCl_2$ and 5 × Buffer (pH 7.5). (All detection kits were provided by Amoy Diagnostics, Xiamen, People's Republic of China). The reaction program contained three stages: (1) 94°C for 5 minutes; (2) ten cycles of 94°C for 15 seconds, 60°C for 20 seconds, and 72°C for 20 seconds (three steps); and (3) 40 cycles of 94°C for 15 seconds, 58°C for 35 seconds, and 72°C for 15 seconds (three steps). The sequence of primers and probes is listed in Table 1. The expression levels of each molecule were classified into high and low expression groups according to the median value.

Table 1 Sequences of the primers and probes

Target gene	Forward primers	Reverse primers*	Probes
TS	GCGCTACAGCCTGAGAGA	CTCTTTAGCATTTGTGGATCCCTT	FAM-CGCCCTCTGCTGACAACCAAAC GTGTGAGGGCG-Dabcyl
TP	CATGTGGCTGCAAGGTGC	CAGCAGCACTTGCATCTGC	FAM-5′-TGCCCCGGACGTGGTCTGGGGCA-3′-Dabcyl
DPD	CCAAAACTTTCTCTCTTGATAAGGAC	AATGCTAGCAATCACAATGTTGTC	FAM-5′-CCCCCAGAATCATCCGGGGG-3′-Dabcyl
β-actin	ATTGCCGACAGGATGCAGA	CAGGAGGAGCAATGATCTTGAT	FAM-5′-CTGCCCTGGCAC CCAGCACAATGGGCAG-3′-Dabcyl

Note: *Reverse transcription primers.
Abbreviations: DPD, dihydropyrimidine dehydrogenase; TP, thymidine phosphorylase; TS, thymidylate synthase.

Clinicopathological variables

Clinical and pathological characteristics of patients with primary liver cancers were collected; characteristics included age, sex, hepatitis-related virus, serum levels of alpha-fetoprotein (AFP), liver damage, histological differentiation, and number of tumors. These variables were stratified by TS, DPD, and TP mRNA expression levels and are listed in Table 2.

TACE treatment efficacy evaluation

Triphasic computed tomography (CT) was used to assess the therapeutic efficacy. However, the lesion evaluation after TACE can be affected by artifacts produced by high concentrations of lipiodol. On the other hand, the heterogeneous deposition of lipiodol within the lesions can make it difficult to judge the viability and necrosis of the tumors correctly.[19,20] Therefore, in this study, we used treatment outcome instead

Table 2 Comparisons of the clinicopathological factors for high and low TS, DPD, and TP mRNA expression

Variables	Low TS (n)	High TS (n)	*P*-value*	Low DPD (n)	High DPD (n)	*P*-value*	Low TP (n)	High TP (n)	*P*-value*
Sex (male/female)	17/3	16/4	0.677	16/4	17/3	0.677	16/4	17/3	0.677
Age (y ± SD)	46.1 ± 2.7	42.8 ± 2.1	0.337	43.5 ± 2.3	45.3 ± 2.5	0.602	43.4 ± 2.4	45.5 ± 2.4	0.543
Liver damage, Child-Pugh classification (A/B)	19/1	18/2	0.548	19/1	18/2	0.548	19/1	18/2	0.548
Smoking status (nonsmokers/smokers)	13/7	13/7	0.629	14/6	12/8	0.507	15/5	11/9	0.185
Alcoholic drinks status (nondrinkers/drinkers)	14/6	13/7	0.736	15/5	12/8	0.311	14/6	13/7	0.736
AFP, ng/mL (<200/≥200)	12/8	5/15	0.025	9/11	8/12	0.749	5/15	12/8	0.025
Virus marker									
HBV Ag±	18/2	19/1	0.548	18/2	19/1	0.548	19/1	18/2	0.548
HCV Ag±	1/19	0/20	0.311	1/19	0/20	0.311	0/20	1/19	0.311
Cancer differentiation (low/median, high)	8/12	14/6	0.057	10/10	12/8	0.525	13/7	9/11	0.204
Tumor number (1/≥2)	14/6	12/8	0.507	14/6	12/8	0.507	13/7	13/7	0.629
Vessel invasion (yes/no)	5/15	11/9	0.053	7/13	9/11	0.519	8/12	8/12	0.629
Intrahepatic metastasis (yes/no)	5/15	6/14	0.723	5/15	6/14	0.723	6/14	5/15	0.723

Note: *Chi-square test.
Abbreviations: DPD, dihydropyrimidine dehydrogenase; SD, standard deviation; TP, thymidine phosphorylase; TS, thymidylate synthase; y, years.

of treatment response to evaluate therapeutic efficacy. Using methods described in a previous study,[21] patient outcome was evaluated based on intrahepatic and extrahepatic disease progression rates, progression-free survival rates, and overall survival rates at 1 and 2 years. Freedom of intrahepatic disease progression was defined as the interval from the date of TACE to the date of occurrence of any new lesions or death. Freedom of extrahepatic disease progression was defined as the interval from the date of TACE to the date of evidence of extrahepatic disease, intrahepatic venous invasion, or biliary invasion detected. Progression-free survival (PFS) was defined from the date of TACE to tumor progression or the last tumor evaluation. Overall survival (OS) was defined as the period from the date of TACE to death or last follow-up. The end date of the follow-up was October 10, 2010, with a median of 18.9 months (range, 1.93 months–73.5 months).

Statistical analysis

Statistical Product and Service Solutions (SPSS) version 16.0 (IBM Corporation, Armonk, NY, USA) was used for data analysis. Continuous variables were expressed as mean ± SD. Chi-square test was used to assess the potential association between mRNA levels of 5-FU-related enzymes and the categorical clinicopathological parameters, while a Student's *t*-test was used to assess continuous variables. Patient outcome, including freedom of intrahepatic or extrahepatic disease progression, PFS, and OS, were calculated by the Kaplan–Meier method and analyzed using the logrank test. Statistically significant variables were included in a multivariate Cox proportional hazards regression model to test for independent prognostic value. *P*-values of <0.05 were considered significant.

Results

Distributions of TS, DPD, and TP mRNA levels in HCC

According to the median values of mRNA levels of TS/TP/DPD, all patients were classified into high (greater than or equal to the median value) and low (less than the median value) expression groups. The median values of 23.6 (3.71–99.5) for TS, 3.10 (0.25–32.6) for DPD, and 4.50 (0.49–20.5) for TP in 40 cancers were selected for cut-off levels separating high and low mRNA expression.

Baseline clinicopathological parameters and mRNA levels of TS, DPD, and TP

Our study found that high TS and low TP groups was significantly associated with HCC with AFP ≥ 200 ng/mL ($P = 0.025$), but not with other host and pathological factors. The high TS group tends to include more patients with low differentiation ($P = 0.057$) and vessel invasion ($P = 0.053$) than the low TS group. There was no significant difference in baseline clinicopathological parameters between patients in the high and low DPD groups (Table 2).

TACE treatment outcomes and mRNA levels of 5-FU-related enzymes

The 1- and 2-year intrahepatic and extrahepatic disease progression rates in the high DPD group were significantly higher than in the low DPD group ($P = 0.037$ and $P = 0.038$, respectively; Table 3, Figure 1A and B). Similarly, PFS rates in the high DPD group at 1 and 2 years (16% and 8%, respectively) were also significantly lower than in the low DPD group (51% and 15%, respectively; $P = 0.023$, Table 3 and Figure 1C). No significant difference in OS rates at 1 and 2 years was found between the low DPD group (70% and 44%, respectively) and the high DPD group (75% and 44%, respectively; $P = 0.850$, Table 3 and Figure 1D). Furthermore, no significant differences were found between progression rates of intrahepatic or extrahepatic tumors, PFS (Figure 2A and 2B), and OS in either group with low or high TS and TP expression levels (Table 3).

Analyses of multivariate Cox proportional hazards model

Parameters that were statistically significant in univariate analysis were further examined using univariate hazards ratio analysis, which revealed that the high DPD group had a higher risk of intrahepatic disease progression (hazard ratio [HR], 2.212; 95% confidence interval [CI], 1.030–4.753; $P = 0.042$), and a higher risk of extrahepatic disease progression (HR, 3.171; 95% CI, 1.003–10.023; $P = 0.049$), as well as a higher risk of total disease progression (HR, 2.308; 95% CI, 1.102–4.836; $P = 0.027$).

Multiple Cox regression with backward elimination for the selection of the prognostic factors, including sex, smoking, drinking, virus infection status, cancer differentiation, and the combination of TS, DPD, and TP was performed. Results revealed that high DPD (HR, 2.335; 95% CI, 1.034–5.230; $P = 0.039$) is an independent prognostic factor for PFS.

Discussion

Although 5-FU is not always the frontline treatment for HCC, patients responded quite well to 5-FU-based hepatic arterial infusion therapy and TACE.[11,12] TACE is effective in

Table 3 Comparison of treatment effectiveness for high and low TS, DPD, and TP mRNA expression

Treatment outcome	Low TS	High TS	*P*-value*	Low DPD	High DPD	*P*-value*	Low TP	High TP	*P*-value*
Intrahepatic disease progression (%)			0.25			0.037			0.644
1 Y	0.70	0.58		0.50	0.78		0.62	0.67	
2 Y	0.92	0.83		0.86	0.89		0.92	0.83	
Extrahepatic disease progression (%)			0.348			0.038			0.293
1 Y	0.25	0.57		0.20	0.64		0.35	0.53	
2 Y	0.60	0.57		0.33	0.82		0.57	0.62	
Progression-free survival (%)			0.381			0.023			0.365
1 Y	0.31	0.34		0.51	0.16		0.39	0.26	
2 Y	0.08	0.14		0.15	0.08		0.08	0.13	
Overall survival (%)			0.389			0.850			0.799
1 Y	0.68	0.78		0.70	0.75		0.70	0.75	
2 Y	0.39	0.50		0.44	0.44		0.44	0.44	

Note: *Student *t*-test.
Abbreviations: DPD, dihydropyrimidine dehydrogenase; TP, thymidine phosphorylase; TS, thymidylate synthase; Y, year.

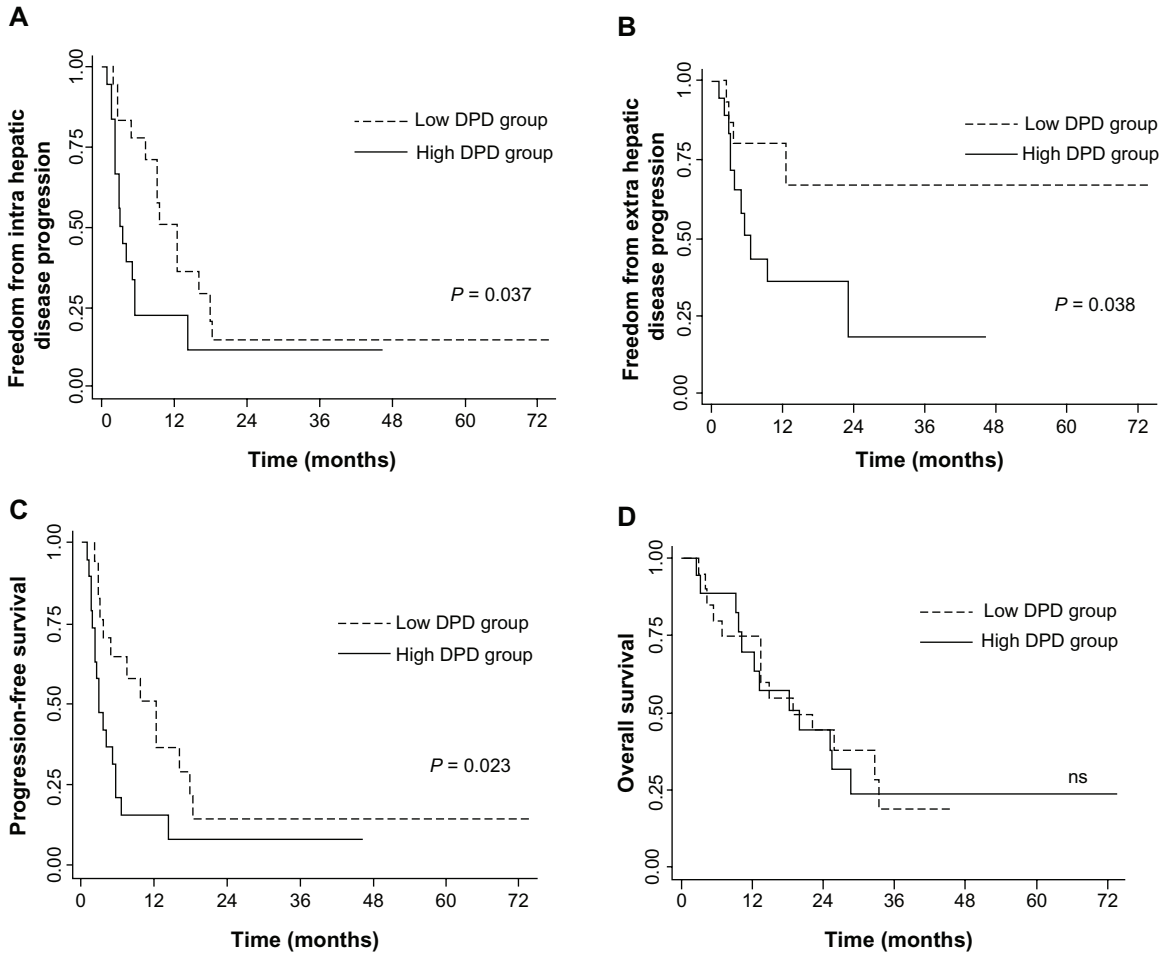

Figure 1 Kaplan–Meier plot of treatment outcome by the two expression DPD groups.
Note: Freedom from intrahepatic disease progression (**A**); freedom from extrahepatic disease progression (**B**); progression-free survival (**C**); overall survival (**D**).
Abbreviations: DPD, dihydropyrimidine dehydrogenase; ns, not significant.

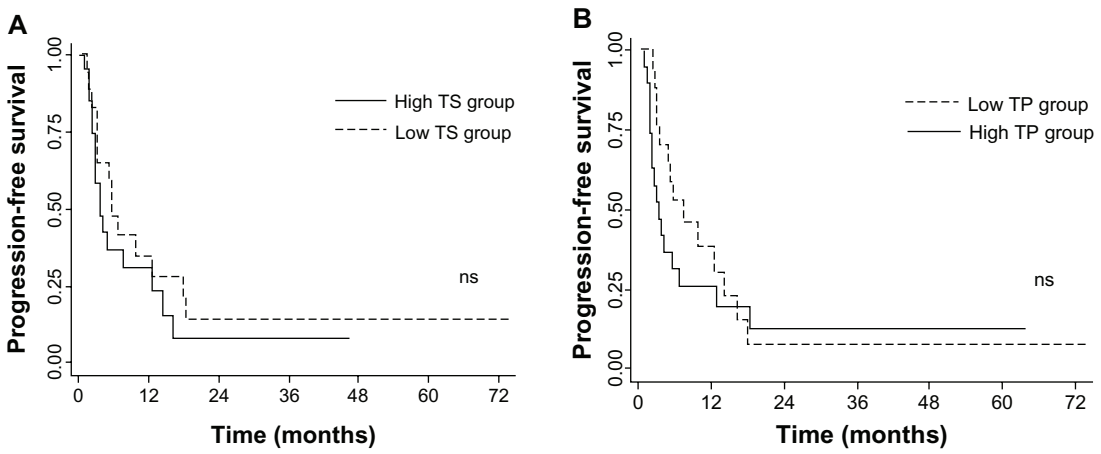

Figure 2 Kaplan–Meier plot of progression-free survival.
Note: (**A**) Expression TS groups; (**B**) expression TP groups.
Abbreviations: ns, not significant; TP, thymidine phosphorylase; TS, thymidylate synthase.

prolonging survival compared with standard supportive care in randomized controlled trials and meta-analyses.[8,22] The main issues with TACE are that it is a nonstandardized procedure with different embolic and chemotherapeutic agents and there is no proven superiority of any chemotherapeutic agent in transarterial therapies or of combined therapy over monotherapy.[22] Thus, further research on how to select the effective chemotherapeutic agents for TACE in HCC is needed. On the other hand, the sensitivity of 5-FU has been demonstrated to be affected by several enzymes, including TS, TP, and DPD.[23] To our knowledge, this is the first study to evaluate the relationships between the gene expression of three major enzymes and 5-FU-based TACE treatment efficacy in HCC patients.

After administration, 80%–90% of 5-FU is degraded by DPD,[24] which catalyzes the first and rate-limiting step of the pyrimidine catabolic pathway.[25] This process generally occurs in all tissues, including tumors, with the highest levels in peripheral blood mononuclear cells and liver,[26] which contain the highest DPD.[27,28] Clinically, DPD activity has been identified as a critical determinant of metabolism and pharmacology of 5-FU,[25] and a congenital deficiency of DPD can result in severe life-threatening toxicity after 5-FU administration.[29] Previous studies reported that DPD overexpression in tumor cells is associated with 5-FU resistance in vitro[30] and in vivo, in colorectal cancer,[13,31] gastric cancer,[32] non–small-cell lung cancer,[17] and oral cancer patients.[33] Our study found that high DPD mRNA expression was significantly related to higher 1-year intrahepatic progression rates (78% vs 50%, $P = 0.037$) and 1-year extrahepatic progression rates (64% vs 20%, $P = 0.038$), higher risk of intrahepatic and extrahepatic disease progression (HR, 2.212 and 3.171, respectively), and lower 1-year PFS rates (16% vs 51%, $P = 0.023$) (Table 3). Furthermore, high DPD mRNA expression level was also found to be an independent prognostic factor for PFS. Consistent with previous studies, our results in HCC patients treated with 5-FU-based TACE supported the hypothesis that high DPD expression level may be a predictive marker of poor treatment outcome for 5-FU-based TACE.

As far as OS is concerned, the results about the prognostic role of DPD are often contradictory; some studies showed the association between DPD expression and cancer patients survival after 5-FU treatment,[14,34] while other studies failed to find this association[35] or had opposite results.[36,37] Our study also didn't find that DPD expression is associated with the survival of HCC patients treated with 5-FU-based TACE. One possible explanation is that patients would receive different treatment strategies after disease progression, which ultimately affects survival prognosis.

Inhibition of TS by the 5-FU metabolite fluorodeoxyuridine monophosphate has been identified as the major mechanism of 5-FU action.[38] Several preclinical and clinical studies demonstrated that high TS levels correlate with 5-FU resistance in various malignancies.[38] On the other hand, TS has also been identified as a key enzyme that affects the prognosis of patients with various cancers.[39] However, our study in HCC patients treated with 5-FU-based TACE failed to prove that TS mRNA expression is a predictive marker for responsiveness to 5-FU, which is consistent with previous studies.[40] Regarding the clinical and pathological parameters, we found that high TS mRNA expression was significantly correlated to high AFP (≥200 ng/mL), and the high TS group had a tendency to include more patients with low differentiation and vessel invasion than the low TS group, but these

differences were not statistically significant. Such results might be attributed by tumor proliferation, which was previously reported associated with intratumoral TS expression,[41] and AFP was reported to enhance growth of human HCC cell lines, which may also contribute to the proliferation of tumor or fetal cells.[42]

The nucleoside cleavage enzyme, TP, is involved in the catalysis of reversible phosphorylation of thymidine to deoxyribose-1-phosphate and thymidine. Clinical studies have demonstrated that low TP expression was associated with a good response to 5-FU[17,23] and a better prognosis.[43] In this study, no difference was observed in the survival of HCC patients treated with 5-FU-based TACE according to the intratumoral TP mRNA expression. Our results are consistent with a study conducted by Soong et al.[36] We also found that low TP mRNA expression was significantly related to high AFP (≥200 ng/mL). A previous study demonstrated that TP confers apoptotic resistance and migration of a cholangiocarcinoma-derived cell line,[44] which may also be induced by the effects of tumor proliferation. However, studies on TP have not produced conclusive results, and further investigation is essential.

Taken together, high DPD mRNA expression is associated with poor treatment outcome in HCC patients treated with 5-FU-based TACE, which may be a predictive marker for treatment resistance of 5-FU in HCC. Although the results of this study were based on a relatively small number of patients and are thus preliminary in nature, some statistically significant correlations were observed. A large-scale study should be conducted to further strengthen our findings.

Acknowledgments

We thank all patients and their families for their participation in this study.

Disclosure

The authors declare that they have no conflicts of interest. This project was supported by the National Natural Science Foundation of China (Grant No 81201718).

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin.* 2005;55(2):74–108.
2. Yeh FS, Yu MC, Mo CC, Luo S, Tong MJ, Henderson BE. Hepatitis B virus, aflatoxins, and hepatocellular carcinoma in southern Guangxi, China. *Cancer Res.* 1989;49(9):2506–2509.
3. Kew MC, Yu MC, Kedda MA, Coppin A, Sarkin A, Hodkinson J. The relative roles of hepatitis B and C viruses in the etiology of hepatocellular carcinoma in southern African blacks. *Gastroenterology.* 1997;112(1):184–187.
4. Parkin DM. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer.* 2006;118(12):3030–3044.
5. Bruix J, Sala M, Llovet JM. Chemoembolization for hepatocellular carcinoma. *Gastroenterology.* 2004;127(5 Suppl 1):179S–188S.
6. De Carlis L, Giacomoni A, Pirotta V, et al. Surgical treatment of hepatocellular cancer in the era of hepatic transplantation. *J Am Coll Surg.* 2003;196(6):887–897.
7. Llovet JM, Real MI, Montana X, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet.* 18 2002;359(9319):1734–1739.
8. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. *Hepatology.* 2003;37(2):429–442.
9. Pinedo HM, Peters GF. Fluorouracil: biochemistry and pharmacology. *J Clin Oncol.* 1988;6(10):1653–1664.
10. Patt YZ, Yoffe B, Charnsangavej C, et al. Low serum alpha-fetoprotein level in patients with hepatocellular carcinoma as a predictor of response to 5-FU and interferon-alpha-2b. *Cancer.* 1993;72(9):2574–2582.
11. Hwang JY, Jang BK, Kwon KM, et al. Efficacy of hepatic arterial infusion therapy for advanced hepatocellular carcinoma using 5-fluorouracil, epirubicin and mitomycin-C. *Korean J Gastroenterol.* 2005;45(2):118–124. Korean.
12. Jang BK, Kwon KM, Chung WJ, et al. Efficacy of hepatic arterial infusion therapy for advanced hepatocellular carcinoma using 5-fluorouracil and cisplatin. *Korean J Hepatol.* 2004;10(4):271–278.
13. Salonga D, Danenberg KD, Johnson M, et al. Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. *Clin Cancer Res.* 2000;6(4):1322–1327.
14. Ichikawa W, Uetake H, Shirota Y, et al. Combination of dihydropyrimidine dehydrogenase and thymidylate synthase gene expressions in primary tumors as predictive parameters for the efficacy of fluoropyrimidine-based chemotherapy for metastatic colorectal cancer. *Clin Cancer Res.* 2003;9(2):786–791.
15. Beck A, Etienne MC, Cheradame S, et al. A role for dihydropyrimidine dehydrogenase and thymidylate synthase in tumour sensitivity to fluorouracil. *Eur J Cancer.* 1994;30A(10):1517–1522.
16. Takahashi T, Yoshida H, Mamada Y, et al. Profiling of fluorouracil-related genes by microdissection technique in hepatocellular carcinoma. *Hepatogastroenterology.* 2007;54(78):1612–1616.
17. Matsuda M, Shiba S, Asakawa M, Kono H, Fujii H. Complete remission of multiple recurrent hepatocellular carcinomas by oral administration of enteric-coated tegafur/uracil in a patient with huge hepatocellular carcinoma extending to the inferior vena cava after hepatic resection: analysis of mRNA expression of fluoropyrimidine metabolism enzymes in the primary tumor. *Int J Clin Oncol.* 2009;14(3):245–248.
18. Baba H, Teramoto K, Kawamura T, Mori A, Imamura M, Arii S. Dihydropyrimidine dehydrogenase and thymidylate synthase activities in hepatocellular carcinomas and in diseased livers. *Cancer Chemother Pharmacol.* 2003;52(6):469–476.
19. Enomoto Y, Itsubo M, Kawabe T, et al. Two cases of transcatheter therapy to hepatocellular carcinoma supplied by the right internal mammary artery. *Nihon Shokakibyo Gakkai Zasshi.* 2000;97(5): 585–589. Japanese.
20. Saccheri S, Lovaria A, Sangiovanni A, et al. Segmental transcatheter arterial chemoembolization treatment in patients with cirrhosis and inoperable hepatocellular carcinomas. *J Vasc Interv Radiol.* 2002;13(10):995–999.
21. Yu SC, Hui JW, Hui EP, et al. Embolization efficacy and treatment effectiveness of transarterial therapy for unresectable hepatocellular carcinoma: a case-controlled comparison of transarterial ethanol ablation with lipiodol-ethanol mixture versus transcatheter arterial chemoembolization. *J Vasc Interv Radiol.* 2009;20(3):352–359.
22. Marelli L, Stigliano R, Triantos C, et al. Transarterial therapy for hepatocellular carcinoma: which technique is more effective? A systematic review of cohort and randomized studies. *Cardiovasc Intervent Radiol.* 2007;30(1):6–25.

23. Metzger R, Danenberg K, Leichman CG, et al. High basal level gene expression of thymidine phosphorylase (platelet-derived endothelial cell growth factor) in colorectal tumors is associated with nonresponse to 5-fluorouracil. *Clin Cancer Res.* 1998;4(10):2371–2376.
24. Fischel JL, Etienne MC, Spector T, Formento P, Renee N, Milano G. Dihydropyrimidine dehydrogenase: a tumoral target for fluorouracil modulation. *Clin Cancer Res.* 1995;1(9):991–996.
25. Diasio RB, Johnson MR. Dihydropyrimidine dehydrogenase: its role in 5-fluorouracil clinical toxicity and tumor resistance. *Clin Cancer Res.* 1999;5(10):2672–2673.
26. Ho DH, Townsend L, Luna MA, Bodey GP. Distribution and inhibition of dihydrouracil dehydrogenase activities in human tissues using 5-fluorouracil as a substrate. *Anticancer Res.* 1986;6(4):781–784.
27. Lu Z, Zhang R, Diasio RB. Dihydropyrimidine dehydrogenase activity in human peripheral blood mononuclear cells and liver: population characteristics, newly identified deficient patients, and clinical implication in 5-fluorouracil chemotherapy. *Cancer Res.* 1993;53(22):5433–5438.
28. Lu Z, Zhang R, Diasio RB. Population characteristics of hepatic dihydropyrimidine dehydrogenase activity, a key metabolic enzyme in 5-fluorouracil chemotherapy. *Clin Pharmacol Ther.* 1995;58(5):512–522.
29. Wei X, McLeod HL, McMurrough J, Gonzalez FJ, Fernandez-Salguero P. Molecular basis of the human dihydropyrimidine dehydrogenase deficiency and 5-fluorouracil toxicity. *J Clin Invest.* 1996;98(3):610–615.
30. Higashiyama M, Kodama K, Yokouchi H, et al. Thymidylate synthase and dihydropyrimidine dehydrogenase activities in non-small cell lung cancer tissues: relationship with in vitro sensitivity to 5-fluorouracil. *Lung Cancer.* 2001;34(3):407–416.
31. Yoshinare K, Kubota T, Watanabe M, et al. Gene expression in colorectal cancer and in vitro chemosensitivity to 5-fluorouracil: a study of 88 surgical specimens. *Cancer Sci.* 2003;94(7):633–638.
32. Ishikawa Y, Kubota T, Otani Y, et al. Dihydropyrimidine dehydrogenase and messenger RNA levels in gastric cancer: possible predictor for sensitivity to 5-fluorouracil. *Jpn J Cancer Res.* 2000;91(1):105–112.
33. Kobayashi H, Koike T, Nakatsuka A, et al. Dihydropyrimidine dehydrogenase expression predicts survival outcome and chemosensitivity to 5-fluorouracil in patients with oral squamous cell carcinoma. *Oral Oncol.* 2005;41(1):38–47.
34. Horiguchi J, Yoshida T, Koibuchi Y, et al. DPD activity and immunohistochemical DPD expression in human breast cancer. *Oncol Rep.* 2004;11(1):65–72.
35. Hakamada Y, Tsuchida A, Arima M, et al. Prognostic predictors in breast cancer patients with postoperative 5-fluorouracil-based chemotherapy. *Int J Mol Med.* 2005;16(2):309–314.
36. Soong R, Shah N, Salto-Tellez M, et al. Prognostic significance of thymidylate synthase, dihydropyrimidine dehydrogenase and thymidine phosphorylase protein expression in colorectal cancer patients treated with or without 5-fluorouracil-based chemotherapy. *Ann Oncol.* 2008;19(5):915–919.
37. Nii A, Shimada M, Ikegami T, et al. Significance of dihydropyrimidine dehydrogenase and thymidylate synthase mRNA expressions in hepatocellular carcinoma. *Hepatol Res.* 2009;39(3):274–281.
38. Van Triest B, Pinedo HM, Giaccone G, Peters GJ. Downstream molecular determinants of response to 5-fluorouracil and antifolate thymidylate synthase inhibitors. *Ann Oncol.* 2000;11(4):385–391.
39. Kornmann M, Schwabe W, Sander S, et al. Thymidylate synthase and dihydropyrimidine dehydrogenase mRNA expression levels: predictors for survival in colorectal cancer patients receiving adjuvant 5-fluorouracil. *Clin Cancer Res.* 2003;9(11):4116–4124.
40. Johnston PG, Benson AB III, Catalano P, Rao MS, O'Dwyer PJ, Allegra CJ. Thymidylate synthase protein expression in primary colorectal cancer: lack of correlation with outcome and response to fluorouracil in metastatic disease sites. *J Clin Oncol.* 2003;21(5):815–819.
41. Nakagawa T, Otake Y, Yanagihara K, et al. Expression of thymidylate synthase is correlated with proliferative activity in non-small cell lung cancer (NSCLC). *Lung Cancer.* 2004;43(2):145–149.
42. Li MS, Li PF, Chen Q, Du GG, Li G. Alpha-fetoprotein stimulated the expression of some oncogenes in human hepatocellular carcinoma Bel 7402 cells. *World J Gastroenterol.* 2004;10(6):819–824.
43. Soong R, Diasio RB. Advances and challenges in fluoropyrimidine pharmacogenomics and pharmacogenetics. *Pharmacogenomics.* 2005;6(8):835–847.
44. Thanasai J, Limpaiboon T, Jearanaikoon P, et al. Effects of thymidine phosphorylase on tumor aggressiveness and 5-fluorouracil sensitivity in cholangiocarcinoma. *World J Gastroenterol.* 16(13):1631–1638.