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Predictive Value of Dihydropyrimidine Dehydrogenase Expression in Tumor Tissue, Regarding the Efficacy of Postoperatively Administered UFT (Tegafur + Uracil) in Patients With p-Stage I Nonsmall-Cell Lung Cancer

TATSUO NAKAGAWA, MD,[1] FUMIHIRO TANAKA, PhD,[1] TETSUYA TAKATA, MD,[1] KATSUNARI MATSUOKA, MD,[1] RYO MIYAHARA, PhD,[1] YOSUKE OTAKE, PhD,[1] KAZUHIRO YANAGIHARA, PhD,[1] MASAKAZU FUKUSHIMAB, PhD,[2] AND HIROMI WADA, PhD[1]*

[1]*Department of Thoracic Surgery, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan*
[2]*Cancer Research Laboratory, Hanno Research Center, Taiho Pharmaceutical Co., Ltd., Hanno, Saitama, Japan*

Background and Objectives: Dihydropyrimidine dehydrogenase (DPD) activity in tumor cells has been suggested to be one of the factors determining the effectiveness of 5-fluorouracil (5-FU). In the present study, we analyzed DPD expression in tumors and investigated retrospectively the relationship between the efficacy of UFT (Tegafur + Uracil) as adjuvant chemotherapy and DPD expression in nonsmall-cell lung cancer (NSCLC).

Methods: DPD expression of 166 resected p-stage I NSCLC was examined immunohistochemically. Patients who were administered UFT alone as adjuvant therapy comprised the UFT group (n = 54), and those who underwent only surgery comprised the control group (n = 112). DPD expression was categorized as either high or low, according to intensity of staining.

Results: DPD expression was high in 98 patients (59.0%) and low in 68 patients (41.0%). Patients with low-DPD tumors who were administered UFT had a significantly better prognosis than those who did not receive adjuvant treatment ($P = 0.021$). No significant difference was found between the two groups of patients with high-DPD tumors ($P = 0.598$).

Conclusions: DPD expression may predict the efficacy of UFT after surgery for p-stage I NSCLC. A prospective study is needed to confirm the role of DPD expression as a predictor of UFT efficacy.

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KEY WORDS: **dihydropyrimidine dehydrogenase; nonsmall-cell lung cancer; adjuvant chemotherapy; prognosis**

Radical surgery is the most effective therapy for patients with localized nonsmall-lung cancer (NSCLC). However, overall prognosis is still poor, with the 5-year survival rate after complete resection being reported as 55–72%, for patients with p-stage I tumors [1–3]. Adjuvant therapies to prevent recurrence and metastasis after surgery are needed.

5-fluorouracil (5-FU) and its derivatives are some of the most widely used chemotherapeutic drugs, especially for the treatment of gastrointestinal tumors. The anticancer effects of 5-FU are thought to be related to its two active metabolites, 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP) and 5-fluoro-uridine-5′-tri-phosphate (FUTP). FdUMP forms a ternary complex with

*Correspondence to: Hiromi Wada, PhD, Department of Thoracic Surgery, Faculty of Medicine, Kyoto University, 53 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8397, Japan. Fax: +81-75-751-4647.
E-mail: wadah@kuhp.kyoto-u.ac.jp

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thymidylate synthase (TS) and 5,10-methylene-tetrahydrofolate (5,10-CH_2FH_2), which blocks TS activity and inhibits the de novo synthesis of thymidylate for DNA synthesis [4]. FUTP is incorporated into RNA, resulting in an alteration of cellular functions in which RNA participates [5].

5-FU is degraded to 2-fluoro-β-alanine mainly in the liver. Dihydropyrimdine dehydrogenase (DPD) is the initial and rate-limiting enzyme in this process [6]. Some experimental studies showed [7–10] that low DPD activity or expression in tumor cells was related to higher 5-FU sensitivity. In some clinical studies, DPD activity in tumors may have correlated with the clinical response to 5-FU-based chemotherapies [11–13].

UFT is an anticancer drug for oral use that contains Tegafur (a 5-FU derivative) and Uracil. UFT has been reported to be an effective postoperative adjuvant therapy for NSLC in randomized prospective studies [14–16]. In our institute, UFT has been used in an adjuvant setting for patients with even early staged NSCLC, and significant efficacy was demonstrated in a retrospective study [17]. In the present study, we investigated DPD expression in resected tumor tissues by immunohistochemical staining, and analyzed retrospectively the relationship between the efficacy of UFT as adjuvant chemotherapy and the expression of DPD in p-stage I NSCLC.

PATIENTS AND METHODS

Patients

The clinical records of patients who underwent complete surgical resection and mediastinal lymph node dissection for p-stage I NSCLC (excluding low-grade malignancies such as carcinoid, mucoepidermoid carcinoma, and adenoid cystic carcinoma) from January 1986 to December 1995 at the Department of Thoracic Surgery of Kyoto University Hospital were retrospectively reviewed. Patients who were treated with UFT alone as a postoperative chemotherapy comprised the UFT group ($n = 54$), and patients who underwent only surgery comprised the control group ($n = 112$). UFT administration was started within 1 month after surgery and continued for more than 3 months. Pathologic stage was classified according to the TNM classification revised in 1997 [18]. Histological type was determined according to the WHO classification [19]. Performance status was determined according to the ECOG Performance Status Scale [20]. Patients who received any clinical therapies prior to the operation or UFT administration for less than 3 months were excluded from this study. Patients dying in the postoperative period (defined as within 30 days if discharged from the hospital or within the same hospitalization) were excluded.

Tissue Samples

Surgical specimens were fixed in 10% formalin, embedded in paraffin, cut into 4-μm sections, and immunohistochemically stained for DPD.

Immunohistochemical Staining for DPD

Peroxidase-blocking reagent was obtained from the DAKO US Co. (Santa Barbara, CA). Normal goat serum, biotinylated goat anti-rabbit IgG, diaminobenzidine tetrahydro-chloride (DAB), and streptavidin-biotinylated peroxidase complex were from the Vector Co., Ltd. (Burlingame, CA). All other chemicals were commercially available products of analytical grade.

DPD expression was evaluated immunohistochemically, using a polyclonal antibody for recombinant human DPD, which was produced by one of us [21]. The procedure was as follows: after tissue sections were deparaffinized and rehydrated, endogenous peroxidase activity and nonspecific binding were blocked. Sections were then incubated with anti-DPD antibody (dilution, 1:2,000) as a primary antibody at 4°C overnight. Biotinylated goat anti-rabbit IgG antibody was applied for 30 minutes at room temperature, followed by streptavidin-biotinylated peroxidase complex for 30 minutes at room temperature. Peroxidase activity was visualized with DAB solution for 3 minutes at room temperature. Counterstaining was performed with hematoxylin (1 minute at room temperature). Sections of human pancreatic cancer tissue obtained from implanted cell line MIAPaCa-2 in nude mice that is positive for DPD were used as positive controls in each staining. The negative control was prepared by omitting the primary antibody for each section.

Evaluation of Staining

Each section was evaluated separately by two of us (T.N. and R.M.) without knowledge of the clinical data. DPD expression was semiquantitated using a visual grading system in which intensity of staining was categorized as grade 0 (negative staining), grade 1+ (weak staining, defined as equal to that of the interstitial background), grade 2+ (moderate staining, defined as an intensity distinct from that of the interstitial background), and grade 3+ (strong staining, defined as an intensity equal to that of the positive control). Specimens classified as grade 0 or 1+ were grouped together and defined as low-DPD tumors, whereas those classified as grade 2+ or 3+ were defined as high-DPD tumors. There was satisfactory agreement (>90%) in the evaluation between two investigators. In those cases where opinions differed, final judgment was determined by consensus.

Statistical Analysis

Comparison of clinicopathologic features between two groups was performed by chi-square test or Student's *t*-test. Survival after surgery was analyzed by the Kaplan-Meier method, and evaluation of the difference was conducted by log-rank test. Multivariable analysis of prognostic factors was conducted by Cox's proportional hazards model. *P*-values less than 0.05 were considered significant.

RESULTS

Clinical Background of Patients

The clinical background of patients is summarized in Table I. There was no significant difference in clinical factors between the UFT group and the control group. The dose and mean duration of UFT administration were 200–600 mg/day/body weight and 22.2 ± 16.3 months (mean $\pm$ SD; range, 3–60 months), respectively.

Expression of DPD

DPD expression was high in 98 patients (59.0%) and low in 68 (41.0%). There was no significant difference between patients with high-DPD and low-DPD tumors in age, gender, histological differentiation, p-T factor, performance status, and UFT administration. The rate of high-DPD tumors was 68.6% for adenocarcinomas, and 35.8% for squamous-cell carcinomas ($P < 0.01$) (Table II).

TABLE I. Patient Background

Factors	Treatment: UFT (n = 54)	Treatment: Surgery alone (n = 112)	*P*
Age	62.2 ± 8.6	64.8 ± 8.5	0.063
Gender			
Male	34	81	0.221
Female	20	31	
Histologic findings			
Adenocarcinoma	37	65	
Squamous cell	13	40	0.321
Others	4	7	
Histologic differentiation			
Well	25	50	
Moderately	20	42	0.974
Poorly	9	20	
P-T factor			
T1	33	62	0.483
T2	21	50	
Performance status			
0	47	102	0.422
≦1	7	10	

TABLE II. Relationship Between Clinical Factors and Dihydropyrimidine Dehydrogenase Expression

Factors	Expression of DPD: Low (n = 68)	Expression of DPD: High (n = 98)	*P*
Age	62.9 ± 8.9	64.7 ± 8.3	0.188
Gender			
Male	52	63	0.094
Female	16	35	
Histologic findings			
Adenocarcinoma	32	70	
Squamous cell	34	19	<0.001
Others	2	9	
Histologic differentiation			
Well	27	48	
Moderately	31	31	0.186
Poorly	10	19	
P-T factor			
T1	43	52	0.193
T2	25	46	
Performance status			
0	64	85	0.123
≦1	4	13	
UFT administration			
Yes	24	30	0.527
No	44	68	

DPD, dihydropyrimidine dehydrogenase.

Survival

The 5-year survival rates of the UFT group and control group were 85.0% and 75.6% ($P = 0.075$), respectively. To see if the expression of DPD could be a predictor for UFT efficacy, we compared the survival rates of the UFT group and control group, stratified according to DPD expression. For the 98 patients who had high-DPD tumors, there was no significant difference in survival between the UFT group and the control group ($P = 0.598$) (Fig. 1). For the 68 patients who had low-DPD tumors, the 5-year survival rate of the UFT group was 91.3%, while that of the control group was 74.2% ($P = 0.021$) (Fig. 2).

A multivariable analysis of seven variables (age, gender, histological type, histological differentiation, p-T factor, performance status, and UFT administration) was performed, stratified according to DPD expression. Administration of UFT and p-T stage were significant prognostic variables in patients with low-DPD tumors ($P < 0.01$) (Table III), but not significant in those with high-DPD tumors.

DISCUSSION

In this study, we evaluated the efficacy of UFT administration after surgery in relation to DPD expression in the tumor tissue of patients with NSCLC. We analyzed DPD

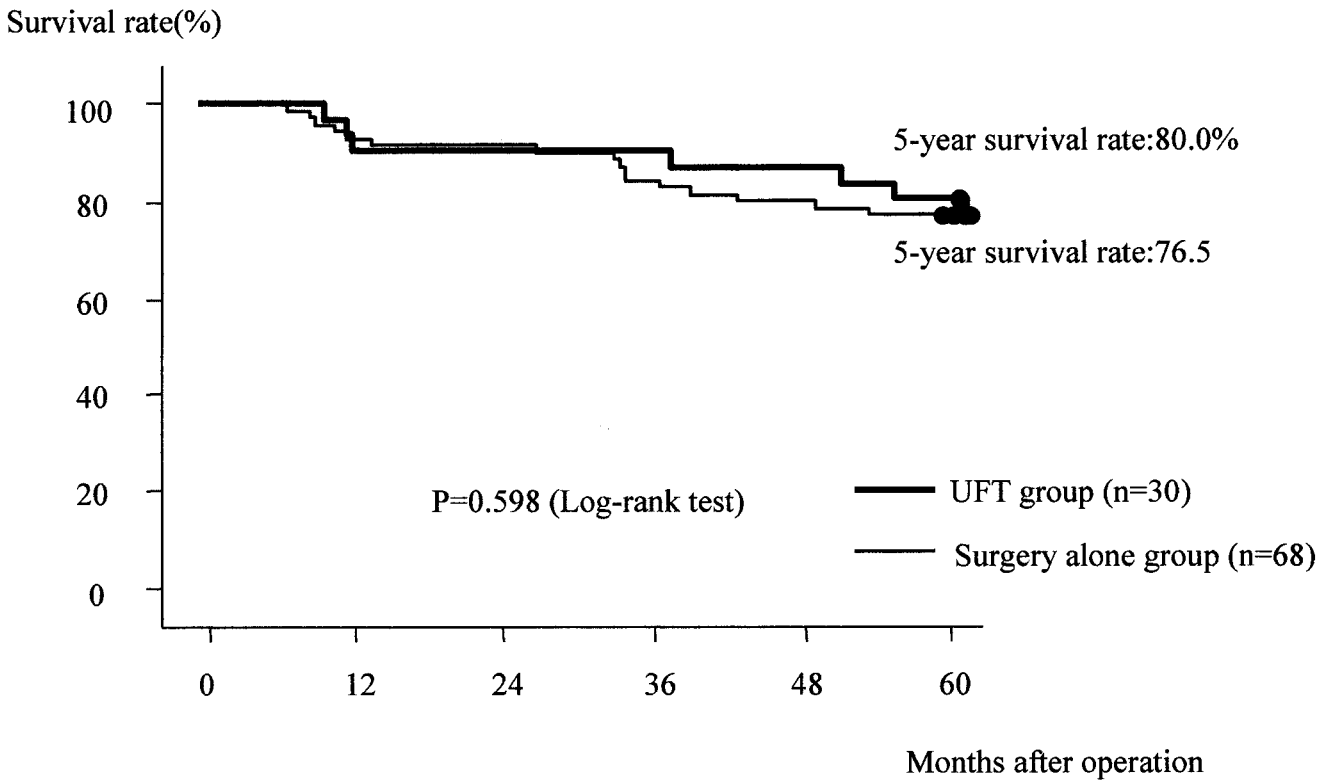

Fig. 1. Comparison of 5-year survival curves between UFT group and control group for patients with high-DPD tumors. There was no significant difference between the two groups ($P = 0.598$).

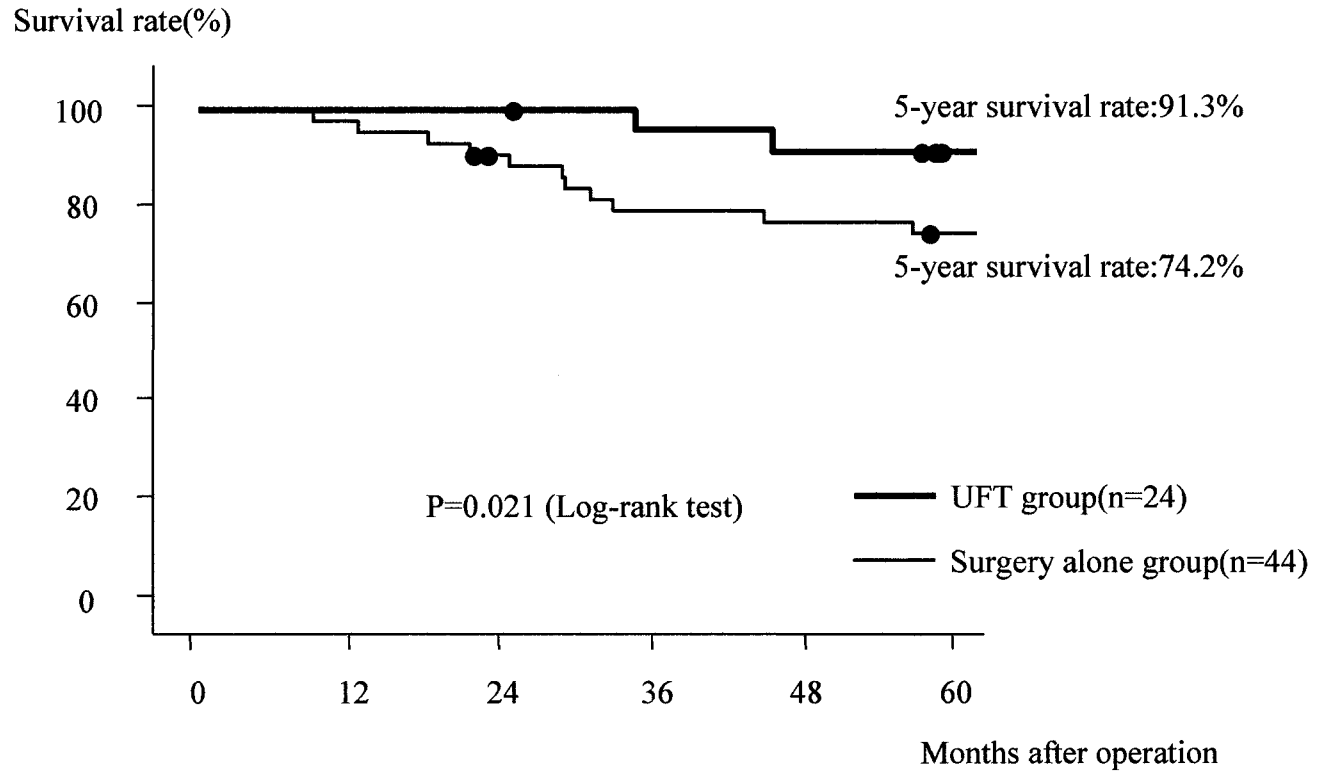

Fig. 2. Comparison of 5-year survival curves between UFT group and control group for patients with low-DPD tumors. The UFT group had a significantly better prognosis than the surgery-alone group ($P = 0.021$).

TABLE III. Multivariate Analysis of Patients With Low-Dihydropyrimidine Dehydrogenase Tumors

Factors	HR	95%CI	*P*
Age	0.939	0.876–1.007	0.076
Gender (male, female)	1.550	0.421–5.710	0.510
Histogic type (ACA,* non-ACA)	0.702	0.190–2.600	0.597
Histogic differentiation (poorly, moderately, well)	0.913	0.425–1.960	0.815
P-T factor (T1, T2)	10.036	2.650–38.001	<0.01
Performance status (0, ≦1)	0.651	0.080–5.309	0.689
UFT administration (yes, no)	10.786	2.086–55.759	<0.01

*adenocarcinoma.

expression in patients with p-stage I NSCLC, because patients with advanced-stage disease tend to undergo various therapies before surgery, which may influence the evaluation of DPD expression on immunohistochemical staining. The results showed that patients with low-DPD tumors treated with surgery and UFT had significantly better survival than patients with low-DPD tumors treated with surgery alone ($P = 0.021$). No significant difference was observed between the two treatment groups for patients with high-DPD tumors ($P = 0.598$). This finding suggests that the expression of DPD in tumors might serve as a possible predictor for the efficacy of UFT administration after complete resection for NSCLC.

DPD is a rate-limiting enzyme that catalyzes 5-FU degradation [6]. Maintaining intratumoral concentration of 5-FU, which may depend on intratumoral DPD activity, may be important in obtaining an effective response to 5-FU chemotherapy. DPD activity or expression in tumor cells has been reported to correlate inversely with 5-FU sensitivity both in vitro [7–9] and in vivo [10]. In clinical studies of patients with head and neck cancer, Etienne et al. [11] found that complete responders to 5-FU-based chemotherapy had significantly lower normalized DPD activity (defined as ratio of tumoral and nontumoral DPD activity) than partial or nonresponding patients. Salonga et al. [12] showed that low DPD expression in colorectal tumors was associated with a better response to 5-FU, and better patient survival. We found only one article that related the efficacy of 5-FU-based adjuvant chemotherapy to DPD activity in tumor tissue [13]. In that article, the authors immunohistochemically assessed the expression of intratumoral DPD in 68 patients with NSCLC treated with oral 5-FU derivatives after surgery, and found that patients with low-DPD tumors had a significantly better prognosis than those with high-DPD tumors. They used the same anti-DPD polyclonal antibody we used in this study. Our present study both supports the results of that article and confirms the usefulness of this anti-DPD polyclonal antibody in the clinical evaluation of DPD. In some studies, DPD was detected by radioenzyme activity assay or reverse transcription-polymerase chain reaction (RT-PCR). These types of assays may be sensitive, but may also be technically difficult. Further, contamination of tumor specimens with normal tissue or blood cells may falsely elevate measurements of intratumoral DPD expression when tissue samples are homogenized, as DPD is expressed in normal tissues, especially in the liver and peripheral mononuclear cells [22]. For these reasons, immunohistochemical staining is preferred and may be useful for evaluating intratumoral DPD expression in a large number of clinical materials.

UFT is a combination drug, containing Tegafur and Uracil at the molar ratio of 1:4. Tegafur, a prodrug of 5-FU, is converted into 5-FU in vivo. Uracil inhibits 5-FU degradation by DPD. The clinical effect of UFT administration for patients with NSCLC has been reported in some prospective randomized studies. The West Japan Study Group for Lung Cancer Surgery conducted a prospective randomized study for completely resected p-stage I–IIIB NSCLC and reported that the prognosis of patients who had been administered UFT (400 mg/body/day) for 1 year after surgery was significantly better than those patients who had received surgical treatment alone [14]. A subsequent study conducted by the same group confirmed the efficacy of adjuvant therapy, including UFT for p-stage I–II NSCLC [15]. Another prospective randomized study conducted by the Study Group of Adjuvant Chemotherapy for Lung Cancer (Chubu, Japan) also reported that both the 5-year survival rate and postoperative disease-free interval of patients who received UFT (8 mg/kg/day for 6 months) after adjuvant intravenous cisplatin (CDDP) and adriamycin (ADM) were significantly better than those of patients who had undergone surgery alone [16]. These results suggest that oral administration of UFT can improve the postoperative survival of patients with NSCLC.

The present study suggests that oral administration of UFT after surgery may improve the survival of patients with p-stage I NSCLC when DPD expression in tumor tissue is low. However, as this study was retrospectively performed, a prospective, randomized study will be

needed to fully elucidate the role of DPD expression in NSCLC as a predictor of UFT efficacy.

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