Low Dihydropyrimidine Dehydrogenase Correlates with Prolonged Survival in Patients with Lung Adenocarcinoma Treated with 5-Fluorouracil

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Abstract. *Background: The enzyme dihydropyrimidine dehydrogenase (DPD) is involved in the metabolism of 5-fluorouracil (5-FU). The aim of this study was to clarify the correlation between the expression of DPD and the efficacy of 5-FU therapy in patients with lung adenocarcinoma (AD). Patients and Methods: We examined surgically resected specimens from 90 stage I to IIIA patients with lung ADs to determine the level of intra-tumoral DPD mRNA. Results: Administration of 5-FU improved the prognosis of patients with low DPD-expressing tumors, whereas it did not do so for patients with high DPD expressing tumors. Patients with low DPD-expressing tumors administered with 5-FU had a significantly better prognosis than those who underwent surgery alone. A Cox proportional hazards regression model revealed that administration of 5-FU was an independent variable to predict prognosis in patients with low DPD-expressing tumors. Conclusion: Quantification of DPD mRNA levels is useful for determining the subgroup of lung AD patients who would benefit most from 5-FU after surgery.*

5-Fluorouracil (5-FU) and its derivatives are widely used for treatment of various types of cancer (1). A recent study showed that postoperative oral administration of tegafur-uracil (UFT) improves survival in patients following resection of stage I lung adenocarcinoma (AD) and its administration has become standard therapy after curative resection in early non-small cell lung cancer (NSCLC) cases (2, 3). Furthermore, a novel oral form of fluorouracil S-1 was shown to have promising effects against advanced NSCLC (4). These findings indicate that 5-FU is effective for NSCLC patients and highlight the importance of detection of biomarkers for prediction of its efficacy for treating NSCLC.

Thymidylate synthase (TYMS), the target enzyme for 5-FU, catalyzes an important process for DNA biosynthesis (5, 6) and we previously reported that the prognosis of NSCLC patients is related significantly to the intratumoral *TYMS* mRNA level (7). Dihydropyrimidine dehydrogenase (DPD) is one of the key enzymes involved in the catabolism of 5-FU and its expression was found useful in predicting the efficacy of 5-FU after surgery for NSCLC based on disease-free interval during short follow-up periods (8-10). In the present study, we examined the efficacy of 5-FU in association with DPD expression in regards to prognosis, including overall survival, in patients with lung ADs over a longer follow-up period.

Patients and Methods

Ninety specimens from lung AD patients determined to be p-stage I to IIIA were obtained during surgical procedures at Osaka University Hospital, Kinki Chuo Chest Medical Center, Toneyama Hospital, and Osaka Prefectural Medical Center for Respiratory and Allergic Disease between January 1999 and March 2003. Quantification of *TYMS* and *DPD* mRNA levels in AD tissues was performed as described previously (7, 10). The obtained copy numbers of *TYMS* and *DPD* were standardized with glyceralde-hydo-3-phosphate dehydrogenase (*GAPDH*) mRNA quantity, used as an endogenous control, with the following equation: 'Result'=Log (*TYMS* or *DPD* RNA copy number in tumor)/(*GAPDH* RNA copy number in tumor)×(6.1×10^9; *GAPDH* RNA copy number in 1 μg of total RNA extracted from the peripheral blood of 30 healthy volunteers).

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Key Words: Lung adenocarcinoma, 5-fluorouracil, dihydropyrimidine dehydrogenase, thymidylate synthase, chemosensitivity.

Table I. *Patient background data.*

Variable	Treatment		*p*-Value
	Surgery alone (control) n=60	5-FU n=30	
Age (years)	63±9.3	62±9.4	0.445
Gender			
Male	35 (58%)	20 (67%)	0.426
Female	25 (42%)	10 (33%)	
Pathologic stage			
I	38 (63%)	20 (67%)	
II	9 (15%)	6 (20%)	0.882
IIIA	13 (22%)	4 (13%)	

5-FU, 5-fluorouracil. *p*-Value, chi-square test or Mann-Whitney *U*-test.

Table II. *Thymidylate synthase (TYMS) and Dihydropyrimidine dehydrogenase (DPD) mRNA levels, and clinicopathologic factors.*

Factor	n	Log TYMS mRNA	*p*-Value	Log DPD mRNA	*p*-Value
Tumor status			0.029		0.054
pT1	49	6.90±0.29		6.89±0.48	
pT2	36	7.03±0.33		6.63±0.54	
pT3	5	7.42±0.54		6.99±0.27	
Nodal status			0.600		0.546
pN0	61	6.96±0.34		6.80±0.57	
pN1	12	7.00±0.36		6.75±0.41	
pN2	17	7.04±0.35		6.80±0.33	

p-Value, chi-square test or Mann-Whitney *U*-test.

Informed consent was obtained from all patients. Those administered UFT following surgery comprised the 5-FU group (n=30), while those who underwent surgery only, comprised the control group (n=60). UFT administration was started within 2 months after surgery and continued for more than 12 months. The dose of UFT was 300-400 mg/day and the mean duration of treatment was 21.5±7.3 months (mean±SD; range 12-26 months). The clinical backgrounds of the patients are summarized in Table I. There was no difference in clinical factors between the groups. The median follow-up period was 78±23 months (range 17-115 months) after surgery.

Chi-square, Mann-Whitney *U*, and Kruskal-Wallis tests were used to compare the results, while survival rates were estimated by the method of Kaplan and Meier and compared using log-rank test, using Statview version 5.0 for Windows (Abacus Concepts, Berkeley, CA, USA). A *p*-value of <0.05 was considered to be statistically significant.

Results

Quantification of *TYMS* and *DPD* mRNA levels in NSCLC tissues was successfully performed for all specimens. Intratumoral *TYMS* and *DPD* mRNA levels ranged from 6.28 to 8.04 (mean±SD; 6.98±0.34) and 5.36 to 8.28 (6.79±0.59), respectively. The results for *TYMS* and *DPD* mRNA levels are summarized in Table II. *TYMS* mRNA levels were associated with tumor status, while *DPD* mRNA levels were not associated with tumor or nodal status.

Thirty-five (39%) of the 90 patients developed distant metastasis after surgery. In regards to tumor stage, 12 (21%) out of 58 patients in stage I, 8 (53%) out of 15 patients in stage II, and 15 (88%) out of 17 patients in stage IIIA suffered from recurrent disease. Categorized by group, 27 (45%) out of 60 patients and 8 (27%) out of 30 in the control and 5-FU groups, respectively, had recurrence. There was no significant difference in overall survival rate between the groups (Figure 1A). Similar to a previous report (7), *TYMS* mRNA levels were significantly correlated to overall survival when dichotomized at the mean *TYMS* mRNA level (Figure 1B).

Next, we evaluated the correlation between *DPD* expression and efficacy of 5-FU. *DPD* mRNA levels were significantly correlated to overall survival in the 5-FU group, but not in the control group when dichotomized at the mean *DPD* mRNA level (Figure 2). In the 5-FU-group, the 5-year survival rate was 92% for the low DPD-expressing subgroup and 68% for the high DPD-expressing subgroup. Patients with low DPD-expressing tumors, who were administered 5-FU had a significantly better prognosis than those who underwent surgery alone (Figure 3A); the 5-year survival rates were 92% for the 5-FU group and 53% for the control group. These findings suggest that the intratumoral *DPD* mRNA level may be a possible predictor for efficacy of 5-FU administration after surgery for NSCLC. On the other hand, patients with high DPD-expressing tumors administered 5-FU had a tendency for a worse prognosis as compared to those who underwent surgery alone (Figure 3B).

We analyzed 5 variables, namely tumor status, nodal metastasis, *TYMS* mRNA expression, *DPD* mRNA expression, and 5-FU administration, using a Cox proportional hazards regression model to determine their effects on overall survival in NSCLC patients (Table IIIA). Multivariate analysis revealed that p-N2 and *TYMS* mRNA expression were independent variables for predicting overall survival (Table IIIB). Furthermore, in patients with low DPD-expressing tumors, multivariate analysis showed that *TYMS* mRNA expression and administration of 5-FU, were each independent variables predicting prognosis (Table IIIC).

Discussion

We performed quantitative assays of intratumoral *TYMS* and *DPD* mRNA levels to assess their association with clinicopathological factors, as well as the feasibility of applying them to predict the efficacy of 5-FU therapy in

patients with NSCLC over a long term. TYMS activity is necessary for cell proliferation because it catalyses an essential step in DNA synthesis, while its overexpression is reported to be associated with tumor proliferation, as well as poor prognosis, in a variety of cancer types (11, 12). As shown in Table IIIB, multivariate analysis revealed that a high level of *TYMS* mRNA was independently correlated to overall survival with a high hazard ratio, indicating that this marker can precisely perform prognosis for patients with lung AD. Determination of gene expression by RT-PCR is a useful technique for small-sized specimens, thus quantification of *TYMS* mRNA levels is clinically sensitive and useful for determining the prognosis of AD patients (7).

As DPD is a rate-limiting enzyme in the catabolism of 5-FU, its high expression in tumors is reported to result in a low sensitivity to 5-FU therapy (13). In the present study, we evaluated the efficacy of 5-FU administration as adjuvant chemotherapy, in relation to intratumoral *DPD* mRNA levels in lung AD patients. Our results revealed that *DPD* expression was significantly inversely correlated to the overall survival of patients administered 5-FU following surgery, indicating that patients with low levels of *DPD* expression in cancer tissue are sensitive to 5-FU. Furthermore, for patients with low DPD-expressing tumors, those administered 5-FU had a significantly better prognosis than those who underwent surgery alone. These findings suggest that the intratumoral *DPD* mRNA level is a possible predictor for the efficacy of 5-FU administration after surgery in lung AD patients. Interestingly, in patients with high DPD-expressing tumors, those administered 5-FU had a tendency for worse prognosis than those who underwent surgery alone (Figure 3B), suggesting that 5-FU may not have benefits for patients with high DPD-expressing tumors. Multivariate analysis showed that administration of 5-FU was an independent variable predicting prognosis of patients with low DPD-expressing lung ADs. Based on these results, determination of *DPD* mRNA levels in lung AD tumors may provide important information for clinicians to decide whether or not to proceed with 5-FU-based chemotherapy for their patients.

Based on our findings for biomarkers associated with 5-FU therapy, it is considered important to evaluate the expressions of TYMS and DPD before establishing a protocol for made-to-order chemotherapy for NSCLC patients (14). In addition, investigation of the effects of more aggressive adjuvant therapy for patients with NSCLC who have elevated *TYMS* or *DPD* mRNA levels is also necessary. Takizawa *et al*. reported that *in vitro* sensitivity to platinum-derived drugs, such as cisplatin and carboplatin, was associated with the expression of *TYMS* and *DPD* in NSCLC specimens (15). They hypothesized that these may be novel markers of DNA repair capacity and may also be linked with chemosensitivity to drugs other than 5-FU. Furthermore, it is important to clarify the roles of TYMS and DPD in regards to chemosensitivity toward various chemotherapy regimens, as their inhibition is now receiving attention for new cancer treatment drugs development. Recently, S-1, a combination of tegafur, gimeracil, and oteracil potassium (Taiho Pharmaceutical), was developed for clinical use (4). Gimeracil is a stronger inhibitor of DPD than uracil when used with UFT. However, Takeda *et al*. reported that a high level of DPD expression predicted resistance to S-1-based chemotherapy in patients with advanced NSCLC (16). Therefore, additional investigations of the effects of new

Table III.

A. Univariate analysis of overall survival in all patients.

Factors	Hazard ratio	95% CI	*p*-Value
Tumor status			
pT3 *vs*. pT1	3.71	1.05-13.2	0.042
pT2 *vs*. pT1	1.91	0.93-3.90	0.079
Nodal status			
pN2 *vs*. pN0	3.33	1.54-7.21	0.002
pN1 *vs*. pN0	1.73	0.67-4.45	0.259
TYMS mRNA			
High *vs*. low	4.17	1.81-9.03	0.001
DPD mRNA			
High *vs*. low	1.09	0.55-2.520	0.804
Administration			
5-FU *vs*. none	1.46	0.68-3.15	0.337

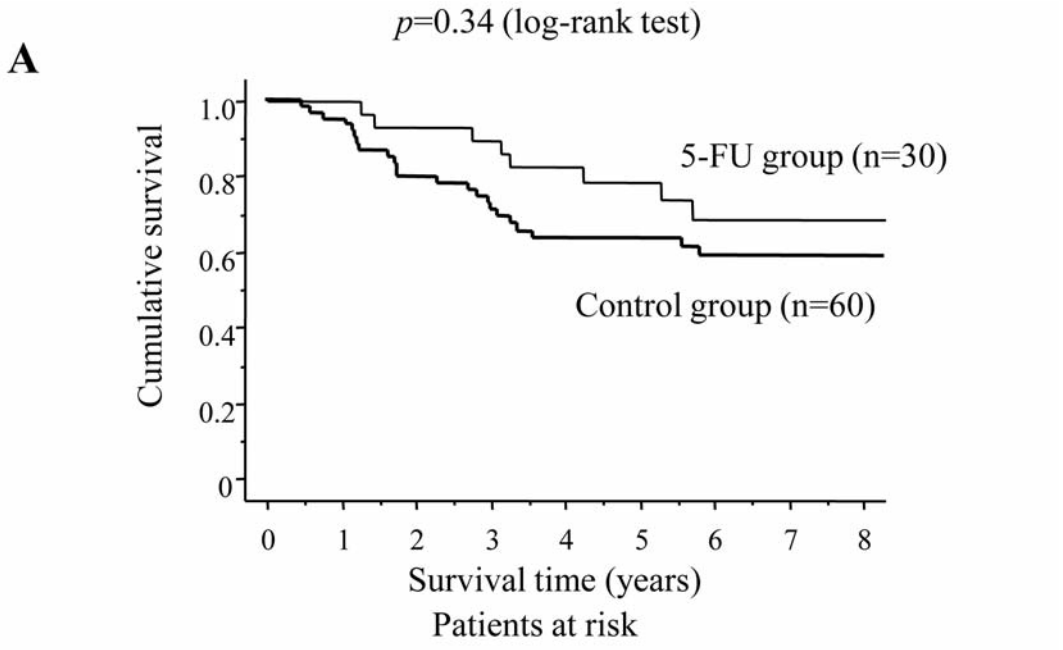
B. Multivariate analysis of overall survival in all patients.

Factor	Hazard ratio	95% CI	*p*-Value
Tumor status			
pT3 *vs*. pT1	2.51	0.69-9.12	0.161
pT2 *vs*. pT1	1.27	0.59-2.75	0.546
Nodal status			
pN2 *vs*. pN0	2.56	1.16-5.66	0.020
pN1 *vs*. pN0	1.41	0.51-3.87	0.511
TYMS mRNA			
High *vs*. low	3.42	1.46-8.02	0.005

C. Multivariate analysis of overall survival in patients with low DPD-expressing tumors.

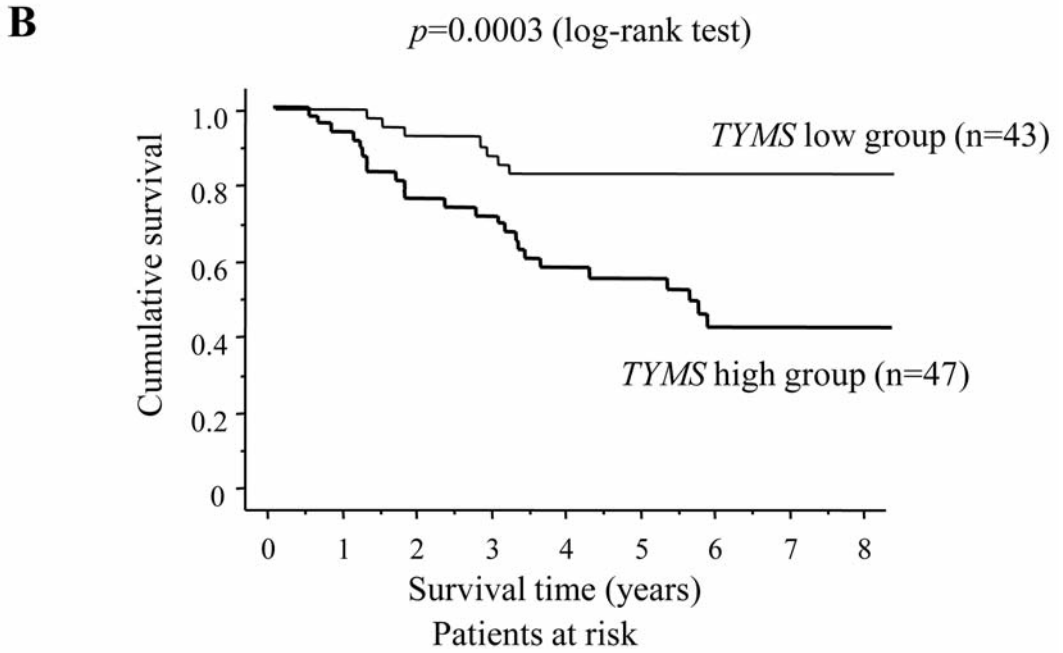
Factor	Hazard ratio	95% CI	*p*-Value
Nodal status			
pN2 *vs*. pN0	1.42	0.42-4.76	0.570
pN1 *vs*. pN0	0.85	0.22-3.27	0.816
TYMS mRNA			
High *vs*. low	5.31	1.17-24.0	0.030
Administration			
5-FU *vs*. none	7.60	1.02-56.7	0.050

CI, Confidence interval. TYMS, Thymidylate synthase. DPD, Dihydropyrimidine dehydrogenase. 5-FU, 5-fluorouracil.



Patients at risk

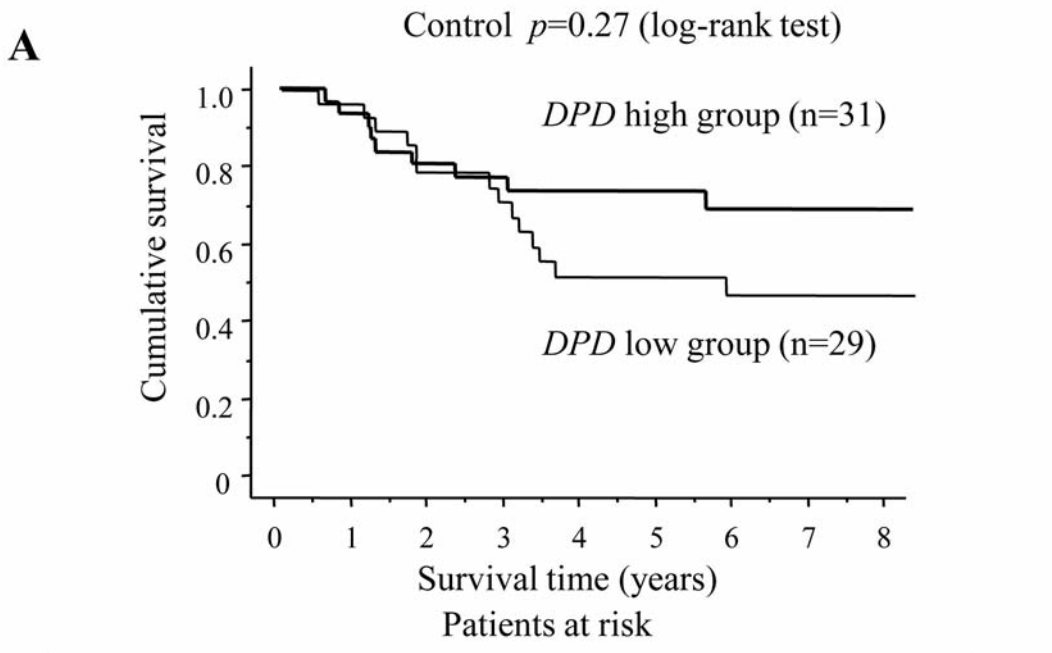
Control	60	47	32	24	12
5-FU	30	27	22	12	6



Patients at risk

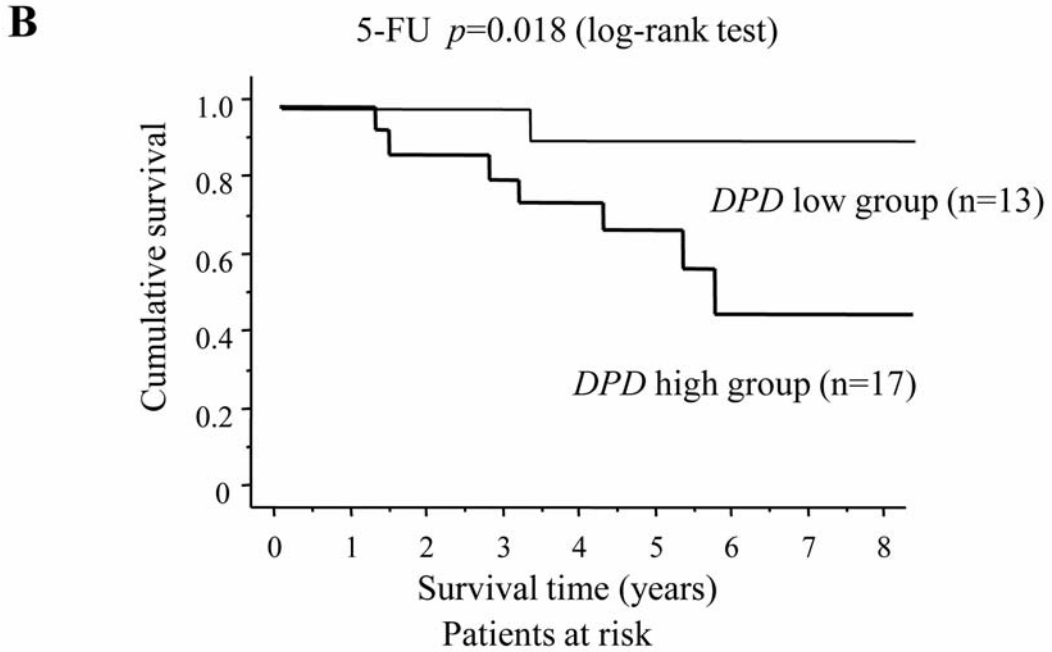
TYMS high	47	35	23	13	8
TYMS low	43	39	31	23	10

Figure 1. *A: Overall survival curves for patients administered and those not administered 5-fluorouracil (5-FU) after surgery. B: Overall survival curves for patients with high and low thymidylate synthase (TYMS) mRNA levels in resected cancer tissues when dichotomized at the mean TYMS mRNA level.*



Patients at risk

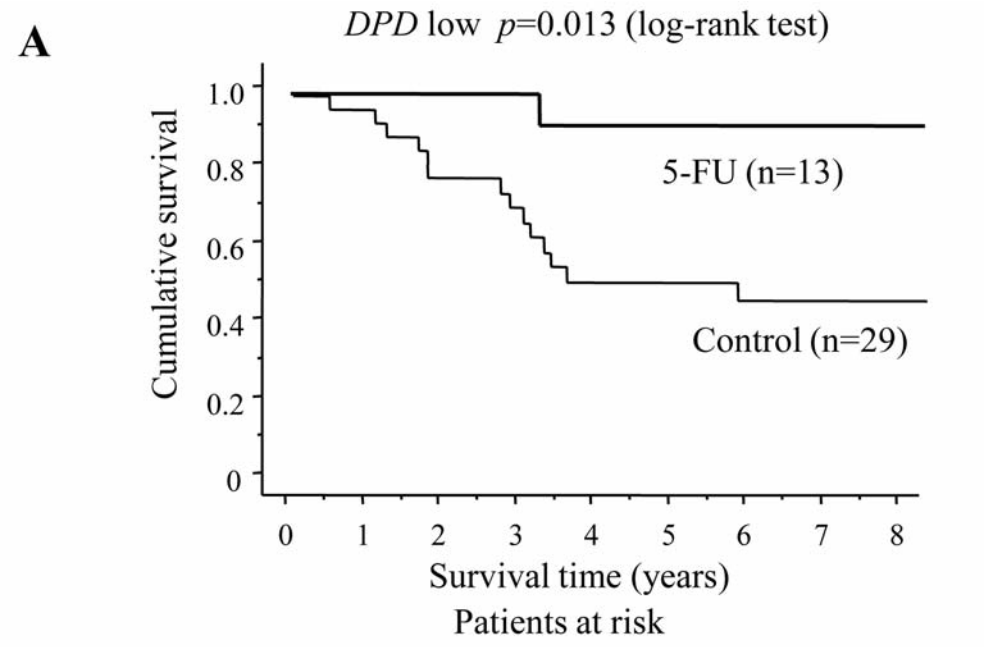
DPD high	31	25	19	14	7
DPD low	29	22	13	10	5



Patients at risk

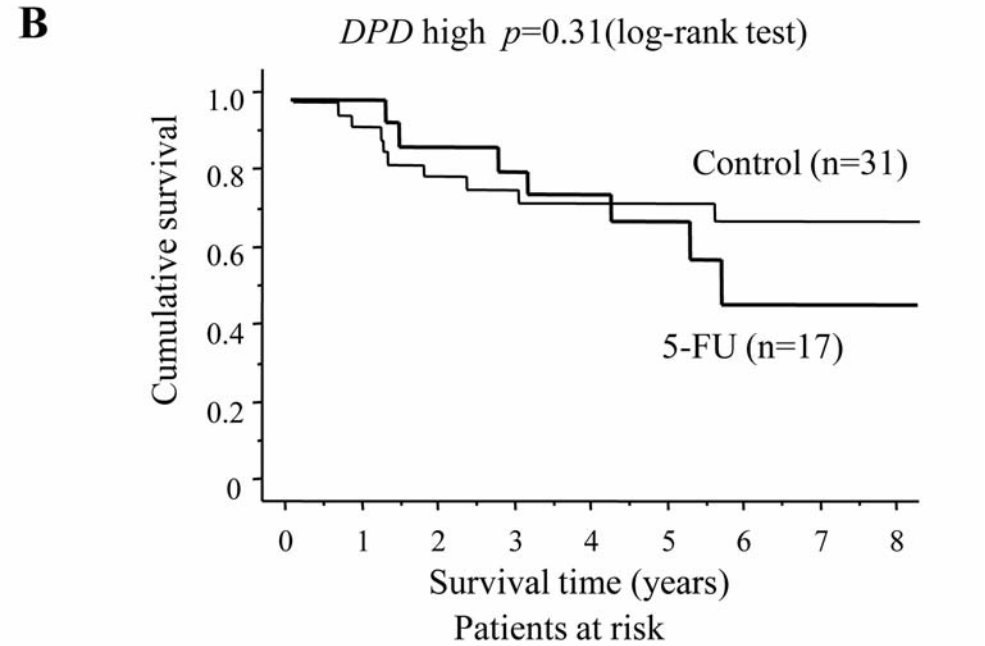
DPD high	17	14	11	3	2
DPD low	13	13	11	9	4

Figure 2. *A: Overall survival curves for patients with low and high dihydropyrimidine dehydrogenase (DPD)-expressing tumors who did not receive 5-fluorouracil (5-FU) when dichotomized at the mean DPD mRNA level. B: Overall survival curves for patients with low and high DPD-expressing tumors who received 5-FU.*



Patients at risk

Control	29	22	13	10	5
5-FU	13	13	11	9	4



Patients at risk

Control	31	25	19	14	7
5-FU	17	14	11	3	2

Figure 3. *A: Overall survival curves for patients with dihydropyrimidine dehydrogenase (DPD)-expressing tumors: comparison between those who underwent surgery alone and those who received 5-fluorouracil (5-FU) when dichotomized at the mean TYMS mRNA level. B: Overall survival curves for patients with high DPD-expressing tumors: comparison between those who underwent surgery alone and those who received 5-FU.*

regimens with other anticancer drugs and molecular targeting therapies for NSCLC patients with high DPD-expressing tumors are necessary.

In conclusion, using real-time RT-PCR, assessment of *TYMS* and *DPD* expressions in tumors from patients with NSCLC can provide precise prognostic information and predict the efficacy of 5-FU therapy after resection.

Conflicts of Interest Statement

The Authors have no conflicts of interest to declare.

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