Thymidine phosphorylase levels and dihydropyrimidine dehydrogenase levels in non-small cell lung cancer tissues

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Abstract. Thymidine phosphorylase (TP) and dihydropyrimidine dehydrogenase (DPD) are the major catabolic enzymes of 5-FU. In this study, we analyzed the concentration of TP and DPD in non-small cell lung cancer tissue by enzyme-linked immunosorbent assay. We measured the TP and DPD levels in 25 adenocarcinoma tissues and 25 squamous cell carcinoma tissues. The mean TP concentration in non-small cell lung cancer tissue was statistically higher than that of normal lung tissue as was the mean DPD concentration. The ratio of the TP level to DPD level in tumor tissue was higher in squamous cell carcinoma than in adenocarcinoma. No significant difference could be detected between the TP level, DPD level, or TP/DPD level and the tumor size or lymph node metastasis. In conclusion, chemotherapy with 5-FU may be more effective in squamous cell lung cancer patients than lung adenocarcinoma patients from the result of the ratio of TP to DPD.

Introduction

Pyrimidine nucleoside phosphorylase (PyNPase), an enzyme that converts 5'-deoxy-5-fluorouridine (5'-DFUR) to 5-fluorouracil (5-FU), has been shown to be identical to a potent angiogenic factor, platelet-derived endothelial cell growth factor (PD-ECGF), which is demonstrated to influence angiogenesis in tumor tissue (1). There are two kinds of PyNPase, thymidine phosphorylase (TP) and uridine phosphorylase, and TP is predominant in humans. On the other hand, dihydropyrimidine dehydrogenase (DPD) is the first and rate-limiting enzyme of the 5-FU catalytic pathway. The effect of 5'-DFUR is thought to be regulated by the balance of TP and DPD, the major catabolic enzyme of 5-FU. We consider that the anticancer effect of 5-FU correlates to the TP level, DPD level, and the ratio of TP to DPD in tumor tissue. There are a few reports about DPD activity in non-small cell lung cancer (2-4).

In this study, we analyzed the concentration of TP and DPD in fresh-frozen non-small cell lung cancer tissues by enzyme-linked immunosorbent assay (ELISA).

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Materials and methods

Tissue samples. We obtained tissue samples from 50 patients with primary non-small cell lung cancer, who underwent lung resection at the Department of Oncological Science, Oita University Faculty of Medicine, between September 1998 and January 2000. The patients consisted of 41 men and 9 women, with a mean age of 70 years, ranging from 45 to 85 years. The tumors were histologically classified as adenocarcinoma in 25 patients and squamous cell carcinoma in 25. The histological diagnosis and postsurgical pathological stage were established according to the TNM (tumor, nodes, metastases) classification of the International Union Against Cancer (UICC) using hematoxylin and eosin-stained formalin-fixed tissue sections. The histological stages of the 50 patients were stage IA (n=16), stage IB (n=11), stage IIA (n=0), stage IIB (n=8), stage IIIA (n=11), stage IIIB (n=3), stage IV (n=1). Prior to surgery, none of the patients had received any treatment for lung cancer. We obtained informed consent for the use of tissue samples from each patient.

Preparation of samples. After the surgical lung resection, we obtained small specimens (~2 mm in diameter) of both tumor tissue and normal lung tissue from the resected lung. The specimen was immediately frozen in liquid nitrogen and then stored at -80˚C until analysis.

Enzyme immunoassay for TP and DPD. The concentrations of TP and DPD in both tumor tissue and normal tissue were quantified in collaboration with Chugai Pharmaceutical Co. Ltd. Reseach Center (Kanagawa, Japan) using sandwich ELISA. In brief, the microplates were coated with 10 μg/ml of either anti-human TP monoclonal antibody 104B or anti-DPD monoclonal antibody 4B9. Each sample was homogenized in a 10-fold volume of buffer, centrifuged at 10,000 x g for 15 min and the supernatant was dispensed on to a plate and incubated at 37˚C for 1.5 h. After washing, the plates were incubated at 37˚C for 1 h with 1 μg/ml of secondary antibodies, either antiTP monoclonal antibody 232-2 or anti-DPD monoclonal antibody 3A5. The plates were further

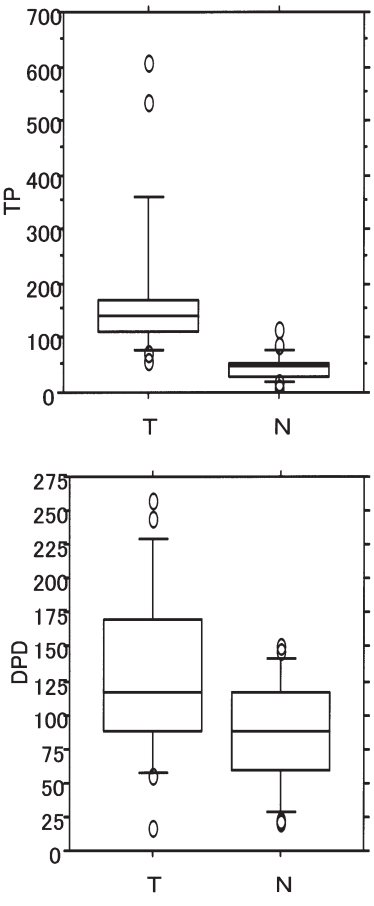

Figure 1. TP and DPD levels in squamous cell carcinoma tissue and normal lung tissue. Both the mean TP and DPD concentrations in squamous cell carcinoma tissue are statistically higher than in normal lung tissue. T, squamous cell carcinoma tissue; N, normal lung tissue.

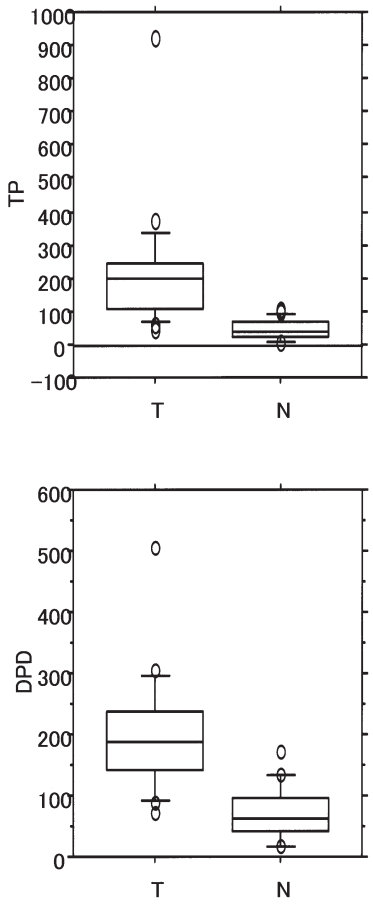

Figure 2. TP and DPD levels in lung adenocarcinoma tissues and normal lung tissues. Both the mean TP and DPD concentrations in lung adenocarcinoma tissue are statistically higher than in normal lung tissue. T, adenocarcinoma tissues; N, normal lung tissues.

reacted with peroxidase-conjugated anti-mouse IgG antiserum and substrate solution. Each concentration obtained from ELISA was adjusted based on the whole protein concentration and was expressed as U/mg protein.

Statistical analysis. We expressed the values of TP and DPD as the mean ± standard deviation. They were compared between the two groups with Student's t-test. A P-value <0.05 was considered significant.

Results

The mean TP concentration was 175±134 U/mg protein in squamous cell carcinoma tissue and 47±23 U/mg protein in normal lung tissue, which was significant (P<0.0001). The mean DPD concentration was 133±63 U/mg protein in squamous cell carcinoma tissue and 84±39 U/mg protein in normal tissue, which was significant (P=0.0017) (Fig. 1).

The mean TP concentration was 213±171 U/mg protein in adenocarcinoma tissue and 47±6 U/mg protein in normal lung tissue, which was significant (P<0.0001). The mean DPD concentration was 198±89 U/mg protein in adenocarcinoma tissue and 69±42 U/mg protein in normal tissue, which was significant (P<0.0001) (Fig. 2).

The ratio of the TP level to DPD level in tumor tissue was higher in squamous cell carcinoma than in adenocarcinoma (not significant) (Fig. 3). No significant difference was detected between the TP level, DPD level, or TP/DPD level and the tumor size or lymph node metastasis (data not shown).

Discussion

The histological type of cancer and patient characteristics influence chemosensitivity to anticancer drugs in cancer treatment. It is problematic to the cancer treatment and quality of life when a patient experiences low sensitivity to an anticancer drug. It is important to select a good response patient to enhance the beneficial effect and to avoid unnecessary treatment in cancer chemotherapy. 5-FU-based oral chemotherapy has been used frequently in a variety of cancer patients. 5'-DFUR is converted to 5-FU by TP and is effective in cancer, so it is more effective in high TP tumor tissue, and depressing the TP level with 5'-DFUR suppresses tumor growth and metastasis (5). DPD, the 5-FU catalytic enzyme present in normal tissue and tumor tissue, is noted to predict the beneficial and adverse effects of 5-FU.

The TP and DPD levels of tumor tissues from patients with various types of cancer have been reported (6-12), but there are a few reports about TP and DPD activity in non-small cell lung cancer (2-4).

In this study, we analyzed the concentrations of TP and DPD and the ratio of TP to DPD in tumor tissue by ELISA in

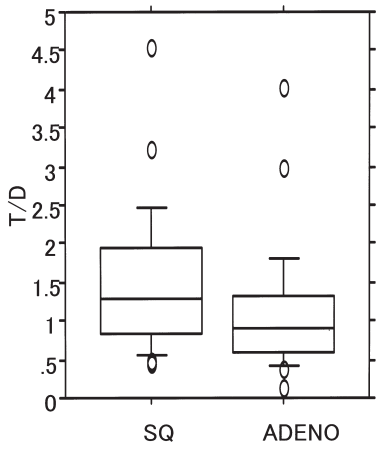

Figure 3. TP level/DPD level ratios in tumor tissue. The ratio of the TP level to the DPD level in tumor tissue is higher in squamous cell lung carcinoma than in lung adenocarcinoma (not significant). T/D, the ratios of TP to DPD; SQ, squamous cell carcinoma tissue; ADENO, adenocarcinoma tissue.

non-small cell lung cancer, as one investigation tool of chemotherapy for lung cancer, which is the biggest cause of cancer mortality. It is known that the protein level of TP and DPD measured by ELISA correlates well with the activity of these enzymes (7,13): the TP level measured in this study indicates TP activity which converts 5'-DFUR to 5-FU in tumor tissue, and the DPD level indicates DPD activity which inactivates 5-FU in tumor tissue. Ishikawa and coworkers reported that the ratio of TP to DPD was useful as an index of the effect of 5'-DFUR (14). Therefore, we measured TP and DPD levels and the ratio of TP to DPD in this study.

In our results, both TP and DPD levels were significantly higher in lung squamous cell carcinoma tissue and lung adenocarcinoma tissue than in normal tissue. The ratio of the TP level to the DPD level in tumor tissue was higher in squamous cell carcinoma than in adenocarcinoma, because there was no difference in the TP level in tumor tissue and normal tissue in squamous cell carcinoma and adenocarcinoma but the DPD level between tumor tissue and normal tissue was significantly higher in adenocarcinoma than in squamous cell carcinoma. This result is similar to Yano and cowerkers' data (2). No significant difference was detected between the TP level, DPD level, or TP/DPD level and the tumor size or lymph node metastasis. Katsumata and coworkers reported that TP expression correlated with the cancer stage (15) but in this study there was no correlation between the TP or DPD level and the tumor size or lymph node metastasis. Kato and coworkers reported that chemotherapy with uracil-tegafur improved survival among stage I lung cancer-resected patients (16). Even in pathological stage I non-small cell lung cancer, some patients die from cancer and some patients are improved by 5-FU-based chemotherapy. It is important to select good response patients and to avoid unnecessary treatment in cancer chemotherapy. It was made clear in this study that TP and DPD levels were different between squamous cell carcinoma and adenocarcinoma but were not different between small and large tumors or no lymph node and lymph node metastasis tumors. From this result, it is expected that chemotherapy with 5-FU will be more effective in squamous cell lung cancer than in lung adenocarcinoma. The correlation of DPD levels with survival in 5-FU-based chemotherapy has been reported (3,4,17). We consider that it is necessary to examine not only DPD levels but also TP levels because we think that they both influence the effect of chemotherapy, independent of the cancer stage. Kato and coworkers reported the effectiveness of 5-FU-based chemotherapy in lung adenocarcinoma patients (16), but we think that its efficacy should also be examined for squamous cell lung carcinoma.

Comparing our results with Mori and coworkers' data (7), measured in the same manner by ELISA, the ratio of TP to DPD in primary lung cancer is higher than that in prostate or bladder cancer, lower than that in esophageal, renal, colorectal, or breast cancer, and the same as that in gastric, pancreatic, cervical, or hepatic cancer. The TP level, DPD level, and the ratio of TP to DPD may be useful parameters for predicting the efficacy of chemotherapy with 5-FU. The efficacy of 5-FU-based chemotherapy is expected to be the same in lung cancer as in gastric, pancreatic, cervical, or hepatic cancer.

We conclude that chemotherapy with 5-FU is more effective in squamous cell lung cancer patients than lung adenocarcinoma patients from the results of the ratio of TP to DPD. Further prospective, randomized, controlled clinical trials are needed to confirm this result.

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