

Regulation of Dihydropyrimidine Dehydrogenase and Pyrimidine Nucleoside Phosphorylase Activities by Growth Factors and Subsequent Effects on 5-Fluorouracil Sensitivity in Tumor Cells

Masatsugu Ueda,^{1,3} Kozo Kitaura,² Osamu Kusada,² Yoshino Mochizuki,² Naomi Yamada,² Yoshito Terai,¹ Koji Kumagai,¹ Ken Ueki¹ and Minoru Ueki¹

¹Department of Obstetrics and Gynecology, Osaka Medical College, 2-7 Daigakumachi, Takatsuki, Osaka 569-8686 and ²Analytical Research Center, Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8731

Dihydropyrimidine dehydrogenase (DPD) and pyrimidine nucleoside phosphorylase (PyNPase) are the first and rate-limiting enzymes that regulate 5-fluorouracil (5-FU) metabolism, and tumoral DPD activity appears to be a promising predictor of 5-FU sensitivity. However, the regulatory mechanisms determining these enzyme activities have not been fully understood. We investigated the biological effects of epidermal growth factor (EGF) and transforming growth factor (TGF)- α on cell growth and tumoral DPD and PyNPase activities, and the subsequent effects on 5-FU sensitivity in uterine cervical carcinoma SKG-IIIb cells. The treatment of tumor cells with EGF or TGF- α resulted in a concentration-dependent increase in tumor cell growth and PyNPase activity, whereas tumoral DPD activity was inhibited. Their stimulatory effects on tumor cell growth correlated well with PyNPase activity, but were inversely related to DPD activity ($P < 0.01$). 5-FU sensitivity of tumor cells increased in the presence of EGF or TGF- α . These growth factors were shown to stimulate the first, rate-limiting enzyme activity in 5-FU anabolism and to inhibit that in 5-FU catabolism, leading to enhancement of the antiproliferative action of 5-FU at achievable therapeutic levels. The tumor environmental factors, EGF and TGF- α , may act as intrinsic regulators of DPD and PyNPase activities that affect the 5-FU sensitivity of individual tumors.

Key words: Dihydropyrimidine dehydrogenase — Pyrimidine nucleoside phosphorylase — Growth factor — 5-Fluorouracil sensitivity — Cervical cancer

5-Fluorouracil (5-FU) has been widely used in the treatment of a variety of neoplastic diseases, particularly cancers of the breast and digestive organs, and is given either alone or in combination with other cytostatics. We have demonstrated that 5-FU-based chemotherapy is also useful for the treatment of uterine cervical cancer.^{1,2)} Two main modes of action have been proposed for 5-FU through its active metabolites, 5-fluoro-dUMP (FdUMP) and 5-fluoro-UTP. FdUMP suppresses thymidylate synthetase (TS) by forming a covalent ternary complex with 5,10-methylenetetrahydrofolate, which inhibits DNA synthesis.³⁾ 5-Fluoro-UTP is incorporated into cellular RNA, resulting in RNA dysfunction.⁴⁾ 5-FU is initially anabolized by pyrimidine nucleoside phosphorylase (PyNPase) in both pathways. Thymidine phosphorylase (ThdPase) converts 5-FU to 5-fluorodeoxyuridine (FdUR), a precursor of FdUMP. Uridine phosphorylase (UrdPase) converts 5-FU to 5-fluorouridine (FUR), a precursor of 5-fluoro-UMP, which is finally metabolized to 5-fluoro-UTP. The former enzyme is identical to platelet-derived endothelial cell growth fac-

tor (PD-ECGF)⁵⁾ and is closely associated with tumor angiogenesis.^{5,6)} 5-FU is initially catabolized to 5-fluorodihydrouracil (DHFU) by dihydropyrimidine dehydrogenase (DPD), mainly in the liver, then dihydropyrimidinase and β -ureido-propionase catalyze the formation of 2-fluoro- β -alanine. Several recent studies^{7–10)} concerned with 5-FU antitumor effects have demonstrated that tumoral DPD activity may influence 5-FU sensitivity. Thus, DPD and PyNPase are considered to be the first and rate-limiting enzymes in the chain of reactions that regulate 5-FU metabolism.

Determination of tumoral DPD has become of clinical interest because elevated intratumoral DPD can influence the tumor response to 5-FU therapy as a result of increased inactivation. Etienne *et al.*⁸⁾ evaluated DPD activity in tumor biopsy specimens from head and neck cancer patients before administration of 5-FU and found that the tumoral/non-tumoral DPD activity ratio was higher in the non-responding patients than in those with a partial or complete response. Moreover, certain biochemical modulations to enhance the antitumor activity of 5-FU by inhibiting intratumoral DPD activity have been attempted.^{11,12)} The underlying differences in tumoral DPD activity result in a variable 5-FU degradation prior to 5-FU

³To whom correspondence and reprint requests should be addressed.

E-mail: gyn017@poh.osaka-med.ac.jp

engagement in the anabolic pathway and therefore affect the 5-FU sensitivity of the tumor. However, it is still unclear why DPD activity varies within an individual tumor and what factors intrinsically regulate its intratumoral activity.

Inflammatory cytokines, tumor necrosis factor α (TNF α), interleukin-1 α (IL-1 α) and interferon γ (INF γ), which are often detected in the tumor environment,¹³ are reported to upregulate PyNPase expression in human and mouse tumor cells and to increase the sensitivity of tumor cells to 5'-deoxy-5-fluoro-uridine (5'-dFUrd), which is biotransformed into 5-FU by PyNPase.^{14, 15} Eda *et al.*¹⁴ indicated that growth factors, such as PD-ECGF and basic fibroblast growth factor, might also be involved in the biochemical modulation of the anti-proliferative activity of 5-FU and 5'-dFUrd. Ishikawa *et al.*¹⁵ demonstrated that some cytokines simultaneously upregulate the potential of Lewis lung carcinoma cells to invade extracellular matrices and their PyNPase activity, modulating the specific action of 5'-dFUrd to selectively kill tumor cells with high invasive and metastatic potential. We have also reported that epidermal growth factor (EGF) and transforming growth factor- α (TGF- α), which are secreted by tumor cells and/or surrounding interstitial cells, stimulate invasive activity and proteinase expression of gynecological tumor cells.^{16, 17} These reports led us to examine whether tumor environmental factors affect the rate-limiting enzyme activity in 5-FU metabolism, which may regulate the antiproliferative action of 5-FU in an individual tumor. In the present study, we investigated the biological effects of EGF and TGF- α on cell growth and tumoral DPD and PyNPase activities, and the effects on 5-FU sensitivity of tumor cells.

MATERIALS AND METHODS

Cell culture SKG-IIIb,¹⁸ originating from a moderately differentiated epidermoid carcinoma of the uterine cervix, was kindly provided by Dr. S. Nozawa (Keio University School of Medicine, Tokyo). SKG-IIIb cells possess EGF receptors with a dissociation constant of 8.3 nM, and the total binding sites amount to 164 fmol/mg protein, as previously described.¹⁶ The cells were maintained as monolayer cultures in Ham's F-12 nutrient mixture (Gibco, Paisley, UK) supplemented with heat-inactivated 10% fetal bovine serum (Gibco) at 37°C in a humidified incubator with 5% CO₂. They were grown to confluence in 25-cm² tissue-culture flasks (Nunc, Roskilde, Denmark), washed with phosphate-buffered saline (PBS), and then harvested after a brief treatment with 0.1% trypsin solution containing 0.02% EDTA (Gibco). The cell viability was determined by Trypan-blue-dye exclusion prior to use. **Chemical reagents** EGF and TGF- α were purchased from Wakunaga Seiyaku Co., Ltd. (Osaka). 3-(4,5-Dimeth-

ylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma Chemical Co. (St. Louis, MO). 5-FU was prepared at Kyowa Hakko Kogyo Co., Ltd. (Tokyo). 5-[6-³H]FU was obtained from Moravек Biochemicals (Brea, CA). All other chemicals used were of the highest purity commercially available.

MTT assay Effects of EGF and TGF- α on the proliferation of SKG-IIIb cells were examined by means of MTT assay with some modifications, as previously described.¹⁹ Cells (5×10^3) in a volume of 100 μ l of growth medium per well were uniformly seeded into 96-well microplates (Corning, New York, NY) and incubated at 37°C for 24 h. The medium was then replaced with 100 μ l of serum-free medium containing various amounts of growth factors. After further incubation at 37°C for 24, 48, and 96 h, 10 μ l of MTT dissolved in PBS at a concentration of 5 mg/ml was added to each well, and the plates were incubated at 37°C for 4 h. The medium was removed, 150 μ l of dimethyl sulfoxide was added to each well, and the plates were agitated for 5 min. The absorbance was then read at 570 nm in a scanning spectrophotometer (Molecular Devices Co., Sunnyvale, CA). In a parallel experiment, the effects of EGF and TGF- α on the antiproliferative action of 5-FU on SKG-IIIb cells were examined. We previously observed that 0.1 to 100 μ g/ml of 5-FU with EGF or TGF- α had cytotoxic effects on SKG-IIIb cells.²⁰ Therefore, for this further investigation, cells were exposed to serum-free medium containing lower concentrations of 5-FU (0.001 to 0.1 μ g/ml) 24 h after plating to confirm the biological effects of these growth factors at achievable therapeutic levels of 5-FU. The cells were cultured at 37°C for 7 days in the presence or absence of growth factors, and cell viability was evaluated by MTT assay. Results are expressed as the relative percentage of absorbance compared to controls.

DPD assay DPD activity was measured according to the method described by Beck *et al.*⁷ with some modifications. Aliquots (6×10^6) of tumor cells in 60 ml of conditioned medium were seeded in a flask and incubated at 37°C for 24 h. The medium was then replaced with the same volume of serum-free medium containing various amounts of growth factors. After further incubation at 37°C with or without growth factors for 24, 48, and 96 h, the cells were harvested, washed with PBS, collected by centrifugation, and stored at -80°C until use. The cell pellet was freeze-thawed twice and centrifuged at 15 000 rpm for 30 min at 4°C. The supernatant was kept on ice until assayed. The assay consisted of incubating 500 μ l of supernatant with 100 μ l of 5-[6-³H]FU and 25 μ l of 6.25 mM NADPH. The duration of incubation was 15, 30, and 45 min at 37°C. The reaction was stopped by addition of 70 μ l of 5% HClO₄ solution to 70 μ l of each sample. The sample was then mixed with 140 μ l of 20 mM phosphate buffer (pH 3.5) and centrifuged at 15 000 rpm for 10 min

at 4°C. Forty microliters of the supernatant was analyzed for the presence of [³H]DHFU and [³H]2-fluoro-3-ureidopropionate ([³H]FUPA) using a high-performance liquid chromatography (HPLC) method. Detection was performed using a radioactive flow monitor (Laytest, Straubenhardt, Germany). DPD activity was calculated by taking into account the sum of DHFU and FUPA peaks, and was expressed as pmol of 5-[6-³H]FU catabolized per minute and per milligram of protein. The protein concentration was determined using the method of Lowry *et al.*²¹⁾

PyNPase assay PyNPase activity was assayed according to the method reported by Horiuchi *et al.*²²⁾ with some modifications. Twenty-four hours after seeding, cells were exposed to various amounts of growth factors for 24, 48, and 96 h. The cells were then harvested, washed with PBS, collected by centrifugation, and stored at -80°C as described above. The cell pellet was sonicated for 15 min on ice in 1 ml of 50 mM potassium phosphate buffer (pH 7.4) containing 12.5 mM 2-mercaptoethanol. This solution was then centrifuged at 105 000g for 60 min at 4°C. One milliliter of the supernatant was gradually mixed with 1.222 ml of saturated ammonium sulfate, kept on ice for 30 min, and centrifuged at 14 000g for 15 min at 4°C. The precipitate was then dissolved in 1 ml of 20 mM Tris-HCl buffer (pH 7.4) containing 5 mM 2-mercaptoethanol, dialyzed for 16 h at 6°C, and used as a source of enzyme assay. The reaction mixture for ThdPase assay contained 100 μl of 180 mM Tris-HCl buffer (pH 7.4), 50 μl of 30 mM 5-FU, 50 μl of 30 mM deoxyribose-1-phosphate, and 100 μl of the enzyme solution from the cells. The reaction was carried out at 37°C for 15 min and was then terminated by the addition of 70 μl of 3 M HClO₄. The reaction mixture for UrdPase assay contained 100 μl of 180 mM Tris-HCl buffer (pH 7.4), 50 μl of 6 mM 5-FU, 50 μl of 6 mM ribose-1-phosphate, and 100 μl of the enzyme solution from the cells. The reaction was done at 37°C for 45 min and was terminated as described above. The sample was then mixed with 50 μl of 0.22 mM 3-methylxanthine as the internal standard. After centrifugation at 15 000 rpm for 30 min at 4°C, 50 μl of the supernatant was added to an HPLC column (YMC-ODS-AM302), and the amount of FUDR or FUR produced was measured with a UV monitor at 269 nm.

Statistical analysis All experiments were performed in triplicate. The significance of differences between groups was calculated by applying a non-parametric test. The Spearman rank correlation coefficient was also used to analyze the relation between two different values. A level of $P < 0.05$ was accepted as statistically significant.

RESULTS

Effects of growth factors on tumor cell growth Fig. 1 shows the dose-response curves of SKG-IIIb cells 24, 48,

and 96 h after the addition of various concentrations of EGF or TGF-α. The growth of SKG-IIIb cells was stimulated by the presence of 0.1 to 100 ng/ml of EGF or of 1 to 100 ng/ml of TGF-α in a concentration-dependent manner ($P < 0.01$).

Effects of growth factors on DPD and PyNPase activities Fig. 2 illustrates the DPD and PyNPase activities of SKG-IIIb cells 24, 48, and 96 h after the addition of various concentrations of EGF or TGF-α. DPD and PyNPase activities are expressed as pmol catabolites formed per minute per milligram protein and nmol anabolites formed per minute per 10⁸ tumor cells, respectively. DPD activity of SKG-IIIb cells was inhibited by EGF or TGF-α in a concentration-dependent manner (Fig. 2, a and d). The 96-

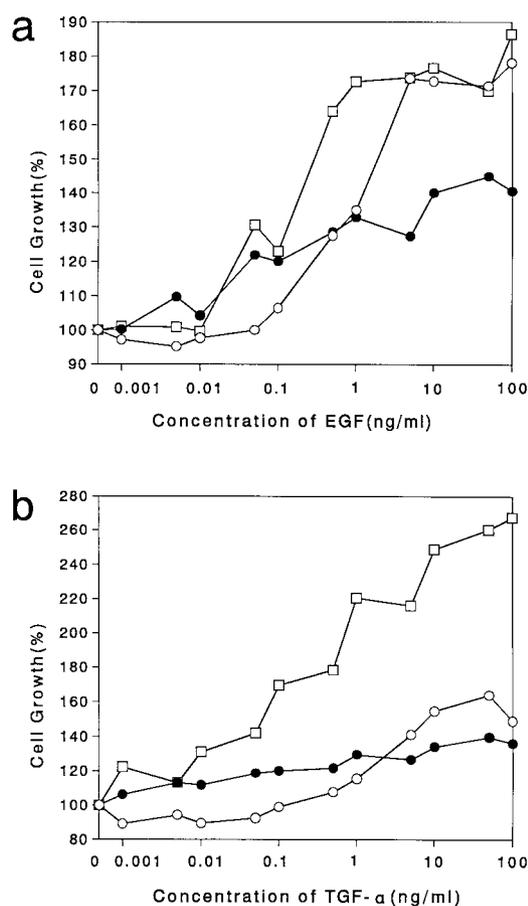


Fig. 1. The dose-response curves of SKG-IIIb cells 24 (●), 48 (□), and 96 (○) h after the addition of EGF (a) and TGF-α (b). Cells (5×10^3) were uniformly seeded into 96-well microplates and incubated for 24 h. The medium was then replaced with serum-free medium containing various amounts of growth factors. After further incubation for 24, 48, and 96 h, cell viability was evaluated by MTT assay. Each point represents the mean of triplicates.

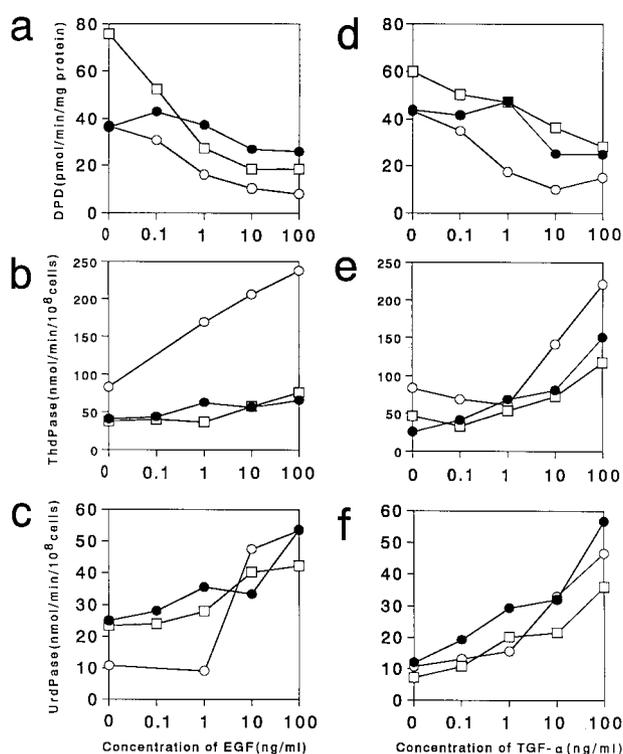


Fig. 2. DPD and PyNPase activities of SKG-IIIb cells 24 (●), 48 (□), and 96 (○) h after the addition of EGF (a, b and c) and TGF- α (d, e and f). Cells (6×10^6) were seeded in a flask and incubated for 24 h. The medium was then replaced with serum-free medium containing various amounts of growth factors. After further incubation for 24, 48, and 96 h, DPD and PyNPase activities of tumor cells were evaluated by an HPLC method. Each point represents the mean of triplicates.

h incubation of tumor cells with 0.1 to 100 ng/ml of either growth factor resulted in a significant decrease of their DPD activity ($P < 0.01$). In contrast, ThdPase and UrdPase activities of SKG-IIIb cells were stimulated by the presence of 10 to 100 ng/ml of EGF or TGF- α in a concentration-dependent manner ($P < 0.01$; Fig. 2, b, c, e and f).

Correlation between tumor cell growth, and DPD and PyNPase activities Fig. 3 shows SKG-IIIb cell growth and DPD, ThdPase and UrdPase activities 96 h after the addition of various concentrations of EGF or TGF- α . Regression analysis with the Spearman rank correlation coefficient on plots of tumor cell growth versus DPD activity showed a significant inverse correlation between them ($P < 0.01$; Fig. 3, a and e). On the other hand, the stimulatory effects of EGF or TGF- α on tumor cell growth correlated well with both ThdPase and UrdPase activities ($P < 0.01$): the greater the cell growth, the higher the ThdPase and UrdPase activities (Fig. 3, b, c, f and g). The correlation of DPD and ThdPase activities further suggested

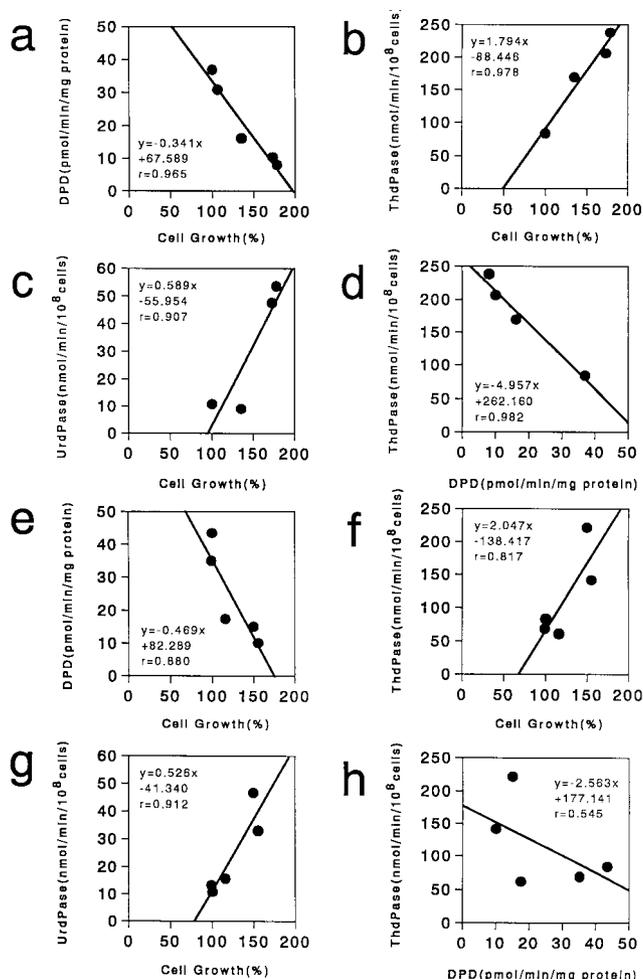


Fig. 3. Correlation between tumor cell growth, and DPD and PyNPase activities 96 h after the addition of EGF (a, b, c and d) and TGF- α (e, f, g and h). a and e, Correlation between tumor cell growth and DPD activity. b, c, f and g, Correlation between tumor cell growth and PyNPase activity. d and h, Correlation between DPD and ThdPase activities. Each point represents the mean of triplicates.

that there was an inverse relation ($P < 0.01$) between these two parameters after a 96-h treatment of tumor cells with EGF or TGF- α (Fig. 3, d and h).

Enhancement of antiproliferative action of 5-FU by growth factors Next, we examined whether 5-FU selectively kills SKG-IIIb cells exposed to EGF and TGF- α . Fig. 4 shows the dose-response curves of SKG-IIIb cells 7 days after the addition of 0.001 to 0.1 $\mu\text{g/ml}$ of 5-FU with growth factors. EGF and TGF- α enhanced the antiproliferative action of 0.001 to 0.01 and 0.001 to 0.05 $\mu\text{g/ml}$ of 5-FU on the cells, respectively ($P < 0.05$).

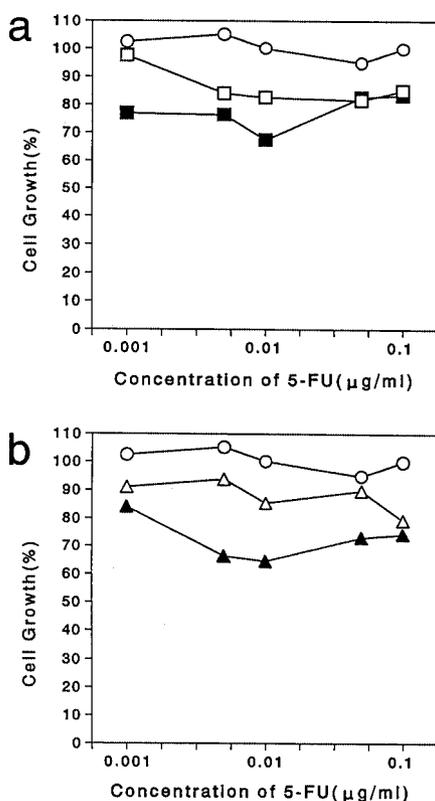


Fig. 4. The dose-response curves of SKG-IIIb cells 7 days after the addition of 5-FU with EGF (a) or TGF- α (b). Twenty-four hours after plating, cells were exposed to various concentrations of 5-FU. After further incubation for 7 days with growth factors, cell viability was evaluated by MTT assay. The relative percentages of cell growth compared to controls in the absence of EGF or TGF- α (○) and in the presence of 10 (□) and 100 (■) ng/ml of EGF or 10 (△) and 100 (▲) ng/ml of TGF- α were plotted. Each point represents the mean of triplicates.

DISCUSSION

The best understood aspect of the cytotoxic action of 5-FU is the FdUMP-mediated inhibition of TS, the final enzyme of the *de novo* pathway that converts 2'-deoxyuridylylate to thymidylate by reductive methylation.²³ Experimental studies performed on colorectal, breast, or head and neck cancer cells have shown that overexpression of TS protein or TS activity is associated with 5-FU resistance.^{7,24,25} However, Etienne *et al.*⁸ reported that there was no relation between tumoral TS activity and 5-FU responsiveness and that TS was not likely to be the main target for 5-FU in head and neck cancer. Although much attention has been paid to the 5-FU activation routes, increased expression of TS is not universally recognized as a determinant factor for 5-FU sensitivity.^{26,27} Moreover, there have been few reports on the role of PyN-

Pase, the first and rate-limiting enzyme in the 5-FU anabolic pathway.^{14,15,28} Since Naguib *et al.*²⁹ first reported that DPD activity was high in the human liver and variable in tumors, the role of 5-FU catabolism at the cellular target level has been of interest. Tumoral DPD activity appears to be a promising predictor of 5-FU sensitivity.⁷⁻¹⁰ The DPD activity in human tumors seems to be highly variable: it ranged from 13 to 193 pmol/min/mg protein in biopsy specimens of 63 head and neck tumors⁸ and from 28 to 207 pmol/min/mg protein in surgical specimens of 60 colorectal tumors.³⁰ However, data on tumoral DPD activity are limited, and the regulatory mechanisms determining DPD activity in human tumors are not fully understood.

Our present results demonstrate that tumoral DPD and PyNPase activities are regulated by growth factors and influence the 5-FU sensitivity of the tumor cells. The treatment of human uterine cervical carcinoma SKG-IIIb cells with EGF or TGF- α resulted in a concentration-dependent increase in tumor cell growth and PyNPase activity, whereas tumoral DPD activity was inhibited by the presence of these growth factors, again in a concentration-dependent manner. Stimulatory effects of EGF and TGF- α on tumor cell growth correlated well with PyNPase activity; however, there was an inverse relation between tumor cell growth and DPD activity. We observed that these growth factors stimulate the first, rate-limiting enzyme activity in 5-FU anabolism and inhibit that in 5-FU catabolism, leading to enhancement of the antiproliferative action of 5-FU at achievable therapeutic levels. EGF and TGF- α , tumor environmental factors, may act as potential intrinsic regulators of DPD and PyNPase activities that affect 5-FU sensitivity in individual tumors.

Previous researchers have hypothesized that DPD activity may be controlled by mechanisms related to tumor growth.³¹⁻³³ Queener *et al.*³¹ demonstrated that DPD activity in rat hepatomas decreased in parallel with the increase in tumor growth rate. In contrast, Williams and Tuchman³² reported that DPD activity in mouse neuroblastoma cells increased during culture, whereas thymidine kinase activity decreased markedly. Takechi *et al.*³³ observed time-dependent increases in DPD activity during the growth of human fibrosarcoma HT-1080 cells and human pancreatic carcinoma MIAPaCa-2 cells in cultures. The inverse relation between tumor cell growth and DPD activity in our study is consistent with the change of DPD activity in rat hepatomas during tumor growth. Generally, proliferating cells are much more sensitive to anticancer agents than nonproliferating cells are. In the case of 5-FU treatment, the antitumor activity of 5-FU has been found to be higher in growing small tumors than in stable large tumors in murine colon carcinomas.³⁴ The present results may provide a partial explanation: decreased DPD and increased PyNPase activities during tumor cell growth

may allow proliferating tumor cells to be more sensitive to 5-FU because of decreased degradation and increased activation of 5-FU.

Inflammatory cytokines, such as TNF α , IL-1 α , and INF γ , that upregulate the expression of type-IV collagenase and PyNPase^{14, 15)} have been detected in various human and mouse tumor tissues.¹³⁾ Some of them are reported to enhance the metastatic potential of tumor cells in either *in vitro* or *in vivo* tumor models.^{15, 35, 36)} In addition, factors that downregulate cytokine expression, such as indomethacin, suppress metastasis.³⁷⁾ Therefore, these tumor environmental factors would enhance the metastatic ability of tumor cells by upregulating type-IV collagenase activity, and 5-FU as well as 5'-dFUr might inhibit metastasis by selectively killing such tumor cells due to the high PyNPase activity. We previously demonstrated that EGF and TGF- α also upregulate invasive activity and proteinase expression of cervical, endometrial and ovarian carcinoma cells.^{16, 17)} Our present results have revealed that these growth factors suppress tumoral DPD activity, thereby making tumor cells more sensitive to 5-FU. More-

over, it has been reported that INF α can induce a marked increase in PyNPase and a decrease in DPD activity.^{28, 38)} Fluoro-pyrimidines may be effective against tumor cells with high invasive and metastatic potential because of low DPD and high PyNPase activities under the influence of various tumor environmental factors that upregulate the invasive phenotype of the cells. The relation between the expression of cytokines and growth factors in tumor tissues and the anti-metastatic action of 5-FU and 5'-dFUr should be further elucidated in *in vivo* tumor models.

ACKNOWLEDGMENTS

We are grateful to Dr. S. Nozawa, Department of Obstetrics and Gynecology, Keio University School of Medicine, for the gift of the SKG-IIIb cell line. We also thank Mr. M. Sato, Medical Planning Section, Kyowa Hakkō Kogyō Co., Ltd., for his interest and advice.

(Received May 29, 2000/Revised July 25, 2000/Accepted August 8, 2000)

REFERENCES

- 1) Ueki, M., Okamura, S. and Maeda, T. Individualization of patients for adjuvant chemotherapy after surgical treatment of cervical cancer. *Br. J. Obstet. Gynaecol.*, **94**, 985–990 (1987).
- 2) Ueda, M., Kumagai, K., Ueki, K., Inoki, C., Orino, I. and Ueki, M. Growth inhibition and apoptotic cell death in uterine cervical carcinoma cells induced by 5-fluorouracil. *Int. J. Cancer*, **71**, 668–674 (1997).
- 3) Langenback, R. J., Danenberg, P. V. and Heidelberger, C. Thymidylate synthetase: mechanism of inhibition by 5-fluoro-2'-deoxyuridylate. *Biochem. Biophys. Res. Commun.*, **48**, 1565–1571 (1972).
- 4) Matsuoka, H., Ueo, H., Sugimachi, K. and Akiyoshi, T. Preliminary evidence that incorporation of 5-fluorouracil into RNA correlates with antitumor response. *Cancer Invest.*, **10**, 265–269 (1992).
- 5) Furukawa, T., Yoshimura, A., Sumizawa, T., Haraguchi, M., Akiyama, S., Fukui, K., Ishizawa, M. and Yamada, Y. Angiogenic factor. *Nature*, **356**, 668 (1992).
- 6) Ueda, M., Ueki, K., Kumagai, K., Terai, Y., Okamoto, Y., Ueki, M. and Otsuki, Y. Apoptosis and tumor angiogenesis in cervical cancer after preoperative chemotherapy. *Cancer Res.*, **58**, 2343–2346 (1998).
- 7) Beck, A., Etienne, M. C., Cheradame, S., Fischel, J. L., Formento, P., Renee, N. and Milano, G. A role for dihydropyrimidine dehydrogenase and thymidylate synthase in tumour sensitivity to fluorouracil. *Eur. J. Cancer*, **30A**, 1517–1522 (1994).
- 8) Etienne, M. C., Cheradame, S., Fischel, J. L., Formento, P., Dassonville, O., Renee, N., Schneider, M., Thyss, A., Demard, F. and Milano, G. Response to fluorouracil therapy in cancer patients: the role of tumoral dihydropyrimidine dehydrogenase activity. *J. Clin. Oncol.*, **13**, 1663–1670 (1995).
- 9) Jiang, W., Lu, Z., He, Y. and Diasio, R. B. Dihydropyrimidine dehydrogenase activity in hepatocellular carcinoma: implication in 5-fluorouracil-based chemotherapy. *Clin. Cancer Res.*, **3**, 395–399 (1997).
- 10) Ishikawa, Y., Kubota, T., Otani, Y., Watanabe, M., Teramoto, T., Kumai, K., Kitajima, M., Takechi, T., Okabe, H. and Fukushima, M. Dihydropyrimidine dehydrogenase activity and messenger RNA level may be related to the antitumor effect of 5-fluorouracil on human tumor xenografts in nude mice. *Clin. Cancer Res.*, **5**, 883–889 (1999).
- 11) Haaz, M. C., Fischel, J. L., Formento, P., Renee, N., Etienne, M. C. and Milano, G. Impact of different fluorouracil biochemical modulators on cellular dihydropyrimidine dehydrogenase. *Cancer Chemother. Pharmacol.*, **38**, 52–58 (1996).
- 12) Takechi, T., Uchida, J., Fujioka, A. and Fukushima, M. Enhancing 5-fluorouracil cytotoxicity by inhibiting dihydropyrimidine dehydrogenase activity with uracil in human tumor cells. *Int. J. Oncol.*, **11**, 1041–1044 (1997).
- 13) Naylor, M. S., Stamp, W. H. S. and Balkwill, F. R. Investigation of cytokine-gene expression in human colorectal cancer. *Cancer Res.*, **50**, 4436–4440 (1990).
- 14) Eda, H., Fujimoto, K., Watanabe, S., Ura, M., Hino, A., Tanaka, Y., Wada, K. and Ishitsuka, H. Cytokines induce thymidine-phosphorylase expression in tumor cells and make them more susceptible to 5'-deoxy-5-fluorouridine. *Cancer Chemother. Pharmacol.*, **32**, 333–338 (1993).

- 15) Ishikawa, T., Ura, M., Yamamoto, T., Tanaka, Y. and Ishitsuka, H. Selective inhibition of spontaneous pulmonary metastasis of Lewis lung carcinoma by 5'-deoxy-5-fluorouridine. *Int. J. Cancer*, **61**, 516–521 (1995).
- 16) Ueda, M., Ueki, M., Terai, Y., Ueki, K., Kumagai, K., Fujii, H., Yoshizawa, K. and Nakajima, M. Biological implications of growth factors on the mechanism of invasion in gynecological tumor cells. *Gynecol. Obstet. Invest.*, **48**, 221–228 (1999).
- 17) Ueda, M., Fujii, H., Yoshizawa, K., Terai, Y., Kumagai, K., Ueki, K. and Ueki, M. Effects of EGF and TGF- α on invasion and proteinase expression of uterine cervical adenocarcinoma OMC-4 cells. *Invasion Metastasis*, **18**, 176–183 (1999).
- 18) Nozawa, S., Udagawa, Y., Ohta, H., Kurihara, S. and Fishman, W. H. Newly established uterine cervical-cancer cell line (SKG-III) with Regan isoenzyme, human chorionic gonadotropin β -subunit and pregnancy-specific β 1-glycoprotein phenotypes. *Cancer Res.*, **43**, 1748–1760 (1983).
- 19) Ueda, M., Fujii, H., Yoshizawa, K., Abe, F. and Ueki, M. Effects of sex steroids and growth factors on migration and invasion of endometrial adenocarcinoma SNG-M cells *in vitro*. *Jpn. J. Cancer Res.*, **87**, 524–533 (1996).
- 20) Ueda, M., Ueki, M., Terai, Y., Morimoto, A., Fujii, H., Yoshizawa, K. and Yanagihara, T. Stimulatory effects of EGF and TGF- α on invasive activity and 5'-deoxy-5-fluorouridine sensitivity in uterine cervical-carcinoma SKG-IIIb cells. *Int. J. Cancer*, **72**, 1027–1033 (1997).
- 21) Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275 (1951).
- 22) Horiuchi, T., Suga, S., Sawada, H., Hashizume, H., Aoyama, H., Ina, K. and Kimura, K. Basic study for the clinical application of fluoropyrimidines to cancer chemotherapy (part I)—metabolism of fluoropyrimidines in cancer patients—. *Jpn. J. Clin. Pharmacol. Ther.*, **16**, 475–478 (1985).
- 23) Spears, C. P., Gustavsson, B. G., Mitchell, M. S., Spicer, D., Berne, M., Bernstein, L. and Danenberg, P. V. Thymidylate synthetase inhibition in malignant tumors and normal liver of patients given intravenous 5-fluorouracil. *Cancer Res.*, **44**, 4144–4150 (1984).
- 24) Johnston, P. G., Drake, J. C., Trepel, J. and Allegra, C. J. Immunological quantification of thymidylate synthase using the monoclonal antibody TS 106 in 5-fluorouracil-sensitive and -resistant human cancer cell lines. *Cancer Res.*, **52**, 4306–4312 (1992).
- 25) Van der Wilt, C. L., Pinedo, H. M., Smid, K. and Peters, G. J. Elevation of thymidylate synthase following 5-fluorouracil treatment is prevented by the addition of leucovorin in murine colon tumors. *Cancer Res.*, **52**, 2922–2928 (1992).
- 26) Peters, G. J., Laurensse, E., Leyva, A., Lankelma, J. and Pinedo, H. M. Sensitivity of human, murine, and rat cells to 5-fluorouracil and 5'-deoxy-5-fluorouridine in relation to drug-metabolizing enzymes. *Cancer Res.*, **46**, 20–28 (1986).
- 27) Aschele, C., Sobrero, A., Faderan, M. A. and Bertino, J. R. Novel mechanism(s) of resistance to 5-fluorouracil in human colon cancer (HCT-8) sublines following exposure to two different clinically relevant dose schedules. *Cancer Res.*, **52**, 1855–1864 (1992).
- 28) Schwartz, E. L., Hoffman, M., O'Connor, C. J. and Wadler, S. Stimulation of 5-fluorouracil metabolic activation by interferon- α in human colon carcinoma cells. *Biochem. Biophys. Res. Commun.*, **182**, 1232–1239 (1992).
- 29) Naguib, F. N. M., El Kouni, A. M. and Cha, S. Enzymes of uracil catabolism in normal and neoplastic human tissue. *Cancer Res.*, **45**, 5405–5412 (1985).
- 30) McLeod, H. L., Sludden, J., Murray, G. I., Keenan, R. A., Davidson, A. I., Park, K., Koruth, M. and Cassidy, J. Characterization of dihydropyrimidine dehydrogenase in human colorectal tumors. *Br. J. Cancer*, **77**, 461–465 (1998).
- 31) Queener, S. F., Morris, H. P. and Weber, G. Dihydrouracil dehydrogenase activity in normal, differentiating, and regenerating liver and in hepatomas. *Cancer Res.*, **31**, 1004–1009 (1971).
- 32) Williams, C. S. and Tuchman, M. Correlation of dihydropyrimidine dehydrogenase, thymidine phosphorylase and thymidine kinase activities in strongly and weakly malignant cultured murine neuroblastoma cells. *Int. J. Cancer*, **43**, 901–904 (1989).
- 33) Takechi, T., Okabe, H., Fujioka, A., Murakami, Y. and Fukushima, M. Relationship between protein levels and gene expression of dihydropyrimidine dehydrogenase in human tumor cells during growth in culture and in nude mice. *Jpn. J. Cancer Res.*, **89**, 1144–1153 (1998).
- 34) Van Laar, J. A. M., Rustum, Y. M., Van der Wilt, C. L., Smid, K., Kuiper, C. M., Pinedo, H. M. and Peters, G. J. Tumor size and origin determine the antitumor activity of cisplatin or 5-fluorouracil and its modulation by leucovorin in murine colon carcinomas. *Cancer Chemother. Pharmacol.*, **39**, 79–89 (1996).
- 35) Okada, Y., Tsuchiya, H., Shimizu, H., Tomita, K., Nakanishi, I., Sato, H., Seiki, M., Yamashita, K. and Hayakawa, T. Induction and stimulation of 92-kDa gelatinase/type-IV collagenase production in osteosarcoma and fibrosarcoma cell lines by tumor-necrosis factor α . *Biochem. Biophys. Res. Commun.*, **171**, 610–617 (1990).
- 36) Bani, M. R., Garofalo, A., Scanziani, E. and Giavazzi, R. Effect of interleukin-1-beta on metastasis formation in different tumor systems. *J. Natl. Cancer Inst.*, **83**, 119–123 (1991).
- 37) Arguello, F., Baggs, R. B., Graves, B. T., Harwell, S. E., Cohen, H. J. and Frantz, C. N. Effect of IL-1 on experimental bone/bone-marrow metastasis. *Int. J. Cancer*, **52**, 802–807 (1992).
- 38) Milano, G., Fischel, J. L., Etienne, M. C., Renee, N., Formento, P., Thyss, A., Gaspard, M. H., Thill, L. and Cupissol, D. Inhibition of dihydropyrimidine dehydrogenase by α -interferon: experimental data on human tumor cell lines. *Cancer Chemother. Pharmacol.*, **34**, 147–152 (1994).