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Cytokines Induce Uridine Phosphorylase in Mouse Colon 26 Carcinoma Cells and Make the Cells More Susceptible to 5′-Deoxy-5-fluorouridine

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The antiproliferative activity of 5-fluorouracil (5-FUra) and 5′-deoxy-5-fluorouridine (5′-dFUrd), used in combination with typical cytokines and growth factors, was investigated in mouse colon 26 carcinoma cells. Tumor necrosis factor α (TNFα), interleukin-1α (IL-1α), and interferon γ (IFNγ) at low doses showing $<50\%$ inhibition of cell growth by themselves enhanced the susceptibility of the cells to the activity of 5′-dFUrd. In particular, a mixture of these cytokines greatly enhanced the activity of 5′-dFUrd and 5-FUra by up to 12.4- and 2.7-fold, respectively, whereas the activity of other cytostatics was only slightly changed (<1.5-fold). Basic fibroblast growth factor also increased the susceptibility, but only to 5′-dFUrd. This preferential enhancement of the activity of 5′-dFUrd would be due to induction by the cytokines of uridine phosphorylase (Urd Pase), by which 5′-dFUrd is converted to 5-FUra. TNFα, IL-1α, IFNγ, and a mixture of these factors increased the enzyme activity by up to 3.7-fold in colon 26 cells. Consequently, the anabolism of 5′-dFUrd to fluoronucleotides and the incorporation of 5-FUra into RNA in colon 26 cells were increased by TNFα treatment. In addition, the increase by the cytokine mixture in the susceptibility to 5′-dFUrd was abolished by an inhibitor of Urd Pase, 2,2′-anhydro-5-ethyluridine. These results indicate that induction of Urd Pase activity by cytokines is a critical event that increases the susceptibility to 5′-dFUrd.

Key words: Cytokine — Uridine phosphorylase — 5-Fluorouracil — 5′-Deoxy-5-fluorouridine — Mouse colon 26 carcinoma

5-Fluorouracil (5-FUra) is an anticancer drug that has been used for the treatment of a variety of neoplastic diseases, particularly cancers of the breast and digestive organs. Recently, a new treatment regimen with this drug and other drugs in combination, such as methotrexate,[1] leucovorin[2,3] and interferon α (IFNα),[4] was found to show superior clinical efficacy. IFNα has been shown to improve the efficacy of 5-FUra in the treatment of colorectal cancer,[4] although the mechanism of the improvement by IFNα has not been fully clarified. IFNα was reported to enhance the antiproliferative activity of fluoropyrimidines in cultures of human cancer cells through induction of thymidine phosphorylase activity[5,6] and through augmentation of thymidylate synthase inhibition.[7,8]

We have investigated the antiproliferative activity of fluoropyrimidines in combination with various agents, particularly to ascertain whether the activity of 5′-deoxy-5-fluorouridine (5′-dFUrd) is modulated by agents that are known to modulate the activity of 5-FUra. 5′-dFUrd is a prodrug of 5-FUra and is used clinically in the treatment of breast, colorectal and gastric cancers.[9] We have shown that this drug is converted to 5-FUra by pyrimidine nucleoside phosphorylases, mainly by uridine phosphorylase (EC 2.4.2.3, Urd Pase) in rodents and by thymidine phosphorylase (EC 2.4.2.4, TdR Pase) in humans.[10–12] In both species, the corresponding enzyme was more abundant in tumors than in normal tissues, with some exceptions.[10–12] Consequently, 5′-dFUrd was shown to be more effective against many murine transplantable tumors in terms of therapeutic indices and is less myelosuppressive than 5-FUra.[13–16]

Recently, we found that inflammatory cytokines, such as tumor necrosis factor α (TNFα), interleukin-1α (IL-1α) and interferon γ (IFNγ), were much more effective in enhancement of the antiproliferative activity of fluoropyrimidines than IFNα. We also found that these cytokines enhanced the activity of 5′-dFUrd towards mouse and human cancer cells to a much greater extent than they enhanced that of 5-FUra. This preferential enhancement of the activity of 5′-dFUrd would be due to induction of TdR Pase in human tumor cells, as observed in the case of IFNα[6] and Urd Pase in mouse colon 26 carcinoma cells. In this paper, we compare the enhancement by cytokines of the antiproliferative activity of cytostatics, particularly 5′-dFUrd and 5-FUra, in cultures of mouse colon 26 cells. We also discuss the mechanism of the preferential increase in the susceptibility to 5′-dFUrd.

MATERIALS AND METHODS

Cells and culture The mouse colon 26 carcinoma cell line used was obtained from Dr. T. Kataoka, the Cancer Chemotherapy Center, Japanese Foundation of Cancer

Research (Tokyo). This cell line was grown on plastic plates in RPMI1640 supplemented with 10% fetal bovine serum in a humidified, 5% CO_2 atmosphere at 37°C, and passaged twice weekly. This cell line was confirmed to be free of mycoplasma by the use of a mycoplasma detection kit (Boehringer Mannheim, Mannheim, Germany).

Reagents 5-FUra was purchased from Kyowa Hakko Co. (Tokyo, Japan), and 5′-dFUrd used was synthesized at F. Hoffmann-La Roche (Basel, Switzerland). 2,2′-Anhydro-5-ethyluridine (ANEUR) was synthesized at Nippon Roche K.K. (Kamakura). The other cytostatic drugs such as ACNU, Ara-C, CDDP, etoposide, doxorubicin, methotrexate, mitomycin C, vincristine and vinblastine were purchased commercially. [6-^{3}H]5-FUra (714 GBq/mmol) and [6-^{3}H]5′-dFUrd (370 GBq/mmol) were purchased from NEN (Dreiech, Germany) and Moravek Biochemicals, Inc. (Brea, CA), respectively. Recombinant TNFα (human, 5×10^7 U/mg), IL-1 α (human, 5×10^7 U/mg; mouse, 5.6×10^8 U/mg), IFN αA/D (human, 1.5×10^8 IU/mg) and IFNγ (mouse, 5×10^6 U/mg) were provided by Drs. W. Lesslauer (F. Hoffmann-La Roche, Basel, Switzerland), W. D. Benjamin (Hoffmann-La Roche, Nutley, NJ), Y. Suhara and Y. Furuichi (Nippon Roche, Kamakura), respectively. Recombinant human basic FGF was purchased from PROGEN (Heidelberg, Germany), and recombinant human platelet derived growth factor-bb (PDGF-bb) was provided by F. Hoffmann-La Roche.

Cytotoxicity assay A single-cell suspension (5×10^2 cells) was added to a microtest plate containing the serially diluted cytostatics. The cells were then cultured at 37°C for 9 days in the presence or absence of cytokines until the cell number in the control culture was ten-fold greater than the initial cell number. The cell growth in monolayers was observed after staining of the cells with crystal violet or measured by using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) method as described elsewhere.[17,18] The IC_{50} values of cytostatics were expressed as the concentrations at which the growth was inhibited by 50% as compared with the control.

Urd Pase assay Cultured cells were collected and sonicated in 10 m*M* Tris-HCl buffer (pH 7.4) containing 15 m*M* NaCl, 1.5 m*M* $MgCl_2$ and 50 μM potassium phosphate. This solution was then centrifuged at 105,000*g* for 90 min. The supernatant was dialyzed overnight against 20 m*M* potassium phosphate buffer (pH 7.4) and 1 m*M* β-mercaptoethanol, and used as a source of crude uridine phosphorylase. The protein concentration was determined by the method of Lowry *et al.*[19] All procedures were done at 4°C. The reaction mixture (120 μl) for the enzyme activity assay contained 183 m*M* potassium phosphate buffer (pH 7.4), 10 m*M* 5′-dFUrd, and the crude enzyme from mouse cancer cells. The reaction was done at 37°C for 60 min and then terminated by the addition of 360 μl of methanol. After removal of the precipitate by centrifugation, an aliquot of the reaction mixture (100 μl) was supplemented with 20 μM 5-chlorouracil as an internal standard and then applied to the high-performance liquid chromatography (HPLC) column (ERC-ODS-1171). The solvent system used was as follows; 50 m*M* sodium phosphate buffer (pH 6.8) containing 5 m*M* 1-decanesulfonic acid-methanol (85:15, v/v). The amount of 5-FUra produced was measured with a UV monitor (280 nm).

Analysis of nucleotides and RNA containing 5-FUra A single-cell suspension of colon 26 cells (4×10^4) was placed in a 6-well plate. After 3 h of culturing, hrTNFα was added to the cells at a concentration of 250 U/ml. The cells were then cultured at 37°C for 3 days. For the last 4 h of the culture, [6-^{3}H]5-FUra (9.25 GBq/mmol) or [6-^{3}H]5′-dFUrd (9.25 GBq/mmol) was added. The cells were harvested and treated with 20% cold TCA. The solution was neutralized with 0.5 *M* tri-octylamine solution containing Freon, and then 5-FUra, 5′-dFUrd, and fluoronucleotides in the aqueous upper layer were fractionated by HPLC [column, YMC Pack AQ-312 120A ODS; eluent, 20 m*M* potassium phosphate buffer (pH 5.0), 5 m*M* heptasulfonic acid and 5% methanol (v/v); flow rate, 1.0 ml/min].[20] TCA insolubles were washed with ice-cold 10% TCA and suspended in 3 ml of AQUASOL-2. The radioactivity was counted with an Aloka liquid scintillation counter.

Statistical analysis Differences in the enzyme activity, IC_{50} and incorporation of labeled fluoropyrimidines among groups were compared by using Student's *t* test. Differences were considered to be significant when the probability (*P*) value was <0.05.

RESULTS

Enhancement of the antiproliferative action of 5′-dFUrd by the cytokines The effects of various cytostatics in combination with a mixture of TNFα, IL-1α and IFNγ were studied in mouse colon 26 cells. Table I shows that the cytokine mixture at a low dose showing 15% growth inhibition on colon 26 cells greatly enhanced the antiproliferative activity for 5′-dFUrd (5.3-fold) but it was not able to do the same for the other cytostatics (<1.5-fold). This preferential enhancement of 5′-dFUrd activity by the cytokines was studied further. As Table II and Fig. 1 show, each of the three cytokines TNFα, IL-1α and IFNγ slightly enhanced the activity of only 5′-dFUrd in colon 26 cells, and their effect appeared to be synergistic. Incubation with the cytokine mixture at higher dose made the cells much more susceptible to 5′-dFUrd (12.4-fold), while the enhancement for 5-FUra activity was only slight (2.7-fold). The cytokine mixture, even at

Table I. Increase of the Antiproliferative Activity of 5-FUra, 5′-dFUrd and Other Antineoplastic Agents by Mixture of Cytokines

Drug	Cell growth inhibition (μM) Cytokines[a] −/+	Increase of susceptibility (fold)[b]
Fluoropyrimidines		
5-FUra	0.45/0.29	1.55*
5′-dFUrd	9.39/1.77	5.31*
Other antineoplastic agents		
ACNU	8.55/6.71	1.27*
Ara-C	0.60/0.61	0.98
CDDP	0.38/0.28	1.36*
Doxorubicin	0.085/0.068	1.25*
Mitomycin-C	0.068/0.045	1.51*
Methotrexate	0.025/0.024	1.04
Vinblastine	0.0092/0.0087	1.06
Vincristine	0.030/0.027	1.11*
Etoposide	0.31/0.27	1.15*

a) Mixture of cytokines, hrTNFα 1000+mrIL-1α 200+mrIFNγ 2 U/ml.
b) The increase of the susceptibility to cytostatics (IC_{50}: fold) by the cytokines was expressed as follows: IC_{50} of cytostatics (without cytokines)/IC_{50} of cytostatics (with cytokines).
* $P<0.05$.
Cell growth inhibition by mixture of cytokines alone: 14.7±5.8%.

lower doses showing no cell growth inhibition by themselves, enhanced the activity of 5′-dFUrd by 5.4-fold. In separate experiments, the growth factors PDGF and bFGF were also tested for their ability to enhance the activity of the fluoropyrimidines, and bFGF was found to be effective. Human rIFNαA/D, that is active against mouse cells as well as human cells,[21] was also tested at a dose of 100 IU/ml, but no enhancement was observed (data not shown).

Induction of Urd Pase activity by cytokines and growth factors To elucidate the mechanism of the preferential enhancement by the cytokines of the activity of 5′-dFUrd on colon 26 cells, we measured pyrimidine nucleoside phosphorylase activity, which converts 5′-dFUrd to 5-FUra, after the cytokine treatment. TNFα, IL-1α and IFNγ all enhanced the enzyme activity, the mixture of all three producing the largest increase of 3.7-fold. The enzyme activity in the cells treated with bFGF was increased by 1.9-fold, while it only increased slightly with PDGF (1.4-fold). Other cytokines and growth factors, such as interleukin-2 (IL-2), granulocyte macrophage-colony stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), transforming growth factor β (TGFβ) and epidermal growth factor (EGF) were also tested at 100 U/ml, but Urd Pase activity in colon 26 cells was basically unchanged by them (data not shown). In a separate experiment, the activity of the enzyme derived from colon 26 cells both treated with and without the cytokine mixture was completely inhibited

Table II. Increase of the Antiproliferative Activity of 5-FUra and 5′-dFUrd and Induction of Uridine Phosphorylase Activity by Cytokines in Mouse Colon 26 Cells

Cytokines (U/ml) Growth factors (ng/ml)		Growth inhibition by cytokine alone (%)	Cell growth inhibition: IC_{50} (μM) of susceptibility to 5-FUra cytokine−/+ (fold)[b]		Increase of uridine phosphorylase activity (fold)[c] (μg/5-FUra/mg protein/h)
				5′-dFUrd	
					Control 243.1±23.1
hrTNFα	100	−1.0±1.6	0.73/0.73 (1.0)	12.6/6.8 (1.9)*	497.1±6.1 (2.0)*
	10	0.3±3.4	0.73/0.74 (1.0)	12.6/9.2 (1.4)*	NT
mrIL-1α	100	6.4±4.1	0.69/0.71 (1.0)	11.0/7.5 (1.5)*	466.9±36.9 (1.9)*
	10	2.9±4.3	0.69/0.71 (1.0)	11.0/6.3 (1.7)*	NT
mrIFNγ	10	17.9±3.6	0.66/0.30 (2.2)*	11.6/1.5 (7.7)*	444.9±15.1 (1.8)*
	1	5.5±2.9	0.66/0.47 (1.4)*	11.6/4.4 (2.6)*	NT
Mixture	#1[a]	37.1±13.1	0.69/0.26 (2.7)*	12.4/1.0 (12.4)*	889.8±96.6 (3.7)*
	#2	8.5±5.1	0.69/0.42 (1.6)	12.4/2.4 (5.4)*	NT
					Control 339.2±15.9
hr-bFGF	100	15.5±6.5	0.47/0.46 (1.0)	6.80/3.67 (1.9)*	660.5±61.0 (1.9)*
hrPDGF	100	−2.2±2.4	0.53/0.54 (1.0)	8.05/7.17 (1.1)*	486.8±50.7 (1.4)*

a) Mixture #1: hrTNFα 100, mrIL-1α 100, mrIFNγ 10 U/ml. Mixture #2: hrTNFα 10, mrIL-1α 10, mrIFNγ 1 U/ml.
b) The increase of the susceptibility to cytostatics (IC_{50}: fold) by the cytokines was expressed as described in Table I (footnote).
c) Cells were exposed to cytokines for 3 days. In this experiment, hrIL-1α was used instead of mrIL-1α.
NT: Not tested. * $P<0.05$.

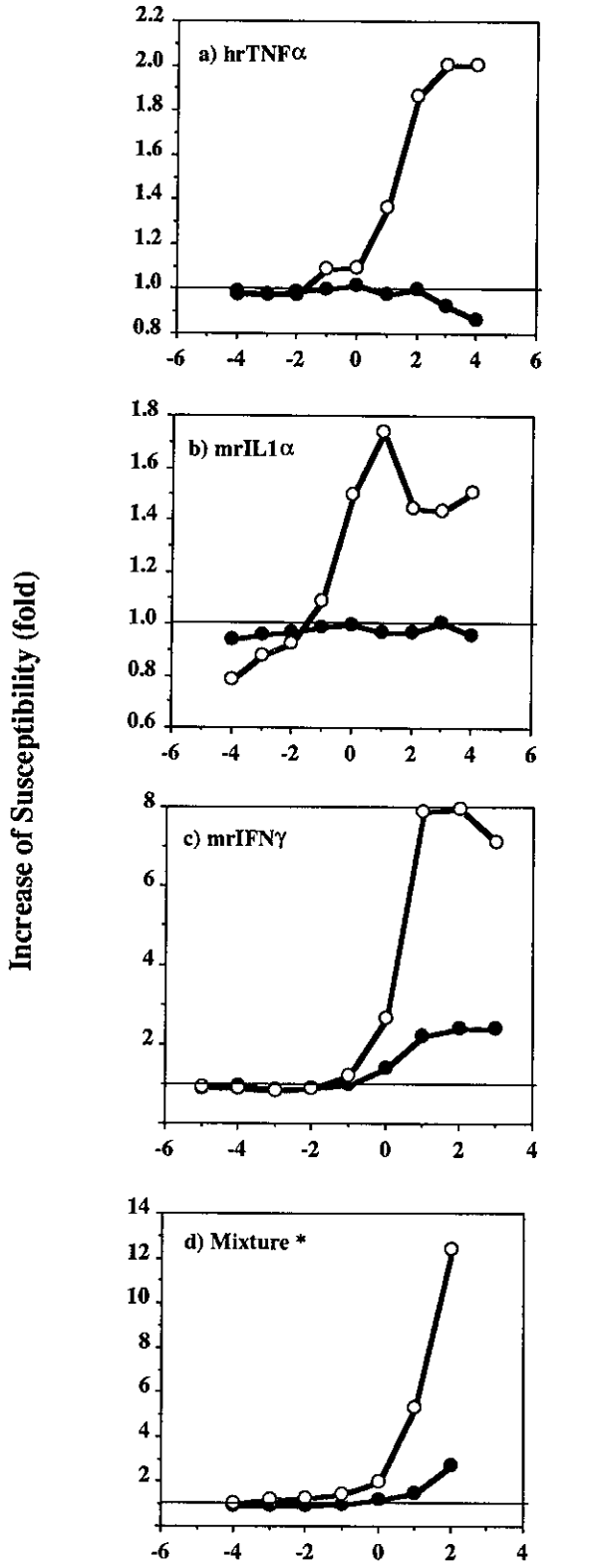

Fig. 1. Enhancement of antiproliferative action of 5′-dFUrd by cytokines in murine colon 26 carcinoma cells. Colon 26 cells were cultured with 5′-dFUrd and 5-FUra for 9 days in the presence or absence of various doses of hrTNFα (a), mrIL-1α (b), mrIFNγ (c) or their mixture (d). IC_{50}s of 5′-dFUrd and 5-FUra either in the presence or absence of the cytokines were measured, and increase of the cell susceptibility (IC_{50}) induced by the cytokines was calculated; 5′-dFUrd (o), 5-FUra (●). The cytokines were used at the doses indicated in the figure except for IFNγ in Fig. 1d(*), where IFNγ were used at one-tenth of the doses indicated.

(>99%) by the enzyme inhibitor 2,2′-anhydro-5-ethyluridine (ANEUR), that is selective to Urd Pase but not to TdR Pase.[22] This result confirmed that the enzyme induced by the cytokine mixture was Urd Pase.

Enhancement of 5′-dFUrd metabolism by hrTNFα Next, we tried to confirm that the increase in Urd Pase activity by cytokines accelerates 5′-dFUrd anabolism. In Fig. 2, we compare concentrations of the metabolites of radiolabeled 5′-dFUrd and 5-FUra, such as nucleotides and RNA containing 5-FUra, in colon 26 cells pretreated with or without TNFα. In the cells pretreated with TNFα, the anabolism of 5′-dFUrd to nucleotides and RNA containing 5-FUra was accelerated to twice that in non-treated colon 26 cells. On the other hand, the anabolism of 5-FUra was not changed by the TNFα treatment.

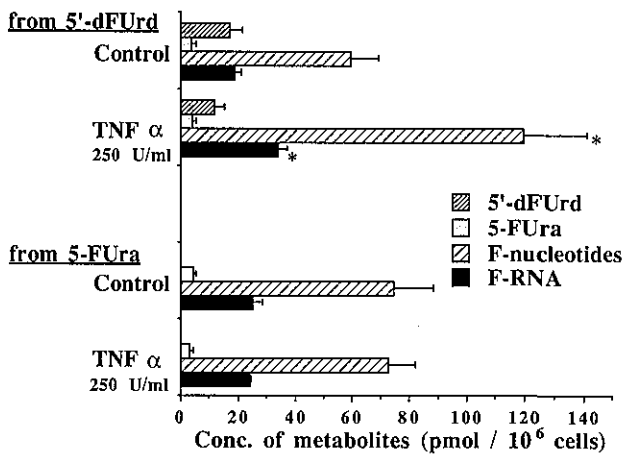

Fig. 2. Incorporation of radiolabeled 5′-dFUrd and 5-FUra into F-containing nucleotides and RNA. Colon 26 cells cultured with or without hrTNFα for 3 days were incubated with radiolabeled 5′-dFUrd (40 μM) and 5-FUra (5 μM) for 4 h. The radioactivity of the fractions of 5-FUra, 5′-dFUrd and F-containing nucleotides and cold TCA insolubles prepared from the cells treated with 5′-dFUrd and 5-FUra was counted. * $P<0.05$ as compared with the control.

Table III. Effects of Cytokines and ANEUR on Cell Susceptibility to 5-FUra and 5′-dFUrd

	Cell growth inhibition (IC_{50}: μM) of	
	5-FUra (fold)[a]	5′-dFUrd (fold)
Control	0.31±0.02 (−)	7.41±0.41 (−)
ANEUR[b]	0.52±0.06 (0.60)[d]	61.19±4.77 (0.12)[d]
Mixture of cytokines[c]	0.15±0.02 (2.07)[d]	1.31±0.07 (5.66)[d]
ANEUR+Cytokines	0.45±0.06 (0.69)[e]	31.91±4.54 (0.23)[d, e]

a) The increase of the susceptibility to cytostatics (IC_{50}: fold) by the cytokines was expressed as described in Table I (footnote).
b) ANEUR (2,2′-anhydro-5-ethyluridine): 200 μM.
c) Mixture of cytokines: hrTNFα 10+mrIL-1α 10+mrIFNγ 10 U/ml.
d) $P<0.05$ vs. control.
e) $P<0.05$ vs. mixture of cytokines.
Cell growth inhibition by ANEUR alone: −2.3±3.9%, mixture of cytokines alone: 22.4±6.7%, ANEUR and mixture of cytokines: 22.6±3.2%.

Urd Pase essential for increase by cytokines in cell susceptibility to fluoropyrimidines The experiment, in which an inhibitor of Urd Pase was used, confirmed that the enzyme activity is involved in the enhancement of 5′-dFUrd and 5-FUra activity by the cytokines (Table III). The Urd Pase inhibitor ANEUR reduced the susceptibility of colon 26 cells to the two fluoropyrimidines, greatly for 5′-dFUrd (0.12-fold) and slightly for 5-FUra (0.60-fold). This is because the enzyme is essential for conversion of 5′-dFUrd to 5-FUra and would accelerate anabolism of 5-FUra through conversion to 5-FUrd. The mixture of TNFα, IL-1α and IFNγ increased the susceptibility to 5-FUra (2.1-fold) and 5′-dFUrd (5.7-fold) as shown in Table I and Fig. 1. However, in the presence of ANEUR, the effect of the cytokines on the susceptibility was not observed for 5-FUra or was very slight for 5′-dFUrd as compared to that in the absence of ANEUR.

DISCUSSION

We have examined cytokines and growth factors for ability to enhance the antiproliferative activity of various cytostatics. The present study showed that inflammatory cytokines, such as TNFα, IL-1α, IFNγ and their mixture, more effectively modulate the activity of fluoropyrimidines than does IFNα in mouse colon 26 cells. They greatly enhanced the activity of 5′-dFUrd and slightly enhanced that of 5-FUra. However, no clear enhancement was observed with other cytostatics. Several laboratories have already demonstrated a marked increase in the activity of 5-FUra by cytokines, such as TNFα and IFNs.[7, 23–30] In particular, Schwartz *et al.* showed that IFNα enhanced the activity of 5-FUra on tumor cells through augmentation of TdR Pase and Urd Pase activity, the levels of which would be associated with the efficacy of 5-FUra.[5] These two pyrimidine nucleoside phosphorylases are essential for conversion of 5′-dFUrd to 5-FUra. Tevaearai *et al.* reported that IFNα enhanced the susceptibility of human tumor cells to 5′-dFUrd as a result of the increase in TdR Pase activity but had no effect on susceptibility to 5-FUra.[6] The present study also showed that other cytokines and the growth factor bFGF augmented Urd Pase activity in mouse colon 26 cells and consequently made them more susceptible to 5′-dFUrd. In separate experiments, we confirmed that the cytokines induce TdR Pase at the levels of enzyme activity and mRNA expression in human tumor cells. The increase in the pyrimidine nucleoside phosphorylases induced by cytokines is considered to be the main event that makes tumor cells more susceptible to 5′-dFUrd as well as to 5-FUra.

The idea that the cytokines induce Urd Pase and make tumor cells more susceptible to 5′-dFUrd and 5-FUra is further supported by the fact that the enhancement of antiproliferative activity was abolished by ANEUR, a specific inhibitor of Urd Pase.[22] 5-FUra is converted to fluoronucleotides, the active metabolites, through its conversion to fluorouridine (FUrd) by Urd Pase and then to fluorouridine monophosphate (FUMP) by uridine kinase and through its direct conversion to FUMP by phosphoribosyl transferase. Therefore, Urd Pase induction would result in the enhancement of 5-FUra activity. However, the other possibility that the cytokines induce other enzymes necessary for the conversion of 5-FUra to the active metabolites still remains. In fact, the degree of Urd Pase induction by the cytokines was not always well correlated with increase in susceptibility to the fluoropyrimidines (Table II). Among the cytokines, only IFNγ greatly increased the susceptibility, although all the cytokines induced Urd Pase to a similar degree. IFNγ

may induce additional enzyme(s) necessary for 5-FUra anabolism.

TdR Pase and Urd Pase, which are key enzymes in the salvage pathway of pyrimidine nucleotide biosynthesis, exist predominantly in humans and rodents, respectively.[10-12] In each species, the corresponding enzymes are abundant in tumor tissues as compared to normal tissues.[10-12] It is of interest that TNFα, IL-1α, IFNγ and bFGF as well as IFNα induce these pyrimidine nucleoside phosphorylases. In addition, we have observed that interleukin-6 (IL-6) and PDGF are capable of inducing TdR Pase in human tumor cells (data not shown). Cytokines and growth factors are produced by various cells such as lymphocytes, macrophages, granulocytes, fibroblasts, endothelial cells, etc., that exist in tumor tissues as infiltrated cells and stroma cells. In addition, many tumor cells are known to produce cytokines and growth factors. In tumor tissues, various factors would induce pyrimidine nucleoside phosphorylases and increase the efficacy of 5′-dFUrd. Furthermore, 5′-dFUrd would have more therapeutic benefits in combination therapy with such inducers.

Urd Pase activity is induced by the cytokines. However, the physiological roles of this enzyme induction have not yet been clarified. Recently, human TdR Pase was reported to be identical to PD-ECGF, platelet-derived endothelial cell growth factor, that has angiogenic activity.[31,32] The enzyme induction by inflammatory cytokines and growth factors may be associated with tumor angiogenesis. Geng *et al.* reported that *ras*-infected NIH-3T3 cells are more susceptible to 5′-dFUrd than are normal NIH3T3 cells as a result of higher Urd Pase activity in the transformed cells.[33] The roles of the pyrimidine nucleoside phosphorylases in tumor angiogenesis and cell transformation should be clarified.

We have shown that 5′-dFUrd is effective only after its conversion to 5-FUra by TdR Pase and Urd Pase, and that it has unique characteristics not found with 5-FUra or other cytostatics. 5′-dFUrd was highly effective on spontaneous metastasis to the lung of Lewis lung carcinoma inoculated s.c. In particular, when it was administered during the process of tumor cell invasion, the activity was much more obvious even at quite a low dose, 1/40 of the inhibitory dose required for the s.c. tumor growth.[34] We also reported that 5′-dFUrd improved cachexia in mouse bearing colon 26 carcinoma, and prolonged the survival period.[35,36] This anticachexia activity was not simply associated with its antitumor activity. The mechanisms of these actions of 5′-dFUrd may reflect the unique characteristics of pyrimidine nucleoside phosphorylases, which are essential enzymes for the conversion of 5′-dFUrd to 5-FUra. Further studies are required.

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