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# Cytokines induce thymidine phosphorylase expression in tumor cells and make them more susceptible to 5′-deoxy-5-fluorouridine

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**Abstract.** The present study shows that various cytokines such as tumor necrosis factor (TNFα), interleukin-1α (IL-1α), and interferon-γ (IFNγ) make tumor cells much more susceptible to the cytostatic 5′-deoxy-5-fluorouridine (5′-dFUrd) than to 5-fluorouracil (5-FUra) and other cytostatics. These three cytokines increased the susceptibility of human cancer cell lines (COLO201, MKN45 and WiDr) but did not affect that of normal fibroblast WI38 cells. The cytokine mixture induced a 50-fold increase in the susceptibility of COLO201 to 5′-dFUrd, whereas a 12-fold increase and a less than 5-fold enhancement in the susceptibility to 5-FUra and other cytostatics, respectively, were observed. The increased susceptibility would be a result of the induction of thymidine phosphorylase (TdR Pase), which is the essential enzyme for the conversion of 5′-dFUrd to 5-FUra. The cytokine mixture increased TdR Pase activity by up to 47 times and greatly induced its mRNA expression in the cancer cell lines. These results suggest that the therapeutic benefit of 5′-dFUrd would be improved by its use in combination with the cytokines.

## Introduction

5-Fluorouracil (5-FUra) has been 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. The efficacy of 5-FUra has been improved through the development of new treatment regimens for combination therapy with this drug. Methotrexate [27], leucovorin [1, 5], and interferon-α (IFNα) [30] improve the therapeutic efficacy of 5-FUra in the treatment of colorectal cancer. Recently, it has been reported that IFNα increases TdR Pase activity in tumor cell cultures and makes the cells more susceptible to 5-FUra [25] and to 5′-deoxy-5-fluorouridine (5′-dFUrd) [29].

5′-dFUrd, a prodrug of 5-FUra, is used clinically in the treatment of breast, colorectal, and gastric cancers. We have shown that 5′-dFUrd is converted to 5-FUra by the pyrimidine nucleoside phosphorylases thymidine phosphorylase (TdR Pase, EC 2.4.2.4) and uridine phosphorylase (Urd Pase, EC 2.4.2.3), both of which exist predominantly in humans and rodents, respectively [12, 14, 17]. The former enzyme was recently shown to be identical to platelet-derived endothelial cell growth factor (PD-ECGF) [10]. The corresponding pyrimidine nucleoside phosphorylase in these two species is more abundant in tumors than in normal tissues except for the intestinal tract of rodents [12, 14, 17] and the liver of humans [31]. Consequently, 5′-dFUrd is more effective than 5-FUra against many murine transplantable tumors, particularly in terms of therapeutic indices, and is less myelosuppressive [2, 4, 6, 21].

We investigated the antiproliferative activity of cytostatics in combination with various cytokines and growth factors in human and murine cancer cells. These studies show that the antiproliferative activity of 5′-dFUrd is enhanced by tumor necrosis factor (TNFα), interleukin-1α (IL-1α) interferon-γ (IFNγ), IL-6, IFNα, platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF), particularly by the first three cytokines. Since the three inflammatory cytokines are often detected in tumor tissues and have been clinically tested, it would be worthwhile to test the antiproliferative activity of either these individual cytokines or their mixture in combination with 5′-dFUrd. In the present report, we show the enhancement induced by TNFα, IL-1α, IFNγ, and a mixture of the three in human tumor and normal cell lines. We also show that the increase in susceptibility to 5′-dFUrd is due to the induction of TdR Pase by the cytokines, as has been observed for 5-FUra [25] and 5′-dFUrd [29] by IFNα. In addition, we demonstrate that the cytokines induce the expression of TdR Pase mRNA, which is detected by Northern-blot hybridization.

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## Materials and methods

*Cells and culture.* Human colon cancer COLO201 and WiDr, gastric cancer MKN45, and normal fibroblast WI38 cell lines were used in this study. These cell lines were purchased from Dainippon Pharmaceuticals (Osaka, Japan) except for MKN45, which was obtained from Immunobiological Laboratories (Fujioka, Japan). All of the cell lines were grown on plastic plates in the following media supplemented with 10% fetal bovine serum in a humidified atmosphere containing 5% $CO_2$ at 37° C and were passaged twice weekly: RPMI1640 for COLO201 and MKN45 cells, minimum essential medium (MEM) with nonessential amino acids for WiDr cells and MEM for WI38 cells. The fetal bovine serum used for the cancer cell lines was heated at 56° C for 30min except for experiments using WI38 cells. We confirmed all of the cell lines to be free of mycoplasma by using a mycoplasma detection kit (Boehringer Mannheim, Germany).

*Reagents.* 5-FUra was purchased from Kyowa Hakko (Tokyo, Japan), and 5′-dFUrd was synthesized at F. Hoffmann-La Roche (Basle, Switzerland). The other cytostatics used were purchased commercially. Recombinant TNFα (human, $5 \times 10^7$ U/mg) and IL-1α (human, $3 \times 10^8$ U/mg) were provided by Dr. W. Lesslauer (F. Hoffmann-La Roche, Basle) and Dr. W. D. Benjamin (Hoffmann-La Roche, Nutley), respectively. Recombinant IFNγ (human, $3.9 \times 10^6$ U/mg) were prepared at Nippon Roche K. K. (Kamakura, Japan).

*Cytotoxicity assay.* A single-cell suspension of cells ($2-25 \times 10^2$ cells) was added to a microtest plate containing serially diluted cytostatics. The cells were then cultured at 37° C for 7–10 days in the presence or absence of cytokines until the cell number in the control culture had increased to 10 times the initial cell number. Cell growth in monolayers was observed after the cells had been stained with crystal violet, whereas cell growth in suspension was observed by the MTT method as described elsewhere [20, 28]. The $IC_{50}$ value for the cytostatics was expressed as the concentration at which growth was inhibited by 50% as compared with the control value.

*TdR 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 μ*M* 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 was used as a source of crude TdR Pase. The protein concentration was determined by the method of Lowry et al. [16]. All procedures were done at 4° C. The reaction mixture (120 μl) for the enzyme-activity assay contained 183 m*M* potassium phosphate (pH 7.4), 10 m*M* 5′-dFUrd, and the crude enzyme from human cells. The reaction was done at 37° C for 60 min and was then terminated by the addition of 360 μl methanol. After removal of the precipitate by centrifugation, an aliquot of the reaction mixture (100 μl) was added together with 20 μ*M* 5-chlorouracil as the internal standard and was then applied to the high-performance liquid chromatograph (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-decane sulfonic acid: methanol (85 : 15, v/v). The amount of 5-FUra produced was measured with a UV monitor (280 nm).

*TdR Pase total RNA and cDNA probe.* Total RNA was prepared by the method of extraction with guanidinium thiocyanate followed by centrifugation in cesium trifluoroacetate solutions as described elsewhere [22]. A 17mer cDNA primer was chemically synthesized, based on the structure disclosed for human PD-ECGF, which is identical to human TdR Pase [10]. Then, the cDNA (203 bp) of PD-ECGF (exon 2) was amplified by polymerase chain reaction (PCR) with the MKN45 cell genome DNA. The $^{32}P$-labeled cDNA probe was prepared using a multiprime labeling kit ($5 \times 10^9$ cpm/μg DNA; Amersham, Buckinghamshire, UK).

*Northern-blot analysis.* Total RNA samples (20 μg/lane) were electrophoresed in a formaldehyde-denaturing agarose gel and blotted onto Hybond-N nylon membranes (Amersham, Buckinghamshire, UK). The RNA on the membranes was prehybridized for 2 h and then hybridized with TdR Pase cDNA. As a control, we carried out hybridization with the human β-actin cDNA probe (Clontech, Palo Alto, Calif.) on the same membrane. Kodak XAR-2 film was used for radioautography.

*Statistical analysis.* Differences in the enzyme activity and $IC_{50}$ values were compared using Student's *t*-test. Differences were considered to be significant when the probability (*P*) value was <0.05.

**Table 1.** Increase in the antiproliferative activity of 5-FUra, 5′-dFUrd, and other antineoplastic agents in COLO201 human colon carcinoma cells

| Drug | Cell-growth inhibition ($IC_{50}$; μ*M*) (–/+ cytokines)[a] | Increase in susceptibility (-fold)[b] |
|---|---|---|
| Fluoropyrimidines: | | |
| 5-FUra | 1.73/0.14 | 12.36* |
| 5′-dFUrd | 47.8/0.96 | 49.79* |
| Other antineoplastic agents: | | |
| ACNU | 174.1/84.1 | 2.07* |
| Ara-C | 0.0865/0.137 | 0.63(*) |
| CDDP | 0.639/0.122 | 5.24* |
| Doxorubicin | 0.052/0.030 | 1.73* |
| Mitomycin C | 0.0084/0.0020 | 4.20* |
| Methotrexate | 0.0059/0.0052 | 1.13 |
| Vinblastine | 0.0017/0.0011 | 1.55* |
| Vincristine | 0.0023/0.0013 | 1.77* |
| Etoposide | 0.58/0.25 | 2.32* |

[a] hrTNFα, 100 U/ml, + hrIL-1α, 100 U/ml, + hrIFNγ, 100 U/ml

[b] The increase in the susceptibility (-fold) to cytostatics induced by the cytokines was expressed as follows: $IC_{50}$ of cytostatics (without cytokines)/$IC_{50}$ of cytostatics (with cytokines). Cell-growth inhibition induced by the mixture of cytokines alone, 27.2% ± 4.2%

* $P < 0.05$

## Results

### *Enhancement of the antiproliferative action of 5′-dFUrd by cytokines*

The antiproliferative activity of some cytostatics is enhanced by TNFα, IL-1α, and IFNγ, particularly by a mixture of these cytokines. As Table 1 shows, a mixture of TNFα, IL-1α, and IFNγ increased the susceptibility of human COLO201 cells to cytostatics, whereas these cytokines themselves inhibited cell growth slightly (27.2% ± 4.2%). The $IC_{50}$ values for 5′-dFUrd and 5-FUra were increased 50 and 12 times, respectively, by the cytokine mixture. However, they produced a less than 5-fold increase in the susceptibility to other cytostatics. This preferential enhancement of 5′-dFUrd and 5-FUra activity was extensively demonstrated in WiDr, COLO201, and MKN-45 cell lines (Table 2). Again, the cancer cells were made more susceptible to 5′-dFUrd by TNFα, IL-1α, IFNγ and their mixture, and the effects appeared to be more obvious at doses at which the cytokines themselves inhibited cell growth. In addition, the degree of enhancement produced by the cytokines varied to some extent from cell line to cell line. On the other hand, the susceptibility to 5′-dFUrd was not changed, even by the cytokine mixture (1.5-fold increase in WI38 cells; data not shown).

**Table 2.** Increase in the antiproliferative activity of 5-FUra and 5′-dFUrd induced by cytokines in COLO201 and WiDr human colon carcinoma cells and in MKN45 human gastric carcinoma cells

| Cytokines (U/ml) | | Growth inhibition by cytokines alone (%) | $IC_{50}$ (μ*M*) and increase (-fold) in susceptibility to: 5-FUra | 5′-dFUrd |
|---|---|---|---|---|
| | | | –/+ cytokine (-fold)[a] | –/+ cytokine (-fold) |
| COLO201: | | | | |
| hrTNFα | 1000 | 9.4 ± 5.0 | 1.49/0.87(1.7)* | 18.4 / 8.95(2.1)* |
| | 100 | 8.1 ± 6.2 | 1.48/1.03(1.4)* | 19.5 /11.2 (1.8)* |
| hrIL-1α | 1000 | 1.6 ± 4.3 | 1.47/1.46(1.0) | 19.1 /18.8 (1.0) |
| | 100 | 2.2 ± 7.9 | 1.57/1.52(1.0) | 19.1 /20.6 (0.9) |
| hrIFNγ | 100 | 29.1 ± 6.7 | 1.40/0.83(1.7)* | 19.4 / 5.10(3.8)* |
| | 10 | 6.4 ± 4.2 | 1.39/1.24(1.1) | 18.0 /13.4 (1.3)* |
| Mixture 1 | 1000+1000+100 | 33.3 ± 23.7 | 1.71/0.52(3.8) | 22.3 / 1.66(16.0)* |
| Mixture 2 | 100+100+10 | 20.6 ± 10.0 | 1.39/0.94(1.5)* | 19.2 / 4.64(4.2)* |
| WiDr: | | | | |
| hrTNFα | 1000 | >60 | | |
| | 100 | 48.0 ± 17.7 | 0.84/0.73(1.2)* | 27.4 /14.8 (2.0)* |
| hrIL-1α | 1000 | 13.6 ± 7.1 | 0.84/0.58(1.5)* | 27.1 /12.4 (2.3)* |
| | 100 | 12.6 ± 7.3 | 0.84/0.59(1.4)* | 27.8 /15.8 (1.8)* |
| hrIFNγ | 100 | >90 | | |
| | 10 | 17.3 ±11.9 | 0.83/0.63(1.3)* | 25.5 / 3.31(7.8)* |
| Mixture | 10+10+1 | 34.0 ± 2.1 | 0.81/0.46(1.8)* | 26.5 / 4.40(6.1)* |
| MKN45: | | | | |
| hrTNFα | 1000 | >90 | | |
| | 100 | 40.0 ± 10.6 | 0.13/0.07(1.9)* | 0.55/ 0.15(4.7)* |
| | 10 | 26.5 ± 11.5 | 0.15/0.09(1.7)* | 0.62/ 0.25(2.8)* |
| hrIL-1α | 1000 | 21.3 ± 9.8 | 0.13/0.09(1.4) | 0.55/ 0.21(3.5)* |
| | 100 | 16.5 ± 6.9 | 0.15/0.08(1.9)* | 0.62/ 0.27(2.3)* |
| hrIFNγ | 100 | 39.0 ± 14.7 | 0.13/0.05(2.7)* | 0.71/ 0.07(10.4)* |
| | 10 | 15.4 ± 5.1 | 0.15/0.07(2.1)* | 0.62/ 0.16(4.1)* |
| Mixture | 10+100+10 | 48.5 ± 12.3 | 0.17/0.07(2.8)* | 0.49/ 0.09(6.6)* |

Mixture of cytokines: hrTNFα, 10 U/ml, + hrIL-1α, 10 U/ml, + hrIFNγ, 1 U/ml (WiDr); hrTNFα, 10 U/ml, + hrIL-1α, 100 U/ml, + hrIFNγ, 10 U/ml (MKN45); hrTNFα, 1000 U/ml, + hrIL-1α, 1000 U/ml, + hrIFNγ, 100 U/ml (COLO201, mixture 1); hrTNFα, 100 U/ml, + hrIL-1α, 100 U/ml, + hrIFNγ, 10 U/ml (COLO201, mixture 2)

[a] The increase in the susceptibility to cytostatics ($IC_{50}$-fold) induced by the cytokines was expressed as described in the footnote to Table 1.

* $P < 0.05$

*Induction of TdR Pase activity by cytokines*

It can be assumed that the cytokines' preferential enhancement of the susceptibility of the cells to 5′-dFUrd results from an increase in TdR Pase activity, which is essential for the conversion of 5′-dFUrd to 5-FUra. Therefore, we measured the enzyme activity of cancer cells treated for 24 h with the cytokines. TNFα, IL-1α, IFNγ, and a mixture of all three enhanced the enzyme activity (Table 3). The activity was also considerably increased (up to 47-fold) by

**Table 3.** Induction of TdR Pase activity in human carcinoma cell lines and normal fibroblasts

| Cytokines (U/ml) | | TdR Pase activity (μg 5-FUra mg protein$^{-1}$ h$^{-1}$) | | | |
|---|---|---|---|---|---|
| | | COLO201 | WiDr | MKN45 | WI38 |
| Control | | 8.9 ± 0.5 | 3.3 ± 0.8 | 17.8 ± 1.7 | 11.7 ± 2.9 |
| hrTNFα | 100 | 7.3 ± 1.6(0.8) | 38.2 ± 3.0(11.6)* | 34.9 ± 1.9(2.0)* | N. T. |
| hrIL-1α | 100 | 6.3 ± 0.0(0.7) (*) | 8.9 ± 1.3(2.7)* | 28.0 ± 3.3(1.6)* | N. T. |
| hrIFNγ | 10 | 13.0 ± 0.7(1.5)* | 21.5 ± 4.6(6.5)* | 35.5 ± 4.4(2.0)* | N. T. |
| Mixture | 100+100+10 | 29.1 ± 1.1(3.3)* | 155.3 ± 14.0(47.1)* | 83.0 ± 3.8(4.7)* | 28.5 ± 0.3(2.4)* |

Mixture of cytokines: hrTNFα, 100 U/ml, + hrIL-1α, 100 U/ml, +hrIFNγ, 10 U/ml. Cells were exposed to cytokines for 24 h. N. T., Not tested

* $P < 0.05$

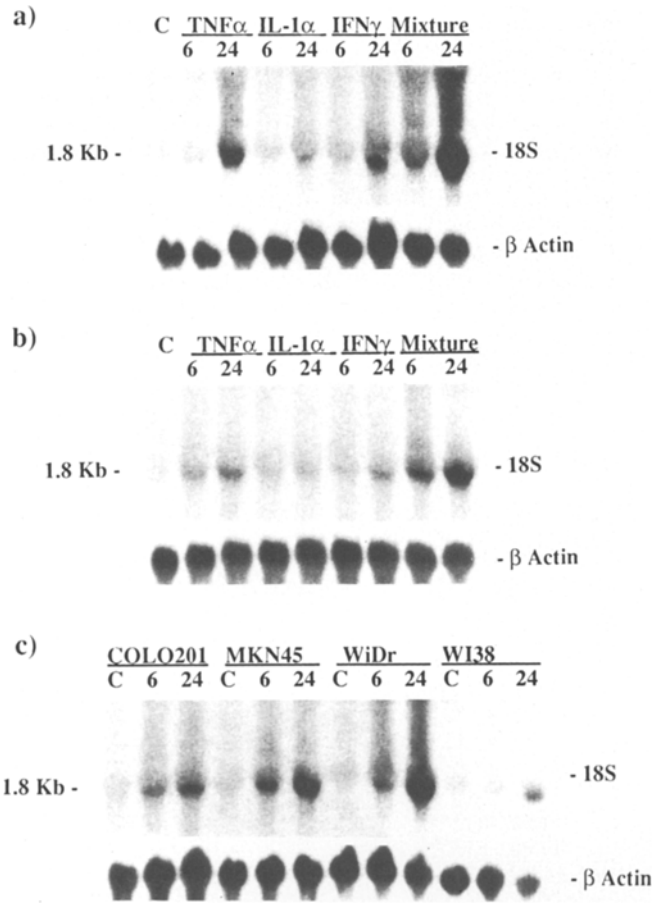


**Fig. 1 a–c.** Induction of TdR Pase mRNA expression by cytokines in human cancer cell lines and normal fibroblasts. **a** WiDr human colon carcinoma cells. **b** MKN45 human gastric carcinoma cells. **c** Three human carcinoma cells and normal fibroblasts. *C*, Control. Cells were exposed to the cytokines (hrTNFα, 100 U/ml; hrIL-1α, 100 U/ml; hrIFNγ, 10 U/ml; and a mixture of them) for 6 or 24 h (**a, b**) or to a mixture of cytokines (hrTNFα, 100 U/ml, +hrIL-1α, 100 U/ml, + hrIFNγ, 10 U/ml; **c**). Total RNA was extracted from cultured cells with guanidinium thiocyanate buffer (pH 7.0) containing 5.5 *M* guanidinium thiocyanate, 25 m*M* sodium citrate, 0.5% sodium *N*-lauroyl sarcosinate, and 0.2 *M* β-mercaptoethanol. The cell lysates were layered on a cushion of a cesium trifluoroacetate-ethylenediaminetetraacetic acid (EDTA) solution (4.15 *M* cesium trifluoroacetate, 0.1 *M* EDTA, pH 7.0, p = 1.50) and centrifuged at 25,000 rpm for 24 h. The pellets were dissolved in 4.0 *M* guanidinium thiocyanate buffer. After precipitation by ethanol, pellets were dissolved in TE buffer (10 m*M* TRIS-HCl and 1 m*M* EDTA, pH 7.5). RNA samples (20 μg/lane) were electrophoresed in a formaldehyde-denaturing agarose gel [1.0% agarose, 2.2 *M* formaldehyde, 20 m*M* 3-(*N*-morpholino) propanesulfonic acid (MOPS), 1 m*M* EDTA, 5 m*M* sodium acetate, pH 7.0] and blotted onto Hybond-N nylon membranes (Amersham, Buckinghamshire, UK). RNA on the membranes was prehybridized and then hybridized with TdR Pase cDNA in a buffer containing 5 × SSPE (900 m*M* NaCl, 50 m*M* sodium phosphate, 5 m*M* EDTA, pH 7.7), 5 × Denhardt's solution (0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone), 0.5% sodium dodecyl sulfate (SDS), and sonicated and denatured salmon-testis DNA (40 μg/ml). Hybridization was carried out at 65° C for 20 h. The membranes were washed in 2 × SSPE, 0.5% SDS for 30 min at 65° C and were then autoradiographed using Kodak XAR-2 film at –80° C in the presence of intensifying screens. We used the human β-actin cDNA probe as a positive control

the mixture of cytokines in WiDr cells, and the effect of the cytokines on enzyme induction appeared to be superadditive. Again, in WI-38 cells, the increase was only slight, even following exposure to the cytokine mixture (2.4-fold).

*Northern-blot analysis of TdR Pase mRNA induction by cytokines*

Enzyme induction by the cytokines was substantiated by the enhancement of mRNA expression as shown by Northern-blot analysis. Figure 1 demonstrates that in terms of levels of mRNA expression, TdR Pase was also induced by each of the three cytokines TNFα, IL-1α, and IFNγ, at least within 6 h after their addition, and that the effect of the cytokines was superadditive. On the other hand, TdR Pase induction was very low in the fibroblast cell line WI38, and the message was detected only at 24 h after the addition of the cytokine mixture (Fig. 1 c). The induction of TdR Pase as shown through mRNA expression coincided well with the observed increase in enzyme activity levels.

## Discussion

Several laboratories have demonstrated a marked synergistic effect between 5-FUra and cytokines such as TNFα and IFN [8, 15, 18, 24], although the mechanism of this synergism has not yet been fully clarified. Elias and Crissman [8] and Elias and Sandoval [9] have suggested that IFNα enhances the activity of 5-FUra through the augmentation of its inhibitory activity on thymidylate synthase, whereas Schwartz et al. [25] and Tevaearai et al. [29] have reported that IFNα enhances the antiproliferative activity of 5-FUra and 5′-dFUrd, respectively, through an increase in the activity of TdR Pase, which is one of the key enzymes in the salvage pathway for pyrimidine nucleotide biosynthesis. The present study showed that TNFα, IL-1α, and IFNγ greatly enhanced the antiproliferative activity of fluoropyrimidines, particularly 5′-dFUrd, also through the induction of TdR Pase as one of the mechanisms.

The induction of TdR Pase explains this perferential increase in the susceptibility of cancer cells to 5′-dFUrd fairly well, because conversion of this drug to 5-FUra can be accelerated by this enzyme. 5-FUra is further anabolized to 2′-deoxy-5-fluorouridine (2′-dFUrd) by TdR Pase and then to the active metabolite 5′-fluorodeoxyuridine monophosphate (FdUMP) by thymidine kinase. Therefore, the increase in 5-FUra susceptibility would also be due to the TdR Pase induction. The degree of the induction of TdR Pase activity by cytokines varied among the three cancer cell lines used in the present study. The cells not responding to cytokines may have previously secreted sufficient levels of the factors as autocrine factors or may not have expressed receptors for the factors. However, the preferential increase in 5′-dFUrd susceptibility induced by the cytokines was not always associated with TdR Pase induction. Other mechanisms by which the cytokines induce other enyzmes necessary for the conversion of 5-FUra to the active metabolites should exist.

Human TdR Pase has recently been reported to be identical to human PD-ECGF [10]. The internal 120 amino acid sequence and its corresponding DNA sequence of TdR Pase were identical to those of PD-ECGF. We confirmed that purified TdR Pase from the human colon cancer xenograft HCT116 has the same amino acid sequence at the N-terminal, with 9 amino acids having thus far been determined (data not shown). Therefore, we synthesized a cDNA probe of PD-ECGF (TdR Pase), with which the induction of TdR Pase mRNA by the cytokines TNFα, IL-1α, and IFNγ was clearly demonstrated in our Northern-blot hybridization analysis. In the human fibroblast cell line WI38, TdR Pase mRNA induction was not obvious. It was slightly induced only by the cytokine mixture. In addition, even the cytokine mixture could not increase the susceptibility of WI38 cells to 5′-dFUrd or 5-FUra. The control mechanisms for TdR Pase induction in cancer cells may be different from those for its induction in fibroblast cells.

Various cytokines are known to exert their biological activity through up- or down-regulation of common enzymes such as the induction of collagenase or the suppression of lipoprotein lipase [3, 7, 13, 19, 23, 26]. The present study shows that TdR Pase is one such enzyme commonly induced by cytokines (TNFα, IL-1α, and IFNγ). In separate experiments, we observed that other cytokines such as IFNα and IL-6 and the growth factor bFGF also induced the enzyme activity to a slight extent, whereas IL-2 did not induce it (data not shown). Additional cytokines and growth factors may exist that induce TdR Pase. TdR Pase may play some physiological role(s) in the responses of tumor cells to cytokines and, possibly, to growth factors. Since this enzyme is identical to PD-ECGF, which has angiogenic activity [11], it may be associated with cytokine- and growth factor-induced tumor angiogenesis.

Cytokines are produced by various cells such as lymphocytes, macrophages, granulocytes, fibroblasts, and endothelial cells, which exist in tumor tissues as infiltrated cells and stroma cells. Even tumor cells themselves are known to produce cytokines. Tumor cells in tissues that might be exposed to various cytokines would have higher levels of TdR Pase. We previously reported that TdR Pase activity was several times higher in human tumor tissues than in normal tissues adjacent to the tumor [12, 17]. The high enzyme activity in tumor tissues may partly be due to the induction of TdR Pase activity by inducers of the enzyme, although the tumor cell itself has higher levels of the enzyme activity than do normal cells. 5′-dFUrd, which is converted to 5-FUra by TdR Pase, would provide more therapeutic benefits if it were used in combination therapy with the TdR Pase-inducing cytokines.

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