

# X-Ray Irradiation Induces Thymidine Phosphorylase and Enhances the Efficacy of Capecitabine (Xeloda) in Human Cancer Xenografts

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## ABSTRACT

Thymidine phosphorylase (dThdPase) is an essential enzyme for the activation of the cytostatics capecitabine (*N*<sup>4</sup>-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine) and its intermediate metabolite 5'-deoxy-5-fluorouridine (5'-dFUrd) to 5-fluorouracil (5-FUra) in tumors. We observed previously that several cytokines and cytostatics up-regulated dThdPase expression and consequently enhanced the efficacy of capecitabine and 5'-dFUrd. In the present study, we found that X-ray irradiation also up-regulated dThdPase expression in several human cancer xenografts. A single-dose local irradiation at 5 Gy increased dThdPase levels by up to 13-fold at 9 days after the irradiation. Whole-body irradiation also up-regulated dThdPase in a tumor, but it did not increase the enzyme level in the liver. We also observed that the irradiation increased the levels of human tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), which is an up-regulator of dThdPase, prior to the dThdPase up-regulation. These results indicate that X-ray irradiation might increase dThdPase levels indirectly through the human TNF- $\alpha$  in the tumor tissue. In the WiDr colon and MX-1 mammary human cancer xenograft models, the combination of a single local X-ray irradiation with either capecitabine or 5'-dFUrd was much more effective than either radiation or chemotherapy alone. In contrast, treatment with X-ray irradiation and 5-FUra in combination showed no clear additive effects. Combined modality treatment of cancer patients with capecitabine and X-ray irradiation would have greater potential usefulness than conventional radiochemotherapy with 5-FUra.

## INTRODUCTION

Capecitabine is a novel fluoropyrimidine carbamate that is being used clinically for the treatment of breast cancer patients who have failed paclitaxel and anthracycline regimens (1), and

it is being assessed for the treatment of other types of cancer. It generates the active drug 5-FUra<sup>2</sup> selectively in tumors by three enzymes located in the liver and in tumors; the final step is the conversion of the intermediate metabolite 5'-dFUrd to 5-FUra by dThdPase in tumors (2, 3). This conversion appeared to be a rate-limiting step for the efficacy of capecitabine. We observed that the conversion was insufficient in a human cancer xenograft line, which was refractory to capecitabine in *in vivo* therapy (4), and that the susceptibility of human cancer xenografts to 5'-dFUrd correlated with their levels of dThdPase expression (5). Therefore, the efficacy of capecitabine and its intermediate 5'-dFUrd would be optimized by selecting patients who have tumors with high levels of dThdPase expression. Another useful approach for optimizing capecitabine and 5'-dFUrd therapy would be a combination therapy with their rational partners, such as up-regulators of dThdPase.

Previously, we reported that several cytokines, such as IL-1 $\alpha$ , TNF- $\alpha$ , IFN- $\gamma$  up-regulated dThdPase mRNA expression in human cancer cells and increased the susceptibility of the tumor cells to 5'-dFUrd (6). Furthermore, we observed in human cancer xenograft models that the anticancer drugs, such as paclitaxel and docetaxel, increased tumor levels of dThdPase expression and consequently showed a synergistic antitumor activity with capecitabine (7). We suggested that the taxanes might increase dThdPase level indirectly through the up-regulation of TNF- $\alpha$ , because the taxanes simultaneously increased tumor levels of human TNF- $\alpha$ . Several factors have been reported to up-regulate TNF- $\alpha$ . It is, therefore, of interest to investigate whether factors other than taxanes increase dThdPase levels in tumors and make the tumors more susceptible to capecitabine. In the present study, we found that X-ray irradiation, which is known to up-regulate TNF- $\alpha$  (8–10), indeed increased tumor levels of both TNF- $\alpha$  and dThdPase. Consequently, the efficacy of X-ray irradiation and either capecitabine or 5'-dFUrd in combination was much better than either treatment in human cancer xenograft models. We describe these results and discuss the potential of this rational combination therapy for cancer patients with either capecitabine or 5'-dFUrd and X-ray irradiation.

## MATERIALS AND METHODS

**Chemicals.** Capecitabine and its intermediate metabolite 5'-dFUrd were obtained from F. Hoffmann-La Roche (Basle, Switzerland). 5-FUra was purchased from Kyowa Hakko Co. (Tokyo, Japan). 2,2'-Anhydro-5-ethyluridine was synthesized by the method described elsewhere (11).

Capecitabine and 5'-dFUrd were dissolved or suspended in 40 mM citrate buffer (pH 6.0) containing 5% gum arabic as the

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<sup>2</sup> The abbreviations used are: 5-FUra, 5-fluorouracil; 5'-dFUrd, 5'-deoxy-5-fluorouridine; TNF, tumor necrosis factor; IL, interleukin.

vehicle and then administered p.o. to mice. 5-FUra was dissolved in saline and given i.p. The fluoropyrimidines used in combination therapy were given at their maximum tolerated doses (4, 5) as described in the tables.

**Animals.** Male and female BALB/c *nu/nu* mice, 5 or 6 weeks of age, were obtained from SLC, Inc. (Hamamatsu, Japan).

**Tumors.** The human cancer lines used were obtained from the following institutions: colon cancer WiDr, HT-29, and cervix cancer SIHA from the American Type Culture Collection (Rockville, MD); gastric cancer MKN-45 from Immunobiological Laboratories, (Fujioka, Japan); and mammary cancer MX-1 from Dr. T. Tashiro (Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan). MX-1 was maintained by continuous *in vivo* passage in BALB/c *nu/nu* mice. The other cancer cell lines were maintained in *in vitro* cultures.

**Human Cancer Xenograft Model.** A single-cell suspension ( $5$  to  $10 \times 10^6$  cells/mouse) of WiDr, HT-29, MKN-45, and SIHA or small pieces of MX-1 was inoculated s.c. into nude mice. The experiments were started when the tumor volume reached  $\sim 0.2$ – $0.5$  cm<sup>3</sup>. The number of animals in each experiment group was specified in the legend of each table and figure. To evaluate the antitumor effect of X-ray irradiation, we measured tumor size and body weight at the time the tumor tissues were excised for measuring dThdPase levels. The tumor volume was estimated by using the following equation,  $V = ab^2/2$ , where  $a$  and  $b$  are tumor length and width, respectively. The tumor volume change was calculated as expressed by the formula  $V_T - V_O$ , where  $V_T$  is the mean volume on any given day and  $V_O$  is the mean volume at the start of the treatment. To evaluate the efficacy of X-ray irradiation or X-ray irradiation combined with fluoropyrimidines, tumor size and body weight were measured twice a week. Carcass body weight was calculated by subtracting the tumor weight, which was estimated from tumor volume, from the body weight. The tumor volume was plotted as a function of time. From these lines, mean estimates were made of growth delay ( $T_{2.5}$ ), where  $T_{2.5}$  is the time taken for a tumor to grow 2.5-fold of the volume on the first day of treatment.

All animal experiments were conducted in accordance with the "Guidelines for the Care and Use of Laboratory Animals in the Nippon Roche Research Center."

**X-Ray Irradiation.** Local X-ray irradiation with tumor or whole-body irradiation was carried out by using an X-ray unit (Hitachi X-ray unit, model MBR-1520R with 0.5 mm Al/0.1 mm Cu filter, 100 kVp, 1.33 Gy/min). Mice bearing the tumors were injected i.p. with pentobarbital (45–55 mg/kg) as an anesthetic agent 20 min before the irradiation to be immobilized. In combination therapy, either capecitabine, 5'-dFUrd, 5-FUra, or the vehicle was administered 1 h before irradiation.

**dThdPase Assays.** Tissues were homogenized in 10 mM Tris buffer (pH 7.4) containing 15 mM NaCl, 1.5 mM MgCl<sub>2</sub>, and 50  $\mu$ M potassium phosphate with a glass homogenizer. The homogenate of tumor tissues was then centrifuged at  $10,000 \times g$  for 20 min at 4°C, and the supernatants were stored at  $-80^\circ\text{C}$  until use. For the measurement of dThdPase activity in the liver, the homogenate of liver was centrifuged at  $105,000 \times g$  for 90 min at 4°C. The supernatants were then dialyzed overnight at

4°C against 20 mM potassium phosphate buffer (pH 7.4) containing 1 mM  $\beta$ -mercaptoethanol and stored at  $-80^\circ\text{C}$  until use. The protein concentration was determined by the methods of Lowry *et al.* (12). The dThdPase level of tumors was measured by ELISA with monoclonal antibodies specific to human dThdPase, as described previously by Nishida *et al.* (13). One unit corresponds to the dThdPase level of the standard enzyme solution (extracts of human colon cancer xenograft HCT 116), which phosphorylates 5'-dFUrd to 5-FUra at the rate of 1  $\mu$ g 5-FUra/h, and the dThdPase levels measured correlated well with those measured by an enzyme activity assay (6, 13). The monoclonal antibodies were not cross-reacted with mouse dThdPase. Because uridine phosphorylase also phosphorylates 5'-dFUrd to 5-FUra, mouse dThdPase activity in the livers was measured by the method described by Eda *et al.* (6), in the presence of 1 mM 2, 2'-anhydro-5-ethyluridine, an uridine phosphorylase-specific inhibitor (14).

**TNF- $\alpha$  Assays.** The supernatants prepared from tumor tissues were examined for their levels of TNF- $\alpha$  with the ELISA method. Human TNF- $\alpha$  was measured using the Quantikine ELISA kit (R&D systems). The ELISA assay is specific for only human TNF- $\alpha$ .

**Statistical Analysis.** Tumor volume change, carcass body weight, and levels of dThdPase were analyzed using Student's *t* test or the Mann-Whitney *U* test, as described in the legends of the tables and figures. Differences were considered to be significant when  $P < 0.05$ .

## RESULTS

**dThdPase and TNF- $\alpha$  Up-Regulation.** Table 1 shows the expression of dThdPase levels in tumor tissues after single-dose local X-ray irradiation at 2.5 or 5 Gy in five human cancer xenograft models. An up-regulation of dThdPase was observed in four of the five human cancer xenograft models studied. We further investigated kinetics of the dThdPase up-regulation by single-dose local X-ray irradiation at 2.5 or 5 Gy in the WiDr human colon tumor xenograft model (Fig. 1). The dThdPase levels in the tumor tissue were maximally increased on day 9 and then gradually decreased up to day 18. After the irradiation, increased tumor levels of human TNF- $\alpha$  expression first were seen, which appears to be followed by the dThdPase up-regulation (Fig. 2). Because the dThdPase and TNF- $\alpha$  levels were detected by the ELISA with monoclonal antibodies specific to human dThdPase and TNF- $\alpha$ , respectively, the measured levels of these two factors corresponded to those of the factors produced in the human cancer cells. The dThdPase up-regulation correlated with the TNF- $\alpha$  up-regulation in each mouse studied (Fig. 3). X-ray irradiation might increase dThdPase levels indirectly through the TNF- $\alpha$  up-regulation. In contrast, human IL-1 $\alpha$ , which also up-regulates dThdPase, was not elevated by the irradiation (data not shown).

**Tumor Preferential dThdPase Up-Regulation.** To investigate whether X-ray irradiation up-regulates dThdPase in normal organs as well as in tumors, we compared the enzyme activity in tumors and in the liver, where the enzyme activity is the highest among normal organs, after the whole-body X-ray irradiation. On day 6 after the irradiation at 5 Gy in mice bearing the WiDr human colon cancer xenograft ( $n = 3$ ), the human

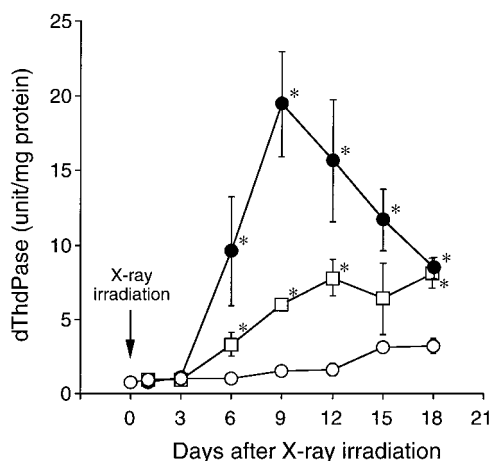
**Table 1** Effect of X-ray irradiation on up-regulation of dThdPase and tumor growth inhibition in several human cancer xenografts

Mice were given single-dose local X-ray irradiation on day 0. Tumor volume was measured on day 6 or day 9 after X-ray irradiation, and tumor tissues ( $n = 3$ ) were excised to measure the levels of dThdPase.

Tumor	Radiation dose (Gy)	dThdPase (units/mg protein) Mean $\pm$ SD		Tumor volume change (mm <sup>3</sup> ) Mean $\pm$ SD	
		on day 6	on day 9	on day 6	on day 9
WiDr	Control	1.0 $\pm$ 0.2	1.5 $\pm$ 0.2	379 $\pm$ 190	490 $\pm$ 149
	2.5	3.3 $\pm$ 0.8 <sup>a</sup>	6.0 $\pm$ 0.3 <sup>a</sup>	255 $\pm$ 27	489 $\pm$ 168
	5	9.6 $\pm$ 3.7 <sup>a</sup>	19.5 $\pm$ 3.5 <sup>a</sup>	242 $\pm$ 44	253 $\pm$ 41
HT-29	Control	1.8 $\pm$ 0.1	1.7 $\pm$ 0.1	198 $\pm$ 14	327 $\pm$ 39
	2.5	5.2 $\pm$ 0.4 <sup>a</sup>	4.8 $\pm$ 0.4 <sup>a</sup>	201 $\pm$ 33	233 $\pm$ 43 <sup>a</sup>
	5	10.1 $\pm$ 1.8 <sup>a</sup>	8.5 $\pm$ 0.8 <sup>a</sup>	97 $\pm$ 77	181 $\pm$ 40 <sup>a</sup>
MKN-45	Control	2.8 $\pm$ 0.1	2.8 $\pm$ 0.4	351 $\pm$ 61	399 $\pm$ 58
	2.5	3.8 $\pm$ 0.3 <sup>a</sup>	3.6 $\pm$ 0.2 <sup>a</sup>	225 $\pm$ 108	340 $\pm$ 99
	5	4.6 $\pm$ 0.7 <sup>a</sup>	4.5 $\pm$ 0.3 <sup>a</sup>	241 $\pm$ 27 <sup>a</sup>	273 $\pm$ 37 <sup>a</sup>
MX-1	Control	6.8 $\pm$ 1.1	ND <sup>b</sup>	1117 $\pm$ 246	ND
	2.5	18.0 $\pm$ 4.8 <sup>a</sup>	ND	540 $\pm$ 113 <sup>a</sup>	ND
	5	27.9 $\pm$ 4.8 <sup>a</sup>	ND	322 $\pm$ 202 <sup>a</sup>	ND
SIHA	Control	60.6 $\pm$ 6.1	60.0 $\pm$ 11.3	244 $\pm$ 104	489 $\pm$ 152
	2.5	67.3 $\pm$ 3.8	59.4 $\pm$ 3.3	144 $\pm$ 170	264 $\pm$ 88
	5	55.3 $\pm$ 11.5	59.9 $\pm$ 4.9	91 $\pm$ 48	64 $\pm$ 18 <sup>a</sup>

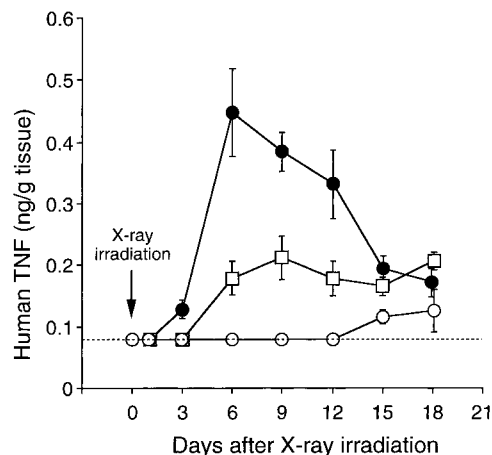
<sup>a</sup> Significantly different from each control;  $P < 0.05$  by Student's  $t$  test.

<sup>b</sup> ND, not done.



**Fig. 1** Effect of local X-ray irradiation on dThdPase up-regulation of the WiDr human colon cancer tumor in nude mice. Mice bearing WiDr tumors were randomized into groups of three mice each, and single-dose local X-ray irradiation was given on day 0. Mice were sacrificed, and tumor tissues were excised at various days after X-ray irradiation. Data points, mean dThdPase values; bars, SD. \*, significantly different from control at each points;  $P < 0.05$  by Student's  $t$  test.  $\circ$ , control;  $\square$ , 2.5 Gy;  $\bullet$ , 5 Gy.

dThdPase levels of tumor tissues were measured by the ELISA with antihuman dThdPase antibody, whereas the mouse dThdPase in the liver was measured by an enzyme activity assay method instead, because an ELISA for mouse dThdPase is not available. X-ray irradiation increased the level of dThdPase expression in the tumor by 9.4-fold as compared with that in the control group (control;  $0.8 \pm 0.0$  unit/mg protein, radiation 5 Gy;  $7.5 \pm 0.7$  units/mg protein). In contrast, it did not increase the dThdPase activity in the liver (control;  $5.5 \pm 0.7$   $\mu$ g 5-FUra/mg protein/h, radiation 5 Gy;  $5.3 \pm 0.6$   $\mu$ g 5-FUra/mg protein/h). dThdPase up-regulation by X-ray irradiation would be tumor selective or human cancer xenograft selective.



**Fig. 2** Effect of local X-ray irradiation on human TNF- $\alpha$  of the WiDr human colon cancer tumor in nude mice. The samples used in the experiment shown Fig. 1 were examined for their levels of human TNF- $\alpha$ . Data points, mean human TNF- $\alpha$  values; bars, SD. Human TNF- $\alpha$  levels below the detection limit were assigned as 80 pg/g of tissue.  $\circ$ , control;  $\square$ , 2.5 Gy;  $\bullet$ , 5 Gy.

**Combination Therapy.** It is expected that the preferential effect of X-ray irradiation on enzyme up-regulation in tumor tissues would result in increasing their susceptibility to capecitabine treatment. To ascertain this possibility, we conducted combination therapy with local X-ray irradiation and capecitabine in the WiDr human colon cancer xenograft, which is a cell line refractory to fluoropyrimidines because of low dThdPase expression. Capecitabine, 5'-dFUrd, and 5-FUra were given at their maximum tolerated dose, whereas the local X-ray irradiation was given at 5 Gy once. The efficacy of local X-ray irradiation in combination with either capecitabine or 5'-dFUrd was much higher than that of either treatment, and tumor regression was observed only in the combination groups (Table 2).

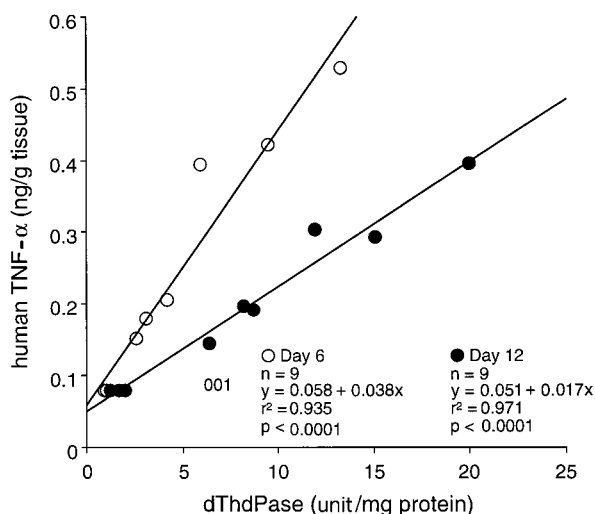


Fig. 3 Correlation between dThdPase and human TNF- $\alpha$  of the WiDr tumor tissues after local X-ray irradiation. Data points, data on days 6 and 12 from individual mice tested in the experiment shown Figs. 1 and 2. Significance was calculated by assigning the value for the human TNF- $\alpha$  level below the detection limit as 80 pg/g of tissue. ○, day 6; ●, day 12.

When delay of tumor growth ( $T_{2.5}$ ) is compared, the efficacy of the combination appears to be more than just additive. In contrast, local X-ray irradiation and 5-FUra in combination showed no clear additive activity. On the other hand, toxicity in terms of weight loss does not appear to be increased in mice given local X-ray irradiation and capecitabine, and the degree of toxicity was similar to that in mice given local X-ray irradiation and 5-FUra. Local X-ray irradiation and either capecitabine or 5'-dFUrd in combination also showed much better antitumor activity than either treatment in the other tumor model studied, the MX-1 human mammary cancer xenograft model (Table 3).

## DISCUSSION

Previously, we suggested that some anticancer drugs, such as taxanes, cyclophosphamide, and mitomycin C, up-regulated dThdPase and TNF- $\alpha$  expression in the WiDr human colon cancer (7). dThdPase is an enzyme essential for the activation of the anticancer drug capecitabine and its intermediate 5'-dFUrd to 5-FUra (2). These anticancer drugs and the others, such as taxanes, therefore showed a synergistic anticancer efficacy in human cancer xenograft models (7). The present study showed that another TNF- $\alpha$  up-regulator, X-ray irradiation, also increased levels of dThdPase in tumors in human cancer xenograft models. Interestingly, the whole-body X-ray irradiation increased tumor levels of dThdPase, but it did not increase the enzyme levels in the liver, a normal organ. Similarly, as was the case with the taxanes (7), the up-regulation of dThdPase by X-ray irradiation appeared to be tumor selective, although the mechanism of the selective dThdPase up-regulation has not yet been clarified. Environmental factors specific to the tumor tissue, such as inflammatory cell infiltration and local cytokine production, might be involved in tumor-selective up-regulation.

The up-regulation of dThdPase was preceded by the

TNF- $\alpha$  up-regulation in the X-ray irradiated mice as well as by the anticancer drug taxanes in the previous study (7), and the degree of the up-regulation of TNF- $\alpha$  was correlated with the dThdPase up-regulation in each mouse studied. In one human cancer xenograft model studied (SIHA cervical cancer), dThdPase levels were not increased after the X-ray irradiation, and human TNF- $\alpha$  levels were below the detection limit (data not shown). We have reported that several cytokines up-regulate dThdPase, such as TNF- $\alpha$ , IL-1 $\alpha$ , and IFN- $\gamma$  *in vitro* (6). These results indicate that X-ray irradiation might up-regulate dThdPase indirectly through the human TNF- $\alpha$  in the tumor tissue, although the mechanism of dThdPase up-regulation should be investigated further. X-ray irradiation was reported to up-regulate IL-1 in cell cultures (15). However, it is not likely to be involved in the up-regulation of dThdPase in the present study, because tumor levels of IL-1 $\alpha$  was not changed at 1–18 days after the X-ray irradiation (data were not shown). X-ray irradiation was also reported to induce TNF- $\alpha$  for 20 h in tumor cell cultures (8). The up-regulation of TNF- $\alpha$  in the WiDr tumors was, however, observed at several days after X-ray irradiation. X-ray irradiation might affect host stromal cells in tumor tissues, resulting in the up-regulation of TNF- $\alpha$  in tumor cells and consequently that of dThdPase. These processes may take 6 days. We also observed a similar delay in the dThdPase up-regulation by taxanes (7). Either docetaxel or paclitaxel up-regulated both TNF- $\alpha$  and dThdPase in human cancer xenografts at 4 or 6 days after their administration.

The efficacy of X-ray irradiation combined with either capecitabine or 5'-dFUrd was much higher than that of either treatment alone in the human cancer xenograft models studied. Their efficacy in tumor growth inhibition appeared to be additive, but the combination delayed tumor growth to a much greater extent than did either treatment alone. X-rays might also enhance the efficacy of capecitabine and 5'-dFUrd through the up-regulation of dThdPase in tumors. Previously, we observed similar results showing that the efficacy of X-ray irradiation and 5'-dFUrd in combination was synergistic in the mice bearing murine colon 26 carcinoma (16). X-ray irradiation would be a rational partner of chemotherapy with capecitabine and with its intermediate metabolite 5'-dFUrd. In contrast, although 5-FUra has been reported to enhance cell killing by radiation in some *in vitro* studies and in several animal tumor models (17–19), the efficacy of 5-FUra and radiation had no clear additive effect in the present study. On the basis of the prior studies, 5-FUra has been used in combination therapy with radiation for the treatment of squamous cell carcinoma (20). From the present study, we may conclude that combined modality treatment of cancer patients with either capecitabine or 5'-dFUrd and X-ray would have greater potential usefulness than the conventional radio-chemotherapy with 5-FUra.

In these days, suicide gene therapy of cancer, which consists of gene transfection for a prodrug activation enzyme and therapy with the prodrug, has been investigated, such as the combination with the cytosine deaminase gene and 5-fluorocytosine and those with the herpes simplex virus thymidine kinase gene and ganciclovir (21–23). In this approach, the prodrugs are expected to be activated to their corresponding active drugs only in cells transfected with the

**Table 2** Antitumor activity of the combination of X-ray irradiation and fluoropyrimidines on the growth of the WiDr human colon cancer xenograft in nude mice

Mice bearing WiDr tumors were randomized into groups of seven mice each, and treatment was started 18 days after the tumor inoculation. The single-dose local tumor irradiation at 5 Gy was given on the first day of treatment (day 0). Fluoropyrimidines were administered p.o. or i.p. at their maximum tolerated doses daily from day 0 for 21 days.  $T_{2.5}$  is the time taken for a tumor to grow to 2.5-fold of the volume on day 0.

Drug mg/kg/day (mmol/kg/day)	Radiation	Efficacy after 3 weeks of treatment (on day 21)				
		Tumor volume change (mm <sup>3</sup> ), Mean ± SD	% of tumor growth inhibition	Carcass body weight (g) Mean ± SD	$T_{2.5}$ (day)	Growth delay <sup>a</sup> (day)
Vehicle	—	700 ± 147	—	22.3 ± 1.7	12.6	—
Capecitabine	539 (1.5)	391 ± 176 <sup>b</sup>	44	21.2 ± 2.8	21.4	8.8
5'-dFUrd	185 (0.75)	567 ± 95 <sup>c</sup>	19	21.0 ± 1.2	14.8	2.2
5-FUra	19.5 (0.15)	568 ± 100 <sup>c</sup>	19	21.6 ± 1.6	14.6	2.0
Vehicle	+ 5 Gy	300 ± 103 <sup>b</sup>	57	23.3 ± 3.0	23.8	—
Capecitabine	539 (1.5)	-77 ± 47 <sup>b,d</sup>	111	22.1 ± 1.6	>42.0	>18.2
5'-dFUrd	185 (0.75)	53 ± 55 <sup>b,d</sup>	92	20.3 ± 2.2 <sup>b</sup>	39.7	15.9
5-FUra	19.5 (0.15)	247 ± 130 <sup>b,d</sup>	65	20.4 ± 2.1	26.3	2.5

<sup>a</sup> Difference in the  $T_{2.5}$  day between each fluoropyrimidine-treated group and its respective vehicle control.

<sup>b</sup> Significantly different from the vehicle group;  $P < 0.05$  by the Mann-Whitney  $U$  test.

<sup>c</sup> Significantly different from the group treated with radiation alone;  $P < 0.05$  by the Mann-Whitney  $U$  test.

<sup>d</sup> Significantly different from the group treated with fluoropyrimidine alone;  $P < 0.05$  by the Mann-Whitney  $U$  test.

**Table 3** Antitumor activity of the combination of X-ray irradiation and fluoropyrimidines on the growth of the MX-1 human mammary cancer xenograft in nude mice

Mice bearing MX-1 tumors were randomized into groups of seven mice each, and treatment was started 17 days after tumor inoculation. The single-dose local tumor irradiation at 5 Gy was given on the first day of treatment (day 0). Fluoropyrimidines were administered p.o. or i.p. at their maximum tolerated doses daily from day 0 for 21 days.  $T_{2.5}$  is the time taken for a tumor to grow to 2.5-fold of the volume on day 0.

Drug mg/kg/day (mmol/kg/day)	Radiation	Efficacy after 3 weeks of treatment (on day 21)				
		Tumor volume change (mm <sup>3</sup> ), Mean ± SD	% of tumor growth inhibition	Carcass body weight (g) Mean ± SD	$T_{2.5}$ (day)	Growth delay <sup>a</sup> (day)
Vehicle	—	3480 ± 982	—	19.7 ± 1.3	6.5	—
Capecitabine	539 (1.5)	1768 ± 252 <sup>b</sup>	49	20.0 ± 1.4	9.4	2.9
5'-dFUrd	185 (0.75)	1580 ± 255 <sup>b</sup>	55	19.2 ± 1.0	11.0	4.5
5-FUra	19.5 (0.15)	2705 ± 703 <sup>c</sup>	22	19.3 ± 1.6	6.5	0.0
Vehicle	+ 5 Gy	1670 ± 713 <sup>b</sup>	52	20.1 ± 1.2	10.9	—
Capecitabine	539 (1.5)	393 ± 416 <sup>b,d</sup>	89	21.5 ± 1.2 <sup>b</sup>	27.2	16.3
5'-dFUrd	185 (0.75)	274 ± 483 <sup>b,d</sup>	92	19.7 ± 1.0	26.6	15.7
5-FUra	19.5 (0.15)	1631 ± 410 <sup>b,d</sup>	53	19.6 ± 1.6	12.6	1.7

<sup>a</sup> Difference in the  $T_{2.5}$  day between each fluoropyrimidine-treated group and its respective vehicle control.

<sup>b</sup> Significantly different from the vehicle group;  $P < 0.05$  by the Mann-Whitney  $U$  test.

<sup>c</sup> Significantly different from the group treated with radiation alone;  $P < 0.05$  by the Mann-Whitney  $U$  test.

<sup>d</sup> Significantly different from the group treated with fluoropyrimidine alone;  $P < 0.05$  by the Mann-Whitney  $U$  test.

prodrug activation enzymes. The combination with dThdPase and capecitabine therapy would be an additional candidate for suicide gene therapy. However, this approach has not yet become reality, because a technology of gene transfection to tumor cells in tissues has not yet been established. In the present study, we demonstrated that X-ray irradiation induced the prodrug activation enzyme dThdPase selectively in tumor tissues. The combination with the dThdPase up-regulation by X-ray irradiation and capecitabine therapy would be an alternative for suicide gene therapy.

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# Clinical Cancer Research

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