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Adenoviral-mediated transfer of *Escherichia coli* uracil phosphoribosyltransferase (*UPRT*) gene to modulate the sensitivity of the human colon cancer cells to 5-fluorouracil

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Abstract

5-Fluorouracil (5-FU) has been used as a chemotherapeutic drug for colorectal cancer. *Escherichia coli* uracil phosphoribosyltransferase (UPRT), a pyrimidine salvage enzyme, converts 5-FU into 5-fluorouridine monophosphate (5-FUMP) at the initial step of 5-FU activation. We investigated the effects of adenoviral-mediated transfer of the *E. coli UPRT* gene into human colon cancer cells on 5-FU metabolism and 5-FU chemosensitivity. Three cell lines were used (HT29, KM12 and SW1116). The intracellular levels of 5-fluorodeoxyuridine monophosphate (5-FdUMP) and 5-FU incorporated into RNA after 5-FU treatment in cells infected with adenovirus containing the *UPRT* gene (AdCA-*UPRT*) were significantly higher than those of non-infected cells. This was accompanied by marked inhibition of thymidylate synthase (TS) in all cell lines. Furthermore, HT29, KM12 and SW1116 infected with AdCA-*UPRT* were, respectively, 13.1-, 30.2- and 70.5-fold more sensitive to 5-FU than non-infected cells. Most importantly, treatment with AdCA-*UPRT* and 5-FU effectively inhibited the growth of HT29-xenografted subcutaneous tumours in nude mice. Therefore, AdCA-*UPRT*/5-FU treatment had the potential to enhance the actions of 5-FU at both the DNA and RNA levels. Treatment augmented the sensitivity of human colon cancer cells to 5-FU both *in vitro* and *in vivo*. We conclude that adenoviral-mediated transfer of the *E. coli UPRT* gene into colon cancer cells can achieve biochemical modulation of 5-FU and this provides a new approach in the treatment of colorectal cancer.

Keywords: Uracil phosphoribosyltransferase; Gene therapy; Adenoviral vector; 5-Fluorouracil

1. Introduction

5-Fluorouracil (5-FU) is a pyrimidine anti-metabolite that has been used as a chemotherapeutic agent for the treatment of colorectal cancer [1]. 5-FU requires enzymatic conversion to a nucleotide, ribosylation and phosphorylation to achieve cytotoxic activity. There are two major active metabolites: 5-fluorodeoxyuridine monophosphate (5-FdUMP) and 5-fluorouridine triphosphate (5-FUTP). More than one metabolic pathway to activate 5-FU has been found. 5-FU is anabolised to 5-fluorouridine monophosphate (5-FUMP) directly by orotate phosphoribosyltransferase (OPRT) or by a combination of uridine phosphorylase and uridine kinase. 5-FUMP is further anabolised to 5-FdUMP and 5-FUTP by subsequent enzyme reactions. 5-FU can also be anabolised to 5-fluorodeoxyuridine by thymidine phosphorylase. 5-Fluorodeoxyuridine is directly converted by thymidine kinase to 5-FdUMP, which blocks the catalytic activity of thymidylate synthase (TS) by forming a covalent ternary complex with 5,10-methylenetetrahydrofolate, which inhibits DNA synthesis [2]. 5-FUTP is incorporated into RNA (F-RNA), resulting in RNA dysfunction [3]. These mechanisms are critical in the tumour-killing effect of 5-FU. Accordingly, 5-FU exerts its cytotoxicity through the actions of its metabolites at both the DNA and RNA levels.

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In contrast, 5-FU is rapidly catabolised to alpha-fluoro-beta-alanine after contact with dihydropyrimidine dehydrogenase (DPD) [4]. DPD is the initial, rate-limiting enzyme in the catabolism of 5-FU and has an important role in regulating the availability of 5-FU for anabolism. In fact, it has been reported that high intratumoural expression of TS or DPD is associated with a low response to 5-FU therapy [5]. Consequently, the efficacy of 5-FU therapy for advanced colorectal cancer was limited to 10–15% [4,6].

Elucidation of the mechanism of 5-FU activation has led to the development of a biochemical modulation therapy [7]. The idea is to combine fluoropyrimidine with a TS or DPD inhibitor, such as 5-FU/leucovorin [8,9] or S-1 [10], respectively. These new strategies increased not only the antitumour effect of 5-FU, but also the adverse side-effects, resulting in their efficacy being limited to approximately 30–40%. Moreover, the antitumour effects were partial and temporary. These approaches in the treatment of unresectable and metastatic colorectal cancer, therefore, remain unsatisfactory.

Most recently, gene therapy has offered an alternative approach to cancer treatment. Investigators have used suicide gene therapies [11–17], in which viral vectors are utilised to transfer genes encoding drug-activating enzymes. Transduction of the *Escherichia coli* cytosine deaminase (*CD*) gene into tumour cells followed by the administration of 5-fluorocytosine (5-FC), the so-called *CD*/5-FC system, is one example. CD converts the non-toxic prodrug 5-FC to the cytotoxic agent 5-FU so the *CD*/5-FC system could produce a high concentration of 5-FU in cells expressing the *CD* gene and the local milieu. Thus, the *CD*/5-FC system has the potential to overcome the low therapeutic index of 5-FU and reportedly, is effective *in vitro* and *in vivo* [11,13,17]. However, despite *CD* expression, a number of tumour cells were 5-FC-resistant, which may be attributable to the lack of anabolizing enzymes of 5-FU and the degradation of the formed 5-FU by DPD.

This led us to the idea of gene therapy using a 5-FU anabolising enzyme gene to enhance the antitumour effect of 5-FU, without increasing its adverse side-effects. *E. coli* uracil phosphoribosyltransferase (UPRT) is a pyrimidine salvage enzyme, corresponding to OPRT in mammalian cells. It converts 5-FU into 5-FUMP in the presence of 5-phosphoribosyl-α-1-diphosphate (PRPP) at the initial step of 5-FU activation [18]. Kanai and colleagues [19] have reported that adenoviral-mediated delivery of the *UPRT* gene sensitised cancer cells to 5-FU. Additionally, Inaba and colleagues [20] have shown that AdCA-*UPRT* infection increased the intracellular level of 5-FU nucleotides compared with AdCA-*lacZ* infection in human gastric cancer cells. However, the effect of the AdCA-*UPRT*/5-FU system on DNA- and RNA-directed action of 5-FU in human cancer cells remains unclear.

In the present study, we aimed to investigate the effect of treatment with or without adenoviral-mediated transfer of the *E. coli UPRT* gene on DNA and RNA by quantifying the intracellular levels of FdUMP and F-RNA, and the per cent TS inhibition in 5-FU-treated human colon cancer cells. Furthermore, the cytotoxic effects of the *UPRT*/5-FU system on cell growth *in vitro*, as well as *in vivo* treatment using nude mice bearing HT29-xenograft subcutaneous (s.c.) tumours, were studied.

2. Materials and methods

2.1. Cell culture

Human colon cancer cell lines HT29, KM12 and SW1116 and human embryonal kidney cell line 293 were cultured in Dulbecco's modified Eagle's medium (DMEM/F12): Nutrient Mixture F-12, Ham 1:1 containing 10% fetal bovine serum at 37°C in a humidified atmosphere of 5% carbon dioxide and 95% air.

2.2. Recombinant adenoviral preparation

Two replication-defective recombinant adenoviruses, AdCA-*lacZ* (containing the *E. coli β-galactosidase* gene) and AdCA-*UPRT* (containing the *E. coli UPRT* gene), were constructed from human adenoviral serotype 5 using homologous recombination as previously described [12,19,21,22]. The *UPRT* gene was driven by the CAG promotor (composed of a cytomegalovirus immediate early enhancer and a chicken *β-actin* promotor) [23]. In brief, the *UPRT* (*upp*) gene (GenBank accession number X57104) was amplified from *E. coli* K12 DHa F′ genomic DNA by polymerase chain reaction (PCR) [19] and its gene fragment was subcloned into the *EcoRI* and *BamHI* sites of pBluescript II SK(+) (Stratagene Corp., La Jolla, CA, USA), resulting in pBluescript II SK(+)-*upp*. The *EcoRI* and *BamHI*-digested *upp* DNA fragment from pBluescript II SK(+)-*upp* was subcloned into the *EcoRI* and *BglII* sites of pCAGGS [23], resulting in pCA-*UPRT*. The CAG-*UPRT*-poly(A) expression cassette was cloned into the *SwaI* site of the pAxcw cosmid [21,22], resulting in pAxCA-*UPRT*. A recombinant adenovirus with the *lacZ* gene under the control of the CAG promoter, AxCA*lacZ* (AdCA–*lacZ*), was constructed as previously described [21]. To produce recombinant adenoviruses, cosmids bearing the expression cassette were cotransfected into 293 cells together with the adenoviral DNA-terminal protein complex digested at the *NsiI* sites to allow homologous recombination, followed by replication and encapsidation of the recombinant adenoviral DNA into the infectious virions [22]. Incorporation of the expression cassette into the isolated

recombinant virus was confirmed by digestion with restriction enzymes [22]. The recombinant viruses were propagated subsequently in 293 cells and harvested by sonication. The viral solutions were stored at −80°C until use. Viral titres were determined by plaque assay using 293 cells [21,22]. Viral stocks used in these experiments did not contain detectable levels of replication-competent virus, as determined by PCR analysis using two pairs of primers specific to adenoviral *E1A* DNA, with coamplification of the *E2B* DNA that was used as an internal control [24].

2.3. In vitro *adenoviral-mediated* lacZ *expression*

During the exponential growth phase, colon cancer cells were plated in 6-well culture plates (Iwaki glass, Tokyo, Japan) at a density of 5×10^5 cells per well 24 h before AdCA-*lacZ* infection. Immediately before infection, the culture medium was aspirated, and suspensions of adenovirus in various amounts (0–100 multiplicity of infection (MOI)) were distributed over the monolayers. After a 24-h incubation, β-D-galactosidase expression was evaluated using X-gal as the substrate as previously described [22]. Blue precipitate in the cell nucleus indicated β-D-galactosidase expression. We used each adenovirus vector at an MOI of 10 in *in vitro* studies for the three human colon cancer cell lines and this was shown to have satisfactory gene transfer without any significant cytotoxicity, as revealed by the AdCA-*lacZ* assay [25] (data not shown).

2.4. Determination of 5-FU metabolism in *AdCA*-UPRT *infected cells*

DNA- and RNA-directed actions of 5-FU were evaluated by quantifying the intracellular levels of 5-FdUMP and F-RNA in adenoviral-infected, or non-infected cells. Ten million cells were infected with either AdCA-*UPRT* or AdCA-*lacZ* at a MOI of 10. After 24 h of incubation, the medium was replaced with fresh medium containing 1 μM of 5-FU and the cells were incubated at 37°C for another 24 h. The cells were then collected and the intracellular levels of 5-FdUMP and F-RNA were determined by the modified Moran and Spears' method [26] and gas chromatography coupled to mass spectrometry [27], respectively.

2.5. Analysis of TS inhibition

TS inhibition was studied in four groups: 5-FU alone (control), leucovorin/5-FU, AdCA-*lacZ*/5-FU, and AdCA-*UPRT*/5-FU. Ten million cells were cultured at 37°C for 24 h. The medium was then replaced with fresh medium containing 5-FU (1 μM) with or without leucovorin. Leucovorin (100 μM) did not show significant cytotoxicity in the three colon cancer cell lines, as revealed by growth assays (data not shown). After another 24-h period of incubation, the cells were collected and TS assays performed by the modified Moran and Spears' method [28]. In brief, an excess amount of [^{3}H]-FdUMP was added to the samples to determine TS activity by binding assay. The samples were homogenised in 200 mM Tris–HCl, 10 mM 2-mercaptoethanol, 100 mM NaF and 15 mM cytidine monophosphate. The suspension was centrifuged (25 000*g*, 60 min). Part of the cytosol fraction was stored at 4°C for the free TS and the rest was incubated with 600 mM NH_4HCO_3, 100 mM 2-mercaptoethanol, 100 mM NaF and 15 mM cytidine monophosphate (pH 8, Buffer A) at 25°C for 4 h to release 5-FdUMP from the TS for the assay of total TS. A volume of 0.05 ml of the cytosol fraction was incubated with 0.05 ml of Buffer A, 10 mg/ml bovine serum albumin, 0.01 ml of coenzyme solution (10 mM tetrahydrofolate, 67 mM formaldehyde, 15 mM sodium ascorbate) and 0.05 ml of [^{3}H]-5-FdUMP (in 5 mM potassium phosphate buffer; pH 7.4) at 30°C for 20 min. After 1 ml of charcoal suspension had been added to the samples, they were centrifuged (2000*g*, 20 min) and the radioactivities in 0.8 ml of the supernatants were determined in a scintilation counter. The results of TS assay were calculated as percentage inhibition of TS, using the following formula: (1-free TS/total TS)×100.

2.6. In vitro *cell growth in the* UPRT/*5-FU system*

The effect of AdCA-*UPRT* infection on the sensitivity of human colon cancer cells to 5-FU was evaluated with an *in vitro* growth assay. Two thousand five hundred cells were cultured at 37°C for 24 h in four groups: 5-FU alone (control), leucovorin/5-FU, AdCA-*lacZ*/5-FU, or AdCA-*UPRT*/5-FU. The medium was then replaced with the fresh medium containing various concentrations of 5-FU (0–1000 μM) with or without leucovorin (100 μM). The cells were cultured at 37°C for 5 days in each group, and the number of viable cells was assessed by the crystal violet staining procedure [25]. Results were expressed as a percentage of the number of cells compared with those of the drug-free controls.

2.7. In vivo *study using the* UPRT/*5-FU system in nude mice*

HT29 cells were suspended in phosphate-buffered solution (PBS) for inoculation into athymic mice. Five-week-old male athymic BALB/cAnNCrj-nu/nu mice (Charles River Co., Kanagawa, Japan) were inoculated s.c. with 1×10^6 HT29 cells. Seven days after inoculation, mice with established subcutaneous tumours were randomly divided into six groups ($n=6$) and the mice in each group were injected intratumorally with a combination of the following preparations: PBS (control), AdCA-*lacZ*, or AdCA-*UPRT* with or without 5-FU.

Viruses (5×10^8 plaque forming units pfu) suspended in 0.1 ml, phosphate-buffered saline (PBS) were injected intratumorally daily for 3 consecutive days and 5-FU (10 mg/kg) was injected intraperitoneally (i.p.) daily for 14 days. Tumour volume (TV) was measured in a blind manner using calipers up to day 35. TV was estimated from the formula, $TV=(a^2\times b)/2$, where a is the width (mm) of the tumour and b is its length (mm), and tumour growth was evaluated by the relative tumour volume ($RTV=TV_n/TV_7$; where TV_n and TV_7 are tumour volumes at day n and day 7, respectively).

2.8. Statistical analysis

Statistical analysis was performed by the one-way ANOVA throughout the study. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Transduction rate with AdCA-lacZ

The number of cells expressing *lacZ* increased in proportion with the MOI in the three human colon cancer cell lines (Fig. 1). The *lacZ* gene was expressed in approximately 20% or more of the cells at an MOI of 1 and was expressed in 90–100% of the cells at an MOI of 100, in each cell line. These results indicated that the human colon cancer cell lines used in the present study were susceptible to adenovirus infection. The transduction efficiencies (%) at MOIs of 1 and 10 by this adenovirus vector system were respectively 20.0±2.7 and

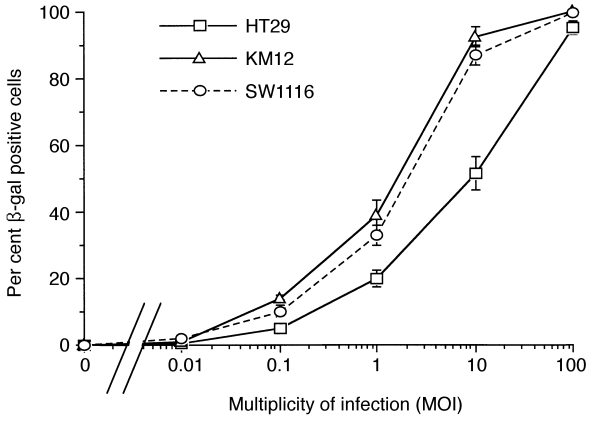

Fig. 1. Transduction efficiency of adenovirus in human colon cancer cells *in vitro*. HT29, KM12 and SW1116 cells were seeded in 6-well plates and infected with AdCA-*lacZ* at MOIs from 0 to 100 plaque forming units (pfu)/cell. Cells expressing the *lacZ* gene exhibited a characteristic blue colour after X-gal staining (β-D-galactosidase (B-gal) positive cells). The number of β-gal-positive cells was determined by counting 100 cells in four independent microscopic fields (original magnification 100×). All values are means ± standard deviation (S.D.) for four experiments.

Table 1
Intracellular levels of 5-FdUMP[a]

Cell line	No infection	AdCA-*lacZ* infection	AdCA-*UPRT* infection
HT29	0.28±0.04	0.36±0.10	2.62±0.58
KM12	0.17±0.29	0.18±0.30	1.48±0.11
SW1116	Less than 0.04	Less than 0.04	0.43±0.38

[a] (pmol/1×10^7 cell). Results are expressed as means±standard deviation (S.D.) of triplicate determinations.

51.5±4.9 in HT29, 38.8±5.0 and 92.5±3.0 in KM12 and 33.3±3.2 and 87.0± in SW1116 as shwn in Fig. 1.

3.2. The enhancing effect of AdCA-UPRT on 5-FU anabolism

Intracellular 5-FdUMP level in AdCA-*UPRT*-infected cells was significantly higher than that in uninfected cells by 9.4 times in HT29 and 8.7 times in KM12 ($P<0.001$ in both cell lines, Table 1), whereas the 5-FdUMP level was only detectable in the AdCA-*UPRT*-infected SW1116. In addition, the intracellular F-RNA level in AdCA-*UPRT*-infected cells was significantly higher than that in uninfected cells by a factor of 14.0 in HT29, 13.3 in KM12 and 15.1 in SW1116 ($P<0.0001$ in all cell lines, Table 2).

3.3. The enhancing effect of AdCA-UPRT infection and 5-FU treatment on TS inhibition

TS inhibition rates (%) in 5-FU alone (control), leucovorin/5-FU and AdCA-*UPRT*/5-FU were respectively 50.3±1.2, 58.9±3.0 and 57.1±3.5 in the HT29 cells, 37.2±1.7, 37.0±1.8 and 47.0±5.8 in the KM12 cells, and 45.2±2.0, 51.9±3.2 and 57.2±0.7 in the SW1116 cells (Fig. 2). TS inhibition was significantly different between 5-FU alone and AdCA-*UPRT*/5-FU treated cells in all cell lines. There were also significant differences in TS inhibition between 5-FU alone and leucovorin/5-FU treatment in HT29 and SW1116 cells ($P<0.01$ in both cell lines) reflecting the biochemical modulation effects of leucovorin. Although there was no difference in TS inhibition between AdCA-*UPRT*/5-FU and leucovorin/5-FU treatment in HT29 cells, TS inhibition in AdCA-*UPRT*/5-FU treated cells was significantly

Table 2
Intracellular levels of F-RNA[a]

Cell line	No infection	AdCA-*lacZ* infection	AdCA-*UPRT* infection
HT29	54.8±1.7	64.4±1.2	764.9±62.4
KM12	46.3±17.1	43.9±7.5	616.0±15.1
SW1116	41.5±2.4	41.5±2.4	627.0±30.8

[a] (ng/mgRNA). Results are expressed as means±standard deviation (S.D.) of triplicate determinations.

higher than in the case of treatment with leucovorin/5-FU in the KM12 and SW1116 cells ($P < 0.01$ and $P < 0.05$, respectively).

3.4. *The effect of AdCA*-UPRT *infection on the sensitivity of cancer cells to 5-FU* in vitro

A growth study was performed to determine whether *UPRT* gene transfer by adenovirus could increase the sensitivity of human colon cancer cell lines to 5-FU. All of the AdCA-*UPRT*-infected cells, HT29 (Fig. 3a), KM12 (Fig. 3b), and SW1116 cells (Fig. 3c), had an increased sensitivity to 5-FU. AdCA-*lacZ* infection, however, did not alter the growth of the three cell lines used in this study. As shown in Table 3, the IC_{50} values (in a representative experiment) of 5-FU in the control and leucovorin-modulated groups were, respectively, 3.53 and 0.73 in HT29 cells, 6.34 and 4.19 in KM12 cells and 2.89 and 0.88 μM in SW1116 cells. In contrast, the IC_{50} values (μM) of 5-FU in the AdCA-*UPRT* infection groups at MOIs of 1 and 10 were, respectively, 0.92 and 0.27 in HT29 cells, 0.71 and 0.21 in KM12 cells and 0.46 and 0.041 in SW1116 cells. Thus, treatment with AdCA-*UPRT*/5-FU showed the MOI-dependent increase in 5-FU sensitivity and the lowest IC_{50}s of the four groups in all the cell lines at the viral dose of an MOI of 10. The transduction efficiencies (%) at MOIs

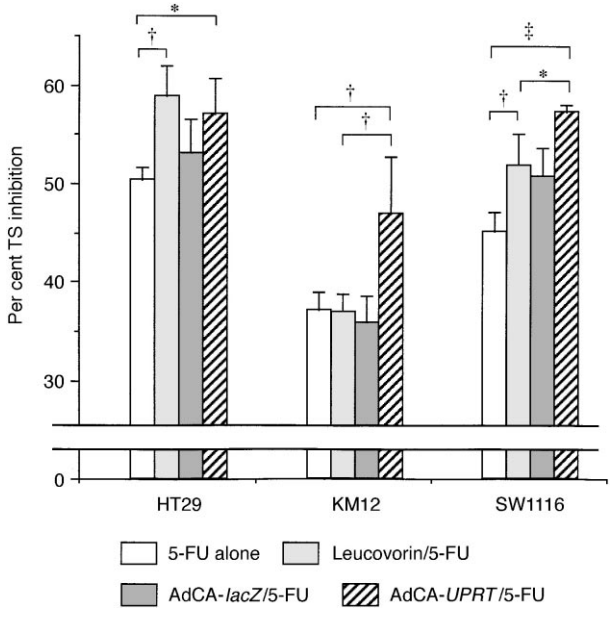

Fig. 2. Effect of AdCA-*UPRT* infection on 5-FU-induced thymidylate synthase (TS) inhibition *in vitro*. HT29, KM12 and SW1116 cells were incubated for 24 h in medium containing 1 μM of 5-FU alone or after infection with either AdCA-*UPRT* or AdCA-*lacZ* at an MOI of 10 or 0, or in medium containing 5-FU (1 μM) and leucovorin (100 μM) without adenovirus infection. TS assay was quantified by the modified Moran and Spears' method. TS inhibition rate was calculated using the following formula: (1-free TS/total TS)×100. All values are means ±standard deviation (S.D.) for three determinations. $*P < 0.05$; $\dagger P < 0.01$; $\ddagger P < 0.0005$.

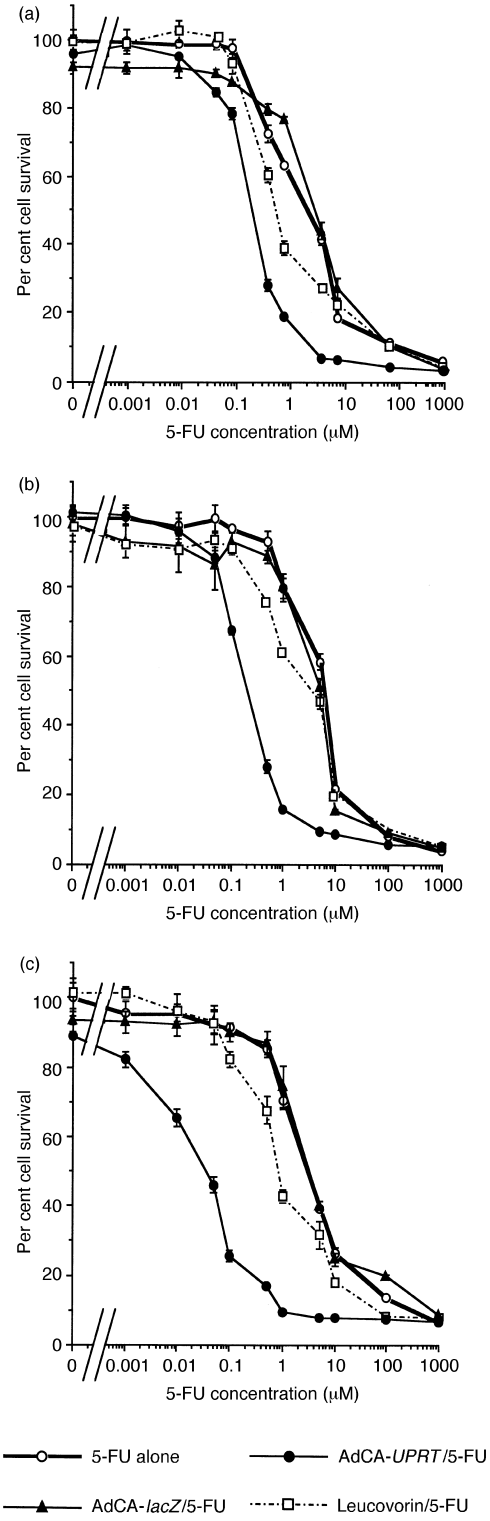

Fig. 3. Effect of AdCA-*UPRT* infection on cancer cell sensitivity to 5-FU *in vitro*. HT29 (a), KM12 (b), and SW1116 (c) cells were incubated for 5 days in medium containing various concentrations of 5-FU alone or after infection with either AdCA-*UPRT* or AdCA-*lacZ* at an MOI of 10 or 0, or in the medium containing various concentrations of 5-FU and leucovorin (100 μM) without adenovirus infection. Each point on the ordinate corresponds to the percentage of surviving cells compared with uninfected cells without 5-FU regarded as 100%. All values are means ±standard deviation (S.D.) of four determinations.

of 1 and 10 by this adenovirus vector system were, respectively, 20.0 and 51.5 in the HT29 cells, 38.8 and 92.5 in the KM12 cells, and 33.3 and 87.0 in the SW1116 cells, as shown in Fig. 1. These results revealed that the degree of increased 5-FU sensitivity correlates with the level of *UPRT* expression.

3.5. *The antitumour effect of* UPRT/*5-FU in nude mice*

A nude mouse model was used in this study. When we started the treatment (day 7), the established subcutaneous tumour was 4–6 mm in length. PBS (control) or adenoviruses were given intratumorally with or without 5-FU. AdCA-*UPRT* treatment together with the systemic administration of 5-FU resulted in a significant suppression of HT29 tumour growth (Fig. 4). Relative tumour volume following treatment with AdCA-*UPRT* and 5-FU was significantly different from that of the other groups from day 14 until the end of the experiment (day 35; $P < 0.0001$). There was no accidental death of the mice and the differences in the change in body weight of each group was less than 20% throughout this study.

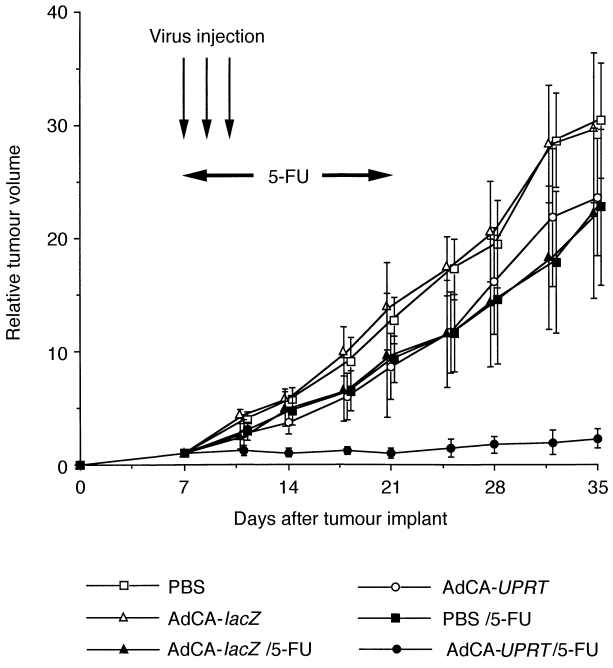

Fig. 4. Effect of AdCA-*UPRT*/5-FU on growth suppression of HT29-xenografted subcutaneous (s.c) tumours in nude mice. Seven days after s.c administration of 1×10^6 HT29 cells, mice with established s.c tumours were randomised into six groups to receive AdCA-*UPRT*, AdCA-*lacZ* or PBS, with or without 5-FU. The vectors or PBS were directly injected into tumours at days 7, 8 and 9. From day 7, 5-FU (10 mg/kg) was administered intraperitonealy (i.p.) into 5-FU treated mice daily for 14 days. All values are means±standard deviation (S.D.) of the relative tumour volume of six mice in each group.

4. Discussion

Increasing tumour cell sensitivity to cytotoxic agents by inserting a gene encoding a drug-activating enzyme is a promising strategy for cancer gene therapy. In the present study, the effects of adenoviral-mediated transfer of the *E. coli UPRT* gene on 5-FU metabolism and the chemosensitivity of three human colon cancer cell lines to 5-FU were investigated. We first examined the effect of AdCA-*UPRT* infection on the DNA- and RNA-directed anabolising pathways of 5-FU by quantifying the intracellular 5-FdUMP and F-RNA levels. It was shown that AdCA-*UPRT* infection increased the intracellular 5-FdUMP and F-RNA levels significantly in all three cell lines (Tables 1 and 2). This result clearly indicated that 5-FU anabolism in AdCA-*UPRT*-infected cells was remarkably enhanced. Furthermore, the effect of *UPRT*/5-FU treatment on cancer cell growth was obvious. The IC_{50} analysis in the *in vitro* cell growth experiment showed that AdCA-*UPRT* infection at a MOI of 10 led to a 13.1- to 70.5-fold increase in the sensitivity of the three cell lines to 5-FU (Table 3). In addition, *in vivo* treatment with *UPRT*/5-FU showed a significant growth suppression of HT29-xenografted s.c. tumours, without adverse side-effects, in nude mice when compared with mice treated with 5-FU alone. These results demonstrated that adenoviral-mediated *UPRT* gene delivery induced a marked augmentation of 5-FU chemosensitivity in human colon cancer cells and limited 5-FU activation in target tumours.

Table 3
IC_{50} of 5-FU (control), leucovorin-modulated and AdCA-*UPRT* infected human colon cancer cells

	The IC_{50} of 5-FU (μM)[a]				
			AdCA-UPRT infection		
Cell line	Control	Leucovorin-modulated	MOI = 1	MOI = 10	IC_{50} ratio[b]
HT29	3.53	0.73	0.92	0.27	13.1
KM12	6.34	4.19	0.71	0.21	30.2
SW1116	2.89	0.88	0.46	0.041	70.5

[a] Results are representative of four independent experiments.
[b] Values were obtained from the IC_{50} of 5-FU treated parental/AdCA-*UPRT* (MOI = 10) infected cells.

An additional study compared the modulating effects of 5-FU alone, leucovorin/5-FU and AdCA-*UPRT*/5-FU on TS inhibition. AdCA-*UPRT*/5-FU treatment induced a stronger TS inhibition than that of 5-FU alone in all cancer cells studied. The inhibitory action in this treatment group is believed to be induced through increased intracellular 5-FdUMP. TS inhibition in the AdCA-*UPRT*/5-FU group was equivalent to that of the leucovorin/5-FU group in HT29 cells and was higher than the leucovorin/5-FU group in KM12 and SW1116 cells. These results indicated that *UPRT*/5-FU therapy might have stronger DNA-directed action by affecting TS inhibition than the leucovorin/5-FU modulation therapy.

5-FU is often given to patients for the treatment of colorectal cancer by either continuous intravenous (i.v.) injection or daily oral administration. Continuous i.r. injection produces a constant 5-FU plasma concentration ranging from 0.5 to 8.0 μM and oral administration produces 0.6 μM of 5-FU plasma concentration [29]. The *in vitro* IC_{50} of 5-FU for AdCA-*UPRT* infected cells was 0.041–0.27 μM. Although the cytotoxicity of 5-FU in the *in vitro* study was different from the *in vivo* study, these IC_{50} values in plasma could be achieved easily in both oral and i.v. administration of 5-FU without side-effects. Therefore, the *UPRT*/5-FU treatment may minimise the systemic side-effects of 5-FU by localising a high concentration of the active metabolites in the tumour, while the plasma concentrations of 5-FU remain low.

The goal of gene therapy is the treatment of patients who have advanced colorectal cancer with surgically unresectable lesions. Lesions such as multiple liver and/or lung metastases, and locally advanced disease in the pelvic space will be indications for this therapy. To be clinically applicable, sufficient gene expression in the target tumours has to be accomplished. Several approaches have been developed in gene transfer systems including DNA-liposome complexes, retroviral vectors, and adenoviral vectors [30]. The adenoviral vector used in this study is believed to be a more suitable system for cancer gene therapy because its gene delivery mechanism is independent of cell replication, despite its immunogenicity. Another critical issue in the development of a tumour-specific gene expression system is to avoid normal tissues undergoing the cytotoxic effects. Recently, an adenoviral vector carrying the transcriptional control elements encoding a suicide gene was reported to be preferentially expressed in tumour cells [12,15]. In colorectal cancer, carcinoembryonic antigen (CEA) is a tumour-associated marker which is elevated in the serum of advanced stage patients [31]. Therefore, a CEA promoter would be useful for discriminating target tumours from normal tissues. This development in adenoviral gene transfer may make i.v. or intra-hepatic-arterial administration of adenoviral vectors possible in the future.

Our data demonstrates that the adenoviral-mediated transfer of the *E. coli UPRT* gene could enhance both the DNA- and RNA-directed activating anabolisms of 5-FU resulting in the sensitising of human colon cancer cells. We suggest that the *UPRT*/5-FU system can be regarded as a new biochemical modulation of fluorouracil therapy for colorectal cancer treatment.

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