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ORIGINAL PAPER

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5-fluorouracil and hydroxyurea enhance adenovirus-mediated transgene expression in colon and hepatocellular carcinoma cells

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Abstract *Purpose*: To investigate the efficient transduction of tumor cells which remains a major limitation of cancer gene therapy.. *Methods*: In this study, we tested whether treatment with antimetabolic drugs 5-FU and hydroxyurea (HU) could improve adenovirus-mediated gene expression in tumor cells. *Results*: We found that 5-FU and HU treatment significantly increased β-gal activity in adenovirus (Ad.CMVBG)-infected human colon carcinoma (LoVo) and hepatocellular carcinoma (SMMC7721) cells in a dose- and time-dependent manner. These increases were maximized at 5.01 ± 0.42-fold and 3.32 ± 0.32-fold for 5-FU (50 μM), and at 6.60 ± 0.50-fold and 4.82 ± 0.43-fold for HU (5 mM) treatment, respectively, after 48 h infection. Transient increases in viral uptake, determined by real-time PCR for viral DNA content and by confocal microscopy for viral particles, were observed in 5-FU or HU-treated cells that partially contribute to the overall increases of β-gal expression. Moreover, mRNA levels for the β-gal gene in infected cells were significantly increased in both LoVo and SMMC7721 cells by 5-FU and HU treatment in contrast to the inhibition of viral DNA replication and the unchanged mRNA levels for α-actin gene. The induction appeared to be the result of enhanced transcription since β-gal mRNA half-life was not affected by drug treatment. However, similar induction was not detected in CMV-β-gal-expressing stable cells, suggesting that an adenovirus-associated mechanism might be involved in this induction. *Conclusions*: Our findings suggest that it may be possible to improve tumor cell transduction by adenovirus using chemotherapy.

Keywords Chemotherapy · Cytosine deaminase · 5-fluorouracil · Gene therapy · Hepatoma

Abbreviations yCD: Yeast cytosine deaminase · 5-FC: 5-fluorocytosine · 5-FU: 5-fluorouracil · HU: Hydroxyurea · β-gal: β-galactosidase · CMV: Cytomegalovirus · Ad.CMVBG: E1-deleted adenovirus containing β-gal gene · PCR: Polymerase chain reaction · CAR: Coxsackie-adenovirus receptor · pfu: Plaque-forming units · MOI: Multiplicity of infection · RT-PCR: Reverse transcription-PCR · DRB: 5,6-dichlorobenzimidazole · FACS: Fluorescence-activated cell sorting

Introduction

E1-deleted adenovirus is the most widely used vector in clinical trials of gene therapy because of its high efficiency for gene transfer, ease of high titer production, and relative safety [1, 2, 3, 4]. However, insufficient transgene expression in tumors remains a key problem [5, 6].

The combination of adenovirus gene therapy and other therapeutic approaches, such as chemotherapy or radiation, has been shown to improve outcomes in a number of in vivo studies and clinical trials [7, 8, 9, 10, 11], suggesting that chemotherapeutic agents and radiation can enhance adenovirus-mediated transgene expression [12, 13, 14, 15, 16] in tumors. Whereas this enhancement could vary among cell types or therapeutic agents, the underlying mechanism needs to be further investigated. As a major determinant of adenovirus entry, cell surface CAR expression has been shown to vary during cell cycle change in response to chemotherapeutic agents, leading to an increased virus uptake

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and enhanced transgene expression [12]. S/G2 arrest, induced by many agents and by adenovirus infection (both wild type and E1-mutant), has been demonstrated to facilitate viral uptake and viral DNA replication [17, 18, 19, 21], thus, an enhanced transgene expression.

Our previous study demonstrated that adenovirus carrying yCD genes selectively transduce colon carcinoma cells and convert prodrug 5-FC to 5-FU in intrahepatic tumor xenografts [22]. However, the overall efficiency of transduction for the initial viral infusion is relatively low. In another study, we found that HU, an agent that blocks viral DNA synthesis, greatly enhanced adenovirus-mediated gene expression in tumor cells (unpublished data). This prompted us to test whether 5-FU and HU, both antimetabolic agents that affect DNA synthesis and the cell cycle, would improve adenovirus-mediated gene expression in tumor cells. It is particularly important in our current yCD enzyme/prodrug strategy where the converted drug as an active form (5-FU) could potentially improve the efficiency for initial viral infusion in intrahepatic tumors.

In this study, we demonstrated that 5-FU and HU significantly enhanced adenovirus-mediated gene expression in human colon and hepatocellular carcinoma cells. The mechanisms involving viral uptake, DNA replication, and transgene transcription were further explored.

Materials and methods

Adenovirus vectors and agents

Ad.CMVBG, an E1-deleted recombinant adenovirus serotype-5 vector containing β-gal gene under the control of CMV promoter, was constructed and propagated by the Vector Core Facility of the University of Michigan (Ann Arbor, Mich., USA). For virus labeling, 1×10^{12} particles were reacted with Cy3 dye (Amersham Pharmacia Biotech, Piscataway, N.J., USA) in 0.1 M sodium carbonate buffer (pH 9.3) as recommended by the manufacturer. Labeled Ad.CMVBG (Cy3-Ad.CMVBG) were then dialyzed against buffer containing 10% glycerol, 10 mM HEPES, and 1 mM $MgCl_2$ (pH 7.4) using Slide-A-Lyzer (MWCO 7000, Pierce, Rockford, Ill., USA). Aliquots were stored at -70 °C in the dark. The OD600 and plaque assays were used to measure the number of viral particles and pfu of stocks, respectively. 5-FU and HU were obtained from Sigma-Aldrich (St. Louis, Mo., USA) and were resuspended in appropriate buffer.

Cell culture, drug treatment, and virus infection

The human colon cancer cell line LoVo was purchased from American Type Culture Collection (ATCC, Manassas, Va., USA), and the human hepatocellular carcinoma cell line SMMC7721 was a kind gift from Z.Y. Tang (Liver Cancer Institute, Fudan University, Shanghai, P.R. China) [23]. Stable cell line for LoVo and SMMC7721 expressing β-gal (LoVo-β-gal and SMMC7721-β-gal respectively) were established by infecting parental LoVo and SMMC7721 cells with a lentivirus vector containing a CMV-driven β-gal gene. Cells were maintained in RPMI media containing 10% FBS and antibiotics at 37 °C with 5% CO_2. LoVo and SMMC7721 cells were preseeded in 24-well plates (1×10^5 cells/well) overnight prior to Ad.CMVBG infection (MOI 100) in the presence or absence of 5-FU (5 μM, 25 μM, 50 μM, and 200 μM) or HU (0.5 mM, 1 mM, 5 mM, and 10 mM) for 12 h, 24 h, and 48 h. Determination of transgene expression, viral uptake, and mRNA levels are described individually below.

Transgene expression by β-gal activity assay

LoVo and SMMC7721 cells were either pre-infected by Ad.CMVBG for 3 h prior to removal of free virus in the media or were infected by Ad.CMVBG for various time points in the presence or absence of 5-FU or HU. β-gal activities in cells were measured by β-Galactosidase Enzyme Assay System (Promega, Madison, Wis., USA) under the manufacture's recommended conditions and were quantified by the absorbance reading at 420 nm using a SPECTRAmax PLUS384 Microplate Spectrophotometer (Molecular Devices Corporation, Sunnyvale, Calif., USA). Drug-induced β-gal expression, which was further normalized by the amount of total cell lysate, is represented as the fold increase compared to the activity from non-drug-treated cells at each time point.

Determination of viral DNA by Real-time PCR

For virus uptake measurement, LoVo and SMMC7721 cells were pre-incubated with or without 5-FU (50 μM) or HU (5 mM) for 36 h before Ad.CMVBG infection for 3 h followed by extensive washing in PBS to remove free virus from media. For replication of viral DNA, cells were pre-infected with Ad.CMVBG for 3 h followed by extensive washing and were then incubated with 5-FU or HU for 48 h. Total cellular DNA was purified using DNeasy Tissue Kit (Qiagen, Valencia, Calif., USA) and was assessed for viral DNA by real-time PCR using primers specific for the β-gal gene sequence (5' primer: CACGGCAGATACACTTGCTG, and 3' primer: ATCGCCATTTGACCACTACC). Viral DNA copies were calculated from a standard curve and were further normalized in relative to the copy numbers for α-actin (5' primer: CGAGATCCCTCCAAAATCAA, and 3' primer: TGTGGTCATGAGTCCTTCCA) by the comparative CT method (PE Applied Biosystems User Bulletin#2). Drug-induced viral uptake was represented as the fold increase in uptake compared to that of non-drug-treated control cells. Real-time PCR was performed using QuantiTect SYBR Green PCR Kit (Qiagen) on the

DNA Engine Opticon System (MJ Research, Waltham, Mass., USA) in the reaction mixture (20 μl) consisting of 10 μl of Mater Mix, 1 μl of each primer (12.5 μM), and 1 μl of sample. The thermal cycling conditions began with denaturation and an enzyme activation step at 95 °C for 15 min and 36 cycles at 94 °C for 15 s, 53 °C for 30 s, and 72 °C for 30 s. Plates were read between annealing and extension in every cycle. The melting curve was performed from 50 °C to 95 °C at 1.0 °C increments. Samples were independently analyzed three times.

Confocal microscopy laser scanning

LoVo and SMMC7721 cells were seeded on 8-well chamber slides (Nalge Nunc International, Naperville, Ill., USA) at 1×10^4 cells/well and were incubated in the presence or absence of 5-FU or HU for 36 h. Cells were then incubated with Cy3-Ad.CMVBG (MOI 40) for 3 h followed by extensive washing in PBS to remove free viral particles. Cells were then fixed in 4% paraformaldehyde solution for confocal microscopy scanning. Image analysis was performed using an Olympus IX SLA Confocal Laser Scanning Biological Microscope equipped with Texas Red laser guns operated by Fluoview software FV-300 (Olympus USA, Melville, N.Y., USA).

Quantification of mRNA by Real-time RT-PCR

LoVo and SMMC7721 cells were pre-infected by Ad.CMVBG as described above followed by incubation in the presence or absence of 5-FU (50 μM) or HU (5 mM) for 0 h, 12 h, 24 h, and 48 h. Stable cell lines LoVo-β-gal and SMMC7721-β-gal were treated by 5-FU or HU under similar condition without viral infection. Total RNAs were purified using the RNeasy Kit in combination with the RNase-Free DNase Set (Qiagen) to remove cellular DNA. The level of mRNA for β-gal was determined by the real-time RT-PCR performed on the DNA Engine Opticon System using QuantiTect SYBR Green RT-PCR kit (Qiagen) under the conditions recommended by manufacturer. The reaction mixture (20 μl) consisted of 1 μl of sample, 10 μl of Master mix (Qiagen, Germany), 0.2 μl of QuantiTect RT Mix, and 1.5 μl of each primers (12.5 μM) described above, and proceeded through reverse transcription (50 °C for 30 min), initial denaturation and enzyme activation (95 °C for 15 min) and 36 PCR cycles (94 °C for 15 s, 53 °C for 30 s and 72 °C for 30 s). The levels of mRNA for β-gal and α-actin were normalized by total RNA amount and were expressed as fold increases in comparison with control cells.

Stability of mRNA for β-gal by measurement of half-life

LoVo and SMMC7721 cells were pre-infected by Ad.CMVBG for 3 h before incubation with 5-FU (50 μM) or HU (5 mM) for 48 h. Cells were then treated with DRB (Sigma) at 100 μg/ml for 0 h, 0.5 h 1 h, 2 h, and 3 h to stop new synthesis of all cellular mRNA prior to total RNA isolation for the real-time RT-PCR quantification of mRNA for β-gal and α-actin genes as described above. The level of mRNA, normalized by total RNAs, was determined as fold increase relative to the level in non-DRB-treated cells at the time when DRB treatment started (arbitrarily set as 1), and was represented as log expression. Half-life for β-gal mRNA was calculated from linear regression fitting curve as the time when the value decreased by half.

Data analysis and statistics

Each experiment was performed four times (each at triplicate). Values are expressed as means ± standard error and were compared by ANOVA analysis. Data were considered significantly different when $P < 0.01$.

Results

Gene expression and viral uptake induced by 5-FU and HU in Ad.CMVBG-infected cells

LoVo and SMMC7721 cells were chosen to represent primary liver tumors and colon cancer metastatic to the liver. We found that β-gal expression in these cells infected by Ad.CMVBG was significantly induced in the presence of 5-FU or HU compared with the expression in untreated control cells in a time- and dose-dependent manner. Induction was observed at 24 h of drug treatment and was maximized at 48 h for both 5-FU at 50 μM (5.01 ± 0.42-fold and 3.32 ± 0.32-fold, respectively) and HU at 5 mM (6.60 ± 0.50-fold and 4.82 ± 0.43-fold, respectively) (Fig. 1). Such induction was not detected when lower doses of 5-FU (5 and 25 μM) or HU (0.2 mM and 1 mM) was used while no significant additional induction was achieved at higher dose (200 μM of 5-FU or 10 mM of HU) (data not shown). Therefore, 5-FU at 50 μM and HU at 5 mM, both clinically achievable concentrations, were utilized in subsequent experiments. These results demonstrated that 5-FU and HU increased transgene expression in Ad.CMVBG-infected cells.

We wished to determine if drug-induced gene expression was due to increased uptake of virus in cells. To test this, LoVo and SMMC7721 cells were infected with Ad.CMVBG, washed, exposed to drugs, and assessed for intracellular viral DNA by real-time PCR. We found that viral DNA content was modestly but significantly increased in both cell lines in response to 5-FU (2.02 ± 0.13-fold and 1.51 ± 0.12-fold, respectively) or HU (2.52 ± 0.20-fold and 1.80 ± 0.13-fold, respectively) treatment (Fig. 2A). This was further supported by the direct evidence from confocal microscopy that enhanced total uptake of a Cy3-labelled Ad.CMVBG was observed in cells pre-treated with 5-FU or HU compared to control cells (Fig. 2B). These results demonstrated that 5-FU and HU facilitated adenovirus entry in cells.

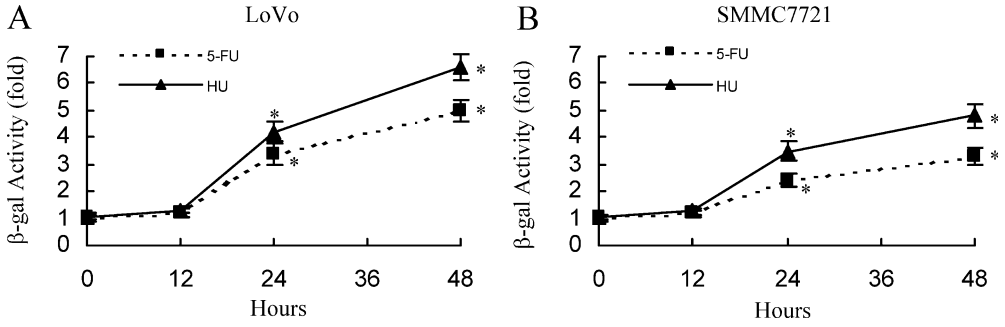


Fig. 1 5-FU and HU induced β-gal activity treatment in Ad-CMVBG-infected cells. LoVo and SMMC7721 cells in 24-well plates (1×10^5 cells/well) were infected by AdCMVBG (MOI 100) in the presence or absence (control) of 5-FU (50 μM) or HU (5 mM) for 0 h, 12 h, 24 h, and 48 h. β-gal activities were measured from cell lysates as described in Materials and Methods. Data are expressed as fold increase relative to control groups for each time point. **A** LoVo cells, **B** SMMC7721 cells. * Significantly different from control ($P < 0.01$, $n = 4$)

5-FU and HU induced β-gal expression by increasing mRNA levels

Although these findings suggested that an increase, of viral uptake played a role in increased gene expression, the overall induction in β-gal activity was greater in response to drug treatment than could be accounted for by increased viral uptake. To determine whether other mechanisms are involved in the induction, cells were infected by Ad.CMVBG before incubation with drugs. We found that β-gal activities in LoVo and SMMC7721 cells were enhanced by 48-h treatment with 5-FU (3.50 ± 0.30-fold and 1.82 ± 0.15-fold, respectively) or HU (4.52 ± 0.35- and 2.71 ± 0.20-fold, respectively) compared with control group (Fig. 3A). This is consistent with the induction observed when drug treatment and virus infection were carried out simultaneously, suggesting that drug-enhanced gene expression involved other cellular mechanisms in addition to the increased virus uptake. Real-time PCR analysis demonstrated that, under control conditions, viral DNA selectively

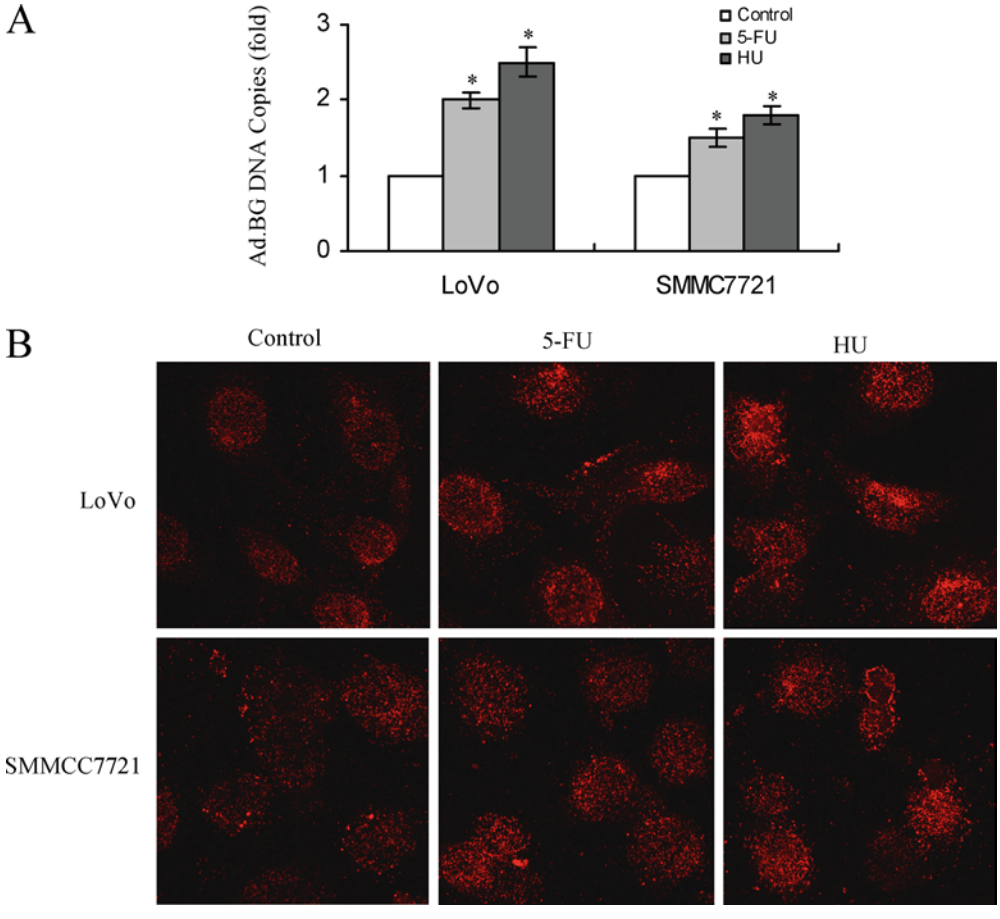


Fig. 2 5-FU and HU increased viral uptake in cells. LoVo and SMMC7721 cells were pre-incubated with or without (control) 5-FU (50 μM) or HU (5 mM) for 36 h prior to AdCMVBG or Cy3-AdCMVBG infection for 3 h followed by extensive washing. **A** Real-time PCR determination of viral DNA content in whole cell lysates by amplification of the β-gal sequence as described in Materials and Methods. Data are expressed as the fold increase relative to control groups. **B** Confocal microscopy of Cy3-AdCMVBG-infected LoVo and SMMC7721 cells with or without drug pre-treatment Images were generated by Olympus IX SLA Confocal Laser Scanning Biological Microscope equipped with Texas Red laser gun (60×). *: Significantly different from controls ($P < 0.01$, $n = 4$)

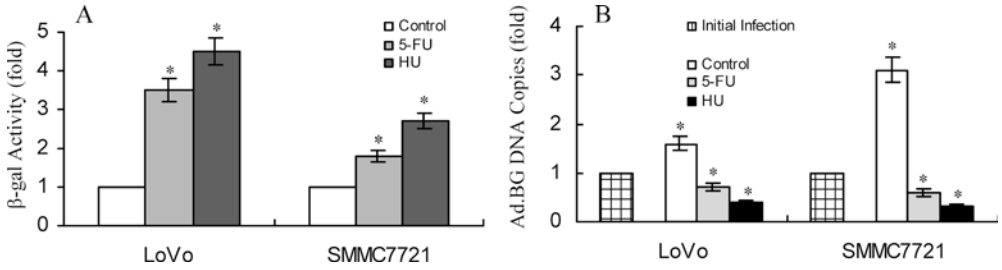
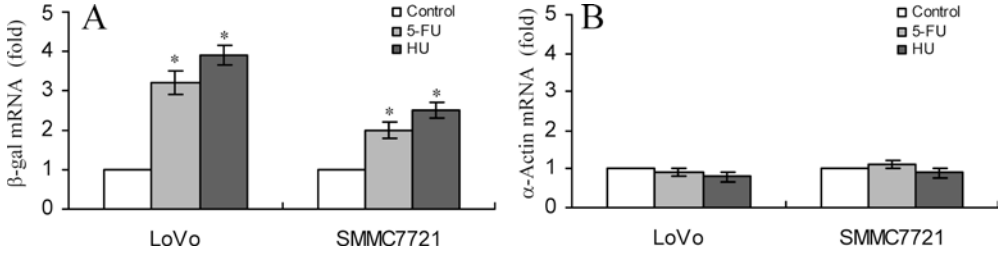


Fig. 3 5-FU and HU inhibited viral DNA replication in Ad-CMVBG-infected cells. LoVo and SMMC7721 cells (MOI 100 for 3 h) were pre-infected by AdCMVBG (MOI 100) for 3 h prior to removal of free virus in media and were then incubated with 5-FU (50 μM) or HU (5 mM) for 48 h while untreated cells were used as controls. Cell lysates were measured for β-gal activity (β-gal assay system) and viral DNA content (Real-time PCR by Opticon System) as described in Materials and Methods. **A** β-gal activity was expressed as fold increase relative to control groups of each cell line. **B** Viral DNA copies were expressed as fold increases relative to the amount in each cell line detected immediately after 3-h infection (initial infection). * Significantly different from controls ($P < 0.01$, $n = 4$)

replicated in both LoVo and SMMC7721 cells at 48 h after infection (1.61 ± 0.14-fold and 3.13 ± 0.25-fold, respectively) compared to cellular DNA (Fig. 3B). As expected, treatment with both 5-FU and HU significantly inhibited viral DNA replication evidenced by the decreased number of viral DNA copies in LoVo (0.72 ± 0.05-fold and 0.41 ± 0.04-fold, respectively) and SMMC7721 (0.63 ± 0.08-fold and 0.32 ± 0.04-fold, respectively) (Fig. 3B). In contrast, β-gal mRNA levels were greatly increased in LoVo and SMMC7721 cells in response to 5-FU (3.25 ± 0.3-fold and 2.02 ± 0.18-fold, respectively) or HU (3.94 ± 0.25-fold and 2.55 ± 0.20-fold, respectively) treatment (Fig. 4A). Importantly, drug treatment had no effect on α-actin mRNA production in both cell lines (Fig. 4B), suggesting no overall change in mRNA levels. Therefore, the drug-induced β-gal activities in virus-infected cells were the results of the elevated β-gal mRNA levels instead of an increased viral DNA content.

Fig. 4 5-FU and HU treatment increased β-gal mRNA levels in AdCMVBG-infected cells. Pre-infected LoVo and SMMC7721 cells were incubated with 5-FU or HU for 48 h as described in Fig 3. Levels of mRNA were determined by real-time RT-PCR using specific primers for β-gal or α-actin gene as described in Materials and Methods. Data are expressed as fold increases relative to the levels from control cells (arbitrarily set as "1"). **A** β-gal mRNA. **B** α-actin mRNA. * Significantly different from controls ($P < 0.01$, $n = 4$)



To determine whether the increase in β-gal mRNA levels was the result of enhanced transcription or of prolonged stability, the half-life of β-gal mRNA was assessed in the presence or absence of drug treatment. We found that neither 5-FU nor HU treatment altered the half-life of β-gal mRNA compared to untreated controls in both LoVo (2.56 ± 0.27 h, 2.48 ± 0.31 h, and 2.75 ± 0.23 h, respectively) and SMMC7721 (1.08 ± 0.11 h, 1.03 ± 0.12 h, and 1.20 ± 0.15 h, respectively) cells (Fig. 5). We also found that neither drug altered the half-life for α-actin mRNA (data not shown). Thus, our result indicated that the elevated mRNA levels in response to drug treatment was not likely the outcome of changes in mRNA turnover but the reflection of an enhanced transcription.

5-FU- and HU-induced transgene transcription required adenovirus infection

To determine whether the drug-induced gene transcription was associated with adenovirus infection rather than the upregulation of CMV promoter activity, stable cell lines expressing CMV-β-gal (LoVo-β-gal and SMMC7721-β-gal) were compared to adenovirus-infected parental cells for β-gal mRNA levels in response to drug treatment. We found that 5-FU or HU treatment significantly increased β-gal mRNA levels in virus-infected cells in a time-dependent manner which occurred at as early as 12-h incubation, and β-gal mRNA levels were further enhanced after a 24-h incubation in both pre-infected LoVo (2.22 ± 0.24-fold and 2.51 ± 0.25-fold, respectively) (Fig. 6A) and pre-infected SMMC7721 (1.60 ± 0.24-fold and 1.80 ± 0.25-fold, respectively) (Fig. 6B) in comparison with non-treated cells. However, similar induction was not detected in stable cell lines where CMV-β-gal gene was presented as part of genomic DNA, suggesting that these drugs enhanced β-gal gene transcription through an adenovirus-associated mechanism in infected cells.

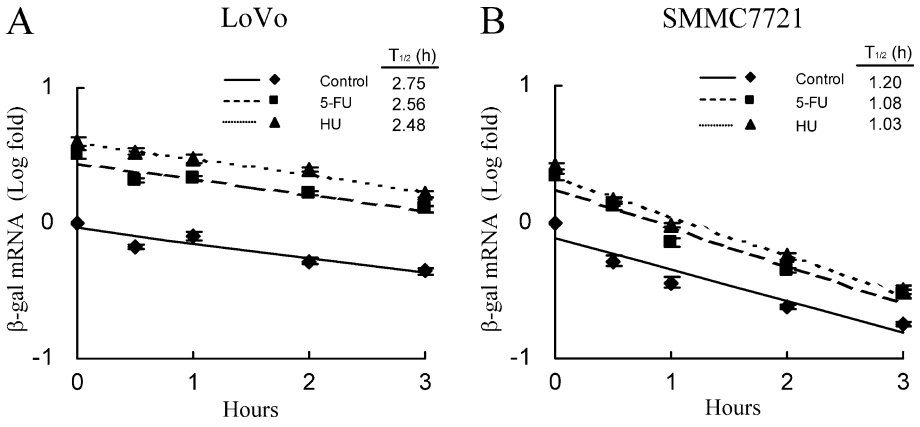


Fig. 5 5-FU and HU did not alter β-gal mRNA half-life in AdCMVBG-infected cells. LoVo and SMMC7721 cells were pre-infected by AdCMVBG (MOI 100) for 3 h followed by the removal of free virus in media and continued incubation in the presence or absence of 5-FU (5 mM) or HU (5 mM) for 48 h. Cells were then incubated with DRB (100 μg/ml) for 0 h, 0.5 h, 1 h, 2 h, and 3 h prior to real-time RT-PCR analysis for β-gal mRNA. The level of mRNA for β-gal normalized to total RNAs was determined as fold increase relative to the level in non-DRB-treated cells and is represented as log expression. Half-life ($T_{1/2}$) was calculated using linear regression as described in Materials and Methods. **A** LoVo cells. **B** SMMC7721 cells ($n=4$)

Discussion

In this study, we demonstrated that the antimetabolic chemotherapeutic agents 5-fluorouracil and hydroxyurea enhanced adenovirus-mediated transgene expression in human colon carcinoma and hepatocellular carcinoma cells. This induction was accompanied by transient increases in viral uptake and was correlated to the induction of gene transcription but not viral DNA replication in infected cells. Our findings are potentially important in combining adenovirus gene therapy and chemotherapy for cancer treatment.

The mechanisms by which chemotherapeutic agents induce adenovirus-mediated transgene expression are complex. Although a transient increase in total viral uptake was observed in response to 5-FU or HU treatment, it did not seem to be exclusively responsible for the overall drug-induced β-gal activity in infected cells evidenced by our study. First, the induction of viral uptake was less than the induction of β-gal activity in infected cells. Second, pre-infected cells responded to drug treatment similarly to cells without viral pre-infection. Third, viral DNA replication was inhibited by 5-FU or HU treatment, further suggesting that the number of viral DNA in infected cells was not a determinant for the transgene expression. We also found that other chemotherapeutic agents including aphidicolin, doxorubicin, and etopside but not cisplatinum, carboplatinum, and nacodazole induced adenovirus-mediated gene expression in various human tumor cells (data not shown) consistent with some previous reports [12, 13, 14, 15]. Systems comparing other drugs should provide insight as to whether these drugs share similar mechanism(s) for inducing adenovirus gene expression.

One major finding in this study is that 5-FU and HU enhanced transcription for transgene in adenovirus-infected cells. Wild-type adenovirus induces the host cell to enter the S phase of the cells cycle, providing an optimal environment for viral gene expression and viral replication [24]. We found that 5-FU or HU treatment elicits an S phase arrest in LoVo and SMMC7721 cells by FACS analysis (data not shown) consistent with previous studies in other cells [25, 26]; thus, it may provide compensatory factors for E1-mutant adenovirus

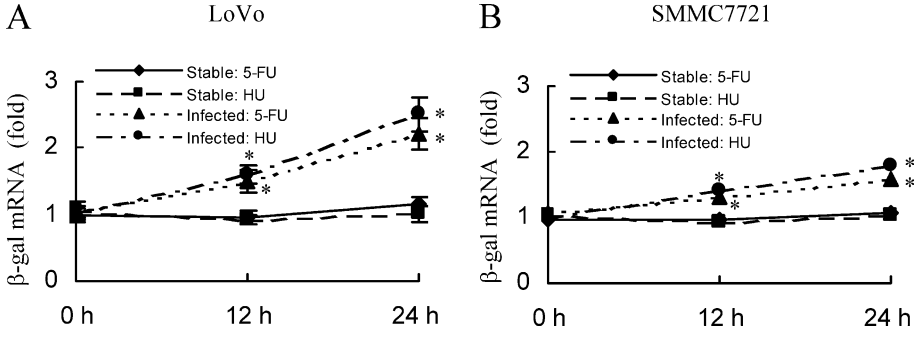


Fig. 6 5 FU and Hy induced β-gal transcription required adenovirus infection. LoVo and SMMC7721 cells, either parental cells that received pre-infection of AdCMVBG (*Infected*) or cells stably expressing CMV-β-gal (*Stable*), were incubated with 5-FU (5 mM) or HU (5 mM) for 0 h, 12 h, and 24 h. β-gal mRNA levels were assessed by real-time RT-PCR and were expressed as fold increase relative to untreated control groups. **A** LoVo cells: **B** SMMC7721 cells. * Significantly different from controls ($P < 0.01$, $n=4$)

vector to improve transgene expression. In particular, the cellular transcription factor E2F, elevated during S phase arrest, has been implicated in activation of a variety of adenovirus promoters as well as the major late promoter [27, 28], leading to enhanced gene transcription. Moreover, our study demonstrated that stable LoVo and SMMC7721 cells expressing CMV-β-gal gene responded to 5-FU and HU treatment by increasing β-gal mRNA levels, although to a lesser extent than did adenovirus-infected cells, at 48 h of incubation (data not shown). Whether 5-FU or HU treatment may activate CMV promoter, which has been suggested for other chemotherapeutic agent [13], remains to be seen.

5-FU is commonly used in clinical treatments for hepato-gastroenteric tumors. Our previous study demonstrated that adenovirus containing CEA-yCD gene selectively transduced intrahepatic colon carcinoma metastases, leading to an efficient prodrug conversion from 5-FC to 5-FU in tumors [22]. Our current findings that that 5-FU and HU induced adenovirus-mediated gene expression in human colon and hepatocellular carcinoma cells would provide us with a new strategy for combining traditional chemotherapy and adenovirus gene therapy that utilizes an enzyme/prodrug approach in the treatment of liver cancer. This is particularly important in improving selectivity and efficacy for repeated adenovirus administration where a high level of 5-FU can be achieved from initial viral infusion.

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