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ORIGINAL ARTICLE

Enhancement of CPT-11 antitumor activity by adenovirus-mediated expression of β–glucuronidase in tumors

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CPT-11 is a clinically important prodrug that requires conversion into the active metabolite SN-38, a potent topoisomerase I poison, for antitumor activity. However, SN-38 is rapidly metabolized to the inactive SN-38 glucuronide (SN-38G) in the liver, which reduces the amount of SN-38 available for killing cancer cells. Here, we investigated if local expression of β-glucuronidase (βG) on cancer cells to catalytically convert SN38G to SN38 could enhance the antitumor activity of CPT-11. βG was tethered on the plasma membrane of three different human cancer cell lines: human colon carcinoma (LS174T), lung adenocarcinoma (CL1-5) and bladder carcinoma (EJ). Surface β-glucuronidase-expressing cells were 20 to 80-fold more sensitive to SN-38G than the parental cells. Intravenous CPT-11 produced significantly greater suppression of CL1-5 and LS174 T tumors that expressed βG as compared with unmodified tumors. Furthermore, an adenoviral vector expressing membrane-tethered βG (Ad.βG) increased the sensitivity of cancer cells to SN-38G even at multiplicity of infections as low as 0.16, indicating bystander killing of non-transduced cancer cells. Importantly, intratumoral injection of Ad.βG significantly enhanced the *in vivo* antitumor activity of CPT-11 as compared with treatment with CPT-11 or Ad vectors alone. This study shows that Ad.βG has potential to boost the therapeutic index of CPT-11. *Cancer Gene Therapy* (2011) **18,** 381–389; doi:10.1038/cgt.2011.3; published online 25 February 2011

Keywords: CPT-11; β-glucuronidase; SN-38G; adenovirus; cancer therapy; prodrug

Introduction

Irinotecan (CPT-11) is a derivative of the natural alkaloid camptothecin that is approved for the treatment of patients with metastatic colorectal cancer. CPT-11 has also demonstrated anticancer activity against a variety of solid tumors in preclinical and clinical trials.[1,4] CPT-11 is a prodrug, which is enzymatically converted by esterases to SN-38,[5,6] a potent topoisomerase I inhibitor that is thought to have the major role in the antitumor activity of CPT-11.[7] However, a large fraction of SN-38 is further metabolized in the liver by members of the UDP-glucuronosyltransferase 1A family to the inactive glucuronide metabolite (SN-38G).[8] The serum concentration of SN-38G can exceed the concentration of SN-38 in the circulation by up to 25-fold,[9–12] thereby reducing the exposure of tumor cells to SN-38.

Prodrug activating gene therapy is an investigational approach to enhance the effectiveness of cancer chemotherapy that entails delivery of an enzyme encoding gene that can catalytically convert an anticancer prodrug to a cytotoxic antineoplastic agent.[13] For example, expression of carboxyesterases in cancer cells can catalyze the hydrolysis of CPT-11 to SN-38 and enhance CPT-11 antitumor activity.[14,15] Glucuronide analogs of anticancer drugs can act as prodrugs that can be activated by the enzyme β-glucuronidase (βG).[16,17] This suggests that another approach to enhance CPT-11 effectiveness might be to express βG in the host to catalyze the hydrolysis of SN-38G back to SN-38. In fact, SN-38G excreted in the bile can be converted back to SN-38 by bacterially produced βG in the intestine, which is thought to contribute to the antitumor effect of CPT-11, but also produces intestinal toxicity.[18] Because systemic administration of βG could expose normal tissues to high concentrations of SN-38, tumor-localized expression of βG by intratumoral injection or by transductional targeting of gene transfer vectors are approaches that appear promising to increase the concentration of SN-38 in tumors without increasing systemic exposure to the drug. Most glucuronides do not readily enter cells by passive diffusion due to the presence of the charged glucuronide moiety.[19] Expression of secreted βG at cancer cells can effectively activate glucuronide prodrugs, but leakage from the tumor microenvironment may allow systemic activation of prodrugs that produces adverse effects.[20] A more selective approach to activate glucuronide prodrugs is to

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anchor βG on the membrane of tumor cells.[21,22] We have previously found that tumor-located expression of membrane-tethered βG greatly sensitized tumor cells to a glucuronide produg of p-hydroxyaniline mustard and produced strong antitumor activity *in vivo*.[23]

In a recent study, we demonstrated that stable expression of a membrane-tethered form of βG on EJ bladder cancer cells increased their sensitivity to SN-38G and enhanced the therapeutic efficacy of CPT-11 *in vivo*.[24] We transduced the tumor cells *ex vivo* with a retroviral vector to achieve βG expression. Because this approach is difficult to use clinically, in the present study we used an adenoviral (Ad) vector for gene transfer into tumors. Ad vectors possess advantages for *in vivo* gene transfer into tumors including their superior *in vivo* gene transfer efficiency of both dividing and non-dividing cells after systemic or intratumoral administration.[25–27] Ad vectors are also being used to deliver prodrug-activating enzymes into tumors in clinical trials.[28] Because it is difficult to transduce all cancer cells in a tumor, we also investigated if tumor-located expression of membrane-tethered βG could sensitize untransduced bystander cancer cells to SN-38G.

Materials and methods

Cell lines

LS174T human colon cancer cells were from the American Type Culture Collection (Manassas, VA). The 293N human embryonic kidney cells were a kind gift from Dr S K Shyue (Academia Sinica, Taiwan). CL1-5 human lung cancer cells were kindly provided by Dr P C Yang (Academia Sinica, Taiwan).[29] EJ human bladder carcinoma cells were a gift from Dr Konan Peck (Academia Sinica, Taiwan).[30]

Cells were infected with retroviral particles expressing membrane-anchored βG and selected with G418 to create stable surface βG-expressing cell lines as previously described.[23] All cells were cultured in RPMI 1640 or Dulbecco's modified Eagle's medium supplemented with 10% bovine calf serum, 2.98 mg ml^{-1} HEPES, 1 mg ml^{-1} sodium bicarbonate, 100 units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin in a humidified atmosphere of 5% CO_2 in air at 37 °C.

Ad vectors

The pAd-CMV plasmids containing membrane-tethered βG or anti-dansyl scFv (αDNS) transgenes[31] were co-transfected with pJM17, which carries the entire Ad5 genome, lacking E1 and E3 functions, in E1-complementing 293N cells to produce Ad expressing membrane-tethered βG (Ad.βG) and Ad.αDNS. Recombinant viruses were propagated in 293 cells, banded in CsCl gradients, dialyzed and stored at 80 °C.[32] Virus titers (plaque forming units) were determined by plaque assay on 293N cells as described.[33] Quantification of replication-competent adenoviruses by E1- and E4-specific quantitative PCR showed that preparations contained less than one copy E1 + (replication-competent adenoviruses) viral genome per 10^8 genomes.

Cell growth rate

Cells (1.5×10^5) per well seeded in triplicate were counted daily for 5 days. Cell growth rates were plotted and calculated with GraphPad Prism Version 5 (GraphPad software, La Jolla, CA) using an exponential growth equation.

Flow cytometry

Cells were stained with a rat antibody against murine βG (7G7)[23] or a rat antibody against HA (Roche, Mannheim, Germany) followed by a goat antibody against rat immunoglobulin G (H + L) conjugated with fluorescein isothiocyanate (Jackson ImmunoResearch Laboratories, PA). Cell viability was confirmed by propidium iodide staining. The surface immunofluorescence of 10 000 viable cells was measured with a FACSCaliber flow cytometer (Becton Dickinson, Franklin Lakes, NJ) and fluorescence intensities were analyzed with Flowjo V7.2.5 (Tree Star, Ashland, OR).

X-Glu and immunofluorescence staining

Cells on glass coverslips were fixed with 2% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min at 4 °C. The cells were incubated with 1.25 mg ml^{-1} 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-Glu) in PBS, pH 5.0, containing 5 mM $K_3Fe(CN)_6$ and 5 mM $K_2Fe(CN)_6$. The cells were then stained with a rabbit antibody against βG at 4 °C for 45 min followed by rhodamine-conjugated goat anti-rabbit immunoglobulin G (Organon Teknika, Malvern, PA) at 4 °C for 30 min. After washing with PBS, the cells were fixed with 2% paraformaldehyde in PBS for 10 min at room temperature. The cells were mounted in GVA mount (Zymed, San Francisco, CA) and observed under a fluorescence microscope equipped with a digital camera.

^{3}H-thymidine incorporation assay

A total of 5000 cells per well were seeded in 96 well microtiter plates. For determination of drug cytotoxicity, cells were treated with graded concentrations of drugs in triplicate. For the bystander killing assay, 250 nM SN-38G or SN-38 was added to defined ratios of βG-expressing and non-expressing cells. In adenovirus transduction experiments, cells that were 48 h post-transduced with Ad.βG or Ad.αDNS at defined multiplicity of infections were treated with 0 nM or 250 nM SN-38G. After 48 h, the cells were washed twice with sterile PBS and then incubated in fresh medium for another 24 h. Fresh medium containing 1 μCi per well, ^{3}H-thymidine was added 16 h before the cells were harvested on glass-fiber filters and the radioactivity was measured on a TopCount scintillation counter. Results are expressed as percent of ^{3}H-thymidine incorporation compared with untreated cells.

Fluorescent immunohistochemistry

Mice bearing 90 ~ 110 mm^3 subcutaneous EJ tumors were intratumorally injected with 10^9 plaque-forming units of Ad.βG, Ad.αDNS or PBS on days 9 and 10 and tumors were obtained for cryosection on day 12. Tissue sections were fixed in 4% paraformaldehyde. Biotin-labeled goat

anti-HA (Vector Laboratories, Burlingame, CA) or biotin-labeled goat anti-rabbit immunoglobulin G (Chemicon International) antibodies prepared in PBS-bovine serum albumin (5%) were applied to the sections overnight at 4 °C. After washing with PBS, tissue sections were stained with streptavidin-labeled rhodamine (Jackson Immuno-Research Laboratories) followed by 4′,6-diamidino-2-phenyl indole staining. Tissue sections were observed under a fluorescence microscope equipped with a charge-coupled devise detector fields at $\times 200$ magnification.

Antitumor activity in vivo

Beige-Scid mice were maintained under specific-pathogen free conditions. Groups of Beige-Scid mice bearing $110 \sim 130\,mm^3$ subcutaneous tumors in their right flank were intravenously injected with PBS or $10\,mg\,kg^{-1}$ CPT-11 on two consecutive days. For adenovirus-mediated gene delivery experiments, mice were intra-tumorally injected with 10^9 plaque forming units per mouse of Ad.βG or Ad.αDNS in PBS on two consecutive days when tumor volume reached $\sim 90\,mm^3$, followed by intravenous administration of PBS or 10 mg/kg or $15\,mg\,kg^{-1}$ CPT-11 for two consecutive days. Body weights and tumor sizes were followed every 2–3 days. Tumor volume was calculated according the formula: length $\times$ width $\times$ height $\times 0.5$.

Statistical significance

Statistical significance of differences between mean values was estimated with GraphPad Prism Version 5 using the unpaired (independent) *t*-test for unequal variances. *P*-values of $\leqslant 0.05$ were considered statistically significant.

Results

Characterization of cells

To determine if expression of βG could enhance the antitumor activity of different tumor types to CPT-11, βG was tethered on three different cancer cell lines by fusing the *βG* gene to the juxtamembrane immunoglobulin-like extracellular domain, transmembrane domain and cytoplasmic tail of murine B7-1.[23] We located βG on the surface of cancer cells because most

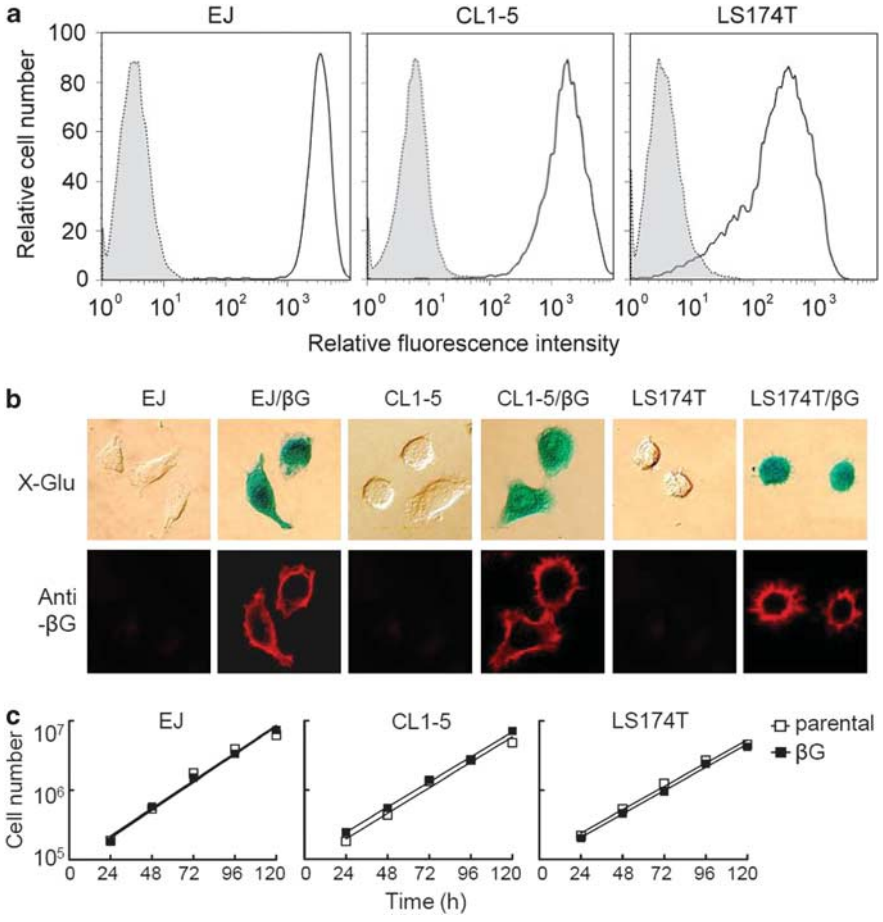

Figure 1 Characterization of cell lines. (**a**) Surface β-glucuronidase (βG)-expressing (solid lines) or non-expressing (shaded area) cells were immunofluorescence stained with a rat anti-βG antibody (7G7), followed by fluorescein isothiocyanate conjugated anti-rat immunoglobulin G (IgG) antibody and then analyzed on a flow cytometer. (**b**) Cells grown on coverslips were incubated with X-Glu solution and then stained with a rabbit antibody against βG, followed by rhodamine-conjugated anti-rabbit IgG antibody. Cells were observed under phase-contrast (upper panels) or under a fluorescence microscope equipped with a charge-coupled devise detector (lower panels). (**c**) Growth rate of cells ($n=3$).

glucuronides do not readily cross the plasma membrane of cells due to the highly polar nature of the glucuronic acid group. Immunofluorescence staining with anti-βG antibody followed by analysis on a flow cytometer showed that high levels of βG accumulated on the surface of EJ bladder cancer cells, CL1-5 lung cancer and LS174T colon cancer (Figure 1a). Biochemical reaction with X-Glu and immunofluorescence staining with an anti-βG antibody further confirmed that functionally active βG was located on the plasma membrane of EJ/βG, CL1-5/βG and LS174T/βG, but not the corresponding wild type cells (Figure 1b). There was no obvious difference in cell growth rate between βG-expressing and non-expressing cells (Figure 1c), indicating that surface expression of βG did not hinder cell growth.

Table 1 Cytotoxicity of CPT-11, SN-38 and SN-38G to human cancer cells

Cell lines	*IC_{50} (nM)*		
	CPT-11	*SN-38*	*SN-38G*
EJ	2100 ± 140	7.7 ± 0.6	>1000
EJ/βG	2100 ± 52	5.3 ± 0.2	29 ± 0.3
CL1-5	2300 ± 170	10 ± 0.3	>1000
CL1-5/βG	2400 ± 67	9.1 ± 0.7	50 ± 6.9
LS174T	750 ± 30	1.9 ± 0.1	>1000
LS174T/βG	610 ± 19	1.5 ± 0.1	12 ± 0.7

Abbreviation: IC, inhibitory concentration.
Results represent mean values of triplicate determinations ± s.d.

In vitro *cytotoxicity of CPT-11, SN-38 and SN-38G*

To verify that membrane-anchored βG could convert SN-38G to SN-38, the viability of cells exposed to graded concentrations of CPT-11, SN-38 and SN-38G for 48 h was estimated by measuring incorporation of ^{3}H-thymidine into cellular DNA. βG-expressing and parental cells displayed similar sensitivities to CPT-11 and SN-38 (Table 1). Treatment of SN-38G did not affect any of the cell lines at concentrations below 1000 nM (Figure 2). By contrast, βG-expressing cells were over 20–80-fold more susceptible to SN-38G in comparison with parental cells, demonstrating that βG-mediated hydrolysis of SN-38G could increase its cytotoxicity.

Surface display of βG can induce potent bystander killing

To investigate whether cell surface-located activation of SN-38G could induce bystander killing of neighboring tumor cells, SN-38G was added to mixtures of wild-type and βG-expressing EJ, CL1-5 and LS-174T tumor cells. Significant killing of the cell population was achieved even when only 5% of the cells expressed βG (Figure 3). These data indicate that activation of SN-38G at the cell surface allows efficient diffusion of SN-38 to neighboring cells.

CPT-11 therapeutic efficacy can be enhanced by βG expression in tumors

To investigate whether CPT-11 therapeutic efficacy could be improved by tumor-located expression of βG, mice bearing established LS174T and LS174T/βG colon or CL1-5 and CL1-5/βG lung cancer xenografts were

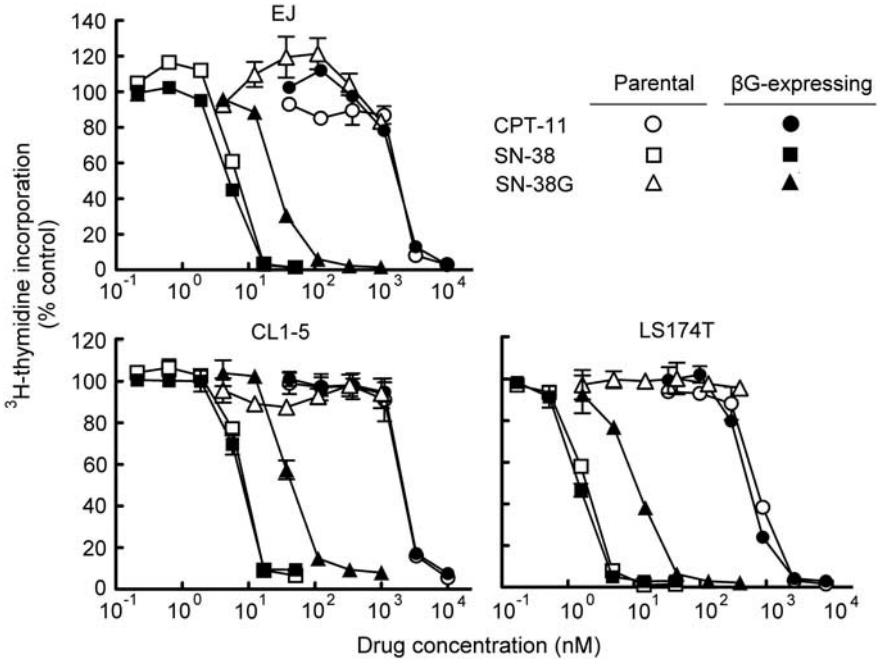

Figure 2 Membrane-tethered β-glucuronidase increases cellular sensitivity to SN-38G. Cancer cell lines were incubated with graded concentrations of CPT-11, SN-38 or SN-38G in triplicate for 48 h. Cells were incubated with fresh medium for an additional 24 h before incorporation of ^{3}H-thymidine was measured. Bars, s.d.

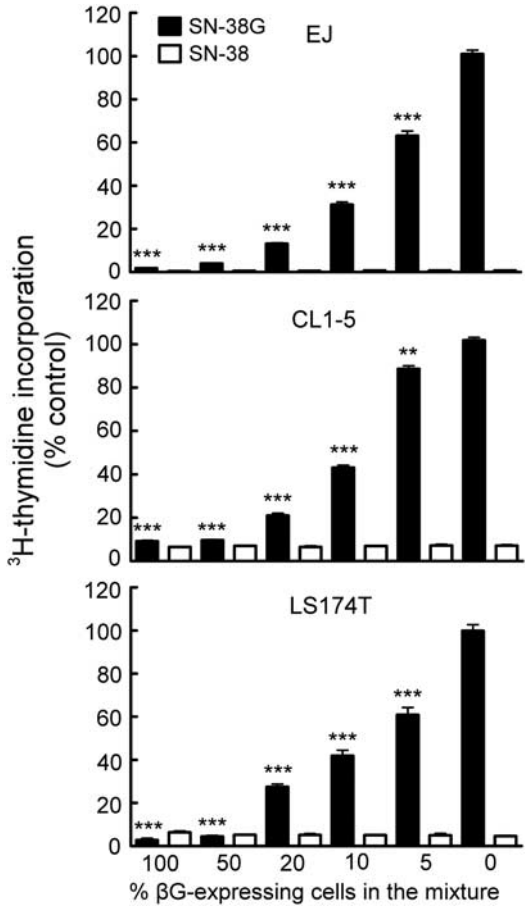

Figure 3 Cell-surface display of β-glucuronidase can provide bystander killing. Defined ratios of βG-expressing and non-expressing cells were treated with 250 nM SN-38 or SN-38G for 48 h. The cells were further incubated for 24 h in fresh medium before incorporation of ^{3}H-thymidine was measured. Bars, s.d. Significantly lower incorporation of ^{3}H-thymidine as compared with parental cells after treatment with SN-38G is indicated. $^{**}P \leqslant 0.005$; $^{***}P \leqslant 0.0005$.

intravenously injected with PBS or 10 mg kg^{-1} CPT-11 on two consecutive days after tumor sizes reached ~130 mm^3. CPT-11 produced significantly greater suppression of βG-expressing tumors as compared with wild-type tumors, especially for CL1-5 lung adenocarcinoma xenografts (Figure 4). There was no obvious difference in the mean body weights between CPT-11-treated mice bearing parental and βG-expressing tumors, indicating that intratumoral activation of SN-38G did not induce additional systemic cytotoxicity.

Ad.βG transduction sensitizes cancer cells to SN-38G
To examine if an adenoviral vector-mediated GDEPT approach could improve CPT-11 treatment, we constructed two recombinant adenoviruses to express membrane-anchored βG (Ad.βG) or a membrane-anchored α-dansyl scFv antibody (Ad.αDNS) as a control. To determine the cytotoxic potency of Ad.βG *in vitro*, EJ bladder cancer cells were transduced with graded concentrations of Ad.βG or Ad.αDNS. Dose-dependent expression of the proteins on cells was observed (Figure 5a). SN-38 treatment had no effect on cells infected with Ad.αDNS whereas Ad.βG-infected cells displayed an increased sensitivity to SN-38G, even at a multiplicity of infection of 0.16 (Figure 5b).

Ad.βG/CPT-11 GDEPT retards tumor growth in vivo
To evaluate the therapeutic efficacy of the combination of Ad.βG and CPT-11 treatment, mice bearing EJ bladder tumors were treated by intratumoral injection with Ad.βG or Ad.αDNS and then with intravenous PBS or 15 mg kg^{-1} CPT-11 over the next two days. Fluorescent immunohistochemical staining of tumor sections with anti-HA antibody demonstrated high expression of βG or αDNS scFv in tumors two days after adenovirus injection (Figure 5c). Significant suppression of tumor growth was observed for the combination treatment of Ad.βG and CPT-11 as compared with the PBS-treated group or combined treatment with Ad.αDNS and CPT-11 (Figure 5d), suggesting that extracellular conversion of SN-38G to SN-38 at tumor sites through GDEPT with βG could be a practical method to enhance CPT-11 efficacy.

Discussion

Prodrug-enzyme combination therapy seeks to enhance the therapeutic index of chemotherapy by selective enzymatic conversion of relatively non-toxic anticancer prodrugs in tumors. Based on this concept, we investigated if tumor-located conversion of an endogenously generated metabolite of CPT-11 to the potent topoisomerase I inhibitor SN-38 could enhance the antitumor efficacy of CPT-11. Tumor cells that expressed βG on their surface displayed increased sensitivity to SN-38G, a major glucuronide metabolite generated after CPT-11 administration. Importantly, improved therapeutic efficacy without increased toxicity was observed in tumors treated with Ad.βG and CPT-11, suggesting that GDEPT is a potential method to deliver βG to tumor sites to enhance the antitumor activity of CPT-11.

Tumor-localized enhancement of CPT-11 antitumor activity by membrane-anchored βG differs in three important ways from most traditional prodrug-enzyme therapies. First, SN-38G is an endogenously generated prodrug. Most commonly investigated anticancer prodrugs, such as ganciclovir, 5-fluorocytosine (5-FC), 4-[N,N-bis(2-iodoethyl) amino] phenoxycarbonyl L-glutamic acid (ZD2767P) and 5-(azaridin-1-yl) 2,4-dinotrobenzamide (CB1954) are chemically synthesized *ex vivo* and then directly administered to patients.[28,34–36] By contrast, SN-38G is generated *in vivo* by UDP-glucuronosyl transferases acting on SN-38, the active metabolite of CPT-11.[8] *In vivo* generation of SN-38G may be particularly advantageous as hydrophilic glucuronide metabolites are rapidly eliminated from the circulation.[37] Continuous generation of SN-38G allows sustained high levels of SN-38G (5–25 fold higher than SN-38) in the circulation of patients.[9–12] Hydrolysis of SN-38G by

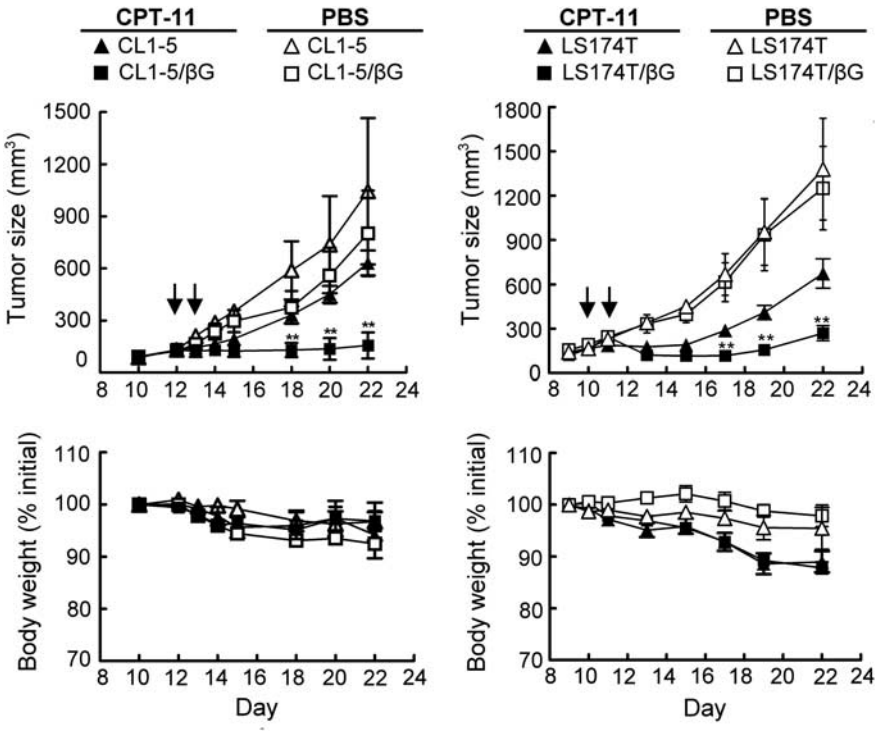

Figure 4 Growth of tumors expressing membrane-bound β-glucuronidase (βG) was suppressed to a greater degree than parental tumors. Beige-Scid mice bearing 110–130 mm^3 subcutaneous tumors were intravenously injected with 10 mg kg^{-1} CPT-11 or PBS on two consecutive days (indicated as arrows). Tumor sizes (top) and body weight (bottom) were measured every 2–3 days. Bars, s.e.m. Significant differences in tumor size between mice bearing mβG-expressing and parental tumors treated with CPT-11. $^{**}P \leqslant 0.005$.

membrane-anchored βG may allow prolonged exposure of tumor cells to SN-38, important for the time-dependent antitumor activity of topoisomerase I inhibitors that are effective against cancer cells during the S phase of the cell cycle.[38]

A second way by which βG activation of SN-38G differs from commonly investigated prodrug/enzyme combinations is that most activating enzymes, such as herpes simplex virus thymidine kinase for ganciclovir, cytosine deaminase for 5-FC, nitroreductase for CB1954 and carboxypeptidase G2 for ZD2767P, are expressed intracellularly because the anticancer prodrugs can readily enter transduced cells by passive or facilitated transport. Glucuronides, on the other hand, do not efficiently enter cells, resulting in poor activation of glucuronide prodrugs when βG is expressed in the cytosol of mammalian cells.[19,39] To overcome poor penetration of glucuronides into cells, we anchored βG on the plasma membrane to allow effective contact with SN-38G. High levels of βG could be expressed on all three tumor cells investigated by fusing an optimized transmembrane domain derived from the B7-1 antigen to the c-terminus of βG.[23,40] Expression of βG on cells allowed effective conversion of SN-38G to SN38 as demonstrated by greatly enhanced cellular sensitivity to SN-38G (Figure 2). We also observed that extracellular conversion of SN-38G to SN-38 provided significant bystander killing of neighboring cells that did not express surface-tethered βG. Expression of as few as 5% βG-expressing cells in the mixture allowed significant killing of non-transduced cells (Figure 3). Adenoviral delivery of βG at a range of multiplicity of infections also showed that potent bystander killing was elicited at even a multiplicity of infection as low as 0.16 (Figure 5b). Thus, generation of SN-38 by a few βG-expressing cells can elicit potent bystander killing. As it is difficult to transduce all malignant cells in a tumor, bystander killing of non-transfected cancer cells may be important for enhancement of CPT-11 antitumor activity by Ad.βG.

A third way in which βG activation of SN-38G differs from other prodrug systems is that βG is an endogenous enzyme whereas other prodrug activating enzymes are typically derived from bacterial (carboxypeptidase G2, nitroreductase), yeast (cytosine deaminase) or viral (herpes simplex virus thymidine kinase) sources.[41] These foreign enzymes do not have human analogs, allowing selective activation of prodrugs at sites of enzyme expression. βG, by contrast, is expressed in most mammalian cells, especially in the liver and kidney tissues in humans. Systemic activation of glucuronides by endogenous βG, however, appears to be limited by the compartmentalization of βG in lysosomes, which effectively limits contact of extracellular glucuronides with endogenous βG in most tissues.[39] βG does accumulate in the interstitial space in tumors due to secretion by tumor-infiltrating monocytes and release from necrotic cancer cells.[42–44] This explains the effectiveness of cancer monotherapy with synthetic glucuronide prodrugs of

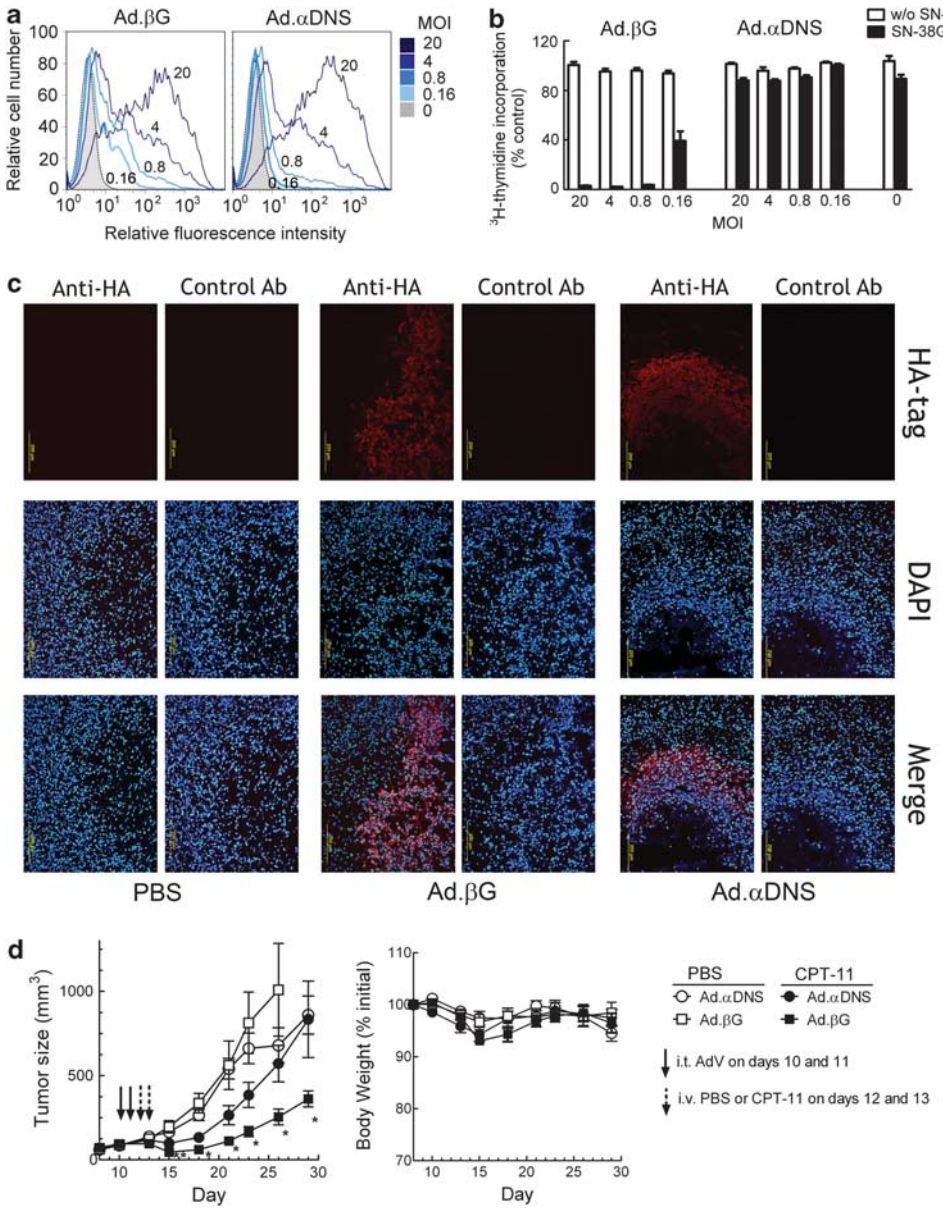

Figure 5 Adenovirus-mediated *βG* gene delivery *in vitro* and *in vivo*. (**a**) Expression level of transduced genes at different multiplicity of infections (MOIs) was detected with anti-hemagglutinin (HA) antibody, followed by fluorescein isothiocyanate-conjugated secondary antibody and then analyzed on a flow cytometer. (**b**) Cells infected with adenoviral vector expressing membrane-tethered β-glucuronidase (Ad.βG) or adenoviral anti-dansyl scFv (Ad.αDNS) at the indicated MOIs were treated with 0 or 250 nM SN-38G for 48 h, followed by ^{3}H-thymidine incorporation assay. Bars, s.d. (**c**) Mice bearing 90–110 mm^3 subcutaneous (s.c.) EJ tumors were intratumorally injected with 10^9 plaque forming units (pfu) Ad.βG, Ad.αDNS or phosphate-buffered saline (PBS) on days 9 and 10 and tumors were obtained for cryosection on day 12. Biotin-labeled goat anti-HA or biotin-labeled goat anti-rabbit immunoglobulin G (control antibody) antibodies were applied to the sections followed by streptavidin-labeled rhodamine (red). The nucleus was stained by 4′,6-diamidino-2-phenyl indole (DAPI) (blue). Sections were observed under a fluorescence microscope equipped with a charge-coupled devise detector. (**d**) Mice bearing 90–110 mm^3 s.c. EJ tumors were i.t injected with 10^9 pfu Ad.βG or Ad.αDNS on days 9 and 10 and intravenously injected with 15 mg kg^{-1} CPT-11 or PBS on days 11 and 12. Bars, s.e.m. Significant differences in size between tumors injected with Ad.βG and Ad.αDNS after CPT-11 treatment are indicated: $^{*}P \leqslant 0.05$; $^{**}P \leqslant 0.005$.

anthracyclines and topoisomerase I inhibitors in mice bearing human xenografts[16,17,42] and suggests that even without Ad.βG transduction of cancer cells, SN-38 generated by deconjugation of SN-38G by βG in the tumor microenvironment may contribute to the anti-tumor activity of CPT-11.[45,46]

Our study suggests several potential methods to further enhance the antitumor efficacy of CPT-11. First, improved βG variants that display enhanced activity at the conditions present in the tumor microenvironment may facilitate the development of highly effective Ad.βG vectors for clinical use.[47] Second, in addition to using

Ad.βG as a delivery vehicle, selective hydrolysis of glucuronide anticancer prodrugs in the tumor microenvironment can be achieved in mice models by systemic administration of E. coli engineered to express high levels of βG,[48] suggesting that such bacteria may also be useful for enhancing the activity of CPT-11. Finally, tumor-located expression of carboxylesterase, responsible for the catalytic hydrolysis of CPT-11 to SN-38, can sensitize tumor cells to CPT-11 *in vitro* and *in vivo*.[49,50] As carboxyesterases act on CPT-11 whereas βG acts on SN-38G, combined treatment with Ad.βG and Ad.carboxyesterase vectors may offer a rationale method to further enhance the antitumor efficacy of CPT-11.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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