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Photochemical Control of the Infectivity of Adenoviral Vectors Using a Novel Photocleavable Biotinylation Reagent

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Summary

We have explored a novel strategy for controlling the infectivity of adenoviral vectors. This strategy involves a method whereby the infectivity of adenoviral vectors is neutralized by treatment of viral particles with a water-soluble, photocleavable biotinylation reagent. These modified viral vectors possess little to no infectivity for target cells. Exposure of these modified viral vectors to 365 nm light induces a reversal of the neutralizing, chemical modification, resulting in restoration of infectivity to the viral vectors. The light-directed transduction of target cells by photoactivatable adenoviral vectors was demonstrated successfully both in vitro and in vivo. This photochemical infectivity trigger possesses great potential, both as a research tool and as a novel tactic for the delivery of gene-transfer agents, since the infectivity of adenoviral vectors can be controlled externally in a versatile manner.

Introduction

Adenoviral vectors have become one of the most useful gene transfer agents [1–5]. They are structurally stable and can be produced to high infectious titers (up to $\sim 10^{12}$ infectious units/ml). Their genome can be easily manipulated to deliver large transgenes. Adenoviral vectors can infect a wide range of cells, including nondividing cells. These characteristics make adenoviral vectors a particularly attractive tool for a wide variety of gene transfer applications. However, the broad target cell range of adenoviral vectors has two consequences that limit their utility for in vivo gene transfer applications, such as gene therapy. First, their broad tropism can result in the delivery of the transgene in a nonspecific manner. Second, large doses of undirected adenoviral vectors would be required to ensure an adequate amount of gene transfer to the target site. Thus, the ability to control the delivery of adenoviral vectors in a controlled or directed manner is of paramount importance in the development of adenovirus-based in vivo gene transfer protocols.

We have explored a novel strategy for controlling the infectivity of adenoviral vectors. This strategy employs a chemical agent that modifies adenoviral vectors in a reversible fashion such that their infectivity is eliminated but can be restored upon application of an external stimulus. This strategy allows adenovirus-mediated gene transduction to be externally controllable.

Results and Discussion

We have created a novel strategy for controlling the infectivity of adenoviral vectors. This strategy involves a method whereby the infectivity of an adenoviral vector is first neutralized by a reversible chemical modification. Reversal of this chemical modification allows for the restoration of infectivity to the adenoviral vector. This strategy had recently been successfully implemented on retroviral vectors derived from amphotropic Moloney murine leukemia virus by using photocleavable biotin (PCB) [6] as the modification reagent [7]. PCB (Figure 1A) contains a biotin moiety linked though a spacer arm to a 1-(2-nitrophenyl)ethyl group, which is derivatized with an NHS (*N*-hydroxysuccinimide) ester. The NHS ester reacts selectively with primary aliphatic amino groups, such as N termini and lysine residues on proteins, which are abundant in viral vectors, to form a carbamate bond. When PCB-biomolecule conjugates are exposed to 300–365 nm light, the PCB moiety undergoes an intramolecular photochemical reaction, which involves the cleavage of the carbamate bond. This results in the regeneration of the primary amino group on the biomolecule and releases the PCB moiety, in which the 1-(2-nitrophenyl)ethyl group is converted to a 2-nitrosoacetophenone derivative [6]. PCB is highly hydrophobic and exhibits low solubility in aqueous media. To reduce hydrophobicity and increase solubility in aqueous media, a water-soluble version of PCB (WSPCB) was synthesized. WSPCB is structurally similar to PCB, but it has a mixed polarity spacer arm between the biotin and 1-(2-nitrophenyl)ethyl moieties (Figure 1A). The synthesis of WSPCB (Figure 1B) involved the production of a mono-biotinylated derivative of 2,2'-(ethylenedioxy)-bis(ethylamine), which was then conjugated to 5-succinylamidomethyl-2-nitroacetophenone. The resulting intermediate was converted to a reactive NHS derivative.

In order to determine if the infectivity of adenoviral vectors could be eliminated by treatment with either

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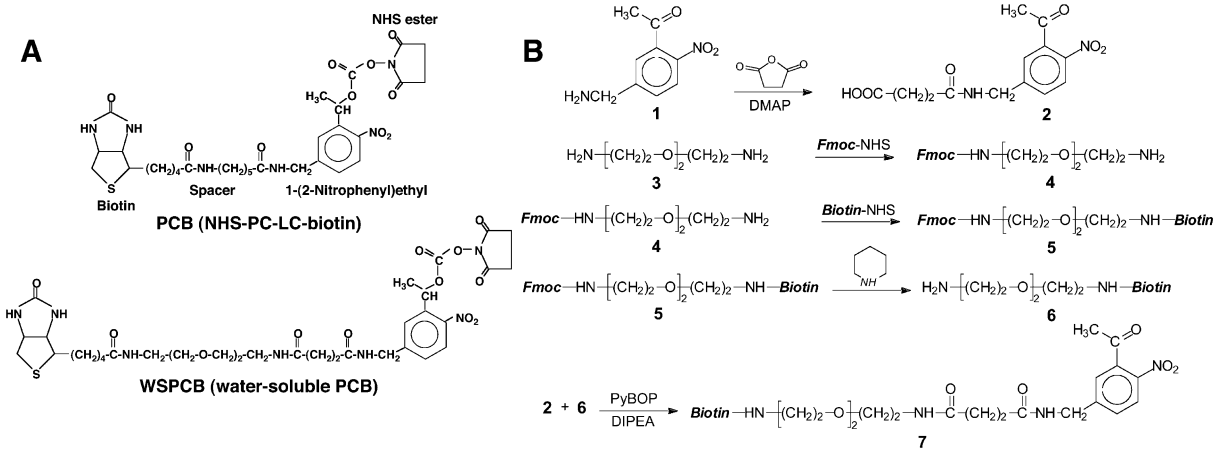

Figure 1. Structures and Synthesis of Photocleavable Biotins

(A) Structures of photocleavable biotin (NHS-PC-LC-Biotin; PCB) and its water-soluble derivative, WSPCB.

(B) Synthesis scheme of WSPCB.

PCB or WSPCB, adenoviral vectors were exposed to various concentrations of PCB or WSPCB. The adenoviral vectors used contain a transducable *lac*Z gene, which provides a simple means of detecting transduced cells. Treatment with WSPCB at 1 mg/ml or greater virtually eliminated the infectivity of these vectors (Figure 2A). In contrast, treatments with PCB were considerably less effective at inhibiting the infectivity of these vectors than with WSPCB at the same concentrations (Figure 2B). Retroviral vectors, when treated with PCB, showed a behavior contrary to this finding [7]. PCB was highly effective at abrogating retroviral infectivity at concentrations of 1–2 mg/ml. This differential sensitivity of retroviral and adenoviral vectors to these two biotinylation reagents may be derived from the vast differences in the structures and properties of the outer surfaces of these viruses. Having observed the sensitivity of the adenoviral vectors to biotinylation by WSPCB, we determined the concentration range in which WSPCB could function to modulate viral infectivity (Figure 2C). Treatment with as low as 0.1 mg/ml WSPCB reduced infectivity by nearly 50%. Treatment with WSPCB at concentrations greater than 0.4 mg/ml virtually eliminated the infectivity of these vectors.

We tested whether the infectivity of the biotinylated adenoviral vector could be recovered via photocleavage of the WSPCB molecule from the viral particles. Adenoviral vectors were treated with various concentrations of WSPCB, and then either kept in the dark or exposed to 365 nm light. These samples were analyzed for their infectivity by using D-17 cells (Figure 3A). WSPCB-treated, nonirradiated adenoviral vectors showed virtually no infectivity. In contrast, when WSPCB-treated viral vectors were exposed to 365 nm light, their infectivity was restored. At an energy output of 16 mW/cm^2, restoration of infectivity occurred within 1 min of exposure to 365 nm light, with maximum recovery occurring after 3 min of irradiation (Figure 3B). Irradiation with 365 nm light beyond 3 min did not enhance the recovery of infectivity. Instead, the infectivity of irradiated vectors decreased slightly with prolonged exposure, possibly because of damage to adenoviral vectors caused by shorter-wavelength radiation emitted from the UV light source.

We next attempted to determine whether the reactivation of adenoviral infectivity could be done in situ in the presence of target cells. Adenoviral vectors treated with 1 mg/ml WSPCB were added to D-17 cells growing in borosilicate glass vials. These vials were either kept in the dark or irradiated with 365 nm light. Infection assays of cells, which had been exposed to WSPCB-treated adenovirus vectors and kept in the dark, showed negligible amounts of infection, while great amounts of infection were observed in cells exposed to WSPCB-treated adenoviral vectors and irradiated with 365 nm light (Figure 4). Irradiation with 365 nm light showed no appreciable effects on cell viability. These experiments were performed by using WSPCB-treated adenoviral vectors, which had been washed of free, unreacted WSPCB by repeated rounds of ultrafiltration. This ensured that the photoactivatable infectivity of adenoviral vectors, shown above, would not be based on the presence of non-virion-associated WSPCB.

The results described above imply that WSPCB is covalently attached to viral particles upon treatment and that the association of WSPCB with virions is responsible for the photoactivatable infectivity of treated vectors. To determine whether WSPCB is associated with virions, and, if so, whether irradiation with 365 nm light causes cleavage of virion-associated WSPCB, Western blotting analysis was performed. Adenoviral vectors were treated with 1 mg/ml WSPCB and either irradiated with 365 nm light or kept in the dark. The resulting viral proteins were separated by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), transferred to a membrane, and probed for conjugated WSPCB with a streptavidin-alkaline phosphatase conjugate (Figure 5). Biotinylation was observed on 84 kDa and 134 kDa proteins in samples derived from WSPCB-treated, nonirradiated adenoviral vectors (lane 1). The amount of WSPCB, bound to these proteins, was markedly reduced when WSPCB-treated viral vectors had been ex-

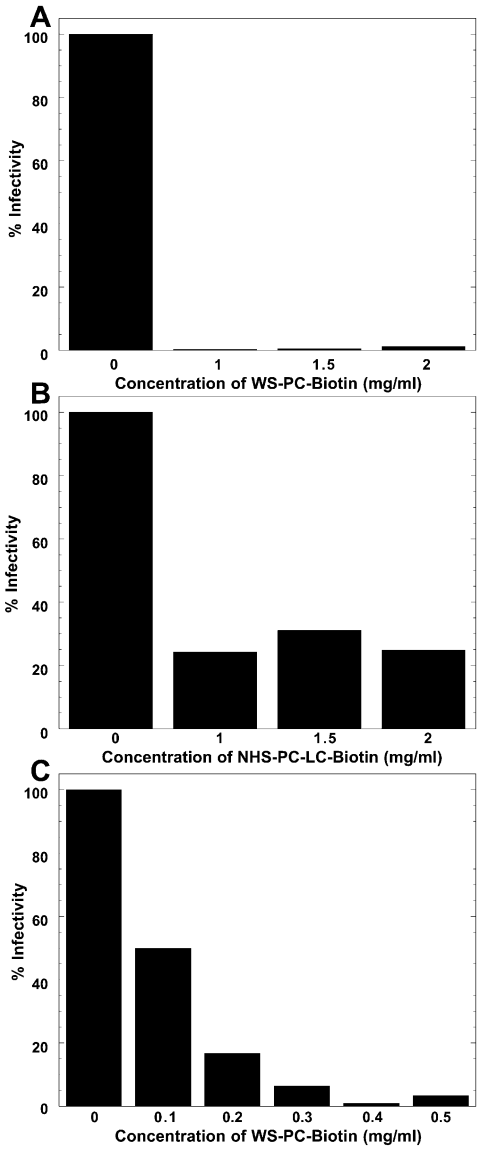

Figure 2. Effect of Treatment with WSPCB or PCB on the Infectivity of Adenoviral Vectors

A stock solution of WSPCB or PCB, both in DMF, was diluted in PBS (pH 7.4). The diluted WSPCB or PCB (25 μl) was allowed to react with adenoviral vectors (2.5×10^6 viral particles in 25 μl PBS) for 2 hr. The infectivity of the resulting viral vectors was assayed by using D-17 cells. DMF at concentrations used during treatment with WSPCB or PCB (up to 8%) showed no effect on infectivity and cell viability. (A and C), WSPCB; (B), PCB.

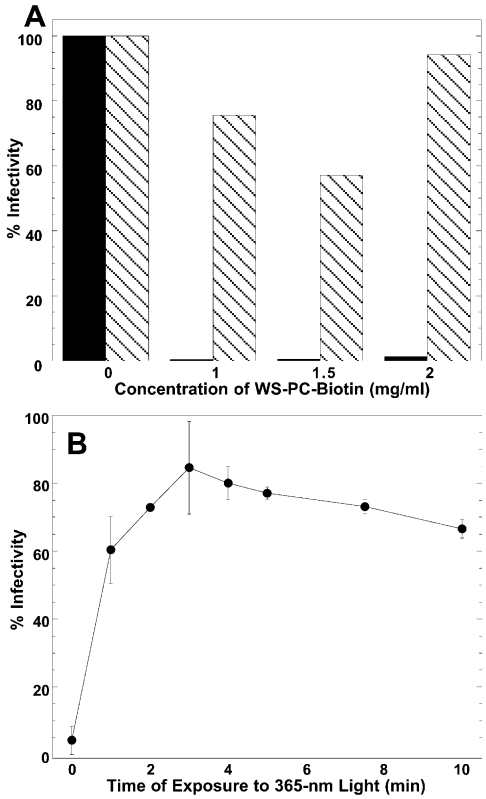

Figure 3. Photoactivation of WSPCB-Treated Adenoviral Vectors

(A) Photoactivation of the infectivity of adenoviral vectors treated with various concentrations of WSPCB. Adenoviral vectors were treated with various concentrations of WSPCB, placed into borosilicate glass vials, and either kept in the dark (solid bars) or irradiated with 365 nm light (16 mW/cm^2) for 3 min (hatched bars). The resulting viral vectors were added to monolayers of D-17 cells (5×10^4 per well) to analyze their infectivity.

(B) Time course of the restoration of the infectivity of WSPCB-treated adenoviral vectors. Adenoviral vectors were treated with 1 mg/ml WSPCB and irradiated with 365 nm light (16 mW/cm^2) for the durations indicated. The infectivity of the irradiated viral vectors was analyzed by using D-17 cells.

posed to 365 nm light (lane 2). These results demonstrate that irradiation of WSPCB-treated adenoviral vectors with 365 nm light cleaves and subsequently liberates WSPCB from virions. These data strongly suggest that the conjugation of WSPCB to adenoviral vectors and cleavage of virion-associated WSPCB is the infectivity-controlling factor.

As shown above, WSPCB is vastly more effective than PCB in inhibiting the infectivity of adenoviral vectors. The primary difference in properties between the two biotinylation reagents, i.e., hydrophilicity, suggests that the mechanism of infectivity inhibition by these reagents is based on the modulation of the function of viral proteins on the outer, solvent-exposed viral surface. Hence we hypothesized that the ability of adenoviral vectors to either bind or enter target cells is disrupted upon treatment with WSPCB. To test this hypothesis, we devised an assay that quantifies the ability of viral particles to be adsorbed by target cells. Adenoviral vectors were treated with 1 mg/ml WSPCB and incubated over a monolayer of D-17 cells in a culture dish or in an empty culture dish for 3 hr. Then, culture supernatants containing unbound viral vectors were collected, exposed to 365 nm light, and added to fresh D-17 cells for analysis of infectious titers. Comparison of the infectious titers of adenoviral vectors incubated with D-17 cells and those incubated in an empty culture dish indicates a percentage of viral vectors that were associated with the D-17 cells (Figure 6). WSPCB-treated adenoviral vectors exhibited minimal levels of cell association. Adenoviral vectors, which had been treated with WSPCB and irradi-

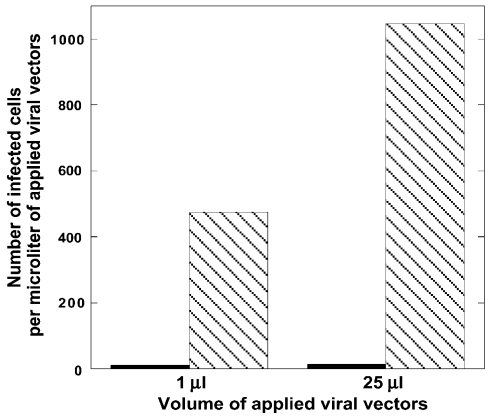

Figure 4. In Situ Photoactivation of WSPCB-Treated Adenoviral Vectors

Adenoviral vectors were treated with 1 mg/ml of WSPCB. The resulting viral vectors were purified away from unreacted WSPCB by ultrafiltration, and small aliquots (1 μl or 25 μl) of the purified viral vectors were placed within each of four 3 ml borosilicate glass vials containing monolayers of D-17 cells (5×10^4 per vial) covered with 500 μl of DMEM/6% FBS. Two of the vials were placed in the dark (solid bars), while the other two vials were exposed to 365 nm light (16 mW/cm^2) for 4 min (hatched bars). At 48 hr after irradiation, cells were stained for the expression of the *lac*Z gene. Shown are the results from two independent experiments with different viral inocula.

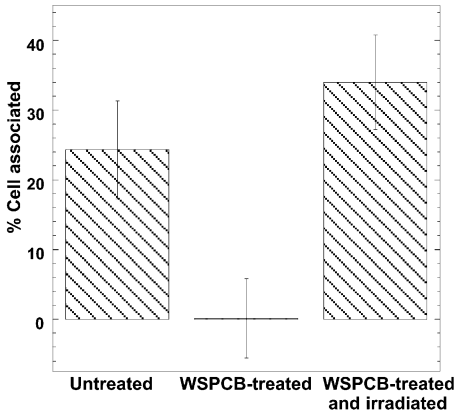

Figure 6. Adsorption Assays of WSPCB-Treated Adenoviral Vectors

Adenoviral vectors were treated with 1 mg/ml WSPCB and incubated for 3 hr over monolayers of D-17 cells grown in a 35 mm culture dish. The same viral vector sample was also incubated in an empty 35 mm culture dish to estimate nonspecific binding of virions to culture dishes. The supernatants (culture medium fractions) containing unbound virions were collected and exposed to 365 nm light (16 mW/cm^2) for 3 min. Adenoviral vectors, which had been treated with 1 mg/ml WSPCB and exposed to 365 nm light prior to the application to D-17 cells or an empty tissue culture dish, were also used in the same manner. Adenoviral vectors without WSPCB treatment were used as controls. All supernatants were subjected to infectivity analysis using fresh D-17 cells. The percentage of cell-associated virions for each sample is calculated as: [(Infectivity remaining in the supernatant after incubation in an empty dish) – (infectivity remaining in the supernatant after incubation with D-17 cells)] / (infectivity remaining in the supernatant after incubation in an empty dish).

ated with 365 nm light prior to incubation with D-17 cells, showed levels of cell association equivalent to those of untreated adenoviral vectors. This data suggests that the conjugation of WSPCB to adenoviral vectors inhibits their infectivity by interfering with their ability to bind to target cells and that irradiation with 365 nm light restores the binding ability of these vectors for target cells by releasing virion-associated WSPCB.

Following the successful development and characterization of photoactivatable adenoviral vectors in an in vitro setting, we tested the potential of these adenoviral vectors to be used for in vivo gene transfer applications using tumors growing in nude mice as viral targets. Athymic nude mice were each injected percutaneously with D-17 cells on each side of the back of the mice just above the upper legs. After the formation of small tumor nodules on both sides of the mice, tumor sites were injected with adenoviral vectors that had been treated with 0.5 mg/ml WSPCB (these treated viral vectors showed photoactivatable infectivity in vitro with cultured D-17 cells; data not shown). The mice were covered in an aluminum foil cloak, which was designed and cut in a way such that only one tumor site was exposed. Exposed tumor sites were irradiated externally with 365 nm light for 4 min through the skin over the tumor nodule. The mice were sacrificed at 48 hr after irradiation, and the tumor nodules, along with adjoining tissue, were collected, sectioned, and stained for the expression of the *lac*Z gene. Sections of tumor sites that were not exposed to 365 nm light show very few, if any, infected cells (Figure 7). However, sections of injected tumor sites that were irradiated with 365 nm light through the skin show a large number of infected cells. This indicates that the infectivity of WSPCB-treated adenoviral vectors can be reactivated in vivo by external photoirradiation. This also demonstrates that the inactivation of viral infectivity with WSPCB is a modification that is not reversed under physiological, unirradiated conditions. Apparently, the skin did not function as a complete barrier to the reactivating light. The skin that was positioned over the irradiated tumor sites was also not noticeably affected by the irradiation. A control

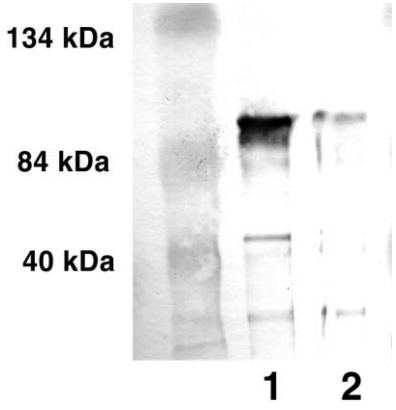

Figure 5. Western Blotting Analysis of WSPCB-Treated Adenoviral Vectors

Adenoviral vectors were treated with 1 mg/ml WSPCB and either kept in the dark (lane 1) or irradiated with 365 nm light (16 mW/cm^2) for 4 min (lane 2). The resulting viral vectors were subjected to SDS-PAGE, and proteins were transferred to polyvinylidene difluoride membrane. Biotinylated viral proteins were detected by using a streptavidin-alkaline phosphatase conjugate (Pierce).

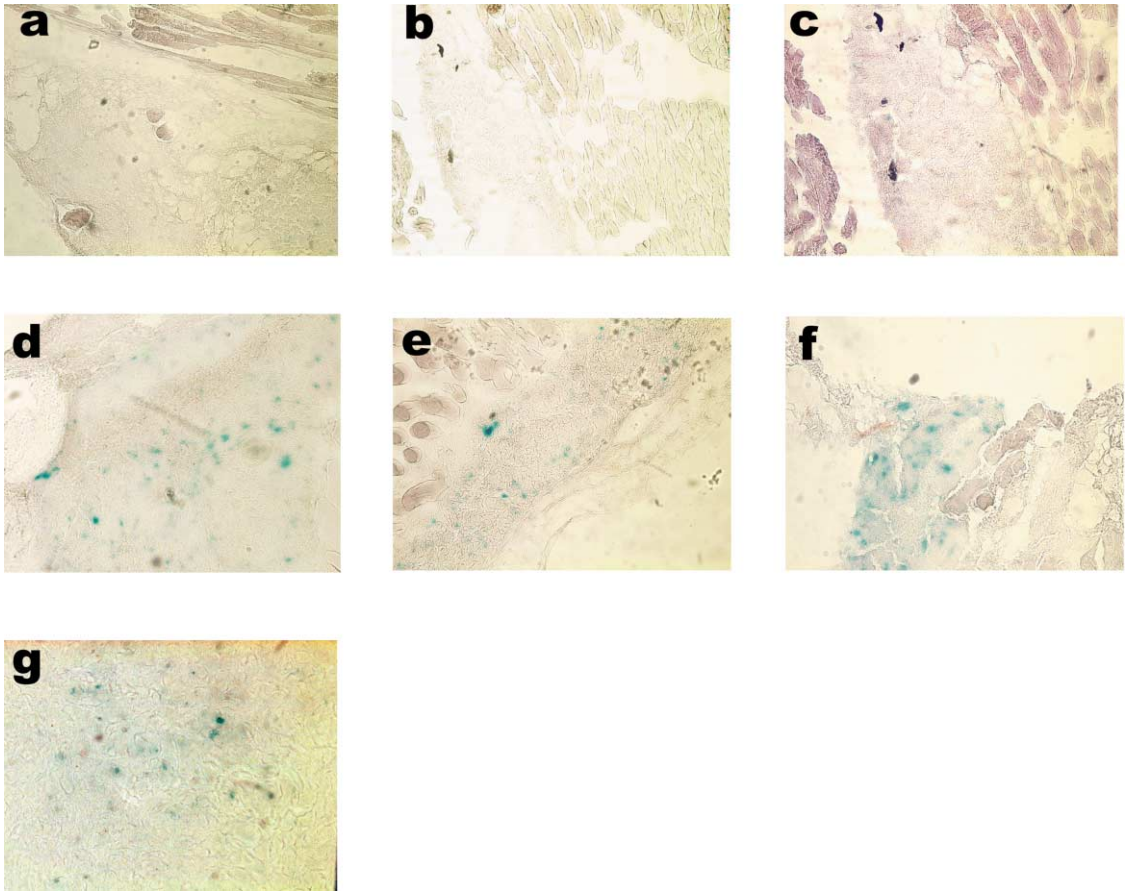

Figure 7. In Vivo Photoactivation of WSPCB-Treated Adenoviral Vectors

Mouse subcutaneous tumor models were prepared by using D-17 cells and athymic nude mice as described in Experimental Procedures. After small tumor nodules (3–5 mm) formed in these mice, each tumor site was injected with WSPCB-treated adenoviral vector (250 μl). Mice were anesthetized and covered with aluminum foil with a hole to expose only the right-hand tumor sites. Exposed tumor sites were irradiated with 365 nm light for two 2 min periods, separated by a 30 s intermission. At 48 hr after irradiation, mice were sacrificed, followed by the collection of tumor sites with adjoining tissue. Tissue was sectioned (50 μm sections), and the resulting tissue sections were stained for the expression of the *lac*Z gene using X-gal as the substrate. The stained tissue sections were viewed under a light microscope. (A–C), no irradiation (control); (D–F), irradiated with 365 nm light. A control experiment was performed in the same manner by injecting unmodified adenoviral vectors into the tumor site (G).

experiment, in which unmodified adenoviral vectors were injected in the same manner, showed a comparable amount of infection, indicating that the reactivation of PC-biotin-modified adenoviral vectors by photoirradiation under in vivo conditions is also highly efficient as seen with in vitro systems. These data demonstrate the potential that WSPCB-treated adenoviral vectors can be used as activatable gene transfer agents in whole animals.

Significance

We have demonstrated a method of making adenoviral vectors selectively activatable by an external stimulus. Conjugation of a PCB derivative, WSPCB, virtually eliminates the infectivity of adenoviral vectors in a reversible manner. Exposure of WSPCB-treated viral vectors to 365 nm light restores infectivity to levels approaching those prior to biotinylation. Inhibition and restoration of the infectivity of adenoviral vectors has successfully been demonstrated both in vitro and in vivo. This infectivity trigger holds considerable potential for the delivery of adenoviral vectors, since the site-specific activation of viral vectors could be mediated by the application of 365 nm light focused on the target sites. The viral surface biotin moiety should also be useful for further modification of the viral surface. For example, biotinylated materials can be attached to viral surface biotin moieties by using streptavidin as a molecular bridge with little effect on the cleavage efficiency of virion-associated PCB by photoirradiation (M.W.P., D.A.H., and T.S., unpublished data). These findings offer a novel strategy to the field of gene therapy by creating a new way to control the activities of viral vectors.

Experimental Procedures

Adenoviral Vectors and Target Cells

The adenoviral vector used in this study, Ad5.CMV-LacZ (Qbiogene, Montreal, Canada), is derived from adenovirus serotype 5 with the deletion of the viral E1A, E1B, and E3 genes. The adenoviral vector carries the bacterial *lac*Z gene (β-galactosidase) under the control

of the human cytomegalovirus immediate-early promoter. This viral vector was produced by using 293A cells (Qbiogene), a subline of 293 cells (human embryonal kidney cells transformed by sheared adenovirus serotype 5 genome), and purified by two rounds of CsCl gradient centrifugation, followed by removal of CsCl by dialysis against 10 mM Tris-Cl (pH 8.0), 2 mM $MgCl_2$, 4% sucrose [8, 9]. The original preparation was diluted to 1×10^{10} viral particles/ml (1×10^9 infectious units/ml) and stored at −70°C until used. The dog osteosarcoma cell line D-17 (ATCC number, CCL-183; American Type Culture Collection) was maintained in DMEM supplemented with 6% FBS (BioWhittaker).

Infectivity Assays

The infectivity of adenoviral vectors was determined by using D-17 cells as targets, which are highly permissive to infection by adenoviral vectors. An adenoviral vector stock was placed over monolayers of 5×10^4 D-17 cells in a 24-well plate and incubated at 37°C for 24 hr. Cells were washed once with culture medium and incubated at 37°C for 24 hr to permit the expression of the *lac*Z gene. Cells were then fixed with 0.5% glutaraldehyde and stained for β-galactosidase activity using X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) as the substrate. Infected, *lac*Z-expressing cells (stained blue) were counted under a light microscope.

Synthesis of Photocleavable Biotinylation Reagents

A photocleavable biotin (PCB; NHS-PC-LC-biotin) (Figure 1A) was synthesized as previously described [6]. A water-soluble derivative of PCB, WSPCB (Figure 1A), was synthesized by using the following procedure (Figure 1B). All chemicals were obtained from Aldrich except benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP), which was from Novabiochem. 5-Aminomethyl-2-nitroacetophenone hydrochloride (1.73 g, 7.5 mmol) (compound 1) was dissolved in 60 ml of dimethylformamide (DMF). To this solution, *N,N′*-diisopropylethylamine (DIPEA; 1.2 ml), 2,6-dimethylaminopyridine (DMAP; 0.46 g, 3.75 mmol), and succinic anhydride (0.75 g, 7.5 mmol) were added. The reaction mixture was stirred at room temperature overnight, added to 120 ml of 0.1 M HCl, and extracted three times, each with 50 ml of chloroform. Organic extracts were combined, dried, and evaporated. Crude products were recrystallized from acetonitrile to give compound 2 (1.2 g, 49% yield). To a stirred solution of 2,2′-(ethylenedioxy)-bis-(ethylamine) (2 g; 13.5 mmol) (compound 3) in 100 ml of acetonitrile, a solution of 9-fluorenylmethoxycarbonyl *N*-hydroxysuccinimide (Fmoc-NHS; 4.95 g, 14.8 mmol) in 50 ml of acetonitrile was added over the course of 30 min. The reaction mixture was stirred for an additional 1 hr, concentrated under reduced pressure, and purified on a silica gel using 0%–6% methanol step-gradient in chloroform/0.8% triethylamine. Fractions containing a mono-Fmoc derivative were pooled and evaporated to give 2.50 g of compound 4 (52% yield). To a stirred solution of compound 4 (2.5 g, 7 mmol) in 50 ml of methanol, a solution of biotin-NHS (2.63 g, 7.7 mmol) in 60 ml of 95% methanol was added over the course of 15 min. After 1 hr at room temperature, thin layer chromatography (chloroform/methanol/acetic acid, 9:1:1 v/v/v) showed complete conversion into compound 5. The mixture was then concentrated under reduced pressure and purified on a silica gel using a 0%–6% methanol step-gradient in chloroform to give 2.2 g of compound 5 (54% yield). Compound 5 (2.2 g, 3.8 mmol) was added to 6 ml of 20% piperidine in DMF. The resulting solution was stirred at room temperature for 10 min, concentrated to about 2 ml under reduced pressure, and added to 20 ml of cold ether. After incubation at −70°C for 30 min, the precipitate was collected by centrifugation. The precipitate (compound 6) was dissolved in 2 ml of methanol, reprecipitated as above, and dried (yield 1.1 g, 82%). Compound 2 (5-succinylamidomethyl-2-nitroacetophenone) (0.59 g, 1.94 mmol) was dissolved in 3 ml of DMF. To this solution, a solution of PyBOP (0.99 g, 1.94 mmol) in 3 ml DMF was added, followed by the addition of *N,N*-diisopropylethylamine (0.68 ml, 3.9 mmol). The resulting solution was stirred at room temperature for 15 min, and then a solution of compound 6 (0.68 g, 1.94 mmol) in 3 ml DMF was added. Stirring continued overnight, and solvents were evaporated under reduced pressure. The residue was purified on a silica gel column using a 0%–20% step-gradient of methanol in chloroform to give 0.75 g of compound 7 (67% yield). Reduction with sodium borohydride and conversion to the target NHS carbonate were carried out as described previously [6].

Treatment of Adenoviral Vectors with PCB and WSPCB

A stock solution of PCB (25 mg/ml in DMF) was diluted in PBS (pH 7.4), and the diluted PCB (25 μl) was added to 2.5×10^6 adenoviral vector particles (2.5×10^5 infectious units) in PBS (25 μl). The biotinylation reactions were performed on ice in the dark for 2 hr and terminated by the addition of 100 μl DMEM/10% FBS. Treatment of adenoviral vectors with WSPCB was performed in the same manner, except that the WSPCB stock solution used had a concentration of 100 mg/ml in DMF.

Photoirradiation of WSPCB-Treated Adenoviral Vectors

Adenoviral vectors were treated with various concentrations of WSPCB as above. WSPCB-treated viral vectors were divided into two groups of borosilicate glass vials. One group of borosilicate glass vials was kept in the dark, while the other group was irradiated for 3 min with 365 nm light using a UV lamp (model B-100 SP, UV Products) equipped with a 160 W mercury vapor bulb, which emits long-wavelength UV light in the 355–375 nm range, peaking at 365 nm. Actual light intensities in this and other experiments were determined by using a UV light meter (model 06-662-65, UV Products). The infectivity of the nonirradiated and irradiated adenoviral vectors was analyzed by using D-17 cells. The time course of the infectivity activation of WSPCB-treated adenoviral vectors upon photoirradiation was investigated in a similar manner, except that the exposure time to 365 nm light was varied.

Western Blotting Analysis

Adenoviral vectors, which had been treated with WSPCB as above, were placed in borosilicate glass vials (1.2×10^9 viral particles per vial). One vial was kept in the dark, while the other vial was exposed for 4 min to 365 nm light as above. Each of these viral samples was centrifuged at 25,000 × g for 2 hr at 4°C to precipitate adenoviral vectors. Viral precipitates were suspended in 10 μl of an SDS sample solution containing 20 mM 2-mercaptoethanol and electrophoresed on 4%–20% polyacrylamide gradient gels [10]. Proteins were transferred from the gel to polyvinylidene difluoride membrane (Millipore) by using a semidry electroblotter. The membrane was blocked with SuperBlock (Pierce) and then incubated for 30 min with a streptavidin-alkaline phosphatase conjugate (Pierce), diluted 5000-fold in SuperBlock. Bound streptavidin-alkaline phosphatase conjugates were visualized by alkaline phosphatase activity using nitroblue tetrazolium chloride/5-bromo-4-chloro-3′-indolylphosphate *p*-toluidine salt (Pierce) as the substrates.

Virus Adsorption Assay

Adenoviral vectors (1.25×10^9 viral particles per reaction) were treated with 1 mg/ml WSPCB as above. The resulting viral vectors were either irradiated with 365 nm light (16 mW/cm^2) for 3 min or kept in the dark. Each of these samples was added to a monolayer of D-17 cells cultured on a culture dish, and the mixtures were incubated at 37°C for 3 hr. The supernatant (culture medium fraction) containing unbound adenoviral vectors was collected and irradiated with 365 nm light for 3 min as above (this irradiation step was omitted for adenoviral vectors that had been irradiated with 365 nm light). The infectivity of the resulting viral vectors was analyzed by using fresh D-17 cells to estimate the amount of adenoviral vectors that remained unbound. Adenoviral vectors without WSPCB treatment were used as controls. Nonspecific binding of adenoviral vectors was estimated by using empty culture dishes.

In Vivo Activation of WSPCB-Treated Adenoviral Vectors

Mouse subcutaneous tumor models were prepared by using D-17 cells and 9-week-old athymic nude mice (Hsd: Athymic Nude-*nu*/*nu*; Harlan). D-17 cells (2×10^7 in 200 μl PBS per injection) were injected percutaneously on each side of the back of the mice just above the upper legs. After 24 hr, small tumor nodules (3–5 mm) formed in these mice on both sides (at both injection sites). Adenoviral vectors were treated with 0.5 mg/ml WSPCB as above. In vitro analysis using D-17 cells showed that the infectivity of the resulting WSPCB-

treated viral vectors was inhibited to near completion and that the infectivity can be reactivated efficiently upon irradiation of 365 nm light.

Each tumor site of the mice was injected with 250 μl of the WSPCB-treated adenoviral vectors (2.3×10^9 infectious units prior to the treatment) prepared above. After briefly massaging the injection sites, each mouse was anesthetized and covered with aluminum foil with a hole to expose only the right-hand tumor sites. Exposed tumor sites were irradiated at a distance of 12 cm with a 365 nm UV lamp (model B-100SP) for two 2 min periods, separated by a 30 s intermission. Mice were maintained for 48 hr and then sacrificed, followed by the collection of tumor sites with adjoining tissue. Tissue was sectioned (50 μm sections) in a CM 1850 Cryostat (Leica) at −14°C. Tissue sections were stained for the expression of the *lac*Z gene using X-gal as the substrate, and the stained tissue sections were viewed under a light microscope. A control experiment was performed in the same manner by injecting an equal amount of unmodified adenoviral vectors into a tumor site.

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