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Advances in Brief

Enhanced Phagemid Particle Gene Transfer in Camptothecin-treated Carcinoma Cells[1]

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Abstract

Engineered phage-based vectors are an attractive alternative strategy for gene delivery because they possess no natural mammalian cell tropism and can be genetically modified for specific applications. Genotoxic treatments that increase the transduction efficiency of single-stranded adeno-associated virus were tested on cells transfected by single-stranded phage. Indeed, green fluorescent protein transgene expression by epidermal growth factor-targeted phagemid particles increased with heat shock, UV irradiation, and camptothecin (CPT) treatment. CPT resulted in transduction efficiencies of 30–45% in certain human carcinoma cell lines and reduced the minimal dose needed to detect green fluorescent protein-expressing cells to as low as 1–10 particles/cell. Targeted phage transduction was effective in many tumor cell lines and in prostate tumor xenografts with CPT treatment. Taken together, these data suggest the feasibility of using phage-based vectors for therapeutic gene delivery to cancer cells.

Introduction

Effective cancer gene therapy will depend on the development of vectors that can deliver therapeutic genes to tumor cells while minimizing vector-associated toxicity. The discovery that modified filamentous bacteriophage vectors are capable of gene delivery to mammalian cells when targeted with growth factors (1–3), antibody (4), or viral capsid protein (5) raises the possibility of developing phage vectors for targeted gene therapy (6). Prokaryotic viral vectors are an attractive alternative to existing animal viral vectors because they lack intrinsic tropism for mammalian cells and can be conveniently produced in bacteria. Moreover, because of their relatively simple genetics, they are amenable to improvement by both rational design and directed evolution. Currently, phage vector transduction efficiency (1–4%) is considerably lower than most viral vectors. However, transduction levels as high as 10% are obtained in human prostate carcinoma cells transfected with multivalent phagemid vectors that are optimized for cell binding and internalization (7). Facilitation of strand conversion might enhance transduction by targeted phages, even further because filamentous phages contain single-stranded DNA. We reasoned, therefore, that genotoxic treatments that are known to increase transduction by the single-stranded DNA vector, AAV[3] (8), might also increase transduction by targeted phage particles. We show here that the same treatments significantly increase the efficiency of EGF-targeted phage transduction in human carcinoma cells. Treatment with the topoisomerase I inhibitor, CPT, increased transduction levels to as high as 45% of treated cells in prostate and lung carcinoma cell lines. Intratumoral injection of EGF-targeted phagemid particles resulted in significant transduction in tumor xenografts that was also enhanced by CPT treatment. These results suggest that targeted phagemid particles may be beneficial for cancer gene therapy, particularly in combination with CPT chemotherapy.

Materials and Methods

Preparation of Phagemid Particles. Multivalent EGF-targeted (pUCMG4CT-EGF) and control (pUCMG4CT) phagemid particles were prepared by rescue with a gene III deleted helper phage, and the noninfectious phagemid particles were titered by measuring single-stranded DNA content as described previously (7).

Cell Culture. All cell lines were obtained from American Type Culture Collection (Rockville, MD). PC-3 (human prostate carcinoma), H596, H460, and H1299 (human lung carcinomas) cells were grown in RPMI 1640. DU145 (human prostate carcinoma), SKOV3 (human ovarian carcinoma), A549 (human lung carcinoma), and CACO-2 (human colon carcinoma) cells were grown in DMEM. ME-180 (human cervical carcinoma) and SAOS (human osteosarcoma) cells were grown in McCoy's 5A medium. SCC-9 (human squamous cell carcinoma) cells were grown in a 1:1 mixture of Ham's F12K and DMEM. U87-MG (human glioblastoma), SKBR3, and MB-231 (human breast carcinomas) cells were grown in minimum essential Eagle's medium. All culture media were supplemented with 10% fetal bovine serum, FBS (Sigma Chemical Co.), 0.1 mM nonessential amino acids, 1 mM pyruvate, 2 mM L-glutamine, and 50 μg/ml gentamicin.

Genotoxic Treatments. Genotoxic treatments were performed 40 h after the addition of phage in medium containing 10% fetal bovine serum. Medium was removed at 40 h after phage addition, and cells were incubated with CPT at 10 μM for 7 h at 37°C, followed by replacement with fresh medium and an additional incubation of 49 h at 37°C or as indicated for time-course experiments. For heat shock experiments, phages were removed, fresh medium was added, and cells were incubated at 42.5°C for 7 h, followed by an additional 49 h at 37°C. For UV treatments, phage-containing medium was removed, and the cells were immediately irradiated using a Stratalinker UV cross-linker (Stratagene, La Jolla, CA) at a dose of 50 J/m^2. Fresh medium was added, and the cells were incubated an additional 49 h at 37°C.

***In Vitro* Phagemid Particle Transfection.** Cells (20,000 cells/well) were plated in 12-well dishes 24 h before phage particle addition. Medium was removed, and phages were added at doses ranging from 10^6 to 10^{11} cfu/ml in medium containing 10% fetal bovine serum. Cells were incubated for 96 h at 37°C, except for time-course experiments, where phages were removed, and cells were analyzed at the indicated times. In some cases, cells were subjected to genotoxic treatments. Cells were washed three times in PBS (Life Technologies, Inc., Rockville, MD) released from the plate with trypsin-EDTA (Life Technologies, Inc.), pelleted, and resuspended in FACS buffer (0.925% formaldehyde, 0.02% sodium azide, and 2% glucose in 10 mM PBS, pH 7.4). FACS analysis (Cytometry Research Services, San Diego, CA) was performed on samples of 10,000 cells using the FITC filter set. Autofluorescent GFP-expressing cells were also visualized directly using an epifluorescent inverted microscope (Nikon Diaphot) and digital camera. Pseudo color was added using Photoshop software (Adobe Systems, San Jose, CA). Phage transfection experiments were performed in duplicate wells, and each experiment was repeated at least three times.

***In Vivo* Phagemid Particle Transfection.** Mice (male *nu/nu*; 43–56 days) obtained from Charles River Laboratories were allowed to acclimate for 1

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[3] The abbreviations used are: AAV, adeno-associated virus; EGF, epidermal growth factor; cfu, colony-forming unit(s); FACS, fluorescence-activated cell sorter; GFP, green fluorescent protein; CPT, camptothecin.

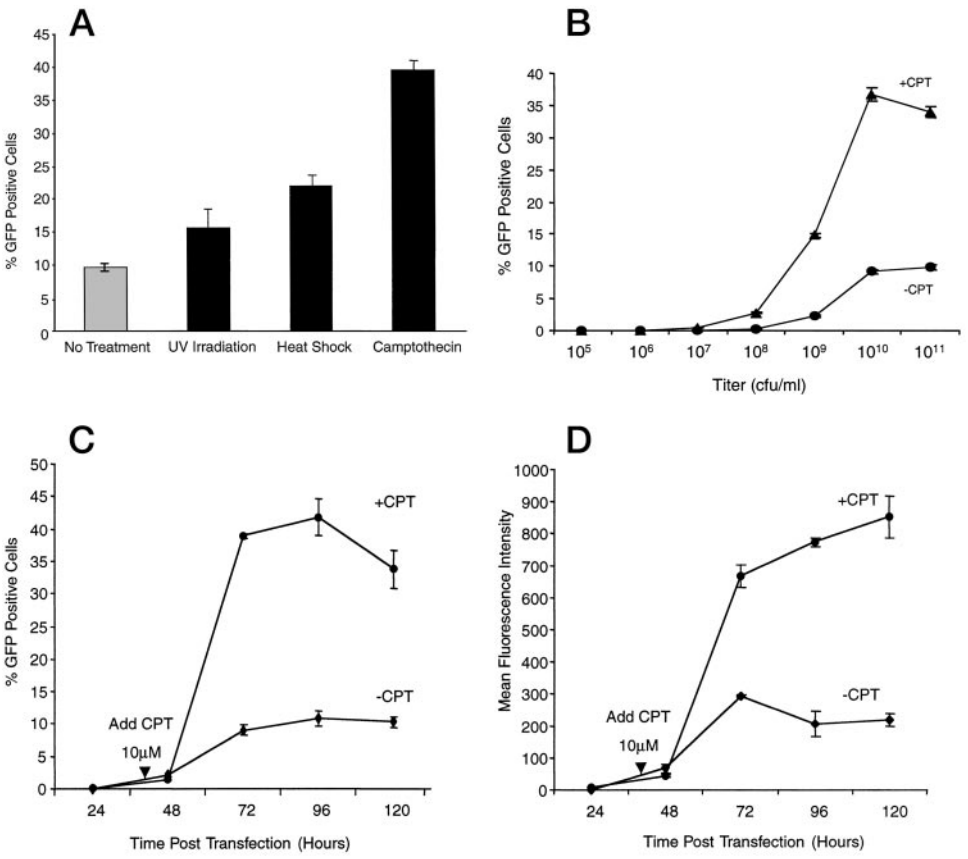

Fig. 1. Genotoxic treatments increase the transduction efficiency of EGF-targeted phagemid particles. *A*, PC-3 cells were transfected with EGF-targeted phagemid particles at 10^{10} cfu/ml as described in the text. Cells received UV irradiation, heat shock, or CPT treatment 40 h after phagemid particle transfection and were harvested for analysis by FACs at 96 h. The percentage of GFP-positive cells increased in treated cells up to 4-fold (CPT) that of transfected cells receiving no treatment. *B*, increase in phagemid vector potency in CPT-treated cells. Transduction by EGF-targeted phagemid particles was compared in CPT-treated cells and cells without CPT treatment over doses ranging 10^5 cfu/ml to 10^{11} cfu/ml. *C*, the effect of CPT on the increase in the percentage of EGF-phagemid-transduced cells is time dependent. EGF-phagemid particles (10^{10} cfu/ml) were added to PC-3 cells, CPT (10 μM) was added at 40 h, and the cells were harvested at various time points for analysis by FACs. The largest increase in the percentage of transduced cells occurs between 48 and 72 h after transfection and starts to decrease between 96 and 120 h in CPT-treated cells. *D*, the expression of the GFP transgene as measured by the mean fluorescence intensity in CPT-treated cells continues to increase with time up to 120 h. GFP expression in cells without CPT treatment reaches a maximum between 48 and 96 h. *Bars*, SE.

week before receiving injections. Mice were mildly anesthetized with Metofane (methoxyfluorane), and PC-3 cells were injected s.c. (1×10^7 cells in 100 μl) into the right flank. Tumors were measured weekly with calipers, and tumor volume was calculated using the formula for a prolate ellipsoid: (width^2 $\times$ length)/2. When tumors reached a volume between 200 and 500 mm^3, daily intratumoral phage injections were administered for 3 consecutive days. One group of mice ($n = 4$) received 3 injections each of targeted pUCMG4CT-EGF (30 μl/injection) for a total dose of 2.7×10^{11} cfu/mouse. The control group ($n = 4$) received three injections each of pUCMG4CT (40 μl/injection) for a total dose of 2.6×10^{11} cfu/mouse. Half of the mice from each group received intratumoral CPT injections (30 μl of a 1 mM stock solution) 2 days after the final phagemid particle injection, and the other half received 30 μl of HBSS (Life Technologies, Inc.). The short-term distribution of intratumorally injected phagemid particles was measured 6 h after injection in a mouse that received two intratumoral injections of 30 μl of pUCMG4CT-EGF/injection and in an HBSS-injected control.

Analysis of Phagemid Particle-treated Tumors. Seven days after the first intratumoral injection of phagemid particles, mice were anesthetized with a lethal dose of sodium pentobarbital and perfused with 4% paraformaldehyde in 0.1 M phosphate buffer for 5 min. After perfusion, tumors were dissected out, frozen in OCT compound (Sakura Finetek, Torrance, CA), and stored at −80°C. Cryostat sections (14-μm-thick) were mounted on charged slides, fixed in 4% paraformaldehyde in PBS for 20 min at room temperature, and rinsed in PBS. Continuous sections throughout the entire tumor were analyzed by alternating detection of GFP immunoreactivity with detection of GFP autofluorescence.

GFP and phage particle immunoreactivity were analyzed as follows. Sections were rinsed three times in PBS and incubated for 20 min in blocking buffer [50 mM Tris Buffered Saline (pH 7.6; Jackson ImmunoResearch, West Grove, PA), 1% BSA (Fraction V; Fisher Biotech), 0.02% Tween 20, and 0.05% sodium azide] containing 10% normal goat serum. Blocking buffer was removed, and slides were incubated for 1 h with rabbit anti-GFP antibody (Molecular Probes, Eugene, OR) at a 1:2000 dilution in blocking buffer or rabbit anti-M13 antibody (Sigma Chemical Co., St. Louis, MO) at a 1:200 dilution in blocking buffer. Endogenous peroxidase was blocked by treating the sections with 3% H_2O_2 (Sigma Chemical Co.) for 5 min. Sections were incubated for 30 min in horseradish peroxidase-conjugated goat antirabbit antibody (Vector Laboratories) at a 1:200 dilution in 50 mM Tris Buffered Saline (pH 7.6), containing 0.02% Tween 20, 0.05% sodium azide, 1.5% normal goat serum, and 5% normal mouse serum (Jackson ImmunoResearch). All steps were separated by washing in 50 mM Tris (pH 7.6) containing 0.02% Tween 20. Sections were developed with diaminobenzidine tetrahydrochloride substrate (DAKO, Carpinteria, CA) for 5 min and counterstained with Mayer's hematoxylin (DAKO) for 30 s, rinsed in tap water for 2 min, dehydrated, cleared, and mounted.

Cells that displayed GFP autofluorescence were detected using an epifluorescent inverted microscope (Nikon Diaphot) equipped with FITC and 4′,6-diamidino-2-phenylindole filters. Tissue sections were mounted in media containing 4′,6-diamidino-2-phenylindole (Vector, Burlingame, CA) to visualize cell nuclei.

Results

Genotoxic Treatments Increase Phagemid Particle-mediated Transduction. We tested the effect of several genotoxic treatments on pUCMG4CT-EGF phagemid particle transduction in PC-3 cells. Heat shock and UV resulted in a 2- and 1.5-fold increase, respectively, in the percentage of phagemid particle-transduced cells (Fig. 1*A*). CPT had the greatest effect on phagemid transduction, resulting in a 4-fold increase. None of the genotoxic treatments increased nonspecific transduction of untargeted control pUCMG4CT particles beyond 0.03% (data not shown). We further analyzed the effect of CPT treatment at phagemid particle doses ranging from 10^5 to 10^{11} cfu/ml. CPT increased both the maximal transduction and the potency of the phagemid particles (Fig. 1*B*). In CPT-treated cells, 10–50-fold lower phagemid particle doses were as effective as higher doses in untreated cells. Increasing the phagemid particle dose from 10^{10} to 10^{11} cfu/ml did not increase transduction levels significantly, whether or not the cells were CPT treated. CPT increased transduction ~10-fold at doses

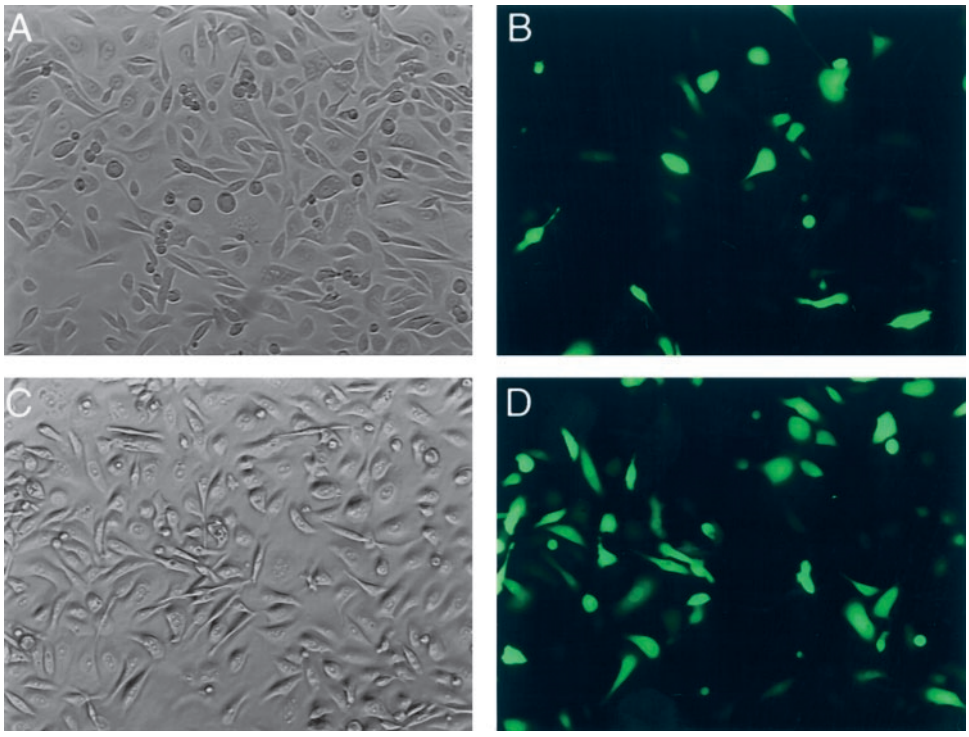

Fig. 2. Direct visualization of GFP autofluorescence in EGF-phagemid particle-transfected PC-3 cells treated with CPT. *A*, phase contrast image of EGF-phagemid-transfected PC-3 cells without CPT. *B*, autofluorescent cells in same field as *A*. *C*, phase contrast image of EGF-phagemid-transfected PC-3 cells with CPT. *D*, autofluorescent cells in the same field as *C*.

up to 10^9 cfu/ml and ~4-fold at higher doses as maximal transduction levels were reached. Significantly, the lowest phagemid dose needed to directly detect transduced cells using fluorescence microscopy was reduced 100-fold from 10^7–10^8 to 10^5–10^6 cfu/ml with CPT treatment thus reducing the multiplicity of infection to 1–10 phagemid particles/cell.

Effect of CPT Is Time Dependent. The effect of CPT treatment increased with time after treatment (Fig. 1, *C* and *D*). The greatest increase in both the percentage of GFP-expressing cells (~4-fold) and the amount of GFP expression/cell (a 3.5-fold increase in mean fluorescence intensity) occurred between 8 and 32 h after CPT was added (48–72 h after pUCMG4CT-EGF phagemid transfection). The percentage of transduced PC-3 cells continued to increase with time up to 96 h after adding phagemid particles and decreased by 120 h. The amount of GFP expressed/cell (measured by mean fluorescence intensity) continued to increase from 72–120 h after transfection. The increase in both the fluorescence intensity and the number of autofluorescing cells with CPT treatment was confirmed by direct observation of cells using epifluorescent microscopy (Fig. 2).

CPT Increases Phagemid Particle-mediated Transduction of Various Human Cancer Cell Lines. The transduction efficiency of pUCMG4CT-EGF phagemid particles and the effect of CPT were assessed on 13 additional EGF receptor-expressing human carcinoma cell lines. Significant levels of transduction were obtained in all cell lines tested (Table 1). Both the phagemid particle transduction efficiency and the effect of CPT varied, depending on the cell line. Relatively high EGF-phagemid-mediated transduction (5–10%) was obtained in two cell lines (PC-3, H1299) in the absence of CPT. In these cell lines, CPT treatment resulted in a 4–8-fold increase in transduction (to ~40%). The transduction efficiency in a second group (ME-180, SAOS, SKBR3, U87 MG, and SSC-9) was lower (about 1–2%), as was the response to CPT (about 1–2-fold). Transduction efficiency in a third group of cell lines (SKOV3, MDA-MB-231, DU-145, CACO-2, H460, and A549) was very low (<0.4%) but increased significantly with CPT treatment (*e.g.*, up to 6% in CACO-2).

Phagemid Particle-mediated Transduction of Human Carcinoma Cells in a Tumor Xenograft Model. We analyzed the effect of CPT on phage-mediated transduction in PC-3 tumor xenografts grown in nude mice. The distribution of phagemid particles at 6 h after intratumoral injection of pUCMG4CT-EGF particles clearly indicated the presence of phagemid particles within the tumor parenchyma in the area corresponding to the injection site and areas lateral to the injection (Fig. 3*A*). At higher magnification, the phagemid particles were detected within the tumor cells and also associated with the extracellular matrix (Fig. 3, *B* and *C*). When tumors were injected with untargeted control phagemid particles, staining was predominantly extracellular and more localized to the injection site (not shown). Phagemid particle staining was not detected in control tumors injected with HBSS.

Table 1 *EGF-phagemid particle transduction of various human carcinoma cell lines*

Cells were plated into 12-well tissue culture dishes (20,000 cells/well) 24 h before addition of PUCMG4CT-EGF phage (1.0×10^{10} cfu/ml). Phages were removed 40 h later, and cells were either treated with 10 μM CPT (+CPT) or left untreated (−CPT) for 7 h. Fresh medium was added, and cells were incubated for an additional 49 h. Cells were removed using trypsin-EDTA, and GFP expression was analyzed by FACS analysis.

Cell line	Tissue	Transduction[a] −CPT	+CPT	Fold Increase
PC-3	Prostate	9.63 ± 0.55	39.50 ± 1.33	4.10
HI299	Lung	5.02 ± 0.53	39.87 ± 3.03	7.95
ME-180	Cervix	1.93 ± 0.37	4.15 ± 0.34	2.15
SKBR3	Breast	1.48 ± 0.32	3.29 ± 0.34	2.22
SAOS	Bone	0.99 ± 0.29	1.44 ± 0.23	1.46
U87-MG	Brain	0.76 ± 0.11	1.98 ± 0.27	2.60
SSC-9	Tongue	0.96 ± 0.30	1.50 ± 0.19	1.55
H596	Lung	0.12 ± 0.01	0.47 ± 0.01	3.92
DU145	Prostate	0.15 ± 0.01	1.85 ± 0.24	12.07
CACO-2	Colon	0.36 ± 0.07	6.52 ± 1.49	18.12
H460	Lung	0.01 ± 0.01	0.21 ± 0.05	21.00
SKOV3	Ovary	0.23 ± 0.6	2.17 ± 0.28	9.42
MB-231	Breast	0.07 ± 0.01	0.81 ± 0.14	12.10
A549	Lung	0.10 ± 0.02	0.95 ± 0.14	9.48

[a] Transduction = % GFP positive cells (mean ± SEM of at least three independent experiments performed in duplicate).

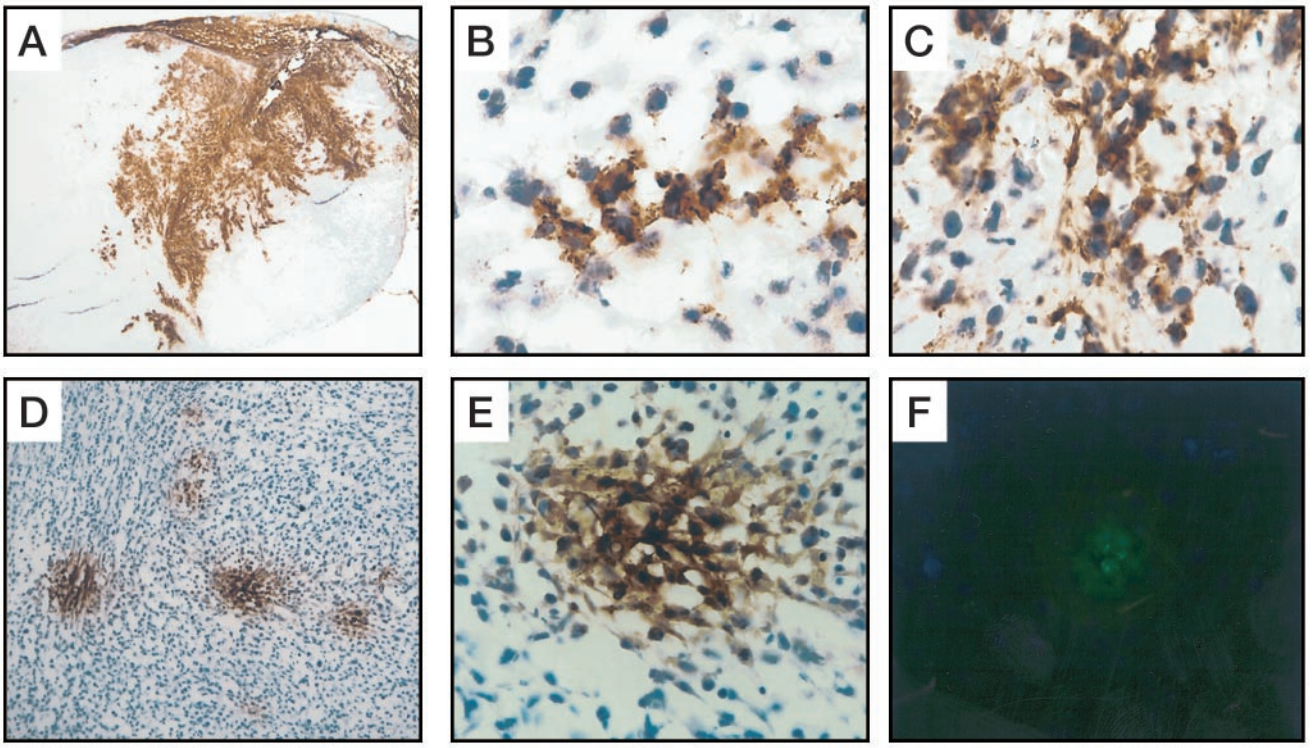

Fig. 3. Local delivery of pUCMG4CT-EGF phagemid particles to PC-3 tumor xenografts. To assess the short-term distribution of phagemid particles, treated tumors were processed 6 h after phagemid particle injection, stained with anti-M13 antibody, and visualized with a horseradish peroxidase-labeled secondary antibody and 3,3′-diaminobenzidine substrate (see text). The anti-phagemid particle immunostaining (*brown*; *A–C*) indicated that pUCMG4CT-EGF phagemid particles localized to the injection site and adjacent tumor parenchyma (*A*, ×20). At higher magnification, the M13 immunoreactivity was clearly localized within the tumor cells and also associated with the extracellular matrix (*B* and *C*, ×1000). Analysis of GFP expression in tumors harvested 7 days after phagemid particle injection demonstrated the presence of groups of GFP-positive cells throughout the tumor (*D*, ×100). Analysis of consecutive sections demonstrated that tumor cells showing intracellular GFP immunoreactivity (*E*, ×1000) were also positive for GFP autofluorescence (*F*, ×1000).

The ability of EGF-targeted phagemid particles to transduce tumor cells in PC-3 tumor xenografts was examined by analyzing GFP transgene expression in tumors harvested 1 week after the first phagemid particle injection. GFP transgene expression was assessed by epifluorescent microscopy and immunostaining with anti-GFP antibody. The results (Fig. 3, *D* and *E*) indicated numerous areas of GFP expression throughout tumors injected with pUCMG4CT-EGF phagemid particles. When GFP immunoreactive areas were examined under higher magnification, they correlated with corresponding areas of GFP autofluorescence in adjacent sections (Fig. 3*F*). GFP-expressing cells were detected in all four mice injected with pUCMG4CT-EGF phagemid particles. A modest increase (2–5-fold) in GFP-expressing cells was observed in CPT-treated tumors injected with pUCMG4CT-EGF phagemid particles compared with tumors receiving only EGF-targeted phagemid particles.

Discussion

We report here that high levels of EGF-targeted phagemid gene delivery to human carcinoma cells can be obtained using the same genotoxic treatments that increase transduction in AAV vector-transfected cells. Of the genotoxic treatments tested, CPT resulted in the greatest improvement in transduction and increased both the percentage of cells transduced and the level of transgene expressed. The increase in transgene expression extended out to 3 days after treatment, indicating that the effect of CPT was long lived. Although the response was varied, a wide variety of carcinoma cell lines were transduced by EGF-targeted particles, and transduction was increased by CPT in each cell line tested. In most cases, phage-mediated transduction in CPT-treated cells ranged from 0.2 to 6.5%. Remarkably, transduction efficiencies of >40% were observed in a prostate and a lung carcinoma cell line indicating that, similar to viral vector transduction, there are cell line-dependent differences in phage transduction efficiency. To our knowledge, these are the highest transduction levels obtained to date using phage-based vectors for targeted gene delivery. Investigation of this cell-dependent response could lead to insights on how to engineer more effective phage vectors.

The highest phage-mediated transduction levels obtained with CPT rival transduction with viral vectors such as adenovirus and are greater than transduction with receptor-targeted DNA conjugates (9, 10). The high levels of phage-mediated transduction relative to DNA conjugates may be because phage, similar to viral vectors, are protected from nucleases by coat proteins. Direct comparison will be needed to determine the relative efficiencies of phage *versus* viral and nonviral systems. Clearly, CPT and other treatments might allow administration of phage vectors at lower doses because phagemid vector potency was 10–50-fold greater in CPT-treated cells. Indeed, in PC-3 cells, a multiplicity of infection as low as 1–10 phagemid particles/cell resulted in detectable levels of transduced cells (by direct observation using fluorescent microscopy) in CPT-treated cultures. Taken together, these data suggest the potential of phage vectors for efficient targeted gene delivery to mammalian cells.

Similar to most gene delivery vehicles, little is known about the specific mechanisms by which targeted phage particles successfully transduce cells. Transduction efficiency is the combined result of intracellular processing, including trafficking, phage uncoating, and strand conversion. Presumably, facilitation of any of these processes might improve transduction efficiency considerably. In the case of the single-stranded viral vector, AAV, genotoxic treatments such as heat shock, UV irradiation, and the topoisomerase I inhibitor, CPT, have been shown to increase transduction (8, 11, 12). The mechanism of

increased transgene expression is not fully understood but is thought to involve activation of the host cell repair machinery in response to DNA damage (11, 13). However, other explanations cannot be ruled out because of the pleiotropic effects of these treatments (*i.e.*, altered endosomal processing and transgene transcription). Cell-specific differences in phagemid-mediated transduction in response to CPT are likely to be caused by differences in sensitivity to genotoxic stress. Thus, further studies are under way to determine the mechanism by which genotoxic treatments increase phagemid particle transduction efficiency and to apply these findings to the design of further improvements in phage-based vectors for gene delivery. As an alternative to rational design, it may be possible to use genetic selection as described by Kassner *et al.* (3) to select more effective phage from combinatorial phage libraries.

Previous studies on the transduction of mammalian cells by phage-based vectors have all been performed *in vitro*. The high levels of phage-mediated transduction obtained in the present study with human prostate carcinoma cells and CPT suggested that it might be possible to transduce tumor cells *in vivo* with phage-based vectors. Our data indicate that targeted phagemid particle binding, internalization, and gene delivery *in vivo* is indeed feasible. The phage-staining pattern and the distribution of GFP expression in treated tumors reported here indicate that the targeted filamentous phage particles penetrated throughout the tumor, perhaps as a result of their elongated geometry (6 nm $\times$ 500-1000 nm), which might allow them to pass through tissues and vasculature in a serpentine manner. The relative low but significant transduction reported here is, to our knowledge, the first demonstration of phage-mediated gene transfer *in vivo*. In these initial studies, direct injection of CPT into tumors after phage injection had a positive effect on phage-mediated transduction. Studies are under way to determine optimal CPT dose and administration using more stable CPT derivatives and to examine the effectiveness of gene transfer with systemically delivered phagemid particles.

As we and others have shown, filamentous phages have little or no tropism for mammalian cells; however, tropism for cells that bear the appropriate receptors can be conferred to phage particles by expression of a targeting ligand on the phage coat (2–4). Thus, the specificity of EGF-targeted phages is expected to be limited to cells expressing EGF receptor, which is elevated in many tumors. Indeed, we did not detect transduction of normal human endothelial cells by EGF-targeted phage particles (data not shown), and in preliminary studies with systemically delivered EGF-phagemid particles, we detected GFP expression in PC-3 tumors but not in normal liver and spleen. Because specificity of the phage particles for tumor cells is determined by the targeting ligand, other targeting ligands could be used to increase specificity for different types of tumors.

Finally, CPT enhancement of gene delivery by targeted phagemid particles has significant implications for the use of phage-based vectors in cancer gene therapy. The observation that CPT and its derivatives are cytotoxic to carcinoma cells and can lead to tumor regression in a variety of tumor models (14, 15) has led to clinical testing of CPT analogues (*i.e.*, topotecan and CPT-11; Ref. 16). The mechanism of the anticancer activity of CPT is thought to involve DNA damage, which eventually leads to cell cycle arrest and apoptosis (17). Because p53 mutants are more resistant to the effects of CPT (18), one approach to overcoming drug resistance is to deliver a wild-type *p53* gene to cells that lack functional p53 protein (19, 20). Indeed, *p53* gene replacement in combination with CPT has been shown to potentiate the effectiveness of the drug *in vivo* (19, 20). In this regard, it is interesting to speculate on the possibility of phagemid particle *p53* gene delivery in combination with chemotherapy because of the potential synergistic effects of phagemid gene delivery and genotoxic agents.

Acknowledgments

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