Selection of Muscle-Binding Peptides from Context-Specific Peptide-Presenting Phage Libraries for Adenoviral Vector Targeting

Debadyuti Ghosh^{1,2} and Michael A. Barry^{1,2,3*}

Center for Cell and Gene Therapy, Baylor College of Medicine, The Methodist Hospital, Texas Children's Hospital, Houston, Texas¹; Departments of Molecular and Human Genetics and Immunology, Baylor College of Medicine, Houston, Texas³; and Department of Bioengineering, Rice University, Houston, Texas²

Received 24 September 2004/Accepted 2 August 2005

Production of cell-targeting vectors in part involves the addition of new targeting ligands to the vector to mediate binding to the cells of interest. For viral vectors, the ideal approach is to genetically engineer new ligands into the capsid proteins of the virus to generate a single agent to mediate therapy. Although this is ideal, this insertion of an exogenous ligand from one structural context into the differing structural context of a capsid protein can ablate the function of the ligand or disrupt viral assembly and function. To address this context problem for adenoviral vectors, we have engineered a “context-specific” peptide-presenting phage library. We have displayed a 12-amino-acid (12-mer) random peptide library between the H and I sheets of the fiber protein of adenovirus type 5 on the pIII protein of fd bacteriophage. This library was used for peptide selection against C2C12 mouse skeletal muscle cells. Five rounds of selection combined with four rounds of clearing on nontarget cells selected one primary peptide designated 12.51, which bound target C2C12 cells approximately 100-fold better than the positive control RGD peptide. Translation of 12.51 back into the fiber protein produced a ligand-modified adenoviral vector that mediated 14-fold-better transduction of target C2C12 cells. These data suggest context-specific peptide-presenting libraries may allow selection of compatible peptide ligands for functional translation into viral vectors for retargeting.

Adenoviruses are arguably one of the most robust vectors for gene delivery. The attraction for the use of these viruses as gene delivery vectors stems from their ability to transduce a wide range of dividing and nondividing cell types. Adenovirus type 2 (Ad2) and Ad5 bind and enter cells through a multistep process usually involving the combined interactions of the fiber and penton base proteins with cellular receptors. The trimeric fiber protein forms a shaft structure at the vertices of the virus icosahedron and is thought to mediate initial cell binding (10). Cells lacking the fiber receptor (the coxsackievirus-adenovirus receptor [CAR]) are relatively resistant to infection (4). Alternate interactions are mediated by an RGD motif in the penton base that binds to α_v integrins. For most cells, this binding is thought to initiate virus internalization after fiber binding (30). However, in cells lacking CAR, the penton base mediates both cell binding and entry, albeit at one-half the level as in cells bearing both receptors (10). Recent work has also identified a third ligand for cell binding in the fiber protein that appears to mediate cell-binding via cellular heparin sulfate proteoglycans (25). Studies have demonstrated that intravenous injection of adenoviral vectors into mice and primates results up to 70% of total gene delivery occurring in the liver (11, 28), with marked nonspecific uptake into Kupffer cells (12, 23). This high level of gene delivery is likely due to the easy access the vector has to liver cells from the blood due to the presence of large fenestrations in the vasculature of the liver.

Adenoviral vectors also transduce a variety of nondividing cells including myoblasts and myofibers. First-generation adenoviral vectors carrying a 6.3-kbp mini-dystrophin gene mediated expression in as many as 50% of myofibers after direct injection into the muscle bed of *mdx* mice (19). This promising result was observed in very young (5- to 7-day-old) mice and used fairly large amounts of Ad ($>10^9$ PFU). Subsequent work showed this high-efficiency delivery occurred only in very young mice (1). This decreased transfection with age appears to be due in part to the loss of the CAR on the muscle cells (7, 24).

One approach to avoid these problems is to develop cell-targeting vectors by replacing the promiscuous or ineffective cell-binding native ligands present on vectors with cell-specific ligands to generate vectors with enhanced cell specificity and activity. Proof of principle for this approach for muscle has been demonstrated by using Ad vectors modified with ligands such as polylysine (5).

Although polylysine does increase transduction, this ligand is nonspecific and increases transduction on many cell types. To identify other ligands for vector targeting, we and others have applied peptide-presenting phage libraries to select cell binding peptides (reviewed in reference 3). Although many cell-binding peptides can be identified from phage libraries, two unpredictable problems can arise when inserting ligands into capsid proteins: (i) insertion of the ligand can destroy capsid and vector function (31), and (ii) the ligand may fail when translated into the heterologous structure of the virus (16). These “context” problems are fundamental, since an ideal candidate peptide ligand may be identified but cannot be

* Corresponding author. Mailing address: One Baylor Plaza, BCM505, Houston, TX 77030. Phone: (713) 798-5959. Fax: (713) 798-1481. E-mail: mab@bcm.edu.

FIG. 1. Phage binding of HI-RGD on an integrin-expressing cell line. HI-RGD phage was bound to integrin-expressing human embryonic kidney 293A cell line compared to control VCSm13 helper phage and HI-BAP phage. A total of 10⁸ input phage of each was bound to 293A cells for 1 h, and titers of collected cell fractions were determined on YT-AMP plates in serial tenfold dilutions.

plates seeded with 293A cells grown to 70% confluence by using Lipofectamine (Invitrogen). About 14 days posttransfection, plaque formation was observed, and plaques were picked, subjected to small-scale amplification. Large-scale amplification was done by using a cell factory (Corning, New York). After a cytopathic effect was observed, cell lysate was collected and subjected to three freeze-thaw cycles. After centrifugation, viral supernatant was purified by two rounds of cesium chloride (CsCl) density gradient ultracentrifugation at 20,000 rpm for 3 h. Collected viruses were desalted by using DG10 desalting columns (Bio-Rad, California). The genomic titer of the viral particles was quantitated by real-time PCR (Rotorgene; Corbett, Sydney, Australia) using Stratagene MasterMix according to manufacturer's recommendations.

Ad transduction on muscle cells. C2C12 myoblasts were grown to 70% confluence on 12-well plates. C2C12 myotubes were differentiated from low-passage (passage 1) C2C12 myoblasts to 50% confluence in the presence of DMEM and 2% horse bovine serum in 24-well plates. On the day of transduction, the medium was removed and replaced with serum-free medium for 2 h to clear receptors. For C2C12 targeting, medium was then removed and wild-type Ad and Ad-HI-12.51 were added at a multiplicity of infection of 1,000 for 30 min in serum-free medium. The media were then aspirated, and the cells were refreshed with serum-containing medium. Transduction was visualized by fluorescence microscopy (Olympus, WA) and was assessed 48 h posttransduction by flow cytometry (Becton Dickinson, California).

RESULTS

Display of peptides within the fiber HI loop on phage. A BAP and RGD integrin-binding motif were incorporated into the HI loop of the Ad5 fiber as negative and positive control cell-binding peptides, respectively (17; data not shown). The H and I sheets along with intervening peptides were then subcloned for display on the N terminus of the mature pIII protein on fd bacteriophage (phage designated HI-BAP and HI-RGD). To determine the functionality of the displayed peptides, HI-BAP was biotinylated with the biotin protein ligase, BirA. Western blotting of HI-BAP and HI-RGD detected with neutravidin and anti-pIII demonstrated that this peptide was biotinylated and therefore functional in the context of the 29-kDa HI-BAP pIII protein (data not shown). To test the functionality of HI-RGD phage for cell binding, phage were tested for their ability to bind integrin-positive 293 cells as described earlier (2). HI-RGD phage bound the cells 60- and

TABLE 1. Sequence diversity of “context-specific” HI loop 12-mer library

H strand sequence	12-mer sequence	I strand sequence
PVTLTITL	FCPESQVKWLTL	GAYYSMSFSW
PVTLTITL	KWQVFFHSEDVG	GAYYSMSFSW
PVTLTITL	AKGKWWAASQK	GAYYSMSFSW
PVTLTITL	VTVKMLLDWLAA	GAYYSMSFSW
PVTLTITL	LRADSVELYIYG	GAYYSMSFSW
PVTLTITL	GMAELGGLEWGM	GAYYSMSFSW
PVTLTITL	ARADRELTKDSW	GAYYSMSFSW
PVTLTITL	RDGCQSPGLGRG	GAYYSMSFSW
PVTLTITL	TSGTLRRSVYIG	GAYYSMSFSW
PVTLTITL	GEDKMRWNRFK	GAYYSMSFSW

200-fold better, respectively, than negative control VCSm13 and HI-BAP phage (Fig. 1). These data demonstrate that both peptide ligands are functionally displayed within the HI loop on phage and suggest this HI loop “context” can be used as a scaffold for random peptide display on phage.

Selection of random peptides against C2C12 mouse myoblasts. Semirandom oligonucleotides bearing 12 NNK codons were inserted into the HI loop between the H and I sheets in place of RGD in pUC118 HI-RGD as in reference (8). The ligation was then transformed into XL1-Blue bacteria by electroporation, followed by infection with helper phage VCSM13 to generate a 12-mer peptide library with approximately 2 × 10⁸ members. Diversity of the library clones was confirmed by sequencing of randomly picked phagemid clones (Table 1). This 12-mer peptide-presenting phagemid library was selected in vitro against C2C12 mouse myoblasts as described earlier (2, 27) with several modifications. To reduce the number of promiscuous peptides that were selected against C2C12 myoblasts, a clearing strategy was applied in which the phage were first adsorbed onto nontarget cells before the supernatant of this reaction was transferred to the target muscle cells. After five rounds of selection, 10 phage clones were picked and sequenced to assess the repertoire of selected peptides (Table 2). A total of 30% of the clones were identical to the 12.51 sequence, 20% were identical to 12.52 sequence, and 1 clone displayed the 12.53 sequence. The remaining clones either had a frameshift, truncation, or deletion in the sequence similar to that frequently observed when phagemid libraries were selected (26). Nevertheless, observation of the same peptide in multiple clones indicated that these peptides were likely selected for their cell-binding abilities (2, 27). BLAST of the 12.51, 12.52, and 12.53 sequences for similar proteins did not generate any matches with known proteins. These observations are consistent with our previous work with long peptide librar-

TABLE 2. Sequences of selected peptides against C2C12 myoblasts after five rounds of selection with four rounds of preclearing

Peptide or other finding	Sequence	Frequency (no. detected/no. tested)
12.51	TARGEHKEELI	3/10
12.52	LRQTGAASAVWG	2/10
12.53	ARRADTQWRGLE	1/10
Frameshifts		1/10
Deletions/truncations		3/10

FIG. 2. Binding of C2C12-selected phage to target C2C12 myoblasts. Peptide-presenting phage clone 12.51 and control phage were bound on C2C12, and phage binding was quantitated determining phage titers as described in the text.

ies where no significant data has been obtained by alignment of peptides to the nucleic acid and protein databases (2, 27).

Cell binding of 12.51 selected peptide on phage. Since the 12.51 sequence was most frequently displayed from the selected clones, the 12.51 phage was tested for its ability to bind C2C12 cells compared to negative control phage, such as HI-BAP and HI-RGD. Phage were bound to C2C12 myoblasts and were collected, and titers were determined as described previously (2, 27). 12.51 phage-selected peptide bound C2C12 myoblasts approximately 100- and 1,000-fold better than HI-RGD and HI-BAP phage, respectively (Fig. 2).

Retargeting of adenoviral gene therapy vectors with muscle-selected peptides. Given that 12.51 appeared to bind C2C12 cells, this peptide was genetically engineered in between the H and I sheets of the Ad5 fiber, and this modified fiber gene was recombined into a dsRed2 red fluorescent protein-expressing Ad5 genome by using Red recombinase system (Fig. 3) (6, 14). This recombinant Ad5 was then transfected into 293 cells and formed plaques within 14 days (data not shown). Given that the virus formed plaques at the same rate as unmodified Ad5, this suggested that the 12.51 peptide was tolerated within the viral context and did not disrupt viral assembly and function. Large-scale amplification and purification yielded normal amounts of viral particles. Particle/PFU ratios were 23 for unmodified Ad and were 64 for Ad-HI-12.51, indicating a slight reduction in transduction of nonmuscle cells by the peptide-modified vector. To assess transduction, unmodified Ad and Ad-HI-12.51 were added to C2C12 myoblasts, and transduction was assessed 48 h later. In this case, Ad-HI-12.51 mediated ~14-fold increase in transduction to C2C12 myoblasts compared to unmodified Ad (Fig. 4A, B, and E). Ad-HI-12.51 mediated twofold-lower transduction on differentiated C2C12 myotubes than against myoblasts (Fig. 4C, D, and F). Nonetheless, the 12.51-modified vector still mediated fourfold higher transduction than unmodified Ad5. These data therefore suggest that 12.51 may be useful for increasing transduction of both myoblasts and differentiated muscle cells. These data suggest that translation of this “context”-selected

FIG. 3. Translation of selected 12.51 peptide into Ad vector. 12.51 peptide flanked by H and I β sheets were cloned into pL29FiberStop shuttle construct by site-directed mutagenesis. After BglII/KpnI digestion, the resulting fragment was recombined into the pAd Easy backbone by red recombinase system, and recombinants were selected on zeocin-resistant plates.

peptide back into its native context in the adenoviral fiber can enhance transduction to target cells.

DISCUSSION

Adenoviruses have proved to be an attractive method for gene delivery due to their ability to transduce a wide variety of dividing and nondividing cells. However, their ability to successfully target a given cell type is limited by its evolution to infect a multitude of cell types. As one approach to develop more specific vectors, we and others have used peptide-presenting phage libraries to identify cell-binding peptides to be engineered into gene therapy vectors (2, 13, 16, 21, 22, 27, 29). However, two problems can arise from

FIG. 4. Transduction of mouse C2C12 myoblasts and myotubes. Wild-type Ad5 or Ad-HI-12.51 expressing red fluorescent protein was applied to the indicated C2C12 myoblasts or myotubes. Cells were transduced with 1,000 particles/cell, and transduction was analyzed 48 h later by fluorescence microscopy (A to D) and flow cytometry (E and F). Flow cytometry results are expressed as the percent positive cells transduced by respective virus after 60 min of incubation. The standard deviation of each is indicated. (A) Ad5 on myoblasts; (B) Ad-HI-12.51 on myoblasts; (C) Ad5 on myotubes; (D) Ad-HI-12.51 on myotubes; (E) transduction of myoblasts measured by flow cytometry; (F) transduction of myotubes measured by flow cytometry.

incorporation of selected peptide ligands into viral vectors for retargeting: the selected peptide ligand can ablate viral assembly or function, and the virus can debilitate the binding and/or internalization ability of the peptide ligand. In order to circumvent this ligand compatibility problem, we generated here a "context-specific" filamentous phage library in which random peptides were displayed between the H and I sheets from the Ad5 fiber protein, a location previously used on the virus for ligand display (20). By this approach, we demonstrate the selection and functional

translation of a skeletal muscle cell-binding peptide back into Ad5 vectors to increase transduction of these cells.

Previous work reported the display of the intact knob domain from the fiber on filamentous phage (18). In our hands, similar display of intact knob phage was toxic to bacteria and with only 1 out of 100 displaying the knob (3). We speculate that this toxicity was due to incompatibility of knob during its secretion from the bacteria on the pIII protein. A more recent study has demonstrated intact knob peptide libraries on lyso-genic lambda phage rather than the secreted filamentous

phage (9). In this case, a 2×10^5 member semirandom 14-mer peptide library was displayed in the HI loop of knob. Selection of this library against NIH 3T3 cells selected peptides that increased transduction on the 3T3 cells when translated into the virus by 100-fold (9). The advantage of this lambda library approach is that legitimate knob trimers can be displayed on the phage. The advantage of the filamentous phage display used in our study is that substantially larger peptide libraries can be generated to theoretically allow more peptide ligands to be screened to perhaps find higher affinity ligands (3). Like all phage-displayed libraries, one limitation of the "context-specific" library is the possible selection of phage particles that are truncated, deleted, or frameshifted of the peptide ligands (8, 26). Work is currently under way to optimize library design to overcome these ligand display problems.

Our data suggest that "context-specific" peptide libraries can be used to identify ligands that are compatible when translated back into the viral capsid context. Ad-HI-12.51 exhibited 14-fold increase in transduction compared to wild-type Ad in vitro. In our studies, we observed poor transduction of mouse muscle cells by unmodified Ad5, which is likely due to the low level of CAR expression on these cells (15). Consequently, the efficacy of Ad transduction is limited. Upon incorporation of selected 12.51 peptide into the Ad HI loop, we observed significant increase in transduction.

In summary, we demonstrate proof of principle for the use of relatively large repertoire "context-specific" peptide-presenting phage libraries as a potential approach to generate and identify compatible ligands when incorporated back into the viral capsid for retargeting. We are currently testing this library for its utility in selecting novel peptides against human skeletal muscle cells and other target cells for gene therapy.

ACKNOWLEDGMENTS

We thank Mary E. Barry for technical assistance. We also thank M. Brandon Parrott and Kristen Adams for the use of plasmids.

This study was supported by grants to M.A.B. from the Muscular Dystrophy Association. D.G. was supported by the Research Training Program in Molecular Virology at Baylor College of Medicine (T32 AI007471).

REFERENCES

1. Ascadi, G., A. Jani, B. Massie, M. Simoneau, P. Holland, K. Blaschuk, and G. Karpati. 1994. A differential efficiency of adenovirus-mediated in vivo gene transfer into skeletal muscle cells of different maturity. *Hum. Mol. Genet.* **3**:579–584.
2. Barry, M. A., W. J. Dower, and S. A. Johnston. 1996. Toward cell-targeting gene therapy vectors: selection of cell-binding peptides from random peptide-presenting phage libraries. *Nat. Med.* **2**:299–305.
3. Barry, M. A., S. Takahashi, and M. B. Parrott. 2002. Selection of peptides on phage, p. 549–579. In J. A. C. Douglas (ed.), *Vector targeting strategies for gene therapy*. Wiley-Liss, Inc., Hoboken, N.J.
4. Bergelson, J. M., J. A. Cunningham, G. Droguett, E. A. Kurt-Jones, A. Krithivas, J. S. Hong, M. S. Horwitz, R. L. Crowell, and R. W. Finberg. 1997. Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5. *Science* **275**:1320–1323.
5. Bouri, K., W. G. Feero, M. M. Myerburg, T. J. Wickham, I. Kovesdi, E. P. Hoffman, and P. R. Clemens. 1999. Polylysine modification of adenoviral fiber protein enhances muscle cell transduction. *Hum. Gene Ther.* **10**:1633–1640.
6. Campos, S. K., and M. A. Barry. 2004. Rapid construction of capsid-modified adenoviral vectors through bacteriophage lambda red recombination. *Hum. Gene Ther.* **15**:1125–1130.
7. Cao, B., R. Pruchnic, M. Ikezawa, X. Xiao, J. Li, T. J. Wickham, I. Kovesdi, W. A. Rudert, and J. Huard. 2001. The role of receptors in the maturation-dependent adenoviral transduction of myofibers. *Gene Ther.* **8**:627–637.
8. Cwirla, S. E., E. A. Peters, R. W. Barrett, and W. J. Dower. 1990. Peptides on phage: a vast library of peptides for identifying ligands. *Proc. Natl. Acad. Sci. USA* **87**:6378–6382.
9. Fontana, L., M. Nuzzo, L. Urbanelli, and P. Monaci. 2003. General strategy for broadening adenovirus tropism. *J. Virol.* **77**:11094–11104.
10. Huang, S., T. Kamata, Y. Takada, Z. M. Ruggeri, and G. R. Nemerow. 1996. Adenovirus interaction with distinct integrins mediates separate events in cell entry and gene delivery to hematopoietic cells. *J. Virol.* **70**:4502–4508.
11. Huard, J., H. Lochmuller, G. Acsadi, A. Jani, B. Massie, and G. Karpati. 1995. The route of administration is a major determinant of the transduction efficiency of rat tissues by adenoviral recombinants. *Gene Ther.* **2**:107–115.
12. Lieber, A., H. C. Meuse, L. Schowalter, D. Kirillova, I. Winther, and M. A. Kay. 1997. The role of Kupffer cell activation and viral gene expression in early liver toxicity after infusion of recombinant adenovirus vectors. *J. Virol.* **71**:8798–8807.
13. Mazzucchelli, L., J. B. Burritt, A. J. Jesaitis, A. Nusrat, T. W. Liang, A. T. Gewirtz, F. J. Schnell, and C. A. Parkos. 1999. Cell-specific peptide binding by human neutrophils. *Blood* **93**:1738–1748.
14. Mercier, G. T., J. A. Campbell, J. D. Chappell, T. Stehle, T. S. Dermody, and M. A. Barry. 2004. A chimeric adenovirus vector encoding reovirus attachment protein sigma1 targets cells expressing junctional adhesion molecule 1. *Proc. Natl. Acad. Sci. USA* **101**:6188–6193.
15. Nalbantoglu, J., N. Larochelle, E. Wolf, G. Karpati, H. Lochmuller, and P. C. Holland. 2001. Muscle-specific overexpression of the adenovirus primary receptor CAR overcomes low efficiency of gene transfer to mature skeletal muscle. *J. Virol.* **75**:4276–4282.
16. Nicklin, S. A., S. J. White, S. J. Watkins, R. E. Hawkins, and A. H. Baker. 2000. Selective targeting of gene transfer to vascular endothelial cells by use of peptides isolated by phage display. *Circulation* **102**:231–237.
17. Parrott, M. B., K. E. Adams, G. T. Mercier, H. Mok, S. K. Campos, and M. A. Barry. 2003. Metabolically biotinylated adenovirus for cell-targeting, ligand screening, and vector purification. *Mol. Ther.* **8**:689–702.
18. Pereboev, A., L. Pereboeva, and D. T. Curiel. 2001. Phage display of adenovirus type 5 fiber knob as a tool for specific ligand selection and validation. *J. Virol.* **75**:7107–7113.
19. Ragot, T. E. A. 1993. Efficient adenovirus-mediated transfer of a human minidystrophin gene to skeletal muscle of *mdx* mice. *Nature* **361**:647–650.
20. Reynolds, P. N., I. Dmitriev, and D. T. Curiel. 1999. Insertion of an RGD motif into the HI loop of adenovirus fiber protein alters the distribution of transgene expression of the systemically administered vector. *Gene Ther.* **6**:1336–1339.
21. Romanczuk, H., C. E. Galer, J. Zabner, G. Barsomian, S. C. Wadsworth, and C. R. O'Riordan. 1999. Modification of an adenoviral vector with biologically selected peptides: a novel strategy for gene delivery to cells of choice. *Hum. Gene Ther.* **10**:2615–2626.
22. Romanov, V. I., D. B. Durand, and V. A. Petrenko. 2001. Phage display selection of peptides that affect prostate carcinoma cells attachment and invasion. *Prostate* **47**:239–251.
23. Schiedner, G., W. Bloch, S. Hertel, M. Johnston, A. Molodtsov, V. Dries, G. Varga, N. Van Rooijen, and S. Kochanek. 2003. A hemodynamic response to intravenous adenovirus vector particles is caused by systemic Kupffer cell-mediated activation of endothelial cells. *Hum. Gene Ther.* **14**:1631–1641.
24. Sewry, C. A., and T. A. Partridge. 1996. The muscular dystrophies. In N. R. A. C. Lemoine (ed.), *Gene therapy*. BIOS Scientific Publishers, Ltd., Oxford, England.
25. Smith, T., N. Idamakanti, H. Kylefjord, M. Rollence, L. King, M. Kaloss, M. Kaleko, and S. C. Stevenson. 2002. In vivo hepatic adenoviral gene delivery occurs independently of the coxsackievirus-adenovirus receptor. *Mol. Ther.* **5**:770–779.
26. Soltes, G., H. Barker, K. Marmai, E. Pun, A. Yuen, and E. J. Wiersma. 2003. A new helper phage and phagemid vector system improves viral display of antibody Fab fragments and avoids propagation of insert-less virions. *J. Immunol. Methods* **274**:233–244.
27. Takahashi, S., H. Mok, M. B. Parrott, F. C. Marini, M. Andreef, M. K. Brenner, and M. A. Barry. 2003. Selection of chronic lymphocytic leukemia (CLL) binding peptides. *Cancer Res.* **63**.
28. Tang, D., S. A. Johnston, and D. P. Carbone. 1994. Butyrate-inducible and tumor-restricted gene expression by adenovirus vectors. *Cancer Gene Ther.* **1**:15–20.
29. White, S. J., S. A. Nicklin, T. Sawamura, and A. H. Baker. 2001. Identification of peptides that target the endothelial cell-specific LOX-1 receptor. *Hypertension* **37**:449–455.
30. Wickham, T. J., P. Mathias, D. A. Cheresch, and G. R. Nemerow. 1993. Integrins avb3 or avb5 promote adenovirus internalization but not virus attachment. *Cell* **73**:309–319.
31. Wickham, T. J., E. Tzeng, L. L. Shears, P. W. Roelvink, Y. Li, G. M. Lee, D. E. Brough, A. Lizonova, and I. Kovesdi. 1997. Increased in vitro and in vivo gene transfer by adenovirus vectors containing chimeric fiber proteins. *J. Virol.* **71**:8221–8229.