Adenovirus Targeting to c-erbB-2 Oncoprotein by Single-Chain Antibody Fused to Trimeric Form of Adenovirus Receptor Ectodomain

Elena A. Kashentseva, Toshiro Seki, David T. Curiel, and Igor P. Dmitriev

Division of Human Gene Therapy, [E. A. K., T. S., D. T. C., I. P. D.], Departments of Medicine, Pathology, and Surgery [D. T. C.], and Gene Therapy Center [D. T. C.], University of Alabama at Birmingham, Birmingham, Alabama 35294-3300

ABSTRACT

The use of adenovirus (Ad) vectors for cancer gene therapy applications is currently limited by several factors, including broad Ad tropism associated with the widespread expression of coxsackievirus and adenovirus receptor (CAR) in normal human tissues, as well as limited levels of CAR in tumor cells. To target Ad to relevant cell types, we have proposed using soluble CAR (sCAR) ectodomain fused with a ligand to block CAR-dependent native tropism and to simultaneously achieve infection through a novel receptor overexpressed in target cells. To confer Ad targeting capability on cancer cells expressing the c-erbB-2/HER-2/neu oncogene, we engineered a bispecific adapter protein, sCARfC6.5, that consisted of sCAR, phage T4 fibrin polypeptide, and C6.5 single-chain fragment variable (scFv) against c-erbB-2 oncoprotein. Incorporation of fibrin polypeptide provided trimerization of sCAR fusion proteins that, compared with monomeric scFv CAR protein, resulted in augmented affinity to Ad fiber knob domain and in increased ability to block CAR-dependent Ad infection. We demonstrated that sCARfC6.5 protein binds to cellular c-erbB-2 oncoprotein and mediates efficient Ad targeting via a CAR-independent pathway. As illustrated in cancer cell lines that overexpress c-erbB-2, targeted Ad, complexed with sCARfC6.5 adapter protein, provided from L5- to 17-fold enhancement of gene transfer compared with Ad alone and up to 130-fold increase in comparison with untargeted Ad complexed with sCAR control protein. The use of recombinant trimeric sCAR-scFv adapter proteins may augment Ad vector potency for targeting cancer cell types.

INTRODUCTION

Ad represents a large family of nonenveloped viruses (1). Human Ad includes 47 known viral serotypes grouped into six distinct subgroups, A to F. Most of the studies on the mechanism of Ad infection have concluded that receptor recognition is one of the key factors that determines cell tropism (2, 3). In this regard, the initial steps of Ad infection involve at least two sequential virus-cell interactions, each mediated by a specific viral capsid protein. Ad infection is initiated by the binding of globular knob domain of trimeric fiber protein to a host cell primary receptor (4, 5). Subsequent interaction of the penton base with αV integrins mediates virion internalization via receptor-mediated endocytosis (6). Fiber receptor for Ad subgroups A, C, D, E, and F has been identified as the CAR (7–9). CAR is an integral membrane protein consisting of two extracellular immunoglobulin-like D1 and D2 domains, a transmembrane region, and a COOH-terminal cytoplasmic domain (8, 10). The extracellular domain of CAR is sufficient for virus attachment and infection (11, 12), whereas both transmembrane and intracellular regions appear to be dispensable for these functions (13). Both structural analysis of fiber knob complexed with CAR D1 domain (14) and knob mutagenesis studies (15) revealed that amino acid residues responsible for CAR binding are located on lateral surfaces formed by the interface of two adjacent knob monomers. These data suggest an avidity mechanism when three CAR molecules could simultaneously bind per one fiber knob trimer, which was recently supported by kinetic analysis of Ad2 knob binding to the CAR D1 domain (16).

Well-characterized Ad serotypes 2 and 5 from subgroup C are predominantly used as vectors for in vitro and in vivo gene delivery (17), because of high infection efficiency in a variety of human cell types and tissues. However, this broad viral tropism is disadvantageous for gene delivery to cancer cell types refractory to Ad infection because of the absence or low levels of CAR expression (18–21). This limitation could be solved by Ad targeting via a nonviral receptor (22, 23). Several strategies have been tested in an effort to target Ad via CAR-independent pathways (24) including chemical conjugation or genetic modification of viral capsid proteins to incorporate targeting ligands and the use of bispecific adapter molecules to provide indirect virus linkage with the cell-surface receptors. The technical achievement of Ad targeting via adapter molecules has been approached by a variety of methods. Bispecific conjugates of antibodies or their Fab fragments were used to achieve linkage between target receptor and v.p. by means of specific recognition through either a fiber knob domain or penton base (reviewed in Refs. 17, 22, 23). Further refinement of this strategy has been accomplished by the engineering of recombinant proteins consisting of an anti-knob scFv fused with human EGF (25) or a scFv against EGFR (26). The original concept of employment of fusion proteins comprising a soluble viral receptor and targeting ligand was proposed for retrovirus targeting to specific cell types (27). Applying this strategy to Ad targeting, we have developed an approach based on the use of sCAR ectodomain fused with EGF, achieving simultaneously the blocking of virus-CAR interaction and the redirection of Ad to cells overexpressing EGFR (28, 29). A similar approach was successfully applied to target Ad to high-affinity Fcγ receptor I-positive human mononcytic cells (30). The use of recombinant adapter molecules eliminates chemical conjugation and provides a high degree of flexibility for ligand substitution and, consequently, expands the targeting capabilities of Ad vectors.

We hypothesized that the predicted 3:1 stoichiometry of CAR-knob binding could provide high-affinity linkage of trimeric sCAR-ligand proteins to v.p. and, thereby, promote the ligand-mediated binding to target receptors. In this study, we describe a novel approach of Ad transductional targeting to cancer cell types expressing c-erbB-2 oncoprotein by means of a recombinant protein adapter. The gene known as c-erbB-2/HER-2/neu, encoding a member of the erbB family of growth factor receptors, is most frequently altered in human cancer and was shown overexpressed in a number of malignancies including tumors that arise in the breast and ovary (31, 32). We engineered a bispecific protein, sCARfC6.5, featuring a unique trimeric design and consisting of sCAR fused with phage T4 fibrin polypeptide and C6.5 scFv against c-erbB-2. We have demonstrated that the sCARfC6.5 protein efficiently blocks Ad native tropism while simultaneously...
mediating virus infection via an alternative CAR-independent pathway, which markedly enhances gene transfer efficiency to cell lines that overexpress c-erbB-2. Our data suggest that the use of this original approach may augment the potency of Ad vectors for cancer gene therapy.

MATERIALS AND METHODS

Cells and Media. The 293 human kidney cell line, transformed with Ad5 DNA, was purchased from Microbix (Toronto, Ontario, Canada). The human breast cancer cell lines MDA-MB-468, AU-565, SK-BR-3, BT-474, and MCF-7 and the ovarian cancer cell line SK-OV-3, established from adenocarcinomas of mammary gland and ovary, respectively, were from the American Type Culture Collection (Manassas, VA). All of the cell lines were maintained in recommended growth media supplied by Mediatech (Herndon, VA) containing 10% FBS (HighClone, Logan, UT) and 2 mM glutamine at 37°C in a humidified atmosphere of 5% CO₂. Infection of the cells with Ad was carried out in the infection medium containing 2% FBS.

Enzymes. Restriction endonucleases, Klenow enzyme, T4 DNA ligase, and proteinase K were from either New England Biolabs (Beverly, MA) or Boehringer Mannheim (Indianapolis, IN).

Antibodies. Murine serum to baculovirus-produced human sCAR protein was generated at the University of Alabama at Birmingham Hyridoma Core Facility. The MAb RmcB (33) to human CAR were produced using hybridoma purchased from American Type Culture Collection and kindly provided by J. T. Douglas (University of Alabama at Birmingham). PentaHis Mabs were from Qiagen Inc. (Valencia, CA). Rabbit serum against phage T4 fibrin protein was kindly provided by V. Mesyanzhinov (Shemykin and Ovchinnicov Institute of Bioorganic Chemistry, Moscow, Russia). Mouse Mabs to the human c-erbB-2/HER-2 neu oncoprotein, Ab-2 (Clone9G6.10), were purchased from NeoMarkers Inc., (Fremont, CA). Normal mouse IgG₁ were from OEM Concepts (Toms River, NJ). Goat antimouse and antirabbit IgG conjugated with alkaline phosphatase were from Sigma Chemical Co. (St. Louis, MO) and Pierce (Rockford, IL), respectively. Streptavidin-alkaline phosphatase conjugate was from Bio-Rad Laboratories (Hercules, CA). Alexa 488-labeled goat antimouse IgG were from Molecular Probes (Eugene, OR).

Viruses. A recombinant Ad5 vector, AdLucGFP, containing double expression cassette consisting of firefly luciferase gene and GFP gene under the control of cytomegalovirus immediate early promoter in place of the E1 region of the Ad genome, was constructed as described by Seki et al. (34). Ad was propagated on 293 cells and purified by centrifugation in CsCl gradients by a standard protocol. The titers of pV. p.v. and infectious v.p. were determined by the use of the methods of Maizel et al. (35) and Mittereder et al. (36), respectively.

Construction of Recombinant Plasmids. To generate the recombinant gene encoding the extracellular domain of human CAR followed by polypeptide sequence derived from bacteriophage T4 fibrin protein (37), PCR was used. Sense primer 5′-GGT GAA AGA TCT GTA ACC AAT AAA ATA AAA AAT GTA GGT ACT ATG ACT ATT GTA GTA CCA CCC GCG CCG CTG GTG ATA AAA AGG GAA CTA GCT CTG CGC TAG 3′-restriction sites and AgeI-fragment was ligated with 775-bp DNA fragment was cloned into AgeI cleaved pFasBacH7a (Life Technologies, Inc.), which resulted in plasmid pFB6bC6.5. Then, plasmid pFB6bC6.5 was digested with SstI and HincII restriction sites, respectively. The PCR product was digested with XbaI and SpeI and a purified 769-bp DNA fragment was cloned into SpeI cleaved pFasBacH7a (Life Technologies, Inc.), which resulted in plasmid pFB6bC6.5. The constructed plasmid, containing recombinant gene encoding sCAR, His6, short linker, fibrin polypeptide, hinge region, and C6.5 scFv, was sequenced to confirm the correct DNA structure. The resultant plasmid, designated pFB6bC6.5, was then used to generate the recombinant baculovirus using the Bac-to-Bac system.

Expression, Purification, and Biotinylation of the Fusion Proteins. The fusion proteins, sCARf and sCARfC6.5, comprised of sCAR-His₆-fibrin and sCAR-His₆-fibrin-hinge-C6.5scFv polypeptide sequences, respectively, were expressed in High Five cells (Invitrogen, Carlsbad, CA) that were infected with recombinant baculoviruses. Recombinant His₆-tagged proteins were purified from dialyzed culture medium by immobilized metal-ion-affinity chromatography on Ni-nitrilotriacetic acid (Ni-NTA)-Sepharose (Qiagen Inc.) as described previously (29). Protein concentrations were determined by the BCA-200 protein assay kit using bovine gamma globulin as the standard (Pierce). Purified sCAR and previously produced sCAR-His₆ (29) proteins were biotinylated using EZ-Link SulfoNHS-LS-Biotinylation kit (Pierce). The degree of biotin-protein incorporation [determined using HABA method (Pierce)] was 0.6 biotin per molecule of scCAR-His₆, monomeric protein and 0.5 biotin per trimeric molecule of sCAR protein.

Protein Electrophoresis and Western blot. To determine whether the recombinant sCAR and sCARfC6.5 fusion proteins could form trimers, they were analyzed by SDS-PAGE. Purified proteins were either boiled in Laemmlı loading buffer prior to electrophoresis to denature proteins to monomers or loaded on the gel without denaturation. The trimeric or monomeric configurations of protein molecules were determined based on their mobilities in the gel. To analyze the composition of sCAR fusion proteins, we used Western blot. Samples of boiled sCARfC6.5 and sCARf proteins separated on 4–15% gradient SDS-PAGE were transferred to polyvinylidene difluoride membrane and probed with murine anti-sCAR serum, PentaHis Mab, or rabbit antifibrin serum. Bound IgG were detected with secondary alkaline phosphatase-conjugated antibodies.

ELISA. Solid-phase binding ELISA was performed as follows. Recombinant Ad5 knob protein, expressed in Escherichia coli as described previously (39), was diluted in 50 mM NaHCO₃ (pH 9.6) at a concentration of 1 μg/ml and was immobilized on Nunc-Maxisorp ELISA plate overnight. The wells were blocked with PBS [10 mM NaHPO₄, 10 mM KH₂PO₄ (pH 7.4), and 136 mM NaCl] containing 0.05% Tween 20 and 2% BSA and then washed with PBS containing 0.05% Tween 20. Biotinylated sCAR-His₆ and sCARf proteins diluted in blocking buffer to concentrations ranging from 0.01 to 25 pmol/ml were added to the wells in 100-μl aliquots. After a 1-h incubation at room temperature, the wells were washed, and bound biotinylated proteins were detected by 45-min incubation with 1:1000 dilution of streptavidin-alkaline phosphatase conjugate (Bio-Rad). The plates were then developed using signal-producing reagent p-nitrophenyl phosphate (Sigma Chemical Co.). Plates were read in a microtiter plate reader, set at 405 nm; results are presented as mean absorbance ± SD.

Indirect Immunofluorescence. The analysis of cell lines for expression levels of CAR and c-erbB-2 oncoprotein was performed by indirect immunofluorescence assay using flow cytometry as follows. Aliquots (100 μl) of cells, resuspended in FACS buffer [10 mM NaHPO₄, 10 mM KH₂PO₄ (pH 7.4), 136 mM NaCl, 1% BSA, and 0.1% NaN₃] at a concentration of 2 × 10⁶ cells/ml were incubated with either RmcB (anti-CAR) or Ab-2 (anti-c-erbB-2) Mab at...
a concentration of 5 μg/ml for 1 h at 4°C. An isotype-matched normal mouse IgG1 was used as a negative control. Cells were washed with FACS buffer by centrifugation and then were incubated with secondary Alexa 488-labeled goat antimouse antibody (Molecular Probes) at a concentration of 5 μg/ml for 1 h at 4°C. Cells were washed with FACS buffer prior to flow cytometry analysis. To validate that C6.5 scFv, incorporated in the context of sCARIC6.5 protein, are able to bind to cellular c-erbB-2, cells were incubated first with sCARIC6.5 or with sCAR protein as a negative control at a concentration of 10 μg/ml. After a 1-h incubation, cells were washed and incubated with primary Rm cB MAb and then with secondary Alexa 488-labeled antibody as described above. Cell samples (10^5 cells/sample) were analyzed by flow cytometry performed at the University of Alabama at Birmingham FACS Core Facility. Data were expressed as the geometric mean fluorescence intensity of the entire gated population. The positive cell population was determined by gating the right-hand tail of the distribution of the negative control sample for each cell line at 1%.

Gene Transfer Assay. The assay of Ad-mediated gene transfer to the cells was performed as follows. Aliquots (3 μl) of AdLucGFP vector were mixed with 6-μl aliquots of sCAR or sCARIC6.5 protein dilutions ranging from 0.2 to 53 pmol or of PBS (10 mM NaHPO4, 10 mM KH2PO4 [pH 7.4], and 136 mM NaCl) for 15 min at room temperature. The virus-sCARprotein-containing complexes were diluted to 1 ml with infection medium containing 2% FBS, and 200-μl aliquots were then added to the cell monolayers (grown in a 24-well plate (3-5 × 10^5 cells/well) at MOI of 100 v.p./cell) and were incubated for 30 min at room temperature to allow virus internalization. Then, infection medium was aspirated, the cells were washed with PBS, and the cells were incubated in a growth medium containing 10% FBS at 37°C to allow expression of the reporter genes. Forty-six h postinfection, cells were lysed and luciferase activity was analyzed by using the Promega (Madison, WI) luciferase assay system and a Berthold (Gäthersburg, MD) luminometer. For inhibition of the Ad infection of 293 cells AdLucGFP vector was mixed with sCAR or sCARIC6.5 protein dilutions (1.1-30 pmol), or with sCAR-His6 protein dilutions (3-230 pmol), or with PBS for 15 min at room temperature. Monolayers of 293 cells were exposed to the virus-sCARprotein-containing complexes at MOI of 13 v.p./cell for 30 min and then were incubated for an additional 20 h at 37°C to allow expression of luciferase gene prior to analysis.

RESULTS

Design and Generation of sCAR Fusion Proteins. To exploit the trivalent nature of CAR-knob interaction for the purposes of Ad targeting, we engineered a recombinant adapter protein consisting of soluble CAR in fusion with a trimerization sequence and a targeting ligand (Fig. 1A). We hypothesized that the predicted 3:1 stoichiometry of CAR-knob binding could provide high-affinity linkage of trimeric sCAR-ligand adapter proteins to virus and thereby block CAR-dependent Ad infection. Our goal was to generate a trimeric sCAR-ligand protein capable of efficiently blocking Ad native tropism while providing a novel target-selective tropism to c-erbB-2-positive cells (Fig. 1B). Because of the absence of a specific cognate ligand for c-erbB-2 oncoprotein, we chose C6.5 scFv as a targeting moiety that binds to c-erbB-2 oncoprotein. Presence of three sCAR domains in the context of trimerized adapter molecule potentially provides high-affinity viral linkage because of the trivalent stoichiometry of CAR-knob binding. The use of trimeric sCARIC6.5 adapter protein might, therefore, allow efficient blocking of Ad infection of Ad-bearing cells. C6.5 scFv targeting moiety of virus-bound adapter protein mediates recognition of c-erbB-2-positive cells, thereby providing novel target-specific Ad tropism.

Characterization of Recombinant sCAR Fusion Proteins. The polypeptide composition of produced fusion proteins was characterized by Western blot analysis. Detection of denatured electrophoto-
with either PBS or one of the sCAR-His₆, sCARf, or sCARfC6.5 proteins at varying concentrations and was used to infect 293 cells, which are known to express a high level of CAR. The ability of sCAR fusion proteins to block viral infection was assessed by sCAR protein dose-dependent impairment of Ad-mediated gene transfer as measured by luciferase activity in infected cells (Fig. 3B). It was shown that sCARfC6.5 as well as sCARf displayed an increased ability to inhibit CAR-dependent Ad infection compared with monomeric sCAR-His₆ protein. The concentrations of sCARfC6.5, sCARf, and sCAR-His₆ needed to block Ad infection by 50% were 3, 6, and 54 nm, respectively. Interestingly, sCARfC6.5 protein displayed Ad infection inhibition efficiency somewhat higher than did sCARf control protein. This experiment validated the utility of trimeric sCAR fusion proteins to block CAR-dependent Ad tropism and, therefore, provided a rationale for additional c-erbB-2 targeting studies.

**Bispecific sCARfC6.5 Protein Binds to Cellular c-erbB-2.** Flow cytometry analysis was performed to validate that C6.5 scFv incorporated into recombinant sCARfC6.5 fusion protein retained its ability to bind c-erbB-2 oncoprotein at the cell surface. The sCARfC6.5 protein was used to bind to c-erbB-2 that was overexpressed on AU-565 breast cancer cells. The MDA-MB-468 breast cancer cell line, previously shown to be c-erbB-2-negative, was used as a control. The sCARfC6.5 protein, bound to c-erbB-2 displayed at the cell surface by c-erbB-2-specific murine antibodies, was detected by a secondary horseradish peroxidase-conjugated goat antiserum. Bound antibody was visualized by enhanced chemiluminescence and autoradiography.

### Analysis of sCAR Fusion Proteins Binding to Ad Fiber Knob

We characterized trimeric sCAR fusion protein with respect to its ability to bind Ad fiber knob compared with monomeric sCAR-His₆ protein generated previously (29). The knob-binding affinities of sCARf and sCARfC6.5 proteins were compared by ELISA using immobilized Ad5 knob expressed in E. coli (39). Compared with monomeric sCAR-His₆ protein, the knob-binding affinity of trimeric sCARf protein was increased at least 20-fold in a range of tested concentrations from 0.1 to 5 pmol/ml (Fig. 3A). This result suggests that, compared with sCAR-His₆ monomer, trimeric sCARf protein possesses augmented ability to provide viral linkage by means of high-affinity binding to Ad fiber knob domain. Therefore, trimeric sCAR fusion proteins might offer improved blocking capability of CAR-dependent virus-cell attachment and viral infection.

To determine whether augmented binding to Ad fiber knob results in increased ability of trimeric sCAR proteins to block Ad infection, we performed an infection inhibition assay. AdLucGFP vector, expressing both luciferase and GFP reporter genes, was preincubated...
Fig. 4. Confirmation of sCARIC6.5 protein binding to cellular c-erbB-2. Trimeric sCARIC6.5 and sCAR fusion proteins were incubated with either c-erbB-2-positive AU-565 or c-erbB-2-negative MB-468 cells. The sCAR fusion proteins bound to cells were probed with anti-CAR RmcB MAb and then detected with secondary Alexa 488-labeled goat antimouse antibodies. Binding of sCARIC6.5 protein (black line) to c-erbB-2-positive AU-565 cells was seen because of the positive staining relative to sCARF control protein (gray line) or anti-CAR MAb alone (spike filled in black). Representative data from two independent experiments are shown.

Augmentation of c-erbB-2-targeted Ad Infection Efficiency. The Ad infection of the cells overexpressing c-erbB-2 oncoprotein mediated by the sCARIC6.5-targeting protein resulted in the enhancement of luciferase reporter gene expression. This increase in reporter activity could result from an increased number of infected cells or, alternatively, from an elevated level of transgene expression caused by the augmented infection of a limited population of cells. To address this issue, cells were infected with AdLucGFP vector, preincubated with PBS (Ad alone), sCARF (untargeted Ad), or sCARIC6.5 (c-erbB-2-targeted Ad) proteins at a sCAR protein:Ad ratio of 2 × 10⁻⁷ pmol/v.p. Ad infection efficiency was monitored by direct visualization of GFP expression by fluorescence microscopy. Fig. 7 shows the results of Ad-mediated GFP reporter gene delivery to three representative cell lines: AU-565, SK-BR-3, and BT-474. Infection with AdLucGFP vector alone resulted in a low percentage of GFP-expressing cells, whereas highly increased numbers of infected cells were detected in the case of c-erbB-2-targeted Ad. In contrast, infection...
with untargeted Ad showed a decreased infection efficiency compared with that of Ad alone, which resulted in few or no fluorescent cells. Infection of c-erbB-2-negative MDA-MB-468 cells with targeted Ad did not show any improvement of infection efficiency compared with that achieved by untargeted Ad or by Ad alone (data not shown). These results were consistent with data obtained for Ad-mediated luciferase gene delivery (Fig. 6).

**DISCUSSION**

One of the major challenges facing Ad gene delivery systems is the modification of viral native tropism to confer targeting capability on selected cell types. The limitation of Ad vectors associated with broad CAR-dependent tropism and inefficient infection of CAR-deficient cells could be solved by Ad targeting to a novel receptor overexpressed in the cells of interest. In this study, we explored the utility of a recombinant adapter protein to achieve modification of Ad tropism. The use of adapter molecules to mediate cell-specific Ad infection relies on the following: (a) the ability to be produced and purified at preparative amounts and retain stable structure; (b) the ability to provide efficient linkage to the v.p. while blocking Ad native tropism; and (c) the ability to achieve binding to cell-specific receptors, thus generating a novel tropism. We previously developed a targeting approach based on the use of the sCAR ectodomain fused with EGFR, which allowed both the blocking of CAR-dependent tropism and the Ad targeting to the cells overexpressing EGFR (29). To extend this approach, we engineered an adapter protein, sCARIC6.5, comprised of sCAR fused with a trimerization domain and a scFv against c-erbB-2 oncoprotein to target cancer cell types, and a sCARF control protein that lacked the anti-c-erbB-2 scFv targeting moiety. A trimerization domain derived from phage T4 fibrin protein was incorporated into the design of the sCAR fusion proteins to achieve tight viral linkage by virtue of trivalent binding to the Ad fiber knob. Both the sCARIC6.5 targeting protein and the control sCARF protein were expressed in insect cells and purified at preparative amounts by affinity chromatography. An analysis of purified fusion proteins showed that sCARF and sCARIC6.5 retain their designed composition and maintain stable trimeric structure. As expected, trimerization of sCAR proteins resulted in the augmentation of knob-binding efficiency by at least 20-fold compared with that achieved by the monomeric sCAR-His8 protein. This result is consistent with a kinetic analysis showing that, in contrast to the high on/off interaction rates between CAR D1 domain and Ad2 fiber knob, the binding of the knob domain to three D1 molecules simultaneously leads to a low overall off rate and $K_d$ of $\sim$1 nM (16). We then evaluated whether trimerization would result in an improved ability of sCAR proteins to block Ad infection. An infection inhibition assay demonstrated that the concentrations (3 and 6 nM, respectively) of trimeric sCARIC6.5 and sCARF proteins that were needed to inhibit Ad-mediated gene transfer by 50% were, respectively, 18- and 9-fold lower than the concentration (54 nM) of monomeric sCAR-His8 protein that was needed. These data proved the utility of trimeric sCAR adapter proteins in blocking CAR-dependent Ad infection. The fact that T4 fibrin forms homotrimers that are resistant to dissociation by SDS and digestion by trypsin (37, 38) suggests that the incorporation of a fibrin polypeptide in the context of sCAR fusion might provide a highly stable trimeric structure compatible with in vitro and likely in vivo, Ad targeting schemes. We showed that, in addition to its ability to block Ad infection, the sCARIC6.5 adapter protein binds to cellular c-erbB-2 oncoprotein and, therefore, enables Ad targeting via a CAR-independent pathway. In ovarian and breast cancer cell lines overexpressing c-erbB-2, the ability of the c-erbB-2 oncoprotein to mediate Ad infection was illustrated by markedly increased levels of gene transfer and numbers of infected cells. Thus, the use of the sCARIC6.5 adapter protein overcomes the barrier of CAR deficiency by retargeting the Ad infection via the c-erbB-2 oncoprotein and provides a 20-fold enhancement of gene transfer efficiency in comparison with that provided by the sCARF control protein. Our observation that the use of the sCARIC6.5 adapter decreased the Ad infection efficiency in c-erbB-2-negative MDA-MB-468 cells that expressed moderate levels of CAR suggests the high specificity of this Ad-targeting approach. Importantly, the presence of three scFvs in each trimeric sCARIC6.5 molecule likely contributes to the increase in apparent receptor affinity attributable to polyclonal binding and contributes, therefore, to the efficiency of Ad targeting. In fact, the presence of additional binding sites prolongs the association of scFv-based molecules with tumor cells in vitro and in vivo. Studies of bivalent diabody molecules that were constructed from C6.5 scFv demonstrated a more highly prolonged association (60-fold) with c-erbB-2
ACKNOWLEDGMENTS

We are grateful to Dr. James D. Marks for the provision of the cDNA for C6.5 scFv, Dr. Robert Finberg (Dana Farber Cancer Institute, Boston, MA) for cDNA for CAR, and Dr. V. Mesyanzhinov for anti-fibrin serum. We thank Dr. Victor Krasnykh (Division of Human Gene Therapy, University of Alabama-Birmingham) for making RmcB antibody, Dr. Victor Krasnykh (Division of Human Gene Therapy, University of Alabama-Birmingham) for the plasmid encoding recombinant CAR, and Dr. V. Mesyanzhinov for anti-fibrin serum. We thank the DNA Sequencing Core and the FACS Core Facility at the University of Alabama at Birmingham for providing assistance. Thanks to Dr. Joel N. Glasgow for fruitful discussions and proofreading of the manuscript.

REFERENCES


Downloaded from cancerres.aacrjournals.org on April 15, 2017. © 2002 American Association for Cancer Research.
Adenovirus Targeting to c-erbB-2 Oncoprotein by Single-Chain Antibody Fused to Trimeric Form of Adenovirus Receptor Ectodomain

Elena A. Kashentseva, Toshiro Seki, David T. Curiel, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/2/609

Cited articles
This article cites 42 articles, 24 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/2/609.full.html#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
/content/62/2/609.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.