Targeted Adenoviral Vectors I: Transductional Targeting

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1. Introduction

Human adenovirus[1] (Ad) has been used extensively to derive replication-incompetent gene delivery vectors to correct genetic disorders and develop candidate vaccines for a variety of infectious diseases and cancer immunotherapy, and as conditionally replicative Ad (CRAd) agents for cancer virotherapy. Adenovirus vectors have been used in 22% of all gene therapy clinical trials, followed by retroviral vectors (19%) and naked/plasmid deoxyribonucleic acid (DNA) (18%).[2,3] A major factor limiting the effectiveness of current-generation Ad vectors is their inability to accomplish specific gene delivery to cells of interest. Indeed, a National Institutes of Health report identified the "need for vector targeting" as a central objective for the field of gene therapy.[4] Extensive studies of interactions between Ad capsid proteins and host cells in vitro revealed that efficient Ad infection requires the presence of sufficient levels of receptors responsible for virus attachment to the cellular membrane and internalization. Adenovirus attachment to the cell is mediated by fiber binding with its C-terminal knob domain to a primary cellular receptor. Subsequent interaction of $\alpha_v\beta_{3/5}$-integrins, the secondary cellular receptor, with an Arg-Gly-Asp (RGD) sequence within a protein loop extended from the penton base is required to trigger endocytosis resulting in virus internalization. Most Ad of species B have been shown to use human membrane cofactor CD46 as the predominant attachment receptor[5] whereas the coxsackievirus group B and Ad receptor (CAR)[6] has been identified as the primary high-affinity receptor for many representatives of species A, C, D, E, and F.[7–9] Therefore, levels of CD46 and CAR expression determine the infection efficacy of Ad serotype 35 of species B and Ad serotype 2 (Ad2) or 5 (Ad5) (both of species C), respectively, which are mostly used for vector construction purposes. Thus, an unfavorable expression pattern of primary Ad receptor in a clinical context would result in an insufficient level of infection of target cells while leading to ectopic virus sequestration by nontarget tissues. The delineation of key steps of the Ad cellular entry pathway in vitro, in which cell attachment is distinct from subsequent virus internalization, suggested that Ad recognition of cognate primary receptor represents a rate-limiting step, which could be intervened in an effort to redirect virus-cell binding via an alternative cellular receptor to confer susceptibility to Ad vector infection. Transductional targeting strategies seek to redirect Ad binding to appropriate nonnative receptors to increase the efficiency of gene transfer to the cell type selected to achieve therapeutic intervention.

Adenoviral Vectors for Gene Therapy. http://dx.doi.org/10.1016/B978-0-12-800276-6.00009-7

2. Adapter-Mediated Ad Vector Targeting Approach

Efforts to redirect Ad vectors via receptors overexpressed on the cells that are refractory to Ad infection mainly focus on incorporating targeting ligands by means of chemical conjugation or genetic modification of viral capsid proteins and using bispecific adapter molecules to mediate virus recognition of target cells. The use of bispecific protein adapters was originally proposed to bridge viral particle and cell surface molecule to overcome inefficient virus infectivity owing to the scarcity of Ad attachment receptor[7–9] or its localization on inaccessible parts of the cell.[10–17] This goal was originally addressed by the development of bispecific antibody (bsAb) conjugates, which are able to bind both the viral capsid protein and the cell surface receptor, allowing indirect linkage between viral particles and cellular receptor (Figure 1).

2.1 Use of Ab Conjugates for Ad Targeting

To construct bsAb adapters, Wickham et al. used monoclonal antibody (mAb) against an FLAG peptide, which was genetically incorporated in place of the deleted RGD sequence in penton base protein, chemically conjugated to mAb with specificities for α_v-integrin receptors or human CD3 to redirect the AdFLAG vector to endothelial and smooth muscle cells or T cells, respectively.[18,19] Although successfully demonstrating the feasibility of in vitro virus retargeting via non–Ad receptors displayed on human venule endothelial cells, intestinal smooth muscle cells, and resting T cells that are normally refractory, this approach was later abandoned, apparently because of reduced virus viability resulting from RGD sequence deletion.[20] An alternative

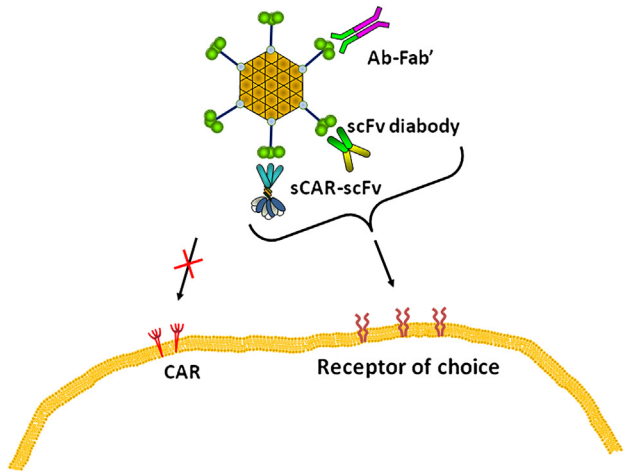

Figure 1 Strategies of Ad vector targeting using bispecific adapters. Adenovirus retargeting from various cell types can be achieved using bispecific adapter proteins. Bispecific adapters consist of Ad fiber knob-binding moiety fused to alternative receptor-binding ligand including Fab antibodies, scFv Ab, and biological ligands. Targeting adapters allow efficient CAR-independent transduction of cells of interest.

approach to provide adapter binding to Ad capsid was proposed by Douglas et al.,[21] based on the use of neutralizing mAb 1D6.14, which blocks binding of the Ad5 fiber knob to CAR. The feasibility of Ad retargeting via a nonviral receptor was demonstrated by conjugating the Fab' fragments of mAb 1D6.14 to folate to allow virus linkage to the folate receptor, which is overexpressed on the surface of a variety of malignant cells. This Fab–folate conjugate was complexed with Ad5 vector carrying the luciferase reporter gene and was shown to redirect virus infection of target cells via the folate receptor at a high efficiency. When complexed with Ad5 carrying the gene for herpes simplex virus thymidine kinase, the Fab–folate conjugate mediated the specific killing of cells that overexpress the folate receptor. This work demonstrated the feasibility of employing an adapter approach both to ablate endogenous viral tropism and to introduce novel tropism in vitro.

Use of the Ab-based Ad5 vector targeting approach was further explored to circumvent the lack of CAR expression and improve gene transfer specifically to tumor cells by generating a bispecific Ab conjugate consisting of anti-knob Fab' fragments conjugated to mAb 425,[22] which was derived against the epidermal growth factor receptor (EGFR), a tumor-associated marker negligibly expressed in normal mitotically quiescent tissues.[23] Targeting of Ad5 vector via EGFR using this Ab conjugate led to enhanced gene transfer relative to untargeted Ad in 7 of 12 human glioma cell lines and 6 of 8 primary glioma samples derived from tumors of various histologies.[24] Furthermore, EGFR retargeting showed marked transduction enhancement in both squamous cell carcinoma of the head and neck cell lines and primary tumor tissue compared with normal tissue from the same patient.[25] These studies illustrated that Ad targeting via EGFR overcomes cell deficiency in CAR expression to achieve an increase in gene transfer efficiency in tumor cell types, and therefore suggests that a bispecific adapter approach could augment Ad vector potency for cancer gene therapy applications.

Based on these essential findings, an adapter approach was employed to promote Ad-mediated gene transfer in dendritic cells (DC) to assess its targeting utility in the context of important therapeutic applications proposed for genetically modified DCs. To this end, Tillman et al.[26] tested Fab' fragment (1D6.14) chemically conjugated to mAb G28-5 agonistically binding DC's receptor CD40.[27] The CD40 receptor is attractive for DC targeting because it has an important role in inducing DC maturation and priming cytotoxic T cells.[28] Ad5 vector retargeting via the CD40 pathway using this bispecific construct dramatically enhanced gene transfer to monocyte-derived DCs (MoDCs) established from peripheral blood of normal human volunteer donors and induced both their phenotypic and functional maturation as demonstrated by increased T cell stimulation in an allogeneic mixed leukocyte reaction and by enhanced interleukin (IL)-12p70 release.[26] To explore the potential of an adapter-mediated targeting approach to enhance the efficacy of DC-based vaccinations in vivo, Tillman et al.[29] employed a similar Fab' conjugated with mAb FGK45[30] against mouse CD40 (mCD40) along with Ad vector encoding a tumor antigen. To this end, AdE7 vector expressing the human papillomavirus type-16 (HPV-16) E7 oncogene, which represents an attractive target for antigen-specific immunity of cervical cancer, was coupled with Fab-anti-murine CD40 and then was used to load bone marrow–derived DCs

(BMDCs) ex vivo. It was shown that subcutaneous injection of BMDCs infected with CD40-targeted AdE7 provided superior protection against HPV-16–induced tumor challenge and improved prophylaxis against outgrowth of established tumors relative to BMDCs infected by untargeted Ad. This study illustrated that Ad-modified DCs may be used in repeated vaccination to establish antigen-specific and $CD8^+$ T cell–dependent protection. These findings suggested that Ad-based DC loading with tumor antigens can elicit productive antitumor immunity and that the enhancement of gene transfer and DC maturation mediated by CD40-targeted Ad complex may facilitate this process.

To further demonstrate the clinical utility of adapter-mediated DC targeting, de Gruijl et al.[31] evaluated CD40-targeted Ad vectors performance in the context of three-dimensional human tissue under physiological and clinically highly relevant conditions. To this end, a human skin explant model was used to test transduction efficiency of cutaneous DC after intradermal injection of Ad5 vector preincubated with antiknob Fab'-G28-5 conjugate.[26] Significantly enhanced transduction efficiency and selectivity and an increased activation state of migrating DC were achieved while extending antigen-specific cytotoxic T lymphocyte (CTL)-stimulatory ability for up to 1 week after the start of migration, in contrast to DC transduced by untargeted Ad. Because DC targeting in vivo might obviate the need for the in vitro culture of autologous DC for adoptive transfer, CD40-targeted Ad vectors constitute a promising new vaccine modality for tumor immunotherapy.

To determine whether an adapter-mediated Ad-targeting approach could maintain fidelity upon systemic vascular administration, Reynolds et al.[32] used a bispecific Ab conjugate to target Ad infection specifically to angiotensin-converting enzyme (ACE), which is preferentially expressed on pulmonary capillary. Administration of ACE-targeted vector complexes via tail vein injection into rats resulted in at least a 20-fold increase in both Ad genome localization and luciferase transgene expression in the lungs whereas luciferase activity in the liver was reduced by over 80% compared with the untargeted vector. This study showed that an adapter-mediated Ad targeting can indeed alter the biodistribution profile of an Ad vector given systemically, thus providing encouraging implications for the further development of targetable, injectable Ad vectors that may enable gene therapy for pulmonary vascular disease. The use of ACE-targeting adapter combined with endothelial-specific transgene expression driven by flt-1 promoter resulted in a synergistic 300,000-fold improvement in the selectivity of luciferase expression for lung versus the usual site of vector sequestration, the liver.[33]

The use of adapter-mediated Ad retargeting toward tumor cells was demonstrated using mAb against the epithelial cell adhesion molecule (EpCAM)[34] conjugated with antifiber knob Fab' fragments.[35] The EpCAM-targeted Ad vectors complexed with this bispecific Ab conjugate showed an improved transduction of primary tumor cells and cell lines established from gastric and esophageal adenocarcinoma compared with normal gastric epithelium.[36] Using a similar approach, chemical conjugation of the antiknob Fab' was achieved with basic fibroblast growth factor (FGF2)[37] in an effort to develop a new treatment approach for Kaposi sarcoma.[38] Of note, use of FGF2-targeted Ad complexes achieved direct therapeutic goals in a murine orthotopic model of human ovarian carcinoma relevant to a current human clinical cancer gene therapy scheme.[39,40]

3. Recombinant Ad Targeting Adapters

Further refinement of adapter approach was accomplished by engineering recombinant proteins consisting of a neutralizing single-chain fragment variable (scFv) Ab S11 against Ad fiber knob fused with human EGF[41] or scFv 425 against EGFR[42] to improve Ad5 infection efficiency in cancer cells. Recombinant adapter molecules such as these have advantages for Ad retargeting, because use of the chemical conjugation of Ab molecules increases the difficulties of producing Ad retargeting complexes, which makes this approach relatively complex and expensive to develop. To further improve vector targeting specificity, the use of native tropism-ablated Ad, which was previously constructed to contain both CAR- and α_V-integrin-binding mutated residues,[43,44] was tested using bispecific scFv adapters targeted toward human EGFR or EpCAM.[45] An elegant study by van Beusechem et al. demonstrated that these native tropism-ablated Ad vectors complexed with bispecific scFv efficiently and selectively targeted both alternative receptors on the surface of human cancer cell lines and primary human tumor specimens. Moreover, EGFR-targeted doubly ablated vectors were selective for human brain tumors versus the surrounding normal brain tissue, resulting in a 5- to 38-fold improved tumor-to-normal brain targeting index compared with nonablated control vectors.[45] Application of EpCAM-targeted double-ablated Ad vector for gastric cancer gene therapy showed a favorable ration of tumor over normal tissue transduction.[46] Of note, the transduction efficiency mediated by EpCAM-targeted native tropism-ablated Ad complexes reached levels similar to or exceeding those achieved with native Ad control for EpCAM-expressing primary human gastric tumors, whereas transduction of gastric epithelium and liver tissue was reduced at least 10-fold.

To achieve targeted genetic modification of hepatic stellate cells (HSCs), Reetz et al.[47] designed a peptide of the nerve growth factor (NGFp) with specific affinity for the p75 neurotrophin receptor (p75NTR) present on HSCs. Coupling of this NGFp to Ad particles was done via chemical conjugation using bifunctional polyethylene glycol (PEG) or by coating with a fusion protein composed of scFv S11 and p75NTR. Coupling of NGFp to Ad via S11 or PEGylation resulted in markedly reduced liver tropism and enhanced gene transfer to HSCs, whereas Ad GFP-S11-NGFp transduced activated HSCs better than Ad GFP-PEG-NGFp. This study contributed to the development of gene transfer system targeted to activated HSCs based on systemically applied Ad vector modified with NGFp.

These successful examples of employing bispecific adapters to achieve receptor-specific Ad gene transfer rationalized further development of the recombinant adapter molecule design. In this regard, Dmitriev et al. proposed using the soluble extracellular CAR domain (sCAR) fused to human EGF as a targeting ligand to engineer a novel class of adapters capable of blocking CAR-dependent Ad tropism while promoting infection of CAR-deficient cell types overexpressing EGFR including human mammary gland, ovarian, epidermoid, squamous, and pancreatic carcinoma cells.[48,49] A similar approach was applied to engineer sCAR ectodomain fused to the Fc region of the human immunoglobulin G1 protein to

target Ad vector via high-affinity Fc_{γ} receptor I while achieving up to a 250-fold increase in transgene expression in CAR-negative human monocytic cell lines expressing the target receptor (CD64).[50] Using noninvasive optical imaging to monitor firefly luciferase (luc) luciferin-dependent bioluminescent activity, Liang et al. showed that systemic vascular administration of Ad5-luc vector coated with the newly generated sCAR-EGF protein resulted in significantly reduced ectopic luc expression in the liver and markedly facilitated luc expression in tumor xenografts displaying elevated EGFR levels compared with sCAR-6His-coated Ad5-luc control.[51] This demonstration of both liver untargeting and tumor retargeting of Ad vector mediated by bispecific recombinant adapter suggested that sCAR-EGF–coated virions could maintain fidelity after systemic delivery, thus providing encouraging implications for the development of targetable, injectable Ad vector systems that may enable gene therapy for cancer. To assess the use of an adapter approach for Ad targeting to colon, lung, and breast epithelial tumors that express carcinoembryonic antigen (CEA), Li et al. used noninvasive optical imaging of bioluminescent luc activity provided by Ad complexed with a bispecific sCAR-MFE protein containing an scFv MFE-23 against CEA.[52] The use of sCAR-MFE adapter resulted in Ad vector retargeting to CEA-positive epithelial tumor cells in cell culture, subcutaneous tumor xenografts, and hepatic tumor grafts while showing greater than 90% reduction of Ad-directed luc expression in the liver after systemic vector administration.

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. These considerations warranted further development of the adapter-mediated Ad targeting approach to improve its potency in the context of systemic applications. One development endeavor was to design bispecific recombinant molecules that have higher binding affinity to viral capsid to maintain fidelity of virus–adapter complexes subsequent to systemic delivery. In this regard, both structural analysis of fiber knob bound to CAR D1 domain[53] and identification of a conserved CAR-binding site on the fiber protein[43] suggested an avidity mechanism when three CAR molecules could simultaneously bind per one fiber knob trimer, which was supported by kinetic analysis of Ad2 knob binding to the CAR D1 domain.[54] Based on these considerations, it was hypothesized that trimeric sCAR-ligand molecules could achieve high-affinity linkage to fiber knob and promote ligand-mediated binding to target receptors.

To test this hypothesis, Kashentseva et al. engineered the sCARfC6.5 adapter protein consisting of sCAR, phage T4 fibritin-derived polypeptide, and C6.5 scFv against c-erbB-2 oncoprotein to confer Ad targeting capability on cancer cells expressing the c-erbB-2/HER-2/neu oncogene.[55] It was demonstrated that incorporation of fibritin polypeptide provided trimerization of sCAR fusion proteins that resulted in increased affinity to Ad fiber knob and augmented the ability to block CAR-dependent Ad infection, compared with monomeric sCAR protein. As illustrated in cancer cell lines that overexpress c-erbB-2, targeted Ad, complexed with sCARfC6.5 adapter protein, provided 1.5- to 17-fold enhancement of gene transfer compared with Ad alone and up to

130-fold increase compared with untargeted Ad complexed with sCARfibritin control protein. In a parallel study, Kim et al. employed an isoleucine GCN4 trimerization domain to improve sCAR binding to fiber knob[56] while engineering recombinant adapters containing a cyclic RGD peptide (cRGD) or the receptor-binding domain of apolipoprotein E to achieve efficient gene transfer in human diploid fibroblasts in vitro. Whereas the trimerized sCAR devoid of targeting ligand provided efficient blocking of ectopic liver gene transfer in normal C57BL/6 mice, addition of either ligand failed to retarget the liver in vivo. To apply gene therapy treatment for hepatic colorectal cancer (CRC) metastatic tumors, which often express both cyclooxygenase-2 (COX-2) and CEA, Li et al. coupled the use of COX-2 promoter for transcriptional control with transductional targeting mediated by a trimerized sCARfMFE adapter containing anti-CEA scFv.[57] This study demonstrated that the use of both transcriptional control and sCARfMFE adapter allowed retargeting of Ad-mediated expression of the herpes simplex virus type 1 thymidine kinase (HSV1-tk) therapeutic gene from normal liver tissue to hepatic CRC tumors after systemic virus injection, which increased the therapeutic efficacy of ganciclovir treatment for hepatic CRC tumors while reducing its hepatic toxicity. These results indicate that trimerized sCAR-ligand proteins can markedly improve Ad targeting potency in vivo owing to its high-affinity binding to fiber knob, which efficiently blocks CAR-dependent viral tropism while conferring a novel cell-binding specificity mediated by trimeric ligand moiety via an alternative tumor-associated receptor.

The sCAR-derived adapters have also been exploited to confer Ad targeting abilities toward dendritic cells (DCs) to orchestrate immune responses in an effort to develop vaccines and potent anticancer immunotherapy. The current procedure of ex vivo loading of autologous DCs with tumor-associated antigen (TAA) and their activation for clinical application is laborious and expensive, and remains poorly standardized. The use of viral vectors represents an attractive alternative approach to loading resident DCs in vivo by targeted TAA delivery and simultaneous activation. The feasibility of sCAR-mediated Ad targeting to DCs was demonstrated by Pereboev et al., by generating sCAR fusion with scFv against human CD40, which was derived using the G28-5 hybridoma cell line[27] and demonstrating highly efficient transduction of immature MoDCs.[58] Using this sCAR-G28 adapter, Asiedu et al.[59] showed that improved transduction of mature rhesus monkey MoDCs with Ad expressing transforming growth factor (TGF)-β1 could significantly suppress alloimmune responses and inhibit proliferation of CD4 and CD8 responder T cells. These results and work by Clement et al.[60] illustrated that adapter-mediated Ad targeting can promote TGF-β1 gene expression in nonhuman primate mature MoDCs to function as alloantigen-specific cellular immunosuppressants, an approach that has the potential to facilitate induction of allograft tolerance in vivo. The study by Brando et al.[61] showed that the bispecific scFv S11-G28 adapter can serve as well to significantly enhance Ad transduction efficiency of human MoDCs while increasing the ability of MoDC to activate CTL in an antigen-specific manner.

Further development of the CD40-targeting approach was achieved using the adapter molecule, CFm40L, which was designed by fusing ectodomains of CAR and

mCD40 ligand (mCD40L) via a trimerization motif.[62] Incorporation of the trimerization motif served to increase fiber knob binding avidity[55] while maintaining the native trimeric CD40L conformation necessary for efficient mCD40 binding and function,[28] which is compatible with its human counterpart owing to the high degree of homology between mouse and human tumor necrosis factor–like CD40L domains.[63] Pereboev et al. showed that gene transfer to mouse BMDC using CFm40L-targeted Ad was over four orders of magnitude more efficient than that for the untargeted Ad5 control, resulting in transduction of 70% of the BMDC compared with undetectable transduction using Ad5 control. Most important, CD40-targeted Ad induced in vivo phenotypical DC maturation, upregulated IL-12 expression, and elicited superior Th and CTL responses against the β-galactosidase model antigen in Balb/c mice. Results of this study demonstrated that Ad-mediated gene transfer to DC can be significantly enhanced using nonnative transduction pathways such as the CD40 pathway, which may have important applications in genetic vaccination for cancer and infectious diseases. To study the effects of adapter-mediated Ad targeting via the CD40 pathway in vivo, Huang et al.[64] compared biodistribution and immune responses after intravenous (i.v.), intradermal (i.d.), and intranasal (i.n.) administration of CFm40L-coated Ad5 and untargeted Ad5 control in Balb/c mice. The CD40-targeted Ad5 injected i.v. revealed increased transgene expression in the lung and thymus, which normally do not sequester significant amounts of virus after systemic administration. After i.d. injection, CD40-targeted Ad showed about 300-fold lower gene transfer signals detected mainly in local draining lymph nodes and skin compared with control Ad5. Of note, undesirable ectopic sequestration of untargeted Ad5, which was detected in brain tissue that showed the second highest gene expression level after the lung, was largely ablated using CD40-targeted Ad complexes. Moreover, CD40 targeting elicited more sustained antigen-specific cellular immune responses (up to 17-fold) against nucleocapsid protein of SARS-CoV, which was used as a model antigen, at later time points (30 days after boosting) after i.d. and i.n. application, but also significantly hampered humoral responses irrespective of the administration route. This study demonstrated that CFm40L adapter-mediated Ad targeting can profoundly alter the patterns of virus biodistribution and immune responses against the transgene after local and systemic administration.

Preclinical evidence of therapeutic use of an adapter-mediated DC targeting approach for cancer immunotherapy was obtained by Hangalapura et al.[65] using Ad encoding the full-length melanoma antigen recognized by T cell-1 (MART-1) coupled with the CFm40L adapter.[62] It was demonstrated that this CD40-targeted Ad-MART-1vector enhanced transduction of conventional and plasmacytoid DC subsets, but not B cells, in suspensions of human melanoma-draining sentinel lymph nodes ex vivo resulting in reduction of regulatory T cell (Tregs) frequencies while facilitating expansion of functional MART-1–specific $CD8^+$ T cells. Further study by Hangalapura et al.[66] demonstrated enhanced transduction and maturation of cultured BMDCs with CFm40L-coupled Ad-GFP-$TRP2_{aa180–188}$ vector encoding the immunodominant H-2Kb–binding epitope of tyrosinase-related protein 2 (TRP2) fused to eGFP compared with untargeted control. The BMDCs transduced with DC-targeted vector ex vivo induced stronger $TRP2_{aa180–188}$–specific $CD8^+$ T-cell responses in

peripheral blood while resulting in improved prophylactic vaccination efficacy in the aggressive and poorly immunogenic murine B16F10 melanoma model.[67] To assess the effect of CD40 targeting on the induction of immunity against weakly immunogenic TAAs, CFm40L-coupled Adgp100 vector encoding a full-length human gp100[68] was employed for i.d. vaccination. These studies revealed that CD40-targeted Adgp100 significantly enhanced the induction of a $gp100_{25–33}$-specific $CD8^+$ T cell response and antitumor efficacy in both prophylactic and therapeutic vaccination settings, which translated into an improved survival of tumor-bearing animals receiving a CFm40L-Adgp100 vaccine. These results thus clearly showed enhanced antitumor efficacy afforded by the CFm40L-mediated in vivo targeting of Ad5-based vaccines encoding weakly immunogenic TAAs to DCs. Taken together, these studies support the use of CFm40L-coupled Ad vectors for in vivo DC targeting to accomplish high-efficacy CTL priming while breaking immune tolerance against TAAs to achieve therapeutic anticancer efficacy in preclinical and clinical studies.

To test whether the CD40-targeting strategy can improve the outcomes of prostate cancer immunotherapy, Williams et al.[69] developed a murine model of prostate cancer by generating derivatives of the mouse RM-1 prostate cancer cell line expressing human prostate–specific membrane antigen (PSMA).[70] To maximize antigen presentation in target cells, both major histocompatibility complex class I and transporter associated with antigen processing protein expression was induced in RM-1 cells by transduction with Ad5-IFN-gamma vector expressing interferon-gamma.[71] Administering DCs infected ex vivo using CD40-targeted Ad5-huPSMA coupled with CFm40L adapter, as well as direct intraperitoneal injection of the vector–adapter complexes, resulted in high levels of tumor-specific CTL responses against RM-1-PSMA cells pretreated with Ad5-IFN-gamma, thus significantly improving the therapeutic antitumor efficacy. These data suggested that DC-targeted Ad delivery of PSMA mediated by CFm40L adapter may be effective clinically for prostate cancer immunotherapy.

The adapter approach was explored to improve Ad vector utility for T lymphocyte–based therapies.[72] To surmount T lymphocyte resistance to Ad infection, Beatty et al.[73] proposed designing sCAR ectodomain fusion with murine interleukin 2 (sCAR-mIL-2) that targets Ad to the murine IL-2 receptor (IL-2R). Interleukin-2R is T lymphocyte specific and highly expressed in therapeutic T lymphocyte populations such as $CD4^+Foxp3^+$ regulatory T lymphocytes and activated $CD4^+$ and $CD8^+$ T lymphocytes.[74] This study showed the use of Ad5 vector coupled with an sCAR-mIL-2 adapter to infect a murine T-cell line, CTLL-2, and activated primary murine T lymphocytes allowed a nine- and fourfold improvement in reporter gene expression levels compared with Ad5 vector alone, respectively. These findings have broad application for the study of T cell biology and genetic modification of T cells for therapeutic use.

The technologies of designed ankyrin repeat proteins (DARPins) and ribosome display were employed to develop a DARPin that binds the Ad5 fiber knob domain with low nanomolar affinity. In particular, Dreier et al.[75] reported a novel design of bispecific adapter protein that chelated the knob in a bivalent or trivalent fashion while providing binding specificity for HER-2, an established cell-surface biomarker of human cancers. This study showed that the efficacy of gene transfer by the adapter–Ad complex increased accordingly with the functional affinity of these molecules, enabling efficient

virus transduction at low stoichiometric adapter-to-fiber ratios. In principle, DARPins can be generated against any target, which makes this versatile adapter approach useful for developing a broad range of disease-specific Ad vector applications. The most recent refinement of DARPin technology allowed the development of a series of adapters that bind the Ad5 fiber knob with such high affinity that they remain fully bound for more than 10 days while blocking Ad native receptor tropism and mediating interaction with a surface receptor of choice.[76] By solving the crystal structure of the complex of the trimeric knob with three bound DARPins at 1.95-A resolution, Dreier et al. used computer modeling to devise a trimeric protein of extraordinary kinetic stability. Specifically, the capsid protein SHP from the lambdoid phage 21 served to bind the knob like a trimeric clamp fused with DARPins of varying specificities, thus allowing Ad5-mediated gene transfer in a HER-2-, EGFR-, or EpCAM-dependent manner with transduction efficiencies comparable to or even exceeding those of Ad5 alone. With these adapters, efficiently produced in *Escherichia coli*, Ad can be conferred new receptor specificities using receptor-binding ligands available for many cell types of choice, which suggests the means to engineer practical and effective Ad targeting approaches.

3.1 Combination of Genetic Capsid Modification and Adapter-Mediated Ad Targeting

To achieve a strong association between viral particles and adapter proteins, several groups proposed combining genetic capsid modification with a targeting adapter approach. To this end, the Ad5 fiber capsid protein was genetically fused to the C-terminal biotin acceptor peptide (BAP).[77] Adenovirus 5 particles bearing this BAP were metabolically biotinylated during vector production by the endogenous biotin ligase in 293 cells to produce covalently biotinylated virions. The resulting biotinylated vector could be retargeted to new receptors by conjugation to biotinylated antibodies using tetrameric avidin ($K_d = 10^{-15}$ M). Campos et al.[78] used a panel of metabolically biotinylated Ad vectors to directly compare targeted transduction mediated through the fiber, protein IX, and hexon capsid proteins using a variety of biotinylated ligands including mAb, transferrin, EGF, and cholera toxin B. This study clearly demonstrated that effective cell targeting could be achieved only when biotinylated fiber protein served for receptor-binding ligand conjugation. In contrast, protein IX and hexon-mediated ligand conjugation with the same ligands failed to provide vector targeting, likely because of aberrant trafficking at the cell surface or inside targeted cells. These data suggested that Ad targeting will likely be the most efficient through fiber modification rather than pIX or hexon protein. Using Ad5 vector containing metabolically biotinylated fiber proteins, Chen et al.[79] showed retargeting to primary cultured human corneal epithelial cells, which was mediated by conjugation with biotinylated EGF, providing up to ninefold increased transduction of EGFR-expressing corneal epithelial progenitor cells while reducing transduction of differentiated corneal epithelial cells. A biotin–avidin linkage was also used to conjugate Ad vectors to ligands that bind with high affinity to ChemR23, $\alpha_v\beta_3$-integrins, and DC-SIGN receptors[80] to improve the efficacy of human MoDCs transduction, maturation, and ability to stimulate cytokine production by autologous memory $CD8^+$ T cells against

the vector-encoded immunodominant human cytomegalovirus pp65 protein compared with untargeted virus. This study expanded the range of receptors that could be employed for DC targeting to facilitate the development of Ad-based vaccines.

An alternative targeting strategy was proposed to combine genetic incorporation of the immunoglobulin (Ig) binding domain of *Staphylococcus aureus* protein A into the Ad fiber protein with targeting ligands fused to the Ig Fc domain to form vector-ligand targeting complexes.[81–83] Korokhov et al.[82] showed that targeting ligands containing the Fc domain and either an anti-CD40 scFv or CD40L form stable complexes with Ad vector incorporating the so-called Cd of *S. aureus* protein A, which resulted in significant augmentation of gene delivery to MoDCs target cells. Using a similar approach of genetic fiber modification to insert a synthetic 33–amino acid IgG-binding domain (Z33) derived from protein A, Volpers et al.[81] demonstrated up to a 77-fold increased gene transfer efficacy in differentiated primary human muscle cells, which was achieved by preincubation of the AdFZ33 vector with mAb directed against neuronal cell adhesion molecule or α_7-integrin. This versatile Ad targeting strategy was employed by Kawashima et al.[84] to demonstrate highly efficient gene transfer in biliary cancer cells using AdFZ33 vector combined with mAb against EpCAM or EGFR compared with the control antibody or without antibody. This study showed that AdFZ33 vector, which was constructed to express uracil phosphoribosyl transferase, complexed with anti-EpCAM or anti-EGFR mAb, remarkably enhanced the sensitivity of biliary cancer cells to 5-fluorouracil but not cells lacking EpCAM or EGFR expression including normal hepatocytes and thus resulting in significantly suppressed growth of biliary cancer xenografts in nude mice. Employment of this versatile IgG-binding Ad vector approach holds promise to solve the problem of structural and biosynthetic compatibility between viral capsid proteins and targeting ligands by allowing direct use of the available repertoire of mAb against cell surface antigens for Ad targeting to a variety of cellular receptors.

Use of the bispecific adapter approach has established several key concepts with respect to the goal of Ad vector retargeting. (1) It was clearly shown that Ad5-based vectors can provide effective gene transfer via CAR-independent cell entry pathways. Thus, virus interaction with its primary attachment receptor does not appear to be essential to attain the effective cell entry. (2) Achievement of CAR-independent infection via alternative cellular receptors allows augmented levels of gene transfer. Indeed, redirecting Ad5 infection via nonviral receptors allows improving the susceptibility of target cells in vitro and in vivo. (3) The targeting use of adapter molecules depends on interaction with viral capsomers. In this regard, the targeting ability of bispecific molecules appears to be the most efficient through Ad fiber interaction rather than pIX or hexon protein.

4. Adenovirus Targeting Using Genetic Modification of Capsid Proteins

As discussed, molecular adapters have allowed modification of Ad tropism and key proof-of-principle demonstrations of targeted gene transfer in both in vitro and in vivo delivery contexts. However, the genetic capsid modification approach is the preferred

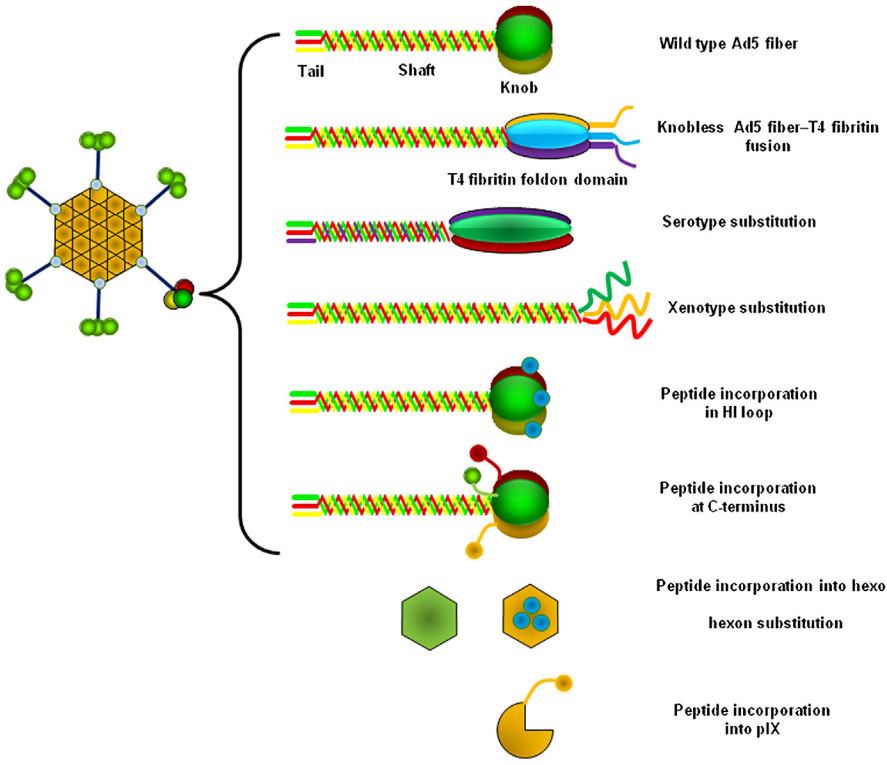

Figure 2 Schematic outline of Ad capsid modifications using genetic incorporation of heterologous ligands. Adenovirus targeting strategies employing manipulation of the Ad genome included peptide incorporation to the HI loop and C-terminus of the knob domain, serotype chimerism, fiber/knob replacement, and combinatorial approaches. Chimeric Ads composed of fiber/knob domains from alternative serotypes, fiber-xenotyped Ad vectors displayed fibers from nonhuman Ad species, and knobless Ad fiber–fibritin fusion. Genetic incorporation of peptides into the HVR of hexon and C-terminus of the pIX.

configuration for clinical applications of targeted Ad vectors. Methods to alter Ad vector tropism have capitalized on the knowledge that viral capsid proteins including fiber, hexon, penton base, and pIX are the key determinants of specificity of Ad infection. On the basis of these considerations, several approaches have been developed to alter Ad5 tropism using genetic capsid modifications (Figure 2).

5. Employment of Chimeric and Mosaic Fibers

In the first instance, the method of Ad retargeting is based on use of Ad capsid pseudotyping using fiber substitution with fiber from different serotypes. These chimeric fibers are primarily derived from viruses that employ different receptors for cell binding including CD46,[85] CD80/CD86,[86] and desmoglein-2.[87,88] In early studies, the

use of a method to construct an Ad5/3 vector containing chimeric fibers composed of the tail and shaft domains of Ad5 and the knob domain of Ad serotype 3 (Ad3) was established.[89] More recent studies demonstrated high transduction efficacy of Ad5/3-based vectors in a variety of Ad5-refractory tumor cell types with low CAR expression including renal cell carcinoma,[90] ovarian cancer,[91] melanoma,[92] and prostate cancer cells.[93] Several fiber chimeric Ad vectors have been developed to achieve tumor- or tissue-specific gene delivery by employing fibers derived from Ad35, Ad40, or Ad19p.[94–98] Over the past decade, additional approaches to Ad retargeting were developed using fibers from nonhuman Ad species. A number of fiber-xenotyped Ad5 vectors were developed based on chimeric fibers with knob domains from aviadenovirus or atadenovirus,[99] canine Ad serotype 2 (CAV-2),[100] canine Ad serotype 1 (CAV-1),[101] and porcine Ad serotype 3 and 4 (PAd3 and PAdV-4).[102,103] In addition to fibers from nonhuman Ad vectors, a fiber-mosaic Ad5 vector encoding two different fibers including the wild-type Ad5 and the receptor-binding molecule of Dearing (T3D) reovirus serotype 3 was constructed. Use of fiber-like σ1 attachment protein provided enhanced infectivity in tissues with low CAR expression and tropism expansion via infection of cells expressing sialic acid and junction adhesion molecule 1.[104]

Ad targeting must embody the concept that tumors are complex tissues that are composed of many interdependent cellular components, including malignant cells, cancer stem (or stem-like) cells, and tumor-associated stromal elements. In this regard, a fiber-mosaic strategy of capsid modification in which single viral particles can incorporate two distinct fiber species was evaluated using mosaic Ad5 vectors with fibers derived from wild-type Ad5 fiber and FF6H protein consisting of the amino-terminal segment of Ad5 fiber sequence genetically fused with the carboxy-terminal portion of the phage T4 fibritin protein, followed by the linker and the 6-His ligand.[105] In another study, Murakami et al. generated a fiber-mosaic Ad vector displaying both Ad5 fiber and a chimeric fiber protein composed of the Ad5 tail domain and the Ad3 shaft and knob domains. The capacity of the dual-fiber Ad vector to transduce distinct cell types in a mixed cell population was demonstrated in vitro. This fiber profile allows the expanded tropism required for an inclusion targeting strategy, which is based on the use of fiber-mosaic viral particles that can infect cells efficiently with a distinctive receptor's repertoire.[106] More recent studies have demonstrated that employment of fibers derived from both Ad5 and Ad3 increased oncolytic potency of CRAd. An experimental therapy study using a human pancreatic tumor xenograft model demonstrated that employment of complex mosaicism increased efficacy of the combination of oncolytic virotherapy with chemotherapy.[107]

6. Employment of Targeting Peptides in Fiber Modification

Several strategies have been developed to alter tropism of Ad5-based vectors to achieve a cell-specific gene delivery by employing fiber modifications using genetic incorporation of targeting motifs. Generally, retargeting strategies have focused on

Ad fiber modifications, because it is the major determinant of Ad tropism. It was shown that insertion of an integrin-binding RGD motif or polylysine peptides into the C-terminus of the fiber knob significantly reduced the transduction efficiency of CAR-positive cells by Ad vectors.[108] In early studies, the use of an exposed HI-loop structure connecting β-sheets H and I in the Ad knob domain as an alternate location for the cysteine-constrained RGD-4C (CDCRGDCFC) peptide insertion was demonstrated by Dmitriev et al.[109] Based on evidence that RGD and polylysine (pK7) motifs bind to different cell surface proteins, cellular integrins, and heparan sulfate–containing receptors, respectively, double-modified Ad fiber knob with RGD and pK7 motifs have been shown to enhance Ad5 infection via CAR-independent pathways and improved gene transfer efficiency.[110] Promising data have been demonstrated in studies in which the phage display technique was used to determine specific binding peptides. The display of polypeptide repertoires on the surface of filamentous phages as well as peptide incorporated Ad libraries was shown to be a valuable method for isolating unique peptides that can be employed for Ad targeting. In vivo selection of phage display libraries and Ad libraries displaying random peptides on the fiber knob techniques were successfully employed to acquire a number tumor-homing peptides with a targeting specificity related to angiogenic blood vessels (CDCRGDCFC and CNGRCVSGCAGRC),[111] tumor lymphatic vessels (CGNKRTRGC),[112] pancreatic cancer (SYENFSA),[113] and renal cell carcinoma (HITSLLS).[98] Phage display technology was used to isolate an HVGGSSV peptide that binds specifically to tax-interacting protein-1 receptor in irradiated tumors.[114] Although these modifications allowed for tropism alterations, in many instances the application of this approach has been limited by incompatibility between the capsid and ligand. In addition, targeting motifs fused to the fiber protein demonstrated decreased binding functionality or an impaired proper protein tertiary structure.[115]

The means of transductional retargeting of the Ad was accomplished by fiber modification approaches allowing incorporation of large and complex targeting moieties and retaining trimerization of the fiber. These modifications are based on the concept of chimeric knobless fiber by replacing the native Ad fiber knob protein with an alternative protein capable of providing trimerization functions and allowing the incorporation of targeting peptides.[116] Initial studies involved generating knobless Ad vectors with trimerization motifs derived from Moloney murine leukemia virus,[117] bacteriophage T4 fibritin,[118] or trimerization motifs derived from reoviral σ1 protein[119] were introduced in place of knob domain followed by the C-terminal Myc-epitope or 6His-tag. All of these fiber-modified vectors were shown to mediate receptor-specific transduction in vitro through interaction with surface-expressed Abs.

Whereas a wide range of targeting moieties have been employed for recombinant Ad vectors, the restricted repertoire of available targeting peptides that are functionally compatible with insertion in the fiber protein has led to the consideration of various Ab species for Ad retargeting purposes. Furthermore, the biosynthesis of candidate Ab species designed for Ad incorporation must be compatible with Ad capsid protein synthesis and assembly. To this point, available Ab species have not proved to be biologically compatible with cytosolic Ad capsid synthesis and assembly, resulting in loss of binding affinities. This loss of binding specificity, in the instance of incorporated

scFv, is likely because Ad capsid proteins are normally synthesized in the cytosol with assembly in the nucleus, whereas scFv molecules are typically routed through the rough endoplasmic reticulum.[120] In this context, the redox state of the cytosol likely results in improper scFv folding that perturbs the structural configuration required for antigen recognition, leading to loss of binding specificity. Despite the demonstrated utility of stabilized scFv with molecular scaffold motifs designed to resist the deleterious effect of the cytosol redox state for Ad retargeting,[116,121] the limited available repertoire of target specificities of this class of scFv practically restricts this approach.

Recent studies validated the use of fiber-based targeting moieties using synthetically constructed monobodies representing single-domain Ab mimics based on the tenth human fibronectin type III domain (10Fn3) scaffold to achieve selectivity of gene transfer using tropism-modified Ad.[122] In contrast to these synthetically constructed monobodies, Kaliberov et al. considered the use of alternate antibody species that might embody a stability profile compatible with the cytosolic biosynthesis of Ad capsid proteins.[123] The discovery of unconventional immunoglobulins derived from the serum of animals in the camelid family (camels and alpacas) that consist of only the two heavy-chains (hcAbs) as the basis of antigen recognition and binding has made possible their use for Ad-mediated gene therapy.[124] Unlike conventional immunoglobulins, hcAbs contain a single variable domain (VHH) linked to two constant domains.[125] Cloned and isolated single-domain antibodies have shown effective targeting in model systems and a remarkable stability profile compared with conventional immunoglobulins and scFvs.[126,127] It was shown that expression of anti-CEA VHH genetically incorporated into a deknobbed Ad5 fiber-fibritin protein did not disrupt the trimerization capability of the Ad fiber and retain antigen recognition functionality. The ability of an anti-CEA VHH fused to fiber-fibritin chimera to provide specific and efficient targeting of the CEA-expressing cancer cells for Ad-mediated gene transfer was also demonstrated.[123]

7. Employment of Alternative Capsid Sites for Ligand Incorporation

Despite the demonstrated use of fiber modification for Ad retargeting, this approach has been limited by incompatibility between the fiber protein and ligand that leads to impaired antigen recognition functionality. Another approach has focused on the development of retargeting Ad using other capsid proteins besides fiber. In early studies, small peptides were incorporated into Ad capsid proteins, such as peptide epitope from the hemagglutinin protein of influenza virus within a penton base.[128]

Evidence shows that the hexon is the most abundant Ad capsid protein, which makes the hexon an attractive site for the presentation of targeting moieties. The tendency of i.v. administered Ad5 to localize in the liver represents a major factor limiting current strategies to accomplish targeting of Ad vector. The major pathway of liver transduction involves interactions of Ad capsid proteins with circulating blood cells and with plasma proteins including several components of complement pathway and

blood coagulation zymogens.[129,130] Although not universally accepted, liver uptake of Ad is mediated by high-affinity interaction between the major protein in the Ad5 capsid, hexon, and γ-carboxylated glutamic acid domain of coagulation factor X (FX). The Ad5–FX complex attaches to hepatocytes through binding of the serine protease domain of FX to cell surface heparan sulfate proteoglycans.[131–133] It was shown that different Ad serotypes interact with FX with distinct affinities. For instance, human Ad serotypes 26, 35, and 48 bind to FX with relatively low affinity compared with Ad5.[131,134–136] More recently, it was shown that ablation of FX binding to Ad5 with modified hexon protein resulted in decreased liver tropism.[130,137,138]

Comparisons of the hexon sequence among different Ad serotypes revealed several unique serotype-specific sequences: hypervariable regions (HVR1–9) at loops 1 and 2, which are exposed on the exterior surface of the hexon molecule. Incorporation of an αv-specific DCRGDCF ligand in the HVR5 of hexon resulted in enhanced transduction of cells with low levels of CAR expression.[139] In another study, the 6-His epitope was incorporated in HVR2 and HVR5.[140] It was shown that HVRs 2, 3, 5, 6, and 7 are amenable to insertion of a 6-His motif. In addition, anti–6-His Ab recognized Ad vectors with 6-His inserted into HVRs 2 and 5.[110] A subsequent study demonstrated that HVR5 of hexon was capable to accommodate a peptide up to 36 amino acids (aa) in length[141] as well as the 71-aa BAP protein[142] with minimal adverse effects on virion stability. It was later shown that substitution of HVR7 of the Ad5 hexon with HVR7 from Ad3 resulted in decreased liver tropism and dramatically altered biodistribution of gene expression. Systemic administration of AdH5/H3CMVLuc, AdH5RoboLuc, and AdH5/H3RoboLuc in C57BL6J mice produced Luc expression in the liver that was 59- and 431- and over 240,000-fold, respectively, lower than wild-type AdH5CMVLuc. The results of these studies suggest that the combination of liver detargeting using a genetic modification of hexon with an endothelium-specific transcriptional control element produces an additive effect in the improvement of Ad5 biodistribution.[143]

The minor capsid protein IX (pIX), which is present in 240 copies in the Ad capsid, was exploited as an anchor for heterologous C-terminal extensions of up to 113 aa in length, which included 75 Å α-helical spacers between pIX protein and peptide ligands. The MYC-tagged-pIX molecules were readily accessible to anti-MYC Ab.[144] In early studies, use of pIX for genetic incorporation of targeting ligands was established by Dmitriev et al.[145] In this study, Ad vectors containing modified pIX carrying a C-terminal Flag epitope along with a heparan sulfate binding motif consisting of either eight consecutive lysines or a polylysine sequence were constructed. The pIX variants were efficiently incorporated into the capsid of Ad particles. Using an anti-Flag Ab, it was shown that modified pIXs are incorporated into virions and display Flag-containing C-terminal sequences on the capsid surface. The incorporation of a polylysine motif into the pIX ectodomain resulted in significant augmentation of Ad fiber knob-independent infection of CAR-deficient cell types.[145] Using this strategy, Ad retargeting was achieved by incorporating large targeting moieties, including eGFP,[146] HSV1-tk,[147] and metallothionein.[148,149]

The use of pIX protein as a platform for presenting scFv or sdAb molecules for Ad retargeting was evaluated. The 13R4 scFv directed against β-galactosidase, which

was selected for its capacity to fold correctly in a reducing environment such as the cytoplasm, was fused with pIX using a 75-Å-spacer sequence.[121] In another study, a single-chain T cell receptor directed against cancer/testis antigen melanoma-associated antigen (MAGE)-A1 in complex with the human leukocyte antigen (HLA) class I molecule of haplotype HLA-A1 was fused with the C terminus of the pIX. Generated particles specifically transduced melanoma cells expressing the HLA-A1/MAGE-A1 target complex with at least 10-fold higher efficiency than control viruses.[150] However, because of the nature of the Ad capsid proteins synthesis and virion assembly, even the endoplasmic reticulum–targeted pIX-scFv proteins were incorporated into the Ad capsid at a low level that was not sufficient to retarget virus infection. In contrast, it was shown that expression of anti-EGFRvIII sdAb on the Ad capsid through fusion to pIX can be used to redirect Ad infection.[120]

8. Conclusion

It is widely acknowledged that improving the therapeutic potential of Ad vectors requires elimination of the natural viral tropism and introduction of a novel mechanism of selective cell recognition that would allow directed virus localization to the target tissue. The strategies described above including the use of bispecific adapter molecules and the genetic incorporation of targeting ligands into capsid proteins were extensively developed to redirect Ad5 infection via nonnative pathways. Targeted Ad vectors hold the promise to expand the types of diseases that can be treated by gene therapy and to make the therapeutic applications of Ad vectors more effective. The increased specificity achieved by targeting virus infection to cells of interest will ultimately allow lower and safer doses of Ad vectors to be provided when regional or systemic delivery is contemplated in the future.

The nature of the virus–host interactions that dictate the fate of systemically administered Ad vectors has come under considerable scrutiny in recent years. Recent studies focused on the biology of interactions between Ad capsid components and host blood factors and their influence on systemic virus biodistribution revealed the ability of the vitamin K–dependent coagulation factors VII, IX, X, and protein C to bind trimeric hexon in the viral capsid and facilitate CAR-independent infection of hepatic cells after intravascular Ad5 vector administration. These efforts serve to highlight the complexity of virus–host interplay in the artificial blood-borne environment and have identified modifications of the fiber and hexon proteins that significantly decrease infection and virus-induced toxicity in the liver. Thus, it is recognized that the infection pathway of systemically administered Ad5 is mediated via multiple mechanisms involving blood factors rather than direct virus interaction with cellular receptors. On this basis, it becomes increasingly apparent that engineering of capsid proteins to overcome ectopic sequestration in the liver coupled with virus retargeting via a nonnative infection pathway represents a rational strategy to direct Ad vector localization to the tissue of interest subsequent to systemic vascular administration. In this regard, genetic engineering of the Ad fiber protein appears the most straightforward way to generate targeted Ad vectors with novel tropism.

Despite major advancements illustrating the potential of genetic Ad targeting in vitro, efforts to employ high-affinity ligands including growth factors and scFvs have mostly been unsuccessful, frustrating targeting of Ad vectors to many attractive cellular markers. On the basis of these deliberations, the use of alternate Ab species that might embody a stability profile compatible with the cytosolic biosynthesis of Ad capsid proteins was considered. Camelid hcAbs possess characteristics ideal for an Ad retargeting strategy: (1) cytosolic stability allowing functional incorporation into the Ad capsid and (2) compatibility with phage biopanning selection to allow target cell specificity. Based on these useful attributes, a number of targeted Ad vectors using genetic incorporation of sdAb into fiber-fibritin or pIX proteins have been developed. This finding provides an important technical approach allowing practical linkage of capsid modification of Ad vector and ligand-based strategies for targeting gene delivery.

Whereas single-component vector systems have been favored for employment in the context of human clinical trials, rigorous analysis of the pharmacodynamics and systemic stability of vector–adapter complexes could provide the rationale for clinical translation. In this respect, previous in vivo studies using various Ad5 fiber knob-binding adapters have provided compelling evidence of reduced ectopic liver transduction and receptor-specific vector delivery to target organs or tumors. The utility of adapter molecules constructed using an anti-Ad5 knob scFv or the sCAR ectodomain is obviously limited to Ad5 and other CAR-binding Ad serotypes. This provides a rationale for the development of a new class of protein adapters capable of Ad vector targeting by virtue of binding to alternative capsid epitopes. The use of such a serotype-independent targeting modality could provide the technical means for testing the ability of vectors derived from representatives of various Ad species to localize to the tissue of interest while overcoming ectopic organ sequestration.

Thus, novel Ad tropism modification maneuvers that embody the concepts of detargeting and retargeting by combining elements of genetic capsid modification and adapter-based approaches have encouraging implications for further development of advanced delivery vehicles.

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