

Chapter 18

Bispecific Antibodies and Gene Therapy

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18.1 Introduction

18.1.1 Gene Therapy

Gene therapy is the transfer of genes into patients' cells for therapeutic purposes (Fig. 18.1). Gene therapy was originally envisioned as a cure for inherited (monogenic) diseases by gene correction, i.e., by replacing or complementing the causative mutated gene with a functional copy. In recent decades, however, gene therapy has been intensively investigated for treatment of many diseases by transfer of diverse classes of therapeutic genes from various species (Table 18.1). Examples are genes encoding pathogen antigens for prevention or treatment of infectious diseases (genetic vaccination); genes encoding agonists or antagonists of vascular growth factors for treatment of cardiovascular diseases; or genes that directly or indirectly mediate tumor cell killing for cancer treatment. Gene therapy drugs consist of the therapeutic gene, which defines the mode of therapeutic action, and the gene transfer vector, which needs to facilitate appropriate stability, delivery, and expression of the therapeutic gene (Fig. 18.1). Indeed, major efforts in gene therapy research focus on vector development, since the delivery of therapeutic genes is complex and critically determines treatment efficacy. Since the 1990s a multitude of gene therapy clinical trials have been performed with thousands of patients and therapeutic efficacy was demonstrated recently. Examples are the restoration of immunity in SCID patients, restoration of some degree of vision in childhood blindness or inhibition of neurodegeneration (Kohn 2010; Roy et al. 2010; Cartier et al. 2009). However, most gene therapy approaches necessitate

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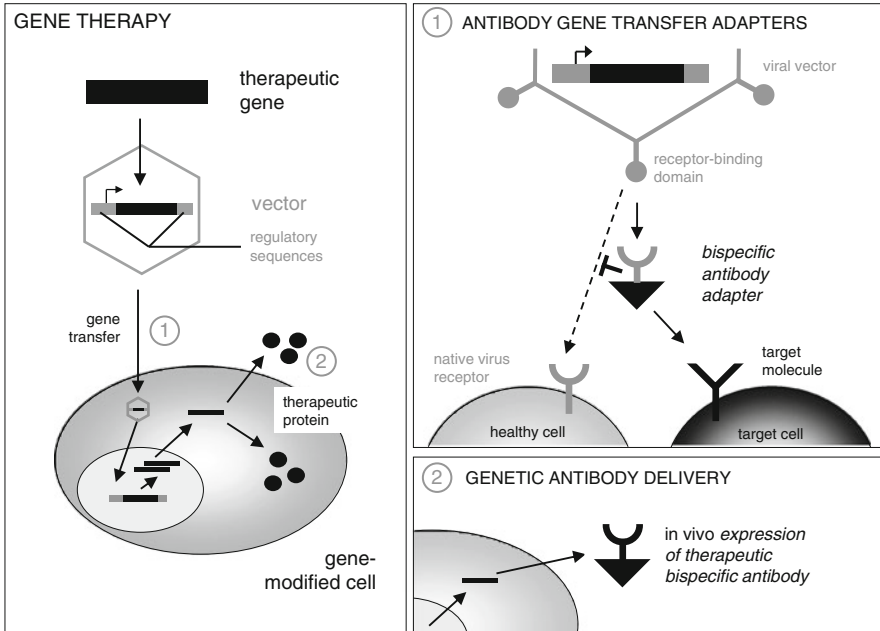


Fig. 18.1 Bispecific antibodies and gene therapy. *Left panel:* For gene therapy, a therapeutic gene is delivered into the patient's cell, where the gene product is expressed and mediates therapeutic activity. Examples are the complementation of the patient's genetic defects or the killing of cancer cells. For delivery and expression, the therapeutic gene is incorporated into a gene transfer vector containing regulatory sequences (e.g., for transcription start and termination). Frequently, the vector is also a means for efficient gene delivery into the patient's cells (e.g., replication-deficient viruses). *Right panels:* Bispecific antibodies can be either a tool for targeting gene transfer vectors to specific cell types (1) or gene transfer can be exploited as a tool for antibody therapy by antibody gene transfer and subsequent synthesis of the antibody in the patient (2)

improved efficacy or selectivity of gene transfer in order to facilitate successful applications in patients.

18.1.2 Gene Therapy Vectors

To ensure proper expression of the therapeutic gene in the patients' cells, a gene therapy vector contains a promoter and a transcription termination/polyadenylation signal (Fig. 18.1). Further regulatory elements can be exploited, for example, to achieve enhanced (introns) or bicistronic (internal ribosome entry sites, IRES) gene expression. Importantly, regulatory elements can be exploited for spatial or temporal control of gene expression. Examples are inducible or cell type-specific promoters or sequences differentially regulating mRNA stability or translation efficiency (Goverdhanan et al. 2005; Dorer and Nettelbeck 2009; Brown and Naldini 2009). To improve stability of the therapeutic DNA, these eukaryotic expression

Table 18.1 Therapeutic genes used in gene therapy

Therapeutic gene	Disease	Activity	References
Correct copy of mutated gene encoding	Monogenetic disease	Gene correction	Kohn (2010), Roy et al. (2010), Margaritis and High (2010), Cartier et al. (2009)
– Cytokine receptor	– Immunodeficiency		
– Retinal pigment epithelium protein	– Childhood blindness		
– Blood coagulation factor	– Hemophilia		
– Transporter <i>ABCD1</i>	– Neurodegenerative disease		
Vascular growth factor gene; soluble vascular growth factor receptor gene	Vascular diseases, cancer	Angiogenesis, anti-angiogenesis	Karvinen and Yla-Herttuala (2010), Brandwijk et al. (2007)
Cytokine gene, co-stimulatory genes, gene encoding T cell receptor	Infectious diseases, cancer	Immunostimulation	Liu et al. (2010), Dotti et al. (2009)
Antibody-encoding gene	Infectious diseases, cancer	Antibody-dependent immune activation, neutralization of pathogens, receptor agonism or antagonism	Watanabe et al. (2010)
Gene encoding antigen	– Infectious diseases	Genetic vaccination	Smits et al. (2009), Rice et al. (2008)
– Pathogen-derived	– Cancer		
– Tumor-associated			
Pro-apoptotic gene	Cancer	Cytotoxic; para- and autokrine apoptosis induction	Griffith et al. (2009)
– Fas, TRAIL			
Suicide gene (encodes prodrug-activating enzyme)	Cancer	Cytocidal in combination with prodrug	Portsmouth et al. (2007)
– HSV thymidine kinase		– Ganciclovir	
– Yeast cytosine deaminase		– 5-FC	
Gene encoding shRNA, miRNA, ribozyme	Various	Inhibition of target gene expression	Singer and Verma (2008)

cassettes are inserted either into circular plasmids, which might be further packaged by non-viral vectors, or into genomes of replication-deficient viral vectors (Table 18.2). Delivery of the resulting gene drug can be achieved by physical methods, for example, plasmid delivery into the skin by a gene gun for genetic vaccination. Alternatively, delivery can be mediated by the vector, for example, by penetration of virus vectors into the target tissue, uptake into cells and transfer of the recombinant virus genome containing the therapeutic gene into the nucleus (viral transduction). To ensure safety, viruses used as gene transfer vectors have been crippled by gene deletions in order to prevent virus replication. Gene transfer vectors determine the fate of the therapeutic gene: retroviral vectors insert their genome and thus the therapeutic gene into the host cell chromosome, whereas plasmids or adenoviral vectors result in episomal gene transfer. Thus, retroviruses facilitate stable gene transfer of transduced cells and their progeny. This is required for corrective treatment of inherited diseases, for example, by ex vivo gene transfer

Table 18.2 Gene transfer vectors used in gene therapy

Gene transfer vector	Delivery	Features	References
Naked plasmid	Injection, pressure-perfusion, gene gun, ultrasound, electroporation,	Regulatory DNA sequences, easy to produce, episomal gene transfer, often inefficient gene transfer	Wells (2004)
Plasmid + non-viral vectors (cationic lipids, liposomes, cationic polymers)	Non-specific uptake or ligand-mediated cell entry	See plasmid, varying efficiency, specific gene transfer possible	Wolff and Rozema (2008)
Retrovirus	Viral transduction, targeting ligand-mediated cell entry possible	Regulatory DNA sequences, stable gene transfer (chromosomal integration, problem of insertional mutagenesis), genetic vector engineering for improved gene transfer	Baum et al. (2006)
Adeno-associated virus (AAV)	Viral transduction, targeting ligand-mediated cell entry possible	Regulatory DNA sequences, episomal gene transfer, long-term gene transfer in postmitotic cells, genetic vector engineering for improved gene transfer	Buning et al. (2008)
Adenovirus	Viral transduction, targeting ligand-mediated cell entry possible	Regulatory DNA sequences, episomal gene transfer, high capacity for foreign DNA, genetic vector engineering for improved gene transfer	McConnell and Imperiale (2004)

into hematopoietic stem cells for treatment of inherited immunodeficiencies (Kohn 2010). In contrast, transient gene transfer is usually sufficient for genetic vaccination or cytoablative cancer therapy. For the latter, however, efficient gene transfer is pivotal and thus vector choice is determined by transduction efficiency. In this regard, conditionally replication-competent viral vectors have been recently engineered allowing for vector spread in tumors and thus amplified gene transfer (Parato et al. 2005; Cody and Douglas 2009). Such replication-competent vectors also mediate tumor cell lysis by virus replication, termed oncolysis or virotherapy. Hence, from the perspective of the virotherapist, insertion of therapeutic genes into the genome of oncolytic viruses is a strategy to complement oncolysis with gene therapy (“arming” of oncolytic viruses).

18.1.3 Targeting Gene Transfer

Many gene therapy applications require the restriction of gene transfer to specific cells. This is obvious for cytoablative gene therapy and for replication-competent vectors. Also effective genetic vaccination can depend on gene transfer into appropriate immune cells, as antigen expression in the wrong cells can trigger tolerance rather than immunity. Consequently, vector targeting is a major challenge for gene therapy research. Targeted gene therapy (or viral replication) can be achieved by inserting cell-binding ligands into the gene transfer vector for targeted cell entry, or by post-entry regulation of therapeutic gene expression using appropriate regulatory sequences, as mentioned above.

18.1.4 Bispecific Antibodies and Gene Therapy

Bispecific antibodies and gene therapy are connected in two ways. First, bispecific antibodies have been developed as promising tools for targeting cell entry of gene transfer vectors: as adapter molecules they link the vector to a marker molecule (specifically) expressed on the target cell surface (Fig. 18.1). Second, gene therapy can be an alternative means for delivery of therapeutic antibodies to patients, i.e., by antibody production in the patients’ cells (genetic antibody therapy, Fig. 18.1). Besides genetic delivery of (established) soluble antibodies, such antibody gene transfer can also facilitate new applications for (bispecific) antibody therapy, for example by expression of membrane-bound or intracellular derivatives. Certainly combination therapies of bispecific antibodies and gene therapy can also be envisioned.

18.2 Bispecific Antibodies Are Tools for Gene Therapy

18.2.1 Adapters for Targeting Cell Entry of Viral Gene Therapy Vectors

Bispecific antibodies have been exploited in gene therapy as tools to direct viral gene transfer vectors to diseased cells. Therefore, an antibody with specificity for a viral surface protein is linked to a second antibody that binds to a cell surface molecule of interest, thus implementing an adapter molecule that binds the gene transfer vector to the target cell (Figs. 18.1 and 18.2). Such modification of virus tropism is required when virus receptor expression is lacking on target cells, preventing gene transfer, or when widespread expression of the native virus receptor on healthy cells leads to adverse side effects and vector sequestration. For the latter, either the viral attachment proteins have been mutated without losing their affinity for the adapter, or the receptor-binding domain of the virus attachment protein is shielded by the adapter. The resulting loss of virus tropism for healthy cells is termed de-targeting. Binding of and entry into target cells in both cases is mediated by the target of the cell-binding moiety of the adapter (re-targeting). Important advantages of the adapter strategy are (a) it does not require modifications to the virus structure, which might well turn out to be detrimental for

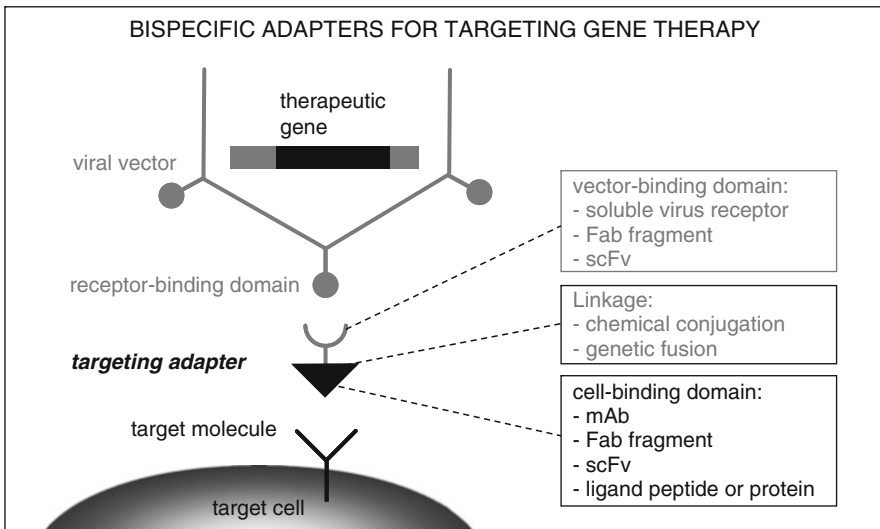


Fig. 18.2 Bispecific antibodies as tools for targeting gene therapy. Bispecific adapters binding to the gene transfer vector via one specificity and to a cell surface molecule with the other are used for delivering therapeutic genes to specific cell types. This strategy is of interest to gene therapy in order to ensure targeted therapy and avoid side effects. These bispecific adapters might contain one antibody or antibody fragment (for either vector or cell binding). Alternatively, they can be bispecific antibodies: chemical conjugate, diabody or tandem scFv

vector assembly, stability, or activity; (b) it is flexible as vector binding to any target molecule, to which an antibody can be raised, is possible by exchange of the adapter's cell-binding moiety and (c) once an effective adapter for a specific vector has been generated, it can be used for transfer of any therapeutic gene by corresponding derivatives of this vector.

18.2.2 Gene Therapy with Adenoviral Gene Transfer Vectors

Bispecific antibodies as adapters for targeting gene transfer have been most intensively investigated with adenoviral vectors. Adenoviruses (Ads, McConnell and Imperiale 2004) possess a double-stranded linear DNA genome covered by a protein capsid, but not a lipid envelope. The receptor-binding spike of the adenoviral capsid, made of the trimeric fiber protein, is responsible for attachment to host cells by binding to the virus receptor, which is the coxsackie-adenovirus receptor (CAR) for the mostly used human Ad serotype 5 (HAdV-5). Virus internalization into the host cell is then mediated by a secondary interaction of a different virus capsid protein, the penton base, with cellular integrins. By separating cell binding from entry, this two-step mechanism facilitates a high degree of flexibility for the nature of initial attachment of Ad vectors to cells. After entry of the vector into the cell, the virus genome is transferred to the nucleus, where viral genes are expressed from the episomal genome. Likewise, therapeutic genes are expressed after transfer of Ad vector genomes into patients' cells. Therefore, essential viral genes are replaced with the therapeutic gene, rendering the vector replication-deficient. More recently, therapeutic genes have been inserted into replication-competent Ads (McConnell and Imperiale 2004). Ads represent prominent gene therapy vectors, as they are stable, can be produced at high titers, possess an effective gene transfer machinery, and are only mildly pathogenic (HAdV-5 causes common cold symptoms) (McConnell and Imperiale 2004). They have been the most frequently used viral vectors in clinical gene therapy trials (Journal of Gene Medicine Clinical Trials Database). These trials have revealed a favorable safety profile of Ad vectors in patients. Cancer gene therapy and genetic vaccination are the regimens where Ad vectors are widely used. One therapeutic approach in cancer gene therapy is molecular chemotherapy, also termed gene-dependent enzyme prodrug therapy (GDEPT). This strategy is based on transfer of a gene encoding a prodrug-activating enzyme, which activates a harmless prodrug into an effective chemotherapeutic drug (Portsmouth et al. 2007). The rationale for this strategy is that tumor-restricted prodrug activation should facilitate effective concentrations of the chemotherapeutic drug in the tumor, which cannot be achieved by conventional systemic infusion of the drug due to dose-limiting side effects. GDEPT and other cytotoxic cancer gene therapies depend on tumor-selective gene transfer which is not provided by unmodified HAdV-5 or other Ad serotypes due to widespread expression of Ad receptors also on healthy cells. Ads are also frequently used as vectors for genetic vaccination, which is most efficient when the antigen gene is transduced into professional antigen-presenting cells (APCs), which provide the

proper signals for activation of immune effector cells. Dendritic cells (DCs) are the most effective APCs, but are difficult to transduce. Though Ads are the most effective gene transfer vectors for DCs, high vector titers are required for efficient DC transduction because of low expression of CAR.

18.2.3 Antibodies for Targeting Adenoviral Gene Transfer

Antibodies are attractive binding molecules for targeting gene transfer vectors based on their high affinity, specificity, and the opportunity to generate antibodies with specificity for virtually any cell surface target molecule. Three strategies have been pursued for insertion of targeting ligands into viral gene transfer vectors: genetic fusion to viral capsid or envelope proteins, complexing with bispecific adapters, or chemical linkage. Major drawbacks of the genetic and chemical strategies are that they are tedious and often interfere with viral functions. Moreover, genetic insertion of antibodies into the adenoviral capsid is hampered by the incompatibility of biosynthesis of capsid and antibody molecules. Ad capsid proteins are synthesized in the cytosol and transferred to the nucleus where viral particle assembly takes place, whereas antibodies are produced via the secretory pathway, which ensures their proper folding. Consequently, genetic fusion of antibodies to Ad capsid proteins has been inefficient and limited to a few cytosolically stable antibody fragments (Hedley et al. 2006; Vellinga et al. 2007; Poulin et al. 2010). In contrast, synthesis of adapter molecules can be separated from virus production. Moreover, the insertion of cell-binding antibodies into adapter molecules is less tedious than the engineering of a complete new virus genome and resulting adapters can be linked to any Ad vector, allowing for better flexibility. For production of adapters, antibody fragments binding to Ad capsid proteins, mostly the fiber, and antibodies or antibody fragments binding to cell surface target molecules of interest have been used (Fig. 18.2). They have been linked by chemical conjugation (see also: Chap. 3) or by genetic fusion, the latter generating tandem scFvs or scDb (see also: Chap. 5). As an alternative to bispecific antibodies, adapters have been generated by linking virus-binding antibody fragments to cell-binding proteins or peptides, or by linking cell-binding antibody fragments to the soluble adenovirus receptor CAR.

18.2.4 Antibody-Derived Chemical Conjugates as Bispecific Adapter Molecules for Targeting Adenoviral Gene Transfer

The adapter strategy for targeting cell entry of Ad vectors has been pioneered by Douglas and co-workers for targeting of folate receptor overexpressing tumor cells (Douglas et al. 1996). To this end, they chemically conjugated folate to the

Fab fragment of a neutralizing anti-Ad fiber monoclonal antibody (MAb). A Fab fragment was used to avoid agglutination of Ad vectors by bivalent antibodies. After complexation to the respective Ad vector, the adapter mediated folate-dependent transfer of a reporter gene or of cytoablative genetic prodrug activation to target cells. The Fab fragment alone inhibited adenoviral transduction, which was expected as it was derived from a neutralizing antibody. Thus the Fab-folate adapter realized targeted gene transfer by both ablating virus binding to the native virus receptor and directing virus attachment to a novel cell surface molecule (Fig. 18.1). Wickham et al. described a bispecific antibody for directing Ad cell binding to integrins. This adapter consisted of the integrin-binding MAb chemically linked to a second MAb with specificity for a peptide tag, which was engineered into the Ad penton base (Wickham et al. 1996). The conjugate mediated enhanced adenoviral transduction of human smooth muscle and endothelial cells, which were only modestly transduced by unmodified HAdV5 vectors. Subsequently, various bispecific antibody conjugates were reported, that consist of a fiber-binding Fab fragment covalently linked to a cell-binding antibody or antibody fragment. Such bispecific antibody conjugates have been reported to re-direct Ad gene transfer to various cell types via binding to different cell surface molecules, including CD40, EpCAM, Tag72, CD70, and ACE (Tillman et al. 1999, 2000; De Gruijl et al. 2002; Brandao et al. 2003; Miller et al. 1998; Haisma et al. 1999; Israel et al. 2001; Reynolds et al. 2000, 2001). These reports confirm the high flexibility of the adapter approach. For example, DCs, as professional antigen-presenting cells, represent targets of interest for gene transfer aiming at genetic vaccination for infectious or malignant diseases. Conjugates of α -fiber Fab and MAbs binding to the DC surface molecule CD40 allowed for efficient Ad gene transfer into mouse and human DCs (Tillman et al. 1999, 2000). With this adapter, improved efficiency and selectivity of Ad gene transfer to DCs was also achieved in situ using human skin explants (De Gruijl et al. 2002). In addition to targeting Ad entry, the α -fiber Fab/ α CD40 mAb adapter triggered DC activation, as required for efficient induction of immune responses, via its CD40-binding activity. Accordingly, the adapter increased the efficiency of tumor vaccination with Ad vector transduced DCs in an animal model (Tillman et al. 2000). Adapter targeting of Ad vectors to cancer cells was demonstrated in cell culture studies with an EGFR-binding α -fiber Fab/MAB conjugate for squamous cell carcinoma, glioblastoma, and osteosarcoma (Miller et al. 1998; Blackwell et al. 1999; Barnett et al. 2002); with an EpCAM-binding Fab/Fab conjugate for various adenocarcinomas (Haisma et al. 1999; Heideman et al. 2001); with a TAG-72-binding α -fiber Fab/MAB conjugate for ovarian cancer (Kelly et al. 2000); and with a CD70-binding α -fiber Fab/MAB conjugate for B cell lymphomas (Israel et al. 2001). As CAR-expression varies on cancer cells, adapters frequently mediated markedly enhanced transduction of cancer cells. Yet another type of antibody-based adapter conjugate has been generated by linking α -fiber Fab fragments to basic fibroblast growth factor for targeting of various cancer cells (FGF2, Goldman et al. 1997; Rogers et al. 1997; Rancourt et al. 1998) to a synthetic lung-homing peptide (Trepel et al. 2000), or to the Hc-fragment of tetanus toxin for targeting neuronal cells (Schneider et al. 2000).

18.2.5 Recombinant Antibody-Derived Bispecific Adapter Molecules for Targeting Adenoviral Gene Transfer

Recombinant bispecific adapter molecules possess attributes that are advantageous for application in vector targeting when compared with chemical conjugates. Foremost, they can be produced by standardized procedures of prokaryotic or eukaryotic expression yielding well-defined molecules. Both tandem single chain variable fragments (tandem scFvs, see also: Chap. 5) and single chain diabodies (scDBs, see also: Chap. 5) have been used for targeting Ad gene transfer. Haisma and co-workers demonstrated that Ad transduction of glioblastoma and carcinoma cells can be increased by complexing the virus with a recombinant tandem α -fiber/ α -EGFR scFv (Haisma et al. 2000). Our group reported in 2001 that a scDB with specificities for the Ad fiber and Endoglin, which is expressed on proliferative endothelium, facilitated targeted transduction of endothelial cells (Nettelbeck et al. 2001). In contrast to the tandem scFv, which was expressed in eukaryotic cells, the scDB was produced in bacteria. Ad transduction was also targeted to gastric cancer cells with a tandem scFv adapter binding to EpCAM (Heideman et al. 2002), to DCs with a CD40-binding tandem scFv adapter (Brandao et al. 2003), to melanoma cells using a scDB adapter binding the melanoma surface antigen HMWMAA (Nettelbeck et al. 2004), or to breast cancer cells with either a tandem scFv or a scDB binding to CEA (Korn et al. 2004). For improved de-targeting, “receptor-blind” Ad mutants were combined with tandem scFv or scDB adapters that were derived from α -fiber scFvs that retained binding to mutant fibers (van Beusechem et al. 2002; Nettelbeck et al. 2004; Carette et al. 2007). These Ad vectors could not bind CAR, even when individual fiber molecules were not protected after complexation with adapters. In consequence, this strategy of combined genetic/immunological tropism-modification implements a further increase in selectivity of gene transfer. Recombinant antibody-derived adapters for targeting adenoviral transduction were also obtained by fusion of α -fiber scFv to ligand proteins (EGF or uPAR, Watkins et al. 1997; Harvey et al. 2010) or to ligand peptides (Nicklin et al. 2000). Alternatively, cell-binding scFvs (α -c-erbB2, α -CD40 or α -Fc γ RI) were fused to monomeric or trimeric soluble CAR (Kashentseva et al. 2002; Pereboev et al. 2002; Kim et al. 2002; Sapinoro et al. 2007). Such sCAR-derived adapters offer the advantage of improving affinity to fiber by sCAR trimerization; however, they naturally cannot bind to “receptor-blind” fiber-mutant viruses. These strategies also demonstrated that the adapter, besides targeting gene transfer, might also influence the outcome of gene therapy in different ways: adenoviral gene transfer to DCs by CD40-binding adapters, but not by the Fc γ RI-binding adapter resulted in DC activation, thus influencing the type of immune response (immunization versus tolerization, Tillman et al. 1999; Sapinoro et al. 2007).

18.2.6 Toward Applications of Bispecific Adapter Molecules in Gene Therapy

In vitro studies with adapter molecules, including various bispecific antibodies, have clearly proven that viral cell entry can be re-directed via novel cell surface receptors, thus reprogramming virus tropism. This has been demonstrated in established cell cultures, freshly purified normal and tumor cells and in tissue explants, as for the demonstration of DC-targeted gene transfer in skin explants (de Gruijl et al. 2002). What are possible applications of bispecific antibodies and other antibody-derived gene transfer adapters? First, due to their modular composition and the opportunity to rapidly (in comparison with genetically engineered viruses) produce new adapters by chemical or genetic means, they facilitate the analysis, comparison, and screening of cell surface molecules for their feasibility as targets for viral gene transfer. Second, applications of adapters for ex vivo gene therapy are of interest. An example is genetic vaccination of cancer or infectious diseases by ex vivo gene transfer into DCs isolated from patients. Gene therapy of inherited diseases by ex vivo gene transfer into hematopoietic (stem) cells is a further application. Here, however, retroviral vectors are preferred over Ad vectors, as they facilitate stable gene transfer and thus prolonged gene correction or replacement (Table 18.2). Of note, adapter-targeting of retroviral gene transfer has been demonstrated recently (see below). Most gene therapy applications, however, require in vivo gene transfer. For establishing adapters for targeting gene transfer, in vivo extensive studies on the stability, efficiency, and selectivity of adapter-vector complexes after in vivo application are needed. Whereas rigorous studies for the evaluation of pharmacologic and therapeutic parameters of adapter-targeted gene transfer are still to be done, initial studies have shown efficacy of adapter-targeting in vivo. In an effort to facilitate gene therapy of pulmonary vascular disease, Reynolds and colleagues investigated a Fab-mAb conjugate adapter that binds angiotensin-converting enzyme (ACE) for targeting of Ad gene transfer to the lung endothelium in rats (Reynolds et al. 2000, 2001). By systemic application of adapter-bound or uncomplexed Ad vector, it was shown that this adapter increased gene transfer to the lung by more than 20-fold. Importantly, gene transfer was directed to endothelial cells. Moreover, gene transfer to the liver, the organ responsible for most Ad-induced side effects, was reduced more than 80%. Hence, this study demonstrated both systemic stability of the adapter-vector complex and adapter-dependent vector de- and re-targeting in vivo. For the Fab-FGF2 adapter, several studies in mice showed adapter-dependent reduction of liver transgene expression after systemic injection of Ad vectors and reduced toxicity of Ad-mediated genetic prodrug activation therapy. Furthermore, this adapter increased therapeutic activity of Ad-mediated genetic prodrug activation of peritoneal malignancies, when the Ad vectors were injected intraperitoneally (Rancourt et al. 1998; Gu et al. 1999;

Printz et al. 2000). In vivo stability of adaptor-vector complexes has also been demonstrated for recombinant proteins. Trimeric, but not monomeric sCAR significantly blocked liver gene transfer by Ads after systemic application of the sCAR-Ad vector complex into mice (Kim et al. 2002). However, in a different study, a sCAR-scFv adapter targeting CEA also reduced liver transduction by Ad vectors after systemic injection of adapter-virus complexes into mice (Li et al. 2007). After systemic injection, this adapter also increased adenoviral transduction of CEA-positive, but reduced transduction of CEA-negative tumors that were grafted to mouse livers. Furthermore, a trimeric derivative of the sCAR-CEA adapter mediated improved targeting of adenoviral gene transfer in vitro and in vivo. In combination with transcriptional targeting using the cox-2 promoter, this trimeric adapter increased therapeutic activity and at the same time reduced liver toxicity of genetic prodrug activation therapy with HSV-tk/GCV (Li et al. 2009). Studies with sCAR-EGF and trimeric sCAR-mCD40L confirm the re-targeting properties of sCAR-derived adapters in vivo (Liang et al. 2004; Huang et al. 2007). In addition to facilitating selective gene transfer, targeting adenoviral cell binding and entry is of interest also for improving oncolytic Ads. Toward this end, adapter molecules are of interest to re-direct the injected virus to target tumors. To also allow for targeting of progeny viruses of oncolytic Ads produced in patients' tumors, genes encoding recombinant bispecific adapters have been inserted into the genome of these viruses. Using a tandem scFv with specificity for the Ad fiber and EGFR, van Beusechem and co-workers demonstrated increased viral spread and oncolysis in two- and three-dimensional tumor cell cultures (van Beusechem et al. 2003; Carette et al. 2007).

18.2.7 Adapter Targeting is Feasible for Several Viral Gene Transfer Vectors

Although most widely investigated for Ad vectors, adapters have been also shown to facilitate targeted cell entry of other viruses. Adeno-associated viruses (AAV) are small non-enveloped viruses that are frequently used for diverse gene therapy applications (Buning et al. 2008). Tropism-modification of AAV vectors was achieved with a bispecific Fab/Fab antibody conjugate. The adapter with specificity for the virus capsid and for integrins facilitated gene transfer into megakaryocytes, which are not permissive to unmodified AAV vectors (Bartlett et al. 1999). For non-human coronaviruses, enveloped RNA viruses that naturally do not enter human cells, infectivity for human cancer cells was established with a bispecific tandem scFv with specificities for a coronavirus surface glycoprotein and EGFR (Wurdinger et al. 2005a, b). The idea of this approach was to selectively kill tumor cells by lytic virus infection rather than viral gene transfer. Similar results were obtained using a recombinant adapter built of soluble

coronavirus receptor fused to the EGFR-binding scFv (Wurdinger et al. 2005a, b). Newcastle disease virus, which is in development for viral oncolysis and gene therapy, has been re-targeted using a recombinant adapter built of a virus-binding scFv and IL-2 (Bian et al. 2005, 2006). Retroviruses are enveloped RNA viruses, which insert their genome after reverse transcription into the chromosome of infected cells. Therefore, retroviral vectors facilitate long-term gene transfer which is especially suitable for gene correction therapy of monogenetic diseases. Adapter targeting of retrovirus cell entry was reported for recombinant proteins built of the virus receptor extracellular domain fused to EGF, VEGF, or an EGFR-specific scFv (Snitkovsky and Young 1998; Boerger et al. 1999; Snitkovsky et al. 2000, 2001).

18.3 Gene Transfer as a Tool for Antibody Therapy: Genetic Antibody Delivery

18.3.1 Genetic Antibody Delivery

Gene therapy can be exploited for expressing antibodies in patients, which might be advantageous for achieving sustained and/or efficient antibody concentrations and/or a favorable antibody biodistribution by local expression. Thus, gene therapy is a tool of interest to overcome rapid antibody clearance or poor access to tumors as reported for antibodies that are injected as proteins. Genetic antibody therapy can be implemented by *in vivo* or *ex vivo* gene transfer (Fig. 18.3), i.e., by direct injection of the gene transfer vector into patients or by gene transfer in cultures of previously isolated cells followed by injection of the resulting genetically engineered cells, respectively. Dependent on the design of the gene transfer vector, genetic antibody application can be transient or permanent, constitutive or inducible, targeted or ubiquitous. For example, retroviral vectors allow for stable gene transfer, inducible promoters facilitate control of antibody expression, and targeted vectors can direct gene transfer to specific cell types (see Sects. 18.1.2 and 18.1.3). Therefore, gene therapy possesses high potential and flexibility for implementing improved antibody delivery for specific applications. However, this area of research is still in its infancy and more widespread investigations are warranted.

With the advent of recombinant DNA technology it became possible to establish novel strategies for antibody production and to engineer antibody properties (for example affinity maturation and humanization), formats (single chain fragments), and fusion proteins (immunotoxins). Recombinant antibodies have been frequently produced in bacteria, but gene transfer into eukaryotic cells has also been utilized for *in vitro* production of immunoglobulins, antibody fragments or antibody fusion proteins. Having established the engineering of recombinant gene constructs for eukaryotic antibody expression, also the *in vivo* production of antibodies became feasible. Examples are the expression of functional recombinant MAbs in mice

GENETIC ANTIBODY THERAPY

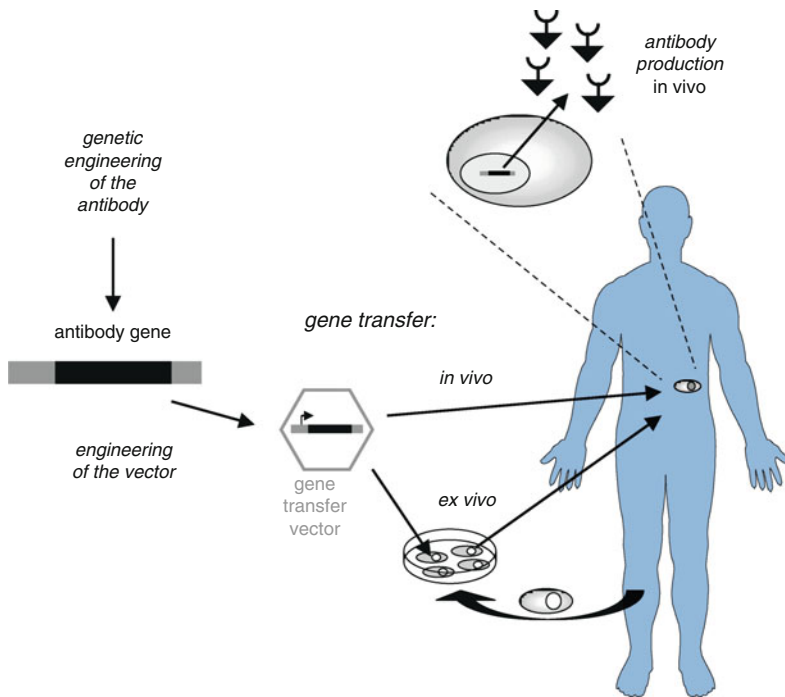


Fig. 18.3 Gene therapy as a tool for antibody delivery: Genetic antibody therapy. For genetic antibody delivery antibody genes, which can be engineered to match specific purposes, are incorporated into gene transfer vectors. These vectors are either directly injected into patients (in vivo gene therapy) or are used for gene transfer into cells previously purified from a patient followed by re-injection of the engineered cells into the patient (ex vivo gene therapy). The antibodies are produced in the patient from cells genetically modified by in vivo or ex vivo gene transfer. Dependent on the vector design, antibody production can be transient or prolonged, constitutive or inducible and show local or systemic activity

after transfer of genetically engineered cells (Noel et al. 1997) or after in vivo gene transfer with an adenoviral or AAV vector (Noel et al. 2002; Jiang et al. 2006; Watanabe et al. 2009; Lewis et al. 2002; Fang et al. 2005, 2007; Skaricic et al. 2008; De et al. 2008; Ho et al. 2009). Toward this end, Fang and coworkers optimized antibody production: they expressed the heavy and light chains of the MAb at equal amounts from a single open reading frame using a “ribosomal skip” sequence. Thereby, serum levels of >1 mg/ml antibody for extended time periods were obtained in mice after injection of a single dose of AAV vector. In a subsequent study, the same group demonstrated that by using an inducible promoter, serum antibody levels after in vivo gene transfer can be repeatedly shut off and on (Fang et al. 2007). This represents a promising strategy to increase safety and/or facilitate dose adaptation in potential future clinical applications of genetic

antibody delivery. De and co-workers combined genetic delivery of a MAb gene by AAV and Ad vectors to achieve both rapid (Ad) and persistent (AAV) antibody production (De et al. 2008).

Functional expression *in vivo* was also demonstrated for recombinant antibody fragments or fusion proteins that contain such fragments after adenoviral gene transfer (Whittington et al. 1998; Arafat et al. 2002; Afanasieva et al. 2003; Kasuya et al. 2005; Liu et al. 2010). The expression of chimeric antigen receptors by T cells and subsequent adoptive T cell therapy is another important application of genetic antibody delivery.

18.3.2 In Vivo Expression of Bispecific Antibodies

Álvarez-Vallina and team have developed genetic delivery of bispecific antibodies by engineered cells. In 2003, they reported anti-tumor activity for a bispecific diabody expressed *in vivo* from irradiated, genetically engineered 293T cells (Blanco et al. 2003). They produced stably transfected 293T cells secreting a diabody with specificity for both CEA and CD3. A second cell line additionally secreted a bivalent CEA-specific diabody fused to the extracellular domain of B7-1. After co-injection with CEA-positive tumor cells into mice, these genetically engineered cells showed anti-tumor activity compared with co-injection of control 293T cells. Subsequent to this proof-of-principle study, the same group engineered a lentiviral gene transfer vector encoding the CEA-CD3 diabody (Compte et al. 2007). This vector facilitated the transduction of different types of hematopoietic cells that showed prolonged secretion of active diabody *in vitro* and antitumor activity *in vivo*. In a follow-up study, the group demonstrated that also the implantation of lentivirally transduced endothelial cells into mice resulted in prolonged production of the CEA/CD3 diabody with therapeutic activity (Compte et al. 2010). This study aims at a therapeutic regimen that allows for the production of therapeutic antibodies from neovessels that have incorporated *ex vivo* engineered endothelial cells.

Genetic delivery of bispecific antibodies has also been reported for intracellular applications: cell surface localization of two membrane proteins, VEGFR2 and Tie-2, could be blocked by expression of a corresponding bispecific, tetravalent antibody targeted to the endoplasmic reticulum (Jendreyko et al. 2003). This intracellular bispecific antibody showed anti-angiogenic activity *in vitro*, which was superior to monovalent control antibodies. A similar construct with specificity for VEGFR2 and Tie-2 mediated anti-angiogenic and anti-tumor activity *in vivo* after adenoviral gene transfer (Jendreyko et al. 2005).

18.4 Conclusions

Proof of principle has been demonstrated in several cell culture studies and animal models for both the utility of bispecific antibodies for targeting gene therapies and the feasibility of gene transfer for delivering recombinant bispecific antibodies.

Based on this fundamental work, bispecific antibody adapters and gene transfer technologies should now be considered for improving therapeutic regimens in gene therapy and antibody therapy, respectively. Cooperation between antibody engineers and gene therapists are warranted to further develop bispecific antibodies and gene transfer vectors for this purpose.

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