Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Virus Research 143 (2009) 184–194

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Review

Adenovirus receptors and their implications in gene delivery

Anurag Sharma[a,b], Xiaoxin Li[a,b], Dinesh S. Bangari[a,b,c], Suresh K. Mittal[a,b,*]

[a] Department of Comparative Pathobiology, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907, USA
[b] Bindley Bioscience Center, Purdue University, West Lafayette, IN 47907, USA
[c] Genzyme Corporation, Department of Pathology, 5 The Mountain Road, Framingham, MA 01701-9322, USA

ARTICLE INFO

Article history:
Available online 26 February 2009

Keywords:
Adenoviral vector
Adenovirus receptor
Gene delivery
Targeting
Tissue tropism

ABSTRACT

Adenoviruses (Ads) have gained popularity as gene delivery vectors for therapeutic and prophylactic applications. Ad entry into host cells involves specific interactions between cell surface receptors and viral capsid proteins. Several cell surface molecules have been identified as receptors for Ad attachment and entry. Tissue tropism of Ad vectors is greatly influenced by their receptor usage. A variety of strategies have been investigated to modify Ad vector tropism by manipulating the receptor-interacting moieties. Many such strategies are aimed at targeting and/or detargeting of Ad vectors. In this review, we discuss the various cell surface molecules that are implicated as receptors for virus attachment and internalization. Special emphasis is given to Ad types that are utilized as gene delivery vectors. Various strategies to modify Ad tropism using the knowledge of Ad receptors are also discussed.

Contents

1. Introduction

Adenoviruses (Ads) are nonenveloped, double-stranded DNA viruses under the family *Adenoviridae* (Berk, 2007; Wold and Horwitz, 2007). Viral particles are 80–120 nm in diameter with icosahedral symmetry and contain a linear genome of ~26–44 kb in size. The Ad capsid is composed of 240 homotrimeric hexons and 12 pentameric pentons located at each vertex of the icosahedral capsid. From the base of each penton extends a homotrimeric fiber. Each fiber monomer is comprised of an amino-terminus that is non-covalently anchored to the penton base, a carboxy-terminus globular domain that binds to the cell surface receptor, and a rod-like shaft that varies in length according to the Ad serotype. Other minor proteins such as IIIa, VI, VIII and IX are also associated with the viral capsid (Berk, 2007; Vellinga et al., 2005; Wold and Horwitz, 2007).

There are more than 100 Ad serotypes including 51 human Ad (HAd) serotypes identified to date. Ads are known to infect a wide variety of vertebrate species that include mammals, fish,

* Corresponding author at: Department of Comparative Pathobiology, Purdue University, West Lafayette, IN 47907, USA. Tel.: +1 765 496 4894; fax: +1 765 494 9830.
E-mail address: mittal@purdue.edu (S.K. Mittal).

0168-1702/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2009.02.010

Table 1
Adenovirus tropism and receptor usage.

HAd Subgroups	Serotypes	Predominant natural tropism	Known receptor/s usage[a]	References
A	12, 18, 31	Gastrointestinal	CAR	Roelvink et al. (1998)
B1	3, 7, 16, 21, 50	Respiratory	CD46, CD80/86, Receptor X, HSPG	Fleischli et al. (2007), Segerman et al. (2003a), Short et al. (2006), Sirena et al. (2004), Tuve et al. (2008)
B2	11, 14, 34, 35	Renal	CD46, CD80/86, Receptor X, HSPG	Fleischli et al. (2007), Segerman et al. (2003a), Segerman et al. (2003b), Short et al. (2006), Tuve et al. (2008)
C	1, 2, 5, 6	Respiratory	CAR, HSPG, MHC-I, VCAM-I, Integrins	Bergelson et al. (1997), Chu et al. (2001), Dechecchi et al. (2000), Hong et al. (1997), Wickham et al. (1993)
D	8, 9, 10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36-39, 42–49, 51	Ocular	CAR, Sialic acid, CD46	Arnberg et al. (2000a), Roelvink et al. (1998)
E	4	Respiratory, Ocular	CAR	Roelvink et al. (1998)
F	40, 41	Gastrointestinal	CAR	Roelvink et al. (1998)

[a] Listed receptors are suggested to be used by one or more serotypes of the subgroup.

birds, amphibians and reptiles (Davison et al., 2003). HAd serotypes are classified into six distinct subgroups (species) (A–F) based on their hemagglutination properties, oncogenic potential in newborn hamsters, genomic organization and DNA homology (Berk, 2007; Fauquet et al., 2005). Subgroup B is further subdivided into B1 and B2 subspecies on the basis of restriction enzyme digestion patterns of their genomes and differences in tissue tropism (Segerman et al., 2003a; Wadell et al., 1980). In immunocompetent individuals, HAds are involved in mostly mild and self-limiting disease, whereas, in children and immunocompromised adults the disease may be acute or even life-threatening (Kojaoghlanian et al., 2003). In general, HAds of different subgroups exhibit distinct tissue tropism and clinical manifestations (Table 1). Typically, HAd subgroup B1, C and E mainly cause respiratory tract infections, whereas those of subgroup D and E lead to ocular infections (Russell, 2005). HAd serotypes from subgroup A and F are responsible for gastrointestinal infections and B2 subgroup HAds cause renal and urinary tract infections (Russell, 2005). Likewise, Ads from nonhuman origin also show distinct tissue tropism. The initial attachment of Ad to its primary receptor, which differs among Ad subgroups (Table 1), is considered as one of the primary determinants to Ad tropism.

Ads have generated immense interest as vectors for therapeutic gene delivery. HAd serotype 5 (HAd5) is the most extensively studied and most commonly used Ad serotype for gene delivery applications. To date, many preclinical studies as well as clinical trials with variable but encouraging results have been conducted or are currently in progress (http://www.wiley.co.uk/genetherapy/clinical/). As of September 2008, nearly 25 percent of 1472 gene therapy clinical trials approved worldwide utilized Ad vectors; most of them were directed towards cancer gene therapy (http://www.wiley.co.uk/genetherapy/clinical/). Popularity of Ad vectors is based on several advantages such as efficient transgene delivery and expression, transduction of both dividing and non-dividing cells, ease of propagation to high titers, episomal persistence of the Ad genome within the nucleus with minimal risk of genomic insertional mutagenesis, relative stability in blood following systemic administration, high capacity to accommodate foreign DNA and significant progress in our understanding of the biology of Ad (Douglas, 2007; Wu et al., 2001). However, despite aforementioned advantages, clinical application of Ad vectors is limited by several disadvantages such as strong immunogenicity of Ad vectors, prevalence of preexisting anti-HAd immunity in human population, lack of specific targeting, rapid blood-clearance and predominant hepatotropism following systemic administration (Douglas, 2007; Wu et al., 2001).

Ad entry into the host cells is mediated through two main events: an initial step of virus attachment to a primary cell surface receptor with the knob domain of the viral fiber followed by secondary interactions between viral capsid components and internalization receptors (Leopold and Crystal, 2007). For a variety of HAd serotypes and few nonhuman Ads, several cellular receptors have been identified or proposed (Zhang and Bergelson, 2005). The identification of cellular receptors used by Ads is necessary for better understanding of viral pathogenesis as well as for the development of novel Ad-based gene delivery vectors. In this review, we discuss the various Ad receptors, their implications in Ad tropism and various strategies to modify Ad-receptor interaction for the development of novel Ad vectors with altered tropism, greater efficacy and safety.

2. Adenoviral receptors

2.1. Coxsackievirus–adenovirus receptor (CAR)

Coxsackievirus–adenovirus receptor is a 46 kDa type I transmembrane glycoprotein that was initially identified as a high affinity attachment receptor for coxsackievirus B as well as HAd serotypes 2 and 5 (Bergelson et al., 1997; Tomko et al., 1997). CAR belongs to the cortical thymocyte marker of the Xenopus (CTX)-subfamily of immunoglobulin (Ig) superfamily and consists of two extracellular Ig-like domains (distal variable type—D1; proximal C2 type—D2), a single-pass hydrophobic transmembrane domain and a long carboxy-terminal cytoplasmic domain (Chretien et al., 1998; Wang and Bergelson, 1999). Among these domains, the D1 domain alone is sufficient for interaction with the Ad fiber knob (Freimuth et al., 1999; Kirby et al., 2000; Wang and Bergelson, 1999). In general, the knob–CAR interaction serves to attach the virion to the host cell surface and subsequently, virus endocytosis is promoted by interaction of virus-CAR complex with additional co-receptors such as integrins (Wickham et al., 1993). However, integrin-independent virus internalization, though at a slower rate, has also been reported (Shayakhmetov et al., 2005a). In addition to HAd2 and HAd5, many HAd serotypes of subgroup A, D, E and F but not of subgroup B recognize CAR (Roelvink et al., 1998). Several Ad vectors derived from nonhuman species have also been investigated as alternative vectors for gene delivery applications (Bangari and Mittal, 2006). Many of these vectors, such as canine, chimpanzee and avian Ads have been shown to interact with CAR, while bovine, porcine and ovine Ads appear to enter the cells in a CAR-independent manner (Bangari and Mittal, 2005; Bangari et al., 2005a; Cohen et al., 2002; Glasgow et al., 2004; Soudais et al., 2000; Tan et al., 2001). Susceptibility of a particular cell type to HAd5 infection has been found to correlate with the expression levels of CAR (Asaoka et al., 2000; Fuxe et al., 2003; Hemmi et al., 1998; Li et al., 1999). Moreover, the induced expression of CAR on a variety of cell types naturally refractory to Ad infection showed improved transduction (Nalbantoglu et al., 2001). Transgenic mice expressing CAR in selected tissues also showed enhanced Ad transduction to the target cells (Bao et al., 2005). Homologues of human CAR are present in several other species

including mice, rats, dogs and pigs with high levels of homology (Bergelson et al., 1998; Fechner et al., 1999; Tomko et al., 1997).

Tissue distribution of CAR is quite complex and developmentally regulated (Philipson and Pettersson, 2004). Though sufficient levels of mRNA encoding CAR have been observed in various tissues (Tomko et al., 2000, 1997), the highest levels have been reported in embryonic tissues and gradually decline after birth in most of the tissues (Raschperger et al., 2006). On polarized epithelial cells, CAR is preferentially expressed at the basolateral surface and is absent from the apical surface that may limit the virus infection across the epithelial surface (Cohen et al., 2001; Walters et al., 1999; Zabner et al., 1997). Studies have localized CAR to the tight junction and/or adherens junction where it is associated with zonula occludens-1 and other tight junction proteins and is engaged in homotypic cell–cell interaction, adhesion and tissue genesis (Cohen et al., 2001; Raschperger et al., 2006). CAR-deficient mice die during embryonic stage due to defects in cardiac development indicating the importance of CAR in organogenesis and embryonic development (Chen et al., 2006; Dorner et al., 2005).

In spite of the wide variation in the fiber knob amino acid sequences among the HAd serotypes compared to HAd5 (29–66%), amino acid residues involved in CAR-binding are well-conserved among the CAR-binding HAd serotypes. By sequence analysis and mutagenesis studies, the key CAR-binding residues on HAd5 and other CAR-binding serotypes were identified on the side of each monomer of the trimeric knob (Kirby et al., 2000, 2001; Roelvink et al., 1999). The crystal structure of HAd12 fiber knob complexed with D1 domain of CAR identified critical regions of knob for CAR-binding (Bewley et al., 1999). CAR D1 molecule binds at the interface between two adjacent HAd12 knob monomers, which is consistent with the observation that most neutralizing antibodies are directed against the trimeric knob (Bewley et al., 1999). However in HAd5 and HAd2, the adjacent monomer may not contribute to CAR-binding, but each monomer of the trimeric knob independently binds to CAR (Kirby et al., 2000). Other CAR-binding serotypes (HAd9 and HAd41) also have conserved residues for CAR-binding and similar crystal structures compared to HAd5 (Kirby et al., 2001; Roelvink et al., 1998, 1999). On the other hand, non-CAR-binding serotypes (HAd3, HAd7, HAd19, HAd30, and HAd35) either lack the conserved CAR-binding residues or the charge/steric hindrance, which hampers the knob–CAR interaction (Burmeister et al., 2004; Durmort et al., 2001; Law and Davidson, 2002, 2005; Leissner et al., 2001).

The shaft domain imparts the Ad fiber protein its length and also determines its flexibility (Chroboczek et al., 1995; Ruigrok et al., 1994). Different Ad serotypes exhibit different fiber-shaft lengths depending on the number of pseudo-repeats within the shaft. In general, a shorter and rigid fiber hinders Ad binding to CAR as well as secondary interactions with integrins (Chroboczek et al., 1995; Shayakhmetov and Lieber, 2000). Interestingly, the charge on hypervariable region (HVR) 1 of the hexon can also influence Ad interaction with its receptor (Crawford-Miksza and Schnurr, 1996). These observations implicate the complex nature of Ad-CAR interaction.

Because of the paucity of CAR on their surface, many primary and cancer cells are refractory to transduction by CAR-binding Ad vectors (Kim et al., 2002). CAR expression levels in cancer tissues inversely correlate with tumor aggressiveness, but induction of CAR on highly tumorigenic cancer cells has tumor suppressor effects. This observation also highlights the need for other Ad vectors with CAR-independent internalization for cancer gene therapy.

2.2. Integrins

Integrins are non-covalently associated heterodimeric cell surface adhesion molecules composed of α and β subunits that play a critical role in a number of host cell functions including cell attachment, migration, growth and differentiation (Luo et al., 2007; Stewart and Nemerow, 2007). There are eighteen different α subunits and eight β subunits, which can form more than twenty α/β heterodimers (Stewart and Nemerow, 2007). Most integrins are ubiquitously expressed on a wide variety of cells, and a broad range of microbial pathogens can recognize them to invade host cells (Hynes, 1992; Stewart and Nemerow, 2007). Multiple types of integrin molecules that include vitronectin receptors αvβ3 and αvβ5 (Wickham et al., 1993), as well as αvβ1 (Li et al., 2001), α3β1 (Salone et al., 2003) and α5β1 (Davison et al., 2001) have been shown to act as secondary receptors for many Ads. Integrins interact with the Arg-Gly-Asp (RGD) or Leu-Asp-Val (LDV) motif displayed on the exposed loops of Ad penton base. In general, Ad–integrin interaction is of relatively low affinity; therefore, high affinity primary fiber-receptor interaction is crucial for efficient Ad infection. Most of the sequenced HAd serotypes, except HAd40 and HAd41, contain the RGD motif in their penton bases and most likely use integrins as co-receptors (Albinsson and Kidd, 1999). The cytoplasmic domains of α and β subunits of integrins interact with a variety of signaling molecules, therefore Ad–integrin interaction promotes activation of p130CAS (Crk-associated substrate), phosphatidylinositol 3 kinase and Rho family of small GTPase, which results in actin polymerization, cytoskeletal rearrangement and enhanced Ad internalization through receptor-mediated endocytosis (Nemerow and Stewart, 1999). The cryoelectron microscopic structural analyses of HAd2 or HAd12 complexed with the αvβ5 integrin revealed its binding to the penton base RGD motif (Chiu et al., 1999; Mathias et al., 1998). These structural findings also suggested that the pentameric spatial arrangement of RGD motifs on the penton base is necessary for receptor clustering and initiation of cell-signaling events required for virus internalization. Besides internalization, the integrin αvβ5 plays a major role in membrane permeabilization and virus escape into the cytosol (Majhen et al., 2009; Wickham et al., 1994). Ad vectors with penton base RGD motif deletion not only showed delayed uptake but also resulted in slow endosomal escape (Shayakhmetov et al., 2005a). Members of the β2 integrin family (αMβ2 and αLβ2) led to the attachment of fiberless HAd2 particles to CAR-deficient monocytic cells, followed by the secondary interaction with αv integrins (Huang et al., 1996).

In order to overcome the limited expression of primary Ad receptors, strategies based on incorporation of additional RGD motifs on Ad fiber knobs have been used to enhance the Ad transduction to a wide variety of cells including endothelial cells, smooth muscle, fibroblasts, numerous tumor cell types, and dendritic cells (DCs) that express low levels of CAR but high levels of integrins (Hidaka et al., 1999; Majhen and Ambriovic-Ristov, 2006; Okada et al., 2001; Staba et al., 2000; Wickham et al., 1997).

2.3. CD46

Membrane cofactor protein (MCP) or CD46 is a ubiquitously expressed type I transmembrane glycoprotein, and its biological function is to prevent complement activation on the autologous tissue by binding and inactivating C3b and C4b (Liszewski et al., 2005). CD46 mainly consists of an amino-terminal extracellular domain comprising of four modules, termed as short consensus repeats (SCR)—SCR I, SCR II, SCR III and SCR IV, one to three Ser-Thr-Pro (STP) rich domain/s, a short region of unknown function, a hydrophobic transmembrane domain and a carboxy-terminal cytoplasmic tail (Russell, 2004). Owing to alternative RNA splicing in the STP region and cytoplasmic domain, four major CD46 isoforms (BC1, BC2, C1 and C2), in addition to minor splice variants, are co-expressed in most tissues (Liszewski et al., 2005). Interestingly, CD46 is also referred to as “pathogen magnet” since besides subgroup B HAds, it also acts as a receptor for a number of other human pathogens

such as measles virus, herpesvirus 6, bovine viral diarrhea virus, *Streptococcus pyogenes*, *Neisseria gonorrhoeae*, *N. meningitides* and *Helicobacter pylori*, each recognizing a different structure on the CD46 ectodomain (Cattaneo, 2004; Lindahl et al., 2000).

Earlier studies had demonstrated that neither subgroup B HAds cross-competed with HAd virions from other serogroups (A, C, D, E and F) for cell receptors, nor they interacted with a soluble recombinant CAR, suggesting that they utilized different cellular receptor/s for internalization (Akiyama et al., 2004; Defer et al., 1990; Stevenson et al., 1995). Subsequently, CD46 was identified as a cellular receptor for the majority of subgroup B HAds including HAd3, HAd7, HAd16, HAd21 and HAd50 (subspecies B1), and HAd11, HAd14, HAd34 and HAd35 (subspecies B2) (Fleischli et al., 2007; Gaggar et al., 2003; Segerman et al., 2003a,b; Sirena et al., 2004). CD46 usage by HAd3 and HAd7 remains controversial (Fleischli et al., 2007; Gustafsson et al., 2006; Marttila et al., 2005; Tuve et al., 2006). These discrepancies could be due to the variation in cell types, CD46 expression levels, different isoforms of CD46, or the involvement of additional receptors. It has been suggested that HAd3 and HAd7 engage CD46 via similar binding sites as those by HAd11 and HAd35, but antibody or soluble CD46 competition experiments showed differences in CD46 binding by HAd3/HAd7 and HAd11/HAd35 (Fleischli et al., 2007). In addition, subgroup D HAds (e.g. HAd37 and HAd49) have also been suggested to use CD46 as an attachment receptor (Lemckert et al., 2006; Wu et al., 2004).

Antibody mapping, competition assays, the use of CD46 mutants and the crystal structure of HAd11 knob complexed with the knob-binding region of CD46 have unraveled the interaction of subgroup B HAds with CD46. It was demonstrated that the SCR II domain was crucial for the binding and infection with HAd35 or HAd11 fiber-bearing vectors, although SCR I is also required to maintain SCR II in a conformation that favors virus binding (Sakurai et al., 2006; Shayakhmetov et al., 2005b). Crystallographic studies have shown that binding of HAd11 to CD46 is accompanied by profound change in CD46 conformation as it gets straightened into a rod-like shape from its bent native form. This conformational change further exposes the hidden residues in CD46 for binding to the HAd11 knob. Three major contact regions (designated as A, B and C) within the HI, DG and IJ loops of HAd11 knob together with residues critical for CD46-binding were identified (Persson et al., 2007). Critical residues of the HAd35 fiber knob likely to be involved in CD46-binding were also identified (Power et al., 2007). The crystal structure of HAd35 fiber knob was solved and a model of the fiber knob complexed with CD46 was generated (Pache et al., 2008). Despite certain structural differences in CD46-binding regions of HAd35 and HAd11, both Ads exhibited similar binding mechanism and affinity.

Numerous studies have suggested the existence of additional receptor/s for some of the subgroup B HAds (Marttila et al., 2005; Segerman et al., 2003a; Tuve et al., 2006). The identity of an additional receptor, which is distinct from CD46, remains elusive and has been referred to as species B HAd receptor (sBAR) or 'receptor X'. This elusive receptor is expressed at high levels on human mesenchymal and undifferentiated embryonic stem cells as well as on a variety of tumor cell lines, which are potential targets for gene therapy and stem cell research (Tuve et al., 2006). An alternative classification of subgroup B HAds based on their receptor usage has also been proposed (Tuve et al., 2006). Group I HAds (HAd16, HAd21, HAd35 and HAd50) almost exclusively use CD46; Group II HAds (HAd3, HAd7 and HAd14) utilize 'receptor X' but not CD46 and Group III HAd (HAd11) use both CD46 and 'receptor X'. Chimpanzee Ad type 1 (AdC1), which is closely related to B2 subgroup HAds, unlike other chimpanzee Ad serotypes (AdC5, AdC6, AdC7 and AdC68), utilizes CD46 for cell entry but not CAR (Tatsis et al., 2007).

Vectors derived from subgroup B HAds or pseudotyped chimeric vectors having fibers from subgroup B HAds can efficiently transduce cell types that are refractory to transduction by traditional HAd5-based vectors including malignant cancer cells, hematopoietic or mesenchymal stem cells, smooth muscle cells, human bone marrow stromal cells, synoviocytes, lymphocytes and DCs. Ad vectors utilizing CD46 as a receptor demonstrated reduced ability to induce interleukin (IL)-12 and other proinflammatory cytokines as compared to CAR-utilizing Ads, thereby dampening the immune response against Ads (Iacobelli-Martinez et al., 2005). Therefore, CD46-utilizing vectors could significantly improve the duration of transgene expression in the target tissues.

Because of the lack of CD46 expression on rodent cells and low homology between human and rodent CD46, rodents do not serve as an ideal model for subgroup B HAds. CD46 transgenic mouse models that have CD46 expression profile similar to monkeys and humans, have been suggested as a suitable preclinical model for CD46-binding Ad vectors (Sakurai et al., 2006; Tatsis et al., 2007; Verhaagh et al., 2006).

2.4. CD80/86

CD80 (B7-1) and CD86 (B7-2) are type I glycoproteins and members of the Ig superfamily comprising of two extracellular Ig-like domains linked to a transmembrane domain and a cytoplasmic tail (Greenwald et al., 2005). Both CD80 and CD86 are expressed on the surface of antigen-presenting cells (APCs), including DCs and B lymphocytes, and act as co-stimulatory signals for activation of cell-mediated immune response by binding to CD28 and cytotoxic T lymphocyte antigen-4 (CTLA-4) molecules (Greenwald et al., 2005). Members of subgroup B HAds (both B1 and B2 Ads) specifically bind to and infect cells that express CD80 and CD86 (Short et al., 2004, 2006). Tropism of subgroup B HAds to the cells of hematopoietic origin and neoplastic cells is due to high levels of CD80/86 expression on these cells (Davidoff et al., 1999; Kanerva et al., 2002; Knaan-Shanzer et al., 2001; Rea et al., 2001; Short et al., 2004).

Since DCs are the most potent APCs, upregulation of CD80/86 molecules on mature DCs and their enhanced transduction by subgroup B HAds further highlight the potential of such vectors for vaccine and cancer gene therapy. Furthermore, transduction of DCs may also allow Ad vector to escape the host immune surveillance and to modulate the host immune responses (Rea et al., 2001). However, the effect of Ad transduction on biological function of CD80/86 and its implication on T cell immune responses are still unknown.

CD80/86 are distinct from the unknown 'receptor X' of subgroup B HAds (Tuve et al., 2006). The involvement of CD80/86, in addition to CD46 and 'receptor X', as receptors for Ad internalization, further adds to the complexity of the receptor usage by subgroup B HAds. Further understanding of subgroup B HAd internalization will pave the way for the design of novel Ad vectors for gene delivery.

2.5. Sialic acid

Sialic acid refers to N- or O-substituted derivatives of neuraminic acid, which are usually found in gangliosides and glycoproteins. Due to their negative charge and external position on glycoproteins and gangliosides as well as on the outer cell membranes, sialic acid has the potential to be the critical component of ligands for recognition by specific viruses. Sialic acid is known to be used by influenza virus, rotavirus, coronavirus and polyomavirus as a cellular receptor, although these viruses greatly differ in their interaction with sialic acid (Dormitzer et al., 2002; Stehle and Harrison, 1997; Weis et al., 1988). Several members of subgroup D HAds (HAd8, HAd19a, HAd37) have tropism for the eyes and are frequently associated with epidemic keratoconjunctivitis (EKC) (Bell et al., 1959; Bennett et al., 1957; Hierholzer et al., 1974; Liszewski et al., 2005; Rekhter et al., 1998). These EKC-causing serotypes were demonstrated to

use sialic acid as a cellular receptor (Arnberg et al., 2000a,b, 2002). On the contrary, closely related HAd9 and HAd19p (subgroup D HAds) do not cause EKC and neither use sialic acid as a receptor. The predicted isoelectric points of the knobs of sialic acid-binding HAd serotypes are at least 2 logs higher than those of other HAds implying that the electric charge can play a key role in knob–sialic acid interactions (Arnberg et al., 2002). The binding of HAd37 to sialic acid was shown to be sensitive to salt and negatively charged compounds that further supported the importance of the electric charge in Ad-receptor interaction (Arnberg et al., 2002). The knob of HAd19p (without ocular tropism) differs from HAd37 knob (with ocular tropism) only at two positions (Glu240Lys and Asp340Asn) that result in partial loss of unusually high positive charge from the HAd37 knob (Burmeister et al., 2004; Huang et al., 1999). The amino acid alignment and crystal structure of subgroup D HAds revealed the conservation of the sialic acid-binding site located on the top of the knob that does not overlap with the CAR-binding site at the side of the knob (Burmeister et al., 2004). Based on these findings, a multivalent sialic acid has been demonstrated to aggregate and neutralize HAd37 virions and has been proposed as a potential antiviral drug for treatment of EKC (Johansson et al., 2007). Furthermore, the sialic acid utilizing Ads or chimeric HAd5 vectors with fiber/knobs derived from subgroup D Ads have demonstrated expanded tropism to cell types such as hematopoietic cells including DCs, otherwise considered refractory to transduction by CAR-utilizing HAd vectors.

2.6. Proteoglycans (PGs)

Proteoglycans (PGs) are ubiquitously expressed glycoproteins that consist of a protein core with one or more covalently attached glycosaminoglycan (GAG) chains. GAGs are long, negatively charged, linear, carbohydrate polymers with variably sulfated repeating disaccharide units. PGs form the major component of extracellular matrix (ECM) and are involved in numerous biological functions such as cellular attachment, proliferation and differentiation, embryonic development, blood coagulation, and receptor-mediated endocytosis (Bishop et al., 2007). Because of the wide prevalence of PGs, several pathogens have evolved to exploit them as an attachment or internalization receptor. Heparan sulfate proteoglycans (HSPGs) were shown to be involved in the attachment and infection of HAd2 and HAd5 and a consensus HSPG-binding sequence [Lys-Lys-Thr-Lys (KKTK) motif] in the fiber shaft was suggested to be responsible for virus interaction with the cell surface HSPGs (Dechecchi et al., 2001, 2000).

Since hepatocytes are rich in the surface expression of HSPGs, enhanced liver transduction was attributed to the fiber-shaft KKTK motif and HSPG interaction. Mutation in the putative HSPG-binding motif of Ad resulted in significant reduction in liver transduction (Smith et al., 2003a,b). This reduction in transduction was more pronounced when KKTK mutation was combined with CAR and/or integrin-binding ablation (Nicol et al., 2004). However, Ad hepatotropism appeared to be receptor-independent and ablation of CAR and/or integrin-binding did not result in significant reduction in HAd5 liver transduction, despite decrease in transduction of hepatocytes in vitro (Nicklin et al., 2005).

Despite several studies on HSPGs and Ad interaction, there is still no clear evidence to implicate the role of KKTK motif in the liver-targeting by Ad vectors. Moreover, the Ad fiber KKTK motif has not been experimentally shown to bind to HSPGs nor Ad have vectors been found to be associated with HSPGs. Due to the remarkably poor hepatotropism, KKTK mutant vectors initially appeared to be good candidates for developing retargeted vectors, but they were unable to efficiently transduce susceptible cells in vitro or in vivo (Smith et al., 2003a,b). Furthermore, incorporation of retargeting ligands such as the integrin-binding RGD motif or the endothelial cell targeting Gln-Pro-Glu-His-Ser-Ser-Thr (QPEHSST) peptide in the HI loop of shaft-mutated Ad fiber fails to improve virus infectivity in cell lines that express high levels of integrins or to the endothelial cells (Bayo-Puxan et al., 2006; Kritz et al., 2007). This diminished retargeting could be either due to the effect of mutation on the fiber structure and/or stability presumably because the KKTK motif is positioned adjacent to the flexibility-imparting domain of the fiber shaft, or mutation in the KKTK motif might interfere with post-attachment processes such as virion endocytosis, endosomal lysis and escape, and nuclear translocation (Di Paolo et al., 2007; Kritz et al., 2007). Since KKTK mutant Ads showed only attenuated transduction of susceptible cells, Di Paolo et al. (2007) employed an indirect alternative strategy to investigate the potential role of the KKTK motif in liver transduction. They generated fiber-shaft chimeric HAd5-based vectors possessing fiber-shaft domain derived from HAd31 or HAd41 that lacked the KKTK motif, but could recognize CAR as an attachment receptor (Di Paolo et al., 2007). No reduction in the efficiency of liver transduction by fiber-shaft chimeric vectors was observed compared to unmodified HAd5 vectors, suggesting that KKTK motif-HSPG interaction is unlikely to mediate Ad hepatotropism. Clearly, further studies are necessary to elucidate the exact role of the KKTK motif in various steps of virus entry. In a suggested alternative CAR-independent pathway, certain blood factors (see below) can act as a bridge to link Ad to hepatocellular HSPGs [or low-density lipoprotein (LDL) receptor-related protein] to mediate enhanced liver transduction (Shayakhmetov et al., 2005b).

A recent study has identified HSPGs as low affinity, sulfation-dependent ligands to HAd3 and HAd35 (both subgroup B HAds) (Tuve et al., 2008). HAd3 interacted with HSPGs via the knob while HAd35 interaction to HSPGs was via other unknown viral protein/s. It was observed that PGs were not the absolute requirement for virus attachment; instead these vectors exploit ubiquitous HSPGs in order to gain better access to other high affinity and preferred attachment receptor/s (such as CD46, and 'receptor X'). Remarkable differences in their binding affinities suggest that HSPGs most likely do not represent the unidentified 'receptor X' for subgroup B HAds.

2.7. Major histocompatibility complex class I (MHC-I)

Major histocompatibility complex class I (MHC-I) molecules are the cell surface peptide-binding and antigen-presenting glycoproteins that consist of a polymorphic heavy chain non-covalently linked to an invariant chain (β-2-microglobulin; β2m) (Bjorkman and Parham, 1990). Reverse antibody biopanning of a phage display library was employed to identify mimotopes of the fiber protein receptor and the α2 domain of the heavy chain of MHC-I was proposed to be involved in primary binding of HAd2 and HAd5 fiber (Hong et al., 1997). The expression of MHC-I on a lymphoblastoid cell line resulted in increased fiber binding and Ad-mediated gene transfer as compared to the cells that lacked MHC-I expression (Hong et al., 1997). However, involvement of MHC-I in Ad attachment to susceptible cells remains unclear (Davison et al., 1999; McDonald et al., 1999). Human leukocyte antigen (HLA, human MHC system) and CAR were co-expressed on Chinese hamster ovary (CHO) cells, and it was found that HAd5 fiber bound to a single high affinity CAR receptor and not to HLA (Davison et al., 1999). It was suggested that MHC-I molecules may play a role in Ad attachment and internalization only in the absence of or low availability of CAR. Alternatively, MHC-I can directly or indirectly assist to increase the CAR accessibility to the Ad fiber (Davison et al., 1999).

2.8. Vascular cell adhesion molecule-1 (VCAM-1)

Vascular cell adhesion molecule-1 (VCAM-1) is a type I membrane sialoglycoprotein expressed by cytokine-activated

endothelium that mediates leukocyte-endothelial cell adhesion and signal transduction, and may play a role in the development of atherosclerosis (Osborn et al., 1989). Similar to CAR, VCAM-1 is also an Ig superfamily protein that shares modest level of homology with CAR (Chu et al., 2001). Ad-mediated gene transfer to vascular endothelium has been observed to be more effective in atherosclerotic vessels as compared to undamaged vessels (Ooboshi et al., 1997; Rekhter et al., 1998). Increased surface expression of VCAM-1 on atherosclerotic vessels was suggested to be responsible, in part, for augmented Ad transduction (Chu et al., 2001). Constitutive expression of VCAM-1 in murine fibroblast (NIH 3T3) cells resulted in modest increase in Ad binding, as compared to HAd5 infection in parental NIH 3T3 cells (Chu et al., 2001).

Other unidentified molecules of Ig superfamily that share some homology with CAR could possibly act as auxiliary low-affinity receptors that may assist to improve Ad-mediated gene transfer to CAR-deficient cells.

3. Role of blood factors in adenoviral tropism

In vivo tropism of Ad differs remarkably from its in vitro tropism. In cell culture systems, Ad follows the classical two-step process for internalization but in vivo, a multitude of host factors significantly modulate the tropism and biodistribution of the systemically inoculated Ad vector. Increased transduction of hepatocytes following systemic administration appeared to be independent of Ad primary receptors as abolition of CAR- and/or integrin-Ad interactions has not been successful to modify Ad tropism (Nicklin et al., 2005). Shayakhmetov et al. utilized *in situ* liver perfusion technique to investigate Ad-mediated hepatocyte transduction in the presence or absence of blood and demonstrated that coagulation factor IX and complement component C4-binding protein can bind to the Ad fiber knob and can act as a link for virus uptake by hepatocytes through HSPG or LDL receptor-related protein (Shayakhmetov et al., 2005b). Furthermore, mutations in the fiber knob that ablate blood factor-binding, significantly reduced the transduction of the liver cells (Shayakhmetov et al., 2005b). In a subsequent study, additional vitamin K-dependent blood coagulation factors (FVII, FIX, FX and protein C) that share a common domain structure, were implicated in HAd5 transduction of hepatocytes (Parker et al., 2006). Downregulation of vitamin K-dependent zymogens by warfarin resulted in remarkable reduction in hepatocellular transduction and FX infusion and restored Ad transduction of hepatocytes (Parker et al., 2006). Recently, two independent studies utilizing cryoelectron microscopy and surface plasmon resonance analysis have demonstrated that HAd5 hexon protein (not fiber protein) binds to FX and this interaction is mainly responsible for Ad localization to hepatocytes (Kalyuzhniy et al., 2008; Waddington et al., 2008). FX-binding sites were identified in the HVR of the hexon protein and mutations in the hexon or swapping of HAd5 HVR with that of non-FX-binding Ad serotype (HAd48) resulted in substantial reduction in HAd5 liver tropism (Kalyuzhniy et al., 2008; Waddington et al., 2008). It was also suggested that FX forms a mesh that covers most of the Ad surface and may sterically inhibit fiber-mediated interactions with other receptors. There was a significant variation among Ad serotypes in their ability to bind FX that correlated with their ability to transduce hepatocytes. These findings provide new insights regarding hepatotropism of Ad vectors and further investigations in this direction would pave the way for the development of safe and tissue-specific Ad vectors for gene delivery.

4. Strategies to modify adenoviral tropism

Systemic administration is necessary to harness the full potential of Ad vectors in gene delivery applications. In vitro tropism of Ad vectors does not necessarily correlate with their in vivo tropism.

Table 2
Some examples of strategies for modification of Ad tropism.

Ad vector	Tropism altering modification	Basis of altered tropism	Cell type	Response	References
Ad5-pk7	Polylysine (pK7) motif on HAd5 knob	Enhanced transduction of cancer cells with high surface HSPGs	Human glioma xenografts in mouse model	Higher transduction and marker gene expression	Zheng et al. (2007)
Ad5/3-RGD	HAd5 with HAd3 knob containing RGD domain	Enhanced transduction of cancer cells with high integrin expression	Human glioma xenografts in mouse model	1000-fold increased infectivity	Ulasov et al. (2006)
Ad5Luc1-CK1	HAd5 containing CAV-1 knob	Enhanced CAV knob-mediated, CAR-independent vector transduction	Ovarian cancer cell lines/ovarian cancer patients Primary tissue slice samples	Superior transduction of cancer cells	Stoff-Khalili et al. (2005)
CAV-2 (canine Ad)	Nonhuman Ad with alternate tropism	Use of alternate/distinct receptors	In vivo mouse respiratory tract Ex vivo human pulmonary epithelia	Efficient transduction of respiratory epithelia Escape HAd5 immunity Low inflammation	Keriel et al. (2006)
HAd5	mEGF-polymer coating	Selective transduction of EGFR rich cancer cells	Peritoneal xenograft model of human ovarian cancer in mouse model	Restricted vector tropism and toxicity and enhanced antitumor efficacy	Morrison et al. (2008)
HAd5	Bispecific antibody (Ab) targeting HAd fiber knob and human endoglin	Bispecific Ab bridge Ad to vascular endoglin upregulated in angiogenic areas of tumors	Primary endothelial cells and HUVEC cell line	Enhanced, selective and CAR-independent transduction of HUVEC	Nettelbeck et al. (2001)

Abbreviations: CAV: canine adenovirus; mEGF: murine epidermal growth factor; EGFR: EGF receptor; HUVEC: human umbilical vein endothelial cells; HSPG: heparan sulfate proteoglycans; Ab: antibody.

Moreover, natural tropism of Ads usually does not always match the therapeutic requirements. Several investigators are developing strategies to ablate the native tropism of Ad vectors and introduce novel tropism towards target cells. Numerous strategies for retargeting Ad vectors have been proposed and investigated with variable efficacies (Table 2). One of the approaches is physical targeting, in which the virus surface is coated with polymers such as polyethylene glycol, poly-[N-(2-hydroxypropyl)methacrylamide] (pHPMA) or biodegradable alginate microparticles (Croyle et al., 2000; Fisher et al., 2001; Kreppel and Kochanek, 2008; Sailaja et al., 2002). This modification ablates the native tropism of the vector besides shielding it from the host immune response. Selective targeting could be achieved by attachment of a variety of targeting ligands (peptides, proteins or antibodies) to these polymers (Eto et al., 2008; Kreppel and Kochanek, 2008; Morrison et al., 2008; Stevenson et al., 2007). Another effective strategy for physical targeting is the use of bispecific adaptor molecules (including bispecific antibodies or fusion proteins) that consist of two components—one that binds with high affinity to the fiber knob and the other that binds with high specificity with a target tissue-specific receptor (Dmitriev et al., 2000; Douglas et al., 1996; Haisma et al., 2000, 1999; Nettelbeck et al., 2001; Parrott et al., 2003). In this strategy, however, the two-component nature of bispecific molecule adds complications in manufacturing such vectors and also in maintaining batch-to-batch homogeneity. Furthermore, as these modifications are not genetic, the progeny virions would be devoid of such modifications. Therefore, genetic modification of the capsid proteins is a more favored option.

In genetic targeting, the fiber knob, being the receptor-seeking moiety, is chosen to incorporate foreign targeting ligands. Two locations (C terminus and HI loop of HAd5 fiber knob) have been identified that accept such modifications with least constraints. Incorporation of RGD or polylysine (pK7) ligands on these locations led to enhancement of Ad infectivity to a wide variety of target cells (for example tumors cells and DCs) that overexpress integrins or HSPGs, respectively (Dmitriev et al., 1998; Koizumi et al., 2003; Wickham et al., 1996; Wu et al., 2002). Besides fiber knob, other capsid proteins such as hexon, penton, pIX or pIII have also been investigated to alter the vector tropism by cell-specific ligand incorporation (Dmitriev et al., 2002; Glasgow et al., 2006; Vellinga et al., 2004; Vigne et al., 1999; Wu et al., 2005). Utilizing affibodies or library screening approaches, cell-specific targeting ligands can be identified that can be incorporated to Ad capsid to form stable virions (Belousova et al., 2008; Henning et al., 2002; Nord et al., 1997).

Another insightful scheme to confer novel tropism to HAd5-derived vectors is the substitution of the fiber/knob with that of other Ad serotypes that utilize non-CAR receptors for their internalization (this approach is also known as pseudotyping). Chimeric HAd5 vectors carrying fiber/knob from several other HAd serotypes (HAd35, HAd37, and HAd41) or nonhuman Ad such as, canine adenovirus (CAV) serotype 1, CAV2, and ovine adenovirus (OAV) serotype 8 have been generated, which showed improved transduction in ovarian cancer, malignant glioma, or head and neck cancer models (Breidenbach et al., 2004; Glasgow et al., 2004; Kanerva et al., 2002; Nakayama et al., 2006; Ni et al., 2006; Nicol et al., 2004; Rea et al., 2001; Stoff-Khalili et al., 2005; Ulasov et al., 2006; Zheng et al., 2007).

Despite their novel tropism and non-HAd5 fiber/knob components, chimeric Ad vectors can still get neutralized by HAd5 hexon-specific antibodies; therefore, some of the rare HAd serotypes (Stone and Lieber, 2006) and nonhuman Ads (Bangari and Mittal, 2006) are being developed and investigated as alternate vectors for gene delivery. These Ads are not prevalent in the human population and have distinct receptor usage, thus offering potential advantage over HAd5-based vectors. Vectors based on nonhuman Ads originally derived from pig (porcine adenovirus serotype 3; PAd3) or cattle (bovine adenovirus serotype 3; BAd3) have been developed (Bangari and Mittal, 2004; Mittal et al., 1995; Reddy et al., 1999a,b). It has been demonstrated that there are no preexisting cross-neutralizing antibodies against PAd3 or BAd3 in humans, and importantly, HAd5-neutralizing antibodies do not cross-neutralize PAd3 or BAd3 (Bangari et al., 2005b; Moffatt et al., 2000). PAd3 and BAd3 vectors can efficiently transduce human and murine cells in culture and internalization of these vectors was CAR- and integrin-independent (Bangari and Mittal, 2005; Bangari et al., 2005a,b). In vivo studies in mice also indicated the altered biodistribution pattern of BAd3 and PAd3 vectors as compared to HAd5 vector (Sharma et al., in press). Similarly, vectors derived from canine Ad, ovine Ad, chimpanzee Ad, murine Ad and fowl Ad are also being developed (Bangari and Mittal, 2006).

Recently, cell-based delivery of Ad vector is emerging as a novel delivery approach in which cells infected with Ad in vitro carry the Ad vector to the target tissue (Power et al., 2007). This non-receptor-mediated Ad transduction system prevents vector neutralization by anti-Ad antibodies and elicits the desired therapeutic effect (Power et al., 2007). Transcriptional targeting is another approach that involves the placement of critical viral transcription units or therapeutic gene with the target tissue-specific regulatory elements (TREs) (Nettelbeck, 2008). As a result, the expression of therapeutic gene and/or virus replication is expected to occur selectively or preferentially in target cells. The early gene 1A (E1A) of Ad is the most common choice to be controlled by TREs as it is expressed first and is essential for viral replication, but other essential early genes (E1B, E2, and E4), either alone or in combination, have also been exogenously controlled to impart tissue specificity to Ad vectors (Doronin et al., 2001; Kawashima et al., 2004; Ko et al., 2005; Kuppuswamy et al., 2005; Li et al., 2005; Rodriguez et al., 1997).

5. Conclusions

Similar to many other viruses, Ads have evolved to utilize redundant receptors abundantly expressed on a wide variety of cells throughout the body for cell invasion. Various studies have unraveled a variety of cell surface molecules involved in Ad entry by demonstrating interaction with viral capsid proteins. In this review, we discussed some of these cellular surface molecules that include CAR, integrins, CD46, CD80/86, sialic acid, proteoglycans (HSPGs), MHC-I and VCAM-1. Identification of additional Ad receptors that have eluded recognition since long will further widen the repertoire of Ad receptors and would be of importance to unravel the complexities of virus tropism and pathogenesis. Moreover, the knowledge of Ad and cell receptor interaction enables us to design specific vectors to target a specific tissue or an organ by ablation of the natural tropism and/or incorporation of new ligands, which may lead to reduction in the vector dose and in vivo toxicity while evading preexisting immune responses to Ad vectors.

To date, numerous strategies have been investigated to modulate the tropism of Ad vectors that have resulted in improved safety and efficacy as evident by promising preclinical as well as clinical data (Aghi and Martuza, 2005; Rein et al., 2006). Differences in the receptor usage by Ad serotypes provide the unique opportunity to exploit the natural diversity in Ad tropism in designing vectors for diverse gene therapy applications. It is critical to identify suitable vector candidates to specifically and efficiently target important cell types for preventive or therapeutic gene delivery applications. Notably, most of the receptors identified to date utilized by Ad belong to Ig superfamily. Additional cell surface components that are similar in structure and share homology with identified receptors may potentially function as at least low affinity attachment receptors either alone or in combinations with multiple molecules, to stabilize the virus particle and facilitate its accessibility to the internalization

receptors on the cell surface. Though the receptor binding is thought to be one of the key determinants of Ad tissue tropism, it is not sufficient to explain all aspects of in vivo host–virus interactions. For instance, enhanced transduction of liver cells or Ad uptake by Kupffer cells appear to be independent of the receptor usage. Better understanding of structural and functional interactions between Ad and host cells/proteins is required for rational design of more effective and safe vectors. In addition, the knowledge of Ad virus–cell interactions could aid in making improvements to other vector systems such as nonviral vectors that utilize Ad translocation pathways to obtain effective gene or drug transfer.

Acknowledgements

This work was funded by Public Health Service grant CA110176 from the National Cancer Institute. We are grateful to Jane Kovach for her secretarial assistance. We apologize to other researchers whose work we failed to include in this review mainly because of unintended overlook.

References

Aghi, M., Martuza, R.L., 2005. Oncolytic viral therapies—the clinical experience. Oncogene 24, 7802–7816.
Akiyama, M., Thorne, S., Kirn, D., Roelvink, P.W., Einfeld, D.A., King, C.R., Wickham, T.J., 2004. Ablating CAR and integrin binding in adenovirus vectors reduces nontarget organ transduction and permits sustained bloodstream persistence following intraperitoneal administration. Mol. Ther. 9, 218–230.
Albinsson, B., Kidd, A.H., 1999. Adenovirus type 41 lacks an RGD alpha(v)-integrin binding motif on the penton base and undergoes delayed uptake in A549 cells. Virus Res. 64, 125–136.
Arnberg, N., Edlund, K., Kidd, A.H., Wadell, G., 2000a. Adenovirus type 37 uses sialic acid as a cellular receptor. J. Virol. 74, 42–48.
Arnberg, N., Kidd, A.H., Edlund, K., Nilsson, J., Pring-Akerblom, P., Wadell, G., 2002. Adenovirus type 37 binds to cell surface sialic acid through a charge-dependent interaction. Virology 302, 33–43.
Arnberg, N., Kidd, A.H., Edlund, K., Olfat, F., Wadell, G., 2000b. Initial interactions of subgenus D adenoviruses with A549 cellular receptors: sialic acid versus alpha(v) integrins. J. Virol. 74, 7691–7693.
Asaoka, K., Tada, M., Sawamura, Y., Ikeda, J., Abe, H., 2000. Dependence of efficient adenoviral gene delivery in malignant glioma cells on the expression levels of the Coxsackievirus and adenovirus receptor. J. Neurosurg. 92, 1002–1008.
Bangari, D.S., Mittal, S.K., 2004. Porcine adenoviral vectors evade preexisting humoral immunity to adenoviruses and efficiently infect both human and murine cells in culture. Virus Res. 105, 127–136.
Bangari, D.S., Mittal, S.K., 2005. Porcine adenovirus serotype 3 internalization is independent of CAR and alphavbeta3 or alphavbeta5 integrin. Virology 332, 157–166.
Bangari, D.S., Mittal, S.K., 2006. Development of nonhuman adenoviruses as vaccine vectors. Vaccine 24, 849–862.
Bangari, D.S., Sharma, A., Mittal, S.K., 2005a. Bovine adenovirus type 3 internalization is independent of primary receptors of human adenovirus type 5 and porcine adenovirus type 3. Biochem. Biophys. Res. Commun. 331, 1478–1484.
Bangari, D.S., Shukla, S., Mittal, S.K., 2005b. Comparative transduction efficiencies of human and nonhuman adenoviral vectors in human, murine, bovine, and porcine cells in culture. Biochem. Biophys. Res. Commun. 327, 960–966.
Bao, Y., Peng, W., Verbitsky, A., Chen, J., Wu, L., Rauen, K.A., Sawicki, J.A., 2005. Human coxsackie adenovirus receptor (CAR) expression in transgenic mouse prostate tumors enhances adenoviral delivery of genes. Prostate 64, 401–407.
Bayo-Puxan, N., Cascallo, M., Gros, A., Huch, M., Fillat, C., Alemany, R., 2006. Role of the putative heparan sulfate glycosaminoglycan-binding site of the adenovirus type 5 fiber shaft on liver detargeting and knob-mediated retargeting. J. Gen. Virol. 87, 2487–2495.
Bell Jr., S.D., Mc, C.D., Murray, E.S., Chang, R.S., Snyder, J.C., 1959. Adenoviruses isolated from Saudi Arabia. I. Epidemiologic features. Am. J. Trop. Med. Hyg. 8, 492–500.
Belousova, N., Mikheeva, G., Gelovani, J., Krasnykh, V., 2008. Modification of adenovirus capsid with a designed protein ligand yields a gene vector targeted to a major molecular marker of cancer. J. Virol. 82, 630–637.
Bennett, F.M., Hamilton, W., Law, B.B., Macdonald, A., 1957. Adenovirus eye infections in Aberdeen. Lancet 273, 670–673.
Bergelson, J.M., Cunningham, J.A., Droguett, G., Kurt-Jones, E.A., Krithivas, A., Hong, J.S., Horwitz, M.S., Crowell, R.L., Finberg, R.W., 1997. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. Science 275, 1320–1323.
Bergelson, J.M., Krithivas, A., Celi, L., Droguett, G., Horwitz, M.S., Wickham, T., Crowell, R.L., Finberg, R.W., 1998. The murine CAR homolog is a receptor for coxsackie B viruses and adenoviruses. J. Virol. 72, 415–419.
Berk, A.J., 2007. Adenoviridae: the viruses and their replication. In: Fields, B.N., Knipe, D.M., Howley, P.M. (Eds.), Fields Virology, fifth ed. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philiadelphia, pp. 2355–2394.
Bewley, M.C., Springer, K., Zhang, Y.B., Freimuth, P., Flanagan, J.M., 1999. Structural analysis of the mechanism of adenovirus binding to its human cellular receptor, CAR. Science 286, 1579–1583.
Bishop, J.R., Schuksz, M., Esko, J.D., 2007. Heparan sulphate proteoglycans fine-tune mammalian physiology. Nature 446, 1030–1037.
Bjorkman, P.J., Parham, P., 1990. Structure, function, and diversity of class I major histocompatibility complex molecules. Annu. Rev. Biochem. 59, 253–288.
Breidenbach, M., Rein, D.T., Wang, M., Nettelbeck, D.M., Hemminki, A., Ulasov, I., Rivera, A.R., Everts, M., Alvarez, R.D., Douglas, J.T., Curiel, D.T., 2004. Genetic replacement of the adenovirus shaft fiber reduces liver tropism in ovarian cancer gene therapy. Hum. Gene Ther. 15, 509–518.
Burmeister, W.P., Guilligay, D., Cusack, S., Wadell, G., Arnberg, N., 2004. Crystal structure of species D adenovirus fiber knobs and their sialic acid binding sites. J. Virol. 78, 7727–7736.
Cattaneo, R., 2004. Four viruses, two bacteria, and one receptor: membrane cofactor protein (CD46) as pathogens' magnet. J. Virol. 78, 4385–4388.
Chen, J.W., Zhou, B., Yu, Q.C., Shin, S.J., Jiao, K., Schneider, M.D., Baldwin, H.S., Bergelson, J.M., 2006. Cardiomyocyte-specific deletion of the coxsackievirus and adenovirus receptor results in hyperplasia of the embryonic left ventricle and abnormalities of sinuatrial valves. Circ. Res. 98, 923–930.
Chiu, C.Y., Mathias, P., Nemerow, G.R., Stewart, P.L., 1999. Structure of adenovirus complexed with its internalization receptor, alphavbeta5 integrin. J. Virol. 73, 6759–6768.
Chretien, I., Marcuz, A., Courtet, M., Katevuo, K., Vainio, O., Heath, J.K., White, S.J., Du Pasquier, L., 1998. CTX, a Xenopus thymocyte receptor, defines a molecular family conserved throughout vertebrates. Eur. J. Immunol. 28, 4094–4104.
Chroboczek, J., Ruigrok, R.W., Cusack, S., 1995. Adenovirus fiber. Curr. Top. Microbiol. Immunol. 199 (Pt 1), 163–200.
Chu, Y., Heistad, D., Cybulsky, M.I., Davidson, B.L., 2001. Vascular cell adhesion molecule-1 augments adenovirus-mediated gene transfer. Arterioscler. Thromb. Vasc. Biol. 21, 238–242.
Cohen, C.J., Shieh, J.T., Pickles, R.J., Okegawa, T., Hsieh, J.T., Bergelson, J.M., 2001. The coxsackievirus and adenovirus receptor is a transmembrane component of the tight junction. Proc. Natl. Acad. Sci. U.S.A. 98, 15191–15196.
Cohen, C.J., Xiang, Z.Q., Gao, G.P., Ertl, H.C., Wilson, J.M., Bergelson, J.M., 2002. Chimpanzee adenovirus CV-68 adapted as a gene delivery vector interacts with the coxsackievirus and adenovirus receptor. J. Gen. Virol. 83, 151–155.
Crawford-Miksza, L., Schnurr, D.P., 1996. Analysis of 15 adenovirus hexon proteins reveals the location and structure of seven hypervariable regions containing serotype-specific residues. J. Virol. 70, 1836–1844.
Croyle, M.A., Yu, Q.C., Wilson, J.M., 2000. Development of a rapid method for the PEGylation of adenoviruses with enhanced transduction and improved stability under harsh storage conditions. Hum. Gene Ther. 11, 1713–1722.
Davidoff, A.M., Stevenson, S.C., McClelland, A., Shochat, S.J., Vanin, E.F., 1999. Enhanced neuroblastoma transduction for an improved antitumor vaccine. J. Surg. Res. 83, 95–99.
Davison, A.J., Benko, M., Harrach, B., 2003. Genetic content and evolution of adenoviruses. J. Gen. Virol. 84, 2895–2908.
Davison, E., Kirby, I., Elliott, T., Santis, G., 1999. The human HLA-A*0201 allele, expressed in hamster cells, is not a high-affinity receptor for adenovirus type 5 fiber. J. Virol. 73, 4513–4517.
Davison, E., Kirby, I., Whitehouse, J., Hart, I., Marshall, J.F., Santis, G., 2001. Adenovirus type 5 uptake by lung adenocarcinoma cells in culture correlates with Ad5 fibre binding is mediated by alpha(v)beta1 integrin and can be modulated by changes in beta1 integrin function. J. Gene Med. 3, 550–559.
Dechecchi, M.C., Melotti, P., Bonizzato, A., Santacatterina, M., Chilosi, M., Cabrini, G., 2001. Heparan sulfate glycosaminoglycans are receptors sufficient to mediate the initial binding of adenovirus types 2 and 5. J. Virol. 75, 8772–8780.
Dechecchi, M.C., Tamanini, A., Bonizzato, A., Cabrini, G., 2000. Heparan sulfate glycosaminoglycans are involved in adenovirus type 5 and 2-host cell interactions. Virology 268, 382–390.
Defer, C., Belin, M.T., Caillet-Boudin, M.L., Boulanger, P., 1990. Human adenovirus-host cell interactions: comparative study with members of subgroups B and C. J. Virol. 64, 3661–3673.
Di Paolo, N.C., Kalyuzhniy, O., Shayakhmetov, D.M., 2007. Fiber shaft-chimeric adenovirus vectors lacking the KKTK motif efficiently infect liver cells in vivo. J. Virol. 81, 12249–12259.
Dmitriev, I., Kashentseva, E., Rogers, B.E., Krasnykh, V., Curiel, D.T., 2000. Ectodomain of coxsackievirus and adenovirus receptor genetically fused to epidermal growth factor mediates adenovirus targeting to epidermal growth factor receptor-positive cells. J. Virol. 74, 6875–6884.
Dmitriev, I., Krasnykh, V., Miller, C.R., Wang, M., Kashentseva, E., Mikheeva, G., Belousova, N., Curiel, D.T., 1998. An adenovirus vector with genetically modified fibers demonstrates expanded tropism via utilization of a coxsackievirus and adenovirus receptor-independent cell entry mechanism. J. Virol. 72, 9706–9713.
Dmitriev, I.P., Kashentseva, E.A., Curiel, D.T., 2002. Engineering of adenovirus vectors containing heterologous peptide sequences in the C terminus of capsid protein IX. J. Virol. 76, 6893–6899.
Dormitzer, P.R., Sun, Z.Y., Wagner, G., Harrison, S.C., 2002. The rhesus rotavirus VP4 sialic acid binding domain has a galectin fold with a novel carbohydrate binding site. EMBO J. 21, 885–897.
Dorner, A.A., Wegmann, F., Butz, S., Wolburg-Buchholz, K., Wolburg, H., Mack, A., Nasdala, I., August, B., Westermann, J., Rathjen, F.G., Vestweber, D., 2005. Coxsackievirus-adenovirus receptor (CAR) is essential for early embryonic cardiac development. J. Cell Sci. 118, 3509–3521.

Doronin, K., Kuppuswamy, M., Toth, K., Tollefson, A.E., Krajcsi, P., Krougliak, V., Wold, W.S., 2001. Tissue-specific, tumor-selective, replication-competent adenovirus vector for cancer gene therapy. J. Virol. 75, 3314–3324.
Douglas, J.T., 2007. Adenoviral vectors for gene therapy. Mol. Biotechnol. 36, 71–80.
Douglas, J.T., Rogers, B.E., Rosenfeld, M.E., Michael, S.I., Feng, M., Curiel, D.T., 1996. Targeted gene delivery by tropism-modified adenoviral vectors. Nat. Biotechnol. 14, 1574–1578.
Durmort, C., Stehlin, C., Schoehn, G., Mitraki, A., Drouet, E., Cusack, S., Burmeister, W.P., 2001. Structure of the fiber head of Ad3, a non-CAR-binding serotype of adenovirus. Virology 285, 302–312.
Eto, Y., Yoshioka, Y., Mukai, Y., Okada, N., Nakagawa, S., 2008. Development of PEGylated adenovirus vector with targeting ligand. Int. J. Pharm. 354, 3–8.
Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U., Ball, L.A., 2005. Virus taxonomy: Eighth Report of the International Committee on the Taxonomy of Viruses. Elsevier Academic Press, San Diego.
Fechner, H., Haack, A., Wang, H., Wang, X., Eizema, K., Pauschinger, M., Schoemaker, R., Veghel, R., Houtsmuller, A., Schultheiss, H.P., Lamers, J., Poller, W., 1999. Expression of coxsackie adenovirus receptor and alphav-integrin does not correlate with adenovector targeting in vivo indicating anatomical vector barriers. Gene Ther. 6, 1520–1535.
Fisher, K.D., Stallwood, Y., Green, N.K., Ulbrich, K., Mautner, V., Seymour, L.W., 2001. Polymer-coated adenovirus permits efficient retargeting and evades neutralising antibodies. Gene Ther. 8, 341–348.
Fleischli, C., Sirena, D., Lesage, G., Havenga, M.J., Cattaneo, R., Greber, U.F., Hemmi, S., 2007. Species B adenovirus serotypes 3,7,11 and 35 share similar binding sites on the membrane cofactor protein CD46 receptor. J. Gen. Virol. 88, 2925–2934.
Freimuth, P., Springer, K., Berard, C., Hainfeld, J., Bewley, M., Flanagan, J., 1999. Coxsackievirus and adenovirus receptor amino-terminal immunoglobulin V-related domain binds adenovirus type 2 and fiber knob from adenovirus type 12. J. Virol. 73, 1392–1398.
Fuxe, J., Liu, L., Malin, S., Philipson, L., Collins, V.P., Pettersson, R.F., 2003. Expression of the coxsackie and adenovirus receptor in human astrocytic tumors and xenografts. Int. J. Cancer 103, 723–729.
Gaggar, A., Shayakhmetov, D.M., Lieber, A., 2003. CD46 is a cellular receptor for group B adenoviruses. Nat. Med. 9, 1408–1412.
Glasgow, J.N., Everts, M., Curiel, D.T., 2006. Transductional targeting of adenovirus vectors for gene therapy. Cancer Gene Ther. 13, 830–844.
Glasgow, J.N., Kremer, E.J., Hemminki, A., Siegal, G.P., Douglas, J.T., Curiel, D.T., 2004. An adenovirus vector with a chimeric fiber derived from canine adenovirus type 2 displays novel tropism. Virology 324, 103–116.
Greenwald, R.J., Freeman, G.J., Sharpe, A.H., 2005. The B7 family revisited. Annu. Rev. Immunol. 23, 515–548.
Gustafsson, D.J., Segerman, A., Lindman, K., Mei, Y.F., Wadell, G., 2006. The Arg279Gln [corrected] substitution in the adenovirus type 11p (Ad11p) fiber knob abolishes EDTA-resistant binding to A549 and CHO-CD46 cells, converting the phenotype to that of Ad7p. J. Virol. 80, 1897–1905.
Haisma, H.J., Grill, J., Curiel, D.T., Hoogeland, S., van Beusechem, V.W., Pinedo, H.M., Gerritsen, W.R., 2000. Targeting of adenoviral vectors through a bispecific single-chain antibody. Cancer Gene Ther. 7, 901–904.
Haisma, H.J., Pinedo, H.M., Rijswijk, A., der Meulen-Muileman, I., Sosnowski, B.A., Ying, W., Beusechem, V.W., Tillman, B.W., Gerritsen, W.R., Curiel, D.T., 1999. Tumor-specific gene transfer via an adenoviral vector targeted to the pan-carcinoma antigen EpCAM. Gene Ther. 6, 1469–1474.
Hemmi, S., Geertsen, R., Mezzacasa, A., Peter, I., Dummer, R., 1998. The presence of human coxsackievirus and adenovirus receptor is associated with efficient adenovirus-mediated transgene expression in human melanoma cell cultures. Hum. Gene Ther. 9, 2363–2373.
Henning, P., Magnusson, M.K., Gunneriusson, E., Hong, S.S., Boulanger, P., Nygren, P.A., Lindholm, L., 2002. Genetic modification of adenovirus 5 tropism by a novel class of ligands based on a three-helix bundle scaffold derived from staphylococcal protein A. Hum. Gene Ther. 13, 1427–1439.
Hidaka, C., Milano, E., Leopold, P.L., Bergelson, J.M., Hackett, N.R., Finberg, R.W., Wickham, T.J., Kovesdi, I., Roelvink, P., Crystal, R.G., 1999. CAR-dependent and CAR-independent pathways of adenovirus vector-mediated gene transfer and expression in human fibroblasts. J. Clin. Invest. 103, 579–587.
Hierholzer, J.C., Guyer, B., O'Day, D., Schaffner, W., 1974. Letter: Adenovirus type 19 keratoconjunctivitis. N. Engl. J. Med. 290, 1436.
Hong, S.S., Karayan, L., Tournier, J., Curiel, D.T., Boulanger, P.A., 1997. Adenovirus type 5 fiber knob binds to MHC class I alpha2 domain at the surface of human epithelial and B lymphoblastoid cells. EMBO J. 16, 2294–2306.
Huang, S., Kamata, T., Takada, Y., Ruggeri, Z.M., Nemerow, G.R., 1996. Adenovirus interaction with distinct integrins mediates separate events in cell entry and gene delivery to hematopoietic cells. J. Virol. 70, 4502–4508.
Huang, S., Reddy, V., Dasgupta, N., Nemerow, G.R., 1999. A single amino acid in the adenovirus type 37 fiber confers binding to human conjunctival cells. J. Virol. 73, 2798–2802.
Hynes, R.O., 1992. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 69, 11–25.
Iacobelli-Martinez, M., Nepomuceno, R.R., Connolly, J., Nemerow, G.R., 2005. CD46-utilizing adenoviruses inhibit C/EBPbeta-dependent expression of proinflammatory cytokines. J. Virol. 79, 11259–11268.
Johansson, S.M., Nilsson, E.C., Elofsson, M., Ahlskog, N., Kihlberg, J., Arnberg, N., 2007. Multivalent sialic acid conjugates inhibit adenovirus type 37 from binding to and infecting human corneal epithelial cells. Antiviral Res. 73, 92–100.
Kalyuzhniy, O., Di Paolo, N.C., Silvestry, M., Hofherr, S.E., Barry, M.A., Stewart, P.L., Shayakhmetov, D.M., 2008. Adenovirus serotype 5 hexon is critical for virus infection of hepatocytes in vivo. Proc. Natl. Acad. Sci. U.S.A. 105, 5483–5488.
Kanerva, A., Mikheeva, G.V., Krasnykh, V., Coolidge, C.J., Lam, J.T., Mahasreshti, P.J., Barker, S.D., Straughn, M., Barnes, M.N., Alvarez, R.D., Hemminki, A., Curiel, D.T., 2002. Targeting adenovirus to the serotype 3 receptor increases gene transfer efficiency to ovarian cancer cells. Clin. Cancer Res. 8, 275–280.
Kawashima, T., Kagawa, S., Kobayashi, N., Shirakiya, Y., Umeoka, T., Teraishi, F., Taki, M., Kyo, S., Tanaka, N., Fujiwara, T., 2004. Telomerase-specific replication-selective virotherapy for human cancer. Clin. Cancer Res. 10, 285–292.
Keriel, A., Rene, C., Galer, C., Zabner, J., Kremer, E.J., 2006. Canine adenovirus vectors for lung-directed gene transfer: efficacy, immune response, and duration of transgene expression using helper-dependent vectors. J. Virol. 80, 1487–1496.
Kim, M., Zinn, K.R., Barnett, B.G., Sumerel, L.A., Krasnykh, V., Curiel, D.T., Douglas, J.T., 2002. The therapeutic efficacy of adenoviral vectors for cancer gene therapy is limited by a low level of primary adenovirus receptors on tumour cells. Eur. J. Cancer 38, 1917–1926.
Kirby, I., Davison, E., Beavil, A.J., Soh, C.P., Wickham, T.J., Roelvink, P.W., Kovesdi, I., Sutton, B.J., Santis, G., 2000. Identification of contact residues and definition of the CAR-binding site of adenovirus type 5 fiber protein. J. Virol. 74, 2804–2813.
Kirby, I., Lord, R., Davison, E., Wickham, T.J., Roelvink, P.W., Kovesdi, I., Sutton, B.J., Santis, G., 2001. Adenovirus type 9 fiber knob binds to the coxsackie B virus-adenovirus receptor (CAR) with lower affinity than fiber knobs of other CAR-binding adenovirus serotypes. J. Virol. 75, 7210–7214.
Knaan-Shanzer, S., Van Der Velde, I., Havenga, M.J., Lemckert, A.A., De Vries, A.A., Valerio, D., 2001. Highly efficient targeted transduction of undifferentiated human hematopoietic cells by adenoviral vectors displaying fiber knobs of subgroup B. Hum. Gene Ther. 12, 1989–2005.
Ko, D., Hawkins, L., Yu, D.C., 2005. Development of transcriptionally regulated oncolytic adenoviruses. Oncogene 24, 7763–7774.
Koizumi, N., Mizuguchi, H., Utoguchi, N., Watanabe, Y., Hayakawa, T., 2003. Generation of fiber-modified adenovirus vectors containing heterologous peptides in both the HI loop and C terminus of the fiber knob. J. Gene Med. 5, 267–276.
Kojaoghlanian, T., Flomenberg, P., Horwitz, M.S., 2003. The impact of adenovirus infection on the immunocompromised host. Rev. Med. Virol. 13, 155–171.
Kreppel, F., Kochanek, S., 2008. Modification of adenovirus gene transfer vectors with synthetic polymers: a scientific review and technical guide. Mol. Ther. 16, 16–29.
Kritz, A.B., Nicol, C.G., Dishart, K.L., Nelson, R., Holbeck, S., Von Seggern, D.J., Work, L.M., McVey, J.H., Nicklin, S.A., Baker, A.H., 2007. Adenovirus 5 fibers mutated at the putative HSPG-binding site show restricted retargeting with targeting peptides in the HI loop. Mol. Ther. 15, 741–749.
Kuppuswamy, M., Spencer, J.F., Doronin, K., Tollefson, A.E., Wold, W.S., Toth, K., 2005. Oncolytic adenovirus that overproduces ADP and replicates selectively in tumors due to hTERT promoter-regulated E4 gene expression. Gene Ther. 12, 1608–1617.
Law, L.K., Davidson, B.L., 2002. Adenovirus serotype 30 fiber does not mediate transduction via the coxsackie-adenovirus receptor. J. Virol. 76, 656–661.
Law, L.K., Davidson, B.L., 2005. What does it take to bind CAR? Mol. Ther. 12, 599–609.
Leissner, P., Legrand, V., Schlesinger, Y., Hadji, D.A., van Raaij, M., Cusack, S., Pavirani, A., Mehtali, M., 2001. Influence of adenoviral fiber mutations on viral encapsidation, infectivity and in vivo tropism. Gene Ther. 8, 49–57.
Lemckert, A.A., Grimbergen, J., Smits, S., Hartkoorn, E., Holterman, L., Berkhout, B., Barouch, D.H., Vogels, R., Quax, P., Goudsmit, J., Havenga, M.J., 2006. Generation of a novel replication-incompetent adenoviral vector derived from human adenovirus type 49: manufacture on PER.C6 cells, tropism and immunogenicity. J. Gen. Virol. 87, 2891–2899.
Leopold, P.L., Crystal, R.G., 2007. Intracellular trafficking of adenovirus: many means to many ends. Adv. Drug Deliv. Rev. 59, 810–821.
Li, E., Brown, S.L., Stupack, D.G., Puente, X.S., Cheresh, D.A., Nemerow, G.R., 2001. Integrin alpha(v)beta1 is an adenovirus coreceptor. J. Virol. 75, 5405–5409.
Li, X., Zhang, Y.P., Kim, H.S., Bae, K.H., Stantz, K.M., Lee, S.J., Jung, C., Jimenez, J.A., Gardner, T.A., Jeng, M.H., Kao, C., 2005. Gene therapy for prostate cancer by controlling adenovirus E1a and E4 gene expression with PSES enhancer. Cancer Res. 65, 1941–1951.
Li, Y., Pong, R.C., Bergelson, J.M., Hall, M.C., Sagalowsky, A.I., Tseng, C.P., Wang, Z., Hsieh, J.T., 1999. Loss of adenoviral receptor expression in human bladder cancer cells: a potential impact on the efficacy of gene therapy. Cancer Res. 59, 325–330.
Lindahl, G., Sjobring, U., Johnsson, E., 2000. Human complement regulators: a major target for pathogenic microorganisms. Curr. Opin. Immunol. 12, 44–51.
Liszewski, M.K., Kemper, C., Price, J.D., Atkinson, J.P., 2005. Emerging roles and new functions of CD46. Springer Semin. Immunopathol. 27, 345–358.
Luo, B.H., Carman, C.V., Springer, T.A., 2007. Structural basis of integrin regulation and signaling. Annu. Rev. Immunol. 25, 619–647.
Majhen, D., Ambriovic-Ristov, A., 2006. Adenoviral vectors—how to use them in cancer gene therapy? Virus Res. 119, 121–133.
Majhen, D., Nemet, J., Richardson, J., Gabrilovac, J., Hajsig, M., Osmak, M., Eloit, M., Ambriovic-Ristov, A., 2009. Differential role of alpha(v)beta(3) and alpha(v)beta(5) integrins in internalization and transduction efficacies of wild type and RGD4C fiber-modified adenoviruses. Virus Res. 139, 64–73.
Marttila, M., Persson, D., Gustafsson, D., Liszewski, M.K., Atkinson, J.P., Wadell, G., Arnberg, N., 2005. CD46 is a cellular receptor for all species B adenoviruses except types 3 and 7. J. Virol. 79, 14429–14436.
Mathias, P., Galleno, M., Nemerow, G.R., 1998. Interactions of soluble recombinant integrin alphav beta5 with human adenoviruses. J. Virol. 72, 8669–8675.
McDonald, D., Stockwin, L., Matzow, T., Blair Zajdel, M.E., Blair, G.E., 1999. Coxsackie and adenovirus receptor (CAR)-dependent and major histocompatibility com-

plex (MHC) class I-independent uptake of recombinant adenoviruses into human tumour cells. Gene Ther. 6, 1512–1519.
Mittal, S.K., Prevec, L., Graham, F.L., Babiuk, L.A., 1995. Development of a bovine adenovirus type 3-based expression vector. J. Gen. Virol. 76 (Pt 1), 93–102.
Moffatt, S., Hays, J., HogenEsch, H., Mittal, S.K., 2000. Circumvention of vector-specific neutralizing antibody response by alternating use of human and non-human adenoviruses: implications in gene therapy. Virology 272, 159–167.
Morrison, J., Briggs, S.S., Green, N., Fisher, K., Subr, V., Ulbrich, K., Kehoe, S., Seymour, L.W., 2008. Virotherapy of ovarian cancer with polymer-cloaked adenovirus retargeted to the epidermal growth factor receptor. Mol. Ther. 16, 244–251.
Nakayama, M., Both, G.W., Banizs, B., Tsuruta, Y., Yamamoto, S., Kawakami, Y., Douglas, J.T., Tani, K., Curiel, D.T., Glasgow, J.N., 2006. An adenovirus serotype 5 vector with fibers derived from ovine atadenovirus demonstrates CAR-independent tropism and unique biodistribution in mice. Virology 350, 103–115.
Nalbantoglu, J., Larochelle, N., Wolf, E., Karpati, G., Lochmuller, H., Holland, P.C., 2001. Muscle-specific overexpression of the adenovirus primary receptor CAR overcomes low efficiency of gene transfer to mature skeletal muscle. J. Virol. 75, 4276–4282.
Nemerow, G.R., Stewart, P.L., 1999. Role of alpha(v) integrins in adenovirus cell entry and gene delivery. Microbiol. Mol. Biol. Rev. 63, 725–734.
Nettelbeck, D.M., 2008. Cellular genetic tools to control oncolytic adenoviruses for virotherapy of cancer. J. Mol. Med. 86, 363–377.
Nettelbeck, D.M., Miller, D.W., Jerome, V., Zuzarte, M., Watkins, S.J., Hawkins, R.E., Muller, R., Kontermann, R.E., 2001. Targeting of adenovirus to endothelial cells by a bispecific single-chain diabody directed against the adenovirus fiber knob domain and human endoglin (CD105). Mol. Ther. 3, 882–891.
Ni, S., Gaggar, A., Di Paolo, N., Li, Z.Y., Liu, Y., Strauss, R., Sova, P., Morihara, J., Feng, Q., Kiviat, N., Toure, P., Sow, P.S., Lieber, A., 2006. Evaluation of adenovirus vectors containing serotype 35 fibers for tumor targeting. Cancer Gene Ther. 13, 1072–1081.
Nicklin, S.A., Wu, E., Nemerow, G.R., Baker, A.H., 2005. The influence of adenovirus fiber structure and function on vector development for gene therapy. Mol. Ther. 12, 384–393.
Nicol, C.G., Graham, D., Miller, W.H., White, S.J., Smith, T.A., Nicklin, S.A., Stevenson, S.C., Baker, A.H., 2004. Effect of adenovirus serotype 5 fiber and penton modifications on in vivo tropism in rats. Mol. Ther. 10, 344–354.
Nord, K., Gunneriusson, E., Ringdahl, J., Stahl, S., Uhlen, M., Nygren, P.A., 1997. Binding proteins selected from combinatorial libraries of an alpha-helical bacterial receptor domain. Nat. Biotechnol. 15, 772–777.
Okada, N., Saito, T., Masunaga, Y., Tsukada, Y., Nakagawa, S., Mizuguchi, H., Mori, K., Okada, Y., Fujita, T., Hayakawa, T., Mayumi, T., Yamamoto, A., 2001. Efficient antigen gene transduction using Arg-Gly-Asp fiber-mutant adenovirus vectors can potentiate antitumor vaccine efficacy and maturation of murine dendritic cells. Cancer Res. 61, 7913–7919.
Ooboshi, H., Rios, C.D., Chu, Y., Christenson, S.D., Faraci, F.M., Davidson, B.L., Heistad, D.D., 1997. Augmented adenovirus-mediated gene transfer to atherosclerotic vessels. Arterioscler. Thromb. Vasc. Biol. 17, 1786–1792.
Osborn, L., Hession, C., Tizard, R., Vassallo, C., Luhowskyj, S., Chi-Rosso, G., Lobb, R., 1989. Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. Cell 59, 1203–1211.
Pache, L., Venkataraman, S., Nemerow, G.R., Reddy, V.S., 2008. Conservation of fiber structure and CD46 usage by subgroup B2 adenoviruses. Virology 375, 573–579.
Parker, A.L., Waddington, S.N., Nicol, C.G., Shayakhmetov, D.M., Buckley, S.M., Denby, L., Kemball-Cook, G., Ni, S., Lieber, A., McVey, J.H., Nicklin, S.A., Baker, A.H., 2006. Multiple vitamin K-dependent coagulation zymogens promote adenovirus-mediated gene delivery to hepatocytes. Blood 108, 2554–2561.
Parrott, M.B., Adams, K.E., Mercier, G.T., Mok, H., Campos, S.K., Barry, M.A., 2003. Metabolically biotinylated adenovirus for cell targeting, ligand screening, and vector purification. Mol. Ther. 8, 688–700.
Persson, B.D., Reiter, D.M., Marttila, M., Mei, Y.F., Casasnovas, J.M., Arnberg, N., Stehle, T., 2007. Adenovirus type 11 binding alters the conformation of its receptor CD46. Nat. Struct. Mol. Biol. 14, 164–166.
Philipson, L., Pettersson, R.F., 2004. The coxsackie-adenovirus receptor—a new receptor in the immunoglobulin family involved in cell adhesion. Curr. Top. Microbiol. Immunol. 273, 87–111.
Power, A.T., Wang, J., Falls, T.J., Paterson, J.M., Parato, K.A., Lichty, B.D., Stojdl, D.F., Forsyth, P.A., Atkins, H., Bell, J.C., 2007. Carrier cell-based delivery of an oncolytic virus circumvents antiviral immunity. Mol. Ther. 15, 123–130.
Raschperger, E., Thyberg, J., Pettersson, S., Philipson, L., Fuxe, J., Pettersson, R.F., 2006. The coxsackie- and adenovirus receptor (CAR) is an in vivo marker for epithelial tight junctions, with a potential role in regulating permeability and tissue homeostasis. Exp. Cell Res. 312, 1566–1580.
Rea, D., Havenga, M.J., van Den Assem, M., Sutmuller, R.P., Lemckert, A., Hoeben, R.C., Bout, A., Melief, C.J., Offringa, R., 2001. Highly efficient transduction of human monocyte-derived dendritic cells with subgroup B fiber-modified adenovirus vectors enhances transgene-encoded antigen presentation to cytotoxic T cells. J. Immunol. 166, 5236–5244.
Reddy, P.S., Idamakanti, N., Babiuk, L.A., Mehtali, M., Tikoo, S.K., 1999a. Porcine adenovirus-3 as a helper-dependent expression vector. J. Gen. Virol. 80 (Pt 11), 2909–2916.
Reddy, P.S., Idamakanti, N., Chen, Y., Whale, T., Babiuk, L.A., Mehtali, M., Tikoo, S.K., 1999b. Replication-defective bovine adenovirus type 3 as an expression vector. J. Virol. 73, 9137–9144.
Rein, D.T., Breidenbach, M., Curiel, D.T., 2006. Current developments in adenovirus-based cancer gene therapy. Future Oncol. 2, 137–143.
Rekhter, M.D., Simari, R.D., Work, C.W., Nabel, G.J., Nabel, E.G., Gordon, D., 1998. Gene transfer into normal and atherosclerotic human blood vessels. Circ. Res. 82, 1243–1252.
Rodriguez, R., Schuur, E.R., Lim, H.Y., Henderson, G.A., Simons, J.W., Henderson, D.R., 1997. Prostate attenuated replication competent adenovirus (ARCA) CN706: a selective cytotoxic for prostate-specific antigen-positive prostate cancer cells. Cancer Res. 57, 2559–2563.
Roelvink, P.W., Lizonova, A., Lee, J.G., Li, Y., Bergelson, J.M., Finberg, R.W., Brough, D.E., Kovesdi, I., Wickham, T.J., 1998. The coxsackievirus-adenovirus receptor protein can function as a cellular attachment protein for adenovirus serotypes from subgroups A, C, D, E, and F. J. Virol. 72, 7909–7915.
Roelvink, P.W., Mi Lee, G., Einfeld, D.A., Kovesdi, I., Wickham, T.J., 1999. Identification of a conserved receptor-binding site on the fiber proteins of CAR-recognizing adenoviridae. Science 286, 1568–1571.
Ruigrok, R.W., Barge, A., Mittal, S.K., Jacrot, B., 1994. The fibre of bovine adenovirus type 3 is very long but bent. J. Gen. Virol. 75 (Pt 8), 2069–2073.
Russell, S., 2004. CD46: a complement regulator and pathogen receptor that mediates links between innate and acquired immune function. Tissue Antigens 64, 111–118.
Russell, W.C., 2005. Adenoviruses. In: Mahy, B.W.J., ter Meulen, V. (Eds.), Topley and Wilson's Microbiology and Microbial Infections, ninth ed. Hodder Arnold, London, pp. 439–447.
Sailaja, G., HogenEsch, H., North, A., Hays, J., Mittal, S.K., 2002. Encapsulation of recombinant adenovirus into alginate microspheres circumvents vector-specific immune response. Gene Ther. 9, 1722–1729.
Sakurai, F., Kawabata, K., Koizumi, N., Inoue, N., Okabe, M., Yamaguchi, T., Hayakawa, T., Mizuguchi, H., 2006. Adenovirus serotype 35 vector-mediated transduction into human CD46-transgenic mice. Gene Ther. 13, 1118–1126.
Salone, B., Martina, Y., Piersanti, S., Cundari, E., Cherubini, G., Franqueville, L., Failla, C.M., Boulanger, P., Saggio, I., 2003. Integrin alpha3beta1 is an alternative cellular receptor for adenovirus serotype 5. J. Virol. 77, 13448–13454.
Segerman, A., Arnberg, N., Erikson, A., Lindman, K., Wadell, G., 2003a. There are two different species B adenovirus receptors: sBAR, common to species B1 and B2 adenoviruses, and sB2AR, exclusively used by species B2 adenoviruses. J. Virol. 77, 1157–1162.
Segerman, A., Atkinson, J.P., Marttila, M., Dennerquist, V., Wadell, G., Arnberg, N., 2003b. Adenovirus type 11 uses CD46 as a cellular receptor. J. Virol. 77, 9183–9191.
Sharma, A., Bangari, D.S., Tandon, M., Pandey, A., HogenEsch, H., Mittal, S.K. Comparative analysis of vector biodistribution, persistence and gene expression following intravenous delivery of bovine, porcine and human adenoviral vectors in a mouse model. Virology, in press 2009 Feb 9 [Epub ahead of print].
Shayakhmetov, D.M., Eberly, A.M., Li, Z.Y., Lieber, A., 2005a. Deletion of penton RGD motifs affects the efficiency of both the internalization and the endosome escape of viral particles containing adenovirus serotype 5 or 35 fiber knobs. J. Virol. 79, 1053–1061.
Shayakhmetov, D.M., Gaggar, A., Ni, S., Li, Z.Y., Lieber, A., 2005b. Adenovirus binding to blood factors results in liver cell infection and hepatotoxicity. J. Virol. 79, 7478–7491.
Shayakhmetov, D.M., Lieber, A., 2000. Dependence of adenovirus infectivity on length of the fiber shaft domain. J. Virol. 74, 10274–10286.
Short, J.J., Pereboev, A.V., Kawakami, Y., Vasu, C., Holterman, M.J., Curiel, D.T., 2004. Adenovirus serotype 3 utilizes CD80 (B7.1) and CD86 (B7.2) as cellular attachment receptors. Virology 322, 349–359.
Short, J.J., Vasu, C., Holterman, M.J., Curiel, D.T., Pereboev, A., 2006. Members of adenovirus species B utilize CD80 and CD86 as cellular attachment receptors. Virus Res. 122, 144–153.
Sirena, D., Lilienfeld, B., Eisenhut, M., Kalin, S., Boucke, K., Beerli, R.R., Vogt, L., Ruedl, C., Bachmann, M.F., Greber, U.F., Hemmi, S., 2004. The human membrane cofactor CD46 is a receptor for species B adenovirus serotype 3. J. Virol. 78, 4454–4462.
Smith, T.A., Idamakanti, N., Marshall-Neff, J., Rollence, M.L., Wright, P., Kaloss, M., King, L., Mech, C., Dinges, L., Iverson, W.O., Sherer, A.D., Markovits, J.E., Lyons, R.M., Kaleko, M., Stevenson, S.C., 2003a. Receptor interactions involved in adenoviral-mediated gene delivery after systemic administration in non-human primates. Hum. Gene Ther. 14, 1595–1604.
Smith, T.A., Idamakanti, N., Rollence, M.L., Marshall-Neff, J., Kim, J., Mulgrew, K., Nemerow, G.R., Kaleko, M., Stevenson, S.C., 2003b. Adenovirus serotype 5 fiber shaft influences in vivo gene transfer in mice. Hum. Gene Ther. 14, 777–787.
Soudais, C., Boutin, S., Hong, S.S., Chillon, M., Danos, O., Bergelson, J.M., Boulanger, P., Kremer, E.J., 2000. Canine adenovirus type 2 attachment and internalization: coxsackievirus-adenovirus receptor, alternative receptors, and an RGD-independent pathway. J. Virol. 74, 10639–10649.
Staba, M.J., Wickham, T.J., Kovesdi, I., Hallahan, D.E., 2000. Modifications of the fiber in adenovirus vectors increase tropism for malignant glioma models. Cancer Gene Ther. 7, 13–19.
Stehle, T., Harrison, S.C., 1997. High-resolution structure of a polyomavirus VP1-oligosaccharide complex: implications for assembly and receptor binding. EMBO J. 16, 5139–5148.
Stevenson, M., Hale, A.B., Hale, S.J., Green, N.K., Black, G., Fisher, K.D., Ulbrich, K., Fabra, A., Seymour, L.W., 2007. Incorporation of a laminin-derived peptide (SIKVAV) on polymer-modified adenovirus permits tumor-specific targeting via alpha6-integrins. Cancer Gene Ther. 14, 335–345.
Stevenson, S.C., Rollence, M., White, B., Weaver, L., McClelland, A., 1995. Human adenovirus serotypes 3 and 5 bind to two different cellular receptors via the fiber head domain. J. Virol. 69, 2850–2857.

Stewart, P.L., Nemerow, G.R., 2007. Cell integrins: commonly used receptors for diverse viral pathogens. Trends Microbiol. 15, 500–507.
Stoff-Khalili, M.A., Rivera, A.A., Glasgow, J.N., Le, L.P., Stoff, A., Everts, M., Tsuruta, Y., Kawakami, Y., Bauerschmitz, G.J., Mathis, J.M., Pereboeva, L., Seigal, G.P., Dall, P., Curiel, D.T., 2005. A human adenoviral vector with a chimeric fiber from canine adenovirus type 1 results in novel expanded tropism for cancer gene therapy. Gene Ther. 12, 1696–1706.
Stone, D., Lieber, A., 2006. New serotypes of adenoviral vectors. Curr. Opin. Mol. Ther. 8, 423–431.
Tan, P.K., Michou, A.I., Bergelson, J.M., Cotten, M., 2001. Defining CAR as a cellular receptor for the avian adenovirus CELO using a genetic analysis of the two viral fibre proteins. J. Gen. Virol. 82, 1465–1472.
Tatsis, N., Blejer, A., Lasaro, M.O., Hensley, S.E., Cun, A., Tesema, L., Li, Y., Gao, G.P., Xiang, Z.Q., Zhou, D., Wilson, J.M., Ertl, H.C., 2007. A CD46-binding chimpanzee adenovirus vector as a vaccine carrier. Mol. Ther. 15, 608–617.
Tomko, R.P., Johansson, C.B., Totrov, M., Abagyan, R., Frisen, J., Philipson, L., 2000. Expression of the adenovirus receptor and its interaction with the fiber knob. Exp. Cell Res. 255, 47–55.
Tomko, R.P., Xu, R., Philipson, L., 1997. HCAR and MCAR: the human and mouse cellular receptors for subgroup C adenoviruses and group B coxsackieviruses. Proc. Natl. Acad. Sci. U.S.A. 94, 3352–3356.
Tuve, S., Wang, H., Jacobs, J.D., Yumul, R.C., Smith, D.F., Lieber, A., 2008. Role of cellular heparan sulfate proteoglycans in infection of human adenovirus serotype 3 and 35. PLoS Pathog. 4, e1000189.
Tuve, S., Wang, H., Ware, C., Liu, Y., Gaggar, A., Bernt, K., Shayakhmetov, D., Li, Z., Strauss, R., Stone, D., Lieber, A., 2006. A new group B adenovirus receptor is expressed at high levels on human stem and tumor cells. J. Virol. 80, 12109–12120.
Ulasov, I.V., Tyler, M.A., Zheng, S., Han, Y., Lesniak, M.S., 2006. CD46 represents a target for adenoviral gene therapy of malignant glioma. Hum. Gene Ther. 17, 556–564.
Vellinga, J., Rabelink, M.J., Cramer, S.J., van den Wollenberg, D.J., Van der Meulen, H., Leppard, K.N., Fallaux, F.J., Hoeben, R.C., 2004. Spacers increase the accessibility of peptide ligands linked to the carboxyl terminus of adenovirus minor capsid protein IX. J. Virol. 78, 3470–3479.
Vellinga, J., Van der Heijdt, S., Hoeben, R.C., 2005. The adenovirus capsid: major progress in minor proteins. J. Gen. Virol. 86, 1581–1588.
Verhaagh, S., de Jong, E., Goudsmit, J., Lecollinet, S., Gillissen, G., de Vries, M., van Leuven, K., Que, I., Ouwehand, K., Mintardjo, R., Weverling, G.J., Radosevic, K., Richardson, J., Eloit, M., Lowik, C., Quax, P., Havenga, M., 2006. Human CD46-transgenic mice in studies involving replication-incompetent adenoviral type 35 vectors. J. Gen. Virol. 87, 255–265.
Vigne, E., Mahfouz, I., Dedieu, J.F., Brie, A., Perricaudet, M., Yeh, P., 1999. RGD inclusion in the hexon monomer provides adenovirus type 5-based vectors with a fiber knob-independent pathway for infection. J. Virol. 73, 5156–5161.
Waddington, S.N., McVey, J.H., Bhella, D., Parker, A.L., Barker, K., Atoda, H., Pink, R., Buckley, S.M., Greig, J.A., Denby, L., Custers, J., Morita, T., Francischetti, I.M., Monteiro, R.Q., Barouch, D.H., van Rooijen, N., Napoli, C., Havenga, M.J., Nicklin, S.A., Baker, A.H., 2008. Adenovirus serotype 5 hexon mediates liver gene transfer. Cell 132, 397–409.
Wadell, G., Hammarskjold, M.L., Winberg, G., Varsanyi, T.M., Sundell, G., 1980. Genetic variability of adenoviruses. Ann. N Y Acad. Sci. 354, 16–42.
Walters, R.W., Grunst, T., Bergelson, J.M., Finberg, R.W., Welsh, M.J., Zabner, J., 1999. Basolateral localization of fiber receptors limits adenovirus infection from the apical surface of airway epithelia. J. Biol. Chem. 274, 10219–10226.
Wang, X., Bergelson, J.M., 1999. Coxsackievirus and adenovirus receptor cytoplasmic and transmembrane domains are not essential for coxsackievirus and adenovirus infection. J. Virol. 73, 2559–2562.
Weis, W., Brown, J.H., Cusack, S., Paulson, J.C., Skehel, J.J., Wiley, D.C., 1988. Structure of the influenza virus haemagglutinin complexed with its receptor, sialic acid. Nature 333, 426–431.
Wickham, T.J., Filardo, E.J., Cheresh, D.A., Nemerow, G.R., 1994. Integrin alpha v beta 5 selectively promotes adenovirus mediated cell membrane permeabilization. J. Cell Biol. 127, 257–264.
Wickham, T.J., Mathias, P., Cheresh, D.A., Nemerow, G.R., 1993. Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment. Cell 73, 309–319.
Wickham, T.J., Roelvink, P.W., Brough, D.E., Kovesdi, I., 1996. Adenovirus targeted to heparan-containing receptors increases its gene delivery efficiency to multiple cell types. Nat. Biotechnol. 14, 1570–1573.
Wickham, T.J., Tzeng, E., Shears II, L.L., Roelvink, P.W., Li, Y., Lee, G.M., Brough, D.E., Lizonova, A., Kovesdi, I., 1997. Increased in vitro and in vivo gene transfer by adenovirus vectors containing chimeric fiber proteins. J. Virol. 71, 8221–8229.
Wold, W.S.M., Horwitz, M.S., 2007. Adenoviruses. In: Fields, B.N., Knipe, D.M., Howley, P.M. (Eds.), Fields Virology, fifth ed. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, pp. 2395–2436.
Wu, E., Trauger, S.A., Pache, L., Mullen, T.M., von Seggern, D.J., Siuzdak, G., Nemerow, G.R., 2004. Membrane cofactor protein is a receptor for adenoviruses associated with epidemic keratoconjunctivitis. J. Virol. 78, 3897–3905.
Wu, H., Han, T., Belousova, N., Krasnykh, V., Kashentseva, E., Dmitriev, I., Kataram, M., Mahasreshti, P.J., Curiel, D.T., 2005. Identification of sites in adenovirus hexon for foreign peptide incorporation. J. Virol. 79, 3382–3390.
Wu, H., Seki, T., Dmitriev, I., Uil, T., Kashentseva, E., Han, T., Curiel, D.T., 2002. Double modification of adenovirus fiber with RGD and polylysine motifs improves coxsackievirus-adenovirus receptor-independent gene transfer efficiency. Hum. Gene Ther. 13, 1647–1653.
Wu, Q., Moyana, T., Xiang, J., 2001. Cancer gene therapy by adenovirus-mediated gene transfer. Curr. Gene Ther. 1, 101–122.
Zabner, J., Freimuth, P., Puga, A., Fabrega, A., Welsh, M.J., 1997. Lack of high affinity fiber receptor activity explains the resistance of ciliated airway epithelia to adenovirus infection. J. Clin. Invest. 100, 1144–1149.
Zhang, Y., Bergelson, J.M., 2005. Adenovirus receptors. J. Virol. 79, 12125–12131.
Zheng, S., Ulasov, I.V., Han, Y., Tyler, M.A., Zhu, Z.B., Lesniak, M.S., 2007. Fiber-knob modifications enhance adenoviral tropism and gene transfer in malignant glioma. J. Gene Med. 9, 151–160.