Adenoviruses

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Contributor: Dragomira Majhen

Adenoviruses represent exceptional candidates for wide-ranging therapeutic applications, from vectors for gene therapy to oncolytics for cancer treatments. The first ever commercial gene therapy medicine was based on a recombinant adenovirus vector, while most recently, adenoviral vectors have proven critical as vaccine platforms in effectively controlling the global coronavirus pandemic.

adenovirus endocytosis vectors gene therapy

1. Introduction

Adenoviruses (AdVs) can cause mild health problems, like acute respiratory, gastrointestinal, and ocular infections, but have no oncogenic potential in humans [1]. To ensure viral progeny, AdVs need to efficiently infect permissive cells that will enable both adenoviral entry and replication of DNA. The life cycle of AdVs can be simply divided into several stages: binding to a receptor expressed on a cell surface, entry into the cell by endocytosis, endosomal escape, trafficking through the cytoplasm, and docking at the nuclear pore complex (NPC), followed by nuclear entry of the adenoviral genome. Once adenoviral DNA enters the nucleus, expression of early and late genes allows assembly of new viral particles and their release from the host cell is enabled.

Thanks to their features, like the relative ease with which their genome can be modified as well as relative tolerance towards genetic manipulation, AdVs have been recognized as promising vectors for gene transfer. Today, according to the Wiley database on Gene Therapy Trials Worldwide, AdVs present 17.5% of vectors used in gene therapy clinical trials (https://a873679.fmphost.com/fmi/webd/GTCT; accessed on 10 August 2021). The first ever commercial gene therapy medicine was recombinant adenovirus encoding human p53 tumor suppressor gene, Gendicine, registered for the treatment of head and neck cancers on the market in China [2]. Besides being vectors for gene transfer, AdVs are widely used as vectors for vaccination. Numerous AdV vaccine candidates for treating infectious diseases and cancer are under investigation, with the most important milestone being achieved when European Medicines Agency recently approved three AdV-vector-based vaccines: one against Ebola (Zabdeno; Ad26.ZEBOV [3]) and two against Covid-19 (ChAdOx1 nCoV-19 [4] and Ad26.COV2.S [5]), demonstrating the efficacy of AdV-based vaccines as tools for controlling pandemic outbreaks.

We distinguish the two types of commonly used adenoviral vectors: replication-defective and oncolytic adenoviruses. Due to molecular engineering, replication-defective AdVs cannot replicate in infected cells, however they provide high expression of transgenes with minimum expression of AdV proteins. Unlike replication-defective AdVs (also known as vectors), oncolytic AdVs selectively replicate in and lyse cancer cells, and are, therefore,

commonly used vectors in clinical trials for cancer gene therapy [6]. The world first oncolytic virus product was also an adenovirus, Oncorine (H101), an oncolytic adenovirus intended to be used in combination with chemotherapy as a treatment for patients with late-stage refractory nasopharyngeal cancer [7]. Currently, a large number of AdV platforms are being developed for oncology applications, for example, Enadenotucirev, a clinical stage oncolytic AdV with broad potential to target solid tumor carcinomas [8]; DNX-2401; Delta-24-RGD oncolytic AdV, developed as immunotherapeutic treatment for recurrent malignant glioma [9]; and TILT-123, a human AdV5/AdV3 chimeric oncolytic adenovirus encoding for tumor necrosis factor alpha and human interleukin 2 aimed at generating an anti-cancer immune response [10].

To fulfil its role as a vector, an AdV needs to successfully deliver its DNA genome to the host nucleus, a process highly influenced by AdV intracellular translocation. In this review, we discuss which factors are involved in AdV endocytosis, focusing mainly on receptors and endocytic scaffold proteins, as well as the influence of a particular type of endocytosis on AdV intracellular trafficking.

Human adenoviruses (HAdVs) are non-enveloped double stranded DNA viruses with icosahedral capsids of approximately 90 nm in diameter and mass of ~150 megadaltons [11]. HAdVs consist of 13 structural proteins that form the outer capsid of icosahedral geometry and the inner core, where double-stranded DNA is bound with the core proteins. Major building blocks of the HAdV capsid are hexon and penton. There are 240 copies of the hexon trimer, and 12 pentons comprising an extended fiber protein non-covalently attached to the penton base protein. The fiber protein is composed of tail, shaft, and knob domains, the latter containing several loops. In addition, capsids comprise minor proteins IIIa, VI, VIII, and IX [12]. HAdVs are classified into seven subgroups, A–G [13], according to hemagglutination and serum neutralization reactions or by genome sequencing and bioinformatics analysis (International Committee on Taxonomy of Viruses—ICTV). HAdV infection starts with binding to the primary receptor at the cell surface. Primary receptor usage differs between different HAdVs serotypes. After initial attaching to the primary receptor, HAdV binds αv integrins on the cell surface through an RGD peptide sequence present in the penton base of the virus [14]. This interaction triggers internalization of HAdV, as well as a number of signaling events that lead to virus cell entry within the endosomal compartment [15]. Partial disassembly of the capsid occurs prior to membrane penetration, however the escape of incoming adenoviruses from endosomes is dependent on the viral membrane lytic protein VI, whose lytic activity is pH-independent [16][17]. Once liberated from the endosome, the fibreless HAdV capsid is transported towards the nucleus along a network of microtubules [18] and motor proteins, such as dynein (unidirectional movement) or kinesin (bidirectional) [19][20]. Final docking of the HAdV capsid occurs at the nuclear pore and the HAdV genome enters the nucleus [21], where it remains episomal. A scheme of HAdV cell entry is presented on **Figure 1**.

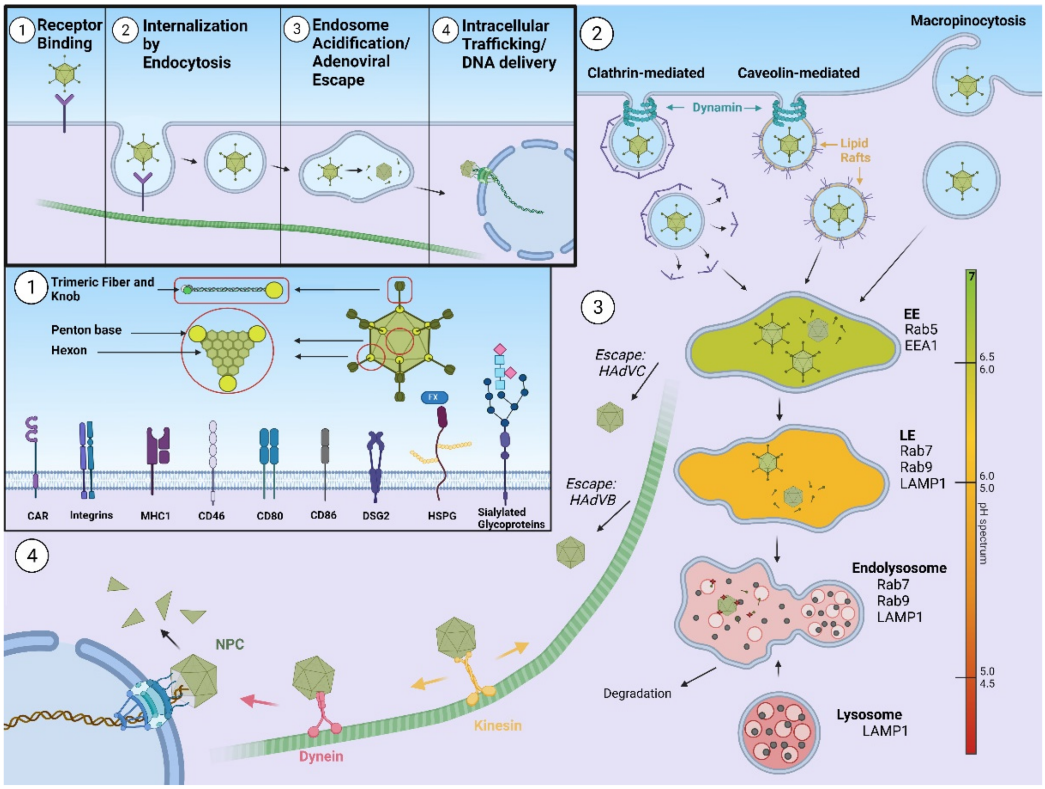

Figure 1. Schematic representation of different steps in adenovirus infection, ultimately leading to delivery of the viral genome to the host cell nucleus. 1. Adenovirus basic structure and various possible binding receptors. 2. Internalization of the virion by different mechanisms of endocytosis. 3. Virion trafficking within vesicles and endosome acidification, followed by virion escape. 4. Docking at the nuclear pore complex and delivery of the adenoviral genome. Abbreviations: CAR – coxsackie and adenovirus receptor; MHC1—major histocompatibility complex class 1; DSG2—desmoglein 2, HSPG—heparan sulfate proteoglycans, FX—factor X, EE—early endosome, LE—late endosome, EEA1—early endosome antigen 1, LAMP1—lysosomal-associated membrane protein 1, HAdVC—human adenovirus subgroup C, HAdVB—human adenovirus subgroup B, NPC—nuclear pore complex.

2. Host Innate Immune Response and Human Adenovirus Escape from Endosome

Due to their pathogen-associated molecular patterns (PAMPs), HAdVs can activate innate immune response. HAdV PAMPs are recognized by pattern-recognition receptors (PRRs), among which the most prominent are Toll-like (TLRs), AIM2-like (ALRs), and NOD-like receptors (NLRs) [22]. Following internalization into the endosome, adenoviral DNA can be recognized by TLR9. Involvement of TLR9 in immune activation has been reported for CAR- and CD46-utilizing HAdV vectors [23]. HAdV detection by NLRP3 containing inflammasome is dependent on HAdV penetration of endosomal membranes, the release of the lysosomal protease cathepsin B into the cytoplasm, and the production of reactive oxygen species. Inflammasome activation by HAdV5 also leads to NLRP3 and cathepsin B-dependent, but caspase-1-independent, necrotic cell death, resulting in the release of the proinflammatory molecule HMGB1. Thus, rupture of cathepsin-enriched lysosomes during HAdV5 cell entry serves

as a danger signal leading to the release of proinflammatory mediators [24]. Additionally, it has been shown that HAdV-induced inflammasome activation can be influenced by HAdV endosomal sorting. HAdV5 vectors possessing human adenovirus type 16 (HAdV16) knob domains (HAdV subgroup B, CD46-utilizing), Ad5f16, have shown greater localization to cathepsin-enriched lysosomes prior to endosomal escape, which led to enhanced cytosolic release of cathepsins and augmented reactive oxygen species production compared to HAdV5, even though both viruses penetrate endosomal membranes with similar efficiency [25].

It has been proposed that HAdV37 can use different pathways to enter cells of different origin which could account for a relative paucity of proinflammatory gene expression upon infection with this virus [26][27]. In the case of HAdV37, the role of dynamin-2, a protein that allows detachment of the endocytic vesicle from the plasma membrane, in triggering innate immune response has also been studied. Overexpression of dynamin-2 prior to viral infection led to a statistically significant increase in CXCL10 and CXCL8, while downregulating dynamin-2 led to statistically significant reductions in CCL2, CCL5, CXCL1, CXCL11, IL-1ra, IL-6, MIF, and Serpin E1 [28]. Connection of virus localization and cytokine expression after HAdV infection was reported also for HAdV35 and HAdV26, both used as vaccine platforms [29][30]. Of note, engagement of CD46 as a cellular receptor appears to dampen the host immune response against the infected cell through downregulation of C/ERPbeta, a situation that could have implications when CD46 binding AdVs, like HAdV35, are considered for gene delivery and vaccine development [31]. It has been shown that HAdV35 and HAdV26 accumulate in the late endosomal compartment more extensively than HAdV5 at 2 to 8 h following infection and that innate immune stimulation by all HAdV vectors was sensitive to inhibitors of endosomal acidification, cathepsin B, and caspase 1 [32]. The role of endosomal escape in HAdV-triggered innate response was seen also with Ad2-ts1 mutant, which is defective in endosomal escape [33]. While HAdV2 induced rapid phosphorylation of p38 and ERK, as well as a significant cytokine response, Ad2-ts1 failed to activate p38 or ERK and induced only a limited cytokine response [34].

Contrary to the neutralizing effect observed in epithelial cells, HAdV5 infection in the presence of antiviral antibodies significantly increased FcR-dependent viral internalization in macrophages. In direct correlation with the increased viral internalization, antiviral antibodies amplified the innate immune response to HAdV5, as determined by the expression of NF-κB-dependent genes, type I IFNs, and caspase-dependent IL-1β maturation. Confocal microscopy revealed subversion of natural tropism and targeting toward LAMP1-positive phagolysosomes in HAdV5-infected macrophages in the presence of antiviral antibodies, but not in epithelial cells. This skewing toward phagolysosomes amplified innate immune response [35].

Taken together, these data suggest that route of entry of endosomal trafficking and escape of HAdVs is a critical factor in the induction of innate immune response. Stalling HAdVs in one of the endocytic vesicles can induce activation of the innate immune system that otherwise would not happen. In addition, a dynein-mediated unidirectional movement towards the perinuclear microtubule minus end results in faster capsid transport towards the nucleus, therefore, kinesin-mediated bidirectional movement could slow down the HAdV and possibly contribute to HAdV stalling [20]. Aberrant recognition by innate immune sensors can be not only deleterious for potential vectors but can also cause undesired host immune response. Therefore, correct intracellular trafficking of the HAdV vector is needed not only to assure functionality in terms of successful delivery of transgene, but also in

order not to provoke unintended immune response and, hence, increase safety of vectors intended for therapeutic applications.

3. Outlook

HAdV infection is a complex process involving several steps: binding to a primary receptor, internalization, escape from the endosome, intracellular trafficking and finally genome delivery to the nucleus. In this review we described how factors involved in almost all those stages can be orchestrated by cellular endocytosis pathways. Endocytosis can change HAdV receptors' expression or influence their recycling, thus, impeding their availability on the cell surface. Aberrant endocytosis itself can change the internalization procedure of an HAdV and force it to use another method of cell entry. This can influence not only transgene expression but can also trigger different host immune response which in some instances can be undesired, but in others might be beneficial. Regardless of the fact that AdVs are considered not to be very dangerous, adenoviral infection is a significant cause of mortality in the immunocompromised individual, so understanding their means of infection and entry may also be critical at the level of developing new antivirals to treat such patients.

In summary, the reasons for the different intracellular traffic of HAdVs of different serotypes may be different: (1) binding to different receptors on the cell surface triggers different signals for HAdVs to enter the cell; (2) HAdVs enter cells with different types of endocytosis; (3) during intracellular trafficking, different HAdVs serotypes are found in different types of endosomes, from which they may have different exit efficiencies; (4) HAdV travel depends on motor proteins and others. When considering the use of HAdV-based vectors for translational therapeutic applications, these represent key considerations when designing constructs for optimal transgene expression in target cells.

References

1. Ghebremedhin, B. Human adenovirus: Viral pathogen with increasing importance. Eur. J. Microbiol. Immunol. 2014, 4, 26–33.
2. Peng, Z. Current status of gendicine in China: Recombinant human Ad-p53 agent for treatment of cancers. Hum. Gene Ther. 2005, 16, 1016–1027.
3. Pollard, A.J.; Launay, O.; Lelievre, J.D.; Lacabaratz, C.; Grande, S.; Goldstein, N.; Robinson, C.; Gaddah, A.; Bockstal, V.; Wiedemann, A.; et al. Safety and immunogenicity of a two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Europe (EBOVAC2): A randomised, observer-blind, participant-blind, placebo-controlled, phase 2 trial. Lancet Infect. Dis. 2021, 21, 493–506.
4. Voysey, M.; Clemens, S.A.C.; Madhi, S.A.; Weckx, L.Y.; Folegatti, P.M.; Aley, P.K.; Angus, B.; Baillie, V.L.; Barnabas, S.L.; Bhorat, Q.E.; et al. Safety and efficacy of the ChAdOx1 nCoV-19

vaccine (AZD1222) against SARS-CoV-2: An interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet 2021, 397, 99–111.

5. Sadoff, J.; Gray, G. Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against Covid-19. N. Engl. J. Med. 2021, 384, 2187–2201.
6. Cunliffe, T.G.; Bates, E.A.; Parker, A.L. Hitting the Target but Missing the Point: Recent Progress towards Adenovirus-Based Precision Virotherapies. Cancers 2020, 12, 3327.
7. Liang, M. Oncorine, the World First Oncolytic Virus Medicine and its Update in China. Curr. Cancer Drug Targets 2018, 18, 171–176.
8. Illingworth, S.; Di, Y.; Bauzon, M.; Lei, J.; Duffy, M.R.; Alvis, S.; Champion, B.; Lieber, A.; Hermiston, T.; Seymour, L.W.; et al. Preclinical Safety Studies of Enadenotucirev, a Chimeric Group B Human-Specific Oncolytic Adenovirus. Mol. Ther. Oncolytics 2017, 5, 62–74.
9. Lang, F.F.; Conrad, C.; Gomez-Manzano, C.; Yung, W.K.A.; Sawaya, R.; Weinberg, J.S.; Prabhu, S.S.; Rao, G.; Fuller, G.N.; Aldape, K.D.; et al. Phase I Study of DNX-2401 (Delta-24-RGD) Oncolytic Adenovirus: Replication and Immunotherapeutic Effects in Recurrent Malignant Glioma. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 2018, 36, 1419–1427.
10. Havunen, R.; Siurala, M.; Sorsa, S.; Grönberg-Vähä-Koskela, S.; Behr, M.; Tähtinen, S.; Santos, J.M.; Karell, P.; Rusanen, J.; Nettelbeck, D.M.; et al. Oncolytic Adenoviruses Armed with Tumor Necrosis Factor Alpha and Interleukin-2 Enable Successful Adoptive Cell Therapy. Mol. Ther. Oncolytics 2017, 4, 77–86.
11. Mangel, W.F.; San Martín, C. Structure, function and dynamics in adenovirus maturation. Viruses 2014, 6, 4536–4570.
12. Nemerow, G.R.; Stewart, P.L.; Reddy, V.S. Structure of human adenovirus. Curr. Opin. Virol. 2012, 2, 115–121.
13. Lefkowitz, E.J.; Dempsey, D.M.; Hendrickson, R.C.; Orton, R.J.; Siddell, S.G.; Smith, D.B. Virus taxonomy: The database of the International Committee on Taxonomy of Viruses (ICTV). Nucleic Acids Res. 2018, 46, D708–D717.
14. Stewart, P.L.; Nemerow, G.R. Cell integrins: Commonly used receptors for diverse viral pathogens. Trends Microbiol. 2007, 15, 500–507.
15. Greber, U.F.; Willetts, M.; Webster, P.; Helenius, A. Stepwise dismantling of adenovirus 2 during entry into cells. Cell 1993, 75, 477–486.
16. Wiethoff, C.M.; Wodrich, H.; Gerace, L.; Nemerow, G.R. Adenovirus protein VI mediates membrane disruption following capsid disassembly. J. Virol. 2005, 79, 1992–2000.
17. Suomalainen, M.; Luisoni, S.; Boucke, K.; Bianchi, S.; Engel, D.A.; Greber, U.F. A direct and versatile assay measuring membrane penetration of adenovirus in single cells. J. Virol. 2013, 87,

12367–12379.

18. Suomalainen, M.; Nakano, M.Y.; Keller, S.; Boucke, K.; Stidwill, R.P.; Greber, U.F. Microtubule-dependent plus- and minus end-directed motilities are competing processes for nuclear targeting of adenovirus. J. Cell Biol. 1999, 144, 657–672.
19. Scherer, J.; Vallee, R.B. Adenovirus recruits dynein by an evolutionary novel mechanism involving direct binding to pH-primed hexon. Viruses 2011, 3, 1417–1431.
20. Zhou, J.; Scherer, J. Role of kinesins in directed adenovirus transport and cytoplasmic exploration. PLoS Pathog. 2018, 14, e1007055.
21. Strunze, S.; Engelke, M.F.; Wang, I.H.; Puntener, D.; Boucke, K.; Schleich, S.; Way, M.; Schoenenberger, P.; Burckhardt, C.J.; Greber, U.F. Kinesin-1-mediated capsid disassembly and disruption of the nuclear pore complex promote virus infection. Cell Host Microbe 2011, 10, 210–223.
22. Hendrickx, R.; Stichling, N.; Koelen, J.; Kuryk, L.; Lipiec, A.; Greber, U.F. Innate immunity to adenovirus. Hum. Gene Ther. 2014, 25, 265–284.
23. Iacobelli-Martinez, M.; Nemerow, G.R. Preferential activation of Toll-like receptor nine by CD46-utilizing adenoviruses. J. Virol. 2007, 81, 1305–1312.
24. Barlan, A.U.; Griffin, T.M.; McGuire, K.A.; Wiethoff, C.M. Adenovirus membrane penetration activates the NLRP3 inflammasome. J. Virol. 2011, 85, 146–155.
25. Barlan, A.U.; Danthi, P.; Wiethoff, C.M. Lysosomal localization and mechanism of membrane penetration influence nonenveloped virus activation of the NLRP3 inflammasome. Virology 2011, 412, 306–314.
26. Lee, J.S.; Mukherjee, S.; Lee, J.Y.; Saha, A.; Chodosh, J.; Painter, D.F.; Rajaiya, J. Entry of Epidemic Keratoconjunctivitis-Associated Human Adenovirus Type 37 in Human Corneal Epithelial Cells. Invest. Ophthalmol. Vis. Sci. 2020, 61, 50.
27. Yousuf, M.A.; Zhou, X.; Mukherjee, S.; Chintakuntlawar, A.V.; Lee, J.Y.; Ramke, M.; Chodosh, J.; Rajaiya, J. Caveolin-1 associated adenovirus entry into human corneal cells. PLoS ONE 2013, 8, e77462.
28. Lee, J.S.; Ismail, A.M.; Lee, J.Y.; Zhou, X.; Materne, E.C.; Chodosh, J.; Rajaiya, J. Impact of dynamin 2 on adenovirus nuclear entry. Virology 2019, 529, 43–56.
29. Custers, J.; Kim, D.; Leyssen, M.; Gurwith, M.; Tomaka, F.; Robertson, J.; Heijnen, E.; Condit, R.; Shukarev, G.; Heerwegh, D.; et al. Vaccines based on replication incompetent Ad26 viral vectors: Standardized template with key considerations for a risk/benefit assessment. Vaccine 2021, 39, 3081–3101.

30. Zahn, R.; Gillisen, G.; Roos, A.; Koning, M.; van der Helm, E.; Spek, D.; Weijtens, M.; Grazia Pau, M.; Radošević, K.; Weverling, G.J.; et al. Ad35 and ad26 vaccine vectors induce potent and cross-reactive antibody and T-cell responses to multiple filovirus species. PLoS ONE 2012, 7, e44115.
31. Iacobelli-Martinez, M.; Nepomuceno, R.R.; Connolly, J.; Nemerow, G.R. CD46-utilizing adenoviruses inhibit C/EBPbeta-dependent expression of proinflammatory cytokines. J. Virol. 2005, 79, 11259–11268.
32. Teigler, J.E.; Kagan, J.C.; Barouch, D.H. Late endosomal trafficking of alternative serotype adenovirus vaccine vectors augments antiviral innate immunity. J. Virol. 2014, 88, 10354–10363.
33. Greber, U.F.; Webster, P.; Weber, J.; Helenius, A. The role of the adenovirus protease on virus entry into cells. EMBO J. 1996, 15, 1766–1777.
34. Smith, J.S.; Xu, Z.; Tian, J.; Palmer, D.J.; Ng, P.; Byrnes, A.P. The role of endosomal escape and mitogen-activated protein kinases in adenoviral activation of the innate immune response. PLoS ONE 2011, 6, e26755.
35. Zaiss, A.K.; Vilaysane, A.; Cotter, M.J.; Clark, S.A.; Meijndert, H.C.; Colarusso, P.; Yates, R.M.; Petrilli, V.; Tschopp, J.; Muruve, D.A. Antiviral Antibodies Target Adenovirus to Phagolysosomes and Amplify the Innate Immune Response. J. Immunol. 2009, 182, 7058.