REVIEW

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Modifications in Adenoviral vectors to enhance its tropism: a literature review

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ABSTRACT

Viruses' high rate of cellular entry raises the possibility that they could be utilized as vectors to introduce new functional copies of a gene into a cell. This review aims to explore modifications in Adenoviral vectors to enhance its tropism. It is feasible to exploit the infection pathway to achieve a therapeutic objective without the following expression of viral genes, which, if expressed, would cause disease and damage. It has been demonstrated that this is achievable. This is performed by swapping a therapeutic gene with an existing harmful gene in the genome of the virus. Due to its unique features, adenovirus, commonly known as Ad, is an intriguing possibility for use as a viral vector in gene therapy. Despite this, therapeutic applications of ad vectors are limited due to their immunogenicity and broad native tropism. Several distinct forms of nonimmunogenic polymers are utilized in the chemical or physical modification of ad vectors to circumvent these obstacles. In this review, many modifications, including capsid pseudotyping, serotype switching, and multiple conjugation-based techniques, are discussed in order to boost the specificity of target adenoviruses.

Keywords: Adenoviral vector, tropism, gene therapy.
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INTRODUCTION

As a result of natural selection, viruses are now perfectly adapted to infect living organisms. In order to invade host cells, replicate their genome, and then spread to new cells, they employ cunning mechanisms. Because viruses are able to enter cells so effectively, they could be used as vectors to transport working genes to cells that possess them. By inserting a therapeutic gene into the viral genome in place of a pathogenic one, one could use the infection pathway to achieve a therapeutic goal without the subsequent expression of viral genes, which would otherwise cause disease and toxicity. Despite this, implementing this plan for gene therapy is challenging and fraught with uncertainty. These viruses are highly efficient transducers because of their highly evolved infection mechanisms; however, these same mechanisms also raise serious safety concerns, such as insertional mutagenesis and acute immune response, which severely limit their clinical application as gene delivery vectors. Due to this, a significant amount of time has been spent on research and development to create safer, more fuel-efficient vehicles.[1,2]

Adenoviruses have been widely recognized as potent gene delivery vectors at least since the 1980s. As it turns out, adenoviruses have all the best features for gene delivery: high packaging capacity, low genotoxic potential, ease of production of high functional titers, and high levels of gene expression in a wide range of cell types. The recombinant adenoviral vector (Ad) was created by deleting important genes that are translated in the early translation phase, specifically the E1A and E1B genes, whose expression permits host cell transformation and activation of other viral gene expression. In order to increase the cloning capacity of this recombinant Adenovirus, further deletion of the E3 gene sequence was performed during the development of this Adenovirus vector.[3,4] With these modifications, it is hoped that the recombinant Adenovirus can express the transgene-encoded protein without expressing the viral protein responsible for pathogenesis. virus. To ensure that no pathogenic Adenoviruses are formed in the host cell, the E2 and E4 genes were eliminated from the Adenovirus vector construct, which has been shown to improve the vector cloning process. This type of Adenovirus vector is referred to as "gutless." In order to produce a new virus, gutless adenovirus needs a helper vector (helper-dependent adenoviral vector). These vectors retain only the terminal ITR required for replication and packaging of the adenoviral genome, resulting in a significant

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increase in cloning capacity. Adenoviruses bind to cells through the interaction of the C-terminal knob domain on the capsid protein with the primary cellular receptor on the host cell. Additionally, the presence of secondary bonds between v3/5-integrins in cells and the Arg-Gly-Asp sequence on the viral capsid protein Ad contributes to the induction of viral endocytosis and subsequent viral internalization. Adenovirus type B also binds to CD46, whereas adenovirus types A, C, D, E, and F have a high affinity for binding to CAR on the surface of the host cell. Consequently, the level of CD46 and CAR expression on the surface of host cells determines the efficacy of Adenovirus infection.[5,6,7]

Adenovirus (Ad) vector-based gene therapy has been extensively developed due to Ad's ability to efficiently infect numerous cell and tissue types. However, this broad tropism poses several challenges, one of which is the difficulty of delivering transgenes to specific cells. For instance, the majority of intravenously administered Ad is stored in the liver, which may not be a desirable target. Ad5 also transduces dendritic cells, which serve as antigen-presenting cells (APC) and enhance the immune response to vectors.[8,9] The immunogenicity and tropism of Ad vectors pose significant challenges to their efficient and safe application in clinical trials, despite the clinical benefits they provide. Adverse immune responses are a major roadblock because they are mounted against ad vectors. Vectors with the ability to target particular cells can eliminate this issue and permit the use of lower total particle doses (thus not being immunogenic).[4] Changes in vector tropism will also increase the use of Adenovirus vectors. Ad, which has modified tropism, can infect cells such as hematopoietic cells that are normally resistant to infection.[5] Modifications must be made to the Adenovirus capsid protein in order to make Ad infection specific to a cell type, so that this Ad vector can be administered to patients in low doses. Capsid pseudotyping, the addition of specific antibodies to the viral capsid, and serotype switching are among the modifications made.[8-15] This review aims to explore modifications in Adenoviral vectors to enhance its tropism.

Capsid Pseudotyping

Combining the functions of Adenovirus vectors with those of other viral types can extend the duration of transgene expression and/or deliver transgenes to specific target cells or tissues. One way to increase the tropism of the Adenovirus vector against a type of cell is to modify the capsid Ad by deleting the capsid protein encoding gene and replacing it with a capsid protein encoding gene from another virus type, in which the capsid constituent proteins of these other viruses contain receptors.[6,16] Binding sites can only interact with receptors on certain cell types. The recombinant Adenovirus that encodes the surface protein of the vesicular stomatitis virus (VSV) G protein is an example of a pseudotype capsid that has been developed in Adenovirus vectors (VSV-G). Several studies have demonstrated, however, that the production of this recombinant Ad is quite challenging due to the presence of toxic components in the VSV-G capsid. The incorporation of inducible promoters is a further modification made to this recombinant Ad vector. Adding an inducible promoter that requires tetracycline as an inducer, it is possible to control the expression of a toxic protein required for the formation of the VSV-G 8 capsid.[8,16]

Serotype Switching

Adenoviruses are grouped into two main groups, namely, Ads that can infect humans and Ads that can't infect humans. Currently there are 55 serotypes of Adenovirus that can infect humans. They were further grouped into seven species namely, A, B, C, D, E, F, and G (Table 1). Data related to genomes and biological characters, Adenovirus species C has been the most complete data, so Ad species C is the most frequently developed as a vector for therapeutic genes (especially Ad2

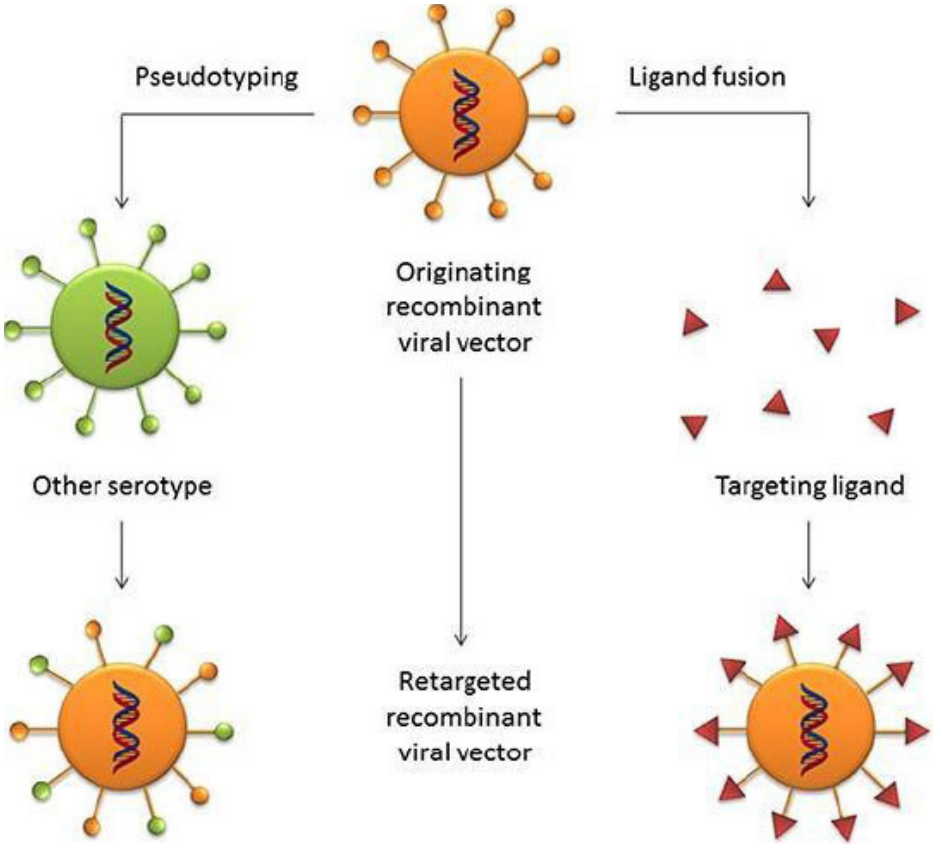

Figure 1. The mechanism for pseudotyping plasmids in viral vectors, such as Adenovirus vectors. Where the gene encoding the protein that makes up the Adenovirus capsid is deleted and replaced with a plasmid encoding gene from another virus with RBD that detects only one type of receptor on a particular cell.[7]

Table 1. Classification of serotypes of seven classes of Adenoviruses that can infect humans.

Species	Serotypes
A	12, 18, 31
B	3, 7, 11, 14, 16, 21, 34, 35, 50, 55
C	1, 2, 5, 6
D	8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 36, 37, 38, 39, 42, 43, 44, 45, 46, 47, 48, 49, 51, 53, 53
E	4
F	40, 41
G	52

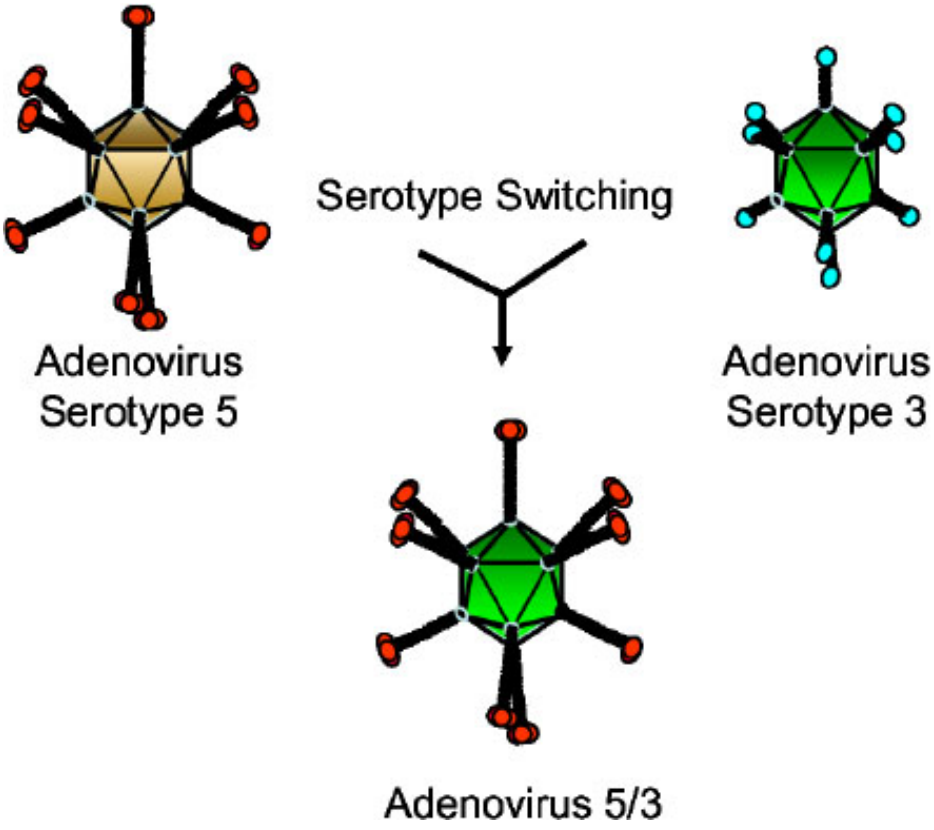

Figure 2. Serotype switching in Adenovirus vector.[9]

and Ad5).[4] In addition, Adenovirus with serotypes that cannot infect humans are also studied as a vector for gene therapy. In addition, Adenovirus with a serotype that cannot infect humans is also studied as a vector for gene therapy.[5,9,10]

Although 51 serotypes of Adenovirus that can infect humans have been characterized, most of the human Adenoviral vectors currently used for gene therapy are based on subgroup C Adenovirus serotypes 2 and 5. However, preclinical and clinical studies with this vector revealed some limitations associated with its use in gene therapy in humans. One of the main limitations is the presence of a pre-existing immune response in humans. Viral infections due to Adenovirus subgroup C are endemic in most of the human population. This is what causes most people in the world to have neutralizing antibodies to the Adenovirus subgroup C.[5]

An alternative strategy that can be done is serotype switching (Figure 2), namely recombinant Adenovirus vector whose capsid protein is derived from Adenovirus with a serotype that often infects humans or the pathological nature is not high, so it does not activate the body's immune system. In a study of replacement of Ad5 with the Ad26 serotype, it showed a reduction in chronic viremia levels in experimental animals. In another study, the hexon hypervariable regions (HVR) in Ad5 were replaced by hexon HVR Ad48 in macaques showing a significant decrease in viremia levels.[9]

In vector helper-dependent adenovirus (HD-Ad), all virus ORFs were removed. There was no Ad protein produced in the HD-Ad vector-transduced cells thereby avoiding the T cell response that could kill the transduced cells. This reduced immunogenicity allows the expression of the transgene in mice and baboons for many years. The HD-Ad system is also uniquely adapted for serotype switching processes, as Ads of certain species can cross-package each other's genomes. Therefore, HD-Ad has the ability to evade cytotoxic T cell responses and neutralizing antibodies.[7] Low immunogenicity not only enhances safety, but also increases persistence in vivo.[10]

Multiple Conjugation-based Strategies

Adapter-based transduction retargeting on the capsid surface of Adenovirus vectors is achieved by associating a foreign targeting entity to the virus, either by covalent or non-covalent interactions. In addition, multiple conjugation-based strategies can be combined to create a multi-component targeting system. Adapters may consist of conjugated Ab fragments, or bispecific antibodies. Bispecific antibodies contain two distinct binding sites, one end specifically binding to the adenovirus plasmid protein and the other end specifically binding to the target cell receptor.[9]

The form of this bispecific antibody can be of various kinds, including scFv-scFv, Fab conjugates and single chain or tandem diabody conformations. With regard to Adenoviral engineering, many of these approaches can be designed for retargeting, as well as de-targeting, of native receptor binding. Tissue-selective antibody conjugation to an Adenoviral vector has been achieved by incorporation of the genetic sequence of the immunoglobulin (Ig) Fc binding domain on the staphylococcal A protein, into the fibre-encoding sequence on the Ad plasmid. This motif was well tolerated when inserted into the HI loop of the Ad5 plasmid. Using this approach,

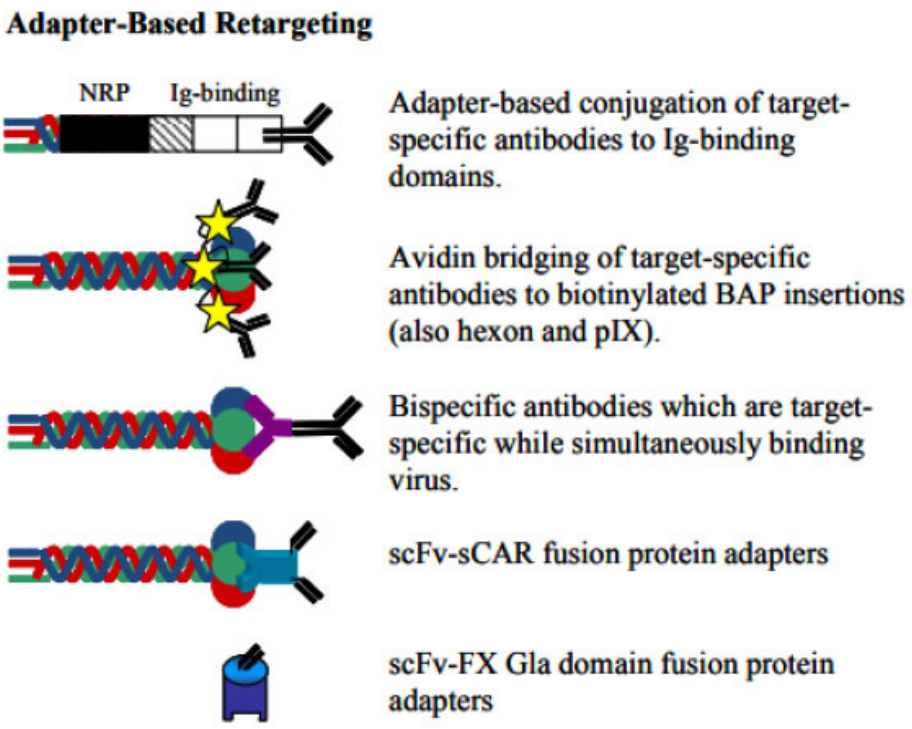

Figure 3. Several strategies involve the addition of antibodies to protein fibers in the capsid of the adenovirus vector to increase the effectiveness of transduction.[9]

a recombinant Adenovirus containing a human monoclonal antibody that binds to the tumor marker epidermal growth factor receptors (EGFR), CD40 and CD40L[9], has been shown to result in increased infectivity in cells expressing similar target receptors. Furthermore, this strategy has been extended to allow targeting to the nerve cell adhesion molecule and the α7 integrin subunit, which is expressed on human primary myoblasts.[2]

Challenges of Modifications in Adenoviral Vectors to Increase Tropism

Despite the benefits and advantages such as high level of transduction efficiency, able to infect to numerous cell types, and the process of transduction that does not need cell division; there are some challenges have to be conquered regarding modifications in adenoviral vectors includes small insert size, immunogenicity, competence of replication, limited targets, until non-integration. Moreover, Adeno-associated virus has risk in integration, expression decline over time due to degradation that leads to episomal loss, small capacity of packaging, low titers, vigorous immune response. Meanwhile, advantages and benefits of application of Adeno-associated virus as vector in gene therapy namely integration into the host genome, no viral genes, ability to transduce cells that still not dividing actively. Furthermore Adeno-associated virus has characteristics of non-inflammatory and non-pathogenic.[5,6,10]

The first challenge is the increased competent replication of Adenoviruses when preparing the products, therefore vector particles containing E1 protein are present in batches. This became the main concern of safety issues as the recombinant vector replication potentially leads to tumor formation. Ultimate new design of packaging cell lines such as PER C6 cells, nullified any DNA sequence overlapping vector and E1 could prevent formation of competent particles formation in product construction. Next challenge is pre-existing immunity to several Adenoviruses in humans due to widespread occurrence of wild-type Adenoviruses in nature and expansive circulating amounts of serotypes in humans, therefore the vector products may be cleared by host immune system before it infects target cells and express desired proteins thus limiting efficacy of product severely. In order to circumvent the pre-existing immunity, non-human origin serotypes could be turned to scarce human serotypes. It can be selected from non-human primates or possibly competent alternative species. Nullifying pre-existing immunity could reposition the Adenoviral vectors as foremost apparatus in succeeding gene editing and development of vaccine products, i.e., Ad26 derived vector in Janssen vaccines and chimpanzee from Oxford had dominant roles in developing safe and effective SARS-CoV-2 vaccines.[12] Nonetheless, campaign of mass vaccination applying these kinds of vectors currently reveal induction of low frequency of severe clotting in humans. Further studies currently held to understand fundamental scientific principles of this scarce phenomenon and further modification of Adenoviral vector in order to elucidate the clotting in foreseeable future.[5,6,10]

CONCLUSION

Adenoviruses are highly desirable vectors for gene therapy because of their sophistication and their ability to successfully cross both extra- and intracellular barriers. Ad vectors have a greater capacity to transport genes than even the most sophisticated nonviral gene delivery techniques. Despite Ads' unparalleled benefits, significant hurdles prevent them from widespread clinical use. These include immunogenicity, which triggers the body's natural innate and adaptive immune responses, and inherent broad tropism, which results in inefficient targeted delivery. There are a number of significant obstacles that make it difficult to fully realize the translational potential of adenoviral vectors, especially when it comes to achieving targeting after its delivery. Although there are still numerous obstacles to overcome, significant progress has been made in recent years, especially with regards to de-targeting Ad5 from its innate hepatotropism. Although studies aiming to further characterize the in vivo biodistribution of these vectors are still in their infancy, it is already obvious that they will provide considerable foundations for the creation of optimized retargeting techniques. Newly discovered disease-specific biomarkers, together with technological advancements and

creative retargeting tactics like capsid pseudotyping, serotype switching and multiple conjugation-based strategies, will allow for the selection of individualized vectors with enhanced efficacy. Overall, the promise of successful retargeted ad distribution free of negative in vivo interactions is becoming increasingly probable.

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Conflicts of interest

There have been no competing interests regarding this manuscript.

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Author contribution

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