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Adenovirus vs AAV Vectors for Gene Delivery: Their Advantages and Disadvantages

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Abstract

Gene therapy is to treat and cure diseases by an introduction of therapeutic genes in defective cells or tissues of human body. Gene delivery system, gene expression system, and therapeutic gene are three core elements for gene therapy. The efficient delivery of therapeutic genes and appropriate gene expression are the crucial issues for therapeutic outcome of gene delivery. Because it can be used in common for the treatment and cure of various diseases, gene delivery system is the most important core element for a successful gene therapy. Viruses are naturally evolved to transfer their genomes into host cells efficiently. This ability has made vectorologists exploit viruses as attractive vehicles for the delivery of therapeutic genes. Viral vectors based on adenovirus (Ad) and adeno-associated virus (AAV) have been often used for gene delivery in laboratory. Ad and AAV vectors derived from human DNA viruses differ greatly in their life cycle, expression level and duration of transgenes, immunogenicity, and vector preparation. Both vectors can be used as effective tools for gene therapy and more recently in functional genomics. Here, the characteristics of Ad and AAV vectors are discussed.

Brief history on development of Ad and AAV vectors

In 1953, adenoviruses (Ads), adenoid-degenerating viruses, were first cultured and reported as distinct viral agents (1). Currently, fifty one human adenovirus serotypes have been isolated. Adenoviruses are pathogenic viruses associated with acute respiratory disease, acute hemorrhagic cystitis, hepatitis, gastroenteritis, and myocarditis. Adenovirus serotye 4 and 7 were shown to be major inducers of a large number of acute febrile respiratory syndromes among military recruits (2, 3). Live, oral enteric-coated Ad4 and Ad7 vaccines were developed to prevent acute respiratory diseases (4, 7). These vaccines have been in use only in military, but not for civilians. More than 10 million people have been immunized safely with live Ad4 and Ad7 vaccines (6, 7). Since Ad4 and Ad7 vaccines can be administered orally, adenovirus was engineered initially as an oral vaccine vehicle to deliver suface antigens of hepatitis B virus (8). Since then, adenoviral vector based on serotype 5 has been developed with the expansion of the gene therapy field. Adenovirus 5 is not associated with serious disease.

Adeno-associated virus (AAV) was first recognized as common contaminants of adenovirus stocks in the

mid-1960s (9) and it was determined that AAV was defective virus. Human diseases associated with AAV have not been reported so far. Efficient replication of AAV occurs in the presence of hepler viruses such as adenovirus and herpes virus. AAVs are extremely widespread in the animal kingdom and may occur in every species that has an adenovirus. AAV was used as a model system to study DNA replication, DNA structure, gene expression, and gene regulation. A potential use of AAV as a gene delivery vehicle was proposed in 1984 (10, 11). Since then, AAV-based vectors have been used as promising vectors for the treatment of various genetic and acquired diseases (12, 13).

Life cycle of Ad and AAV

Adenoviruses are nonenveloped icosahedral double-stranded DNA viruses with the ability to infect many cell types. Adenoviral genome is about 36 kb in size. The viruses are replicated in the nucleus of the cell without integration into the host DNA. Viral and cellular proteins are required for DNA replication. After replication, the viruses are assembled in the cytoplasm and the host cells are lysed to release the virus.

AAV is also nonenveloped icosahedral DNA virus like adenovirus, but contains single-stranded DNA as its genome. The size of AAV genome is about 4.7 kb. Viral replication occurs in the nucleus of the cell in the presence of helper virus such as adenovirus (14). Without helper virus, AAV integrates into chromosome 19 of the host genome. The site-specific integrating property is unique to AAV (15). The integrated AAV genome can be rescued when adenovirus is infected.

Current status of Ad and AAV vectors

Adenovirus vectors can be prepared to be either replication-competent or defective. Adenoviruses engineered as tools for gene therapy are generally designed to be defective for replication. Currently, three different kinds of replication-defective adenoviral vectors have been developed. First generation adenoviral vector is defected in E1 region of adenoviral genome in which E1A is essential for virus replication (16). This vector grows only in cells such as the 293 cells constitutively expressing E1A and E1B genes (17). When this vector was used in animal, the inflammatory reaction occurred. It is believed that inflammation results from continued low-level expression of viral genes in the absence of the E1A and E1B genes. In order to eliminate leakiness of expression of other adenoviral genes, especially those that result in immunogenic coat proteins expressed from late genes in first generation adenoviral vector, second generation adenoviral vector was developed that contains either a temperature-sensitive DNA binding-protein gene (E2A) or selective deletion of E4 gene (18). The 3rd generation adenovirus vector contains only the necessary cis-acting elements, the two origins of DNA replication at each of the two physical ends of the virus, and the packaging sequences from within the first 500 base pairs of the left-hand end (19). The 3rd generation adenoviral vector (gutless adenovirus vector) requires a complementing helper virus, which provides all the required early and late gene products, including structural proteins, for the assembly of progeny virus particles. It was assumed that the adenovirus genes eliminated from the vector construct, the less immunogenic and toxic it might become,

although these assumptions have not yet been validated sufficiently. The coding capacity for the 1st generation adenoviral vector is up to 8.3 kb, while the gutless vector could contain inserts of more than 30 kb.

Unlike adenoviral delivery systems, the AAV original vector composition (145 bp terminal repeat constructs flanking the transgene of interest) appears to be the final version. AAV vector based on serotype 2 (AAV2 vector) has been used for gene delivery. However, preexisting immunity to natural infection of AAV2 could limit the extended use of this vector. Currently, eight different kinds of AAV vectors based on different serotypes of AAV isolated from human and primates have been developed (20-24). The characteristics of 8 AAV vectors are summarized in Table 1.

A major goal in developing a targeted vector is to reduce the level of transduction of nontarget cells and thereby to decrease side-effect and at the same time to increase therapeutic effect. Recently, much effort has been given to create a targetable AAV2 vector. Two approaches have been used to modify AAV's virion: chemically cross-linked bifunctional antibodies (25) and genetic manipulation of the capsid gene (26).

Table 1. Characteristics of AAV serotype vectors

AAV Vectors (Year of vector development)	Sources	Amino acid homology of capsid gene	Cross reactivity to human or mouse anti-AAV2 antibody	Cellular receptors (Heparin binding)	Characteristics
AAV2 (1984)	human	100	+++	*HSPG, FGFR, α vβ 5 integrin (+++)	efficient transduction to liver, muscle, neuron, retina
AAV1 (1999)	primate	83	++	(+++)	better transduction effciency for muscle cells than AAV2
AAV3 (1997)	human	82	++	(+++)	antigenically similar to AAV1 and AAV2
AAV4 (1997)	primate	40	-	(-)	different transduction efficiency for CNS from AAV2
AAV5 (1999)	human	44	-	(-)	50 fold more transduction efficiency in mouse lung cells than AAV2
AAV6 (1998)	human	80	++	(+++)	>99% amino acid homology with AAV1
AAV7 (2002)	primate	78	-	nd	better transduction effciency for muscle cells than AAV2
AAV8 (2002)	primate	79	-	nd	10-100 fold higher transduction effciency for hepatocyte than other AAV serotypes

*HSPG; heparansulfate proteoglycan, FGFR; fibroblast growth factor receptor

Advantages and disadvantages of Ad and AAV vectors in gene delivery

The use of adenoviral vectors for gene therapy has several advantages over other commonly used gene delivery vectors. Adenoviral vectors have high transduction efficiency in a broad range of cell types, including both actively replicating cells and terminally differentiated quiescent cells such as hepatocyte, muscle, and neuron. The 1st generation adenoviral vector can express transgenes highly and transiently. One can easily obtain high titers of adenoviral stock (10^{13} pfu/ml). Large DNA inserts (more than 8.3 kb) can be accommodated in adenoviral vectors. A major drawback is that adenoviral vector is highly immunogenic. This results in inflammatory and toxic reactions, and depletion of transduced cells in vivo. On the other hand, the immunogenicity of adenoviral vector can be advantageous for cancer immuno-gene therapy and for therapeutic vaccine vehicle against infectious pathogens, because adenoviral vector by itself can function as an adjuvant. The presence of preexisting antibody or humoral immune response to adenoviral vector may neutralize adenoviral vector particles before or during the gene transfer processes. Thus, 1st generation adenoviral vector is a suitable gene delivery system for the treatment of diseases, such as cancer and cardiovascular diseases, which do not necessarily require long-term expression of therapeutic genes. Since 3rd generation adenoviral vector can express transgene more than 6 months and appears not to induce cellular immunity, the vector fits with genetic diseases which require long-term expression of therapeutic gene. However, helper adenovirus which is used for the preparation of 3rd generation adenoviral vector should be completely eliminated.

Unlike adenoviral vector, AAV vector does not induce cellular immunity, although it induces humoral immunity and transduction efficiency is affected by the presence of preexisting antibody. AAV vector expresses transgene more than 1.6 year in muscle cells and can transduce dividing and nondividing cells like adenoviral vector. AAV is not pathogenic and heat-stable. These are the major advantages of AAV vector. Major limitations of AAV vector are variations of infectivity of AAV onto different cell types and the size of therapeutic gene that can be packaged. The stably integrating property of AAV vector contributes to long-term expression of transgene, but may cause cell mutagenesis. It is tedious and difficult to obtain high titers of AAV stocks. AAV vector is beneficial to gene therapy of genetic diseases such as hemophilia, muscular dystrophy, and cystic fibrosis which require long-term transgene expression. The major characteristics of Ad and AAV vectors are summarized in Table 2.

Conclusion

The improvement of Ad and AAV vectors has allowed for a broad range of therapeutic applications using gene transfer technology. Gene transfer technology has a more active role in clinical trials, and there has a dramatic increase in the number of preclinical and clinical studies in gene therapy using Ad and AAV vectors for last five years (27). Furthermore, since these vectors are highly efficient at gene transfer in a broad spectrum of cell types and species, and have been used, both in vitro and in vivo, Ad and AAV vectors can be used to achieve gain or loss of function in functional genomics (28). However, the degree of Ad and AAV vectors-development is not still sufficiently adequate to meet all the requirements for ideal gene delivery vehicle, in particular for human gene therapy. Thus, much effort should be given to further improve gene delivery efficiency and production methods of Ad and AAV vectors.

Table 2. Characteristics of Ad and AAV vectors

Characteristics	AAV vector	Adenovirus Vector		
		1st generation	2nd generation	3rd generation (gutless)
Wild type virus Genome size	single-stranded DNA 4.7 kb	double-stranded DNA 36 kb		
Pathogenicity	None	Not lethal		
Insert size	5 kb (10 kb)	8.3 kb	10 kb	more than 30 kb
Location	nuclear	nuclear		
Titer	10^{13} particles/ml	10^{13} particles/ml		
Stability	very stable, heat resistant	Stable, heat-sensitive		
Host range	Nondividing & Dividing cells (hepatocyte, neuron, muscle, retina)	Nondividing & Dividing cells		
Vector production	tedious	easy	easy	tedious
Administration	In vivo, Ex vivo	In vivo, Ex vivo		
Expression period	Long (years)	Short (2 weeks in mice)	Short (6 weeks in mice)	Long (more than 6 months)
Expression level	Moderate, Slow	Srong, Immediate		
Safety	Insertional mutagenesis	Inflammation	Reduced inflammation	Yes
Humoral immunity	Yes	Yes		
Cellular immunity	No	Yes	Yes, but less than 1st	No
Preexisting antibody	Yes, but depends on AAV serotypes	Yes	Yes	Yes
Clinical Experiences	AAV-CFTR and AAV-FIX in Phase I & II	Ad-p53 in Phase III. Ad-TK, Ad-CD, Ad-IL2 in Phase I	Ad-OTC in Phase I (terminated)	Ad-FIX in Phase I

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