The Libyan International Medical University

Faculty of Basic Medical Science

Adeno-Associated Virus for Gene Therapy

Sama Ahmed Elmehdawi

Supervised by: Dr. Amal Alnaili

Assisted by: Dr. Nawar Montasser

Report Submitted to fulfill the requirements for Scientific Research Activity

Date of Submission: 12/3/ 2020

Abstract:

Adeno-Associated Virus (AAV) was discovered during the 1960s and since then, it has become a revolutionary viral vector in gene therapy and gene delivery. AAV is a non-enveloped virus that can be engineered to deliver DNA to target cells, and has attracted a significant amount of attention in the field. The ability to generate recombinant AAV particles lacking any viral genes and containing DNA sequences of interest for various therapeutic applications has thus far proven to be one of the safest strategies for gene therapies.

Introduction:

Human Gene therapy can be described as the delivery of genetic material to patients' cells with a therapeutic purpose. Two distinctive classes of vectors mediate the transfer of these materials which include non-viral and viral. (1)

Non-Viral Vectors

Non-viral methods may be divided into three categories; inorganic particles, synthetic/natural biodegradable particles, and physical methods. These methods have become ubiquitous because of their ability to transfer large genes, their relative safety, and site-specificity. However, they may be restricted by their relatively poor transgene expression, and low transfection efficiency. (2)

Viral Vectors

Viruses have advanced to become quite efficient at the transfer of genetic material to particular cell types or tissues resulting in the expression of therapeutic genes while averting immunosurveillance by an infected host. These characteristics make viruses appealing vectors, for gene therapy. A number of factors must be considered regarding any viral vector including the capability to attach and enter the target cell, successful transfer to the nucleus, the extent of expression inside the nucleus for a sustained period of time, and a general lack of toxicity. There

are various viral agents which have been used for gene transfer such as herpes simplex virus, retrovirus, adenovirus, and adeno-associated virus (AAV). Each selected with some precise attributes that would make them more or less appropriate for the task, depending on the preferred profile. (3)

Adeno-associated virus (AAV) is amongst the most studied and used gene therapy vectors. It was first discovered as a contaminant of adenovirus preparations. Due to its twelve human serotypes (AAV serotype 1 to AAV-12) the virus has a wide range of tropism but AAV2 is the most notably studied. AAV is a member of the family Parvoviridae and is belongs within the genus Dependovirus. It is a small (25-nm), nonenveloped virus that consists of a linear single-stranded DNA genome.

The genome of the AAV consists of three genes, Rep (Replication), Cap (Capsid), and aap (assembly) which produce at least nine gene products by the use of three promoters, differential splicing, and alternative translation start sites. These coding sequences are flanked via inverted terminal repeats (ITRs). ITRs has a major role in replication in which it serves as a primer for second-strand synthesis through DNA polymerase. The Rep gene mediates replication and packaging via encoding four proteins (Rep78, Rep68, Rep52, and Rep40). The Cap expression provides the viral capsid proteins (vp; VP1/VP2/VP3), which protect the viral genome, in addition to mediating cell binding and internalization. Whereas the aap gene encodes the assembly-activating protein (AAP). Which is thought to provide a scaffolding characteristic for capsid assembly. (4)

The AAV lifecycle starts with the infection of the target cell mainly through the attachment to the cell surface heparan sulfate proteoglycan (HSPG) which serves as a receptor for the virus. Fibroblast growth factor receptor, αvβ5 integrin, hepatocyte growth factor receptor, and laminin receptor have additionally been implicated as coreceptors or facilitators of AAV access into target cells. After successful infection, and in the presence of a helper virus (adenovirus or herpesvirus), the AAV undergoes genome replication, viral gene expression, and virion formation. Certain adenoviral genes were identified including E1a, E1b, E2a, E4, and VA associated genes that provide helper functions in AAV gene expression.

There is restricted AAV replication in the absence of adenovirus or herpesvirus, the AAV gene

expression is repressed, and the AAV genome can set up latency via integrating into a 4-kb region on chromosome 19. The latent AAV genome may be rescued upon superinfection by adenovirus. (5)

The aim of this report is to discuss AAV biology, viral structure, and cell entry mechanisms as well as discusses successful application of the AAV vector for inherited disorders, outlining advantages, and disadvantages.

Materials and methods:

The most extensively used approach to construct and purify recombinant AAV particles for preclinical programs is the triple transfection approach using HEK293 cells which are engineered to provide adenovirus helper genes in trans. This technique requires three plasmids:
The first plasmid carries the Rep and Cap genes which form the proteins essential for replication and capsid formation
Whilst the second plasmid carries the required adeno helper genes, these include E4orf6, E2a, and VA RNA while E1a and E1b55k are typically expressed via the packaging cell line itself.
Finally, the third plasmid is the vector DNA plasmid which consists of the inverted terminal repeat trangene cassette.
The cell homogenate is purified following 48-72 h transfection, subsequently AAV quality control is assessed which includes infectious and transducing properties, genome titer, and integrity of the packaged AAV genome.
In addition the AAV capsid can be modified to extend its tropism towards specific tissues. (6)

Results/Discussion:

A successful gene therapy method must provide the target tissue with the proper amount of a therapeutic gene accomplishing long-term gene expression without significant toxicity. (6) The adeno associated virus has become amongst the most commonly used viral vectors. This is probably the result of numerous factors. First, AAV transgene expression persists for years or a

life time whereas almost all other viral vectors cause an initial burst of transgene expression which usually disappears after a rather brief period of time, measured in weeks. Second, AAV vectors usually do not induce a deleterious immune response. This characteristic depends on the efficient MOI of the vector used as well as the site of administration. Another major positive characteristic is the poor uptake of AAV via dendritic cells, also the small capacity of the genome means that no viral genes persist.

Finally, the AAV-based vectors are capable of transducing a wide range of host cells which include both dividing and non-dividing cell types. (5)

AAVs many advantages have resulted in the successful treatment of many genetic diseases some of which may include:

Hemophilia B

A blood clotting disorder resulting from a mutation in the gene encoding coagulation factor IX (fix) leading to its deficiency.

long-term expression of fix was achieved through AAV-based therapy, without major liver toxicity nor fix-specific antibodies detected following liver- or muscle-directed injections.

Leber congenital amaurosis (LCA)

A severe form of inherited retinal blindness which occurs in infants and children as a result of mutations within the RPE65 (retinal pigment epithelium-specific 65-kDa) gene. Numerous independent studies using rAAV2/2 expressing RPE65 complementary DNA (cDNA) have provided initial evidence of efficacy and short-term safety in this disorder. (6)

Lipoprotein lipase deficiency (LPL)

Is an uncommon inherited disease characterized by chylomicronaemia, severe hypertriglyceridaemia, and risk of recurrent pancreatitis or other complications. Incorporation of episomal copies of functional LPL genes into muscle cells deficient of active LPL, through Intramuscular administration of AAV1-LPLS447X gene therapy was generally well tolerated and was accompanied by a decrease in pancreatitis incidence,moreover two years after administration signs of clinical improvement continued. (7)

Rheumatoid arthritis

It is an inflammatory autoimmune disease of the joints. Recent therapy approaches include the blocking off of host reactions towards itself, most important of which is through the inhibition of the effects of the cytokine tumor necrosis factor alpha (TNF-α). For prolonged inhibition of TNF an alternative approach emerged involving the construction of AAV vectors which expressed TNF inhibitors. (5)

Pulmonary fibrosis

Idiopathic pulmonary fibrosis was the target in this therapy. It is acknowledged that telomeres serve as protecting structures on the ends of chromosomes. One of the causes of the development of pulmonary fibrosis is due to the presence of short telomeres. These short telomeres lead to cellular apoptosis due to the cessation of cell division.

Delivery of telomerase via AAV serotype 9 resulted in the repair of these short telomeres, hence improving lung function, as well as decreasing inflammation and fibrosis at 1–3 weeks after vector therapy. It is of importance to note that after 8 weeks of gene therapy pulmonary fibrosis either improved or disappeared. AAV9 therapy resulted in longer telomeres and improved proliferation of ATII cells, in addition to decreasing apoptosis, DNA damage, and senescence. (6)

α1-antitrypsin deficiency (AAT)

Alpha-1 antitrypsin (AAT) deficiency, characterized by low plasma levels of the serine protease inhibitor AAT, is one of the causes of secondary emphysema resulting from the inadequate protection of the lung from neutrophil proteases.(8)

Treatment with rAAV2/8-ACE2 resulted in a significant elevation in ACE2 expression and protein activity, which was limited to the liver sparing other organs. (6)

Despite the tremendous progress made in the use of AAV vectors for human gene therapy and its successful application in several diseases, there are still some hurdles to overcome. For instance the host immune response remains a concern therefore several approaches have emerged to reduce the response. One such approach is decreasing the vector dose needed for a therapeutic

response. The modification of the surface capsid through the addition of particular ligands for the attachment to target tissues, as well as the discovery of more AAV serotypes are one possibility. Another area of concern with great potential for improvement is the route of administration. Especially for the application of AAV vectors in the central nervous system (CNS). The vector administration currently requires an open neurosurgical operation. The development of AAV vectors with the capability to cross the blood-brain barrier would highly facilitate CNS gene therapy. (5)

Furthermore another notable drawback associated with AAV in comparison to the other viral vectors is its small packaging size, limiting the size of the transgene transported. However, with time and smart engineering techniques it is possible to conquer these obstacles. (6)

Conclusion:

Since the discovery of AAV, its unique biology, simple structure, and no known disease associations, as well as its broad tropism, and efficient transduction with stable and long-term transgene expression have made AAV vectors the leading gene delivery vectors in clinical development. However For the final application of AAV to the clinic, there are still many hurdles to overcome. With the rapid advances in AAV therapy, we can optimistically anticipate these hurdles to be overcome in the foreseeable future to pave the way for the final application of AAV therapy to human gene therapies.

References:

1. Özduman K, Wollmann G, Piepmeier JM. Gene Therapy for Meningiomas. In: Meningiomas. Elsevier; 2010. p. 681–90.

2. Ramamoorth M, Narvekar A. Non viral vectors in gene therapy-an overview. J Clin diagnostic Res JCDR. JCDR Research \& Publications Private Limited; 2015;9(1):GE01.

3. Robbins PD, Ghivizzani SC. Viral vectors for gene therapy. Pharmacol Ther. Elsevier; 1998;80(1):35–47.

4. Naso MF, Tomkowicz B, Perry WL, Strohl WR. Adeno-associated virus (AAV) as a vector for gene therapy. BioDrugs. Springer; 2017;31(4):317–34.

5. Daya S, Berns KI. Gene therapy using adeno-associated virus vectors. Clin Microbiol Rev. Am Soc Microbiol; 2008;21(4):583–93.

6. Rajapaksha I, Angus P, Herath C. Adeno-Associated Virus (AAV)-Mediated Gene Therapy for Disorders of Inherited and Non-Inherited Origin. In: In Vivo and Ex Vivo Gene Therapy for Inherited and Non-Inherited Disorders. IntechOpen; 2018. Chapter. Pg 1-20.

7. Gaudet D, Méthot J, Kastelein J. Gene therapy for lipoprotein lipase deficiency. Curr Opin Lipidol. LWW; 2012;23(4):310–20.

8. Chiuchiolo MJ, Crystal RG. Gene therapy for alpha-1 antitrypsin deficiency lung disease. Ann Am Thorac Soc. American Thoracic Society; 2016;13(Supplement 4):S352–69.