

# Adenovirus vector for gene therapy in liver diseases

## 1. Introduction

Adenoviruses are a vector that can be used in gene therapy to treat a variety of conditions, such as cancers and congenital diseases. They are one of the more common viruses to use for gene therapy because of the wide range of tissues it can affect, relatively well-mapped genome, ease of gene editing, and lack of viral replication in hosts (Singh 2018). This gene therapy technology is used to help express biological functions that are missing from unhealthy host genes, such as missing enzymes. This is accomplished by taking genes that code for those enzymes and incorporating into the target cells of the host. Diseases such as ornithine transcarbamylase deficiency or phenylketonuria are potentially curable using gene therapy to incorporate the necessary genes. This technology works by using altered adenoviruses to target the liver cells of infants. These adenoviruses must then attach and enter the cells in order to incorporate the necessary genes for enzyme production that were missing.

## 2. Gene therapy using adenovirus

### *2.1 Ornithine Transcarbamylase Deficiency Disease and Phenylketonuria*

The liver disease, ornithine transcarbamylase deficiency, is a genetic disorder that causes a lack of the enzyme called ornithine transcarbamylase (Lichter et al.

2014). The liver cells of the infants suffering from ornithine transcarbamylase deficiency or phenylketonuria are treated using the adenovirus as a delivery vehicle. The gene that is being delivered by the adenovirus involves production of the enzymes that are missing from those that suffer from ornithine transcarbamylase deficiency and phenylketonuria. These disorders can be improved by increasing enzyme production through gene therapy. This enzyme is necessary for the break down and removal of nitrogen from the body. The removal of excess ammonia in the body is important because it is a neurotoxin. Those that suffer from this disease experience developmental delay, learning disabilities, and even death if it is not treated early on (Lichter et al. 2014). Even when infants are treated early on, usually further treatment is needed later because current treatment does not have lasting effects. (Lichter et al. 2014). Currently, treatment still usually requires a liver transplant for the infants.

Phenylketonuria is a disease that involves the lack of an enzyme called phenylalanine hydroxylase (Sumailly et al. 2017). This causes phenylalanine to build up in the body. Phenylalanine comes from diet, and failure to break down this protein can cause learning disabilities and seizures (Sumailly et al. 2017).

While this condition can be remedied with diet changes, it must be caught early on in order to make the necessary changes.

Gene therapy for both of these conditions involves inserting genes into the adenovirus genome that can enhance production of ornithine transcarbamylase or phenylalanine hydroxylase depending on what gene is inserted into the adenovirus vector. Improving the production of these enzymes can improve the conditions of those that suffer from these disorders. However, the genes of the adenovirus and of the patient must first be altered to allow these changes to take place.

## *2.2 Alteration of adenovirus and patient DNA*

The adenovirus is first altered to include the necessary genes. A type of the adenovirus called a replication defective mutant is given the healthy genes that the patient is missing (Wold 2013). The E1A and E1B genes are removed, and the healthy genes are inserted in place of those genes (Wold 2013). The missing E1A gene is important for additional transcription units, so viral replication is therefore stopped, which is beneficial for gene therapy (Singh 2018). This is because while the adenovirus protein transcription occurs, there is no viral progeny being created. This allows the enzymes added to the adenovirus genome to be produced in the host's liver cells, while controlling viral replication. It is also altered to contain the nucleases necessary for insertion of adenovirus into the patient's DNA. The adenovirus is first altered to have its disease-causing parts removed so that it cannot harm the patient. The adenovirus is then edited to contain the genes for producing the enzymes ornithine transcarbamylase or phenylalanine hydroxylase

for breaking down ammonia or phenylalanine, respectively. Alteration of the patient's DNA is also required, however. The recombinant DNA is inserted into the patient's genome so that production of the necessary enzymes can occur. This is achieved by including DNA snipping nucleases into the adenovirus delivery vehicle. Once the adenovirus genome has been edited and the necessary DNA snipping nucleases have been added, it is prepared to be entered into the liver cells (Wold 2013).

### 3. Attachment and entry of altered adenovirus onto cells

The adenovirus must first attach to a receptor on the liver cell and then interact with a coreceptor for the capsid to be taken into the cell.

However, the receptor attachment of adenoviruses requires not just receptor contact, but also interactions with integrin proteins. The adenovirus capsid has 12 pentons at each end of its icosahedral capsid (Sharma et al. 2009). It also has many protruding fibers, which the virus uses for attachment onto host cells.

#### *3.1 Receptor Attachment and Entry into Cell*

The adenovirus attaches to the Coxsackievirus and adenovirus receptor (Car) of the liver cells (Wold 2013). This receptor is not sufficient for entry into the host cell, however. The receptor must also interact with integrin proteins, such as Alpha V integrin, for entry (Wold 2013). The cell absorbs the proteins which is what

helps to initiate entry into the host cell (Sharma et al. 2009). This involves a process known as receptor-mediated endocytosis. Endocytosis of the virus particle involves the import of the virus into the cell through clathrin-coated pits, which puts the particle into an endosome (Wold 2013). Once inside the endosome, acidification occurs inside and begins breaking down the outer proteins of the virus particle (Sharma 2009). This causes the pentons of the adenovirus to break off, resulting in penton toxicity. This toxicity causes the breakdown of the endosome phospholipid bilayer, allowing the adenovirus capsid to enter the cytoplasm of the cell (Sharma et al. 2009). The adenovirus can then find the microtubules that makes up the cytoskeleton of the cell. The microtubules are made of motor proteins called kinesins and dyneins. Kinesins move along the microtubules through hydrolysis of adenosine triphosphate molecules, giving them energy to walk along the filaments. This moves the adenovirus capsid to the nucleus of the cell, where the viral replication can occur.

#### 4. Issues with immune system's response to treatment

A concern with gene therapy using viruses is that the immune system will attack the adenovirus and prevent healthy genes from reaching the liver cells. This is a common issue with many treatments using viruses as gene therapy. Toth (2013) conducted a study that concluded that mice injected with recombinant adenovirus that targeted the Kupffer cells of the liver had strong immunogenic

responses to the virus. The immune responses shown in the mice began immediately after injection of the virus, resulting in inflammatory responses that killed the mice (Toth 2013). This study showed that adenoviruses are highly immunogenic. If the recombinant adenovirus acted similarly immunogenic in a human host as it did in mice, there would be huge risks that would outweigh the potential benefits. This is troubling for the purpose of using adenoviruses as a vector for gene therapy.

The use of adenoviruses for gene therapy has risks of being ineffective because of its immunogenicity. However, ornithine transcarbamylase deficiency alone has around a 50% mortality rate in infants (Donovan 2021). Even if survival occurs, there are many complications regarding the health of the infant. The high mortality rate of this disease is a heavy factor in deciding whether to choose gene therapy as a potential future or to rely on current treatments.

## 5. Conclusion

It is important to understand the entry and attachment of the adenovirus vector because of complications of the virus's immunogenicity. While there are concerns over the effectiveness of the treatments due to the immune systems of humans, iECURE, a company researching adenovirus gene therapy, has seen promising results from their trials (Pratap 2021). The trials may point to a viable

treatment for these high mortality diseases, so knowledge of the vector's entry and attachment will better aid those endeavors.

## Literature Cited

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