



Viral Vectors as a Gene Delivery Vehicle

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Abstract

The field of gene and cell therapy is experiencing a renaissance, with innovations in the use of viral vectors as a gene delivery vehicle being a key factor in the field's growth. However, the long-term impact of viral vectors requires continuing research into their design that addresses both efficacy and the problems associated with immunogenicity and toxicity.

Key points:

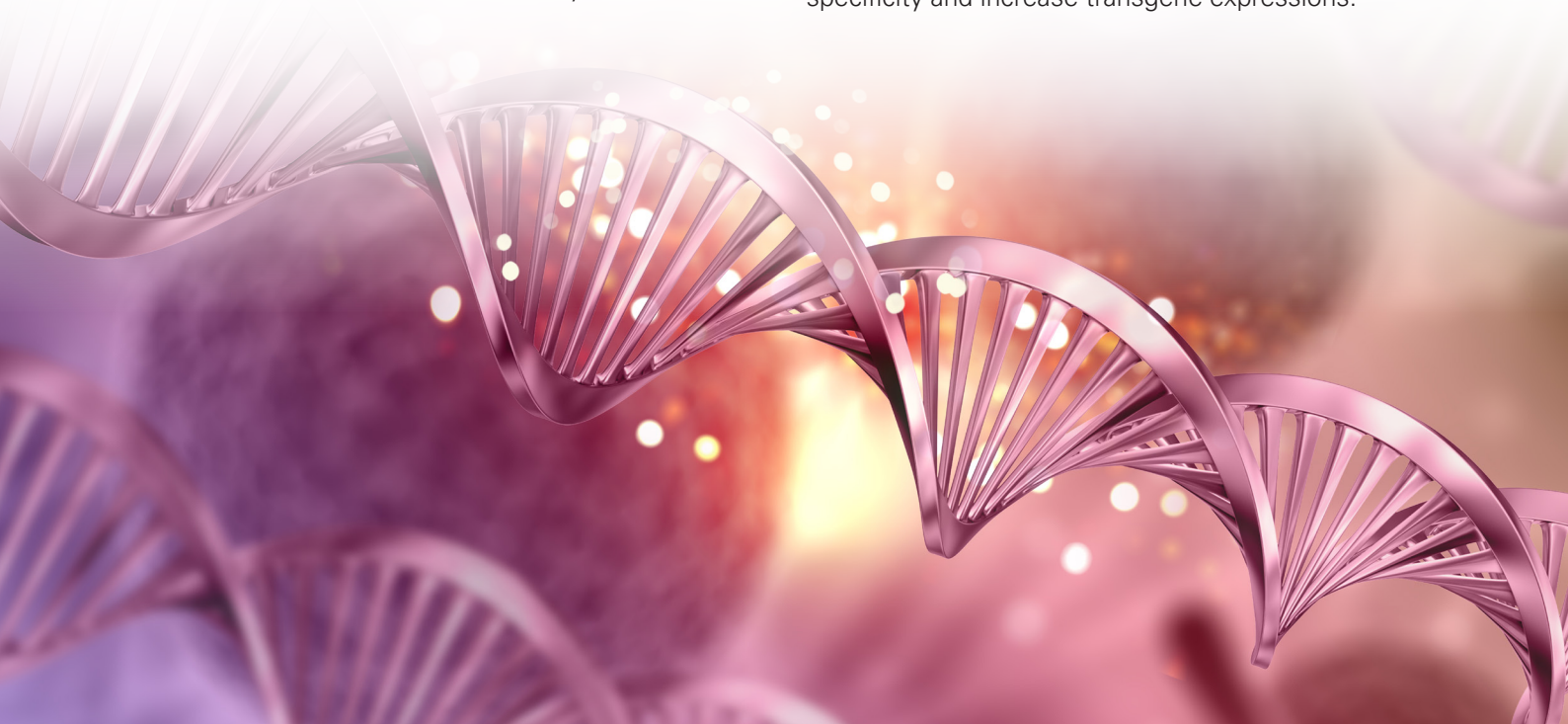
- Leveraging knowledge of genetic disease mechanisms to develop gene therapies has been challenging; however, recent advances in viral-mediated gene delivery have shown great promise in improving the health of patients.
- Viral vectors have proved to be the most efficient mechanism of gene delivery by taking advantage of a virus's natural ability to infiltrate human cells and introduce their genetic material.
- Several different viruses have been used as vectors for gene therapy including adenoviruses (AVs), adeno-associated viruses (AAVs), retroviruses (RVs), lentiviruses (LVs) and herpes simplex viruses (HSVs), with each virus having its advantages and limitations.
- Safety issues with early viral vectors for gene therapy, including a death and several instances of viral-mediated leukemia, cast doubt on the ability of gene therapy to safely cure or mitigate disease. However, these setbacks prompted further research and the development of viral vectors which overcame many of the safety concerns.
- With current viral vectors, cytopathic and severe immunogenic responses have been reduced and better control of cell tropism and transgene expression has been achieved.
- Continuing advances in viral vector design are expected to lead to improvements in the efficacy of gene therapies and a reduction in safety concerns.

Introduction

Genes are composed of DNA that encodes proteins that make up the form and function of a person's body. While there is natural variability in gene sequences that make us individuals (i.e., hair and eye color), certain mutations in genes that encode critical functions can result in severe disease (e.g., sickle cell anemia and cystic fibrosis). While these genetic diseases were once thought incurable, the growth in understanding of genetics and molecular biology and the development of molecular tools have given hope to these patients that these diseases could be treated by correction of the defective genes via gene therapy. The field of gene therapy, however, has been marked by many transitions since it was first envisioned in the early 1970s,

and over the years scientists have faced multiple challenges in creating powerful technology that allows the introduction of a transgene adequately and safely into human cells.

Although several methods for introducing genetic material into a cell have been tried and developed, viral vector-mediated gene delivery has proved to be the most efficient, with almost **70% of gene therapy trials being based on viral vectors**. Viral vectors are particularly effective for targeted gene therapy due to their natural ability to invade host cells – a critical aspect of achieving therapeutic efficacy. Furthermore, viral vector expression genomes can be adjusted using tissue-specific promoters to enhance target specificity and increase transgene expressions.



Viral vector-mediated gene therapies have been introduced into the host by one of three mechanisms:

IN VIVO: In this method, the viral vector is administered by direct intravenous injection into patients which infects and delivers the gene into host cells. While in vivo delivery has proven successful in mitigating disease, it has also resulted in severe life-threatening immunogenic responses and cases of insertional mutagenesis and oncogenesis.

IN SITU: In this method, the viral vector is administered directly into the target tissue. This avoids some of the immunogenicity issues from systemic in vivo delivery but is limited to targets that can be readily accessed such as melanomas.

Viral biology

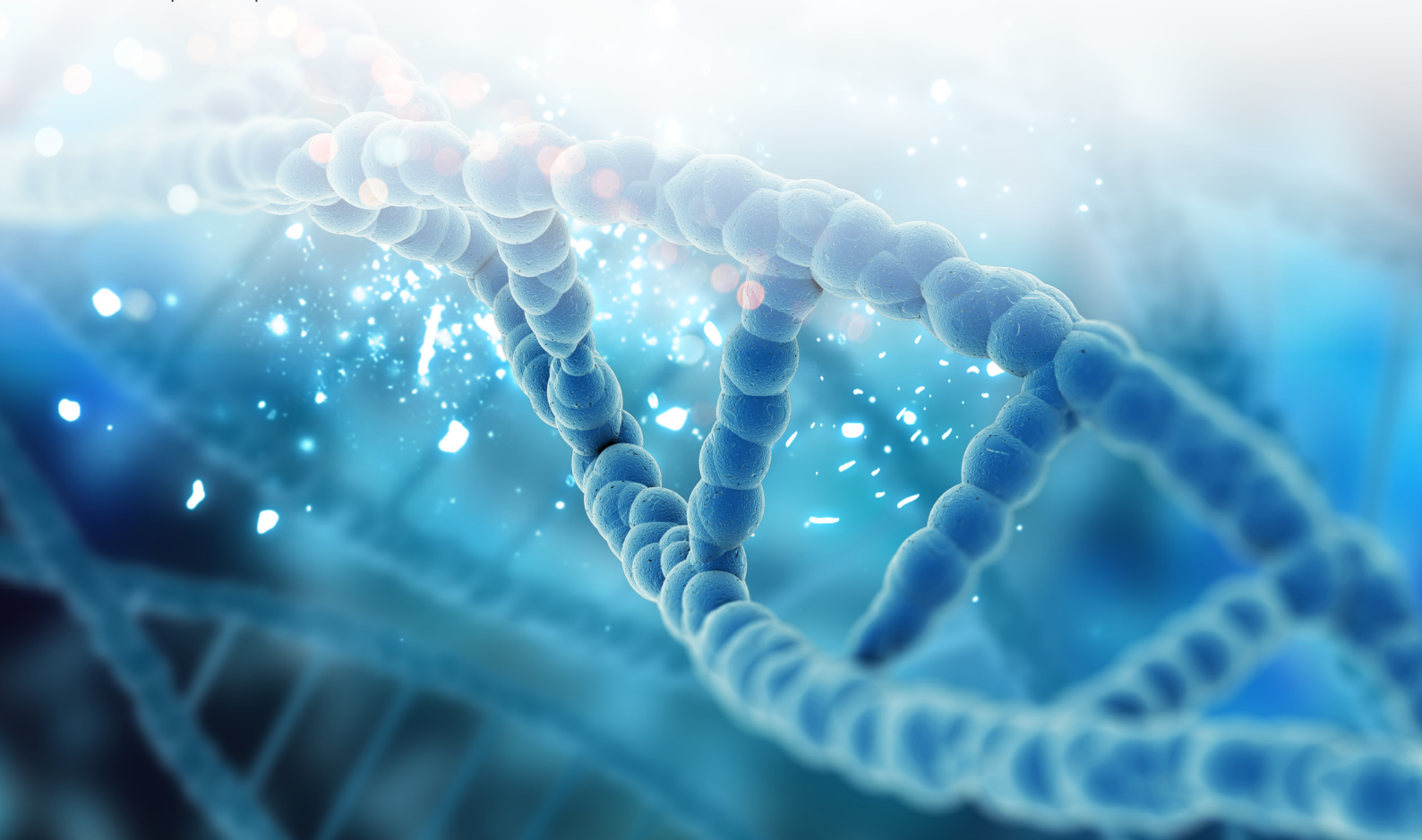
As stated previously, while many different mechanisms of gene delivery have been developed, viruses have proved to be the most efficient due to their natural ability to enter cells and introduce their genetic material. Furthermore, viruses have a level of cell tropism, unlike many of the non-viral delivery mechanisms, that allow directed delivery of genetic material to particular target cells or sites within the body. These distinct features give viral-mediated gene delivery the potential to treat a wide spectrum of diseases. The most promising viruses being developed as viral vectors include:

- Adenoviruses (AVs)
- Adeno-associated viruses (AAVs)
- Retroviruses (RVs)
- Lentiviruses (LVs)
- Herpes simplex viruses (HSVs)

EX VIVO: In this method, the patient's cells are extracted and cultured outside the body before they are infected and genetically modified by the viral vector. Once modified, the cells are reinserted into the patient. This is often referred to as cell therapy. The most common cell types used for ex vivo delivery are T-cells (CAR-T therapy) or hematopoietic cells. Ex vivo delivery avoids any issues regarding immunogenicity but is often dependent on eliminating the patient's current T-cell or hematopoietic cell population in order for the therapy to be successful, and this presents its own safety issues.

With DNA viruses, like AAVs, AVs, and HSVs, the delivered genetic material remains largely episomal, meaning it is not integrated into the host cell chromosome and thus gets diluted as the cell divides. With RNA viruses like RVs and LVs, their genetic material is integrated into the host cell chromosome in a non-random manner and gets propagated as the cell divides, giving longer-term expression than episomally delivered genes.

Both DNA and RNA viral vectors, however, have distinct disadvantages. DNA viruses tend to be larger viruses that can generate immune responses that can cause reactogenicity as well as neutralizing antibodies that limit the efficiency of gene delivery. RNA viruses, while smaller and less immunogenic, can cause unintended changes in gene expression in the host cell due to integration in the chromosome, and this has the potential to promote oncogenesis in patients.



Viral vectors

All viral vectors consist of genetic material (double-stranded DNA, single-stranded DNA, or RNA) encased in a protective protein shell or capsid. Most RNA viral vector capsids are further encased in a viral envelope which is composed of membrane lipids and proteins. The size of the capsid largely dictates the amount of genetic material and the size of the transgene that can be contained within the viral vector. Larger viruses such as AVs can carry transgenes of up to 36 kb while smaller viruses like AAVs can only accommodate a transgene of 3-4 kb. Furthermore, the capsid and/or envelope proteins can provide cell tropism and antigen recognition. Despite the differences in the size or nature of the genetic material, all viral vectors share these common characteristics:

1. **Safety and stability** – The viral vector needs to be infectious in order to deliver the transgene and have the desired therapeutic effect. However, viruses often cause cytopathic effects following infection due to replication; therefore, to prevent these harmful effects, the viral vectors are rendered replication-deficient by deleting the parts of the viral genome responsible for replication. These deletions also serve to make room in the genome for the transgene. Essentially, each viral vector particle is designed to infect a single cell to provide the transgene.
2. **Controlled transgene expression** – Once the viral vector has provided the transgene, the efficacy of the gene therapy depends on the ability of the transgene to be expressed at an appropriate level and time, and for an appropriate duration. The genomes of viral vectors have included various regulatory elements (promoters and enhancers) that drive transgene expression to the desired levels. Care must be taken to ensure overexpression of the transgene does not occur, as this has the potential to cause inflammation and other toxicities.
3. **Controlled cell tropism** – Viral cell tropism is defined as the ability of a virus to infect different cell types. For many viruses, particularly AAVs, cell tropism is determined by the affinity of proteins in the capsid for receptors on particular host cell types. Small variations in AAV capsid proteins (AAV serotypes) can result in different affinities and thus preferences for different cell types. Often, specific AAV serotypes are chosen as the basis for a gene therapy in order to target particular cell types. In addition, cell tropism can also be controlled at the genetic level by choosing transgene promoters that are only induced in certain cell types.

Unfortunately, immunogenicity remains an issue. For viral vector-based gene therapies, immunogenicity has the potential to reduce therapeutic efficacy by increasing the chances of the host's immune system detecting and destroying the introduced therapy. This is particularly a problem for larger viruses like AVs and HSVs which tend to be more immunogenic.

Conclusion

Viral vector design for gene therapy has come a long way since its inception and continues to evolve as knowledge of viral biology grows. However, while great strides have been made in the design of viral vectors to reduce cytopathic effects, immune responses against vectors and transgene products remain a significant barrier to achieving desirable efficacy. A better understanding of immunogenicity and formulation of more targeted therapies hold huge potential for the treatment of a wider range of conditions.

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