Retrovirus Vectors and Human GMOs

Find Out More

Talking Points: Retrovirus Vectors and Human GMOs

by Tony Ghepardo, author of: *We Poisoned Our Grandchildren*.

Retrovirus

- What are retroviruses?
- Why are retroviruses used in genetic engineering?
- Why are retroviral vectors necessary?
- What dangers do retroviruses pose?
- Gene Therapy

What are retroviruses?

"REPLICATION OF RETROVIRUSES". Feb 2010.

<https://www.lehigh.edu/~jas0/V07.html>.

Retrovirus virions contain reverse transcriptase, that uses the viral RNA as a template to synthesize DNA. This enzyme produces a ds DNA copy of the viral genome, and this gets incorporated into a (random?) [indeterminate] [arbitrary] chromosomal site in the host cell nucleus.

The most widespread retrovirus in humans is HIV.

Why are Retroviruses used in genetic engineering?

Wikipedia. "vector". 24 July 2018.

<https://en.wikipedia.org/wiki/Vector_(molecular_biology)>.

Vector, a vector is a DNA molecule used as a delivery vehicle to artificially carry foreign genetic material into another cell.

Burnie, David. B.S. "Retrovirus." 24 July 2018.

<http://autocww.colorado.edu/~toldy3/E64ContentFiles/VirusesMoneransAndProtists/RetrovirusB1.html>.

Retroviruses make suitable vectors because they have the chemical apparatus that is needed to splice genes into particular target cells.

Why are retroviral vectors necessary?

National Center for Biotechnology Information, U.S. National Library of Medicine. "The use of retroviral vectors for gene therapy-what are the risks? A review of retroviral pathogenesis and its relevance to retroviral vector-mediated gene delivery."

24 July 2018.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC515179/>.

Retroviral life cycle

It is the unique nature of the retroviral life cycle, combined with the simplicity and advantageous arrangement of the retroviral genome, which has made retroviruses so attractive as vectors for gene therapy. The principal feature of the retroviral life cycle that is of interest is the ability of the retrovirus to copy its RNA genome into a double-stranded DNA form which is then efficiently and exactly integrated into the host cell genome.

Otherwise, an article full of weasel words.

National Center for Biotechnology Information, U.S. National Library of Medicine. "The use of retroviral vectors for gene therapy-what are the risks? A review of retroviral pathogenesis and its relevance to retroviral vector-mediated gene delivery."

24 July 2018.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC515179/>.

Boundless.com. "Retroviruses: Double-Stranded RNA Viruses." 24 July 2018.

<https://courses.lumenlearning.com/boundless-microbiology/chapter/retroviruses-double-stranded-rna-viruses/>.

Retroviruses are proving to be valuable research tools in molecular biology and have been successfully used in gene delivery systems.

Chaudhari, Sweena. "Retroviruses - Friends or Foes?" 24 July 2018.

<https://bitesizebio.com/25991/retroviruses-friends-or-foes/>.

Retroviruses are commonly used to introduce genes into mammalian cells to express or knockdown genes of interest. In addition, retroviruses are being developed for use in gene therapy. Though the use of retroviruses raises safety issues, new molecular and genetic techniques of vector design circumvent concerns and make them widely available for safe use in the lab and as potential gene therapy agents.

What dangers do retroviruses pose?

Boundless.com. "Retroviruses: Double-Stranded RNA Viruses." 24 July 2018.

<https://courses.lumenlearning.com/boundless-microbiology/chapter/retroviruses-double-stranded-rna-viruses/>.

- retroviruses infect a host cell with their genome, and then are reverse transcribed into double stranded DNA, with the DNA then integrated into the home cell genome.

[This mechanism thwarts natural selection by posing a gene drive danger. The engineered gene is always dominant whether it is beneficial or detrimental.]

- When integrated into a host genome, a retrovirus is hard to detect and can lay dormant for prolonged periods, having no discernible effect on the host.

- Retroviruses can be human pathogens, and cause many diseases, but have also proven to be invaluable tools when used by molecular biologists.

A special variant of retroviruses are endogenous retroviruses, which are integrated into the genome of the host and inherited across generations. Endogenous retroviruses are a type of transposon.

National Center for Biotechnology Information, U.S. National Library of Medicine.

"DNA Transposons: Nature and Applications in Genomics." Apr 2010.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2874221/>.

Transposable elements (TEs) are defined as DNA sequences that are able to move from one location to another in the genome.

For example, transposable elements comprise approximately 45% of the human genome. From bacteria to humans, transposable elements have accumulated over time and continue to shape genomes through their mobilization.

- The reverse transcription of HIV viral RNA to DNA is error prone, causing HIV to have a high mutation rate

Special concerns for using retroviral vectors

Chaudhari, Sweena. "Retroviruses - Friends or Foes?" 24 July 2018.

<https://bitesizebio.com/25991/retroviruses-friends-or-foes/>.

In 1990, Nobel laureate Howard Temin, who co-discovered the reverse transcriptase enzyme, detailed the risks and safety considerations associated with retroviral vectors, but stated that "safety considerations should not hold up further human trials of retrovirus vectors".

In 1992, in a pre-clinical trial in rhesus monkeys, a retrovirally-transduced bone marrow transplant led to development of fatal lymphomas in 3 out of 10 recipients, the cause being formation of replication-competent viruses.

Increasing safety

Research and development of viral vector strategies and newer technologies to increase safety include:

Self-inactivation

Higher biosafety is ensured in newer systems due to genetic modifications in the transfer plasmid making it easy to integrate into the host genome but prevent activation afterwards (called a SIN or self-inactivating vector).

Tropism

Depending on the type of viral envelope proteins, three types of viruses can be made:

- Pantropic - can infect cells of all species
- Ecotropic - can infect cells of mouse and rat origin
- Amphotropic - can infect human, mouse and rat cells

Ecotropic viruses are the safest to use, as they cannot infect human cells. (Though consider the fact that ecotropic and amphotropic viruses are less stable than pantropic viruses).

Containment

All retroviral work is carried out in a BSL2 (or BSL3 for certain experiments like work with HIV) containment facility and is strictly governed by institutional guidelines.

Gene Therapy

Chaudhari, Sweena. "Retroviruses - Friends or Foes?" 24 July 2018.

<https://bitesizebio.com/25991/retroviruses-friends-or-foes/>.

Retroviruses are commonly used to introduce genes into mammalian cells to express or knockdown target genes. In addition, retroviruses are being developed for use in gene therapy. Though the use of retroviruses raises safety issues, new vector designs circumvent concerns and make them widely available for safe use in the lab and as potential gene therapy agents.

Pros and cons of retroviral vectors

Compared to other modes of gene delivery such as classical chemical based transfection, electroporation, and microinjection, retroviruses have the following characteristics:

Pros:

- The DNA integrates into the host cell genome and replicates stably with genomic DNA instead of remaining in a plasmid form in the cytoplasm
- They have low immunogenicity
- Lentiviruses (as opposed to standard modified retroviruses like MMLV) can infect both dividing and quiescent cells

Cons:

- Smaller insert carrying capacity
- Risk of insertional mutagenesis - accidental insertion of retroviral DNA into an unintended site causing disruption of host gene function
- Lower titers (than other viral vectors)
- Low replication efficiency in self-inactivating (SIN) vectors

Some of the risks of gene therapy:

- The immune system may respond to the working gene copy that has been inserted by causing inflammation.
- The working gene might be slotted into the wrong spot.
- The working gene might produce too much of the missing enzyme or protein, causing other health problems.
- Other genes may be accidentally delivered to the cell.
- The deactivated virus might target other cells as well as the intended cells.
- The deactivated virus may be contagious.

Burnie, David. B.S. "Retrovirus." 24 July 2018.

<http://autocww.colorado.edu/~toldy3/E64ContentFiles/VirusesMoneransAndProtists/RetrovirusB1.html>.

Retroviruses used in gene therapy are genetically engineered to prevent them from replicating. However, there is still a slight possibility that these genetically engineered retroviruses may insert genes in an inappropriate region of DNA, triggering cancer or other problems. In early 2003, the United States Food and Drug Administration (FDA) halted 27 gene therapy clinical trials that used a retrovirus to ferry genes into blood-producing cells. Two children involved in the trials became ill with a condition resembling leukemia, and the FDA decided it was unsafe to continue using this procedure.

"Gene Therapy". Last updated: May 2011.

<https://www.betterhealth.vic.gov.au/health/conditionsandtreatments/gene-therapy>.

Gene Therapy

A person born with adenosine deaminase (ADA) deficiency lacks an important enzyme of their immune system. This means that infections are likely and can even be fatal. ADA deficiency was the first genetic disorder to undergo experimental gene therapy trials in 1990. It was chosen because a single, relatively uncomplicated gene causes it. The results were promising.

[The Human Genome Project (HGP) 1990 - 2003.]

Seven out of 10 infants treated to date have restored immune function, but two of the children treated initially have developed a form of leukaemia. The leukaemia in these two patients was caused when the virus used to deliver the therapeutic gene activated a cancer-causing gene.

After the first boy developed leukaemia in October 2002 and the second in January 2003, clinical trials of the gene therapy being conducted in a number of countries were halted. These have now been resumed, but only for patients with no other treatment options. Work is continuing to make the therapy as safe as possible.

Gene therapy is currently an experimental discipline and much research remains to be done before this approach to the treatment of disease will realise its full potential. Between 1989 and 2010, 1698 clinical gene therapy trials were initiated or approved worldwide. So far, less than one per cent of these have shown clinical benefit.

Gene Therapy. net. "Retroviral Vectors". (c) 2018.

<http://www.genetherapynet.com/viral-vector/retroviruses.html>.

One of the problems of gene therapy using retroviruses is that the integrase enzyme can insert the genetic material of the virus in any arbitrary position in the genome of the host.

U.S. Department of Health & Human Services. "Is gene therapy safe?" Published: 14 Aug 2018.

<https://ghr.nlm.nih.gov/primer/therapy/safety>.

Several studies have already shown that this approach can have very serious health risks, such as toxicity, inflammation, and cancer. Because the techniques are relatively new, some of the risks may be unpredictable; however, medical researchers, institutions, and regulatory agencies are working to ensure that gene therapy research is as safe as possible.