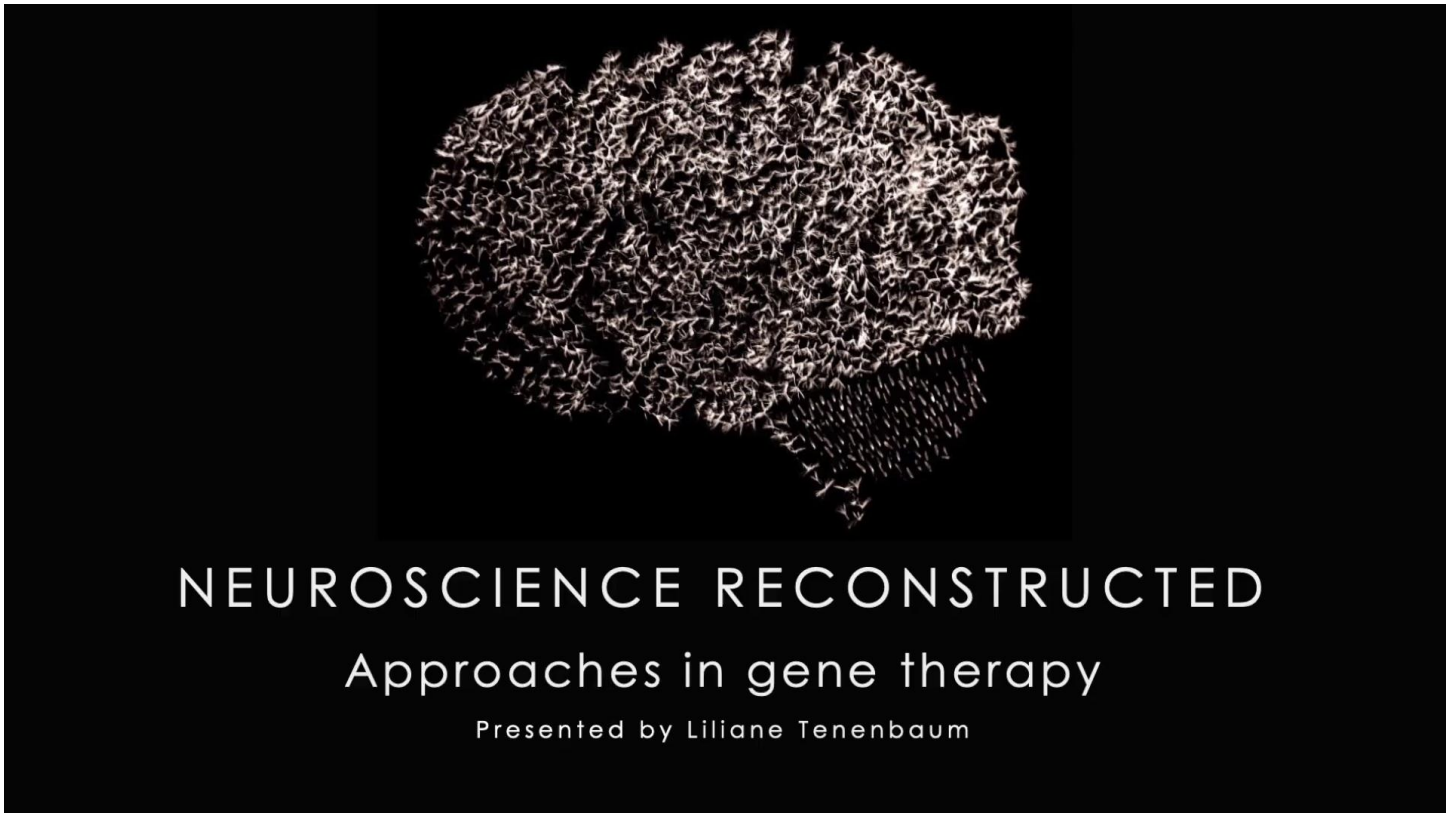

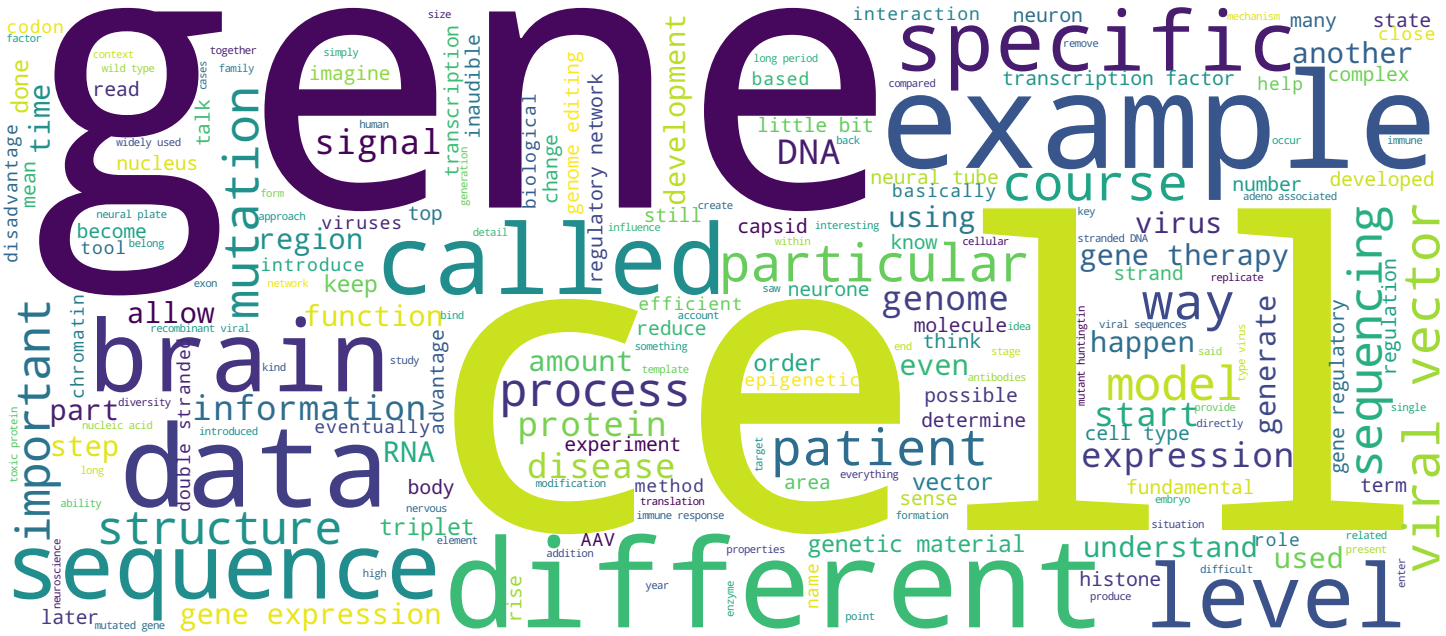

Search MOOC

Video

EPFL

Approaches

The approach depends on the characteristics of the disease:

- **Spinal muscular atrophy (monogenic; recessive mutation)**
  - **Replacing** the mutated *SMN* gene with a healthy gene
  - **Editing** the mutated *SMN* gene
- **Huntington disease (monogenic; dominant mutation)**
  - **Reducing** mutant *htt* mRNA: ex. siRNA, antisense oligonucleotides
  - **Editing** the mutated *HTT* gene

The approach, as you can imagine, will depend on the characteristic of the disease and also on the technical challenge. We cannot do everything in all cases. Let's go back to our example, which is spinal muscular atrophy. It is a monogenic disease with a recessive mutation. Since the mutation is recessive, we can simply replace the mutated gene and add a healthy gene that will make the job. But we could also use genome editing, use CRISPR/Cas9 to precisely repair the mutated gene. When we have dominant mutation, the challenge starts to be a little bit more complicated. You cannot anymore replace the mutated toxic protein. You have to inactivate or reduce the amount of the toxic protein in order to have a therapeutic effect. You can reduce the amount of mutant huntingtin Messenger RNA by using small interfering RNAs or antisense oligonucleotides. You can also use genome editing. When you use genome editing, you want to reduce the expression of the mutant huntingtin because correcting the gene is very difficult. As I told you before, it's a repetition of triplets, and it's just the number of triplets that is different. It's very difficult to find a unique sequence that will allow you to remove a few of the triplets.

Notes

Summary

0m 06s

Approaches

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  - **Functional improvement** by introducing a "gene-drug": enzymes involved in dopamine biosynthesis, neurotrophic factor

Advantages of gene transfer over conventional drugs:

- *Accessibility*: direct gene expression in the brain of drugs blocked by the blood-brain barrier
- *Long-term treatment:* single treatment instead of chronic medication
- *More physiological delivery*

Finally, when you deal with multifactorial diseases, the situation is even more complex. We cannot target a mutation, but we can still use replacement gene therapy in order to obtain a functional improvement. We can talk, in this case, of a gene drug, a gene that is not introduced to correct the mutation, but that will enhance a function that is progressively lost due to the degeneration of the dopaminergic neurons that occurs in Parkinson's disease. You will tell me, what is the advantage of a gene transfer? Can't you use conventional drugs to provide precursors of dopamine like it is actually done by giving L-DOPA, which is a dopamine precursor to a patient. There are several advantages. First of all, many drugs do not pass the blood brain barrier, so you cannot give them orally. You have to introduce them directly in the brain. Gene therapy has another advantage. We will see later that the vectors used now in gene therapy, the viral vectors, allow, after a single injection, to obtain transgene expression for very, very long period, probably lifelong. This is a big advantage compared to chronic medication which give rise to undesired effect after a long periods.

Notes

Summary

2m 21s

Approaches

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- *Accessibility*: direct gene expression in the brain of drugs blocked by the blood-brain barrier
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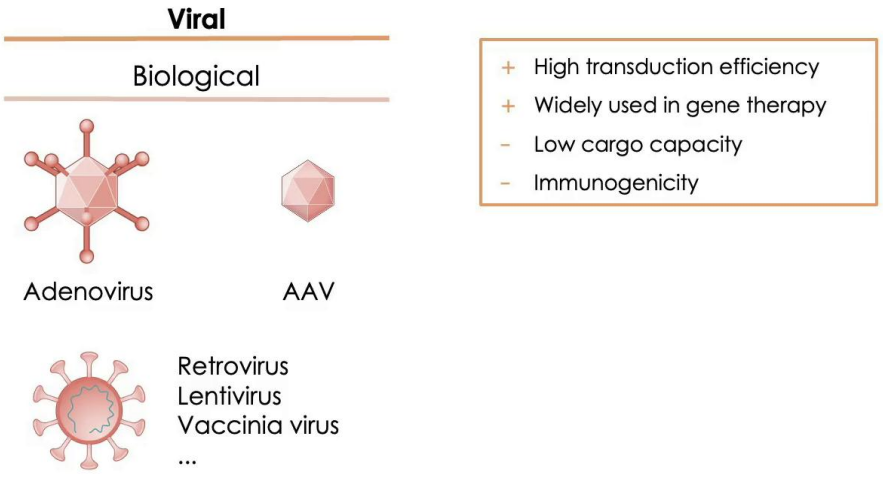
Also, as compared to drugs, the delivery of the factor will be more physiological in the sense that it is the cells of the patient which will synthesise the enzyme or the neurotrophic factor, and the level of expression will be close to a physiological level.

Notes

Summary

4m 21s

Delivery vectors



Adapted from Mirón-Barroso S et al. Nanotechnology-Based Strategies to Overcome Current Barriers in Gene Delivery. *Int J Mol Sci*. 2021;22(16):8537. Published 2021 Aug 9. doi:10.3390/ijms22168537

There are several types of vectors that have been developed to transport genetic material into cells. The most widely used and the most efficient are derived from viruses. We have double stranded DNA viruses. Their genome is a double stranded DNA ready to be transcribed and translated. Then we have retroviruses and lentiviruses, which are RNA viruses. They have two strands of RNA. Of course, I am citing only the mostly used viral vectors, but many others have been developed but were not so much used as these three families. We have also adeno-associated viruses, which do not belong to adenovirus family. This is often taught because of their name. But they belong to the family of the parvoviruses. Parvo coming from Greek and meaning small. It's one of the smallest virus. It's called adeno-associated because it needs adenovirus or another big virus to help them replicate, otherwise, they don't replicate. AAV also have DNA genome, but it is a single strand. These viral vectors have advantages and disadvantages. The main advantage is that they are really efficient in transducing cells.

Notes

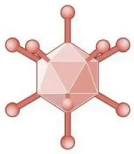
Summary

4m 51s

Delivery vectors

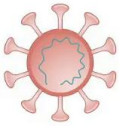
Viral

Biological



Adenovirus

AAV



Retrovirus
Lentivirus
Vaccinia virus
...

\+ High transduction efficiency
\+ Widely used in gene therapy
\- Low cargo capacity
\- Immunogenicity

See the supplementary information for more on delivery vectors

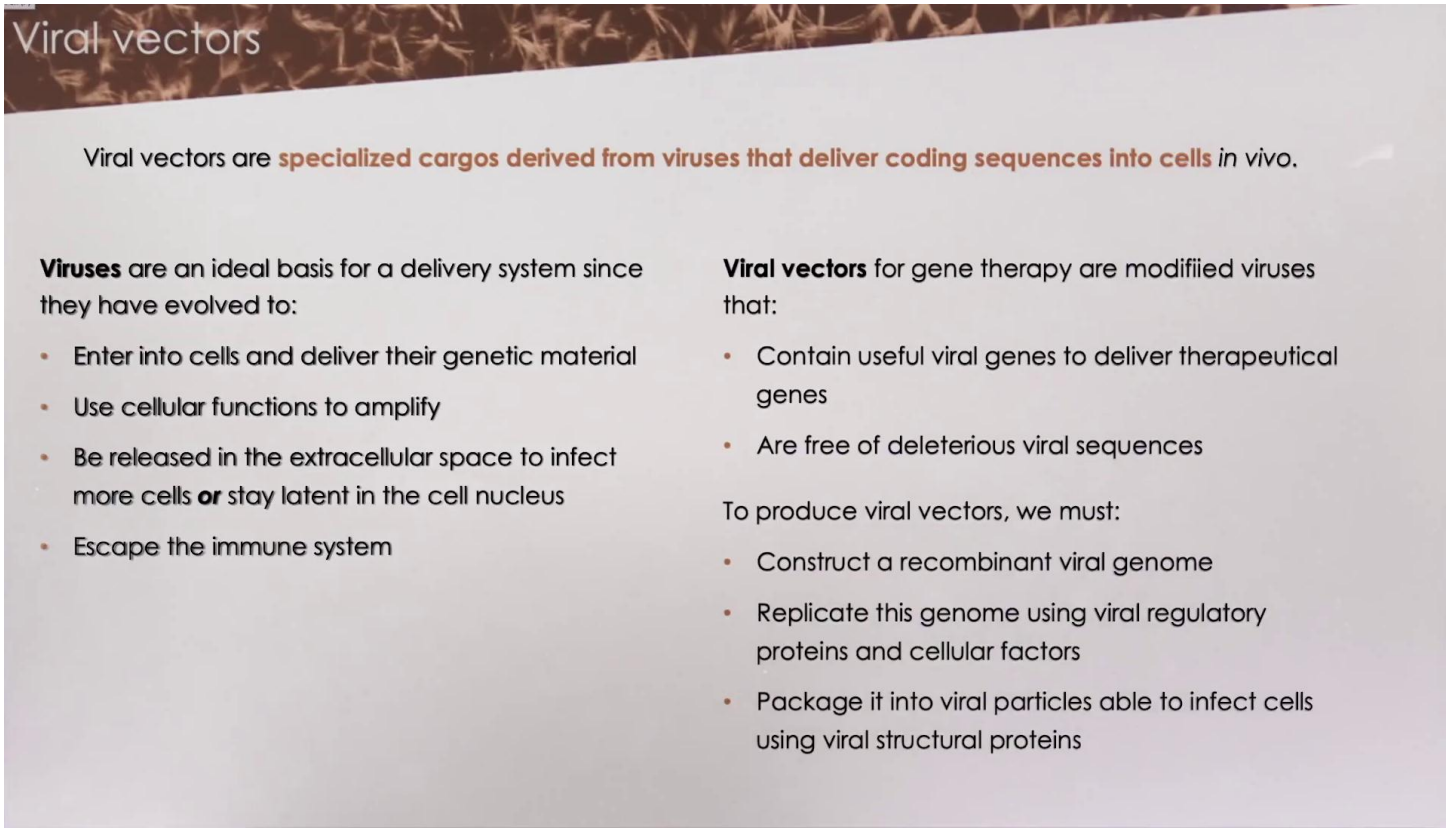
Adapted from Mirón-Barroso S et al. Nanotechnology-Based Strategies to Overcome Current Barriers in Gene Delivery. *Int J Mol Sci*. 2021;22(16):8537. Published 2021 Aug 9. doi:10.3390/ijms22168537

Transduction means introducing genetic material into the nucleus of the cells when the process is done by a virus, in contrast to transfection, which is used when it's a naked genetic material that is introduced into the cell. The other advantage that came with decades of use is that they are widely used, they are very well characterised, and their production methods have been really optimised. But the disadvantages is that they have a low cargo capacity. That means that the amount of genetic material that can be packaged into these viruses cannot exceed the size of the wild type virus, and this can be limiting for large genes. Finally, the very limiting factor is the immunogenicity. Since they all have foreign proteins, viral proteins in their capsid for adenovirus and AAV, and they have, in addition to a capsid, an envelope made of lipoproteins. The body will react by eliciting an immune response. It's even worse. It will be exacerbated if we are using a virus that the patient has been in contact with. It is said that the patient is seropositive when the patient have antibodies because he has been already infected with the wild type virus. That's an important factor to take into account.

Notes

Summary

7m 19s



That's why we will focus on viral vectors. If you think, of course, viruses, you can understand that they are an ideal basis to deliver nucleic acids into cells because they have evolved over millions of years to do so. They are able to enter the cells, they live on the genome. They use cellular functions to amplify, and then the progeny is released into the extracellular space. They can infect new cells, and they have also developed strategies to escape the immune system. Now, to make a viral vector, we will need to keep some of these properties, but to get rid of those we don't want. Of course, we want to keep the ability to enter the cells and reach the nucleus, but we don't want an amplification of the recombinant viral genome, in this case, since we are going to introduce our therapeutic gene into the genome of the virus. We have to remove the deleterious viral sequences, ideally, all the coding viral sequences. But we have to keep what is necessary for replication and packaging of the recombinant genome because we will have to produce outside the body. In culture, we will have to produce a large amount of recombinant viral vectors. But the final product should be devoid of anything toxic and anything that can allow the viral vector to replicate in vivo in the patient.

Notes

Summary

9m 43s