Gene Therapy

Oliver, Gian-Marco, Anshak
4d

Table of Contents

Preface

Our motivation why we chose our topic

We had originally chosen a different topic concerning computational applications of genetics.
Since we were unable to locate specialists in that topic, we were forced to shift our focus to a different subject proposed by Anshak, which was gene therapy.
We have chosen the topic because it has shown potential for making a bio-term paper and also seemed like an interesting topic for us to work on.

What is especially interesting?

It is interesting because it shows a big potential in giving us the ability to treat inherited disorders, types of cancer and certain viral infections. It still remains a risky form of treatment at the moment and test still have to be conducted to ensure the procedures safety and efficiency.

Our questions with respects for the topic

How soon will this form of treatment take until it is enough understood to be used in healthcare?
How does a virus have to be modified so that it doesn't trigger an immune system response?

Introduction

What is Gene Therapy?

Gene therapy is a method of transferring genetic material via vectors into cells or tissues in order to cure or prevent a disease. Initially developed to treat single gene hereditary diseases (e.g. haemophilia, muscular dystrophy), it can now treat polygenic and non-inherited diseases (e.g. cancer, hepatitis C) as well.

Recent Events

In April 2016, the second gene therapy treatment called *Strimvelis* in Europe was approved by the European Commision. This was the very first **ex-vivo** *(see "description of engineering technique")* gene therapy to treat children born with an inherited disorder *adenosine deaminase (ADA)*, which causes *severe combined immunodeficiency (SCID)*. Patients with ADA-SCID are incapable of developing a healthy immune system, eventually leading to fatal illnesses as they become prone to common everyday sickness.

The very first **in-vivo** *(see "description of engineering technique")* gene therapy to be approved by the US Food and Drug Administration (FDA) was also announced recently. In December 2017, *Luxturna* (Voretigene neparvovec) FDA approved this therapy to treat *Leber's congenital amaurosis (LCA)*; an inherited eye disease which causes severe blindness within the first few months of life.

Scientific History

The concept of gene therapy was first mentioned by authors Friedmann and Roblin in the Science Magazine. Stanfield Rogers was also quoted in their article "Gene therapy for human genetic disease?" for proposing that "exogenous good DNA be used to replace the defective DNA in those who suffer from genetic defects." In 1984, a group from Harvard Medical School, Boston successfully designed a retrovirus vector system. With this system, foreign genes could be efficiently inserted into chromosomes of mammals.

The first attempt to modify human DNA in 1980 by Martin Cline was an unsuccessful one. The National Institute of Health (NIH) approved the first gene therapy research in the US in 1990. Ashanti DeSilva, a 4 year old child that had *ADA-SCID*, was treated resulting with promising yet temporary effects.

Where and why is the technique used?

At the moment, gene therapy is used to treat and cure heritable and non-heritable diseases (e.g. haemophilia, cystic fibrosis and leukemia). However, the techniques that are used for gene therapy have the potential to engineer humans further. Infertility can be treated and athletes may start gene doping in order to enhance their performances, but gene therapy also has the potential to alter physical features of humans.

Are there alternative treatments?

Stem cell therapy is one alternative for treating leukemia, a disease that gene therapy also treats. There are several types of stem cell therapies, each with a different method of curing patients.

Description of engineering technique

Types of gene therapies

- **In vivo:** therapeutic gene carrying vector is injected into patient's body and then the vector enters the infected cell.
  Ex vivo: infected cells are treated with therapeutic genes outside patient's body and later transferred back to the home body. (Usually same as **in-vitro**)
- **Somatic (SCGT):** therapeutic genes are transferred into somatic (non-gamete) cells. These modifications cannot be inherited by offspring.
  Germline (GGT): modifications of gametes (sperm and egg cells) that occur by interpolation of therapeutic genes. These changes are heritable.

Explanation of the applied technique

Once the infected part of the patient's body has been identified, the corresponding therapeutic gene must be chosen to treat it. This therapeutic gene must then be transferred via a vector. An ideal vector should be able to transduce the infected cells efficiently and not activate an immune system response. Several vectors have been developed for specific diseases as a universal vector for all diseases does not exist. There are 2 types of vectors:

Viral Vectors

Viruses are infectious agents that can transfer their genetic information into their host's cells very efficiently. The pathogenic genes of the virus are replaced with the therapeutic gene and now the virus becomes a harmless viral vector. This viral vector is then introduced into the targeted cell, where the viral vector then releases the therapeutic gene, which then enters the nucleus and integrates into the genome or remains in an extrachromosomal form. If this vectors can infect cells that must be able to multiply, then they can only be performed ex-vivo. If they can infect quiescent cells

too, then in-vivo therapy is also possible. The structure and types of viral vectors are on the next page.

Non-viral Vectors

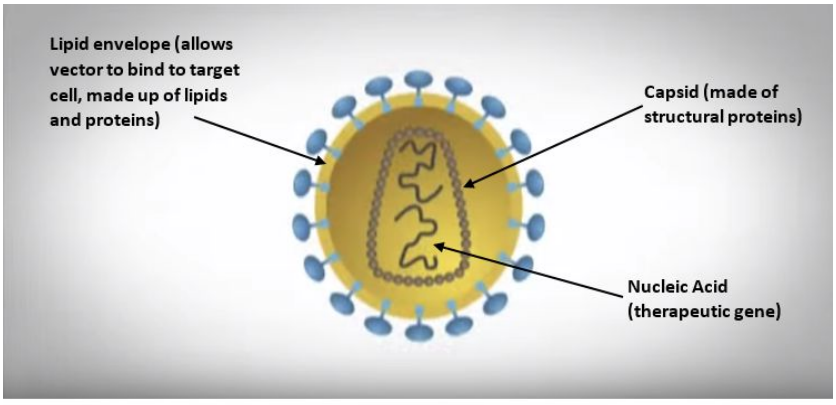
Non-viral vectors are not derived from a virus. In this type of gene therapy, a double stranded DNA structure called a plasmid carries the therapeutic gene as part of its genome. Plasmids can directly enter cells and tissues, however it is very inefficient. Hence, physical and chemical methods exist to increase the efficiency of this DNA delivery (e.g. electro transference and lipoplexes). In some aspects non-viral vector gene therapy is advantageous over viral vector gene therapy:

- Non-viral vectors do not have a certain size limit for the therapeutic gene
- Non-viral vectors can not trigger any immune responses

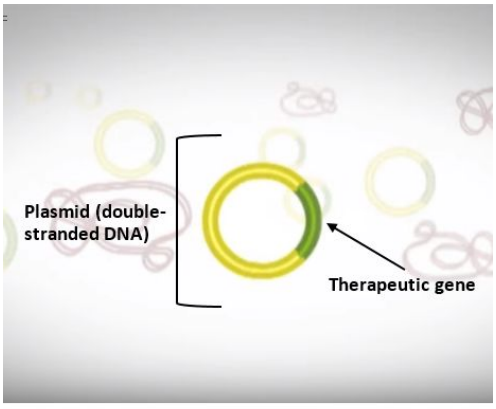
Whereas the only disadvantage is the lower in-vivo transfer efficiency.

Type of Viral Vector	Types of Gene Therapy	Therapeutic Gene Form	Size (kb*)
Retroviral Vector (RV)	Ex-vivo	RNA genome is reverse transcribed and integrates into host cell's genome	8
Lentiviral Vector (LV)	In-vivo, Ex-vivo	RNA genome is reverse transcribed and integrates into host cell's genome	8
Adenoviral Vector (AdV)	In-vivo, Ex-vivo	Double stranded DNA is released into nucleus and stays in extrachromosomal form	37
Adeno-Associated Vector (AAV)	In-vivo, Ex-vivo	Single stranded DNA is released into nucleus, becomes double stranded and stays in extrachromosomal form	4.5

*kb refers to kilobases



Structure of a Viral Vector



Structure of a Non-viral Vector

Documentation

After some research, we chose Dr. Andrea Banfi as our expert and interviewee. He is director of the cell and gene therapy group at the University Hospital in Basel, where he focuses on blood vessel growth and potential new therapies for related diseases.

Our Questions

1. *What are scientists (in Basel and globally) researching/developing at the moment?*
   In Basel, the development of gene therapy is not the main focus of research, but it is used as a tool for different forms of treatment. Globally, it is a lot more important. There it was a popular subject when it first came up 15-20 years ago. It's lost interest over time but now it's regaining a lot of interest: "It's going through a renaissance".

2. *How soon will this form of treatment take until it is enough understood to be used in healthcare according to you?*
   Gene therapy is already in use in some countries and was approved by their governments (the U.S. for example). But it still has to be expanded upon to improve the effectiveness and reduce the risks of gene therapy.

Dr. Andrea Banfi's Lab, University Hospital Basel

3. *How has CRISPR influenced gene therapy?*
   CRISPR hasn't influenced gene therapy yet as it itself is a fairly new technology which we've only had for a couple of years. Whether it will prove to be a useful addition to the technology still needs to be figured out.

4. *Where do the therapeutic genes, that are carried by the viral vectors, come from? What happens with the genes of the virus?*
   The origin of the therapeutic genes depends of the sickness which is treated.
   If it is a genetic disease then a gene from a healthy individual is extracted to be carried by the virus. Viruses are the most efficient way to transfer a gene into other cells, because that is what they improved over millions of years. Thus they have become rather efficient at it. One can remove the coding sequences in the viruses genes, which leaves the genes which are responsible to make it work. Therefore, one can implant the genes into that empty shell of the virus to make it implant the desired genetic data.

5. *Could this technology be used in a dangerous way? Isn't it potentially dangerous?*
   Being based on genetic vectors rather than viruses that can self-replicate, gene therapy does not pose a viable use for a weapon. It is dangerous when the dosage of the vectors is too high, causing an allergic reaction, an immune response or when it is injected into the wrong location.

6. *What according to you are some other disadvantages of gene therapy?*
   The interaction of the genetic vectors with the immune system is the main issue of gene therapy. It is very challenging to make the immune system accept the vectors without destroying them. You can solve this by helping the body understand that the vectors pose an advantage to the body.
   Another problem is if the therapeutic genes are introduced into cells which don't possess the broken data in the first place, they may replace a vital part of the genome, causing a miss- or nonsense. This specitivity of the viruses to only make them enter certain cells is a complicated aspect, which also poses a big challenge in developing gene therapy.

7. *In your opinion, how will gene therapy influence the human race?*
   According to Dr. Banfi, the main aspect it will influence is eugenics. He also mentions that we could alter the human race physically, but every nation worldwide has agreed to limit genetic alterations like with gene therapy to non-reproductive cells as not to have the genetic alteration carry on over generations, forever staining the human genome with artificially introduced genes.

Discussion

What progress has been made using gene therapy?

As already discussed in the Introduction, successful application of gene therapy has only happened quite recently, as it is a really new treatment, with much more to discover and learn and many hurdles to overcome. Many treatments have been **approved**, but only few operations have been completed so far. But there have been many advancements, especially in 2017, including the fore-mentioned first in-vivo treatment to be approved by the FDA in the U.S., a boy was cured of sickle-cell disease using experimental gene therapy, 2 types of cancer had gene-therapy treatments approved and executed. Many other treatments have also been approved in 2017.

Future research steps for gene therapy?

Because this field of medicine is still in its infancy, even speculation is difficult. As Dr. Banfi stated, CRISPR is revolutionary, but it's unclear whether it will influence gene therapy. After that's said, we do believe that gene therapy is at a turning point, and many new and exciting developments are made every day.

Ethical Aspects

As we saw in question 5, the current sentiment among the nations of the world is that permanent change to humans (gene manipulation in human gametes) is unethical. The question still remains whether gene therapy overall is ethical. So little is currently known about the limits of gene manipulation that there is no answer at the moment. Would making a superhuman soldier, as fictional and impossible as it may seem, be allowed? How much change is allowed? At some point the leaders of the world will have to decide.

Currently, gene therapy is only used sparingly, but with live-saving effects. If made more efficient, many of humanity's problems could be solved.

But as will everything concerning gene therapy at this moment in time, we can only speculate.

Summary

Gene therapy is a new treatment in which a non-reproducing virus with correct DNA is sent to human cells with genetic defects in its DNA. The vector (custom virus) the enters the nucleus to replace the incorrect gene. This procedure has recently been gaining a lot of traction, and is being approved and on a few occasions even successfully performed globally. But many hurdles still have to be overcome and extensive research has to be done. Luckily, progress is being made.

References

- AGTC, “Gene Therapy Explained” <https://www.youtube.com/watch?v=xOQFJJOBGM0>
- Kurzgesagt - In a Nutshell, “Genetic Engineering Will Change Everything Forever – CRISPR” <https://www.youtube.com/watch?v=jAhjPd4uNFY>
- Wikipedia, “Gene Therapy” <https://en.wikipedia.org/wiki/Gene_therapy>
- Wikipedia, “Strimvelis” <https://en.wikipedia.org/wiki/Strimvelis>
- National Institute of Health, “Adenosine deaminase deficiency” <https://ghr.nlm.nih.gov/condition/adenosine-deaminase-deficiency>
- Luxturna, “Luxturna” <https://luxturna.com/#isi>
- Wikipedia, “Leber's congenital amaurosis” <https://en.wikipedia.org/wiki/Leber%27s_congenital_amaurosis>
- Wikipedia, “Voretigene neparvovec” <https://en.wikipedia.org/wiki/Voretigene_neparvovec>
- Science Magazine, “Gene Therapy for Human Genetic Disease?” <http://science.sciencemag.org/content/178/4061/648>
- Constance L. Cepko, Bryan E. Roberts & Richard C. Mulligan, “Construction and applications of a highly transmissible murine retrovirus shuttle vector” <https://www.cell.com/cell/pdf/0092-8674(84)90440-9.pdf?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2F0092867484904409%3Fshowall%3Dtrue>
- Wikipedia, “Cell Therapy” <https://en.wikipedia.org/wiki/Cell_therapy>
- Victor Sanchez Clares, “Gene Therapy” <https://www.youtube.com/watch?v=znZVsNBkrQs>
- Stem Cell Center of Competence, “Dr. Andrea Banfi” <http://www.baselstemcells.ch/banfi.html>