Gene therapy in optic nerve disease

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“Now, just relax your back-bone & let yourself unwind...”
"ON SECOND THOUGHT, LET'S GO WITH GENE THERAPY."
What is gene therapy?

- Gene therapy (GT) is a technique that uses genes via DNA to treat or prevent a disease.
- GT can be used to correct a specific gene defect where the gene function and defect is known (defective gene replacement).
- GT can be used to change host gene expression to slow a disease course.
- The long term goals of GT are:
  - The expressed gene results in a functioning product
  - To maintain continued expression of the transfected gene
What is gene therapy?
Three elements needed for GT:
- Specific known gene product
- Vector to deliver the gene (DNA) to cells
- The target cells to receive the DNA
What is a vector?

A vector carries a specific gene’s DNA to be incorporated into the host DNA.

Currently the most effective vectors are viruses.
  - Recombinant adeno-associated viruses (AAV)
  - Lentiviruses (retroviruses)

AAV is a small double stranded DNA virus that cause no known human disease.

The most common virus use is “rep” deficient, meaning it does not replicate in the host cell.
AAV vectors have been shown to transfect different retinal cells (photoreceptors, RPE cells, Mueller cells, and RGC’s).

It is the RGC’s in which gene therapy is directed to cause optic nerves to improve functions.

The most direct way to transfect RGC’s is via intravitreal administration.
Adenoviral vectors
Vectors

- Another vector for GT are lentiviruses which are retroviruses (like HIV) that can deliver viral RNA into host DNA.

- They do not need active cell division to work and result in long term production of a defective gene.


- However, mutagenesis has been seen in animal studies with these type of vectors (Donahue RE et al. Helper virus induced T cell lymphoma in nonhuman primates after retroviral mediated gene transfer. *J Exp Med* 1992; 176: 1125-1135.)
Lentiviruses
Other vectors

- **Plasmids:**
  - Circular DNA molecules shared by bacteria to share DNA
  - Gene therapy plasmids are sometimes packaged inside liposomes which then fuse with cell membranes.
  - The disadvantage of plasmids and liposomes are that they are much less efficient than viruses at getting genes into cells.
  - The advantages are that they can carry larger genes, and most don't trigger an immune response.
Plasmids

Inserting a DNA Sample into a Plasmid
Plasmids
What makes a vector successful?

- The vector needs to target the correct cell type (i.e. RGC not cardiac for example).
- The vector has to be able to integrate its carrier gene into the designated cell type DNA.
- Once within the host DNA, the DNA must be read and transcribed into a functioning product.
- Lastly, the vector must avoid side effects:
  - Toxicity
  - Immune reaction
Viral vectors

• Advantages of viral vectors:
  • They are very good at targeting and entering cells.
  • Some target specific types of cells.
  • They can be modified so that they do not replicate and destroy cells.

• Disadvantages of viral vectors:
  • They can carry a limited amount of genetic material (smaller gene products).
  • They can cause immune responses in patients leading to an illness or cause cell death in cells that have incorporated the vector.
Risks/limitations of gene therapy

- Side effects of vectors used
- Not all cells transfected maintain function
- Cells may have variable duration of function (CF)
- Insertional mutagenesis
  - Possibility of vector induced mutagenesis (leukemia in SCIDS)
- AAV has limited amount of passenger DNA that it can contain.
- Immune toxicity:
  - Immune response to the vector or the new transgene product
Gene therapy and the eye

- Unlike other organs, the eye is small so there is less risk to other structures.
- Most target cells in the eye are not undergoing cell division so less risk of mutagenesis.
- More direct access via vitreous or intra-retinal.
Optic nerve and gene therapy

- Numerous causes to optic neuropathy
  - Glaucoma
  - AION
  - Traumatic optic neuropathy
  - Compressive optic neuropathy
  - Optic neuritis
  - Genetic optic neuropathies
  - Many others

- Why bother with GT and optic nerve disease?

- No current treatment to reverse optic nerve injury
Optic nerve and gene therapy

- How do you approach GT in optic neuropathies?
- If there is not a gene missing, what are you using GT for?
- The answer is GT can be used to promote substances that prevent cell death (neuro-protective agents).
- In terms of a genetic disease, GT is used to introduce the abnormal DNA into the cell to produce the normal DNA.
Gene therapy and Leber’s Hereditary Optic Neuropathy

- Leber’s Hereditary Optic Neuropathy (LHON):
  - Maternally inherited mitochondrial DNA (mtDNA) gene defect affecting electron transport chain Complex 1.
  - Defective mitochondria lead to lack of energy production for cell function thus leading to eventual cell death via accumulation of free radicals.
  - Occurs as a result of a single point mutations
  - 95% of cases are related to three different mutations at specific nucleotide positions
    - 11778 (50%) ND4
    - 14484 ND6
    - 3460 ND1
  - Unfortunately, most of the time occurs in younger individuals.
  - There is incomplete penetrance with M>F.
The powerful mitochondria

- Functions:
  - Energy production via oxidative phosphorylation for cell function and cell maintenance
  - Regulate free radicals and homeostasis of other minerals, metals, proteins, carbs and fats.
  - Control apoptosis
Mitochondrial DNA

Human mtDNA
16569 bp
LHON DNA point mutations
oxidative phosphorylation
reactive oxygen species

Gene therapy for mitochondrial diseases: Leber Hereditary Optic Neuropathy as the first candidate for a clinical trial

Hélène Cwerman-Thibault, Sébastien Augustin, Sami Ellouze, José-Alain Sahel, Marisol Corral-Debrinski

LHON and gene therapy

- The rat model offers promising human studies.
- Currently, there is active recruitment for gene therapy for the 11778 gene mutation in humans.
Glaucoma and gene therapy

- Prevalence of open angle glaucoma in US in 2004 were 2 million people.

- Worldwide, glaucoma is the leading cause of irreversible blindness and the number of people with glaucoma worldwide will increase to 111.8 million in 2040.
Glaucoma and gene therapy

• Likely has multiple risk factors
  • Genetic
    • GLC1A through GLC1N in POAG
  • Environmental

• Goal of GT is to slow the rate of RGC loss
  • Preventing RGC apoptosis
  • Stimulating neuro-protective agents
  • Specific gene therapy in the future
Preventing cell apoptosis in glaucoma

- IAP’s (inhibitors of apoptosis proteins) include:
  - Anti-caspase activity as caspase has been shown to be pro-apoptotic in glaucoma models.
  - XIAP has been shown in rats to prevent retinal ischemia in high IOP (Renwick J, Narang MA, Coupland SG, et al. XIAP-mediated neuroprotection in retinal ischemia. Gene Ther 2006; 13:339–347).
Baculoviral IAP repeat-containing-4 protects optic nerve axons in a rat glaucoma model

XIAP has been shown in rats to prevent retinal ischemia in high IOP

Neuro-protection in glaucoma

- Neurotrophic factors are used to sustain RGC life
  - Brain derived neurotrophic factor (BDNF)
    - Protein that helps support the survival of neurons
Gene therapy with brain-derived neurotrophic factor as a protection: retinal ganglion cells in rat glaucoma model

**Table 3. Percent Axon Loss Relative to Control**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glaucoma</th>
<th>n</th>
<th>No Glaucoma</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>52.3 ± 27.1</td>
<td>25</td>
<td>0.0 ± 12.0</td>
<td>9</td>
</tr>
<tr>
<td>AAV-BDNF</td>
<td>32.3 ± 23.0</td>
<td>27</td>
<td>7.9 ± 13.8</td>
<td>10</td>
</tr>
<tr>
<td>AAV-GFP (all)</td>
<td>52.3 ± 24.2</td>
<td>30</td>
<td>8.7 ± 12.3</td>
<td>13</td>
</tr>
<tr>
<td>AAV-GFP (peak IOP &lt; 43.6)</td>
<td>45.1 ± 24.7</td>
<td>19</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Data are the mean percentage ± SD

Neuro-protection in glaucoma

- Ciliary-derived neurotrophic factor (CNTF):
  - Protein with neuroprotective properties that increases RGC survival following injury

Effect of CNTF on retinal ganglion cell survival in experimental glaucoma

GT in glaucoma

- Although numerous groups have shown animal model benefits of certain therapies, there have been a few human studies but nothing has been published.

- Clinicaltrials.gov reveals one completed study with CNTF and glaucoma performed by Dr. Jeff Goldberg now in UCSD but no published details are available.
Traumatic Optic Neuropathy most commonly occurs after a MVA.


Many cases have NLP vision at onset of trauma with poor visual prognosis.

There is no current proven treatment for TON (observation vs steroid vs surgery) and decision of any treatment is felt to be made on an individual case basis.
Traumatic optic neuropathy

- The animal model used for TON are the crush or nerve transection models.
- Studies done aimed to provide neuro-protection by injecting AAV with brain derived neurotrophic factor (BDNF).
  - 76% of RGC remained alive at 2 weeks after axotomy
  - >90% of these neurons are lost without treatment.
Effect of TrkB gene transfer on the protection of axotomized RGCs in vivo.

Traumatic optic neuropathy

- Prevention of RGC loss after nerve transection has also been seen with adenovirus delivery of X-Chromosome-liked inhibitor of apoptosis, a gene that inhibits cell apoptosis.

TON and GT

• Once again, animal models have shown some benefit to gene therapy however there have been no human studies performed.
Optic neuritis and gene therapy

- Optic neuritis is the initial presenting condition in 25% of MS patients and will occur in 50% of all MS patients throughout the disease course.

- The annual incidence is around 5 per 100,000 (Rodriguez et. al Neurology 1995).

- ON results in permanent axonal loss thus reducing number of neurons resulting in permanent nerve damage.

- Intravenous steroids for three days improve the speed of recovery of ON but not the visual outcomes (see ONTT results)

- No way to reverse optic nerve damage in ON.
Optic neuritis

- GT has been applied to the animal ON model.
- The animal model used ON/MS induce oligodendrocyte immune mediated damage via free radicals.
- GT has been guided as to reduce free radical formation via insertion of catalases which break down free radical formation.
Adenoviral gene therapy with catalase suppresses experimental optic neuritis

ON and GT

- Only other GT studies in optic neuritis used other methods to lower free radical exposure and maintaining mitochondrial function:

- Though optic neuritis is not uncommon, gene therapy has been less frequently studied.

- There are no human studies.
AION and gene therapy

- NAION is the most common acute optic nerve disorder above the age of 50.
- The annual incidence has been reported to be 2.6 (Lensworth and Arnold JNO 1994) to 10.3 per 100,000 individuals (Hattenhauer et. al AJO 1997).
- There is no cure to AION.
- GT has been studied the least in AION.
A CNTF implant was used by Dr. Jeff Goldberg but no results were published.

The Quark study phase I and IIa have occurred which have looked at QPI-1007, a synthetic small interfering RNA that is designed to temporarily inhibit expression of the pro-apoptotic protein, Caspase 2.

They report “The study was not designed for determining the efficacy of the drug...the proportion of NAION patients improving by ≥3 lines (≥ 15 letters) at month 3 was 51.7% (n=29) compared with 39.7% (n=121) of IONDТ historical controls”.

Current phase II/III is still in the works.
Conclusions

- Optic nerve diseases are a common cause of permanent, irreversible loss of vision.
- Methods of GT are directed at targeting specific gene defects and targeting known genes functions, both to improve nerve function and thus vision.
- Most commonly used vectors are adenoviruses.
- LHON gene therapies are already being used in human clinical trials.
- Though many GT animal models have been used in glaucoma, no human studies have been conducted.
Conclusions

- In TON, animal models have been promising, but there are yet any human trials with neuro-protection.

- The above goes for optic neuritis.

- In AION, unpublished data exists for CNTF and there is current investigation being done in humans.

- In the next decade, hopefully we will see more laboratory work being translated to human treatments.
Thank you...
References


References


References

