Gene and Stem Cell Therapy for Usher Syndrome

Ian C. Han, MD
Assistant Professor
Wynn Institute for Vision Research
Department of Ophthalmology and Visual Sciences
9th Annual USH Connections Conference | July 15, 2017
Financial Disclosures

NONE
Objective

- Describe the treatment strategy for curing vision loss from Usher syndrome
Outline

- Basic eye anatomy
- Basic cell biology
- Treatment based on disease severity
- Gene therapy
- Stem cell therapy
- Surgery to deliver genes and stem cells
Why do photoreceptors degenerate?

- Genetic variants
The best treatment strategy for Usher syndrome depends on the severity of disease.
Treatment

Disease Course

Mild | Moderate | Severe

Gene therapy | Cell therapy | Cell therapy

Gene therapy
Gene Therapy
Gene Therapy

• Replace or correct the gene variant
• Requires viable cells to make the gene product (proteins)
• Several ways to deliver gene therapy
Gene therapy using an adenovirus vector
104 Genes

- 75% have cDNAs that will fit into AAV (less than ~5Kb)
- The remainder will fit into HDAd (~35Kb)
## Usher Genes

<table>
<thead>
<tr>
<th>USH# Nomenclature</th>
<th>Gene</th>
<th>Clinical Type</th>
<th>Protein</th>
<th>cDNA Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Usher Type 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USH1B</td>
<td>MYO7A</td>
<td>USH Type 1</td>
<td>Myocin 7A</td>
<td>6,645</td>
</tr>
<tr>
<td>USH1C</td>
<td>USH1C</td>
<td>USH Type 1</td>
<td>Harmonin</td>
<td>2,697</td>
</tr>
<tr>
<td>USH1D</td>
<td>CDH23</td>
<td>USH Type 1</td>
<td>Cadherin-Like 23</td>
<td>10,062</td>
</tr>
<tr>
<td>USH1F</td>
<td>PCDH15</td>
<td>USH Type 1</td>
<td>Protocadherin 15</td>
<td>5,871</td>
</tr>
<tr>
<td>USH1G</td>
<td>USH1G</td>
<td>USH Type 1</td>
<td>Scaffold Protein Containing Ankyrin Repeats and SAM Domain</td>
<td>1,683</td>
</tr>
<tr>
<td>USH1J</td>
<td>CIB2</td>
<td>USH Type 1</td>
<td>Calcium and Integrin Binding Family Member 2</td>
<td>561</td>
</tr>
<tr>
<td><strong>Usher Type 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USH2A</td>
<td>USH2A</td>
<td>USH Type 2</td>
<td>Usherin</td>
<td>15,606</td>
</tr>
<tr>
<td>USH2C</td>
<td>GPR98</td>
<td>USH Type 2</td>
<td>Monogenic Audiogenic Seizure Susceptibility 1 Homolog</td>
<td>18,918</td>
</tr>
<tr>
<td>USH2D</td>
<td>DFNB31</td>
<td>USH Type 2</td>
<td>Whirlin</td>
<td>2,721</td>
</tr>
<tr>
<td><strong>Usher Type 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USH3A</td>
<td>CLRN1</td>
<td>USH Type 3</td>
<td>Clarin-1</td>
<td>735</td>
</tr>
<tr>
<td>USH3B</td>
<td>HARS</td>
<td>USH Type 3</td>
<td>Histidyl-tRNA Synthetase</td>
<td>1,527</td>
</tr>
<tr>
<td>N/A</td>
<td>ABHD12</td>
<td>USH Type 3-Like</td>
<td>Abhydrolase Domain Containing Protein 12</td>
<td>1,212</td>
</tr>
</tbody>
</table>
HDAd can solve the large gene problem

- USH2A is the second most common inherited retinal disease gene
- 92% of Usher is caused by genes that won’t fit into AAV (e.g. MYO7A, USH2A, PCDH15, CDH23)
Progress in Gene Therapy for Usher syndrome at the WIVR

- We have manufactured every known Usher gene
- We are currently testing these gene products in cell, tissue, and animal models
- We are testing HDAd gene delivery
Human Retinal Organ Culture
What is involved in testing a gene before human clinical studies?
What is involved in testing a gene before human clinical studies?

- FDA regulations to ensure that genes are safe before it reaches a human eye.
Multiple New Studies at the WIVR Per Year

• We can package one product per month in the cGMP facility

• We can re-use the FDA IND documents (each trial is very similar in rationale and design)

• The rate limiting steps are now generating pre-clinical data and conducting the clinical trials themselves
Good Manufacturing Practices, Open-source, Non-profit, FDA-registered
What about treatment for more severe disease?
Treatment

Disease Course

Mild  Moderate  Severe

Gene therapy  Cell therapy

Gene therapy

AAV

Cell therapy
Structure of the Retina

Light

Nerve fibers

To optic nerve

Ganglion cell

Amacrine cell

Bipolar cell

Horizontal cell

Cone

Rod

Pigment epithelium

Choroid

Sclera
Photoreceptors
Structure of the Retina

Light

Nerve fibers

To optic nerve
- Ganglion cell
- Amacrine cell
- Bipolar cell
- Horizontal cell

Gono Therapy

Pigment epithelium

Choroid

Sclera
Stem Cells
Stem Cell Therapy
What are stem cells?

- Multipotent, undifferentiated cells
- Can be directed to change into specific cells in the body
Three Key Strategic Decisions

• Autologous cells or not
• Polymer supported or not
• Blind or sighted eyes
Our Strategic Decisions

- Autologous cells
- Polymer supported
- Blind eyes
Induced Pluripotent Stem Cells

- iPSCs can be used to create very authentic photoreceptor precursor cells suitable for autologous transplantation, BUT . . .
- They still harbor the mutation(s) that caused the disease in the first place
- Fortunately, CRISPR/CAS9 genome editing can be used to correct the mutations in the iPSCs before differentiating them into retinal cells
Skin fibroblasts
(from 3mm punch biopsy)
GMP cell lines have been generated from 35 patients with severe visual loss.

**Skin fibroblasts**
(from 3mm punch biopsy)
Pluripotency Factors

Skin fibroblasts
(from 3mm punch biopsy)
Pluripotency Factors

Skin fibroblasts
(from 3mm punch biopsy)

IPSCs
3D Differentiation

D30
Our Strategic Decisions

- Autologous cells
- Polymer supported
- Blind eyes
Why Polymer Supported?

• Cells with a scaffold of support have upwards of 50 fold higher rate of survival than non-supported cells.
500,000 cells
A) Step 1: Sample Collection
Skin Biopsy

B) Step 2: Isolate Fibroblasts

C) Step 3: iPSC generation (i) and clonal expansion (ii)

D) Step 4: CRISPR Correction of Patient Specific iPSCs

E) Step 5: Validate CRISPR Corrected Patient Specific iPSCs
Whole Genome Sequencing

F) Step 6: iPSC (i) to photoreceptor cell (ii) differentiation

G) Step 7: Scaffold Fabrication (i) and photoreceptor cell loading (ii)

H) Step 8: Graft Transplantation

Summary of steps required for PR Graft production and delivery
How do we delivery gene and stem cell therapy?

• Modern retinal surgery techniques
Vitrectomy surgery
Surgery for gene therapy

Video courtesy Steve Russell, MD
Surgery for stem cell therapy

• How do we deliver the polymer and stem cells under the center of the retina?
500,000 cells
Loading the polymer

Video courtesy Elliott Sohn, MD
5 mm polymer under a pig retina
OCT scan of a polymer transplanted in a pig model of retinitis pigmentosa
Microscopic Sections of 3D polymer
Summary of our treatment strategy
Treatment

Disease Course

Mild  Moderate  Severe

Gene therapy  AAV  Cell therapy

Gene therapy  Cell therapy

Cell therapy
Blood Sample

Skin Biopsy

Genetic Testing

Establish Cell Lines

CRISPR-corrected Autologous Cells

Evaluate Mutations

Test Efficacy of Gene and Drug Therapies

Patient

Therapy

Patient
Acknowledgements


Stephen A. Wynn Institute for Vision Research
Chunhua Jiao

Steve Russell

Emily Kaalberg

Elliott Sohn

Surgery Team