Gene Therapy for Glaucoma

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Disclosure block: Alcon (C, R), Allergan (C, R), Bausch & Lomb (C, R), Inspire (F, C, P, R), Johnson & Johnson (C, R), Lens AR, Inc (F), Pfizer (R), QLT (C, R), Santen (F, C, R), Merck (C, R), Altheos (C), Amakem (C), WARF (P)
Outflow Enhancement

- **Trabecular meshwork:** altering cytoskeleton / cell adhesion / cell contractility in the TM - enhance conventional outflow facility

- **Uveoscleral pathway:** prostaglandins / altering matrix metalloproteinase / collagen / extracellular matrix pathway to reset uveoscleral outflow at a higher level
Integrin signaling

Rho GTPases

Integrins

Actin cytoskeleton

Myosin II-driven force

ERK, JNK, PI-3K...

Membrane

Adhesion plaque

Integrin signaling

ECM

External force
Arachidonic Acid Cascade (Partial)

ESSENTIAL FATTY ACIDS → PHOSPHOLIPIDS → ARACHIDONIC ACID

- Phospholipase A<sub>2</sub>
- Cyclooxygenase

PGG<sub>2</sub> → PGG<sub>2</sub> → PGG<sub>2</sub> → PGG<sub>2</sub>

PGD<sub>2</sub> → PGE<sub>2</sub> → PGF<sub>2α</sub> → PGF<sub>2α</sub>

PGF<sub>2α</sub> → Prostacyclin synthase

PGI<sub>2</sub> → Thromboxane synthase

TXA<sub>2</sub>
Uveoscleral Outflow in Cynomolgus Monkeys

5th Day of Treatment bid With 2 μg PGF$_{2\alpha}$-IE

<table>
<thead>
<tr>
<th>PGF$_{2\alpha}$</th>
<th>Control</th>
<th>PG/Cont</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.101 ± 0.141</td>
<td>0.489 ± 0.070</td>
<td>2.329* ± 0.235</td>
</tr>
</tbody>
</table>

Data are mean ± SEM; μl/min; n=9; *P<0.00001.
MMP-3 in PG vs Non-PG Treated Monkey Eyes

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Vehicle</th>
<th>PGF$_{2\alpha}$-IE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciliary Muscle</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>Iris</td>
<td>0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>Sclera</td>
<td>0.00</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Optical Density (OD units ± SD)
Small Molecules
Signal Pathways

- Receptors
- Mediators

Gene Products

- Proteins
- Enzymes
Gene Therapy

Strategy: Reprogram target cells to make more or less of something

Method: Viral vector incorporating the gene of interest

Consequences: Up or down regulate a biochemical / physiological process

Obstacles: Duration of expression, viral toxicity, turning gene on/off, immune/inflammatory responses, localization of transfection and/or gene activity
Human anterior segments perfused with AdGFPCald
Perfused at 2.5µl/min

$10^7$ pfu AdGFPCald; vehicle

Baseline 0.20±0.03µl/min/mmHg (n=11)

Change from baseline = 49.0±24.8% AdGFPCald (p<0.09); 0.6±7.8% vehicle (p<0.9)
Outflow Facility (μl/min/mmHg) after Ad vectors in Monkey Organ Culture

<table>
<thead>
<tr>
<th>N=8</th>
<th>Treated</th>
<th>Control</th>
<th>T/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.35±0.04</td>
<td>0.48±0.07</td>
<td>0.78±0.08*</td>
</tr>
<tr>
<td>AdGFPCald or AdGFP</td>
<td>1.03±0.30</td>
<td>0.67±0.17</td>
<td>1.47±0.17*</td>
</tr>
<tr>
<td>Ad/BL</td>
<td>2.63±0.47*</td>
<td>1.34±0.20</td>
<td>2.01±0.19*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N=4</th>
<th>Treated</th>
<th>Control</th>
<th>T/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.31±0.09</td>
<td>0.41±0.10</td>
<td>0.82±0.16</td>
</tr>
<tr>
<td>AdGFPC3 or AdGFP</td>
<td>0.66±0.14</td>
<td>0.39±0.06</td>
<td>1.79±0.38</td>
</tr>
<tr>
<td>Ad/BL</td>
<td>2.44±0.54</td>
<td>1.09±0.20</td>
<td>2.25±0.28*</td>
</tr>
</tbody>
</table>

Data are mean±s.e.m. Significantly different from 1.0 by the two-tailed paired t-test; *P<0.05

Caldesmon

C3
Long term transgene expression with viral vectors

634, OD, FIV.GFP, day 515, SLE - quiet, IOP normal

673, OS, scAAV.GFP, day 641, SLE - quiet, IOP normal
GFP expression in transduced monkey eye

Monkey #1, 167 days post initial injection, FIV-BOVPGF-GFP, SLE - quiet
Steroid-induced Elevated IOP Model

Gerometta, IOVS 2009

Courtesy Terete Borrás
Single injection of MMP1 Gene Therapy vectors into Steroid Sheep Model

Prednisolone

AdhNull     Control

AdhMMP1 mutant
no active enzyme

AdhMMP1

Courtesy Terete Borrás
Effects of prostaglandin pathway lentiviral vectors on IOP in domestic cats.

(a) Five-month IOP data from the two experimental groups (COX-2co + PGFS + FPRco and COX-2co + FPRco) with the largest IOP-reduction effect throughout the course of the study. Each right eye received a prostaglandin pathway vector (dashed lines) and was compared at each time point to its paired intra-animal control left eye, which received the control vector (solid lines). Each point is an average of the IOPs of the three animals in each experimental group at the given time after injection. Error bars represent the standard deviation of the mean IOP of the three animals.

(b) Mean IOP differences (right eyes relative to control left eyes) over the course of study for each treatment group. Groups treated with at least COX-2co and FPRco demonstrated the greatest average IOP reductions relative to paired control left eyes.
Injection of FIV-BOVPGF decreases IOP, monkey #1

* After virus injection, $p < 0.0001$, Wilcoxon signed rank test
ANOVA: *different from opposite eye corrected for BL (p=0.0115)
** different from opposite eye corrected for BL (p=0.0029) and
different from data >5m (p=0.0173)
ECT

6 mm in length and consists of genetically-modified human cells packaged in a semi-permeable hollow fiber membrane with a suture loop at one end to anchor the implant to the sclera. The implant is surgically placed in the vitreous body. The implant is sutured in a manner that allows for its retrieval when desired. The surgical procedure is performed as an out-patient procedure in about 25 minutes.
Encapsulated Cell Technology (Neurotech)

- ECT is a unique technology that allows for the sustained, long term delivery of therapeutic factors to the back of the eye.

- ECT implants consist of cells that have been genetically modified to produce a specific therapeutic protein and are encapsulated in a semi-permeable hollow fiber membrane.

- The cells continuously produce the therapeutic protein which diffuses out of the implant at the target site.
Delivery Opportunities

Canaloplasty

iTrack ophthalmic microcatheter with illuminated beacon tip.

Ultrasound image of the canal following viscodilation.

http://www.isciencesinterventional.com/us/asatanimation.htm
Knowledge of mechanisms of neuronal death and its prevention, delay, or reversal is now sufficient so that we can envision glaucoma therapy directed at retinal ganglion cells and axons.
Neuroprotection/Rescue / Regeneration for Glaucoma

Knowledge of mechanisms of neuronal death and its prevention, delay, or reversal is now sufficient so that we can envision glaucoma therapy directed at retinal ganglion cells and axons.

<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
Gene therapy strategies for glaucoma

<table>
<thead>
<tr>
<th>Strategies</th>
<th>Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Increase conventional outflow</td>
<td>• Actin cytoskeleton/cell adhesion/cellular contractility/relaxation/TM architecture</td>
</tr>
<tr>
<td>• Increase uveoscleral outflow</td>
<td>• wNT pathway</td>
</tr>
<tr>
<td>• Decrease aqueous humor production</td>
<td>• PG receptor – MMP regulation</td>
</tr>
<tr>
<td>• Neuroprotection, rescue, regeneration &amp; targeting</td>
<td>• ECM mechanisms – TGFβ2 – cochlin</td>
</tr>
<tr>
<td>– RGC axons</td>
<td>• Neurotrophins</td>
</tr>
<tr>
<td>– RGC soma</td>
<td>• Bcl-2 family (Bax, Bad pro-apoptotic; Bcl-2 and Bcl-xl pro-survival)</td>
</tr>
</tbody>
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