A Glaucoma

COCH Transgene Expression in Cultured Human Trabecular Meshwork Cells and Its Effect on Outflow Facility in Monkey Organ Cultured Anterior Segments

Eun Suk Lee,*1,2 B’Ann T. Gabelt,1 Jennifer A. Faralli,3 Donna M. Peters,1,3 Curtis R. Brandt,1,4 Paul L. Kaufman,1 and Sanjoy K. Bhattacharya*1,5

PURPOSE. To determine the effects of COCH transgene expression on cultured human trabecular meshwork (HTM) cell morphology and on outflow facility (OF) in monkey organ cultured anterior segments (MOCAS).

METHODS. An adenoviral (Ad) vector expressing both cochlin (COCH) and green fluorescent protein (GFP) (AdCOCHGFP) or GFP alone (AdGFP) was used to transduce cultured HTM cells (multiplicity of transduction, 2.8 and 28). COCH transgene expression in transduced HTM cells and the culture medium was verified by Western blot analysis and immunofluorescence detection 5 days after transduction. MOCAS were used to test the effect of Ad vectors (2.8 × 1010 viral particles per segment) on OF. The morphology of transduced MOCAS was evaluated by light microscopy.

RESULTS. Western blot analysis showed a viral vector dose-dependent expression of cochlin in transduced cells and the culture medium. There was no notable morphologic change in transduced cells. In MOCAS, cochlin expression was detectable in the medium by 3 days after transduction. A 35% decrease in OF in AdCOCHGFP-transduced MOCAS was detected after 3 days, decreasing by 76% after 12 days when compared to control segments injected with AdGFP. Anterior segment pressure (ASP) more than doubled (P < 0.05) in segments injected with AdCOCHGFP at 12 days after transduction. Light microscopy revealed normal angle structures in transduced segments.

CONCLUSIONS. Ad vector delivery of the COCH transgene resulted in cochlin expression in HTM cells and MOCAS. Cochlin expression was effective in decreasing OF and increasing ASP in MOCAS, suggesting possible involvement of cochlin in IOP elevation in vivo. COCH gene delivery has potential for use in developing a glaucoma model. (Invest Ophthalmol Vis Sci. 2010;51:2060–2066) DOI:10.1167/iovs.09-4521

Glaucomas are a group of irreversible blinding eye diseases. The projected number of people affected with glaucoma worldwide in 2010 is approximately 60.5 million. Primary open-angle glaucoma (POAG) is the most common form of glaucoma, where intraocular pressure (IOP) is a causal risk factor for optic neuropathy and lowering IOP has beneficial effects. Elevation in IOP occurs because of an imbalance between aqueous humor production and outflow. This imbalance occurs in part in glaucomatous eyes because of an increased resistance to aqueous humor outflow, particularly within the trabecular meshwork (TM) of the anterior segment. Changes within the extracellular matrix (ECM) of the TM, especially the increased deposition of ECM proteins, are thought to contribute to the increased resistance to outflow.

Cochlin is an ECM protein that was identified in human glaucomatous but not in control TM tissue by proteomic analysis and was associated with TM protein and mucopolysaccharide deposits. It is also expressed in glaucomatous DBA/2 mice but not in wild-type (CD1 or C57Bl/6j) mice. Structurally, cochlin contains a signal peptide for secretion into the ECM, a factor C homology (FCH) domain, and two von Willebrand factor A-like (vWFA) domains. It undergoes multimerization in response to applied shear stress, which is a property of the vWF domain. Cochlin deposits are also found in the inner ear where it is associated with progressive hearing loss disorder DFNA9, a factor C homology (FCH) domain, and two von Willebrand factor A-like (vWFA) domains. It undergoes multimerization in response to applied shear stress, which is a property of the vWF domain.

In the TM, cochlin is secreted into the ECM. Its expression in anterior segment organ culture models is increased after TGFβ2-treatment and is associated with anterior segment pressure (ASP) elevation and outflow facility (OF) reduction. Whether cochlin overexpression alone is sufficient to elevate IOP (in the anterior segment culture context: ASP) or is a consequence of other TGFβ2-induced changes relevant to IOP elevation is not yet known. Injection of siRNA against cochlin in the New Zealand White rabbits decreases IOP by approximately 25% for 78 hours, suggesting that cochlin expression can influence IOP (Graham FL, et al. IOVS 2007;48:ARVO E-Abstract 4808). We have shown that exogenous cochlin also results in aggregation of isolated primary TM cells.

From the Departments of 1Ophthalmology and Visual Sciences, 3Pathology and Laboratory Medicine, and 4Medical Microbiology, University of Wisconsin, Madison, Wisconsin; the 2Department of Ophthalmology, Institute of Vision Research, Yonsei University College of Medicine, Seoul, Republic of Korea; and the 5Bascom Palmer Eye Institute, University of Miami, Miami, Florida.

Supported by National Institutes of Health Grants RO1 EY002698, RO1 EY017006, RO1 EY016236, P30 EY016665, P51 RR000167, S10 RR019382, P30 EY01480, R03 EY15266, and RO1 EY016112; unrestricted grants from Research to Prevent Blindness (RPB); an RPB Career Award (SKB); a faculty research grant 6-2009-0515 from Yonsei University College of Medicine for 2009 (ESL), and funds from the Ocular Physiology Research and Education Foundation (PLK).

Submitted for publication August 20, 2009; revised October 28, 2009; accepted November 4, 2009.

Disclosure: E.S. Lee, None; B.T. Gabelt, None; J.A. Faralli, None; D.M. Peters, None; C.R. Brandt, None; P.L. Kaufman, None; S.K. Bhattacharya, None

*Each of the following is a corresponding author: Sanjoy K. Bhattacharya, University of Miami, Bascom Palmer Eye Institute, 1638 NW 10th Avenue, 706, Miami, FL 33136; sbhattacharya@med.miami.edu. Eun Suk Lee, Yonsei University, Department of Ophthalmology and Visual Science, Sadaemungu Shinchondong, Seoul 120-752, Korea; eslec.korea@gmail.com.
In the present study, we determined whether adenovirus-mediated expression of cochlin alone is sufficient to alter the morphology of cultured human trabecular meshwork (HTM) cells and/or alter ASP and OF in monkey organ cultured anterior segments (MOCAS).

**METHODS**

**Construction of Recombinant Replication-Deficient Adenoviral Vector Carrying Human Cochlin and GFP Genes**

An adenoviral vector construction system (AdEasy Adenoviral system: Stratagene, La Jolla, CA) was used. Cochlin (COCH) cDNA (GenBank accession number AF006740; [http://www.ncbi.nlm.nih.gov/Genbank](http://www.ncbi.nlm.nih.gov/Genbank); provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD) was subcloned into the shuttle vector, pShuttle-CMV, with an influenza virus hemagglutinin (HA) tag fused to the carboxyl terminus of COCH. This dual-expression vector also contained the gene for GFP downstream from an internal ribosome entry site (IRES) element, so that COCH and GFP could be expressed from the same transcript. The resultant shuttle-COCH/ was then used to generate adenoviral recombinants through homologous recombination with the adenoviral backbone vector, pAdEasy-1, in BSK183 bacterial cells (Stratagene). For packaging, the COCH vector DNA was linearized by digestion with PacI. The linearized DNA was then transduced into HEK295 cells (Lipofectamine Plus; Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. The cultures were transfected at 37°C with 5% CO2 for 3 hours, at which time the medium was replaced with DMEM with 10% fetal bovine serum (FBS). A separate adenovirus vector expressing GFP alone (AdGFP) was used as the control. Virus titers were determined by spectrophotometric absorbance at 260 nm of viral particles (VPs), as described previously.18

**Transduction of HTM Cells**

HTM cells were isolated, as previously described.19–21 from a 17-year-old male donor and a 27-year-old female donor, with no known history of ocular disease. All experiments were performed with cultures between passages 5 and 7. The cells were grown to confluence in low-glucose DMEM (Sigma-Aldrich, St. Louis, MO) containing 15% FBS, 2 mM l-glutamine, 1% amphotericin B (Mediatech, Herndon, VA), 0.05% gentamicin (Mediatech), and 1 ng/mL FGF-2 (Peprotech, Rocky Hill, NJ). Cultures were maintained for one additional week after confluence, at which time FGF-2 was removed from the medium and the culture medium was replaced with DMEM containing 5% FBS. The cells were then used when the medium was replaced with AdGFP or AdCOCHGFp. The cultures were transduced at multiplicities of transduction (MOT) of 2.8 and 28 in DMEM containing 10% FBS and 2 mM l-glutamine. Controls were nontransduced cells that were incubated with fresh DMEM containing 10% FBS.

**Morphology of HTM Cell Cultures Transduced with Adenovirus Vectors**

Light microscope images of transduced cultures were captured with an inverted microscope (Axiovert 200; Carl Zeiss Meditec, Inc., Thornwood, NY) equipped with a high-resolution color camera (AxioCam; Carl Zeiss Meditec, Inc.), on the day of and 5 days after transduction. All images were processed with the system software (Axiovision 4.2; Carl Zeiss Meditec, Inc.).

**Western Blot Analysis**

To confirm expression of the cochlin protein, both HTM cell lysates and medium were collected 5 days after transduction. The HA-cochlin in the medium was isolated by immunoprecipitation with anti-HA Sepharose (Covance, Emeryville, CA), collected by centrifugation, and resuspended in Laemmli sample buffer22 containing 2% β-mercaptoethanol. Cell lysates were prepared by scraping the cells from culture dishes into 1× phosphate-buffered saline (PBS). Microscopic examination confirmed that >99.99% of the cells were removed from the dish. The cells were then pelleted by centrifugation at 2500 rpm for 3 minutes and resuspended in sample buffer containing 2% β-mercaptoethanol. The cells were lysed by sonication for 12 cycles on ice. Both cell lysates and medium were boiled for 5 minutes and were electrophoresed in 10% SDS-polyacrylamide gels. The proteins were then electrophoretically transferred to nitrocellulose, blocked with 5% non-fat dry milk in 1× buffer (Genius buffer I; 100 mM maleic acid, 150 mM NaCl [pH 7.5]; Technne, Princeton, NJ) with 0.3% vol/vol Tween-20 and probed with polyclonal anti-cochlin antibody (Santa Cruz Biotechnology, Santa Cruz, CA). The membrane was washed and incubated with secondary donkey anti-goat IgG conjugated with horseradish peroxidase (HRP; Santa Cruz Biotechnology) and then developed with the enhanced chemiluminescence (ECL) system (Amersham Biosciences, Piscataway, NJ), according to the manufacturer’s instructions and exposed to x-ray film. Cochlin expression in the medium from MOCAS was also analyzed by Western blot analysis. For these studies medium was collected overnight on days 0, 3, 6, 9, and 12 (one sample was collected at 11 days) and in some cases 24 days after transduction. All medium/perfusate was removed from the dish on the day before the collection day. On the designated collection day, the medium was aspirated into a syringe, transferred to a tube, and frozen at −70°C until it was analyzed. The average ± SEM duration of the medium collection period was 24 ± 2 hours. The HA-cochlin in medium was isolated by immunoprecipitation, as described for the cell culture medium.

**ASP and OF in MOCAS**

Eyes from rhesus monkeys (Macaca mulatta) and cynomolgus monkeys (Macaca fascicularis) from either the Wisconsin National Primate Research Center or Dr. Kaufman’s colony at the University of Wisconsin School of Medicine and Public Health, which were being euthanized for other protocols (adhering to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research) were obtained fresh and placed in organ culture, usually less than 2 hours after enucleation. The anterior segments were prepared for organ culture as described in detail in a previous report.23 Briefly, the eyes were bisected at the equator. The lens, iris, and ciliary body were removed from the anterior segment. The anterior segment was then mounted in the organ culture dish and secured with tissue glue and a plastic ring lined with aquaporin silicone. Medium was infused at a constant rate of 2.5 μL/min except when OF was being measured. Baseline (BL) OF was measured after overnight equilibration by using the two-level constant pressure perfusion technique.23 Briefly, fluid flow from an external reservoir was measured for approximately 1 hour, alternating every 4 minutes between two different pressures 10 mm Hg apart. OF was calculated as the change in flow divided by the change in pressure and sequentially averaged. Medium infusion continued at a constant rate of 2.5 μL/min when OF was not being measured. ASP in MOCAS was monitored (Biotec transducers/HSE Tam-D amplifier; Harvard Apparatus, Holliston, MA) and recorded every 15 minutes throughout the experiment, except at those times when infusion was temporarily stopped to take OF measurements or to replenish the medium in the infusion syringe (roughly once every 72 hours). It is important to note that we have compared the change after adjusting BL values; therefore the differences in BL between test and control groups are not expected to affect the measurable outcome. Anterior segments were injected via the inflow port with ~80 μL containing 2.8 × 1010 VPs of AdCOCHGFp, whereas the contralateral anterior segments received the same dose of AdGFP. Studies were usually terminated after 12 days but in some cases were maintained for up to 24 days.

**GFP Expression and Cochlin Localization in the TM of MOCAS**

At the endpoint of the cochlin OF studies, all MOCAS were perfused at ~8 mm Hg with 5 mL of 4% paraformaldehyde for 30 minutes, cut into
Results

COCH Transgene Expression in Cultured HTM Cells

HTM cells transduced with AdCOCHGFP at either MOT 2.8 or 28 did not show any morphologic changes compared with nontransduced or AdGFP transduced control cells transduced at MOT 28 (Figs. 1A–D). The morphology of AdGFP-transduced cells at the lower MOT (2.8) was also unchanged compared with nontransduced cells (data not shown). Transduction of HTM cells by AdGFP or AdCOCHGFP was confirmed by GFP expression (Figs. 1E–H). Western blot analysis also confirmed the expression of cochlin and showed a viral vector dose-dependent expression of a ~60- to 66-kDa protein corresponding to cochlin 5 days after transduction in AdCOCHGFP-transduced cells and medium but not in nontransduced or AdGFP-transduced cells or medium (Fig. 2). Lysates from cells transduced with AdCOCHGFP also showed a ~40-kDa isoform band of cochlin.8,25 Lower molecular weight bands in the AdCOCHGFP transduced cells (shown) and medium (not shown) detected by anti-cochlin antibody may represent cochlin degradation products.8,26

Cochlin Expression in MOCAS

At the conclusion of the experiment, GFP fluorescence in the MOCAS was examined (Fig. 3) to confirm the efficient transduction by either AdCOCHGFP or AdGFP. Although most GFP expression occurred in the TM (Fig. 3), occasional fluorescence was also visible in the corneal endothelium and the remnant of the ciliary body. Cochlin expression was also confirmed by using anti-cochlin staining in paired anterior segment transduced with AdGFP (Figs. 3A–D) or AdCOCHGFP (Figs. 3E–H). The paired control segments, transduced with AdGFP, showed prominent GFP expression (Fig. 3B) but failed to demonstrate any positive staining for cochlin (Fig. 3C). In contrast, the TM of anterior segments transduced with AdCOCHGFP demonstrated strong, positive staining for cochlin (Fig. 3G) in areas showing GFP expression (Fig. 3F), indicating robust cochlin expression.

Tissue cellularity was similar for segments transduced with AdGFP or AdCOCHGFP (Figs. 3A, 3E) as demonstrated by the presence of cell nuclei (Figs. 3D, 3H), an intact Schlemm’s canal and organized trabecular beams in both AdGFP and AdCOCHGFP transduced segments (Fig 3).

Transduction of the COCH gene with adenoviral vectors also resulted in the secretion of the expressed cochlin into the medium (Fig. 4). Peak cochlin secretion into the medium was

Data Analysis

ASP and OF data are expressed as the mean ± SEM and were analyzed by the two-tailed paired t-test for ratios compared to 1.0 (OF) or for differences compared with 0.0 (ASP). For data analysis, only segments demonstrating GFP expression and BL OF of less than 0.75 μL/min/mm Hg were included. Mean ASP ± SEM for the designated time points was usually calculated by averaging 40 consecutive pressure measurements at least 2 hours after any system stoppage. For unpaired group analysis, all segments were analyzed by the two-tailed unpaired t-test. For paired group analysis, only contralateral anterior segments from a given monkey were compared by the two-tailed paired t-test (all analyses: SPSS 11.5; SPSS, Chicago, IL). AdCOCHGFP and AdGFP transduced segments were compared with each other after normalization for BL differences. AdCOCHGFP segments were defined as responders when an OF decrease of at least 50% was observed at 12 days after AdCOCHGFP transduction after normalization for BL. Repeated-measures ANOVA (RMANOVA) was performed to evaluate the time-course of OF responses for AdCOCHGFP and AdGFP.
detected 3 days after transduction which was the earliest time point examined. Cochlin expression persisted for up to 24 days (data not shown) after transduction, although the level of expression in the medium was weaker than that observed at 5 days. Cochlin was absent in medium collected before transduction or in medium from AdGFP-transduced segments (Fig. 4).

**Effect of Cochlin Expression on OF in MOCAS**

Only segments that had normal BL OF in the range of 0.20 to 0.75 μL/min/mm Hg were considered. Nine AdCOCHGF- and seven AdGFP-transduced segments originating from nine rhesus and two cynomolgus monkeys were included for data analysis. Of these 16 segments, there were five in contralateral pairs. Table 1 shows the effect of Ad-transduced expression of cochlin in MOCAS on OF. Unpaired and paired analysis showed that BL OF for AdCOCHGF (0.44 ± 0.04 and 0.42 ± 0.05) was lower than that of AdGFP (0.52 ± 0.05 and 0.56 ± 0.05) segments (both P < 0.01). The analysis showed that a reduction in OF in AdCOCHGF was detected 3 days after transduction (35% decrease, unpaired, P < 0.001) compared with AdGFP segments after a correction for BL facility. OF decreased further over the 12-day duration of the study (76% decrease, unpaired, P < 0.001). A similar trend was found when paired segments were analyzed. In this instance, a 55% reduction occurred at 12 days after transduction (P < 0.05). When only responder AdCOCHGF segments were included, OF was decreased by 83% and 68%, for unpaired (n = 7) and paired analysis (n = 4), respectively, 12 days after transduction. Time course analysis for the OF response after transduction revealed that, whereas OF was not changed in AdGFP-transduced segments, OF in AdCOCHGF-transduced segments was significantly decreased 9 to 12 days after transduction when compared to BL (RMANOVA, unpaired segments, Fig. 5, P = 0.001; paired segments, data not shown, P = 0.022).

**FIGURE 3.** Cochlin localization in AdCOCHGF-transduced MOCAS. (A–D) AdGFP-transduced segment. (E–H) AdCOCHGF-transduced segment. The hematoxylin-eosin-stained anterior eye segments (H&E) are shown to demonstrate cellularity. Paraffin-embedded sections (5 μm) showed GFP expression (green channel; B, F) and were labeled for cochlin (red channel, Alexa Fluor; C, G). Merged images including DAPI-stained cell nuclei (blue channel) are shown in (D) and (H). Note that only segments transduced with AdCOCHGF showed cochlin (G). Bar, 50 μm.

Change in ASP was evaluated in a similar manner. Although BL ASP before adenovirus injection was not significantly different between AdGFP and AdCOCHGF-transduced segments, ASP posttransduction in AdCOCHGF-transduced segments was elevated by approximately 140% to 170% (unpaired and paired, P < 0.05) by day 12 when compared with AdGFP-transduced segments after correction for BL. There was no detectable ASP increase in two segments (Fig. 6), and so the results were also evaluated using only responder segments. When the responder segments were evaluated, the magnitude of ASP increase in AdCOCHGF-injected segments was approximately 150% to 210% (unpaired and paired, P < 0.05). The two pairs of segments that did not demonstrate any ASP elevation after transduction (Fig. 6) had detectable levels of cochlin in the tissue and medium.

**DISCUSSION**

This study demonstrated, for the first time, that cochlin expression alone results in an OF decrease and subsequent ASP increase. Previously, we demonstrated that TGFβ2-induced ASP elevation was associated with an increase in cochlin secretion into the medium and expression in the tissue of monkey and pig organ-cultured anterior segments treated with TGFβ2. Since TGFβ2 has been shown to increase TM ECM protein production leading to ECM remodeling, previously reported reductions in the outflow of aqueous humor and elevation in ASP may be attributable to the production of these other proteins. Whether the increase in IOP in human and DBA/2J mice as well as in the organ culture systems is due solely to the overexpression of cochlin or interactions between cochlin and other proteins in the ECM is still unknown.

The magnitude and time of onset of the ASP change in seven responders was quite variable despite the fact that all segments in this study were injected with a fixed amount of virus (2.8 × 10¹⁵ VPs/segment; Fig. 6) and cochlin was consistently expressed (data not shown). In five of the nine MOCAS that showed GFP and cochlin expression, the ASP increase was gradual, whereas two responders (segments 1 and 3, Fig. 6) showed a decrease in OF with subsequent ASP increase after day 9. The gradual change in ASP suggests that the effect may be due to the time it took to reach a critical level of cochlin expression in the TM. This notion would be consistent with the fact that the maximum secretion of cochlin into the medium occurred at 3 days after AdCOCHGF transduction.

Alternatively, the variability in the responses may be because the efficiency of vector delivery and transduction in organ culture and/or the tissue susceptibility varied between segments. Several factors govern gene expression from a viral vector, including local fluid dynamics at the time of injection and intrinsic properties of the host tissue, not all of which are...
well understood. Relatively fresh tissue often appears to possess greater viral load tolerance and greater expression levels compared with older tissue as exemplified by comparing different reports. In one study, approximately 3 $\times 10^{10}$ VPs were well tolerated in vivo and showed consistent expression for more than 2 years, while in another study, $10^9$ pfu ($\sim 10^{10}$ VP) caused 100% loss of TM cells in human organ culture. The levels of transduction obtained in organ culture with adenoviral vectors may vary experimentally, and this could be a factor in the variability of the results observed. In studies conducted with MOCAS treated with TGFβ2 as well as in the current studies of cochlin overexpression, ASP elevation required some time to develop. However, in both experiments, the time-dependent reduction in OF was approx-

**TABLE 1. Effect of Cochlin on Outflow Facility of MOCAS**

<table>
<thead>
<tr>
<th>Unpaired Segments</th>
<th>Paired Segments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outflow Facility</strong></td>
<td><strong>Outflow Facility</strong></td>
</tr>
<tr>
<td><strong>AdCOCHGFp</strong></td>
<td><strong>AdGFp</strong></td>
</tr>
<tr>
<td>BL</td>
<td>0.44 ± 0.04</td>
</tr>
<tr>
<td>Rx(d3)</td>
<td>0.40 ± 0.06</td>
</tr>
<tr>
<td>Rx(d3)/BL</td>
<td>0.91 ± 0.11</td>
</tr>
<tr>
<td>Rx(d6)</td>
<td>0.35 ± 0.07</td>
</tr>
<tr>
<td>Rx(d6)/BL</td>
<td>0.81 ± 0.18</td>
</tr>
<tr>
<td>Rx(d9)</td>
<td>0.27 ± 0.06</td>
</tr>
<tr>
<td>Rx(d9)/BL</td>
<td>0.63 ± 0.15</td>
</tr>
<tr>
<td>Rx(d12)</td>
<td>0.15 ± 0.04</td>
</tr>
<tr>
<td>Rx(d12)/BL</td>
<td>0.37 ± 0.10</td>
</tr>
<tr>
<td><strong>Responder</strong></td>
<td><strong>Responder</strong></td>
</tr>
<tr>
<td>BL</td>
<td>0.46 ± 0.05</td>
</tr>
<tr>
<td>Rx(d3)</td>
<td>0.38 ± 0.07</td>
</tr>
<tr>
<td>Rx(d3)/BL</td>
<td>0.81 ± 0.11</td>
</tr>
<tr>
<td>Rx(d6)</td>
<td>0.28 ± 0.06</td>
</tr>
<tr>
<td>Rx(d6)/BL</td>
<td>0.58 ± 0.09*</td>
</tr>
<tr>
<td>Rx(d9)</td>
<td>0.21 ± 0.06</td>
</tr>
<tr>
<td>Rx(d9)/BL</td>
<td>0.45 ± 0.11†</td>
</tr>
<tr>
<td>Rx(d12)</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>Rx(d12)/BL</td>
<td>0.28 ± 0.05</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM. AdCOCHGFp to AdGFp ratio (AdCOCHGFp/AdGFp) was significantly different from 1.0 by the two-tailed, two-sample t-test for unpaired segments or by the paired t-test for paired segments as indicated. For unpaired segments: all, n = 9 for AdCOCHGFp (8 for day 12); n = 7 for AdGFp (6 for day 12); responder, n = 7 for AdCOCHGFp; n = 5 for AdGFp. For paired segments: all, n = 5 (5 contralateral pairs included in the unpaired 16 segments); responder, n = 4 (5 contralateral pairs included in the unpaired 12 responder segments). BL, baseline; Rx, treatment with adenovirus as indicated.

* P < 0.01.
† P < 0.001.
‡ P < 0.05.

**FIGURE 5.** Time-course of OF response normalized to that of BL for AdCOCHGFp (n = 8) and AdGFp (n = 6)-transduced segments. Data are the mean ± SEM. The ratio was significantly changed over time in the AdCOCHGFp-transduced segments (RMANOVA, P = 0.001), whereas that of the AdGFp-transduced segments was not changed (P > 0.05).

**FIGURE 6.** Change in ASP for individual AdCOCHGFp (n = 9)- and AdGFp (n = 7)-treated MOCAS 12 days after transduction (8 days after transduction in segment 1 and 11 days after transduction in segment 9). Data are expressed as the percentage change in ASP compared with BL. The mean ratio was subjected to a two-tailed paired t-test; *P < 0.05.
Imately 40% after 5 to 9 days. Cochlin induction by TGFβ2 was slower, suggesting that more complex interactions may be involved in the ASP elevation compared with that after COCH transduction alone. In contrast, OF gradually increased with time in control AdGFP-transduced MOCAS. There is normally a gradual increase in OF and a decrease in ASP over several days that is probably due to washout of ECM and/or increased permeability of the thin sclera.23,35 Future investigations may unravel further details of the processes involved in IOP and ASP elevation.

The intersegment variability in the OF changes and ASP in response to cochlin expression also suggests that differences in cochlin levels within the tissue and in cochlin–protein interactions in different TM segments play a role. We identified annexin A2 as a molecule that may interact with cochlin in the glaucomatous TM.17 Annexin A2 is a membrane binding protein that has been implicated to play a role in exocytosis.36 However, not all functions of annexin A2 are known and its role in the TM remains unclear. The role of annexin A2 in dynamic remodeling of the cytoskeleton,37 may be linked to the cochlin-annexin A2 interaction in the TM. The presence of cochlin may also contribute to collagen degradation,9 leading to alteration of TM ECM and consequently to modulation of OF. However, at present there is no direct evidence that cochlin expression affects collagen degradation in vivo.

The observed effect of cochlin is not explicable simply due to overexpression of cochlin causing clogging of the TM. In biological systems, more than a 15% variation in total protein expression under only slightly different conditions (for example, circadian fluctuations) is not uncommon, and it is unlikely that overexpression of a single protein would exceed the same level of the natural variation in total protein expression. Perfusion experiments performed using human organ culture anterior segments (HOCAS) with BSA and myocilin also suggest that expression of a single protein alone does not always lead to blockage of the outflow pathway. Expression of myocilin alone, even at a much higher protein level of 25 μg/ml, was insufficient to decrease OF in HOCAS unless aqueous humor was also present.38,39

The present study also suggests that COCH gene delivery to the TM may be used to develop a glaucoma model in vivo. However, it is not known whether transient cochlin expression in the TM and secretion into the aqueous humor is sufficient to maintain permanent changes in OF and IOP. If prolonged cochlin expression is necessary to maintain IOP elevation and OF reduction in vivo, transgene delivery by lentivectors (Buie LK, et al. J OVS 2007;48:ARVO E-Abstract 2050)40 and scAAV vectors15 should be considered, since adenoviruses, such as those used in the present study, provide only transient gene delivery.51,52 Further investigations are needed to elucidate the biology of the cochlin effect on OF and IOP and perhaps reveal approaches that may be developed for glaucoma therapy.

References


