Glaucoma

Stanniocalcin-1 Is an Ocular Hypotensive Agent and a Downstream Effector Molecule That Is Necessary for the Intraocular Pressure-Lowering Effects of Latanoprost

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PURPOSE. To identify downstream signaling molecules through which intraocular pressure (IOP) is lowered following treatment with the prostaglandin analog latanoprost.

METHODS. Total RNA and protein isolated from primary human Schlemm’s canal cells (n = 3) treated with latanoprost (free acid; 100 nM) were processed for quantitative PCR and Western blot analysis. IOP was evaluated in stanniocalcin-1 (STC-1−/−) and wild-type mice following treatment with latanoprost or Rho kinase inhibitor Y27632. Human anterior segment pairs (n = 8) were treated with recombinant STC-1 (5, 50, or 500 ng/mL) and pressure was recorded using custom-designed software. The effect of recombinant STC-1 (0.5 mg/mL) on IOP was evaluated in wild-type mice. Tissue morphology was evaluated by light and transmission electron microscopy.

RESULTS. Increased STC-1 mRNA (4.0- to 25.2-fold) and protein expression (1.9- to 5.1-fold) was observed within 12 hours following latanoprost treatment. Latanoprost reduced IOP in wild-type mice (22.0% ± 1.9%), but had no effect on STC-1−/− mice (0.5% ± 0.7%). In contrast, Y27632 reduced IOP in both wild-type (12.5% ± 1.2%) and in STC-1−/− mice (13.1% ± 2.8%). Human anterior segments treated with STC-1 (500 ng/mL) showed an increase in outflow facility (0.15 ± 0.03 to 0.27 ± 0.09 μl/min/mm Hg) while no change was observed in paired vehicle-treated controls. Recombinant STC-1 reduced IOP in wild-type mice by 15.2% ± 3.0%. No observable morphologic changes were identified between treatment groups when evaluated by microscopy.

CONCLUSIONS. Latanoprost-induced reduction of IOP is mediated through the downstream signaling molecule STC-1. When used by itself, STC-1 exhibits ocular hypotensive properties.

Keywords: Schlemm’s canal, latanoprost, Rho kinase inhibitor Y27632, ocular hypotension, stanniocalcin-1

Glaucoma is the leading cause of irreversible visual impairment, projected to affect nearly 80 million people worldwide by the year 2020 and increasing to 110 million by 2040.1–3 Glaucoma is characterized by loss of retinal ganglion cells, axonal attenuation, and optic nerve atrophy.4 While there are no cures for the disease, treatment regimens that include medical, laser, and surgical therapies have proven useful in slowing disease progression. These therapies are all directed at reducing elevated intraocular pressure (IOP), the most prevalent and only treatable risk factor for glaucoma. Prostaglandin analogs such as latanoprost (Xalatan; Pfizer, Inc., New York City, NY, USA); bimatoprost (Lumigan; Allergan, Inc., Irvine, CA, USA); or travoprost (Travatan; Alcon Laboratories, Inc., Fort Worth, TX, USA) are typically the initial agents prescribed in the medical management of elevated IOP due to their high responder rate and IOP-lowering effects.5–7

Prostaglandin analogs reduce IOP primarily by increasing outflow facility via the uveoscleral pathway. This occurs by matrix metalloproteinase remodeling of the extracellular matrix leading to subsequent changes in the resistance of outflow in the ciliary muscle.8–11 Several studies have also suggested that the conventional outflow pathway, which consists of the trabecular meshwork, Schlemm’s canal, collector channels, and aqueous veins, may be a secondary pathway by which prostaglandin analogs reduce pressure.7,9,12–18

Studies using prostaglandin analogs in general, and latanoprost in particular, have found that binding of these drugs to the FP receptor leads to activation of the phosphatidylinositol and protein kinase C pathways, an increase in calcium release and subsequent phosphorylation of myosin light chain kinase in the iris.7,19–21 While latanoprost binds to FP and FP-like receptors in cells of the conventional outflow pathway,22 what happens following receptor activation and the identity of critical effector molecules necessary for increasing outflow facility are unknown. Identifying latanoprost-induced effector molecules will allow for a better understanding of the pathophysiology behind the treatment of ocular hypertension.

While generally well tolerated, prostaglandin analogs do exhibit notable side effects including conjunctival hyperemia, ocular surface irritation, pigmentation of the iris and...
periorcular skin, and hypertrichosis. Therefore, elucidating downstream signaling partners of latanoprost will help identify targets for development of novel treatments potentially leading to improved IOP reduction with fewer side effects. In light of this, we sought to identify downstream signaling molecules following latanoprost treatment with reference to IOP reduction. In order to identify downstream effector molecules, we treated Schlemm’s canal cells with latanoprost and analyzed gene and protein expression at several different time points. We identified stanniocalcin-1 (STC-1), a multifunctional phosphoglycoprotein hormone with anti-inflammatory, antiapoptotic, antioxidative damage, and neuroprotective properties, as one of the most differentially expressed molecules at the RNA level. In this study, we validated STC-1 upregulation and assessed its role as an effector molecule in latanoprost-mediated IOP regulation.

METHODS

Validation of STC-1 Expression

Cell Culture. Primary human Schlemm’s canal cell lines were grown to confluence in six-well plates (BD Falcon, Franklin Lakes, NJ, USA) containing Dulbecco’s modified Eagle’s medium (DMEM; Life Technologies, Grand Island, NY, USA); 10% fetal bovine serum (Mediatech, Manassas, VA, USA); and 1% penicillin/streptomycin (Life Technologies) in 5% CO2 at 37°C. Confluent cells were washed twice with phosphate-buffered saline and incubated in serum-free DMEM for 24 hours to synchronize the growth potential of the cells. Cells were incubated with latanoprost (de-esterified free-acid form at 100 nM final concentration; Cayman Chemical, Ann Arbor, MI, USA) or vehicle (ethanol, final dilution 1:1000) in DMEM containing 1% penicillin/streptomycin.

RNA Analysis. Primary Schlemm’s canal cells were grown to confluence, treated with 100 nM latanoprost for 6 hours, and harvested. Total RNA was extracted using an RNA isolation kit (RNeasy Total RNA Isolation Kit; Qiagen, Hilden, Germany). Approximately 250 ng of total RNA was reverse transcribed into cDNA using a synthesis kit (Script cDNA; Bio-Rad Laboratories, Inc., Hercules, CA, USA). Quantitative real-time polymerase chain reaction (qPCR) reaction using STC-1 forward, 5’-AGGCGGACGAGAAATGACT-3’; reverse, 5’-GGTTGGGCCAGAACCACCT-3’; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward, 5’-CTCTGACTTCAACAGC-3’; reverse, 5’-TGCTAGCAAATTCCGT-3’ primers were performed on a PCR system using a master mix (Roche Light Cycler 480 with SYBR Green I; Roche, Indianapolis, IN, USA). We performed qPCR amplification with a pre-denaturation step at 95°C followed by 45 cycles of denaturation at 95°C, annealing at 63°C, and extension at 72°C. Fold change was calculated after normalization with GAPDH.

Protein Analysis. Primary Schlemm’s canal cells were grown to confluence, treated with 100 nM latanoprost for 15 minutes, 1, 2, 4, or 6 hours, and harvested. Schlemm’s canal cell pellets were suspended in ice-cold lysis buffer (50 mM Tris pH 8.0, 0.5% sodium dodecyl sulfate, 0.5% Triton X-100, 137 mM NaCl, 3 mM KCl, 8 mM Na2HPO4-7H2O, 1 mM KH2PO4, protease inhibitors; Roche) and passed repeatedly through a 21-gauge needle for homogenization. Lysate was centrifuged at 13,000g for 10 minutes, and total protein was quantified by the Bradford assay. Cell lysates containing 20 μg total protein were mixed with reducing lane marker buffer (ThermoFisher Scientific, Waltham, MA, USA) containing 50 mM Tris-HCl, 2% mercaptoethanol, 2% glycerol, 0.1% SDS, and harvested. Total protein were analyzed by 4% to 15% SDS-PAGE gradient gel (Bio-Rad Laboratories, Inc.). Proteins were transferred to polyvinylidene difluoride membrane (Millipore Corp., Billerica, MA, USA) in 1X transfer buffer (50 mM Tris, 384 mM glycine, 0.01% SDS, 20% methanol). Membranes were blocked in 20 mM Tris (pH 7.5), 150 mM NaCl, 0.05% Tween-20, and 2% instant nonfat dry milk. Blots were probed with rabbit monoclonal anti-human STC-1 (Novus Biologicals, Littleton, CO, USA) and mouse monoclonal anti-human GAPDH (Novus Biologicals). Secondary antibodies used were horseradish peroxidase-linked anti-rabbit or antimouse, respectively (GE Healthcare, Piscataway, NJ, USA). Antibody/antigen complexes were detected using ECL Western blot signal detection reagent (GE Healthcare). Chemiluminescence film (BioMax XAR; Eastman Kodak, Rochester, NY, USA) was used to visualize protein signals. Each film was digitized with a photographic scanner (Epson Perfection 2400; Epson America, Inc., Long Beach, CA, USA). The band intensities for Western blot analysis were quantified using ImageJ software (http://rsb.info.nih.gov/ij/index.html in the public domain by the National Institutes of Health, Bethesda, MD, USA) and normalized to GAPDH.

Animal Experiments

All animal studies and treatment protocols were approved by the Mayo Clinic (Rochester, MN, USA) Institutional Animal Care and Use Committee and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. We obtained STC-1+/− and littermate wild-type mice from the Sheikh-Hamad laboratory, Baylor College of Medicine, and bred at Mayo Clinic. Mice, aged 5 to 8 months, were utilized in the experiments. A handheld rebound tonometer (Icare TonoLab; Colonial Medical Supply, Franconia, NH, USA) was used to measure IOP in conscious mice. For IOP measurements, the tonometer was held perpendicular to the cornea according to the manufacturer’s instructions. The tonometer records six readings from the same eye, discards the highest and lowest values, and shows the average of the remaining four values as a single IOP reading. Three independent measurements were obtained daily at similar time points and were averaged to obtain the daily IOP value for each eye. After 1 week of baseline IOP measurements, STC-1+/− mice were treated with latanoprost (n = 10) or Rho kinase inhibitor Y27632 (Enzo Life Sciences, Farmingdale, NY; n = 10). Congenic wild-type controls were treated with latanoprost (n = 8) or Y27632 (n = 10). Treatments were daily in one eye for 7 consecutive days with 5 μL of latanoprost-free acid (100 μM solved in 1:1000 DMSO in PBS) or 10 μM Y27632 (dissolved in phosphate-buffered saline). In the contralateral eye, vehicle was added daily in the same proportion as the treated eye for 7 consecutive days. Additionally, wild-type mice (n = 7; Charles Rivers Laboratories, Wilmington, MA, USA) were treated with 5 μL of topicaly administered recombinant human STC-1 (0.5 mg/mL; Biobvendor Research & Diagnostic Products, Asheville, NC, USA) or vehicle (phosphate-buffered saline) daily for 7 days to examine the effect of STC-1 on IOP. In all animals, the right eye served as the vehicle control eye while the left eye received study drug (latanoprost, Y27632, or STC-1). We recorded IOP in both eyes three times daily at 1, 4, and 23 hours following treatment.

Ex Vivo Human Anterior Segment Culture

Anterior segments from human donor eyes (age 75.5 ± 17.5 years, range: 51 to 98 years; n = 8) were perfused in culture with DMEM within 10.2 ± 4.4 hours of death as previously described. After achieving a stable baseline pressure, one anterior segment from each pair received recombinant human STC-1 at concentrations of 5, 50, or 500 ng/mL (dissolved in
H₂O), while the fellow eye received vehicle and served as the control. We added STC-1 and vehicle using a gravity-driven constant pressure method of anterior chamber exchange followed by continuous perfusion. Hourly pressure readings were obtained from the average of 60, one-minute pressure measurements using a custom-designed software program. The experimental eye was typically the right eye and the control was the left eye.

Tissue Preparation and Microscopy
For human anterior segments, selected wedges of tissue 180º apart that included the trabecular meshwork and Schlemm's canal were isolated and fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). Tissue wedges were dehydrated in a series of ascending ethanol concentrations, cleared with 100% acetone, infiltrated and embedded in Epon araldite, and sectioned at 0.5 μm. Eye tissue sections were stained with toluidine blue and examined using a light microscope (Nikon Corp., Tokyo, Japan). Additional tissue wedges were sectioned at 100 nm, placed on copper film grids, and stained with uranyl acetate and lead citrate. Tissue sections on copper film grids were examined using a transmission electron microscope (JEOL-1400; JEOL USA, Inc., Peabody, MA, USA).

For mouse eye histopathology, whole eyes were enucleated from euthanized mice following termination of the experiment. Eyes were placed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) overnight, and processed for light microscopy as described above for human anterior segment wedges. Additional 100-nm sections were placed on copper film grids, and stained with uranyl acetate and lead citrate. Digital images of toluidine blue-stained sections and transmission electron–processed sections were obtained following examination under a light microscope (Nikon Corp.) and a transmission electron microscope (JEOL USA, Inc.).

Statistics
Prior to initiation of statistical analysis, all data sets were evaluated for distribution assessment using the Shapiro-Wilk test. For animal studies, significance of IOP change was assessed between experimental and vehicle-treated control eyes using Student’s paired t-test for data sets with normal distribution and Wilcoxon sign-rank test for nonparametric data sets. Variations in daily IOP are graphically presented as distribution and Wilcoxon sign-rank test for nonparametric statistical significance was analyzed using a Student’s two-tailed t-test. All values are expressed as mean ± standard deviation. Statistical calculations were performed using a statistical software package (JMP; SAS Institute, Inc., Cary, NC, USA).

For human anterior segment studies, the effect of latanoprost was expressed as the change in outflow facility (C) for each anterior segment. Results from each pair of anterior segments were combined into a group mean for each drug, and statistical significance was analyzed using a Student’s two-tailed paired t test. All values are expressed as mean ± standard deviation.

Results
STC-1 Is Upregulated in Latanoprost-Treated Primary Human Schlemm's Canal Cells
Preliminary findings from confluent primary human Schlemm's canal cell lines treated with latanoprost identified STC-1, a secreted phosphoglycoprotein hormone with multiple functions, as a gene consistently upregulated following latanoprost treatment. Quantitative PCR studies in human Schlemm's canal cells showed an 18.1 ± 4.3-fold (n = 3) induction. Cell lysates isolated from latanoprost-treated primary human Schlemm's canal cells at various time points showed an increase of STC-1 protein expression of 1.9-fold at 2 hours and approximately 5.0-fold at 4 and 6 hours, correlating with increased mRNA expression levels identified by qPCR (Fig. 1).

IOP Recordings in STC-1 Knockout Mice Following Treatment With Latanoprost
Because of its multifunctional properties and ability to act in an autocrine and paracrine fashion similar to prostaglandin analogs, we examined the role of STC-1 in downstream signaling following latanoprost treatment in vivo using STC-1-/- mice and congenic wild-type controls. Assessment of baseline IOPs for 6 days showed no significant difference between STC-1-/- mice (16.6 ± 0.5, n = 10) and wild-type controls (16.2 ± 0.3, n = 8; P = 0.1). Histologic examination of STC-1-/- mice by light and transmission electron microscopy showed a normal-appearing ocular anatomy, an open angle with appropriate trabecula within the trabecular meshwork, and intact inner and outer walls of Schlemm's canal, all similar to wild-type controls (Fig. 2). With no observable morphologic differences between STC-1-/- and wild-type mice, we proceeded to assess the effect of latanoprost treatment on IOP in these mice. Topical eye treatment with latanoprost (100 nM) reduced IOP in wild-type controls by 3.8 mm Hg (P < 0.001, n = 8), which correlated to a 22.0% ± 1.9% decrease in IOP when compared to the fellow contralateral eyes treated with vehicle alone (Fig. 3). Consistent IOP change was identified throughout the once daily dose as determined by IOP pressure monitoring at 1, 4, and 25 hours after treatment (Table). In contrast, STC-1-/- mice did not show any significant reduction in IOP after topical latanoprost treatment (0.5% ± 0.7%, P = 0.34, n = 10) at any time point throughout the 7-day treatment regime. To determine if this was unique to latanoprost, we treated STC-1-/- with Y27632, a Rho kinase inhibitor that increases outflow through both the uveoscleral and the conventional outflow pathway. Treatment of either wild-type (n = 10) or STC-1-/- (n = 10) mice with Y27632 resulted in significant reductions of IOP 12.5% ± 1.2% and 13.1% ± 2.8%, respectively (P < 0.0001, n = 10; Fig. 4; Table). These data suggest that STC-1 has a unique and key role in latanoprost signaling mediated IOP reduction.

STC-1 Perfusion in Ex Vivo Human Anterior Segment Culture
To determine if STC-1 would influence IOP reduction by itself, we perfused human anterior segments with several concentrations of recombinant STC-1 for 24 hours. Perfusion with either 5 ng/mL (0.15 ± 0.04 to 0.15 ± 0.04 μL/min/mm Hg, n = 2, P = 0.20) or 50 ng/mL (0.14 ± 0.05 to 0.18 ± 0.07 μL/min/mm Hg, n = 4, P = 0.20) had no significant effect on outflow facility. However, all anterior segments perfused with STC-1 at 500 ng/mL had decreased pressure and increased outflow facility (0.15 ± 0.05 to 0.27 ± 0.09 μL/min/mm Hg, n = 5, P = 0.02) compared to baseline (Fig. 5). Paired controls treated with vehicle showed no change in outflow facility from their baseline values. To determine whether the changes in pressure could be secondary to morphologic changes, microscopic analysis was performed. Morphologic analysis showed viable and healthy cells in the trabecular meshwork and Schlemm’s canal of control and treated eyes (Fig. 6). No major disruptions of the juxtanaculicular tissue or the
basement membrane of Schlemm’s canal inner and outer walls were observed. These results indicate that STC-1 may be a molecule within the latanoprost signaling pathway that can be therapeutically targeted to lower IOP.

Effect of Recombinant STC-1 on IOP

To determine if STC-1 has ocular hypotensive activity in vivo, wild-type C57Bl/6 mice were treated with recombinant STC-1 (0.5 mg/mL) or vehicle daily for 7 days (Fig. 7). Stanniocalcin-1 reduced IOP by 15.2% when compared to vehicle-treated contralateral eyes (range of IOP reduction from 5.1% to 21.4%). Following withdrawal of treatment, IOP returned to baseline. These results suggest that STC-1 has IOP-lowering properties when used as a standalone agent.

DISCUSSION

Prostaglandin analogs like latanoprost are a first-line medical therapy for IOP reduction due to their once daily dosing,
greater response rates, and greater amount of IOP reduction compared to other classes of pressure-lowering medications. Like all IOP-lowering medications, latanoprost has notable side effects. Identifying the critical effector molecules in the signaling pathway of latanoprost will provide insights into additional molecules to target for IOP reduction, potentially with fewer side effects. In the current study, we identified STC-1 as a transcript and protein that is highly induced following treatment of human Schlemm’s canal cells with latanoprost. Topical latanoprost significantly reduced IOP in wild-type controls while STC-1/C0 mice demonstrated no IOP reduction to topical latanoprost. In contrast, Rho kinase inhibitor Y27632 demonstrated IOP reduction in both wild-type and STC-1/C0 mice, indicating that STC-1 is a unique and important downstream signaling molecule necessary for the ocular hypotensive properties of latanoprost. Additionally, recombinant STC-1 by itself also increased outflow facility in human anterior segments and reduced IOP in vivo in wild-type mice. Together, these results suggest that STC-1 is a critical and unique effector molecule for the latanoprost signaling pathway that is necessary for latanoprost-induced IOP reduction and that STC-1 by itself can act as an ocular hypotensive agent.

Stanniocalcin was first described in fish as a 50-kDa homodimeric glycoprotein that is secreted from the corpuscles of Stannius into the bloodstream in the setting of hypercalcemia to regulate calcium excretion at the gills and gut. In mammals, two homologues of STC have been identified, STC-1 and STC-2. STC-1 is the most studied of the two mammalian forms, having a 50% amino acid homology with its fish counterpart. STC-1 is a secreted, homodimeric phosphoglycoprotein that has preserved protein structure similarity between mammals and fish including the conservation of 11 cysteine residues. It is expressed in a wide variety of tissues most notably bone, skeletal muscle, heart, thymus, and spleen. Functionally, STC-1 has been associated with calcium uptake, hypoxic preconditioning, hypoxic stress properties through suppression of reactive oxygen species. Additionally, STC-1 has also been shown to be neuroprotective for neurons, photoreceptors, and retinal ganglion cells and has been linked to anti-inflammatory effects by inhibiting macrophage chemotaxis.
modulating transendothelial migration of leukocytes and reducing T cell infiltration. 23,45,49,50

In treating wild-type mice with topical latanoprost, we observed a reduction in IOP by approximately 22.0% ± 1.9%, consistent with previous reports, 30 while the STC-1−/− mice showed no response (0.55% ± 0.7%). Mice that were STC-1−/− were responsive to Rho kinase inhibitor Y27632, which confirmed that STC-1−/− mice have normal functioning outflow pathways, but that elimination of STC-1 expression in these mice renders topical latanoprost ineffective.

The current study adds Schlemm’s canal cells to the list of cells that express STC-1 and shows that its expression is highly

![Graph](https://via.placeholder.com/150)

**FIGURE 3.** Effect of latanoprost treatment in STC-1−/− mice. STC-1−/− mice (n = 10) and congenic controls (n = 8) were treated once daily with 100 μM latanoprost. The daily IOP was recorded as the average of IOP measurements at 1, 4, and 25 hours following latanoprost treatment. While congenic control mice show IOP reduction with latanoprost, STC-1−/− do not, suggesting STC-1 is an important effector molecule for latanoprost-induced IOP reduction.

![Graph](https://via.placeholder.com/150)

**FIGURE 4.** Rho kinase inhibitor Y27632 reduces IOP in STC-1−/− mice. STC-1−/− mice (n = 10) and congenic controls (n = 10) were treated once daily with 10 mM Y27632. The daily IOP was recorded as the average of IOP measurements at 1, 4, and 25 hours following Y27632 treatment. Both STC-1−/− mice and congenic controls showed IOP reduction following treatment with Y27632.
FIGURE 5. Perfusion with STC-1 shows increase in outflow facility and decrease in pressure. (A) Outflow facility of human anterior segments (n = 8) following perfusion with 5, 50, or 500 ng/mL of recombinant human STC-1. (B) Representative graph of an eye pair perfused with 500 ng/mL STC-1. *P ≤ 0.05.

FIGURE 6. Histologic analysis of human anterior segment ocular tissue following treatment with recombinant STC-1. (A, B) Representative sections (3 μm) of recombinant STC-1 and vehicle-treated eyes that were stained with toluidine blue. (C, D) Transmission electron micrographs showing ultrastructure of recombinant STC-1 and vehicle-treated eyes. Recombinant human STC-1 and vehicle-treated eyes had similar morphology and ultrastructural appearance suggesting no apparent detrimental side effects of recombinant STC-1 treatment. AC, anterior chamber; JCT, juxtaocular region; TM, trabecular meshwork; SC, Schlemm’s canal.
influenced by latanoprost. This is an important finding as little is known about the molecular events that connect latanoprost treatment with IOP reduction. Latanoprost has been shown to phosphorylate myosin light chain kinase in the iris, indicating a role for latanoprost in cell relaxation. However, STC-1 is the first individual molecule that has been identified as a key downstream effector of latanoprost signaling. In addition to its variety of functional activities, our results also suggest that STC-1 has ocular hypotensive properties, since addition of recombinant STC-1 to human anterior segment cultures decreased pressure and increased outflow facility, and when applied topically to wild-type mice reduced IOP in vivo.

Despite the benefits of latanoprost in lowering IOP, long-term treatment with latanoprost can often cause significant side effects, which can be contraindicative for its prolonged usage. Identification of STC-1 as a downstream signaling molecule through which latanoprost executes its IOP-lowering effects, and the fact that recombinant STC-1 shows ocular hypotensive properties, makes this an attractive therapeutic target. Utilization of a specific downstream molecule such as STC-1 within the latanoprost signaling cascade may eliminate some of the major side effects while providing a specialized therapeutic strategy.

In summary, STC-1 is a necessary downstream signaling molecule for latanoprost-mediated lowering of IOP. Additionally, STC-1 demonstrates ocular hypotensive properties when used by itself, mimicking the effect of latanoprost. Given its novel role in latanoprost-mediated IOP reduction, STC-1 may be considered a promising candidate molecule for devising future therapeutic regiments to reduce IOP.

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