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Suppression of Epithelial to Mesenchymal Transition Markers in Mouse Lens by a Smad7-based Recombinant Protein

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Conflict of Interest Statement

MLH, MGP, and BS declare no potential conflicts of interest. JMP and XJW are inventors of PCT/US18/68089 published as WO 2019/133950. DDW and XJW have shares of Allander Biotechnologies, LLC., which develops Smad7-based therapies.

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Abstract

Cataracts, a clouding of the eye lens, are a leading cause of visual impairment and are responsible for one of the most commonly performed surgical procedures worldwide. Although generally safe and effective, cataract surgery can lead to a secondary lens abnormality due to transition of lens epithelial cells to a mesenchymal phenotype (EMT) and opacification of the posterior lens capsular bag. Occurring in up to 40% of cataract cases over time, posterior capsule opacification (PCO) introduces additional treatment costs and reduced quality of life for patients. Studies have shown that PCO pathogenesis is driven in part by TGF- β , signaling through the action of the family of Smad coactivators to effect changes in gene transcription. In the present study, we evaluated the ability of Smad-7, a well characterized inhibitor of TGF- β -mediated Smad signaling, to suppress the EMT response in lens epithelial cells associated with PCO pathogenesis. Treatment of lens epithelial cells with a cell-permeable form of Smad7 variant resulted in suppressed expression of EMT markers such as alpha smooth muscle actin and fibronectin. A single application of cell-permeable Smad7 variant in the capsular bag of a mouse cataract surgery model resulted in suppression of gene transcripts encoding alpha smooth muscle actin and fibronectin. These results point to Smad7 as a promising biotherapeutic agent for prevention or substantial reduction in the incidence of PCO following cataract surgery.

Keywords

Aldose reductase; cataract; epithelial-to-mesenchymal transition; lens; Smad

1. Introduction

Cataract surgery is one of the most commonly performed surgical operations in the world. Despite usually excellent postsurgical outcomes in the short term following cataract surgery, up to 40% of patients develop over months to years a secondary opacification of the lens, referred to as posterior capsular opacification (PCO) [1, 2]. PCO is thought to arise from a small number of lens epithelial cells (LECs) that escape removal during lens extraction and therefore can remain adherent to the inner surface of the lens capsular bag. In some patients, these residual LECs may undergo an epithelial-to-mesenchymal transition (EMT), resulting in a myofibroblastic phenotype that leads to deposition of extracellular matrix proteins, capsular wrinkling, and light scattering along the visual axis. Collectively, these conditions are known as PCO. Although PCO can be treated by ablating the posterior capsule with a Nd:YAG laser (YAG capsulotomy), this procedure adds significant financial burden, reduced quality of life, and increased risk for subsequent development of retinal detachments and

intraocular pressure spikes [2]. Therefore, new therapies to reduce or eliminate the risk for PCO development would be a significant improvement for cataract patients.

Research on the EMT process in LECs has found that signal transduction mediated by transforming growth factor- β (TGF- β) depends on various members of the SMAD protein family to effect changes in transcription of genes encoding EMT biomarkers α -smooth muscle actin (α SMA), and fibronectin [3–5]. Inhibition of this signaling pathway has been shown to reduce TGF- β -induced upregulation of EMT markers [6]. Smad7 is an inhibitor of the TGF- β /SMAD signaling [7]. Han and coworkers recently demonstrated prophylactic and therapeutic effects of a cell-permeable Smad7 biologic against TGF- β -mediated changes to oral epithelial cells in a mouse model of radiation-induced oral mucositis [8]. Smad7 has not yet been tested as a TGF- β /SMAD pathway inhibitor in LECs.

Another key player in PCO pathogenesis is aldose reductase (AR), normally cited for its role in the polyol pathway in converting glucose to sorbitol while producing NADP⁺ and NADH [9]. NADH oxidase produces reactive oxygen species (ROS), which can increase EMT [10]. AR has been studied to better understand this link to PCO and has been found to be critical in influencing PCO [5, 11, 12]. Studies of LEC in cell culture models have demonstrated that AR inhibition prevents PCO biomarkers through suppression of SMAD signaling [12].

Several groups, including our own, have turned to animal models as a means to look at drug effects in a more clinically-relevant experimental setting. Recent work by our lab [12] and others [6] has shown PCO biomarkers α SMA and fibronectin are upregulated following a modified extracapsular lens extraction (ECLE) surgical procedure in the mouse. Studies from the laboratories of Ramana and Srivastava demonstrated that AR inhibition prevents PCO biomarker α SMA expression in pig eye capsular bags. [13] In the current study, we take advantage of our AR-transgenic mouse model, which has been shown to have a robust EMT response to lens extraction, to investigate whether the inhibitory Smad7 protects against EMT [12]. Evidence is presented that a single treatment of Smad7-based protein is sufficient to reduce the expression of EMT markers in this cataract mouse model.

2. METHODS

2.1 Transgenic Mice

Throughout all experiments, compliance was maintained with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All mice were handled in strict accordance with good animal practice, and all animal work was approved by the University of Colorado Institutional Animal Care and Use Committee. Lens phenotypes of the AR-Tg strain of mice designed for over-expression of AR (PAR40), have been previously described [14].

2.2 Smad7-based Recombinant Protein

We used a Tat-protein construct comprising Smad7 residues 203-426 fused to an amino-terminal Tat cell penetration domain (RKKRRQRRR) and a carboxy-terminal HA affinity epitope (designated as Tat-Smad7(203-426)-HA). The Smad7 fragment is designed to include the PY domain that is responsible for Smurf2-mediated TGF- β receptor degradation

and the MH2 domain that is responsible for transcriptional repression of Smad2/3 responsive genes [15, 16]. Sequences coding for this polypeptide were cloned into the pGEX-6p-1 expression vector, which was transformed into BL-21 Star *Escherichia coli* host cultures for protein over-expression. Recombinant protein was purified from host cell lysates essentially as described [17].

2.2 Extracapsular Lens Extraction

The modified extracapsular lens extraction procedure was performed based on previously published techniques [12, 18]. Mice were anesthetized using 5% isoflurane via inhalation. One eye of each mouse was dilated using a 1:4 mixture of 2% phenylephrine (Paragon Biotech Ins, Portland, OR) and 0.5% tropicamide (Akron, Lake Forest, IL), followed by one drop of ophthalmic proparacaine solution (Sandoz, Princeton, NJ), followed by betadine (Alcon, Fort Worth, TX). Both the cornea and the anterior capsule were incised along a central axis using a disposable ophthalmic knife (Alcon, Fort Worth, TX). The lens is separated from the capsular bag by hydro-dissection using saline injected with a bent cannula (Alcon, Fort Worth, TX). Angled jeweler forceps were used to gently remove the lens, and further flushed with saline to remove any remaining lens material. At this step, an injection of either 1X phosphate buffered saline (PBS), or 1 µg of Tat-Smad7(203-426)-HA was performed, followed by a 1-minute pause of surgery. As no intraocular lens was used to replace the native lens, viscoelastic was injected into the open chamber to regain and maintain ocular structure. A 10-0 nylon suture was used to close the corneal incision. All mice were euthanized 5 days postoperatively and capsules removed from both the surgical and the contralateral, control eye. Each experimental condition was performed in parallel.

2.3 Quantitative RT-PCR

Lens epithelial capsule were removed from eyes and placed into 500µL QIAzol® (Qiagen, Austin, TX) and frozen at –80°C. For control eyes, the whole lens was first removed, then ciliary processes and zonular fibers were removed, followed by capsular bag dissection from the lens fiber mass. For surgically treated eyes, we enucleated the eye, and made incisions posteriorly to locate and extract the capsular bag. Lens capsular bags so removed were placed into 500µL QIAzol (Qiagen, Austin, TX) and stored at –80°C until used for PCR analysis. Once the lens capsular tissue was thawed in QIAzol® (Qiagen, Austin, TX) 100 µL of chloroform was added and vortexed. The layers were then centrifuged at 4°C, for 15 min and the RNA was extracted from aqueous layer using a RNeasy Micro kit (Qiagen, Austin, TX) according to the manufacturer's protocol. cDNA synthesis was performed using iScript™ Advance cDNA Synthesis Kit for RT-qPCR (Biorad, Hercules CA) followed by QRT-PCR using iTaq™ Universal SYBR Green Supermix (Biorad, Hercules, CA) and amplified on CFX Connect (Biorad, Hercules, CA). The primers used in this study (Integrated DNA Technologies, Coralville IA) were αSMA (forward 5'-CTGTTATAGGTGGTTTCGTGGA-3'; reverse 5'-GAGCTACGAAGTGCCTGAC-3'), and fibronectin (forward 5'-TTGTTCTAGACACTGGAGAC-3'; reverse 5'-GAGCTATCCAATTTACCTTCAGA-3'). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control (forward 5'-CTGGAGAACATGCCAAGTA-3'; reverse 5'-TGTTGCTGTAGCCGTATTCA-3'). Data were analyzed using the 2^{–Ct} method.

2.4 Immunofluorescence

Surgically treated eyes were fixed in 4% paraformaldehyde for 30 min prior to incubation with increasing concentrations of sucrose (Thermo Fisher, Waltham, MA) dissolved in 1X (PBS) at 10%, 20%, and 30% sucrose solutions for 4 hours each. The tissue was then embedded in optimum cutting temperature (OCT) media (Tissue Tek, Torrance, CA) and frozen to -80°C before 10 μm sections were cut using a Microm HM550 cryostat (Thermo Fisher Scientific, Waltham, MA). OCT was removed by placing glass slide (Thermo Fisher, Waltham, MA) dissolved in acetone at -20°C for 10 min, and then blocked with 5% bovine serum albumin with 0.4% Triton X-100 for 30 min at room temperature. Sections were stained with αSMA ab32575 (Abcam, Cambridge, MA) for 1 hour at a 1:500 dilution. Slides were washed three times with PBS then incubated with a 1:1000 dilution of the anti-Rabbit IgG secondary antibody conjugated to Alexa-Fluor 488 (Thermo Fisher Scientific, Waltham, MA) for 30 min at room temperature. Slides were washed three times and mounted with DAPI containing mounting media (Vector Laboratories, Burlingame, CA) and cover slipped.

2.5 Confocal microscopy

The sections were imaged using a Nikon Eclipse Ti (Nikon, Tokyo) with a Nikon C2 camera (Nikon, Tokyo) equipped with 405/488/561/640 nm lines. Intensity of the laser and NIS Elements software settings were constant between specimens and acquisition in comparison slides. All stains were performed at the same time between comparisons. Images were processed after imaging to optimize brightness and contrast for viewing on diverse computer screens and all comparisons were adjusted in the same manner.

2.6 Culture of primary lens epithelial cells and Immunofluorescence

Primary lens epithelial cells were obtained from 4–6 capsular bags from untreated AR-Tg mouse eye. LEC cells were collected from capsular bag with 0.05% Trypsin-EDTA treatment at 37°C , 10 min. Cultures were maintained at 37°C , 5% CO_2 in DMEM (Dulbecco's modified eagle medium; Corning Cellgro, Manassas, VA) with 10% Fetal Bovine Serum (Gemini Bio products, West Sacramento, CA) with 100U Penicillin and 100 μg Streptomycin (Corning) and 0.25 μg AmphotericinB (Lonza) per mL. Six-well plates were seeded with 3×10^5 cells/well for 24 hours. Media was then removed, and wells were washed twice with PBS. Serum free DMEM was then added to each well before a treatment of Tat-Smad7(203-426)-HA at 1 $\mu\text{g}/\text{mL}$. Approximately 20 min later, TGF- β 2 (Novus Biologicals, Centennial, CO) was then added to control and all treatment wells at 10 ng/mL. Cells were harvested 48 hours later using 100 μL of RIPA Buffer (Pierce Biotechnology, Rockford, IL) with proteinase inhibitor (Pierce Biotechnology, Rockford, IL). Cells were left on ice for 10 min before being centrifuged. Protein levels were estimated using the bicinchoninic acid (BCA) method (Pierce Biotechnology, Rockford, IL) according to the manufacturer's protocol in preparation for running samples on western blots.

For Tat-Smad7(203-426)-HA protein penetration and EMT marker immunofluorescence, a slide chamber was seeded with 5,000 cells per well and incubated in the same conditions as described above. The wells were then rinsed with 1X PBS and then serum-free DMEM was added before Tat-Smad7(203-426)-HA at a final concentration of 1 $\mu\text{g}/\text{mL}$ was applied.

After 20 min, TGF- β 2 was added to a final concentration of 10 ng/mL. After one hour of treatment, wells were rinsed with 1X PBS, followed by 4% paraformaldehyde for 10 min. Cells were then washed again with 1X PBS before blocking with 5% bovine serum albumin containing 0.4% Triton-X in Tris-buffered saline and Tween 20 (TBST). Cells were then stained with either antibody to recognize the HA-epitope (#3724; Cell Signaling at 1:50 dilution, Danvers, MA), α SMA (ab32575 at 1:500 dilution, Abcam, Cambridge, MA), or fibronectin (NBP1-91258 at 1:150 dilution, Novus, Centennial, CO). After incubation with the primary antibody for 1 hours at 37° or overnight at 4° (HA antibody), slides were washed with 1X PBS before applying 1:1000 secondary antibody conjugated to Alexafluor 488 (Thermo Fisher Scientific, Waltham, MA) and mounted in DAPI fluoromount (Electron Microscopy Science, Hatfield, PA).

2.7 Western Blot

Samples were diluted 1:1 in a loading buffer solution of 95:5 Laemmli Sample Buffer (Biorad, Hercules, CA) to 2-mercaptoethanol (Sigma Aldrich, St. Louis MO) with a total of 20 μ g of protein per well. Samples were run in Mini-PROTEAN Precast Gels (Biorad, Hercules, CA). Gels were transferred onto a methanol-activated PVDF membrane (GE, Piscataway NJ). Membranes were blocked with 5% non-fat dry milk in TBST and probed with the same primary antibodies as used in immunofluorescence. After three washes with TBST, peroxidase-conjugated goat anti-mouse IgG secondary antibody and peroxidase-conjugated goat anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) at 1:5000 dilution in blocking buffer was used before a final wash step. A 1:1 mix of peroxidase buffer and Luminol/Enhancer Solution (Thermo Fischer Scientific, Waltham, MA) was applied before imaging with Biorad ChemiDoc XRS+ imaging system. Images were analyzed using ImageLabTM Software Version 3.0.

2.8 Statistical analysis

All statistical analysis was performed using Graphpad Prism version 5.03 (Graphpad Software, La Jolla) using two-tailed Student's t-test or one-way ANOVA on repeated measures with Tukeys multiple comparison. Replicates were considered significant where * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$.

3. RESULTS

3.1 Exogenous Tat-Smad7(203-426)-HA penetration of primary lens epithelial cells

Research has shown Smad7 to be effective in downregulating TGF- β signaling in oral epithelial cells in vitro and in vivo [8]. In order to test the ability of Smad7 to influence signaling in primary lens epithelial cells, we first wanted to confirm the ability of Tat-Smad7(203-426)-HA to penetrate primary LECs. This was accomplished by exposing LEC primary cultures from AR-Tg mice to quantities of recombinant Tat-Smad7(203-426)-HA containing the Tat cell penetration peptide domain at the N-terminus as well as an HA epitope tag at the C-terminus for immunolocalization [8] (Fig 1B). Immunostaining for the HA epitope showed a strong signal throughout the cytoplasm. This observation demonstrates that recombinant Tat-Smad7(203-426)-HA is capable of gaining access to the intracellular compartment of LECs, a critical requirement to be effective as an inhibitor of Smad

signaling downstream from the TGF β receptor complex [19]. This observation was critical to support the possible use of Tat-Smad7(203-426)-HA as a therapeutic protein in vivo.

3.2 Tat-Smad7(203-426)-HA reduces TGF- β 2 induction of α SMA in primary lens epithelial cells

The abundance of gene transcripts encoding the EMT marker α SMA has been previously reported to increase after cataract surgery in a mouse model [12]. Given the prospect of using Smad7-based protein as a therapeutic agent in the lens, we wanted to elucidate its effects on EMT changes within lens epithelial cells. We subjected primary lens epithelial cells from AR-Tg mice to serum free media, and treated them with Tat-Smad7(203-426)-HA for 20 min before adding TGF- β 2 (10 ng/mL) for 4 hours. As shown in Fig 2, α SMA immunostaining was very substantially decreased in Tat- Smad7-treated cells as compared to vehicle-treated control cells (compare figs 2B and 2C). Similarly, immunostaining for fibronectin, another marker of EMT, was also decreased after Tat- Smad7 treatment compared to vehicle control (Fig 2E and 2F). To quantify these changes, we carried out densitometry western blot using cells harvested after 48 hours of treatment. As shown in Fig 3, Tat-Smad7(203-426)-HA treatment resulted in a statistically-significant decrease in the abundance α SMA as compared to control. These data support the prospective use of Tat-Smad7(203-426)-HA in vivo.

3.3 Tat-Smad7(203-426)-HA intraocular injection during a mock cataract surgery in a mouse model reduces expression of α SMA

LEC remaining in the capsular bag after lens extraction develop an EMT phenotype associated with increased levels of α SMA detected by immunostaining and qPCR measurements [12]. To test the ability of Tat-Smad7(203-426)-HA to inhibit EMT pathways in vivo, we conducted immunostaining for α SMA in eyes 5 days after treatment with an intraocular injection of Tat-Smad7(203-426)-HA during a lens extraction surgery (Fig 4). Compared to eyes treated with an intraocular injection of PBS during surgery (Fig 4A), eyes treated with an intraocular injection of Tat-Smad7(203-426)-HA (1 μ g) during surgery showed an appreciably thinner concentration of lens epithelial cells with α SMA signal inside the capsular bag (Fig 4B). These results are consistent with results obtained in cell culture models, demonstrating that a single dosing with Tat-Smad7(203-426)-HA is sufficient to suppress the upregulation of the EMT marker α SMA. To quantify the effect of Tat-Smad7(203-426)-HA therapy, we isolated RNA from capsular bags dissected from the eyes of our 5 day post-surgically treated groups. Unoperated contralateral eyes were used to recover lens capsules as controls. Based on qPCR results, gene transcript levels for EMT markers α SMA and fibronectin were found to increase in the lens extracted eyes as compared to unoperated eyes. As in tissue culture experiments, Tat-Smad7(203-426)-HA treatment prevented an increase in mRNA expression of the EMT markers α SMA and fibronectin (Figs 5A, 5B).

4. DISCUSSION

Cataract surgery is one of the most commonly performed surgical operations in the world. Given the sheer numbers of cataract procedures delivered each year worldwide, PCO is

a major problem as it is the most common complication of cataract surgery, occurring in up to 40% of patients over time. Treatment for PCO requires follow up clinic visits for Nd:YAG laser capsulotomies to clear the visual axis. This procedure itself, although safe, has associated complications and financial burden. A wide variety of strategies have been explored for preventing PCO, including improvements in the design of intraocular lens materials and their manufacturing, novel devices to remove adherent epithelial cells, and cytotoxic agents to eliminate epithelial cells immediately after surgery [2]. Unfortunately, none of these innovations has proven effective in the clinic.

PCO is thought to be caused by residual lens epithelial cells left over following cataract surgery. These residual cells undergo EMT, resulting in a myofibroblastic phenotype. By virtue of the observation that aldose reductase inhibitors can suppress signaling pathways associated with PCO, aldose reductase has been implicated as being involved in PCO pathogenesis. However, a functional mechanism to explain a role for AR has been elusive. Evidence suggests that enhanced flux of substrates through the polyol pathway leads to oxidative stress through a change in redox balance involving NADPH/NADP⁺ and NAD⁺/NADH [20]. Reduced NADPH levels from AR catalytic activity could lead to reduced steady-state levels of glutathione, a usually abundant antioxidant, due to limiting amounts of NADPH required by glutathione reductase to recycle oxidized glutathione (GS-SG) back to its reductive form (GSH). Blockade of the catalytic activity of AR with AR inhibitors would thus be expected to spare NADPH levels and protect against oxidative stress. However, our prior in vitro experiments demonstrated that AR also plays a noncatalytic role in TGF- β signaling through its physical interactions with Smad2 and Smad3, key components of the Smad-signaling pathway in lens epithelial cells. Lens epithelial cells from AR-transgenic mice demonstrate elevated levels of TGF- β signaling, leading to EMT [12]. The current studies show that a Smad7 variant has promising inhibitory effects on the TGF- β signaling pathways that may be invoked in the pathogenesis of PCO in our surgical mouse model.

This study provides both an in vitro and a surgical in vivo demonstration of the ability of Smad7-based protein to suppress EMT as measured by the abundance of biomarkers such as α SMA and fibronectin. Smad7-based recombinant protein containing the TAT cell penetration peptide domain was shown to penetrate primary lens epithelial cells where it had the effect of decreasing α SMA protein expression. Furthermore, we employed a surgical extracapsular lens extraction in a mouse model to test the efficacy of Smad7-based protein treatment on suppression of the EMT response. Despite the lack of a drug delivery method to ensure prolonged exposure of lens tissue to the therapeutic protein, we observed that one dose of Smad7-based protein was sufficient to have a significant effect on markers of EMT expressed by LEC in both cell culture and surgical mouse models.

These results suggest that Smad7 may have sufficient effect to suppress the EMT markers α SMA and fibronectin after a single treatment. As the development of lens changes in human cataract patients can take months-to-years before YAG capsulotomy is required to restore clear vision, further studies will be required to establish the boundaries of a treatment window for PCO prevention in humans. Additional studies are also needed to establish an effective means of delivering therapeutic amounts of Smad7-based protein to the lens capsular bag. We believe that further research to prevent PCO has the potential to

substantially reduce the financial burden and threatened quality of life among the millions of cataract patients worldwide.

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Abbreviations

AR	aldose reductase
AR-Tg	AR transgenic mouse
αSMA	α -smooth muscle actin
EMT	epithelial-to-mesenchymal transition
LEC	lens epithelial cell
TGF-β	transforming growth factor-beta isoform
PCO	posterior capsule opacification

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Highlights

- Lens epithelial cells undergo EMT following lens removal in a mouse model of cataract.
- A variant of Smad7 containing the Tat cell penetration domain was shown to enter into lens epithelial cells in culture.
- The cell permeable variant of Smad7 suppresses EMT in cultured mouse lens epithelial cells.
- One dose of Smad7-based protein was sufficient to significantly suppress markers of EMT in a mouse cataract surgical model

Fig 1. Penetration of Tat-Smad7(203-426)-HA into lens epithelial cells. Epithelial cells from lenses of AR-Tg mice were incubated in the presence of TGF- β 2 (10 ng/ml) and Tat-Smad7(203-426)-HA (1 μ g/ml) containing an HA peptide epitope domain at the C-terminus or vehicle control. After 1 hour incubation, cells were immunostained with antibodies directed to the HA domain. Nuclei were visualized with DAPI staining. (A) Vehicle control; (B) Tat-Smad7(203-426)-HA-treated cells. Images are representative of at least three independent experiments.

Fig 2.

Expression of EMT markers in lens epithelial cells. Expression of α SMA (A-C) and fibronectin (D-F) in AR-Tg primary lens epithelial cells in culture. Panels A,D: Cultures were treated with PBS vehicle control. Panels B, C, E, F: Cultures were treated with TGF- β 2 (10 ng/mL). Panels C and F: Cultures were treated with Tat-Smad7(203-426)-HA (2 μ g/mL) 20 min after TGF- β 2. TGF- β 2 resulted in induction of α SMA protein signal (B), as well as fibronectin protein signal (E). Tat-Smad7(203-426)-HA treatment substantially suppressed the induction of α SMA (C) and fibronectin (F). DAPI (blue) shows nuclear staining. Images are representative of at least three independent experiments.

Fig 3.

Suppression of TGFβ-induced αSMA expression in LEC by Tat-Smad7 (203-426)-HA. TGFβ-treated cells (10 ng/ml) were incubated with vehicle (control) or Tat-Smad7(203-426)-HA (1 μg/ml) and extracted 48 h later for Western blot analysis of αSMA and GAPDH protein levels. Blots were imaged using HRP and analyzed in ImageLab with αSMA normalized to GAPDH. Tat-Smad7(203-426)-HA treatment decreased αSMA protein expression ($p < 0.01$, **) as determined using Student's two-tailed t-test. Values are mean $\pm$ SEM.

Fig 4.

Therapeutic effect of Tat-Smad7(203-426)-HA in an in vivo cataract extraction model. Lens capsular bags were recovered 5 days after extracapsular lens extraction and processed for immunostaining for the abundance of α SMA expression (green). (A) α SMA expression was widespread throughout cells in the capsular bag in the untreated eye; (B) Eyes treated with 1 μ g Tat-Smad7(203-426)-HA at the time of lens extraction showed a much restricted population of immunopositive cells (B). Scale bar= 200 μ m. In both panels, dotted and dashed lines trace the anterior and posterior capsules, respectively.

Fig 5.

Effect of Tat-Smad7(203-426)-HA on expression of αSMA and fibronectin in an *in vivo* cataract extraction model. Levels of mRNA transcripts encoding the EMT markers αSMA (A) and fibronectin (B) were measured in lens capsular material from unoperated lenses (control) or lenses recovered 5 days after ECLE surgery and treatment with either Smad7 or PBS vehicle. Compared to unoperated control, ECLE induces an increase in αSMA and FN mRNA expression. (A) Intracapsular injection of Tat-Smad7(203-426)-HA (1 μg) immediately after lens extraction (Smad7) largely suppresses induction of αSMA. (B) Tat-Smad7(203-426)-HA treatment reduced fibronectin expression but to an extent not sufficient for statistical significance. N=4 for all treatment groups. Values are mean ± SEM where * p<0.05, ** p<0.01, and *** p<0.001.