Pharmacological Regulation of SPARC by Lovastatin in Human Trabecular Meshwork Cells

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Purpose. Statins have been shown to increase aqueous outflow facility. The matricellular protein SPARC (secreted protein acidic and rich in cysteine) is a critical mediator of aqueous outflow and intraocular pressure (IOP). Here, we examine the effects of lovastatin on SPARC expression in trabecular meshwork (TM) cells, exploring the molecular mechanisms involved.

Methods. Primary cultured human TM cells were incubated for 24, 48, and 72 hours with 10 μM lovastatin. In separate cultures, media was supplemented with either farnesyl pyrophosphate (FPP) or geranylgeranyl pyrophosphate (GGPP) for the duration of the 72-hour time point experiment. Trabecular meshwork cells were also pretreated for 24 hours with lovastatin followed by 24-hour stimulation with 3 ng/mL TGF-β2. Cell lysates and media were harvested and relative mRNA and protein level changes were determined. Krüppel-like factor 4 (KLF4) localization in normal human anterior segments was examined by immunofluorescence. Adenovirus expressing human KLF4 was used and relative changes in SPARC mRNA and protein levels were assessed.

Results. Incubating TM cells with lovastatin suppressed SPARC mRNA and protein levels. This effect was reversed upon media supplementation with GGPP but not FPP. Pretreating cells with lovastatin inhibited TGF-β2 induction of SPARC. The KLF4 transcription factor was expressed throughout the TM and the inner and outer walls of Schlemm's canal. Lovastatin treatment upregulated KLF4 mRNA and protein levels. Overexpression of KLF4 downregulated SPARC expression.

Conclusions. Collectively, our data identify lovastatin as an important pharmacological suppressor of SPARC expression in TM cells, and provide further insight into the molecular mechanisms mediating statin enhancement of aqueous outflow facility.

Keywords: statins, SPARC, KLF4, TGF-β2, glaucoma, trabecular meshwork

Primary open-angle glaucoma (POAG) is a leading cause of irreversible blindness worldwide.[1] Among persons of African American or Latino descent in the United States, POAG represents the leading cause of preventable blindness.[1–3] Progressive optic nerve damage and the resultant visual field loss in POAG are believed to be due to an elevated intraocular pressure (IOP) beyond the structural and/or vascular capabilities of the optic nerve to withstand. The elevated IOP of POAG is caused by impaired aqueous drainage through the trabecular meshwork (TM).[4]

Between 80% and 90% of aqueous outflow occurs through the TM ("conventional" pathway), with the remaining 10% to 20% exiting through the ciliary body face ("uveoscleral" pathway).[5] The juxtacanicular (JCT) region, which includes the inner wall of Schlemm's canal and underlying JCT TM, is the anatomic location of highest outflow resistance.[6,7] Outflow facility studies using human anterior segment perfusion systems with either glycosaminoglycan degradation enzymes, matrix metalloproteinases, or inhibition of glycosaminoglycan sulfation, strongly indicate that the balance between ECM synthesis and degradation regulates aqueous outflow.[8–10]

Matricellular proteins are nonstructural secreted glycoproteins that facilitate cellular control over their surrounding ECM.[11] Secreted protein acidic and rich in cysteine (SPARC) is the prototypical matricellular protein and has been associated with increased tissue fibrosis and aberrant tissue remodeling, processes that perturb ECM homeostasis and may have a role in glaucoma pathogenesis.[12] SPARC is expressed throughout the TM with prominent levels within the JCT region.[13] Quantitative proteomic studies have demonstrated that SPARC is the most highly upregulated protein in human TM cells following TGF-β2 treatment.[14] TGF-β2 is elevated up to 3-fold in the aqueous humor of patients with POAG.[15–18] In perfused cadaveric human anterior segments and live rats, TGF-β2 increases IOP and causes alterations of JCT ECM.[19,20] We have previously shown that SPARC-null mice have a 15% to 20% lower IOP, with more uniform outflow and decreased collagen fibril diameter compared with control wild-type mice.[21,22] We have further demonstrated that SPARC overexpression elevates IOP with a corresponding qualitative increase in the JCT ECM of perfused cadaveric human anterior segments.[23] Together, these data suggest that SPARC may function as an important node in the regulation of outflow facility and IOP, and have provided an impetus for the identification of novel pharmacological molecules and/or signaling pathways that modulate SPARC expression.

HMG-CoA (3-hydroxy-3-methyl-glutaryl-CoA) reductase inhibitors, or statins, are a widely prescribed class of cholesterol-lowering medications that have been shown to exert beneficial effects independent of their lipid-lowering properties, so-called

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"pleiotropic" effects.[24] Growing clinical data have suggested that statins may be protective against the development and progression of open-angle glaucoma in specific patient populations.[25–29] Studies using organ-cultured porcine anterior segments have demonstrated that statins increase aqueous outflow facility.[30] Statins have also been shown to suppress ECM synthesis in various nonocular tissues.[31–33] The Krüppel-like factor 4 (KLF4) transcription factor is induced by statins and functions as an important mediator of statin-related vasoprotection.[34,35] Recent work in other ocular and non-ocular tissues has identified SPARC as a downstream target of KLF4.[36,37] We hypothesized that statins may suppress SPARC expression in TM cells via the induction of KLF4.

Materials and Methods

Trabecular Meshwork Cell Culture

Primary human trabecular meshwork (TM) cells were isolated, in accordance with the tenets of the Declaration of Helsinki, and cultured as previously described.[38] Independent primary human TM cell lines were generated from donors ranging in age from 35 to 70 years and no known history of ocular disease. Cell cultures were maintained, unless otherwise stated, in Dulbecco's modified Eagle's medium (Life Technologies, Grand Island, NY) supplemented with 20% fetal bovine serum, 1% L-glutamine (2 mM), and gentamicin (0.1 mg/mL) at 37°C in a 10% CO_2 atmosphere. Trabecular meshwork cells from passage 4 to 5 cultures were used.

RNA Isolation and Real-Time Gene Expression Assays

Total RNA was isolated using the RNAqueous-4PCR kit (Life Technologies) as described by the manufacturer. Following isolation, RNA was treated with a commercial kit (RNase-free DNase I; Life Technologies) to remove DNA contaminants. RNA concentration was assessed via spectrophotometry. Total RNA (500 ng) was then reverse transcribed using the M-MLV reverse transcription kit (Promega, Madison, WI). Quantitative real-time PCR for SPARC (Hs00234160_m1), KLF4 (Hs00358836_m1), and internal control GAPDH (Hs02758991_g1) was performed using predesigned gene expression assays as described by the manufacturer's instructions (TaqMan; Life Technologies). Levels of SPARC and KLF4 were normalized to GAPDH using the formula $2^{-\Delta Ct}$. The relative fold change in expression between control and treatment groups was then assessed using the $2^{-\Delta\Delta Ct}$ method.

Lovastatin Time Course Experiments

Trabecular meshwork endothelial cells at 90% to 100% confluency (equal between control and treatment groups) were cultured in serum-free media for 19 hours, and subsequently incubated for 24, 48, or 72 hours in serum-free media containing 10 μM activated lovastatin (Calbiochem #438186, San Diego, CA) or vehicle (dimethyl sulfoxide 1% vol/vol). Cell culture media containing lovastatin or vehicle was replaced every 24 hours for the 48- and 72-hour time points. For isoprenoid supplementation experiments, primary human TM cells were treated with 10 μM lovastatin or DMSO vehicle with concurrent addition of either 10 μM farnesyl pyrophosphate (FPP), 10 μM geranylgeranyl pyrophosphate (GGPP), or vehicle for 72 hours (Sigma-Aldrich, St. Louis, MO).

Adenoviral-Mediated Infection Experiments

Primary human TM endothelial cells at 70% to 90% confluency were infected in 2% FBS media with either Ad-KLF4 (multiplicity of infection [MOI] = 50; SignaGen Laboratories, Rockville, MD) or Ad-Null control virus (MOI = 50; SignaGen Laboratories). Multiplicity of infection is defined as the ratio of infectious viral units to cells.[39] After 18 hours, an equal volume of 10% FBS media was added to each well and cells were incubated for an additional 24 hours. Cells were then washed once with PBS and incubated for 24 hours in serum-free media containing either 3 ng/mL activated, recombinant human TGF-β2 (R&D Systems, Minneapolis, MN), or 4 mM HCl solution containing 0.1% human serum albumin as the vehicle.

TGF-β2 Stimulatory Experiments

Trabecular meshwork cells at 90% to 100% confluency (equal between control and treatment groups) were cultured in serum-free media for 19 hours, and subsequently incubated for 24 hours in serum-free media containing either 10 μM lovastatin or vehicle (dimethyl sulfoxide 1% vol/vol). Cells were then treated for an additional 24 hours with either 3 ng/mL activated, recombinant human TGF-β2 or 4 mM HCl solution containing 0.1% human serum albumin as vehicle.

Immunoblot Analysis

For SPARC detection, conditioned media from TM cell cultures was harvested and centrifuged at 2300*g* for 10 minutes at 4°C. The supernatant was then concentrated (Amicon Ultra-4 Filter Unit, 10 kDa; Millipore Corp., Milford, MA), and protein content quantified (DC Protein Assay; Bio-Rad Laboratories, Inc., Hercules, CA). For KLF4 protein detection, TM cells were lysed for 3 minutes on ice with cold 1× RIPA buffer containing 0.5% aprotinin, 0.1% EDTA, 1% EGTA, 0.5% phenylmethanesulfonyl fluoride, and 0.01% Leupeptin. Samples were then centrifuged at 18000*g* for 15 minutes at 4°C and supernatant protein content was isolated and quantified. In all experiments, equal amounts of protein were treated with 6× reducing buffer and boiled for 5 minutes. Samples were then electrophoresed in 10% SDS-polyacrylamide gels, and proteins were transferred to nitrocellulose membranes (0.45-μm pore size; Invitrogen, Carlsbad, CA). Membranes were blocked for 1 hour at room temperature in a 1:1 mixture of 1× TBS-T (20 mM Tris-HCl [pH 7.6], 137 mM NaCl, 0.1% Tween-20) and blocking buffer (Rockland, Inc., Gilbertsville, PA), followed by overnight incubation at 4°C with the indicated primary antibody at 1:10,000 for SPARC (Hematologic Technologies, Inc., Essex Junction, VT); 1:1000 for KLF4 (Cell Signaling Technology, Inc., Danvers, MA); and 1:1000 for GAPDH (Trevigen, Inc., Gaithersburg, MD). Following overnight incubation with the primary antibody, the membranes were washed three times with 1× TBS-T and incubated for 1 hour at room temperature with dye-conjugated affinity purified 680 anti-mouse or 800 anti-rabbit IgG antibodies, respectively (IRDye; 1:10,000 dilution; Rockland, Inc.). The membranes were then washed three times with 1× TBS-T and scanned, and integrated band intensities were calculated using an infrared imaging system (Odyssey; LI-COR Biosciences, Lincoln, NE). For evaluation of KLF4 expression in normal human TM tissue, samples were isolated from donors aged 42 and 47 years and processed as above, with β-actin loading control at 1:1000 dilution (Cell Signaling Technology, Inc.).

Immunofluorescence

Human donor eyes (aged 44 and 69 years) were immersion-fixed in 10% neutral buffered formalin, sequentially dehydrated in ethanol solutions (75%, 85%, 95%, 100%), and embedded in paraffin. Six micron sections were mounted on poly-L-lysine-coated glass slides and baked at 60°C for 2 hours. Slides were

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deparaffinized in xylene, sequentially rehydrated in ethanol solutions, and washed three times for 10 minutes in phosphate-buffered saline with 0.1% Tween-20 (PBS-T). Following 1 hour incubation in 10% goat serum, tissues were permeabilized for 5 minutes in 0.2% Triton-100 in 1× PBS and again washed three times in PBS-T. The sections were then incubated at 4°C overnight either in primary KLF4 monoclonal antibody (Thermo Scientific, Billerica, MA) diluted 1:200 in PBS or in PBS alone. Slides were washed three times in PBS-T prior to incubation in 1:200 goat anti-mouse 594 AlexaFluor secondary IgG (Invitrogen) and then washed three more times following incubation. Nuclei were stained in DAPI antifade reagent (SlowFade Gold; Invitrogen). Labeled tissues were imaged and analyzed by fluorescent light microscopy using a Zeiss Observer3.1.

Statistics

Statistical analysis was performed using graphing and statistics software (GraphPad Prism 6; GraphPad, La Jolla, CA). A two-tailed Student's *t*-test was used for comparing differences between two groups. Comparisons between multiple groups were made using one-way ANOVA followed by Tukey's HSD post hoc test for multiple comparisons. Differences were considered statistically significant for $P < 0.05$.

Results

Lovastatin Suppresses SPARC Expression in a Time-Dependent Manner

To determine whether statins regulate SPARC expression in human TM cells, SPARC mRNA and protein levels were analyzed after 24, 48, and 72 hours of incubation with lovastatin. Real-time quantitative PCR analysis of SPARC mRNA levels revealed a statistically significant reduction at 24 and 72 hours (Fig. 1A). Immunoblot analysis of the TM-conditioned media demonstrated significant decreases in SPARC protein levels at 48 and 72 hours (Fig. 1B). Cells treated with lovastatin appeared elongated by 24 hours and remained so through 72 hours (data not shown), which is consistent with changes to TM cellular morphology observed and well-documented in previous reports.[30,40]

GGPP Supplementation Reverses Lovastatin-Induced SPARC Suppression

In addition to reducing cholesterol biosynthesis, lovastatin has also been shown to suppress the synthesis of intermediaries such as the isoprenoids FPP and GGPP.[41,42] Isoprenoid supplementation has been shown to reverse lovastatin-induced cytoskeletal changes in cultured porcine and human TM cells, suggesting their involvement in regulating downstream RhoA GTPase control over cellular tone and ECM deposition.[30,40,43] We examined whether lovastatin-induced SPARC suppression could be reversed with concurrent addition of either 10 μM FPP or GGPP for 72 hours. Immunoblot analysis of the TM-conditioned media demonstrated full recovery in SPARC protein levels at 72 hours when GGPP, but not FPP, had been supplemented in the media (Fig. 2).

Lovastatin Inhibits SPARC Induction Under TGF-β2 Stimulatory Conditions

TGF-β2 is an important regulator of ECM synthesis and deposition in the TM.[19,20,44,45] In TM cells, SPARC is the most highly upregulated protein by TGF-β2.[14] We next investigated whether lovastatin could suppress SPARC induction under TGF-β2 stimulatory conditions. Analysis of SPARC mRNA and protein levels revealed a significant suppression in TGF-β2–mediated induction of SPARC (Figs. 3A, 3B).

Evaluation of KLF4 Expression in Human TM

SPARC has been identified as an important downstream target of KLF4 in ocular and nonocular tissues.[36,37] KLF4 expression in the human trabecular meshwork is unknown. To characterize its expression, immunoblot analysis and immunofluorescent staining of KLF4 in normal cadaveric human TM tissue was performed. Immunoblot analysis of human TM tissue demonstrated KLF4 expression (Fig. 4A). Immunofluorescent staining identified KLF4 expression throughout the TM as well as the inner and outer walls of Schlemm's canal (Fig. 4B).

Lovastatin Treatment Upregulates KLF4 Expression

To determine whether lovastatin has an effect on KLF4 expression in human TM cells, mRNA and protein levels were quantified 24, 48, and 72 hours after initiation of lovastatin treatment. KLF4 mRNA and protein levels were significantly increased at each time point measured (Figs. 5A, 5B).

Effect of TGF-β2 Stimulation on KLF4 Expression

We next assessed whether TGF-β2 stimulation affects KLF4 expression under basal and lovastatin conditions. KLF4 mRNA levels were not significantly affected by TGF-β2 under both basal and lovastatin conditions (Fig. 6A). While TGF-β2 did not affect KLF4 protein levels under lovastatin conditions, a small, statistically significant 2.4-fold increase in KLF4 protein expression was seen under basal conditions (Fig. 6B).

Overexpression of KLF4 Suppresses SPARC Under Basal and TGF-β2 Stimulatory Conditions

To assess the effect of KLF4 on SPARC expression, human TM cells were infected with control adenovirus or virus expressing human KLF4. Overexpression of KLF4 led to an increase in SPARC mRNA levels under basal conditions but decrease under TGF-β2 stimulation (Fig. 7A). By contrast, overexpression of KLF4 significantly suppressed SPARC protein levels under both basal and TGF-β2 stimulatory conditions (Fig. 7B).

Discussion

Growing clinical evidence has suggested a protective role for statins in the development and progression of open-angle glaucoma in specific patient populations.[25–29] Studies of organ cultured porcine eye anterior segments have shown that statins increase aqueous outflow facility.[30] Matricellular proteins, and in particular SPARC, have emerged as important modulators of outflow facility and IOP.[21–23] Here, we identify lovastatin as a pharmacologic suppressor of SPARC expression in TM cells and provide evidence suggesting that these effects may be mediated via the upregulation of transcription factor KLF4 (Fig. 8).

In the present study, we demonstrated that lovastatin suppressed SPARC levels in a time-dependent pattern in cultured TM cells. The reversibility of lovastatin-induced SPARC suppression upon supplementation with equimolar GGPP—but not FPP—suggests that GGPP is an important intermediary in this process. Additionally, the pretreatment of cells with lovastatin significantly inhibited TGF-β2–mediated

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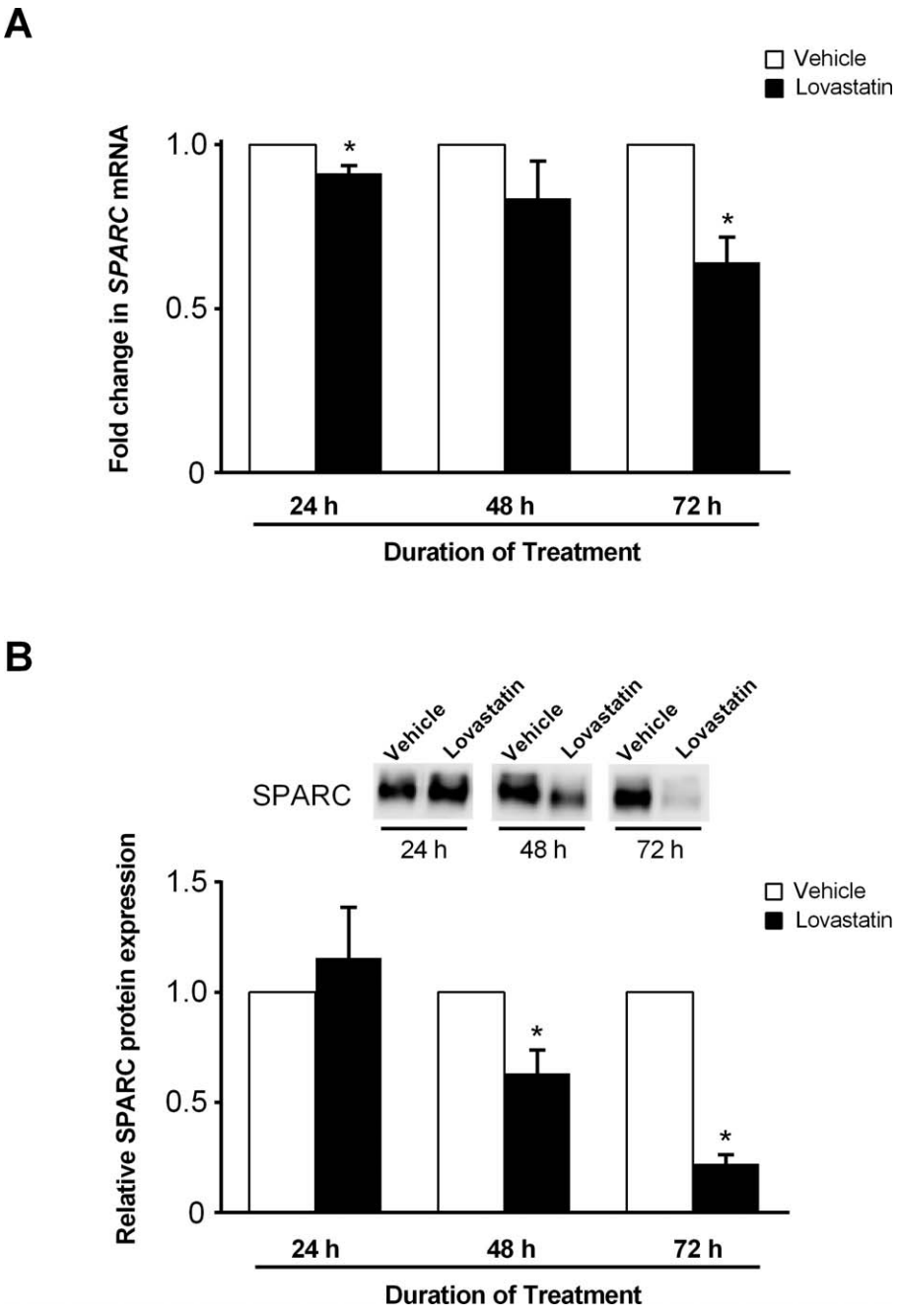

Figure 1. Lovastatin suppresses SPARC expression. (**A**) *SPARC* mRNA levels ($n = 4$). (**B**) Representative immunoblot and densitometric analysis of SPARC protein levels ($n = 8$ for 24 hours, $n = 5$ for 48 hours and 72 hours) from conditioned media of primary human TM cells cultured for 24, 48, and 72 hours with either 10 μM lovastatin or DMSO vehicle. All data are expressed as the mean ± SEM (*$P < 0.05$ versus its time-corresponding vehicle control; n refers to the number of independent experiments performed using n different primary human TM cell strains).

induction of SPARC. Transforming growth factor-β2 has been implicated in the pathogenesis of POAG, with multiple studies demonstrating elevated levels of TGF-β2 in the aqueous humor of POAG patients.[15–18] Importantly, TGF-β2 has been shown to increase ECM accumulation in the TM and aqueous outflow resistance.[19,20,44,46] Current work from our laboratory strongly suggests that SPARC is critical for TGF-β2–mediated ECM accumulation and ocular hypertension in mice (Swaminathan SS, Oh D-J, Kang MH, Shepard AR, Pang I-H, unpublished observations, 2013). Pharmacological modulation of SPARC expression may serve as an important therapeutic avenue for the reestablishment of a functional aqueous drainage system through the TM.

In other ocular and nonocular tissues, the transcription factor KLF4 regulates SPARC expression.[36,37] Immunofluorescent analysis of KLF4 in cadaveric human anterior segments revealed expression throughout the TM as well as inner and outer walls of Schlemm's canal, suggesting that KLF4 is biologically relevant to transcriptional signaling in the conventional outflow pathway. Incubation of TM cells with lovastatin resulted in a significant induction in KLF4 mRNA and protein expression. The mechanisms mediating KLF4 upregulation by

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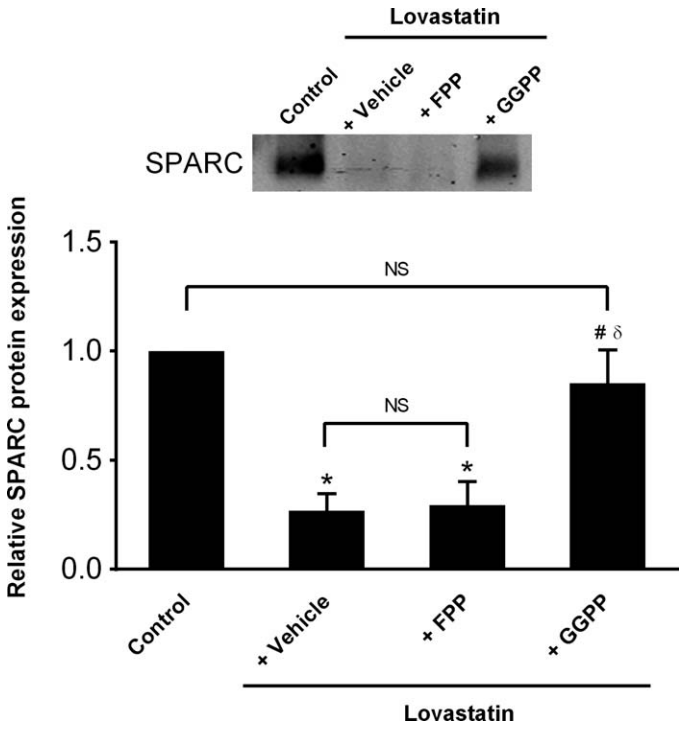

FIGURE 2. Geranylgeranyl pyrophosphate, but not FPP, supplementation reverses lovastatin-induced suppression of SPARC protein levels. SPARC levels ($n = 6$) were assessed in the conditioned media of primary human TM cells incubated for 72 hours with 10 μM lovastatin or DMSO vehicle with concurrent addition of either 10 μM FPP, 10 μM GGPP, or vehicle. All data are expressed as the mean ± SEM (*$P < 0.05$ versus control; #$P < 0.05$ versus lovastatin + vehicle; δ$P < 0.05$ versus lovastatin + FPP; n refers to the number of independent experiments performed using n different primary human TM cell strains).

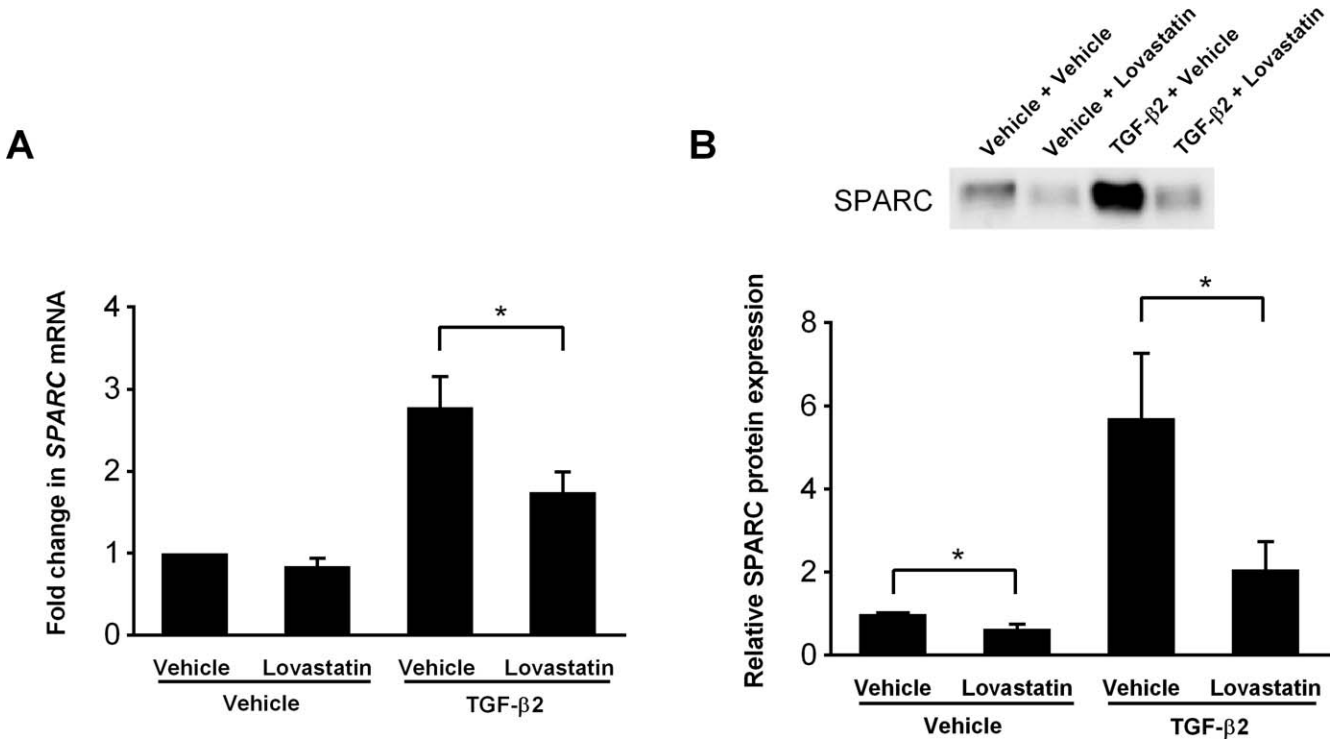

FIGURE 3. Lovastatin inhibits TGF-β2 induction of SPARC. (**A**) *SPARC* mRNA expression ($n = 5$). (**B**) Representative immunoblot and densitometric analysis of SPARC protein levels ($n = 6$) from conditioned media of primary human TM cells pretreated for 24 hours with 10 μM lovastatin or DMSO vehicle followed by 24-hour stimulation with either 3 ng/mL activated, recombinant human TGF-β2, or HCl vehicle. All data are expressed as the mean ± SEM (*$P < 0.05$; n refers to the number of independent experiments performed using n different primary human TM cell strains).

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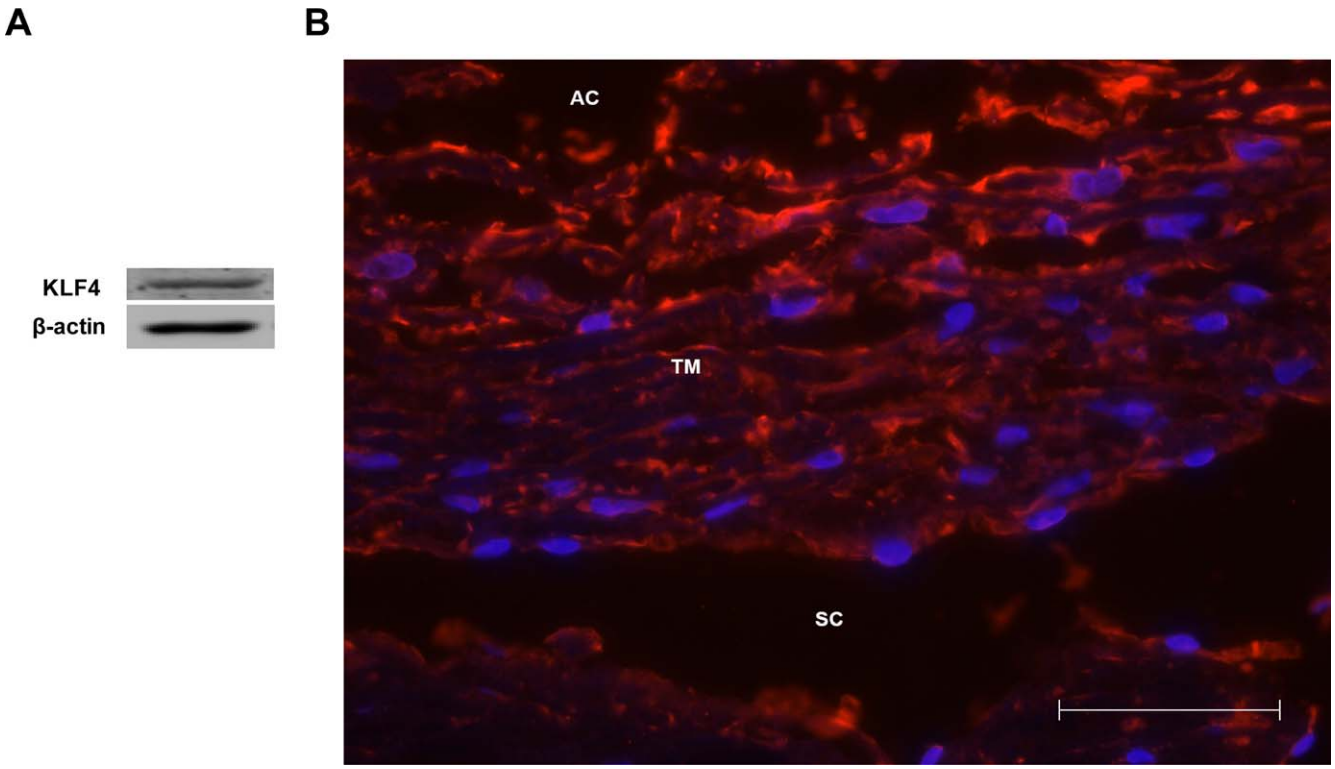

FIGURE 4. KLF4 expression in human TM. (**A**) Representative immunoblot showing detection of KLF4 in normal cadaveric human TM tissue from a 42-year-old donor. (**B**) Immunolocalization of KLF4 in human trabecular meshwork from an 84-year-old donor. Representative images demonstrate KLF4 expression (*red*) throughout the TM as well as inner and outer walls of Schlemm's canal. Cell nuclei stained with DAPI (*blue*). AC, anterior chamber; SC, Schlemm's canal. *Scale bar*: 50 μm.

lovastatin in TM cells remain unknown. However, in vascular endothelial cells, the MEK5/ERK5/MEF2 pathway is an important regulator of KLF4 induction.[34,35] Further clarification of the role of this signaling pathway in TM cells is needed.

Overexpression of KLF4 reduced SPARC protein levels under basal and TGF-β2 stimulatory conditions, suggesting that KLF4 may be an attractive target for therapeutic modulation of SPARC. Interestingly, under basal conditions, KLF4 overexpression upregulated SPARC mRNA levels by 1.3-fold, while suppressing SPARC protein levels by 3.3-fold. These results suggest that posttranscriptional mechanisms may play an important role in KLF4-mediated regulation of SPARC. Micro-

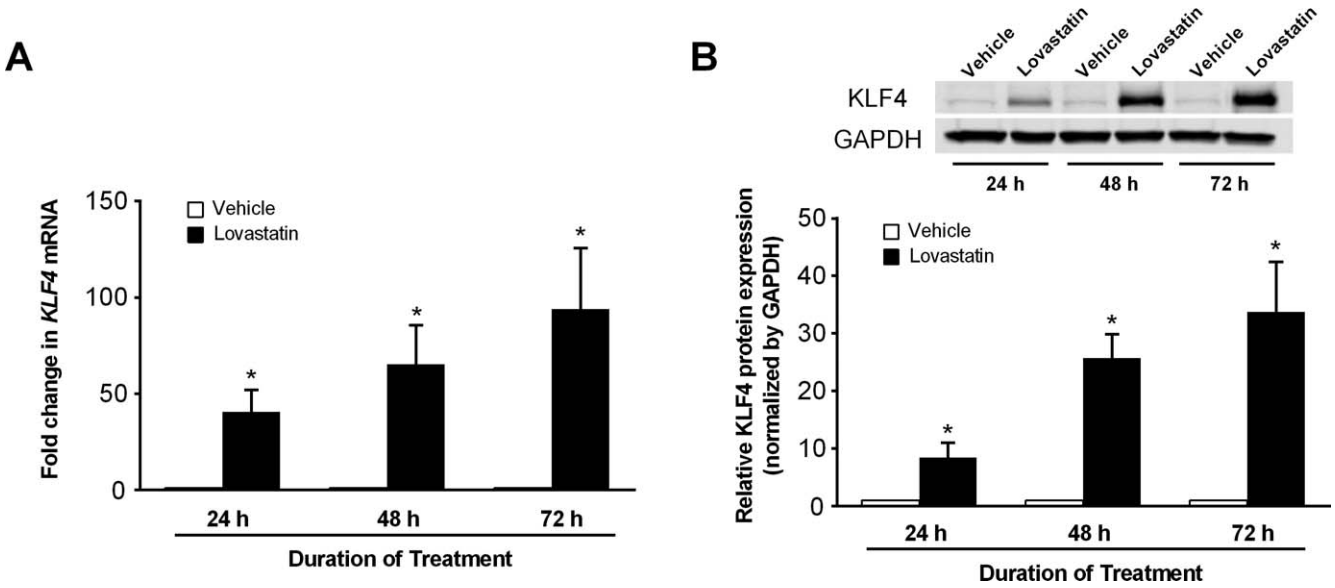

FIGURE 5. Lovastatin induces expression of KLF4. (**A**) *KLF4* mRNA levels ($n = 5$). (**B**) Representative immunoblot and densitometric analysis of KLF4 protein expression ($n = 4$) from cell lysates of primary human TM cells incubated for 24, 48, and 72 hours with either 10 μM lovastatin or DMSO vehicle. All data are expressed as the mean ± SEM (*$P < 0.05$ versus its time-corresponding vehicle control; n refers to the number of independent experiments performed using n different primary human TM cell strains).

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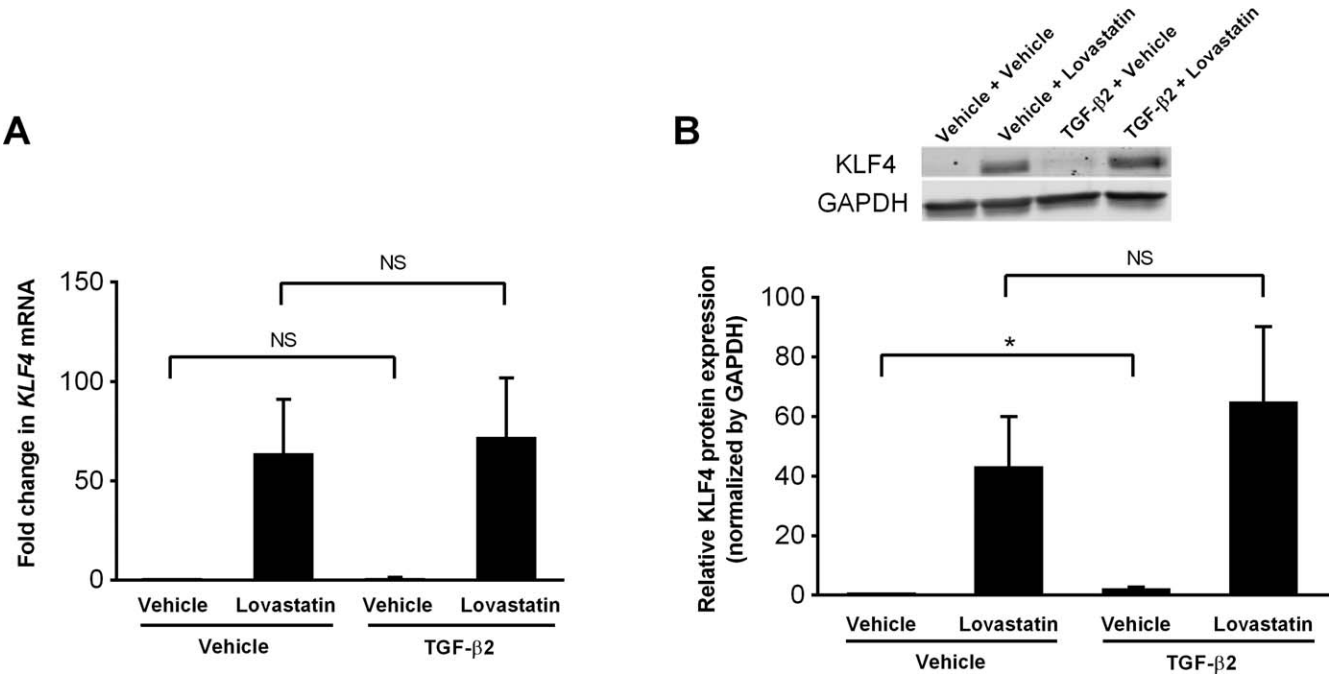

Figure 6. Effect of TGF-β2 stimulation on KLF4 expression. (**A**) *KLF4* mRNA levels ($n = 6$). (**B**) Representative immunoblot and densitometric analysis of KLF4 protein levels ($n = 4$) from cell lysates of primary human TM cells pretreated for 24 hours with 10 μM lovastatin or DMSO vehicle followed by 24-hour stimulation with either 3 ng/mL activated, recombinant human TGF-β2, or HCl vehicle. All data are expressed as the mean ± SEM (*$P < 0.01$; n refers to the number of independent experiments performed using n different primary human TM cell strains).

RNAs are small, single-stranded noncoding RNAs that modulate posttranscriptional gene expression. KLF4 has been shown to regulate the expression of various microRNAs.[47–49] Further investigation is necessary to assess whether microRNA modulation by KLF4 functions as an important posttranscriptional regulatory mechanism for SPARC expression. Under TGF-β2 stimulatory conditions, KLF4 overexpression suppressed both SPARC mRNA and protein levels. Activation of the SMAD3 transcription factor is critical for TGF-β2–mediated induction of SPARC.[50] In nonocular tissues, KLF4 can directly interact with SMAD3 and inhibit its ability to bind target gene promoters.[51] Additional studies would be necessary to

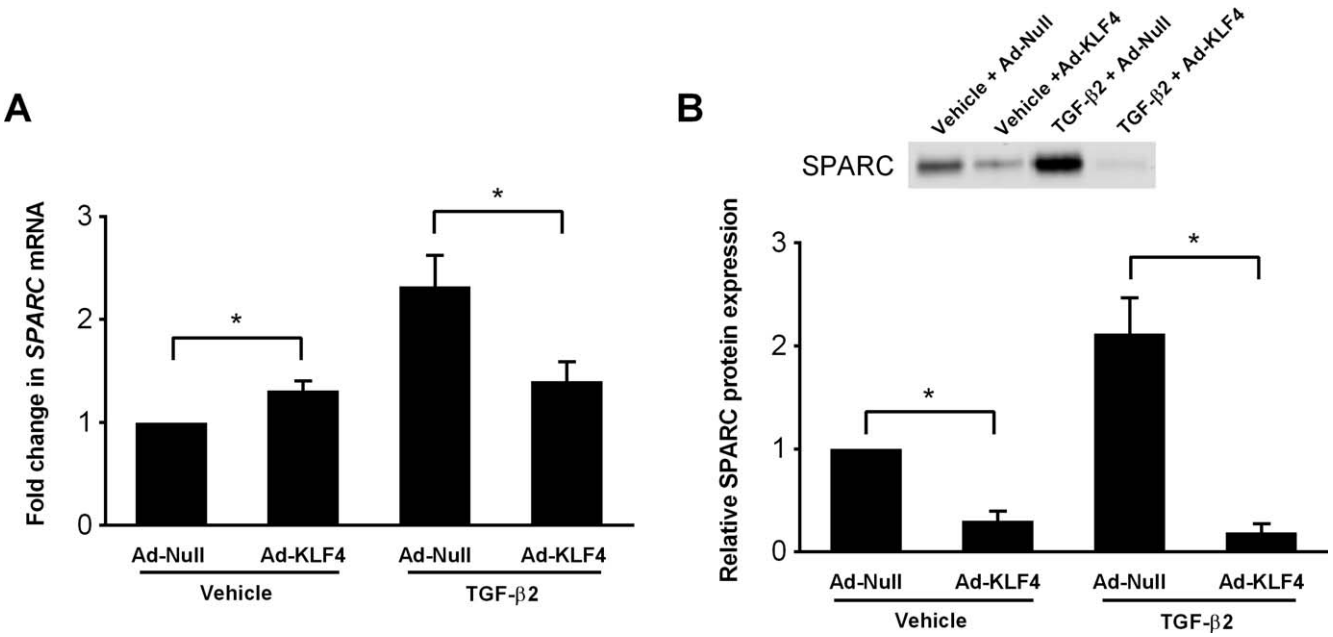

Figure 7. Overexpression of KLF4 suppresses SPARC levels under basal and TGF-β2 stimulatory conditions. (**A**) *SPARC* mRNA expression ($n = 4$). (**B**) Representative immunoblot and densitometric analysis of SPARC protein levels ($n = 4$) from conditioned media of primary human TM cells infected with either Ad-Null or Ad-KLF4 adenovirus followed by 24-hour stimulation with either 3 ng/mL activated, recombinant human TGF-β2 or HCl vehicle. All data are expressed as the mean ± SEM (*$P < 0.05$; n refers to the number of independent experiments performed using n different primary human TM cell strains).

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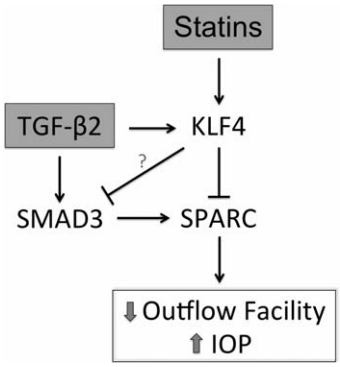

Figure 8. Working model for the regulation of SPARC by lovastatin.

determine whether KLF4 binds and inhibits SMAD3 activity in TM cells.

Rho GTPase functions as an important mediator of ECM induction by TGF-β2.[52] In TM cells, inhibition of Rho GTPase suppresses TGF-β2-mediated ECM production.[52] Statins have been shown to indirectly inhibit Rho GTPase signaling via the prevention of isoprenoid intermediate synthesis.[53,54] In non-ocular tissues, RhoA inhibits KLF4 DNA binding.[55] Thus, indirect inhibition of RhoA by statins may allow for enhanced DNA binding of KLF4 and transcriptional suppression of SPARC. It is possible that Rho signaling may have a role in KLF4 regulation of SPARC in TM cells.

Collectively, the results presented here identify lovastatin as a novel pharmacological modulator of SPARC expression and provide further insight into the molecular mechanisms mediating the effects of statins on outflow facility.

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