Increased RhoA and RhoB Protein Accumulation in Cultured Human Trabecular Meshwork Cells by Lovastatin

Cynthia L. Von Zee,1,2 Michael P. Richards,1 Ping Bu,5 Jay I. Perlman,3,4,5 and Evan B. Stubbs, Jr2,5,6

PURPOSE. This study aimed to determine the effect of lovastatin on Rho G-protein expression and activation in human trabecular meshwork (TM) cells.

METHODS. Confluent cultures of low-passage (primary) or transformed (GT3M) human TM cells were incubated overnight with vehicle (0.01% ethanol) or activated lovastatin (10 μM). Changes in Rho mRNA, protein content, and activation were quantified by qRT-PCR, immunoblotting, and ELISA, respectively. Factin organization was determined using Alexa Fluor 488–conjugated phalloidin.

RESULTS. Low-passage or transformed TM cells treated with lovastatin exhibited marked increases in RhoA and RhoB mRNA and protein content. Actinomycin D prevented lovastatin-dependent increases in RhoB, but not RhoA, protein accumulation. In contrast, cycloheximide prevented lovastatin from increasing both RhoA and RhoB. Supplementation with mevalonate or geranylgeranyl pyrophosphate prevented, whereas inhibition of geranylgeranyl transferase mimicked, the effects of lovastatin on RhoA and RhoB accumulation. The effect of lovastatin was dose dependent, with newly synthesized protein accumulating in the cytosol. The amount of functionally active (GTP-bound) RhoA in cell lysates was significantly reduced by lovastatin. Lovastatin altered the morphology of TM cells by disrupting F-actin organization.

CONCLUSIONS. Lovastatin enhances the accumulation of RhoA and RhoB in human TM cells, in part, by limiting geranylgeranyl isoprenylation of these G-proteins. We propose that post-translational geranylgeranyl transferase regulates a receptor of both RhoA and RhoB protein expression and processing in human TM cells. Increased accumulation of unprenylated forms of RhoA and RhoB may disrupt Rho-dependent regulation of TM cell cytoskeletal organization.

S tatins are a group of potent 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitors that are widely used clinically as cholesterol-lowering agents.1 Recently, statins have been reported to have beneficial effects that extend beyond their ability to reduce serum lipids. As a result, statins are now being aggressively evaluated as therapeutic agents for the management of a wide variety of disorders,2–4 including those affecting vision.5 Long-term statin therapy in patients with cardiovascular problems has been associated with a lower incidence and slowed progression of primary open angle glaucoma.6 Nagaoka et al.7 reported that systemic administration of simvastatin lowers intraocular pressure (IOP) in humans, whereas Song et al.8 found an increase in aqueous humor (AH) outflow in vitro after lovastatin treatment. Our own preliminary studies support a protective role of simvastatin during experimental ischemic-reperfusion retinal injury (Bu P, et al. IOVS 2007;48:ARVO E-Abstract 208). The mechanism(s) by which statins increase AH outflow, reduce IOP, and protect against retinal injury remain unclear.

HMG-CoA reductase is the rate-limiting enzyme in the mevalonate-cholesterol biosynthetic pathway.9,10 In addition to biosynthesizing cholesterol, the mevalonate pathway produces 15-carbon farnesyl (F) and 20-carbon geranylgeranyl (GG) pyrophosphate (PP) intermediates required for post-translational membrane targeting and functional activation of regulatory G-proteins, including Ras and Rho.11,12 Depletion of mevalonate by HMG-CoA reductase inhibition markedly limits the availability of FPP and GGPP isoprenoid intermediates.13 This limitation reduces farnesyl- and geranylgeranyl-transferase catalyzed isoprenylation of G-proteins.14,15 As a consequence, unprenylated forms of G-proteins have a reduced ability to associate with target membranes. Importantly, post-translationally immature forms of G-proteins may maintain partial function16,17 and interfere with the activity of mature membrane-anchored proteins.

Disruption of Rho G-protein mediated signaling events in trabecular meshwork (TM) cells may be of central importance in statin-dependent regulation of AH outflow. Rho G-proteins are a well-characterized family (RhoA-C, Rac1-3, and Cdc42) of small monomeric GTP binding proteins implicated in regulating a variety of TM cellular functions, including actin cytoskeletal organization and cell contractility.18 Inhibition of Rho with C3 exoenzyme, or expression of a dominant-negative mutant RhoA GTPase, in human eye anterior segments has been shown to increase AH outflow.19,20 The interaction of RhoA with Rho kinase effectors (ROCK1 [ROKβ, p160ROCK] or ROCK2 [ROKα, Rho Kinase]) is believed to regulate actin polymerization,21–23 making it a putative pharmacologic target for the regulation of AH outflow.24 Both ROCK isoforms are expressed in human TM cells.25,26 Selective inhibitors of Rho kinases are currently being evaluated as novel therapeutic agents for the management of ocular hypertensive disorders.27

Given the importance of the signaling pathways governed by Rho proteins in TM cells and their role in regulating AH outflow, we investigated in this study the consequence of
disrupting Rho isoprenylation on human TM cell function. Inhibition of HMG-CoA reductase with lovastatin elicits a marked and selective increase in Rhoa and RhoB mRNA and G-protein content in human TM cells, in part, by limiting geranylgeranyl isoprenylation of these G-proteins (Von Zee CL, et al. IOVS 2008;49:ARVO E-Abstract:1628). We propose that post-translational geranylgeranylation facilitates RhoA and RhoB protein expression and processing in TM cells. We speculate that increased accumulation of unprenylated inactive (GDP-bound) forms of RhoA and RhoB may disrupt Rho-dependent regulation of TM cell cytoskeletal organization.

**Materials and Methods**

**Preparation and Culture of Human TM Cells**

The use of human cadaver material in this study was approved by the Edward Hines, Jr. VA and Loyola University (Chicago, IL) institutional review boards. Fresh cadaver corneoscleral rims were obtained (Illinois Eye Bank, Chicago, IL) at time of corneal transplant, and low-passage (primary) human TM cells were prepared using a collagenase-free procedure, as previously described. TM tissue harvested from four separate donors were used to establish four separate low-passage TM cell lines. Individual TM cell lines were restricted to one to four passages. The purity of low-passage TM cell cultures typically exceeded 95%, as routinely determined by cell morphology (adherent flattened non-spindle shaped soma with prominent lamellipodia). Low-passage TM cell cultures exhibiting more than 5% spindle-shaped morphologic characteristics were considered contaminated with fibroblasts and discarded. Simian virus 40 (SV40)-transformed human TM cells from a male glaucomatous patient (GTM3) were a generous gift from A. F. Clark (Alcon Laboratories, Ft. Worth, TX). Human low-passage TM cells were established on cell culture flasks (Falcon Primaria; BD Biosciences, San Jose, CA) in Eagle’s minimal essential medium containing 10% FCS, 1% sodium bicarbonate, 292 mmol/L sodium lactate, and 110 mmol/L sodium bicarbonate and supplemented with a commercial cocktail of protease inhibitors (Roche Applied Science, Indianapolis, IN). Particulate (crude membranes) and soluble (cytosolic) subcellular fractions were prepared as previously described. TM cell lysates were centrifuged at 100,000 g for 30 minutes using an ultracentrifuge (Beckman Coulter, Fullerton, CA). The supernatant (soluble cytosolic fraction) was collected and stored at −80°C until use. Protein concentrations in cell lysates (typically 7 to 8 mg/mL) and prepared subcellular fractions were measured by the BCA method (Pierce Biotechnology, Rockford, IL) using bovine serum albumin as the standard.

**Immunoblot Analysis**

Proteins (20 μg per lane) in cell lysates or subcellular fractions were resolved by SDS-PAGE and transferred onto nitrocellulose membranes as previously described. Washed membranes were blocked and incubated overnight at 4°C in the presence of a 1:10,000 dilution of mouse anti-Rho (A,B,C) monoclonal (clone 55; Millipore, Temecula, CA). To determine which Rho isoforms were most affected by treatment, immunoblots were stripped and re-stained with Rho isoform-selective antibodies (1:200 dilution of mouse anti-RhoA monoclonal [clone 26C4] or rabbit anti-RhoB polyclonal [119]) from Santa Cruz Biotechnology (Santa Cruz, CA). Immunoblots were similarly stripped and stained with a 1:10,000 dilution of rabbit anti-GAPDH polyclonal (Trevesgen, Gaithersburg, MD) primary antibody as a protein loading control. Washed membranes were incubated using a 1:10,000 dilution of goat anti-rabbit IgG secondary antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), respectively. Immunostained proteins were visualized by ECL. Relative changes in pan-Rho, RhoA, RhoB, or GAPDH total protein content was determined by densitometry.

**Rho Activation Assay**

The levels of active GTP-bound RhoA protein in lysates prepared from lovastatin-treated cells was determined by ELISA using a commercially available G-LISA activation assay kit (BK124; Cytoskeleton Inc., Denver, CO). Briefly, cell lysates (150 μg protein) were incubated for 30 minutes at 2°C in microtiter wells pre-coated with an RBD domain of RhoA primary antibody as a protein loading control. Washed membranes were incubated using a 1:10,000 dilution of goat anti-mouse IgG secondary antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), respectively. Immunostained proteins were visualized by ECL. Relative changes in pan-Rho, RhoA, RhoB, or GAPDH total protein content was quantified by densitometry.

**Real Time RT-PCR**

Total RNA was extracted from low-passage or transformed human TM cells using reagent (TRizol; Invitrogen) and 5 μg was reverse-transcribed (Super Script III First Strand Synthesis system; Invitrogen). RhoA, RhoB, and GAPDH-specific cDNA sequences were amplified by real-time (IQ SYBR Green Supermix; Bio-Rad, Hercules, CA) quantitative RT-PCR using a PCR detection system (Mini-Opticon; Bio-Rad). The following human-specific primer pairs were used: RhoA (forward, 5'-TGGAAAATCTATGTGGCGGATATCG; reverse, 5'-TCTGCTTCCTTCAG-GTTAACCGG) and RhoB (forward, 5'-AGGACTCAAGTGTTATAGGCC; reverse, 5'-CAGCCATTCTGGGATATCG) and GAPDH (forward, 5'-GGTTTAACCGG; reverse, 5'-TGAAAACTATGTGGCGGATATCG; reverse, 5'-GCC; reverse, 5'-CAGCCATTCTGGGATATCG).
Alteration of Rho G–Protein Subcellular Localization and Functional Activation by Lovastatin

In resting GTM3 cells, Rho was found to be predominantly associated within cell membranes with little expressed in cell cytosol (Fig. 3). Lovastatin elicited a dose-dependent decrease in membrane-associated Rho proteins, with a disproportionate accumulation of both RhoA and RhoB in the cell cytosol (Fig. 3). Notable increases in soluble RhoA were observed beginning at ~100 nM lovastatin with a half-maximal effectiveness of ~400 nM. By comparison, higher concentrations of lovastatin (>1 μM) were needed to alter soluble RhoB content in these cells. At 1 μM lovastatin, a lightly immunostained RhoB doublet was appreciated, the significance of which remains unclear. Membrane-associated RhoB G-proteins were not detectable in vehicle-treated cells.

By altering the subcellular localization of Rho, lovastatin may influence the amount of functionally active (GTP-bound) Rho G–proteins present in GTM3 cells. Measurable quantities of GTP-bound RhoA were present in vehicle-treated low-passage human TM cells cultured in the presence of protease inhibitors. Proteins in cell lysates (20 μg/lane) were resolved on 4–20% SDS-polyacrylamide gels, transferred to nitrocellulose membranes, and immunostained for the Rho subfamily of G-proteins. (A) Western immunoblot of pan-Rho, RhoA, and RhoB protein expressed in cell lysates from a single experiment performed in triplicate. Levels of GAPDH, a housekeeping enzyme, are shown for comparison as loading controls. (B) Quantitative densitometric comparison of the results shown in (A) normalized to GAPDH and expressed as the mean ± SD. Vehicle (open bar), lovastatin (hashed bar), lovastatin + mevalonate (solid bar), mevalonate (gray bar). *P < 0.01 (one-way ANOVA with Dunnett’s post hoc analysis).
levels of RhoA-specific and RhoB-specific mRNA expressed in GTM3 cells were increased twofold and 48-fold, respectively, by lovastatin (Table 1). Co-incubation with mevalonate (5 mM) prevented lovastatin-dependent changes in RhoA or RhoB mRNA expression (Table 1). Lovastatin treatment of low-passage human TM cells elicited similar increases in both RhoA (twofold) and RhoB (sevenfold) mRNA content (Table 1).

Effect of Lovastatin on Transcriptional and Translational Regulation of Rho G–Protein Expression

Lovastatin increases in RhoA content in GTM3 cells were not affected by pretreatment (0.5 μg/mL, 1 hour) with actinomycin D (Fig. 5), an inhibitor of gene transcription. In contrast, actinomycin D ablated lovastatin-dependent increases in RhoB expression (Fig. 5). The constitutive expression of GAPDH was unaltered by actinomycin D. Inhibiting de novo protein syn-

thesis with cycloheximide (1.4 μg/mL, 1 hour pretreatment) blocked lovastatin-dependent increases in both RhoA and RhoB protein expression (Fig. 6).

Increase of Rho Content by Inhibiting Protein Geranylgeranylation with Lovastatin

Supplementing GTM3 cell cultures with geranylgeranyl pyrophosphate, but not farnesyl pyrophosphate, prevented lovastatin increases in RhoA and RhoB content (Fig. 7). Lovastatin-dependent increases in RhoA and RhoB content in low-passage human TM cells were similarly prevented by GGPP supplementation (Fig. 8).

The mechanism by which geranylgeranyl isoprenoid supplementation affects lovastatin-dependent Rho G–protein expression was determined by treating GTM3 cell cultures with specific inhibitors of farnesyl transferase (FTI-277) or geranylgeranyl transferase-I (GGTI-298). The content of RhoA and RhoB in FTI-277 treated GTM3 cells was similar to vehicle-treated controls (Fig. 9). In contrast, GTM3 cells treated with GGTI-298 exhibited a significant increase in RhoA and RhoB content, mimicking the effect of lovastatin. Confluent cultures of low-passage human TM cells treated with GGTI-298 similarly exhibited increases in RhoA and RhoB content (Fig. 10). The effect of lovastatin on the levels of FPP or GGPP in low-passage or transformed human TM cells was not determined.

Functional Disruption of Filamentous Actin Organization by Lovastatin

Transformed GTM3 cells grown to confluency exhibit prominent phallolidin-positive stress fiber organization (Fig. 11). Cells treated with lovastatin showed marked changes in morphology including a loss of phallolidin-positive stress fiber staining (Fig. 11B). Supplementation with geranylgeranyl pyrophosphates prevented lovastatin-dependent changes in cell morphology and in phallolidin-positive stress fiber staining (Fig. 11C), whereas treating cells with GGTI-298 (10 μM) qualitatively mimicked the effect of lovastatin (Fig. 11D). Low-passage hu-
man TM cell cultures also displayed prominent phalloidin-positive stress fiber organization (Fig. 12A). Similar to GTM3 cells, prominent changes in human TM cell morphology and stress fiber organization were evident after 48 hours of lovastatin treatment (Fig. 12B) and were preventable by co-incubation with mevalonate (Fig. 12C).

**DISCUSSION**

In this study, we report the novel finding that lovastatin elicits a marked and selective increase in the content of RhoA and RhoB G-proteins in low-passage (primary) and transformed (GTM3) human trabecular meshwork (TM) cells. Inhibition of HMG-CoA reductase with lovastatin leads to enhanced accumulation of RhoA and RhoB, in part, by limiting geranylgeranyl isoprenylation of these G-proteins. We propose that post-translational geranylgeranylation facilitates RhoA and RhoB protein expression and processing in human TM cells. We speculate that increased accumulation of unprenylated inactive (GDP-bound) forms of RhoA and RhoB may disrupt Rho-dependent regulation of TM cell cytoskeletal organization.

Disruption of Rho G-protein mediated signaling events in TM cells may be of central importance in statin-dependent regulation of AH outflow. The mechanism by which statins increase AH outflow and reduce IOP remains unclear. Competitive inhibition of HMG-CoA reductase with mevastatin triggers an adaptive response in cultured mammalian cells that results in a marked 200-fold increase in the expression of the reductase protein. Upregulation of reductase expression by lovastatin involves several levels of control including induction of transcription, enhanced mRNA translation, and slowing of reductase degradation, consistent with classical feedback-

### Table 1. Lovastatin Increases Rho mRNA Content in TM Cells

<table>
<thead>
<tr>
<th>TM Cell Type</th>
<th>Treatment</th>
<th>RhoA mRNA Levels</th>
<th>RhoB mRNA Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transformed</td>
<td>Vehicle</td>
<td>1.0 ± 0.3</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Lovastatin</td>
<td>2.3 ± 0.6*</td>
<td>47.8 ± 12.4**</td>
</tr>
<tr>
<td></td>
<td>Mevalonate</td>
<td>1.1 ± 0.4</td>
<td>0.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Lovastatin + Mevalonate</td>
<td>1.4 ± 0.2</td>
<td>3.1 ± 3.3</td>
</tr>
<tr>
<td>Low Passage</td>
<td>Vehicle</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Lovastatin</td>
<td>2.2</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Transformed (GTM3, n = 3) or low passage (primary, n = 2) human TM cells were incubated (24–48 hours) in the presence of vehicle (0.01% ethanol) or lovastatin (10 μM), and RhoA-specific or RhoB-specific mRNA content was determined by qRT-PCR. Data shown are mean ± SD (transformed) or average (low passage) fold-changes expressed relative to GAPDH-specific mRNA content. *P < 0.05; **P < 0.01 (one-way ANOVA with Dunnett’s post hoc analysis).

**FIGURE 5.** Actinomycin D inhibits lovastatin-dependent increases in RhoA and RhoB protein content. Confluent GTM3 cells were pretreated (1 hour) with (+) or without (−) 0.5 μg/mL actinomycin D (Act D) and incubated for an additional 24 hours with vehicle (0.01% ethanol) or lovastatin (10 μM) as indicated. (A) Western immunoblot of RhoA and RhoB protein expressed in cell lysates (20 μg protein/lane) prepared from a single experiment performed in triplicate. GAPDH content is shown for comparison as a loading control. (B) Quantitative densitometric comparison of the results shown in (A) expressed as the mean ± SD. Vehicle (open bar), lovastatin (batched bar). **P < 0.01 (one-way ANOVA with Dunnett’s post hoc analysis).

**FIGURE 6.** Cycloheximide prevents lovastatin increases in RhoA and RhoB protein content. Confluent GTM3 cells were pretreated (1 hour) with (+) or without (−) 1.4 μg/mL cycloheximide (Cyclohex) and incubated for an additional 24 hours with vehicle (0.01% ethanol) or lovastatin (10 μM) as indicated. (A) Western immunoblot of RhoA and RhoB protein expressed in cell lysates (20 μg protein/lane) prepared from a single experiment performed in triplicate. GAPDH content is shown for comparison as a loading control. (B) Quantitative densitometric comparison of the results shown in (A) expressed as the mean ± SD. Vehicle (open bar), lovastatin (batched bar). **P < 0.01 (one-way ANOVA with Dunnett’s post hoc analysis).
regulatory control of a rate-limiting enzyme. However, more recent evidence suggests the regulation of protein expression by lovastatin may not be uniquely restricted to HMG-CoA reductase. Holstein et al. reported that HMG-CoA reductase inhibition leads to upregulation of additional proteins modified by the mevalonate-cholesterol pathway, including members of the Rho subfamily of G-proteins.35 Our own experience working with HCT116 colon carcinoma cells confirms the ability of statins to increase Rho protein expression (Deadwyler GD,}

**FIGURE 7.** Geranylgeranyl, but not farnesyl, pyrophosphate prevents lovastatin increases in RhoA and RhoB protein content. Confluent GTM3 cells were incubated (24 hours) with vehicle (0.01% ethanol), lovastatin (10 μM), lovastatin plus farnesyl-PP (Lov + FPP, 10 μM each) or lovastatin plus geranylgeranyl-PP (Lov + GGPP, 10 μM each) as indicated. (A) Western immunoblot of RhoA and RhoB protein expressed in cell lysates (20 μg protein/lane) prepared from a single experiment performed in triplicate representative of two separate experiments. GAPDH content is shown for comparison as a loading control. (B) Quantitative densitometric comparison of the results shown in (A) expressed as the mean ± SD. Vehicle (open bar), lovastatin (hatched bar), lovastatin + farnesyl-PP (gray bar), lovastatin + geranylgeranyl-PP (solid bar). **P < 0.01 (one-way ANOVA with Dunnett’s post hoc analysis).

**FIGURE 8.** Geranylgeranyl pyrophosphate supplementation prevents lovastatin increases in RhoA and RhoB protein content in low-passage human TM cells. Confluent cultures of low-passage human TM cells were incubated (48 hours) with vehicle (0.01% ethanol), lovastatin (10 μM), or lovastatin plus geranylgeranyl-PP (Lov + GGPP, 10 μM each) as indicated. Western immunoblot of RhoA and RhoB protein expressed in cell lysates (10 μg protein/lane) prepared from a single experiment performed in duplicate. GAPDH content is shown for comparison as a loading control.

**FIGURE 9.** Inhibition of geranylgeranyl transferase, but not farnesyl transferase, increases RhoA and RhoB protein content. Confluent GTM3 cells were incubated (24 hours) with vehicle (0.01% ethanol), lovastatin (10 μM), farnesyl transferase inhibitor (FTI-277, 10 μM), or geranylgeranyl transferase inhibitor (GGTI-298, 10 μM) as indicated. (A) Western immunoblot of RhoA and RhoB protein expressed in cell lysates (20 μg protein/lane) prepared from a single experiment performed in triplicate. GAPDH content is shown for comparison as a loading control. (B) Quantitative densitometric comparison of the results shown in (A) expressed as the mean ± SD. Vehicle (open bar), lovastatin (hatched bar), FTI-277 (gray bar), GGTI-298 (solid bar). **P < 0.01 (one-way ANOVA with Dunnett’s post hoc analysis).

**FIGURE 10.** Inhibition of geranylgeranyl transferase increases RhoA and RhoB protein content in low-passage human TM cells. Confluent cultures of low-passage human TM cells were incubated (48 hours) with vehicle (0.01% ethanol), lovastatin (10 μM), or geranylgeranyl transferase inhibitor (GGTI-298, 10 μM) as indicated. Western immunoblot of RhoA and RhoB protein expressed in cell lysates (10 μg protein/lane) prepared from a single experiment performed in duplicate. GAPDH content is shown for comparison as a loading control.

Post-translational isoprenylation facilitates membrane targeting and functional activation of newly synthesized Rho proteins. Limiting the availability of essential FPP and GGPP metabolic intermediates by HMG-CoA reductase inhibition severely impairs protein isoprenylation. Restoration of protein isoprenylation by the addition of mevalonate prevents lovastatin increases in Rho protein expression. One anticipated consequence of impaired protein isoprenylation is the measurable loss of membrane-associated Rho, possibly resulting in an increased accumulation of soluble unprenylated protein. However, the disproportionate accumulation of RhoA and RhoB proteins in the cytosol of lovastatin treated cells observed here, we argue, is suggestive of a more complex mechanism regulating Rho expression. Holstein et al. proposed a role of isoprenoid pyrophosphates as active repressors of Rho G-protein expression. Consistent with this thesis, supplementation of TM cell cultures with GGPP, but not FPP, prevented lovastatin increases in both RhoA and RhoB. Rho subfamily of G-proteins are modified largely by transferase-catalyzed geranylgeranylation, which may explain the isoprenoid selectivity.

Whereas isoprenoid pyrophosphates may act as repressors of Rho expression, isoprenylation itself may target Rho proteins for degradation. Under conditions where isoprenoid availability was not limited, we found that selective inhibition of geranylgeranyl transferase markedly increases Rho protein expression, similar to that seen for lovastatin. Post-translational geranylgeranylation may serve to facilitate Rho protein degradation in human TM cells.

The mevalonate-cholesterol pathway may also regulate transcriptional and translational expression of Rho. Statin depletion of mevalonate reportedly increases RhoB mRNA. We observed, in a mevalonate-preventable manner, lovastatin increases in RhoA mRNA and RhoB mRNA. By inhibiting transcription, we were able to prevent lovastatin from increasing RhoB, but not RhoA, protein accumulation. By comparison, inhibiting de novo protein synthesis with cycloheximide prevented lovastatin from increasing RhoA and RhoB protein. These findings collectively support a negative role of isoprenoid metabolic intermediates or isoprenylated proteins themselves on RhoB promoter activity. The regulation of RhoA expression by lovastatin in TM cells most likely occurs by a post-transcriptional mechanism, possibly by enhancing RhoA mRNA stability.

The functional relevance of these findings is underscored by studies showing that unprenylated forms of G-proteins are not biologically inert. Interference of Ras-signaling by functionally active unprenylated Ras G-proteins suggest functional consequences. However, the majority of soluble unprenylated Rho proteins that accumulate in lovastatin-treated TM cells did not appear to be functionally active (GTP-bound). TM cells treated with lovastatin or geranylgeranyl transferase inhibitor exhibited decreased actin stress fiber organization coincident with increased unprenylated Rho protein accumulation. Isoprenoid supplementation prevented lovastatin dissociation of filamentous actin. It remains to be elucidated whether increased stability of unprenylated inactive (GDP-bound) Rho proteins leads to changes in Rho signaling.

**Acknowledgments**

The authors thank Beatrice Yue and Xiang Shen (University of Illinois Chicago) and Charles Bouchard (Loyola University Chicago) for assistance procuring and culture of human TM cells, and Mary Ellen Moore and Bilquis Basith for helpful discussion.

**Figure 11.** Lovastatin disrupts filamentous actin organization. GTM3 cells cultured in chambered coverslips were incubated (24 hours) with (A) vehicle (0.01% ethanol), (B) lovastatin (10 μM), (C) lovastatin plus geranylgeranylfPP (10 μM each), or (D) geranylgeranyl transferase inhibitor (GGTI-298, 10 μM), and subsequently stained with Alexa Fluor 488–conjugated phalloidin. Shown are confocal photomicrographs of stained cultures representative of 2 to 12 separate coverslips. Bar, 20 μm.

**Figure 12.** Lovastatin disrupts filamentous actin organization in low-passage human TM cells. Low-passage human TM cells cultured in chambered coverslips were incubated (48 hours) with (A) vehicle (0.01% ethanol), (B) lovastatin (10 μM), or (C) lovastatin (10 μM) plus mevalonate (5 mM), and subsequently stained with Alexa Fluor 488–conjugated phalloidin. Shown are confocal photomicrographs of stained cultures representative of two to eight separate coverslips. Bar, 20 μm.
References


