Simvastatin Modulates Angiotensin II Signaling Pathway by Preventing Rac1-Mediated Upregulation of p27

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Abstract. Recent experimental observations have suggested that statins may exert modulatory effects on a number of pathobiological processes beyond their cholesterol-lowering properties. Some of the pleiotropic effects of statins seem to be mediated by their ability to block the synthesis of isoprenoid intermediates, which serve as important lipid attachments required for the proper function and activation of the small GTP-binding proteins. The current study explored the modulatory effects of simvastatin (SMV) on the angiotensin II (Ang II)-induced Rac1-mediated, upregulation of cyclin-dependent kinase inhibitor p27. Ang II (100 nM) stimulation of rat mesangial cells induced a significant increase in p27 protein expression. Co-treatment of cells with SMV (1 μM) inhibited Ang II–induced upregulation of p27 protein. Addition of mevalonate (200 μM) or geranylgeranyl pyrophosphate (5 μM) reversed the inhibitory effect of SMV on p27 protein expression, suggesting that the effect of SMV is geranylgeranyl dependent. This study also provides evidence for a sequential link between Ang II stimulation and downstream activation of Rac1, intracellular H₂O₂ production, and Akt kinase leading to upregulation of p27 protein in mesangial cells. It was also shown that SMV, by inhibiting Rac1 activity, reversed Ang II–induced increase in intracellular H₂O₂ production, Akt activation, and p27 protein expression. The data presented in this study not only elucidate Ang II–mediated signaling cascade in mesangial cells but also demonstrate for the first time the modulatory effects of SMV on Ang II–induced signaling pathway at the cell cycle level.

The 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, or statins, are potent inhibitors of cholesterol biosynthesis that are used extensively in the treatment of patients with hypercholesterolemia (1,2). Several studies have demonstrated the beneficial effects of statins in reducing cardiovascular-related morbidity and mortality (3,4). Recently, it was also suggested that statins may confer renoprotection in a variety of glomerular diseases, including diabetic nephropathy (5,6). It is usually assumed that the beneficial effects of statins result from the competitive inhibition of cholesterol synthesis. However, statins may also exert additional effects on cell signaling pathways by preventing the synthesis of various isoprenoids derived from the mevalonate (MEV) pathway, such as farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) (7,8). Both FPP and GGPP are important lipid attachments required for the subcellular localization and function of a variety of proteins, including small GTPase-binding proteins (9). In support of cholesterol-independent or pleiotropic properties of statins in diabetic nephropathy, we and others recently showed that some of the beneficial effects of statin therapy in diabetic milieu may be mediated via modulation of small GTPase proteins (10,11).

The Rho family of small GTPases are 20- to 40-kD monomeric G proteins that can cycle between two interconvertible forms: GDP-bound (inactive) and GTP-bound (active) states (12,13). Activation of transmembrane growth factor receptors can promote the exchange of GDP to GTP on Rho proteins, causing membrane translocation and activation of GTP-bound Rho proteins. Recent studies in a variety of cell types have demonstrated that the Rho family of small GTPases may play a crucial role in regulating cellular hypertrophy (12–15). For instance, it has been shown that Rac1, a member of Rho family of small GTPases, is critical for the signal transduction leading to cardiac myocytes and mesangial cell (MC) hypertrophy via activation of reactive oxygen species (ROS) (14,16).

Glomerular cell hypertrophy is a characteristic lesion of early stages of diabetic nephropathy (17–19). Intra renal angiotensin II (Ang II), hyperglycemia, and TGF-β hierarchically and/or coordinately have been suggested as the mediators of cell hypertrophy in diabetic nephropathy (20–22). Ang II, an octapeptide hormone, exerts both hemodynamic (leading to increased glomerular capillary pressure) and nonhemodynamic effects (stimulation of cellular hypertrophy and extracellular matrix expansion). Mediators of Ang II–induced MC hypertrophy in diabetic milieu have not fully been identified. However, several observations have shown the pivotal role of p27,
a member of CIP/KIP family of cyclin-dependent kinase inhibitors (CDKI), in glucose and Ang II–induced cellular hypertrophy (23–27).

In the current study, we examined the modulatory effects of simvastatin (SMV) on a novel signaling pathway by which 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors may ameliorate the detrimental effects of Ang II, independent of their cholesterol-lowering properties, on MC hypertrophy. To this aim, we investigated the modulatory effect of SMV on the Ang II–induced Rac1-regulated, NADPH-dependent oxidase signaling pathway involving Akt activation and upregulation of CDKI p27.

Materials and Methods

Reagents and antibodies

DMEM/F12, FBS, and PBS were obtained from Invitrogen (Carlsbad, CA). Angiotensin, diphenylene iodonium (DPI), MEV, GGPP, and FPP were purchased from Sigma (St. Louis, MO). Anti-Akt and anti phospho-Akt (Ser473) were commercially purchased (Cell Signal Technology, Beverly, MA). Akt1 cDNA allelic pack and Rac Activation Assay Kits were obtained from Upstate Biotechnology (Lake Placid, NY). Anti-p27 antibody was purchased from BD Biosciences Pharmingen (San Diego, CA), and fluorescence probe 2′, 7′-dichlorofluorescein diacetate (DCF-DA) was obtained from Molecular Probes (Eugene, OR). SMV and MEV were chemically activated as described previously (10).

Cell Culture and Transfection

Rat glomerular mesangial cells were grown in DMEM/F12 medium containing 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified incubator at 37°C under 5% CO2. Transfections of different mutants were performed as described previously (10). Briefly, in transfection studies, cells were grown to 50% confluence and then transfected with 1 μg of fusion plasmid DNA using LipofectAMINE Reagent according to the manufacturer’s protocol (Invitrogen). Transfected cells were grown in 800 μg/ml G418 (Invitrogen) until colonies were formed. Subsequently, the colonies were grown in growth medium containing 800 μg/ml G418 and incubated at 37°C and 5% CO2 until the cells achieve ~70% confluence. The confluent cells were trypsinized and grown in growth medium containing 10% FBS and 800 μg/ml G418. Plasmids containing wild-type Rac1 (pcDNA3 Rac1) and dominant negative Rac1 construct (pcDNA3 Rac1N17) were gifts of Dr. Jacob Sznajder (Northwestern University, Chicago, IL).

Western Blotting

For each experiment, a total of 5 × 105 cells were seeded, and at subconfluence (~70%), cells were made quiescent for 48 h. Cells were rinsed twice with ice-cold PBS and scraped in 500 μl of ice-cold lysis buffer (20 mM Tris [pH 7.5], 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM PMSF, 10 μg/ml leupeptin, 100 μg/ml aprotinin, and 1 mM Na3VO4). The samples were centrifuged and dissolved in SDS sample buffer (187.5 mM Tris [pH 6.8], 6% SDS, 30% glycerol, 150 mM dithiothreitol, and 0.03% bromphenol blue) and boiled for 5 min. Protein concentrations were determined by the BCA protein assay (Pierce, Rockford, IL). Equal amounts of protein were separated on SDS–polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were probed with primary antibodies at 4°C overnight (at a dilution of 1:100 for p27, 1:500 for AKT, and 1:500 for Rac1) and incubated with the appropriate secondary antibodies for 1 h. Immunoreactive bands were visualized by enhanced chemiluminescence reaction. Each blot is representative of at least three similar experiments.

Rac1 Activation Assay

Rac1 activity was determined by a pull-down assay according to the instructions by the manufacturer (Rac activity assay kit; Upstate Biotechnology). In brief, 105 cells were grown in 10-cm dishes, washed in cold PBS, and lysed in ice-cold MLB buffer (25 mM HEPES [pH 7.5], 150 mM NaCl, 1% NP-40, 10 mM MgCl2, 2.0 mM EDTA, and 2% glycerol). Samples were centrifuged and incubated for 60 min at 4°C with 10 μl of PAK-1 PBD-agarose to precipitate GTP-bound Rac1. Precipitated complexes were washed three times in MLB buffer and resuspended in 30 μl of 2× Laemmli buffer. Total lysates and precipitates were analyzed by performing SDS-PAGE and Western blot analysis using mouse monoclonal antibody against Rac1 (at a dilution of 1:500; Upstate Biotechnology).

Detection of Intracellular H2O2

The H2O2-sensitive fluorescence probe DCF-DA was used to assess the generation of intracellular H2O2. This compound is converted by intracellular esterases to 2′,7′-dichlorofluorescin, then oxidized by H2O2 to the highly fluorescent 2′,7′-dichlorofluorescin. Cells were grown to near confluence in coverglass chambers and then made quiescent by serum starvation for 48 h. Cells were stimulated with Ang II (100 nM) for 1 h and incubated with 10 μM DCF-DA for 30 min at 37°C. The DCF fluorescence was visualized at an excitation wavelength of 488 nm and emission at 520 nm using a bandpass filter on Zeiss LSM510 laser scanning confocal microscope.

Confocal Laser Scanning Fluorescence Microscopy

MC were grown on glass coverslips. The cells were fixed with 3.7% formaldehyde and permeabilized with 0.3% Triton X-100 in PBS for 10 min at room temperature. Cells were then incubated with anti-P27 Kip1 antibody for 2 h at room temperature. Coverslips were washed and incubated with TRITC-conjugated secondary antibody (Zymed Laboratories, South San Francisco, CA). After DAPI staining (3 μg/ml), the coverslips were mounted on glass slides with antifade mounting media (Molecular Probes) and examined using a confocal fluorescence microscope (Zeiss LSM510).

[3H]Leucine Incorporation

MC were plated in 24-well plates, serum starved for 48 h, and then exposed to Ang II (100 nM) in the presence or absence of SMV (1 μM) for 24 h with addition of 1 μCi of [3H]leucine for 6 h (Amer sham, Piscataway, NJ) and then processed for determination of incorporated radioactivity after precipitation with 10% TCA as described previously (10).

Statistical Analyses

ANOVA with a Student-Newman-Keuls test was used to evaluate differences between two or more different experimental groups. Results are expressed as mean ± SEM. P ≤ 0.05 was considered as statistically significant.

Results

Effect of SMV on Ang II–Induced p27 Protein Expression

Ang II–mediated MC hypertrophy is characterized by the induction of CDKI p27 at the cell cycle level (23–27). To
define the modulatory effect of SMV on Ang II–induced upregulation of p27 protein expression, cultured rat MC were made quiescent by serum deprivation for 48 h and exposed to Ang II (100 nM) in the presence or absence of SMV (1 μM) for 6 h. As shown in Figure 1, Ang II–treated MC exhibited a significant increase in p27 protein expression that was reversed with co-treatment of cells with SMV as determined by Western blot analysis. For determining the role of various isoprenoids derived from MEV in regulating the inhibitory effect of SMV on Ang II–induced upregulation of p27 protein, Ang II–stimulated MC were co-treated with 1 μM SMV and MEV (200 μM) or various isoprenoids intermediaries (geranylgeranyl pyrophosphate [GGPP; 5 μM], farnesyl pyrophosphate [FFP; 5 μM], and squalene [SQ; 5 μM]). As shown in Figure 1, co-treatment of cells with MEV reversed the inhibitory effect of SMV on p27 protein expression. In addition, co-treatment of cells with GGPP but not with FPP or SQ reversed the inhibitory effect of SMV, suggesting that the modulatory effect of SMV is geranylgeranyl-dependent and independent of cholesterol biosynthesis as SQ, an immediate precursor of cholesterol, failed to reverse the effect of SMV on p27 protein expression.

**Modulatory Effect of SMV on Ang II–Induced Rac1 Activation**

We recently reported that SMV modulates the activation of the Rho family of small GTPases in diabetic milieu (10). To
decipher whether Rac1, a member of the Rho family of small GTPases, mediates Ang II–induced increase in p27 protein expression, rat MC were exposed to Ang II (100 nM), and the temporal profile of Ang II–induced Rac1 activation was determined. Rac1 activation was measured by an affinity pull-down assay using GST fusion protein PAK1-PBD, which recognizes only the active form of Rac1 (GTP-Rac1). Ang II stimulation of MC increased Rac1 activity by approximately threefold in MC after 5 min (Figure 2A). For studying the effect of SMV on Ang II–induced Rac1 activity, cells stimulated with Ang II were co-treated with SMV (1 μM). Co-treatment of Ang II–stimulated MC with SMV reversed Ang II–induced increase in Rac1 activity, suggesting a novel role for SMV in modulating Ang II–induced Rac1-mediated signaling pathway in MC (Figure 2B). The inhibitory effect of SMV on Rac1 activity was reversed when cells were co-treated with MEV (200 μM), indicating that the effect of SMV on Ang II–induced Rac1 activity is MEV dependent. As shown in Figure 2B, total Rac1 protein levels were unchanged.

For establishing a sequential link between Rac1 activity and Ang II–induced upregulation of p27 protein, MC were transfected with dominant-negative (N17 Rac) and wild-type Rac1 and stimulated with Ang II (100 nM). As shown in Figure 3, Ang II stimulation failed to increase p27 protein expression in dominant negative Rac1 transfected cells, indicating a critical role for Rac1 activation in Ang II–induced upregulation of p27 in MC.

The protein abundance of p27 is known to be mainly regulated by posttranslational mechanisms (28,29). Whereas P27 needs to be transported into the nucleus to exert its effect on the cell cycle (30), degradation of p27 by a ubiquitin-dependent pathway requires cytoplasmic localization of p27 (31,32). Thus, the next question that we addressed was the role of Rac1 on the Ang II–induced nuclear translocation of p27. To this
end, we investigated the subcellular localization of p27 in response to Ang II using immunofluorescence microscopy. DAPI was used as a marker for nuclear staining. As shown in Figure 4, quiescent cells exhibited nuclear and weak cytoplasmic staining of p27. Upon stimulation with Ang II, intense nuclear staining of p27 was detected. However, cells transfected with dominant negative Rac1 mainly displayed diffuse cytoplasmic staining of p27, suggesting that Ang II-induced nuclear translocation of p27 from the cytoplasm is Rac1 mediated. Thus, these data confirm that Rac1 plays a major role in Ang II signaling pathway in MC.

Effect of SMV on NADPH-Mediated $H_2O_2$ Production

One of the recent discoveries on the role of ROS in cell signaling pertains to the observations that ROS can act as an integral part of signaling pathways as they fulfill the important prerequisites for intracellular messengers (33–37). For determining whether Ang II stimulates NADPH oxidase activity and intracellular $H_2O_2$ production, MC were grown and made quiescent in culture medium that contained 0.1% FBS. Cells were then stimulated with 100 nM Ang II for 1 h. Medium was replaced with Hanks’ solution containing $H_2O_2$ sensitive fluorophore DCF-DA (10 μM). Ang II stimulation caused a robust increase in DCF-DA fluorescence in MC (Figure 5, A and B). For determining whether NADPH oxidase is the source of $H_2O_2$ production, Ang II–stimulated MC were preincubated with DPI (10 μmol/L), a molecule that competitively inhibits flavin-containing enzymes such as NADPH oxidase. Preincubation with DPI resulted in complete inhibition of Ang II–induced increase in DCF-DA fluorescence (Figure 5C), suggesting that a flavin-containing enzyme is the source for intracellular $H_2O_2$. Ang II–induced increase in $H_2O_2$ was also inhibited when cells were preincubated with 10 μM losartan, a specific AT1 receptor blocker, indicating that this induction was AT1 receptor dependent (Figure 5D). Similar results were obtained when cells were treated with 1 μM SMV (Figure 5E), suggesting that SMV inhibits Ang II–induced upregulation of NADPH oxidase activity and intracellular $H_2O_2$ production.

Because Rac1 proteins are essential in NADPH oxidase signaling pathway, its role in activation of the NADPH complex by Ang II was also investigated. For determining whether Rac1 regulates Ang II–induced upregulation of $H_2O_2$, cells were transfected with dominant-negative and wild-type Rac1 and stimulated with Ang II (100 nM). As shown in Figure 5F, Ang II stimulation did not increase $H_2O_2$ production in dominant-negative Rac1 transfected MC as determined by DCF-DA fluorescence, indicating a sequential link between Rac1 activity and intracellular $H_2O_2$ production.

Effect of SMV on Ang II–Induced Akt Activity

The list of redox-sensitive targets of ROS include the mitogen-activated protein kinase family, stress-activated protein kinases, NF-κB, caspases, and Akt (37,38). Growing evidence indicates that Akt, a serine-threonine kinase, is a potential target of Ang II–induced hypertrophy of vascular smooth muscle cell and MC (16,37,38). For studying the effect of Ang II on Akt activation and the modulatory effects of SMV on Akt signaling pathway, serum-starved MC were stimulated with Ang II (100 nM), and total and phosphorylated Akt (Ser473) were detected using antibodies against total and phospho-Akt (Ser473; Cell Signal Technology). Ang II–stimulated MC showed an approximately threefold increase in the ratio of phospho-Akt/total Akt after 20 min (Figure 6A). As shown in Figure 6B, co-treatment of cells with SMV (1 μM) inhibited Ang II–induced upregulation of phospho-Akt. The inhibitory effect of SMV was reversed when cells were co-treated with MEV (200 μM).

For determining whether Rac1 regulates Ang II–induced upregulation of phospho-Akt, MC were transfected with dominant-negative and wild-type Rac1 and stimulated with Ang II (100 nM). As shown in Figure 7, Ang II stimulation did not increase phospho-Akt protein expression in MC transfected with dominant-negative Rac1, indicating that Ang II–induced...
Akt phosphorylation is mediated by a Rac1-dependent pathway.

For determining whether Akt signaling pathway mediates Ang II–induced upregulation of p27, MC were transfected with dominant-negative (K179M) and wild-type Akt1 (wtAkt) and stimulated with Ang II (100 nM). As shown in Figure 8, Ang II stimulation did not increase p27 protein expression in MC transfected with dominant-negative Akt1 cells, indicating that Akt activation is necessary for the Ang II–induced upregulation of p27 in MC.

To gain further insights into the role of Akt on the nuclear translocation of p27, we performed immunofluorescent microscopy of p27. As shown in Figure 9, MC stimulated with Ang II exhibited intense nuclear staining of p27. However, cells transfected with dominant-negative Akt showed diffuse cytoplasmic staining of p27, suggesting that Akt is also necessary for the Ang II–induced nuclear translocation of p27 from the cytoplasm.

**Effect of SMV on Ang II–Induced MC Hypertrophy**

Previous studies have shown the pivotal role of Ang II in MC hypertrophy as measured by [3H]leucine incorporation (23–25). The role of SMV on the Ang II–induced de novo protein synthesis in MC was examined using [3H]leucine incorporation. Serum-starved cultured rat MC were made quiescent by serum deprivation for 48 h and then exposed to 100 nM Ang II for 24 h. The incorporation of [3H]leucine was compared in cells exposed to Ang II in the presence and absence of SMV (1 μM). As shown in Figure 10, exposure of MC to Ang II increased [3H]leucine incorporation by 195 ± 3% compared with control cells (P < 0.05). Co-treatment of cells with SMV attenuated Ang II–induced increase in [3H]leucine uptake (128 ± 2%). Cells co-treated with SMV and GGPP (5 μM) exhibited increased [3H]leucine incorporation (170 ± 8%), indicating that the inhibitory effect of SMV on Ang II–induced [3H]leucine uptake is geranylgeranyl dependent.

**Discussion**

This study demonstrate that Ang II stimulation in mesangial cells via activation of Rac1GTPase protein, intracellular H₂O₂ activation, and increased Akt activity leads to increased p27 protein expression. Our data also provide evidence for the modulatory effect of SMV on Ang II–induced Rac1-regulated, NADPH-dependent oxidase signaling pathway involving Akt activation and CDKI p27 in mesangial cells (Figure 11).

Previous studies from our laboratory and others have indi-
cated that statins modulate DNA synthesis and cellular proliferation by preventing prenylation of small GTPase proteins such as Ras and Rho GTPases (10,39,40). The posttranslational lipid modification (isoprenylation) of small GTPase proteins is necessary for the translocation of Rho GTPases from the cytosol to the membrane, where activation of these proteins takes place. Statins, by inhibiting isoprenylation of small GTPase proteins, prevent membrane translocation and activity of small GTPases and thus interfere in a number of cellular processes, such as apoptosis, differentiation, cellular proliferation, and hypertrophy (41–43).

The critical role of p27 in cellular hypertrophy induced by Ang II or high glucose has been shown in a variety of cell types (23,24). For instance, Wolf et al. (23) demonstrated that the expression of p27 was upregulated in proximal tubular cells stimulated by Ang II and that p27 antisense oligonucleotide reversed the Ang II–mediated cellular hypertrophy. It has also been reported that MC isolated from p27−/− mice did not undergo cellular hypertrophy under high glucose conditions (25,26). More recently, the significance of p27 in the progression of diabetic nephropathy in vivo has also been established (44). However, the underlying upstream mediators of Ang II–induced MC hypertrophy are not fully identified. Gorin et al. (24) recently suggested the involvement of Rac1 GTPase in Ang II–induced signaling pathway in MC hypertrophy. Several other studies have also indicated the involvement of Rho A and Rho-kinase in Ang II–induced hypertrophy in vascular smooth muscle cells (16,45). In this study, we not only demonstrated the role of Rac1 on Ang II–induced signaling pathway at the cell cycle level, but we also showed that SMV, by inhibiting Rac1 activity, downregulates Ang II–induced increase in p27 protein levels. The data presented in this study also suggest that

![Figure 9. Effect of Akt on the nuclear translocation of p27.](image)

(Left) DAPI was used as a marker for nuclear staining. (Middle) Immunofluorescent microscopy of p27 using anti-p27 antibody. (Right) Merged images. After stimulation with Ang II, MC displayed intense p27 nuclear staining of p27. MC transfected with dominant-negative Akt1(K199M) showed diffuse cytoplasmic staining. Thus, the data indicate that Akt activation promotes nuclear translocation of p27. Images were visualized using a Zeiss LSM510 confocal laser scanning microscope (n = 3).
the effect of SMV on p27 is cholesterol independent as cotreatment of cells with SQ, an immediate precursor of cholesterol in the MEV pathway, did not reverse the inhibitory effect of SMV on p27 protein expression. However, co-treatment of MC with GGPP reversed the inhibitory effect of SMV, indicating that the effect of SMV on Ang II–induced upregulation of p27 protein is geranylgeranyl dependent. Furthermore, this study showed that Rac1 activation is required for Ang II–induced upregulation of p27, as MC transfected with dominant-negative Rac1 failed to increase p27 protein expression in response to Ang II.

Our data also suggest that Ang II–induced signaling pathway in MC involves ROS and increased NADPH oxidase activity. When NADPH oxidase activity was inhibited by DPI, the Ang II increase in H₂O₂ production was eliminated. Thus, our results confirm previous observations regarding the involvement of ROS in Ang II–mediated signaling in MC (24,46,47). This study, however, provides new insights by establishing a sequential link between Ang II–induced Rac1 activity and an increase in intracellular H₂O₂ production as dominant-negative Rac1 transfected MC failed to increase H₂O₂ production. Furthermore, the data presented in this study indicate that SMV, by inhibiting activation of Rac1, prevents Ang II–induced intracellular H₂O₂ production and NADPH-dependent oxidase activity in MC.

We also assessed the contribution of Akt activation on Ang II–induced upregulation of p27 and the effect of SMV on Ang II–induced Akt signaling pathway. Our data indicate that Ang II increased phospho-Akt (active form) in a time-dependent manner. SMV inhibited the activation of Akt, and the inhibitory effect of SMV on Akt phosphorylation was MEV dependent. Moreover, we provided further evidence that Akt activation is required for Ang II increase in p27 protein expression as MC transfected with dominant-negative Akt1 did not express upregulation of p27 protein in response to Ang II. These data support the previous reports indicating that Ang II stimulation induces cellular hypertrophy by activating Akt pathway, probably through phosphorylation of p27 at serine residues (48–50).

The expression of p27 is transcriptionally and posttranslationally regulated (28–32). Although transcriptional regulation of p27 has been reported by Forkhead transcription factor, the p27 expression is known to be mainly regulated by posttranslational mechanisms (30–32). The best characterized mechanism of p27 degradation is ubiquitin-dependent degradation (28–32). It is generally believed that degradation of p27 requires cytoplasmic localization of p27. To provide further evidence for the pivotal role of Rac and Akt on the Ang II–induced upregulation of p27, we performed several immunohistochemical analyses using confocal laser scanning fluorescence microscopy. Together, our data indicate that both Rac1 and Akt are necessary for the Ang II–induced nuclear translocation of p27. The data presented in this study demonstrate that dominant-negative mutants of Rac and Akt promote degradation of p27 with the subsequent decrease in p27 protein levels.

Lipophilic statins, such as SMV, are much more widely taken up by passive diffusion into a broad range of tissues and cells as compared with hydrophilic statins. This distinction among statins could influence the ability of statins to exert their pleiotropic effects on the basis of the ability of nonhepatic cells to transport the different members of the statin family into the cell according to their hydrophobicity. Therefore, further stud-
ies are planned to examine the class effect of various statins on Ang II signaling pathway.

In conclusion, on the basis of our findings, we propose that Ang II–induced MC hypertrophy involves several mediators that include small GTPase protein, Rac1, activation of ROS, phosphorylation of Akt, and increased expression of CDKI p27. This study also demonstrates for the first time the modulatory effect of SMV on Ang II–mediated Rac1-regulated, NADPH and Akt kinase-dependent signaling pathway involved in the upregulation of CDKI p27 protein expression in MC.

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References

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