HMG-CoA reductase inhibitor simvastatin mitigates VEGF-induced “inside-out” signaling to extracellular matrix by preventing RhoA activation

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The guanine nucleotide-binding protein Rho family, consisting of Rho, Rac, and Cdc42, are 20- to 40-kDa monomeric G proteins that can cycle between two interconvertible forms: GDP bound (inactive) and GTP bound (active) states (34, 46). Several growth factors can promote the exchange of GDP to GTP on Rho proteins resulting in membrane translocation and activation of GTP-bound Rho proteins. The Rho family of small GTPases are involved in a number of essential biological activities of the cell including actin stress fiber formation, cell motility, and cell aggregation (1, 37). More recently, Rho GTPases have also emerged as key regulators in the regulation of integrin-mediated signaling (4, 11, 12).

Integrins are heterodimeric transmembrane molecules that consist of α- and β-subunits. The main ligands for integrins are extracellular matrix adhesive proteins and cellular counter receptors. High-affinity ligand binding requires that integrins to become activated by undergoing conformational changes regulated by “inside-out” signaling. In turn, integrin ligation triggers “outside-in” signaling that regulate, among many other functions, gene regulation and cell motility (13, 14, 18, 42, 44). Integrin β-subunit cytoplasmic domains are required for integrin activation, whereas α cytoplasmic domain usually plays a regulatory role. Once integrins are activated, one of the earliest changes initiated by their activation is tyrosin phosphorylation of proteins such as talin, paxilin, and the cytosolic focal adhesion kinase (FAK) (31, 38). FAK phosphorylation is considered to be a critical step in the integrin-mediated signaling events. In this study, we report a critical role for β1-integrin activation and FAK phosphorylation in VEGF signaling to ECM.
A growing body of evidence is suggesting that HMG-CoA reductase inhibitors (statins), by inhibiting mevalonate (MEV) biosynthesis, may also exert modulatory effects on several signaling pathways beyond their cholesterol-lowering properties (27, 36, 47). For instance, we and others (6, 7, 55) previously provided evidence that some of the beneficial effects of statins in the diabetic milieu may be mediated through their modulatory effects on several signaling pathways. In the present study, we extend our previous observations and describe the underlying signaling mechanism through which VEGF leads to increased type IV collagen accumulation in MC. We also explored the effect of MEV depletion by using simvastatin (SMV), a hydrophobic statin, on VEGF-induced signaling pathway.

**Materials and Methods**

**Reagents and antibodies.** Recombinant rat VEGF165 was obtained from R&D Systems (Minneapolis, MN). DMEM/F12, FBS, and PBS were purchased from Invitrogen (Carlsbad, CA). MEV was purchased from Sigma (St. Louis, MO). Rho A activation assay kit was obtained from Upstate Biotechnology (Lake Placid, NY). Rho A antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The monoclonal anti-β1-integrin subunit (active conformation) antibody, clone HUTS-4, and function-blocking monoclonal mouse anti-β1 integrin antibody were purchased from Chemicon (Temecula, CA). The monoclonal anti-β1-integrin subunit (active conformation) antibody, clone HUTS-21 was purchased from BD Pharmingen, San Diego, CA), and collagen IV antibody was from Novus Biologicals (Littleton, CO), and cytochalasin D was obtained from Sigma. Rho-damine-phalloidin was purchased from Molecular Probes (Eugene, OR). SMV was kindly provided by Merck (West Point, PA). Both SMV and MEV were chemically activated as described previously (6, 7, 55).

**Cell culture and transfection.** Early passaged (passage 3–10) rat glomerular MC (6, 7, 55) 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 RhoA mutants were performed as described previously (6, 7, 55). Briefly, in transfection studies, cells were grown to 50–60% confluence and then transfected with 1 μg of fusion plasmid DNA using lipofectamine according to the manufacturer’s protocol (Invitrogen). Subsequently, the transfected colonies were grown in growth medium containing 800 μg/ml G418 until the cells achieve ~70% confluence. Plasmids containing wild-type RhoA (pcDNA3 RhoA) and dominant negative RhoA construct (pcDNA3 RhoA N17) were generous gifts of Dr. J. Szajdjer (Northwestern University, Chicago, IL). In transfection studies, control experiments were performed using lipofectamine alone as an additional control group.

**Rho A activity pull-down assay.** RhoA activity was measured by a pull-down assay according to the instructions by the manufacturer (Rho Activity Assay Kit, Upstate Biotechnology). Briefly, 107 cells were grown in 100-mm dishes, washed in ice-cold PBS twice, and lysed in ice-cold MLB buffer (25 mM HEPES, pH 7.5, 150 mM NaCl, 1% NP-40, 10 mM MgCl2, 1.0 mM EDTA, and 2% glycerol). The samples were centrifuged and incubated for 45 min at 4°C with 10 μl of rhotekin agarose to precipitate GTP-bound RhoA. Precipitated complexes were washed three times in MLB buffer and resuspended in 30 μl of 2X Laemmli buffer. Total and precipitates were analyzed by performing SDS-PAGE and Western blot analysis using monoclonal anti-RhoA antibody at a dilution of 1:500.

**Confocal laser-scanning fluorescence microscopy.** MC were grown on glass coverslips. The cells were fixed with 3% paraformaldehyde and permeabilized with 0.2% Triton X-100 in PBS for 10 min at room temperature. The cells were incubated with anti-phospho-FAK antibody for 1 h at room temperature and then incubated with FITC-conjugated secondary antibody (Zymed Laboratories, San Francisco, CA). To detect F-actin, cells were incubated with rhodamine-phalloidin (1:100) for 30 min at 37°C after fixation and permeabilization. The coverslips were mounted on glass slides with antifade mounting media (Molecular Probes) and examined using a confocal fluorescence microscope (Zeiss LSM510, Thornwood, NY).

**Flow cytometric analysis.** Near-confluent growth-arrested MC were pretreated with SMV or vehicle control for 12–18 h, after which VEGF (50 ng/ml) was added to the medium. At the end of the indicated times, cells were harvested by HyQTase (Hyclone, Logan, UT) in their culture media and washed twice with 1× DPBS without Ca2+ and Mg2+ (400 g, 5 min, room temperature). Mesangial cells were then resuspended and fixed with 3.7% formaldehyde. Cells were then incubated with total anti-integrin antibody (Chemicon), monoclonal anti-β1-integrin subunit (active conformation) antibodies, clones HUTS-21 (BD Pharmingen) and HUTS-4 (Chemicon), in blocking buffer (2% goat serum in DPBS without Ca2+ and Mg2+) for 45 min on ice. Cells were then incubated with secondary antibodies for 1 h on ice. Flow cytometric analysis was performed using a Beckman Coulter EPICSXL-MCL (Becton, Dickinson, CA).

**[3H]Proline incorporation.** The collagen synthesis was examined by [3H]proline incorporation. For these experiments, 5 × 105 cells were seeded in 24-well plates in DMEM/F12 medium containing 10% FBS. At confluence, cells were starved with serum-free medium for 48 h, and then the cells were exposed to various concentrations of VEGF in the presence or absence of SMV for 24 h; 2 μCi of [3H]proline (Amershams, Piscataway, NJ) and ascorbic acid (50 μg/ml) were added to each well for the last 12 h of incubation. The cells were washed twice with ice-cold PBS, precipitated twice with ice-cold 10% TCA (Sigma), solubilized in 2 ml 0.2 N NaOH containing 0.1% Triton X-100. The samples were taken to liquid scintillation counting. Additional cells seeded in parallel were scraped off the plate to detect the protein concentration. Proline incorporation was expressed as counts per minute (cpm) per microgram of protein.

**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 added 0.5 ml of the ice-cold lysis buffer [50 mM Tris·HCl, pH 7.5, 150 mM NaCl, 100 μg/ml PMSF, 0.1% SDS, 1% Nonidet P-40 (NP-40), 0.5% sodium deoxycholate, 10 μg/ml aprotinin, 2 μg/ml leupeptin, 10 mM EDTA], incubated for 20 min on ice, and then were scraped and centrifuged. Protein concentrations were determined by the BCA protein assay (Fierce, Rockford, IL), and then separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were probed with primary antibodies diluted in TBS-T containing 5% nonfat milk at 4°C overnight. The membranes were incubated with the appropriate secondary antibodies for 1 h at room temperature. Immunoreactive bands were visualized by ECL reaction. Each blot is representative of at least three similar independent experiments.

For detection of occupied β1 integrins, cells were plated on collagen-coated slides before VEGF stimulation. Cells were incubated with HUTS-21 or HUTS-4 antibodies for 30 min at 37°C. Cells were rinsed with PBS, lysed in SDS sample buffer, and bound antibody was detected by Western blot analysis.

**Statistical analyses.** Data are expressed as means ± SE. ANOVA with a Student-Newman-Keuls test was used to evaluate differences between two or more different experimental groups. A value of P ≤ 0.05 was considered significant.

**Results**

**Effects of VEGF and SMV on cell-associated collagen synthesis.** VEGF-induced cell-associated collagen synthesis in MC was measured by [3H]proline incorporation. Serum-starved cultured rat MC were exposed to incremental concentrations of VEGF for 24 h. As shown in Fig. 1, VEGF...
stimulation caused a significant increase in [3H]proline incorporation in a dose-dependent manner. Cotreatment of cells with SMV (1 μM) prevented VEGF-induced (50 ng/ml) increase in [3H]proline uptake. The addition of MEV (300 μM) reversed the effect of SMV, indicating that the inhibitory effect of SMV on VEGF-induced [3H]proline incorporation was MEV dependent. We next tested whether the inhibitory effect of SMV on VEGF signaling was mediated by isoprenoids derived from the MEV pathway. To this end, VEGF-stimulated MC were incubated with SMV (1 μM) and cotreated with either geranyl geranyl pyrophosphate (GGPP) or squalene (SQ), two important isoprenoids derived from HMG-CoA reductase. We also investigated the effect of SQ, an immediate precursor of cholesterol, to test whether the inhibitory effect of SMV on VEGF signaling is independent of cholesterol biosynthesis. The data on Fig. 1 show that cotreatment of cells with GGPP but not with SQ reversed the inhibitory effect of SMV, suggesting that the modulatory effect of statins on VEGF signaling is geranyl geranyl dependent but independent of cholesterol synthesis since cotreatment of cells with SQ failed to reverse the effect of SMV. Taken together, these findings indicate that the inhibitory effect of SMV on VEGF signaling is MEV and geranyl geranyl dependent.

We next examined the effect of VEGF and SMV on type IV collagen protein levels by Western blot analysis. As shown in Fig. 2, cells exposed to incremental concentrations of VEGF exhibited significant increase in type IV collagen protein levels. Cotreatment of VEGF-stimulated cells with SMV (1 μM) abrogated the effect of VEGF (50 ng/ml) on cell layer collagen type IV protein levels. The inhibitory effect of SMV was reversed when cells were cotreated with MEV (300 μM).

Effects of VEGF and SMV on RhoA activity. We recently reported that SMV modulates the activation of the Rho family of small GTPases in the diabetic milieu (6, 7, 55). To decipher whether RhoA, a member of the Rho family of small GTPases, mediates VEGF-induced collagen accumulation, MC were exposed to VEGF (50 ng/ml), and RhoA activity was measured by an affinity pull-down assay using GST fusion protein rhotekin, which recognizes only the active form of RhoA (GTP-RhoA). VEGF stimulation significantly increased RhoA activity in MC after 20 min (Fig. 3). To examine the effect of
SMV on VEGF-induced RhoA activity, cells stimulated with VEGF were cotreated with SMV (1 μM). As shown in Fig. 3, cotreatment of VEGF-stimulated cells with SMV inhibited VEGF-induced increase in RhoA activity without significantly changing total RhoA protein levels. The inhibitory effect of SMV on RhoA activity, and the addition of MEV reversed the inhibitory effect of SMV. B: densitometric analysis of GTP-RhoA (n = 3, *P < 0.05 vs. control).

To establish a link between RhoA activation and VEGF-induced type IV collagen protein levels, MC were transfected with dominant-negative mutant (N17RhoA) and wild-type (wt) RhoA. The data in Fig. 4 show that VEGF (50 ng/ml) stimulation failed to increase cell layer collagen IV protein levels in dominant-negative RhoA-transfected cells, indicating a critical role for RhoA activation in VEGF-induced collagen synthesis.

**Effects of VEGF and SMV on the actin cytoskeleton remodeling.** To examine whether VEGF signaling in MC involves actin cytoskeleton remodeling and to elucidate the modulatory effect of SMV on VEGF-induced cytoskeletal remodeling, MC were incubated with VEGF (50 ng/ml) for 30 min in the presence or absence of SMV (1 μM) and the actin cytoskeleton was visualized by rhodamine phalloidin staining using scanning confocal electron microscopy. MC treated with VEGF exhibited a significant increase in actin stress fiber formation (Fig. 5B). Upon treatment with SMV (1 μM), the density of stress fibers was significantly decreased (Fig. 5C). Because RhoA is one of the major regulators of actin stress fiber formation, we further examined whether RhoA mediates VEGF-induced stress fiber formation. As shown in Fig. 5D,
MC transfected with dominant-negative RhoA also showed a significant reduction in VEGF-induced density of stress fibers, indicating that VEGF-induced actin stress fiber formation was mediated by a RhoA-dependent pathway.

Effects of VEGF and SMV on tyrosine phosphorylation of FAK. FAK, a cytoplasmic protein tyrosine kinase, binds directly to the cytoplasmic domain of \( \beta_1 \)-integrin subunits and plays an important role in the integrin-mediated signaling pathway (31, 38). To examine the potential involvement of FAK phosphorylation in VEGF-induced signaling, we performed laser-scanning confocal immunofluorescent microscopy using anti-phosphorylated FAK (Tyr397) antibody. MC stimulated with VEGF (50 ng/ml) exhibited a significant increase in FAK Tyr397 phosphorylation (Fig. 6). However, in cells cotreated with SMV (1 \( \mu \)M), VEGF-induced FAK phosphorylation was significantly reduced (Fig. 6C). MC transfected with dominant-negative mutant of RhoA also showed a significant decrease in VEGF-induced FAK phosphorylation (Fig. 6D) indicating the central role of RhoA in VEGF-induced FAK phosphorylation.

We also assessed VEGF-induced total and phosphorylated FAK protein expression by Western blot analysis. Whereas VEGF stimulation (50 ng/ml) significantly increased phospho-FAK protein levels, SMV (1 \( \mu \)M) and MC transfected with N17RhoA exhibited significantly lower protein levels of phospho-FAK. Total FAK protein levels were unchanged as shown in Fig. 7. Thus our data indicate that VEGF signaling pathway involves FAK phosphorylation, and RhoA mediates VEGF-induced FAK phosphorylation.

For determining whether actin cytoskeleton and FAK phosphorylation are involved in VEGF-induced cell-associated collagen synthesis, MC were treated with cytochalasin D (Cyto D; 1.0 \( \mu \)g/ml). Cyto D, a specific inhibitor of filament actin cytoskeleton, has also been shown to abolish FAK phosphorylation (25). As shown in Fig. 8, VEGF (50 ng/ml) stimulation did not increase the levels of cell layer collagen type IV in MC treated with Cyto D, indicating that VEGF-induced actin cytoskeleton reorganization is necessary for VEGF-induced collagen synthesis.

Effects of VEGF and SMV on VEGF-induced \( \beta_1 \)-integrin activation. Growing evidence has indicated that \( \beta_1 \)-integrins mediate growth factor-dependent expansion of ECM in MC (23, 26). However, whether VEGF-induced mesangial matrix expansion is also mediated by \( \beta_1 \)-integrin activation and whether RhoA activation is necessary for VEGF-induced \( \beta_1 \)-integrin activation have not yet been explored. Accordingly, we examined the effect of VEGF and SMV on \( \beta_1 \)-integrin activation. Integrins can switch between active and inactive conformations. In the inactive state, integrins have a low affinity for ligands. Intracellular signaling events such as protein kinase C stimulation can prime the integrins, which result in a conformational change that makes the extracellular domain competent for ligand binding by exposing the ligand-binding
To address the effect of VEGF and SMV on β1-integrin activation, serum-starved MC were stimulated with VEGF (50 ng/ml) and β1-integrin activation was detected by flow cytometry using an antibody (HUTS-21) specific for the active conformation of β1-integrins, for 30 min at 37°C. Cells were then lysed in SDS sample buffer and bound antibody was detected by Western blot analysis as previously described (48). The results in Fig. 9C indicate that stimulation of MC with VEGF caused a significant increase in binding of HUTS-4 as well as HUTS-21 (data not shown). Cotreatment of cells with SMV (1 μM) inhibited VEGF-induced increase in β1 integrin activation. The inhibitory effect of SMV was reversed when the cells were cotreated with MEV (300 μM). The data in Fig. 9C also indicate that RhoA activation is necessary to stimulate β1-integrin activation since VEGF stimulation failed to activate β1-integrin in MC transfected with a dominant-negative mutant of RhoA, N17RhoA, indicating that VEGF-induced β1-integrin activation is mediated by a RhoA-dependent pathway. This experiment also provides strong evidence for the proposed VEGF-induced “inside-out” signaling pathway since our data indicate that RhoA activation is necessary before β1-integrin activation.

To further ascertain that β1-integrin activation is necessary for VEGF-induced collagen synthesis, MC were treated with a monoclonal function-blocking anti-β1 integrin antibody (20 μg/ml) and stimulated with VEGF (50 ng/ml). As shown in Fig. 10, VEGF stimulation did not increase the protein levels of cell layer collagen type IV in MC when β1-integrin activation was blocked, indicating that β1-integrin activation is necessary for VEGF-induced cell layer collagen synthesis.

DISCUSSION

This study describes the signaling events that are responsible for VEGF-induced collagen IV accumulation in MC. Our data suggest that VEGF triggers an inside-out signaling pathway in MC which is characterized by RhoA activation, actin cytoskeleton remodeling, intracellular FAK phosphorylation, and β1-integrin activation leading to increased type IV collagen synthesis.
thesis. Thus the results of the present study provide a missing piece of the mechanistic puzzle concerning VEGF-induced mesangial matrix expansion. Furthermore, our data also provide strong evidence that SMV, by preventing RhoA activation, inhibits VEGF signaling pathway and enhanced collagen synthesis in MC.

Statins are commonly used drugs in the treatment of patients with hypercholesterolemia that have been suggested to exert significant pleiotropic effects on cell signaling pathways largely by preventing mevalonic acid biosynthesis (27, 36, 47, 50, 51). MEV is necessary for the posttranslational lipid modification (isoprenylation) of small GTPase proteins, a process essential for the proper translocation of Rho GTPases from the cytosol to the membrane where activation of these proteins takes place (3, 15, 43). Previous studies from our laboratory and others indicated that statins modulate several cellular processes by preventing prenylation of small Rho GTPases such as RhoA and Rac1 (6, 7, 20, 27, 29, 36, 49, 55). In support of a modulatory effect of statins on ECM accumulation, Kim et al. (20) reported that lovastatin inhibited high glucose-induced overexpression of fibronectin. Similarly, Nishimura et al. (29) showed that pravastatin prevented serum-induced type IV collagen secretion. The data presented in this study are consistent with these previous results by establishing the inhibitory effects of statins on ECM expansion. In addition, we clearly characterized the sequential activation of various components of VEGF-induced signaling pathway in the current study and identified the modulatory effect of HMG-CoA reductase inhibitors and MEV depletion on VEGF signaling.

To decipher VEGF signaling, we initially explored the role of RhoA activation on VEGF-induced signaling pathway in MC. We showed that RhoA activity is required for VEGF-induced collagen synthesis since MC transfected with dominant-negative RhoA mutant failed to increase cell layer type IV collagen protein levels in response to VEGF. Cotreatment of MC with SMV also inhibited VEGF-induced collagen accumulation by preventing RhoA activation. The addition of MEV reversed the inhibitory effect of SMV on VEGF-induced RhoA activity and collagen accumulation, indicating that the effect of SMV on VEGF-induced collagen accumulation was MEV dependent. Taken together, our data suggest that RhoA mediates VEGF-induced collagen synthesis; and SMV inhibits VEGF signaling to ECM by preventing RhoA inactivation.

The list of cellular events instigated by actin cytoskeletal reorganization is rapidly growing. For instance, Hubchak et al. (17) showed that TGF-β1-induced collagen accumulation is associated with cytoskeleton reorganization. Moreover, Cyto D, an inhibitor of actin cytoskeletal assembly, has been previously reported to disrupt the formation of fibronectin networks (30, 54). In this study, we report that VEGF induces actin cytoskeletal rearrangement in MC, and disruption of actin cytoskeleton with Cyto D reduces VEGF-stimulated collagen type IV synthesis. Furthermore, our data indicate that VEGF-induced actin cytoskeletal remodeling is mediated by RhoA.
activation which leads to β₁-integrin activation. Thus the results of this study show that actin cytoskeletal remodeling, regulated by RhoA activation, is required for VEGF-induced collagen synthesis.

Integrins, a group of heterodimeric transmembrane receptors, play a pivotal role in ECM assembly and cell-ECM interactions (32, 35). A critical role of integrins is to provide a link between ECM and the cytoskeleton, whereby ECM ligand-integrins interactions can activate intracellular signaling transduction cascades resulting in enhanced gene expression and cellular differentiation ("outside-in" signaling) (13, 42). Interestingly, several recent studies indicated that signals from within the cells can also propagate through integrins and regulate ECM remodeling through an, as yet, incompletely understood mechanism termed "inside-out" signaling (14, 18, 19, 39, 44). Activation of integrins through inside-out signaling seems to be mediated by increased affinity and/or avidity state of integrins (44). The affinity state of integrin is regulated by its conformational changes induced by a number of signaling pathways or proteins that interact with the cytoplasmic domains of the integrins. Structural studies suggest that modulation of integrin affinity involves changes in the spatial relationship of the cytoplasmic and/or transmembrane domains of the α- and β-subunits (52). The β cytoplasmic domain is critical for recruitment of integrins to focal contacts since its truncation/mutation impairs this process (33). Several previous studies suggested a central role for β₁-integrin activation in mesangial matrix accumulation (21, 22, 24). In this study, we assessed the contribution of β₁-integrin activation on VEGF signaling to ECM. Our data indicate that VEGF stimulation significantly increases β₁-integrins activity. Moreover, the results of this study suggest that SMV inhibits increased affinity of β₁-integrins via a MEV-dependent pathway. Thus the data presented in this study provide strong evidence indicating that β₁-integrins activation is required for VEGF-induced collagen synthesis since a specific β₁-integrin blocker prevented increased protein levels of cell layer collagen type IV. Our study also provides the first evidence that VEGF-induced activation of β₁-integrins in MC is mediated by RhoA activation because VEGF stimulation failed to increase β₁-integrin activity in cells transfected with dominant-negative mutant of RhoA. Furthermore, our data clearly demonstrate that RhoA activation precedes β₁-integrin activation consistent with an inside-out signaling. This adds to the growing body of evidence of the importance of RhoA activation in inside-out signaling.

Several cytoplasmic proteins including talin, α-actin, and FAK bind to the β₁ cytoplasmic domain of integrins and contribute to integrin cytoskeletal interactions (2, 10, 31, 38, 53). However, earlier studies on integrin-dependent cell adhesion and signaling demonstrated that integrin clustering could also trigger increased tyrosine phosphorylation of a 120-kDa tyrosine kinase known as FAK (2, 10, 53). FAK activation as demonstrated by an increase in phosphorylation on Tyr 397 is an integral component of the integrin signaling pathway (10, 53). To explore whether FAK phosphorylation was involved in VEGF-induced signaling pathway, we examined the effect of VEGF and SMV on FAK phosphorylation. The data presented in this study suggest that FAK phosphorylation is involved in the inside-out VEGF-dependent signaling by transmitting RhoA activation and actin cytoskeletal cues to ECM leading ultimately to collagen accumulation. Our data indicate that VEGF stimulation increased FAK Tyr 397 phosphorylation, and FAK phosphorylation was inhibited by SMV. We also showed that VEGF failed to increase FAK phosphorylation in MC transfected with dominant-negative mutant of RhoA. Thus our results suggest that VEGF, via a RhoA-dependent pathway, mediates tyrosine phosphorylation of FAK.

In conclusion, we demonstrated that VEGF-induced collagen accumulation involves several mediators that include activation of small GTPase protein, RhoA, reorganization of the actin cytoskeleton, FAK phosphorylation, and activation of β₁ integrins. Based on these data, we propose an inside-out signaling model for VEGF-induced mesangial matrix expansion. This model provides several novel targets in the therapeutic approach to the abnormal mesangial matrix expansion.
observed in the diabetic milieu. Our study also provides a new rationale to the use of statins, independent of their cholesterol-lowering properties, in the early stages of DN.

GRANTS

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