Smooth muscle cell (SMC) differentiation is regulated by a complex array of local environmental cues, but the intracellular signaling pathways and the transcription mechanisms that regulate this process are largely unknown. We and others have shown that serum response factor (SRF) contributes to SMC-specific gene transcription, and because the small GTPase RhoA has been shown to regulate SRF, the goal of the present study was to test the hypothesis that RhoA signaling is a critical mechanism for regulating SMC differentiation. Coexpression of constitutively active RhoA in rat aortic SMC cultures significantly increased the activity of the SMC-specific promoters, SM22 and SM α-actin, whereas coexpression of C3 transferase abolished the activity of these promoters. Inhibition of either stress fiber formation with the Rho kinase inhibitor Y-27632 (10 μM) or actin polymerization with latrunculin B (0.5 μM) significantly decreased the activity of SM22 and SM α-actin promoters. In contrast, increasing actin polymerization with jasplakinolide (0.5 μM) increased SM22 and SM α-actin promoter activity by 22-fold and 13-fold, respectively. The above interventions had little or no effect on the transcription of an SRF-dependent c-fos promoter or on a minimal thymidine kinase promoter that is not SRF-dependent. Taken together, the results of these studies indicate that in SMC, RhoA-dependent regulation of the actin cytoskeleton selectively regulates SMC differentiation marker gene expression by modulating SRF-dependent transcription. The results also suggest that RhoA signaling may serve as a convergence point for the multiple signaling pathways that regulate SMC differentiation.

Vascular smooth muscle cell (SMC)\(^3\) differentiation is an important process during vasculogenesis and angiogenesis, and it is recognized that alterations in SMC phenotype play a role in the progression of several prominent cardiovascular disease states including atherosclerosis, hypertension, and restenosis (1–3). It is well established that SMC do not terminally differentiate and that SMC phenotype is regulated by a complex array of local environmental cues including humoral factors, cell-cell and cell-matrix interactions, inflammatory stimuli, and mechanical stresses (reviewed in Refs. 4 and 5). However, the mechanisms by which these diverse signals regulate SMC phenotype and the transcription mechanisms that ultimately regulate SMC differentiation are largely unknown. It is clear that the identification of the signaling and transcription pathways that control SMC-specific gene expression will be an important step toward our understanding of blood vessel development and the role played by SMC during the development of cardiovascular disease.

To date, no transcription factors have been identified that specify SMC lineage or by themselves can explain SMC-specific gene expression. However, serum response factor (SRF), a MADS box transcription factor, has been shown to contribute to the regulation of most SMC differentiation marker genes and may be a key transcriptional regulator of SMC differentiation (6–9). Interestingly, CArG cis-elements (CC(A/T))\(_6\)GG) that bind SRF are present in the promoters of virtually all of the SMC-specific marker genes including SM α-actin, SM myosin heavy chain, SM22, h-caldesmon, calponin, and telokin. The CArGs within any given SMC-specific promoter are highly conserved among species and have been shown to be required for the high level of activity that these promoters exhibit in SMC cultures (7, 8, 10–14). Results from transgenic studies demonstrate that CArG elements within the SM22 and SM α-actin promoters were also required for the expression of these genes in vivo (8–10). Indeed, we have shown that interactions among multiple CArG elements are important for the in vivo expression of SM α-actin and that a CArG element within the SM α-actin first intron functions as a SMC-specific enhancer-like element (10). Because SRF is ubiquitously expressed, the mechanisms whereby it contributes to SMC differentiation are somewhat unclear. However, evidence suggests that other factors including additional cis- and trans-regulatory elements may mediate cell-type-specific regulation by SRF (6, 15–22).

The CArG motif was first identified as the core sequence of the serum response element (SRE) within early response genes such as c-fos, and much of what is known regarding the signaling pathways that regulate SRF-dependent transcription resulted from studies on the c-fos promoter (reviewed in Ref. 23). During serum- or growth factor-mediated stimulation, SRF forms an initiation complex with a protein from the ternary complex factor family whose members (Elk-1, SAP-1, and Net/Erp) are regulated by MAP kinase phosphorylation (24–26). The ternary complex is stabilized by protein interactions between SRF and the ternary complex factor and by interactions between the ternary complex factor and the Ets cis-element that flanks the SRE CArG (27). Recent evidence suggests that SRF-dependent transcription is also regulated by the small factors.
GTP-binding protein RhoA, which is required for serum-induced c-fos expression, and may be important for regulating CARG-dependent genes in the skeletal muscle as well (28–31). The effects of RhoA on SRF-dependent transcription are not dependent upon the presence of an Ets domain or on the known SRF accessory proteins suggesting that RhoA activates SRF-dependent transcription in a ternary complex-independent manner. Importantly, the CARG elements within the SMC-specific promoters are not flanked by a consensus Ets domain and do not appear to be regulated by an Ets-dependent mechanism.

Recently, Sotiropoulos et al. (32) have shown that in NIH 3T3 cells, the effects of RhoA on SRF-mediated transcription may be secondary to the actions of RhoA on the actin cytoskeleton. These results are of particular importance because they are the first to suggest that SRF-dependent transcription is positively regulated through increases in actin polymerization. The exact mechanisms for SRF activation remain to be identified, and the significance of these findings in regard to the expression of SRF-dependent gene targets is unclear because an artificial c-fos promoter (lacking a consensus Ets domain) was used as the primary measure of Rho-dependent SRF activation.

In the present studies, we extend the observations of Sotiropoulos et al. (32) by demonstrating in SMC that multiple SMC-specific promoters are regulated via RhoA-dependent changes in actin dynamics. In addition, we demonstrate that these RhoA-induced changes are at least partially mediated by Rho kinase. Because virtually all of the SMC-specific promoters identified to date are regulated by SRF, these results suggest that RhoA signaling regulates SMC phenotype. Moreover, because many of the environmental factors that regulate SMC phenotype have also been shown to activate RhoA, these results also suggest that RhoA may help to integrate multiple environmental signals that regulate SMC differentiation.

**MATERIALS AND METHODS**

**Cell Culture Transient Transfections and Reporter Assays—**SMC from rat thoracic aorta were isolated, cultured, and transfected as described (88, 293, 34). In short, cells were maintained in 10% serum and were transfected 24 h after plating at 70–80% confluency with 2 μg of reporter plasmid DNA, using the transfection reagent Superfect (Qiagen) as per protocol. The SM22 promoter (from −450 to +88), the SM α-actin promoter (p2600Int, from −2560 to +2784, and p125, from −125 to +44), and the c-fos promoter (from −356 to +109) that were used in this study have been described previously (8–10, 35). Coexpression experiments were carried out by including 0.25 μg of the following expression constructs: empty Prk5 vector; Prk5 containing constitutively active RhoA (L63), Cdc42 (L61), or Rac (L61); or Prk5 containing C3 transerase (expression plasmids were a generous gift from Alain Hall, University College, London, United Kingdom). Cells expressing C3 were maintained in 10% serum, whereas cells expressing the small G proteins were maintained in defined serum-free medium. After 48 h, cell lysates were prepared for the measurement of CAT activity using standard methods (34). The CAT activity of each sample was normalized to the protein concentration of each cell lysate as measured by the Bradford assay. A promoterless CAT construct was also transfected to serve as a base-line indicator of CAT activity, and the activity of each promoter construct was expressed relative to promoterless activity. Additionally, an SV40 promoter-CAT construct with enhancer (Promega) served as a positive control of transfection and CAT activity. All CAT activities represent at least three independent experiments with each construct tested in triplicate per experiment. Relative CAT activity data are expressed as the means ± S.D. computed from the results obtained from each set of transfection experiments. We did not cotransfect a viral promoter/LacZ construct as a control for transfection efficiency because we have previously shown that such constructs exhibit unknown and variable squelching effects on the SM-specific promoters, presumably due to competition for common transcription factors (34). Moreover, we have found that the inclusion of such controls is unnecessary in that variations in transfection efficiency among independent experimental samples are routinely very small (<10%) (34).

**RESULTS**

**SMC-specific Promoter Activity Was Regulated by RhoA Signaling—**Current evidence suggests that signaling through RhoA is required for SRE-dependent c-fos expression and regulates several CARG-containing genes in the skeletal muscle (28–31). To determine whether RhoA was important for CARG-dependent SMC-specific gene regulation, we cotransfected CAT reporter constructs driven by the SM22 and SM α-actin promoters into rat aortic SMC along with an expression vector containing C3 transerase, which specifically ADP-ribosylates and irreversibly inhibits RhoA. The SM22 promoter (from −450 to +88) and the SM α-actin promoter (from −2600 to +2754) used in these studies were shown to direct SMC-specific regulation of these genes in transgenic models (8–10). C3 transerase expression completely abolished the activity of both promoters, indicating that RhoA activity is required for the expression of these constructs in SMC (Fig. 1A). To test the involvement of RhoA more directly, we also cotransfected a constitutively active RhoA (L63) into serum-starved SMC. L63 RhoA trans-activated the SM22 and the full-length SM α-actin promoters by 10-fold and 6-fold, respectively (Fig. 1B). Constitutively active RhoA also dramatically increased the activity of a minimal SM α-actin promoter (−125 to +44) that is regulated by two CARG elements within this short promoter region but had no effect on the same construct that contained mutations to the CARG elements (data not shown). These results indicate that the effects of RhoA on SMC-specific gene expression were mediated by a CARG/SRF-dependent mechanism. Significantly, L63 RhoA had relatively modest effects on the activity of an Ets-containing SRE-dependent c-fos promoter in SMC.

Because the Rho family members Cdc42 and Rac have also been shown to up-regulate the SRF activity of c-fos, we also tested whether the constitutively active forms of these proteins would regulate SMC differentiation marker gene expression. Although all three small GTPases slightly but significantly increased c-fos promoter activity (p < 0.01), SM α-actin promoter activity was not affected by constitutively active Rac and was slightly inhibited by constitutively active Cdc42 (Fig. 1C).

**Drug Treatment and F-Actin Visualization—**Latrunculin B and cytochalasin D were purchased from Calbiochem (San Diego, CA). Jaspaklindole was purchased from Molecular Probes (Eugene, OR). Y-27632 was a generous gift from Akiko Yoshimura (Welfide Corporation, Osaka, Japan). Before drug treatment, SMC were transfected with SMC-specific reporter constructs and c-fos reporter constructs as described above. Immediately after the removal of the Superfect transfection reagent, cells were either treated with latrunculin B (0.5 μM), jasplakindole (0.5 μM), cytochalasin D (2 μM), Y-27632 (10 μM), or vehicle. SMC treated with latrunculin B or Y-27632 were maintained in 10% serum, and those treated with jasplakindole or cytochalasin D were maintained in defined serum-free medium. After 12 h, cells were harvested, and reporter assays were performed as described above. Drug-induced effects on actin polymerization were measured in parallel experiments for all groups. At 0, 5, 10, and 24 h after drug treatment, cells were rinsed three times with ice-cold phosphate-buffered saline, fixed with 4% paraformaldehyde, stained with FITC-phalloidin, and visualized with a fluorescent microscope. SMC transfected with a constitutively active Myc-tagged L63 RhoA were immunostained with an anti-C-Myc antibody and counterstained with FITC-phalloidin.

**Measurement of Endogenous Actin Expression—**Control- or Y-27632-treated SMC were maintained in minimal methionine medium containing 10% fetal calf serum for 12 h. Cells were then switched to fresh medium containing 50 μCi/ml[^35S]methionine and were incubated for an additional 12 h. Cells were then rinsed three times with ice-cold phosphate-buffered saline and lysed in loading buffer. Equal amounts of DNA were loaded onto tube gels, and actin isoforms were visualized by standard two-dimensional gel electrophoresis as described previously (36). Multiple labeling experiments were performed, and three separate gels were run for each treatment group per experiment. The ratio of radiolabeled SM α-actin to nonmuscle β-actin was determined by densitometry using ImageQuant software (Molecular Dynamics, Sunnydale, CA).

**REFERENCES**

1. MATSUMOTO, M., A. KURASHIGE, M. INOUE, Y. KAMADA, H. MIYAKE, AND R. NAKAI. 1983. Expression and genetic mapping of a c-fos-related cDNA from mouse embryonic RNA. J. Biol. Chem. 258: 7577–7581.
2. MATSUMOTO, M., A. KURASHIGE, M. INOUE, Y. KAMADA, H. MIYAKE, AND R. NAKAI. 1983. Expression and genetic mapping of a c-fos-related cDNA from mouse embryonic RNA. J. Biol. Chem. 258: 7577–7581.
RhoA regulated Stress Fiber Formation in SMC—Sotiropoulos et al. (32) have suggested that RhoA activation regulates gene expression through its effects on the actin cytoskeleton. Therefore, to test the effects of RhoA activation on actin polymerization, we transfected a Myc-tagged L63 RhoA expression plasmid into SMC that were then stained for F-actin stress fibers with FITC-phalloidin. Fig. 2B shows that untransfected SMC maintained in 10% serum had fairly well developed stress fibers (small arrows). However, SMC expressing L63 RhoA had dramatically increased actin stress fiber networks (large arrows), demonstrating that RhoA activation regulates stress fiber formation in SMC.

SMC Differentiation Marker Gene Transcription Was Regulated by Actin Polymerization—Our results suggested that RhoA activation regulates both SMC-specific gene transcription and stress fiber formation in SMC. To test whether the effects of RhoA on SMC-specific promoter activity were mediated by its effects on actin dynamics, we treated SMC with latrunculin B, a toxin that disrupts actin polymerization. Latrunculin B treatment (0.5 mM) of SMC in 10% serum disrupted stress fiber formation for up to 12 h (Fig. 3A). After this time, actin polymerization gradually returned, and by 24 h SMC treated with latrunculin B were virtually indistinguishable from untreated cells. In addition, latrunculin B also had little effect on total cellular protein, which indicated that this drug was not overtly cytotoxic under these conditions. Latrunculin B did not affect c-fos promoter activity but dramatically attenuated the activities of the SM22 and SMα-actin promoters (Fig. 3B). Importantly, latrunculin B also inhibited trans-activation of the SMα-actin promoter by L63 RhoA as well as L63 RhoA-induced stress fiber formation.

The results presented in Fig. 3 suggest that RhoA activation induces SMC-specific gene expression by increasing actin polymerization. Therefore, to further test whether SM α-actin transcription was sensitive to actin dynamics, we treated SMC with jasplakinolide, a toxin known to increase actin polymerization (37). SMC treated with 0.5 mM jasplakinolide and labeled with FITC-phalloidin had very disorganized actin structures.
and contained large amorphous actin aggregates (data not shown). These effects are consistent with the known actions of this toxin, which sever F-actin, creating new nucleation sites that support actin polymerization (37). Jasplakinolide increased SM22 and SM α-actin promoter activities by 22-fold and 14-fold, respectively, but increased c-fos promoter activity by only 3-fold. Cytochalasin D had similar effects increasing the activities of the SM22 and SM α-actin promoters but having no effect on the c-fos promoter. The fact that cytochalasin D (which disrupts actin polymerization) increased SMC-specific promoter activity suggests that cytochalasin-induced SRF activity is not directly regulated by increased actin polymerization per se and that additional mechanisms that sense actin dynamics in SMC may be involved in regulating SMC-specific transcription. Interestingly, Sotiropoulos et al. (32) made similar observations in their studies on the c-fos SRE and suggested that SRF-dependent transcription may be negatively regulated by monomeric G-actin and that cytochalasin D may sequester monomeric G-actin in a manner that effectively reduces the free pool. To determine whether the effects of cytochalasin D were independent of those mediated by RhoA, we treated SMC transfected with L63 RhoA with cytochalasin D. The treatment of SMC with cytochalasin D prevented both the L63 RhoA-induced increase in differentiation marker gene expression (Fig. 4B) and L63 RhoA-induced stress fiber formation (data not shown).

Transcriptional Regulation by RhoA in SMC Was Partially Mediated by Rho Kinase—It is well known that RhoA-dependent activation of Rho kinase induces actin polymerization and stress fiber formation in many cell types (reviewed in Ref. 38). However, it is unclear whether the activity of Rho kinase correlates with the effects of RhoA on the transcription of c-fos (39–42). Our results indicated that in SMC, the SMC-specific promoters were significantly more sensitive than the c-fos promoter was to activation by RhoA signaling. Therefore, to test whether SMC-specific transcription was regulated through the activation of Rho kinase, we treated SMC with the highly specific Rho kinase inhibitor Y-27632 (43). The micrograph in Fig. 5A shows that 10 μM Y-27632 significantly inhibited stress fiber formation in SMC and also led to the appearance of long thin cellular extensions. Y-27632 also reduced the activity of transiently transfected SMC-specific promoters by 50–60% (Fig. 5B), although it had little effect on the c-fos promoter or on the minimal thymidine kinase (TK) promoter that does not contain CArG elements. Fig. 5C is an autoradiograph from a representative two-dimensional gel that illustrates the effects of Y-27632 on the expression of endogenous actin isoforms. Interestingly, the results showed that all of these CArG-containing actin genes were down-regulated to some extent. However, the synthesis of the SM-specific α-actin isoform was inhibited to a greater extent than the ubiquitously expressed β-actin isoform (α: β synthesis ratios were reduced by greater than 40% in Y-27632-treated SMC, p < 0.01). Taken together, these results suggest that Rho kinase plays an important role in the regulation of SMC differentiation marker gene expression and that ubiquitously expressed CArG-dependent genes, such as c-fos and nonmuscle β-actin, may be less sensitive to this regulatory pathway.

DISCUSSION

A major goal of our laboratory has been to elucidate control processes that regulate SMC differentiation by identifying the mechanisms that coordinate the transcription of SMC differentiation marker genes. The focus of the present study...
was to investigate the role of RhoA signaling in the regulation of SMC-specific gene transcription. Because RhoA activates SRF, a transcription factor that has been shown to regulate virtually all of the SMC-specific marker genes identified to date (7, 8, 10–14), we hypothesized that RhoA signaling contributes to the coordinate regulation of SMC-specific gene expression.

The data presented indicate that: 1) RhoA activation is important for the expression of several SMC-specific marker genes; 2) regulation of actin polymerization by RhoA (but not by Cdc42 or Rac) may be an important determinant of SMC-specific gene transcription; 3) RhoA-dependent regulation of SMC-specific gene transcription is mediated at least in part by Rho kinase; and 4) in SMC, the CARG-dependent SMC-specific genes (i.e. SM22, SM α-actin) and the CARG-dependent genes (i.e. c-fos, β-actin) that are ubiquitously expressed are differentially regulated by RhoA.

Results presented in Fig. 1 provide strong evidence that RhoA activity is required for SMC-specific promoter activity because C3 transferase, which ADP-ribosylates and irreversibly inactivates RhoA, completely inhibited the activities of the SM22 and SM α-actin promoters. Constitutively active RhoA up-regulated several SMC-specific CARG-dependent genes, further indicating that RhoA activation is important for this response. RhoA activation has been shown to have similar effects on the SRF-dependent activity of several CARG-dependent skeletal muscle-specific genes (30, 31), suggesting that RhoA activation may be a common signaling pathway that regulates muscle-specific gene expression. In fact, RhoA also regulates SRF expression (probably through CARG elements within the SRF promoter) and may be part of an important positive feedback mechanism for maintaining high levels of SRF expression in all three muscle cell types (44, 45). Although our results indicate that RhoA-dependent activation of SRF plays a major role in SMC-specific transcription, other mechanisms must be involved that distinguish SRF-dependent transcriptional regulation in SMC from SRF-dependent transcriptional regulation in the other muscle cell types. For example, it is well known that in cardiac and skeletal muscles, SRF interacts with other...
cardiac and skeletal muscle-specific factors, such as myoD, GATA-4, and Nkx2.5, to regulate cell-type-specific expression (22, 46–48). However, factors that confer SMC-specific gene regulation have not been identified in SMC. We have recently provided evidence that an SRF-associated protein that is selectively expressed in SMC may contribute to SMC-specific gene regulation by SRF (6). Several splice variants of SRF have also been described recently, but a detailed description of the expression patterns and activities of these variants has not been reported, which makes it difficult to determine whether alternative splicing of SRF contributes to cell-type-specific gene regulation (49, 50).

Our results also indicate that multiple SMC-specific differentiation marker genes are regulated by RhoA-induced changes in the actin cytoskeleton. Constitutively active RhoA trans-activated the SM22 and SM α-actin promoters but had little effect on the CARG-containing c-fos promoter. Inhibiting actin polymerization with latrunculin B blocked trans-activation by constitutively active RhoA, which supports a model in which RhoA-mediated increases in F-actin promote SMC differentiation marker gene transcription. Our observation that jasplakinolide, an actin polymerizing agent, dramatically increased SMC-specific promoter activity is also consistent with such a model. The constitutively active forms of Cdc42 and Rac did not stimulate SMC-specific promoter activity, suggesting that actin polymerization at the cell periphery does not regulate SMC-specific transcription and suggests that separate pools of actin may differentially regulate SMC function. Our data also indicate that signaling through Rho kinase is significant, but because Y-27632 only inhibited promoter activity by 50–60%, we cannot rule out the involvement of other Rho effectors, such as PKN, citron kinase, rhotekin, and the diaphanous family of proteins.

The precise mechanisms by which actin dynamics affect SRF-dependent transcription are currently unclear. Based in part on the observed effects of cytochalasin D, Sotiropoulos (32) has suggested a model whereby G-actin inhibits SRF directly or sequesters cofactors required for SRF activation. Our data indicate that cytochalasin D-induced activation of SRF-dependent SMC-specific transcription does not require an intact actin stress fiber network. However, we did show that the expression of constitutively active Rhos in cytochalasin D-treated SMC did not result in stress fiber formation or in an increase in SM α-actin promoter activity above that seen with cytochalasin D alone. These data indicate that in SMC these interventions probably converge at some level and that RhoA must affect actin polymerization to increase SMC marker gene transcription. There is some evidence to suggest that nuclear actin may regulate transcription by helping to modify chromatin structure (51), and it has been shown that G-actin shuttles through the nucleus (52) and binds specifically to important nuclear proteins including the SWI/SNF complex (53, 54). It is also important to consider that the observed effects of RhoA activation (and of the cytoskeletal inhibitors) may be partially due to secondary changes in cell morphology that occur under these conditions. In addition, the pharmacological mechanisms of the cytoskeletal inhibitors used in this study are only partially understood, and undescribed side effects of these drugs may have affected SMC differentiation marker gene expression.

The signal transduction pathways that regulate SMC differentiation are only partially understood, but results from the present studies suggest that RhoA activation may serve as a convergence point for multiple environmental factors known to regulate SMC-specific gene expression and differentiation. For example, integrin-matrix interactions, mechanical stretch, and contractile agonists, such as endothelin-1 and angiotensin II, are known to regulate SMC-specific gene expression as well as the activity of RhoA (40, 55–57). Whether these environmental factors regulate SMC differentiation through RhoA-dependent signaling mechanisms and/or changes in actin dynamics will be an important area for future studies.

RhoA activation is also required for growth factor-induced expression of c-fos, and recent evidence suggests that RhoA activation may mediate the growth response of SMC to angiotensin II, thrombin, and mechanical stretch (40, 55, 58). The data presented in this manuscript, however, demonstrate that c-fos expression in SMC is not dramatically up-regulated by constitutively active RhoA or by drug-induced modulation of the actin cytoskeleton, indicating that the RhoA signaling pathways that regulate SMC differentiation marker gene expression diverge from those that regulate cell growth and proliferation. SMC proliferation and differentiation are not necessarily mutually exclusive (4), and it will be very important to further clarify the specific signals that differentiate these two SRF-dependent pathways. Indeed, Seasholtz et al. (58) found that constitutively active RhoA only potentiates SMC proliferation in the presence of activated Ras, and results from Alberts et al. (28) indicate that additional signaling through the SAPK/JNK pathway may be important for the Rho-mediated regulation of c-fos. Integrin signaling also requires Rho activation (59–61), and it interesting to speculate that Rho and integrin pathways may converge (perhaps at the level of the actin cytoskeleton) to regulate SMC growth and/or differentiation.

In summary, the data presented herein indicate that RhoA signaling plays a major role in the SRF-dependent regulation of SMC differentiation marker gene expression. Although the exact mechanisms involved in controlling the transcriptional activation of SRF are not completely clear, the activation of Rho kinase and RhoA-specific changes in actin polymerization seem to be involved. Interestingly, most SMC-specific differentiation marker genes identified to date code for contractile or contractile-associated proteins (4). Therefore, RhoA signaling may also help to determine SMC phenotype (and possibly the phenotype of other muscle cells as well) by increasing contractile protein gene expression. Moreover, when coupled with extensive evidence that RhoA increases SMC contractility (43, 62), our data suggest that short term regulation of SMC contractile force may be coupled to long term regulation of SMC contractile-associated gene expression by a RhoA-dependent mechanism. Finally, given the importance of SMC growth and differentiation during blood vessel development and cardiovascular pathophysiology, further studies to identify the mechanisms that regulate RhoA-dependent changes in SMC differentiation marker gene expression may provide novel insights regarding the mechanisms that contribute to the abnormal regulation of SMC differentiation characteristic of vascular diseases such as atherosclerosis.

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REFERENCES
1. Ross, R. (1993) Nature 362, 801–809
2. Kocher, O., and Gabbiani, G. (1986) Hum. Pathol. 17, 875–880
3. Kocher, O., Gabbiani, F., Gabbiani, G., Reddy, M. A., Cukay, M. S., Peters, H., and Huttner, I. (1991) Lab. Invest. 65, 459–470
4. Owens, G. K. (1995) Physiol. Rev. 75, 487–517
5. Owens, G. K., and Wise, G. (1997) Agents Actions Suppl. 48, 3–24
6. Mack, C. P., Thompson, M. M., Lawrenz-Smith, S., and Owens, G. K. (2000) Circ. Res. 86, 221–232
7. Madsen, C. S., Hershey, J. C., Hautmann, M. B., White, S. L., and Owens, G. K. (1997) J. Biol. Chem. 272, 6332–6340
8. Kim, S., Ip, H. S., Lu, M. M., Clendenin, C., and Parmacek, M. S. (1997) Mol. Cell. Biol. 17, 2266–2278
9. Li, L., Liu, Z., Mercer, B., Overbeek, P., and Olson, E. N. (1997) Dev. Biol. 187, 311–321
10. Mack, C. P., and Owens, G. K. (1999) Circ. Res. 84, 852–861
11. Yano, H., Hayashi, K., Momiyama, T., Saga, H., Haruna, M., and Sobue, K. (1995) J. Biol. Chem. 270, 23661–23666
12. Herring, B. P., and Smith, A. F. (1997) Am. J. Physiol. 272, C1394–C1404
13. Momiyama, T., Hayashi, K., Obata, H., Chimori, Y., Nishida, T., Ito, T., Kamikawa, W., Matsuda, H., and Sobue, K. (1998) Biochem. Biophys. Res. Commun. 242, 429–435
14. Miano, J. M., Carlson, M. J., Spencer, J. A., and Misra, R. P. (2000) J. Biol. Chem. 275, 9614–9622
15. Carson, J. A., Schwartz, R. J., and Booth, F. W. (1996) Am. J. Physiol. 270, C1624–C1633
16. Catala, F., Wanner, R., Barton, P., Cohen, A., Wright, W., and Buckingham, M. (1995) Mol. Cell. Biol. 15, 4585–4596
17. Chen, C. Y., and Schwartz, R. J. (1996) Mol. Cell. Biol. 16, 6372–6384
18. Crusiman, R., Masutani, H., Leibovitch, M. P., Robin, P., Soudant, I., Trouche, D., and Harel-Bellan, A. (1996) J. Biol. Chem. 271, 5258–5264
19. Lee, Y., Nadal-Ginard, B., Mahdavi, V., and Izumo, S. (1997) Mol. Cell. Biol. 17, 2745–2755
20. Sartorelli, V., Kurabayashi, M., and Kedes, L. (1993) Circ. Res. 73, 395–406
21. Sprenkle, A. B., Murray, S. F., and Glembotski, C. C. (1995) Circ. Res. 77, 1060–1069
22. Sartorelli, V., Webster, K. A., and Kedes, L. (1990) Genes Dev. 4, 1811–1822
23. Treisman, R. (1992) Trends Biochem. Sci. 17, 423–428
24. Price, M. A., Rogers, A. E., and Treisman, R. (1990) EMBO J. 9, 2589–2601
25. Hill, C. S., Marais, R., John, S., Wynne, J., Dalton, S., and Treisman, R. (1993) Cell 73, 395–406
26. Giovane, A., Pintzas, A., Maira, S. M., Sobieszczuk, P., and Wasylyk, B. (1994) Genes Dev. 8, 1502–1513
27. Macleod, K., Leprince, D., and Stehelin, D. (1992) Trends Biochem. Sci. 17, 421–424
28. Alberts, A. S., Geneste, O., and Treisman, R. (1998) Cell 92, 475–487
29. Hill, C. S., Wynne, J., and Treisman, R. (1995) Cell 81, 1159–1170
30. Wei, L., Zhou, W., Cruissen, J. D., Johansen, F. E., Prywes, R., Rainsouramah, D., and Schwartz, R. J. (1998) J. Biol. Chem. 273, 30287–30294
31. Takano, H., Komuro, I., Oka, T., Shigijima, I., Hario, Y., Mizuno, T., and Yasaki, Y. (1994) Mol. Cell. Biol. 14, 5380–5389
32. Sotiriouluou, A., Gineitis, D., Copeland, J., and Treisman, R. (1999) Cell 98, 159–169
33. Blank, R. S., McQuinn, T. C., Yin, K. C., Thompson, M. M., Takevasu, K., Schwartz, R. J., and Owens, G. K. (1993) J. Biol. Chem. 268, 864–869
34. Shimizu, R. T., Blank, R. S., Jervis, R., Lawrence-Smith, S. C., and Owens, G. K. (1995) J. Biol. Chem. 270, 7631–7643
35. Ginty, D. D., Wilson, R. N., and Weinberg, R. A. (1986) Mol. Cell. Biol. 6, 4305–4316
36. Blank, R. S., and Owens, G. K. (1990) J. Cell Physiol. 142, 635–642
37. Bubb, M. R., Spector, I., Beyer, B. B., and Fosen, K. M. (2000) J. Biol. Chem. 275, 5163–5170
38. Hall, A. (1998) Science 279, 509–514
39. Sahai, E., Albers, A. S., and Treisman, R. (1998) EMBO J. 17, 1350–1361
40. Yamakawa, T., Tanaka, S., Numaguchi, K., Yamakawa, Y., Motley, E. D., Ichihara, S., and Inagami, T. (2000) Hypertension 35, 313–318
41. Sahai, E., Ishizaki, T., Narumiya, S., and Treisman, R. (1999) Curr. Biol. 9, 136–145
42. Chihara, K., Amano, M., Nakamura, N., Yano, T., Shihata, M., Tokui, T., Ichikawa, H., Ikebe, R., Ikebe, M., and Kaibuchi, K. (1997) J. Biol. Chem. 272, 25121–25127
43. Uehata, M., Ishizaki, T., Sato, H., Uno, T., Kahara, H., Morishita, T., Yamakawa, H., Yamagami, K., Inui, J., Maekawa, M., and Narumiya, S. (1997) Nature 389, 990–994
44. Belaguli, N. S., Schildmeyer, L. A., and Schwartz, R. J. (1997) J. Biol. Chem. 272, 18222–18231
45. Spencer, J. A., and Misra, R. P. (1996) J. Biol. Chem. 271, 16535–16543
46. Chen, C. Y., and Schwartz, R. J. (1997) Mol. Endocrinol. 11, 812–822
47. Sepulveda, J. L., Belaguli, N., Nigam, V., Chen, C. Y., Nemer, M., and Schwartz, R. J. (1998) Mol. Cell. Biol. 18, 3405–3415
48. Chen, C. Y., Cruissen, J., Majesky, M., Topouzis, S., McGinniss, T., Frankovsky, M. J., and Schwartz, R. J. (1996) Dev. Genet. 19, 119–130
49. Belaguli, N. S., Zhou, W., Trinh, T. H., Majesky, M. W., and Schwartz, R. J. (1999) Mol. Cell. Biol. 19, 4582–4591
50. Kemp, P. R., and Metcalfe, J. C. (2000) Biochem. J. 345, 445–451
51. Rungger, D., Rungger-Brandle, E., Chapignon, C., and Gabbiani, G. (1979) Nature 282, 320–321
52. Wada, A., Fukuda, M., Mishima, M., and Nishida, E. (1998) EMBO J. 17, 1635–1641
53. Van Etten, R. A., Jackson, P. K., Baltimore, D., Sanders, M. C., Matsudaira, P. T., and Janmey, P. A. (1994) J. Cell Biol. 124, 325–340
54. Zhao, K., Wang, W., Rando, O. J., Xue, Y., Swider, K., Kuo, A., and Crabtree, G. R. (1998) Cell 95, 625–636
55. Numaguchi, K., Egochi, S., Yamakawa, T., Motley, E. D., and Inagami, T. (1999) Circ. Res. 85, 5–11
56. Fleming, I. N., Elliott, C. M., and Exton, J. H. (1996) J. Biol. Chem. 271, 33067–33073
57. Kim, J. H., Cho, Y. S., Kim, B. C., Kim, Y. S., and Lee, G. S. (1997) Biochem. Biophys. Res. Commun. 232, 223–226
58. Seasholtz, T. M., Majumdar, M., Kaplan, D. D., and Brown, J. H. (1999) Circ. Res. 84, 1186–1193
59. Renshaw, M. W., Toksoz, D., and Schwartz, M. A. (1996) J. Biol. Chem. 271, 21691–21694
60. Hotchin, N. A., and Hall, A. (1995) J. Cell Biol. 131, 1857–1865
61. Barry, S. T., Flinn, H. M., Humphries, M. J., Critchley, D. R., and Ridley, A. J. (1997) Cell Adhes. Commun. 4, 387–398
62. Somlyo, A. P., and Somlyo, A. V. (2000) J. Physiol. (Lond.) 522, 177–185