Studies spanning the last two decades have begun to uncover the molecular mechanisms underlying smooth muscle cell (SMC) differentiation, specifically the critical role of the ubiquitously expressed serum response factor (SRF) transcription factor. SRF selectively binds to conserved cis-regulatory “CarG box” elements, CC(A/T)6GG consensus sequence, in the promoters or first introns of numerous smooth muscle-selective genes. The SMC contractile marker genes smooth muscle alpha actin (SmαA), smooth muscle myosin heavy chain (SM-MHC), and smooth muscle 22 alpha (SM22α) all require multiple CarG boxes for proper transcriptional activation. On SRF binding, the coactivator myocardin is recruited to the promoter through direct interaction with SRF and is required for expression of CarG containing SMC-selective genes. In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Sayers et al detail the in vivo regulation of a SMC-selective CarG-arrestin (CRP2) gene, focal adhesion kinase related nonkinase (FRNK), that plays a critical role in CarG-dependent contractile gene expression during SMC differentiation and after vascular injury.

See accompanying article on page 2115

Unlike other muscle cell types, adult SMCs exist in a highly plastic nonterminally differentiated state. SMCs can assume a proliferative and migratory synthetic phenotype, allowing for both complete investment of the endothelial tube during vascular development and repair of the vessel wall after arterial injury. Various environmental cues direct SMCs to phenotypically modulate into a highly contractile state that is necessary for proper vascular function. Important to these processes is focal adhesion kinase (FAK) signaling, whereby integrins and growth factors stimulate the formation of focal adhesions required for cellular migration and proliferation. FRNK is an endogenous dominant interfering mutant that serves to inhibit FAK signaling and is specifically expressed in SMCs, mainly in the large arteries. The comprehensive study conducted by Sayers et al describes the in vivo regulation of FRNK expression and illustrates the important role FRNK plays in SMC differentiation. The authors elegantly show that FRNK is specifically expressed in SMCs and transiently expressed during postnatal development. FRNK overexpression in 10T1/2 smooth muscle-like progenitor cells leads to induction of SmαA, SM-MHC, and SM22α promoter-reporter activity. Transforming growth factor beta (TGF-β), a growth factor present in the SMC environment during development and vascular injury, increases FRNK promoter activity and expression. Notably, TGF-β stimulation in concert with FRNK overexpression results in a greater induction of SM22α promoter activity and protein expression. Homozygous deletion of the FRNK gene ablates this response and leads to increased SMC proliferation. After carotid ligation, FRNK−/− mice show a dramatic reduction in SmαA protein in neointimal SMCs at 14 days after injury, whereas wild-type mice display elevated SmαA levels associated with injury resolution and SMC phenotypic modulation to the contractile state. Altogether, these data suggest that FRNK inhibition of FAK signaling contributes to SMC differentiation during postnatal development and after acute vascular injury.

Although most SMC-selective genes fall under the SRF/myocardin mode of regulation, this mechanism is not the only means through which SMC-selective genes are transcriptionally regulated. It has been shown that aortic carboxypeptidase-like protein (ACLP), a protein secreted by SMCs, and cysteine-rich protein 2 (CRP2), a bridging factor between SRF and other transcription factors, are two important SMC-selective genes that are transcriptionally activated independently of SRF. Of major interest in the study conducted by Sayers and colleagues is the finding that FRNK plays a critical role in the regulation of SRF/myocardin/CARG-dependent contractile gene expression, yet the FRNK promoter itself is regulated in an SRF/myocardin/CARG-independent manner. The authors demonstrate that SRF overexpression in SRF−/− ES cells does not induce FRNK promoter activity, and that myocardin overexpression in 10T1/2 cells does not stimulate FRNK mRNA production. These data indicate that FRNK is transcriptionally regulated through other mechanisms that have yet to be discovered. Whereas the majority of SMC CARG-regulated genes are contractile and cytoskeletal proteins, CARG-independent genes such as ACLP, CRP2, and FRNK have diverse functions but are all involved in the induction of CARG-dependent gene expression and SMC phenotypic modulation. The question that begs to be answered is, “What regulates ACLP, CRP2, and FRNK?” Studies have shown that CRP2 is positively regulated by TGF-β, and although not previously shown in SMCs, ACLP expression in preadipocytes is upregulated after TGF-β stimulation. Perhaps each of these CARG-independent genes is transcriptionally

See accompanying article on page 2115

Unlike other muscle cell types, adult SMCs exist in a highly plastic nonterminally differentiated state. SMCs can assume a proliferative and migratory synthetic phenotype, allowing for both complete investment of the endothelial tube during vascular development and repair of the vessel wall after arterial injury. Various environmental cues direct SMCs to phenotypically modulate into a highly contractile state that is necessary for proper vascular function. Important to these processes is focal adhesion kinase (FAK) signaling, whereby integrins and growth factors stimulate the formation of focal adhesions required for cellular migration and proliferation. FRNK is an endogenous dominant interfering mutant that serves to inhibit FAK signaling and is specifically expressed in SMCs, mainly in the large arteries. The comprehensive study conducted by Sayers et al describes the in vivo regulation of FRNK expression and illustrates the important role FRNK plays in SMC differentiation. The authors elegantly show that FRNK is specifically expressed in SMCs and transiently expressed during postnatal development. FRNK overexpression in 10T1/2 smooth muscle-like progenitor cells leads to induction of SmαA, SM-MHC, and SM22α promoter-reporter activity. Transforming growth factor beta (TGF-β), a growth factor present in the SMC environment during development and vascular injury, increases FRNK promoter activity and expression. Notably, TGF-β stimulation in concert with FRNK overexpression results in a greater induction of SM22α promoter activity and protein expression. Homozygous deletion of the FRNK gene ablates this response and leads to increased SMC proliferation. After carotid ligation, FRNK−/− mice show a dramatic reduction in SmαA protein in neointimal SMCs at 14 days after injury, whereas wild-type mice display elevated SmαA levels associated with injury resolution and SMC phenotypic modulation to the contractile state. Altogether, these data suggest that FRNK inhibition of FAK signaling contributes to SMC differentiation during postnatal development and after acute vascular injury.

Although most SMC-selective genes fall under the SRF/myocardin mode of regulation, this mechanism is not the only means through which SMC-selective genes are transcriptionally regulated. It has been shown that aortic carboxypeptidase-like protein (ACLP), a protein secreted by SMCs, and cysteine-rich protein 2 (CRP2), a bridging factor between SRF and other transcription factors, are two important SMC-selective genes that are transcriptionally activated independently of SRF. Of major interest in the study conducted by Sayers and colleagues is the finding that FRNK plays a critical role in the regulation of SRF/myocardin/CARG-dependent contractile gene expression, yet the FRNK promoter itself is regulated in an SRF/myocardin/CARG-independent manner. The authors demonstrate that SRF overexpression in SRF−/− ES cells does not induce FRNK promoter activity, and that myocardin overexpression in 10T1/2 cells does not stimulate FRNK mRNA production. These data indicate that FRNK is transcriptionally regulated through other mechanisms that have yet to be discovered. Whereas the majority of SMC CARG-regulated genes are contractile and cytoskeletal proteins, CARG-independent genes such as ACLP, CRP2, and FRNK have diverse functions but are all involved in the induction of CARG-dependent gene expression and SMC phenotypic modulation. The question that begs to be answered is, “What regulates ACLP, CRP2, and FRNK?” Studies have shown that CRP2 is positively regulated by TGF-β, and although not previously shown in SMCs, ACLP expression in preadipocytes is upregulated after TGF-β stimulation. Perhaps each of these CARG-independent genes is transcriptionally
activated through similar TGF-β-stimulated mechanisms or by the same transcription factors (i.e., the SMAD family of transcription factors) to work in concert to enhance TGF-β induction of SMC contractile genes. Importantly, it has been shown that myocardin participates in SMAD-dependent activation of SMMHC, SMαA, ACLP, and CArG-independent regulation of SM22α. Interestingly, overexpression of ACLP provokes preadipocytes to transdifferentiate into SMCs, whereas CRP2 protein transducted into cardiomyocytes leads to the transcription of SMC-selective CArG-dependent genes. Although unknown, FRNK overexpression might also be sufficient to direct cell transdifferentiation into the SMC phenotype. These three CArG-independent genes could potentially be critical upstream signals that together cue a cell to express SMC contractile and cytoskeletal proteins required for SMC maturation and differentiation.

An important protein in SMCs that functions, in essence, as a toggle between the synthetic and contractile phenotypes is the CArG-binding transcription factor SRF. This ubiquitously expressed transcription factor uniquely binds not only CArG DNA sequences in contractile and cytoskeletal genes, but also to CArG DNA in the promoters of early-onset genes involved in SMC growth and migration. Of significant interest in the research of Sayers et al is the tightly controlled endogenous expression of FRNK during postnatal development, whereby FRNK mRNA is briefly upregulated between days 4 and 10, peaking at day 7. FRNK protein exhibits a short half-life of 4.5 hours, which allows for a controlled and rapid inhibition of FAK signaling and a significant increase in contractile gene expression. This rigid, temporal regulation of FRNK expression might also function as a key molecular switch, whereby FRNK protein can direct a proliferating cell to alter its gene expression and transform into a quiescent, contractile SMC. It is unknown whether FRNK expression and subsequent FAK inhibition lead to changes in SRF activity or myocardin cofactor recruitment, but it is evident that SRF binding is mediated through changes in environmental cues and that TGF-β stimulation of SMCs increases SRF expression. It could be possible that FRNK is functioning upstream of SRF binding, or that FRNK and SRF protein, both modulated by TGF-β, work in parallel for complete SMC differentiation. Future studies are necessary to determine the specific molecular mechanisms through which FRNK acts as an important SMC phenotypic modulator.

Acknowledgments

We thank Dr Sean Garvey, PhD and Monica Lee, ME for their insightful feedback and review of this commentary.

Sources of Funding

Research in the author’s laboratory is funded by NIH RO1 HL081682, AHA Scientist Development Grant.

Disclosures

None.

References


"FRNKly, Smooth Muscle, I Don't Give a CArG!": A Novel Mechanism for Smooth Muscle Cell Differentiation
Julia A. Lemmon and Brian R. Wamhoff

doi: 10.1161/ATVBAHA.108.176875
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/28/12/2091

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/