Preventing Stenosis by Local Inhibition of KCa3.1
A Finger on the Phenotypic Switch

Karen M. Lounsbury

The sources of genetic alterations that underlie phenotypic switching of vascular smooth muscle cells (VSMCs) during stenosis have recently been the subject of intense study. It is becoming increasingly clear that transcriptional control of ion channels plays an important role not only in expression of the differentiated phenotype, but also in the development and maintenance of the proliferative phenotype (see reviews1,2). Functional expression of voltage-dependent calcium channels (VDCC) and large-conductance Ca2+-activated K+ channels (BKCa) is known to be necessary for the maintenance of vascular smooth muscle cell (VSMC) differentiation.3–6 More recently, upregulation of the intermediate-conductance Ca2+-activated K+ channel, KCa3.1 (IKCa1, encoded by KCNN4) and store-operated Ca2+ channels such as TRPC (transient receptor potential) have been linked to the proliferative phenotype.7–9 Moreover, selective inhibition of KCa3.1 channels using TRAM-34 has been shown to inhibit growth factor–mediated proliferation of VSMCs and to prevent the development of restenosis.10 In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Tharp and colleagues contribute to this accumulating evidence through their discovery that local delivery of TRAM-34 via balloon catheter prevents phenotypic switching of coronary artery VSMCs and limits subsequent restenosis. Their findings encourage further investigation of TRAM-34 as a therapy for the prevention of angioplasty-induced restenosis and provide a means for selective delivery that may prevent untoward side-effects associated with systemic administration of TRAM-34. Although the significant players in phenotypic switching are becoming more apparent, many questions remain with respect to how the altered array of ion channels is translated into signals affecting the cell proliferation machinery.

See accompanying article on page 1084

The study by Tharp et al used a model of porcine coronary VSMC phenotypic modulation after balloon angioplasty, a model that closely resembles human postangioplasty restenosis. Laser-capture microdissection was used to isolate medial VSMCs for examination of mRNA levels of relevant molecules and immunostaining confirmed changes at the protein level. In agreement with the authors’ findings using a rat model of arterial injury,8 angioplasty induced expression of KCa3.1 and caused downregulation of repressor element 1–silencing transcription factor (REST), smooth muscle myosin heavy chain (SMMHC), and myocardin. Delivery of TRAM-34 via coating the angioplasty balloon reversed these effects, and, most importantly, reduced restenosis in response to angioplasty.

These findings support the emerging model of VSMC phenotypic switching characterized by the routing of transcriptional activity through a combination of intracellular signaling pathways and chromatin organization as outlined in the Figure. Maintenance of the differentiated phenotype is accomplished through VDCC function and BKCa activation by Ca2+ sparks released from ryanodine receptors (RYR).11 Ca2+ influx through VDCCs activates the Rho GTPase and Ca2+/CaM-dependent protein kinase (CaMK).12,13 Rho kinase (ROK) can induce expression of myocardin, a cofactor that directs SRF transcription toward differentiation markers such as smooth muscle α actin (SMA) and SMMHC.14–16 CaMK mediates transcription of c-fos and TRPC through its activation of serum response factor (SRF) and Ca2+/cAMP-response element binding protein (CREB).17,18 Signaling through growth factor receptors (GFR), activation of Gq-coupled receptors including the angiotensin II type 1 receptor (ATIR), and Ca2+ influx through store-operated Ca2+ channels (ie, TRPC) all contribute to the activation of extracellular signal regulated kinase (ERK). ERK activation leads to Elk-1 phosphorylation, which can displace myocardin from SRF.19 ERK also induces transcription of proliferative genes such as the AP-1 family member, c-fos.20–22 Increased production of c-fos leads to induction of KCa3.1 expression through AP-1 promoter elements.23 KCa3.1 function results in voltage-independent membrane hyperpolarization, possibly important in enhancing Ca2+ signaling through TRPC channels.24 Blockage of KCa3.1 with TRAM-34 prevents downregulation of myocardin and SMMHC, thus promoting the differentiated phenotype. TRAM-34 also prevents downregulation of REST, which blocks transcription of KCa3.1 through repression at the RE1 site.25 A key area for future investigation is to understand why increased activity of KCa3.1 is necessary for activation and maintenance of the proliferative phenotype. One attractive possibility is that the voltage-independent hyperpolarization promotes influx of Ca2+ through store-operated channels which is necessary for maintenance of signaling through the Ras/ERK and phospholipase C (PLC) mitogenic pathways. Another possibility is that TRAM-34 directly alters the opening of nonselective ion channels, such as TRP, a property which has been reported in microglial cells and should be ruled out in the current system.26

From the Department of Pharmacology, University of Vermont, Burlington.

Correspondence to Karen Lounsbury, Department of Pharmacology, University of Vermont, Given Building, 89 Beaumont Avenue, Burlington, VT 05405. E-mail Karen.lounsbury@uvm.edu


Arterioscler Thromb Vasc Biol is available at http://atvb.ahajournals.org

DOI: 10.1161/ATVBAHA.108.164988
In addition to supporting an important role for KCa3.1 in the proliferative switch, the effectiveness of the localized delivery of TRAM-34 in the study by Tharp et al has important practical clinical implications. TRAM-34 is a derivative of the triarylmethane, clotriamazole, which selectively blocks KCa3.1 with a KD of 20 nmol/L. TRAM-34 has been shown to have therapeutic effectiveness in the treatment of sickle cell anemia and as an immunosuppressant. As such, systemic administration of TRAM-34 has the potential to produce undesired immunosuppression in patients treated with angioplasty. The delivery by balloon catheter has the potential advantage of selectively affecting the area of angioplasty injury while avoiding systemic complications. Continued study of the mechanisms linking KCa3.1 activity to VSMC phenotype switching and the clinical applications of direct delivery of TRAM-34 as a preventative for restenosis are thus clearly warranted.

Disclosures

None.

References


Preventing Stenosis by Local Inhibition of \( \text{K}_{\text{Ca}3.1} \): A Finger on the Phenotypic Switch

Karen M. Lounsbury

*Arterioscler Thromb Vasc Biol.* 2008;28:1036-1038
doi: 10.1161/ATVBAHA.108.164988

*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/28/6/1036

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/