Vascular smooth muscle cells (VSMCs) undergo phenotypic switching in response to vascular injury. A hallmark feature of VSMC phenotypic switching is altered expression of VSMC-selective genes that define the contractile phenotype, including SMα-actin, SMC myosin heavy chain (SM-MHC), Calponin, SM22α, and H-caldesmon. Although expression of no single marker is specific for SMC lineage, SM-MHC appears to be the most specific marker to date. After acute vascular injury of an intact blood vessel, the expression of SMC differentiation markers are transiently decreased 7 days after vascular injury, when VSMCs are proliferating and migrating into the neointima. Expression of SMC marker proteins is largely regulated at the gene transcription level in vitro and in vivo. Transcriptional regulation of many of these contractile proteins involves the transcription factor serum response factor (SRF), which binds CArG cis regulatory elements. Recent studies have shown that myocardin and myocardin-related factors (MRTFA, MRTFB or MKL1, MKL2) function as SRF coactivators and are required for SRF-CArG-dependent regulation of VSMC-selective genes. SRF activity itself is also regulated by posttranscriptional mechanisms such as phosphorylation and alternative splicing.

Intracellular gp91phox-related NAD(P)H oxidase (Nox) is a family of enzymes that generate ROS in a controlled manner. In the mouse, the Nox family includes Nox 1, Nox 2, Nox 3, and Nox 4. In humans the Nox family consists of Nox1 to Nox5 and the dual oxidases Duox 1 and Duox 2. Nox 1 and Nox 4 are both expressed in human and rodent aortic VSMCs. Nox 1, the first identified mammalian homologue of gp91phox, is involved in signal transduction leading to hypertrophy and cell proliferation. Nox 1 can form a complex with p22phox which enhances its stability and superoxide production in vitro. Distantly related to gp91phox, Nox 2, Nox 4 is expressed in a variety of cells including those in the vasculature. Nox 4, unlike other members of Nox family, produces large amounts of hydrogen peroxide constitutively. Nox 4 also functions as a complex with p22phox on internal membranes to produce ROS. Nox 4, unlike Nox 1, does not reside in the plasma membrane, and is not controlled by Rac or the cytosolic Nox components.

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, in the article “Nox4 Is Required for Maintenance of the Differentiated Vascular Smooth Muscle Cell Phenotype”, Clemans et al have published their novel findings implicating Nox 4 as a regulator of VSMC differentiation marker gene expression. In their study, Nox 4 mRNA and protein levels were more abundant in early versus late passage rat thoracic aortic VSMC culture when VSMC SM-MHC, Calponin, SMα-actin, and H-caldesmon were also abundantly expressed. Downregulation of Nox 4 with siRNA to Nox 4 resulted in a significant decrease in baseline ROS levels and decreased SMα-actin, SM-MHC, and calponin mRNA and protein levels in VSMCs in culture. Nox 4 colocalized with the SMα-actin–based stress fibers in early passage VSMCs in culture. In late passage VSMC cells, Nox 4 localized to focal adhesions. In contrast, Nox 1 increased in late passed VSMC culture when differentiation markers were low. Furthermore, transfection of early passed VSMCs treated with siRNA to Nox 4 reduced SRF levels, a factor necessary for expression of differentiation marker genes in SMCs. In vivo studies in the rat carotid artery balloon injury model, demonstrated that Nox 4 appeared in the neointima 7 days after injury, preceding re-expression of SM-MHC 10 days after injury. Both Nox 4 and SM-MHC were prominently expressed on post injury day 15.

These intriguing findings suggest an important role for Nox 4 in the regulation of VSMC phenotypic switching in response to vascular injury and raise interesting questions about the mechanisms whereby Nox 4 might regulate VSMC marker gene expression. Previous studies by Li et al demonstrate a role for Nox 4 in the regulation of differentiation in other cell systems. Nox 4 is the principal Nox isoform

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Reactive oxygen species (ROS), like superoxide anions and hydrogen peroxide, have been implicated in a variety of processes involved in vascular lesion formation such as proliferation, differentiation, and apoptosis. Classically described as microbial byproducts of oxidative metabolism, they also function as intracellular second messengers in processes involved in vascular lesion formation such as proliferation, differentiation, and apoptosis. Classically described as microbial byproducts of oxidative metabolism, they also function as intracellular second messengers in

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expressed in undifferentiated embryonic stem cells, differentiating embryoid bodies (EB), and neonatal cardiomyocytes. Downregulation of Nox 4 in EBs decreased the expression and delayed p38 MAPK phosphorylation, decreased the expression of Nkx2.5 and myocyte enhancer factor 2 C (MEF2C), restricted MEF2C to the cytoplasm, inhibited ventricular myosin regulatory light chain 2 (MLC2v) expression, inhibited myofibrillogenesis, and impaired beating activity of EB. H2O2 administrated to EBs with Nox 4 knockdown, rescued the beating activity.15 In VSMCs, p38 MAPK is activated by H2O2 and is required for activation of the SMC marker gene promoters.19,20 Nox 4 has also been shown to mediate transforming growth factor (TGF)-β1 induced superoxide production and Smad 2/3 transcription factor activation of SMα-actin expression, and differentiation of cardiac fibroblasts into myofibroblasts.21,22

The study by Clempus in this issue and prior studies suggest that the cellular localization of ROS is an important determinant of its cellular function. Hilenski et al have previously demonstrated that Nox 1 and Nox 4 have distinct subcellular localizations in VSMCs. Nox 4 was localized to the nuclei and focal adhesions which are major sites for PI3 kinase and Src. Nox 1 had a surface distribution along the cellular margins where it colocalized with caveolae. Such compartmentalization is suggested to affect Nox 4-induced ROS activity in VSMCs by spatial detention as well as by functional alterations when interacting with focal adhesions or with actin cytoskeleton.23 In the present study, Nox 4 colocalized with stress fibers in early passaged and with focal adhesions in late passaged VSMCs, suggesting that local increased concentrations of ROS in the actin cytoskeleton leads to activation of cellular signals that result in increased VSMC marker gene expression. Whereas Nox 4-generated ROS localized in focal adhesions may activate signals that decrease VSMC marker gene expression.

VSMC gene expression is known to be largely regulated at the transcriptional level.4 In the current issue, Clempus et al demonstrate that inhibition of Nox 4 in early passaged VSMCs leads to a decrease in SRF, an important regulator of SMC marker gene transcription. Interestingly, ROS regulates the expression of other transcription factors that modulate VSMC marker gene expression such as Smad 2/3 regulation in fibroblasts and Id3 in VSMCs.9,22,24 The mechanisms whereby Nox 4-induced ROS expression in specific cellular compartments regulate the expression and activation of transcription factors that may activate or inhibit VSMC differentiation gene expression are unknown. ROS is known to activate intracellular signaling pathways such as Src, Ras, JAK2, Pyk2, PI3K, MAPK, protein phosphatases, phospholipases, and intracellular cation regulation pathways. These may be a few of several potential pathways that could be differentially regulated by ROS generated in different compartments of the cell. Selective pathway activation by ROS generated in actin stress fibers may lead to maintenance of VSMC marker gene expression through increased expression of SRF or other transcription factors that activate VSMC differentiation genes (Figure, A), whereas ROS generated in focal adhesions may activate a different pathway leading to inhibition of SRF or other activating factors or induction of transcription factors that repress VSMC gene expression.
(Figure, B). Further studies are needed to define the specific pathways that are regulated by ROS in different cellular compartments and determine whether these pathways regulate VSMC marker gene expression both in vitro and in vivo. The answer to these and other intriguing questions raised by this novel report may provide important insight into the link between oxidative stress and VSMC phenotypic switching in response to vascular injury.

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Disclosures

None.

References


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