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Redox control of vascular smooth muscle cell function and plasticity

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Abstract

Vascular smooth muscle cells (SMC) play a major role in vascular diseases, such as atherosclerosis and hypertension. It has long been established in vitro that contractile SMC can phenotypically switch to function as proliferative and/or migratory cells in response to stimulation by oxidative stress, growth factors, and inflammatory cytokines. Reactive oxygen species (ROS) are oxidative stressors implicated in driving vascular diseases, shifting cell bioenergetics, and increasing SMC proliferation, migration, and apoptosis. In this review, we summarize our current knowledge of how disruptions to redox balance can functionally change SMC and how this may influence vascular disease pathogenesis. Specifically, we focus on our current understanding of the role of vascular nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX) 1, 4, and 5 in SMC function. We also review the evidence implicating mitochondrial fission in SMC phenotypic transitions and mitochondrial fusion in maintenance of SMC homeostasis. Finally, we discuss the importance of the redox regulation of the soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP)-protein kinase G (PKG) pathway as a potential oxidative and therapeutic target for regulating SMC function.

Introduction

Vascular smooth muscle cells (SMC) regulate arterial vascular tone and provide structural stability for blood flow regulation needed for delivery of oxygen to tissues. SMC have classically been defined by expression of the following SMC contractile- or contraction-associated proteins: smooth muscle myosin heavy chain 11 (MYH11), smooth muscle α-actin (ACTA2), smooth muscle 22 α/transgelin (TAGLN), and H1-calponin (CNN1) [1]. It has been well-established that in response to growth factors, cytokines, and reactive oxygen species (ROS), cultured SMC can downregulate their contractile genes and phenotypically switch to a more “synthetic” state where cells have an increased proliferative and/or migratory capacity (reviewed in refs. [1–5]). Notably, the advent of rigorous SMC lineage tracing mouse models has provided in vivo evidence that SMC phenotypic switching can

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

occur in the context of disease and has greatly expanded upon this classic two-state contractile-synthetic model of SMC function. Specifically, these studies provide evidence that: (1) in vascular disease, SMC contractile genes, such as ACTA2, can be expressed by other cell types making lineage tracing essential for accurate identification of SMC in vivo [6–9]; (2) SMCs undergo oligoclonal proliferation in diseases, such as atherosclerosis and pulmonary arterial hypertension [10–15]; (3) in atherosclerosis, SMCs can express marker genes and take on functions ascribed to other cell types to become, for example, macrophage and/or myofibroblast like cells [12, 14, 16]; and (4) SMCs can have both beneficial and detrimental roles in atherosclerotic disease progression [11, 12, 17].

An impaired SMC redox balance, exacerbated by the presence of excessive ROS, is a contributing factor to SMC phenotypic switching and has been associated with increased SMC proliferation, migration, apoptosis, and shifts in cell bioenergetics [3, 18–22]. Importantly, ROS levels are elevated in atherosclerosis, systemic hypertension, and pulmonary arterial hypertension, diseases in which SMC are major players [23–25]. It is thus likely that both extra- and intracellular sources of ROS acting on SMC contribute to vascular disease pathogenesis. Therefore, a greater understanding of the redox signaling pathways that influence SMC function and phenotypic state in the context vascular disease is of critical importance.

In this mini review, we have focused on our current understanding of how redox signaling can functionally change SMC and how this in turn may impact vascular disease development. Moving forward, we encourage more in-depth studies on the mechanisms of redox signaling on both SMC function and SMC phenotypic fate in vascular disease pathogenesis.

ROS and SMC function

Overproduction of ROS, leading to oxidative stress, has been implicated in numerous vascular pathologies [23–25]. ROS, such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), can induce SMC proliferation, migration, and apoptosis [3, 18–22]. While there are several producers of vascular ROS, studies in SMC biology have largely focused on nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX) and mitochondrial mediated ROS generation. NOX proteins facilitate NADPH-mediated reduction of oxygen (O_2) to superoxide (O_2^-) [26, 27]. Even the dismutated product of superoxide, namely H_2O_2, can acquire oxidative reactivity in the presence of peroxidase enzymes, Fenton chemistry, and low pKa of cysteine residues on proteins; [28] thereby taking on major mediator activity in NOX signaling. NOX1, NOX4, and NOX5 isoforms are present within SMC [29, 30]. Both NOX1 and NOX4 are bound to p22phox at cell membranes [29, 30]. In addition, the cytosolic side of NOX interacts with Rho GTPase RAC1 and p47phox/Nox organizer 1 (NOXO1) and NOX activator 1 (NOXA1). NOX4 binds with polymerase-δ activating protein 2 (POLDIP2) in the cytosol, which is thought to be involved in NOX4-mediated organization of the cell cytoskeleton [30]. Vascular NOX proteins also localize to different cellular compartments. NOX1 localizes to caveolae and endosomes on the plasma membrane; whereas, NOX4 localizes to the nucleus, endoplasmic reticulum (ER), and mitochondria [30]. NOX5 is the most unique vascular NOX in that it is expressed in human

and porcine, but not mouse or rat, SMC [31–33]; localizes to the plasma and ER membrane [29, 30]; and contains EF hand motifs activated by intracellular calcium [29, 30].

Evidence suggests that NOX4 is involved in maintenance of SMC quiescence while NOX1 has a role in modulating SMC function. Rat aortic SMCs treated with platelet derived growth factor beta (PDGF-BB) or angiotensin II (Ang II), which induce SMC proliferation and hypertrophy, respectively, leads to downregulation of NOX4 expression and upregulation of NOX1 expression [34, 35]. PDGF-BB-induced increased NOX1 expression and H_2O_2 production in SMC has been shown to cause downstream activation of c-Jun N-terminal kinase (JNK), as well as cyclin D and extracellular signal-regulated kinase (ERK)1/2 signaling enhancing SMC migration and proliferation, respectively [29]. Ang II-induced SMC hypertrophy is regulated by NOX1 activation of Ras, p38 mitogen kinase activated protein kinase (MAPK)/protein kinase B (Akt), and epidermal growth factor (EGF) receptor pathways [29]. Interestingly, evidence suggests that basal production of H_2O_2 is the purview of NOX4, while both basal and Ang II-stimulated O_2^- production are the purview of NOX1 in rat aortic SMC [35]. Collectively, this indicates in all likelihood that NOX1 and NOX4 have highly specialized roles within SMC.

In cultured rat SMC, *Nox1* mRNA expression was found to be enhanced in late passage SMC that express low levels of ACTA2, a hallmark of a phenotypically modulated SMC [36]. Cultured *Nox1*$^{-/-}$ mouse SMC exhibit decreased proliferative capacity and PDGF-BB-induced migratory capacity, while overexpression of NOX1 in SMC had the opposite effects [19]. Similarly, transgenic mice that over-express human *NOX1* specifically in SMC had increased Ang II-induced vascular O_2^- production, hypertension, and vessel wall hypertrophy [37]. Apparently inconsistent findings were reported in studies with global *Nox1* knockout mice which had decreased neointima formation and cell proliferation after femoral wire injury, reduced Ang II-induced vessel wall hypertrophy, and decreased susceptibility to aortic dissection [19, 38, 39]. However, the extent to which loss-of-SMC-produced NOX1 contributed to these observations is indeterminable. Overall, the data suggest NOX1 may be important in SMC function and potentially phenotypic plasticity in vascular disease.

In contrast to NOX1, NOX4 function has been associated with SMC contractile proteins in vitro. NOX4 colocalizes with ACTA2, MYH11, and CNN1 in differentiated rat aortic SMC [36]. In high-passage SMC with low levels of ACTA2 expression, NOX4 has been observed to relocate to focal adhesions [36]; siRNA knockdown of *Nox4* in cultured rat aortic SMC led to decreased expression of ACTA2, MYH11, and CNN1, suggesting a relationship between NOX4 and SMC contractile protein expression [36]. siRNA knockdown of *Nox4* in rat aortic SMC also results in decreased levels of serum response factor (SRF), a factor required for CArG box dependent expression of SMC contractile proteins [36]. In agreement with in vitro observations, NOX4 and CNN1 are coordinately expressed within the neointima and vessel wall 15 days post rat carotid balloon injury [36, 40]. Furthermore, studies by Tong et al.[41] in mice with TAGLN-driven overexpression of a human dominant negative mutant of *NOX4* showed decreased carotid wire-injury-induced neointima formation, as compared to controls. These same mice also had reduced atherosclerotic lesion sizes after 12 weeks of Western diet feeding [42]. These data would support the hypothesis

that SMC NOX4 expression may contribute to maintenance of SMC in a quiescent contractile state.

There is also evidence, however, that NOX4 may have stimuli-specific effects on SMC proliferation and apoptosis. Ismail et al.[43] showed that transforming growth factor beta 1 (TGF-β1), but not PDGF-BB or interferon gamma (IFNγ), increased NOX4 expression in cultured human pulmonary artery SMC. siRNA knockdown of *NOX4* resulted in decreased baseline and TGF-β1-induced cell proliferation of human pulmonary artery SMC, as measured by MTT assay [44]; it should be noted this metabolic assay quantifies the reduction of a tetrazolium dye to formazan, indicating cell viability as a surrogate measure of cell proliferation. Moreover, this same group showed that NOX4 was increased in the media of human donor pulmonary arteries, hypoxia can induce NOX4 expression in cultured human pulmonary artery SMC, and that siRNA knockdown of *NOX4* resulted in decreased hypoxia-induced proliferation [43, 44]. SMC NOX4 has also been associated with increased ROS production and decreased SMC viability. For example, overexpression of *NOX4* in cultured mouse SMC increased both H_2O_2 production and SMC apoptosis [22]. Additionally, siRNA knockdown of *NOX4* in human SMC decreased ROS levels and proliferation [45]. Taken together, this infers that NOX4 regulation of SMC function may therefore be dependent on the environmental stimuli and origin of the SMC being studied.

It is important to state that an inherent difficulty in studying NOX proteins is the diversity of activities these proteins can elicit depending on cell type, tissue, or disease state in which they are expressed (reviewed in refs. [23–26]). This is quite apparent in studies involving assessment of vascular NOX4 in vivo. Gray et al.[46] showed that global $Nox4^{-/-}ApoE^{-/-}$ mice treated with streptozotocin (STZ) to induce diabetes and diabetes-induced atherosclerosis had no difference in lesion burden as compared to wild-type STZ-diabetic controls. However, Schürmann et al.[47] showed that inducible global $Nox4^{-/-}ApoE^{-/-}$ mice subjected to an accelerated atherosclerosis model attained with partial carotid ligation and western diet feeding had increased aortic atherosclerotic lesion burden relative to similarly treated controls. It is reasonable to presume that the apparent contradictory results observed are due to the differences in disease model and experimental design used in the studies. While these global transgenic studies can inform as to the net effect of NOX4 in normal and disease development, their design does not yield information on cell-specific functions of NOX proteins. Therefore, the continued focused use of SMC-specific NOX mouse models in the context of vascular disease will be essential for understanding the specific contribution of SMC NOX proteins to SMC function, SMC phenotypic state, and vascular disease development.

Finally, in comparison to NOX1 and NOX4, not much is known about NOX5 function. As previously noted, NOX5 is present in human and porcine cells, but not in rat or mouse cells [31–33]. In a small sampling of non-diseased and diseased human coronary arteries, it was discovered that NOX5 expression was increased in diseased human coronary arteries and present within atherosclerotic lesions [48]. In culture, human aortic SMC treated with IFNγ upregulated NOX5 production [49]. With respect to the function of SMC-produced NOX5, siRNA knockdown of *NOX5* in cultured human aortic SMC was found to impair PDGF-BB mediated proliferation and ROS production [50]. Likewise, work by Gole et al.[32] showed

that adenoviral shRNA knockdown of *NOX5* impaired basic fibroblast growth factor (bFGF)-induced porcine coronary SMC migration. Therefore, it is highly feasible that NOX5 is important in human-SMC responses to stress and SMC phenotype modulation.

Mitochondrial fission and SMC function

Mitochondria are the crucial energy producing organelles of the cell. In normal physiological conditions, SMC mitochondria ATP generation is accompanied by low levels of O_2^- and H_2O_2 production [51, 52]. Increased ROS levels and production in response to pathogenic stimuli causes mitochondrial damage, leading to a shift in mitochondria state from fusion to fission [51, 53]. Mitochondria fission is characterized by the fragmentation of mitochondria and mitochondrial networks in response to stress, facilitating the removal of severely stress-damaged mitochondria and the generation of new mitochondria [51]. Fission is a process regulated primarily by GTPase dynamin-related protein 1 (DRP1, gene name *DNM1L*) with assistance from other cofactor proteins (i.e., Mid49, Mid51, and Mff) [51, 54].

SMC phenotypic transitions, specifically in response to PDGF-BB treatment in vitro, have been shown to involve mitochondria shifts from fusion to fission. SMC DRP1 expression has been linked most closely to enhanced SMC proliferation and migration [55–57]. PDGF–BB treatment of human SMC results in increased phosphorylation of serine 616 of DRP1, which activates mitochondrial fission [55, 58]. Moreover, PDGF-BB treatment induces DRP1-dependent increases in SMC proliferation [55, 58, 59] and mitochondrial ROS production [55]. This stands in agreement with findings of increased DRP1 and serine 616 phosphorylated DRP1 levels, as well as increased mitochondrial fission and SMC proliferation rates in SMC derived from patients with pulmonary arterial hypertension [58]. Finally, adenoviral-mediated overexpression of a dominant negative mutant of DRP1 in a mouse femoral wire injury model showed decreased neointima formation and vessel wall ROS levels, suggesting DRP1-mediated mitochondrial fission may be important for vessel wall cell response to injury [55].

PDGF-BB mediates a shift in SMC metabolism from glycolysis to fatty-acid oxidation that is regulated in part by DRP1 [59, 60]. This is evidenced by impairment of the metabolic switch in SMC when PDGF-BB treatment has been combined with the selective DRP1 inhibitor Mdivi-1, 2-deoxyglucose, a non-hydrolyzable glucose that inhibits glycolysis, and/or the phosphoinositide 3-kinase inhibitor LY-294002 [59, 60]. Pharmacological stimulation of SMC glucose oxidation with trimetazidine has similarly been shown to reverse hypoxia-induced mitochondrial fission in human pulmonary artery SMC [61]. Interestingly, when SMC were treated with PDGF-BB, pharmacological inhibition of mitochondrial fission did not impact SMC autophagy and vacuole formation, a process important for the degradation and clearance of SMC contractile proteins [56, 60].

In addition to SMC proliferation, contractile, and osteogenic stimuli also induce mitochondrial fission. Liu et al. [62] have demonstrated ex vivo that rat mesenteric arteries contracted with a high-potassium solution (KPSS) have mitochondria fission occurring in vessel wall cells. Furthermore, SMC treated with KPSS in vitro showed increases in both

cytosolic Ca^{2+} and mitochondrial ROS production, effects which were ameliorated by the addition of either Mdivi-1 or nitroglycerin [62]. Separately, work by Rogers et al.[63] has shown that treatment of human SMC with calcification-inducing osteogenic media increases DRP1 levels. Furthermore, Mdivi-1 inhibition of DRP1 impairs osteogenic media inducing cultured human SMC calcification [63]. Taken together, mitochondrial fission seems to be an essential process in SMC response to environmental stimuli and for SMC phenotypic switching. However, further studies are warranted to understand the direct mechanisms of connection between SMC response to environmental stimuli, mitochondrial fission, mitochondrial ROS generation, calcium signaling, and SMC function.

Mitochondrial fusion and SMC homeostasis

Mitochondrial fusion produces an interconnected mitochondrial network that is important in the maintenance of SMC homeostasis and quiescence [51, 64]. In response to cell energy demands and oxidative stress, mitofusion allows for complementation between healthy mitochondria and stress damaged mitochondria, diluting and mitigating damage for maintenance of cell homeostasis [51, 64]. Mitofusion occurs at both the inner and outer mitochondrial membranes with mitofusin 1 (MFN1) and mitofusin 2 (MFN2) acting at the outer mitochondrial membrane [51].

MFN2 is the most extensively studied in vascular SMC. Rat aortic SMC treated with PDGF-BB, bFGF, and endothelin-1 (ET-1) have decreased MFN2 expression levels [21, 65]. Overexpression of MFN2 in cultured rat or rabbit aortic SMC has been shown to impair SMC proliferation and arrest growth of SMC in cell cycle G_0/G_1 phase [21, 65–67]. Zhou et al.[67] discovered that MFN2 serine 442, which contains a protein kinase A (PKA) phosphorylation site, is an important residue for MFN_2^- mediated impairment of SMC proliferation [67]. MFN2 binding to Ras has been shown in aortic SMC over-expressing MFN2 [65]. Overexpression of MFN2 in SMC also decreases pAKT and pERK levels, implicating MFN2 as an inhibitor of the Ras-Raf-ERK1/2 signaling pathway [21, 65, 68].

MFN2 overexpression in cultured rat SMC has also been shown to increase mitochondrial mediated SMC apoptosis [21]. Rat SMC treated with H_2O_2 causes upregulation of MFN2 and caspases 3 and 9, markers of cell death [21]. Knockdown of *Mfn2* by siRNA protects against H_2O_2 mediated apoptosis [21]. Overexpression of MFN2 in balloon injured rat carotid arteries led to a decrease in media and neointimal cell proliferation, increased medial cell death (TUNEL+), and reduced neointima formation as compared to controls [21]. Likewise, in hypercholesterolemic rabbits, overexpression of MFN2 has been shown to impair atherosclerotic lesion formation, though cell viability was not assessed [66]. Combined, these data suggest that MFN2 regulates SMC homeostasis and viability.

However, contradictory evidence exists that similar phenotypes occur when MFN2 levels are reduced. For example, work by Ding et al.[69] showed that shRNA knockdown of *MFN2* in human SMC decreased SMC viability, as measured by NADPH reduction of tetrazolium to formazan. Similarly, Zhang et al.[70] found that siRNA knockdown of *Mfn2* in rat pulmonary artery SMC decreases SMC proliferation; viability, as measured by MTT assay; and pAKT levels. Despite the apparent contradiction between studies, there is a general

consensus that MFN2 is involved in cultured SMC viability, proliferation, and expression of pAKT and pERK1/2. We speculate that the quantity of MFN2 produced by SMC may therefore be a crucial determinant in regulation of SMC viability and proliferative capacity.

In addition to its role in mitochondrial fusion, MFN2 has been shown to link mitochondria with the endoplasmic reticulum (ER) [71, 72]. The transfer of calcium (Ca^{2+}) between mitochondria and ER is important for regulating mitochondrial bioenergetics and cell viability [71–74]. The mitochondria-ER link promotes Ca^{2+} flux into the mitochondria, an activity that is associated with increased ATP generation and anti-proliferative activity in SMC [75, 76]. Li et al.[72] showed in cultured rat aortic SMC that there is increased mitochondrial fusion and mitochondria-ER links when SMC are in the G_0/G_1 versus S phase. Moreover, mitochondria-ER links were shown to be increased in rat aortic SMC when MFN2 was overexpressed and decreased with siRNA *Mfn2* knockdown [72]. Separately, Morales et al.[76] showed that glucagon-like peptide-1 (GLP-1) enhancement of rat aortic SMC glucose uptake increased MFN2 levels. They also showed that MFN2 and PKA activity were important for GLP-1 mediated Ca^{2+} transfer from the ER to the mitochondria [76].

In both cardiac and skeletal muscles, NO and carbon monoxide have been shown to induce cGMP-PKG-dependent induction of PGC1α and mitochondrial fusion [77–79]. While there is no direct evidence that the sGC-cGMP-PKG pathway impacts mitochondrial bioenergetics, studies have shown that siRNA knockdown of *PGC1α* in human pulmonary artery SMC decreases *MFN2* mRNA expression and vice versa [80]. Taken together, it is interesting to speculate on ROS regulation of sGC-cGMP-PKG as a master upstream regulator of SMC mitochondrial bioenergetics, as well as SMC function and phenotypic state.

The sGC-cGMP-PKG pathway

Excessive ROS generation can drive vascular pathogenesis and SMC phenotypic transitions by uncoupling the nitric oxide (NO)—soluble guanylate cyclase (sGC)—cyclic guanosine monophosphate (cGMP)—protein kinase G (PKG) pathway [81–83]. Oxidation of sGC heme iron (Fe^{2+} to Fe^{3+}) impairs binding of NO to sGC [84–87]. H_2O_2 and peroxynitrite ($ONOO^-$) are particularly strong oxidants that predispose the sGC enzyme to heme loss, insensitivity to NO signaling, and degradation via the ubiquitin protein degradation pathway [88, 89]. Uncoupling of the pathway, which is central to maintaining SMC in their quiescent state and mediating vessel dilation [90–93], has been implicated in several vascular diseases and has been specifically targeted in treatment of pulmonary arterial hypertension [94–96]. For example, in a rat carotid artery balloon injury model it was reported that sGC expression decreased by ~90% in the vessel wall, as compared to uninjured controls [97]. Likewise, inducible SMC-specific sGC knockout mice showed severely elevated blood pressure at baseline [98].

Physiological restoration of sGC to its NO-sensitive Fe^{2+} state requires redox regulation of the enzyme's heme iron. Recently it was discovered that NADH cytochrome B5 reductase 3 (CYB5R3) in SMC functions as a regulator of sGC redox state and NO sensitivity; a

mechanism previously unknown [99]. It was shown that CYB5R3 complexes with the oxidized sGC enzyme and is necessary for NO to signal cGMP production by the enzyme under oxidative conditions [99]. Circumvention of impaired NO-sGC signaling due to functional loss-of-sGC's heme iron is the focus of a pipeline of pharmacological compounds that activate oxidized or heme-deficient sGC thus reactivating the sGC-cGMP signaling cascade. Indeed, Cinaciguat, or BAY 58–2667, is one of these pharmacological compounds and was recently shown to prevent neointima formation following wire injury and to decrease SMC migration and proliferation, as measured by in vitro scratch wound healing assay [100]. These sGC activators and the discovery of the sGC reductase CYB5R3 represent novel strategies and targets for modulating SMC function and treating vascular disease.

Concluding remarks

Oxidative stress and SMCs are integral contributors to various vascular diseases, including atherosclerosis, pulmonary arterial hypertension, and systemic hypertension [23–25]. As described herein, changes to SMC redox state can shift SMC from contractile to phenotypically diverse cells in vitro and drive changes in mitochondrial bioenergetics; this in turn can impact vascular disease. In addition, ROS can target and modulate sGC redox state in SMC, thus impacting the activation of downstream signaling proteins and kinases (i.e., PKG, PGC1α) that have been implicated in modulating SMC state, mitochondrial fission, and mitochondrial fusion. One possible connection between the pathways discussed here is that PDGF-BB-induced increases in NOX1 expression resulting in production of O_2^- and H_2O_2 could in turn oxidize sGC and thereby inhibit cGMP production and PKG activation (Fig. 1). It is also plausible that this PDGF-BB-mediated increase in SMC ROS production results in terminally damaged mitochondria, causing increased phosphorylation of DRP1 serine 616 and SMC mitochondrial fission (Fig. 1). Finally, it has been shown that the sGC-cGMP-PKG pathway regulates cytosolic Ca^{2+} [101] and that mitofusion protein MFN2 is a crucial mediator of Ca^{2+} flux between the mitochondria and ER [71–74]. It is therefore interesting to speculate that Ca^{2+} flux may be the downstream convergence point for these pathways and the currency for SMC phenotypic switching. However, the rate limiting factors and direct pathway integrations that factor into SMC responses to environmental cues and the balancing of SMC between quiescence, motility, proliferation, and viability remain incompletely understood and warrant further inquiry.

Another critical next step in the field will be to move beyond a two-state view of SMC plasticity. Indeed, recent SMC lineage tracing studies have demonstrated that SMC can become foam cell, mesenchymal stem cell, or myofibroblast-like in atherosclerosis, with these phenotypic states likely having both beneficial and detrimental effects on disease progression [9, 11, 14, 17]. It is reasonable to predict that similar observations will be made in other vascular diseases. Therefore, the key challenges to the field moving forward will be to discern (1) if and how SMC produce and respond to ROS in vivo in a manner that directly contributes to SMC phenotypic state and function and; (2) to what extent, if any, this contributes to disease progression and/or resolution.

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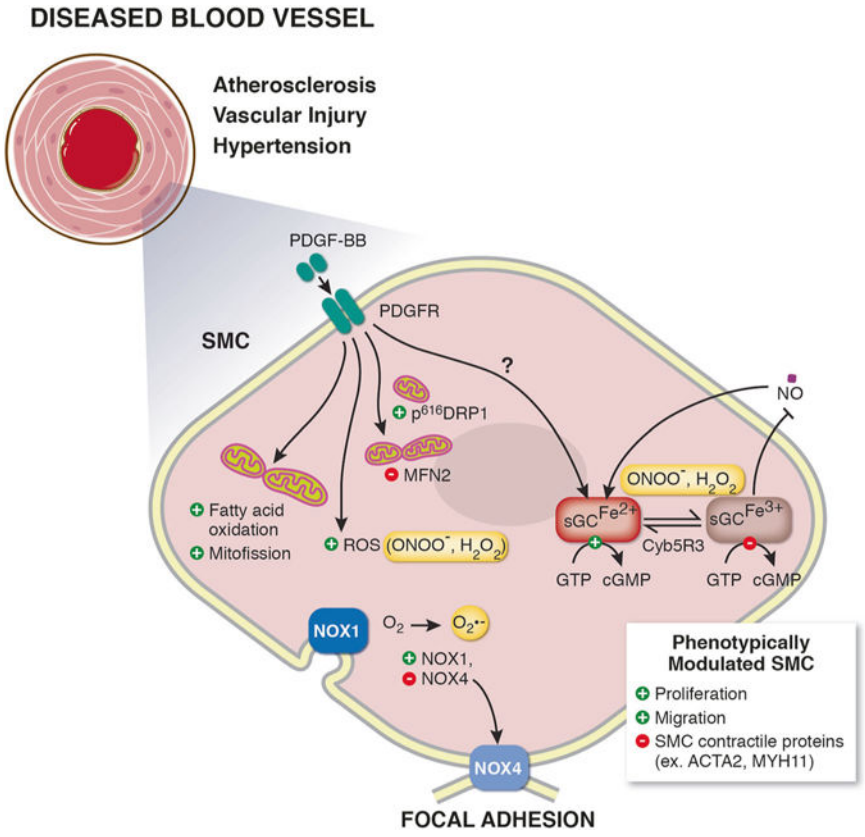

Fig. 1.

Schematic illustration showing mechanisms of smooth muscle cell (SMC) phenotypic switching. In response to vascular disease, SMC phenotypically switch from contractile cells to proliferative and migratory SMC characterized by downregulation of SMC contractile proteins (ex. smooth muscle α actin (ACTA2), smooth muscle myosin heavy chain 11 (MYH11)). SMC mitogen platelet derived growth factor beta (PDGF-BB) is known to induce SMC phenotypic switching. PDGF-BB can change cell bioenergetics, including shifting SMC metabolism to fatty-acid oxidation, increasing reactive oxygen species (ROS) production, and inducing mitochondrial fission, via increasing phosphorylation of dynamin-related protein 1 (DRP1) and decreasing mitofusin 2 (MFN2) levels. Vascular nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX) NOX1 and NOX4 generate superoxide (O_2^-) and are known to drive SMC switching. The soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP)-protein kinase G (PKG) pathway signaling maintains SMC quiescence and mediates physiological vessel dilation. Excessive ROS generation results in oxidation of the sGC heme iron (Fe^{2+} to Fe^{3+}) inhibiting binding of nitric oxide (NO) to sGC. Restoration of the sGC heme iron back to its NO-sensitive Fe^{2+} state is done endogenously by NADH cytochrome B5 reductase 3 (Cyb5R3)