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Discoveries

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Angiotensin II, NADPH Oxidase, and Redox Signaling in the Vasculature

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Abstract

Significance: Angiotensin II (Ang II) influences the function of many cell types and regulates many organ systems, in large part through redox-sensitive processes. In the vascular system, Ang II is a potent vasoconstrictor and also promotes inflammation, hypertrophy, and fibrosis, which are important in vascular damage and remodeling in cardiovascular diseases. The diverse actions of Ang II are mediated *via* Ang II type 1 and Ang II type 2 receptors, which couple to various signaling molecules, including NADPH oxidase (Nox), which generates reactive oxygen species (ROS). ROS are now recognized as signaling molecules, critically placed in pathways activated by Ang II. Mechanisms linking Nox and Ang II are complex and not fully understood. ***Recent Advances:*** Ang II regulates vascular cell production of ROS through various recently characterized Noxs, including Nox1, Nox2, Nox4, and Nox5. Activation of these Noxs leads to ROS generation, which in turn influences many downstream signaling targets of Ang II, including MAP kinases, RhoA/Rho kinase, transcription factors, protein tyrosine phosphatases, and tyrosine kinases. Activation of these redox-sensitive pathways regulates vascular cell growth, inflammation, contraction, and senescence. ***Critical Issues:*** Although there is much evidence indicating a role for Nox/ROS in Ang II function, there is still a paucity of information on how Ang II exerts cell-specific effects through ROS and how Nox isoforms are differentially regulated by Ang II. Moreover, exact mechanisms whereby ROS induce oxidative modifications of signaling molecules mediating Ang II actions remain elusive. ***Future Directions:*** Future research should elucidate these issues to better understand the significance of Ang II and ROS in vascular (patho) biology. *Antioxid. Redox Signal*. 19, 1110–1120.

Introduction

ANGIOTENSIN II (ANG II), the major bioactive peptide of the renin–angiotensin system (RAS), plays a major role in the regulation of vascular function and structure. It is a multifunctional vasoactive peptide produced systemically and locally within the vascular wall. Ang II is a potent vasoconstrictor that also has mitogenic, proinflammatory, and profibrotic actions. These effects are elicited through myriad-signaling pathways, many of which involve reactive oxygen species (ROS), particularly superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) (41, 47, 71). Under physiological conditions, ROS production is tightly controlled, and ROS play an important role as a signaling molecule in the control of endothelial function and vascular tone. In pathological conditions, when ROS bioavailability is increased (oxidative stress), Ang II signaling is altered, leading to endothelial dysfunction, vascular remodeling, and inflammation, important processes underlying vascular injury in cardiovascular disease.

Ang II exerts its diverse actions *via* two G-protein-coupled receptors, Ang II type 1 (AT_1R) and type 2 (AT_2R) receptors (27, 41, 47, 71). The AT_1R mediates most of the known actions of Ang II. The AT_2R is associated with antiproliferative, proapoptotic, and vasodilatory actions of Ang II and tends to counteract AT_1R effects. Signaling pathways elicited by Ang II/AT_1R involve interactions with several heterotrimeric G-proteins coupled to second messengers and cytosolic proteins, including phospholipase C (PLC), phospholipase A2 (PLA2), phospholipase D (PLD), and protein kinase C (PKC) (71). In addition, Ang II/AT_1R signals through activation of many receptor and nonreceptor tyrosine kinases and serine threonine kinases, important in cell growth, hypertrophy, and inflammation. Growing evidence indicates that many of the

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pathways through which Ang II signals involve activation of NADPH oxidase (Nox), which is a major source of ROS in vascular cells.

Nox and Ang II

Griendling and colleagues were among the first to demonstrate that Ang II activates vascular Nox (31, 75). Using rat aortic vascular smooth muscle cells (VSMCs), they showed that treatment of VSMCs with Ang II for 4–6 h caused a nearly threefold increase in intracellular $O_2^{\bullet -}$ formation as detected by the lucigenin assay (31). This, derived from activation of both NADPH and NADH oxidases (31, 75) and p22phox, was found to be obligatory in this process (75). The pathophysiological significance of these cell-based findings was confirmed in rat studies where Ang II-induced hypertension was associated with increased vascular generation of Nox-derived $O_2^{\bullet -}$ and endothelial dysfunction, effects that were blocked by losartan, an AT_1R blocker (59, 62). These early studies paved the way for the field relating to Ang II, oxidative stress, and hypertension.

Mechanisms whereby Ang II regulates Nox are complex and occur at the gene, transcriptional, and post-transcriptional levels and involve numerous intermediate signaling molecules (*e.g.*, c-Src, PKC, receptor tyrosine kinases, protein disulfide isomerase, and polymerase delta-interacting protein 2 [Poldip2]) and scaffolding proteins/platforms (*e.g.*, lipid rafts, caveolae, actin, caveolin, and cortactin) (23, 36, 46). More recently, it has been shown that Nox-derived ROS, in turn, regulate Ang II receptors (58). Hence, there is a feed-forward system where Ang II regulates ROS-generating Nox, which regulates expression and activation of AT_1R (Fig. 1).

Nox Family Oxidases

Nox was originally considered to be expressed only in phagocytic cells involved in host defense and innate immunity. The prototypical reduced nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase, Nox2, that is found in phagocytes, comprises five components, (phox for phagocyte oxidase), p47phox, p67phox, p40phox, p22phox, and gp91phox (4), and the small G protein Rac 1/2. It is now clear that there is a family of NAD(P)H oxidases, called Noxs, the primary function of which is to catalyze the transfer of electrons from NADPH to molecular oxygen *via* the catalytic subunit (Nox). The mammalian Nox family comprises seven members: Nox1, Nox2, Nox3, Nox4, Nox5, Duox1, and Duox2 (28, 67). All are transmembrane proteins that have a core catalytic subunit (Nox) and numerous regulatory subunits. Unlike phagocytic NAD(P)H oxidase, which is activated only upon stimulation and which generates $O_2^{\bullet -}$ in a burst-like manner extracellularly, nonphagocytic Noxs are constitutively active, produce $O_2^{\bullet -}$ intracellularly in a slow and sustained fashion, and act as intracellular signaling molecules, influencing not only transcription factors but also other molecules involved in inflammation, cell growth, and contraction, such as mitogen-activated protein (MAP) kinases, tyrosine kinases, and protein phosphatases (15, 16).

Nox1, Nox2, Nox4, and Nox5 have been identified in cardiovascular and renal tissue (39). Although all Noxs have the same function to generate ROS, mechanisms of activation, subunit requirements, and intracellular distributions vary between isoforms (Fig. 2). Nox1 and Nox2 are constitutively associated with p22phox, and the full activation of Nox1/p22phox and Nox2/p22phox requires interaction with other cytosolic subunits, including p47phox (or its homolog

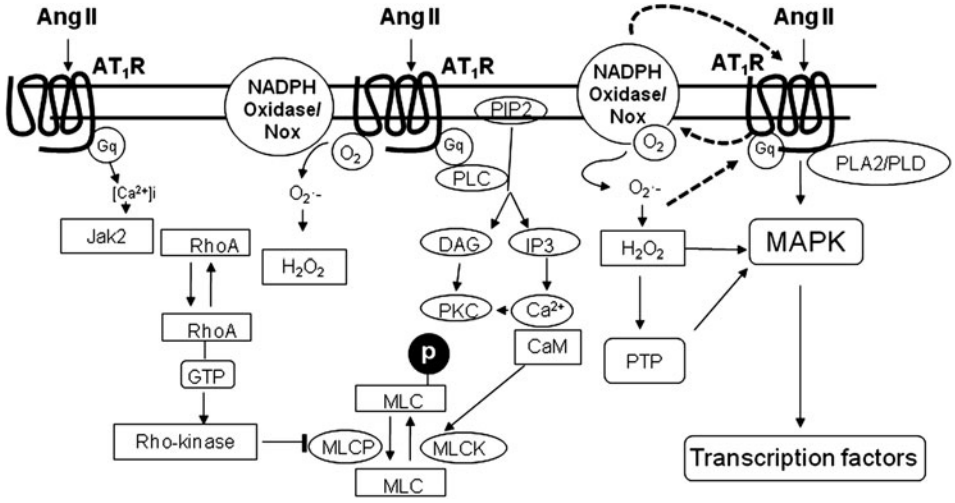

FIG. 1. Role of Nox-derived ROS in Ang II-mediated effects in vascular cells. Ang II binds to its AT_1R, which couples to heterometric Gq proteins, to activate PLC, leading to generation of IP3 and DAG, resulting in increased $[Ca^{2+}]_i$ that triggers phosphorylation of MLC_{20} and stimulation of contraction. Ang II also induces contraction through the RhoA/Rho-kinase pathway that increases Ca^{2+} sensitivity by inhibiting MLCP. Ang II/AT_1R stimulates Nox-derived ROS formation, which regulates MAPKs, tyrosine kinases, PTPs, and transcription factors. Formation of ROS through Ang II/AT_1R further regulates AT_1R through a feed-forward mechanism (*dashed lines*). Ang II, angiotensin II; AT_1R, Ang II type 1 receptor; $[Ca^{2+}]_i$, intracellular free-calcium concentration; DAG, diacylglycerol; IP3, inositol-3-phosphate; MAPKs, mitogen-activated protein kinases; MLCK, myosin light-chain kinase; MLCP, myosin light-chain phosphatase; Nox, NADPH oxidase; PLC, phospholipase C; PTPs, protein tyrosine phosphatases; ROS, reactive oxygen species.

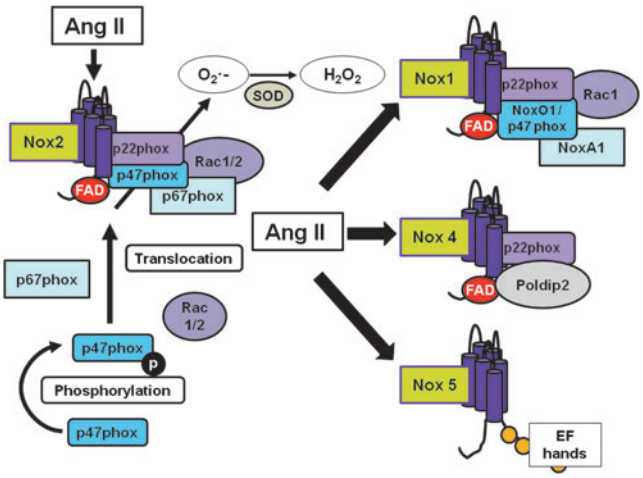

FIG. 2. Nox activation and differences between Nox homologs. Nox comprises a complex of membrane and cytosolic subunits. Nox2 is the classical prototype. Membrane proteins are p22phox and the Nox subunit, and form a noncovalent heterodimer. These proteins possess the electron transport apparatus and may act as a physical conduit for the electron transfer that occurs across the membrane. The cytosolic proteins (p47phox, p67phox, NoxO1, NoxA1, and rac 1/2) are cofactors for enzymatic activity and are used to initiate and/or regulate electron transfer. To Nox2 be activated, p47phox is phosphorylated and translocates from the cytosol to the membrane with the other cytosolic subunits (p67phox and rac1/2). Nox1 can also be activated in a similar way to Nox2, but possesses other cytosolic subunits such as NoxO1 and NoxA1. Nox4 does not require any cytosolic subunit to be activated. Nox4 is constitutively active in cells, and its activity is controlled by Poldip2. On the other hand, Nox5 activation is not dependent on any subunit, but because of calcium-binding domains (EF hands), its activity is controlled by calcium and calmodulin. NoxA1, NADPH activator 1; NoxO1, NADPH organizer 1; Poldip2, polymerase (DNA-directed) delta-interacting protein 2.

NADPH organizer 1 [NoxO1]), p67phox (or homolog NADPH activator 1 [NoxA1]), and Rac (21, 42, 65). Nox4 is constitutively active and associates with p22phox, but does not require other oxidase subunits for its activation, whereas Nox5 functions independently of any Nox subunits and is activated in a calcium–calmodulin-dependent manner (21, 65).

Hyperactivation of Noxs leads to excessive ROS generation that disrupts redox networks, normally regulated by thiol-dependent antioxidant systems. This results in oxidative stress, triggering molecular processes, which, in the vasculature, contributes to vascular damage (Fig. 3). All vascular cell types, including endothelial cells, VSMCs, and adventitial cells (fibroblasts and adipocytes), are capable of producing ROS through Noxs. Noxs have been extensively reviewed (21, 37, 39, 65), and only an overview of recent developments related to Ang II is discussed here.

Regulation of Noxs and Nox Subunits by Ang II

Ang II is functionally associated with Nox1, Nox2, and Nox5 and variably with Nox4 in the vasculature. The biological significance of different Noxs being activated by the same vasoactive agent still awaits clarification, but their differential tissue distribution, cellular localization, and subcellular compartmentalization probably play a major role in Nox-specific actions elicited by Ang II (5). Ang II-activated Nox1 appears to be important in VSMCs from large arteries, but Nox2 may be more important in small-resistance arteries, especially in humans (31, 68, 75). Nox4 abundance is greater in endothelial cells than in VSMCs, at least in basal conditions, although there is probably important cross-talk between Noxs in different cell types (Fig. 4). Regulation also seems to differ in pathological conditions. For example, while Ang II increased expression of all Noxs in VSMCs from normal Wistar Kyoto rats (WKY), only Nox1 was influenced in VSMCs from spontaneously hypertensive rats (SHR) (7, 19, 66). This may be important in vascular dysfunction associated with

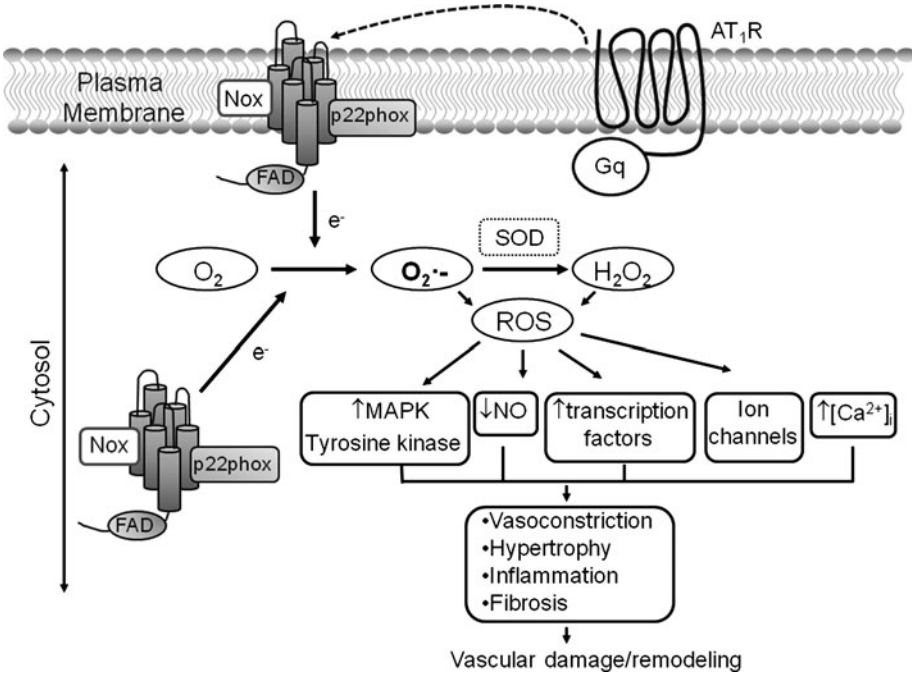

FIG. 3. Ang II-stimulated redox-sensitive pathways that promote vascular remodeling. Generation of ROS by plasma membrane-associated Noxs and cytosolic Noxs in response to Ang II-AT_1R signaling leads to activation of multiple pathways that promote vascular injury and remodeling. e^-, electron; NO, nitric oxide; SOD, superoxide dismutase.

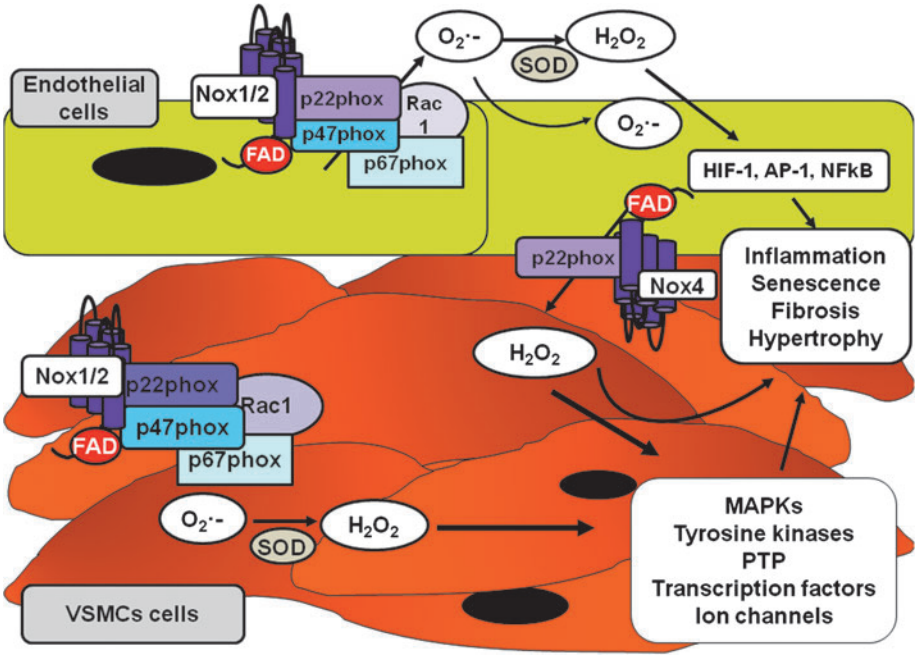

FIG. 4. Distribution of vascular Noxs in endothelial and VSMCs and downstream signaling pathways regulated by Nox-derived ROS. In basal conditions, Nox4 expression is greater in endothelial cells than in VSMCs. Nox1 and Nox2 are expressed in VSMCs from small arteries, whereas Nox1, but not Nox2, is expressed in VSMCs from large arteries. These Noxs are upregulated in pathological conditions. The exact distribution of vascular Nox isoforms *in vivo* still awaits confirmation. VSMCs, vascular smooth muscle cells.

hypertension. In transgenic mice, in which Nox1 is overexpressed in vascular smooth muscle (SMCnox1), ROS production is enhanced in response to Ang II, causing endothelial nitric oxide synthase (NOS) uncoupling and decreased nitric oxide bioavailability, with resultant impaired vasorelaxation (19).

In vitro studies have indicated that Ang II is a potent stimulator of vascular Noxs (70). It induces activation (phosphorylation) of the oxidase subunits; it increases expression of Nox isoforms and Nox subunits (p47phox, p67phox, and p22phox); and it stimulates ROS production in cultured VSMCs and in intact arteries (76). Mechanisms linking Ang II to the enzyme and upstream signaling molecules modulating NAD(P)H oxidase in vascular cells have not been fully elucidated, but phospholipases, PKC, Src tyrosine kinases, phosphatidylinositol 3-kinase (PI3K), and Rac may be important (8, 64). Ang II stimulates Nox activity through various phospholipases, including PLC, PLA2, and PLD (8, 64). We showed that c-Src is critically placed between the AT_1R and Nox, where it stimulates phosphorylation of p47phox and activation of the oxidase (73). These processes involve cortactin interaction with actin, which may act as a scaffolding network for Nox subunit trafficking to the cell membrane to assemble the functionally active oxidase complex (72). Lipid rafts/caveolae play an important role in agonist-stimulated activation of vascular Noxs and may act as signaling platforms to integrate redox events (13). In pathological conditions, where the RAS is upregulated, for example, hypertension, diabetes, and atherosclerosis, activation of Noxs by Ang II is augmented, leading to increased ROS generation and oxidative stress (11, 45).

Nox-Derived ROS As Signaling Molecules in the Vasculature

The dynamics and chemical properties of ROS dictate the biological role that they will play. The more reactive the species, the shorter is the half-life and the more rapidly it will interact with other molecules. ROS can also be electrically charged or electrically neutral, hydrophobic, or hydrophilic. Such characteristics determine their ability to cross membranes and/or to move in the environment between aqueous and lipophilic environments.

ROS are produced as intermediates in reduction–oxidation (redox) reactions, leading from O_2 to H_2O. The major mechanism for ROS generation begins with the reduction of O_2 by the addition of one electron, to generate $O_2^{\bullet -}$, considered the primary ROS. $O_2^{\bullet -}$ interacts with other molecules to produce secondary ROS, directly or through enzyme- or metal-catalyzed reactions (35). Reduction of $O_2^{\bullet -}$ leads to formation of H_2O_2, which is further converted to secondary metabolites such as highly reactive hydroxyl $HO^{\bullet}$. Although the favored reaction is the generation of H_2O_2, $O_2^{\bullet -}$ also reacts with nitric oxide ($NO^{\bullet}$) to form peroxinitrite ($ONOO^-$), with transition metals, such as iron found in iron/sulfur center-containing proteins, or it may be protonated to the hydroperoxyl radical ($H_2O^{\bullet}$). $H_2O^{\bullet}$ is particularly important in lipid peroxidation and atherogenesis.

Of the ROS generated in vascular cells, $O_2^{\bullet -}$ and H_2O_2 appear to be especially important. In biological systems, $O_2^{\bullet -}$ is short-lived owing to its rapid dismutation to H_2O_2. Dismutation can be spontaneous (rate constant $= 8 \times 10^4$/mol/s) or enzymatic *via* superoxide dismutase (SOD) (rate constant $= 2 \times 10^9$/mol/s) (26). Superoxide reacts with $NO^{\bullet}$ to form $ONOO^-$ with a rate constant of $4–16 \times 10^9$/mol/s (3), resulting in $NO^{\bullet}$ quenching and resultant decreased in $NO^{\bullet}$ bioavailability. Redox-regulated uncoupling of NOS also contributes to decreased $NO^{\bullet}$ and increased generation of $O_2^{\bullet -}$ (Fig. 5). In addition, oxidants react with FeS_4 or with protein thiols such as cysteine residues, an effect that is increased by metabolic stress (10). Although $O_2^{\bullet -}$ has the capacity to react with many molecules, the preferred reaction is dismutation to H_2O_2 because of the fast reaction rate of SOD. Three mammalian SOD isoforms have been identified:

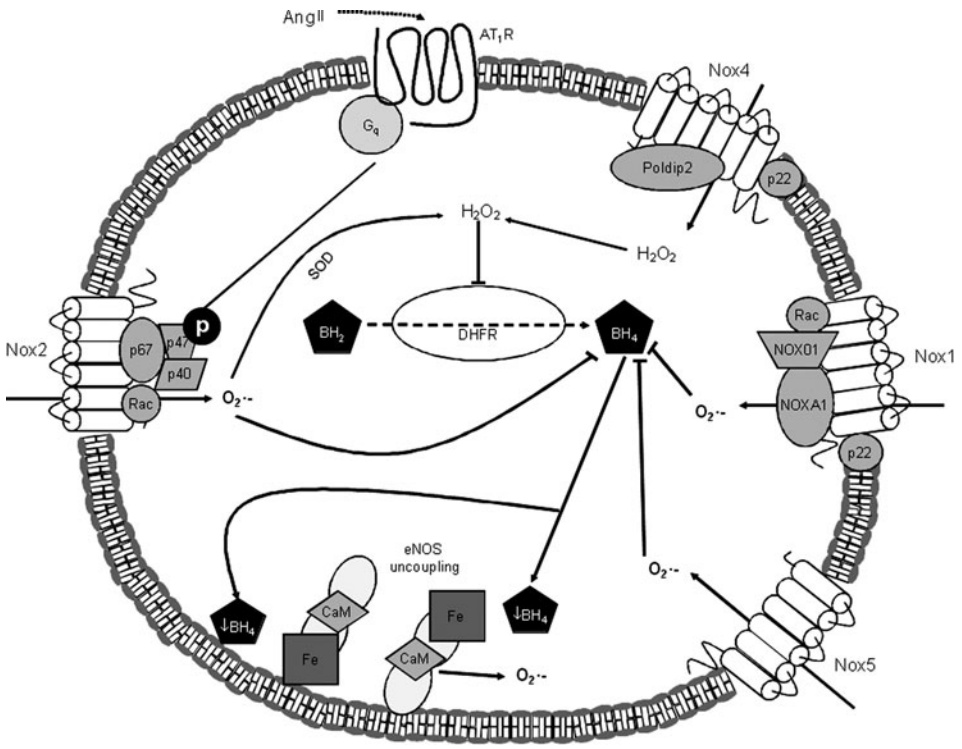

FIG. 5. Nox and eNOS by Ang II in endothelial cells. Ang II stimulates production of $O_2^{\bullet -}$ by Nox1, Nox2, and Nox5 through the AT_1R. Additionally, Ang II stimulates production of H_2O_2 directly through Nox4, and indirectly through the SOD-mediated conversion of $O_2^{\bullet -}$ produced by Nox1, 2, and 5. The $O_2^{\bullet -}$-mediated oxidation and inactivation of the eNOS cofactor BH_4 promotes uncoupling of eNOS, leading to eNOS-mediated production of $O_2^{\bullet -}$. Additionally, H_2O_2 inhibits DHFR, an enzyme that catalyzes the conversion of BH_2 to BH_4, which further reduces BH_4 bioavailability, leading to eNOS uncoupling. BH_2, dihydrobiopterin; BH_4, tetrahydrobiopterin; CaM, calmodulin; DHFR, dihydrofolate reductase; eNOS, endothelial nitric oxide synthase; Fe, eNOS heme domain; H_2O_2, hydrogen peroxide; $O_2^{\bullet -}$, superoxide; p22, p22 *phox* (Nox subunit); p40, p40 *phox* (Nox subunit); p47, p47 *phox* (Nox subunit); p67, p67 *phox* (Nox subunit); Rac, Rho-like GTPase Rac.

copper/zinc SOD (SOD1), manganese-containing mitochondrial SOD (Mn-SOD, SOD2), and extracellular SOD (EC-SOD, SOD3) (48). The major vascular SOD is EC-SOD. The negative charge on $O_2^{\bullet -}$ makes it unable to cross cellular membranes, except possibly through ion channels, such as chloride channel 3 (CLC-3). CLC-3 transports $O_2^{\bullet -}$ out of endosomes into the cytoplasm in endothelial cells and has been implicated to play an important role in VSMC regulation (51). H_2O_2 has a longer lifespan than $O_2^{\bullet -}$, is relatively stable, and is easily diffusible within and between cells. The main source of H_2O_2 in vascular tissue is the dismutation of $O_2^{\bullet -}$: $2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$.

H_2O_2 is tightly regulated by intracellular and extracellular enzymes, including catalase, glutathione peroxidase, thioredoxin, and other peroxyredoxins, which convert H_2O_2 to water and O_2 and other metabolites. Although both $O_2^{\bullet -}$ and H_2O_2 have been suggested to act as signaling molecules, it is mainly H_2O_2 that is considered a signaling molecule because of its relative stability, tight regulation, subcellular localization, and ability to react reversibly with cysteine residues (25).

ROS-specific effects are mediated in large part through the oxidative modification of thiols on cysteine residues within redox-sensitive target proteins (24). Of all the unique cysteine residues in the human genome, 20,000–40,000 are highly sensitive to oxidations (1). Oxidation of thiols leads to structural changes in target molecules that result in activation or inactivation of signaling proteins. For example, protein tyrosine phosphatases (PTPs) are inactivated by oxidation, leading to increased phosphorylation of downstream proteins. In addition to protein oxidation, ROS induce oxidative damage through lipid peroxidation of cellular membranes and DNA breaks (1). Through these mechanisms, $O_2^{\bullet -}$ and H_2O_2 influence many signaling molecules important in the regulation of vascular function, including MAP kinases, nonreceptor tyrosine kinases, receptor tyrosine kinases, PTPs, and redox-sensitive transcription factors (17, 40) (Fig. 6). Activation of these molecules participates in cell growth, migration, expression of proinflammatory genes, production of extracellular matrix proteins, and contraction, processes important in the regulation of vascular function and tone.

The distinct chemical properties between $O_2^{\bullet -}$ and H_2O_2 and their different sites of distribution mean that different species of ROS activate diverse signaling pathways, which lead to divergent, and potentially opposing, biological responses. For example, in the vasculature, increased $O_2^{\bullet -}$ levels inactivate the vasodilator $NO^{\bullet}$, leading to endothelial dysfunction and vasoconstriction (61), whereas H_2O_2 acts as a direct vasodilator in some vascular beds, including cerebral, coronary, and mesenteric arteries (54).

The specificity of signaling through ROS is related to many factors, including subcellular localization of Nox isoforms, binding of Noxs to scaffolding proteins, and

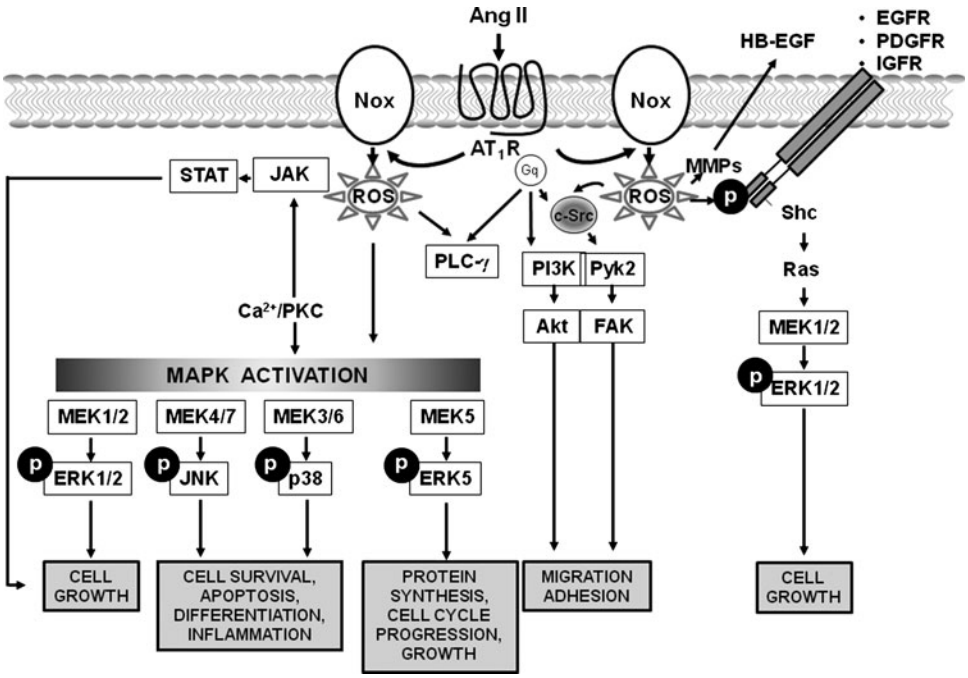

FIG. 6. Ang II-mediated redox-sensitive growth signaling in VSMCs. In VSMCs, binding of Ang II to the AT_1R leads to increased ROS generation, in part, *via* activation of c-Src-induced Nox activation. ROS stimulate nonreceptor tyrosine kinases such as FAK, JAK and PI3K, and PLC/PKC as well as receptor tyrosine kinases, such as EGFR, IGFR, and PDGFR. Redox signaling in turn regulates downstream MEK cascades, leading to phosphorylation of MAPKs, which induce growth, apoptosis, differentiation, migration, and inflammation of VSMCs. Ang II/AT_1R also induces MMP-mediated extracellular release of HB-EGF, which then stimulates EGFR transactivation and ERK1/2 MAPK activation. EGFR, epidermal growth factor receptor; FAK, focal adhesion kinase; HB-EGF, heparin-binding epidermal growth factor; IGFR, insulin-like growth factor receptor; JAK, Janus-activated kinase; JNK, c-Jun NH2-terminal kinase; MEK, MAPK/ERK kinase; MMP, matrix metalloproteinase; p, phosphorylation; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PLC, phospholipase C; STAT, signal transducers and activators of transcription.

compartmentalization within intracellular organelles, for example, the mitochondria, the nucleus, and the endoplasmic reticulum (ER). Nox2 is membrane-associated, whereas Nox4 has been identified in the mitochondria, the ER, and the nucleus. Mitochondrial Nox4 expression is increased in kidneys from diabetic rats (29, 30, 78). It has been proposed that localization of Nox4 to the mitochondria creates a short paracrine loop, whereby ROS production by mitochondrial Nox4 regulates or is regulated by ROS generated by the mitochondrial respiratory chain (3). Furthermore, Nox subunits or Nox isoforms may interact with other proteins to facilitate localized ROS generation.

Ang II Signaling and ROS in the Vasculature

Ang II mediates effects *via* complex intracellular signaling pathways that are stimulated after binding of the peptide to its cell-surface receptors. Both receptors play a role in regulating VSMC function, although they differ in their actions.

Redox-sensitive growth signaling by Ang II in vascular cells

Ang II stimulates cell growth through phosphorylation of tyrosine kinases, activation of MAP kinases, mobilization of intracellular calcium (Ca^{2+}), and production of ROS (77) (Fig. 6). Ang II, *via* AT_1R, induces phosphorylation of multiple tyrosine kinases, including c-Src, Janus family kinases (JAK), focal adhesion kinase (FAK), protein tyrosine kinase 2 (Pyk2), p130Cas, and PI3K (56). c-Src, which regulates Nox, is a critically important kinase involved in trophic and contractile actions of Ang II (12). c-Src is upstream from many growth-signaling molecules, including PLC-γ, Pyk2, FAK, JAK, Shc, MAP kinases, and PI3K (12).

Of the many growth-signaling molecules, the MAP kinase family is best characterized (38). In VSMCs, Ang II activates all four of the major MAP kinases, extracellular signal-regulated kinases (ERK1/2), p38MAP kinase, c-Jun N-terminal kinases (JNK), and ERK5. ERK1/2, phosphorylated by MAP kinase kinase 1/2 (MEK1/2) (MAP/ERK kinase), is a key growth-signaling kinase, whereas JNK and p38MAP kinase, phosphorylated by MEK4/7 and MEK3/6, respectively, influence cell survival, apoptosis, differentiation, and inflammation. ERK5 is involved in protein synthesis, cell cycle progression, and cell growth. Activation of these MAP kinases requires ROS and Nox activation, because Nox inhibitors and various antioxidants attenuate Ang II-induced activation of MAP kinases (22, 63, 69). MAP kinase phosphorylation may not be directly influenced by ROS, but rather by changes in activation of upstream PTPs. PTPs possess highly conserved cysteine residues that are highly sensitive to oxidation (53).

Ang II transactivation of receptor tyrosine kinases: role of ROS

Ang II also activates receptor tyrosine kinases, classically linked to growth-signaling pathways, even though it may not bind directly to these receptors. This process of transactivation has been demonstrated for epidermal growth factor (EGF) receptor, platelet-derived growth factor receptor, subtype β, and insulin-like 1 growth factor receptor (16) (Fig. 6). Mechanisms whereby Ang II-induces receptor tyrosine kinase transactivation include activation of Pyk2 and Src, metalloprotease-dependent shedding of heparin-binding EGF-like growth factor and ROS (50). In rat VSMCs, Ang II, through increased oxidative stress and activation of c-Src, transactivates EGF-R, which leads to MAP kinase activation and cell growth, an effect that is augmented in SHR (43).

Ang II signaling, RhoA/Rho kinase, and ROS

Activation of RhoA and its downstream target Rho-kinase is increasingly being recognized as an important mechanism of vasoconstriction by Ang II and accordingly has been implicated in the pathophysiology of hypertension and other vascular diseases (44, 57). RhoA, a member of the Rho family of small GTPase-binding proteins, is abundantly expressed in VSMCs and participates in vasoconstriction *via* phosphorylation of myosin light chain and sensitization of contractile proteins to Ca^{2+}. RhoA/Rho-kinase also influences VSMC migration, a process that is redox sensitive, since apocynin, which inhibits Nox activity and ROS generation, blocked Ang II-stimulated effects (55). In the renal vasculature, alpha(2)-adrenoceptors potentiate renal vascular responses to Ang II. This interaction, which influences vascular resistance, involves RhoA/Rho-kinase, ROS, and Nox2, since Ang II effects were attenuated by Y27632 (Rho-kinase inhibitor), tempol (superoxide dismutase mimetic), and gp91ds-tat (Nox2 inhibitor) (34).

RhoA and Nox also regulate microparticle release from Ang II-stimulated endothelial cells (9). Microparticles are submicron fragments that arise from plasma membrane blebbing and subsequently shed from activated or apoptotic cells. They impair angiogenesis, promote oxidative stress, and influence vasodilation, and they themselves produce ROS (49). Studies in cultured endothelial cells and in Ang II-infused mice demonstrated that fasudil and apocynin, inhibitors of Rho kinase and Nox, respectively, blunted microparticle formation (6). These processes have been implicated in Ang II-mediated oxidative damage of the endothelium.

Ang II, transcription factors, inflammation, and ROS

Important downstream targets of signaling pathways are transcription factors, which are highly redox sensitive. Many transcription factors important in inflammation, cell growth, and fibrosis are activated by Ang II through mechanisms that involve Nox and ROS. Among transcription factors that are activated and sensitive to both Ang II and ROS, there is the activator protein 1 (AP-1), nuclear factor kappa B, cyclic AMP response element-binding protein (CREB), and hypoxia-inducible factor 1 (HIF-1) (6, 74). Mitochondrial-generated ROS have also been shown to be essential intermediates for HIF-1 activation in VSMCs (18, 60).

Transcription factors act as signal integrators regulating processes related to vascular inflammation. Responses to proinflammatory stimuli, such as Ang II and ROS, lead to activation of transcription factors important in the production of proinflammatory cytokines and interleukins. These processes in turn influence leukocyte adherence, chemotaxis, and the inflammatory response, which underlie vascular damage in cardiovascular disease.

Ang II signaling, ROS, and vascular cell senescence

In addition to regulating cell growth, Ang II influences other components of the life cycle of cells, including senescence. Senescence is defined as a form of irreversible arrest in cell proliferation and is associated with a characteristic gene and protein phenotype different to that of proliferating cells. Senescent cells characteristically express senescence-associated β- galactosidase and pShc-66 (1, 15). As cells reach the end of their lifespan, they undergo replicative senescence, driven by telomere dysfunction, a process that occurs with normal aging. In pathological conditions, such as hypertension and atherosclerosis, vascular cells undergo premature senescence, which can be triggered by Ang II and oxidative stress (52). Such processes contribute to vascular remodeling and early vascular aging. Clinical studies using angiotensin receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors (ACEis) demonstrated protective effects against age-related cardiovascular changes (52). *In vitro* studies supported these findings showing a link between Ang II stimulation and vascular cell senescence, an effect blocked by ARBs and ACEis (14). In AT_1R knockout mice, lifespan was prolonged by almost 30% compared to genetically matched wild-type controls (2, 20). This enhanced longevity was associated with improved cardiovascular morphology, reduced ROS production, attenuated mitochondrial loss, and enhanced expression of survival genes (nicotinamide phosphoribosyltransferase [Nampt] and sirtuin-3 [Sirt3]) (2).

Molecular mechanisms through which Ang II regulates vascular cell senescence seem to involve signaling pathways similar to those that control cell growth. In addition, ROS are emerging as important modulators of senescence (32). This is evidenced by studies demonstrating that H_2O_2 induces vascular cell senescence, that Nox and ROS production are increased in senescent cells, and that antioxidants inhibit Ang II-induced senescence (32, 33). ROS induce DNA damage, which is critically involved in Ang II-mediated redox-triggered cell senescence. Both telomere-dependent and telomere-independent mechanisms have been linked to Ang II-mediated senescence (32). Telomere-related effects influence replicative senescence, whereas telomere-independent processes may contribute to premature senescence. The net effect of Ang II may relate to the concentration and chronicity of Ang II stimulation.

Conclusions

Over the recent past, our views of Ang II have changed from being a simple vasoconstrictor to that of a complex pleiotropic factor, involved in vascular hypertrophy, fibrosis, inflammation, and aging. These effects are mediated through diverse signaling pathways involving PLC/PKC/Ca^{2+} mobilization, PLA2, PLD, MAP kinases, tyrosine kinases, proto-oncogene expression, RhoA/Rho-kinase, inflammation, and

cell cycle modulation. Common to these pathways are ROS, derived in large part from vascular Noxs. Through increased generation of ROS and activation of redox-sensitive transcription factors, Ang II promotes expression of cell adhesion molecules and induces synthesis of proinflammatory mediators and growth factors. These molecular and cellular processes facilitate increased vascular permeability, leukocyte recruitment, calcification, and vascular fibrosis, leading to vascular injury, structural remodeling, and premature aging. Targeting some of these molecular events with novel therapeutic strategies, possibly at the level of Nox-derived ROS, may regress or prevent arterial remodeling and aging and thereby provide important vascular protection in cardiovascular and age-related diseases.

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References

1. Al Ghouleh I, Khoo NK, Knaus UG, Griendling KK, Touyz RM, Thannickal VJ, Barchowsky A, Nauseef WM, Kelley EE, Bauer PM, Darley-Usmar V, Shiva S, Cifuentes- Pagano E, Freeman BA, Gladwin MT, and Pagano PJ. Oxidases and peroxidases in cardiovascular and lung disease: new concepts in reactive oxygen species signaling. *Free Radic Biol Med* 51: 1271–1288, 2011.
2. Benigni A, Corna D, Zoja C, Sonzogni A, Latini R, Salio M, Conti S, Rottoli D, Longaretti L, Cassis P, Morigi M, Coffman TM, and Remuzzi G. Disruption of the Ang II type 1 receptor promotes longevity in mice. *J Clin Invest* 119: 524–530, 2009.
3. Block K, Gorin Y, and Abboud HE. Subcellular localization of Nox4 and regulation in diabetes. *Proc Natl Acad Sci U S A* 106: 14385–14390, 2009.
4. Bokoch GM and Zhao T. Regulation of the phagocyte NAD(P)H oxidase by Rac GTPase. *Antioxid Redox Signal* 8: 1533–1548, 2006.
5. Brandes RP and Schröder K. Differential vascular functions of Nox family NAD(P)H oxidases. *Curr Opin Lipidol* 19: 513–518, 2008.
6. Brasier AR. The nuclear factor-kappaB-interleukin-6 signalling pathway mediating vascular inflammation. *Cardiovasc Res* 86: 211–218, 2010.
7. Briones AM, Tabet F, Callera GE, Montezano AC, Yogi A, He Y, Quinn MT, Salaices M, and Touyz RM. Differential regulation of Nox1, Nox2 and Nox4 in vascular smooth muscle cells from WKY and SHR. *J Am Soc Hypertens* 5: 137–153, 2011.
8. Brown DI and Griendling KK. Nox proteins in signal transduction. *Free Radic Biol Med* 47: 1239–1253, 2009.
9. Burger D, Montezano AC, Nishigaki N, He Y, Carter A, and Touyz RM. Endothelial microparticle formation by angiotensin II is mediated via Ang II receptor type I/NADPH 18 oxidase/Rho kinase pathways targeted to lipid rafts. *Arterioscler Thromb Vasc Biol* 31: 1898–1899, 2011.
10. Burgoyne JR, Haeussler DJ, Kumar V, Ji Y, Pimental DR, Zee RS, Costello CE, Lin C, McComb ME, Cohen RA, and Bachschmid MM. Oxidation of HRas cysteine thiols by metabolic stress prevents palmitoylation *in vivo* and contributes to endothelial cell apoptosis. *FASEB J* 26: 832–841, 2012.
11. Callera GE, Montezano AC, Yogi A, Tostes RC, and Touyz RM. Vascular signaling through cholesterol-rich domains: implications in hypertension. *Curr Opin Nephrol Hypertens* 16: 90–104, 2007.
12. Callera GE, Yogi A, Briones AM, Montezano AC, He Y, Tostes RC, Schiffrin EL, and Touyz RM. Vascular proinflammatory responses by aldosterone are mediated via c-Src trafficking to cholesterol-rich microdomains: role of PDGFR. *Cardiovasc Res* 91: 720–731, 2011.
13. Camici GG, Cosentino F, Tanner FC, and Lüscher TF. The role of p66Shc deletion in ageassociated arterial dysfunction and disease states. *J Appl Physiol* 105: 1628–1631, 2008.
14. Capettini LS, Montecucco F, Mach F, Stergiopulos N, Santos RA, and da Silva RF. Role of renin-angiotensin system in inflammation, immunity and aging. *Curr Pharm Des* 18: 963–970, 2012.
15. Cosentino F, Francia P, Camici GG, Pelicci PG, Lüscher TF, and Volpe M. Final common molecular pathways of aging and cardiovascular disease: role of the p66Shc protein. *Arterioscler Thromb Vasc Biol* 28: 622–628, 2008.
16. Cruzado MC, Risler NR, Miatello RM, Yao G, Schiffrin EL, and Touyz RM. Vascular smooth muscle cell NAD(P)H oxidase activity during the development of hypertension: Effect of angiotensin II and role of insulinlike growth factor-1 receptor transactivation. *Am J Hypertens* 18: 81–87, 2005.
17. Dikalov SI, Dikalova AE, Bikineyeva AT, Schmidt HH, Harrison DG, and Griendling KK. Distinct roles of Nox1 and Nox4 in basal and angiotensin II-stimulated superoxide and hydrogen peroxide production. *Free Radic Biol Med* 45: 1340–1351, 2008.
18. Dikalova AE, Bikineyeva AT, Budzyn K, Nazarewicz RR, McCann L, Lewis W, Harrison DG, and Dikalov SI. Therapeutic targeting of mitochondrial superoxide in hypertension. *Circ Res* 107: 106–116, 2010.
19. Dikalova AE, Góngora MC, Harrison DG, Lambeth JD, Dikalov S, and Griendling KK. Upregulation of Nox1 in vascular smooth muscle leads to impaired endothelium-dependent relaxation via eNOS uncoupling. *Am J Physiol Heart Circ Physiol* 299: H673–H679, 2010.
20. Donnini S, Terzuoli E, Ziche M, and Morbidelli L. Sulfhydryl angiotensin-converting enzyme inhibitor promotes endothelial cell survival through nitric-oxide synthase, fibroblast growth factor-2, and telomerase cross-talk. *J Pharmacol Exp Ther* 332: 776–784, 2010.
21. Dutta S and Rittinger K. Regulation of NOXO1 activity through reversible interactions with p22 and NOXA1. *PLoS One* 5: e10478.
22. Ebrahimian T, Li MW, Lemarié CA, Simeone SM, Pagano PJ, Gaestel M, Paradis P, Wassmann S, and Schiffrin EL. Mitogen-activated protein kinase-activated protein kinase 2 in angiotensin II-induced inflammation and hypertension: regulation of oxidative stress. *Hypertension* 57: 245–254, 2011.
23. Fernandes DC, Manoel AH, Wosniak J Jr., and Laurindo FR. Protein disulfide isomerase overexpression in vascular smooth muscle cells induces spontaneous preemptive NADPH oxidase activation and Nox1 mRNA expression: effects of nitrosothiol exposure. *Arch Biochem Biophys* 484: 197–204, 2009.

24. Flohé L, Toppo S, Cozza G, and Ursini F. A comparison of thiol peroxidase mechanisms. *Antioxid Redox Signal* 15: 763–780, 2011.
25. Freinbichler W, Colivicchi MA, Stefanini C, Bianchi L, Ballini C, Misini B, Weinberger P, Linert W, Varešlija D, Tipton KF, and Della Corte L. Highly reactive oxygen species: detection, formation, and possible functions. *Cell Mol Life Sci* 68: 2067–2079, 2011.
26. Fridovich I. Superoxide anion radical (O2-.), superoxide dismutases, and related matters. *J Biol Chem* 272: 18515–18517, 1997.
27. Garrido AM and Griendling KK: NADPH oxidases and angiotensin II receptor signaling. *Mol Cell Endocrinol* 302: 148–158, 2009.
28. Geiszt M. NAD(P)H oxidases: New kids on the block. *Cardiovasc Res* 71: 289–299, 2006.
29. Gordillo G, Fang H, Park H, and Roy S. Nox-4-dependent nuclear H_2O_2 drives DNA oxidation resulting in 8-OHdG as urinary biomarker and hemangioendothelioma formation. *Antioxid Redox Signal* 12: 933–943, 2010.
30. Graham KA, Kulawiec M, Owens KM, Li X, Desouki MM, Chandra D, and Singh KK. NADPH oxidase 4 is an oncoprotein localized to mitochondria. *Cancer Biol Ther* 10: 223–231, 2010.
31. Griendling KK, Minieri CA, Ollerenshaw JD, and Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 74: 1141–1148, 1994.
32. Herbert KE, Mistry Y, Hastings R, Poolman T, Niklason L, and Williams B. Angiotensin II-mediated oxidative DNA damage accelerates cellular senescence in cultured human vascular smooth muscle cells via telomere-dependent and independent pathways. *Circ Res* 102: 201–208, 2008.
33. Imanishi I, Hano T, and Nishio I. Angiotensin II accelerates endothelial progenitor cell senescence through induction of oxidative stress. *J Hypertens* 23: 97–104, 2005.
34. Jackson EK, Gillespie DG, Zhu C, Ren J, Zacharia LC, and Mi Z. Alpha2-adrenoceptors enhance angiotensin II-induced renal vasoconstriction: role for NADPH oxidase and RhoA. *Hypertension* 51: 719–726, 2008.
35. Jacob C, Jamier V, and Ba LA. Redox active secondary metabolites. *Curr Opin Chem Biol* 15: 149–155, 2011.
36. Jagadeesha DK, Takapoo M, Banfi B, Bhalla RC, and Miller FJ Jr. Nox1 transactivation of epidermal growth factor receptor promotes N-cadherin shedding and smooth muscle cell migration. *Cardiovasc Res* 93: 406–413, 2012.
37. Katsuyama M, Matsuno K, and Yabe-Nishimura C. Physiological roles of NOX/NADPH oxidase, the superoxide-generating enzyme. *Clin Biochem Nutr* 50: 9–22, 2012.
38. Knock GA and Ward JP. Redox regulation of protein kinases as a modulator of vascular function. *Antioxid Redox Signal* 15: 1531–1547, 2011.
39. Lassegue B and Clempus RE. Vascular NAD(P)H oxidases: specific features, expression, and regulation. *Am J Physiol Regul Integr Comp Physiol* 285: R277–R297, 2003.
40. Lassègue B, Sorescu D, Szöcs K, Yin Q, Akers M, Zhang Y, Grant SL, Lambeth JD, and Griendling KK. Novel gp91(phox) homologues in vascular smooth muscle cells: nox1 mediates angiotensin II-induced superoxide formation and redox-sensitive signaling pathways. *Circ Res* 88: 888–894, 2010.
41. Lemarié CA and Schiffrin EL: The angiotensin II type 2 receptor in cardiovascular disease. *J Renin Angiotensin Aldosterone Syst* 11: 19–31, 2010.
42. Leto TL, Morand S, Hurt D, and Ueyama T. Targeting and regulation of reactive oxygen species generation by Nox family NADPH oxidases. *Antioxid Redox Signal* 11: 260, 2009.
43. Li Y, Lévesque LO, and Anand-Srivastava MB. Epidermal growth factor receptor transactivation by endogenous vasoactive peptides contributes to hyperproliferation of vascular smooth muscle cells of SHR. *Am J Physiol Heart Circ Physiol* 299: H1959–H1967, 2010.
44. Loirand G and Pacaud P. The role of Rho protein signaling in hypertension. *Nat Rev Cardiol* 7: 637–647, 2010.
45. Lu J, Mitra S, Wang X, Khaidakov M, and Mehta JL. Oxidative stress and lectin-like ox-LDLreceptor LOX-1 in atherogenesis and tumorigenesis. *Antioxid Redox Signal* 15: 2301–2333, 2011.
46. Lyle AN, Deshpande NN, Taniyama Y, Seidel-Rogol B, Pounkova L, Du P, Papaharalambus C, Lassègue B, and Griendling KK Poldip2, a novel regulator of Nox4 and cytoskeletal integrity in vascular smooth muscle cells. *Circ Res* 105: 249–259, 2009.
47. Mehta PK and Griendling KK: Angiotensin II cell signalling: physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol* 292: C82–C97, 2007.
48. Mendez JI, Nicholson WJ, and Taylor WR. SOD isoforms and signaling in blood vessels: evidence for the importance of ROS compartmentalization. *Arterioscler Thromb Vasc Biol* 25: 887–888, 2005.
49. Meziani F, Tesse A, and Andriantsitohaina R. Microparticles are vectors of paradoxical information in vascular cells including the endothelium: role in health and diseases. *Pharmacol Rep* 60: 75–84, 2008.
50. Mifune M, Ohtsu H, Suzuki H, Nakashima H, Brailoiu E, Dun NJ, Frank GD, Inagami T, Higashiyama S, Thomas WG, Eckhart AD, Dempsey PJ, and Eguchi S. G protein coupling and second messenger generation are indispensable for metalloprotease-dependent, heparin binding epidermal growth factor shedding through angiotensin II type-1 receptor. *J Biol Chem* 280: 26592–26599, 2005.
51. Miller FJ Jr., Filali M, Huss GJ, Stanic B, Chamseddine A, Barna TJ, and Lamb FS. Cytokine activation of nuclear factor kappa B in vascular smooth muscle cells requires signaling endosomes containing Nox1 and ClC-3. *Circ Res* 101: 663–671, 2007.
52. Min LJ, Mogi M, Iwai M, and Horiuchi M. Signaling mechanisms of angiotensin II in regulating vascular senescence. *Ageing Res Rev* 8: 113–121, 2009.
53. Monteiro HP, Arai RJ, and Travassos LR. Protein tyrosine phosphorylation and protein tyrosine nitration in redox signaling. Antioxid Redox Signal 10: 843–889, 2008.
54. Montezano AC, Burger D, Paravicini TM, Chignalia AZ, Yusuf H, Almasri M, He Y, Callera GE, He G, Krause KH, Lambeth D, Quinn MT, and Touyz RM. Nicotinamide adenine dinucleotide phosphate reduced oxidase 5 (Nox5) regulation by angiotensin II and endothelin-1 is mediated via calcium/calmodulin-dependent, rac-1-independent pathways in human endothelial cells. *Circ Res* 106: 1363–1373, 2010.
55. Montezano AC, Callera GE, Yogi A, He Y, Tostes RC, He G, Schiffrin EL, and Touyz RM. Aldosterone and angiotensin II synergistically stimulate migration in vascular smooth muscle cells through c-Src-regulated redox-sensitive RhoA pathways. *Arterioscler Thromb Vasc Biol* 28: 1511–1518, 2008.

56. Montezano AC and Touyz RM. Reactive oxygen species and endothelial function—role of nitric oxide synthase uncoupling and Nox family nicotinamide adenine dinucleotide phosphate oxidases. *Basic Clin Pharmacol Toxicol* 110: 87–94, 2012.
57. Nguyen Dinh Cat A and Touyz RM. Cell signaling of angiotensin II on vascular tone: novel mechanisms. *Curr Hypertens Rep* 13: 122–128, 2011.
58. Nishida M, Kitajima N, Saiki S, Nakaya M, and Kurose H. Regulation of Angiotensin II receptor signaling by cysteine modification of NF-κB. *Nitric Oxide* 25: 112–117, 2011.
59. Paravicini TM and Touyz RM. Redox signaling in hypertension. *Cardiovasc Res* 71: 247–258, 2006.
60. Patten DA, Lafleur VN, Robitaille GA, Chan DA, Giaccia AJ, and Richard DE. Hypoxiainducible factor-1 activation in nonhypoxic conditions: the essential role of mitochondrialderived reactive oxygen species. *Mol Biol Cell* 21: 3247–3257, 2010.
61. Queisser N, Fazeli G, and Schupp N. Superoxide anion and hydrogen peroxide-induced signaling and damage in angiotensin II and aldosterone action. *Biol Chem* 391: 1265–1279, 2010.
62. Rajagopalan S, Kurz S, Münzel T, Tarpey M, Freeman BA, Griendling KK, and Harrison DG. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest* 97: 1916–1923, 1996.
63. Rasmussen HH, Hamilton EJ, Liu CC, and Figtree GA. Reversible oxidative modification: implications for cardiovascular physiology and pathophysiology. *Trends Cardiovasc Med* 20: 85–90, 2010.
64. Seshiah PN, Weber DS, Rocic P, Valppu L, Taniyama Y, and Griendling KK. Angiotensin II stimulation of NAD(P)H oxidase activity: upstream mediators. *Circ Res* 91: 406–413, 2002.
65. Streeter J, Thiel W, Brieger K, and Miller FJ Jr. Opportunity Nox: the future of NADPH oxidases as therapeutic targets in cardiovascular disease. *Cardiovasc Ther* 2012. [Epub ahead of print]; doi: 10.1111/j.1755–5922.2011.00310.x.
66. Tabet F, Schiffrin EL, Callera GE, He Y, Yao G, Ostman A, Kappert K, Tonks NK, and Touyz RM. Redox-sensitive signaling by angiotensin II involves oxidative inactivation and blunted phosphorylation of protein tyrosine phosphatase SHP-2 in vascular smooth muscle cells from SHR. *Circ Res* 103: 149–154, 2008.
67. Touyz RM, Briones AM, Sedeek M, Burger D, and Montezano AC. NOX Isoforms and Reactive Oxygen Species in Vascular Health. *Mol Interv* 11: 27–35, 2011.
68. Touyz RM, Chen X, Tabet F, Yao G, He G, Quinn MT, Pagano PJ, and Schiffrin EL. Expression of a functionally active gp91phox-containing neutrophil-type NAD(P)H oxidase in smooth muscle cells from human resistance arteries: regulation by angiotensin II. *Circ Res* 90: 1205–1213, 2002.
69. Touyz RM, Cruzado M, Tabet F, Yao G, Salomon S, and Schiffrin EL. Redox-dependent MAP kinase signaling by Ang II in vascular smooth muscle cells: role of receptor tyrosine kinase transactivation. *Can J Physiol Pharmacol* 81: 159–167, 2003.
70. Touyz RM and Schiffrin EL. Ang II-stimulated superoxide production is mediated via phospholipase D in human vascular smooth muscle cells. *Hypertension* 34: 976–982, 1999.
71. Touyz RM and Schiffrin EL: Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. *Pharmacol Rev* 52: 639–672, 2000.
72. Touyz RM, Yao G, Quinn MT, Pagano PJ, and Schiffrin EL. p47p hox associates with the cytoskeleton through cortactin in human vascular smooth muscle cells: role in NAD(P)H oxidase regulation by angiotensin II. *Arterioscler Thromb Vasc Biol* 25: 512–518, 2005.
73. Touyz RM, Yao G, and Schiffrin EL. c-Src induces phosphorylation and translocation of p47phox: role in superoxide generation by angiotensin II in human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 23: 981–987, 2003.
74. Ungvari Z, Bailey-Downs L, Gautam T, Sosnowska D, Wang M, Monticone RE, Telljohann R, Pinto JT, de Cabo R, Sonntag WE, Lakatta EG, and Csiszar A. Age-associated vascular oxidative stress, Nrf2 dysfunction, and NF-{kappa}B activation in the nonhuman primate Macaca mulatta. *J Gerontol A Biol Sci Med Sci* 66: 866–875, 2011.
75. Ushio-Fukai M, Zafari AM, Fukui T, Ishizaka N, and Griendling KK. p22phox is a critical component of the superoxide-generating NADH/NADPH oxidase system and regulates angiotensin II-induced hypertrophy in vascular smooth muscle cells. *J Biol Chem* 271: 23317–23321, 1996.
76. Virdis A, Neves MF, Amiri F, Touyz RM, and Schiffrin EL. Role of NAD(P)H oxidase on vascular alterations in angiotensin II-infused mice. *J Hypertens* 22: 535–542, 2004.
77. Wassmann S and Nickenig G. Pathophysiological regulation of the AT1-receptor and implications for vascular disease. *J Hypertens Suppl* 24: S15–S21, 2006.
78. Zhang L, Nguyen MV, Lardy B, Jesaitis AJ, Grichine A, Rousset F, Talbot M, Paclet MH, Qian G, and Morel F. New insight into the Nox4 subcellular localization in HEK293 cells: first monoclonal antibodies against Nox4. *Biochimie* 93: 457–468, 2011.

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Abbreviations Used

ACEi = angiotensin-converting enzyme inhibitor
Ang II = angiotensin II
AP-1 = activator protein 1
ARB = angiotensin receptor blocker
AT_1R = Ang II type 1 receptors
AT_2R = Ang II type 2 receptors
BH_2 = dihydrobiopterin
BH_4 = tetrahydrobiopterin
$[Ca^{2+}]_i$ = intracellular free-calcium concentration
CaM = calmodulin
CIHR = Canadian Institute of Health Research
CLC-3 = chloride channel 3
DAG = diacylglycerol
DHFR = dihydrofolate reductase
EC-SOD (SOD3) = extracellular superoxide dismutase
EGF = epidermal growth factor
eNOS = endothelial nitric oxide synthase
ER = endoplasmic reticulum
ERK1/2 = extracellular signal-regulated kinase1/2
FAK = focal adhesion kinase
$H_2O^{\bullet}$ = hydroperoxyl radical
H_2O_2 = hydrogen peroxide
HB-EGF = heparin-binding EGF-like growth factor
HIF-1 = hypoxia-inducible factor 1
IGF-1 = insulin-like 1 growth factor
IP3 = inositol-3-phosphate
JAK = Janus family kinases
JNK = c-Jun N-terminal kinases
MAP = mitogen-activated protein
MAPK = mitogen-activated protein kinase
MEK1/2 = MAP kinase kinase 1/2
MLC = myosin light chain
MLCP = myosin light-chain phosphatase
MLCK = myosin light-chain kinase
MMP = matrix metalloproteinase
Mn SOD (SOD2) = manganese-containing mitochondrial SOD
NAD(P)H = reduced nicotinamide adenine dinucleotide phosphate
Nampt = nicotinamide phosphoribosyltransferase
NFκB = nuclear factor kappa B
$NO^{\bullet}$ = nitric oxide
Nox = NADPH oxidase
NoxA1 = NADPH activator 1
NoxO1 = NADPH organizer 1
$O_2^{\bullet-}$ = superoxide anion
$ONOO^-$ = peroxinitrite
PDGFR = platelet-derived growth factor receptor
Phox = phagocyte oxidase
PI3K = phosphatidylinositol 3-kinase
PKC = protein kinase C
PLA2 = phospholipase A2
PLC = phospholipase C
PLD = phospholipase D
Poldip2 = polymerase delta-interacting protein 2
PTP = protein tyrosine phosphatases
Pyk2 = protein tyrosine kinase 2
RAS = renin–angiotensin system
ROS = reactive oxygen species
SHR = spontaneously hypertensive rats
Sirt3 = sirtuin-3
SMCnox1 = smooth muscle cell Nox1
SOD = superoxide dismutase
SOD1 = copper/zinc superoxide dismutase 1
STAT = signal transducers and activators of transcription
VSMCs = vascular smooth muscle cells
WKY = Wistar Kyoto rats