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Nox enzymes and oxidative stress in atherosclerosis

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1. ABSTRACT

Oxidative stress is a major contributor to the etiology of all severe vascular pathologies, such as atherosclerosis. NADPH oxidases (Nox) are a class of multicomponent enzymes whose unique function is the generation of reactive oxygen species (ROS) in the vascular cells and in circulating immune cells interacting with blood vessels. Physiological production of Nox-derived ROS contributes to the maintenance of vascular homeostasis. In pathological states, hyperactivity of Nox induces oxidative stress. Nox-derived ROS interact and stimulate other enzymatic sources of oxygen/nitrogen reactive intermediates, and amplify the initial response to insults. In atherosclerosis, Nox-induced lipid peroxidation is highly deleterious and expands the free radical reactions initially produced by activated Nox. Therefore, understanding the molecular mechanisms of Nox regulation, vascular and subcellular compartmentalization of ROS production and its subsequent biological significance, may lead to a focused and effective anti-oxidative stress therapy. We present here, recent advances in Nox regulation in the vasculature and discuss novel potential intrinsic feedback mechanisms and current and pharmacological perspectives to target Nox, which may have an impact in vascular health and disease.

2. INTRODUCTION

Cardiovascular disorders (CVD) are the leading cause of mortality in Western societies and an expanding cause of death in the developing countries. CVD include the coronary heart disease, cerebrovascular disease, and peripheral vascular disease, wherein the blood flow to the heart, the brain, and peripheral vasculature are severely compromised. The end product of most CVD is the formation of atheromatous plaques, which can occlude partially or totally the arterial lumen and disrupt the perfusion of the affected tissues causing organ injury and failure. In addition, plaque vulnerability and rupture (atherothrombosis) represents a life threatening complication of atherosclerosis resulting in thrombus formation and thrombi release that ultimately may obstruct the blood vessels in various locations (1).

Atherosclerosis is generally viewed as the outcome of a lipid disorder and a chronic inflammatory reaction of large- and medium-sized arteries (2). It is characterized by progressive lipid accumulation in the vessel's intima, dysfunctions of endothelial cells (ECs) and smooth muscle cells (SMCs), and a robust participation of extravasated immune cells. These characteristics illustrate that atherosclerosis is a multifactorial vascular disorder

portrayed by complex interactions and cross talk between the resident cells of the vascular wall, the cells of the immune system and the factors they produce. Moreover, a complex interplay between predetermined and variable risk factors converges to plaque development and ultimately rupture and downstream cardiovascular complications and events (3).

In the early stages of atherogenesis, endothelial dysfunction triggers a chronic inflammatory process in the vascular wall characterized by decreased nitric oxide (NO) bioavailability, induction of cell adhesion molecules, cytokines, and chemokines that facilitate the attachment, trapping, and transmigration of monocytes through the endothelial layer into the underlying intima. Consecutively, the monocytes differentiate into macrophages that via the non-regulated scavenger-receptor mechanism take up oxidized low-density lipoproteins (oxLDL) and become foam cells (4).

Phenotypic alterations in SMCs are equally important in the initiation and progression of the atherosclerotic lesion, and ultimately contribute to artery wall thickening. In atherosclerosis, SMCs undergo hypertrophy, synthesize excess extracellular matrix and inflammatory cytokines, proliferate and migrate from the media towards the vessel's intima. Other cell types involved in development of atherosclerotic plaque comprise platelets, neutrophils, dendritic cells, mast cells and T lymphocytes, which are recruited into the lesion following exposure to chemotactic stimuli (4). Of particular importance is that oxidative stress contributes, at least in part, in all the pathological processes leading to disease progression (5,6).

3. IS OXIDATIVE STRESS IN ATHEROSCLEROSIS A CAUSE OR A CONSEQUENCE OF VASCULAR INJURY?

Oxidative stress is a pathological phenomenon resulting from the imbalance in the production of reactive oxygen species (ROS) and the ability of biological systems to detoxify the reactive intermediates; it represents a distinctive attribute of all major cardiovascular diseases. The role of ROS in atherosclerosis is generally accepted (2). However, numerous clinical trials failed to demonstrate that the antioxidant therapy improve the health of patients with cardiovascular diseases (7,8). Therefore, many questions arise in relation to our current understanding of the molecular processes involved in ROS formation and action. Thus far, various pharmacological interventions have been used to suppress oxidative stress-induced damage in the cardiovascular system namely antioxidant supplements containing vitamins (e.g., vitamins C and E) and polyphenols or selective inhibitors of various enzymatic sources of ROS (9). These pharmacological approaches have numerous drawbacks such as insufficient concentration of active compounds at the site of ROS formation, or vitamins themselves becoming radicals with pro-oxidant activity or not being efficient scavengers (i.e. vitamins C and E) for hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). Moreover, vitamin E accumulates in lipid membranes and lipoproteins and therefore its access to the oxidative events occurring in the cytoplasm or the extracellular space is considerably limited. Furthermore, the reaction between superoxide ($O_2^{\bullet-}$) and NO leading to NO deficiency and peroxynitrite anion (ONOO-) formation is not significantly affected by vitamins C and E.

To counteract the deleterious effects of ROS (and to comprehend the failure of the antioxidant therapy) one has to ponder the diversity of enzymatic and non-enzymatic sources of ROS, their distinct vascular distribution and subcellular compartmentalization, and complex regulation during various stages of the disease process (10).

Most of the current knowledge on ROS comes from studies on animal models or *in vitro* experiments on various cell-types and isolated tissues; yet, the precise role of the oxidative stress in the onset and progression of atherosclerosis in general and in humans, in particular, remains a debatable issue.

The oxidative stress-induced vascular insults theory in humans is supported by clinical data, which validate that the transition from a physiological to a moderate oxidative status and ultimately severe oxidative stress is associated with atherosclerosis and numerous pathological conditions that predispose to lesion formation. Clinical and experimental studies revealed that in diabetes, hypertension, and hypercholesterolemia, ROS overproduction occurs early in the disease process and is associated with a deregulation of the antioxidant system (11).

Several lines of evidence (*in vivo* and *in vitro* models) highlight the critical role of oxidative stress in endothelial dysfunction and atherosclerotic lesion formation indicating that ROS-induced ECs dysfunction is the primary step and the major contributor to the etiology of all severe vascular pathologies (12,13). Reduced endothelial NO bioavailability, caused by its inactivation by $O_2^{\bullet-}$ and the consequent ONOO- formation, leads to impaired vascular relaxation, enhanced endothelial transcytosis, up-regulation of pro-inflammatory molecules, and the alteration of EC fibrinolytic activity. Furthermore, persistent oxidative stress renders endothelial NO synthase (eNOS) "uncoupled", a dysfunctional state in which the enzyme ceases to generate NO and produces $O_2^{\bullet-}$ as an alternative (14).

Oxidation of macromolecules especially of LDL (oxLDL) plays a key role in all stages of atherogenesis such as fatty streak formation, development of complex lesion, and plaque rupture. Mechanistically, LDL is oxidatively modified in the vascular wall by iron-dependent or lipoxygenase-catalyzed oxidation, reaction with myeloperoxidases-derived HOCl, or direct oxidation by reactive nitrogen species, such as nitrogen dioxide radical ($\bullet NO_2$), nitryl chloride (NO_2Cl), and ONOO-. Consecutively, oxLDL further stimulates the production of ROS and the expression of many pro-inflammatory gene products including cell adhesion molecules, cytokine, and

chemokines, which promote the recruitment of immune cells to the lesion and SMCs migration and proliferation within the arterial intima (15). In addition, the uptake of oxLDL by macrophages and SMC to form lipid-loaded foam cells has been reported to be highly controlled by ROS, which obstructs the reverse cholesterol transport system (16). Other than ECs and SMCs, oxidizing agents modulate the function of various signaling molecules in fibroblasts, which promote the inflammatory reaction of vascular adventitia (17).

Besides vascular resident cells, transvasaeted and infiltrated immune cells i.e. monocytes/macrophages, platelets, neutrophils, and T lymphocytes are important sources of ROS within the atherosclerotic plaque. They further promote oxidative alterations of LDL and extracellular matrix constituents, platelets aggregation, thrombogenesis, or act as signaling molecules regulating redox-sensitive pro-inflammatory pathways (18). In this context, although oxidative injury may not the sole etiology of atherosclerosis, it amplifies inflammatory responses to vascular insults.

4. MECHANISMS OF OXIDATIVE STRESS IN ATHEROSCLEROSIS

Thus far, the mechanisms underlying the oxidative stress in vascular pathology, comprise the overproduction of ROS, alterations in the endogenous antioxidant system, and the production of various oxygen intermediates such as peroxynitrite and hydroxyl radicals that cannot be efficiently neutralized by the naturally occurring antioxidant mechanisms (19,20).

In the cardiovascular system, the cellular ROS-generating enzyme systems that can contribute to oxidative stress are dedicated enzymes such as NADPH oxidases and enzymes that produce ROS as a byproduct of cellular respiration and metabolism including, the mitochondrial respiratory chain, lipo-/cyclooxygenases, dysfunctional nitric oxide (NO) synthases, cytochrome P450 reductases, and xanthine oxidase (21).

4.1. NADPH oxidases

NADPH oxidases (Nox) represent a class of hetero-oligomeric enzymes comprising seven members (Nox1-5 and Duox1/2), whose primary function is the generation of ROS in a highly regulated manner both in physiological and pathological conditions (22). Of particular importance is that Nox-derived ROS interact and stimulate other enzymatic sources of oxygen/nitrogen reactive intermediates, and generally amplify the initial response to insults (23,24). Moreover, different subtypes of Nox are expressed in the cardiovascular cells (ECs, SMCs, vascular and cardiac fibroblasts, cardiac myocytes, pericytes) and in the circulating cells interacting with the blood vessels (monocytes/macrophages, neutrophils, lymphocytes, platelets, dendritic cells) (25).

Studies in cell culture and animal models provide evidence for the critical role of Nox-dependent oxidative stress in atherosclerosis. Nox activity and expression is induced by a plethora of agonists associated with atherosclerotic lesion formation. Among these factors, there are angiotensin II (AngII), high glucose, oxLDL, platelet-derived growth factor (PDGF), thrombin, tumor necrosis factor α (TNFα), interferon γ (IFNγ), and pathological shear stress (26-28). From human studies, there is evidence that chronic and acute overproduction of Nox-derived ROS in pathological states play a key role in the disease onset and progression (29).

4.2. Mitochondrial electron transport chain

Mitochondrial electron transport chain represents a major source of $O_2^{\bullet-}$ and consequently H_2O_2. It has been estimated that during oxidative phosphorylation process, 1% - 2% of O_2 is incompletely reduced to $O_2^{\bullet-}$ which is effectively neutralized by manganese superoxide dismutase. Under pathological conditions uncoupling of the mitochondrial electron transport complexes I-IV (NADH-ubiquinone oxidoreductase, succinate-ubiquinone oxidoreductase, ubiquinole-cytochrome c reductase, cytochrome c oxidase) leads to increased $O_2^{\bullet-}$ production. In the absence of protective histone-like proteins and of DNA damage-repair enzymatic machinery, the mitochondrial DNA is prone to oxidative damage, which ultimately leads to mitochondrial loss of functions that triggers apoptotic events. Defects in mitochondrial DNA resulting in altered mitochondrial electron transport chain enzyme activity and persistent oxidative stress have been ample reported in diabetes, atherosclerosis, obesity, and cigarette smoking (30).

4.3. Lipoxygenases

Lipoxygenases are non-heme containing dioxygenases, which oxidize polyunsaturated fatty acids to hydroperoxy fatty-acid derivatives. Evidence exist that 12/15-lipoxygenase (LO) and its products, 12-hydroxyeicosatetraenoic acid (12-HpETE) and 15-HpETE, are implicated in pathological processes leading to atherosclerosis (31). Knockout of 12/15-LO gene causes significant inhibition of early atherosclerotic lesions in ApoE-deficient (ApoE-/-) mice (32,33). The 12/15-LO product, 12-HpETE acid increases monocyte adhesion to human ECs and mediates inflammatory processes leading to atherosclerosis (34). The 12/15-LO activation induces SMCs growth, hypertrophy, and inflammatory gene expression whereas pharmacological inhibition of 12/15-LO reduces blood pressure in hypertensive rats and prevents intimal hyperplasia in balloon-injured rat carotid arteries (35-37).

4.4. Uncoupled NO synthases

Uncoupled NO synthases (endothelial/inducible – eNOS/iNOS) are another important sources of ROS in vasculature. Physiologically, the enzyme produces NO and L-citruline by transferring electrons from a heme group in the oxygenase domain to the substrate L-arginine. In pathological conditions, such as atherosclerosis, diabetes, hypertension, and hypercholesterolemia, the lack of essential co-factors/substrate (i.e., 5,6,7,8-tetrahydrobiopterin-BH4, L-arginine), renders the enzyme dysfunctional. Two different dysfunctional states have been described for NOS: “total uncoupled” when the enzyme

produces $O_2^{\bullet-}$ and "partial uncoupled" in which case the enzyme generates both $O_2^{\bullet-}$ and NO, leading to a condition favoring production of ONOO-. Thus, incapacitating NO-generating eNOS/iNOS is a major contributor to the onset of oxidative stress and a key pathological trigger of atherosclerosis (38).

4.5. Xanthine oxidoreductase

Xanthine oxidoreductase, a molybdenum-containing enzyme, catalyzes the oxidation of hypoxanthine to xanthine, with the subsequent production of ROS and uric acid. The enzyme complex exists in separate but interconvertible forms, xanthine dehydrogenase (XD) and xanthine oxidase (XO). Reduction of O_2 by either form of the enzyme yields to $O_2^{\bullet-}$ and H_2O_2 with xanthine and hypoxanthine as substrates. Noteworthy XD preferentially reduces NAD^+ whereas XO specifically reduces O_2. Conversion of XD to XO is stimulated by ischemia/reperfusion, exposure to inflammatory cytokines or by oxidation of critical cysteine residues by reactive oxygen/nitrogen intermediates. The XO is expressed in the plasma and the membrane of ECs and is not present in SMCs (39,40).

Clinical evidence exists that XO-induced oxidative stress is correlated with coronary artery diseases, whereas treatment with allopurinol (a pharmacological inhibitor of XO), attenuates oxidative stress, induces vasorelaxation and improves endothelial function in hypertensive patients (41). Yet, NO-dependent endothelial function is unaffected by allopurinol in hypercholesterolemic subjects (42).

5. Phagocyte-type Nox: role in cardiovascular diseases

The structure, function, and regulation of Nox have been originally described in professional phagocytes (neutrophils, monocytes/macrophages). In these cells, in cooperation with myeloperoxidases, NADPH oxidase plays a major role in host defense against invading microbes through the production of toxic ROS such as hypochlorous acid (HOCl), a highly reactive oxidant.

During phagocytosis, macrophages also produce considerable amounts of NO. Consequently, the Nox-derived superoxide ($O_2^{\bullet-}$) reacts with NO generating ONOO-, a highly cytotoxic chemical species which directly attack and oxidize biological molecules in invading microorganisms, resulting in molecular damage and microbial death (43).

Structurally, the phagocytic Nox contains five subunits: a membrane-associated cytochrome b558 (named for the spectral absorption at 558 nm), consisting of heavily glycosylated 91-kDa protein (gp91phox, also termed Nox2) and non-glycosylated 22-kDa subunit (p22phox) in a 1:1 complex, and three cytosolic regulatory components, p40phox, p47phox, and p67phox. Besides "Phox" proteins, activation of Nox involves a low-molecular-weight GTP-binding protein (Rac1/2, Rap 1A). In resting phagocytes, the enzyme complex is dissociated, but upon exposure to microorganisms or inflammatory mediators is rapidly activated.

Two major mechanisms regulate Nox activity: p47phox phosphorylation and Rac-GTPase (44). Serine phosphorylation of p47phox represents the limiting step required for the complex activation; it triggers assembly of cytosolic subunits, its translocation to the membrane and association with cytochrome b558. Rac interacts directly to p67phox and activates Nox in its GTP-bound active state, only. In addition to Nox2-containing NADPH oxidase, in macrophages, Nox1 and Nox4 have been shown to be essential inducible isoforms, which participate in the oxidation processes in the vascular wall (45, 46).

Genetic defects in the genes encoding four of the "Phox" proteins (gp91phox, p22phox, p47phox and p67phox) cause chronic granulomatous disease (CGD), which is a rare inherited disorder of the innate immune system. In CGD dysfunctional phagocyte Nox are unable to produce ROS, thus leading to life-threatening bacterial and fungal infections (43). Although the phagocyte-type Nox-derived ROS has an important role in pathogenesis of vascular diseases (e.g. atherosclerosis), a correlation between defective Nox2-dependent oxidative burst in CGD patients and cardiovascular abnormalities was not demonstrated yet.

In addition to macrophages, dendritic cells (DCs) and lymphocytes, which usually reside in the adventitia of normal arteries, were found in the arterial atherosclerotic lesions. Apart from being involved in innate immunity, Nox2-containing NADPH oxidase also controls adaptive immunity, and antigen presentation by DCs is a key process in adaptive immune responses. The antigens are partially degraded and processed in the DCs endosomes and then presented by major histocompatibility complex (MHC) class I molecules to CD8+ T lymphocytes. It has been reported that Nox2 is a critical regulator of antigen processing during cross-presentation by DCs; the Nox2-derived ROS maintain an alkaline pH in the endosome lumen. Knockout of Nox2 results in enhanced endosomal acidification that promotes increased antigen degradation and less cross-presentation (47). In addition, Nox2-deficient T cells elicit enhanced activation of mitogen-activated protein kinases cascades in response to T-cell receptor stimulation (48). Because DCs and T lymphocytes are important constituents of atheroma and Nox2 up-regulation is correlated with macrophage infiltration in complicated lesions, the study of the genetic defects of the Nox subunits and their potential consequences in cardiovascular diseases is of particular importance and has to be further investigated.

6. Non-phagocyte type Nox: expression pattern in the vascular cells

Non-phagocyte Nox enzyme family consists of 7 members (Nox1-5, Duox1/2), each with a distinct cell and tissue distribution. Nox enzymes are classified into three major categories, as a function of the additional domains to the prototypical catalytic subunit Nox2. The first category comprises Nox1, Nox3, and Nox4 subtypes, which have a similar structural organization and molecular weight with Nox2.

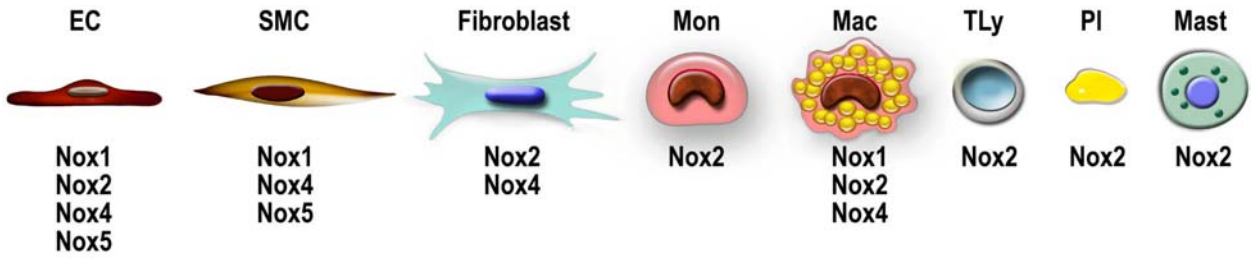

Figure 1. Distinct expression of Nox isoforms in the cells involved in atheroma formation. Resident vascular cells, the endothelial cells (EC), smooth muscle cells (SMC) and adventitial fibroblasts; the cells of the immune system, monocytes (Mon) that turn into macrophages and foam cells (Mac) within the intima, T lymphocytes (TLy), platelets (Pl), and mast cells (Mast).

Nox5, the second group of the Nox family, posses in addition to the Nox2-type catalytic core, an extra amino-terminal calmodulin-like domain that contains four Ca^{2+}-binding EF-hands structures (49). Two types of Nox5 have been described, Nox5-S and Nox5-L. The latter possesses an amino-terminal calmodulin-like domain that contains four Ca^{2+}-binding EF-hands structures, whereas Nox5-S is lacking this domain. Hitherto, four splice variants of Nox5-L, namely Nox5α, Nox5β, Nox5γ, and Nox5δ have been identified in humans. In particular, the *Nox5* gene is not present in the rodent's genome.

The Nox5-like dual oxidases 1 and 2 (Duox1/2) are the third category of Nox. Duox possess, in addition to the Nox5-based structure, an extracellular peroxidase domain that uses the H_2O_2 generated by its Nox catalytic core. Duox enzymes are involved in thyroid hormone synthesis and in cooperation with lactoperoxidase were suggested to play a role in host defense in various tissues (50).

Different subtypes of Nox along with their regulatory subunits are expressed in the cardiovascular cells including ECs, SMCs, vascular and cardiac fibroblasts, cardiac myocytes, and pericytes (38). The ECs contain Nox1, Nox2, Nox4 (most abundant isoform), and Nox5-based NADPH oxidases. In SMCs, a high expression of Nox1, Nox4, and Nox5 and a low level of Nox2 have been detected. Adventitial and cardiac fibroblasts contain mainly Nox2 and Nox4 (51) (Figure 1).

Nox subtypes are differentially distributed within the cellular compartments; moreover, they control specific ROS-mediated signal transduction pathways. Nox1 and Nox2 are localized in caveolae, in the plasma membrane, and endosomes. Nox4 has been detected in focal adhesions, the nucleus, endoplasmic reticulum, and mitochondria (52,53). Nox5 is expressed in the perinuclear compartment, colocalized with markers for the endoplasmic reticulum, and in the plasma membrane (54,55).

6.1. Nox-induced oxidative stress signals in the vascular cells

In addition to their capacity to alter cell functions by reacting indiscriminately with a large majority of macromolecules which cause irreversible damage of DNA, proteins, carbohydrates, and lipids constituents, ROS are key regulators of signal transduction.

Nox enzymes catalyze the formation of $O_2^{\bullet-}$ by one-electron reduction of molecular oxygen (O_2) using NADPH as an electron donor. Chemical conversion of $O_2^{\bullet-}$ or interactions with other biological molecules generates a large spectrum of second messengers which transduce important physiological and pathological signals. Spontaneous or enzymatic dismutation of $O_2^{\bullet-}$ produces H_2O_2, which consecutively may be reduced via the Haber-Weiss reaction to HO•, hitherto, the most powerful oxidizing agent identified in biological systems.

Alternatively, $O_2^{\bullet-}$ can react with NO by a process that is highly regulated by the rate of diffusion of both radicals, the result of which is the formation of ONOO-, a highly potent oxidant. Nox-generated ROS may lead to the production of several other radicals, including lipid peroxidation products (6,56).

Evidence exists that, in cardiovascular cells, changes in the redox state alter directly or indirectly the activities of several intracellular signaling molecules (57). The biological targets of redox signaling comprise a large spectrum of molecules including enzymes (especially protein kinases, phosphatases, and phospholipases), transcription factors, peptides, ion channels and transporters, lipids, carbohydrates, and other oxygen-based species (58). Transient inhibition of protein tyrosine phosphatases (PTPs) through the reversible oxidization of their catalytic cysteine suppresses protein dephosphorylation and represents a major mechanism by which H_2O_2 regulates various cellular processes. In addition, $O_2^{\bullet-}$ and H_2O_2 modify the activity of the mitogen-activated protein kinase (MAPKs) family and of different receptor and non-receptor protein tyrosine kinases (PTKs) (59).

Besides protein kinases, phosphatases and transcription factors, Nox-derived ROS are the key regulators of intracellular Ca^{2+} and K^+ concentrations by a mechanism that involves reversible thiol oxidation of the cysteine residues present on ion channels and transporters (60,61).

Enzymatic and non-enzymatic lipid peroxidation represents an important mechanism of the oxidative stress-mediated vascular injury. Peroxidation of lipids has deleterious effects because the formation of these products extends the free radical reactions. Under an oxidative environment, both circulating or intima-infiltrated lipoproteins and plasma membrane phospholipids are susceptible to ROS attack. The reaction between Nox-derived ROS and polyunsaturated fatty acids (PUFAs) generates fatty acid peroxyl radical (R-COO-) that can attack adjacent fatty acid chains and initiate the production of other lipid radicals by a chain reaction mechanism (58).

The lipid peroxidation comprises three sequential stages: initiation, propagation, and termination. Hydrogen atom abstraction from the double bonds of the PUFAs represents the initiation step of lipid peroxidation. Several oxygen-derived reactive intermediates can abstract the first hydrogen atom including hydroxyl radical (HO•), hydroperoxyl radical (HO_2•) and to a lesser extent $O_2^{\bullet-}$ or H_2O_2 (non-radical) (62). As recently reviewed by Riahi et al. (63), nonenzymatic peroxidation pathway of *n*-3 and *n*-6 PUFAs generates 4-hydroxy-*2E*-hexenal (4-HHE) and 4-hydroxy-*2E*-nonenal (4-HNE), whereas enzymatically regulated peroxidation pathways involves different lipoxygenases (e.g. 12/15-LO) that ultimately produce 4-HNE and 4-hydroxy- *2E*,6*Z*-dodecadienal (4-HDDE). Notably, 4-HDDE is exclusively derived from 12-HpETE, the 12-LO metabolite of arachidonic acid, whereas 4-HNE is the end peroxidation product of 15-LO metabolites of arachidonic acid, linoleic acid, and other *n*-6 PUFAs.

Hydroxyalkenals have been implicated in various pathophysiological interactions due to their chemical reactivity and formation covalent adducts with proteins, nucleic acids and phospholipids. The progressive accumulation of these adducts can alter normal cell functions and ultimately may lead to cell death, unless the cells are equipped with an efficient enzymatic neutralizing system of these hydroxyalkenals (e.g., glutathione peroxidase, fatty aldehyde dehydrogenase) (64).

Lipid peroxidation products affect the cell membrane structure, causing changes in its fluidity and permeability, alterations of ion transport and inhibition of metabolic processes (65). In addition, lipid peroxidation products directly affect mitochondrion function and can induce further increases in ROS generation. In particular, 4-HNE induces vascular SMCs apoptosis through an increased mitochondrial production of ROS (66). In addition, break down of lipid peroxides in the presence of reduced metals give rise to reactive aldehyde products, including malondialdehyde (MDA), 4-HNE, 4-HHE and acrolein, all having detrimental effects on the cells. The chemical adduct reactivity of the three 4-hydroxyalkenals correlates directly to their hydrophobicity [LogP(o/w)]: 4-HHE (0.89) < 4-HNE (2.45) < 4-HDDE (3.48) (67). Still, at low non-cytotoxic concentrations these molecules could function as signaling molecules, as it has been shown for 4-HNE and to some extent for 4-HHE (68).

Distinct to lipoxygenase-mediated lipid peroxidation, there is evidence that Nox-derived ROS are effective triggers of lipid peroxides formation (69). Based on the fact that Nox enzymes generate mainly $O_2^{\bullet-}$ and H_2O_2 which are not able to abstract hydrogen atoms *per se* from the double bonds of the PUFAs, additional cooperative processes are involved in the formation of oxygen-derived molecules with a higher chemical reactivity. As mentioned, HO• and HO_2• are the most important initiators of lipid peroxidation. Therefore, protonation of Nox-derived $O_2^{\bullet-}$ to afford HO_2• represents a critical step by which Nox interfere with lipid peroxidation chain reactions. Moreover, H_2O_2 may be reduced to HO•, in the presence of free transition metal ions (e.g., Fe^{2+}, Cu^{2+}) via the Haber-Weiss reaction (70).

In addition to being involved in lipid peroxides formation, Nox enzymes themselves are targets of lipid peroxidation products action. Evidence exists that 4-HNE increases the activity of Nox and that the pro-inflammatory effect of 4-HNE is mediated, at least in part, by Nox-derived ROS in murine macrophages (71).

13-hydroperoxyoctadecadienoic acid (13-HPODE) is also implicated in the pathophysiology of atherosclerosis. 13-HPODE, a constituent of oxLDL, can induce cytotoxicity of vascular SMCs, a condition that facilitate plaque destabilization and/or rupture. Consistent with this hypothesis, Li et al. (72) showed that 13-HPODE and 9-HPODE increases $O_2^{\bullet-}$ production and is cytotoxic for vascular SMCs. The 13-HPODE-induced increase in $O_2^{\bullet-}$ was blocked by knock-down of p22phox, suggesting that the $O_2^{\bullet-}$ was produced by activated Nox.

Siems et al. (73) established that 4-HNE inhibits Nox-dependent $O_2^{\bullet-}$ formation in phorbol myristate acetate (PMA)-stimulated human neutrophils by 4-HNE-binding to -SH and -NH2 groups. Therefore, a possible explanation of enzyme inhibition may be the tendency of 4-hydroxyalkenals to form covalent adducts with macromolecules, rather than the alosteric inhibition of the enzyme complex. Likewise, at low concentration, 4-HHE elicits antioxidant activities by inducing the expression of heme oxygenase-1 through the redox-activation of Nrf2 transcription factor in human umbilical vein ECs (74).

6.2. Regulation of vascular Nox activity and expression

The activity of Nox1 and Nox2 isoforms is highly controlled by phosphorylation cascades involving cytosolic regulatory subunits that trigger the assembly of the enzyme complex. Besides p40phox, p47phox, and p67phox components, two different homologues have been identified in vascular cells, the Nox organizer 1 (Noxo1) - the analog of p47phox, and Nox activator 1 (Noxa1) - the analog of p67phox.

Unlike phagocytic cells, different functional aspects are involved in the regulation of vascular Nox activity. Thus, Noxo1 is pre-localized within the membrane together with Nox1 and p22phox distinct to p47phox, which is localized in the cytosol (35). To function, Nox1-4 enzymes require the p22phox subunit, whereas Nox5

isoforms are activated directly by calcium (75). The polymerase delta interacting-protein 2 (Polidp2) has been identified as a novel partner for Nox4-p22phox complex, which enhances enzyme stability and activity. In addition, it was suggested that owing to strategic nuclear localization, the Nox4-p22phox-Polidip2 complex might have a role in the regulation of key nuclear process such as redox modification of DNA or associated proteins, DNA synthesis and repair (76). Nevertheless, as reviewed by Miller (77) many questions arise in relation to the nuclear compartmentalization and function of Nox4.

In aortic SMCs, Nox1 activity is controlled by a ClC-3 anion transporter, which is required for charge neutralization of the electron flow generated by Nox1 across the membrane of early endosomes (78). Recently, Chu et al. (79) showed that ClC-3 is necessary for the activation of SMCs by TNF-α (and not by thrombin), and deficiency of ClC-3 markedly reduces neointimal hyperplasia following vascular injury in mice aorta.

Pathways linked to phosphorylation of Nox1 and Nox2 regulatory subunits and their assembly into active complexes comprise, among others, phospholipases (PLCβ/γ, PLD), arachidonic acid metabolites, protein kinase C (PKC), phosphatidylinositol 3-kinase (PI3K), GTP-binding proteins (Ras, Rac1/2), members of the mitogen-activated protein kinase (MAPK) family (p38MAPK, ERK1/2), and non-receptor protein tyrosine kinases (80,81). In contrast to Nox1 and Nox2, which require the regulatory subunits for their function, Nox4 produces ROS constitutively, and variations in protein level directly affect the activity of Nox4 (82). Nox5 activity is Ca^{2+}-sensitive. Moreover, activation mechanisms involving PKC- and the proto-oncogenic tyrosine kinase c-Abl phosphorylation of Nox5 have been reported (55,83). In addition, chaperone proteins (e.g., protein disulfide isomerase) are important regulators on Nox activity (84).

Changes in the gene expression of the Nox isoforms are critical for their function. Evidence exist that multiple transcription factors are coordinately involved in the regulation of Nox expression and function. In the myelomonocytic cell lineage, Nox2 transcription is controlled by PU.1, Elf-1, IRF-1 (interferon regulatory factor-1), and ICSBP (interferon consensus sequence binding protein) (85).

In human colon epithelial Caco-2 cells, Nox1 is transcriptionally regulated by GATA-binding factors (86), whereas in murine macrophages, the induction of Nox1 by lipopolysaccharide (LPS) is partially mediated by CCAAT/enhancer-binding protein (C/EBP)β and C/EBPδ (87).

In human aortic SMCs, AP-1 is an important regulator of the genes coding for p22phox (*CYBA*), Nox1, and Nox4 transcription (88, 89). STAT1 and STAT3 physically interact with the promoter of human Nox1 and Nox4 genes in SMCs exposed to interferon (IFN) γ and JAK/STAT-dependent mechanisms are involved in the modulation of Nox-derived $O_2^{\bullet-}$ production. In addition, the promoter activities of the genes coding for p22phox, p47phox (*NCF1*), and p67phox (*NCF2*), are significantly enhanced in SMCs overexpressing STAT1/STAT3, a finding that indicates the presence of functionally GAS/ISRE elements (90). Ets1, a critical mediator of vascular inflammation and remodeling, regulates *NCF1* transcription in response to AngII in SMCs (91). In addition, growth-promoting transcription factor E2F physically interacts and controls the transcription of Nox4 promoter in A7r5 cells and primary mouse aortic SMCs (92).

Reportedly, NF-kB signaling represents a central mechanism in the control of the transcription of various Nox subunits. Anrather et al. (93) have shown that, in murine monocytes, the expression of the gene coding for gp91phox/Nox2 (*CYBB*) is induced by NF-kB. Moreover, the up-regulation of p47phox and p22phox expression by LPS/IFNγ was blunted in IkBα-overexpressing cells indicating the involvement of the NF-kB pathway in the regulation of these components. Gauss et al. (94) reported similar results in TNFα-exposed human monocytes/macrophages. These observations are consistent with our previous study demonstrating the role of NF-kB in the regulation of Nox activity and p22phox transcription in human aortic SMCs (95). Recently, we reported that NF-kB is also a regulator of Nox1- and Nox4-containing NADPH oxidase in human aortic SMCs (96).

Hypoxia sensing and associated signaling events represent key features in vascular cell physiology and pathology (97). Evidence exists that hypoxic conditions stimulate the expression of Nox activity (98). In a recent study, Diebold et al. (99) have found that hypoxia induces Nox4 mRNA and protein levels in pulmonary artery SMCs and in pulmonary vessels in mice exposed to hypoxic conditions. The response is dependent on HIF-1α, which interacts with the corresponding elements in the Nox4 promoter. Consequently, the HIF-1α dependent up-regulation of Nox4 by may be an essential mechanism to preserve ROS level after hypoxia and the hypoxia-induced proliferation of pulmonary artery SMCs. Furthermore, ATF (activating transcription factor)-1, a transcription factor of the CREB (CRE-binding protein)/ATF family, plays a pivotal role in the up-regulation of Nox1 in rat vascular SMCs (100). Pendyala et al. (101) have demonstrated that Nrf2, a critical transcriptional regulator of antioxidant genes, also controls Nox4 expression in mouse lung and human lung endothelium in response to hyperoxia.

6.3. PPARs in the vasculature: redox-signaling regulators?

Peroxisome proliferator-activated receptors (PPARs) are members of a superfamily of nuclear hormone receptors. They have 3 isoforms, PPARα, PPARβ/δ, and PPARγ, which act in concert with Retinoid X Receptor (RXR) as ligand-activated transcription factors. PPARs play key roles in the regulation of energy homeostasis, fatty acid metabolism and inflammation. Agonists of PPARα and PPARγ are currently used therapeutically. The former lowers plasma triglycerides, VLDL, increases HDL cholesterol, and the latter affects free fatty acid metabolism,

thus reducing insulin resistance and blood glucose level (102,103). Activation of PPARβ/δ enhances glucose tolerance, insulin-stimulated glucose disposal, cholesterol efflux and oxygen consumption (104).

PPARs regulate gene expression by dimerizing with RXR and binding to specific DNA sequence elements termed PPRE (Peroxisome Proliferator Response Element). Besides their metabolic actions, PPARs also control immune and inflammatory responses, and regulate cell proliferation, differentiation, and survival.

PPAR ligands can be either synthetic, such as peroxisome proliferators, hypolipidemic agents, anti-inflammatory or insulin-sensitizing drugs, or endogenous ligands, most of which are fatty acids or their derivatives (105).

Clinical and experimental data indicate that PPARs agonists modulate ROS production in blood vessels, but the precise function of PPARs in the regulation of Nox enzymes is scantily elucidated. The protective cardiovascular effects of PPARα and PPARγ activators have been demonstrated (102,106-108). Moreover, combined low doses of PPARα and PPARγ agonists attenuate the development of hypertension, correct vascular structural abnormalities, and improve endothelial function, oxidative stress, and vascular inflammation (109). The PPARγ agonist, rosiglitazone, has been reported to prevent high-glucose-induced oxidative stress and Nox hyperactivity in ECs and to reduce the hyperglycemia-induced Nox expression in diabetic mice (110,111). In addition, pioglitazone treatment prevents hypertension and renal oxidative stress, both by reducing free-radical production and by increasing NO production/availability (112). Thus far, the involvement of the PPARβ/δ isoform in the regulation of Nox enzymes has not been investigated.

Activation of PPARα and PPARγ inhibits inflammatory responses by preventing the activation of nuclear transcription factors, NF-kB, AP-1, and STAT1/3 (112,114). Since these transcription factors are also key regulators of some Nox isoforms (39,88-90,95), we can hypothesize the existence of an auto-regulatory mechanism by which diverse PPAR isoforms control the transcription of Nox in a negative feedback loop.

Interestingly, Teissier et al. (69) have reported that PPARα induces Nox activity and expression in macrophages, leading to the generation of oxLDL with PPARα activation properties. These findings lead to the novel concept that a "controlled oxidative stress" mediated by Nox activation might also generate certain anti-inflammatory activities. In addition, the promoter activities of the genes coding for p22phox, Nox1, and Nox4, were significantly enhanced in human aortic ECs and SMCs overexpressing hPPARα, hPPARβ/δ, hPPARγ or hRXRα; a finding that indicates the presence of functionally PPRE elements. Moreover, the transcriptional activities of each component were significantly up-regulated by PPARα, PPARβ/δ or PPARγ agonists. Based on the fact, that RXRα up-regulates various Nox transcription, the contribution of other heterodimerization partners, distinct to PPARs, such as subfamily 1 nuclear receptors including CAR (constitutive androstane receptor), FXR (farnesoid X receptor), LXR (liver X receptor), PXR (pregnane X receptor), RAR (retinoic acid receptor), TR (thyroid hormone receptor), and VDR (vitamin D receptor) should be considered (Manea et al. unpublished data). Further extensive studies will elucidate the intimate relation between Nox and PPAR/RXR system in vascular health and disease (Figure 2).

6.4. Nox-derived ROS in cardiovascular pathology

6.4.1. Nox subtypes-specific effects

In physiological conditions, Nox-derived ROS contribute to the maintenance of vascular tone and regulate key processes such as cell growth, proliferation, differentiation, apoptosis, cytoskeletal organization, and cell migration. In pathological conditions excessive Nox-dependent ROS formation, which is frequently correlated with the up-regulation of different Nox isoforms, promotes oxidative injury to the cells of the cardiovascular system (6).

As recently reviewed by Rivera et al. (51), transgenic and knockout mice provided much of our current knowledge about the role of Nox enzymes in vascular physiology and pathology. Compelling evidence demonstrates that Nox-derived ROS play an important role in vascular inflammation and injury and that genetic ablation of Nox components (p47phox, Nox1, Nox2) protects the vascular cells against the detrimental effects of oxidative stress. ApoE-/- mice develop atherosclerotic lesions that cover the entire spectrum of human lesions, including fatty streaks, intermediate lesions, fibrous plaques, and vulnerable plaques exhibiting necrotic core and intra-plaque hemorrhage (115). Numerous studies performed on ApoE-/- mice clearly depicts that changes in Nox activity and expression occur early in atherogenesis, and hyperactivity of Nox associated with the up-regulation of various isoforms marks all the stages of the plaque formation (42). In addition, Nox1, Nox2, and Nox4 are activated and up-regulated in the blood vessels of ApoE-deficient mice and diabetic animals (116,117). ApoE/p47phox double-knockout mice have significantly less atherosclerosis compared with ApoE-/- mice. Moreover, aortic $O_2^{\bullet-}$ levels are lower in p47phox-/- mice than in wild-type mice. Moreover, aortic SMCs from p47phox-/- mice exhibit a decreased proliferative response to growth factors compared with that of the SMCs of wild-type mice (12).

Hypertension represents a major risk factor for atherosclerosis and its complications and several reports demonstrate that oxidative stress is both cause and consequence of hypertension (61). In mice, Nox1 deficiency decreases AngII-induced blood pressure, media hypertrophy, and extracellular matrix accumulation, but not cell proliferation (118,119). Consistent with these findings, AngII-infused mice overexpressing Nox1 in vascular smooth muscle cells display an elevation of blood pressure, medial hypertrophy and significant up-regulation of $O_2^{\bullet-}$ formation (120). Furthermore, overexpression of Nox1 in

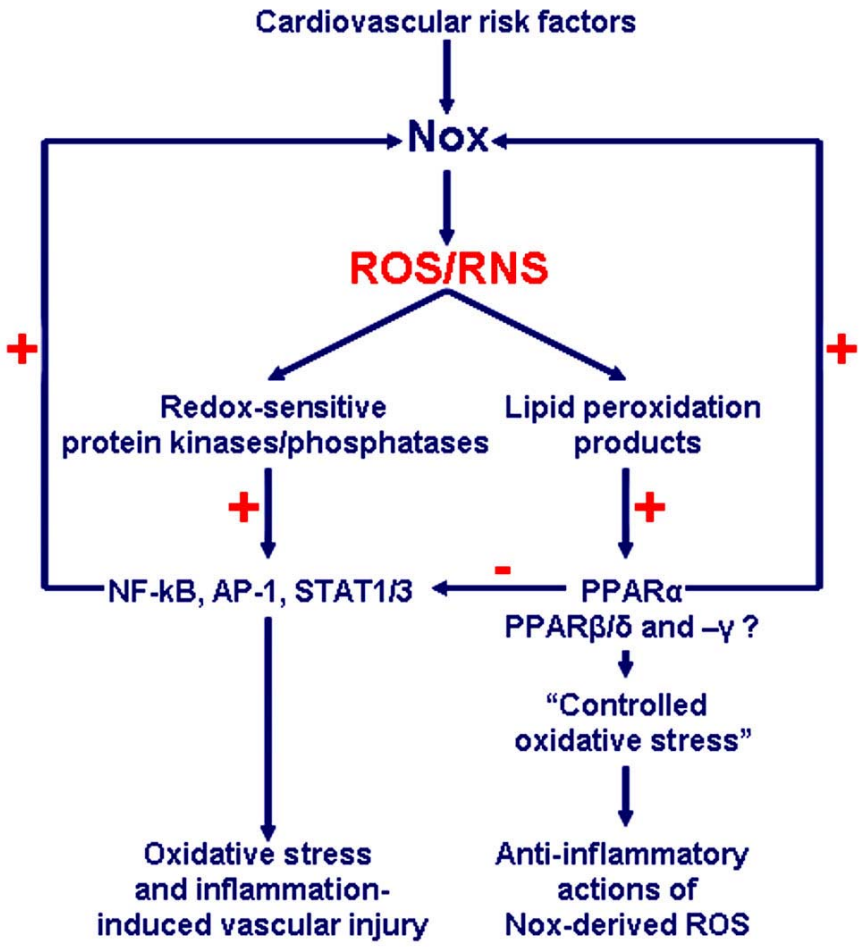

Figure 2. Diagrammatic representation of Nox activation in vascular cells and the hypothetical mechanism whereby Nox-derived ROS, besides inducing transcription factor - mediated oxidative stress, could elicit anti-inflammatory activities *via* PPARs. Nox mediates the signals of several cardiovascular risk factors generating reactive oxygen/nitrogen species (ROS/RNS) which activates redox signaling/transcription pathways and promote lipid peroxidation. In addition, Nox-derived ROS may produce endogenous ligands (i.e. lipid peroxidation products) for PPARα, PPARβ/δ or PPARγ whose activation inhibit key regulators of Nox isoforms and pro-inflammatory genes (NF-kB, AP-1, and STAT1/3), thus determining a state of "controlled oxidative stress" with anti-inflammatory action. Consequently, Nox-generated ROS may have a dual role: they either induce oxidative vascular injury or may elicit anti-inflammatory functions *via* PPARs activation.

vascular SMCs leads to enhanced production of ROS in response to ANG II, causes eNOS uncoupling and the ensuing decrease in NO bioavailability, resulting in impaired vasorelaxation (121).

ROS-mediated protein oxidation is significantly diminished in Nox2-/- mice compared with wild-type mice, and this is accompanied by reduced neointimal proliferation (monitored by intimal thickness and the intimal/medial ratio). In addition, Nox2 deficiency leads to reduced cell proliferation and leukocyte accumulation, indicating that Nox2-mediated oxidation has a requisite role in the cell response to injury (122).

The physiological and pathological functions of Nox4 and Nox5 isoforms, in vivo, are less understood, because there are few atherosclerosis-related studies on Nox4 deficient mice, and the Nox5 gene is not present in the rodent's genome. Therefore, much of the current knowledge comes from in vitro experiments on various cell-types and isolated tissues. Recently, Zhang et al. (123) have generated a Nox4-null mouse model and a cardiomyocyte-targeted Nox4-transgenic model to elucidate the effects of Nox4 during cardiac stress. In contrast to the effects of Nox2 and other ROS sources, the increase in cardiomyocyte Nox4 resulted in protection against pressure overload-induced adverse cardiac remodeling. The authors conclude that Nox4 facilitates the preservation of myocardial capillary density during pressure overload by regulating stress-induced cardiomyocyte HIF1 activation and release of vascular endothelial growth factor (VEGF), resulting in increased paracrine angiogenic activity.

Notably, in a recent study Guzik et al. (124) reported a strong association between Nox5 and atherosclerotic lesion progression. Furthermore a specific expression pattern was reported; with Nox5 being expressed mainly by the endothelium in the early stages of the disease while its expression is significantly increased in SMCs underlying fibro-lipid atherosclerotic lesions.

The mechanisms of Nox isoforms regulation were intensively investigated in cell culture and isolated tissues. Both catalytic (i.e., Nox1-5, p22phox) and cytosolic regulatory (i.e., p40phox, p47phox, p67phox, and Noxa1) components of the Nox complex have been shown to be up-regulated by vasoactive agents, inflammatory cytokines, growth factors, high glucose, modified lipids and lipoproteins, hyperinsulinemia, homocysteine, and mechanical stress (125-128).

Taken together these data indicates that Nox1, Nox2, Nox4, and Nox5 are important regulators of cellular pathways mediating ROS-dependent physiological and pathological processes. Given that the different Nox subtypes are expressed concurrently in the vascular cells, and that several isoforms are similarly regulated, their subcellular localization might be an essential factor in determining Nox functions.

6.4.2. Consequences of Nox-related genetic variants in CVD

Genetic studies provided conclusive support that several Nox-related polymorphisms are associated with an increased susceptibility for cardiovascular disorders. Thus far, much attention has been paid to the *CYBA* gene encoding the p22phox essential subunit. The p22phox protein is ubiquitously expressed in cardiovascular cells and represents the α-subunit of the membrane-associated cytochrome b558 that serves as final electron transporter from NADPH to O_2 in both phagocyte-type and non-phagocyte Nox systems (129). Furthermore, increases in p22phox expression associated with elevated ROS production, correlate with severe oxidative stress and various vascular pathological states (38,130). The p22phox protein forms stable and functional heterodimers with Nox1, Nox2 or Nox4, a critical condition for Nox activity as demonstrated by siRNA-based knock-down of p22phox expression (131).

The *CYBA* gene is located on chromosome 16q24 and some allelic polymorphisms are independently associated with cardiovascular risk factors and disease incidence (i.e., hypertension, coronary artery disease, myocardial infarction, cerebrovascular disease, diabetic and non-diabetic nephropathy) (132). Several polymorphisms were detected in the exonic (i.e., C242T, A640G, C549T) and promoter (i.e., -930A/G, -675A/T, -852C/G, -536C/T) regions of the *CYBA* gene, which potentially affect the p22phox expression and consequently the Nox activity. Therefore, the occurrence of a particular allele may predispose to oxidative stress and cardiovascular disease development (133).

The C242T polymorphism results in the replacement of histidine with tyrosine located in the putative heme-binding site (134). The functional aspects of this polymorphism were intensively investigated. Guzik et al. (135) reported that the T allele is associated with a significant down-regulation of Nox activity and reduced oxidative stress in blood vessels. The data regarding the association of *CYBA* C242T polymorphism with diverse vascular pathologies are conflicting; some studies report that the T allele confer protection against coronary artery disease (CAD) others show no statistic significant association (136-138). In contrast, the frequency of the T mutant allele was found to be significantly higher in CAD group as compared to normal subjects (139). Moreover, the C242T *CYBA* polymorphism has been reported to be associated with essential hypertension, with the subjects carrying the CC genotype exhibiting pronounced features of Nox-dependent oxidative stress and endothelial damage (140).

Several other polymorphisms associated with essential hypertension and diabetes have been reported to affect the p22phox mRNA processing and stability or *CYBA* transcriptional activity. These include the A640G polymorphism located in the 3- untranslated region of *CYBA* gene and -930A/G or -675A/T polymorphisms located in the promoter sequence (141-143). Hitherto, reports indicating the existence of functional Nox1-5 polymorphisms with a relevant impact on vascular pathology are missing.

6.5. Pharmacological targeting of Nox activity and expression

The Nox-derived ROS may have both beneficial and deleterious effects. We can safely assume that these effects are function of the expression pattern and regulation of various Nox isoforms, their subcellular compartmentalization, and the rate of ROS generation. Moreover, Nox-derived ROS can stimulate other enzymatic sources of ROS such as eNOS/iNOS, mitochondrial dysfunction, oxidative activation of xanthine oxidase, cytochrome P-450 uncoupling, dysregulation of several peroxisomal oxidases, and generally intensify the initial response to vascular insults (36, 37). Consequently, the co-expression/co-localization of Nox with other enzymatic/non-enzymatic sources of reactive oxygen intermediates at the site of injury is of particular importance. Therefore, inhibition of these enzyme complexes is an attractive therapeutic strategy to counteract the oxidative stress and prevent the escalation of cardiovascular diseases (CVD).

Several drugs that interfere with Nox activation or expression, such as HMG-CoA reductase inhibitors (statins), angiotensin converting enzyme (ACE) inhibitors, AT1-receptor blockers, and calcium channel blockers, reduce vascular oxidative stress, improve endothelial function, and slow down cardiovascular disease progression (10). Recently, Fortuño et al. (144) have shown that losartan metabolite EXP3179 blocks Nox-mediated $O_2^{\bullet-}$ production (inhibiting PKC), which confers, to losartan the capacity

to reduce oxidative stress mediated by phagocytic cells in hypertensive patients.

Statins, in addition to their hypolipemiant effect, inhibit vascular ROS production by preventing the isoprenylation of p21Rac, a low-molecular-weight GTP-binding protein that is critical for Nox assembly and activation. Statins improve endothelial function, inducing the expression of eNOS and the resulting level of bioactive NO (145). Thus, the pleiotropy of some conventional cardiovascular drugs may be employed as a pharmacological strategy to correct the cardiovascular risk factors and to hinder the acceleration of CVD and its downstream complications.

Numerous reports assert that the pharmacological inhibition of Nox complexes is more effective in modulating ROS production than scavenging of ROS by antioxidant vitamins (75,146-148). Vendrov et al. (149) have demonstrated that GKT136901, a specific inhibitor of Nox1/4 activity, attenuates ROS generation and atherosclerosis. Furthermore, Wind et al. (150) report that the Nox inhibitor, VAS2870, reduces oxidative stress and endothelial dysfunction in aortas of aged spontaneously hypertensive rats.

Besides pharmacological interventions, modulation of the upstream regulators of Nox may represent a novel and efficient strategy to attenuate the pathological effects of oxidative stress. We have reported that in human aortic SMCs under pro-inflammatory conditions, Jak/signal transducer and activator of transcription (STAT) signaling represents an important mechanism that mediates up-regulation of Nox1 and Nox4 expression, as well as Nox-derived O2•- production (90). Since Jak2 transduces the signals of various cardiovascular risk factors and during atherogenesis regulates processes such as inflammation, cell growth, proliferation, and migration, pharmacological manipulation of this signaling pathway may represent a novel strategy to reduce oxidative stress and inflammation in atherosclerosis (151,152).

To obtain additional insights into the potential of pharmacological approaches to manipulate Nox activity, we have investigated the effect of Jak2 inhibition on atherosclerotic lesion formation, Nox expression and function employing hypercholesterolemic ApoE-/- mice. Treatment of ApoE-/- mice fed a high-fat, cholesterol-rich diet, with tyrphostin AG490, a highly specific Jak2 pharmacological inhibitor, greatly reduced the up-regulated aortic Nox activity and decreased the mRNA levels and protein expression of each Nox isoform. Morphometric analysis revealed a marked reduction of atherosclerotic lesions in the aorta of AG490-treated animals (Fenyo et al., unpublished data). Similar effects were found employing the protein tyrosine kinase (PTK) pharmacological inhibitor, WP1066 (Manea et al., unpublished data).

Natural compounds that inhibit PTKs and vascular ROS production have been extensively investigated. The first inhibitor discovered was quercetin, a plant-derived flavonoid (153). However, quercetin is highly cytotoxic, and inhibits, in addition, cAMP-dependent kinase, protein kinase C, and other ATP-dependent enzyme systems. The isoflavone genistein has been showed to be a more selective PTKs inhibitor than quercetin and a potent antioxidant (154). Other natural occurring compounds including erbstatin, herbimycin A, levendustin A, and (+) aeroplysinin-1, were also reported to be effective inhibitors of PTKs (155). Tyrphostins, the generic name for 'tyrosine phosphorylation inhibitor', represent a family of chemically modified derivates of erbstatin (156). Of particular importance is that all PTK blockers, except erbstatin, were found to be ATP competitors. Owing to these pharmacological features, tyrphostins have been found to be largely nontoxic agents (157).

WP1066 is a derivate of caffeic acid and a novel analog of the Jak2 inhibitor AG490, Similar to the latter, WP1066 inhibits the phosphorylation of Jak2, but unlike AG490, WP1066 also degrades Jak2 protein, thus blocking more effectively its down-stream signaling events (158).

A significant up-stream regulator of Nox enzymes is c-Src, a non-receptor PTK. Touyz et al. (159) have reported that c-Src activation increases p47phox phosphorylation and Nox-derived O2•- in human vascular SMCs exposed to AngII. In addition, c-Src inhibition decreases the up-regulated expression of Nox2, p22phox, and p47phox. In human pulmonary ECs, a c-Src-dependent tyrosine phosphorylation of p47phox regulates hyperoxia-induced NADPH oxidase activation and ROS production (160). Consistent with this data, c-Src-mediated phosphorylation of Noxa1 has been shown to regulate Nox1 activity in human embryonic kidney 293 cells (161,162). Recently, we found that pharmacological inhibition of c-Src [Src I1: 6,7-Dimethoxy-*N*-(4-phenoxyphenyl)-4-quinazolinamine] in ApoE-/- mice fed a high-fat, cholesterol-rich diet, reduces the extent of atherosclerotic lesions, aortic Nox-dependent O2•- production, Nox1, Nox2, and Nox4 gene and protein expression, as well as the protein level of CD68, a macrophage-specific marker. Furthermore, a significant improvement of vascular redox state associated with a considerable decreases in Nox expression and activity were detected in the aorta of diabetic C57Bl/6 mice treated with Src I1 (Manea et al., unpublished data). Since pharmacological inhibitors of Jak2 and c-Src are promising drugs in cancer therapy (163) these enzymes may be also candidates to modulate therapeutically the oxidative stress and inflammation in atherosclerosis and its complications (Figure 3).

7. CONCLUSIONS

Clinical and experimental data demonstrate that overactivity of the different vascular Nox isoforms

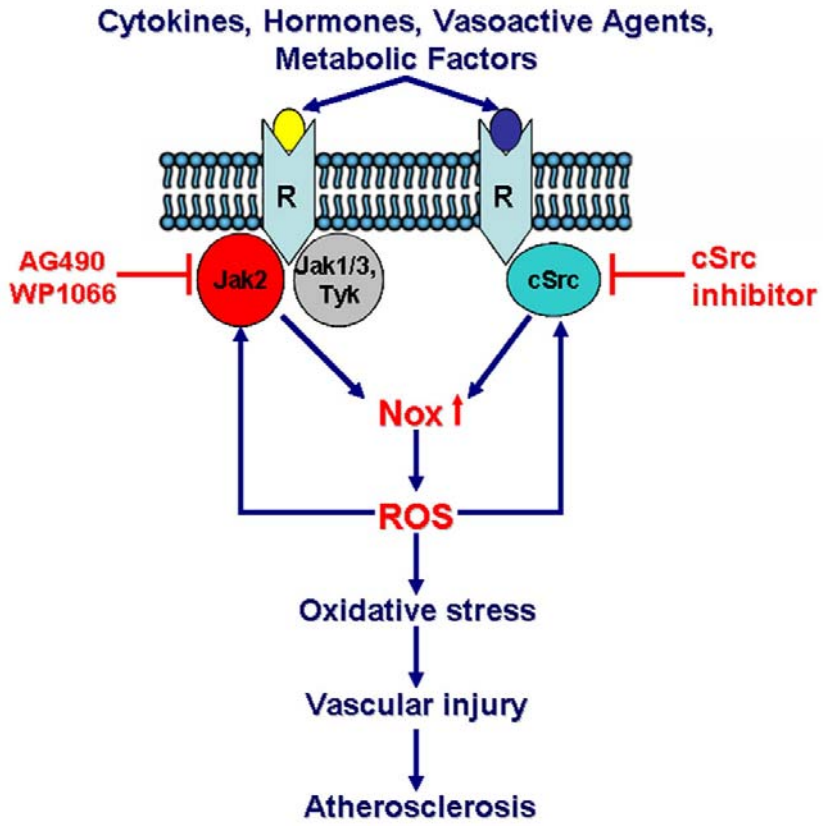

Figure 3. Schematic representation of the possible pharmacological manipulation of Jak2- and cSrc-related signalling pathways in atherosclerosis. Binding of pro- atherogenic factors (i.e. cytokines, hormones, vasoactive agents, metabolic factors) to their specific receptors (R) activate the redox-sensitive protein tyrosine kinases Jak2 and cSrc which in turn up-regulate Nox. Excessive production of Nox-derived ROS induces oxidative stress, which triggers key pathological aspects of atherosclerotic plaque formation such as endothelial dysfunction, inflammation, phenotypic switch of vascular SMCs or matrix remodelling. Hence, pharmacological inhibition of Jak2 (tyrphostin AG490, caffeic acid derivate WP1066) or cSrc that obstructs the activation of oxidative stress (e.g. Nox enzymes) and inflammation-related genes can potentially impede the development of atherosclerotic lesions.

associated with alterations in the antioxidant system triggers oxidative-stress-induced endothelial dysfunction and initiates the chain of critical events that contribute to major cardiovascular pathologies namely atherosclerosis, hypertension, congestive heart failure, ischemia-reperfusion injury, and diabetes-associated vascular complications. Therefore, to develop focused and effective anti-oxidative stress therapy further studies are crucial to reveal and understand the ROS-dependent signal transduction mechanisms, the molecular features of Nox regulation, vascular localization and subcellular compartmentalization of ROS production and its subsequent biological significance.

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9. REFERENCES

1. M. Simionescu: Implications of early structural-functional changes in the endothelium for vascular disease. *Arterioscler Thromb Vasc Biol* 27, 266-274 (2007)

2. I.M. Fearon, and S.P. Faux: Oxidative stress and cardiovascular disease: novel tools give (free) radical insight. *J Mol Cell Cardiol* 47, 372-381 (2009)

3. M. Simionescu, D. Popov and A. Sima: Endothelial transcytosis in health and disease. *Cell Tissue Res* 335, 27-40 (2009)

4. A.V. Sima, C.S. Stancu and M. Simionescu: Vascular endothelium in atherosclerosis. *Cell Tissue Res* 335, 191-203 (2009)

5. D.D. Heistad, Y. Wakisaka, J. Miller, Y. Chu and R. Pena-Silva: Novel aspects of oxidative stress in cardiovascular diseases. *Circ J* 73, 201-207 (2009)

6. A. Manea: NADPH oxidase-derived reactive oxygen species: involvement in vascular physiology and pathology. *Cell Tissue Res* 342:325-339 (2010)

7. S. Yusuf, G. Dagenais, J. Pogue, J. Bosch, and P. Sleight: Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med* 342, 154-160 (2000)

8. MRC/BHF Heart Protection Study of cholesterol-lowering therapy and of antioxidant vitamin supplementation in a wide range of patients at increased risk of coronary heart disease death: early safety and efficacy experience. *Eur Heart J* 20, 725-741 (1999)

9. M. Olukman, C.E. Orhan, F.G. Celenk and S. Ulker: Apocynin restores endothelial dysfunction in streptozotocin diabetic rats through regulation of nitric oxide synthase and NADPH oxidase expressions. *J Diabetes Complications* 24, 415-423 (2010)

10. U. Förstermann: Oxidative stress in vascular disease: causes, defense mechanisms and potential therapies. *Nat Clin Pract Cardiovasc Med* 5, 338-349 (2008)

11. N.R. Madamanchi, R.H. Zhou, A.E. Vendrov, X.L. Niu and M.S. Runge: Does oxidative DNA damage cause atherosclerosis and metabolic syndrome?: new insights into which came first: the chicken or the egg. *Circ Res* 107, 940-942 (2010)

12. A.E. Vendrov, Z.S. Hakim, N.R. Madamanchi, M. Rojas, C. Madamanchi and M.S. Runge: Atherosclerosis is attenuated by limiting superoxide generation in both macrophages and vessel wall cells. *Arterioscler Thromb Vasc Biol* 27, 2714-2721 (2007)

13. E. Dejana, M. Simionescu and H. Wolburg: Endothelial cell biology and pathology. *Cell Tissue Res* 335, 1-3 (2009)

14. S. Ryoo, C.A. Lemmon, K.G. Soucy, G. Gupta, A.R. White, D. Nyhan, A. Shoukas, L.H. Romer and DE. Berkowitz: Oxidized low-density lipoprotein-dependent endothelial arginase II activation contributes to impaired nitric oxide signaling. *Circ Res* 99, 951-960 (2006)

15. R. Zhao, X. Ma, X. Xie and G.X. Shen: Involvement of NADPH oxidase in oxidized LDL-induced upregulation of heat shock factor-1 and plasminogen activator inhibitor-1 in vascular endothelial cells. *Am J Physiol Endocrinol Metab* 297, 104-111 (2009)

16. E.J. Harvey and D.P. Ramji: Interferon-gamma and atherosclerosis: pro- or anti-atherogenic? *Cardiovasc Res* 67, 11-20 (2005)

17. G. Csányi, W.R. Taylor and P.J. Pagano: NOX and inflammation in the vascular adventitia. *Free Radic Biol Med* 47, 1254-1266 (2009)

18. E. Galkina and K. Ley: Immune and inflammatory mechanisms of atherosclerosis. *Annu Rev Immunol* 27, 165-197 (2009)

19. M.Y. Lee, A. San Martin, P.K. Mehta, A.E. Dikalova, A.M. Garrido, S.R. Datla, E. Lyons, K.H. Krause, B. Banfi, J.D. Lambeth, B. Lassègue and K.K. Griendling: Mechanisms of vascular smooth muscle NADPH oxidase 1 (Nox1) contribution to injury-induced neointimal formation. *Arterioscler Thromb Vasc Biol* 29, 480-487 (2009)

20. T. Kondo, M. Hirose and K. Kageyama: Roles of oxidative stress and redox regulation in atherosclerosis. *J Atheroscler Thromb* 16, 532-538 (2009)

21. G. Zalba, A. Fortuño, G. San José, M.U. Moreno, O. Beloqui and J. Díez: Oxidative stress, endothelial dysfunction and cerebrovascular disease. *Cerebrovasc Dis* 24, 24-29 (2007)

22. J. D. Lambeth: NOX enzymes and the biology of reactive oxygen. *Nat Rev Immunol* 4, 181-189 (2004)

23. M. Schrader and H.D. Fahimi: Peroxisomes and oxidative stress. *Biochim Biophys Acta* 1763, 1755-1766 (2006)

24. R.A. Cohena and X. Tong: Vascular oxidative stress: the common link in hypertensive and diabetic vascular disease. *J Cardiovasc Pharmacol* 55, 308-316 (2010)

25. A. Manea, M. Raicu and M. Simionescu: Expression of functionally phagocyte-type NAD(P)H oxidase in pericytes: effect of angiotensin II and high glucose. *Biol Cell* 97, 723-734 (2005)

26. A. Manea, L.I. Tanase, M. Raicu and M. Simionescu: Transcriptional regulation of NADPH oxidase isoforms, Nox1 and Nox4, by nuclear factor-kappaB in human aortic smooth muscle cells. *Biochem Biophys Res Commun* 396, 901-907 (2010)

27. S.W. Chung, J.W. Park, S.A. Lee, S.K. Eo and K. Kim: Thrombin promotes proinflammatory phenotype in human vascular smooth muscle cell. *Biochem Biophys Res Commun* 396, 748-754 (2010)

28. J. Hwang, A. Saha, Y.C. Boo, G.P. Sorescu, J.S. McNally, S.M. Holland, S. Dikalov, D.P. Giddens and K.K. Griendling, D.G. Harrison and H. Jo: Oscillatory shear stress stimulates endothelial production of O2- from p47phox-dependent NAD(P)H oxidases, leading to monocyte adhesion. *J Biol Chem* 278, 47291-47298 (2003)

29. C.P. Judkins, H. Diep, B.R. Broughton, A.E. Mast, E.U. Hooker, A.A. Miller, S. Selemidis, G.J. Dusting, C.G. Sobey and G.R. Drummond: Direct evidence of a role for Nox2 in superoxide production, reduced nitric oxide bioavailability, and early atherosclerotic plaque formation in ApoE-/- mice. *Am J Physiol Heart Circ Physiol* 298, 24-32 (2010)

30. V.M. Victor, N. Apostolova, R. Herance, A. Hernandez-Mijares and M. Rocha: Oxidative stress and mitochondrial dysfunction in atherosclerosis: mitochondria-targeted antioxidants as potential therapy. *Curr Med Chem* 16, 4654-4667 (2009)

31. Y. Huo, L. Zhao, M.C. Hyman, P. Shashkin, B.L. Harry, T. Burcin, S.B. Forlow, M.A. Stark, D.F. Smith, S. Clarke, S. Srinivasan, C.C. Hedrick, D. Praticò, J.L. Witztum, J.L. Nadler, C.D. Funk and K. Ley: Critical role of macrophage 12/15-lipoxygenase for atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 110, 2024-2031 (2004)

32. T. Cyrus, J.L. Witztum, D.J. Rader, R. Tangirala, S. Fazio, M.F. Linton and C.D. Funk: Disruption of the 12/15-lipoxygenase gene diminishes atherosclerosis in apo E-deficient mice. *J Clin Invest* 103, 1597–1604 (1999)

33. R. Natarajan and J.L. Nadler: Lipoxygenases and lipid signaling in vascular cells in diabetes. *Front Biosci* 8, 783-795 (2003)

34. K.B. Reilly, S. Srinivasan, M. E. Hatley, M.K. Patricia, J. Lannigan, D.T. Bolick, G. Vandenhoff, H. Pei, R. Natarajan, J.L. Nadler and C.C. Hedrick: 12/15-Lipoxygenase activity mediates inflammatory monocyte/endothelial interactions and atherosclerosis in vivo. *J Biol Chem* 279, 9440-9450 (2004)

35. K. Nozawa, M.L. Tuck, M. Golub, P. Eggena, J.L. Nadler and N. Stern: Inhibition of lipoxygenase pathway reduces blood pressure in renovascular hypertensive rats. *Am J Physiol* 259, 1774–1780 (1990)

36. M.A. Reddy, Y.S. Kim, L. Lanting and R. Natarajan: Reduced growth factor responses in vascular smooth muscle cells derived from 12/15-lipoxygenase-deficient mice. *Hypertension* 41, 1294 –1300 (2003)

37. J.L. Gu, H. Pei, L. Thomas, J.L. Nadler, J.J. Rossi, L. Lanting and R. Natarajan: Ribozyme-mediated inhibition of rat leukocyte-type 12-lipoxygenase prevents intimal hyperplasia in balloon-injured rat carotid arteries. *Circulation* 103:1446 – 1452 (2001)

38. N.R. Madamanchi, A. Vendrov and M.S. Runge: Oxidative stress and vascular disease. *Arterioscler Thromb Vasc Biol* 25, 29-38 (2005)

39. D. Harrison, K.K. Griendling, U. Landmesser, B. Hornig and H. Drexler: Role of oxidative stress in atherosclerosis. *Am J Cardiol* 91, 7A–11A (2003)

40. S. Martinez-Hervas, J.T. Real, C. Ivorra, A. Priego, F.J. Chaves, F.V. Pallardo, J.R. Viña, J. Redon, R. Carmena and J.F. Ascaso: Increased plasma xanthine oxidase activity is related to nuclear factor kappa beta activation and inflammatory markers in familial combined hyperlipidemia. *Nutr Metab Cardiovasc Dis* 20, 734-739 (2010)

41. N. Jia, P. Dong, Y. Ye, C. Qian and Q. Dai: Allopurinol Attenuates Oxidative Stress and Cardiac Fibrosis in Angiotensin II-Induced Cardiac Diastolic Dysfunction. *Cardiovasc Ther* doi: 10.1.111/j.1755-5922.2.010.0.0243.x (2010)

42. J.G. O'Driscoll, D.J. Green, J.M. Rankin and R.R. Taylor: Nitric oxide-dependent endothelial function is unaffected by allopurinol in hypercholesterolaemic subjects. *Clin Exp Pharmacol Physiol* 26, 779-783 (1999)

43. J. El-Benna, P.M. Dang and M.A Gougerot-Pocidalo: Role of the NADPH oxidase systems Nox and Duox in host defense and inflammation. *Expert Rev Clin Immunol* 3, 111-115 (2007)

44. C.R. Hoyal, A. Gutierrez, B.M. Young, S.D. Catz, J.H. Lin, P.N. Tsichlis and B.M. Babior: Modulation of p47PHOX activity by site-specific phosphorylation: Akt-dependent activation of the NADPH oxidase. *Proc Natl Acad Sci U S A* 100, 5130-5135 (2003)

45. U. Maitra, N. Singh, L. Gan, L. Ringwood and L. Li: IRAK-1 contributes to lipopolysaccharide-induced reactive oxygen species generation in macrophages by inducing NOX-1 transcription and Rac1 activation and suppressing the expression of antioxidative enzymes. *J Biol Chem* 284, 35403-35411 (2009)

46. C.F. Lee, M. Qiao, K. Schröder, Q. Zhao and R. Asmis: Nox4 is a novel inducible source of reactive oxygen species in monocytes and macrophages and mediates oxidized low density lipoprotein-induced macrophage death. *Circ Res* 106, 1489-1497 (2010)

47. A. Savina, C. Jancic, S. Hugues, P. Guermonprez, P. Vargas, I.C. Moura, A.M. Lennon-Duménil, M.C. Seabra, G. Raposo and S. Amigorena: NOX2 controls phagosomal pH to regulate antigen processing during crosspresentation by dendritic cells. *Cell* 126, 205-218 (2006)

48. S.H. Jackson, S. Devadas, J. Kwon, L.A. Pinto and M.S. Williams: T cells express a phagocyte-type NADPH oxidase that is activated after T cell receptor stimulation. *Nat Immunol* 5, 818-827 (2004)

49. J.D. Lambeth: Nox enzymes, ROS, and chronic disease: an example of antagonistic pleiotropy. *Free Radic Biol Med* 43, 332-347 (2007)

50. M. Geiszt, J. Witta, J. Baffi, K. Lekstrom and T.L. Leto: Dual oxidases represent novel hydrogen peroxide sources supporting mucosal surface host defense. *FASEB J* 17, 1502-1504 (2003)

51. J. Rivera, C.G. Sobey, A.K. Walduck and G.R. Drummond: Nox isoforms in vascular pathophysiology:

insights from transgenic and knockout mouse models. *Redox Rep* 15, 50-63 (2010)

52. T. Ago, J. Kuroda, J. Pain, C. Fu, H. Li and J. Sadoshima: Upregulation of Nox4 by hypertrophic stimuli promotes apoptosis and mitochondrial dysfunction in cardiac myocytes. *Circ Res* 106, 1253-1264 (2010)

53. J. Kuroda and J. Sadoshima: NADPH oxidase and cardiac failure. *J Cardiovasc Transl Res* 3, 314-320 (2010)

54. R.S. BelAiba, T. Djordjevic, A. Petry, K. Diemer, S. Bonello, B. Banfi, J. Hess, A. Pogrebniak, C. Bickel and A. Görlach: NOX5 variants are functionally active in endothelial cells. *Free Radic Biol Med* 42, 446-459 (2007)

55. L. Serrander, V. Jaquet, K. Bedard, O. Plastre, O. Hartley, S. Arnaudeau, N. Demaurex, W. Schlegel and K.H. Krause: NOX5 is expressed at the plasma membrane and generates superoxide in response to protein kinase C activation. *Biochimie* 89, 1159-1167 (2007)

56. J.F. Turrens: Mitochondrial formation of reactive oxygen species. *J Physiol* 552, 335-344 (2003)

57. H. Liu, R. Colavitti, I.I. Rovira and T. Finkel: Redox-dependent transcriptional regulation. *Circ Res* 97, 967-974 (2005)

58. B. Shao and J.W. Heinecke: HDL, lipid peroxidation, and atherosclerosis. *J Lipid Res* 50, 599-601 (2009)

59. N.K. Tonks: Protein tyrosine phosphatases: from genes, to function, to disease. *Nat Rev Mol Cell Biol* 7, 833-846 (2006)

60. S. Belia, F. Santilli, S. Beccafico, L. De Feudis, C. Morabito, G. Davi, G . Fanò and M.A. Mariggiò: Oxidative-induced membrane damage in diabetes lymphocytes: effects on intracellular Ca(2 +) homeostasis. *Free Radic Res* 43, 138-148 (2009)

61. A.M. Briones and R.M. Touyz: Oxidative stress and hypertension: current concepts. *Curr Hypertens Rep* 12, 135-142 (2010)

62. M. Guichardant, S. Bacot, P. Molière and M.Lagarde: Hydroxy-alkenals from the peroxidation of n-3 and n-6 fatty acids and urinary metabolites. *Prostaglandins Leukot Essent Fatty Acids* 75, 179-182 (2006)

63. Y. Riahi, G. Cohen, O. Shamni and S. Sasson: Signaling and cytotoxic functions of 4-hydroxyalkenals. *Am J Physiol Endocrinol Metab* 299, 879-886 (2010)

64. D. Demozay, J.C. Mas, S. Rocchi and E. Van Obberghen: FALDH reverses the deleterious action of oxidative stress induced by lipid peroxidation product 4-hydroxynonenal on insulin signaling in 3T3-L1 adipocytes. *Diabetes* 57, 1216-1226 (2008)

65. A. Catalá: Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. *Chem Phys Lipids* 157, 1-11 (2009)

66. J.Y. Lee, G.Y. Jung, H.J. Heo, M.R. Yun, J.Y. Park, S.S. Bae, K.W. Hong, W.S. Lee and C.D. Kim: 4-Hydroxynonenal induces vascular smooth muscle cell apoptosis through mitochondrial generation of reactive oxygen species. *Toxicol Lett* 166, 212-221 (2006)

67. A. Negre-Salvayre, N. Auge, V. Ayala, H. Basaga, J. Boada, R. Brenke, S. Chapple, G. Cohen, J. Feher, T. Grune, G. Lengyel, G.E. Mann, R. Pamplona, G. Poli, M. Portero-Otin, Y. Riahi, R. Salvayre, S. Sasson, J. Serrano, O. Shamni, W. Siems, R.C. Siow, I. Wiswedel, K. Zarkovic and N. Zarkovic: Pathological aspects of lipid peroxidation. *Free Radic Res* 44, 1125-1171 (2010)

68. J.D. Coleman, K.S. Prabhu, J.T. Thompson, P.S. Reddy, J.M. Peters, B.R. Peterson, C.C. Reddy and J.P. Vanden Heuvel: The oxidative stress mediator 4-hydroxynonenal is an intracellular agonist of the nuclear receptor peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta). *Free Radic Biol Med* 42, 1155-1164 (2007)

69. E. Teissier, A. Nohara, G. Chinetti, R. Paumelle, B. Cariou, J.C. Fruchart, R.P. Brandes, A. Shah and B. Staels: Peroxisome proliferator-activated receptor alpha induces NADPH oxidase activity in macrophages, leading to the generation of LDL with PPAR-alpha activation properties. *Circ Res* 95, 1174-1182 (2004)

70. R.M. Touyz: Reactive oxygen species in vascular biology: role in arterial hypertension. *Expert Rev Cardiovasc Ther* 1, 91-106 (2003)

71. M.R. Yun, H.M. Park, K.W. Seo, S.J. Lee, D.S. Im and CD. Kim: 5-Lipoxygenase plays an essential role in 4-HNE-enhanced ROS production in murine macrophages via activation of NADPH oxidase. *Free Radic Res* 44, 742-750 (2010)

72. W.G. Li, L.L. Stoll, J.B. Rice, S.P. Xu, F.J. Miller Jr, P. Chatterjee, L. Hu, L.W. Oberley, A.A. Spector and N.L. Weintraub: Activation of NAD(P)H oxidase by lipid hydroperoxides: mechanism of oxidant-mediated smooth muscle cytotoxicity. *Free Radic Biol Med* 34, 937-946 (2003)

73. W.G. Siems , E. Capuozzo , D. Verginelli, C. Salerno , C. Crifò and T. Grune: Inhibition of NADPH oxidase-mediated superoxide radical formation in PMA-stimulated human neutrophils by 4-hydroxynonenal--binding to -SH and -NH2 groups. *Free Radic Res* 27, 353-358 (1997)

74. A. Ishikado, Y. Nishio, K. Morino, S. Ugi, H. Kondo, T. Makino, A. Kashiwagi and H. Maegawa: Low concentration of 4-hydroxy hexenal increases heme oxygenase-1 expression through activation of Nrf2 and antioxidative activity in vascular endothelial cells. *Biochem Biophys Res Commun* 402, 99-104 (2010)

75. S. Selemidis, C.G. Sobey, K. Wingler, H.H. Schmidt and G.R. Drummond: NADPH oxidases in the vasculature: molecular features, roles in disease and pharmacological inhibition. *Pharmacol Ther* 120, 254-291 (2008)

76. A.N. Lyle, N.N. Deshpande, Y. Taniyama, B. Seidel-Rogol, L. Pounkova, P. Du, C. Papaharalambus, B. Lassègue and K.K. Griendling: Poldip2, a novel regulator of Nox4 and cytoskeletal integrity in vascular smooth muscle cells. *Circ Res* 105, 249-259 (2009)

77. F.J. Miller Jr: Nox4 - Walking the Walk with Poldip2. *Circ Res* 105, 209–210 (2009)

78. F.J. Miller Jr, M. Filali, G.J. Huss, B. Stanic, A. Chamseddine, T.J. Barna and F.S. Lamb: 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)

79. X. Chu, M. Filali, B. Stanic, M. Takapoo, A. Sheehan, R. Bhalla, F.S. Lamb and F.J. Miller Jr: A Critical Role for ClC-3 in Smooth Muscle Cell Activation and Neointima Formation. *Arterioscler Thromb Vasc Biol* 31, 345-351 (2011)

80. T. Yamamori, O. Inanami, H. Nagahata and M. Kuwabara: Phosphoinositide 3-kinase regulates the phosphorylation of NADPH oxidase component p47(phox) by controlling cPKC/PKCdelta but not Akt. *Biochem Biophys Res Commun* 316, 720-730 (2004)

81. L. E. Kilpatrick, S. Sun, H. Li, T.C. Vary and H.M. Korchak: Regulation of TNF-induced oxygen radical production in human neutrophils: role of delta-PKC. *J Leukoc Biol* 87, 153-164 (2010)

82. L. Serrander, L. Cartier, K. Bedard, B. Banfi, B. Lardy, O. Plastre, A. Sienkiewicz, L. Fórró, W. Schlegel and K.H. Krause: NOX4 activity is determined by mRNA levels and reveals a unique pattern of ROS generation. *Biochem J* 406, 105-114 (2007)

83. A. El Jamali, A.J. Valente, J.D. Lechleiter, M.J. Gamez, D.W. Pearson, W.M. Nauseef and R.A. Clark: Novel redox-dependent regulation of NOX5 by the tyrosine kinase c-Abl. *Free Radic Biol Med* 44, 868-881 (2008)

84. M. Janiszewski, L.R. Lopes, A.O. Carmo, M.A. Pedro, R.P. Brandes, C.X. Santos and F.R. Laurindo: Regulation of NAD(P)H oxidase by associated protein disulfide isomerase in vascular smooth muscle cells. *J Biol Chem* 280, 40813-40819 (2005)

85. R. Kakar, B. Kautz and E.A. Eklund: JAK2 is necessary and sufficient for interferon-gamma-induced transcription of the gene encoding gp91PHOX. *J Leukoc Biol* 77, 120-127 (2005)

86. A.C. Brewer, E.C. Sparks and A.M. Shah: Transcriptional regulation of the NADPH oxidase isoform, Nox1, in colon epithelial cells: role of GATA-binding factor(s). *Free Radic Biol Med* 40, 260-274 (2006)

87. U. Maitra, N. Singh, L. Gan, L. Ringwood and L. Li: IRAK-1 contributes to lipopolysaccharide-induced reactive oxygen species generation in macrophages by inducing NOX-1 transcription and Rac1 activation and suppressing the expression of antioxidative enzymes. *J Biol Chem* 284, 35403-35411 (2009)

88. A. Manea, S.A. Manea, A.V. Gafencu, M. Raicu and M. Simionescu: AP-1-dependent transcriptional regulation of NADPH oxidase in human aortic smooth muscle cells: role of p22phox subunit. *Arterioscler Thromb Vasc Biol* 28, 878-885 (2008)

89. M. Raicu and A. Manea: Activator protein-1 regulates Nox1 and Nox4-containing NADPH oxidase transcription in human vascular smooth muscle cells. *Annals of RSCB* XV, 11-17 (2010)

90. A. Manea, L.I. Tanase, M. Raicu and M. Simionescu: Jak/STAT signaling pathway regulates Nox1 and Nox4-based NADPH oxidase in human aortic smooth muscle cells. *Arterioscler Thromb Vasc Biol* 30, 105-112 (2010)

91. W. Ni, Y. Zhan, H. He, E. Maynard, J.A. Balschi and P. Oettgen: Ets-1 is a critical transcriptional regulator of reactive oxygen species and p47(phox) gene expression in response to angiotensin II. *Circ Res* 101, 985-994 (2007)

92. L. Zhang, O.R. Sheppard, A.M. Shah and A.C. Brewer: Positive regulation of the NADPH oxidase NOX4 promoter in vascular smooth muscle cells by E2F. *Free Radic Biol Med* 45, 679-685 (2008)

93. J. Anrather, G. Racchumi and C. Iadecola: NF-kappaB regulates phagocytic NADPH oxidase by inducing the expression of gp91phox. *J Biol Chem* 281, 5657-5667 (2006)

94. K.A. Gauss, L.K. Nelson-Overton, D.W. Siemsen, Y. Gao, F.R. DeLeo and M.T. Quinn: Role of NF-kappaB in transcriptional regulation of the phagocyte NADPH oxidase by tumor necrosis factor-alpha. *J Leukoc Biol* 82, 729-741 (2007)

95. A. Manea, S.A. Manea, A.V. Gafencu and M. Raicu: Regulation of NADPH oxidase subunit p22(phox) by NF-kB in human aortic smooth muscle cells. *Arch Physiol Biochem* 113, 163-172 (2007)

96. A. Manea, L.I. Tanase, M. Raicu and M. Simionescu: Transcriptional regulation of NADPH oxidase isoforms, Nox1 and Nox4, by nuclear factor-kappaB in human aortic smooth muscle cells. *Biochem Biophys Res Commun* 396, 901-907 (2010)

97. M.S. Wolin: Subcellular localization of Nox-containing oxidases provides unique insight into their role in vascular oxidant signaling. *Arterioscler Thromb Vasc Biol* 24, 625-627(2004)

98. P. Goyal, N. Weissmann, F. Grimminger, C. Hegel, L. Bader, F. Rose, L. Fink, H.A. Ghofrani, R.T. Schermuly,

H.H. Schmidt, W. Seeger and J. Hänze: Upregulation of NAD(P)H oxidase 1 in hypoxia activates hypoxia-inducible factor 1 via increase in reactive oxygen species. *Free Radic Biol Med* 36, 1279-1288 (2004)

99. I. Diebold, A. Petry, J. Hess and A. Görlach: The NADPH oxidase subunit NOX4 is a new target gene of the hypoxia-inducible factor-1. *Mol Biol Cell* 21, 2087-2096 (2010)

100. M. Katsuyama, C. Fan, N. Arakawa, T. Nishinaka, M. Miyagishi, K. Taira and C. Yabe-Nishimura: Essential role of ATF-1 in induction of NOX1, a catalytic subunit of NADPH oxidase: involvement of mitochondrial respiratory chain. *Biochem J* 386, 255-261 (2005)

101. S. Pendyala and V. Natarajan: Redox regulation of Nox proteins. *Respir Physiol Neurobiol* 174, 265-271 (2010)

102. B. Staels and J.C. Fruchart: Therapeutic roles of peroxisome proliferator-activated receptor agonists. *Diabetes* 54, 2460-2470 (2005)

103. B. Staels: PPAR agonists and the metabolic syndrome. *Therapie* 62, 319-326 (2007)

104. S.K. Das and R. Chakrabarti: Role of PPAR in cardiovascular diseases. *Recent Pat Cardiovasc Drug Discov* 1, 193-209 (2006)

105. P. Gervois, J.C. Fruchart and B. Staels: Inflammation, dyslipidaemia, diabetes and PPARs: pharmacological interest of dual PPARalpha and PPARgamma agonists. *Int J Clin Pract Suppl* 143, 22-29 (2004)

106. E.L. Schiffrin, F. Amiri, K. Benkirane, M. Iglarz and Q.N. Diep: Peroxisome proliferator-activated receptors: vascular and cardiac effects in hypertension. *Hypertension* 42, 664-668 (2003)

107. Q.N. Diep, K. Benkirane, F. Amiri, J.S. Cohn, D. Endemann and E.L. Schiffrin: PPAR alpha activator fenofibrate inhibits myocardial inflammation and fibrosis in angiotensin II-infused rats. *J Mol Cell Cardiol* 36, 295-304 (2004)

108. H. Toba, S. Miki, T. Shimizu, A. Yoshimura, R. Inoue, N. Sawai, R. Tsukamoto, M. Murakami, Y. Morita, Y. Nakayama, M. Kobara and T. Nakata: The direct antioxidative and anti-inflammatory effects of peroxisome proliferator-activated receptors ligands are associated with the inhibition of angiotensin converting enzyme expression in streptozotocin-induced diabetic rat aorta. *Eur J Pharmacol* 549, 124-132 (2006)

109. C. De Ciuceis, F. Amiri, M. Iglarz, J.S. Cohn, R.M. Touyz and E.L. Schiffrin: Synergistic vascular protective effects of combined low doses of PPARalpha and PPARgamma activators in angiotensin II-induced hypertension in rats. *Br J Pharmacol* 151, 45-53 (2007)

110. G. Ceolotto, A. Gallo, I. Papparella, L. Franco, E. Murphy, E. Iori, E. Pagnin, G.P. Fadini, M. Albiero, A. Semplicini and A. Avogaro: Rosiglitazone reduces glucose-induced oxidative stress mediated by NAD(P)H oxidase via AMPK-dependent mechanism. *Arterioscler Thromb Vasc Biol* 27, 2627-2633 (2007)

111. J. Hwang, D.J. Kleinhenz, H.L. Rupnow, A.G. Campbell, P.M. Thulé, R.L. Sutliff and C.M. Hart: The PPARgamma ligand, rosiglitazone, reduces vascular oxidative stress and NADPH oxidase expression in diabetic mice. *Vascul Pharmacol* 46, 456-462 (2007)

112. A.D. Dobrian, S.D. Schriver, A.A. Khraibi and R.L. Prewitt: Pioglitazone prevents hypertension and reduces oxidative stress in diet-induced obesity. *Hypertension* 43, 48-56 (2004)

113. R. Genolet, W. Wahli and L. Michalik: PPARs as drug targets to modulate inflammatory responses? *Curr Drug Targets Inflamm Allergy* 3, 361-375 (2004)

114. W. Huang and C.K. Glass: Nuclear receptors and inflammation control: molecular mechanisms and pathophysiological relevance. *Arterioscler Thromb Vasc Biol* 30, 1542-1549 (2010)

115. Y. Nakashima, A.S. Plump, E.W. Raines, J.L. Breslow and R. Ross: ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler Thromb. Vasc Biol* 14, 133-140 (1994)

116. X. Xu, X. Gao, B.J. Potter, J.M. Cao and C. Zhang: Anti-LOX-1 rescues endothelial function in coronary arterioles in atherosclerotic ApoE knockout mice. *Arterioscler Thromb Vasc Biol* 27, 871-877 (2007)

117. H. Ding, M. Hashem and C. Triggle: Increased oxidative stress in the streptozotocin-induced diabetic apoE-deficient mouse: changes in expression of NADPH oxidase subunits and eNOS. *Eur J Pharmacol* 561, 121-128 (2007)

118. G. Gavazzi, C. Deffert, C. Trocme, M. Schäppi, F.R. Herrmann and K.H. Krause: NOX1 deficiency protects from aortic dissection in response to angiotensin II. *Hypertension* 50, 189-196 (2007)

119. K. Matsuno, H. Yamada, K. Iwata, D. Jin, M. Katsuyama, M. Matsuki, S. Takai, K . Yamanishi, M. Miyazaki, H. Matsubara and C. Yabe-Nishimura: Nox1 is involved in angiotensin II-mediated hypertension: a study in Nox1-deficient mice. *Circulation* 112, 2677-2685 (2005)

120. A. Dikalova, R. Clempus, B. Lassègue, G. Cheng, J. McCoy, S. Dikalov, A. San Martin, A. Lyle, D.S. Weber, D. Weiss, W.R. Taylor, H.H. Schmidt, G.K. Owens, J.D. Lambeth and K.K. Griendling: Nox1 overexpression potentiates angiotensin II-induced hypertension and vascular smooth muscle hypertrophy in transgenic mice. *Circulation* 112, 2668-2676 (2005)

121. A.E. Dikalova, M.C. Gó ngora, D.G. Harrison, J.D. Lambeth, S. Dikalov and K.K. Griendling: Upregulation of Nox1 in vascular smooth muscle leads to impaired endothelium-dependent relaxation via eNOS uncoupling. *Am J Physiol Heart Circ Physiol* 299, 673-679 (2010)

122. Z. Chen, J.F. Keaney Jr, E. Schulz, B. Levison, L. Shan, M. Sakuma, X. Zhang, C. Shi, S.L. Hazen and D.I . Simon: Decreased neointimal formation in Nox2-deficient mice reveals a direct role for NADPH oxidase in the response to arterial injury. *Proc Natl Acad Sci U SA* 101, 13014-13019 (2004)

123. M. Zhang, A.C. Brewer, K. Schröder, C.X. Santos, D.J. Grieve, M. Wang, N. Anilkumar, B. Yu, X. Dong, S.J. Walker, R.P. Brandes and A.M. Shah: NADPH oxidase-4 mediates protection against chronic load-induced stress in mouse hearts by enhancing angiogenesis. *Proc Natl Acad Sci U SA* 107, 18121-18126 (2010)

124. T.J. Guzik, W. Chen, M.C. Gongora, B. Guzik, H.E. Lob, D. Mangalat, N. Hoch, S. Dikalov, P. Rudzinski, B. Kapelak, J. Sadowski and D.G. Harrison: Calcium-dependent NOX5 nicotinamide adenine dinucleotide phosphate oxidase contributes to vascular oxidative stress in human coronary artery disease. *J Am Coll Cardiol* 52, 1803-1809 (2008)

125. K. Wingler, S. Wünsch, R. Kreutz, L. Rothermund, M. Paul and H.H. Schmidt: Upregulation of the vascular NAD(P)H-oxidase isoforms Nox1 and Nox4 by the renin-angiotensin system in vitro and in vivo. *Free Radic Biol Med* 31, 1456-1464 (2001)

126. L. Papatheodorou and N. Weiss: Vascular oxidant stress and inflammation in hyperhomocysteinemia. *Antioxid Redox Signal* 9, 1941-1958 (2007)

127. A.V. Sima, G.M. Botez, C.S. Stancu, A. Manea, M. Raicu and M. Simionescu: Effect of irreversibly glycated LDL in human vascular smooth muscle cells: lipid loading, oxidative and inflammatory stress. *J Cell Mol Med* 14, 2790-2802 (2010)

128. A.C. Montezano, D. Burger, T.M. Paravicini, A.Z. Chignalia, H. Yusuf, M. Almasri, Y. He, G.E. Callera, G. He, K.H. Krause, D. Lambeth, M.T. Quinn and R.M. Touyz: 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)

129. G. Zalba, G. San José, F.J. Beaumont, M.A. Fortuño, A. Fortuño and J. Díez: Polymorphisms and promoter overactivity of the p22(phox) gene in vascular smooth muscle cells from spontaneously hypertensive rats. *Circ Res* 88, 217-222 (2001)

130. H. Azumi, N. Inoue, S. Takeshita, Y. Rikitake, S. Kawashima, Y. Hayashi, H. Itoh and M. Yokoyama: Expression of NADH/NADPH oxidase p22phox in human coronary arteries. *Circulation* 100, 1494-1498 (1999)

131. T. Kawahara, D. Ritsick, G. Cheng and J.D. Lambeth: Point mutations in the proline-rich region of p22phox are dominant inhibitors of Nox1- and Nox2-dependent reactive oxygen generation. *J Biol Chem* 280, 31859-31869 (2005)

132. G. San José, A. Fortuño, O. Beloqui, J. Díez and G. Zalba: NADPH oxidase CYBA polymorphisms, oxidative stress and cardiovascular diseases. *Clin Sci (Lond)* 114, 173-182 (2008)

133. M.U. Moreno, G. San José, J. Orbe, J.A. Páramo, O. Beloqui, J. Díez and G. Zalba: Preliminary characterisation of the promoter of the human p22(phox) gene: identification of a new polymorphism associated with hypertension. *FEBS Lett* 542, 27-31 (2003)

134. M.C. Dinauer, E.A. Pierce, G.A. Bruns, J.T. Curnutte and S.H. Orkin: Human neutrophil cytochrome b light chain (p22-phox). Gene structure, chromosomal location, and mutations in cytochrome-negative autosomal recessive chronic granulomatous disease. *J Clin Invest* 86, 1729–1737 (1990)

135. T.J. Guzik, N.E. West, E. Black, D. McDonald, C. Ratnatunga, R. Pillai and K.M. Channon: Functional effect of the C242T polymorphism in the NAD(P)H oxidase p22phox gene on vascular superoxide production in atherosclerosis. *Circulation* 102, 1744-1747 (2000)

136. N. Inoue, S. Kawashima, K. Kanazawa, S. Yamada, H. Akita and M. Yokoyama: Polymorphism of the NADH/NADPH oxidase p22phox gene in patients with coronary artery disease. *Circulation* 97, 135–137 (1998)

137. A. Gardemann, P. Mages, N. Katz, H. Tillmanns and W. Haberbosch: The p22*phox* A640G gene polymorphism but not the C242T gene variation is associated with coronary heart disease in younger individuals. *Atherosclerosis* 145, 315–323 (1999)

138. H. Cai, N. Duarte, D.E. Wilcken and X.L. Wang: NADH/NADPH oxidase p22 phox C242T polymorphism and coronary artery disease in the Australian population. *Eur J Clin Invest* 29, 744-748 (1999)

139. S. Nasti, P. Spallarossa, P. Altieri, S. Garibaldi, P. Fabbi, L. Polito, L. Bacino, M. Brunelli, C. Brunelli, A. Barsotti and G. Ghigliotti: C242T polymorphism in CYBA gene (p22phox) and risk of coronary artery disease in a population of Caucasian Italians. *Dis Markers* 22, 167-173 (2006)

140. M.U. Moreno, G. San José, A. Fortuño, O. Beloqui, J. Díez and G. Zalba: The C242T CYBA polymorphism of NADPH oxidase is associated with essential hypertension. *J Hypertens* 24, 1299-1306 (2006)

141. G. San José, M.U. Moreno, S. Oliván, O. Beloqui, A. Fortuño, J. Díez and G. Zalba: Functional effect of the p22phox -930A/G polymorphism on p22phox expression and NADPH oxidase activity in hypertension. *Hypertension* 44, 163-169 (2004)

142. S.C. Lim, S.K. Goh, Y.R. Lai, W.W. Tee, A. Koh, X.H. Xu, Y.S. Wu, E. Yap, T. Subramaniam and C.F. Sum: Relationship between common functional polymorphisms of the p22phox gene (-930A > G and +242C > T) and nephropathy as a result of Type 2 diabetes in a Chinese population. *Diabet Med* 23, 1037-1041 (2006)

143. M.U. Moreno, G. San José, A. Fortuño, O. Beloqui, J. Redón, F.J. Chaves, D. Corella, J. Díez and G. Zalba: A novel CYBA variant, the -675A/T polymorphism, is associated with essential hypertension. *J Hypertens* 25, 1620-1626 (2007)

144. A. Fortuño, J. Bidegain, P.A. Robador, J. Hermida, J. López-Sagaseta, O. Beloqui, J. Díez and G. Zalba: Losartan metabolite EXP3179 blocks NADPH oxidase-mediated superoxide production by inhibiting protein kinase C: potential clinical implications in hypertension. *Hypertension* 54, 744-750 (2009)

145. S. Wolfrum, K.S. Jensen and J.K. Liao: Endothelium-dependent effects of statins. *Arterioscler Thromb Vasc Biol* 23, 729-736 (2003)

146. J.D. Lambeth, K.H. Krause and R.A. Clark: NOX enzymes as novel targets for drug development. *Semin Immunopathol* 30, 339-363 (2008)

147. V. Jaquet, L. Scapozza, R.A. Clark, K.H. Krause and J.D. Lambeth: Small-molecule NOX inhibitors: ROS-generating NADPH oxidases as therapeutic targets. *Antioxid Redox Signal* 11, 2535-2552 (2009)

148. P.M. Seitz, R. Cooper, G.J. Gatto Jr, F. Ramon, T.D. Sweitzer, D.G. Johns, E.A. Davenport, R.S. Ames and L.A. Kallal: Development of a high-throughput cell-based assay for superoxide production in HL-60 cells. *J Biomol Screen* 15, 388-397 (2010)

149. A.E. Vendrov, N.R. Madamanchi, X.L. Niu, K.C. Molnar, M. Runge, C. Szyndralewiez, P. Page and M.S. Runge: NADPH oxidases regulate CD44 and hyaluronic acid expression in thrombin-treated vascular smooth muscle cells and in atherosclerosis. *J Biol Chem* 285, 26545-26557 (2010)

150. S. Wind, K. Beuerlein, M.E. Armitage, A. Taye, A.H. Kumar, D. Janowitz, C. Neff, A.M. Shah, K. Wingler and H.H. Schmidt: Oxidative stress and endothelial dysfunction in aortas of aged spontaneously hypertensive rats by NOX1/2 is reversed by NADPH oxidase inhibition. *Hypertension* 56, 490-497 (2010)

151. M.B. Marrero: Introduction to JAK/STAT signaling and the vasculature. *Vascul Pharmacol* 43, 307-309 (2005)

152. S.A. Manea, A. Manea and C. Heltianu: Inhibition of JAK/STAT signaling pathway prevents high-glucose-induced increase in endothelin-1 synthesis in human endothelial cells. *Cell Tissue Res* 340, 71-79 (2010)

153. M. Sánchez, M. Galisteo, R. Vera, I.C. Villar, A. Zarzuelo, J. Tamargo, F. Pérez-Vizcaíno and J. Duarte: Quercetin downregulates NADPH oxidase, increases eNOS activity and prevents endothelial dysfunction in spontaneously hypertensive rats. *J Hypertens* 24, 75-84 (2006)

154. J.W. Xu, K. Ikeda and Y. Yamori: Genistein inhibits expressions of NADPH oxidase p22phox and angiotensin II type 1 receptor in aortic endothelial cells from stroke-prone spontaneously hypertensive rats. *Hypertens Res* 27, 675-683 (2004)

155. A. Levitzki: Tyrphostins: tyrosine kinase blockers as novel antiproliferative agents and dissectors of signal transduction. *FASEB J* 6, 3275-3282 (1992)

156. P. Yaish, A. Gazit, C. Gilon and A. Levitzki: Blocking of EGF-dependent cell proliferation by EGF receptor kinase inhibitors. *Science* 242, 933-935 (1988)

157. Y. Kuwano, T. Kawahara, H. Yamamoto, S. Teshima-Kondo, K. Tominaga, K. Masuda, K. Kishi, K. Morita and K. Rokutan: Interferon-gamma activates transcription of NADPH oxidase 1 gene and upregulates production of superoxide anion by human large intestinal epithelial cells. *Am J Physiol Cell Physiol* 290, 433-443 (2006)

158. A. Ferrajoli, S. Faderl, Q. Van, P. Koch, D. Harris, Z. Liu, I. Hazan-Halevy, Y. Wang, H. M. Kantarjian, W. Priebe and Z. Estrov: WP1066 disrupts Janus kinase-2 and induces caspase-dependent apoptosis in acute myelogenous leukemia cells. *Cancer Res* 67, 11291-11299 (2007)

159. R.M. Touyz, G. Yao and E.L. Schiffrin: 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)

160. A.K. Chowdhury, T. Watkins, N.L. Parinandi, B. Saatian, M.E. Kleinberg, P.V. Usatyuk and V. Natarajan: Src-mediated tyrosine phosphorylation of p47phox in hyperoxia-induced activation of NADPH oxidase and generation of reactive oxygen species in lung endothelial cells. *J Biol Chem* 280, 20700-20711 (2005)

161. J.S. Kim, B.A. Diebold, B.M. Babior, U.G. Knaus and G.M. Bokoch: Regulation of Nox1 activity via protein kinase A-mediated phosphorylation of NoxA1 and 14-3-3 binding. *J Biol Chem* 282, 34787-34800 (2007)

162. D. Gianni, B. Bohl, S.A. Courtneidge and G.M. Bokoch: The involvement of the tyrosine kinase c-Src in the regulation of reactive oxygen species generation mediated by NADPH oxidase-1. *Mol Biol Cell* 19, 2984-2994 (2008)

163. S. Verstovsek: Therapeutic potential of Janus-activated kinase-2 inhibitors for the management of myelofibrosis. *Clin Cancer Res* 16, 1988-1996 (2010)

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