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NADPH oxidases: key modulators in aging and age-related cardiovascular diseases?

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Abstract

Reactive oxygen species (ROS) and oxidative stress have long been linked to aging and diseases prominent in the elderly such as hypertension, atherosclerosis, diabetes and atrial fibrillation (AF). NADPH oxidases (Nox) are a major source of ROS in the vasculature and are key players in mediating redox signalling under physiological and pathophysiological conditions. In this review, we focus on the Nox-mediated ROS signalling pathways involved in the regulation of 'longevity genes' and recapitulate their role in age-associated vascular changes and in the development of age-related cardiovascular diseases (CVDs). This review is predicated on burgeoning knowledge that Nox-derived ROS propagate tightly regulated yet varied signalling pathways, which, at the cellular level, may lead to diminished repair, the aging process and predisposition to CVDs. In addition, we briefly describe emerging Nox therapies and their potential in improving the health of the elderly population.

Keywords

aging; cardiovascular diseases; reduced nicotinamide–adenine dinucleotide phosphate (NADPH) oxidase; reduced nicotinamide–adenine dinucleotide phosphate oxidase therapeutics (Nox therapeutics)

INTRODUCTION

Aging is the predominant risk factor in cardiovascular diseases (CVDs) [1] and the latest statistics show that of the 83.6 million American adults with some form of CVD, 42.2 million are estimated to be ≥60 years of age [2]. Currently, CVD is the leading cause of death in the U.S.A.; approximately 66 % of CVDs occurring in people 75 years of age or more [2]. It is projected that by 2030 nearly 40.5 % of the U.S.A. population will have more than one type of CVD [3]. With increasing age, clinical manifestations and prognosis of vascular diseases worsen as a result of molecular changes associated with the aging process, often similar to specific pathophysiological changes observed in disease. Moreover, aging is a cumulative multifactorial process and not linked to a single cause. To date, multiple theories of biological aging are discussed within the literature, e.g. the 'wear and tear' theory, auto-immunity theory, programmed cellular theory, somatic mutation theory or

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homoeostatic theory have been discussed previously [4,5]. However, many of these viewpoints share ‘oxidative stress’ as the common denominator in aging. The free radical theory of aging suggests that accumulated damage caused by free radicals and reactive oxygen species (ROS) accelerates the aging process [6]. Although there is still considerable debate that a single theory can explain the aetiology of aging, the free radical theory of aging still draws considerable attention and intrigue. It is now generally accepted that superoxide anion and other ROS can exert beneficial/protective effects under normal conditions, via the promotion of antioxidant enzyme expression and through adaptive cellular signalling responses. Harmful effects, on the other hand, are the result of aberrant redox signalling and direct damage to biologically sensitive targets. Typically, this oxidative damage results in a pro-senescent phenotype, which is one mechanism explaining biological aging [7,8]. Although senescence can be triggered by telomere attrition, mitogen-mediated extracellular signalling or decreased replicative capacity, oxidative stress remains a prominent instigator of this phenotype [9–11]. While associated, biological aging, senescence and longevity are distinct processes. Therefore, an improved understanding of the biology governing age-related decline of cellular function and homoeostasis is imperative.

Furthermore, although differences in cardiovascular function between young and old have been extensively described in the literature, the majority of the *in vivo* studies related to CVD have been performed in young animals. Hence, it is important to understand how ROS contributes to aging and is, in turn, a critical contributor to age-related diseases. Perspectives on the role of NADPH oxidase (Nox)-derived ROS in aging and the potential signalling mechanisms that underlie these age-related changes as well as how they relate to CVD in older population are the focus of the present review. The potential for currently available Nox therapeutic strategies to intervene in the cellular aging process and thereby slow the onset of age-related CVD are also discussed.

OXIDATIVE STRESS THEORY, AGING AND NADPH OXIDASES

The free radical theory of aging, when first proposed by Harman [6], suggested that longevity is governed by the production of endogenous oxygen radicals in cells, resulting in a pattern of cumulative damage in a random and indiscriminate manner. This theory, therefore, implied that an overall reduction in oxidative stress, either by reducing the pro-oxidant load or by increasing antioxidant defences or both, protects against aging and increases lifespan.

Although cellular defences [including catalase, Cu/Zn superoxide dismutase (SOD1) and glutathione peroxidases (Gpxs)] combating oxidative damage are normally plentiful, they are not entirely effective, as oxidatively damaged macromolecules still accumulate with biological aging [12]. There is supporting evidence from studies in *Caenorhabditis elegans* and *Drosophila*, two well-established models to study aging, which revealed that decreased ROS production may improve resistance to oxidative stress and therefore increase lifespan [13,14]. In mammals, however, studies are largely correlative, based on findings of increased oxidatively damage DNA and/or proteins in aged individuals [15]. Moreover, studies performed in vertebrate animals using transgenic and knockout mouse models [e.g. SOD, Gpx, thioredoxin 2 (Trx2) and catalase] provide conflicting evidence regarding effects on

lifespan or longevity [16–24]. It is worth noting that antioxidant enzyme systems are not the only factors involved in cellular homoeostasis. Other enzymes, such as Noxs and myeloperoxidase (MPO), historically assumed to generate ROS for destructive purposes (i.e. in host defence), are now known to serve as important signal-transducing enzymes. Their activation is elicited in response to a variety of growth factors, cytokines and G-protein-coupled receptors [25–30]. ROS are also produced via a variety of other enzyme systems including xanthine oxidase (XO), cytochrome P450 and by uncoupling of nitric oxide (NO) synthase (NOS), yet Noxs appear to predominate in cellular ROS production. ROS have also emerged as locally produced in multiple cellular compartments including at the plasma membrane and within the mitochondrion, peroxisomes and the cytoplasm. Furthermore, basal ROS mediate important biological functions by regulating the activity of diverse intracellular signalling pathways involved in growth, apoptosis, survival, metabolism and migration [26–28,31–34]. It stands to reason therefore that specific signal transduction by any one Nox in a particular cell is a consequence of its co-localization with upstream and downstream effectors of each pathway. A protective role for ROS nothwith-standing, a wide array of studies suggests that ROS participate in biological aging and age-related CVDs such as atherosclerosis, hypertension, stroke and atrial fibrillation (AF) [35–51].

Despite evidence suggesting a causal link between aging and disease, little is known regarding the molecular mechanisms that dictate age-related processes and their association with diseases. A number of studies, at both the cellular and the organismal levels, have reported increased lifespan with increased resistance to oxidative stress. However, whether this may be attributed to a common signal transduction pathway that regulates aging and response to oxidative stress or a variety of pathways is yet to be determined. Logic informs us that multiple pathways are most likely involved. Figure 1 illustrates a few of the important signalling pathways implicated in the process of aging. Indeed, one mechanistic link between aging and oxidants comes from studies showing that mice lacking $p66^{Shc}$ have a ~30 % increase in lifespan, which is correlated to reduced intracellular ROS. These studies also showed that oxidative regulation of p53 and FKHRL1 [forkhead-box protein O3 (FOXO3)] is mediated by $p66^{Shc}$ [52–55]. Other proteins and pathways linked to aging are SIRT1 (sirtuin 1; mammalian homologue of Sir2 protein found in lower organisms) [56–59], Klotho [60–62], AMP-activated protein kinase (AMPK) [63], protein skinhead 1 (SKN-1) [homologue of nuclear respiratory factor 1] [64], Clock 1 (clk-1) [65,66], insulin/insulin-like growth factor (IGF)-1 signalling [67] and mammalian target of rapamycin (mTOR) signalling [68,69].

Mammalian NADPH oxidase

The NADPH oxidase family of enzymes are considered the ‘professional’ enzymatic source of ROS in vascular cells and are therefore known to be a major contributor to vascular dysfunction and disease [43,44,47,48,70–76]. In this regard, Nox isozymes plausibly play a prominent role in age-related susceptibility to vascular occlusive diseases such as coronary artery disease (CAD) and peripheral artery disease (PAD), hypertension and diabetes, through redox signalling pathway activation [29,30,45,46,49,50,77–86]. The structure and function of Nox isozymes are complex and more thoroughly reviewed elsewhere [43,44,47,48,74,87]. It is the aim of the current review to outline their role in ROS

production and their ability to contribute to the aging process. In brief, the Nox family consists of seven isoforms [Nox1–5 and Duox1–2 (dual oxidase 1–2)], of which Nox1, Nox2, Nox4 and Nox5 are known to be expressed in human vascular tissue [88]. Moreover, Nox enzymes are varied in their cellular expression and are identified by their catalytic core, and extensive evidence demonstrates their contribution to vascular dysfunction [77]. Importantly, Nox1, Nox2 and Nox4 isoforms, but not Nox5, require the smaller membrane-bound $p22^{phox}$ component for membrane stabilization and activity [89]. Nox1 and Nox2 are regulated by sometimes distinct, otherwise interchangeable, cytosolic components loosely termed as activators or organizers, which are generally recruited to the membrane components following cellular stimuli to assemble an active Nox [90–92]. To date, Nox2 remains the most extensively studied family member in a variety of physiological and pathophysiological signalling pathways. The active Nox2 enzyme complex comprises the catalytic Nox2 subunit in association with $p22^{phox}$ to form the cytochrome B_{558} complex, $p47^{phox}$ (organizer), $p67^{phox}$ (activator), $p40^{phox}$ and the small Rho-family GTP-binding protein Rac1/2 [30]. Arguably, Nox2 is the most widely distributed of the isoforms, present in phagocytes, heart, lung and the vasculature, among multiple other tissues, and is suggested to be increased in aging vascular cells [93]. Similarly, the active Nox1 system is composed of the Nox1 catalytic subunit, $p22^{phox}$ and in its canonical subunits NoxO1 ($p47^{phox}$ homologue), NoxA1 ($p67^{phox}$ homologue) and Rac1/2 [94]. Nox1 is highly expressed in the colon, but is currently also known to be present in the heart, lungs and blood vessels. In contrast, the Nox4 system is composed of the Nox4 catalytic subunit and $p22^{phox}$, with the only other known associated protein described for its activity being Poldip2 [95]. It is widely held that Nox4's activity is regulated transcriptionally, and, interestingly, it is up-regulated in aging VSMCs (vascular smooth muscle cells) [96]. Incidentally, Nox4 is expressed in all three main vascular cell types: the endothelial cell, smooth muscle cell and fibroblast. Another distinct feature of Nox4 compared with other Nox is that it is constitutively active and appears to preferentially produce H_2O_2 over superoxide anion. It has been suggested that Nox4 may act as an oxygen sensor [97]. Finally, Nox5, which does not require $p22^{phox}$ for membrane stabilization, is regulated by calcium binding through EF-hand motifs [98]. Although Nox enzymes have been implicated to play a crucial role in several signalling pathways, a lack of crystal structure for Nox isoenzymes as well as structural similarities among Nox family members have confounded progress towards the generation of specific antibodies and small-molecule inhibitors. Therefore, when implicating Nox isoenzymes in any study, it is critical that key positive controls are used to account for antibody limitations, as is discussed by Baniulis et al. [99]; namely neutrophil homogenates for Nox2, colon homogenates for Nox1, kidney homogenates for Nox4 or heterologous overexpression cell systems. In addition, mRNA analyses [78,100,101] are highly recommended to corroborate changes detected at the protein level.

ROS production by activated NADPH oxidase is mediated by a wide variety of factors including mechanical forces, environmental factors (e.g. hypoxia) and cytokines and hormones such as angiotensin II (AngII), aldosterone, endothelin-1 (ET-1), platelet-derived growth factor (PDGF), TGFβ (transforming growth factor β) and TNFα (tumour necrosis factor α) [71,75,82,102–106]. In the light of this broad and often deleterious role for Nox,

the quest for efficacious and isoform-selective Nox inhibitors is expected to come into even greater focus in Nox-related disease research, i.e. advances in biochemical characterization and ROS detection agents are anticipated to facilitate viable therapy development [107–110]. As Nox-derived ROS are an important culprit in the free radical theory of aging, selective inhibition is anticipated to be a viable therapeutic strategy in age-related disease, and the roles of Nox in the aging process are expected to be explored in further depth in the coming years as a consequence of an increasingly aging population in the developed world.

ROLE OF ROS SIGNALLING IN PROTEINS INVOLVED IN AGING

$p66^{Shc}$ adaptor protein

$p66^{Shc}$ protein is one of the three isoforms encoded by the mammalian Shc locus. Shc proteins ($p66^{Shc}$, $p52^{Shc}$ and $p46^{Shc}$) are cytoplasmic signal transducers involved in the transmission of mitogenic signals from activated receptors to Ras proteins. Although the three splice variants carry similar structural domains, $p66^{Shc}$ contains an additional collagen-homology region in the N-terminus, allowing $p66^{Shc}$ to function in intracellular pathway(s) that regulate ROS metabolism and apoptosis [52,111]. Whereas activation with growth factors (such as epidermal growth factor receptor, EGF) induce tyrosine phosphorylation and Ras activation, environmental stress factors, such as H_2O_2 or UV irradiation, induce serine phosphorylation on $p66^{Shc}$ through protein kinase Cβ (PKCβ) [112] and JNK [113], causing increased apoptosis. Deletion of $p66^{Shc}$ protein was shown to reduce oxidative stress and high-fat-induced atherogenesis and increase cellular resistance to apoptosis as well as life expectancy by 30 % [52,53]. Since that study, several others have reported a link between the redox regulation of $p66^{Shc}$ protein and downstream signalling via $p53/p21^{cip}$ and FOXO3 to mechanisms that modulate longevity [54,55]. Increasing evidence supports that oxidative stress-induced serine phosphorylation of $p66^{Shc}$ leads to mitochondrial accumulation of $p66^{Shc}$, where it binds to cytochrome *c* via the CH2–PTB domain. This, in turn, leads to redox activation and the transfer of electrons to O_2, leading to generation of mitochondrial H_2O_2. Increased mitochondrial H_2O_2 results in opening of permeability transition pore (PTP) leading to increased swelling and apoptosis [112,114]. Pinton et al. [112] showed that oxidative-stress-mediated activation of PKCβ leads to phosphorylation of $p66^{Shc}$, which effects translocation of $p66^{Shc}$ to the mitochondrial membrane. This process is mediated by prolyl isomerase 1 (Pin1). Another study describing a mechanistic pathway underlying the apoptogenic effect of $p66^{Shc}$ suggested that UV-irradiation-induced dissociation of $p66^{Shc}$ and mitochondrial heat-shock protein (Hsp) 70 results in decreased membrane potential and increased trans-mitochondrial permeability, leading to apoptosis and decreased lifespan [115]. Many other studies strengthen the link between $p66^{Shc}$, mitochondrial oxidative damage and aging by providing evidence of phosphorylation of protein kinase B/Akt, up-regulation of p53-dependent apoptosis, inactivation of FOXO3a and suppression of MnSOD [54,55,116,117]. Most of those studies have linked mitochondrial ROS as a causal source of $p66^{shc}$-mediated oxidative damage. Only one previous study showed that NADPH oxidase-dependent superoxide production in macrophages of $p66^{Shc}$-knockout mice was attenuated by 40 % [118]. In that study, Tomilov et al. [118] demonstrated that the deficit in NADPH oxidase-dependent ROS production was independent of expression levels of Nox2 subunits (i.e. Rac1/2, $p40^{phox}$, $p47^{phox}$, $p67^{phox}$,

p22^{phox} and gp91^{phox}), but rather governed by reduced phosphorylation and membrane translocation of p47^{phox} (organizing subunit of Nox2 complex) in macrophages isolated from p66^{Shc}-knockout mice, as well as in p66^{Shc} siRNA-transfected RAW264.7 macrophages. The authors also observed decreased activation of PKCδ, Akt and extracellular-signal-regulated kinase (ERK) in the p66^{Shc} mutants, suggesting that a Shc-dependent defect in signalling via PKCδ, Akt and ERK activation contributed to the defect in p47^{phox} phosphorylation and Nox2-dependent superoxide production. With that said, these studies, taken together, suggest the potential for p66^{Shc}/Nox2 signalling pathways in oxidative-stress-mediated effects on inflammation, atherosclerosis and longevity.

p53/p21 pathway

The redox-sensitive transcription factor p53 functions as a longevity-associated gene due to its strong tumour-suppressor activity [119]. In unstressed mammalian cells, p53 has a short half-life and is maintained at low levels by ubiquitination catalysed by Mdm2 [120,121], COP1 (constitutively photomorphogenic 1) [122] and Pirh2 (p53-induced protein with a RING-H2 domain) [123]. The stability and activity of p53 are subject to diverse covalent post-translational modifications such as ubiquitination [124,125], phosphorylation [126], acetylation [127], NEDDylation [128], SUMOylation [129] and methylation [130]. Among these post-translational modifications, ROS have been implicated in the phosphorylation of p53 mediated through p38α MAPK (mitogen-activated protein kinase) [131,132] and Polo-like kinase 3 (Plk3) [133]. Moreover, p53 itself is redox-active due to the presence of cysteines that contain redox-sensitive thiol groups [134]. In human p53, two clusters of cysteines in the DNA-binding domain exist and these are essential for the specific binding of p53 to its consensus sequence for transcription of p53-dependent genes [135,136]. Importantly, the role of p53 in premature aging is evident from several mouse studies where persistent low-level activation and increased expression, by ROS signalling, telomere erosion or DNA damage, promotes senescence or irreversible cell cycle arrest [137–139]. In turn, vascular cell senescence is one proposed mechanism surrounding unstable atherosclerotic plaque progression [140]. The ability for p53 to control senescence is consistent with its function to restrain vascularized tumour development, but the precise role for maintenance of longevity requires further investigation [141]. Whereas ROS are demonstrated to promote p53 activation, p53 has also been shown to enhance ROS signalling, presumably via up-regulation of the p67^{phox} subunit as identified previously [142]. However, p53 is also known to participate in the induction of vascular cell apoptosis, which could enhance disease progression [34,143]. But, as p53 also promotes the expression of a number of antioxidant genes, it remains to be clearly demonstrated whether this could account for p53's ability to control oxidative stress and therefore dictate whether a cell becomes apoptotic or senescent [144,145]. Induction of senescence by p53 is associated with the regulation of p53-dependent genes which participate in cell cycle arrest. Of these, p21^{cip} was identified as the key factor governing the switch to a senescent phenotype. p21^{cip} or cyclin-dependent kinase inhibitor 1, functions to inhibit cell cycle progression by placing a block on the G_1- to S-phase transition of the cell cycle [146,147]. Therefore, activation of p53 by ROS and downstream up-regulation of p21^{cip} appears to play a key role in the adaptive response of the vasculature in disease. This is evident in a nutrient deprivation model *in vitro* where serum-starved human endothelial cells underwent p53/p21^{cip}-

dependent cell cycle arrest via Nox2-derived superoxide anion [148]. Furthermore, Nox1- and Nox4-derived ROS were demonstrated to play a role in lung fibroblast senescence via p53-dependent $p21^{cip}$ up-regulation following matricellular CCN1 exposure [149]. Moreover, Kodoma et al. [150] support these findings wherein Nox1- and Nox4-derived ROS play central roles in p53 activation, leading to premature fibroblast senescence. In that study, siRNA against Nox1 and/or Nox4 inhibited Ras-induced premature senescence. This was the first piece of evidence that suppression of selective Nox isoforms could be instrumental in the maintenance of vascular longevity. Finally, redox activation of $p53/p21^{cip}$ signalling leading to senescence is evident in murine models of hypertension [151,152], diabetes [153,154], atherosclerosis [155,156] and pulmonary hypertension [121,157].

Klotho

The *Klotho* gene was first identified by Kuro-o et al. [60] in 1997, as a putative anti-aging gene, since Klotho-null mice displayed phenotypes resembling premature human aging, including vascular calcification, pulmonary emphysema, skin atrophy, muscle atrophy, osteoporosis, arteriosclerosis and cognitive impairment, among others [60,158]. Several studies have linked Klotho's influences to intracellular signalling pathways involved in oxidative stress responses and aging via inhibition of IGF-1 [61,62,159] and $p53/p21^{cip}$ [160] and activation of FOXO3 [62]. Klotho is a transmembrane protein that functions as a FGF23 co-receptor with FGFR and is critical in maintaining proper calcium, phosphate and vitamin haemostasis [60]. Although the *Klotho* gene is mainly expressed in kidneys and the choroid plexus of the brain, the secreted soluble form may cause paracrine effects in distant organs. Recent studies show that Klotho exerts a protective effect against oxidative damage associated with age-related and retinal and neurodegenerative disorders, such as age-related macular degeneration (AMD) and Alzheimer's disease [161,162]. These studies suggest that binding of the secreted Klotho protein to the membrane results in phosphorylation and translocation of FOXO3a to the nucleus, leading to transcriptional activation of MnSOD and peroxiredoxin-2 (Prx2), and thereby increasing resistance to oxidative stress [62]. Klotho is also shown to be protective of arterial calcification by preventing differentiation of VSMCs to an osteoblast-like phenotype and by restoring FGF23 signalling [163,164]. Another study by Saito et al. [165] showed the involvement of ROS and iron metabolism in AngII-mediated down-regulation of renal Klotho expression. Although Klotho is not known to be expressed in blood vessels but is a circulating hormone, it reportedly protects against endothelial dysfunction in age-associated disorders through endothelium-derived NO production [166–169]. The role of Klotho in age-related CVD was initially based on the development of atherosclerosis in Klotho-deficient mice [60]. Since then it has been suggested that Klotho may have a protective role in the cardiovascular system through endothelium-derived NO production and regulation of angiogenesis [166–168]. Subsequent population-based studies in humans have identified a functional variant of Klotho, i.e. KL-VS, which is associated with the early-onset occult CAD [170] and cardiovascular risk factors such as high-density lipoprotein cholesterol, high blood pressure and stroke [171]. Although a number of studies show that Klotho protein is protective under oxidative stress, the precise mechanisms underlying these phenomena are not completely understood [172]. A study recently showed that the protective effect of Klotho against oxidative damage is a consequence of increased endogenous antioxidant capacity (increased SOD2 and

thioredoxin) via increased nuclear factor erythroid-derived 2-related factors 1 and 2 (Nrf1/2) transcriptional activity [173,174]. In a study, Wang et al. [175] showed that direct delivery of Klotho suppresses AngII-induced Nox2-derived superoxide anion production and oxidative damage and attenuates apoptosis via the cAMP/PKA pathway in rat aortic smooth muscle cells (RASMCs). This study suggests the potential cross-talk between Klotho and Nox2 signalling pathways in oxidative-stress-mediated effects on endothelial dysfunction, hypertension, atherosclerosis and lifespan.

IGF-1 pathway

The *C. elegans* homologue of IGF-1, *daf-2,* was one the early genes to be identified as a longevity-related gene [67]. IGF-1 is synthesized by almost all tissues and is involved in cell growth, differentiation and transformation processes. It is also well known that IGF-1 exerts pleiotropic effects by virtue of its binding to both IGF-1 receptor (IGF-1R) and insulin–IGF-1 hybrid receptors [176]. Several studies have shown that oxidative stress such as that caused by H_2O_2 and AngII stimulates IGF-1 and IGF-1R, which is mediated by a tyrosine kinase-dependent redox-sensitive mechanism [177,178]. A number of studies in *C. elegans*, since the discovery of IGF-1 as a longevity-related gene, have shown that attenuation of IGF-1 signalling increases lifespan. This involves cross-talk with several other genes/pathway(s) implicated in aging, such as FOXO transcription factor (*daf-16*) [179], heat-shock transcription factor (HSF)-1 [180], SKN-1, an Nrf-like xenobiotic factor [64] and mTOR pathway. These transcription factors, in turn, modulate a diverse set of genes that act cumulatively to produce large beneficial effects on the lifespan of *C. elegans*. Indeed, mutations known to impair IGF-1R in several centenarian human cohorts strengthen the case that insulin/IGF-1 signalling is negatively associated with lifespan [181,182]. On the other hand, studies in rodents and humans have raised reservations regarding the role IGF-1 plays in aging. Studies show that mice deficient in IGF-1 die shortly after birth, whereas some that live to adulthood have retarded growth [183]. In humans, an age-related decline in endocrine IGF-1 levels is reported, and a large body of evidence suggests that IGF-1 improves cardiovascular function and is atheroprotective [184–187]. IGF-1 overexpression in the heart prevented myocardial cell death after infarction and reduced ventricular dilation, hypertrophy and diabetic cardiomyopathy via attenuation of p53 activation and AngII production, thereby leading to reduced ROS and cellular damage [188–191]. Another study showed that anti-atherogenic effects of IGF-1 infusion in the pro-atherogenic ApoE-knockout mouse model occur via suppression of oxidative stress and up-regulation of vascular eNOS expression leading to increased NO bioavailability [187]. Csiszar et al. [192] reported that in Ames dwarf long-lived mice, low IGF-1 serum concentrations were associated with high oxidative stress in the vasculature and reduced eNOS expression, leading to endothelial dysfunction. More importantly, exogenous IGF-1 enhanced expression of antioxidant enzymes such as MnSOD, Cu/ZnSOD, Gpx1 in explants of mouse aortas and human coronary arterial endothelial cells [192]. In a study, Bailey-Downs et al. [193] showed that IGF-1-deficient mice were also lacking in Nrf-2 and Nrf-2-driven antioxidant genes, NAD(P)H:quinone oxidoreductase1 (NQO1) and heme oxygenase-1 (HMOX-1), thereby impairing the ability of vascular cells to respond to oxidative stress caused by high glucose, oxidized LDL (low-density lipoprotein) and/or H_2O_2. Endocrine IGF-1 deficiency in the vasculature promotes adverse vascular phenotype under disease states and results in

accelerated vascular impairments in aging [193]. Altogether, these studies suggest that IGF-1 has antioxidant-like effects.

Thus, it appears important to note that opposing effects of IGF-1 signalling are at work in different phases of the human life cycle. This disparity in IGF-1-mediated effects is probably a result of the difference between autocrine and paracrine effects of IGF-1. Interestingly, Li et al. [194] used the LID (liver IGF-1-deficiency) mouse model to examine the mechanism involved in IGF-1-deficiency-induced effect on cardiac aging. They showed that severe IGF-1 deficiency reduced aging-associated cardiac contractile dysfunction, prolonged relaxation and ablated intracellular Ca^{2+} dysfunction. Moreover, the IGF-1-deficiency-elicited effects on cardiac aging were correlated with antagonism against aging-induced reduction in Akt, AMPK phosphorylation, Klotho and up-regulated p53 [194]. A similar study involving the response to a challenge with pro-oxidant paraquat in LID mice demonstrated that IGF-1 deficiency effectively enhanced resistance to oxidative-stress-induced cardiac dysfunction, attenuated ROS and carbonyl production, ultimately resulting in improved survival rate against paraquat-induced mortality [195]. In experimental aortic abdominal constriction (AAC) studies in mice, liver-specific IGF-1 deficiency mitigated AAC-induced cardiac hypertrophy and contractile changes via attenuating AAC-induced Akt phosphorylation and glucose transporter 4 (GLUT4) up-regulation and rescuing down-regulated *miR-1* and *miR-133a* levels [196]. Therefore, whether reduced IGF-1 levels are negatively or positively correlated to age-associated decline in cardiovascular physiological functions is yet to be fully elucidated.

NOX IN AGING

Progressive anatomical, mechanical and biochemical changes occur in the heart as an adaptive response with increasing age. Aging is accompanied by an increase in arterial systolic pressure, reduced or maintained diastolic pressure, increased pulse pressure, aortic dilatation and wall thickening [197]. However, one of the major caveats in the study of aging and its effect on CVDs is that multifactorial changes that occur during the aging process are indistinguishable from vascular phenotypes such as endothelial dysfunction, increased collagen deposition, fibrosis and vessel remodelling presented in CVDs. In one of the early studies carried out in individuals who died from causes other than CVDs, it was reported that aging human hearts are characterized by an increase in the volume fraction of myocytes, myocyte loss, cellular hypertrophy and reduction in the ventricular mass. These cellular processes represent underlying causes for the onset of myocardial dysfunction and failure in the aging population [198]. Wang et al. [199] examined the involvement of Nox in age-associated cardiac remodelling in a rodent model of aging and found that age-dependent increases in blood pressure, cardiomyocyte hypertrophy, coronary artery remodelling and cardiac fibrosis were associated with increased myocardial Nox2 activity. The increase in Nox2-dependent ROS were accompanied by increased renin–angiotensin–aldosterone system (RAAS) activation in the myocardium, increased expression of connective tissue growth factor (CTGF) and TGFβ1 and a significant activation of matrix metalloproteinase (MMP)-2 and membrane type-1 (MT1)-MMP, leading to age-associated cardiac remodelling [199]. It is widely acknowledged that Nox4 is localized to the mitochondria of many different cell types and that mitochondrial oxidative stress promotes aging. In this regard,

Ago et al. [200] reviewed the role of mitochondrial Nox4 in the aging process of the heart. It was speculated that oxidant stress in mitochondria builds when antioxidants are unable to counteract the ROS produced by Nox4, triggering the aging process of the heart. It is tempting, therefore, to speculate that Nox4 may be the initiator of mitochondrial 'stress' and oxidant production or vice versa. However, Nox4 has been identified to be beneficial against disease-induced cardiac dysfunction and hypertrophy and is therefore assumed to be 'protective' [44]. Whatever the relationship, it appears that these two very important sources of ROS could be linear in their role. Taken together, these studies suggest that NADPH oxidase-derived ROS play a pivotal role in the process of aging and age-related cardiovascular changes, whether these are in conjunction with or independent of the mitochondrion. However, despite their importance in vascular function and disease, very few studies provide mechanistic insight into the role of Nox in the aging process. Figure 2 depicts the proposed effects of Nox-derived ROS on redox-sensitive longevity signalling in the vessel wall.

NOX IN AGE-ASSOCIATED CVD

Atherosclerosis

Age is an important independent risk factor for the development of atherosclerosis, which persists even when other risk factors such as hypertension, diabetes, smoking and LDL cholesterol levels are controlled. This may be attributable to characteristic features of aging vessels, including reduced medial VSMC number, increased collagen deposition and breakdown of the elastic lamellae, which collectively can lead to medial thickening and vessel remodelling. These changes are likely to further promote increased extracellular matrix and vascular stiffness, subsequently leading to systolic hypertension. Adhesion and recruitment of pro-inflammatory molecules, also a result of luminal and lamellae damage, logically result in lipid accumulation and foam cell formation following monocyte infiltration [201–204]. As a consequence, the effects of atherosclerosis are superimposed on normal aging of the underlying vessel and therefore it is of utmost importance to unravel the molecular mechanisms involved. Towards that end, it is now generally agreed that elevated vascular ROS triggers an atherosclerotic process by oxidatively modifying lipoproteins causing activation of pro-inflammatory signalling and endothelial dysfunction resulting in further elevated oxidative stress. Pro-oxidant stimuli further result in reduced cell proliferation, irreversible growth arrest and apoptosis, elevated DNA damage, epigenetic modifications and telomere shortening, all of which are distinguishing features of cellular senescence as well as accelerated vascular aging associated with atherosclerosis [205]. Many of these cellular events are modulated by ROS-mediated signal transduction catalysed by Nox [74,206]. Because all cells comprising the lining and vessel wall harbour one or the other isoform of Nox, singularly or collectively they provide a mechanism of localized or paracrine release of ROS, which can influence redox-sensitive signalling pathways with fundamental effects on atherogenesis. One of the first points of evidence for NADPH oxidase-derived superoxide anion as a clinical risk factor for atherosclerosis was reported by Guzik et al. [71]. This study, based on clinical samples, showed that NADPH oxidase is the main source of superoxide production in human saphenous veins, which is associated with impaired NO-mediated endothelial function and increased atherosclerotic risk factors. Two

other studies identified the cellular sources of intracellular superoxide anion production within atherosclerotic and non-atherosclerotic human coronary arteries and found increased expression of p22^{phox}, Nox2 (gp91^{phox}) and Nox4 in the shoulder regions of the plaque, whereas expression of Nox1 was reduced [72,78,207]. Interestingly, Sorescu et al. [78] noted a strong association of Nox2 to plaque macrophage content and Nox4 to SMCs and advanced fibrocellular plaques, but not in more advanced atherosclerotic lesions [78]. More importantly, intense ROS in the plaque regions corresponded to increased expression of p22^{phox} and Nox2, implicating NADPH oxidase-derived ROS in plaque rupture [78]. Further, Barry-Lane et al. [79] demonstrated the role of p47^{phox} subunit in the development of atherosclerosis and showed that p47$^{phox-/-}$/ApoE$^{-/-}$ mice have significantly lower aortic superoxide production, decreased proliferative response to growth factors and lesion formation compared with ApoE$^{-/-}$ mice. A later study by the same group showed that the atheroprotective effect of p47^{phox} deficiency in ApoE-null mice was a result of a decrease in Nox2-derived ROS from monocytes/macrophages that reduced levels of oxidized LDL as well as from that of vessel wall cells, which attenuated the expression of cellular adhesion molecules [208]. Several studies further strengthen the involvement of Nox2 in atherogenesis. Judkins et al. [209] demonstrated that Nox2 expression was up-regulated in the aortic endothelium of ApoE$^{-/-}$ mice, before the appearance of lesions. They also showed that the absence of Nox2 in Nox2$^{-/y}$/ApoE$^{-/-}$ mice was manifested by a reduced lesion area in the descending aorta by 50 % and was associated with decreased aortic ROS and increased NO bioavailability [209]. These studies provide a strong rationale for Nox-dependent ROS as potential players contributing to premature aging of the vasculature via propagation of atherosclerosis.

Previously, it was reported that an endothelium-specific increase in Nox2-derived superoxide anion production is sufficient to increase macrophage recruitment and endothelial cell activation, leading to an increased atherosclerotic plaque initiation. However, this early event does not alter the progression of atherosclerosis [210]. Sheehan et al. [80] investigated the role of Nox1-smooth muscle cell component in atherogenesis and demonstrated that deletion of Nox1 reduced diet-induced lesion formation by 28 %, decreased macrophage infiltration and ROS levels compared with ApoE$^{-/-}$ mice. They showed that loss of Nox1 attenuated cell proliferation and increased collagen content within the neointima following carotid ligation. A greater reduction in lesion formation in ApoE/Nox2 double null mice observed in previous studies compared with Nox1$^{-/y}$/ApoE$^{-/-}$ was explained by the absence of Nox1 in circulating cells [80]. These studies linked the increased expression of NADPH oxidase and intracellular superoxide anion production to oxidative modification of LDL and plaque instability via superoxide anion-induced expression of matrix-degrading proteases, such as MMP-2 and MMP-9 as well as associated SMCs and endothelial dysfunction. Additional evidence comes from a study showing the involvement of Nox1-derived ROS in diabetes mellitus-induced atherosclerosis. That study demonstrated that deletion of Nox1 reduced the development of aortic atherosclerosis in the diabetic ApoE$^{-/-}$ mice. This decrease in atherosclerosis was associated with reduced ROS formation, attenuation of chemokine expression, vascular adhesion of leucocytes, macrophage infiltration and reduced expression of pro-inflammatory and profibrotic markers [211].

Not much is known regarding the role of Nox5 in atherosclerosis, primarily because of the limitation posed by Nox5's absence in rats and mice. However, Guzik et al. [81] showed that expression of Nox5 is dramatically increased in atherosclerotic human coronary artery vessel wall which was associated with a 7-fold increase in calcium-dependent NADPH oxidase activity. Interestingly, Nox5 was localized to the endothelial cells in the early lesions and in the smooth muscle layer in advanced lesions [81]. Supporting evidence comes from a study showing that endothelial Nox5 is activated by AngII and ET-1 through Ca^{2+}/calmodulin-dependent and Rac-1-independent mechanisms, leading to increased ROS generation and ERK1/2-regulated growth and inflammatory signal transduction associated with endothelial dysfunction and vascular pathologies [212]. Nevertheless, the affected signal transduction pathways intertwining vascular aging and atherogenesis are poorly understood and are largely derived from studies on cellular senescence. ROS-mediated oxidative DNA damage is known to promote cellular senescence, which occurs both in the mitochondrial DNA and in the nuclear DNA, as well in both telomeric and non-telomeric regions of VSMC and macrophages in plaques [73,213]. Other than directly causing DNA damage, ROS-induced activation of p53 has been shown to promote DDR (DNA damage recognition and repair) and reduce the expression of the IGF-1R in VSMCs as well as the subsequent Akt survival signals [214]. Since both p53 and IGF-1 have been associated with longevity, signal transduction by these molecules might be responsible for NADPH oxidase-mediated damage in atherosclerosis.

Hypertension

Hypertension is one of the most common diseases in the elderly. The prevalence of hypertension increases markedly with advancing age, primarily a result of elevations in blood pressure attributed to alterations in the structure, function and rigidity of vessel wall [215], as well as from a shift in control by the autonomic nervous system [216]. Several studies have shown that aging and hypertension are associated with the impairment of endothelium-dependent vascular relaxation and endothelial dysfunction. Both are known to increase the risk of cardiovascular and cerebrovascular diseases [71,75,217,218]. Oxidative stress is well established as a key regulator of age-induced endothelial dysfunction and RAAS. These influence the sympathetic and parasympathetic arms of the autonomic nervous system and promote hypertension. Moreover, it is well known that RAAS activation may itself potentiate oxidative stress [29,70]. Several clinical and animal model studies of hypertension have shown that increased NADPH oxidase-derived ROS is associated with endothelial dysfunction [76,102,103,105]. Zalba et al. [102] showed that endothelium-dependent relaxation in response to acetylcholine was significantly attenuated in 30-week-old compared with 16-week-old hypertensive rats. This impaired NO-dependent relaxation in AngII-mediated hypertension was linked to increased NADPH oxidase activity and expression of $p22^{phox}$ mRNA [102]. Another study of aging in a spontaneously hypertensive rat (SHR) model showed that RAAS attenuation *in vivo* by temocaprilat, hydralazine and olmesartan reversed age-related advanced cardiac hypertrophy due to suppression of cardiac oxidative stress by attenuating the expression of Nox2 oxidase components, $p22^{phox}$, $p47^{phox}$ and Nox2 [219]. Another study demonstrated the role of MAPK-activated protein kinase 2 (MK2) in AngII-induced hypertension. Ebrahimian et al. [82] showed that MK2, which is a direct downstream target of p38 MAPK, mediates an AngII-induced increase in

Nox2-dependent superoxide production and activation of pro-inflammatory molecules through up-regulation of the p47^{phox} subunit of NADPH oxidase and suppressed antioxidant enzyme catalase. The AngII-mediated up-regulation of MK2 resulted in a vicious cycle that potentiated inflammatory responses to AngII and led to increased VSMC proliferation [82]. Other than Nox2, several studies have demonstrated a role for Nox1 in systemic hypertension. Using transgenic mice overexpressing Nox1 in the smooth muscle cells, Dikalova et al. [220] provided a causal link between Nox1-derived superoxide production and increased systolic blood pressure and aortic hypertrophy in response to AngII. Matsuno et al. [83] generated Nox1$^{-/y}$ mice and showed that Nox1 in AngII-mediated hypertension underlies the pressor response. Similar studies showed that lack of Nox1 attenuated AngII-induced ROS generation and Ca^{2+} signalling in VSMCs and Nox1-derived ROS regulated cell-surface expression of AT1R (AngII type 1 receptor) through mechanisms involving caveolin phosphorylation [221]. Although all the above studies suggest that hypertension, NADPH oxidase-derived ROS and oxidative stress are linked, none of them specifically examine the so-named longevity genes in hypertension. Wang and Sun [222] studied the role of Klotho in the pathogenesis of hypertension. In this study, they showed that *Klotho* gene delivery decreased aortic Nox2 expression and NADPH oxidase activity in SHRs, whereas renal expression of NOS, Nox1 and Nox4 remained unaltered. Further mechanistic studies delving into signalling that confer specificity of Nox inhibition by Klotho in systemic hypertension are warranted. Similarly, the role of Nox in redox signalling with age-related hypertension-linked vessel remodelling remains to be investigated.

One of the common forms of hypertension that is increasingly being diagnosed in elderly population is cor pulmonale or pulmonary arterial hypertension (PAH) [223–225]. PAH is a haemodynamic and pathophysiological condition defined by an increase in mean pulmonary artery pressure (mPAP) ≥25 mmHg at rest. It is the consequence of an increasing pulmonary vascular resistance (PVR) that leads to right ventricular (RV) overload, hypertrophy, reduction in cardiac output and eventually RV failure and death [226]. It is characterized by an intense and characteristic remodelling of small pulmonary arteries, due to structural and functional changes in endothelial, smooth muscle and adventitial fibroblast layers of the vessel wall [227]. One of the key environmental factors that cause extensive irreversible medial and adventitial thickening of vessel wall and muscularization of previously non-muscularized distal vessels is chronic hypoxia (CH). Several studies have implicated ROS in acute hypoxic vasoconstriction and vascular remodelling associated with CH and a pivotal role of NADPH oxidase-derived ROS in this process is now well established. In one of the initial studies, Liu et al. [84] showed that CH-induced increase in superoxide anion production was attenuated in Nox2-null mice. This was associated with a decrease in pathological changes seen in CH-induced vascular remodelling such as mean RV pressure, medial wall thickening of small pulmonary arteries and right heart hypertrophy. They also linked increased ROS production to increased p47^{phox} expression and activity, rather than increased mRNA expression levels of NADPH oxidase subunits, Nox2, p22^{phox}, p40^{phox}, Rac1 and Rac2 [84]. Later studies demonstrated that in the lung vasculature, Nox4-derived ROS play a crucial role in mediating PASMC and fibroblast proliferation-associated vascular remodelling in PAH. This was linked to increased Nox4 mRNA and protein expression [85,228–230]. It was suggested that the difference in localization of Nox2 and Nox4, to the

endothelial and smooth muscle cell layers respectively, is responsible for the differential role of Nox2 and Nox4 in hypoxia-induced pulmonary hypertension. Although it is known that RV function is the critical determinant of lifespan in patients with PAH, not much is known about the role Nox isoforms play in causing RV failure independent of an elevated pulmonary artery pressure. Using a pulmonary artery banding (PAB) model in mice, which is a surgical model of chronic progressive RV pressure overload not associated with structural alterations of the lung circulation, we recently showed PAB results in an increase in Nox4-derived ROS production, which is responsible for cardiac dysfunction and RV failure. We also showed that expression of antioxidant enzymes, SOD, catalase and Gpx did not change [45]. All of these studies elegantly link Nox-derived ROS to structural changes associated with PAH and subsequent RV failure, comparative studies in young and old animals are necessary to understand how these processes are modulated in aging heart.

Atrial fibrillation

AF is the most common type of cardiac arrhythmia. AF refers to abnormal rhythm of the heartbeat caused by perturbed electrophysiology of the myocardium. The incidence of AF increases quite dramatically with age (1.5 % at 50–59 years to 23.5 % at 80–89 years) [231] and even more so with prior history of cardiac surgery and cardiopulmonary diseases [232–235]. A number of studies have shown that oxidative stress plays a vital role in the pathogenesis of AF [236–239]. It is also noteworthy that oxidative stress is also involved in the pathogenesis of several predisposing factors and conditions associated with AF. These include hypertension, CHD (chronic heart disease), obesity and diabetes, among many others. There is a large body of evidence showing the role that the NADPH oxidase family of enzymes plays in the development of AF. Both myocardial Nox2 and myocardial Nox4 have been shown to participate in ROS-mediated effects in AF [38,39,86,240,241]. Initial studies in animal models suggested a Rac1-dependent effect of Nox-derived ROS leading to AF [86,242], which were supported by a number of clinical studies showing a correlation among Nox2 up-regulation, oxidative stress and AF [38,39,243]. Kim et al. [38] suggested that NADPH oxidase-derived ROS play an important role in the atrial oxidative injury associated with AF. In this study, they reported up-regulation of NADPH oxidase subunits $p22^{phox}$, $p47^{phox}$ $p67^{phox}$ and Nox2, but not of Nox1 or Nox4, in tissue homogenates and isolated atrial myocytes from the right atrial appendage of patients undergoing cardiac surgery [38]. Importantly, Reilly et al. [241] suggested that an initial insult caused by Nox activation probably accounts for early development of AF, whereas ROS derived from mitochondria and uncoupled eNOS are involved in long-term AF. The change in the sources of ROS with atrial remodelling therefore could explain why statins are effective in the primary prevention of AF, but not in its management [241]. *In vitro* studies using HL-1 atrial myocytes have implicated Nox2 and Nox4 in TGFβ1-mediated myofibril degradation via calpain activation leading to calcium overload. Collectively, these were shown to elicit electrical and structural remodelling of cardiac tissue in AF [240,244]. Although these studies suggest a causal link between oxidative stress, the role of Nox and AF, the downstream signalling cascade by which atrial structural remodelling contributes to the pathogenesis of AF has not yet been described. A study by Adam et al. [245] demonstrated a marked up-regulation of CTGF, which is associated with increased fibrosis and tissue AngII concentration. With regard to an underlying mechanism, this study suggested that AngII up-

regulated CTGF via activation of Rac1 and NADPH oxidase, leading to up-regulation of N-cadherin and connexin43 (Cx43) and therefore contributing to structural remodelling associated with AF [245].

Nox therapeutics in treatment of CVDs

From the aforementioned studies, it is apparent that NADPH oxidase-derived ROS play a pivotal role in age-associated cardiovascular changes and CVDs. Therefore, Nox could be important drug targets for the treatment of atherosclerosis, hypertension, stroke, AF, diabetes and many other diseases of the elderly. The role of Nox as drug targets in CVDs has been extensively reviewed [246,247]. Despite a large armamentarium of drugs currently approved for specific therapy such as AngII receptor antagonists (azilsartan and benicar), renin inhibitor (aliskiren), calcium channel blockers (amlodipine), synthetic prostacyclin and its analogues (epoprostenol, treprostinil, beraprost, iloprost and selexipag), ET-1 receptor antagonists (bosentan, ambrisentan and macinentan) and type 5 phosphodiesterase (PDE5) inhibitors (sildenafil, tadalafil, vardenafil and riociguat) for PAH, blood thinners (aspirin, warfarin, dabigatran, rivaroxaban and apixaban) and dofetilide and dronedarone for AF, CVDs still contribute to a high level of mortality in the elderly. The beneficial effects of these drugs may, in part, come from their ability to reduce oxidative stress, either directly via attenuating the NADPH oxidase-derived ROS or indirectly by improving NO bioavailability [248,249]. Although antioxidants such as vitamins A, E and C, polyphenols and flavonoids have been used as therapeutic agents with some success to reduce oxidant stress and blood pressure [250], most of the large clinical trials have failed to demonstrate a beneficial cardiovascular effect [251,252]. Compared with the antioxidants, which degrade free radical intermediates and prevent further oxidation of molecules, inhibitors of Nox isoforms decrease the rate of enzymatic ROS formation. These inhibitors either modify Nox expression or target assembly and activation of Nox complexes, thereby reducing the immediate oxidant environment and suppressing ROS-induced signalling cascades.

Although some classical Nox inhibitors (AEBSF, apocynin, PR-39 and S17834) and two commonly used drugs, HMG-CoA reductase inhibitors (statins) and angiotensin receptor antagonists [ARBs (angiotensin II receptor blockers) and ACE (angiotensin-converting enzyme) inhibitors], are regularly being used in animal models and treatment therapy respectively, most of the currently available Nox inhibitors including the above-mentioned are not isoform-specific and may potentially affect both physiological and pathophysiological signalling pathways. Therefore, the development of selective and isoform-specific Nox inhibitors that preferably target only pathological Nox signalling has become a foremost objective for both academia and pharmaceutical companies. A number of different small-molecule inhibitors have become available that could become useful in their own right or by way of their derivatives [253–263]. Nox1/4 inhibitor GKT 136901 was reported to inhibit NADPH oxidase-dependent aortic ROS formation, attenuate CD44- and HA-dependent inflammatory cell recruitment and aortic lesion area in atherosclerotic lesions of $ApoE^{-/-}$ mice [264]. Similarly, GKT137831 inhibited ROS production and attenuated diabetes-induced macrophage infiltration, inflammation and fibrosis associated with atherosclerotic plaque formation in $ApoE^{-/-}$ mice as well as attenuated hypoxia-induced H_2O_2 release and improved indices of ventricular remodelling and pulmonary artery wall

thickening in a mouse hypoxia model of PAH [211,265]. Like the first-generation GKT inhibitor, issues of Nox specificity are still a concern with regard to untoward and off-target effects of these drugs. Novel approaches and rational drug design strategies have yielded isoform selectivity for Nox2 and been discussed in greater detail [107,108,247,258,266]. Assuredly with these innovative approaches, new isoform-specific Nox therapeutics are expected to gain greater traction. That said, peptidic inhibitors still appear to offer the greatest level of Nox selectivity [267–270] and have exhibited high levels of potency in cell-free and whole-cell assays. For this reason, they have been widely employed in animal studies. Admittedly, an inherent concern in the pharmaceutical industry regarding oral bioavailability of these potential therapeutics has stunted development, in spite of the potential for alternative modes of delivery and stabilization to increase clinical application [107,271].

CONCLUSIONS AND PERSPECTIVES

A higher level of care, treatment options and better socioeconomic conditions have resulted in a dramatic increase in the quality and lifespan of the elderly population with cardiovascular complications over the last half-century. Since the free radical theory of aging was first proposed, tremendous progress has been made to identify and understand the molecular mechanisms involved in aging and age-related CVDs. A seminal role of Nox in CVD is quite evident. Despite these advances, manifestation of combined aetiologies (co-morbidities) together with age-related changes further complicates our understanding of the pathophysiological and pathobiological factors contributing to decline in the older patient. Indeed, much more work needs to be done to determine whether there is a unified effect of ROS on longevity and CVD. That is, whether specific Nox isoforms are responsible for modulating CVD and the biology of the aging process and whether these are both modulated by a common gene or set of longevity genes are yet to be fully appreciated. Furthermore, with Nox-targeted therapies showing promising results in animal models and pre-clinical trials, it is possible that, in the near future, we may be able to adapt these interventions to further improving cardiovascular health and/or slow the aging process.

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Abbreviations

AAC	aortic abdominal constriction
AEBSF	4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride
AF	atrial fibrillation
AKT	synonym for Protein kinase B

AngII	angiotensin II
ApoE	apolipoprotein E
CAD	coronary artery disease
CCN1 or CYR61	cysteine-rich angiogenic inducer 61
FGF	fibroblast growth factor
CD44	cluster of differentiation 44
CH	chronic hypoxia
CTGF	connective tissue growth factor
CVD	cardiovascular disease
DUOX	dual oxidase
eNOS	endothelial nitric oxide synthase
ERK	extracellular-signal-regulated kinase
ET-1	endothelin-1
FGF-R	fibroblast growth factor receptor
FOXO3	forkhead-box protein O3
GKT	GenKyotex
Gpx	glutathione peroxidase
HA	hyaluronic acid
HMG-coA	3-hydroxy-3-methyl-glutaryl-coenzyme A
IGF-1	insulin-like growth factor
IGF-1R	IGF-1 receptor
JNK	c-Jun N-terminal kinases
KL-VS	variant of Klotho
LDL	low-density lipoprotein
LID	liver IGF-1-deficiency
MAPK	mitogen-activated protein kinase
Mdm2	mouse double minute 2 homolog
MK2	mitogen-activated protein kinase-activated protein kinase 2
MMP	matrix metalloproteinase
mTOR	mammalian target of rapamycin
NOS	nitric oxide synthase
Nox	NADPH oxidase(s)

Nrf	nuclear factor erythroid-derived 2-related factor
PAB	pulmonary artery banding
PAH	pulmonary arterial hypertension
PASMC	pulmonary artery smooth muscle cell
Pin1	prolyl isomerase 1
PKA	protein kinase A
PKC	protein kinase C
PKCδ	Protein Kinase C delta
Poldip2	polymerase (DNA-directed), delta interacting protein 2
Shc	src homology 2 domain containing
PR-39	proline-arginine (PR)-rich antibacterial peptide
PTB	phospho tyrosine binding
RAAS	renin–angiotensin–aldosterone system
Ras	Rat sarcoma
RING	Really Interesting New Gene
ROS	reactive oxygen species
RV	right ventricular
S17834	1,4-dimethyl-2,3,5,6-tetraiodobenzene
SHR	spontaneously hypertensive rats
Sir2	silent information regulator 2
SKN-1	protein skinhead 1
SMC	smooth muscle cell
SOD	superoxide dismutase
TGFβ	transforming growth factor β
Trx2	Thioredoxin2
VSMC	vascular smooth muscle cell

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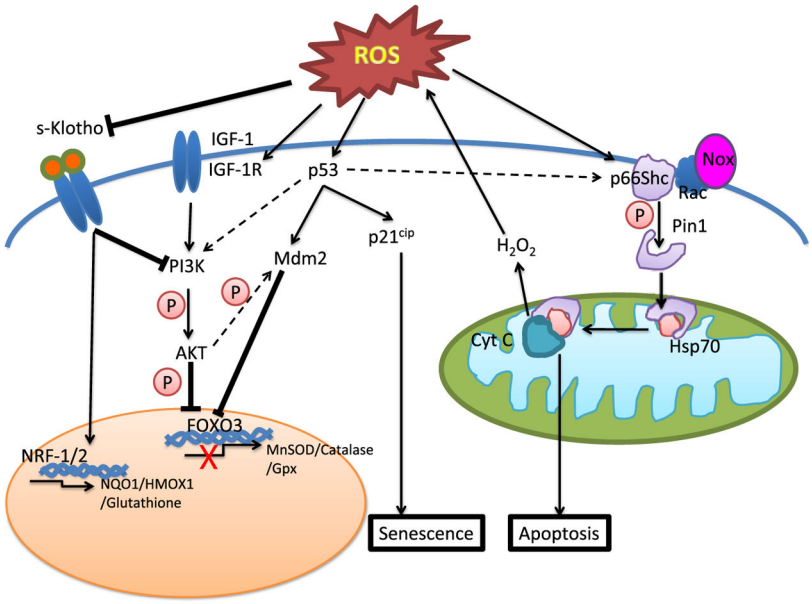

Figure 1. Prominent redox-sensitive signalling pathways involved in aging

Schematic representation of known redox-sensitive signalling pathways which regulate cellular aging and age-related CVDs. Arrows denote positive activation of downstream targets. Blunt-ended arrow denotes inhibitory effects. Dashed arrow denotes cross-talk between pathways. Secreted Klotho prolongs lifespan by increasing the expression of antioxidant genes via Nrf-1/2 or by inhibiting IGF-1 signalling. Binding of growth hormones, such as IGF-1 to their respective receptors or oxidative stress leads to autophosphorylation of *β*-subunits on the IGF-1R, leading to subsequent activation of PI3K/Akt pathway. Akt-mediated phosphorylation of FOXO3 results in nuclear exclusion and inhibition of FOXO3-mediated transcription of target antioxidants, such as MnSOD and Gpx. Oxidative stress induces phosphorylation and translocation of $p66^{Shc}$ via PKC*β* and Pin1 to the mitochondrial inner membrane leading to cytochrome *c*-mediated apoptosis. ROS also causes post-translational modification of p53 resulting in its activation triggering $p21^{cip}$-mediated cell senescence. Akt activation or p53-induced transcriptional activation of Mdm2 leads to ubiquitination-dependent degradation of FOXO3. Abbreviations: PI3K, phosphoinositide 3-kinase; s-Klotho, secreted Klotho.

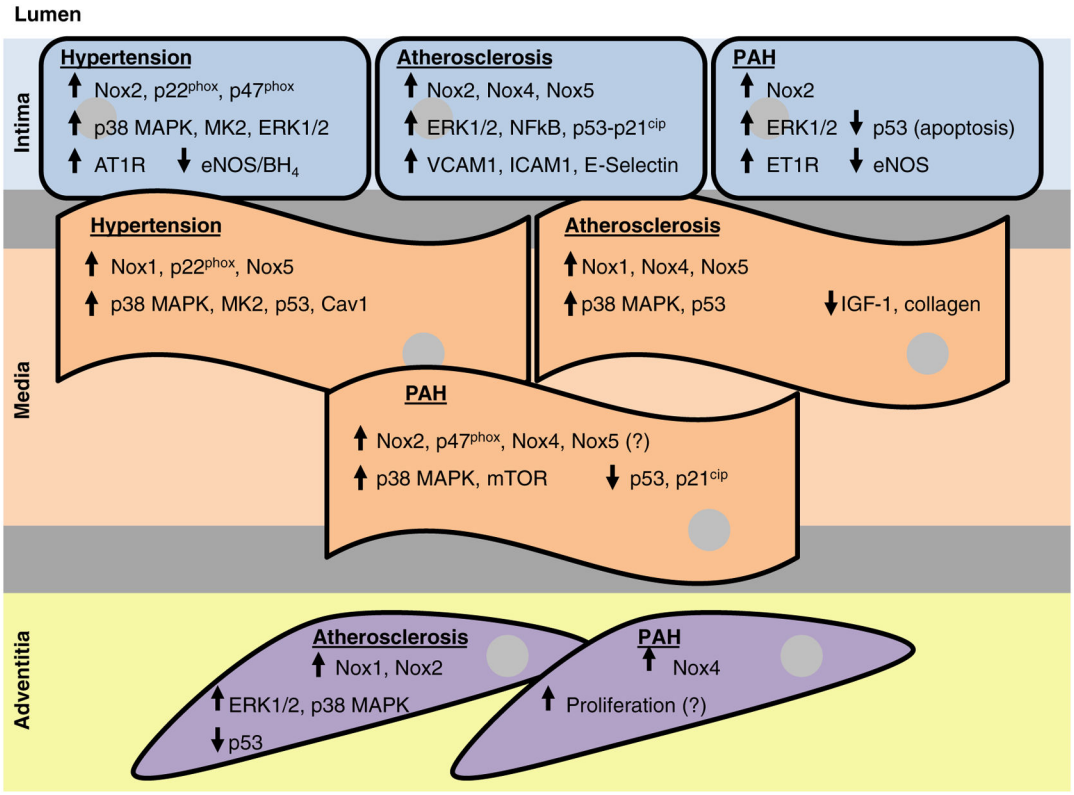

Figure 2. Effects of Nox-derived ROS on redox-sensitive longevity signalling in the vessel wall

Summary of the effects of hypertension, atherosclerosis and PAH on age-related redox-sensitive signalling pathways linked to the different vascular cells comprising the vessel wall (intima: endothelial cells; media: VSMCs; adventitia: fibroblasts/myofibroblasts) as a consequence of Nox-derived ROS. Arrows depict either increased or decreased protein expression or signalling pathway activation. A question mark (?) depicts a potential effect wherein more conclusive evidence is required to fully support the observations in the literature.