Isoform-selective NADPH oxidase inhibitor panel for pharmacological target validation

NOX Inhibitors: From Bench to Naxibs to Bedside

Mahmoud H. Elbatreek, Hermann Mucke, and Harald H. H. W. Schmidt

Contents

M. H. Elbatreek (✉)
Department of Pharmacology and Personalised Medicine, School of MeHNS, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt
e-mail: melbatreek@ppmlab.net

H. Mucke
H. M. Pharma Consultancy, Vienna, Austria

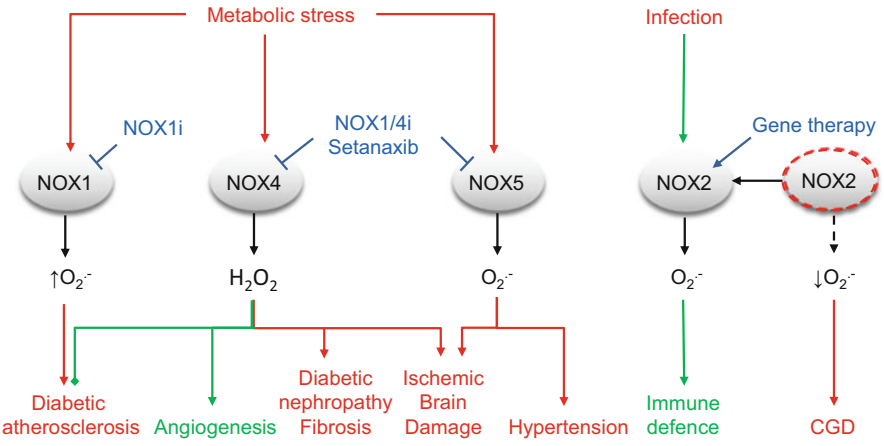
H. H. H. W. Schmidt
Department of Pharmacology and Personalised Medicine, School of MeHNS, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands

Handbook of Experimental Pharmacology, https://doi.org/10.1007/164_2020_387

Abstract

Reactive oxygen species (ROS) are ubiquitous metabolic products and important cellular signaling molecules that contribute to several biological functions. Pathophysiology arises when ROS are generated either in excess or in cell types or subcellular locations that normally do not produce ROS or when non-physiological types of ROS (e.g., superoxide instead of hydrogen peroxide) are formed. In the latter scenario, antioxidants were considered as the apparent remedy but, clinically, have consistently failed and even sometimes induced harm. The obvious reason for that is the non-selective ROS scavenging effects of antioxidants which interfere with both qualities of ROS, physiological and pathological. Therefore, it is essential to overcome this "antidote or neutralizer" strategy. We here review the most promising alternative approach by identifying the disease-relevant enzymatic sources of ROS, target these selectively, but leave physiological ROS signaling through other sources intact. Among all ROS sources, NADPH oxidases (NOX1-5 and DUOX1-2) stand out as their sole function is to produce ROS, whereas most other enzymatic sources only produce ROS as a by-product or upon biochemical uncoupling or damage. This qualifies NOXs as the main potential drug-target candidates in diseases associated with dysfunction in ROS signaling. As a reflection of this, the development of several NOX inhibitors has taken place. Recently, the WHO approved a new stem, "naxib," which refers to *NA*DPH o*x*idase inh*ib*itors, and thereby recognized NOX inhibitors as a new therapeutic class. This has been announced while clinical trials with the first-in-class compound, setanaxib (initially known as GKT137831) had been initiated. We also review the differences between the seven NOX family members in terms of structure and function in health and disease and then focus on the most advanced NOX inhibitors with an exclusive focus on clinically relevant validations and applications.

Graphical Abstract



Therapeutically relevant NADPH oxidase isoforms type 1, 2, 4, and 5 (NOX1, NOX2, NOX4, NOX5). Of note, NOX5 is not present in mice and rats and thus pre-clinically less studied. NOX2, formerly termed gp91^{phox}, has been correlated with many, too many, diseases and is rather relevant as genetic deficiency in chronic granulomatous disease (CGD), treated by gene therapy. Overproduction of ROS through NOX1, NOX4, and NOX5 leads to the indicated diseases states including atherosclerosis (red), a condition where NOX4 is surprisingly protective.

Keywords

Mechanism-based redox therapeutics · NADPH oxidases · NOX inhibitors · Setanaxib · Reactive oxygen species

1 The NADPH Oxidase Family of Enzymes

NADPH oxidases (NOXs) are transmembrane enzymes that transfer electrons from NADPH in the cytoplasm across the cell membrane resulting in the formation of reactive oxygen species (ROS) (Cross and Segal 2004; Panday et al. 2015). The NOX family consists of seven members, NOX1–5 and the dual oxidases DUOX1–2. These enzymes are different in terms of enzyme complex composition, tissue and cellular distributions, subcellular localizations, mechanisms of activation, and the ROS type they produce. Thus, they are implicated in diverse physiological functions and disease conditions (Altenhofer et al. 2015; Casas et al. 2015; Elbatreek et al. 2019).

NOX2 (formerly known as gp91^{phox}) was the first NOX family member to be discovered (Rossi and Zatti 1964; Segal and Jones 1978). Other NOXs were discovered later and share certain sequence homology with this isoform, 56% for NOX1(aka Mox1), 58% for NOX3 (aka MOX2), 39% for NOX4 (aka Renox), 27% for NOX5, 57% for DUOX1 (aka ThOX1), and 43% for DUOX2 (aka ThOX2) (Cheng et al. 2001; De Deken et al. 2000; Suh et al. 1999). With respect to structure, all the family members possess a catalytic subunit, NOX, which is formed of six- or seven-transmembrane helices in NOX1–5 and DUOX1–2, respectively. NOX subunit binds two heme cofactors and allows for NADPH oxidation through a FAD/NADPH-binding domain in the cytosolic C-terminus (Cheng et al. 2001; Meitzler and Ortiz de Montellano 2009, 2011). In the case of NOX5 and DUOX1–2, NOX also binds to an intracellular Ca^{2+}-binding EF-hand region (Banfi et al. 2001, 2004b; Rigutto et al. 2009). Besides, NOXs differ in their membrane or cytosolic binding partners that are required for the enzymatic activity.

In NOX1–4, a membrane-bound subunit, p22^{phox}, is required for stabilization, whereas DUOX1 and 2 associate with the membrane maturation factors DUOXA1 and 2, respectively (Ambasta et al. 2004; Grasberger and Refetoff 2006; Parkos et al. 1987; Ueno et al. 2005). NOXs are also associated with cytosolic activator proteins (NOXA1 for NOX1 and NOX3, p67^{phox} and p40^{phox} for NOX2) which increase

enzymatic ROS-forming activity and organizer proteins (NOXO1 for NOX1 and NOX3, $p47^{phox}$ for NOX2) that help tether the activators with the NOX subunit (Banfi et al. 2003, 2004a; Volpp et al. 1988; Wientjes et al. 1993). In addition, other binding proteins help regulate NOX activity such as the small GTPase, RAC1, for NOX1–3 (Cheng et al. 2006; Diebold and Bokoch 2001; Ueyama et al. 2006), polymerase δ-interacting protein 2 (POLDIP2) for NOX4 (Lyle et al. 2009), and heat shock protein 90 (HSP90) for NOX1–3 and NOX5 (Chen et al. 2011).

Regarding their tissue and cellular distribution, the seven NOXs are widely expressed throughout different tissues (Fig. 1). NOX1 is predominantly expressed in colon epithelium (Szanto et al. 2005) and also in the uterus (Banfi et al. 2000; Suh et al. 1999), placenta (Cui et al. 2006), prostate (Banfi et al. 2000; Suh et al. 1999), pancreas (Xia et al. 2019), retina (Manea et al. 2005), keratinocytes (Chamulitrat et al. 2003), endothelium (Gray et al. 2013), and vascular smooth muscle cells (Lassegue et al. 2001). NOX2 is expressed in phagocytes which are present in numerous tissues and is often called "the phagocyte NADPH oxidase" (Bedard and Krause 2007); however, it can also be detected in other cell types including cardiomyocytes (Krijnen et al. 2003), skeletal muscle (Henriquez-Olguin et al. 2019), endothelial cells (Gorlach et al. 2000), hepatocytes (Reinehr et al. 2005), and neurons (Fan et al. 2019). NOX3 is highly abundant in the inner ear (Banfi et al. 2004a) in addition to other fetal tissues (Banfi et al. 2004a; Cheng et al. 2001), while NOX4 is highly expressed in kidney cells (Geiszt et al. 2000; Gorin et al. 2003; Jha et al. 2016), endothelium (Van Buul et al. 2005), vascular smooth muscle cells (Hoidal et al. 2003), cardiomyocytes (Brewer et al. 2011), fibroblasts (Cucoranu et al. 2005), adipocytes (Den Hartigh et al. 2017), and neurons (Casas et al. 2017). NOX5, which is absent in rodents, shows substantial expression in the testis, spleen, and lymph nodes (Banfi et al. 2001) and is also detected in the endothelial cells (BelAiba et al. 2007), vascular smooth muscle cells (Jay et al. 2008), kidney (Holterman et al. 2014; Jha et al. 2017a), and white blood cells (Manea et al. 2015). DUOX1–2 are predominantly found in the thyroid gland (De Deken et al. 2000) in addition to the lung epithelia (Fischer 2009) and prostate (D. Wang et al. 2005). DUOX1 is also expressed in epidermal keratinocytes (Ko et al. 2014) and DUOX2 in salivary ducts and the gastrointestinal tract (El Hassani et al. 2005; Geiszt et al. 2003b).

The subcellular localization/compartmentalization varies between NOXs in different cell types; however, the data are limited by the lack of high-quality antibodies against these enzymes (Zhang et al. 2019). NOX1 is localized in the endoplasmic reticulum, caveolae, and nucleus (Chamulitrat et al. 2003; Hilenski et al. 2004; Janiszewski et al. 2005), while NOX2 is present at the plasma membrane, perinuclear cytoskeleton, and endoplasmic reticulum (Huang et al. 1995; Krijnen et al. 2003; Segal and Jones 1978; Van Buul et al. 2005). There is barely any information about the subcellular localization of NOX3; however, one study showed the co-localization of NOX3 and $p22^{phox}$ in the plasma membrane of transfected HEK-293 cells (Nakano et al. 2007). NOX4 and NOX5 are localized at the cell membrane, nucleus, endoplasmic reticulum, and mitochondria (Ago et al. 2010; BelAiba et al. 2007; Case et al. 2013; Hilenski et al. 2004; Marzaioli et al. 2017;

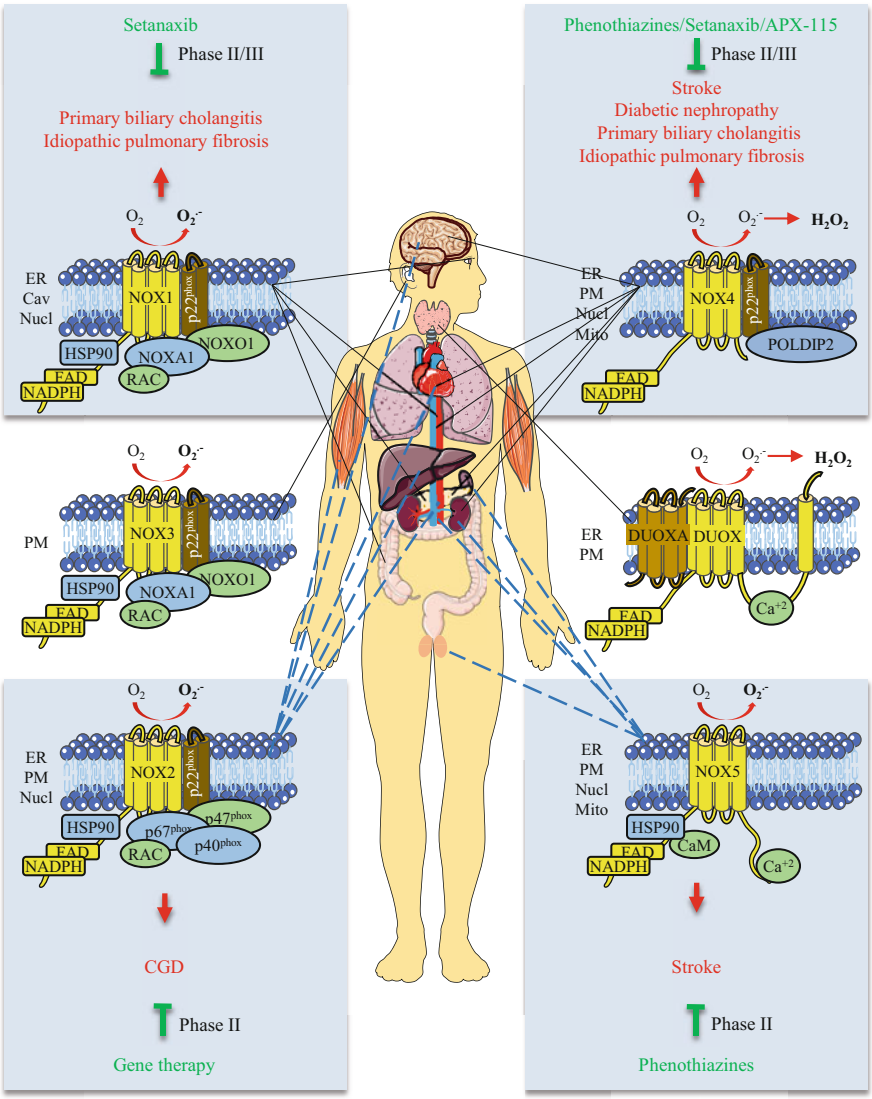


Fig. 1 NADPH oxidases and their clinical relevance. Seven NADPH oxidases, NOX1–5 and DUOX1–2, exist and possess a catalytic transmembrane subunit, NOX, which allows for NADPH oxidation through a FAD/NADPH-binding domain. NOXs have different membrane or cytosolic binding partners ($p22^{phox}$, DUOXA, NOXA1, NOXO1, RAC, HSP90, $p67^{phox}$, $p40^{phox}$, $p47^{phox}$, POLIDIP2) that are required for the enzymatic activity. NOXs are expressed in many organs: NOX1 in the colon, blood vessels, retina, and pancreas; NOX2 in the blood vessels, neurons, skeletal muscles, liver, and heart; NOX3 in the inner ear; NOX4 in the neurons, heart, blood vessels, kidney, and pancreas; NOX5 in the spleen, testis, kidney, and blood vessels; and DUOXs in the thyroid gland. NOXs are also expressed in different subcellular locations including endoplasmic reticulum (ER), caveolae (Cav), nucleus (Nucl), plasma membrane (PM), and mitochondria (Mito). The therapeutically relevant NOXs (in light blue boxes) include NOX1 and NOX4 being tested in

Matsushima et al. 2013; Perrotta et al. 2011; Van Buul et al. 2005; Wu et al. 2010; Yu et al. 2014) and DUOX1–2 at the apical membrane and endoplasmic reticulum (De Deken et al. 2000, 2002; Schwarzer et al. 2004).

Given all these structural characteristics, dissimilar tissue distribution, and subcellular localization of the NOX family members, they show distinct modes of activation (reviewed in (Brandes et al. 2014)) except NOX4 (Zhang et al. 2019) and the DUOXs (Aliasgharzadeh et al. 2019; Azmoonfar et al. 2018; Farhood et al. 2019) which are constitutively active and regulated at the expressional level. Additionally, NOXs differ in their ROS product, i.e., NOX1–3 and NOX5 produce superoxide, while NOX4 and DUOX1–2 produce hydrogen peroxide (Altenhofer et al. 2015). Overall, full characterization of the NOX enzymes is still deficient, yet by generating reliable high-quality antibodies and isoform-specific NOX inhibitors, it might be achievable.

2 NADPH Oxidases in Physiology

Being the sole and primary function of the NOX enzymes, ROS production should not be viewed mainly as disease trigger and metabolic waste. Indeed, ROS from NOXs among others contribute to several physiological functions such as host defense, angiogenesis, cell survival, tissue regeneration, hearing, hormone synthesis and sensitivity, vasodilation, and cell signaling (Elbatreek et al. 2019; Jiang et al. 2011). These functions need to be taken also into consideration as potential sources of side effects when NOX inhibitors are used therapeutically.

With respect to individual NOXs, NOX1-derived ROS slow down apoptosis of gastric mucosal cells and thereby regulate their growth (Teshima et al. 2000). In the colon, NOX1 is a part of the innate immune response, promotes cell proliferation and differentiation, stimulates mucosal wound repair, and prevents inflammation (Coant et al. 2010; Geiszt et al. 2003a; Kajla et al. 2012; Kato et al. 2016; Moll et al. 2018; Rokutan et al. 2006). Moreover, NOX1 plays a role in cell signaling via inhibiting protein tyrosine phosphatases and thus inactivation of peroxiredoxin 1, an enzyme that metabolizes/detoxifies hydrogen peroxide, thereby allowing the localized and transient accumulation of hydrogen peroxide for cell signaling (Woo et al. 2010). In the brain, NOX1 is suggested to suppress neuronal differentiation via inhibiting excessive neurite outgrowth (Ibi et al. 2006).

NOX2 is a key player in the innate host defense against infection. Mutations in genes encoding components of the NOX2 enzyme complex lead to chronic

Fig. 1 (continued) primary biliary cholangitis and idiopathic pulmonary fibrosis, NOX4 also in diabetic nephropathy and stroke, NOX2 in chronic granulomatous disease (CGD), and NOX5 in stroke. NOX inhibitors including setanaxib, APX-115, and phenothiazines and NOX2 gene therapy are being tested in Phase II/III clinical trials for these indications. Abbreviations: CaM, calmodulin; HSP90, heat shock protein 90; NOXA1, NADPH oxidase activator 1; NOXO1, NADPH oxidase organizer 1; POLDIP2, polymerase δ-interacting protein 2

granulomatous disease (CGD) which is characterized by immunodeficiency and recurrent and life-threatening infections (Panday et al. 2015). ROS from NOX2 can kill the attacking microorganisms directly by oxidative damage of proteins, lipids, and DNA and indirectly by activation of downstream signaling (Iles and Forman 2002). In addition to host defense, NOX2 might be involved in learning and memory as CGD patients show cognitive deficits and NOX2 mutant mice have mild memory impairment (Kishida et al. 2006; Pao et al. 2004). NOX2 also might have a protective function against colon inflammation as CGD patients also exhibit non-infective colitis (Pao et al. 2004). Moreover, NOX2 mediates the renal vasoconstriction effect of angiotensin and thus regulates the normal renal blood flow (Haque and Majid 2004) and enhances skeletal muscle metabolism and insulin sensitivity (Henriquez-Olguin et al. 2019). Apart from NOX2, the key physiological roles of NOX3 are mainly known in the inner ear. Mutation in the *NOX3* gene results in a lack of otoconia formation and vestibular dysfunction as shown in "head-tilt" mutant mice (Paffenholz et al. 2004). Also, recently, NOX3, together with NOX5, has been suggested to induce differentiation of human oligodendrocytes (Accetta et al. 2016).

NOX4 has a plethora of physiological and protective roles. This is probably explained by its constitutive activity, wide distribution, and production of hydrogen peroxide which is an omnipresent signaling molecule (Elbatreek et al. 2019; Guo and Chen 2015; Veal and Day 2011; Zhang et al. 2019). However, knocking out the *NOX4* gene in mice and rats does not result in an obvious phenotype or affect the life span of the animals (Kleinschnitz et al. 2010; Rezende et al. 2017). NOX4 enhances hormone-stimulated sodium and water transport in the kidney (Feraille et al. 2014; Lu et al. 2016), adipocytes differentiation (Schroder et al. 2009), insulin sensitivity in the liver and adipose tissue (Mahadev et al. 2004; Taniguchi et al. 2006), glucose-stimulated insulin secretion (Plecita-Hlavata et al. 2020), autophagy in cardiomyocytes (Kouroku et al. 2007), hippocampal neurogenesis, memory formation (Choi et al. 2019; Yoshikawa et al. 2019), angiogenesis, and vasodilation (Burgoyne et al. 2007; Drummond et al. 2000). NOX4 also activates downstream redox-sensitive proteins that play important roles in cell proliferation, migration, and apoptosis (Guo and Chen 2015). Further, NOX4 protects the vasculature from ischemic and inflammatory stress such as in atherosclerosis (Gray et al. 2016; Schroder et al. 2012).

NOX5 is the least studied NOX, and its physiological roles are not fully understood due to its absence in rodents. However, it has been suggested to regulate cell signaling and function (Fulton 2009) and contribute to sperm motility and viability (Ghanbari et al. 2018), as well as vascular smooth muscle cells contraction (Montezano et al. 2018). DUOX enzymes appear to be important for thyroid hormone synthesis. Mutations in the *DUOX2* lead to disruption of thyroid hormone synthesis and hypothyroidism (Moreno et al. 2002). DUOXs also play a role in host defense in the gastrointestinal tract and lung epithelia (van der Vliet et al. 2018).

Most of the abovementioned functions of NOXs derive from preclinical data and the physiological roles of NOXs in humans remain poorly understood. While the

biological effects of NOXs are important for health, dysfunctions in these enzymes may lead to pathology.

3 NADPH Oxidases in Pathology

Several pathophysiological roles have been validated for NOX enzymes, and thus several diseases are largely based on NOX dysregulation (Casas et al. 2015; Dao et al. 2015) (Fig. 1).

3.1 NOX1

NOX1 is involved in fibrotic diseases in many organs (Kato and Hecker 2020). Current clinical studies to target NOX1, together with NOX4, are focused on idiopathic pulmonary fibrosis and primary biliary cholangitis (a fibrotic orphan disease) (Table 1). Moreover, NOX1 seems a clinically relevant target in GI disorders. On the one hand, defects in *NOX1* are found in patients with very-early-onset inflammatory bowel diseases (Hayes et al. 2015; Scherz-Shouval and Elazar 2011). Indeed, some variants in *NOX1* are associated with complete loss of function of the gene product and with loss of ROS production in IBD patients (Schwerd et al. 2018). On the other hand, NOX1 overexpression is associated with colon and gall bladder cancers (Wang et al. 2019; Juhasz et al. 2017; Laurent et al. 2008). Besides GI-related disorders, diabetic vascular complications including diabetes-accelerated atherosclerosis (Gray et al. 2013) and retinopathy (Wilkinson-Berka et al. 2014) are potential conditions for clinical testing of NOX1 inhibitors.

3.2 NOX2

NOX2 genetic defects or inhibition are associated with immune deficiency and increased risk of infection, particularly in diabetes (Gray et al. 2013). Mutations in *CYBB*, *CYBA*, *NCF1*, *NCF2*, and *NCF4* genes, encoding NOX2, p22phox, p47phox, p67phox, and p40phox, respectively, cause CGD. Around 70% of CGD cases are due to mutations in *CYBB* (called X-linked CGD) resulting in decreased NOX2 expression, activity, or both. Therefore, CGD leads to immunodeficiency and increases susceptibility to recurrent and life-threatening infections due to fungal or bacterial pathogens (Giardino et al. 2017; O'Neill et al. 2015). As a key enzyme of the innate and inflammatory response, NOX2 has been suggested to be involved in an excessive and unlikely number of disease models (Casas et al. 2015; Elbatreek et al. 2019) which might indicate a possible positive publication bias, as shown by a meta-analysis of NOX2 studies in stroke (Kleikers et al. 2015), or an epiphenomenon without therapeutic relevance.

Table 1 NOX inhibitors and gene therapy for CGD and their clinical status

	Isoform	Indication	Clinical trial	Results/status
NOX inhibitors				
GKT137831 (GKT-831 or setanaxib)	1,4	Type 2 diabetes mellitus nephropathy	Phase I (NCT03740217)	Safe
			A double-blind, placebo-controlled, randomized, multicenter, parallel group, Phase II (NCT02010242)	Reduced several secondary efficacy endpoints. However, improvements in albuminuria, the study's primary efficacy endpoint, was not achieved after 12 weeks of treatment
		Type 1 diabetes mellitus nephropathy	A double-blind, placebo-controlled, randomized, multicenter, with two parallel arms Phase II (U1111-1187-2609)	Ongoing in Australia and expanded to Europe and New Zealand
		Primary biliary cholangitis/ cirrhosis (PBC)	A double-blind, placebo-controlled, randomized, multicenter, parallel group, Phase II (NCT03226067)	Achieved rapid, dose- and time-dependent reductions in markers of cholestatic bile duct and liver injury. These reductions in disease activity were highly significant for both ALP and GGT
		Idiopathic pulmonary fibrosis (IPF)	A double-blind, placebo-controlled, randomized, multicenter, parallel group, Phase II (NCT03865927)	Not yet recruiting
APX-115	1,2,4	Type 1 diabetes mellitus nephropathy	Phase II (10.4062/ biomolther.2019.188)	Not yet recruiting
Perphenazine	4,5	Stroke	Phase II, repo-stroke (repo-trial.Eu) (EduraCT no. 2019–000474-31)	Not yet recruiting
Gene therapy				
Lentiviral gene therapy	2	X-linked chronic granulomatous disease (X-CGD)	Phase I/II, non-randomized, multicenter, open-label (NCT02234934 and NCT01855685)	The primary objective (to assess the safety and evaluate the efficacy and stability of biochemical and functional reconstitution in the

(continued)

Table 1 (continued)

	Isoform	Indication	Clinical trial	Results/status
				progeny of engrafted cells at 12 months) was met in six of the nine patients
			Phase I/II, non-randomized, open-label (NCT03645486)	Recruiting
			Phase I/II, non-randomized, monocentric open-label (NCT02757911)	Recruiting
Retroviral gene therapy			Phase I/II, non-randomized, single center, uncontrolled, open-label (NCT00778882)	In progress
			Phase I/II, non-randomized, open-label (NCT01906541)	Recruiting

3.3 NOX3

Being expressed in the inner ear, NOX3 appears to be a key target for hearing loss. Preclinical data show that noise exposure causes overexpression of NOX3 that results in cochlear inflammation, apoptosis, and eventually hearing loss (Dhukhwa et al. 2019). NOX3 also was shown to be associated with drug-induced hearing loss (Rybak et al. 2012). Genetic clinical studies show that NOX3 is associated with noise-induced hearing loss (Zhao et al. 2020) and pulmonary hypertension as shown in a recent GWAS (Yin et al. 2018). Further clinical and therapeutic validation of NOX3 in these conditions needs to be investigated.

3.4 NOX4

Preclinical data suggest that NOX4 is involved in many diseases including diabetic kidney disease (Jha et al. 2014, 2016), cancer (Lin et al. 2017), fibrosis of the liver (Lan et al. 2015) and lung (Carnesecchi et al. 2011), and ischemic stroke (Casas et al. 2017, 2019a; Kleinschnitz et al. 2010). Genetic clinical data shows that NOX4 is associated with an increased risk of stroke (He et al. 2018). Diversely, the role of NOX4 in cardiovascular disorders such as hypertension and atherosclerosis is likely limited. Indeed NOX4 seems protective in diabetes-accelerated atherosclerosis (Gray et al. 2016; Schurmann et al. 2015) and myocardial infarction-induced cardiac remodeling (Mongue-Din et al. 2017). Clinical studies on NOX4 are focused on

stroke for acute indications and diabetic kidney disease and fibrotic conditions for chronic indications. Yet, due to the dual effects of NOX4 and its plentiful biological functions in many organs, chronic NOX4 inhibition seems a less attractive approach. In cancer, however, targeting NOX4 needs to be examined given its metabolic, anti-apoptotic, and pro-angiogenic properties.

3.5 NOX5

NOX5 appears as a promising target in cardiovascular diseases, i.e., hypertension and atherosclerosis (Guzik et al. 2008; Touyz et al. 2019). Our recent findings show that NOX5 levels in endothelial microparticles are increased in a subgroup of hypertensive patients leading to eNOS uncoupling and endothelial dysfunction. NOX5 might also be a potential target in stroke (Casas et al. 2019b), myocardial infarction (Hahn et al. 2012), cancer (Antony et al. 2017), diabetic nephropathy (Jha et al. 2017b), aortic aneurysm (Guzik et al. 2013), and hemorrhagic transformation (Won et al. 2011).

3.6 DUOXs

The clinical relevance of targeting DUOX isoforms is not yet clear. Preclinical evidence suggests that DUOXs might contribute to immune and allergic disorders (van der Vliet et al. 2018) and can be targeted for radiation-induced thyroid cancer (Ameziane-El-Hassani et al. 2015). Similar to *NOX1*, mutations in *DUOX2* are found in patients with very-early-onset inflammatory bowel diseases (Hayes et al. 2015).

Taken together, given the diverse effects of the NOX enzymes both in physiology and disease, benefit-risk assessments should be considered as exemplified by NOX2 inhibition which is associated with immunodeficiency and infection (Gray et al. 2013; Panday et al. 2015). Also, inhibition of DUOX2 can result in hypothyroidism and bowel inflammation (Hayes et al. 2015). Similarly, inhibiting NOX1 might enhance gut inflammation (Schwerd et al. 2018), and inhibiting NOX4 might promote atherosclerosis (Gray et al. 2016) and enhance the risk of kidney fibrosis (Nlandu Khodo et al. 2012) and liver cancer (Crosas-Molist et al. 2017). Acute indications such as ischemic stroke are likely to have, however, a low risk-benefit profile.

4 NADPH Oxidases Inhibitors

Despite the fact that NOX inhibitors are already in the clinic, the field has still to be considered relatively immature. There are no compounds available that deserve the term NOS isoform specific. Most compounds are pan-NOX inhibitors. Two recent analyses identified compounds with some isoform preference (Augsburger et al.

2019; Dao et al. 2019), and it has been shown that by using a panel of marginally selective inhibitors, specific isoforms, such as NOX4, could be validated pharmacologically (Dao et al. 2019). Considering the critical roles of NOXs in the pathogenesis of many diseases, they have been suggested as promising therapeutic targets. Several small molecules have been thought to inhibit NOX activity; however, majority were unspecific due to off-target effects. These molecules include, for example, diphenyleneiodonium (DPI) and apocynin. The former is a flavoprotein inhibitor and thus inhibits many other enzymes besides NOXs, while the latter has non-specific ROS scavenging properties (Altenhofer et al. 2015). Likewise, some other recently developed NOX inhibitors are unspecific such as VAS2870, ML-171, and GKT136901 (Augsburger et al. 2019; Dao et al. 2019). Only a few compounds are claimed to be specific NOX inhibitor in preclinical studies including GSK2795039 which selectively inhibits NOX2 (Hirano et al. 2015), GLX7013114 for NOX4 (Wang et al. 2018), and Ewha-18278 (APX-115) for NOX1, NOX2, and NOX4 (Cha et al. 2017).

NOX inhibitors currently being tested in the clinical phase are focused on fibrotic and neurovascular disease indications with NOX1, 4, and 5 as the main isoforms to be targeted. GKT137831 (setanaxib or GKT-831) claimed as a NOX1/4 dual inhibitor and a partial Nox5 inhibitor is the first-in-class NOX inhibitor to reach the clinical trial stage (Table 1). Setanaxib was safe and showed encouraging pharmacokinetic properties during Phase I study. Subsequently, it was tested in Phase II clinical trial for nephropathy in type 2 diabetes patients, yet the primary efficacy endpoint, i.e., albuminuria reduction, was not achieved. However, several other secondary efficacy endpoints were reached such as maximal inhibition of the renin-angiotensin-aldosterone system. In another Phase II trial focused on primary biliary cholangitis, setanaxib has succeeded and met its primary and secondary efficacy endpoints. Two additional Phase II clinical trials using setanaxib are ongoing, for idiopathic pulmonary fibrosis and kidney disease in type 1 diabetes. The second NOX inhibitor to reach the clinical trial stage is APX-115 which is moving from Phase I to II for diabetic kidney disease (Lee et al. 2020). Phenothiazines, already marketed for some indications, i.e., antipsychotic, show pan NOX inhibition activity in some preclinical studies (Seredenina et al. 2015, 2016). One clinical trial is planned to repurpose phenothiazines into stroke (Repo-Stroke).

Based on preclinical data, further indications for NOX inhibitors might also have potential toward the clinical application. For example, in ischemic retina disease and diabetic retinopathy, setanaxib and its analogue GKT136901 showed favorable effects (Appukuttan et al. 2018; Jiao et al. 2019; J L Wilkinson-Berka et al. 2013). Also, in cardiovascular disorders, including diabetes-associated atherosclerosis and hypertensive cardiac remodeling and hypertrophy, and liver fibrosis, setanaxib attenuated inflammatory and fibrotic markers (Gray et al. 2013; Sun et al. 2017; Zeng et al. 2019; Zhao et al. 2015) even when the treatment was delayed (Gray et al. 2017). VAS2870 which is a pan NOX inhibitor showed vascular protective effects in pulmonary hypertension (Li et al. 2019) and Alzheimer's disease (Abubaker et al. 2019). ML090 which has preferential activity toward NOX5 was beneficial in stroke

(Casas et al. 2019b; Dao et al. 2019). Collectively, NOX inhibition seems a promising therapeutic strategy with a broad range of clinical applications and warrants further investigations.

5 Advanced Therapies

Currently, the only known cure for CGD is allogeneic hematopoietic stem cell transplantation which is a high-risk procedure and associated with severe disability or death (Kang et al. 2011b). Only one drug is approved to treat/manage CGD, interferon gamma-1b that reduces the frequency and severity of serious infections associated with the disease (Miller et al. 2009). Current clinical research suggests that gene therapy holds great promise in curing CGD obviating the need for a transplantation donor and eliminating the risks associated with stem cell transplantation (Keller et al. 2018). What also makes gene therapy an attractive treatment for CGD is that restoration of normal NOX activity in only 10–20% of circulating neutrophils is sufficient to achieve significant clinical benefit (Keller et al. 2018). Early clinical trials on gene therapy for CGD were mainly based on γ-retroviral vectors that can only infect mitotically active cell types (Escors and Breckpot 2010). These studies failed to show efficacy and were associated with insertional mutagenesis, due to upregulation of proto-oncogene expression (Kang et al. 2011a; Keller et al. 2018). To overcome the latter issue, self-inactivating (SIN) retroviral vectors have been developed (Thornhill et al. 2008) and are being tested in clinical trials (NCT01906541). More recent clinical trials are using SIN lentiviral vectors (complex retroviruses), which unlike γ-retroviral vectors are capable of transducing quiescent cells and devoid of insertional toxicities (Escors and Breckpot 2010). The preliminary results from these lentiviral gene therapy trials (NCT02234934 and NCT01855685) are encouraging (Kohn et al. 2020) (Table 1).

6 Conclusions

NOX enzymes are primary sources of ROS, and their activation results in the activation of secondary ROS sources, i.e., ROS-dependent ROS production or the kindling-bonfire sequence. These secondary ROS sources include uncoupled nitric oxide synthase (NOS), xanthine oxidase, and dysfunctional mitochondria (Zhang et al. 2019). Therefore, NOX inhibition might represent an intelligent therapeutic strategy in ROS-related diseases as it targets the origin. However, none of the ROS sources act on their own, and different ROS forming enzymes will affect different targets. Thus, combinations are most likely more effective that single target strategies, which may lead to better efficacy and reduced side effects. As NOX inhibitors have entered clinical trials, two main aspects should be considered, specificity and isoform selectivity. Most of the NOX inhibitors in development are non-specific even the most advanced ones, setanaxib and GKT136901, have ROS scavenging activities (Augsburger et al. 2019; Dao et al. 2019). Isoform selectivity

of the NOX inhibitors is also important given the physiological tissue- and cell-specific effects of NOXs. Applying a NOX inhibitor panel approach could be an option for NOX target validation (Dao et al. 2019). Further lead optimization of the current NOX inhibitors might help find isoform-selective compounds. Finally, ROS have important beneficial signaling functions. Thus, acute interventions such as in stroke (NOX4 and NOX5) appear safer than chronic therapies suppressing NOX1 or NOX4. Clinical trials in both directions are under way (NCT03865927, EudraCT No. 2019-000474-31) and will answer this by the early 2020s.

Acknowledgments This review project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 777111 (REPO-TRIAL). This reflects only the author's view, and the European Commission is not responsible for any use that may be made of the information it contains.

Conflict of Interest None

References

Abubaker AA, Vara D, Visconte C, Eggleston I, Torti M, Canobbio I, Pula G (2019) Amyloid peptide beta1-42 induces integrin alphaiibbeta3 activation, platelet adhesion, and thrombus formation in a NADPH oxidase-dependent manner. Oxidative Med Cell Longev 2019:1050476. https://doi.org/10.1155/2019/1050476

Accetta R, Damiano S, Morano A, Mondola P, Paterno R, Avvedimento EV, Santillo M (2016) Reactive oxygen species derived from NOX3 and NOX5 drive differentiation of human oligodendrocytes. Front Cell Neurosci 10:146. https://doi.org/10.3389/fncel.2016.00146

Ago T, Kuroda J, Pain J, Fu C, Li H, Sadoshima J (2010) Upregulation of Nox4 by hypertrophic stimuli promotes apoptosis and mitochondrial dysfunction in cardiac myocytes. Circ Res 106 (7):1253–1264. https://doi.org/10.1161/CIRCRESAHA.109.213116

Aliasgharzadeh A, Farhood B, Amini P, Saffar H, Motevaseli E, Rezapoor S et al (2019) Melatonin attenuates upregulation of Duox1 and Duox2 and protects against lung injury following chest irradiation in rats. Cell J 21(3):236–242. https://doi.org/10.22074/cellj.2019.6207

Altenhofer S, Radermacher KA, Kleikers PW, Wingler K, Schmidt HH (2015) Evolution of NADPH oxidase inhibitors: selectivity and mechanisms for target engagement. Antioxid Redox Signal 23(5):406–427. https://doi.org/10.1089/ars.2013.5814

Ambasta RK, Kumar P, Griendling KK, Schmidt HH, Busse R, Brandes RP (2004) Direct interaction of the novel Nox proteins with p22phox is required for the formation of a functionally active NADPH oxidase. J Biol Chem 279(44):45935–45941. https://doi.org/10.1074/jbc.M406486200

Ameziane-El-Hassani R, Talbot M, de Souza Dos Santos MC, Al Ghuzlan A, Hartl D, Bidart JM et al (2015) NADPH oxidase DUOX1 promotes long-term persistence of oxidative stress after an exposure to irradiation. Proc Natl Acad Sci U S A 112(16):5051–5056. https://doi.org/10.1073/pnas.1420707112

Antony S, Jiang G, Wu Y, Meitzler JL, Makhlouf HR, Haines DC et al (2017) NADPH oxidase 5 (NOX5)-induced reactive oxygen signaling modulates normoxic HIF-1alpha and p27(Kip1) expression in malignant melanoma and other human tumors. Mol Carcinog 56(12):2643–2662. https://doi.org/10.1002/mc.22708

Appukuttan B, Ma Y, Stempel A, Ashander LM, Deliyanti D, Wilkinson-Berka JL, Smith JR (2018) Effect of NADPH oxidase 1 and 4 blockade in activated human retinal endothelial cells. Clin Exp Ophthalmol 46(6):652–660. https://doi.org/10.1111/ceo.13155

Augsburger F, Filippova A, Rasti D, Seredenina T, Lam M, Maghzal G et al (2019) Pharmacological characterization of the seven human NOX isoforms and their inhibitors. Redox Biol 26:101272. https://doi.org/10.1016/j.redox.2019.101272

Azmoonfar R, Amini P, Saffar H, Rezapoor S, Motevaseli E, Cheki M et al (2018) Metformin protects against radiation-induced pneumonitis and fibrosis and attenuates upregulation of dual oxidase genes expression. Adv Pharm Bull 8(4):697–704. https://doi.org/10.15171/apb.2018.078

Banfi B, Maturana A, Jaconi S, Arnaudeau S, Laforge T, Sinha B et al (2000) A mammalian H+ channel generated through alternative splicing of the NADPH oxidase homolog NOH-1. Science 287(5450):138–142. https://doi.org/10.1126/science.287.5450.138

Banfi B, Molnar G, Maturana A, Steger K, Hegedus B, Demaurex N, Krause KH (2001) A Ca(2+)-activated NADPH oxidase in testis, spleen, and lymph nodes. J Biol Chem 276 (40):37594–37601. https://doi.org/10.1074/jbc.M103034200

Banfi B, Clark RA, Steger K, Krause KH (2003) Two novel proteins activate superoxide generation by the NADPH oxidase NOX1. J Biol Chem 278(6):3510–3513. https://doi.org/10.1074/jbc.C200613200

Banfi B, Malgrange B, Knisz J, Steger K, Dubois-Dauphin M, Krause KH (2004a) NOX3, a superoxide-generating NADPH oxidase of the inner ear. J Biol Chem 279(44):46065–46072. https://doi.org/10.1074/jbc.M403046200

Banfi B, Tirone F, Durussel I, Knisz J, Moskwa P, Molnar GZ et al (2004b) Mechanism of Ca2+ activation of the NADPH oxidase 5 (NOX5). J Biol Chem 279(18):18583–18591. https://doi.org/10.1074/jbc.M310268200

Bedard K, Krause KH (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 87(1):245–313. https://doi.org/10.1152/physrev.00044.2005

BelAiba RS, Djordjevic T, Petry A, Diemer K, Bonello S, Banfi B et al (2007) NOX5 variants are functionally active in endothelial cells. Free Radic Biol Med 42(4):446–459. https://doi.org/10.1016/j.freeradbiomed.2006.10.054

Brandes RP, Weissmann N, Schroder K (2014) Nox family NADPH oxidases: molecular mechanisms of activation. Free Radic Biol Med 76:208–226. https://doi.org/10.1016/j.freeradbiomed.2014.07.046

Brewer AC, Murray TV, Arno M, Zhang M, Anilkumar NP, Mann GE, Shah AM (2011) Nox4 regulates Nrf2 and glutathione redox in cardiomyocytes in vivo. Free Radic Biol Med 51 (1):205–215. https://doi.org/10.1016/j.freeradbiomed.2011.04.022

Burgoyne JR, Madhani M, Cuello F, Charles RL, Brennan JP, Schroder E et al (2007) Cysteine redox sensor in PKGIa enables oxidant-induced activation. Science 317(5843):1393–1397. https://doi.org/10.1126/science.1144318

Carnesecchi S, Deffert C, Donati Y, Basset O, Hinz B, Preynat-Seauve O et al (2011) A key role for NOX4 in epithelial cell death during development of lung fibrosis. Antioxid Redox Signal 15 (3):607–619. https://doi.org/10.1089/ars.2010.3829

Casas AI, Dao VT, Daiber A, Maghzal GJ, Di Lisa F, Kaludercic N et al (2015) Reactive oxygen-related diseases: therapeutic targets and emerging clinical indications. Antioxid Redox Signal 23 (14):1171–1185. https://doi.org/10.1089/ars.2015.6433

Casas AI, Geuss E, Kleikers PWM, Mencl S, Herrmann AM, Buendia I et al (2017) NOX4-dependent neuronal autotoxicity and BBB breakdown explain the superior sensitivity of the brain to ischemic damage. Proc Natl Acad Sci U S A 114(46):12315–12320. https://doi.org/10.1073/pnas.1705034114

Casas AI, Hassan AA, Larsen SJ, Gomez-Rangel V, Elbatreek M, Kleikers PWM et al (2019a) From single drug targets to synergistic network pharmacology in ischemic stroke. Proc Natl Acad Sci U S A 116(14):7129–7136. https://doi.org/10.1073/pnas.1820799116

Casas AI, Kleikers PW, Geuss E, Langhauser F, Adler T, Busch DH et al (2019b) Calcium-dependent blood-brain barrier breakdown by NOX5 limits postreperfusion benefit in stroke. J Clin Invest 130:1772–1778. https://doi.org/10.1172/JCI124283

Case AJ, Li S, Basu U, Tian J, Zimmerman MC (2013) Mitochondrial-localized NADPH oxidase 4 is a source of superoxide in angiotensin II-stimulated neurons. Am J Physiol Heart Circ Physiol 305(1):H19–H28. https://doi.org/10.1152/ajpheart.00974.2012

Cha JJ, Min HS, Kim KT, Kim JE, Ghee JY, Kim HW et al (2017) APX-115, a first-in-class pan-NADPH oxidase (Nox) inhibitor, protects db/db mice from renal injury. Lab Investig 97 (4):419–431. https://doi.org/10.1038/labinvest.2017.2

Chamulitrat W, Schmidt R, Tomakidi P, Stremmel W, Chunglok W, Kawahara T, Rokutan K (2003) Association of gp91phox homolog Nox1 with anchorage-independent growth and MAP kinase-activation of transformed human keratinocytes. Oncogene 22(38):6045–6053. https://doi.org/10.1038/sj.onc.1206654

Chen F, Pandey D, Chadli A, Catravas JD, Chen T, Fulton DJ (2011) Hsp90 regulates NADPH oxidase activity and is necessary for superoxide but not hydrogen peroxide production. Antioxid Redox Signal 14(11):2107–2119. https://doi.org/10.1089/ars.2010.3669

Cheng G, Cao Z, Xu X, van Meir EG, Lambeth JD (2001) Homologs of gp91phox: cloning and tissue expression of Nox3, Nox4, and Nox5. Gene 269(1–2):131–140. https://doi.org/10.1016/s0378-1119(01)00449-8

Cheng G, Diebold BA, Hughes Y, Lambeth JD (2006) Nox1-dependent reactive oxygen generation is regulated by Rac1. J Biol Chem 281(26):17718–17726. https://doi.org/10.1074/jbc.M512751200

Choi SA, Kim YH, Park YH, Yang HJ, Jeong PS, Cha JJ et al (2019) Novel crosstalk between Vps26a and Nox4 signaling during neurogenesis. Cell Death Differ 26(9):1582–1599. https://doi.org/10.1038/s41418-018-0226-0

Coant N, Ben Mkaddem S, Pedruzzi E, Guichard C, Treton X, Ducroc R et al (2010) NADPH oxidase 1 modulates WNT and NOTCH1 signaling to control the fate of proliferative progenitor cells in the colon. Mol Cell Biol 30(11):2636–2650. https://doi.org/10.1128/MCB.01194-09

Crosas-Molist E, Bertran E, Rodriguez-Hernandez I, Herraiz C, Cantelli G, Fabra A et al (2017) The NADPH oxidase NOX4 represses epithelial to amoeboid transition and efficient tumour dissemination. Oncogene 36(21):3002–3014. https://doi.org/10.1038/onc.2016.454

Cross AR, Segal AW (2004) The NADPH oxidase of professional phagocytes--prototype of the NOX electron transport chain systems. Biochim Biophys Acta 1657(1):1–22. https://doi.org/10.1016/j.bbabio.2004.03.008

Cucoranu I, Clempus R, Dikalova A, Phelan PJ, Ariyan S, Dikalov S, Sorescu D (2005) NAD(P)H oxidase 4 mediates transforming growth factor-beta1-induced differentiation of cardiac fibroblasts into myofibroblasts. Circ Res 97(9):900–907. https://doi.org/10.1161/01.RES.0000187457.24338.3D

Cui XL, Brockman D, Campos B, Myatt L (2006) Expression of NADPH oxidase isoform 1 (Nox1) in human placenta: involvement in preeclampsia. Placenta 27(4–5):422–431. https://doi.org/10.1016/j.placenta.2005.04.004

Dao VT, Casas AI, Maghzal GJ, Seredenina T, Kaludercic N, Robledinos-Anton N et al (2015) Pharmacology and clinical drug candidates in redox medicine. Antioxid Redox Signal 23 (14):1113–1129. https://doi.org/10.1089/ars.2015.6430

Dao VT, Elbatreek MH, Altenhofer S, Casas AI, Pachado MP, Neullens CT et al (2019) Isoform-selective NADPH oxidase inhibitor panel for pharmacological target validation. Free Radic Biol Med 148:60–69. https://doi.org/10.1016/j.freeradbiomed.2019.12.038

De Deken X, Wang D, Many MC, Costagliola S, Libert F, Vassart G et al (2000) Cloning of two human thyroid cDNAs encoding new members of the NADPH oxidase family. J Biol Chem 275 (30):23227–23233. https://doi.org/10.1074/jbc.M000916200

De Deken X, Wang D, Dumont JE, Miot F (2002) Characterization of ThOX proteins as components of the thyroid H(2)O(2)-generating system. Exp Cell Res 273(2):187–196. https://doi.org/10.1006/excr.2001.5444

Den Hartigh LJ, Omer M, Goodspeed L, Wang S, Wietecha T, O'Brien KD, Han CY (2017) Adipocyte-specific deficiency of NADPH oxidase 4 delays the onset of insulin resistance and

attenuates adipose tissue inflammation in obesity. Arterioscler Thromb Vasc Biol 37 (3):466–475. https://doi.org/10.1161/ATVBAHA.116.308749
Dhukhwa A, Bhatta P, Sheth S, Korrapati K, Tieu C, Mamillapalli C et al (2019) Targeting inflammatory processes mediated by TRPVI and TNF-alpha for treating noise-induced hearing loss. Front Cell Neurosci 13:444. https://doi.org/10.3389/fncel.2019.00444
Diebold BA, Bokoch GM (2001) Molecular basis for Rac2 regulation of phagocyte NADPH oxidase. Nat Immunol 2(3):211–215. https://doi.org/10.1038/85259
Drummond GR, Cai H, Davis ME, Ramasamy S, Harrison DG (2000) Transcriptional and posttranscriptional regulation of endothelial nitric oxide synthase expression by hydrogen peroxide. Circ Res 86(3):347–354. https://doi.org/10.1161/01.res.86.3.347
El Hassani RA, Benfares N, Caillou B, Talbot M, Sabourin JC, Belotte V et al (2005) Dual oxidase2 is expressed all along the digestive tract. Am J Physiol Gastrointest Liver Physiol 288(5):G933–G942. https://doi.org/10.1152/ajpgi.00198.2004
Elbatreek MH, Pachado MP, Cuadrado A, Jandeleit-Dahm K, Schmidt H (2019) Reactive oxygen comes of age: mechanism-based therapy of diabetic end-organ damage. Trends Endocrinol Metab 30(5):312–327. https://doi.org/10.1016/j.tem.2019.02.006
Escors D, Breckpot K (2010) Lentiviral vectors in gene therapy: their current status and future potential. Arch Immunol Ther Exp 58(2):107–119. https://doi.org/10.1007/s00005-010-0063-4
Fan LM, Geng L, Cahill-Smith S, Liu F, Douglas G, McKenzie CA et al (2019) Nox2 contributes to age-related oxidative damage to neurons and the cerebral vasculature. J Clin Invest 129 (8):3374–3386. https://doi.org/10.1172/JCI125173
Farhood B, Aliasgharzadeh A, Amini P, Saffar H, Motevaseli E, Rezapoor S et al (2019) Radiation-induced dual oxidase upregulation in rat heart tissues: protective effect of melatonin. Medicina 55(7):317. https://doi.org/10.3390/medicina55070317
Feraille E, Dizin E, Roth I, Derouette JP, Szanto I, Martin PY et al (2014) NADPH oxidase 4 deficiency reduces aquaporin-2 mRNA expression in cultured renal collecting duct principal cells via increased PDE3 and PDE4 activity. PLoS One 9(1):e87239. https://doi.org/10.1371/journal.pone.0087239
Fischer H (2009) Mechanisms and function of DUOX in epithelia of the lung. Antioxid Redox Signal 11(10):2453–2465. https://doi.org/10.1089/ARS.2009.2558
Fulton DJ (2009) Nox5 and the regulation of cellular function. Antioxid Redox Signal 11 (10):2443–2452. https://doi.org/10.1089/ars.2009.2587
Geiszt M, Kopp JB, Varnai P, Leto TL (2000) Identification of renox, an NAD(P)H oxidase in kidney. Proc Natl Acad Sci U S A 97(14):8010–8014. https://doi.org/10.1073/pnas.130135897
Geiszt M, Lekstrom K, Brenner S, Hewitt SM, Dana R, Malech HL, Leto TL (2003a) NAD(P)H oxidase 1, a product of differentiated colon epithelial cells, can partially replace glycoprotein 91phox in the regulated production of superoxide by phagocytes. J Immunol 171(1):299–306. https://doi.org/10.4049/jimmunol.171.1.299
Geiszt M, Witta J, Baffi J, Lekstrom K, Leto TL (2003b) Dual oxidases represent novel hydrogen peroxide sources supporting mucosal surface host defense. FASEB J 17(11):1502–1504. https://doi.org/10.1096/fj.02-1104fje
Ghanbari H, Keshtgar S, Kazeroni M (2018) Inhibition of the CatSper channel and NOX5 enzyme activity affects the functions of the progesterone-stimulated human sperm. Iran J Med Sci 43 (1):18–25
Giardino G, Cicalese MP, Delmonte O, Migliavacca M, Palterer B, Loffredo L et al (2017) NADPH oxidase deficiency: a multisystem approach. Oxidative Med Cell Longev 2017:4590127. https://doi.org/10.1155/2017/4590127
Gorin Y, Ricono JM, Kim NH, Bhandari B, Choudhury GG, Abboud HE (2003) Nox4 mediates angiotensin II-induced activation of Akt/protein kinase B in mesangial cells. Am J Physiol Renal Physiol 285(2):F219–F229. https://doi.org/10.1152/ajprenal.00414.2002
Gorlach A, Brandes RP, Nguyen K, Amidi M, Dehghani F, Busse R (2000) A gp91phox containing NADPH oxidase selectively expressed in endothelial cells is a major source of oxygen radical generation in the arterial wall. Circ Res 87(1):26–32. https://doi.org/10.1161/01.res.87.1.26

Grasberger H, Refetoff S (2006) Identification of the maturation factor for dual oxidase. Evolution of an eukaryotic operon equivalent. J Biol Chem 281(27):18269–18272. https://doi.org/10.1074/jbc.C600095200

Gray SP, Di Marco E, Okabe J, Szyndralewiez C, Heitz F, Montezano AC et al (2013) NADPH oxidase 1 plays a key role in diabetes mellitus-accelerated atherosclerosis. Circulation 127 (18):1888–1902. https://doi.org/10.1161/CIRCULATIONAHA.112.132159

Gray SP, Di Marco E, Kennedy K, Chew P, Okabe J, El-Osta A et al (2016) Reactive oxygen species can provide atheroprotection via NOX4-dependent inhibition of inflammation and vascular remodeling. Arterioscler Thromb Vasc Biol 36(2):295–307. https://doi.org/10.1161/ATVBAHA.115.307012

Gray SP, Jha JC, Kennedy K, van Bommel E, Chew P, Szyndralewiez C et al (2017) Combined NOX1/4 inhibition with GKT137831 in mice provides dose-dependent reno- and atheroprotection even in established micro- and macrovascular disease. Diabetologia 60 (5):927–937. https://doi.org/10.1007/s00125-017-4215-5

Guo S, Chen X (2015) The human Nox4: gene, structure, physiological function and pathological significance. J Drug Target 23(10):888–896. https://doi.org/10.3109/1061186X.2015.1036276

Guzik TJ, Chen W, Gongora MC, Guzik B, Lob HE, Mangalat D et al (2008) Calcium-dependent NOX5 nicotinamide adenine dinucleotide phosphate oxidase contributes to vascular oxidative stress in human coronary artery disease. J Am Coll Cardiol 52(22):1803–1809. https://doi.org/10.1016/j.jacc.2008.07.063

Guzik B, Sagan A, Ludew D, Mrowiecki W, Chwala M, Bujak-Gizycka B et al (2013) Mechanisms of oxidative stress in human aortic aneurysms--association with clinical risk factors for atherosclerosis and disease severity. Int J Cardiol 168(3):2389–2396. https://doi.org/10.1016/j.ijcard.2013.01.278

Hahn NE, Meischl C, Kawahara T, Musters RJ, Verhoef VM, van der Velden J et al (2012) NOX5 expression is increased in intramyocardial blood vessels and cardiomyocytes after acute myocardial infarction in humans. Am J Pathol 180(6):2222–2229. https://doi.org/10.1016/j.ajpath.2012.02.018

Haque MZ, Majid DS (2004) Assessment of renal functional phenotype in mice lacking gp91PHOX subunit of NAD(P)H oxidase. Hypertension 43(2):335–340. https://doi.org/10.1161/01.HYP.0000111137.15873.4a

Hayes P, Dhillon S, O'Neill K, Thoeni C, Hui KY, Elkadri A et al (2015) Defects in NADPH oxidase genes NOX1 and DUOX2 in very early onset inflammatory bowel disease. Cell Mol Gastroenterol Hepatol 1(5):489–502. https://doi.org/10.1016/j.jcmgh.2015.06.005

He W, Wang Q, Gu L, Zhong L, Liu D (2018) NOX4 rs11018628 polymorphism associates with a decreased risk and better short-term recovery of ischemic stroke. Exp Ther Med 16 (6):5258–5264. https://doi.org/10.3892/etm.2018.6874

Henriquez-Olguin C, Boronat S, Cabello-Verrugio C, Jaimovich E, Hidalgo E, Jensen TE (2019) The emerging roles of nicotinamide adenine dinucleotide phosphate oxidase 2 in skeletal muscle redox signaling and metabolism. Antioxid Redox Signal 31(18):1371–1410. https://doi.org/10.1089/ars.2018.7678

Hilenski LL, Clempus RE, Quinn MT, Lambeth JD, Griendling KK (2004) Distinct subcellular localizations of Nox1 and Nox4 in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 24(4):677–683. https://doi.org/10.1161/01.ATV.0000112024.13727.2c

Hirano K, Chen WS, Chueng AL, Dunne AA, Seredenina T, Filippova A et al (2015) Discovery of GSK2795039, a novel small molecule NADPH oxidase 2 inhibitor. Antioxid Redox Signal 23 (5):358–374. https://doi.org/10.1089/ars.2014.6202

Hoidal JR, Brar SS, Sturrock AB, Sanders KA, Dinger B, Fidone S, Kennedy TP (2003) The role of endogenous NADPH oxidases in airway and pulmonary vascular smooth muscle function. Antioxid Redox Signal 5(6):751–758. https://doi.org/10.1089/152308603770380052

Holterman CE, Thibodeau JF, Towaij C, Gutsol A, Montezano AC, Parks RJ et al (2014) Nephropathy and elevated BP in mice with podocyte-specific NADPH oxidase 5 expression. J Am Soc Nephrol 25(4):784–797. https://doi.org/10.1681/ASN.2013040371

Huang J, Hitt ND, Kleinberg ME (1995) Stoichiometry of p22-phox and gp91-phox in phagocyte cytochrome b558. Biochemistry 34(51):16753–16757. https://doi.org/10.1021/bi00051a024

Ibi M, Katsuyama M, Fan C, Iwata K, Nishinaka T, Yokoyama T, Yabe-Nishimura C (2006) NOX1/NADPH oxidase negatively regulates nerve growth factor-induced neurite outgrowth. Free Radic Biol Med 40(10):1785–1795. https://doi.org/10.1016/j.freeradbiomed.2006.01.009

Iles KE, Forman HJ (2002) Macrophage signaling and respiratory burst. Immunol Res 26 (1–3):95–105. https://doi.org/10.1385/IR:26:1-3:095

Janiszewski M, Lopes LR, Carmo AO, Pedro MA, Brandes RP, Santos CX, Laurindo FR (2005) Regulation of NAD(P)H oxidase by associated protein disulfide isomerase in vascular smooth muscle cells. J Biol Chem 280(49):40813–40819. https://doi.org/10.1074/jbc.M509255200

Jay DB, Papaharalambus CA, Seidel-Rogol B, Dikalova AE, Lassegue B, Griendling KK (2008) Nox5 mediates PDGF-induced proliferation in human aortic smooth muscle cells. Free Radic Biol Med 45(3):329–335. https://doi.org/10.1016/j.freeradbiomed.2008.04.024

Jha JC, Gray SP, Barit D, Okabe J, El-Osta A, Namikoshi T et al (2014) Genetic targeting or pharmacologic inhibition of NADPH oxidase nox4 provides renoprotection in long-term diabetic nephropathy. J Am Soc Nephrol 25(6):1237–1254. https://doi.org/10.1681/ASN.2013070810

Jha JC, Thallas-Bonke V, Banal C, Gray SP, Chow BS, Ramm G et al (2016) Podocyte-specific Nox4 deletion affords renoprotection in a mouse model of diabetic nephropathy. Diabetologia 59(2):379–389. https://doi.org/10.1007/s00125-015-3796-0

Jha JC, Banal C, Okabe J, Gray SP, Hettige T, Chow BSM et al (2017a) NADPH oxidase Nox5 accelerates renal injury in diabetic nephropathy. Diabetes 66(10):2691–2703. https://doi.org/10.2337/db16-1585

Jha JC, Banal C, Okabe J, Gray SP, Hettige T, Chow BSM et al (2017b) NADPH oxidase Nox5 accelerates renal injury in diabetic nephropathy. Diabetes 66(10):2691–2703. https://doi.org/10.2337/db16-1585

Jiang F, Zhang Y, Dusting GJ (2011) NADPH oxidase-mediated redox signaling: roles in cellular stress response, stress tolerance, and tissue repair. Pharmacol Rev 63(1):218–242. https://doi.org/10.1124/pr.110.002980

Jiao W, Ji J, Li F, Guo J, Zheng Y, Li S, Xu W (2019) Activation of the NotchNox4reactive oxygen species signaling pathway induces cell death in high glucose treated human retinal endothelial cells. Mol Med Rep 19(1):667–677. https://doi.org/10.3892/mmr.2018.9637

Juhasz A, Markel S, Gaur S, Liu H, Lu J, Jiang G et al (2017) NADPH oxidase 1 supports proliferation of colon cancer cells by modulating reactive oxygen species-dependent signal transduction. J Biol Chem 292(19):7866–7887. https://doi.org/10.1074/jbc.M116.768283

Kajla S, Mondol AS, Nagasawa A, Zhang Y, Kato M, Matsuno K et al (2012) A crucial role for Nox 1 in redox-dependent regulation of Wnt-beta-catenin signaling. FASEB J 26(5):2049–2059. https://doi.org/10.1096/fj.11-196360

Kang HJ, Bartholomae CC, Paruzynski A, Arens A, Kim S, Yu SS et al (2011a) Retroviral gene therapy for X-linked chronic granulomatous disease: results from phase I/II trial. Mol Ther 19 (11):2092–2101. https://doi.org/10.1038/mt.2011.166

Kang EM, Marciano BE, DeRavin S, Zarember KA, Holland SM, Malech HL (2011b) Chronic granulomatous disease: overview and hematopoietic stem cell transplantation. J Allergy Clin Immunol 127(6):1319–1326.; quiz 1327-1318. https://doi.org/10.1016/j.jaci.2011.03.028

Kato K, Hecker L (2020) NADPH oxidases: pathophysiology and therapeutic potential in age-associated pulmonary fibrosis. Redox Biol 33:101541. https://doi.org/10.1016/j.redox.2020.101541

Kato M, Marumo M, Nakayama J, Matsumoto M, Yabe-Nishimura C, Kamata T (2016) The ROS-generating oxidase Nox1 is required for epithelial restitution following colitis. Exp Anim 65(3):197–205. https://doi.org/10.1538/expanim.15-0127

Keller MD, Notarangelo LD, Malech HL (2018) Future of care for patients with chronic granulomatous disease: gene therapy and targeted molecular medicine. J Pediatric Infect Dis Soc 7 (suppl_1):S40–S44. https://doi.org/10.1093/jpids/piy011

Kishida KT, Hoeffer CA, Hu D, Pao M, Holland SM, Klann E (2006) Synaptic plasticity deficits and mild memory impairments in mouse models of chronic granulomatous disease. Mol Cell Biol 26(15):5908–5920. https://doi.org/10.1128/MCB.00269-06

Kleikers PW, Hooijmans C, Gob E, Langhauser F, Rewell SS, Radermacher K et al (2015) A combined pre-clinical meta-analysis and randomized confirmatory trial approach to improve data validity for therapeutic target validation. Sci Rep 5:13428. https://doi.org/10.1038/srep13428

Kleinschnitz C, Grund H, Wingler K, Armitage ME, Jones E, Mittal M et al (2010) Post-stroke inhibition of induced NADPH oxidase type 4 prevents oxidative stress and neurodegeneration. PLoS Biol 8(9):e1000479. https://doi.org/10.1371/journal.pbio.1000479

Ko E, Choi H, Kim B, Kim M, Park KN, Bae IH et al (2014) Testosterone stimulates Duox1 activity through GPRC6A in skin keratinocytes. J Biol Chem 289(42):28835–28845. https://doi.org/10.1074/jbc.M114.583450

Kohn DB, Booth C, Kang EM, Pai SY, Shaw KL, Santilli G et al (2020) Lentiviral gene therapy for X-linked chronic granulomatous disease. Nat Med 26(2):200–206. https://doi.org/10.1038/s41591-019-0735-5

Kouroku Y, Fujita E, Tanida I, Ueno T, Isoai A, Kumagai H et al (2007) ER stress (PERK/eIF2alpha phosphorylation) mediates the polyglutamine-induced LC3 conversion, an essential step for autophagy formation. Cell Death Differ 14(2):230–239. https://doi.org/10.1038/sj.cdd.4401984

Krijnen PA, Meischl C, Hack CE, Meijer CJ, Visser CA, Roos D, Niessen HW (2003) Increased Nox2 expression in human cardiomyocytes after acute myocardial infarction. J Clin Pathol 56(3):194–199. https://doi.org/10.1136/jcp.56.3.194

Lan T, Kisseleva T, Brenner DA (2015) Deficiency of NOX1 or NOX4 prevents liver inflammation and fibrosis in mice through inhibition of hepatic stellate cell activation. PLoS One 10(7):e0129743. https://doi.org/10.1371/journal.pone.0129743

Lassegue B, Sorescu D, Szocs K, Yin Q, Akers M, Zhang Y et al (2001) Novel gp91(phox) homologues in vascular smooth muscle cells: nox1 mediates angiotensin II-induced superoxide formation and redox-sensitive signaling pathways. Circ Res 88(9):888–894. https://doi.org/10.1161/hh0901.090299

Laurent E, McCoy JW 3rd, Macina RA, Liu W, Cheng G, Robine S et al (2008) Nox1 is over-expressed in human colon cancers and correlates with activating mutations in K-Ras. Int J Cancer 123(1):100–107. https://doi.org/10.1002/ijc.23423

Lee SR, An EJ, Kim J, Bae YS (2020) Function of NADPH oxidases in diabetic nephropathy and development of nox inhibitors. Biomol Ther 28(1):25–33. https://doi.org/10.4062/biomolther.2019.188

Li T, Luo XJ, Wang EL, Li NS, Zhang XJ, Song FL et al (2019) Magnesium lithospermate B prevents phenotypic transformation of pulmonary arteries in rats with hypoxic pulmonary hypertension through suppression of NADPH oxidase. Eur J Pharmacol 847:32–41. https://doi.org/10.1016/j.ejphar.2019.01.020

Lin XL, Yang L, Fu SW, Lin WF, Gao YJ, Chen HY, Ge ZZ (2017) Overexpression of NOX4 predicts poor prognosis and promotes tumor progression in human colorectal cancer. Oncotarget 8(20):33586–33600. https://doi.org/10.18632/oncotarget.16829

Lu X, Wang F, Liu M, Yang KT, Nau A, Kohan DE et al (2016) Activation of ENaC in collecting duct cells by prorenin and its receptor PRR: involvement of Nox4-derived hydrogen peroxide. Am J Physiol Renal Physiol 310(11):F1243–F1250. https://doi.org/10.1152/ajprenal.00492.2015

Lyle AN, Deshpande NN, Taniyama Y, Seidel-Rogol B, Pounkova L, Du P et al (2009) Poldip2, a novel regulator of Nox4 and cytoskeletal integrity in vascular smooth muscle cells. Circ Res 105(3):249–259. https://doi.org/10.1161/CIRCRESAHA.109.193722

Mahadev K, Motoshima H, Wu X, Ruddy JM, Arnold RS, Cheng G et al (2004) The NAD(P)H oxidase homolog Nox4 modulates insulin-stimulated generation of H2O2 and plays an integral

role in insulin signal transduction. Mol Cell Biol 24(5):1844–1854. https://doi.org/10.1128/mcb.24.5.1844-1854.2004

Manea A, Raicu M, Simionescu M (2005) Expression of functionally phagocyte-type NAD(P)H oxidase in pericytes: effect of angiotensin II and high glucose. Biol Cell 97(9):723–734. https://doi.org/10.1042/BC20040107

Manea A, Manea SA, Gan AM, Constantin A, Fenyo IM, Raicu M et al (2015) Human monocytes and macrophages express NADPH oxidase 5; a potential source of reactive oxygen species in atherosclerosis. Biochem Biophys Res Commun 461(1):172–179. https://doi.org/10.1016/j.bbrc.2015.04.021

Marzaioli V, Hurtado-Nedelec M, Pintard C, Tlili A, Marie JC, Monteiro RC et al (2017) NOX5 and p22phox are 2 novel regulators of human monocytic differentiation into dendritic cells. Blood 130(15):1734–1745. https://doi.org/10.1182/blood-2016-10-746347

Matsushima S, Kuroda J, Ago T, Zhai P, Park JY, Xie LH et al (2013) Increased oxidative stress in the nucleus caused by Nox4 mediates oxidation of HDAC4 and cardiac hypertrophy. Circ Res 112(4):651–663. https://doi.org/10.1161/CIRCRESAHA.112.279760

Meitzler JL, Ortiz de Montellano PR (2009) Caenorhabditis elegans and human dual oxidase 1 (DUOX1) "peroxidase" domains: insights into heme binding and catalytic activity. J Biol Chem 284(28):18634–18643. https://doi.org/10.1074/jbc.M109.013581

Meitzler JL, Ortiz de Montellano PR (2011) Structural stability and heme binding potential of the truncated human dual oxidase 2 (DUOX2) peroxidase domain. Arch Biochem Biophys 512(2):197–203. https://doi.org/10.1016/j.abb.2011.05.021

Miller CH, Maher SG, Young HA (2009) Clinical use of interferon-gamma. Ann N Y Acad Sci 1182:69–79. https://doi.org/10.1111/j.1749-6632.2009.05069.x

Moll F, Walter M, Rezende F, Helfinger V, Vasconez E, De Oliveira T et al (2018) NoxO1 controls proliferation of colon epithelial cells. Front Immunol 9:973. https://doi.org/10.3389/fimmu.2018.00973

Mongue-Din H, Patel AS, Looi YH, Grieve DJ, Anilkumar N, Sirker A et al (2017) NADPH oxidase-4 driven cardiac macrophage polarization protects against myocardial infarction-induced remodeling. JACC Basic Transl Sci 2(6):688–698. https://doi.org/10.1016/j.jacbts.2017.06.006

Montezano AC, De Lucca Camargo L, Persson P, Rios FJ, Harvey AP, Anagnostopoulou A et al (2018) NADPH oxidase 5 is a pro-contractile nox isoform and a point of cross-talk for calcium and redox signaling-implications in vascular function. J Am Heart Assoc 7(12):e009388. https://doi.org/10.1161/JAHA.118.009388

Moreno JC, Bikker H, Kempers MJ, van Trotsenburg AS, Baas F, de Vijlder JJ et al (2002) Inactivating mutations in the gene for thyroid oxidase 2 (THOX2) and congenital hypothyroidism. N Engl J Med 347(2):95–102. https://doi.org/10.1056/NEJMoa012752

Nakano Y, Banfi B, Jesaitis AJ, Dinauer MC, Allen LA, Nauseef WM (2007) Critical roles for p22phox in the structural maturation and subcellular targeting of Nox3. Biochem J 403(1):97–108. https://doi.org/10.1042/BJ20060819

Nlandu Khodo S, Dizin E, Sossauer G, Szanto I, Martin PY, Feraille E et al (2012) NADPH-oxidase 4 protects against kidney fibrosis during chronic renal injury. J Am Soc Nephrol 23(12):1967–1976. https://doi.org/10.1681/ASN.2012040373

O'Neill S, Brault J, Stasia MJ, Knaus UG (2015) Genetic disorders coupled to ROS deficiency. Redox Biol 6:135–156. https://doi.org/10.1016/j.redox.2015.07.009

Paffenholz R, Bergstrom RA, Pasutto F, Wabnitz P, Munroe RJ, Jagla W et al (2004) Vestibular defects in head-tilt mice result from mutations in Nox3, encoding an NADPH oxidase. Genes Dev 18(5):486–491. https://doi.org/10.1101/gad.1172504

Panday A, Sahoo MK, Osorio D, Batra S (2015) NADPH oxidases: an overview from structure to innate immunity-associated pathologies. Cell Mol Immunol 12(1):5–23. https://doi.org/10.1038/cmi.2014.89

Pao M, Wiggs EA, Anastacio MM, Hyun J, DeCarlo ES, Miller JT et al (2004) Cognitive function in patients with chronic granulomatous disease: a preliminary report. Psychosomatics 45 (3):230–234. https://doi.org/10.1176/appi.psy.45.3.230

Parkos CA, Allen RA, Cochrane CG, Jesaitis AJ (1987) Purified cytochrome b from human granulocyte plasma membrane is comprised of two polypeptides with relative molecular weights of 91,000 and 22,000. J Clin Invest 80(3):732–742. https://doi.org/10.1172/JCI113128

Perrotta I, Sciangula A, Perrotta E, Donato G, Cassese M (2011) Ultrastructural analysis and electron microscopic localization of Nox4 in healthy and atherosclerotic human aorta. Ultrastruct Pathol 35(1):1–6. https://doi.org/10.3109/01913123.2010.510261

Plecita-Hlavata L, Jaburek M, Holendova B, Tauber J, Pavluch V, Berkova Z et al (2020) Glucose-stimulated insulin secretion fundamentally requires H2O2 signaling by NADPH oxidase 4. Diabetes 7(12):e009388. https://doi.org/10.2337/db19-1130

Reinehr R, Becker S, Eberle A, Grether-Beck S, Haussinger D (2005) Involvement of NADPH oxidase isoforms and Src family kinases in CD95-dependent hepatocyte apoptosis. J Biol Chem 280(29):27179–27194. https://doi.org/10.1074/jbc.M414361200

Rezende F, Schurmann C, Schutz S, Harenkamp S, Herrmann E, Seimetz M et al (2017) Knock out of the NADPH oxidase Nox4 has no impact on life span in mice. Redox Biol 11:312–314. https://doi.org/10.1016/j.redox.2016.12.012

Rigutto S, Hoste C, Grasberger H, Milenkovic M, Communi D, Dumont JE et al (2009) Activation of dual oxidases Duox1 and Duox2: differential regulation mediated by camp-dependent protein kinase and protein kinase C-dependent phosphorylation. J Biol Chem 284(11):6725–6734. https://doi.org/10.1074/jbc.M806893200

Rokutan K, Kawahara T, Kuwano Y, Tominaga K, Sekiyama A, Teshima-Kondo S (2006) NADPH oxidases in the gastrointestinal tract: a potential role of Nox1 in innate immune response and carcinogenesis. Antioxid Redox Signal 8(9–10):1573–1582. https://doi.org/10.1089/ars.2006.8.1573

Rossi F, Zatti M (1964) Biochemical aspects of phagocytosis in polymorphonuclear leucocytes. NADH and NADPH oxidation by the granules of resting and phagocytizing cells. Experientia 20(1):21–23. https://doi.org/10.1007/bf02146019

Rybak LP, Mukherjea D, Jajoo S, Kaur T, Ramkumar V (2012) siRNA-mediated knock-down of NOX3: therapy for hearing loss? Cell Mol Life Sci 69(14):2429–2434. https://doi.org/10.1007/s00018-012-1016-3

Scherz-Shouval R, Elazar Z (2011) Regulation of autophagy by ROS: physiology and pathology. Trends Biochem Sci 36(1):30–38. https://doi.org/10.1016/j.tibs.2010.07.007

Schroder K, Wandzioch K, Helmcke I, Brandes RP (2009) Nox4 acts as a switch between differentiation and proliferation in preadipocytes. Arterioscler Thromb Vasc Biol 29 (2):239–245. https://doi.org/10.1161/ATVBAHA.108.174219

Schroder K, Zhang M, Benkhoff S, Mieth A, Pliquett R, Kosowski J et al (2012) Nox4 is a protective reactive oxygen species generating vascular NADPH oxidase. Circ Res 110 (9):1217–1225. https://doi.org/10.1161/circresaha.112.267054

Schurmann C, Rezende F, Kruse C, Yasar Y, Lowe O, Fork C et al (2015) The NADPH oxidase Nox4 has anti-atherosclerotic functions. Eur Heart J 36(48):3447–3456. https://doi.org/10.1093/eurheartj/ehv460

Schwarzer C, Machen TE, Illek B, Fischer H (2004) NADPH oxidase-dependent acid production in airway epithelial cells. J Biol Chem 279(35):36454–36461. https://doi.org/10.1074/jbc.M404983200

Schwerd T, Bryant RV, Pandey S, Capitani M, Meran L, Cazier JB et al (2018) NOX1 loss-of-function genetic variants in patients with inflammatory bowel disease. Mucosal Immunol 11 (2):562–574. https://doi.org/10.1038/mi.2017.74

Segal AW, Jones OT (1978) Novel cytochrome b system in phagocytic vacuoles of human granulocytes. Nature 276(5687):515–517. https://doi.org/10.1038/276515a0

Seredenina T, Chiriano G, Filippova A, Nayernia Z, Mahiout Z, Fioraso-Cartier L et al (2015) A subset of N-substituted phenothiazines inhibits NADPH oxidases. Free Radic Biol Med 86:239–249. https://doi.org/10.1016/j.freeradbiomed.2015.05.023

Seredenina T, Nayernia Z, Sorce S, Maghzal GJ, Filippova A, Ling SC et al (2016) Evaluation of NADPH oxidases as drug targets in a mouse model of familial amyotrophic lateral sclerosis. Free Radic Biol Med 97:95–108. https://doi.org/10.1016/j.freeradbiomed.2016.05.016

Suh YA, Arnold RS, Lassegue B, Shi J, Xu X, Sorescu D et al (1999) Cell transformation by the superoxide-generating oxidase Mox1. Nature 401(6748):79–82. https://doi.org/10.1038/43459

Sun Q, Zhang W, Zhong W, Sun X, Zhou Z (2017) Pharmacological inhibition of NOX4 ameliorates alcohol-induced liver injury in mice through improving oxidative stress and mitochondrial function. Biochim Biophys Acta Gen Subj 1861(1 Pt A):2912–2921. https://doi.org/10.1016/j.bbagen.2016.09.009

Szanto I, Rubbia-Brandt L, Kiss P, Steger K, Banfi B, Kovari E et al (2005) Expression of NOX1, a superoxide-generating NADPH oxidase, in colon cancer and inflammatory bowel disease. J Pathol 207(2):164–176. https://doi.org/10.1002/path.1824

Taniguchi CM, Emanuelli B, Kahn CR (2006) Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol 7(2):85–96. https://doi.org/10.1038/nrm1837

Teshima S, Kutsumi H, Kawahara T, Kishi K, Rokutan K (2000) Regulation of growth and apoptosis of cultured Guinea pig gastric mucosal cells by mitogenic oxidase 1. Am J Physiol Gastrointest Liver Physiol 279(6):G1169–G1176. https://doi.org/10.1152/ajpgi.2000.279.6.G1169

Thornhill SI, Schambach A, Howe SJ, Ulaganathan M, Grassman E, Williams D et al (2008) Self-inactivating gammaretroviral vectors for gene therapy of X-linked severe combined immunodeficiency. Mol Ther 16(3):590–598. https://doi.org/10.1038/sj.mt.6300393

Touyz RM, Anagnostopoulou A, Camargo LL, Rios FJ, Montezano AC (2019) Vascular biology of superoxide-generating NADPH oxidase 5-implications in hypertension and cardiovascular disease. Antioxid Redox Signal 30(7):1027–1040. https://doi.org/10.1089/ars.2018.7583

Ueno N, Takeya R, Miyano K, Kikuchi H, Sumimoto H (2005) The NADPH oxidase Nox3 constitutively produces superoxide in a p22phox-dependent manner: its regulation by oxidase organizers and activators. J Biol Chem 280(24):23328–23339. https://doi.org/10.1074/jbc.M414548200

Ueyama T, Geiszt M, Leto TL (2006) Involvement of Rac1 in activation of multicomponent Nox1- and Nox3-based NADPH oxidases. Mol Cell Biol 26(6):2160–2174. https://doi.org/10.1128/MCB.26.6.2160-2174.2006

Van Buul JD, Fernandez-Borja M, Anthony EC, Hordijk PL (2005) Expression and localization of NOX2 and NOX4 in primary human endothelial cells. Antioxid Redox Signal 7(3–4):308–317. https://doi.org/10.1089/ars.2005.7.308

van der Vliet A, Danyal K, Heppner DE (2018) Dual oxidase: a novel therapeutic target in allergic disease. Br J Pharmacol 175(9):1401–1418. https://doi.org/10.1111/bph.14158

Veal E, Day A (2011) Hydrogen peroxide as a signaling molecule. Antioxid Redox Signal 15 (1):147–151. https://doi.org/10.1089/ars.2011.3968

Volpp BD, Nauseef WM, Clark RA (1988) Two cytosolic neutrophil oxidase components absent in autosomal chronic granulomatous disease. Science 242(4883):1295–1297. https://doi.org/10.1126/science.2848318

Wang D, De Deken X, Milenkovic M, Song Y, Pirson I, Dumont JE, Miot F (2005) Identification of a novel partner of duox: EFP1, a thioredoxin-related protein. J Biol Chem 280(4):3096–3103. https://doi.org/10.1074/jbc.M407709200

Wang X, Elksnis A, Wikstrom P, Walum E, Welsh N, Carlsson PO (2018) The novel NADPH oxidase 4 selective inhibitor GLX7013114 counteracts human islet cell death in vitro. PLoS One 13(9):e0204271. https://doi.org/10.1371/journal.pone.0204271

Wang FT, Hassan M, Ansari KH, Xu GL, Li XP, Fan YZ (2019) Upregulated NOX1 expression in gallbladder cancer-associated fibroblasts predicts a poor prognosis. Oncol Rep 42 (4):1475–1486

Wientjes FB, Hsuan JJ, Totty NF, Segal AW (1993) p40phox, a third cytosolic component of the activation complex of the NADPH oxidase to contain src homology 3 domains. Biochem J 296 (Pt 3):557–561. https://doi.org/10.1042/bj2960557

Wilkinson-Berka JL, Deliyanti D, Rana I, Miller AG, Agrotis A, Armani R et al (2013) NADPH oxidase, NOX1, mediates vascular injury in ischemic retinopathy. Antioxid Redox Signal 20 (17):2726–2740. https://doi.org/10.1089/ars.2013.5357

Wilkinson-Berka JL, Deliyanti D, Rana I, Miller AG, Agrotis A, Armani R et al (2014) NADPH oxidase, NOX1, mediates vascular injury in ischemic retinopathy. Antioxid Redox Signal 20 (17):2726–2740. https://doi.org/10.1089/ars.2013.5357

Won SJ, Tang XN, Suh SW, Yenari MA, Swanson RA (2011) Hyperglycemia promotes tissue plasminogen activator-induced hemorrhage by increasing superoxide production. Ann Neurol 70(4):583–590. https://doi.org/10.1002/ana.22538

Woo HA, Yim SH, Shin DH, Kang D, Yu DY, Rhee SG (2010) Inactivation of peroxiredoxin I by phosphorylation allows localized H(2)O(2) accumulation for cell signaling. Cell 140 (4):517–528. https://doi.org/10.1016/j.cell.2010.01.009

Wu RF, Ma Z, Liu Z, Terada LS (2010) Nox4-derived H2O2 mediates endoplasmic reticulum signaling through local Ras activation. Mol Cell Biol 30(14):3553–3568. https://doi.org/10.1128/MCB.01445-09

Xia D, Halder B, Godoy C, Chakraborty A, Singla B, Thomas E et al (2019) NADPH oxidase 1 mediates caerulein-induced pancreatic fibrosis in chronic pancreatitis. Free Radic Biol Med 147:139–149. https://doi.org/10.1016/j.freeradbiomed.2019.11.034

Yin C, Li K, Yu Y, Huang H, Yu Y, Wang Z et al (2018) Genome-wide association study identifies loci and candidate genes for non-idiopathic pulmonary hypertension in Eastern Chinese Han population. BMC Pulm Med 18(1):158. https://doi.org/10.1186/s12890-018-0719-0

Yoshikawa Y, Ago T, Kuroda J, Wakisaka Y, Tachibana M, Komori M et al (2019) Nox4 promotes neural stem/precursor cell proliferation and neurogenesis in the hippocampus and restores memory function following trimethyltin-induced injury. Neuroscience 398:193–205. https://doi.org/10.1016/j.neuroscience.2018.11.046

Yu P, Han W, Villar VA, Yang Y, Lu Q, Lee H et al (2014) Unique role of NADPH oxidase 5 in oxidative stress in human renal proximal tubule cells. Redox Biol 2:570–579. https://doi.org/10.1016/j.redox.2014.01.020

Zeng SY, Yang L, Yan QJ, Gao L, Lu HQ, Yan PK (2019) Nox1/4 dual inhibitor GKT137831 attenuates hypertensive cardiac remodelling associating with the inhibition of ADAM17-dependent proinflammatory cytokines-induced signalling pathways in the rats with abdominal artery constriction. Biomed Pharmacother 109:1907–1914. https://doi.org/10.1016/j.biopha.2018.11.077

Zhang Y, Murugesan P, Huang K, Cai H (2019) NADPH oxidases and oxidase crosstalk in cardiovascular diseases: novel therapeutic targets. Nat Rev Cardiol 17(3):170–194. https://doi.org/10.1038/s41569-019-0260-8

Zhao QD, Viswanadhapalli S, Williams P, Shi Q, Tan C, Yi X et al (2015) NADPH oxidase 4 induces cardiac fibrosis and hypertrophy through activating Akt/mTOR and NFkappaB signaling pathways. Circulation 131(7):643–655. https://doi.org/10.1161/CIRCULATIONAHA.114.011079

Zhao T, Wang Y, Li Z, Xu X, Lei S, Huang L et al (2020) Associations of noise kurtosis, genetic variations in NOX3 and lifestyle factors with noise-induced hearing loss. Environ Health 19 (1):13. https://doi.org/10.1186/s12940-020-0566-3