Atherosclerosis is the key component of most cardiovascular diseases, including stroke and myocardial infarction.1–3 An inflamed endothelium recruits inflammatory cells, such as monocytes, via the expression of various mediators and chemokines.4–6 This, in addition to the accumulation of such as monocytes, via the expression of various mediators as nonradical molecules, such as hydrogen peroxide (H$_2$O$_2$).

myeloperoxidase,21 xanthine oxidase (XO),22 and importantly oxygen and nitrogen species play a fundamental role in vascular cell homeostasis and eventually affects the development of atherosclerosis.14 Fine-tuning of cellular redox status is a prerequisite for the well-being of vascular system. Although too much oxidative stress can be detrimental, some basal levels are crucial for proper cell signaling. Recently, a number of publications in ATVB and other journals have demonstrated substantial progress in research into oxidative stress vascular disease, especially atherosclerosis.14–17 In the present article, we highlight these updated publications, providing insights into the mechanisms of reactive oxygen species (ROS) generation in pathophysiological conditions of the vessel wall, and the contribution of redox imbalance to lesion formation via influencing vascular cell (dys)functions.

Oxidative stress is defined as a cellular condition where the damaging effect of oxidant is greater than the beneficial effect of antioxidants. Major oxidants are based on O$_2$ molecules, which are taken in during respiration, with higher reactivity than molecular O$_2$, and are known as ROS.14 ROS are, thus, broadly defined as oxygen-containing chemical species with higher reactive properties. Major ROS include superoxide (O$_2^−$) and hydroxyl (HO$^·$) free radicals, as well as nonradical molecules, such as hydrogen peroxide (H$_2$O$_2$).

Primary sources of oxidative stress in vessel wall are mitochondria,18 uncoupled nitric oxide synthase,19 lipoxygenase,20 myeloperoxidase,21 xanthine oxidase (XO),22 and importantly NAD(P)H oxidases23 (Figure 1). However, the influence of ROS-producing enzymes, especially NADPH oxidases, in the development of atherosclerosis is ambiguous.23 Nox4-derived mitochondrial ROS were detrimental in older mice with atherosclerosis;24 and dominant negative mutant form of Nox4 decreased atherosclerosis formation.25 On the other hand, Nox4 knockout aggravates atherosclerosis,26 especially in diabetic mice.27

High levels of oxidative stress can be counteracted by complex antioxidant cell systems that are crucial for the maintenance of redox balance. The key player that plays a primary role in the regulation of antioxidant gene response is nuclear factor erythroid 2–related factor 2 (Nrf2),28 encoded by the Nfe2l2 gene. However, depending on the mouse model used and cell type analyzed or even sex of animals, Nrf2 showed both pro- and antiatherogenic properties.29 Wire injury induces higher neoimotima formation in Nfe2l2−/− mice than in control animals.30,31 Global knockout of Nfe2l2 in Apoe−/− mice decreased the formation of atherosclerotic lesions.32–35 Transplantation of Nfe2l2−/− bone marrow to Apoe−/− or Ldr−/− recipients attenuated atherosclerosis, what underlines proatherogenic activity of Nrf2 in myeloid cells. However, increased formation of plaque in Ldr−/− mice transplanted with Nfe2l2−/− bone marrow was also reported.37 On the other hand, activation of Nrf2 in SMCs38,39 or ECs40 was protective against atherosclerosis. Furthermore, knockouts of potent antioxidant enzymes, for example, Gpx1,41 Prdx2,42 Hmox1,43 can also aggravate plaque formation. Importantly, basal levels of ROS were crucial for the activation of endoplasmic reticulum (ER) stress response,44 maintenance of SMC contractile phenotype,45 or differentiation of SMC from stem cells.46,47 Thus, the regulation of oxidative stress is complex, and investigation of its role in the pathogenesis of atherosclerosis remains an important subject of many studies. Therefore, the aim of this article is to summarize the latest advances in the research on the role of oxidative stress in the modulation of cells that can affect the development of atherosclerosis.

**Endothelial Cells**

The healthy endothelium is key for the functional maintenance of vascular system.48,49 Sustained ROS levels can contribute to the endothelial dysfunction, and further to its senescence and activation of an inflammatory response, and in turn lead to the development of atherosclerosis. NADPH oxidases, especially Nox4, play an ambiguous role in the development of atherosclerosis. Craige et al50 reported recently that Apoe−/− mice with endothelial-specific Nox4 overexpression (Apoe−/− Nox4$^{OE}$) showed significantly smaller lesions than control Apoe−/− animals. Apoe−/− Nox4$^{OE}$ aortas contained, however, similar numbers of macrophages and did not differ from Apoe−/− mice in expressions of macrophage or inflammatory markers, that is, E- and P-selectin, VCAM1 (vascular
cell adhesion molecule 1), and ICAM1 (intercellular adhesion molecule 1). On the contrary, Apoe−/− Nox4−/− aortas contained higher Treg numbers, while numbers of effector T cells and plasma CXCL9 (chemokine [C-X-C motif] ligand 9) concentration were lower than in Apoe−/− controls. Both mouse lung ECs and human aortic ECs overexpressing Nox4 showed attenuated CXCL9 induction after interferon-γ.50

In another study, downregulation of Nox4 in human aortic ECs, cultured under high glucose conditions, resulted in the increase of profibrotic CTGF (connective tissue growth factor), while endothelial H2O2 decreased.51 On the other hand, Nox4 overexpression caused a significant reduction in p-SMAD3 (phosphorylated mothers against decapentaplegic homolog 3). In diabetic Apoe−/− mice, aortic expression of both Nox1 and Nox4 increases after 10 weeks of diabetes mellitus. Further development of atherosclerosis in diabetic Apoe−/− mice led to the decline in Nox4 expression, while levels of Nox1 remained high. Importantly, Nox4 expression in carotid plaques is lower in patients with cardiovascular events or diabetes mellitus. Nox4 deletion in diabetic Apoe−/− mice increased, while deletion of Nox1 in diabetic Apoe−/− attenuated plaque formation in comparison to control diabetic Apoe−/− mice. Nox4-deficient Apoe−/− diabetic mice showed lower levels of aortic H2O2 while superoxide levels were increased. Nox1 deletion resulted in a decrease in both superoxide and tyrosine nitration. Furthermore, diabetic Nox4−/− Apoe−/− showed elevated serum and aortic MCP-1 (monocyte chemotactic protein 1) concentrations, as well as macrophage accumulation in the vessel wall. Nox4 knockdown also led to the increase of fibrillar collagens I and III in plaques, which was associated with elevated transforming growth factor-β expression and p-SMAD3 levels.51

Nox4 and H2O2 regulate the response to ER stress, which is crucial for the induction of unfolded protein response.44 Importantly, both ER stress and response to it are activated during the development of atherosclerosis.52 Wu et al44 showed that tunicamycin-induced ER stress elevated H2O2 in ER in the Nox4-dependent way. Increased H2O2 concentrations led in turn to the oxidation of sarco/endoplasmic reticulum Ca2+-ATPase and an increase in cytosolic calcium concentration. High levels of Ca2+ caused next the activation of RasGRF (Ras-specific guanine nucleotide releasing factor), which then induced via Ras UPR (unfolded protein response), the unspliced XBP1 (X-box-binding protein 1).53 However, while spliced XBP1 (XPB1s), which positively regulates UPR, contributes to EC apoptosis and atherosclerosis formation, the unspliced XBP1 (XBPlu) can induce the antioxidant response.54 Interestingly, disturbed flow elevated expression of antioxidant genes in ECs in XBPlu- and HDAC3 (histone deacetylase 3)-dependent manner. Increased expression of XBPlu and HDAC3 in HUVECs has decreased with KDR (kinase insert domain receptor) or PI3K (phosphatidylinositol-4,5-bisphosphate 3-kinase) inhibition. What is more, spliced XBPlu (XPB1s), which positively regulates UPR, contributes to EC apoptosis and atherosclerosis formation, the unspliced XBPlu (XBPlu) can induce the antioxidant response.54 Interestingly, disturbed flow elevated expression of antioxidant genes in ECs in XBPlu- and HDAC3 (histone deacetylase 3)-dependent manner. Increased expression of XBPlu and HDAC3 in HUVECs has decreased with KDR (kinase insert domain receptor) or PI3K (phosphatidylinositol-4,5-bisphosphate 3-kinase) inhibition. What is more, spliced XBPlu induction was mediated by the increased stability of Nrf2 transcription factor and upregulation of its target—Hmox154 (Figure 2B).

Laminar shear stress increases expression of transcription factor EB (TFEB) in cultured human ECs.55 TFEB is a basic helix–loop–helix transcription factor, which regulates lysosomal biogenesis.56 Laminar shear stress induced TFEB translocation to the nucleus and decreased mTOR (mechanistic target of rapamycin) activity in ECs. Interestingly, atherosclerosis-resistant regions of mouse aorta had higher TFEB levels than atherosclerosis-prone ones. TFEB overexpression reduced levels of H2O2 and superoxide, as well as expression of SELE, MCP1, VCAM1, IL1B, IL6, and IL8, in ECs and human coronary artery ECs. ECs overexpressing TFEB also had a higher expression of HMOX1, SOD2, and TXN1. Importantly, TFEB can bind to its putative binding sites in the HMOX1 promoter and SOD2 intron 2. However, knockdown only of HMOX1 in TFEB-overexpressing EC potentently attenuated the anti-inflammatory effect of TFEB. Furthermore, although TFEB is known to regulate autophagy,56 ATG5 silencing or pharmacological inhibition of autophagy did not block TFEB anti-inflammatory activity. mTie2-TFEB mice overexpressing TFEB in ECs showed less leukocyte rolling after lipopolysaccharide treatment than wild-type (WT) animals. Finally, Apoe−/− mTie2-TFEB mice on high cholesterol diet had less atherosclerotic lesions than Apoe−/− control animals.55

Nrf2 in steady state conditions is sequestered by Keap1 and directed to the proteasomal degradation.57 Therefore, the regulation of Nrf2 activity is of potential clinical interest.
Recent work by Xie et al\textsuperscript{58} showed that hydrogen sulfide could enhance Nrf2 activity in diabetic atherosclerotic mice and ECs treated with high glucose and oxidized low-density lipoproteins (LDLs). Streptozotocin-treated \textit{Ldlr}\textsuperscript{−/−} mice on a high-fat diet had less atherosclerotic lesions when treated with GYY4137—H\textsubscript{2}S donor. H\textsubscript{2}S treatment reduced ROS levels and VCAM1 and ICAM1 expressions and enhanced Nrf2 nuclear translocation in both diabetic atherosclerotic mice and ECs treated with high glucose and oxidized LDLs. H\textsubscript{2}S did not show the protective effect in \textit{Ldlr}\textsuperscript{−/−} \textit{Nfe2l2}−/− mice or in ECs with silenced \textit{Nfe2l2}. Interestingly, H\textsubscript{2}S enhanced Nrf2 activity by S-sulphydrylation of Keap1 at Cys151.\textsuperscript{58}

Interestingly, lysophosphatidylcholines (LPC)—proinflammatory lipids—can contribute to the ROS formation in atherosclerosis.\textsuperscript{59} The concentration of LPC, as well as expression of \textit{Pla2g7} and \textit{Pla2g4c}, were higher in \textit{Apoe}−/− aortas than in control ones. LPC quickly induced both mitochondrial ROS and to lesser levels cytoplasmatic ROS. Inhibition of NADPH oxidases further increased ROS levels. LPC increased cytoplasmatic and mitochondrial Ca\textsuperscript{2+}, what was crucial for the induction of mitochondrial ROS production. LPC elevated mitochondrial proton leak but not ATP production and did not affect levels of mitochondrial superoxide dismutase or uncoupling protein 3. Furthermore, LPC increased

Figure 2. Endoplasmic reticulum (ER) stress leads to the activation of Nox4 on ER surface but not cell membrane and increases H\textsubscript{2}O\textsubscript{2} levels. Increased oxidative stress inactivates sarco/endoplasmic reticulum Ca\textsuperscript{2+}~ATPase (SERCA), what causes the increase of cytoplasmatic Ca\textsuperscript{2+}; activation of RasGRF, and Ras. Ras induces then unfolded protein response, namely BiP (binding immunoglobulin protein), CHOP (CCAT enhancer-binding protein homologous protein), and phosphorylation of elf2\textsubscript{α} (A). Disturbed flow causes ligand-independent activation of KDR, which induces formation of mTORC2-Akt1-XBP1u-HDAC3 complex and increases Akt1 phosphorylation, what contributes to the enhances stability of nuclear factor erythroid 2–related factor 2 (Nrf2). Nrf2, the regulator of antioxidant response, induces HMOX1 expression, which protects endothelial cells from oxidative stress (B).
expression of proatherogenic genes, such as ICAM1, IL6, and MMP2, what was attenuated by mitoTEMPO—mitochondrial ROS scavenger. Finally, mitoTEMPO inhibited LPC-induced monocyte adhesion to ECs in vitro, and leukocyte rolling and numbers of total and inflammatory monocytes in Apoe−/− high-fat diet mice.29

Mitochondrial oxidative stress can be decreased by thioredoxin 2 (Txn2) that acts together with thioredoxin reductase 2 (Txnrd2) and peroxiredoxin 3 (Prdx3).60 Importantly, the endothelial-specific knockout of Txnrd2 impaired angiogenesis and arteriogenesis after femoral artery ligation and vascular function.61 Lack of Txnrd2 in ECs rendered them more proinflammatory and prothrombotic. Embryonic endothelial progenitor cells deficient in Txnrd2 showed higher intracellular ROS levels and more positive mitochondrial potential. Such cells had also decreased angiogenic properties in vitro, what could be reversed by Txnrd2 re-expression.61 Therefore, the imbalance of redox-mediated signaling and the consequence results in endothelial dysfunction that is a key event for the development of atherosclerosis.

Smooth Muscle Cells

Fine-tuning of SMC proliferation and differentiation status is crucial for the development of atherosclerosis62 because they can contribute to the formation of neointima but, on the other hand, can stabilize the plaque and prevent its rupture.11,63 Oxidative stress in atherosclerosis results primarily from the activity of NAD(P)H oxidases.64 However, their role in SMCs, especially in the context of atherosclerosis, remains ambiguous. Mice lacking Nox1 developed less neointima in femoral arteries after the wire injury than control animals.65 On the other hand, SMC-specific overexpression of Nox1 did not enhance neoplasia formation. Nox1-deficient SMCs were further characterized by lower proliferation rate and migration than WT SMCs. Overexpression of Nox1 slightly enhanced both of the latter characteristics. Lower migration rate in Nox1-deficient cells was associated with changes in the regulation of actin cytoskeleton, that is, cofilin phosphorylation and mDia1 expression increased while PAK1 levels were lower.66 Furthermore, expression of Nox1 was higher in neointimal SMCs than in normal medial cells.66 Elevated levels of Nox1 were associated with ERK1/2 (extracellular signal-regulated kinases 1/2) activation and enhanced MMP-9 (matrix metalloproteinase 9) staining against Nox1. When Nox4 was inhibited, levels of p47phox were elevated on later stages of atherosclerosis.26 Areas of aortic atherosclerotic lesions, staining with DHE, levels of DNA oxidative damage, or macrophage infiltration were similar in aged Apoe−/− and Apoe−/−Ncf1−/− animals. What is more, SMCs from both types of aged mice showed similar upregulation of superoxide and H2O2 when treated with thrombin. Importantly, neither xanthine oxidation inhibition, nor lipoxigenase inhibition but Nox1/4 inhibitor attenuated ROS induction after stimulation with thrombin. The mitochondrial source of elevated ROS in aged SMCs was confirmed with the analysis of H2O2 levels in isolated mitochondria. Both SMC in aortas of aged animals or mitochondria in SMC from aged mice showed high staining against Nox4. When Nox4 was inhibited, levels of mitochondrial ROS in aged SMCs decreased. Interestingly, elevated levels of Nox4 were also detected in samples from older human donors. Last but not least, treatment with mitoTEMPO reduced thrombin-induced VCAM1 upregulation in SMCs in vitro, but also aortic wall stress and lesion area, and prevented oxidative damage in Apoe−/− mice.24

SMC-specific expression of a human-dominant negative form of NOX4 (NOX4 P437H) resulted in decreased neointima formation after carotid artery denudation in FVB/N mice26 or lower atherosclerosis in Apoe−/− mice.72 Protective effect of dominant negative NOX4 in Apoe−/− mice was also associated with lower numbers of infiltrating macrophages. Overexpression of NOX4 P437H resulted in inhibition of soluble epoxide hydroxylase-2 expression and decreased proinflammatory signaling and levels of VCAM1, MCP1, and ICAM1, what was mirrored by lower macrophage adhesion.72 Importantly, soluble epoxide hydroxylase-2 downregulation reduced the expression of thrombospondin-1,72 which was crucial for the observed effects of NOX4 P437H on proliferation and migration in Apoe−/− mice.25 On the other hand, Nox4 in Apoe−/− mice had a protective effect both in mice with spontaneous atherosclerosis development or after partial carotid artery ligation.26 Nox4 deletion led to the decrease in H2O2 in mouse aortas, associated with the increase in superoxide levels. Microarray analysis of gene expression in WT or Nox4-deficient animals showed increased proinflammatory signaling in aortas from the Nox4-deficient mice.26

Nox4 was protective also in diabetic Apoe−/− mice with streptozotocin-induced diabetes mellitus. Such mice had lower
aortic expression of smooth muscle contractile markers, while levels of PDGF (platelet-derived growth factor), vimentin, and osteopontin increased.\textsuperscript{27} \textit{Nofx} deletion further increased aortic expression of PDGF, collagen I, and Ki-67. SMCs isolated from \textit{Nofx}-deficient animals had lower expression of smooth muscle contractile markers, produced less H$_2$O$_2$ but more superoxide and upregulated \textit{Nofx}. Silencing of the latter gene resulted in the attenuation of PDGF-BB and osteopontin production and Ki-67 expression levels. Abnormal expression of PDGF-BB in \textit{Nofx}$^−/−$ SMCs could also be reversed by PDGF inhibition or treatment with H$_2$O$_2$.\textsuperscript{27} What is more, \textit{Nofx} together with Nrf2 play significant roles in the CD38 signaling pathway, which is crucial for the maintenance of SMC contractile phenotype.\textsuperscript{45} Coronary arterial myocytes isolated from \textit{Cd38}$^−/−$ mice had lower calponin and SM22α expression, accompanied by an increase in vimentin and PCNA (proliferating cell nuclear antigen). 7-Ketocholesterol, known atherogenic stimulus, further exacerbated the effect of \textit{Cd38} knockout. Lack of \textit{Cd38} caused a decrease in Nrf2 expression and activity. \textit{Nfe2l2} silencing in coronary arterial myocytes enhanced reduction of contractile markers caused by 7-ketocholesterol. CD38 activity is crucial for the induction of \textit{Nofx} and superoxide production, which is then necessary for the Nrf2 translocation to the nucleus and maintenance of arterial myocytes contractile phenotype.\textsuperscript{45}

Interestingly, \textit{Nfe2l2} overexpression in human aortic SMCs resulted in an increase of \textit{Gclc}, \textit{Gclm}, and \textit{Hmox1} expression and decreased SMC proliferation.\textsuperscript{33} The latter effect could be partially reversed with tin protoporphyrin IX, which is an HMOX1 (heme oxygenase 1) inhibitor. Furthermore, \textit{Ad Nfe2l2} reduced oxidative stress, macrophage infiltration, and MCP-1 levels in balloon-injured aortas in rabbits. Finally, \textit{Nfe2l2} gene transfer in vivo decreased both SMC proliferation and apoptosis, and therefore, it had no effect on neointimal neoplasia.\textsuperscript{38} What is more, PDGF induces Nrf2 nuclear translocation and increases expression of its target genes: \textit{Nqo1}, \textit{Hmox1}, and \textit{Txnr1}.\textsuperscript{31} \textit{Nfe2l2} silencing enhances PDGF-induced migration of SMCs. Furthermore, it leads to prolonged activation of Rac1, which can increase NADPH oxidase activity and ROS production. \textit{Nfe2l2} knockout also caused enhanced PDGF signaling, namely ERK1/2 phosphorylation, which can be inhibited with antioxidants, such as N-acetylcysteine. Femoral injury in \textit{Nfe2l2}$^−/−$ mice resulted in decreased lumen area and higher neointima formation, with no significant changes in media and with greater ERK1/2 activation in neointima.\textsuperscript{31} Sulforaphane, which is Nrf2 activity inducer, reduces neointima formation in injured femoral arteries of mice fed a Western diet.\textsuperscript{39} In vitro, sulforaphane reduced leptin-induced SMC proliferation, cyclin D1 expression, and phosphorylation of both p70S6 kinase and ribosomal S6 protein.\textsuperscript{40} Ashino and et al\textsuperscript{40} showed that injured femoral arteries contained regions of TUNEL$^+$ (terminal deoxynucleotidyl transferase dUTP nick-end labeling) apoptotic cells that overlapped with regions rich in \textit{Nrf2}$^−/−$ cells. Silencing of \textit{Keap1} in rat SMC resulted in higher Nrf2 levels and activity, increased caspase-3/7 activity, and increased apoptosis. \textit{Nfe2l2} silencing rescued the proapoptotic phenotype of \textit{Keap1}-deficient cells. Finally, wire injury of femoral arteries in \textit{Nfe2l2}$^−/−$ mice led to the enhanced formation of neointima, which contained less apoptotic cells. Therefore, authors proposed that Nrf2 protect from neointima also because of the enhancement of SMC apoptosis.\textsuperscript{30}

Interestingly, although \textit{Arg7}$^−/−$ SMCs had higher Nrf2 activity, expression of its target genes, and resistance to oxidative stress, they showed accelerated senescence.\textsuperscript{73} The latter effect was related to the defective autophagy and accumulation of \textit{Sqstm1}/p62, which is a Nrf2 target, but also contributes to its activation.\textsuperscript{74} In vivo, deletion of \textit{Arg7} in SMCs caused enhanced senescence and promoted neointima formation.\textsuperscript{73} On the other hand, carotid artery ligation in \textit{Sqstm1}$^−/−$ mice caused more neointima formation.\textsuperscript{75} SMCs isolated from \textit{Sqstm1}$^−/−$ aortas proliferated and migrated faster.\textsuperscript{75} Overexpression of HMOX1, enzyme degrading toxic heme to biliverdin, carbon monoxide and ferrous iron, and target of Nrf2 activity in SMCs decreased their migration in response to PDGF-BB.\textsuperscript{76} Inhibitory effect of heme oxygenase-1 depended on its enzymatic activity and was mimicked by carbon monoxide. Increased activity of heme oxygenase-1 led to the upregulation of both \textit{Vegfa} (vascular endothelial growth factor A) and \textit{Vegfr1} (vascular endothelial growth factor receptor 1). The latter one formed a complex with PDGFRβ, which attenuated PDGFRβ signaling.\textsuperscript{76} Activation of another Nrf2 target—\textit{Nadph} quinone oxidoreductase-1—by β-lapachone reduced neointima formation after balloon injury in rat carotid arteries and inhibited PDGF-induced SMC proliferation. Increased \textit{Nqo1} (4-nitroquinoline 1-oxide) activity caused AMPK activation mediated by LKB1 but not CaMKKK (Ca$^{2+}$/calmodulin-dependent protein kinase kinase beta).\textsuperscript{77} Taken together, Nrf2 is a crucial player in maintaining the hemostasis of the vessel wall via influencing inflammatory response, SMC proliferation, and neointimal formation.

Increased SMC apoptosis may lead to the plaque instability and rupture.\textsuperscript{78} Wnt/β-catenin/WISP-1 (WNT1-inducible-signaling pathway protein 1) represents example of the pathway that can protect SMCs from apoptosis\textsuperscript{79} and oxidative stress but, on the other hand, enhance SMC migration and contribute to intimal thickening.\textsuperscript{80} Mill et al\textsuperscript{79} showed recently that macrophage-derived Wnt5a could protect SMC from H$_2$O$_2$-induced apoptosis. Exogenous Wnt5a increased amounts of active and nuclear β-catenin in SMC and induced TCF (transcription factor) signaling. However, TCF pathway was attenuated when cells were costimulated with H$_2$O$_2$, but this effect was dependent on the active β-catenin. Protective activity of Wnt5a against H$_2$O$_2$-induced activity was lost after \textit{Lrp5/6} silencing, which suggests that it is regulated by Wnt/\textit{Frizzled}/\textit{Lrp5/6} pathway. Importantly, Wnt5a induced cytotoxic protective WISP-1 in CREB-dependent manner. Interestingly, SMC in stable plaques in human coronary arteries showed higher staining for WISP-1 than those in unstable plaques.\textsuperscript{79} Wnt2 also induced WISP-1 expression in SMC in β-catenin-dependent manner.\textsuperscript{80} Levels of Wnt2 and WISP-1 were elevated in ligated carotid arteries. Furthermore, WISP-1 overexpression promoted intimal thickening, which was reduced in \textit{Wnt2}$^−/−$ animals.\textsuperscript{80}

Finally, although high glutathione levels and, that is, high ratio of reduced to oxidized glutathione are considered protective, too much of it can also lead to the enhanced...
vascular remodeling. Network analysis of gene expression in human carotid neointima showed that the most significantly downregulated network was the one with GPX1 in its hub.81 Furthermore, Apoe−/− mice with deleted Gpx1 were characterized with increased atherosclerotic plaque, which contained more SMCs but not macrophages. However, increased macrophage infiltration in Gpx1−/−Apoe−/− was previously reported by Torzewski et al.41 Furthermore, balloon angioplasty and stenting in Gpx1+/−Apoe−/− mice decreased Gpx1 expression. Global knockout of Gpx1 in Apoe−/− mice caused an increase in superoxide levels in media but not adventitia or endothelium. Effect of lack of Gpx1 on SMCs was mediated by the high activity of ROS1 receptor tyrosine kinase. Inhibition of tyrosine kinase activity or ROS1 silencing could decrease enhanced proliferation and migration of Gpx1−/−Apoe−/− SMCs. Gpx1−/− SMCs contained more glutathione, which resulted from its higher synthesis. Elevated levels of glutathione caused reductive stress that contributed to S-glutathionylation and inactivation of SHP-2 (tyrosine-protein phosphatase nonreceptor type 11) phosphatase, what then inhibited ROS1 inactivation81 (Figure 3). Interestingly, Izawa et al82 reported that buthionine sulfoximine, which is glutathione inhibitor, elevated vessel wall superoxide levels, but on the other hand decreased angiotensin II–induced vessel remodeling.

Monocytes/Macrophages
Monocytes and macrophages play a significant role in the initiation and development of atherosclerosis.4,83,84 At the beginning of atherosclerosis, monocytes, which are attracted by the chemokines secreted by resident vascular cells, migrate into the subendothelial area where they differentiate into macrophages on growth factors stimulation.53 In the atherosclerotic lesions, macrophages ingest oxidized LDL through scavenger receptors and become lipid-laden foam cells.85 Under prolonged ER stress and extracellular stimuli, foam cells eventually undergo apoptosis and lead to the development of atherosclerosis. Although it has been well established that oxidative stress is involved in regulating monocyte migration, differentiation, and macrophage functions in atherosclerosis,86–88 its origins and regulation in this process remain poorly understood.

Nox are primary sources of oxidative stress in macrophages. Although there is still no evidence showing that endogenous Nox in macrophage has a direct impact on the progress of atherosclerosis, many studies have revealed a significant role of Nox-derived ROS in regulation of monocyte differentiation and macrophage functions.89,90 A recent study shows that tumor necrosis factor–like weak inducer of apoptosis (TWEAK), a proinflammatory cytokine, together with fibroblast growth factor–inducible 14 (Fn14) are colocalized with Nox2 in human advanced atherosclerotic plaques.91 In vitro experiments showed that TWEAK/Fn14 axis regulates Nox2-dependent ROS production in macrophages. Deletion of TWEAK in Apoe−/− mice reduces ROS production in macrophages within atherosclerotic plaques, suggesting a possible role of TWEAK/Fn14 and downstream Nox2-derived ROS in atherosclerosis.91 Another study shows that Rac2 can modulate atherosclerotic calcification through regulating interleukin-1β (IL-1β) production in macrophages.92 Rac2 can modulate Rac1 activity, which in turn promotes ROS production via Nox,93 leading to the production of macrophage IL-1β and subsequent vascular SMC calcium deposition in atherosclerotic lesions.92 These studies suggest that a role
of Nox regulated by different signaling pathways may contribute to the development of atherosclerosis, although more cell-specific experiments are still needed to provide further evidence.

Although numerous studies have focused on Nox, mitochondria are also an important source of oxidative stress in macrophages. Early studies have observed an increase in mitochondrial ROS and damage in human atherosclerosis. Recent studies indicate an important role of macrophage mitochondrial oxidative stress in atherosclerosis. Wang et al used a mitochondrial catalase transgenic mouse, which can quench mitochondrial ROS and protect against mitochondrial ROS-induced damage in vivo. Both mouse models, including transplantation of mitochondrial catalase transgenic bone marrow cells into Ldlr knockout mice and macrophage-specific mitochondrial catalase transgenic mice in Ldlr knockout background, showed decreased inflammatory monocyte infiltration, mitochondrial ROS in lesional macrophages, and attenuation of atherosclerosis lesions. Further studies suggest that macrophage mitochondrial ROS promotes MCP-1 production, which affects monocyte infiltration and lesional inflammation. Another recent study in human monocyte/macrophage provides further evidence of mitochondrial ROS regulation in human macrophages. Shirai et al isolated monocytes from patients with atherosclerotic coronary artery disease and showed that monocyte-derived macrophages from the patients produced more IL-6 and IL-1β, which was highly dependent on mitochondrial ROS but not Nox2. Neither Nox2 siRNA knockdown by nor Nox2 inhibition by gp91dstat had any effects on cytokine production. However, mitoTEMPO, a mitochondria-target ROS scavenger, significantly reduced IL-6 and IL-1β production in macrophages from the patients, indicating a critical role of ROS from mitochondria, but not Nox2, in regulating cytokine production in macrophages derived from atherosclerotic patients. Besides, Tumurkhuu et al demonstrated 8-oxoguanine glycosylase, a major DNA glycosylase responsible for removing mitochondrial oxidative stress–induced DNA damage, plays a protective role in atherosclerosis by preventing excessive inflammascase activation in macrophages, further supporting the critical role of macrophage mitochondrial oxidative stress in promoting atherosclerosis.

Another potential source of cellular oxidative stress is the XO. XO inhibitors have been reported to inhibit macrophage ROS formation, inflammatory cytokine release, and atherosclerosis. However, XO breaks down hypoxanthine and xanthine to uric acid and produces ROS, both of which may affect the function of macrophages. A recent report has elucidated that XO-dependent generation of ROS, rather than uric acid, mediates inflammatory cytokine production in macrophages. However, there is still a lack of solid evidence demonstrating the role of macrophage XO in atherosclerosis.

Macrophage not only serves as a source of oxidative stress in atherosclerosis but also itself can modulate or be affected by extracellular oxidative stress. Hemopexin, a hemoglobin scavenger protein, can transport heme into macrophages, thereby, inhibiting heme-mediated ROS production and ROS-mediated oxidative damage. Mehta et al have recently reported a role of hemopexin and macrophage in the regulation of oxidative stress in atherosclerosis. ApoE−/− mice deficient in hemopexin had higher oxidative stress, more macrophage infiltration, and atherosclerotic plaque. Hemopexin deficiency results in the dysfunction of uptake and metabolism of heme in macrophages. The accumulation of heme causes oxidative stress, leading to dysfunctional HDL, abnormal macrophage function, and atherosclerosis aggravation. Recent research by Korytowski et al showed that atherogenic acute family protein D1, which transport both cholesterol and 7-hydroperoxycholesterol to mitochondria of macrophages under oxidative stress, induces mitochondrial lipid peroxidative damage that impairs reverse cholesterol transport in macrophages. Once cholesterol import exceeds export in macrophages, lipid-overloaded macrophages accumulate in atherosclerotic plaques, which obstruct blood flow and advance the progress of atherosclerosis, in which ROS exerts its effects on the most events, if not all.

### Stem/Progenitor Cells

Stem/progenitor cells are characterized by the unique capacity for unlimited growth and self-renewal while maintaining the potential to differentiate into specialized cells. Vascular tissue–resident or adult stem cells have been discovered and display variable capacities for differentiation. Stem/progenitor cells can differentiate into vascular cell lineages, which may contribute to the regenerative process and could be useful for the treatment of atherosclerosis. Recently, several publications in ATVB and other journals have demonstrated the progress in research on the role of stem/progenitor cells in atherosclerosis. As mentioned earlier, oxidative stress response is a key event in the development of atherosclerosis, in which stem/progenitor cells sense the signal of ROS and other related species. One of the primary roles of ROS is to promote stem cell differentiation into SMCs important for both of neointimal formation after angioplasty and plaque stability. Thus, it would be crucial to understand the mechanisms of stem cell differentiation.

There is evidence demonstrating the factors responsible for stem cell differentiation. For example, Wang et al revealed that shear stress induced and suppressed angiogenic growth factors and SMC-associated growth factors, respectively. In addition to shear stress, growth factors and cytokines have been shown to directly regulate SMC differentiation, and the expression levels of cytokines and growth factors are likewise altered during differentiation of mesenchymal stem cells, for example. Importantly, it was found that oxidative stress is essential for stem cells to differentiate into SMCs. ROS are highly reactive molecules that are generated, for example, after interaction of integrins, extracellular matrix, and cytokines. They act as second messengers and mediate a host of cellular processes, including vascular physiology and pathogenesis, including hypertension, restenosis, and atherosclerosis. Xiao et al demonstrated that Nox4-derived H₂O₂ is integral to the differentiation of stem cells into SMCs. Silencing of Nox4 suppressed differentiation, while sustained Nox4 signaling enhanced differentiation of SMC gene markers. Nox4 translocation from the cytoplasm to the nucleus resulted in upregulation of H₂O₂, which in turn led to
induction and phosphorylation of SRF (serum response factor) and its translocation into the nucleus. Phosphorylated SRF binds to the CArg element on the promoter–enhancer regions of SMC-specific genes, recruiting myocardin to the promoter to form a SRF/myocardin complex. This complex was shown to be essential for regulating early-stage Nox4-mediated stem cell differentiation (Figure 4). Furthermore, Nrf3, a member of the cap N collar family of transcription factors, is now considered to be a crucial transcription factor in regulating SMC differentiation by modulating the balance of ROS generation. Pepe et al recently demonstrated that Nrf3 is indispensable for stem cell differentiation toward SMCs. Usually, Nrf3 resides in the ER. During the early stages of SMC differentiation, Nrf3 can directly bind to the promoter region of SMC-specific genes (ie, αSMA, and SM22α) that promotes the formation of the SRF/myocardin complex. Cytoplasmic Nrf3, on the other hand, is able to promote Nox4-mediated ROS production, which drives SMC differentiation. Together, signal pathways mediated by Nox/Nrf3 affect stem/progenitor differentiation into SMCs that influence neointimal formation and plaque stability.

Summary

In pathophysiology of the vessel wall, excessive concentrations of lipids result in free radical formation, and interaction of these molecules with the endothelial wall of the arteries leads to endothelial activation, an early sign of vascular inflammation. An inflamed endothelium recruits inflammatory cells, such as monocytes, via the expression of various mediators and chemokines. This, in addition to the imbalance of ROS generation, leads to the disabling of monocytes into foam cells, which consume dead cells and lipids. This debris eventually develops into a sclerotic, fibrofatty plaque, which decreases the compliance of the vessel, increases the possibility of embolus or thrombus development, through plaque rupture, and finally increases the risk of multiple comorbidities. In all of these processes, ROS plays a significant role in homeostasis of vascular cells and the pathogenesis of atherosclerosis.

Regulation of antioxidant response is complex and, therefore, difficult to target. While high levels of factors responsible for the resolution of the excessive oxidative stress can be beneficial in one cell type, they may be detrimental to the others. The best example shown was Nrf2, whose high levels in SMCs were protective, while its deficiency in myeloid cells caused attenuation of atherosclerosis or its aggravation. On the other hand, while glutathione is a potent antioxidant, its high levels in SMCs caused attenuated inactivation of ROS1 kinase and led to increasing in SMC proliferation and migration. Furthermore, as evidenced in stem cells, ROS were crucial for their differentiation to SMC. ROS and oxidative stress are involved in regulation of many pathways, for example, stem cell differentiation, response to ER stress, and control of inflammation. Therefore, potential atherosclerosis therapies involving the regulation of oxidative stress levels would require precise control.
targeting of certain types of ROS in particular cells and what is more at the specified stage of the disease.

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**Disclosures**

None.

**References**


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