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REVIEW

Redox regulation of endothelial cell fate

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Abstract Endothelial cells (ECs) are present throughout blood vessels and have variable roles in both physiological and pathological settings. EC fate is altered and regulated by several key factors in physiological or pathological conditions. Reactive nitrogen species and reactive oxygen species derived from NAD(P)H oxidases, mitochondria, or nitric oxide-producing enzymes are not only cytotoxic but also compose a signaling network in the redox system. The formation, actions, key molecular interactions, and physiological and pathological relevance of redox signals in ECs remain unclear. We review the identities, sources, and biological actions of oxidants and reductants produced during EC function or dysfunction. Further, we discuss how ECs shape key redox sensors and examine the biological functions, transcriptional responses, and post-translational modifications evoked by the redox system in ECs. We summarize recent findings regarding the mechanisms by which redox signals regulate the fate of ECs and address the outcome of altered EC fate in health and disease. Future studies will examine if the redox biology of ECs can be targeted in pathophysiological conditions.

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Abbreviations

3-MST	3-Mercaptopyruvate sulfurtransferase
AICAR	5-Amino-4-imidazole carboxamide riboside
AMPK	Adenosine monophosphate-activated protein kinase
BAT	Brown adipose tissue
BH4	Tetrahydrobiopterin
BMP	Bone morphogenetic protein
CAT	Catalase
CBS	Cystathionine β-synthase
CEP	ω-(2-Carboxyethyl)pyrrole
CSE	Cystathionine γ-lyase
EPA	Eicosapentaenoic acid
ER	Endoplasmic reticulum
ETC	Mitochondrial electron-transport chain
FoxO	Forkhead homeobox type O
Gpx	Glutathione peroxidase
GR	Glutathione reductase
Grx	Glutaredoxin
GTPCH I	GTP-cyclohydrolase I
H_2O_2	Hydrogen peroxide
HIF-1	Hypoxia-inducible factor 1
HUVEC	Human umbilical vein endothelial cells
ICAM-1	Intercellular adhesion molecule-1
IKK	IκB kinase
JNK	c-Jun N-terminal kinase
Keap1	Kelch-like ECH-associated protein 1
LDLR	Low-density lipoprotein receptor
LKB1	Liver kinase B1
MCP	Monocyte chemoattractant protein

NaHS	Sodium hydrosulfide
NO	Nitric oxide
NO_2-FAs	Nitro-fatty acids
NOS	Nitric oxide synthase
NOX	NADPH oxidase
Nrf2	Nuclear factor erythroid-2-related factor 2
$O_2^{\cdot-}$	Superoxide anion
OGD	Hypoxia and glucose deprivation
PAG	DL-propargylglycine
PI3K	Phosphoinositide 3-kinase
Prx	Peroxiredoxins
PTEN	Phosphatase and tensin homolog
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RSH	Thiol
RSO2H	Sulfinic acids
RSO3H	Sulfonic acids
RSOH	Sulfenic acids
RS-SG	*S*-glutathionylation
RS-SR, RS-SR'	Disulfide bonds
SIRT1	Sirtuin 1
SOD	Superoxide dismutase
SM	Smooth muscle
SREBP2	Sterol regulatory element binding protein 2
TP	Thromboxane receptor
Trx	Thioredoxin
VEGF	Vascular endothelial growth factor
VEGFR	VEGF receptor
VCAM-1	Vascular cell adhesion molecule-1
WAT	White adipose tissue

Introduction

Endothelial cells (ECs) are continuously exposed to circulating blood, which maintains the structure and function of blood vessels [1]. EC fate significantly affects the initiation and progression of diseases, including atherosclerosis [2], hypertension [3], aging [4], inflammation [5], tumor angiogenesis [6], and obesity [7]. The redox system is responsible for EC function and dysfunction [8, 9]. Indeed, modest and controlled generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in ECs is required for numerous vital signaling pathways involved in cell survival, proliferation, activation, stress response, cell motility, vasodilation, and angiogenesis. High levels of oxidants or reductants in ECs have detrimental effects, such as promoting oxygen toxicity. Thus, it is critical to address the important roles of redox homeostasis in the regulation of EC fate, including EC survival, apoptosis, proliferation, migration, senescence, and transdifferentiation. We will also review several pivotal ROS/RNS-sensitive molecules, such as adenosine monophosphate-activated protein kinase (AMPK), phosphatase and tensin homolog deleted from chromosome 10 (PTEN), and hypoxia-inducible factors (HIF), which are involved in redox-regulated EC fate. Specifically, all of the aforementioned molecules can act as 'redox sensors and modulators' due to redox modifications of their cysteine residues, which are critically important for the regulation of protein function.

Redox biology of blood endothelial cells

Executors in the redox system

Key oxidants in ECs: $O_2^{\cdot-}$, H_2O_2, ·OH, NO, and $ONOO^-$

The redox state is characterized by the reduction–oxidation reaction. The redox system includes oxidants and reductants. Reactive oxidants include ROS and RNS. ROS are key components of EC oxidants and are implicated in stress-related conditions and diseases. ROS have recently been shown to be influential players in the biology of ECs [10]. The major ROS in ECs are superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) [11], and hydroxyl radicals (·OH) (Fig. 1). Among these ROS, H_2O_2 is a freely diffusible and stable ROS, whereas $O_2^{\cdot-}$ is a short-lived and highly reactive oxidant. The half-life of the $O_2^{\cdot-}$ ranges from a few nanoseconds to milliseconds.

Reactive nitrogen species in ECs include nitric oxide (NO), nitric dioxide (NO_2), and the much more powerful oxidant, peroxynitrite anion ($ONOO^-$). $ONOO^-$ is generated from a rapid reaction between NO, which is highly diffusible and has a short half-life of 3–4 s, and $O_2^{\cdot-}$ [12, 13] (Fig. 1). $ONOO^-$ has a half-life of approximately 1 s at physiological conditions and reacts with other molecules to form additional RNS, such as NO_2 and N_2O_3. RNS in ECs can react with functional proteins, including protacyclin synthase (PGIS) [14, 15]. The effects of RNS are diverse and may be similar to or opposite from those of ROS.

Key reductants in ECs: H_2S, GSH

Hydrogen sulfide (H_2S) is the third most recognized endogenous gaseous signaling molecule, after NO and carbon monoxide (CO) [16] [17]. H_2S gas is primarily generated from L-cysteine in mammals by the three enzymes, cystathionine γ-lyase (CSE, EC 4.4.1.1) [18, 19], cystathionine β-synthase (CBS, EC 4.2.1.22) [20, 21], and 3-mercaptopyruvate sulfurtransferase (3-MST, EC 2.8.1.2) [22–24] (Fig. 1). Both CSE and CBS predominantly exist in the cytosol, whereas 3-MST is mainly localized in mitochondria and the cytoplasm [25]. CBS was also recently shown to localize in the mitochondrial outer membrane of the

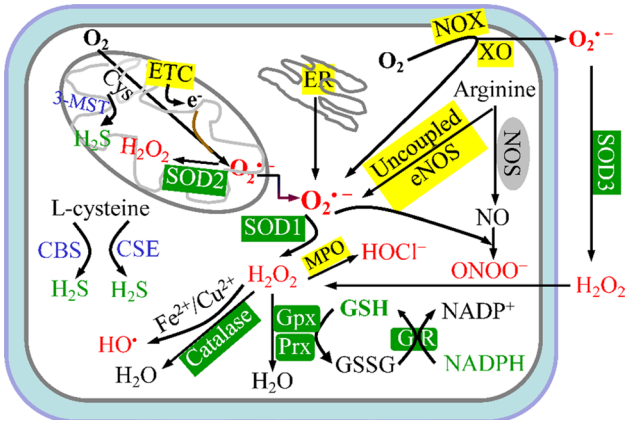

Fig. 1 Redox homeostasis in the endothelial cell. Mitochondrial electron-transport chain (*ETC*), membrane-bound NADPH-oxidase (*NOX*) complex, uncoupled endothelial nitric-oxide synthase (*eNOS*), endoplasmic-reticulum (*ER*) stress, and xanthine oxidase (*XO*) are the five major intracellular sources (shown in yellow background) of reactive oxygen species (ROS). Superoxide anion ($O_2^{\cdot-}$) is the predominant ROS and is rapidly converted into hydrogen peroxide (H_2O_2) by superoxide dismutases (*SODs*). Alternatively, $O_2^{\cdot-}$ can generate peroxynitrite ($ONOO^-$) by reacting with nitric oxide (*NO*). H_2O_2 can be catalyzed to ·OH in the presence of Fe^{2+} or Cu^{2+} ions or may be converted to H_2O and O_2 through a reaction catalyzed by catalase, glutathione peroxidase (*Gpx*), or peroxiredoxins (*Prx*). To maintain redox homeostasis, living cells engage powerful scavenger antioxidant enzyme systems (shown in *green* background) to eliminate intracellular oxidants (shown in *red*). Major reductants are shown in *green*. Endogenous H_2S is produced from L-cysteine (*Cys*) by three enzymes, which are cystathionine γ-lyase (CSE), cystathionine β-synthase (*CBS*), and 3-mercaptopyruvate sulfurtransferase (*3-MST*). *GR* glutathione reductase, *GSH* reduced glutathione, *GSSG* oxidized glutathione

colon cancer-derived epithelial cell line HCT116 [20]. CSE is predominantly expressed in the endothelial layer of blood vessels [26] and in the heart [27], whereas CBS is highly concentrated in the brain [28]. CSE can be physiologically activated by calcium-calmodulin [26]. In addition, lipophilic atorvastatin, but not hydrophilic pravastatin, enhances net production of H_2S in perivascular adipose tissue by inhibiting H_2S mitochondrial oxidation [29].

H_2S exists in solution as both H_2S (about 20 %) and hydrosulfide anion (HS^-) (about 80 %) at pH 7.4. In contrast, the completely deprotonated sulfide (S^{2-}) is found at exceptionally lower levels at physiological pH [30]. Thus, the term H_2S will be employed to refer to the total sulfide pool ($H_2S + HS^- + S^{2-}$) [31, 32]. H_2S serves as an electron donor [22] and functions as a reductant [33], altering the cellular redox status. Emerging data suggest that H_2S is an antioxidant signaling molecule at lower physiological concentrations [34, 35]. However, H_2S is a lethal toxin at higher concentrations due to the inhibition of cytochrome-*c* oxidase in mitochondrial complex IV and prevention of cellular respiration [36]. Similar to NO, high concentrations (100–250 μM) of H_2S promote oxidative stress and reduced survival of ECs and vascular smooth muscle cells (VSMCs) [39]. In contrast, low concentrations (about 30 μM) of H_2S protect ECs against various stressors, such as H_2O_2 [38], high glucose [46], and hyperhomocysteinemia [34]. Low concentrations of H_2S exert distinct physiological functions [35, 47], including vasodilation [26, 41], EC migration and proliferation [20, 41], inhibition of inflammation [48], and stimulation of cellular bioenergetics [20, 22] (Table 1). There are several mechanisms involved in H_2S function [49]. For example, H_2S that is released from ECs can parallel and complement NO [50]. Recently, it was reported that cytoprotective function of H_2S is eNOS-NO dependent [51]. Moreover, H_2S is an endothelium-derived hyperpolarizing factor that mediates endothelium-dependent vasorelaxation [45]. H_2S promotes Nrf2 localization to the nucleus, which induces expression of multiple cellular antioxidants. The predominant function of H_2S in ECs appears to be sulfhydration of target proteins. Sulfhydration is the conversion of cysteinyl thiolates (Cys-SH) to cysteinyl persulfide (Cys-S-SH) by the addition of H_2S-derived sulfur [52, 53] (Fig. 2). H_2S acts as a prominent physiological endothelium-derived hyperpolarizing factor by covalently sulfhydrating the ATP-sensitive potassium channel to induce vessel relaxation [44]. H_2S regulates the activity of vascular endothelial growth factor receptor 2 (VEGFR2) and several other molecules by breaking intrinsic inhibitory disulfide bonds, such as that between Cys1045 and Cys1024 of VEGFR2 [40]. H_2S also *S*-sulfhydrates the C226 and C613 residues in Kelch-like ECH-associated protein-1 (Keap1), which is a redox-sensitive ubiquitin ligase substrate adaptor that represses Nrf2. This activity may reduce the C226-C613 disulfide bridge formed by H_2O_2 [54]. H_2S was recently demonstrated to reversibly oxidize free cysteine thiols, but not disulfide bonds, in PTEN. In addition, H_2S inactivates PTEN via polysulfide formation [31], although it is not clear if this modification occurs in ECs. Therefore, H_2S may oxidize free cysteine thiols by sulfhydration at high concentration, while reducing disulfide bonds at low doses (Fig. 2).

Another critical low-molecular-weight reductant in ECs is reduced glutathione (γ-glutamyl-cysteinyl-glycine, GSH). The glutathione/glutathione disulfide (GSH/GSSG) molecules represent the most abundant thiol-redox system in ECs [55] (Fig. 1). Intracellular GSH is differentially distributed in various subcellular compartments of the cytosol, mitochondria, ER, and nucleus. The cytosol contains more than 70 % of total cellular GSH. The redox state of a cell is generally indicated by the ratio of GSH to GSSG. One versatile property of GSH is its antioxidant function, which maintains redox balance. Interestingly, GSH regulates EC fate and functions, including EC apoptosis [56], angiogenesis [57], and EC-dependent vasodilation [58]. The major

Table 1 Hydrogen sulfide functions in endothelial cells

Donor or knockout model	H_2S function	Mechanisms	References
Na2S (IK-1001)	Vasorelaxation	Improves EC function	[37]
NaHS	Anti-oxidant	Increases expression of SOD, CAT, GPx, and GST	[38]
		Increases Trx-1; decreases NOX4	[34]
	Pro-angiogenic effect	Activates Akt; breaks the Cys1045-Cys1024 disulfide bond in VEGFR2	[39, 40]
	Angiogenesis and vasorelaxation	Increases pAkt-Ser473, peNOS-Ser1177, pVEGFR2, pVASP-Ser239	[41] [42]
GYY-4137	Vasodilator, anti-hypertension	Opens ATP-sensitive K^+ channels in VSMC	[32]
	Pro-angiogenesis	Inhibits soluble fms-like tyrosine kinase-1 and endoglin	[18]
DATS	Attenuates LV dys-function following HF	Decreases angiostatin, increases VEGF, peNOS, and consequent angiogenesis	[43]
$CSE^{-/-}$	Anti-hypertension	Endothelium-dependent vasorelaxation; hyperpolarization by sulfhydrating ATP-sensitive K^+ channels;	[19, 26] [44, 45]
	Anti-atherosclerosis	Decreases levels of total cholesterol, LDL/HDL-cholesterol; increases GSH level, SOD activity, inhibits H_2O_2 production, NF-κB activation, and ICAM-1 expression in aorta or VSMC in response to atherogenic diet; suppresses proliferation of intimal cells in aortic root	[19]
CBS knock down/inhibition	Angiogenesis	Increases cell proliferation and migration of tumor cells and ECs	[20]

CBS cystathionine-β-synthase, *CSE* cystathionine γ-lyase, *DATS* diallyl trisulfide, *GYY4137* morpholin-4-ium 4 methoxyphenyl(morpholino) phosphinodithioate, *Trx-1* thioredoxin-1, *LV* left ventricular, *HF* heart failure

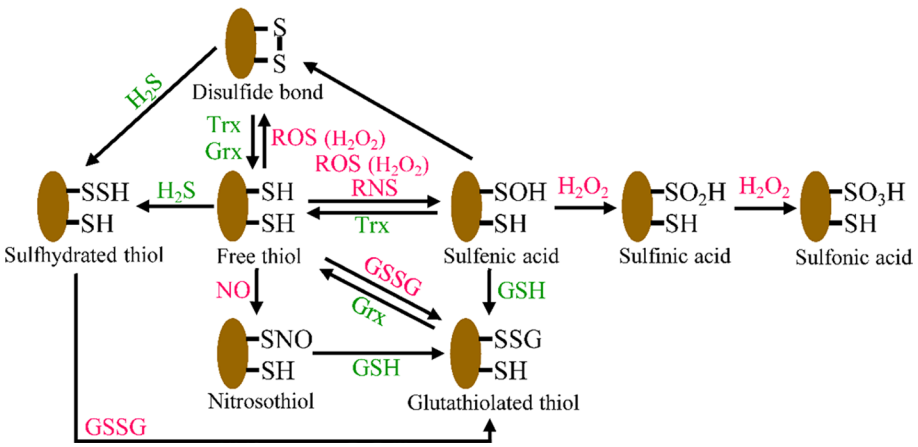

Fig. 2 Reversible and irreversible redox modifications of protein cysteines in ECs. Oxidation of cysteine thiol (*RSH*) by ROS or RNS leads to the generation of highly reactive sulfenic acid (*RSOH*), which can react with another thiol to produce a disulfide bond (*RSSR*) or with GSH to become *S*-glutathionylated (*RSSG*). This oxidative modification is reversible, and reduction is catalyzed by the Trx or Grx system. Further oxidation of RSOH to sulfinic acid (RSO_2H) and sulfonic acid (RSO_3H) is thought to be generally irreversible in vivo. Free thiols can also be modified by sulfhydration or *S*-nitrosylation

molecular mechanisms by which GSH regulates redox modification of redox-sensitive cysteines are thiol-disulfide exchange and protein *S*-glutathiolation [59]. These modifications control a variety of activities, including EC differentiation, proliferation, and apoptosis. For example, *S*-glutathiolation of Cys^{118} in p21Ras causes activation of p21Ras and downstream phosphorylation of Erk and Akt in ECs [60].

Redox homeostasis in ECs

Endothelial cells possess modest levels of intracellular oxidants and reductants. The EC redox status is balanced by oxidant-generating systems and antioxidant systems. Five major systems are responsible for the generation of vascular endothelial ROS, the mitochondrial electron-transport chain (ETC) [10, 61–63], the membrane-bound NAD(P)

H-oxidase (NOX) complex [64, 65], uncoupled endothelial nitric-oxide synthase (eNOS) [66–68], endoplasmic-reticulum (ER) stress [69, 70], and xanthine oxidase (XO) [71, 72] (Fig. 1). The electrons from the mitochondrial ETC can be captured by O_2 to form the superoxide anion ($O_2^{\cdot-}$) [73]. Moreover, the mitochondrial ETC produces $O_2^{\cdot-}$ primarily at complexes I and III [74]. Then, $O_2^{\cdot-}$ can be rapidly converted into H_2O_2 by superoxide dismutase 2 (SOD2, Mn-SOD) in mitochondria. H_2O_2 diffuses out of the mitochondrion in human coronary arteriolar endothelial cells and elicits vasodilation in response to shear stress [75]. Complex III is the main site of ROS production in human umbilical vein endothelial cells (HUVECs) during hypoxia-reoxygenation [76].

Nitric oxide in ECs is mainly produced by eNOS and inducible nitric oxide synthase (iNOS). eNOS function is altered by several oxidants, including $ONOO^-$ [66] and hypochlorous acid (HOCl), which is the major oxidant of myeloperoxidase (MPO) [77]. Normal eNOS function requires the essential co-factor, tetrahydrobiopterin (BH4), which is synthesized by GTP-cyclohydrolase I (GTPCH I) in a rate-limiting step. $ONOO^-$ elevated by high levels of glucose activates the 26S proteasome [78–80] or directly oxidizes and releases zinc ions from GTPCH I. In addition, $ONOO^-$ enhances the ubiquitination and degradation of GTPCH I, which leads to BH4 deficiency [81] and subsequent eNOS uncoupling and dysfunction.

During steady-state cellular conditions, intracellular oxidants levels are tightly regulated by intricate antioxidative systems to maintain redox homeostasis. Major antioxidants in ECs include low-molecular-antioxidants, such as H_2S, reduced GSH, vitamin C, and vitamin E, noncatalytic antioxidant proteins, such as thioredoxin (Trx) [82], glutaredoxin (Grx), peroxiredoxins (Prx) [83], and metallothioneins (MTs) [84], and antioxidant enzymatic defense systems, including SODs, catalase (CAT), glutathione peroxidase (Gpx), and glutathione reductase (GR) (Fig. 1). For example, aged *GPx-1*$^{-/-}$ mice demonstrate elevated oxidant formation as compared with their wild-type littermates, leading to decreased NO bioavailability due to eNOS uncoupling and consequent EC dysfunction [85]. Recent data also indicate that H_2S is a powerful reducing agent [86] that plays a pivotal role in maintaining redox homeostasis through the following distinct mechanisms: (1) H_2S directly scavenges endogenous oxidant species, including $ONOO^-$ [87], $O_2^{\cdot-}$, and H_2O_2 [38]; and (2) H_2S increases the expression of antioxidative enzymes, including SOD, CAT, and Gpx [38]. In addition, GSH maintains the intracellular redox balance by scavenging ROS that are involved in Gpx-mediated hydroperoxide metabolism and GR-catalyzed and NADPH-dependent GSH regeneration (Fig. 1). ROS production within mitochondria can be prevented by uncoupling protein (UCP) 2. For example, the AMPK activator, 5-amino-4-imidazole carboxamide riboside (AICAR), upregulates AMPK-mediated mitochondrial UCP2 via p38 signaling, which attenuates $O_2^{\cdot-}$ and prostacyclin synthase nitration in aortic ECs of diabetic mice [88]. In general, oxidized/reduced GSH, oxidized/reduced Trx, oxidized/reduced Grx, and oxidized/reduced Prx are important redox-buffering systems [83].

General mechanisms of redox-mediated regulation of ECs

Key transcription factors involved in the redox regulation of ECs

Several key transcription factors in ECs, including forkhead box O (FoxO), nuclear factor erythroid-2-related factor 2 (Nrf2), and hypoxia-inducible factor 1 (HIF-1), are redox-sensitive. They act as distinct redox sensors and regulators. The redox state regulates the levels and activities of these transcription factors, which control EC fate and may inversely modulate redox homeostasis.

FoxO-mediated redox regulation in ECs The FoxO family consists of many transcription factors, including FoxO1, FoxO3, FoxO4, and atypical FoxO6. The FoxO proteins have diverse physiological functions, including the regulation of EC fate. Among the FoxO family members, FoxO1 [89, 90], FoxO3a [91], and FoxO4 are implicated in EC biology. FoxO1 and FoxO3a are the most abundant FoxO isoforms in mature ECs [89]. EC redox regulates the activities of FoxOs through multiple posttranslational modifications, including phosphorylation, acetylation, and ubiquitination [92] (Fig. 3). For example, FoxO3a can be phosphorylated by the phosphoinositide 3-kinase (PI3K)/Akt kinase, leading to cytoplasmic retention and defective FoxO3a nuclear transcriptional activity [91]. FoxO1 has a similar profile in response to Akt activation [93]. FoxO1 is also a physiological substrate of protein kinase A-α (PKA-α) [94]. In

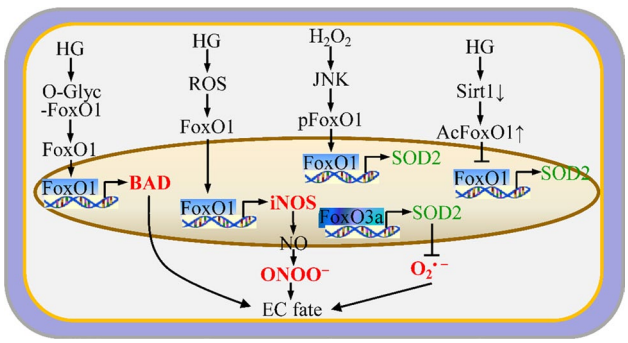

Fig. 3 FoxO-mediated redox system and EC fate. FoxOs regulate EC fate by affecting apoptosis, survival, proliferation, and migration due to the expression of FoxO target genes that are associated with redox balance

contrast, c-Jun NH2-terminal kinase (JNK) phosphorylates FoxO1 at a distinct residue to promote nuclear import and transcriptional activation [90]. In addition, deacetylation of FoxO1 by Sirtuin 1 (SIRT1) promotes FoxO1-mediated transcription of target genes, such as SOD2 [95]. The redox state mutually regulates the biological functions of FoxOs. For example, H_2O_2 induces JNK-mediated nuclear translocation of FoxO1 in cultured ECs [90]. Deletion of FoxO1/FoxO3a/FoxO4 in ECs leads to the reduction of the NOX subunits, *p22phox*, *p67phox*, and *NOX2*, as well as decreased production of ROS, including $O_2^{\cdot-}$ and H_2O_2 [25]. Moreover, FoxO3a functions as a direct transcriptional regulator of a group of antioxidant genes, including *SOD2* [96]. However, activation of endothelial FoxO1 by hyperglycemia-mediated ROS promotes the expression of inducible NOS (iNOS), which mediates NO–$ONOO^-$ production [97]. FoxOs might regulate EC fate by maintaining redox homeostasis. Potente et al. [89] demonstrated that overexpression of constitutively active FoxO1 or FoxO3a significantly blocks EC migration and consequent tube formation in vitro. In addition, Tanaka et al. [97] found that activation of FoxO1 in response to high glucose results in LDL oxidation and eNOS dysfunction in ECs. Activation of FoxO1 [98] and FoxO3 [99] induces apoptosis in HUVECs. Thus, FoxO proteins play essential roles in the response to physiologic oxidative stress and thereby regulate EC survival. Loss of FoxO3 decreases the expression of SOD2 and CAT, leading to excessive amounts of ROS and EC dysfunction. These experiments suggest that FoxO-mediated repression of ROS determines EC fate.

Nrf2-mediated redox regulation in the maintenance of EC fate Nrf2 is a ubiquitously expressed transcription factor that can be induced by endogenous or exogenous stimulators in the cardiovascular system [100, 101]. Nrf2 activates antioxidant responsive element (ARE)-dependent gene expression to maintain cellular redox homeostasis [84]. Nrf2 is usually sequestered in the cytoplasm by binding with Kelch-like ECH-associated protein 1 (Keap1). Keap1 is a redox-sensitive substrate adaptor for the Cul3-dependent E3 ligase and a mediator of Nrf2 proteasomal degradation [102, 103]. H_2O_2 can enhance the formation of an intramolecular disulfide bridge between C226 and C613, which is associated with Keap1 inhibition [54]. H_2S may decrease the C226-C613 disulfide in Keap1 to generate a free thiol and an *S*-sulfhydrated moiety [54]. Nrf2 plays important roles as an oxidant sensor and a regulator of redox homeostasis in ECs. Nrf2 combats detrimental levels of intracellular ROS by transcribing genes that encode several key antioxidant molecules, including Gpx, heme oxygenase-1 (HO-1) [104], and GSH. Expression of Nrf2 protects human aortic endothelial cells (HAECs) from H_2O_2-mediated cell death [104]. This finding suggests that Nrf2 might be a regulatory factor that controls EC fate by modulating redox homeostasis. Chen et al. further showed that overexpression of Nrf2 in HAECs suppresses TNF-α-induced expression of monocyte chemoattractant protein (MCP)-1 and vascular cell adhesion molecule-1 (VCAM-1), which are redox-sensitive inflammatory molecules. Moreover, expression of an upstream kinase for p38 MAP kinase, a constitutively active form of MKK6, partially reversed Nrf2-mediated inhibition of VCAM-1 expression. These results suggest that p38 MAP kinase mediates in part the anti-inflammatory actions of Nrf2 in ECs [104]. In addition, the shear stress in ECs that is generated by circulating blood increases intracellular ROS levels [105], which further stimulates nuclear translocation of Nrf2 and subsequent induction of HO-1 [106]. *Nrf2* deletion inhibits both basal and inducible expression of antioxidant genes, increasing oxidative stress. Global deletion of *Nrf2* inhibits EC sprouting and vascular density. This also occurred with EC-specific deletion of *Nrf2* without affecting the surrounding non-vascular tissue [107]. Collectively, these reports suggest that Nrf2 may be a potent regulator of EC fate due in part to antioxidant activities.

Role of HIF-1 in the redox regulation of EC fate Hypoxia-inducible factor-1 (HIF-1) is a heterodimeric transcription factor composed of the HIF-1α and HIF-1β subunits. Both subunits contain the basic helix-loop-helix and PAS domains [108], which mediate heterodimerization and binding to DNA [109]. The redox system controls the expression, stability, and activity of HIF-1 in ECs, which ultimately affects EC fate. *S*-nitrosylation by NO stabilizes the expression and activity of HIF-1 [110]. Hypoxia induces HIF-1 expression in ECs, resulting in reduced levels of the angiogenic factor interleukin-8 (IL-8). The downregulation of IL-8 by HIF-1 appears to be due to reduced expression of Nrf2 [111].

Redox modifications of cysteine residues in proteins in ECs

Post-translational protein modifications are dynamic, fine-tuned regulatory mechanisms that impact a wide variety of cellular processes. Recently, oxidative modification of cysteine thiols has been shown to be a central mechanism for dynamic post-translational regulation of several key proteins [112], such as AMPK, PTEN, HIF-1, eNOS, and VEGFR2. These modifications usually function as redox sensors and switches in ECs. There are five major redox modifications of protein cysteine in ECs (Fig. 2). The first redox modification is the reversible disulfide bond (RS-SR') [113]. For example, H_2O_2 enhances disulfide formation between Cys124 in the active site of PTEN and a vicinal Cys71, leading to oxidative inactivation of PTEN. This modification contributes to the angiogenesis mediated by H_2O_2 [114]. The second redox modification is *S*-nitrosylation (also called *S*-nitrosation, R-SNO), which is generally

mediated by NO [115]. For example, *S*-nitrosylation of eNOS at Cys99 is associated with monomerization and reduced enzymatic activity [116]. The third redox modification is *S*-glutathiolation (also known as *S*-glutathionylation, RS-SG). *S*-glutathiolation occurs through thiol-disulphide exchange with GSSG or through a reversible reaction between an oxidant-mediated protein thiyl radical and GSH during redox stress [59, 117–119]. For example, H_2O_2 induces *S*-glutathiolation of AMPKα at Cys304, which stimulates AMPK activation [120]. In addition, either vascular endothelial growth factor (VEGF) or H_2O_2 induce *S*-glutathiolation of sarco/endoplasmic-reticulum-Ca^{2+} ATPase-2b at Cys674, which mediates EC migration [121, 122]. Interestingly, oxidized low-density lipoproteins and $ONOO^-$ stimulate *S*-glutathiolation of p21Ras at Cys118, causing insulin resistance in ECs [60, 123]. Recently, Chen et al. demonstrated that GSSG induces *S*-glutathiolation of human eNOS at Cys689 and Cys908. This causes eNOS uncoupling and inhibition in association with reduction of NO and induction of $O_2^{\cdot-}$ [124]. The fourth redox modification is sulfhydration (R-SSH) by H_2S. The disulfide bond between Cys1045 and Cys1024 in VEGFR2 is disrupted by sulfhydration with H_2S [40]. The fifth redox modification involves sulfenylation (R-SOH), sulfinic acid (R-SO2H), and sulfonic acid (R-SO3H). The general mechanism is that ROS react with H_2O_2 target proteins that contain redox-sensitive cysteine residues (R-SH) to produce the reversible sulfenic-(R-SOH) and the irreversible sulfinic-(R-SO2H) and sulfonic-(R-SO3H) acid derivatives [125]. The protein sulfenic acid derivative can further react with NO to generate nitrosothiol (R-SNO) or with another R-SH to yield a disulfide bond (R-SS-R) [126] (Fig. 2).

In addition to the direct redox modification of protein cysteines, several central molecules in ECs are also regulated by redox homeostasis via signaling pathways. For example, NO activates AMPK through a process mediated by the Ca^{2+}/calmodulin-dependent protein kinase kinase [127]. In addition, berberine-elevated $ONOO^-$ stimulates AMPK activation in bovine aortic endothelial cells via phosphorylation of liver kinase B1 (LKB1) at Ser307 [128].

Regulation of EC fate by redox homeostasis

Redox systems, including ROS/RNS, regulate oxygen sensing, vascular tone, apoptosis, cell growth, proliferation, migration, senescence, and inflammatory responses in vascular ECs. As reviewed above, ECs can cope with redox stress to maintain redox homeostasis. These coping strategies involve unique regulatory mechanisms through which endogenous antioxidant defense systems are activated. In addition, a growing body of literature supports the notion that redox homeostasis is an important regulator of EC fate. However, most studies do not clarify the underlying molecular mechanisms. Here, we introduce several potential or well-documented redox-sensitive molecules that participate in the regulation of EC fate. For example, AMPK that has pleiotropic roles in vascular biology [129, 130], including regulation of EC fate, is the major redox sensor in ECs [64, 131].

Redox regulation of EC survival and apoptosis

Redox balance is required for the survival of ECs. In contrast, redox imbalance promotes apoptosis of ECs. We previously showed that $ONOO^-$ dose-dependently (10 μM) inhibits the activity of Akt [132], which is a pro-growth and pro-survival signal in ECs. $ONOO^-$ stimulated by high glucose significantly increases phosphorylation of Ser428 in the tumor suppressor LKB1, which phosphorylates PTEN at Ser380/Thr382/383 in vitro. Increased phosphorylation of PTEN increases PTEN activity. The enhanced lipid phosphatase activity of PTEN inhibits basal and insulin-stimulated phosphorylation of protein kinase B/Akt at Ser473. Thus, Akt activity is reduced, promoting EC apoptosis in response to high glucose in vitro and hyperglycemia in vivo [133]. The exposure of cultured HUVECs to a low concentration of $ONOO^-$ (5 μM) significantly increases the phosphorylation of protein kinase C ζ (PKCζ) at Thr410, which promotes nuclear import of PKCζ. Phosphorylated PKCζ directly phosphorylates Ser248 of LKB1 in vitro and in intact cells, leading to LKB1 nuclear export, PTEN activation, and consequent EC apoptosis [134]. These results suggest that PKCζ mediates LKB1-dependent inhibition of Akt in response to $ONOO^-$, which leads to endothelial apoptosis. In contrast, a high concentration of $ONOO^-$ (>50 μM) oxidizes and inactivates PTEN in ECs (Song et al., unpublished data) or in a cell-free system [31]. Very recently, Ang II was demonstrated to induce EC apoptosis and dysfunction via NOX2 activation by kynureinine pathway [135].

AMPK activation by oxidative stress inhibits apoptosis in multiple types of cells, including ECs [131, 136]. AMPK activation is regulated by redox, such as that produced by H_2O_2 [137], and helps to mediate redox balance [131]. We showed that a low concentration (<10 μM) of $ONOO^-$ enhances the activity of AMPK and its substrate enzymes, including eNOS and acetyl-CoA carboxylase, in ECs [132, 138]. In addition, we demonstrated that $ONOO^-$-dependent AMPK activation occurs subsequently to hypoxia–reoxygenation [139], and in ECs treated with metformin [138], simvastatin [140], or berberine [128]. Moreover, AMPK activation by $ONOO^-$ occurs independently of changes in the AMP/ATP ratio [138, 139]. Ido et al. [136] were the first to report that AMPK activation protects ECs from

apoptosis induced by hyperglycemia-derived oxidative stress. The combination of hypoxia and glucose deprivation (OGD) profoundly activates AMPK within 30 min. AMPK activity reaches a maximum within 2 h of OGD in cultured ECs. However, EC apoptosis is clearly elevated after 6 h of OGD treatment. Furthermore, AMPK inhibition by Compound C or due to overexpression of a dominant-negative AMPK (AMPK-DN) aggravates EC apoptosis in response to OGD. In contrast, pharmacological AMPK activation by AICAR or genetic activation by overexpression of constitutively active AMPK mitigates EC apoptosis in response to OGD. This appears to be due to increased expression of survival factors. AMPK activation activates IκKα and NF-κB nuclear translocation, which enhances the expression of anti-apoptotic Bcl-2 and survivin. In addition, genetic deletion of *AMPKα1* but not *AMPKα2* blunts OGD-augmented activation of NFκB, reducing expression of Bcl-2 and survivin and increasing EC apoptosis [141]. In addition, knockdown of *AMPKα1* in HUVECs downregulates antioxidant molecules, such as SOD2 and CAT, and increases production of ROS in response to low oxygen [142]. AMPKα activation by C-peptide, AICAR, or metformin prevents high glucose-stimulated ROS production, mitochondrial fission, destruction of the mitochondrial membrane potential, and EC apoptosis. Moreover, C-peptide replacement therapy blocks hyperglycemia-induced AMPKα inhibition, ROS generation, and mitochondrial deformation in the aorta of diabetic mice [143].

The FoxO transcription factor family plays important roles in the regulation of EC survival by controlling the expression of the apoptotic factor BAD. Hyperglycemia enhances *O*-glycosylation of FoxO1 in human aorta ECs and promotes nuclear localization of FoxO1. Nuclear FoxO1 increases the expression of BAD, which leads to EC apoptosis during hyperglycemia [144]. Moreover, FoxO1 expression is deregulated in the aorta of streptozotocin-induced diabetic mice and diabetic *db/db* mice. FoxO1 deregulation during hyperglycemia and effects on EC survival are reversed by endothelin-1 [144]. Furthermore, deletion of *FoxO1/FoxO3a/FoxO4* in ECs inhibits apoptosis induced by TNFα, FFA, H_2O_2, 7-keto-cholesterol, and free cholesterol [25].

Redox and the cell cycle in ECs

Cellular redox status oscillates during the cell cycle [145, 146]. For example, entry into early G1 is linked to GSH recruitment and sequestration in the nucleus [55]. Redox regulation of the cell cycle involves modification of cell cycle regulatory proteins, such as transcription factors, cyclins, cyclin-dependent kinases, and cyclin-dependent kinase inhibitors [146]. The GSH levels in cultured ECs are elevated during the initial exponential growth phase and decline as cells become confluent. Thus, inhibition of GSH synthesis and GSH deprivation in human microvascular ECs delay S-to-G2 transition [55]. Importantly, growth factor-stimulated ROS generation and redox regulation of p16, p27, and cyclin D1 drive differentiated cells into the cell cycle [147, 148].

Redox regulation of EC migration and angiogenesis

Angiogenesis is the formation of new blood vessels and occurs by EC sprouting from existing vessels. Angiogenesis is a critical event during embryonic development and multiple disease processes. Cell migration and adhesion are essential for the physiological and pathological functions of ECs. Angiogenesis is initiated by EC sprouting, which is driven and guided by a VEGF gradient. Increasing evidence indicates that the redox system tightly regulates EC migration and angiogenesis [149]. VEGF is a well-known mediator of EC migration. Interestingly, VEGF augments mitochondria-derived ROS and Rac-1 activation in HUVECs, which promotes EC migration [150]. However, activation of the thromboxane A2 receptor (TP) blocks VEGF-induced EC migration and angiogenesis by inhibiting Akt and eNOS phosphorylation [151], possibly by the RNS-dependent Rho/Rho-associated kinase/LKB1/PTEN pathway [152]. FoxO1 over-expression impairs cell migration and tube formation in primary ECs, whereas knockdown of FoxO1 by siRNA augments angiogenesis [89]. We recently demonstrated that *AMPKα2* deletion or inhibition of AMPKα2 impairs expression of UCP2. There are also concomitant increases in ROS and $ONOO^-$, which ultimately inhibits endothelial tube formation. Furthermore, ischemia significantly enhances UCP2 expression, eNOS phosphorylation at Ser 1177, and angiogenesis in ischemic adductor muscles from the thighs of wild-type mice but not in those from either $AMPK\alpha1^{-/-}$ or $AMPK\alpha2^{-/-}$ mice [153]. These results suggest that AMPK-dependent UCP2 expression in ECs enhances angiogenesis in vivo through redox balance. In contrast, AMPKα1 activation by transforming growth factor (TGF)-β-activated kinase 1 stimulates EC proliferation, migration, and subsequent VEGF-induced angiogenesis via SOD2 upregulation and $O_2^{\cdot-}$:H_2O_2 balance [154].

Ischemic retinopathy increases EC levels of ROS (H_2O_2 and $O_2^{\cdot-}$), oxidative stress-mediated lipid oxidation, and subsequent activation of the ataxia telangiectasia mutated (ATM) protein due to phosphorylation of ATM at Ser1987 [155]. ATM activation induces pathological neoangiogenesis associated with EC proliferation and decreases apoptosis independent of VEGF signaling or the response to DNA damage [156]. In contrast, *ATM* deletion downregulates the activity of SOD2, CAT, and Gpx1, ultimately upregulating the amounts of cytosolic and mitochondrial ROS in ECs. In addition, *ATM* deletion inhibits neovascular tuft

formation in response to ischemic retinopathy [31]. These results indicate that pathological neoangiogenesis, but not normal physiological neoangiogenesis, requires the ATM-mediated anti-oxidative response. Jansen et al. [157] demonstrated that the transfer of microRNA-126 (miR-126) via endothelial microparticles (MPs) released from apoptotic ECs stimulated EC migration and proliferation, which are both important steps for re-endothelialization. However, the miR-126 level in endothelial microparticles was significantly downregulated by high glucose, possibly due to the presence of ROS.

Thus, ROS act as "double-edged swords" in angiogenesis. Pathological angiogenesis appears to be stimulated by a certain amount of ROS. However, excessive amounts of ROS appear to suppress physiological or regenerative angiogenesis.

H_2S is required for angiogenesis. For example, *CSE*-deleted mice show impaired wound healing probably due to disrupted angiogenesis [158].

Redox regulation of EC inflammation

Endothelial cell inflammatory activation is an early event that is tightly associated with insulin resistance and atherosclerosis. EC inflammation is regulated by redox signaling. AMPK activation in ECs is linked with anti-inflammatory actions. For example, AMPK activation inhibits fatty acid, palmitate, or cytokine TNF-α-induced NF-κB transactivation and VCAM-1 mRNA expression in cultured HUVECs [159]. Furthermore, AMPK activation by high-molecular-weight adiponectin enhances NO production and represses TNF-α-stimulated NF-κB activation [160]. Recently, Bess et al. [161] elucidated the molecular mechanisms by which AMPK mediates the anti-inflammatory actions of NO in EC. AMPKα2 directly phosphorylates IκB kinase at Ser177 and Ser181, leading to the inhibition of IκB kinase [162]. Inhibition of IκB kinase suppresses phosphorylation of IκB and p65 and inactivates NF-κB. Therefore, NO-mediated AMPK activation suppresses IL-1β and TNF-α-induced activation of NF-κB, E-selectin expression, and monocyte adhesion in human endothelial cells [161]. Deletion of *FoxO1/FoxO3a/FoxO4* in ECs suppresses the expression of inflammatory cytokines, such as MCP-1, IL-1β, and IL-6, in response to lipopolysaccharide due to the inhibition of NF-κB transcriptional activity [25].

In addition, deletion of *Hmox1* (encoding HO-1) in ECs increases ROS levels and expression of proinflammatory molecules, including VCAM-1, intercellular adhesion molecule-1 (ICAM-1), and E-selectin, as compared with that of wild-type ECs. In contrast, HO-1 downregulates RelA phosphorylation at Ser276 and subsequently eliminates the proinflammatory phenotype of activated ECs [163]. Very recently, Xiao et al. demonstrated for the first time that disrupted blood flow promotes the assembly and activation of the NLRP3 inflammasome, which is mediated by sterol regulatory element-binding protein 2 (SREBP2). Cleavage of pro-caspase-1 and pro-IL-1β is also increased in ECs via upregulation of NOX2. The activated NLRP3 inflammasome perturbs EC function and increases inflammation [164]. These results suggest that NOX-mediated redox signaling activates the NLRP3 inflammasome in ECs as in monocytes [165] and lung cells [166].

NaHS, an H_2S donor, upregulates HO-1 and eliminates TNF-α-induced ROS production and NF-κB activation. Furthermore, NaHS inhibits TNF-α-induced expression of ICAM-1 and VCAM-1 mRNA and protein in a dose-dependent manner. NaHS also inhibits mRNA expression of P-selectin and E-selectin and blocks the adhesion of U937 monocytes to HUVECs. Overall, H_2S attenuates TNF-α-induced inflammatory signaling in vascular ECs [167].

Redox and EC senescence

Chronic diseases, such as diabetes, accelerate endothelial dysfunction during aging [168], a process that is associated with cellular senescence. However, the mechanisms underlying this process are unclear. High glucose downregulates Sirt1 via ROS with consequent induction of acetylated FoxO1, which reduces SOD2 in association with EC senescence [95]. Deletion of *FoxO1/FoxO3a/FoxO4* prevents EC senescence via eNOS-derived induction of NO [25]. Burger et al. sequentially passaged primary mouse ECs to induce senescence and discovered that cells maintain the phenotypic characteristics of ECs from passage 4 through passage 21. However, ECs from passage 21 have features of senescence, including increased staining for senescence-associated β-galactosidase, a greater percentage of cells in G1/G0 phase, and increased phosphorylation of p66Shc [169]. Furthermore, the number of MPs from passage 21 ECs is higher than that of passage 4 ECs (approximately 2.2-fold increase versus passage 4). The increased number of MPs is blocked by the Rho kinase inhibitor Fasudil. Exposure of passage 4 ECs to MPs shifts cells from a proliferating to a non-proliferating phenotype and increased staining for senescence-associated β-galactosidase. MPs increase the production of $O_2^{\cdot-}$ and H_2O_2, which is blocked by apocynin, a NOX inhibitor, and rotenone, a mitochondrial oxidase inhibitor, but not by allopurinol, an XO inhibitor. In addition, MPs increase expression of $p21^{cip1}$ and $p16^{ink4a}$ and stimulate phosphorylation of p66Shc in ECs. Pretreatment with the ROS scavenger sodium 4,5-dihydroxybenzene-1,3-disulfonate (tiron) abrogates the pro-senescent effects of MPs [169]. These results suggest that MPs promote EC senescence through NOX- and mitochondrial-derived ROS. In addition, $ONOO^-$ in ECs enhances EC senescence via

post-translational oxidative modifications of intracellular regulatory proteins [43]. These redox-sensitive processes may be important in vascular aging.

Redox signaling and EC transdifferentiation

Endothelial cells are derived from several cell types, including embryonic stem cells [170, 171], induced pluripotent stem cells [172], fibroblasts [173, 174], mesenchymal stem cells [175], adipose-derived stem cells [176–178], and glioblastoma cells [179]. Furthermore, the local environment, such as fluid shear stress [180–182], affects EC differentiation of stem cells. In addition, ECs and blood cells are closely intermixed in the extra-embryonic blood islands of mammals and express the same molecular markers [183]. Thus, they may be derived from a common precursor called the hemangioblast. Myers et al. [184] found that bone morphogenetic protein (BMP) signaling mediates erythroid differentiation. Erythroid lineage and BMP signaling constrain EC fate in *Xenopus* blood islands. Our laboratory showed that *AMPKα1* deletion increases the number of reticulocytes in blood [185] and impairs angiogenesis due to elevated oxidative stress [153]. However, the mechanisms by which AMPKα1 may control EC and blood-cell lineage through redox balance remain elusive.

ECs can also transdifferentiate into other cell types, including smooth muscle (SM)-like cells [186], mesenchymal cells [187], and blood cells. Arciniegas et al. [186] reported that adult bovine ECs can transdifferentiate into SM-like cells in vitro. They showed that TGF-β1 induced α-SM-actin expression in aortic ECs. Cooley et al. [188–190] demonstrated that, during the process of tubulogenesis in three-dimensional matrices of fibrin or type I collagen, HUVECs show increased expression of lymphatic vascular endothelial hyaluronan receptor, PROX1, and vascular endothelial growth factor-3, which are markers of lymphatic endothelial cells. Expression of blood vascular markers, such as VEGFC and CD44, are decreased in ECs during tubulogenesis. These results indicate that vascular ECs can switch to a lymphatic-like phenotype. The induction of this phenotype is reversible and is partially inhibited by co-culture of HUVECs with perivascular cells [191]. Medici et al. [192] reported that HUVECs that express constitutively active activin-like kinase-2 (mutant R206H) show an endothelial-to-mesenchymal transition (EndMT) and acquisition of a stem cell-like phenotype. Treatment with TGF-β2 or BMP4 promotes transdifferentiation of ECs into stem-like cells. These stem-like cells can be further differentiated into osteoblasts, chondrocytes, or adipocytes. More importantly, EndMT may contribute to cardiac fibrosis, leading to a progressive stiffening of the ventricular walls [193]. Later, Gupta et al. [194] demonstrated that capillary ECs within white and brown fat may transdifferentiate into preadipocytes. Thus, vascular ECs possess significant plasticity, which may be highly relevant to the function of ECs. However, the mechanisms by which redox regulates the EC-lineage switch remain largely unknown.

Redox regulation of EC fate in the pathogenesis of disease

In addition to the regulatory functions of the redox system during physiological conditions, excessive or sustained redox imbalance is implicated in the pathogenesis of various diseases, such as cardiovascular disease, cancer, obesity, and diabetes.

EC redox imbalance in cardiovascular diseases

An unbalanced redox status in ECs leads to endothelium dysfunction, which is strongly associated with cardiovascular diseases, such as atherosclerosis, hypertension, diabetic cardiovascular complications [195], and ischemia–reperfusion injury.

EC redox imbalance and atherogenesis

Atherosclerosis is a chronic inflammatory disease and is strongly associated with EC dysfunction [196], particularly during the early stages of atherosclerotic disease. Redox imbalance in ECs plays a pivotal role in the initiation, promotion, and progression of atherosclerosis [197, 198]. A number of key signaling pathways in the EC redox system are highly relevant to atherogenesis (Fig. 4).

Atherogenesis preferentially affects branches and curvatures in the arterial tree. Notably, disturbances in blood flow at vascular branches and curvatures promote inflammation of ECs due to SREBP2-mediated NLRP3 inflammasome activation. This process accelerates atherosclerosis [164]. Furthermore, the SREBP2-NOX2-NLRP3 inflammasome was shown to be elevated and activated in the atherosclerosis-prone intima of the mouse aortic arch but not in the thoracic aorta. EC-SREBP2(N) overexpression in $ApoE^{-/-}$ mice augments atherosclerosis in the aortic arch and in thoracic aorta in response to a high-fat diet [164].

High glucose is the major source of ROS/RNS in the ECs of diabetic models [14]. For example, 3-nitrotyrosine (a biomarker of $ONOO^{-}$) is significantly increased in the aortas of streptozotocin-induced diabetic mice [133]. AMPK activation protects ECs against redox imbalance, such as that which occurs during diabetes. Recently, we demonstrated that deletion of *AMPKα2* upregulates NOX2/4 and $O_2^{\cdot-}$ in ECs via NF-κB activation, leading to EC dysfunction and increased atherosclerosis in low-density-lipoprotein-receptor-knockout ($LDLR^{-/-}$) mice

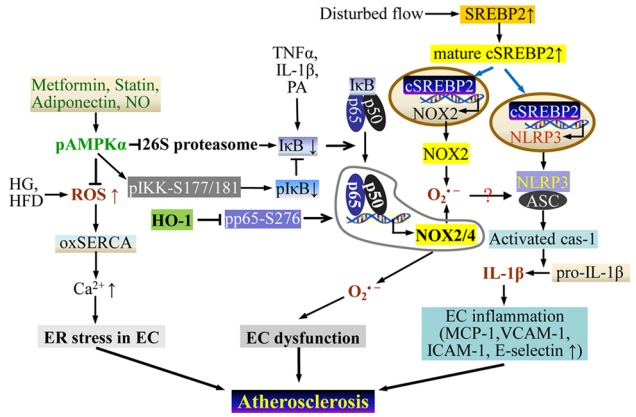

Fig. 4 Redox-regulated EC fate in atherogenesis. AMPK activation in ECs inhibits NF-κB activation by suppressing 26S-proteasome activity or by directly phosphorylating IκK. This ameliorates the ER stress that is induced by ROS due to high glucose or a high-fat diet. Disturbed blood flow activates the NLRP3 inflammasome in ECs through SREBP2-mediated upregulation of NOX2. The activated NLRP3 inflammasome instigates EC inflammation, which accelerates atherogenesis. *Cas-1* caspase-1; other abbreviations as indicated in the text

[199]. Interestingly, reduction of AMPKα2 enhances the oxidation and inactivation of SERCA and increases ER stress in ECs, which further accelerates atherosclerosis in LDLR$^{-/-}$ mice [2]. Activation of AMPK mitigates ER stress due to oxidized LDL/ROS in ECs and attenuates atherosclerosis in *ApoE*$^{-/-}$ mice fed a high-fat diet [200]. Activation of the thromboxane receptor induces phosphorylation of PKCζ at Thr410 and promotes membrane translocation, activating NOX2 in response to elevated $O_2^{\cdot-}$ and $ONOO^-$. These activities cause eNOS to become uncoupled in ECs [201]. Thus, TP activation may be associated with atherothrombotic diseases. In addition, the development of atherosclerosis in the aorta, aortic arch, and descending aorta in response to a western-type diet is mitigated in *LDLR*$^{-/-}$ mice that have EC-specific deletions in *FoxO1/FoxO3a/FoxO4* compared to that of *LDLR*$^{-/-}$ controls. Attenuated atherosclerosis may be attributed to reduced levels of ROS and increased levels of eNOS-derived NO, both of which inhibit inflammation [25]. A western-type diet can promote insulin resistance, which activates FoxO1/FoxO3 early during the process of atherogenesis. Activation of FoxO1/FoxO3 is believed to contribute to atherosclerosis [25]. These results strongly support therapeutic inhibition of FoxOs in ECs as a strategy for treating and/or preventing diabetes-associated cardiovascular diseases.

H_2S is anti-atherosclerotic [202]. For example, in one study, treatment of *ApoE*$^{-/-}$ mice with exogenous H_2S decreased the size of atherosclerotic lesions, whereas CSE inhibition with DL-propargylglycine (PAG) increased the size of these lesions [203]. In addition, although a high level of early mortality was observed in *CBS*$^{-/-}$ mice, a zinc-inducible human *CBS* transgene blocked this effect in *CBS*$^{-/-}$/*ApoE*$^{-/-}$ mice. During pregnancy and weaning, zinc was provided in the drinking water, activating the transgene. Homocysteine levels were only slightly elevated, preventing early mortality. Zinc was removed from the drinking water after the mice were weaned, resulting in severe hyper-homocysteinemia and accelerated atherosclerosis. This study was consistent with prior observations in conventional *CBS*$^{-/-}$/*ApoE*$^{-/-}$ mice. The size of the lesions and the increased levels of tumor necrosis factor, MCP-1, and Ly-6-Chi monocytes/macrophages were proportional to the homocysteine levels. However, insights from other studies suggested that the major factor was the decrease in H_2S. In vitro studies showed that elevated plasma homocysteine inhibited CBS and impaired H_2S production. However, H_2S supplementation completely reversed the inflammation caused by hyper-homocysteinemia. Chemical inhibition of cortical thickness in *ApoE*$^{-/-}$ mice caused a marked increase in atherosclerosis. This excess lesion formation was decreased (by 38 %) with supplementation of sodium hydrosulfide (NaHS), an H_2S donor. *CSE*$^{-/-}$ mice fed an atherogenic diet displayed increased lesional oxidative stress and elevated expression of ICAM-1 in aortae, resulting in the development of early fatty streak lesions in the aortic root [42]. *CSE*$^{-/-}$/*ApoE*$^{-/-}$ mice developed more severe atherosclerosis. Treatment with NaHS, but not *N*-acetylcysteine, retarded the development of atherosclerosis in *CSE*$^{-/-}$ mice, suggesting that H_2S employs an anti-atherosclerotic mechanism distinct from that which is activated by antioxidants.

EC redox imbalance and hypertension

Redox imbalance leads to EC dysfunction [8], which may contribute to impaired EC-dependent relaxation. eNOS uncoupling and dysfunction are implicated in hypertension, in part due to decreased production of NO and increased levels of $O_2^{\cdot-}$. GTPCH1 is the rate-limiting enzyme for de novo synthesis of BH4, the essential cofactor for eNOS. We demonstrated that inhibition or knock down of endothelial GTPCH1 causes an imbalance of NO and $O_2^{\cdot-}$, which increases systolic, diastolic, and mean blood pressures in C57BL6 mice [204]. In addition, $ONOO^-$-mediated tyrosine nitration of PA700, a 26S proteasome regulatory subunit, activates the 26S proteasome and accelerates GTPCH1 degradation, BH4 deficiency, and endothelial dysfunction, which is highly associated with angiotensin II-induced hypertension [205]. Furthermore, NOX-derived $O_2^{\cdot-}$ impairs vasodilation by inactivating NO [206].

Some drugs or agents that alter redox signaling also regulate EC-dependent relaxation. For example,

eicosapentaenoic acid (EPA, 20:5 n-3), an ω-3 polyunsaturated fatty acid that is abundant in fish oils, has cardiovascular-protective effects [207]. We demonstrated that EPA activates AMPK and its substrate eNOS via UCP2 upregulation. Further, EPA improves high-fat/high-cholesterol-induced endothelium-dependent relaxation of the aortic rings. These effects of EPA appear to be dependent on AMPKα1 or eNOS [208]. Thus, fish oils may maintain normal blood pressure via improved redox balance due to NO release.

Moderate levels of the reductant H_2S are required to maintain normal blood pressure. Two of the major enzymes in the trans-sulfuration pathway, CSE and CBS, metabolize homocysteine and serve as major sources of H_2S. H_2S is a physiologic vasorelaxant that opens ATP-sensitive K^+ channels in VSMCs [209]. CSE and H_2S levels are repressed in pulmonary-hypertensive rats [210]. Administration of the CSE inhibitor PAG leads to hypertension in rats [211] and pregnant mice [18]. Deficiency of either CSE or CBS markedly impairs cellular production of H_2S. *CSE*-deleted mice have dramatically reduced levels of H_2S in the serum, heart, and aorta. In addition, they show diminished endothelium-dependent vasorelaxation, which results in pronounced hypertension [19, 26]. However, H_2S levels are higher in patients with coronary artery disease, peripheral arterial disease, or vascular diseases compared to patients who do not have vascular diseases [212].

Redox regulation of EC fate and cancer development

Tumor angiogenesis is essential for tumor development [213]. EC fate, including migration, proliferation, apoptosis, and the formation of tight junctions, is important for tumor angiogenesis and metastasis [214]. Redox systems, including ROS/RNS, regulate angiogenesis during cancer development [215, 216]. Elevated production of ROS mediated by NOX4 and the mitochondrial electron chain increases the expression of HIF-1 and VEGF in ovarian and prostate cancer cells. Increased levels of endogenous ROS are required for angiogenesis and tumor growth [215]. Increased production of ROS enhances tumor angiogenesis and tumor growth of ovarian cancer cells via the recruitment and proliferation of ECs. B16 melanoma cell subcutaneous implants display increased amounts of intracellular H_2O_2 and activated ATM kinase in ECs. In addition, tumor angiogenesis is enhanced in this in vivo model compared with that of normal subcutaneous tissue. However, endothelial-specific deletion of *ATM* significantly blocks pathological neoangiogenesis in association with increased apoptosis and reduced proliferation due to elevated production of ROS [156].

Interestingly, several oxidized products are increased in cancer tissue. For example, the end products of lipid oxidation by oxidative stress, ω-(2-carboxyethyl)pyrrole (CEP) and other related pyrroles, accumulate at high levels in highly vascularized tumors, such as murine and human melanomas [217]. Elevated CEP is recognized by Toll-like receptor 2, but not Toll-like receptor 4, and scavenger receptors on ECs, leading to Rac1 activation, EC migration, and tumor angiogenesis independent of VEGF. Moreover, neutralization of endogenous CEP diminishes the vascularization of melanomas [217].

In addition, H_2S enhances angiogenesis. The H_2S-generating enzyme CBS is selectively upregulated in human colon cancers, resulting in a high rate of H_2S production. CBS knockdown by shRNA or pharmacological inhibition with aminooxyacetic acid inhibits EC migration in colon cancers or co-cultures. These results indicate that endogenous H_2S promotes tumor angiogenesis in human colon cancers [20].

Redox regulation of EC fate in obesity and diabetes

Obesity is characterized by the hypertrophy and hyperplasia of adipocytes in white adipose tissue (WAT). Angiogenesis plays a critical role in the modulation of adipogenesis and the development of obesity [7, 218, 219]. Angiogenic vessels provide nutrients and oxygen to fat tissue, enrich the concentrations of growth factors and cytokines in plasma [220], and increase the numbers of circulating stem cells, including bone marrow-derived stem cells [221, 222]. Angiogenesis also increases the delivery of EC-derived growth factors and cytokines to adipocytes, increasing the growth and expansion of fat tissue in a paracrine manner [223]. ECs from different fat depots have distinct characteristics. Visceral adipose tissue (VAT) has a higher vascular density and number of ECs than that of the corresponding subcutaneous adipose tissue (SAT) from obese human subjects. ECs from VAT display remarkable levels of angiogenesis and inflammation with reduced expression of metabolism-associated genes, including endothelial lipase and PPARγ. VAT-derived ECs express increased levels of cellular senescence markers, such as IGFBP3 and γH_2AX, and express lower levels of SIRT1 [224]. In contrast, human SAT embedded in Matrigel has a stronger angiogenic capacity than VAT. Moreover, the angiogenic capacity of SAT, but not VAT, is inversely associated with insulin sensitivity [225]. Modulation of EC fate is highly associated with angiogenesis. However, because EC fate is tightly regulated by redox, the development of adipose tissue may be affected by the redox state. The growth of adipose tissue in mice was inhibited by angiogenesis inhibitors, such as TNP-470 [226], angiostatin (a plasminogen fragment containing kringle domains 1–4), and endostatin (a C-terminal fragment of collagen XVIII). The mechanisms

implicate decreased proliferation and increased apoptosis of ECs [227]. Tam et al. [228] further demonstrated that formation of new vessels in fat tissues during diet-induced obesity largely depends on angiogenesis rather than de novo vasculogenesis. Anti-angiogenic treatment with monoclonal antibodies that block VEGFR2, but not VEGFR1, may limit the expansion of adipose tissue. Notably, brown adipose tissue (BAT) is probably the most highly vascularized tissue in the body, as each adipocyte is encircled by capillaries [229, 230]. Thus, EC fate may tightly regulate BAT development and function, as well as beige adipocytes differentiation in WAT [231].

Diabetes is well known to alter EC fate and function, since ECs are primary target of hyperglycemia-mediated oxidative damage [101]. For example, gestational diabetes mellitus weakens Nrf2 antioxidant signaling, and consequently induces oxidative stress in fetal ECs in utero [232], which may result in the increased risk of type 2 diabetes in offspring [233]. Additionally, EC fate also affects diabetes progression. Emerging evidence suggests that vascular ECs regulate the activities of insulin associated with metabolism [234, 235]. For example, adipose tissues from eNOS-transgenic mice that were provided a high-fat diet displayed higher levels of PPAR-α and PPAR-γ, increased expression of mitochondrial proteins, higher metabolic rates, and decreased hypertrophy of white adipocytes. Consistent with these alterations, eNOS transgenic mice were resistant to diet-induced obesity and hyper-insulinemia [236]. In addition, NO bioavailability associated with EC function directly stimulates EC insulin transport [237]. These results indicate that adipose tissue mass and diabetes may be regulated by vascular EC fate.

Obesity and most obesity-associated disorders, including diabetic complications, cardiovascular disorders, and malignancies [238], are associated with pathological angiogenesis [223, 239]. Thus, the modulation of EC fate-associated angiogenesis is a promising therapeutic approach for anti-obesity and insulin sensitivity. In particular, the stimulation of angiogenesis in metabolically active BAT or during beige adipocytes induction may facilitate energy expenditure [240, 241]. However, the mechanisms by which redox regulation of EC fate mediates the BAT/WAT switch [242] remain largely unknown.

Therapeutic strategies for redox regulation of EC fate

Endothelial cell fate is a critical determinant of overall health and the development of diseases, such as cardiovascular disease, cancer, obesity, and diabetes. Thus, the normalization of EC function is a potential approach for preventing and treating many human diseases.

Strategies for maintaining redox homeostasis in ECs

Exogenous and direct exposure to oxidants or reductants is an important strategy for maintaining endogenous redox balance in ECs. Nitro-fatty acids (NO_2-FAs) are nitrated derivatives of unsaturated fatty acids that form in human blood and tissues [243]. NO_2-FAs release NO and possess antioxidant functions [244]. Therefore, NO_2-FAs may be promising pharmacological tools as NO donors to prevent inflammatory diseases associated with redox imbalance [245]. In addition, NO_2-FAs may function independently of NO. For example, nitrated oleic acid upregulates HO-1 and HIF1-α and activates Ca^{2+}/calmodulin-dependent protein kinase kinase-β in bovine aortic endothelial cells. This leads to the activation of AMPK and eNOS, which are tightly associated with EC function [246].

H_2S is a chemical reductant with anti-oxidant function. Because H_2S has vasculoprotective functions, including vasorelaxation, prevention of inflammation, anti-proliferation, and anti-thrombotic effects, it is a potential alternative approach for treating hypertension [247]. This is supported by the physiologic role of H_2S as a regulator of blood pressure. Several H_2S donors demonstrate preventive and therapeutic effects. For example, morpholin-4-ium 4 methoxyphenyl (morpholino) phosphinodithioate (GYY4137) is a water-soluble, slow-release formulation of H_2S. Chronic administration of GYY4137 during a 14-day period elicited antihypertensive effects in a rat model of spontaneous hypertension [32]. Sodium hydrosulfide (NaHS), an H_2S donor, increased collateral vessel growth and capillary density in a rat model of hind-limb ischemia via upregulation of VEGF in skeletal muscles and phosphorylated VEGFR2 in neighboring vascular ECs [42]. NaHS administration blocked atherogenesis [19]. Recently, H_2S-donating agents in combination with aspirin, the so-called HS-ASA hybrids, have been shown to have anti-cancer potential [248]. Importantly, a number of human intervention trials currently involve various H_2S donors. For example, NaHS controls EC redox homeostasis and has been used in clinical trials [249]. Interestingly, naturally occurring polysulfides in garlic yield biologically meaningful amounts of H_2S by interacting with GSH in red blood cells [250]. These insights may help explain the cardiovascular protective outcomes of several randomized, controlled trials that utilized garlic extracts [251, 252].

Regulation of redox-sensitive signaling molecules

Activation or inhibition of endogenous oxidants or reductants would help create a balanced redox system and may be a promising strategy for preventing or curing diseases associated with altered EC fate. For example, several inhibitors of NOX have been developed to specifically target

dysfunctional NOX [253]. The polyphenolic derivative S17834, benzo(b)pyran-4-one, inhibits NOX activity in EC membranes and in atherogenesis [254]. The NOX inhibitor also decreases Sirt-1-mediated acetylation of p53 on lysine-382 and ultimately reduces signaling pathways that promote apoptosis of ECs [255]. Redox sensors and modulators normalize the redox state and functions of ECs in vivo. For example, AMPK activation occurs in response to metformin, which is widely prescribed for type-2 diabetes [78]. In addition, gene therapy and peptide/protein therapy are potential strategies for modulating redox signaling. Gene therapy targets include enzymes involved in redox homeostasis and those that control EC fate. The delivery of genes that encode antioxidant-defense enzymes, such as SOD, CAT, Gpx, or eNOS, ameliorates ROS production and atherogenesis in animal models [256, 257]. Delivery or knockdown of genes that encode redox-sensitive transcriptional factors, such as FoxOs, NF-κB, and Nrf-2, has also been successfully employed in animal models [258]. However, the delivery method is often a limiting factor for gene therapy. Nanoparticles targeted to ECs of the desired tissue or organ may provide a promising therapeutic intervention with increased precision and efficacy [259–261]. The administration of nanoparticles that delivered antioxidants, such as CAT and SOD, to ECs was recently shown to elicit favorable outcomes [262].

Conclusions and perspectives

Redox molecules are created during normal EC metabolism. Redox systems play pivotal roles in the modulation of EC fate. Thus, it is important to understand the molecular mechanisms that regulate EC fate. Although great progress has been made in the last two decades, the redox mechanisms that regulate EC fate remain unclear. Future research into redox regulation of EC fate should focus on the following.

1. An emerging feature of redox systems is the spatial and temporal compartmentalization [83]. Recent research shows that different redox-signaling and redox-buffering systems are spatially segregated and have unique compartmentalized functions. Furthermore, oxidative signals selectively oxidize target molecules. The maintenance of compartmentalized redox homeostasis is pivotal for EC fate and function. Thus, determining how this compartmentalized nature of cellular redox systems is linked to EC fate will be critical for fully understanding how EC fate promotes disease development and progression.
2. Intriguingly, many of the molecules that are highly associated with redox regulation of EC fate appear to be modified at redox-sensitive cysteine residues. Such molecules include FoxOs, Nrf2, HIF-1, AMPK, and PTEN. Thus, these molecules may function as 'redox sensors.' Understanding the mechanisms by which these molecules act as 'redox sensors' and regulators of EC fate is an important area of research.
3. The potential physiological and clinical significance of H_2S has received considerable attention in recent years. However, the molecular targets and molecular mechanisms of H_2S action in ECs remain unknown. Novel pharmacological agents that are developed based on the principles of H_2S donation may be able to prevent disease onset by balancing the redox system.
4. Further investigations must be performed to understand the molecular mechanisms by which the redox system regulates EC fate during the development and switching of white adipocytes and beige adipocytes.
5. Additional techniques should be developed to target or deliver genes that encode redox sensors and modulators to ECs.

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Conflict of interest The authors declare that there are no conflicts of interest.

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