Cell. Mol. Life Sci. (2015) 72:3281–3303
DOI 10.1007/s00018-015-1928-9

Cellular and Molecular Life Sciences

REVIEW

ROS signaling and redox biology in endothelial cells

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Received: 25 February 2015 / Revised: 29 April 2015 / Accepted: 7 May 2015 / Published online: 14 May 2015

Abstract The purpose of this review is to provide an overview of redox mechanisms, sources and antioxidants that control signaling events in ECs. In particular, we describe which molecules are involved in redox signaling and how they influence the relationship between ECs and other vascular component with regard to angiogenesis. Recent and new tools to investigate physiological ROS signaling will be also discussed. Such findings are providing an overview of the ROS biology relevant for endothelial cells in the context of normal and pathological angiogenic conditions.

Keywords Reactive Oxygen Species · Redox signaling · Angiogenesis · Genetically-encoded sensors · Redox compartmentalization · Pathological angiogenesis

Introduction

Reactive Oxygen Species, also known as ROS, are an important constituent of cellular physiology since they are normally produced in response to disparate exogenous and endogenous stimuli and they are continuously generated, transformed and consumed in tissues during the normal metabolic activity [1, 2]. A growing body of evidence suggests the existence of an intricate balance between ROS-producing and ROS-scavenging systems that are tightly regulated over time and space and interact with the cellular redox-sensitive elements to shape and finely modulate downstream signaling events in a cell-specific and context-specific manner [3]. In this respect, endothelial cells (ECs), which form blood vessels and the circulatory system, are quite peculiar. Indeed, compared to other cells, they act as an interface between the tissues of different origin and circulating elements such as oxygen, metabolites, and fluid stress. Indeed ECs are constantly exposed to a different array of biochemical and mechanical stimuli and actively adapt their biological responses to preserve the homeostasis of the vascular function [4]. ROS might function as a double-edged sword in endothelial cells. A moderate increase of ROS may promote cell proliferation and survival. However, when the burden of ROS reaches a certain level (the toxic threshold), it may overwhelm the antioxidant capacity of the cell and trigger cell death. Under physiological conditions, normal endothelial cells maintain redox homeostasis with a low level of basal ROS by controlling the balance between ROS generation (pro-oxidants) and elimination (antioxidant capacity). Cells in normal vessels can tolerate a certain level of exogenous ROS stress owing to their 'reserve' antioxidant capacity, which can be mobilized to prevent the ROS level from reaching the cell death threshold. Although high levels or prolonged formation of ROS is generally associated to vascular dysfunctions such as diabetes, carcinogenesis atherosclerosis and aging due to DNA, protein and lipid damage, appropriate levels of ROS have been shown to be indispensible mediators of physiological processes and redox-regulated cellular signaling pathways in endothelial cells.

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ROS signaling: principles, facts and locations

Despite the term ROS includes radicalic (e.g., hydroxyl radical, $OH^{\cdot}$) and non-radicalic (e.g., hydrogen peroxide, H_2O_2) molecules derived from incomplete reduction of oxygen (O_2), it is important to recognize that different species possess distinct reactivity, half-life and molecular targets that influence their selectivity and dictate their biological relevance in a specific context [5]. Among the diverse ROS, hydrogen peroxide (H_2O_2) fulfills most if not all the prerequisites for serving as an intracellular second messenger and appears to be the evolutionarily selected ROS for redox signaling purposes. Indeed, this molecule is almost ubiquitously generated through direct activation of specific systems or alternatively by enzymatic conversion of other precursors (i.e., superoxide anion, O_2^{-}), has a relatively long half-life and can reach target molecules located far from the site of its generation, exerting short-term and prolonged effects in different signaling pathways [6, 7]. Understanding the chemistry of redox signaling is essential to define the specificity and the principles of downstream modifications that represent the real interface between ROS and redox-sensitive targets. It is now well established that under appropriate circumstances H_2O_2 can react with and modify specific redox-reactive cysteine residues (with pKa values between 4 and 5) within target proteins, inducing a covalent modification that is able to influence the activity, stability or subcellular localization of a great variety of molecules, including enzymes, transcription factors, ion channels, cytoskeletal proteins, tyrosine kinase receptors, protein kinases and phosphatases [8–14]. It is important to mention that other amino acids such as methionine and histidine can be oxidized by ROS, but little experimental evidence has been provided and some of the underlying mechanisms as well as the biological consequences of their modifications in redox signaling are still largely unknown. Since the most extensively studied and predominant post-translational modification of redox-sensitive elements involves the reversible oxidation of cysteines, we will mainly discuss the general mechanisms and the potential implication of cysteine-based redox signaling in functional angiogenesis. Cysteine is one of the more readily oxidized amino acids though its relative high pKa values (8–9 at physiological pH) make it a slowly reacting nucleophile due to the prevalence of the protonated thiol form (-SH) in the sulfur atoms. Interestingly, the proximity of positively charged amino acids (Arg, His, Lys) in the local microenvironment of the protein can lower the pKa of specific cysteines (3–4 at physiological pH) greatly increasing their dissociation rate into the thiolate form (S-) and therefore their susceptibility to ROS-induced modifications [15–17]. Therefore, it seems that only a limited group of reactive cysteines has been evolutionarily selected to participate in redox signaling events although the existence of additional determinants and mechanisms is under investigation and remains still elusive. It is, however, now well recognized that most of the biological oxidants, including H_2O_2, react exclusively with the thiolate anion form [15, 17, 18]. Mild oxidation of redox-sensitive cysteines generates reactive sulfenic acid (SOH) that can either form intramolecular/intermolecular disulfide bonds or undergo further oxidation to sulfinic (SO_2H) or sulfonic (SO_3H) acid forms [19]. With the exception of sulfonic acid formation, the sulfenic acid and to a lesser extent the sulfinic acid formation can be reversed by specific reducing systems including peroxiredoxins and thioredoxins [15], determining spatial and temporal control of the associated signaling event [3]. Therefore, in contrast with pathological alterations (pathological angiogenesis), the general principles that drive redox signaling events require the controlled, specific and reversible interaction between physiological amounts of ROS (i.e., H_2O_2) and an appropriate biologically relevant target (thiolate anion of a reactive cysteine residue) that should intercept the peroxide molecules avoiding the kinetic competition of highly efficient scavenging systems or the prolonged accumulation of potentially harmful byproducts [20].

Redox compartmentalization

Different functions of the mammalian cells including the ECs are optimized by clear separation of compatible reactions and chemistries into defined subcellular compartments limited by lipid-containing membranes or even into specialized microdomains located within a certain cellular region [21]. Accumulating knowledge of redox processes in the cytoplasm, nucleus, mitochondria and the secretory pathway of mammalian cells indicates that peculiar redox features have co-evolved with specific residing elements that receive and transduce redox-related signals under tightly controlled spatiotemporal regulation. The emerging concept known as “redox hypothesis” assumes that different redox couples, in their thiol/disulfide forms, are confined in specific subcellular compartments and maintained at distinct steady-state redox potentials that confer them independent properties in terms of specificity and reactivity towards a defined redox event, promoting the regulation of multiple biological processes [22, 23]. Within compartments, the redox couples appear to be controlled by the opposing action of reductive (i.e., Thioredoxin and Glutathione systems) and oxidative systems (i.e., NADPH Oxidases and mitochondrial ETC) that ultimately dictate the redox state of the oxidizable thiols located within

susceptible proteins. Importantly, the redox signaling and control systems co-evolved in specialized redox environments that ensure optimal efficiency while disruption of these complex redox circuits can impair redox control and lead to oxidative stress [24]. Intuitively, defining the mechanisms through which the regulated/reversible oxidation of thiols is exerted within different subcellular compartments will provide a better comprehension of pathophysiological mechanisms associated with oxidative stress [2]. Despite the great relevance of this aspect, it has been historically difficult to achieve a comprehensive knowledge of the interrelation between the production of a certain ROS molecule and the functional modification of the major redox couples or sensitive proteins residing within a certain organelle. The detailed study of these processes would therefore require appropriate tools to investigate local and specific changes with high spatial and temporal resolution that only recently became available.

Experimental approaches to investigate redox compartmentalization

To study the link between ROS formation and redox signaling, it would be desirable to identify the ROS molecule, the redox couple and the putative target proteins that participate to a certain biological process and define their relationship in a spatiotemporal context. A great variety of techniques have been developed to measure ROS generation, to study the redox status (i.e., redox potentials) of specific redox couples or to identify modifications of thiols groups within redox-sensitive proteins [21]. For example, small chemical probes have been extensively used to detect the generation of Superoxide anion (O_2^{-}), Hydroxyl radical ($OH^{\cdot}$), H_2O_2 or less defined ROS-induced alterations (DCFH-DA) in different types of cells; similarly, high-performance liquid chromatography (HPLC) has been employed to estimate GSH/GSSG and NAD(P)H/$(NAD(P)^+$ redox potentials while the combination of redox western blot, chemical labeling of thiols groups (ICAT) and mass spectrometry techniques has been exploited to gain information on the redox status of cysteines residing within proteins of interest [25]. Although widely used, these methods still suffer from general limitations and cannot provide conclusive or totally reliable information of a redox signaling event. In particular, even when sufficient specificity is achieved, the main pitfalls are related with the disruption of cell integrity that precludes dynamic measurements in intact cells and generates experimental artifacts with partial or complete loss of compartment-specific information. Conversely, when organelle-specific probes are employed in living cells, the lack of reversibility poses serious limitations for the temporal analysis of a given process [26]. In the following section, some of the most common fluorescent probes, falling into the categories of chemical and genetically encoded molecules, will be briefly described.

Chemical probes for ROS detection

Several small molecule fluorescent probes are currently available, though few of them can be targeted into specific subcellular organelles and possess sufficient sensitivity and selectivity for the detection of a definite ROS molecule generated at physiological levels. These molecules can be grouped into two main classes, "oxidant sensitive" or "non-redox" dyes. The former includes commonly used probes that become fluorescent after being oxidized while the latter comprises molecules wherein the nucleophilic attack by the oxidant releases a masked fluorophore. Probably the most widespread-used detection method for H_2O_2 in cultured cells is based on the oxidation of dihydrochlorofluorescein (H_2DCFH) to the fluorescent derivative dichlorofluorescein (DCF). However, artifactual oxidation of the probe can occur without the formation of H_2O_2 and is more generally related to the activation of poorly defined oxidative processes [27–29] or the induction of apoptotic cell death leading to cytochrome c release from the mitochondrial inner membrane [30]. Other oxidant-sensitive indicators include MitoSOX Red, a hydroethidine derivative that is uptaken by actively respiring mitochondria and generates 2-hydroxiethium after reacting with superoxide, or the reduced MitoTracker CM-H_2TM/XROS dyes, that become fluorescent, positively charged and readily accumulate into well-energized mitochondria upon oxidation by still undefined ROS molecules. However, the major drawback for the oxidant-sensitive class of sensors is a general lack of specificity, a low sensitivity and the absence of reversibility [31]. New generation of chemical probes with improved sensitivity and specificity such as Amplex Red (dihydroxyphenoxazine derivative), Peroxy Green1 or Peroxy Crimson1 (Boronate derivatives) was subsequently introduced allowing detection of physiological amounts of endogenous H_2O_2 produced in response to growth factor stimulation [27, 32]. Importantly, the attachment of appropriate functional groups has enabled compartment-specific localization of peroxide generation as for the nuclear-Peroxy Emerald1 (nucPE1) [5], mitochondrial-Peroxy Yellow1 (MitoPY1) [33], or even measurement of redox events in living organisms as for Mito-Boronic acid B (MitoB) or SHP-Mito [34, 35]. Nevertheless, intrinsic limitations related to slow reaction rates, lack of reversible behavior and the inability to generate animal models with stable transgenesis raise important concerns on the applicability of these

compounds to signaling events and the correct interpretation of the experimental information provided by their use in living systems.

Genetically encoded redox sensors

One of the most innovative, exciting and biologically relevant tools for rapid, real-time and reversible detection of specific redox events with high spatiotemporal resolution has been represented by the introduction of genetically encoded redox-sensitive proteins. Many of the limitations associated with the use of chemical probes were already overcome by the development of the first GFP-based redox-sensitive probes. By introducing reactive cysteines in strategic positions of either the GFP or YFP β-barrel scaffold, the groups of Jakob Winther and James Remington, respectively, created the redox-sensitive yellow fluorescent protein (rxYFP) [36] and the redox-sensitive GFP (roGFP) [37]. In these sensors, the redox status of the sensitive cysteines depends on the equilibration with the GSSG/GSSH redox couples through the thiol–disulfide exchange catalyzed by the enzyme glutaredoxin (Grx), while the fuorescent properties of the cromophore region are accordingly affected by redox events that modify the steady-state level of the oxidized or reduced susceptible cysteines. Although both allow specific and non-invasive analysis of the dithiol–disulfide equilibrium with the glutathione system, the rxYFP has quite limited dynamic range, is sensitive to pH and chloride anions and does not possess a clear ratiometric beahavior. Moreover, its equilibration rate with the glutathione system depends on the availability or the activity of Grx isoforms. This limitation was partially overcome by fusing the rxYFP to Grx1 to eliminate the dependency on endogenous Grx (rxYFP-Grx1) [38]. In contrast, the roGFP class of sensors has two distinct excitation peaks around 400 and 480 nm with a single emission wavelength around 520 nm, that shift in opposite directions when the sensitive cysteines located on β-strands 7 and 10 exist in the dithiol or disulfide forms. When the vicinal cysteines are oxidized, the formation of a disulfide bridge induces several rearrangements in the β-strands and other conformational changes that ultimately influence the protonation status of specific phenolic groups residing within the cromophore region, producing divergent changes in the excitation peaks. This behavior can be exploited for ratiometric measurements that are insensitive to experimental artifacts related to different expression levels of the sensor, photobleaching or changes in the cellular morphology [26]. Further implementation and genetic engineering of the prototypical roGFP by the group of Tobias Dick led to the generation of additional variants (six so far), among which the roGFP2 is the most used and best characterized due to its pH insensitivity, its high dynamic range and a suitable midpoint redox potential (−280 mV) for measurement of redox events in most of the organelles of mammalian cells. By fusing redox-active enzymes with redox-sensitive roGFP2 moiety, the same group created redox-relay based sensors that are able to rapidly equilibrate with defined redox pairs such as GSSG/2GSH (Grx1-roGFP2) or H_2O_2/H_2O (roGFP2-Orp1) in a reversible, kinetically efficient and specific way [39–41]. Not surprisingly this class of sensors has been successfully employed to study redox changes in different subcellular compartments (cytosol, mitochondria, ER) and various experimental conditions in both cultured cells [22, 42–45] and living organisms [45–47].

Another H_2O_2-specific sensor, called HyPer, was developed by the group of Sergei Lukyanov by inserting a circularly permuted YFP (cpYFP) into the regulatory domain of the OxyR transcription factor from E.coli [48]. Similar to the roGFPs, HyPer exhibits submicromolar affinity for H_2O_2, can be targeted in different subcellular districts [49] or even into specialized intracellular microdomains [50] and allows ratiometric and reversible measurements. Engineered variants with expanded dynamic range (HyPer2) [51] or improved kinetic response (HyPer3) [52] have been introduced and successfully employed in intact cells [53] and model organisms [54]. However, as for other cpFPs, the HyPer excitation ratio is quite sensitive to pH fluctuations so that an independent method for monitoring the pH changes in a given experimental setting should be devised to avoid artifactual results [26, 55]. Similar limitations have been reported for another sensor named cpYFP that was initially targeted into the mitochondria and appeared to detect superoxide production derived from the mitochondrial electron transport chain activity [56]. However, the underlying chemistry was not sufficiently clarified and the presumed specificity of cpYFP for superoxide has been debated for a long time [57] leading to a passionate controversy that only recently was solved by showing that cpYFP responds to pH rather than superoxide changes [57, 58]. Another rapidly expanding class of redox sensors is based on fluorescent resonance energy transfer (FRET) that exploits structural changes induced by different redox states to modify the distance between a FRET donor and acceptor. Though quite limited experimental information is currently available on FRET-based redox sensors, novel variants such as rOHser [59] [60] or CY-RL7 [61] have been recently introduced and targeted to the cytosol, mitochondria, endoplasmic reticulum and nuclei of different cell types [62] or even fused to the dual oxidase activator (DuoxA1) for detection of redox events occurring at the level of the plasma membrane [63]. Despite the great advantages and the exceptional contribution provided by the genetically encoded biosensors in the study of redox events, it should

be recognized that intrinsic limitations are still present such as the restricted number of different fluorophores that are currently available or potential alterations of cell physiology (e.g., signaling pathways) that might derive from the introduction of the sensor in a living system [25]. For these reasons, care should be taken for the interpretation of the results and additional methods should be used to further support the experimental hypotheses under investigation.

Mechanisms of ROS production and scavenging in endothelial cells

ECs form an active monolayer organ that enfolds the inner surface of the blood vessels in the cardiovascular system representing a natural barrier between the blood and the other tissues. ECs represent also an active platform of interaction with numerous cellular elements including leukocytes, vascular smooth muscle cells, pericytes and stem cells. ECs are metabolically active cells constantly exposed to different biochemical and biomechanical stimuli. They are very plastic in the way they adapt to these physiological conditions by preserving the integrity and homeostasis of blood vessels [64]. In several pathological conditions, this homeostasis is perturbed leading to vascular-related diseases [65]. Indeed, it is now well recognized that altered redox homeostasis plays a key role in the pathogenesis of endothelial dysfunctions including atherosclerosis [66], hypertension [67, 68] and diabetes [69], while the appropriate and controlled production of ROS is essential for the activation of signaling pathways involved in normal endothelial functions [70]. Indeed, ROS signaling in endothelial cells drives the formation of new vessels during development, a process called angiogenesis [71, 72]. Redox signaling events in the cardiovascular system rely on the precise, site-specific and reversible modifications of target proteins induced by either reactive oxygen (ROS) or nitrogen species (RNS). The principal mechanisms by which ROS and RNS generate specific biological effects in the endothelial cells include the post-translational modification of cysteine thiols within the active site or regulatory domains of target proteins, the oxidation of iron-sulfur cluster-containing centers and the S-glutathionylation or S-nitrosylation of protein thiolate anions (anionic forms of the sulfur atom contained in cysteine or methionine residues). In line of principle, a fine-tuned regulation of redox signaling depends on the complex interrelation between ROS/RNS production and their elimination exerted by scavenging systems or other regulators that control the intensity of ROS/RNS release or temporally regulate the effect of a certain modification associated to a biological event. The following section will describe the main ROS sources in the vascular system.

NADPH oxidases

NADPH oxidases (NOXs) represent a large family of professional enzymes with oxidase activity that has evolved as specialized sources of ROS production and essential regulators of vascular function and dysfunction [73]. Since the discovery of the phagocytic NADPH oxidase (Nox2), six additional members have been so far identified (Nox1, Nox3, Nox4, Nox5, Duox1 and Duox2) and functionally characterized in mammals. Each of them possesses specific regulatory mechanisms, downstream targets and differential expression in tissues and cellular compartments [72].

All NOXs are membrane-spanning proteins with a core catalytic subunit (Nox1-5, Duox1-2) and a number of accessory subunits (i.e., p47phox, p67phox, p22phox, p40phox, Rac1-2, DuoxA1-2, NoxO1 and NoxA1) that facilitate the assembly of the functional enzyme. In response to specific upstream stimuli, the NOXs transfer electrons from the substrate NADPH to the molecular Oxygen, leading to either superoxide (O_2^-) or H_2O_2 production [74–77]. Importantly, ROS production from NADPH oxidases can occur either in the extracellular space or inside the cellular milieu, depending on the biological membrane wherein the enzyme is located, ranging from plasma membrane, endosomes, endoplasmic reticulum, nucleus, caveolae and mitochondria [77, 78]. Nox4 is the far most abundant isoform in the endothelial cells where in concert with Nox2 seems to be responsible for the basal tone of ROS derived from normal metabolic activity [79]. However, Nox1, Nox2, Nox4 and Nox5 have been shown to mediate physiological and pathological events in the vascular system [76]. Recent evidences suggest that NOXs regulate multiple redox-sensitive signaling pathways of the endothelial cells, including inhibition of tyrosine phosphatases [80], activation of specific transcription factors [81] and enzymes [82] or modulation of ion channels [83] in response to activating stimuli. Nox1 activity has been implicated in cell signaling, cell growth, cell motility and angiogenesis but also in pathologic alterations such as atherosclerosis, hypertension, inflammation and cancer [72, 84–86]. It has been recently demonstrated that NOX5 activation is required for stromal derived factor 1α (SDF-1α)-induced JNK3 activation and angiogenesis in bovine aortic endothelial cells [87].

It is known that Nox2-derived ROS are important mediators of p38 MAPK-dependent proliferation and VEGF-induced migration. Moreover, NOx2 activation appears to affect NO bioavailability and regulate the expression of adhesion molecules during angiogenesis and inflammation [76, 88, 89]. The precise role of NOX4 in physiological and pathological events is still controversial since under certain conditions this isoform was shown to promote oxidative stress and tumorigenesis [90] or positively regulate the

neovascularization process during oxygen-induced retinopathy [91],while evidence from Nox4−/− mice pointed out its protective role from ischemic and pro-inflammatory stress [79]. Similarly, Nox4 was shown to counteract hypertensive alterations [92] and promote angiogenesis through eNOS activation [82] or HIF1α stabilization [93].

Shear stress

ECs lining the lumen of the blood vessels are constantly exposed to blood flow that produces mechano-stress important to shape the vasculature and maintain ECs homeostasis. Interestingly, NOXs-derived ROS production has been shown to play a pivotal role in transducing the mechano signal, a process that modifies redox balance in response to altered mechanical load of the vascular system [94]. Depending on the pattern and the type of the stress, the activation of specific NOXs enzymes can be either beneficial or detrimental [95, 96]. Indeed, in some cases, NOXs-dependent ROS formation has been shown to promote cellular adaptation to physical forces and vascular protection during physiological mechano-stress (laminar shear stress) [8] while conversely disturbed blood flow (turbulent flow, oscillatory high rates) or extensive stretch has been associated to prominent ROS production due to NOXs overactivation (e.g., Nox1, Nox2), and subsequent oxidative damage [97, 98]. However, additional studies will be needed to better define the specificity of NOXs-dependent redox signaling in mechano-transduction events.

Mitochondria

The mitochondria are considered the prominent sources of endogenous ROS under physiological conditions and deregulated mitochondrial ROS production is associated to many cardiovascular alterations [99]. The mitochondrial electron transport chain machinery (mETC), located in the inner mitochondrial membrane, is considered main responsible for mitochondrial ROS production. More specifically, NADH or $FADH_2$ molecules generated in the Krebs cycle are used as electron donors by four complexes in the mETC catalyzing the reduction of molecular oxygen to water through four single-electron transfer reactions. The controlled electrons flow is generally used to build up a chemiosmotic proton gradient across the inner mitochondrial membrane that ultimately drives ATP synthesis at the level of Complex-V (ATP synthase). At several sites along the respiratory chain, especially in the Complex I and III, the electrons can be diverted to reduce molecular oxygen or other electron acceptors and generate free radicals (e.g., O_2^{-}). In addition to the inner membrane, ROS can be additionally produced in the intermembrane space (IMS) by the cytochrome c interacting protein p66shc, in the matrix by metabolic enzymes such as aconitase, PDH, α-KGDH, or even in the outer mitochondrial membrane (OMM) by the flavoprotein monoamine oxidases (MAO A and MAO B) [100].

A minor fraction of the superoxide derived from the mETC can leak into the cytosol through the pore-component VDAC, while the vast majority is enzymatically converted into H_2O_2 by the Mn-SOD (SOD2). Since considerable amounts of peroxide can be generated during mETC activity, Catalase and Glutathione peroxidases cooperate to prevent excessive H_2O_2 production and cellular toxicity, therefore shaping intensity and duration of peroxide levels for signaling purposes. Laminar shear stress was recently shown to promote mitochondrial ROS production and modulate the mitochondrial bioenegetic profile [101]; It was also shown that several cell types respond to acute hypoxia with a transient increase in mitochondrial superoxide that might be used to stabilize Hif1α and contribute to preconditioning [102]; it has been recently proposed that mitochondrial ROS represent an important mechanism of eNOS activation during mitochondrial depolarization in isolated rat cerebral arteries [103]. Compelling evidence emphasized the role of mitochondria as master regulators of redox signaling through the use of ROS-dependent diffusible signals for inter-organelle communication [104]. Indeed, physical relocalization of the mitochondrial network in a perinuclear cluster has been shown to promote hypoxia-induced transcription in the nucleus [105], while the use of mathematical models suggests that the dynamics of mitochondrial network might influence the production of intracellular ROS and the sensitivity to oxidative stress [106]. However, mitochondrial ROS can also be associated to detrimental conditions since hyperglycemia has been shown to increase mitochondrial ROS generation in cultured cells [107, 108] and in mice models of type2 diabetes [107, 109].

Uncoupled eNOS

Nitric oxide ($NO^{\cdot}$) is a gaseous transmitter that stimulates vascular growth and remodeling. In mammals, three different isoforms of nitric oxide synthase have been described (NOS1-3). Among them, the endothelial (eNOS or NOS3) and the neuronal (nNOS or NOS1) enzymes mediate constitutive $NO^{\cdot}$ production, while iNOS activity can be induced in response to pro-inflammatory or pro-angiogenic stimuli. NOSs are homodimeric oxidoreductases that upon activation transfer electrons from the NADPH to the heme iron leading to the oxidation of the guanidine group of L-arginine forming L-citrulline and $NO^{\cdot}$ [110]. The presence of specific cofactors, such as BH4, L-

arginine and probably COQ10, is critical for keeping the enzyme activity coupled to NO production (coupled NOS) since their limited availability promotes the stabilization of a monomeric form with prevalent generation of O_2^- instead of NO· [111–113]. When uncoupled, eNOS can become a powerful source of O_2^- in the endothelium, leading to peroxynitrite formation (ONOO-) and dysfunctional alteration of the vascular system including hypertension or diabetes [114, 115]. However, controlled production of NO· regulates important processes of the vascular integrity and function such as vasodilation, endothelial cell proliferation and angiogenesis [71, 116]. Interestingly, the peculiar subcellular localization of the eNOS, located in the caveolae and the Golgi network membranes, might have important implications in redox signaling events [117, 118]. As for the reversible oxidation of cysteines to sulfenic acid, the formation of S-nitrosothiols is increasingly recognized as a post-translational modification that can alter structure and function of target proteins [119, 120]. Indeed, it has been shown that different endothelial cell lines activate eNOS in response to bradykinin leading to increased EGFR S-nitrosylation and subsequent activation of pro-angiogenic signaling [121]. Importantly, changes in the local redox potential can in turn induce reversible modification of eNOS activity through S-glutathionylation of specific cysteines [122] or dramatically affect NO· bioavailability [115, 123].

Xanthine oxidoreductase

Xanthine oxidase (XO) and xanthine dehydrogenase (XDH) are convertible forms of the enzyme xanthine oxidoreductase (XOR) that catalyze the reduction of hypoxanthine to uric acid in the purine degradation pathway, generating H_2O_2 and O_2^- as byproducts [124]. XDH is the predominant form in well-oxygenated tissues and is converted into XO by reversible thiol oxidation or proteolytic modification under pathological settings including ischemia, inflammatory states and hypoxia [125]. Though mainly expressed by epithelial cells in the liver, intestine and mammary gland, damaged cells can release XDH into the circulation where it can be converted into the XO form. Due to its high affinity for the glycosaminoglycans present on their cell surface, XO can bind to endothelial cells and promote vascular dysfunction [126] [127]. Early reports showed that hypoxia mediates the activation of XDH/XO in primary lung microvascular endothelial cells (LMVEC) through sequential activation of JAK-STAT pathway [128]. It has been recently shown that XO, activated in response to intermittent hypoxia, can promote HIF-2α degradation through ROS-dependent activation of calpains [129]. Recent evidence also showed that XO-derived ROS are required for mediating endothelial cell migration in response to the heparin-binding growth factor, pleiotrophin [130].

Thioredoxin system

The Thioredoxin system includes two isoforms of thioredoxins (Trx1 and Trx2), an interacting protein with negative modulatory functions named Thioredoxin-interacting protein (TXNIP) and Thioredoxin Reductases (TrxR1, TrxR2, TrxR3) that use NADPH equivalents to regenerate the reduced form of thioredoxins once oxidized. Trxs are critical antioxidant enzymes protecting against non-radical oxidants through maintenance of an appropriate protein thiol–disulfide status. They both exert essential roles in vascular growth and remodeling through redox-dependent regulation of signaling events or cellular stress responses [131]. Trx1 is mainly localized in the cytoplasm but can translocate to the nucleus in a context-dependent way, while Trx2 is exclusively localized in the mitochondria [132] [133]. Using knockout mice and human microvascular cells (HME) silenced for TXNIP, it has been demonstrated that this protein is required for VEGF-mediated activation of VEGFR2 and angiogenesis both in vitro and in vivo [134]. It has been also shown that PARP1 inhibition in HUVEC induced TXNIP translocation to the plasma membrane, an event associated with VEGFR2 activation, suggesting that cellular relocalization of TXNIP can mediate nuclear-to-plasma membrane communication [135]. Similarly, it has been shown that TXNIP promotes Trx1 translocation to the plasma membrane leading to VEGFR2 transactivation in response to TNF-α induced ROS production [136]. TXNIP was also recently found to be essential for VEGFR2 internalization in Rab5 endosomes and subsequent activation of endothelial angiogenesis [136], confirming its role in normal vascular growth. Early reports evidenced a direct implication of Trx-1 in HIF1-α increased expression and activity during hypoxic conditions [137]. More recently, Takagi et al. [138] observed that Trx-1-dependent HIF1-α activation was associated with neoangiogenic events in human endothelial cells, suggesting a key role of Trx1 in the regulation of HIF1-α-dependent signaling related to pathological angiogenesis. On the other hand, however, Trx-1 might exert a beneficial effect promoting angiogenesis during tissue ischemia as evidenced by a recent study wherein adenoviral-mediated Trx1 overexpression in diabetic rats conferred protection against myocardial infarction [139]. In an earlier work, it was demonstrated that transgenic mice with increased expression of the mitochondrial Trx-2 isoform had enhanced arteriogenesis and angiogenesis compared to WT littermates, after femoral artery ligation-induced ischemia [140].Interestingly, the mitochondrial thioredoxin reductase (TrxR2) has recently emerged as a central player in the control of

mitochondrial H_2O_2 levels by functional interaction with the thioredoxin and peroxiredoxin axis [141]. In a recent work, it has been shown that TrxR2 deficiency prevented HIF1-α stabilization and decreased VEGF levels, ultimately impairing tumor growth and vascularization [142] [143]. Overall, these observations demonstrate that different members of the Thioredoxin system play an important role in vascular function and dysfunction, though the specific contribution of each component in physiological or pathological contexts still needs to be fully elucidated.

Peroxiredoxins

The peroxiredoxin family includes six different members (Prx1-6) that catalyze the reduction of sulfenic acid residues to the corresponding thiols using either glutathione or ascorbic acid as reductants. Over-oxidation of the peroxidatic cysteine in the active site of Prxs to sulfinic acid requires an ATP-dependent process wherein sulfiredoxin restores back the thiol form [144]. By exerting a tight control over intracellular peroxide levels and redox-sensitive target proteins, Prxs play an essential role in many signaling pathways related to cell growth and cell survival. Prx1 has been shown to positively regulate VEGFR expression in endothelial cells through TL4-mediated HIF1-α activation [145]. [146, 147]. [148]. It is becoming increasingly clear that by controlling intracellular H_2O_2 levels and their effects on sensitive target proteins, Prxs can act as sensors and fine regulators of the associated redox signaling events [149]. It has been recently reported that Prx-2 plays an important role in redox signaling events since its catalytic activity was shown to be necessary for preventing ROS-dependent VEGFR2 inactivation in endothelial cells [150]. Recent evidence suggests that Prx-1 [151] and Prx-2 [101] can be positively regulated by laminar shear stress, with important implications for the endothelial cell metabolism and redox balance. In another pathological context, Prx-1 was found to be overexpressed in human prostate cancer specimens and implicated in positive regulation of tumor growth via TLR4-mediated regulation of tumor vasculature [152]. [153]. [154]. In summary, depending on the redox state of the endothelial cells, Prxs might mediate positive or negative regulation over angiogenesis and attenuate or worsen potential vascular dysfunctions in a context-specific way accounting for the dynamic and complex regulation of the endothelial redox signaling and balance.

Glutathione system

The glutathione system includes reduced (GSH) and oxidized (GSSG) forms of glutathione and additional components that regulate its synthesis, recycling and utilization in downstream biological processes. Gamma-glutamate cysteine ligase (γ-GCL) and glutathione synthetase (GS) are responsible for de novo glutathione synthesis, while glutathione reductase (GSR) and gamma glutamyl transpeptidase (γ-GGT) are the enzymes required for its regeneration and recycling, respectively [71]. A complex network of glutathione-dependent enzymes including glutathione peroxidases (GPXs) and glutathione s-transferases (GSTs) is directly involved in mechanisms of ROS detoxification, while other enzymes such as Glutaredoxins (Grxs) counteract oxidative damage or oxidative-induced modifications in redox-sensitive proteins by adding or removing glutathione equivalents from specific residues [155].

The tripeptide glutathione (GSH) and its metabolism represent the primary antioxidant system in cells and tissues and critically control several aspects of cellular functions including proliferation, survival and signaling events through direct post-translational modification (i.e., glutathionylation) of target proteins [156]. Given the importance of redox homeostasis in the vascular system, it is not surprising that altered glutathione metabolism or signaling can lead to severe dysfunctions of vascular growth, repair and remodeling. The existence of distinct GSH pools within the subcellular organelles supports an elegant mode for independent redox regulation of metabolic processes, including those that control cell fate [23, 156].

Recent studies started to investigate the potential mechanisms through which glutathione might regulate angiogenesis. It has been shown that ICAM-1 was able to promote VEGF-induced eNOS activity and angiogenesis, by increasing the glutathione levels [157]. Later studies evidenced that changes in the GSSG/GSH ratio were able to control HIF-1α activity, since more oxidizing states of glutathione pool were associated to increased HIF-1α stabilization and transactivation of target genes [158].

A direct involvement of S-glutathionylation in the regulation of endothelial cells function has been recently described in human microvascular endothelial cells (HME), wherein VEGF stimulation was able to induce S-glutathionylation of the LMW-PTP, promoting FAK activation and subsequent cell migration [159]. Using mice model of hind limb ischemia, it has been shown that Grx1 overexpression was able to increase the expression of soluble VEGFR1 (sFlt) through a direct NF-kB deglutathionylation, an event associated with decreased pathologic neovascularization [160]. Recent studies provided evidence of a direct mechanism through which glutathione status can affect NO bioavailability, since reversible s-glutathionylation of the eNOS was able to induce uncoupling of the enzyme acting as a redox-dependent switch that might regulate cellular signaling, endothelial function and vascular tone [122] [161]. Using HUVEC cells, it has been shown that BSO-induced glutathione

depletion was able to attenuate TNF-α induced endothelial activation triggering an adaptive response mediated by increased Nrf2 nuclear translocation and subsequent HO-1 and NQO1 transactivation [162]. In a recent work, the protective action of GSH on oxidative stress-induced damage was confirmed in brain endothelial cells. Here, GSH was able to attenuate H_2O_2-dependent generation of NO, ROS production and formation of oxidized 8-hydroxy-2′-deoxyguanosine (8-OHdG) [163]. It has been shown that human brain vascular endothelial cells (HBVEC) are protected from fatty acid-induced oxidative stress by high levels of GSH and this occurs through increased PI3K activation and Akt phosphorylation [164].

Mediators of redox signaling in angiogenesis

It is increasingly recognized that tightly controlled ROS production can regulate several aspects of vascular function, including endothelial cell activation during inflammation, angiogenesis vascular growth and remodeling [165]. Compelling evidence indicates that local and controlled ROS production can positively or negatively modulate the expression or the activity of crucial angiogenic mediators, acting on specific signaling pathways. Potential mechanisms include direct activation of tyrosine kinase receptors (i.e., VEGFR2), modulation of functional regulators (i.e., kinases, phosphatases or other cofactors), modification of ion channels properties (i.e., SERCA pumps), regulation of enzymes (i.e., eNOS and sGC) and alteration of transcription factors activity (i.e., Nrf2 or NF-kB).

Growth factor, cytokines and ROS signaling

VEGF is a prominent inducer and regulator of angiogenesis and vasculogenesis under physiological and pathological settings. Extensive research in the past decades has clarified that upon binding on VEGFR-1 and VEGFR2, this growth factor promotes endothelial cell migration, proliferation and survival, activating downstream effectors including protein kinase-C (PKC), c-Src, PI3K-Akt, phospholipase-C (PLC) MAPK/ERK1/2 cascade and p38-MAPK. Among the molecular mechanisms regulating VEGF-VEGFR axis functions, it has been recently reported that in B1647 leukemia cells, VEGFR2 physically interacts with caveolin-1 in caveolae/lipid rafts, leading to negative modulation of redox signal cascades linked to cell proliferation and glucose uptake [166]. It has also been reported that extracellular H_2O_2 generated by SOD3 enzymes localized in caveolae/lipid rafts was able to sustain VEGF signaling through oxidative inactivation of PTP1B and DEP1 phosphatases in ischemic mice models [167]. Interestingly, the same group demonstrated that the adaptor protein p66Shc is an important regulator of ROS-dependent VEGFR2 phosphorylation in caveolae/lipid rafts, since VEGF administration was able to induce p66Shc phosphorylation and donwstream p38 MAPK activation promoting EC proliferation, migration and capillary tube formation [168]. In another study, VEGF signaling has been shown to induce ROS production through NOX2 and NOX4 activation, promoting leukemic cells survival from apoptotic cell death [169]. It has also been shown that VEGF was able to induce dimerization of the CD146 cell adhesion molecule in response to NOX4-dependent ROS production [170]. The importance of VEGF–NOX functional interaction was further confirmed in another study wherein NOX2- and NOX4-dependent ROS generation was able to induce reversible s-glutathionylation of SERCA and consequent Ca^{2+} influx, a necessary event for VEGF-induced endothelial cell migration [171]. However, it has been recently reported that knockdown of NOX1 but not NOX2 or NOX4 impairs angiogenic processes in mice models [172]. Other experimental work indicates that in rat models of retinopathy NOX4 activation mediates VEGFR2-dependent neovascularization through ROS-dependent activation of STAT3 while in human ECs, the same molecular pathway induced cell proliferation [91]. According to recent evidence, NOX4 activation might also be an important protective mechanism against hypoxia/reoxygenation injury, leading to decreased apoptosis and increased endothelial cells proliferation and migration through upregulation of VEGF/HIF1-α signaling [173]. In another functional study, NOX4-derived ROS production was found to be necessary for HIF1-α and VEGF-mediated pro-angiogenic events induced by insulin administration in human microvascular endothelial cells (HMVEC) [93]. Increased NOX1-dependent ROS production has been shown to promote tumor angiogenesis and tumor growth in prostate cancer cells upregulating Akt and ERK1/2 pathways [174]. Other potential mechanisms of VEGF redox signaling have been recently explored. Compartment-specific ROS production has been proposed to play an important role in VEGF signaling. Indeed, intermittent high glucose stimulation of human retinal endothelial cells (HRECs) has been shown to increase mitochondrial ROS production from the mETC, a necessary event for increased VEGF expression and cell proliferation [175]. Further implication of mitochondrial ROS was more recently confirmed in a study wherein cultured HUVECs stimulated with physiological amounts of VEGF exhibited significant mitochondrial ROS production and increased cell migration [176]. It has also been shown that VEGF stimulation was able to induce localized formation of sulfenic acid residues in target proteins located at the leading edge of endothelial cells, promoting directional migration and post-ischemic angiogenesis [20]. Less conventional regulators

of VEGF functions have been also recently identified. Indeed, it has been evidenced that redox-mediated CD40/CD40L interactions were able to enhance VEGF-induced angiogenesis of HUVEC cells [177]. More recently, Murdoch et al. [160] reported that Grx-1 overexpression was able to attenuate the post-ischemic neovascularization in transgenic mice exposed to hind limb ischemia surgery through deglutathionylation of p65, enhanced activation of NF-kB and increased secretion of soluble VEGF receptor-1 (sFlt). Moreover, s-glutathionylation of LMW-PTP was also shown to be an important regulatory mechanism for VEGF-induced FAK activation and endothelial cell migration of human microvascular endothelial cells [159]. It has been recently reported that Thioredoxin-interacting protein (TXNIP) expression was required for VEGF-induced VEGFR2 activation and subsequent angiogenic response both in vitro and in vivo [134]. On the other hand, the activity of antioxidant enzymes has been shown to play an important role in VEGF signaling. In this context, recent studies demonstrated that Prx2 prevented the oxidative inactivation of VEGFR2 in response to H_2O_2 in human aortic endothelial cells (HAEC) [150].

Another important soluble factor in causing ROS production and signaling in ECs is tumor necrosis factor alpha (TNF-α), a pleiotropic inflammatory cytokine. TNF-α has been shown to both be secreted by endothelial cells and to induce intracellular ROS formation possibly via Rac/NADPH oxidase [178]. Alternatively, TNF-α can stimulate superoxide production in ECs, via CAPK (ceramide-activated protein kinase), XO and encoupled eNOS. Importantly, TNF-α expression induces the production of ROS resulting in endothelial dysfunction in many pathophysiological conditions [179].

Protein tyrosine phosphatases (PTPs) and ROS signaling

The vast majority of cellular signaling events rely on the tight regulation between phosphorylation events of target proteins controlled by multiple protein kinases and dephosphorylation events catalyzed by specific protein phosphatases leading to positive or negative modulation of the kinase activity. Importantly, several lines of investigation suggest that many phosphatases can be regulated by reversible oxidation of sensitive cysteines located within their catalytic site, leading to efficient and temporally regulated kinase-dependent signaling [14, 180]. By virtue of a remarkably low pk_a (near 6.0 at physiological pH), the catalytic cysteines of protein phosphatases are prevalent in the thiolate form and therefore particularly susceptible to oxidative modification leading to sulfonic acid formation (Cys-SOH) [14]. Several studies have been aimed to clarify the processes involved in reactivation of oxidized phosphatases and it is now becoming increasingly clear that irreversible inactivation of tyrosine phosphatases due to higher oxidative states is prevented by the rapid formation of a sulfenylamide species (as for PTP1B) or by direct conjugation with either glutathione (S-glutathionylation) [181] or nitric oxide (S-nitrosylation) [182]. Reactivation of PTP1B and PTEN has also been shown to occur via the Thioredoxin system [183]. Earlier studies reported that mitochondrial SOD2 activity was necessary to induce H_2O_2-dependent PTEN inactivation leading to increased expression of VEGF through PI3K-Akt pathway overactivation [184]. In a more recent report, SOD1 activity was found to be essential for H_2O_2-mediated inactivation of phosphatases during growth factor signaling [185]. The role of mitochondria-derived ROS was further confirmed in a recent study wherein p66Shc-induced ROS production in response to PDGF stimulation was found to be important for PTP1B, SHP-2 and DEP1inactivation, promoting downstream signaling and cell proliferation [186]. It has also been shown that Trx1 selectively reactivated PTP1B but not SHP2 activity, potentiating PDGF-β receptor-dependent signaling and promoting cell proliferation [187]. Recent evidence suggests that redox regulation of tyrosine phosphatases might play an important role in cell adhesion and migration [188]. A functional interrelation between NOXs and tyrosine phosphatases has been recently reported since siRNA-mediated down-regulation of NOX4 impaired cell growth and motility of U87 glioblastoma cells, while conversely NOX4-derived ROS promoted proliferation and migration through PTP1B oxidative inactivation [189]. Moreover, it has also been shown that TGF-β-mediated EMT in pancreatic cancer cells is strongly dependent on NOX4-mediated ROS generation and subsequent PTPT-1B inactivation [190].

Protein kinases (PKs) and ROS signaling

Protein kinases represent one of the largest families of enzymes in the higher organisms and essential components of most cell signaling pathways. In addition to indirect regulation derived from PTPs inactivation, functional regulation of both receptor and non-receptor tyrosine kinases can be mediated by direct oxidative modification on specific amino acidic residues. Oxidant sensing and signaling from kinase activation represents an important interface between oxidant signals and phospho-regulated pathways [191]. Early evidence for ROS-dependent regulation of kinase activity initially focused on the oxidation of c-Src tyrosine kinase on two specific cysteine residues, an event that was associated with increased Tyr416 phosphorylation and the induction of a super-activation state [192]. This observation has been further confirmed in a great variety of different models wherein c-Src has been shown to mediate cell

proliferation, adhesion, survival, cytoskeletal organization and migration through the regulation of Ras/MAPK, FAK and PI3K/Akt pathways [193–195]. Conversely, other reports provided evidence that H_2O_2-mediated oxidation of the conserved Cys277 induces c-Src inactivation through increased Tyr527 phosphorylation, suggesting that redox changes can exert opposing effects on c-Src activity in a context- and cell-dependent way [196]. It has been recently shown that hyperglycemia can potentiate IGF-1-mediated c-Src activation through NOX-4-dependent ROS generation in vascular smooth muscle cells [197], suggesting that different functional or dysfunctional states in the vascular system can regulate the activity of this tyrosine kinase. Compelling evidence suggests that redox events might also modify the activity of several members of the MAPK family, including JNK, ERK1/2 and p38 MAPK, whose tight regulation is critically important for cell growth and cell fate decision [198, 199]. Earlier reports demonstrated [200] that specific cysteines of the ERK2 kinase were susceptible to oxidation in presence of low H_2O_2 doses associated with cell proliferation, while higher amounts were able to oxidatively modify the corresponding cysteines of JNK2 and p38MAPK, with different impacts on cell viability. Therefore, it seems likely that different MAPKs possess peculiar susceptibility to redox changes that might dictate the biological outcome downstream their ROS-induced activation. A recent study demonstrated that continuous activation of endothelial cells induced by TNF-α was able to promote the angiogenesis of endothelial cells as a consequence of sustained p38-MAPK activation induced by NOX-4-dependent ROS [201]. As a stress sensor, p38-MAPK can respond to altered redox states directly linked to endothelial dysfunction. Indeed, it has been shown that HUVECs stimulated with TGF-β undergo significant apoptosis as a consequence of NOX-4-induced ROS production and subsequent p38 activation, an effect that was reverted by pharmacologic inhibition of p38 [202]. Additional Serine/Threonine kinases including protein kinases A/C/G, (PKA, PKC, PKG), JNK and Akt have been shown to undergo redox-dependent modification of their catalytic activity that can influence the related signaling pathways [203]. All 12 PKC isoenzymes contain cysteines in both the regulatory and catalytic domains, whose specific redox modification exerts either positive or negative effects on their enzymatic activity [204]. Although the precise mechanisms through which ROS can activate specific PKC isoforms are still unknown, the intensity, the duration and the localized production of oxidative bursts might play an important role. Recent evidence suggests that the oxidative modification of PKA and PKG on susceptible cysteines induces the formation of an intermolecular disulfide bridge, promoting the cyclic nucleotide-independent activation of the enzyme [205]. The redox regulation of Akt was initially proposed by Ushio-Fukai et al. [206] as a positive regulator of its activity in VSMCs stimulated with Angiotensin-II or submicromolar amounts of H_2O_2. More recent studies, however, indicate that the functional consequence of Akt oxidation is rather an isoform specific event, since PDGF-induced ROS production was shown to be responsible for the direct oxidation of three cysteines in important domains of the Akt2 isoform, leading to kinase inactivation, whereas in the same conditions Akt1 and Akt3 remained active [207]. Recent studies suggest also that the kinase activity of JNK or Akt enzymes might be influenced by S-nitrosylation acting as a negative modulator of their enzymatic activity [208, 209].

Accumulating evidence from the last decade indicates that AMP kinase (AMPK) might represent a key regulator of important ROS-mediated cellular functions. Indeed, using HEK293 cells, it has been shown that H_2O_2 can induce AMPK activation by oxidizing Cys299 and Cys304 residues located in the AMPK-α subunit, while cysteine to alanine mutation partially or totally abrogated the AMPK-enhanced activity, respectively [210]. By directly sensing changes in the energetic and redox balance, AMPK might act as a sensor that integrates metabolic cues and redox-dependent processes and trigger appropriate downstream pathways [211, 212]. A recent study showed that hypoxia can activate AMPK through ROS-dependent Ca^{2+} release from the STIM1-CRAC channel [213], while Wang et al. [214] reported that treatment of bovine aortic endothelial cells (BAEC) with the glycolysis inhibitor 2-Deoxyglucose induces autophagy through mitochondrial ROS-dependent AMPK activation.

Among the most relevant receptor tyrosine kinases, the platelet-derived growth factor receptor (PDGFR), the insulin receptor (IRK), the VEGFR2, and the EGFR have been shown to undergo direct oxidation in specific cysteine residues [215]. Early studies performed on NIH3T3 fibroblasts reported that upon stimulation with PDGF, NOX-dependent ROS production promotes PDGFR autophosphorylation and downstream signaling [216], while more recent evidence suggests that PDGFR kinase activity might be important for controlled H_2O_2 production in response to PDGF treatment [217]. Although the redox regulation of the IR seems to be mainly related to oxidative inactivation of PTP1B, PTEN or SHP2 phosphatases through NOX-4-dependent ROS production [218–220], the inactivation of Prx1 represents an additional regulatory mechanism [221]. However, further studies would be necessary to substantiate and better clarify the molecular mechanisms through which ROS induce direct IRK activation in different cellular and pathophysiological contexts.

It has been recently shown that exogenous H_2O_2 or EGF administration can promote the oxidation of Cys797 located in the EGFR kinase active site and the consequent formation of a sulfenic acid residue [222], confirming that

direct redox regulation can contribute to propagate EGFR-dependent signaling. Among the three different RTKs that mediate the biological effects of VEGF (VEGFR1-3), the only form that appears too susceptible to redox regulation is the VEGFR2 [193]. Early studies demonstrated that H_2O_2 was able to both increase VEGFR2 mRNA levels [223] and enhance its phosphorylation due to NOX-derived ROS [224] and inactivation of PTP1B phosphatase [225]. The presence of redox-active cysteines in the VEGFR2 kinase domain that might be responsible of its direct oxidation has also been described, though the specific residues were not identified [226]. A later study reported that H_2O_2 administration can induce the formation of an intramolecular disulfide bridge between Cys1199 and Cys1209 exerting a negative effect on the VEGFR2 activity, rescued by the antioxidant enzyme PrxII [150]. Later studies evidenced that VEGFR2 can also be regulated by extracellular SOD (SOD3) activity leading to H_2O_2 accumulation in lipid/caveolae rafts and increased VEGFR2 phosphorylation due to PTP1B and DEP1 inactivation [167]. More recent evidence suggests that the mitochondrial regulator p66shc plays an important role in mediating ROS-dependent VEGFR2 phosphorylation in caveolae lipid/rafts of endothelial cells stimulated with VEGF [168]. Similarly, using HCAEC cells, it has been shown that VEGFR2 can be modified and trigger downstream signaling pathways (i.e., Src-PI3K-Akt) as a consequence of VEGF-induced NOX-dependent ROS generation [194]. Finally, another study reported that FGFR1 oxidation on Cys277 might negatively modulate downstream signaling events, providing evidence of a complex and probably context-dependent regulation of RTKs by redox changes [227].

Transcription factors (TF) and ROS signaling

Redox regulation of transcription factors such as HIF1, Ets-1, NF-kB and Nrf2 plays an essential role in vascular function and dysfunction. [228]. The hypoxia-inducible factors (HIFs) transcriptionally regulate several target genes in response to changes in the oxygen levels. HIF1 is a heterodimeric protein composed by an oxygen-regulated HIF-1α and a constitutive oxygen-insensitive HIF-1β subunit. Under hypoxic conditions, HIF-1α degradation is prevented, leading to heterodimerization with the HIF-1β subunit and functional activation of downstream targets. Among the disparate target genes, VEGF and VEGFR are the most relevant for the regulation of angiogenic processes [229]. Importantly, pro-inflammatory cytokines, RNS and ROS have also been shown to promote HIF1-α stabilization through oxygen-independent mechanisms. Earlier studies reported that HIF1-α can be S-nitrosylated at the level of Cys533 leading to enhanced stability and transcriptional activity [230], an observation confirmed by a later study [231]. NOX-derived ROS have also been shown to promote HIF1-α stabilization under both hypoxic or normoxic conditions in endothelial cells [232] and prostate cancer cells [233]. Intriguingly, recent evidence indicates that mitochondria-derived ROS might induce HIF1-α stabilization in melanoma cells [234], while a later study from Calvani et al. [235] proposed a biphasic regulation of HIF-1 in melanoma cells survival wherein mitochondria-derived ROS mediated early HIF1 activation, while NOX-dependent ROS promoted its prolonged stabilization. Together these studies highlight the multifaceted and complex role exerted by redox changes and ROS on HIF1 regulation, with important implications for normal and pathologic angiogenesis.

NF-kB is another heterodimeric transcription factor that regulates the expression of genes involved in cell proliferation, cell survival, inflammation and angiogenesis. Under basal conditions, NF-kB is retained as a cytosolic inactive dimer due to the interaction with IkBs inhibitors [236]. Stress conditions induce IKK-dependent IkB phosphorylation promoting its proteasomal degradation and the subsequent NF-kB translocation into the nucleus, where activation of target genes occurs. Redox regulation of NF-kB is intricate since oxidation of the Cys62 in the p50 subunit occurs in the cytosol leading to nuclear translocation while its reduction mediated by Trx1 or Ref1 is required for DNA-binding and gene transactivation [237, 238]. Exogenous addition of H_2O_2 has been shown to promote alternative phosphorylation of IkB enhancing NF-kB activation in some cell systems while conversely the same modification can exert inhibitory effects in other experimental systems [237]. The IKK complex contains redox-sensitive cysteines (Cys178 in IKKα and Cys179 in IKKβ) that mediate redox regulation of NF-kB activity and generally inhibit its activation [236], while in certain cell types the same modification seems to promote NF-kB activation [239]. Interestingly, both s-nitrosylation and S-glutathionylation modifications have been shown to negatively regulate the NF-kB activation, an effect reversed by either Grx1 or reducing agents [240]. In the endothelial cells, the NF-kB pathway has often been associated with pathological alterations such as atherosclerosis [241], pro-inflammatory states [242] and impaired vascular function in type2 diabetes [243]. In contrast, other recent reports suggest that increased NF-kB can attenuate the post-ischemic revascularization in mice models of hind limb ischemia [160] or positively contribute to vascular remodeling during angiogenesis and vascular regression through transcriptional modulation of pro-apoptotic (FasL) and anti-apoptotic (cFLIP) genes [244]. In summary, the functional interaction between ROS and the NF-kB pathway is intricate and context dependent, since

ROS can be generated and interact with different effector molecules leading to either activation or inhibition of the NF-kB signaling. Similarly, the biological outcome of ROS-dependent regulation appears to be strongly influenced by the duration of the oxidant stimulus and the specific cellular context. With this respect, further studies are required to elucidate the complexity of this process and its interrelation with normal or pathological angiogenesis.

The nuclear factor erythroid 2-related factor (Nrf2) is a master regulator of redox homeostasis that transcriptionally controls the expression of several antioxidant and phase-II enzymes in response to oxidative stress and inflammation. Under basal conditions, Nrf2 is sequestered in the cytosol by the negative regulator Kelch-like ECH-associated protein1 (Keap1) that promotes its proteasomal degradation and prevents the activation of target genes. Key cysteine residues in Keap1 (Cys151, Cys273, Cys288 and Cys613) can be susceptible to oxidative modification or conjugation with electrophiles, leading to Keap1–Nrf2 complex disruption, nuclear translocation of Nrf2 and association with Maf proteins culminating in the transactivation of ARE-containing genes [245]. Among the approximately 600 cytoprotective genes induced by Nrf2, the inducible heme oxygenase (HO-1), the catalytic subunit of glutamate–cysteine ligase (GCLC), NAD(P)H:quinone oxidoreductase 1 (NQO1) and glutathione *S*-transferase (GST) are the most relevant for the vascular system.

Earlier studies reported that Nrf2 activation conferred protection from H_2O_2-induced cytotoxicity and exerted anti-inflammatory effects in HAECs [246]. Another recent study confirmed the importance of Nrf2 in maintaining the functional integrity of the vasculature, since disruption of its signaling by siRNA-mediated knockdown severely impaired the angiogenic processes in human coronary arterial endothelial cells stimulated with VEGF [247]. Recent evidence suggests that Nrf2 pathway is an important regulator of angiogenesis in endothelial cells and bone marrow-derived progenitor cells (PAC), while its deficiency enhanced the muscle blood flow in a mice model of hind limb ischemia [248]. A recent report indicates that Nrf2 can positively regulate the ECs sprouting and vascularization by suppressing Dll/Notch4 pathway in vitro and in vivo [249]. Another study confirmed that Nrf2 is a potent regulator of hypoxia-induced angiogenesis in cardiac microvascular endothelial cells (CMECs) [250]. Interestingly, a functional crosstalk between the Nrf2 pathway and the unfolded protein response has been recently documented in several endothelial cells wherein oxidized phospholipids were able to induce ATF4 and VEGF upregulation through Nrf2-dependent activation [251]. Another recent study reported that Nrf2 activation can promote retinal vascular development and protect the retina from hyperoxia-induced oxidative stress in C57BL/6 mice models [252]. In the context of tumor angiogenesis and malignant progression, it has been recently shown that downregulation of Nrf2 can block the angiogenesis in glioblastoma cells through inhibition of the hypoxia-induced activation of HIF1-α [253]. Notably, this observation was partially confirmed by another study wherein shRNA-mediated Nrf2 knockdown in human colon cancer cells was able to impair tumor growth and tumor angiogenesis in mice xenografts [254]. Therefore, even the redox regulation of Nrf2-associated events seems to produce complex and opposite biological effects that largely depend on the cell type and the physiopathological context.

Hypoxia

Short exposure to hypoxia/reoxygenation, either directly or indirectly, produces ROS that induce oxidative stress associated with angiogenesis or tumor vascularization. ROS can cause tissue injury on one hand and promote tissue repair on the other side by promoting angiogenesis [255]. It thus appears that after causing injury to the cells, ROS promptly initiate the tissue repair process by triggering angiogenic response. Recently, it has been reported that redox signaling may influence neovascularization that occurs during pathological conditions. Tumor angiogenesis is regulated by several growth factors (EGF, TGF-alpha, beta-FGF, VEGF). It has been clearly demonstrated that induction of VEGF by the tumor is triggered by tissue hypoxia. Hypoxia exerts its pro-angiogenic action through various angiogenic factors also in wound healing and tissue regeneration [256, 257].

Redox signaling in endothelial pathologies

ROS signaling and its deregulation (e.g., oxidative stress) are often associated with endothelial dysfunction, with involvement in the pathogenesis of several vascular-related diseases such as hypertension, diabetes or atherosclerosis. Also neoangiogenesis-related pathologies ranging from tumorigenesis and eye diseases (e.g., retinopathies) are greatly characterized by oxidative stress conditions and altered ROS signaling.

The role of redox signaling in tumor angiogenesis is not yet completely characterized. Indeed, converse mechanisms are postulated about how ROS recruit new blood vessels for tumor progression by promoting VEGF secretion [258]. Therefore, analysis and characterization of molecules that sustain redox signaling is a new opportunity for setting up innovative strategies of anti-cancer therapy. In tumor vessel endothelial cells, the increase in ROS generation from metabolic abnormalities and oncogenic signaling may trigger a redox adaptation response, leading

to an upregulation of antioxidant capacity and a shift of redox dynamics with high ROS generation and elimination to maintain the ROS levels below the toxic threshold. As such, tumor angiogenic cells would be more dependent on the antioxidant system and more vulnerable to further oxidative stress induced by exogenous ROS-generating agents or compounds that inhibit the antioxidant system. A further increase of ROS stress in these cancer cells using exogenous ROS-modulating agents is likely to cause elevation of ROS above the threshold level, leading to cell death. This might constitute a biochemical basis to design therapeutic strategies to selectively kill tumor angiogenic cells using ROS-mediated mechanisms [259].

In human retinopathies, abnormal angiogenesis is a hallmark and a cause of this pathology. Here, high oxygen tension induces ROS production that cannot be scavenged by antioxidants. As a result, superoxide anion and hydrogen peroxide levels have shown to be substantially elevated in animal models of diabetic retinopathy or retinopathy of prematurity. Even normal retinas seem to be particularly sensitive to oxidative stress, due to a high consumption of oxygen, high levels of oxidation substrates and exposure to light. Interestingly, it has been demonstrated that ischemic retinopathy is substantially diminished in ATM-deficient mice in which excessive ROS are inhibited [260]. ATM deficiency also lowered tumor angiogenesis and enhanced the antiangiogenic action of VEGF blockade. These data suggest that pathological neoangiogenesis requires DNA damage response (DDR)-mediated oxidative defense and that agents that promote excessive ROS generation may have beneficial effects in the treatment of neovascular disease [261].

Another aspect related to oxidative stress and retinopathies is the production of oxidized lipids. One of the main consequences of ROS production is peroxidation of lipids which results in their functional modification. Peroxidation affects cellular membrane lipids, in particular polyunsaturated fatty acid (PUFA). These oxidation reactions can generate secondary compounds that are even more destructive than ROS themselves. DHA, one of the easily oxidizable PUFAs, represents 60 % of the PUFAs in the retina and the product of its oxidation serves as a biomarker for oxidative stress-induced injury in age-related macular degeneration [65].

Altogether, these findings make very urgent to improve the technologies and model to study ROS signaling in vivo with the idea to design new therapeutic strategies aiming at ROS signaling modulation in different pathological contests.

Acknowledgments We apologize to the many researchers whose work was not cited in this review due to space limitations. We would like to thank all members of Santoro laboratory for support and discussion. Work in Santoro's laboratory is supported by grants from European Community (ERC and Marie Curie IRG), HFSP, Telethon, FWO and AIRC.

References

1. Murphy MP, Holmgren A, Larsson NG, Halliwell B, Chang CJ, Kalyanaraman B, Rhee SG, Thornalley PJ, Partridge L, Gems D, Nystrom T, Belousov V, Schumacker PT, Winterbourn CC (2011) Unraveling the biological roles of reactive oxygen species. Cell Metab 13(4):361–366. doi:10.1016/j.cmet.2011.03.010
2. Schieber M, Chandel NS (2014) ROS function in redox signaling and oxidative stress. Curr Biol CB 24(10):R453–R462. doi:10.1016/j.cub.2014.03.034
3. Finkel T (2011) Signal transduction by reactive oxygen species. J Cell Biol 194(1):7–15. doi:10.1083/jcb.201102095
4. De Bock K, Georgiadou M, Schoors S, Kuchnio A, Wong BW, Cantelmo AR, Quaegebeur A, Ghesquiere B, Cauwenberghs S, Eelen G, Phng LK, Betz I, Tembuyser B, Brepoels K, Welti J, Geudens I, Segura I, Cruys B, Bifari F, Decimo I, Blanco R, Wyns S, Vangindertael J, Rocha S, Collins RT, Munck S, Daelemans D, Imamura H, Devlieger R, Rider M, Van Veldhoven PP, Schuit F, Bartrons R, Hofkens J, Fraisl P, Telang S, Deberardinis RJ, Schoonjans L, Vinckier S, Chesney J, Gerhardt H, Dewerchin M, Carmeliet P (2013) Role of PFKFB3-driven glycolysis in vessel sprouting. Cell 154(3):651–663. doi:10.1016/j.cell.2013.06.037
5. Dickinson BC, Tang Y, Chang Z, Chang CJ (2011) A nuclear-localized fluorescent hydrogen peroxide probe for monitoring sirtuin-mediated oxidative stress responses in vivo. Chem Biol 18(8):943–948. doi:10.1016/j.chembiol.2011.07.005
6. Bae YS, Oh H, Rhee SG, Yoo YD (2011) Regulation of reactive oxygen species generation in cell signaling. Mol Cells 32(6):491–509. doi:10.1007/s10059-011-0276-3
7. Veal E, Day A (2011) Hydrogen peroxide as a signaling molecule. Antioxid Redox Signal 15(1):147–151. doi:10.1089/ars.2011.3968
8. Breton-Romero R, Gonzalez de Orduna C, Romero N, Sanchez-Gomez FJ, de Alvaro C, Porras A, Rodriguez-Pascual F, Laranjinha J, Radi R, Lamas S (2012) Critical role of hydrogen peroxide signaling in the sequential activation of p38 MAPK and eNOS in laminar shear stress. Free Radic Biol Med 52(6):1093–1100. doi:10.1016/j.freeradbiomed.2011.12.026
9. Evangelista AM, Thompson MD, Weisbrod RM, Pimental DR, Tong X, Bolotina VM, Cohen RA (2012) Redox regulation of SERCA2 is required for vascular endothelial growth factor-induced signaling and endothelial cell migration. Antioxid Redox Signal 17(8):1099–1108. doi:10.1089/ars.2011.4022
10. Fernandes R, Hosoya K, Pereira P (2011) Reactive oxygen species downregulate glucose transport system in retinal endothelial cells. Am J Physiol Cell Physiol 300(4):C927–C936. doi:10.1152/ajpcell.00140.2010
11. Kalwa H, Sartoretto JL, Martinelli R, Romero N, Steinhorn BS, Tao M, Ozaki CK, Carman CV, Michel T (2014) Central role for hydrogen peroxide in P2Y1 ADP receptor-mediated cellular responses in vascular endothelium. Proc Natl Acad Sci USA 111(9):3383–3388. doi:10.1073/pnas.1320854111
12. Knock GA, Ward JP (2011) Redox regulation of protein kinases as a modulator of vascular function. Antioxid Redox Signal 15(6):1531–1547. doi:10.1089/ars.2010.3614
13. Grant CM (2011) Regulation of translation by hydrogen peroxide. Antioxid Redox Signal 15(1):191–203. doi:10.1089/ars.2010.3699

14. Ostman A, Frijhoff J, Sandin A, Bohmer FD (2011) Regulation of protein tyrosine phosphatases by reversible oxidation. J Biochem 150(4):345–356. doi:10.1093/jb/mvr104
15. Roos G, Messens J (2011) Protein sulfenic acid formation: from cellular damage to redox regulation. Free Radic Biol Med 51(2):314–326. doi:10.1016/j.freeradbiomed.2011.04.031
16. Roos G, Foloppe N, Messens J (2013) Understanding the pK(a) of redox cysteines: the key role of hydrogen bonding. Antioxid Redox Signal 18(1):94–127. doi:10.1089/ars.2012.4521
17. Paulsen CE, Carroll KS (2013) Cysteine-mediated redox signaling: chemistry, biology, and tools for discovery. Chem Rev 113(7):4633–4679. doi:10.1021/cr300163e
18. Forman HJ, Ursini F, Maiorino M (2014) An overview of mechanisms of redox signaling. J Mol Cell Cardiol 73:2–9. doi:10.1016/j.yjmcc.2014.01.018
19. Steinberg SF (2013) Oxidative stress and sarcomeric proteins. Circ Res 112(2):393–405. doi:10.1161/CIRCRESAHA.111.300496
20. Kaplan N, Urao N, Furuta E, Kim SJ, Razvi M, Nakamura Y, McKinney RD, Poole LB, Fukai T, Ushio-Fukai M (2011) Localized cysteine sulfenic acid formation by vascular endothelial growth factor: role in endothelial cell migration and angiogenesis. Free Radical Res 45(10):1124–1135. doi:10.3109/10715762.2011.602073
21. Jones DP, Go YM (2010) Redox compartmentalization and cellular stress. Diabetes Obes Metab 12(Suppl 2):116–125. doi:10.1111/j.1463-1326.2010.01266.x
22. Kojer K, Bien M, Gangel H, Morgan B, Dick TP, Riemer J (2012) Glutathione redox potential in the mitochondrial intermembrane space is linked to the cytosol and impacts the Mia40 redox state. EMBO J 31(14):3169–3182. doi:10.1038/emboj.2012.165
23. Go YM, Jones DP (2013) Thiol/disulfide redox states in signaling and sensing. Crit Rev Biochem Mol Biol 48(2):173–181. doi:10.3109/10409238.2013.764840
24. Murphy MP (2012) Mitochondrial thiols in antioxidant protection and redox signaling: distinct roles for glutathionylation and other thiol modifications. Antioxid Redox Signal 16(6):476–495. doi:10.1089/ars.2011.4289
25. Kaludercic N, Deshwal S, Di Lisa F (2014) Reactive oxygen species and redox compartmentalization. Front Physiol 5:285. doi:10.3389/fphys.2014.00285
26. Meyer AJ, Dick TP (2010) Fluorescent protein-based redox probes. Antioxid Redox Signal 13(5):621–650. doi:10.1089/ars.2009.2948
27. Rhee SG, Chang TS, Jeong W, Kang D (2010) Methods for detection and measurement of hydrogen peroxide inside and outside of cells. Mol Cells 29(6):539–549. doi:10.1007/s10059-010-0082-3
28. Kalyanaraman B, Darley-Usmar V, Davies KJ, Dennery PA, Forman HJ, Grisham MB, Mann GE, Moore K, Roberts LJ 2nd, Ischiropoulos H (2012) Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. Free Radic Biol Med 52(1):1–6. doi:10.1016/j.freeradbiomed.2011.09.030
29. Karlsson M, Kurz T, Brunk UT, Nilsson SE, Frennesson CI (2010) What does the commonly used DCF test for oxidative stress really show? Biochem J 428(2):183–190. doi:10.1042/BJ20100208
30. Burkitt MJ, Wardman P (2001) Cytochrome C is a potent catalyst of dichlorofluorescin oxidation: implications for the role of reactive oxygen species in apoptosis. Biochem Biophys Res Commun 282(1):329–333. doi:10.1006/bbrc.2001.4578
31. Winterbourn CC (2014) The challenges of using fluorescent probes to detect and quantify specific reactive oxygen species in living cells. Biochim Biophys Acta 1840(2):730–738. doi:10.1016/j.bbagen.2013.05.004
32. Lin VS, Dickinson BC, Chang CJ (2013) Boronate-based fluorescent probes: imaging hydrogen peroxide in living systems. Methods Enzymol 526:19–43. doi:10.1016/B978-0-12-405883-5.00002-8
33. Dickinson BC, Lin VS, Chang CJ (2013) Preparation and use of MitoPY1 for imaging hydrogen peroxide in mitochondria of live cells. Nat Protoc 8(6):1249–1259. doi:10.1038/nprot.2013.064
34. Cocheme HM, Quin C, McQuaker SJ, Cabreiro F, Logan A, Prime TA, Abakumova I, Patel JV, Fearnley IM, James AM, Porteous CM, Smith RA, Saeed S, Carre JE, Singer M, Gems D, Hartley RC, Partridge L, Murphy MP (2011) Measurement of H2O2 within living *Drosophila* during aging using a ratiometric mass spectrometry probe targeted to the mitochondrial matrix. Cell Metab 13(3):340–350. doi:10.1016/j.cmet.2011.02.003
35. Cocheme HM, Logan A, Prime TA, Abakumova I, Quin C, McQuaker SJ, Patel JV, Fearnley IM, James AM, Porteous CM, Smith RA, Hartley RC, Partridge L, Murphy MP (2012) Using the mitochondria-targeted ratiometric mass spectrometry probe MitoB to measure H2O2 in living *Drosophila*. Nat Protoc 7(5):946–958. doi:10.1038/nprot.2012.035
36. Ostergaard H, Henriksen A, Hansen FG, Winther JR (2001) Shedding light on disulfide bond formation: engineering a redox switch in green fluorescent protein. EMBO J 20(21):5853–5862. doi:10.1093/emboj/20.21.5853
37. Dooley CT, Dore TM, Hanson GT, Jackson WC, Remington SJ, Tsien RY (2004) Imaging dynamic redox changes in mammalian cells with green fluorescent protein indicators. J Biol Chem 279(21):22284–22293. doi:10.1074/jbc.M312847200
38. Bjornberg O, Ostergaard H, Winther JR (2006) Mechanistic insight provided by glutaredoxin within a fusion to redox-sensitive yellow fluorescent protein. Biochemistry 45(7):2362–2371. doi:10.1021/bi0522495
39. Morgan B, Sobotta MC, Dick TP (2011) Measuring E(GSH) and H2O2 with roGFP2-based redox probes. Free Radic Biol Med 51(11):1943–1951. doi:10.1016/j.freeradbiomed.2011.08.035
40. Gutscher M, Pauleau AL, Marty L, Brach T, Wabnitz GH, Samstag Y, Meyer AJ, Dick TP (2008) Real-time imaging of the intracellular glutathione redox potential. Nat Methods 5(6):553–559. doi:10.1038/nmeth.1212
41. Gutscher M, Sobotta MC, Wabnitz GH, Ballikaya S, Meyer AJ, Samstag Y, Dick TP (2009) Proximity-based protein thiol oxidation by H2O2-scavenging peroxidases. J Biol Chem 284(46):31532–31540. doi:10.1074/jbc.M109.059246
42. Morgan B, Ezerina D, Amoako TN, Riemer J, Seedorf M, Dick TP (2013) Multiple glutathione disulfide removal pathways mediate cytosolic redox homeostasis. Nat Chem Biol 9(2):119–125. doi:10.1038/nchembio.1142
43. Bhaskar A, Munshi M, Khan SZ, Fatima S, Arya R, Jameel S, Singh A (2015) Measuring glutathione redox potential of HIV-1-infected macrophages. J Biol Chem 290(2):1020–1038. doi:10.1074/jbc.M114.588913
44. Albrecht SC, Sobotta MC, Bausewein D, Aller I, Hell R, Dick TP, Meyer AJ (2014) Redesign of genetically encoded biosensors for monitoring mitochondrial redox status in a broad range of model eukaryotes. J Biomol Screen 19(3):379–386. doi:10.1177/1087057113499634
45. Back P, De Vos WH, Depuydt GG, Matthijssens F, Vanfleteren JR, Braeckman BP (2012) Exploring real-time in vivo redox biology of developing and aging *Caenorhabditis elegans*. Free Radic Biol Med 52(5):850–859. doi:10.1016/j.freeradbiomed.2011.11.037
46. Barata AG, Dick TP (2013) In vivo imaging of H2O2 production in *Drosophila*. Methods Enzymol 526:61–82. doi:10.1016/B978-0-12-405883-5.00004-1

47. Breckwoldt MO, Pfister FM, Bradley PM, Marinkovic P, Williams PR, Brill MS, Plomer B, Schmalz A, St Clair DK, Naumann R, Griesbeck O, Schwarzlander M, Godinho L, Bareyre FM, Dick TP, Kerschensteiner M, Misgeld T (2014) Multiparametric optical analysis of mitochondrial redox signals during neuronal physiology and pathology in vivo. Nat Med 20(5):555–560. doi:10.1038/nm.3520
48. Belousov VV, Fradkov AF, Lukyanov KA, Staroverov DB, Shakhbazov KS, Terskikh AV, Lukyanov S (2006) Genetically encoded fluorescent indicator for intracellular hydrogen peroxide. Nat Methods 3(4):281–286. doi:10.1038/nmeth866
49. Malinouski M, Zhou Y, Belousov VV, Hatfield DL, Gladyshev VN (2011) Hydrogen peroxide probes directed to different cellular compartments. PLoS One 6(1):e14564. doi:10.1371/journal.pone.0014564
50. Mishina NM, Tyurin-Kuzmin PA, Markvicheva KN, Vorotnikov AV, Tkachuk VA, Laketa V, Schultz C, Lukyanov S, Belousov VV (2011) Does cellular hydrogen peroxide diffuse or act locally? Antioxid Redox Signal 14(1):1–7. doi:10.1089/ars.2010.3539
51. Markvicheva KN, Bilan DS, Mishina NM, Gorokhovatsky AY, Vinokurov LM, Lukyanov S, Belousov VV (2011) A genetically encoded sensor for H2O2 with expanded dynamic range. Bioorg Med Chem 19(3):1079–1084. doi:10.1016/j.bmc.2010.07.014
52. Bilan DS, Pase L, Joosen L, Gorokhovatsky AY, Ermakova YG, Gadella TW, Grabher C, Schultz C, Lukyanov S, Belousov VV (2013) HyPer-3: a genetically encoded H(2)O(2) probe with improved performance for ratiometric and fluorescence lifetime imaging. ACS Chem Biol 8(3):535–542. doi:10.1021/cb300625g
53. Sartoretto JL, Kalwa H, Romero N, Michel T (2013) In vivo imaging of nitric oxide and hydrogen peroxide in cardiac myocytes. Methods Enzymol 528:61–78. doi:10.1016/B978-0-12-405881-1.00004-5
54. Mishina NM, Markvicheva KN, Bilan DS, Matlashov ME, Shirmanova MV, Liebl D, Schultz C, Lukyanov S, Belousov VV (2013) Visualization of intracellular hydrogen peroxide with HyPer, a genetically encoded fluorescent probe. Methods Enzymol 526:45–59. doi:10.1016/B978-0-12-405883-5.00003-X
55. Roma LP, Duprez J, Takahashi HK, Gilon P, Wiederkehr A, Jonas JC (2012) Dynamic measurements of mitochondrial hydrogen peroxide concentration and glutathione redox state in rat pancreatic beta-cells using ratiometric fluorescent proteins: confounding effects of pH with HyPer but not roGFP1. Biochem J 441(3):971–978. doi:10.1042/BJ20111770
56. Wang W, Fang H, Groom L, Cheng A, Zhang W, Liu J, Wang X, Li K, Han P, Zheng M, Yin J, Mattson MP, Kao JP, Lakatta EG, Sheu SS, Ouyang K, Chen J, Dirksen RT, Cheng H (2008) Superoxide flashes in single mitochondria. Cell 134(2):279–290. doi:10.1016/j.cell.2008.06.017
57. Schwarzlander M, Logan DC, Fricker MD, Sweetlove LJ (2011) The circularly permuted yellow fluorescent protein cpYFP that has been used as a superoxide probe is highly responsive to pH but not superoxide in mitochondria: implications for the existence of superoxide 'flashes'. Biochem J 437(3):381–387. doi:10.1042/BJ20110883
58. Schwarzlander M, Wagner S, Ermakova YG, Belousov VV, Radi R, Beckman JS, Buettner GR, Demaurex N, Duchen MR, Forman HJ, Fricker MD, Gems D, Halestrap AP, Halliwell B, Jakob U, Johnston IG, Jones NS, Logan DC, Morgan B, Muller FL, Nicholls DG, Remington SJ, Schumacker PT, Winterbourn CC, Sweetlove LJ, Meyer AJ, Dick TP, Murphy MP (2014) The 'mitoflash' probe cpYFP does not respond to superoxide. Nature 514(7523):E12–E14. doi:10.1038/nature13858
59. Zhao BS, Zhang G, Zeng S, He C, Chen PR (2013) Probing subcellular organic hydroperoxide formation via a genetically encoded ratiometric and reversible fluorescent indicator. Integr Biol: Quant Biosci Nano Macro 5(12):1485–1489. doi:10.1039/c3ib40209f
60. Zhao BS, Liang Y, Song Y, Zheng C, Hao Z, Chen PR (2010) A highly selective fluorescent probe for visualization of organic hydroperoxides in living cells. J Am Chem Soc 132(48):17065–17067. doi:10.1021/ja1071114
61. Kolossov VL, Leslie MT, Chatterjee A, Sheehan BM, Kenis PJ, Gaskins HR (2012) Forster resonance energy transfer-based sensor targeting endoplasmic reticulum reveals highly oxidative environment. Exp Biol Med (Maywood) 237(6):652–662. doi:10.1258/ebm.2012.011436
62. Abraham BG, Santala V, Tkachenko NV, Karp M (2014) Fluorescent protein-based FRET sensor for intracellular monitoring of redox status in bacteria at single cell level. Anal Bioanal Chem 406(28):7195–7204. doi:10.1007/s00216-014-8165-1
63. Enyedi B, Zana M, Donko A, Geiszt M (2013) Spatial and temporal analysis of NADPH oxidase-generated hydrogen peroxide signals by novel fluorescent reporter proteins. Antioxid Redox Signal 19(6):523–534. doi:10.1089/ars.2012.4594
64. De Bock K, Georgiadou M, Carmeliet P (2013) Role of endothelial cell metabolism in vessel sprouting. Cell Metab 18(5):634–647. doi:10.1016/j.cmet.2013.08.001
65. Kim YW, Byzova TV (2014) Oxidative stress in angiogenesis and vascular disease. Blood 123(5):625–631. doi:10.1182/blood-2013-09-512749
66. Li H, Horke S, Forstermann U (2014) Vascular oxidative stress, nitric oxide and atherosclerosis. Atherosclerosis 237(1):208–219. doi:10.1016/j.atherosclerosis.2014.09.001
67. Su Q, Qin DN, Wang FX, Ren J, Li HB, Zhang M, Yang Q, Miao YW, Yu XJ, Qi J, Zhu Z, Zhu GQ, Kang YM (2014) Inhibition of reactive oxygen species in hypothalamic paraventricular nucleus attenuates the renin-angiotensin system and proinflammatory cytokines in hypertension. Toxicol Appl Pharmacol 276(2):115–120. doi:10.1016/j.taap.2014.02.002
68. Zuo L, Rose BA, Roberts WJ, He F, Banes-Berceli AK (2014) Molecular characterization of reactive oxygen species in systemic and pulmonary hypertension. Am J Hypertens 27(5):643–650. doi:10.1093/ajh/hpt292
69. Kaneto H, Katakami N, Matsuhisa M, Matsuoka TA (2010) Role of reactive oxygen species in the progression of type 2 diabetes and atherosclerosis. Mediators Inflamm 2010:453892. doi:10.1155/2010/453892
70. Ray PD, Huang BW, Tsuji Y (2012) Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 24(5):981–990. doi:10.1016/j.cellsig.2012.01.008
71. Bir SC, Kolluru GK, Fang K, Kevil CG (2012) Redox balance dynamically regulates vascular growth and remodeling. Semin Cell Dev Biol 23(7):745–757. doi:10.1016/j.semcdb.2012.05.003
72. Takac I, Schroder K, Brandes RP (2012) The Nox family of NADPH oxidases: friend or foe of the vascular system? Curr Hypertens Rep 14(1):70–78. doi:10.1007/s11906-011-0238-3
73. Manea A (2010) NADPH oxidase-derived reactive oxygen species: involvement in vascular physiology and pathology. Cell Tissue Res 342(3):325–339. doi:10.1007/s00441-010-1060-y
74. Takac I, Schroder K, Zhang L, Lardy B, Anilkumar N, Lambeth JD, Shah AM, Morel F, Brandes RP (2011) The E-loop is involved in hydrogen peroxide formation by the NADPH oxidase Nox4. J Biol Chem 286(15):13304–13313. doi:10.1074/jbc.M110.192138
75. De Deken X, Corvilain B, Dumont JE, Miot F (2014) Roles of DUOX-mediated hydrogen peroxide in metabolism, host defense, and signaling. Antioxid Redox Signal 20(17):2776–2793. doi:10.1089/ars.2013.5602

76. Konior A, Schramm A, Czesnikiewicz-Guzik M, Guzik TJ (2014) NADPH oxidases in vascular pathology. Antioxid Redox Signal 20(17):2794–2814. doi:10.1089/ars.2013.5607
77. Lassegue B, Griendling KK (2010) NADPH oxidases: functions and pathologies in the vasculature. Arterioscler Thromb Vasc Biol 30(4):653–661. doi:10.1161/ATVBAHA.108.181610
78. Anilkumar N, San Jose G, Sawyer I, Santos CX, Sand C, Brewer AC, Warren D, Shah AM (2013) A 28-kDa splice variant of NADPH oxidase-4 is nuclear-localized and involved in redox signaling in vascular cells. Arterioscler Thromb Vasc Biol 33(4):e104–e112. doi:10.1161/ATVBAHA.112.300956
79. Schroder K, Zhang M, Benkhoff S, Mieth A, Pliquett R, Kosowski J, Kruse C, Luedike P, Michaelis UR, Weissmann N, Dimmeler S, Shah AM, Brandes RP (2012) Nox4 is a protective reactive oxygen species generating vascular NADPH oxidase. Circ Res 110(9):1217–1225. doi:10.1161/CIRCRESAHA.112.267054
80. Chen K, Kirber MT, Xiao H, Yang Y, Keaney JF Jr (2008) Regulation of ROS signal transduction by NADPH oxidase 4 localization. J Cell Biol 181(7):1129–1139. doi:10.1083/jcb.200709049
81. Diebold I, Flugel D, Becht S, Belaiba RS, Bonello S, Hess J, Kietzmann T, Gorlach A (2010) The hypoxia-inducible factor-2alpha is stabilized by oxidative stress involving NOX4. Antioxid Redox Signal 13(4):425–436. doi:10.1089/ars.2009.3014
82. Craige SM, Chen K, Pei Y, Li C, Huang X, Chen C, Shibata R, Sato K, Walsh K, Keaney JF Jr (2011) NADPH oxidase 4 promotes endothelial angiogenesis through endothelial nitric oxide synthase activation. Circulation 124(6):731–740. doi:10.1161/CIRCULATIONAHA.111.030775
83. Tung JJ, Kitajewski J (2010) Chloride intracellular channel 1 functions in endothelial cell growth and migration. J Angiogenesis Res 2:23. doi:10.1186/2040-2384-2-23
84. Gray SP, Di Marco E, Okabe J, Szyndralewiez C, Heitz F, Montezano AC, de Haan JB, Koulis C, El-Osta A, Andrews KL, Chin-Dusting JP, Touyz RM, Wingler K, Cooper ME, Schmidt HH, Jandeleit-Dahm KA (2013) NADPH oxidase 1 plays a key role in diabetes mellitus-accelerated atherosclerosis. Circulation 127(18):1888–1902. doi:10.1161/CIRCULATIONAHA.112.132159
85. Lee JH, Joo JH, Kim J, Lim HJ, Kim S, Curtiss L, Seong JK, Cui W, Yabe-Nishimura C, Bae YS (2013) Interaction of NADPH oxidase 1 with Toll-like receptor 2 induces migration of smooth muscle cells. Cardiovasc Res 99(3):483–493. doi:10.1093/cvr/cvt107
86. Paletta-Silva R, Rocco-Machado N, Meyer-Fernandes JR (2013) NADPH oxidase biology and the regulation of tyrosine kinase receptor signaling and cancer drug cytotoxicity. Int J Mol Sci 14(2):3683–3704. doi:10.3390/ijms14023683
87. Pi X, Xie L, Portbury AL, Kumar S, Lockyer P, Li X, Patterson C (2014) NADPH oxidase-generated reactive oxygen species are required for stromal cell-derived factor-1alpha-stimulated angiogenesis. Arterioscler Thromb Vasc Biol 34(9):2023–2032. doi:10.1161/ATVBAHA.114.303733
88. Jiang F, Zhang Y, Dusting GJ (2011) NADPH oxidase-mediated redox signaling: roles in cellular stress response, stress tolerance, and tissue repair. Pharmacol Rev 63(1):218–242. doi:10.1124/pr.110.002980
89. Lassegue B, San Martin A, Griendling KK (2012) Biochemistry, physiology, and pathophysiology of NADPH oxidases in the cardiovascular system. Circ Res 110(10):1364–1390. doi:10.1161/CIRCRESAHA.111.243972
90. Gregg JL, Turner RM 2nd, Chang G, Joshi D, Zhan Y, Chen L, Maranchie JK (2014) NADPH oxidase NOX4 supports renal tumorigenesis by promoting the expression and nuclear accumulation of HIF2alpha. Cancer Res 74(13):3501–3511. doi:10.1158/0008-5472.CAN-13-2979
91. Wang H, Yang Z, Jiang Y, Hartnett ME (2014) Endothelial NADPH oxidase 4 mediates vascular endothelial growth factor receptor 2-induced intravitreal neovascularization in a rat model of retinopathy of prematurity. Mol Vis 20:231–241
92. Ray R, Murdoch CE, Wang M, Santos CX, Zhang M, Alom-Ruiz S, Anilkumar N, Ouattara A, Cave AC, Walker SJ, Grieve DJ, Charles RL, Eaton P, Brewer AC, Shah AM (2011) Endothelial Nox4 NADPH oxidase enhances vasodilatation and reduces blood pressure in vivo. Arterioscler Thromb Vasc Biol 31(6):1368–1376. doi:10.1161/ATVBAHA.110.219238
93. Meng D, Mei A, Liu J, Kang X, Shi X, Qian R, Chen S (2012) NADPH oxidase 4 mediates insulin-stimulated HIF-1alpha and VEGF expression, and angiogenesis in vitro. PLoS One 7(10):e48393. doi:10.1371/journal.pone.0048393
94. Johnson BD, Mather KJ, Wallace JP (2011) Mechanotransduction of shear in the endothelium: basic studies and clinical implications. Vasc Med 16(5):365–377. doi:10.1177/1358863X11422109
95. Chiu JJ, Chien S (2011) Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. Physiol Rev 91(1):327–387. doi:10.1152/physrev.00047.2009
96. White SJ, Hayes EM, Lehoux S, Jeremy JY, Horrevoets AJ, Newby AC (2011) Characterization of the differential response of endothelial cells exposed to normal and elevated laminar shear stress. J Cell Physiol 226(11):2841–2848. doi:10.1002/jcp.22629
97. Lu X, Guo X, Wassall CD, Kemple MD, Unthank JL, Kassab GS (2011) Reactive oxygen species cause endothelial dysfunction in chronic flow overload. J Appl Physiol (1985) 110(2):520–527. doi:10.1152/japplphysiol.00786.2009
98. Montezano AC, Touyz RM (2012) Oxidative stress, Noxs, and hypertension: experimental evidence and clinical controversies. Ann Med 44(Suppl 1):S2–16. doi:10.3109/07853890.2011.653393
99. Chouchani ET, Pell VR, Gaude E, Aksentijevic D, Sundier SY, Robb EL, Logan A, Nadtochiy SM, Ord EN, Smith AC, Eyassu F, Shirley R, Hu CH, Dare AJ, James AM, Rogatti S, Hartley RC, Eaton S, Costa AS, Brookes PS, Davidson SM, Duchen MR, Saeb-Parsy K, Shattock MJ, Robinson AJ, Work LM, Frezza C, Krieg T, Murphy MP (2014) Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature 515(7527):431–435. doi:10.1038/nature13909
100. Finkel T (2012) Signal transduction by mitochondrial oxidants. J Biol Chem 287(7):4434–4440. doi:10.1074/jbc.R111.271999
101. Breton-Romero R, Acin-Perez R, Rodriguez-Pascual F, Martinez-Molledo M, Brandes RP, Rial E, Enriquez JA, Lamas S (2014) Laminar shear stress regulates mitochondrial dynamics, bioenergetics responses and PRX3 activation in endothelial cells. Biochim Biophys Acta 1843(11):2403–2413. doi:10.1016/j.bbamcr.2014.07.003
102. Hernansanz-Agustin P, Izquierdo-Alvarez A, Sanchez-Gomez FJ, Ramos E, Villa-Pina T, Lamas S, Bogdanova A, Martinez-Ruiz A (2014) Acute hypoxia produces a superoxide burst in cells. Free Radic Biol Med 71:146–156. doi:10.1016/j.freeradbiomed.2014.03.011
103. Katakam PV, Wappler EA, Katz PS, Rutkai I, Institoris A, Domoki F, Gaspar T, Grovenburg SM, Snipes JA, Busija DW (2013) Depolarization of mitochondria in endothelial cells promotes cerebral artery vasodilation by activation of nitric oxide synthase. Arterioscler Thromb Vasc Biol 33(4):752–759. doi:10.1161/ATVBAHA.112.300560
104. Kotiadis VN, Duchen MR, Osellame LD (2014) Mitochondrial quality control and communications with the nucleus are important in maintaining mitochondrial function and cell health.

Biochim Biophys Acta 1840(4):1254–1265. doi:10.1016/j.bbagen.2013.10.041
105. Al-Mehdi AB, Pastukh VM, Swiger BM, Reed DJ, Patel MR, Bardwell GC, Pastukh VV, Alexeyev MF, Gillespie MN (2012) Perinuclear mitochondrial clustering creates an oxidant-rich nuclear domain required for hypoxia-induced transcription. Sci Signal 5 (231):ra47. doi:10.1126/scisignal.2002712
106. Park J, Lee J, Choi C (2011) Mitochondrial network determines intracellular ROS dynamics and sensitivity to oxidative stress through switching inter-mitochondrial messengers. PLoS One 6(8):e23211. doi:10.1371/journal.pone.0023211
107. Shah GN, Morofuji Y, Banks WA, Price TO (2013) High glucose-induced mitochondrial respiration and reactive oxygen species in mouse cerebral pericytes is reversed by pharmacological inhibition of mitochondrial carbonic anhydrases: implications for cerebral microvascular disease in diabetes. Biochem Biophys Res Commun 440(2):354–358. doi:10.1016/j.bbrc.2013.09.086
108. Patel H, Chen J, Das KC, Kavdia M (2013) Hyperglycemia induces differential change in oxidative stress at gene expression and functional levels in HUVEC and HMVEC. Cardiovasc Diabetol 12:142. doi:10.1186/1475-2840-12-142
109. Dymkowska D, Drabarek B, Podszywalow-Bartnicka P, Szczepanowska J, Zablocki K (2014) Hyperglycaemia modifies energy metabolism and reactive oxygen species formation in endothelial cells in vitro. Arch Biochem Biophys 542:7–13. doi:10.1016/j.abb.2013.11.008
110. Forstermann U, Sessa WC (2012) Nitric oxide synthases: regulation and function. Eur Heart J 33 (7):829–837, 837a–837d. doi:10.1093/eurheartj/ehr304
111. Crabtree MJ, Hale AB, Channon KM (2011) Dihydrofolate reductase protects endothelial nitric oxide synthase from uncoupling in tetrahydrobiopterin deficiency. Free Radic Biol Med 50(11):1639–1646. doi:10.1016/j.freeradbiomed.2011.03.010
112. Mugoni V, Postel R, Catanzaro V, De Luca E, Turco E, Digilio G, Silengo L, Murphy MP, Medana C, Stainier DY, Bakkers J, Santoro MM (2013) Ubiad1 is an antioxidant enzyme that regulates eNOS activity by CoQ10 synthesis. Cell 152(3):504–518. doi:10.1016/j.cell.2013.01.013
113. Tejero J, Stuehr D (2013) Tetrahydrobiopterin in nitric oxide synthase. IUBMB Life 65(4):358–365. doi:10.1002/iub.1136
114. Heiss C, Rodriguez-Mateos A, Kelm M (2014) Central role of eNOS in the maintenance of endothelial homeostasis. Antioxid Redox Signal. doi:10.1089/ars.2014.6158
115. Cassuto J, Dou H, Czikora I, Szabo A, Patel VS, Kamath V, Belin de Chantemele E, Feher A, Romero MJ, Bagi Z (2014) Peroxynitrite disrupts endothelial caveolae leading to eNOS uncoupling and diminished flow-mediated dilation in coronary arterioles of diabetic patients. Diabetes 63(4):1381–1393. doi:10.2337/db13-0577
116. Priya MK, Sahu G, Soto-Pantoja DR, Goldy N, Sundaresan AM, Jadhav V, Barathkumar TR, Saran U, Jaffar Ali BM, Roberts DD, Bera AK, Chatterjee S (2014) Tipping off endothelial tubes: nitric oxide drives tip cells. Angiogenesis. doi:10.1007/s10456-014-9455-0
117. Siddiqui MR, Komarova YA, Vogel SM, Gao X, Bonini MG, Rajasingh J, Zhao YY, Brovkovych V, Malik AB (2011) Caveolin-1-eNOS signaling promotes p190RhoGAP-A nitration and endothelial permeability. J Cell Biol 193(5):841–850. doi:10.1083/jcb.201012129
118. Tian J, Hou Y, Lu Q, Wiseman DA, Vasconcelos Fonsesca F, Elms S, Fulton DJ, Black SM (2010) A novel role for caveolin-1 in regulating endothelial nitric oxide synthase activation in response to H2O2 and shear stress. Free Radic Biol Med 49(2):159–170. doi:10.1016/j.freeradbiomed.2010.03.023
119. Schulman IH, Hare JM (2012) Regulation of cardiovascular cellular processes by S-nitrosylation. Biochim Biophys Acta 1820(6):752–762. doi:10.1016/j.bbagen.2011.04.002
120. Duran WN, Beuve AV, Sanchez FA (2013) Nitric oxide, S-nitrosation, and endothelial permeability. IUBMB Life 65(10):819–826. doi:10.1002/iub.1204
121. Moraes MS, Costa PE, Batista WL, Paschoalin T, Curcio MF, Borges RE, Taha MO, Fonseca FV, Stern A, Monteiro HP (2014) Endothelium-derived nitric oxide (NO) activates the NO-epidermal growth factor receptor-mediated signaling pathway in bradykinin-stimulated angiogenesis. Arch Biochem Biophys 558:14–27. doi:10.1016/j.abb.2014.06.011
122. Chen CA, Wang TY, Varadharaj S, Reyes LA, Hemann C, Talukder MA, Chen YR, Druhan LJ, Zweier JL (2010) S-glutathionylation uncouples eNOS and regulates its cellular and vascular function. Nature 468(7327):1115–1118. doi:10.1038/nature09599
123. Sugiyama T, Michel T (2010) Thiol-metabolizing proteins and endothelial redox state: differential modulation of eNOS and biopterin pathways. Am J Physiol Heart Circ Physiol 298(1):H194–H201. doi:10.1152/ajpheart.00767.2009
124. Kelley EE, Khoo NK, Hundley NJ, Malik UZ, Freeman BA, Tarpey MM (2010) Hydrogen peroxide is the major oxidant product of xanthine oxidase. Free Radic Biol Med 48(4):493–498. doi:10.1016/j.freeradbiomed.2009.11.012
125. Battelli MG, Polito L, Bolognesi A (2014) Xanthine oxidoreductase in atherosclerosis pathogenesis: not only oxidative stress. Atherosclerosis 237(2):562–567. doi:10.1016/j.atherosclerosis.2014.10.006
126. Battelli MG, Bolognesi A, Polito L (2014) Pathophysiology of circulating xanthine oxidoreductase: new emerging roles for a multi-tasking enzyme. Biochim Biophys Acta 1842(9):1502–1517. doi:10.1016/j.bbadis.2014.05.022
127. Lee MC, Velayutham M, Komatsu T, Hille R, Zweier JL (2014) Measurement and characterization of superoxide generation from xanthine dehydrogenase: a redox-regulated pathway of radical generation in ischemic tissues. Biochemistry 53(41):6615–6623. doi:10.1021/bi500582r
128. Wang G, Qian P, Jackson FR, Qian G, Wu G (2008) Sequential activation of JAKs, STATs and xanthine dehydrogenase/oxidase by hypoxia in lung microvascular endothelial cells. Int J Biochem Cell Biol 40(3):461–470. doi:10.1016/j.biocel.2007.08.008
129. Nanduri J, Vaddi DR, Khan SA, Wang N, Makerenko V, Prabhakar NR (2013) Xanthine oxidase mediates hypoxia-inducible factor-2alpha degradation by intermittent hypoxia. PLoS One 8(10):e75838. doi:10.1371/journal.pone.0075838
130. Tsirmoula S, Lamprou M, Hatziapostolou M, Kieffer N, Papadimitriou E (2015) Pleiotrophin-induced endothelial cell migration is regulated by xanthine oxidase-mediated generation of reactive oxygen species. Microvasc Res 98C:74–81. doi:10.1016/j.mvr.2015.01.001
131. Dunn LL, Buckle AM, Cooke JP, Ng MK (2010) The emerging role of the thioredoxin system in angiogenesis. Arterioscler Thromb Vasc Biol 30(11):2089–2098. doi:10.1161/ATVBAHA.110.209643
132. Go YM, Son DJ, Park H, Orr M, Hao L, Takabe W, Kumar S, Kang DW, Kim CW, Jo H, Jones DP (2014) Disturbed flow enhances inflammatory signaling and atherogenesis by increasing thioredoxin-1 level in endothelial cell nuclei. PLoS One 9(9):e108346. doi:10.1371/journal.pone.0108346
133. Goy C, Czypiorski P, Altschmied J, Jakob S, Rabanter LL, Brewer AC, Ale-Agha N, Dyballa-Rukes N, Shah AM, Haendeler J (2014) The imbalanced redox status in senescent endothelial cells is due to dysregulated Thioredoxin-1 and NADPH oxidase 4. Exp Gerontol 56:45–52. doi:10.1016/j.exger.2014.03.005

134. Abdelsaid MA, Matragoon S, El-Remessy AB (2013) Thioredoxin-interacting protein expression is required for VEGF-mediated angiogenic signal in endothelial cells. Antioxid Redox Signal 19(18):2199–2212. doi:10.1089/ars.2012.4761
135. Spindel ON, Yan C, Berk BC (2012) Thioredoxin-interacting protein mediates nuclear-to-plasma membrane communication: role in vascular endothelial growth factor 2 signaling. Arterioscler Thromb Vasc Biol 32(5):1264–1270. doi:10.1161/ATVBAHA.111.244681
136. World C, Spindel ON, Berk BC (2011) Thioredoxin-interacting protein mediates TRX1 translocation to the plasma membrane in response to tumor necrosis factor-alpha: a key mechanism for vascular endothelial growth factor receptor-2 transactivation by reactive oxygen species. Arterioscler Thromb Vasc Biol 31(8):1890–1897. doi:10.1161/ATVBAHA.111.226340
137. Welsh SJ, Bellamy WT, Briehl MM, Powis G (2002) The redox protein thioredoxin-1 (Trx-1) increases hypoxia-inducible factor 1alpha protein expression: Trx-1 overexpression results in increased vascular endothelial growth factor production and enhanced tumor angiogenesis. Cancer Res 62(17):5089–5095
138. Takagi Y, Kikuta K, Moriwaki T, Aoki T, Nozaki K, Hashimoto N, Miyamoto S (2011) Expression of thioredoxin-1 and hypoxia inducible factor-1alpha in cerebral arteriovenous malformations: possible role of redox regulatory factor in neoangiogenic property. Surg Neurol Int 2:61. doi:10.4103/2152-7806.80356
139. Samuel SM, Thirunavukkarasu M, Penumathsa SV, Koneru S, Zhan L, Maulik G, Sudhakaran PR, Maulik N (2010) Thioredoxin-1 gene therapy enhances angiogenic signaling and reduces ventricular remodeling in infarcted myocardium of diabetic rats. Circulation 121(10):1244–1255. doi:10.1161/CIRCULATIONAHA.109.872481
140. Dai S, He Y, Zhang H, Yu L, Wan T, Xu Z, Jones D, Chen H, Min W (2009) Endothelial-specific expression of mitochondrial thioredoxin promotes ischemia-mediated arteriogenesis and angiogenesis. Arterioscler Thromb Vasc Biol 29(4):495–502. doi:10.1161/ATVBAHA.108.180349
141. Sibbing D, Pfeufer A, Perisic T, Mannes AM, Fritz-Wolf K, Unwin S, Sinner MF, Gieger C, Gloeckner CJ, Wichmann HE, Kremmer E, Schafer Z, Walch A, Hinterseer M, Nabauer M, Kaab S, Kastrati A, Schomig A, Meitinger T, Bornkamm GW, Conrad M, von Beckerath N (2011) Mutations in the mitochondrial thioredoxin reductase gene TXNRD2 cause dilated cardiomyopathy. Eur Heart J 32(9):1121–1133. doi:10.1093/eurheartj/ehq507
142. Beck H, Hellfritsch J, Kirsch J, Schneider M, Perisic T, Wortmann M, Frijhoff J, Dagnell M, Fey T, Esposito I, Kolle P, Pogoda K, Ingold I, Angeli JP, Kuhlencordt P, Ostman A, Pohl U, Conrad M (2015) Knockout of mitochondrial thioredoxin reductase stabilizes prolyl hydroxylase 2 and inhibits tumor growth and tumor-derived angiogenesis. Antioxid Redox Signal. doi:10.1089/ars.2014.5889
143. Chen B, Wang W, Shen T, Qi R (2013) Thioredoxin1 down-regulates oxidized low-density lipoprotein-induced adhesion molecule expression via Smad3 protein. PLoS One 8(9):e76226. doi:10.1371/journal.pone.0076226
144. Rhee SG, Woo HA (2011) Multiple functions of peroxiredoxins: peroxidases, sensors and regulators of the intracellular messenger H(2)O(2), and protein chaperones. Antioxid Redox Signal 15(3):781–794. doi:10.1089/ars.2010.3393
145. Riddell JR, Maier P, Sass SN, Moser MT, Foster BA, Gollnick SO (2012) Peroxiredoxin 1 stimulates endothelial cell expression of VEGF via TLR4 dependent activation of HIF-1alpha. PLoS One 7(11):e50394. doi:10.1371/journal.pone.0050394
146. Kisucka J, Chauhan AK, Patten IS, Yesilaltay A, Neumann C, Van Etten RA, Krieger M, Wagner DD (2008) Peroxiredoxin1 prevents excessive endothelial activation and early atherosclerosis. Circ Res 103(6):598–605. doi:10.1161/CIRCRESAHA.108.174870
147. Park JG, Yoo JY, Jeong SJ, Choi JH, Lee MR, Lee MN, Hwa Lee J, Kim HC, Jo H, Yu DY, Kang SW, Rhee SG, Lee MH, Oh GT (2011) Peroxiredoxin 2 deficiency exacerbates atherosclerosis in apolipoprotein E-deficient mice. Circ Res 109(7):739–749. doi:10.1161/CIRCRESAHA.111.245530
148. Kang DH, Lee DJ, Kim J, Lee JY, Kim HW, Kwon K, Taylor WR, Jo H, Kang SW (2013) Vascular injury involves the overoxidation of peroxiredoxin type II and is recovered by the peroxiredoxin activity mimetic that induces reendothelialization. Circulation 128(8):834–844. doi:10.1161/CIRCULATIONAHA.113.001725
149. Randall LM, Ferrer-Sueta G, Denicola A (2013) Peroxiredoxins as preferential targets in H2O2-induced signaling. Methods Enzymol 527:41–63. doi:10.1016/B978-0-12-405882-8.00003-9
150. Kang DH, Lee DJ, Lee KW, Park YS, Lee JY, Lee SH, Koh YJ, Koh GY, Choi C, Yu DY, Kim J, Kang SW (2011) Peroxiredoxin II is an essential antioxidant enzyme that prevents the oxidative inactivation of VEGF receptor-2 in vascular endothelial cells. Mol Cell 44(4):545–558. doi:10.1016/j.molcel.2011.08.040
151. Mowbray AL, Kang DH, Rhee SG, Kang SW, Jo H (2008) Laminar shear stress up-regulates peroxiredoxins (PRX) in endothelial cells: PRX 1 as a mechanosensitive antioxidant. J Biol Chem 283(3):1622–1627. doi:10.1074/jbc.M707985200
152. Riddell JR, Bshara W, Moser MT, Spernyak JA, Foster BA, Gollnick SO (2011) Peroxiredoxin 1 controls prostate cancer growth through Toll-like receptor 4-dependent regulation of tumor vasculature. Cancer Res 71(5):1637–1646. doi:10.1158/0008-5472.CAN-10-3674
153. Tao RR, Wang H, Hong LJ, Huang JY, Lu YM, Liao MH, Ye WF, Lu NN, Zhu DY, Huang Q, Fukunaga K, Lou YJ, Shoji I, Wilcox CS, Lai EY, Han F (2014) Nitrosative stress induces peroxiredoxin 1 ubiquitination during ischemic insult via E6AP activation in endothelial cells both in vitro and in vivo. Antioxid Redox Signal 21(1):1–16. doi:10.1089/ars.2013.5381
154. Stacey MM, Vissers MC, Winterbourn CC (2012) Oxidation of 2-cys peroxiredoxins in human endothelial cells by hydrogen peroxide, hypochlorous acid, and chloramines. Antioxid Redox Signal 17(3):411–421. doi:10.1089/ars.2011.4348
155. Mieyal JJ, Chock PB (2012) Posttranslational modification of cysteine in redox signaling and oxidative stress: focus on s-glutathionylation. Antioxid Redox Signal 16(6):471–475. doi:10.1089/ars.2011.4454
156. Zhang H, Forman HJ (2012) Glutathione synthesis and its role in redox signaling. Semin Cell Dev Biol 23(7):722–728. doi:10.1016/j.semcdb.2012.03.017
157. Langston W, Chidlow JH Jr, Booth BA, Barlow SC, Lefer DJ, Patel RP, Kevil CG (2007) Regulation of endothelial glutathione by ICAM-1 governs VEGF-A-mediated eNOS activity and angiogenesis. Free Radic Biol Med 42(5):720–729. doi:10.1016/j.freeradbiomed.2006.12.010
158. Tajima M, Kurashima Y, Sugiyama K, Ogura T, Sakagami H (2009) The redox state of glutathione regulates the hypoxic induction of HIF-1. Eur J Pharmacol 606(1–3):45–49. doi:10.1016/j.ejphar.2009.01.026
159. Abdelsaid MA, El-Remessy AB (2012) S-glutathionylation of LMW-PTP regulates VEGF-mediated FAK activation and endothelial cell migration. J Cell Sci 125(Pt 20):4751–4760. doi:10.1242/jcs.103481
160. Murdoch CE, Shuler M, Haeussler DJ, Kikuchi R, Bearelly P, Han J, Watanabe Y, Fuster JJ, Walsh K, Ho YS, Bachschmid MM, Cohen RA, Matsui R (2014) Glutaredoxin-1 up-regulation induces soluble vascular endothelial growth factor receptor 1, attenuating post-ischemia limb revascularization. J Biol Chem 289(12):8633–8644. doi:10.1074/jbc.M113.517219

161. Oelze M, Kroller-Schon S, Steven S, Lubos E, Doppler C, Hausding M, Tobias S, Brochhausen C, Li H, Torzewski M, Wenzel P, Bachschmid M, Lackner KJ, Schulz E, Munzel T, Daiber A (2014) Glutathione peroxidase-1 deficiency potentiates dysregulatory modifications of endothelial nitric oxide synthase and vascular dysfunction in aging. Hypertension 63(2):390–396. doi:10.1161/HYPERTENSIONAHA.113.01602
162. Speciale A, Anwar S, Ricciardi E, Chirafisi J, Saija A, Cimino F (2011) Cellular adaptive response to glutathione depletion modulates endothelial dysfunction triggered by TNF-alpha. Toxicol Lett 207(3):291–297. doi:10.1016/j.toxlet.2011.09.017
163. Song J, Kang SM, Lee WT, Park KA, Lee KM, Lee JE (2014) Glutathione protects brain endothelial cells from hydrogen peroxide-induced oxidative stress by increasing nrf2 expression. Exp Neurobiol 23(1):93–103. doi:10.5607/en.2014.23.1.93
164. Zhou HG, Liu L, Zhang Y, Huang YY, Tao YH, Zhang S, Su JJ, Tang YP, Guo ZL, Hu RM, Dong Q (2013) Glutathione prevents free fatty acids-induced oxidative stress and apoptosis in human brain vascular endothelial cells through Akt pathway. CNS Neurosci Ther 19(4):252–261. doi:10.1111/cns.12068
165. Bir SC, Shen X, Kavanagh TJ, Kevil CG, Pattillo CB (2013) Control of angiogenesis dictated by picomolar superoxide levels. Free Radic Biol Med 63:135–142. doi:10.1016/j.freeradbiomed.2013.05.015
166. Caliceti C, Zambonin L, Rizzo B, Fiorentini D, Vieceli Dalla Sega F, Hrelia S, Prata C (2014) Role of plasma membrane caveolae/lipid rafts in VEGF-induced redox signaling in human leukemia cells. BioMed Res Int 2014:857504. doi:10.1155/2014/857504
167. Oshikawa J, Urao N, Kim HW, Kaplan N, Razvi M, McKinney R, Poole LB, Fukai T, Ushio-Fukai M (2010) Extracellular SOD-derived H2O2 promotes VEGF signaling in caveolae/lipid rafts and post-ischemic angiogenesis in mice. PLoS One 5(4):e10189. doi:10.1371/journal.pone.0010189
168. Oshikawa J, Kim SJ, Furuta E, Caliceti C, Chen GF, McKinney RD, Kuhr F, Levitan I, Fukai T, Ushio-Fukai M (2012) Novel role of p66Shc in ROS-dependent VEGF signaling and angiogenesis in endothelial cells. Am J Physiol Heart Circ Physiol 302(3):H724–H732. doi:10.1152/ajpheart.00739.2011
169. Maraldi T, Prata C, Caliceti C, Vieceli Dalla Sega F, Zambonin L, Fiorentini D, Hakim G (2010) VEGF-induced ROS generation from NAD(P)H oxidases protects human leukemic cells from apoptosis. Int J Oncol 36(6):1581–1589
170. Zhuang J, Jiang T, Lu D, Luo Y, Zheng C, Feng J, Yang D, Chen C, Yan X (2010) NADPH oxidase 4 mediates reactive oxygen species induction of CD146 dimerization in VEGF signal transduction. Free Radic Biol Med 49(2):227–236. doi:10.1016/j.freeradbiomed.2010.04.007
171. Evangelista AM, Thompson MD, Bolotina VM, Tong X, Cohen RA (2012) Nox4- and Nox2-dependent oxidant production is required for VEGF-induced SERCA cysteine-674 S-glutathiolation and endothelial cell migration. Free Radic Biol Med 53(12):2327–2334. doi:10.1016/j.freeradbiomed.2012.10.546
172. Garrido-Urbani S, Jemelin S, Deffert C, Carnesecchi S, Basset O, Szyndralewiez C, Heitz F, Page P, Montet X, Michalik L, Arbiser J, Ruegg C, Krause KH, Imhof BA (2011) Targeting vascular NADPH oxidase 1 blocks tumor angiogenesis through a PPARalpha mediated mechanism. PLoS One 6(2):e14665. doi:10.1371/journal.pone.0014665
173. Wang J, Hong Z, Zeng C, Yu Q, Wang H (2014) NADPH oxidase 4 promotes cardiac microvascular angiogenesis after hypoxia/reoxygenation in vitro. Free Radic Biol Med 69:278–288. doi:10.1016/j.freeradbiomed.2014.01.027
174. Li Q, Fu GB, Zheng JT, He J, Niu XB, Chen QD, Yin Y, Qian X, Xu Q, Wang M, Sun AF, Shu Y, Rui H, Liu LZ, Jiang BH (2013) NADPH oxidase subunit p22(phox)-mediated reactive oxygen species contribute to angiogenesis and tumor growth through AKT and ERK1/2 signaling pathways in prostate cancer. Biochim Biophys Acta 1833(12):3375–3385. doi:10.1016/j.bbamcr.2013.09.018
175. Sun J, Xu Y, Sun S, Sun Y, Wang X (2010) Intermittent high glucose enhances cell proliferation and VEGF expression in retinal endothelial cells: the role of mitochondrial reactive oxygen species. Mol Cell Biochem 343(1–2):27–35. doi:10.1007/s11010-010-0495-5
176. Wang Y, Zang QS, Liu Z, Wu Q, Maass D, Dulan G, Shaul PW, Melito L, Frantz DE, Kilgore JA, Williams NS, Terada LS, Nwariaku FE (2011) Regulation of VEGF-induced endothelial cell migration by mitochondrial reactive oxygen species. Am J Physiol Cell Physiol 301(3):C695–C704. doi:10.1152/ajpcell.00322.2010
177. Chakrabarti S, Rizvi M, Morin K, Garg R, Freedman JE (2010) The role of CD40L and VEGF in the modulation of angiogenesis and inflammation. Vascul Pharmacol 53(3–4):130–137. doi:10.1016/j.vph.2010.05.003
178. Stanley AC, Wong CX, Micaroni M, Venturato J, Khromykh T, Stow JL, Lacy P (2014) The Rho GTPase Rac1 is required for recycling endosome-mediated secretion of TNF in macrophages. Immunol Cell Biol 92(3):275–286. doi:10.1038/icb.2013.90
179. Zhang H, Park Y, Wu J, Chen X, Lee S, Yang J, Dellsperger KC, Zhang C (2009) Role of TNF-alpha in vascular dysfunction. Clin Sci (Lond) 116(3):219–230. doi:10.1042/CS20080196
180. Miki H, Funato Y (2012) Regulation of intracellular signalling through cysteine oxidation by reactive oxygen species. J Biochem 151(3):255–261. doi:10.1093/jb/mvs006
181. Boivin B, Yang M, Tonks NK (2010) Targeting the reversibly oxidized protein tyrosine phosphatase superfamily. Sci Signal 3(137):pl2. doi:10.1126/scisignal.3137pl2
182. Chen YY, Chu HM, Pan KT, Teng CH, Wang DL, Wang AH, Khoo KH, Meng TC (2008) Cysteine S-nitrosylation protects protein-tyrosine phosphatase 1B against oxidation-induced permanent inactivation. J Biol Chem 283(50):35265–35272. doi:10.1074/jbc.M805287200
183. Schwertassek U, Haque A, Krishnan N, Greiner R, Weingarten L, Dick TP, Tonks NK (2014) Reactivation of oxidized PTP1B and PTEN by thioredoxin 1. FEBS J 281(16):3545–3558. doi:10.1111/febs.12898
184. Connor KM, Subbaram S, Regan KJ, Nelson KK, Mazurkiewicz JE, Bartholomew PJ, Aplin AE, Tai YT, Aguirre-Ghiso J, Flores SC, Melendez JA (2005) Mitochondrial H2O2 regulates the angiogenic phenotype via PTEN oxidation. J Biol Chem 280(17):16916–16924. doi:10.1074/jbc.M410690200
185. Juarez JC, Manuia M, Burnett ME, Betancourt O, Boivin B, Shaw DE, Tonks NK, Mazar AP, Donate F (2008) Superoxide dismutase 1 (SOD1) is essential for H2O2-mediated oxidation and inactivation of phosphatases in growth factor signaling. Proc Natl Acad Sci USA 105(20):7147–7152. doi:10.1073/pnas.0709451105
186. Frijhoff J, Dagnell M, Augsten M, Beltrami E, Giorgio M, Ostman A (2014) The mitochondrial reactive oxygen species regulator p66Shc controls PDGF-induced signaling and migration through protein tyrosine phosphatase oxidation. Free Radic Biol Med 68:268–277. doi:10.1016/j.freeradbiomed.2013.12.022
187. Dagnell M, Frijhoff J, Pader I, Augsten M, Boivin B, Xu J, Mandal PK, Tonks NK, Hellberg C, Conrad M, Arner ES, Ostman A (2013) Selective activation of oxidized PTP1B by the thioredoxin system modulates PDGF-beta receptor tyrosine kinase signaling. Proc Natl Acad Sci USA 110(33):13398–13403. doi:10.1073/pnas.1302891110
188. Frijhoff J, Dagnell M, Godfrey R, Ostman A (2014) Regulation of protein tyrosine phosphatase oxidation in cell adhesion and

migration. Antioxid Redox Signal 20(13):1994–2010. doi:10.1089/ars.2013.5643
189. Mondol AS, Tonks NK, Kamata T (2014) Nox4 redox regulation of PTP1B contributes to the proliferation and migration of glioblastoma cells by modulating tyrosine phosphorylation of coronin-1C. Free Radic Biol Med 67:285–291. doi:10.1016/j.freeradbiomed.2013.11.005
190. Hiraga R, Kato M, Miyagawa S, Kamata T (2013) Nox4-derived ROS signaling contributes to TGF-beta-induced epithelial-mesenchymal transition in pancreatic cancer cells. Anticancer Res 33(10):4431–4438
191. Corcoran A, Cotter TG (2013) Redox regulation of protein kinases. FEBS J 280(9):1944–1965. doi:10.1111/febs.12224
192. Giannoni E, Buricchi F, Raugei G, Ramponi G, Chiarugi P (2005) Intracellular reactive oxygen species activate Src tyrosine kinase during cell adhesion and anchorage-dependent cell growth. Mol Cell Biol 25(15):6391–6403. doi:10.1128/MCB.25.15.6391-6403.2005
193. Truong TH, Carroll KS (2013) Redox regulation of protein kinases. Crit Rev Biochem Mol Biol 48(4):332–356. doi:10.3109/10409238.2013.790873
194. Lee M, Choy WC, Abid MR (2011) Direct sensing of endothelial oxidants by vascular endothelial growth factor receptor-2 and c-Src. PLoS One 6(12):e28454. doi:10.1371/journal.pone.0028454
195. Gianni D, Taulet N, DerMardirossian C, Bokoch GM (2010) c-Src-mediated phosphorylation of NoxA1 and Tks4 induces the reactive oxygen species (ROS)-dependent formation of functional invadopodia in human colon cancer cells. Mol Biol Cell 21(23):4287–4298. doi:10.1091/mbc.E10-08-0685
196. Giannoni E, Chiarugi P (2014) Redox circuitries driving Src regulation. Antioxid Redox Signal 20(13):2011–2025. doi:10.1089/ars.2013.5525
197. Xi G, Shen X, Maile LA, Wai C, Gollahon K, Clemmons DR (2012) Hyperglycemia enhances IGF-I-stimulated Src activation via increasing Nox4-derived reactive oxygen species in a PKCzeta-dependent manner in vascular smooth muscle cells. Diabetes 61(1):104–113. doi:10.2337/db11-0990
198. Son Y, Kim S, Chung HT, Pae HO (2013) Reactive oxygen species in the activation of MAP kinases. Methods Enzymol 528:27–48. doi:10.1016/B978-0-12-405881-1.00002-1
199. Breton-Romero R, Lamas S (2013) Hydrogen peroxide signaling mediator in the activation of p38 MAPK in vascular endothelial cells. Methods Enzymol 528:49–59. doi:10.1016/B978-0-12-405881-1.00003-3
200. Galli S, Antico Arciuch VG, Poderoso C, Converso DP, Zhou Q, de Kier Bal, Joffe E, Cadenas E, Boczkowski J, Carreras MC, Poderoso JJ (2008) Tumor cell phenotype is sustained by selective MAPK oxidation in mitochondria. PLoS One 3(6):e2379. doi:10.1371/journal.pone.0002379
201. Rajashekhar G, Kamocka M, Marin A, Suckow MA, Wolter WR, Badve S, Sanjeevaiah AR, Pumiglia K, Rosen E, Clauss M (2011) Pro-inflammatory angiogenesis is mediated by p38 MAP kinase. J Cell Physiol 226(3):800–808. doi:10.1002/jcp.22404
202. Yan F, Wang Y, Wu X, Peshavariya HM, Dusting GJ, Zhang M, Jiang F (2014) Nox4 and redox signaling mediate TGF-beta-induced endothelial cell apoptosis and phenotypic switch. Cell Death Dis 5:e1010. doi:10.1038/cddis.2013.551
203. Cosentino-Gomes D, Rocco-Machado N, Meyer-Fernandes JR (2012) Cell signaling through protein kinase C oxidation and activation. Int J Mol Sci 13(9):10697–10721. doi:10.3390/ijms130910697
204. Giorgi C, Agnoletto C, Baldini C, Bononi A, Bonora M, Marchi S, Missiroli S, Patergnani S, Poletti F, Rimessi A, Zavan B, Pinton P (2010) Redox control of protein kinase C: cell- and disease-specific aspects. Antioxid Redox Signal 13(7):1051–1085. doi:10.1089/ars.2009.2825
205. Burgoyne JR, Eaton P (2013) Detecting disulfide-bound complexes and the oxidative regulation of cyclic nucleotide-dependent protein kinases by H2O2. Methods Enzymol 528:111–128. doi:10.1016/B978-0-12-405881-1.00007-0
206. Ushio-Fukai M, Alexander RW, Akers M, Yin Q, Fujio Y, Walsh K, Griendling KK (1999) Reactive oxygen species mediate the activation of Akt/protein kinase B by angiotensin II in vascular smooth muscle cells. J Biol Chem 274(32):22699–22704
207. Wani R, Qian J, Yin L, Bechtold E, King SB, Poole LB, Paek E, Tsang AW, Furdui CM (2011) Isoform-specific regulation of Akt by PDGF-induced reactive oxygen species. Proc Natl Acad Sci USA 108(26):10550–10555. doi:10.1073/pnas.1011665108
208. Yasukawa T, Tokunaga E, Ota H, Sugita H, Martyn JA, Kaneki M (2005) S-nitrosylation-dependent inactivation of Akt/protein kinase B in insulin resistance. J Biol Chem 280(9):7511–7518. doi:10.1074/jbc.M411871200
209. Pei DS, Song YJ, Yu HM, Hu WW, Du Y, Zhang GY (2008) Exogenous nitric oxide negatively regulates c-Jun N-terminal kinase activation via inhibiting endogenous NO-induced S-nitrosylation during cerebral ischemia and reperfusion in rat hippocampus. J Neurochem 106(4):1952–1963. doi:10.1111/j.1471-4159.2008.05531.x
210. Zmijewski JW, Banerjee S, Bae H, Friggeri A, Lazarowski ER, Abraham E (2010) Exposure to hydrogen peroxide induces oxidation and activation of AMP-activated protein kinase. J Biol Chem 285(43):33154–33164. doi:10.1074/jbc.M110.143685
211. Cardaci S, Filomeni G, Ciriolo MR (2012) Redox implications of AMPK-mediated signal transduction beyond energetic clues. J Cell Sci 125(Pt 9):2115–2125. doi:10.1242/jcs.095216
212. Burgoyne JR, Oka S, Ale-Agha N, Eaton P (2013) Hydrogen peroxide sensing and signaling by protein kinases in the cardiovascular system. Antioxid Redox Signal 18(9):1042–1052. doi:10.1089/ars.2012.4817
213. Mungai PT, Waypa GB, Jairaman A, Prakriya M, Dokic D, Ball MK, Schumacker PT (2011) Hypoxia triggers AMPK activation through reactive oxygen species-mediated activation of calcium release-activated calcium channels. Mol Cell Biol 31(17):3531–3545. doi:10.1128/MCB.05124-11
214. Wang Q, Liang B, Shirwany NA, Zou MH (2011) 2-Deoxy-D-glucose treatment of endothelial cells induces autophagy by reactive oxygen species-mediated activation of the AMP-activated protein kinase. PLoS One 6(2):e17234. doi:10.1371/journal.pone.0017234
215. Nakashima I, Kato M, Akhand AA, Suzuki H, Takeda K, Hossain K, Kawamoto Y (2002) Redox-linked signal transduction pathways for protein tyrosine kinase activation. Antioxid Redox Signal 4(3):517–531. doi:10.1089/15230860260196326
216. Catarzi S, Biagioni C, Giannoni E, Favilli F, Marcucci T, Iantomasi T, Vincenzini MT (2005) Redox regulation of platelet-derived-growth-factor-receptor: role of NADPH-oxidase and c-Src tyrosine kinase. Biochim Biophys Acta 1745(2):166–175. doi:10.1016/j.bbamcr.2005.03.004
217. Bouzigues CI, Nguyen TL, Ramodiharilafy R, Claeson A, Tharaux PL, Alexandrou A (2014) Regulation of the ROS response dynamics and organization to PDGF motile stimuli revealed by single nanoparticle imaging. Chem Biol 21(5):647–656. doi:10.1016/j.chembiol.2014.02.020
218. Bashan N, Kovsan J, Kachko I, Ovadia H, Rudich A (2009) Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. Physiol Rev 89(1):27–71. doi:10.1152/physrev.00014.2008
219. Goldstein BJ, Mahadev K, Wu X, Zhu L, Motoshima H (2005) Role of insulin-induced reactive oxygen species in the insulin

signaling pathway. Antioxid Redox Signal 7(7–8):1021–1031. doi:10.1089/ars.2005.7.1021
220. Iwakami S, Misu H, Takeda T, Sugimori M, Matsugo S, Kaneko S, Takamura T (2011) Concentration-dependent dual effects of hydrogen peroxide on insulin signal transduction in H4IIEC hepatocytes. PLoS One 6(11):e27401. doi:10.1371/journal.pone.0027401
221. Woo HA, Yim SH, Shin DH, Kang D, Yu DY, Rhee SG (2010) Inactivation of peroxiredoxin I by phosphorylation allows localized H(2)O(2) accumulation for cell signaling. Cell 140(4):517–528. doi:10.1016/j.cell.2010.01.009
222. Paulsen CE, Truong TH, Garcia FJ, Homann A, Gupta V, Leonard SE, Carroll KS (2012) Peroxide-dependent sulfenylation of the EGFR catalytic site enhances kinase activity. Nat Chem Biol 8(1):57–64. doi:10.1038/nchembio.736
223. Gonzalez-Pacheco FR, Deudero JJ, Castellanos MC, Castilla MA, Alvarez-Arroyo MV, Yague S, Caramelo C (2006) Mechanisms of endothelial response to oxidative aggression: protective role of autologous VEGF and induction of VEGFR2 by H2O2. Am J Physiol Heart Circ Physiol 291(3):H1395–H1401. doi:10.1152/ajpheart.01277.2005
224. Ushio-Fukai M (2007) VEGF signaling through NADPH oxidase-derived ROS. Antioxid Redox Signal 9(6):731–739. doi:10.1089/ars.2007.1556
225. Nakamura Y, Patrushev N, Inomata H, Mehta D, Urao N, Kim HW, Razvi M, Kini V, Mahadev K, Goldstein BJ, McKinney R, Fukai T, Ushio-Fukai M (2008) Role of protein tyrosine phosphatase 1B in vascular endothelial growth factor signaling and cell-cell adhesions in endothelial cells. Circ Res 102(10):1182–1191. doi:10.1161/CIRCRESAHA.107.167080
226. Boivin B, Zhang S, Arbiser JL, Zhang ZY, Tonks NK (2008) A modified cysteinyl-labeling assay reveals reversible oxidation of protein tyrosine phosphatases in angiomyolipoma cells. Proc Natl Acad Sci USA 105(29):9959–9964. doi:10.1073/pnas.0804336105
227. Kemble DJ, Sun G (2009) Direct and specific inactivation of protein tyrosine kinases in the Src and FGFR families by reversible cysteine oxidation. Proc Natl Acad Sci USA 106(13):5070–5075. doi:10.1073/pnas.0806117106
228. Thomas SR, Witting PK, Drummond GR (2008) Redox control of endothelial function and dysfunction: molecular mechanisms and therapeutic opportunities. Antioxid Redox Signal 10(10):1713–1765. doi:10.1089/ars.2008.2027
229. Semenza GL (2014) Hypoxia-inducible factor 1 and cardiovascular disease. Annu Rev Physiol 76:39–56. doi:10.1146/annurev-physiol-021113-170322
230. Li F, Sonveaux P, Rabbani ZN, Liu S, Yan B, Huang Q, Vujaskovic Z, Dewhirst MW, Li CY (2007) Regulation of HIF-1alpha stability through S-nitrosylation. Mol Cell 26(1):63–74. doi:10.1016/j.molcel.2007.02.024
231. Lima B, Lam GK, Xie L, Diesen DL, Villamizar N, Nienaber J, Messina E, Bowles D, Kontos CD, Hare JM, Stamler JS, Rockman HA (2009) Endogenous S-nitrosothiols protect against myocardial injury. Proc Natl Acad Sci USA 106(15):6297–6302. doi:10.1073/pnas.0901043106
232. Kietzmann T, Gorlach A (2005) Reactive oxygen species in the control of hypoxia-inducible factor-mediated gene expression. Semin Cell Dev Biol 16(4–5):474–486. doi:10.1016/j.semcdb.2005.03.010
233. Jung SN, Yang WK, Kim J, Kim HS, Kim EJ, Yun H, Park H, Kim SS, Choe W, Kang I, Ha J (2008) Reactive oxygen species stabilize hypoxia-inducible factor-1 alpha protein and stimulate transcriptional activity via AMP-activated protein kinase in DU145 human prostate cancer cells. Carcinogenesis 29(4):713–721. doi:10.1093/carcin/bgn032
234. Comito G, Calvani M, Giannoni E, Bianchini F, Calorini L, Torre E, Migliore C, Giordano S, Chiarugi P (2011) HIF-1alpha stabilization by mitochondrial ROS promotes Met-dependent invasive growth and vasculogenic mimicry in melanoma cells. Free Radic Biol Med 51(4):893–904. doi:10.1016/j.freeradbiomed.2011.05.042
235. Calvani M, Comito G, Giannoni E, Chiarugi P (2012) Time-dependent stabilization of hypoxia inducible factor-1alpha by different intracellular sources of reactive oxygen species. PLoS One 7(10):e38388. doi:10.1371/journal.pone.0038388
236. Hoesel B, Schmid JA (2013) The complexity of NF-kappaB signaling in inflammation and cancer. Mol Cancer 12:86. doi:10.1186/1476-4598-12-86
237. Morgan MJ, Liu ZG (2011) Crosstalk of reactive oxygen species and NF-kappaB signaling. Cell Res 21(1):103–115. doi:10.1038/cr.2010.178
238. Gloire G, Piette J (2009) Redox regulation of nuclear post-translational modifications during NF-kappaB activation. Antioxid Redox Signal 11(9):2209–2222. doi:10.1089/ARS.2009.2463
239. Madamanchi NR, Runge MS (2013) Redox signaling in cardiovascular health and disease. Free Radic Biol Med 61:473–501. doi:10.1016/j.freeradbiomed.2013.04.001
240. Reynaert NL, van der Vliet A, Guala AS, McGovern T, Hristova M, Pantano C, Heintz NH, Heim J, Ho YS, Matthews DE, Wouters EF, Janssen-Heininger YM (2006) Dynamic redox control of NF-kappaB through glutaredoxin-regulated S-glutathionylation of inhibitory kappaB kinase beta. Proc Natl Acad Sci USA 103(35):13086–13091. doi:10.1073/pnas.0603290103
241. Hajra L, Evans AI, Chen M, Hyduk SJ, Collins T, Cybulsky MI (2000) The NF-kappa B signal transduction pathway in aortic endothelial cells is primed for activation in regions predisposed to atherosclerotic lesion formation. Proc Natl Acad Sci USA 97(16):9052–9057
242. DangLi R, HeKong W, JiQin L, MingHua Z, WenCheng Z (2012) ROS-induced ZNF580 expression: a key role for H2O2/NF-kappaB signaling pathway in vascular endothelial inflammation. Mol Cell Biochem 359(1–2):183–191. doi:10.1007/s11010-011-1013-0
243. Kassan M, Choi SK, Galan M, Bishop A, Umezawa K, Trebak M, Belmadani S, Matrougui K (2013) Enhanced NF-kappaB activity impairs vascular function through PARP-1-, SP-1-, and COX-2-dependent mechanisms in type 2 diabetes. Diabetes 62(6):2078–2087. doi:10.2337/db12-1374
244. Aurora AB, Biyashev D, Mirochnik Y, Zaichuk TA, Sanchez-Martinez C, Renault MA, Losordo D, Volpert OV (2010) NF-kappaB balances vascular regression and angiogenesis via chromatin remodeling and NFAT displacement. Blood 116(3):475–484. doi:10.1182/blood-2009-07-232132
245. Fourquet S, Guerois R, Biard D, Toledano MB (2010) Activation of NRF2 by nitrosative agents and H2O2 involves KEAP1 disulfide formation. J Biol Chem 285(11):8463–8471. doi:10.1074/jbc.M109.051714
246. Chen XL, Dodd G, Thomas S, Zhang X, Wasserman MA, Rovin BH, Kunsch C (2006) Activation of Nrf2/ARE pathway protects endothelial cells from oxidant injury and inhibits inflammatory gene expression. Am J Physiol Heart Circ Physiol 290(5):H1862–H1870. doi:10.1152/ajpheart.00651.2005
247. Valcarcel-Ares MN, Gautam T, Warrington JP, Bailey-Downs L, Sosnowska D, de Cabo R, Losonczy G, Sonntag WE, Ungvari Z, Csiszar A (2012) Disruption of Nrf2 signaling impairs angiogenic capacity of endothelial cells: implications for microvascular aging. J Gerontol Ser A Biol Sci Med Sci 67(8):821–829. doi:10.1093/gerona/glr229
248. Florczyk U, Jazwa A, Maleszewska M, Mendel M, Szade K, Kozakowska M, Grochot-Przeczek A, Viscardi M, Czauderna S, Bukowska-Strakova K, Kotlinowski J, Jozkowicz A, Loboda A,

Dulak J (2014) Nrf2 regulates angiogenesis: effect on endothelial cells, bone marrow-derived proangiogenic cells and hind limb ischemia. Antioxid Redox Signal 20(11):1693–1708. doi:10.1089/ars.2013.5219

249. Wei Y, Gong J, Thimmulappa RK, Kosmider B, Biswal S, Duh EJ (2013) Nrf2 acts cell-autonomously in endothelium to regulate tip cell formation and vascular branching. Proc Natl Acad Sci USA 110(41):E3910–E3918. doi:10.1073/pnas.1309276110
250. Kuang L, Feng J, He G, Jing T (2013) Knockdown of Nrf2 inhibits the angiogenesis of rat cardiac micro-vascular endothelial cells under hypoxic conditions. Int J Biol Sci 9(7):656–665. doi:10.7150/ijbs.5887
251. Afonyushkin T, Oskolkova OV, Philippova M, Resink TJ, Erne P, Binder BR, Bochkov VN (2010) Oxidized phospholipids regulate expression of ATF4 and VEGF in endothelial cells via NRF2-dependent mechanism: novel point of convergence between electrophilic and unfolded protein stress pathways. Arterioscler Thromb Vasc Biol 30(5):1007–1013. doi:10.1161/ATVBAHA.110.204354
252. Uno K, Prow TW, Bhutto IA, Yerrapureddy A, McLeod DS, Yamamoto M, Reddy SP, Lutty GA (2010) Role of Nrf2 in retinal vascular development and the vaso-obliterative phase of oxygen-induced retinopathy. Exp Eye Res 90(4):493–500. doi:10.1016/j.exer.2009.12.012
253. Ji X, Wang H, Zhu J, Zhu L, Pan H, Li W, Zhou Y, Cong Z, Yan F, Chen S (2014) Knockdown of Nrf2 suppresses glioblastoma angiogenesis by inhibiting hypoxia-induced activation of HIF-1alpha. Int J Cancer J Int du Cancer 135(3):574–584. doi:10.1002/ijc.28699
254. Kim TH, Hur EG, Kang SJ, Kim JA, Thapa D, Lee YM, Ku SK, Jung Y, Kwak MK (2011) NRF2 blockade suppresses colon tumor angiogenesis by inhibiting hypoxia-induced activation of HIF-1alpha. Cancer Res 71(6):2260–2275. doi:10.1158/0008-5472.CAN-10-3007
255. Carmeliet P, Jain RK (2011) Molecular mechanisms and clinical applications of angiogenesis. Nature 473(7347):298–307. doi:10.1038/nature10144
256. Brakenhielm E, Cao R, Cao Y (2001) Suppression of angiogenesis, tumor growth, and wound healing by resveratrol, a natural compound in red wine and grapes. FASEB J 15(10):1798–1800
257. Muliyil S, Narasimha M (2014) Mitochondrial ROS regulates cytoskeletal and mitochondrial remodeling to tune cell and tissue dynamics in a model for wound healing. Dev Cell 28(3):239–252. doi:10.1016/j.devcel.2013.12.019
258. Catalano V, Turdo A, Di Franco S, Dieli F, Todaro M, Stassi G (2013) Tumor and its microenvironment: a synergistic interplay. Semin Cancer Biol 23(6 Pt B):522–532. doi:10.1016/j.semcancer.2013.08.007
259. Gorrini C, Harris IS, Mak TW (2013) Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discovery 12(12):931–947. doi:10.1038/nrd4002
260. Okuno Y, Nakamura-Ishizu A, Otsu K, Suda T, Kubota Y (2012) Pathological neoangiogenesis depends on oxidative stress regulation by ATM. Nat Med 18(8):1208–1216. doi:10.1038/nm.2846
261. Alitalo AK, Proulx ST, Karaman S, Aebischer D, Martino S, Jost M, Schneider N, Bry M, Detmar M (2013) VEGF-C and VEGF-D blockade inhibits inflammatory skin carcinogenesis. Cancer Res 73(14):4212–4221. doi:10.1158/0008-5472.CAN-12-4539