



**HAL**  
open science

## Physiological role of reactive oxygen species as promoters of natural defenses

Jérôme Roy, Jean-Marie Galano, Thierry Durand, Jean-Yves Le Guennec,  
Jetty Chung-Yung Lee

► **To cite this version:**

Jérôme Roy, Jean-Marie Galano, Thierry Durand, Jean-Yves Le Guennec, Jetty Chung-Yung Lee. Physiological role of reactive oxygen species as promoters of natural defenses. *FASEB Journal*, 2017, 31 (9), pp.3729 - 3745. 10.1096/fj.201700170R . hal-01822212

**HAL Id: hal-01822212**

**<https://hal.umontpellier.fr/hal-01822212>**

Submitted on 14 Apr 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Physiological role of reactive oxygen species as promoters of natural defenses

Jérôme Roy,<sup>\*,†,1</sup> Jean-Marie Galano,<sup>‡</sup> Thierry Durand,<sup>‡</sup> Jean-Yves Le Guennec,<sup>†</sup> and Jetty Chung-Yung Lee<sup>§</sup>

\*Centre de Recherche du Centre Hospitalier de l'Université de Montréal and Montreal Diabetes Research Center, Department of Nutrition, Université de Montréal, Montreal, Québec, Canada; †Centre National de la Recherche Scientifique Unité Mixte de Recherche 9214, Inserm Unité 1046 Physiologie et Médecine Expérimentale du Cœur et des Muscles, and ‡Institut des Biomolécules Max Mousseron, Centre National de la Recherche Scientifique Unité Mixte de Recherche 5247, École Nationale Supérieure de Chimie de Montpellier, Université de Montpellier, Montpellier, France; and §School of Biological Sciences, The University of Hong Kong, Hong Kong, China

**ABSTRACT:** It has been 60 yr since the discovery of reactive oxygen species (ROS) in biology and the beginning of the scientific community's attempt to understand the impact of the unpaired electron of ROS molecules in biological pathways, which was eventually noted to be toxic. Several studies have shown that the presence of ROS is essential in triggering or acting as a secondary factor for numerous pathologies, including metabolic and genetic diseases; however, it was demonstrated that chronic treatment with antioxidants failed to show efficacy and positive effects in the prevention of diseases or health complications that result from oxidative stress. On the contrary, such treatment has been shown to sometimes even worsen the disease. Because of the permanent presence of ROS in organisms, elaborate mechanisms to adapt with these reactive molecules and to use them without necessarily blocking or preventing their actions have been studied. There is now a large body of evidence that shows that living organisms have conformed to the presence of ROS and, in retrospect, have adapted to the bioactive molecules that are generated by ROS on proteins, lipids, and DNA. In addition, ROS have undergone a shift from being molecules that invoked oxidative damage in regulating signaling pathways that impinged on normal physiological and redox responses. Working in this direction, this review unlocks a new conception about the involvement of cellular oxidants in the maintenance of redox homeostasis in redox regulation of normal physiological functions, and an explanation for its essential role in numerous pathophysiological states is noted.—Roy, J., Galano, J.-M., Durand, T., Le Guennec, J.-Y., Lee, J. C.-Y. Physiological role of reactive oxygen species as promoters of natural defenses. *FASEB J.* 31, 3729–3745 (2017). www.fasebj.org

**KEY WORDS:** oxidative stress · redox pathologies · redox homeostasis · redox signaling

In redox biology, oxidative stress is defined as the increase of reduction potential or a large decrease in the reducing capacity of cellular redox couples (1). One infamous group of these molecules that is responsible for oxidative stress is the reactive oxygen species (ROS). As the name suggests, these are free radical species of oxygen that are in a more reactive state than molecular oxygen and can be reduced to varying degrees. Molecular oxygen is a diradical, containing 2 unpaired electrons with parallel

spin configurations. Because electrons must have opposite spin to occupy the same orbit, electrons added to molecular oxygen must be transferred one at a time during its reduction (2), which results in several high-reactive intermediates (3). These ROS molecules are a relative new concept and this area of research in science was first described only 60 yr ago. Scientists have extended their insights into the complex effects of free radicals and ROS within the biological systems.

In 1954, Commoner *et al.* (4) used electron spin resonance spectroscopy to generate the first data that showed that skeletal muscle contains free radicals. For the first time, the presence of ROS in biological materials was discovered. Two years later, Harman (5, 6) hypothesized that endogenous oxygen radicals may be formed as byproducts of enzymatic reactions *in vivo*. He proposed that traces of iron would catalyze oxidative reactions *in vivo* and that peroxidative chain reactions were possible by analogy to the principle of *in vitro* polymer chemistry. Harman (5, 6) described free radicals that were produced during aerobic respiration as a molecule of evils that may account for

**ABBREVIATIONS:** ATII, angiotensin II; bFGF, basic fibroblast growth factor; BMP, bone morphogenetic protein; CaMKII, calmodulin kinase II; DDA, dendrogenin; HIF-1, hypoxia-inducible factor 1; Mφ, macrophage; nNOS, neuronal NOS; NOX, NADP oxidase; PUFA, polyunsaturated fatty acid; RAR-α, retinoic acid receptor-α; ROS, reactive oxygen species; RyR2, ryanodine receptor 2; SOD, superoxide dismutase; T<sub>reg</sub>, regulatory T; XO, xanthine oxidase

<sup>1</sup> Correspondence: CRCHUM and Montreal Diabetes Research Center, Department of Nutrition, Université de Montréal, 900 rue Saint-Denis, Tour Viger, Montréal, QC H2X 0A9, Canada. E-mail: jerome.roy1902@gmail.com

doi: 10.1096/fj.201700170R

gross cellular damage, mutagenesis, cancer, or the degenerative process of biological aging.

Thereafter, the free radical theory of aging was born. At that time, ROS were considered by scientists as molecules that were uniformly injurious and damaging to tissues and organs. This idea has been entrenched in the minds of exercise physiologists for years. The theory gained credibility in 1969 when McCord and Fridovich (7) discovered the enzyme, superoxide dismutase (SOD), which brought the free radical theory in living organisms into a new era and that eventually convinced scientists that ROS are important for biology systems. This discovery provided the first compelling evidence of the *in vivo* generation of  $O_2^{\cdot-}$ , but through the subsequent elucidation of elaborate antioxidant defenses system (8). In addition, the localization of SOD was used as a tool to locate subcellular sites of  $O_2^{\cdot-}$  generation, which led to the demonstration that mitochondria are the principal source of endogenous oxidants (9). In the 1970s, Chance discovered that intact cells (10) and mitochondria (11) were also a primary site of endogenous oxidant generator. These findings established the missing links of the rate of living theory. Furthermore, discoveries that showed ROS are produced in the body indirectly were fundamental. Thereafter, this became the starting point of a colossal number of research studies on the sources of  $O_2^{\cdot-}$  production and its pathological and physiological roles; however, for a long time, there were doubts about the existence of ROS and their *in vivo* effects. At the beginning of the 1990s, the existence of ROS became indisputable with the use of the electron spin resonance technique that can trap electron spins from ROS molecules.

After the discovery of SOD, extensive research was conducted (12–14) and the scientific community impetuously developed multiple *in vitro* experiments to investigate, in priority, the negative effects of ROS and oxidative damage inflicted by radicals upon DNA (15), proteins (16), lipids (17), and other components of the cell. Therefore, the first part of this review focuses on the involvement of ROS as an essential trigger or secondary factor in the development of pathology highlighting inflammation and cardiovascular disease, and in the second part, we present the physiological implications of ROS as a trigger of molecular signaling.

## INVOLVEMENT OF ROS IN THE DEVELOPMENT OF PATHOLOGY

ROS, such as  $O_2^{\cdot-}$ ,  $^1O_2$ ,  $H_2O_2$ ,  $\cdot OH$ ,  $\cdot ONOO^-$ , and hypochlorite, are known to be produced as byproducts of oxidative metabolism in which energy activation and electron reduction are involved. It is well understood that the production is enhanced during inflammation, aging, radiation exposure, endotoxic shock, and ischemia/reperfusion in the heart, intestine, liver, kidney, and brain. Mechanisms of ROS produced at the cellular level are not well understood; therefore, it is important to follow these mechanisms for the development of therapeutic strategies at cellular sites of dysfunction. Human tissues have a substantial ability to tolerate ROS under normal conditions. When production of ROS exceeds the capacity

of antioxidant defenses, oxidative stress is inflicted, which leads to harmful effects on the function and structural integrity of biological tissues. These ROS free radicals are reactive intermediates and can trigger rapid chain reactions and cause damage to macromolecules, such as lipids, proteins, carbohydrates, and nucleic acids (18). As a consequence of the oxidative damage to these macromolecules, lipid peroxide, carbonyl, and glycated compounds are formed, as well as DNA base modification/strand breakage that subsequently leads to the loss of the functional and structural efficiency of proteins and DNA mutation (18). Considering the continuous generation of ROS, organisms have evolved complex enzymatic defenses against the attacks of free radicals, also termed antioxidant defense (catalase, SOD, glutathione). These enzymes and antioxidant molecules alone, however, are unable to control the oxidative damage and, instead, remove or repair with the aid of other enzymes, such as thioredoxin reductase and methionine sulfoxide reductase (19). Despite these antioxidant protections, it is impossible to escape the oxidative stress that results from an imbalance of oxidative and antioxidant molecules in favor of ROS that potentially leads to biological injuries (20), including the disruption of the redox signal (21). If not regulated properly, it becomes chronic and leads to aging and several age-related diseases and pathologies (22). However, these pathologies, in particular, inflammatory disorders and cardiac alterations presented below, are known to be dependent on ROS production as essential to, a trigger of, or a secondary factor in the genesis of disease.

## Inflammatory disorders

An inflammatory phenomenon is linked to overproduction of ROS after stimulation of the expression of essential enzymes, such as eNOS (expressed in endothelial cell) and neuronal NOS (nNOS; expressed in cardiomyocytes), xanthine oxidase (XO), NADP oxidase (NOX), cyclooxygenase 1 and inducible enzymes NOX2, iNOS, and cyclooxygenase 2. The origin of the overproduced ROS is related to cytokines that are produced during inflammation as well as phagocytic cells, fibroblasts, and chondrocytes. As presented below, overproduction of ROS can be deleterious for acute and chronic inflammatory disease, which is known to be dependent on ROS production.

### Acute inflammatory diseases

Zazzo (23) indicated the implications of ROS in the pathophysiology of acute inflammatory diseases that include systemic inflammatory response syndrome, such as toxic shock, acute respiratory distress syndrome, vast burns, polytrauma, acute renal insufficiencies, and ischemia/reperfusion. For example, during acute respiratory distress syndrome, damage undergone by the endothelium of lung capillaries is a result of a massive activation of neutrophils that also induces excessive ROS within the inflammatory site (24).

This overproduction of ROS *via* activation of neutrophils by NOX is initiated by blood-borne chemotactic

factors that are released after an inflammatory event, such as myocardial ischemia/reperfusion (25). In addition, during this inflammatory reaction, phagocytosis and overconsumption of oxygen take place. In 1994, Babior (26) described such reactions as a burst of respiration as a result of an increase in metabolic activity (50 times more than the normal condition). The burst is defined as a massive production of ROS in inflammation where neutrophils and macrophages (Mφs) produce large quantities of ROS, especially  $O_2^{\cdot-}$ , to activate NOX.

Excess production of ROS is associated with the increment of procoagulative microparticles (*i.e.*, formation of microthrombosis), which triggers systemic inflammatory response syndrome (27) as described in Fig. 1. However, even if this burst of ROS plays a major role in inflammatory diseases and the oxidative damage may have the unwanted consequence of neutrophil activation; its importance in the physiological role of neutrophils to defend against infections, as in lung infection, cannot be ignored.

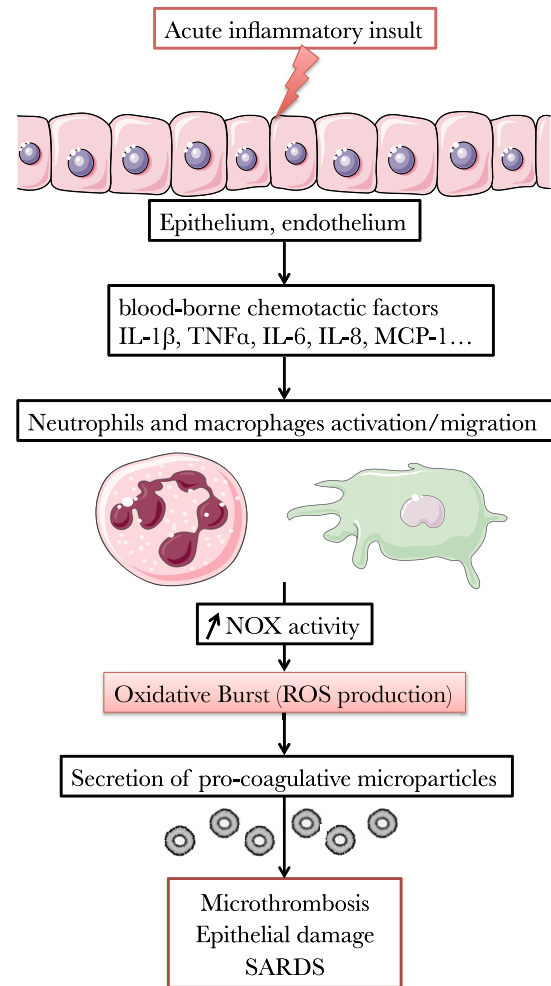
### Chronic inflammatory diseases

**Intestinal disease** Chronic inflammatory disease of the intestine is often associated with an increase of ROS, mainly by  $O_2^{\cdot-}$ , produced by intestinal cells. It is suggested that the lipid peroxidation that follows is responsible for the change in intestinal epithelium function; however, we do not know whether ROS produced during this inflammation is the cause or the consequence of the inflammation (28).

**Renal disease** Molecular oxidation induced by ROS occurs in the tubules and renal glomerulus. Hypochlorite ions that are produced by enzymatic systems end in the formation of chlorinated proteins and lipids, which leads to tubule and renal glomerulus dysfunction. Through several observations, Klebanoff (29) suggested that myeloperoxidase takes part in renal dysfunction. Moreover, myeloperoxidase induces and aggravates the formation of autoantibody in necrotizing glomerulonephritis (30).

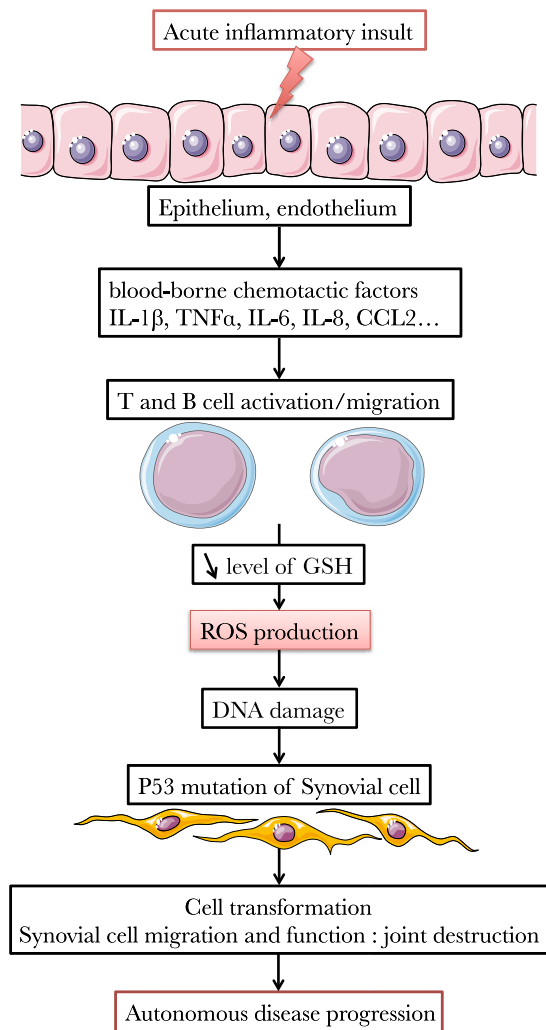
**Osteoarthritis** Maneesh *et al.* (31) showed oxidative stress to be associated with patients who present with degenerative osteoarthritis. Degenerative osteoarthritis is an articular inflammatory phenomenon in which ROS are formed by inflammatory cells by the activation of synovocytes, endothelial cells, and chondrocytes. ROS are essentially produced by NOX, iNOS, and eNOS. Furthermore, ROS are a trigger factor that induces the degradation of collagen and proteoglycans to increase metalloproteases synthesis and the apoptosis of chondrocytes, which leads to cartilage annihilation.

**Rheumatoid arthritis** Rheumatoid arthritis is a systemic autoimmune disease that is characterized by chronic joint inflammation with infiltration of Mφs and activated T cells (32). The pathogenesis of this disease is linked essentially with the formation of ROS at the site of inflammation. T cells that are isolated from the synovial fluid of patients with rheumatoid arthritis showed signs of decreased levels of intracellular glutathione and impaired



**Figure 1.** Impact of neutrophil activation and burst ROS production on severe acute respiratory distress syndrome (SARDS). An initial inflammation insult to the lung results in increased expression and release of blood-borne chemotactic factors, such as IL-1 $\beta$ , TNF- $\alpha$ , and IL-6, and of chemokines, such as IL-8 and monocyte chemoattractant protein 1 (MCP-1). This leads to the activation and recruitment of neutrophils and Mφs into areas of inflamed sites. Activated cells are capable of increasing NOX2 activity to induce bursts of ROS production. These bursts secrete procoagulative microparticles (granule contents), cause bystander damage to host cells (endothelial and epithelial cells), and cause microthrombosis. Disruption of the endothelial-epithelial barrier allows protein-rich fluid to enter the alveolar space, which eventually results in alveolar flooding and respiratory failure. This burst of ROS is a trigger to exaggerate the inflammatory response and, of note, the occurrence of SARDS.

phosphorylation of the adaptor protein linker for T cells (33). Altered subcellular localization of T cells has been shown to cause modification of intracellular glutathione levels (Fig. 2). Migration of monocytes and lymphocytes into the rheumatoid arthritis synovium is mediated by the abnormal expression of several adhesion molecules, including VCAM-1 (34). Although malignant tumors of the synovium are rare, it has been hypothesized that the presence of transformed cells (P53 mutation) in the synovium of patients with rheumatoid arthritis caused by ROS may lead to progressive joint destruction without malignant degeneration (35).



**Figure 2.** Impact of lymphocyte activation on autonomous disease progression. An initial inflammation insult results in increased expression and release of blood-borne chemotactic factors, such as IL-1 $\beta$ , TNF- $\alpha$ , and IL-6, and of chemokines, such as IL-8 and monocyte chemoattractant protein 1. This leads to the activation and recruitment of T lymphocytes into the inflamed site. T and B cells show signs of decreased intracellular glutathione (GSH) levels, which induces ROS production that causes DNA damage to host cells (synovial cell of rheumatoid arthritis patients), eventually contributing to malignant tumors (P53 mutation). This action by ROS production may lead to progressive joint destruction without malignant degeneration, may exaggerate the inflammatory response, and may contribute to the pathogenesis of autonomous disease (rheumatoid arthritis).

## Cardiac pathogenesis

ROS-induced oxidative stress in cardiac and vascular myocytes is associated with cardiovascular tissue injury (36). Chronic overproduction of ROS within the mitochondria of cardiac cells leads to mitochondrial DNA damage, and the accumulation of mutations causes cellular injuries and, consequently, crucial cardiac remodeling. Nonetheless, ROS play a role in the development of cardiovascular diseases, such as atherosclerosis, ischemic heart disease, hypertension,

cardiomyopathies (hypertrophic and dilated), cardiac hypertrophy, congestive heart failure, and blocks of conduction or still cardiac infarction (37). In addition, abnormalities in myocyte function as a result of increased oxidative stress are associated with the effects of ROS in subcellular organelles, which is considered the first step to the development of cardiac dysfunction.

## Arrhythmias

Abnormalities of Ca<sup>2+</sup> homeostasis are a fundamental feature of heart failure with contractile and energetic dysfunction, arrhythmia, transcriptional changes, and mitochondrial ROS production. Redox signaling has a significant impact on Ca<sup>2+</sup> homeostasis of myocytes (38). In myocytes, ROS can target the protein involved in excitation-contraction coupling. Mechanisms that are implicated in these abnormalities include ryanodine receptor 2 (RyR2) protein hyperphosphorylation by PKA, and calmodulin kinase II (CaMKII). Oxidation-enhanced activation of PKA/CaMKII could potentially contribute to RyR2 dysfunction (39). In addition, nNOS colocalizes with RyR2, and it was reported that deficient nNOS-mediated RyR2 S-nitrosylation promotes thiol oxidation *via* XO-dependent ROS, which leads to increased diastolic Ca<sup>2+</sup> and arrhythmia (40).

In the atrium, oxidative stress has been shown to take part in atrial fibrillation, and NOX2 has been implicated in this process. Indeed, NOX is a multimeric complex that is expressed in several tissues and cells—phagocytes, endothelial, epithelial, smooth muscle, and cardiac cells—and there are 7 enzymes: NOX 1–5 for NAD(P)H oxidase and DUOX 1 and 2 for dual oxidase. All multisubunit enzymes of NOX oxidize the soluble coenzyme NADPH, which results in the formation of O<sub>2</sub><sup>•-</sup>. In phagocytic cells, NOX plays a key role in the defense against pathogens. In other cells, NOX participates in cell signaling, during which it releases O<sub>2</sub><sup>•-</sup> extracellularly for phagocytic cells or intracellularly for nonphagocytic cells. NOX2-derived ROS production was increased in the right atrial appendages of patients who underwent cardiac bypass surgery who developed postoperative atrial fibrillation (41). It is also interesting to observe that NOX2-dependent CaMKII oxidation promotes sinus node dysfunction *via* the apoptosis of sinoatrial cells in a mouse model of angiotensin II (ATII)-induced arrhythmias (42). It should be noted that other redox-sensitive mechanisms, such as the effects on L-type Ca<sup>2+</sup> channels, plasmalemma Ca<sup>2+</sup>-ATPase, Na<sup>+</sup>/Ca<sup>2+</sup> exchanger, K<sup>+</sup> channels, and Na<sup>+</sup> channels, can also contribute to the occurrence of arrhythmias (43).

## Atherosclerosis

Animal experiments revealed significant amounts of iron pool in atherosclerotic lesions, which indicates that the iron-catalyzed formation of free radicals may act as the trigger factor in the process and development of



atherosclerosis (44). In human endothelial cells, increased levels of  $[Ca^{2+}]_i$  were observed that could potentially induce oxidative stress and can thus be an additional contributing factor in atherosclerosis progression. Furthermore, up-regulation of cholesterol and the enhanced uptake of oxidized LDL seems to be a key step in the development of atherosclerosis (45). Oxidized LDL accumulates in Mφs and form foam cells, which establishes the initiation of atheroma formation (24). Subsequently, overproduction of ROS facilitates the activation of cells that are involved in atherosclerosis and the formation and progression of lesions (46).

### Ischemia/reperfusion

During an ischemia/reperfusion period, plates of accumulated atheroma reduce the diameter of the vessel and increases rigidity, which leads to occlusion. In the coronary artery, a rupture, obstruction, or vascular occlusion, called stenosis, blocks the distributions of  $O_2$  in the irrigation area by the vessel, which leads to an ischemic event. During ischemia/reperfusion, the cellular source of ROS within heart tissue includes cardiac myocytes, endothelial cells, and neutrophils. Within the cardiac myocytes, ROS can be produced by several sources, such as the mitochondrial respiratory chain, NOX (47), XO (48), and uncoupled NOS. Despite the low oxygen tension during ischemia, moderate ROS generation is thought to occur in the mitochondria (49–51). It has been recognized that mitochondrial complex I takes part in ischemia/reperfusion (52) and, currently, it is known that the major ROS generators in the mitochondria are the ubiquinone–ubiquinol mobile electron carriers, popularly known as coenzyme Q10. Ubiquinone normally accepts electrons from complexes I and III of the electron transport chain and transfers them to complex IV and cytochrome *c*. During an infarct, when oxygen is absent, there is no final electron acceptor for complex IV. Consequently, the ubiquinol pool is highly reduced and increases the level of ubisemiquinone radical (*i.e.*, the reduced form of ubiquinone). When oxygen returns to the mitochondria during reperfusion, ubisemiquinone donates electrons directly to the oxygen-generating  $O_2^-$ . This free radical reacts rapidly with the neighboring molecules, which leads to lipid peroxidation.

After ischemia, the massive burst of ROS during reperfusion originates from a different cellular source, but it has thus far not been identified. Moreover, the massive production of ROS during an ischemia/reperfusion event, in turn, leads to tissue injury that causes serious complications in organ transplantation, stroke, and myocardial infarction (51). The process of ischemia/reperfusion was intensively studied in human myocardial infarction and observed injuries were attributed to ROS released by neutrophil activation (53). In cardiomyocytes and inflammatory cells, NOX2 levels are increased early after acute myocardial infarction in both humans (54) and animal models (55).

NOX activity might also be activated by NADPH, and it has been suggested that increased NADPH levels fuel  $O_2^-$  production in heart failure (56). Numerous studies have investigated the deleterious effects of ischemia/reperfusion-induced ROS production by using various pharmacologic interventions (57). Of note, antioxidant treatment ameliorates both leukocyte adhesion and leukocyte-mediated heart injury in the postischemic period (58). To be complete, treatment with a synthetic SOD mimetic was shown to ameliorate tissue damage in a rat model of ischemia/reperfusion injury (59).

More recently, studies have demonstrated that NOX-derived ROS can promote autophagy (60), with NOX2 and NOX4 representing the isoforms of implicated NOX. NOX2- and NOX4-dependent autophagy plays an important role in the elimination of pathogens by phagocytes and in the regulation of vascular cell and cancer cell survival. Of interest, the authors found that the regulatory role for ROS from NOX2 complexes is also important in autophagy regulation in cardiomyocytes. NOX promotes the activation of autophagy and survival in cardiomyocytes in response to nutrient deprivation and ischemia *via* the protein kinase RNA-like endoplasmic reticulum kinase activation signaling pathway.

### Cardiac hypertrophy

Mounting evidence has strongly implicated ROS signaling in the development of cardiac hypertrophy, which can either be compensatory and adaptive, or a maladaptive precursor to cardiac failure (61–64). Many extracellular factors can induce hypertrophy of cardiomyocytes, and several of the downstream signaling pathways that mediate the hypertrophic growth response to these factors can be activated directly or indirectly by ROS (65). For example, cardiomyocyte hypertrophy induced by GPCR agonists, such as ATII,  $\alpha$ -adrenoceptor agonists, and endothelin-1, has been shown to involve endogenous ROS generation and activation of ERK1/2 and NF- $\kappa$ B (66). ATII induces cardiac hypertrophy *via* a G-protein-linked pathway that involves the generation of ROS and ROS-associated activation of several downstream signals, including MAPKs (67). Of interest, antioxidants were shown to inhibit ROS and block ATII-induced cardiac hypertrophy (68).

TNF- $\alpha$ -induced cardiomyocyte hypertrophy has also been reported to be associated with ROS-dependent activation of NF- $\kappa$ B. In addition, NOX2 has been confirmed to be involved in the cultured cardiomyocyte hypertrophy that is induced by endothelin-1 (69) and ATII (70), which further provides evidence that Akt activation may also be involved. In addition, redox signaling was also implicated in pressure overload-induced cardiac hypertrophy (62). Furthermore, mechanical strain may act as at least one prohypertrophic stimulus during pressure overload and, consistent with NOX2 reports, it has been shown that mechanical stress-induced cardiomyocyte hypertrophy may involve Rac1–ROS-dependent pathways that activate ERK1/2 (64) and p38 MAPK (71).

## Vascular disease

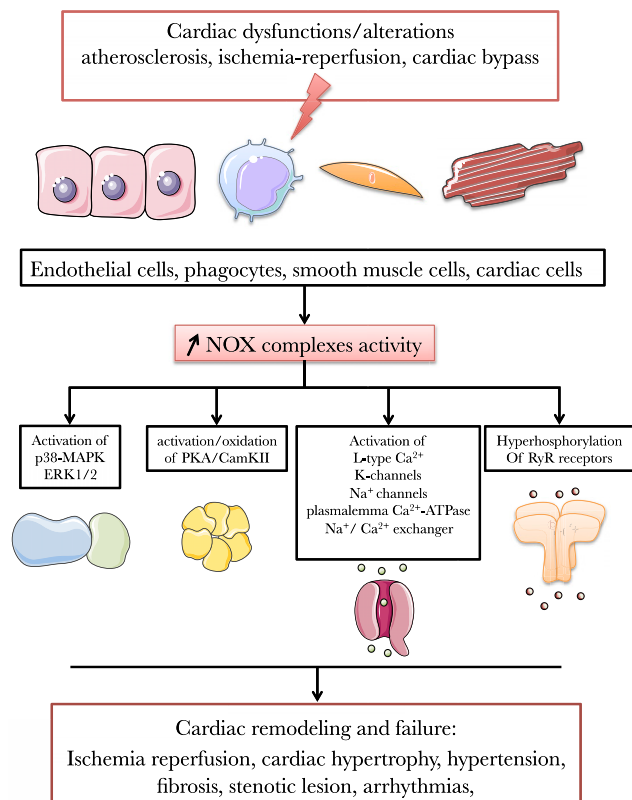
Substantial evidence suggests the involvement of ROS generation in hypertension (72). ROS are generated within endothelial and vascular smooth muscle cells of the vascular wall, as well as by adventitia fibroblasts (73–75). The relationship between ROS and hypertension was suggested by Landmesser *et al.* (76), but it was some 40 yr later that this association was investigated in greater detail when it was demonstrated that ATII-mediated hypertension in rats increased vascular  $O_2^{\cdot -}$  production *via* NOX activation (77).

In the vascular system, ROS production *via* NOX is triggered by stimulation of neuro-humoral vasoconstrictor agents, such as ATII, endothelin-1, and norepinephrine. The action of ATII *via* angiotensin type 1 receptors plays an important role in vasoconstriction (78). Furthermore, enzymatic reduction of molecular oxygen by eNOS no longer couples to L-arginine, which results in the generation of deleterious  $O_2^{\cdot -}$  rather than protective NO (79). This eNOS uncoupling contributes to increase ROS production and endothelial dysfunction that have been observed in various vascular diseases (80), including hypertension (81). Muslin (82) has also shown that increased production of ROS is responsible for the activation of redox-sensitive p38 MAPK, which might be involved in the functional and structural changes that are associated with hypertension. To be complete, it is important to note that hypertension is associated with renal function. In hypertension, nNOS activity is impaired and the effect of NO donors is reduced, which leads to increased tubuloglomerular feedback and decreased renal blood flow and glomerular filtration (83). Overall, these functional changes cooperatively increase blood pressure acutely, whereas long-term hypertension is likely a result of tissue damage and remodeling.

As summarized in Fig. 3, the NOX complex is an important factor that is involved in heart dysfunction. In the short-term, ROS can increase blood pressure by stimulating the heart rate and blood pressure, which may also contribute to chronic hypertension by inducing myocardial hypertrophy. In extreme cases, ROS may be responsible for the emergence of stenotic lesions and fibrosis.

## Other pathologies

Numerous pathologies are known to be dependent on ROS production and alterations and are cataloged in different reviews (22, 84, 85). Most chronic diseases are related to inflammatory rheumatism, inflammatory chronic diseases of the digestive system, bronchopulmonary diseases, skin infection, and chronic viral infections with oxidative stress. Diseases that are associated with ROS include cataract (86), cancer (84, 85), diabetes (87) and insulin resistance (88, 89), obstructive sleep apnea (90), HIV infection (91), asthma (92), psoriasis (93), and chronic granulomatous (94). Moderate levels of ROS have also been observed in neurologic disorders, such as Parkinson's disease (95),



**Figure 3.** Involvement of NOX complex activities in disease progression. Heart dysfunction or an alteration event, such as atherosclerosis, ischemia/reperfusion, myocardial infarction, and cardiac bypass, results in the increased activity of the NOX complex to host cells (endothelial cells, phagocytes, smooth muscle cells, and cardiac cells). This activation induces oxidation, activation, modulation, or phosphorylation for complex proteins (p38-MAPK/ERK1/2), channels (*i.e.*, L-type Ca<sup>2+</sup>, K channels, Na<sup>+</sup> channels, plasmalemma Ca<sup>2+</sup>-ATPase, and Na<sup>+</sup>/Ca<sup>2+</sup> exchanger), receptors (RyR2) and protein kinase (PKA and CaMKII). These chronic actions may lead to progressive cardiac remodeling and failure, such as cardiac hypertrophy, hypertension, fibrosis, stenotic lesion, and arrhythmias.

Alzheimer's disease, Huntington's disease, and in patients with either familial and sporadic amyotrophic lateral sclerosis (96) and schizophrenia (97). Aside from intrinsic causes in oxidative stress, extrinsic causes, such as smoking, alcohol, UVR, pollution, and intensive sport and psychosocial stress, are contributors to human diseases.

Nevertheless, scientific studies that demonstrate a role for oxidative stress in the aging process cannot be neglected. Indeed, multicellular organisms generally undergo qualitative changes with time (aging) that are associated with the progressive degeneration of biological functions, increased susceptibility to diseases, and increased probability of death within a period. This process, known as the theory of aging, may be defined as a progressive decline in the physiological functions of an organism after the reproductive phase of life. Harman (5), in 1956, proposed that free radicals play a role in the ageing process (*i.e.*, the accumulation of oxidized products in the body and the weakening of the antioxidant defense system contribute to aging of

tissues, organs, and organisms). In addition, there are various indirect manifestations of oxidative stress in old age, including lipid peroxidation, DNA oxidation, protein oxidation, and a shift in the redox states of thiol/disulfide redox couples, such as glutathione, cysteine, and albumin. These manifestations suggest that the rate of ROS production per time unit increases with age; however, this conclusion must be tested experimentally.

## PHYSIOLOGICAL IMPLICATIONS OF ROS

ROS are an essential part of many metabolic pathways. In fact, ROS are the spark of basic energy-producing processes. It is evident that oxidative stress takes part in several pathologies; however, numerous studies have highlighted the physiological role of ROS as promoters of natural defenses (22, 84). This may explain, in part, why many intervention studies with chronic antioxidants have failed to show efficacy and positive effects in the prevention of diseases or their complications. There is now a large body of evidence that shows that living organisms have not only adapted to an unfriendly coexistence with ROS but have developed advantageous mechanisms by which these molecules can be used.

With the goal of developing procedures to ameliorate undesirable ROS production for therapy in pathologies, instead, it would be important to identify the molecular effectors of redox biology that are involved in normal biological and physiological responses. By doing so, a new theory about the involvement of cellular oxidants in the maintenance of redox homeostasis to keep normal physiological function should be established. For 2 decades, ROS have undergone a shift from being considered molecules that invoke damage in oxidative stress to regulating signaling pathways that impinge on normal physiological and redox biological responses, as explained below.

### Implication of ROS in skeletal muscle function

In the 1970s, researchers reported for the first time that lipid peroxidation is increased during exercise in humans and rats (98, 99). In the early 1980s, researchers began to understand the biological importance of this finding by identifying the first link between ROS and muscle function (100). Clearly, the idea that ROS are involved in normal muscle contraction dates to the 1990s (101). Reid *et al.* reported, for the first time in muscle, that low levels of ROS that are present in skeletal muscle under basal conditions are a requirement for normal movement and that antioxidant-mediated depletion of ROS from unfatigued skeletal muscle results in the inhibition of their contraction. After this finding, several researchers elucidated this hypothesis in studies on the relationship between ROS production and antioxidants, and proposed that exercise itself can be considered an antioxidant (102). Consequently, ROS that are produced in exercise have a physiological role, and it is conspicuous that they behave as signals to modulate adaptations of muscle to exercise.

### Implication of ROS in excitation-contraction coupling

In cardiac muscle, studies suggest that ROS production has physiological effects in the excitation-contraction coupling protein process. Of note, Sánchez *et al.* (103) provided indirect evidence that during tachycardia, NOX2 contributes to RyR2 redox modifications, such as S-glutathionylation, that can sustain faster Ca<sup>2+</sup> release during increased cardiac activity and in contractile force during exercise. Recently, Prosser *et al.* (104) confirmed these results and reported an important physiological role for acute stretch-induced activation of NOX2 in the mechanotransduction of Ca<sup>2+</sup> release, hence the contractile force of the cardiomyocytes. The authors found that ROS produced by NOX2 are strategically localized to the sarcolemma and T-tubule membranes to permit rapid redox modification of RyR2 and the regulation of cardiac Ca<sup>2+</sup> signaling, thereby tuning RyR2 Ca<sup>2+</sup> signaling sensitivity. This may be an important physiological mechanism involved in the stretch-induced augmentation of contractile activity.

### Impact of ROS in programmed cell death and cancer

Other than muscle exercise, the perception started to change with the assumption that ROS were inevitably deleterious to numerous tissues and the unquestionable beneficial effects of antioxidants. As direct exposure of cells to ROS, such as H<sub>2</sub>O<sub>2</sub>, caused multiple intracellular alterations, including the elevation of cytosolic Ca<sup>2+</sup>, depletion of ATP, oxidation of NADH, and reduction of glutathione, it is evident that ROS contribute to cell death whenever they are generated in the apoptosis process. In addition, studies suggest that increases in cellular ROS production observed in apoptotic processes are triggered by various stimuli, including APO-1/Fas/CD95 ligands (105–107). Even so, the impact of ROS in programmed cell death suggests that they are deleterious and have no positive effects. Whether and how these ROS contribute to the induction of cell death depends on the signaling and execution pathways that are activated (108). The process that leads to proliferation or cell death depends on the condition of the ROS-producing cell. For example, in cancer, ROS production defends the organism by attacking the DNA of the cancer cell, even if it is limited compared with the proliferation of the normal cell (109), and, as mentioned previously, ROS are potential carcinogens, as they facilitate mutagenesis, tumor promotion, and progression (110).

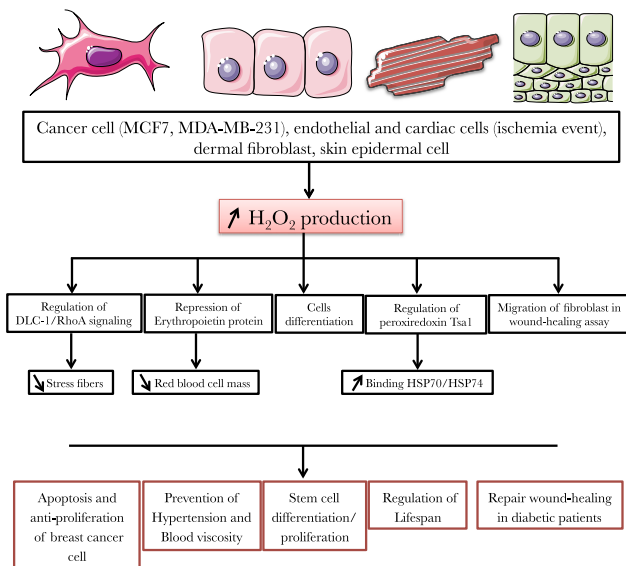
Conversely, Cao *et al.* (111) recently observed that oridonin, a natural diterpenoid that is isolated from an herb (*Rabdosia rubescens*), regulated retinoic acid receptor- $\alpha$  (RAR- $\alpha$ ) and contributed to the pathogenesis of various diseases, including cancer, especially acute promyelocytic leukemia. Of interest, oridonin stabilizes RAR- $\alpha$  protein by increasing cellular ROS levels *via* the activation of the NF- $\kappa$ B signaling pathway. Oridonin increased intracellular ROS levels, whereas pretreatment with the ROS scavenger, N-acetyl-L-cysteine,



dramatically abrogated RAR- $\alpha$  stabilization, which indicates the positive role of ROS in oridonin-induced RAR- $\alpha$  stabilization (111). In addition, Ma *et al.* (112) observed that ROS production ( $H_2O_2$ ) inhibits proliferation and induces apoptosis in MCF-7 breast cancer cells *via* the modulation of cell cycle and apoptosis-related genes, and inhibits migration by decreasing stress fibers *via* DLC1/RhoA signaling. The production and migration of the fibers, which are primarily composed of actin and myosin, were also suppressed by  $H_2O_2$  in a more aggressive breast cancer cell line (MDA-MB-231). These results suggest that that proliferation of breast cancer cells is modulated directly by ROS *via* the modulation gene that encodes for the cellular cycle and apoptosis (112). These two recent findings changed the views of ROS as a deleterious molecule and even assigned it a potential essential role in the diminution of the progression in some cancers (Fig. 4).

### ROS formation as a sensor for changes in oxygen concentration

Oxygen homeostasis is maintained in higher organisms by the tight regulation of red blood cells and *via* respiratory ventilation (113). Growing evidence indicates that an alteration in oxygen concentration is sensed independently by different ROS-producing proteins, including cytochrome *b*. Other studies suggest that a change in the rate of



**Figure 4.** A new role for  $H_2O_2$  signaling. In certain situations, such as an ischemia event or cancer, endothelial cells, cardiac cells, dermal fibroblasts, and skin epidermal cells can increase  $H_2O_2$  production. This activity can induce activation, repression, differentiation, modulation, or migration for protein (erythropoietin and peroxiredoxin), cell (stem cell and fibroblast), and signaling pathways (DLC-1/RhoA). These actions may lead to the regulation of lifespan, a decrease in the proliferation of cancer cells (breast cancer), prevention of hypertension and blood viscosity, an increase in stem-cell differentiation/proliferation, and repair of wound healing in patients with diabetes, with  $H_2O_2$  as the trigger messenger in physiological function.

mitochondrial ROS may play a vital role in oxygen sensing by the carotids in the regulation of arterial blood oxygen (114).

In the event of hypoxia, the hormone erythropoietin, which is mainly produced by kidney and liver cells, regulates the total mass of erythrocytes—defined by red blood cell mass—in circulation. It is clear, that the modification in oxygen tension is sensed by changes in ROS production (115, 116). In addition, the expression of erythropoietin protein or mRNA was found to be strongly repressed by ROS when normoxic cells were treated with catalase *via* the stimulation of erythropoietin (117). This study strongly suggests that ROS are involved in the regulation of red blood cell mass and ventilation. Indeed, during an ischemia event, ROS repress erythropoietin protein, which is also known to increase red blood cell mass, and prevent hypertension and augmentation of blood viscosity (Fig. 4). The mechanism seems to be associated with the transcription factor, hypoxia-inducible factor 1 (HIF-1). When under normoxic conditions, it is rapidly mediated by the  $O_2$ -dependent degradation domain *via* the ubiquitin-proteasome pathway (118). The number of target genes that are activated by HIF-1 continues to increase and includes genes of protein products involved in angiogenesis, energy metabolism, erythropoiesis, cell proliferation and viability, vascular remodeling, and vasomotor responses, indicating the role of ROS in these contexts in response to hypoxia (119).

Urao *et al.* (120) used transgenic mice with endothelial cell-specific overexpression of human catalase and examined whether endogenous ROS in endothelial cells is required for neovascularization after hindlimb ischemia. In this study, they found a significant decrease in the expression of redox-sensitive VCAM-1 and monocyte chemoattractant protein-1, which is required for inflammatory cell recruitment to ischemic tissues (120). Of interest, the researchers also observed a significant decrease in eNOS phosphorylation, which is known as a key regulator of angiogenesis and  $H_2O_2$  to increase eNOS expression. They concluded that ROS, in particular  $H_2O_2$ , are positive effectors in postischemic reparative neovascularization, which aligns with previous reports that NOX2-derived ROS (121), NOX4-derived  $H_2O_2$  (122, 123), or  $H_2O_2$  derived from myeloid cells (124) are required for such a response. In addition, Kim *et al.* (125) showed that angiotensin 1, which is known to play a role in angiogenesis after induction by NO (126), generates  $H_2O_2$  and modulates the activation of p44/42 MAPK and p38 MAPK, thereby playing a critical role in tubule formation, cell migration, and angiogenesis (Fig. 4).

### ROS-mediated amplification of immune responses

For 20 yr, the immune response has been known as a redox-regulated process through the activation of T lymphocytes, which is significantly enhanced by ROS or by a shift in the intracellular glutathione redox state (127). Superoxide and/or physiologically relevant

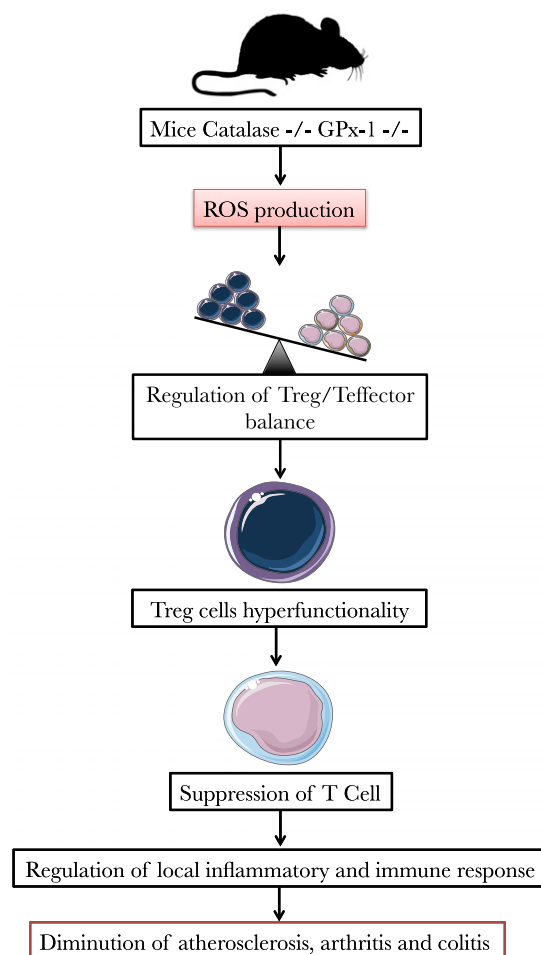
concentrations of  $H_2O_2$  were shown to increase the production of IL-2 in various experimental studies (128). In addition, pharmacologic or genetic manipulation and dampening of the mitochondrial ROS generation can diminish T-cell activation *in vitro* and *in vivo*; however, NOX can be invoked in response to mitochondrial ROS to further sustain ROS levels to maintain T-cell activation (129). One study showed that uncoupling protein 2-knockout mice featured increased levels of mitochondrial ROS and increased immunity to bacterial pathogens (130). These findings suggest a moderate elevation of ROS in the immune system that might enhance normal immune function.

Recently, research has focused on the later stages of the immune response in diseases, which can involve not only the promotion of inflammation, but also its resolution. Among the regulators of inflammation that have garnered attention are regulatory T ( $T_{reg}$ ) cells.  $T_{reg}$  cells are an important subset of T cells that lend themselves to immune tolerance, and data indicate that  $T_{reg}$  cells are relevant, notably, for atherosclerosis. A recent study has suggested a link between ROS and  $T_{reg}$  cells in which the commitment of the  $T_{reg}$  lineage is dependent on localized production of ROS and that scavenging ROS decreased the  $T_{reg}$ /T-cell effector balance. These studies have focused on ROS production that can determine T-cell fate, thereby potentiating  $T_{reg}$  production and decreasing arthritis (131, 132). In 2014, researchers investigated mice with elevated levels of ROS as a result of the deficiency of both GPx-1 and catalase on the  $T_{reg}$  function. The group found that dextran sodium sulfate-induced colitis was attenuated and  $T_{reg}$  cells were hyperfunctional in GPx-1- and catalase-knockout mice. This finding (Fig. 5) suggests that the function of regulatory lymphocytes is closely related to ROS levels and that inflammation may be attenuated appropriately in elevated ROS conditions (133). Taken together, as the endothelium is a major regulator of local inflammatory and immune responses and predominantly secretes ROS into the extracellular space, these new data suggest that endothelial-derived ROS could influence T-cell fate by potentiating  $T_{reg}$  differentiation (Fig. 5).

Moreover, there is evidence that the intracellular redox state also modulates the immunologic functions of Mφs. Hamuro *et al.* (134) reported that Mφs vary strongly in their release of prostaglandins, IL-6, and -12, depending on the intracellular content of glutathione. These data further display a new picture of ROS involvement in the regulation and limitation of inflammatory responses.

### Redox regulation of cell adhesion and migration

Controlled changes in the adhesive properties of cells and tissues play an important role in many biological processes. Cell adhesion plays a substantial role in embryogenesis, cell growth, differentiation, wound repair, and other processes; therefore, changes in the adhesive properties of cells and tissues are suggested to regulate redox tightly (135). Expression of cell adhesion molecules



**Figure 5.** Regulation of inflammatory and immune response by production of ROS. ROS production can be induced genetically in mice without catalase and GPx protein (catalase<sup>-/-</sup> GPx<sup>-/-</sup>). The production of ROS regulates  $T_{reg}$ /T effector cell balance, which induces hyperfunctionality of  $T_{reg}$  cells.  $T_{reg}$  cells suppress T cells, which induces the regulation of the local inflammatory and immune response. For this transgenic mouse model, ROS production is an important effector limiting inflammatory responses that can regulate the development of atherosclerosis, arthritis, and colitis.

is stimulated by bacterial LPS and by various cytokines, such as TNF- $\alpha$  or IL-1 (136). ROS-treated endothelial cells induce the phosphorylation of the focal adhesion kinase, pp125FAK, a cytosolic tyrosine kinase that has been implicated in the oxidant-mediated adhesion process (137). Adhesion of leukocytes to endothelial cells is also enhanced by ROS (2.5-fold increase compared with control) (138). Of interest, this adherence was independent of the XO concentration and abolished by catalase but not by SOD, which suggests that ROS are the effective agents.

Recently, Falanga *et al.* (139) studied the influence of ROS production on cell migration by using a wound-healing assay, notably in patients with diabetes for whom the healing process is slow and impaired. The authors investigated the effect of the high-glucose environment and basic fibroblast growth factor (bFGF) on human dermal fibroblast migration. It is known that bFGFs multiply and play critical roles in the wound-healing process, and that a

decrease in expression may disrupt the normal healing process in patients with diabetes (140). They found that bFGF significantly increased the migration of fibroblasts simultaneously with an increase in intracellular ROS (141). The study indicated ROS production (initiated by H<sub>2</sub>O<sub>2</sub>) as the major element to induce the migration of fibroblast *via* bFGFs in the presence of high-level glucose, which is essential for the wound-healing process in patients with diabetes (Fig. 4).

More recently, Chandrasekaran *et al.* (142) hypothesized that ROS-mediated signaling is linked to bone morphogenetic protein (BMP) receptor activation to dendritic growth. In cultures of rat sympathetic neurons that were exposed to different antioxidants, BMP-induced dendritic growth was blocked in a concentration-dependent manner without altering axonal growth or neuronal cell survival (142). In addition, BMPs up-regulated the expression of NOX2 in different cell types, and small interfering RNA knockdown of NOX2, but not NOX4, significantly decreased BMP7-induced dendritic growth. Collectively, these data support the hypothesis that ROS are involved in downstream signaling events that mediate BMP7-induced dendritic growth in sympathetic neurons, and suggest that ROS-mediated signaling positively modulates dendritic complexity in peripheral neurons.

### Implication of ROS in stem-cell differentiation

ROS are also essential for stem-cell differentiation. Stem cells need to self-renew to maintain both the stem-cell pool and to differentiate to generate specialized tissues. The best-studied example of stem-cell characterization is the hematopoietic stem cell, which differentiates to provide myeloid and lymphoid progenitors throughout a lifespan. Juntilla *et al.* (143), observed that mouse hematopoietic stem cells that are deficient in proteins involved in signal transduction pathways (both AKT1 and AKT2) have reduced levels of ROS and impaired differentiation. Furthermore, numerous studies seem to indicate the role of ROS in differentiation processes. Owusu-Ansah and Banerjee (144) found that ROS triggered differentiation, whereas decreasing ROS impaired differentiation, in *Drosophila* hematopoietic progenitors. In 2012, Malinska *et al.* (145) observed that ROS synthesis in mitochondria by complex I can trigger muscle differentiation of human bone marrow mesenchymal stem cells. In epidermis, Hamanaka *et al.* (146) demonstrated that lowering mitochondrial ROS prevented differentiation in this cell process and, surprisingly, that it can be restored by supplementing with exogenous H<sub>2</sub>O<sub>2</sub>.

Similar observations were made of the regenerative capacity of spermatogonial and neural stem cells. The authors observed that spermatogonial stem cells that were depleted in ROS stopped proliferation but enhanced self-renewal when ROS levels were increased, and also induced the phosphorylation of stress kinases, p38 MAPK and JNK (147). As a follow-up to this report, the authors investigated ROS function in primary brain-derived

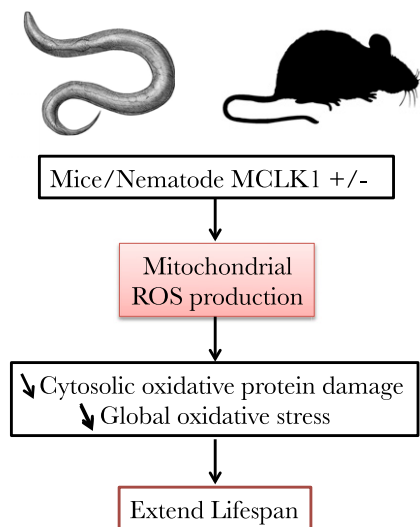
neural progenitors. They discovered that pharmacologic and genetic manipulations that diminished cellular ROS levels interfered with normal neural stem cells and/or multipotent progenitor functions both *in vitro* and *in vivo*. This study identified a redox-mediated regulatory mechanism of neural stem cells function, which may have significant implications for brain injury, disease, and repair (148).

These data indicate that the generation of low levels of ROS are physiologically required to activate proliferative pathways, serving as a trigger signal to support stem-cell proliferation. In contrast, high levels of ROS impair stem-cell function by activating signaling pathways that limit self-renewal but do not necessarily cause cellular damage.

### Implication of ROS in the regulation of aging

It is well understood that the production of ROS is enhanced during aging, but even if researchers attempted intervention studies for the reduction of ROS levels, the outcome is rather mixed and it is not clear whether ROS-induced damage is the underlying cause of aging (149). On the contrary, recent evidence suggests that ROS signaling is required for the maintenance of tissues, and that ROS elevation can activate cellular stress pathways to dampen tissue degeneration and promote healthy aging (150). The initial studies to support the theory of aging—the deleterious effects of ROS—comes from the observation that hypoxia increased the replication of human diploid fibroblasts with the lifespan (151). During hypoxia, ROS decrease, consequently leading to less accumulation of oxidative damage to increase the replication of the human fibroblast lifespan, but later studies have demonstrated that an augmentation of ROS production during hypoxia resulted in the activation of HIF to increase the lifespan (152). After this first contradiction of the theory of aging, Van Raamsdonk and Hekimi (153) observed that the deletion of SOD in mouse mitochondrial matrix elevated mitochondrial DNA damage, as well as cancer incidence, but did not accelerate aging and, instead, extended lifespan (154). Extended lifespan as a result of increased mitochondrial ROS seems to be dependent on glucose restriction (155), mitochondrial electron transport mutation (156) and diminished IGF signaling (157). At the protein level, the long-lived mitochondrial mutant in *Caenorhabditis elegans* seems to increase the replicative lifespan by ROS-dependent activation of HIF (156).

In other animal models (nematodes and mice), reduced activity of MCLK1 (heterozygous COQ7), a mitochondrial enzyme that is required for ubiquinone biosynthesis, was observed to have increased lifespan and mitochondrial ROS (158). Mice with long life were associated with less oxidative damage in the cytosolic proteins, which supports the idea that elevated ROS levels are paradoxically protective *via* the induction of stress pathways (159). The recent data in *C. elegans* and mouse models suggest the physiological role of ROS in the lifespan (Fig. 6). More recently, Hanzén *et al.* (160) reported a new concept in lifespan-protein quality control. The study revealed the role of peroxiredoxin,



**Figure 6.** Regulation of lifespan by ROS production. ROS production can be induced genetically in mice and nematodes by reduction of MCLK1, a mitochondrial enzyme that is required for ubiquinone biosynthesis (MCLK1<sup>+/-</sup>). This diminution can increase mitochondrial ROS production. Animal models show the lifespan to be associated with less oxidative damage to cytosolic proteins.

Tsa1 (the cytosolic peroxiredoxin in yeast), that facilitated the binding of Hsp70/104 chaperones to damaged proteins that were formed with aging *via* H<sub>2</sub>O<sub>2</sub>-specific redox switch in the Tsa1 peroxidatic cysteine (160). These data showed conceptually the new role for H<sub>2</sub>O<sub>2</sub> signaling in proteostasis and lifespan control (Fig. 4).

### Central role of ROS in lipid metabolism

Among the targets of ROS, unsaturated fatty acids, without doubt, are most attacked because of the abundance of double bonds in their structure. Unsaturated fatty acids are highly susceptible to abstract hydrogen atoms of the methylene (CH<sub>2</sub>) group by ·OH and potentially initiate peroxidation. For several reasons, oxidation of lipids by ROS has been largely ignored by investigators, and the effects of the nonenzymatic products of these lipids remain largely unexplored. The reasons for this paucity of investigation could be a result of the rate of lipid oxidation *via* free radicals *in vivo*, which previously was thought to be negligible, or of the previously held idea that any form of lipid peroxidation is undesirable, as it is unconditionally toxic. Of the nonenzymatic oxygenated metabolites investigated, isoprostanes, namely 15-F<sub>2t</sub>-isoprostanes (15-F<sub>2t</sub>-IsoPs) from arachidonic acid [ $\omega$ -6 polyunsaturated fatty acid (PUFA)], are commonly used as biomarkers of lipid peroxidation *in vivo* (161, 162). More recently, it has been shown that they are biologically active (163) as mediators of oxidant injury. They are vasoconstrictors in many species, and in various vascular beds (164) modulate platelet activity (165) and monocyte adhesion (166), as well as induce proliferation of endothelial and smooth muscle cells (167). In addition, oxidative stress is a

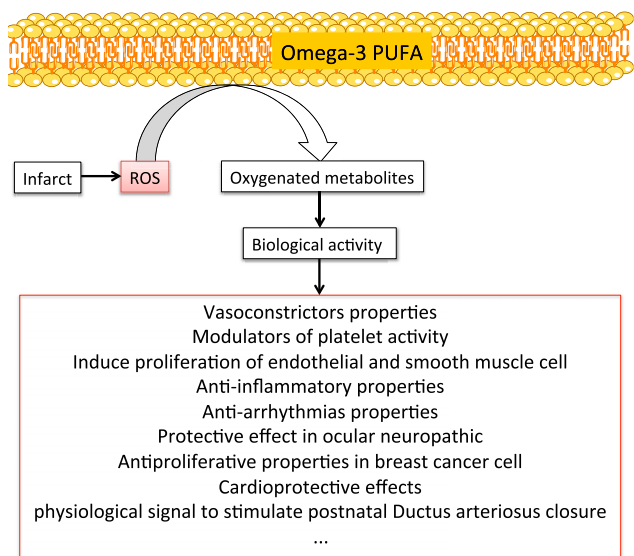
feature of numerous pathologic conditions that occur in the perinatal period. For instance, newborns are subjected to oxidative stress that results from the rapid transition from a low-oxygen environment *in utero* to a relatively high-oxygen environment at birth. In 2012, Comporti *et al.* (168–170) showed that isoprostane levels are increased shortly after birth in response to increased oxygen tension mediated *via* activation of the thromboxane A<sub>2</sub> receptor and that isoprostane may serve as a novel physiological signal to stimulate postnatal ductus arteriosus closure.

As for  $\omega$ -3 PUFA oxidation by ROS, Sethi's group (171) demonstrated, for the first time, that the metabolites generated could contribute to anti-inflammatory activities. The researchers showed that preincubation of endothelial cells with oxidized  $\omega$ -3 PUFAs reduced the adhesion of monocytic cells to endothelial cells, but native  $\omega$ -3 PUFAs had no effect. The researchers hypothesized that the reduced expression of adhesion molecules, such as VCAM-1, by endothelial cells decreased the interaction of phagocytes *via* the action of anti-inflammatory properties of the oxidized  $\omega$ -3 PUFAs. In 2014, Jamil *et al.* (172) investigated the role of oxygenated metabolites of eicosapentaenoic acid from nonenzymatic oxidation, namely 5-F<sub>3t</sub>-IsoPs, in the regulation of glutamatergic neurotransmission. In this study, the group revealed the beneficial role of this compound by reducing excitatory neurotransmitter release, thereby slowing the progression of ocular neuropathic disease by modulating K<sup>+</sup>-induced glutamate release by 5-epi-5-F<sub>3t</sub>-IsoP in isolated bovine retina (172).

Recently, Roy *et al.*, showed that the oxidation of  $\omega$ -3 PUFAs by ROS releases 4(RS)-4-F<sub>4t</sub>-NeuroP from docosahexaenoic acid, which is necessary to prevent isoproterenol-induced arrhythmias in mice with myocardial infarction (173), prevent early arrhythmias in rats after an ischemia/reperfusion period (174), or prevent breast cancer proliferation (175). As previously observed in different oxidative conditions (176), the researchers proposed that in oxidative stress conditions, such as ischemic events, 4(RS)-4-F<sub>4t</sub>-NeuroP is responsible for the antiarrhythmic properties of  $\omega$ -3 PUFAs by countering the cellular stress by ROS. They demonstrated that 4(RS)-4-F<sub>4t</sub>-NeuroP could mediate the cardioprotective effect of  $\omega$ -3 PUFAs by stabilizing the RyR2 complex (177). This discovery created a new perspective on products of nonenzymatic oxygenated metabolites of fatty acids as potent mediators in diseases that involve ROS production as a trigger factor. Overall, effects of nonenzymatic metabolites of  $\omega$ -3 and  $\omega$ -6 PUFAs by ROS oxidation are described in Roy *et al.* (178). These findings are relevant to the potential link between oxidative stress and the physiological role of peroxidation in lipids (Fig. 7).

In addition to unsaturated lipids of  $\omega$ -3 and  $\omega$ -6 PUFAs, antioxidant properties of cholesterol were noticed when the double-binding ligand became oxidized (179). In particular, the cholesterol-containing compounds, epoxide-bearing substances (produced by autoxidation *via* nonenzymatic mechanisms), are unstable because of the high





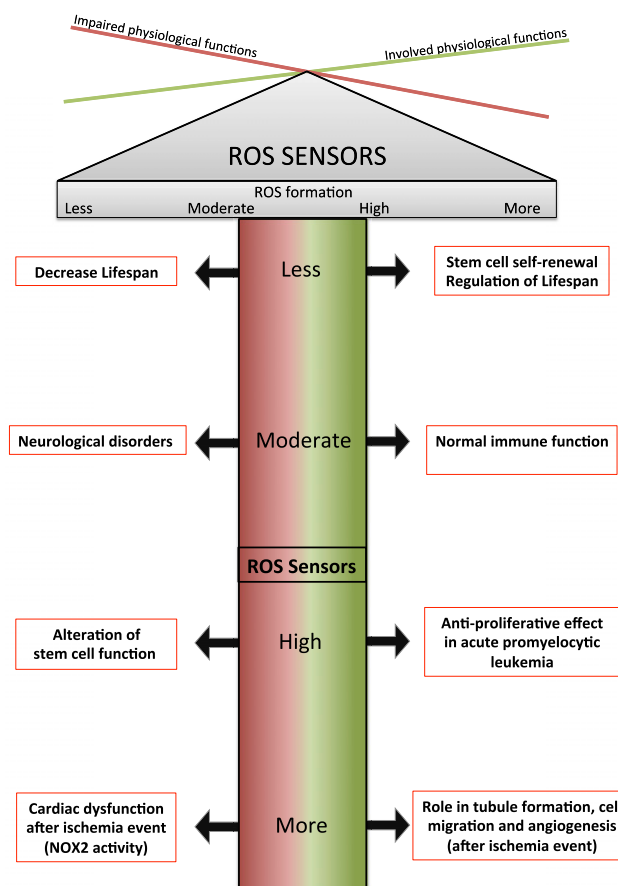
**Figure 7.** Physiological function of oxygenated metabolites of PUFAs. Under intense oxidative stress (an ischemia/reperfusion event), ROS can induce lipoperoxidation of PUFAs. This nonenzymatic oxidation induces the synthesis of oxygenated metabolites, which are biologically active. Oxygenated metabolites of PUFAs may exert vasoconstrictive properties, modulate platelet activity and the proliferation of endothelial and smooth muscle cells, and show that anti-inflammatory, antiarrhythmic, and antiproliferative properties have an effect on ocular neuropathic, cardioprotective, and physiological signals to stimulate postnatal ductus arteriosus closure.

reactivity of the epoxide ring toward nucleophiles, including amines, thiols, and the hydroxyl group, thus exhibiting antioxidant properties. Researchers showed that these oxidation products, such as in 5,6-epoxysterols, could be obtained *via* a nucleophilic substitution mechanism from cholesterol. Of interest, additional evidence points to the existence of active metabolites of cholesterol, namely, oxysterols 5,6a-EC. In 2013, researchers demonstrated an important role in carcinogenesis for dendrogenin (DDA), a natural metabolite in mammals that results from the enzymatic conjugation of 5,6 $\alpha$ -EC with histamine (180). This oxysterol of DDA was not detected in cancer cell lines and was 5-fold lower in human breast tumors compared with normal tissues, which suggests dysregulation of DDA metabolism during carcinogenesis. De Medina *et al.* (181) established that DDA is a selective inhibitor of cholesterol epoxide hydrolase that can trigger tumor redifferentiation and growth control in mice as well as improved animal survival. The properties of DDA and its decreased level in tumors suggests important physiological functions in maintaining cell integrity, differentiation, and, possibly, immune system surveillance.

## CONCLUSIONS

Although the discovery of free radicals raised several interests, it was not until 50–60 yr later that the existence of free radicals in living organisms was demonstrated

and their responsibility for the theory of aging was suggested (4, 5, 7). The balance between the oxidative compounds that are derived from molecular oxygen and the antioxidant defenses in the body gives rise to a subtle harmony that allows ROS to exercise their physiological role without causing collateral damage to cells. This balance can be broken, for example, after an intense physical effort, which causes, then, cellular damage as a result of ROS, but the extent of damage depends on the nature of the ROS and its place of production. Even if it remains difficult to demonstrate the implication of direct or indirect oxidative stress in numerous pathologies, as described in this review, it is indisputable and collectively admitted that ROS play a fundamental role in numerous situations as ROS sensors (Fig. 8). However, the link between oxidative stress and pathologies is complex to determine. Of note, it is difficult to know whether oxidative stress is the origin or the consequence of the pathologies to which it is



**Figure 8.** Involvement of ROS sensors. In numerous situations, it has been proposed that ROS formation at diverse levels (less, moderate, high, more) and to the same degree may take part in physiological functions but can also impair physiological functions. During ischemia/reperfusion, a burst of ROS can induce cardiac dysfunction (by NOX2 activity), but in other cases, is potentially responsible for tubule formation, cell migration, and angiogenesis. In the same physiological situation, during aging, ROS seem to regulate and decrease lifespan. Collectively, new data suggest that ROS can be considered to be ROS sensors.



bound. In addition, as explained in this review and depicted in Fig. 8, the level of ROS formation can be in different situations, in same (theory of aging) situation, or in an impaired physiological situation.

Addressing the regulatory role of ROS is certainly methodologically complex and not easily applicable to field studies because of the intrinsic properties of ROS and because the technical tools that have been developed to this point are neither standardized nor optimized for daily use, which would be of interest in preventive medicine. Yet research on chemicals with pro-oxidant activity increased dramatically, and, presently, the study of oxidative stress in physiology and redox status regulation has gained an important role in medicine, biochemistry, physiology, pharmacology, ecotoxicology, and, more recently, in the evolution of ecology. ROS work as redox messengers in regulatory processes in which the signal is delivered *via* redox chemistry (84, 182). The organism's response to a social or nonsocial environmental stimulus depends on a cascade of processes, starting from the perception of the stimulus to its translation into hormonal secretions, which, in turn, regulate the response itself. For example, research in the area of behavioral endocrinology has contributed to the identification of several mechanisms that regulate the extent and rate at which organisms respond to environmental influences (183). Because of these signaling properties in cell communication, ROS might also be important regulators of the way organisms respond to their environment. As such, the number of publications that involve ROS has increased over the last 10 yr (~10,000 articles published in 2005 compared with ~25,000 in 2015). FJ

## ACKNOWLEDGMENTS

J.R. was supported by the Fondation pour la Recherche Médicale (FRM 40199). The authors declare no conflicts of interest.

## AUTHOR CONTRIBUTIONS

J. Roy, J.-M. Galano, T. Durand, J. C.-Y. Lee, and J.-Y. Le Guennec designed the review (parts) and wrote the paper; and J. Roy created all figures.

## REFERENCES

- Genestra, M. (2007) Oxyl radicals, redox-sensitive signalling cascades and antioxidants. *Cell. Signal.* **19**, 1807–1819
- Sen, C. K. (1995) Oxidants and antioxidants in exercise. *J. Appl. Physiol.* **79**, 675–686
- Yu, B. P. (1994) Cellular defenses against damage from reactive oxygen species. *Physiol. Rev.* **74**, 139–162
- Commoner, B., Townsend, J., and Pake, G. E. (1954) Free radicals in biological materials. *Nature* **174**, 689–691
- Harman, D. (1956) Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* **11**, 298–300
- Harman, D. (1981) The aging process. *Proc. Natl. Acad. Sci. USA* **78**, 7124–7128
- McCord, J. M., and Fridovich, I. (1969) Superoxide dismutase. An enzymic function for erythrocyte (hemocuprein). *J. Biol. Chem.* **244**, 6049–6055
- Lin, Y. J., Seroude, L., and Benzer, S. (1998) Extended life-span and stress resistance in the *Drosophila* mutant methuselah. *Science* **282**, 943–946
- Bae, Y. S., Sung, J. Y., Kim, O. S., Kim, Y. J., Hur, K. C., Kazlauskas, A., and Rhee, S. G. (2000) Platelet-derived growth factor-induced H<sub>2</sub>O<sub>2</sub> production requires the activation of phosphatidylinositol 3-kinase. *J. Biol. Chem.* **275**, 10527–10531
- Sies, H., and Chance, B. (1970) The steady state level of catalase compound I in isolated hemoglobin-free perfused rat liver. *FEBS Lett.* **11**, 172–176
- Chance, B., and Oshino, N. (1971) Kinetics and mechanisms of catalase in peroxisomes of the mitochondrial fraction. *Biochem. J.* **122**, 225–233
- Beckman, K. B., and Ames, B. N. (1998) The free radical theory of aging matures. *Physiol. Rev.* **78**, 547–581
- Finkel, T., and Holbrook, N. J. (2000) Oxidants, oxidative stress and the biology of ageing. *Nature* **408**, 239–247
- Balaban, R. S., Nemoto, S., and Finkel, T. (2005) Mitochondria, oxidants, and aging. *Cell* **120**, 483–495
- Dizdaroglu, M. (1992) Oxidative damage to DNA in mammalian chromatin. *Mutat. Res.* **275**, 331–342
- Davies, K. J. (1987) Protein damage and degradation by oxygen radicals. I. General aspects. *J. Biol. Chem.* **262**, 9895–9901
- Burton, K. P., Morris, A. C., Massey, K. D., Buja, L. M., and Hagler, H. K. (1990) Free radicals alter ionic calcium levels and membrane phospholipids in cultured rat ventricular myocytes. *J. Mol. Cell. Cardiol.* **22**, 1035–1047
- Halliwell, B., and Gutteridge, J. M. C. (1989) *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford
- Höhn, A., König, J., and Grune, T. (2013) Protein oxidation in aging and the removal of oxidized proteins. *J. Proteomics* **92**, 132–159
- Sies, H. (1997) Oxidative stress: oxidants and antioxidants. *Exp. Physiol.* **82**, 291–295
- Jones, D. P. (2006) Redefining oxidative stress. *Antioxid. Redox Signal.* **8**, 1865–1879
- Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., and Telser, J. (2007) Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* **39**, 44–84
- Zazzo, J. F. (2002) Stress oxydant au cours des états inflammatoires aigus et des états d'agression: implications pour la pratique Clinique [in French]. *Nutrition clinique et métabolique.* **16**, 268–274
- Babior, B. M. (2000) Phagocytes and oxidative stress. *Am. J. Med.* **109**, 33–44
- Entman, M. L., and Ballantyne, C. M. (1993) Inflammation in acute coronary syndromes. *Circulation* **88**, 800–803
- Babior, B. M. (1994) Activation of the respiratory burst oxidase. *Environ. Health Perspect.* **102** (Suppl 10), 53–56
- Morel, N., Morel, O., Delabranche, X., Jesel, L., Sztark, F., Dabadie, P., Freyssinet, J.-M., and Toti, F. (2006) Microparticles during sepsis and trauma. A link between inflammation and thrombotic processes [in French]. *Ann. Fr. Anesth. Reanim.* **25**, 955–966
- Reimund, J.-M. (2002) Stress oxydant au cours des syndromes inflammatoires chroniques [in French]. *Nutr. Clin. Metab.* **16**, 275–284
- Klebanoff, S. J. (2005) Myeloperoxidase: friend and foe. *J. Leukoc. Biol.* **77**, 598–625
- Beauvillain, C., Jeannin, P., Delneste, Y., Renier, G., Subra, J.-F., and Chevallier, A. (2012) Autoanticorps anticytoplasme des polynucléaires neutrophiles (ANCA): cibles antigéniques, méthodes diagnostiques. *EMC-Biol. Med. (Paris)* **7**, 1–14
- Maneesh, M., Jayalekshmi, H., Suma, T., Chatterjee, S., Chakrabarti, A., and Singh, T. A. (2005) Evidence for oxidative stress in osteoarthritis. *Indian J. Clin. Biochem.* **20**, 129–130
- Bauerová, K., and Bezek, A. (1999) Role of reactive oxygen and nitrogen species in etiopathogenesis of rheumatoid arthritis. *Gen. Physiol. Biophys.* **18**, 15–20
- Maurice, M. M., Nakamura, H., van der Voort, E. A., van Vliet, A. I., Staal, F. J., Tak, P. P., Breedveld, F. C., and Verweij, C. L. (1997) Evidence for the role of an altered redox state in hyporesponsiveness of synovial T cells in rheumatoid arthritis. *J. Immunol.* **158**, 1458–1465
- Cunnane, G., Fitzgerald, O., Beeton, C., Cawston, T. E., and Bresnihan, B. (2001) Early joint erosions and serum levels of matrix metalloproteinase 1, matrix metalloproteinase 3, and tissue inhibitor of metalloproteinases 1 in rheumatoid arthritis. *Arthritis Rheum.* **44**, 2263–2274

35. Tak, P. P., Zvaifler, N. J., Green, D. R., and Firestein, G. S. (2000) Rheumatoid arthritis and p53: how oxidative stress might alter the course of inflammatory diseases. *Immunol. Today* **21**, 78–82
36. Dhalla, N. S., Temsah, R. M., and Netticadan, T. (2000) Role of oxidative stress in cardiovascular diseases. *J. Hypertens.* **18**, 655–673
37. Kukreja, R. C., and Hess, M. L. (1992) The oxygen free radical system: from equations through membrane-protein interactions to cardiovascular injury and protection. *Cardiovasc. Res.* **26**, 641–655
38. Zima, A. V., and Blatter, L. A. (2006) Redox regulation of cardiac calcium channels and transporters. *Cardiovasc. Res.* **71**, 310–321
39. Sag, C. M., Köhler, A. C., Anderson, M. E., Backs, J., and Maier, L. S. (2011) CaMKII-dependent SR Ca leak contributes to doxorubicin-induced impaired Ca handling in isolated cardiac myocytes. *J. Mol. Cell. Cardiol.* **51**, 749–759
40. Gonzalez, D. R., Beigi, F., Treuer, A. V., and Hare, J. M. (2007) Deficient ryanodine receptor S-nitrosylation increases sarcoplasmic reticulum calcium leak and arrhythmogenesis in cardiomyocytes. *Proc. Natl. Acad. Sci. USA* **104**, 20612–20617
41. Kim, Y. M., Guzik, T. J., Zhang, Y. H., Zhang, M. H., Kattach, H., Ratnatunga, C., Pillai, R., Channon, K. M., and Casadei, B. (2005) A myocardial Nox2 containing NAD(P)H oxidase contributes to oxidative stress in human atrial fibrillation. *Circ. Res.* **97**, 629–636
42. Swaminathan, P. D., Purohit, A., Soni, S., Voigt, N., Singh, M. V., Glukhov, A. V., Gao, Z., He, B. J., Luczak, E. D., Joiner, M. L., Kutschke, W., Yang, J., Donahue, J. K., Weiss, R. M., Grumbach, I. M., Ogawa, M., Chen, P.-S., Efimov, I., Dobrev, D., Mohler, P. J., Hund, T. J., and Anderson, M. E. (2011) Oxidized CaMKII causes cardiac sinus node dysfunction in mice. *J. Clin. Invest.* **121**, 3277–3288
43. Santos, C. X. C., Anilkumar, N., Zhang, M., Brewer, A. C., and Shah, A. M. (2011) Redox signaling in cardiac myocytes. *Free Radic. Biol. Med.* **50**, 777–793
44. Yuan, X.-M., and Li, W. (2003) The iron hypothesis of atherosclerosis and its clinical impact. *Ann. Med.* **35**, 578–591
45. Podrez, E. A., Abu-Soud, H. M., and Hazen, S. L. (2000) Myeloperoxidase-generated oxidants and atherosclerosis. *Free Radic. Biol. Med.* **28**, 1717–1725
46. Beaudoux, J. L., Delattre, J., Therond, P., Bonnefont-rousset, D., Legrand, A., and Peynet, J. (2006) Le stress oxydant, composante physiopathologique de l'athérosclérose [in French]. *Immunol. Biol. Spec.* **21**, 144–150
47. Griendling, K. K., Sorescu, D., and Ushio-Fukai, M. (2000) NAD(P) H oxidase: role in cardiovascular biology and disease. *Circ. Res.* **86**, 494–501
48. Berry, C. E., and Hare, J. M. (2004) Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. *J. Physiol.* **555**, 589–606
49. Lombardi, V., Valko, L., Stolic, S., Valko, M., Ondrejicková, O., Horáková, L., Placek, J., and Troncone, A. (1998) Free radicals in rabbit spinal cord ischemia: electron spin resonance spectroscopy and correlation with SOD activity. *Cell. Mol. Neurobiol.* **18**, 399–412
50. Becker, L. B. (2004) New concepts in reactive oxygen species and cardiovascular reperfusion physiology. *Cardiovasc. Res.* **61**, 461–470
51. Kasparová, S., Brezová, V., Valko, M., Horecký, J., Mlynárik, V., Liptaj, T., Vancová, O., Ulicná, O., and Dobrota, D. (2005) Study of the oxidative stress in a rat model of chronic brain hypoperfusion. *Neurochem. Int.* **46**, 601–611
52. González-Flecha, B., Cutrin, J. C., and Boveris, A. (1993) Time course and mechanism of oxidative stress and tissue damage in rat liver subjected to *in vivo* ischemia-reperfusion. *J. Clin. Invest.* **91**, 456–464
53. Thiagarajan, R. R., Winn, R. K., and Harlan, J. M. (1997) The role of leukocyte and endothelial adhesion molecules in ischemia-reperfusion injury. *Thromb. Haemost.* **78**, 310–314
54. Krijnen, P. A., Meischl, C., Hack, C. E., Meijer, C. J. L. M., Visser, C. A., Roos, D., and Niessen, H. W. M. (2003) Increased Nox2 expression in human cardiomyocytes after acute myocardial infarction. *J. Clin. Pathol.* **56**, 194–199
55. Raha, M., Kimura, S., Yoneyama, H., Kosaka, H., Nishiyama, A., Fukui, T., and Abe, Y. (2001) Effects of furosemide on the tubular reabsorption of nitrates in anesthetized dogs. *Eur. J. Pharmacol.* **428**, 113–119
56. Gupte, S. A., Okada, T., McMurtry, I. F., and Oka, M. (2006) Role of pentose phosphate pathway-derived NADPH in hypoxic pulmonary vasoconstriction. *Pulm. Pharmacol. Ther.* **19**, 303–309
57. Chen, Z., Siu, B., Ho, Y. S., Vincent, R., Chua, C. C., Hamdy, R. C., and Chua, B. H. (1998) Overexpression of MnSOD protects against myocardial ischemia/reperfusion injury in transgenic mice. *J. Mol. Cell. Cardiol.* **30**, 2281–2289
58. Serrano, C. V., Jr., Mikhail, E. A., Wang, P., Noble, B., Kuppusamy, P., and Zweier, J. L. (1996) Superoxide and hydrogen peroxide induce CD18-mediated adhesion in the postischemic heart. *Biochim. Biophys. Acta* **1316**, 191–202
59. Salvemini, D., Wang, Z. Q., Zweier, J. L., Samouilov, A., Macarthur, H., Misko, T. P., Currie, M. G., Cuzzocrea, S., Sikorski, J. A., and Riley, D. P. (1999) A nonpeptidyl mimic of superoxide dismutase with therapeutic activity in rats. *Science* **286**, 304–306
60. Sciarretta, S., Yee, D., Ammann, P., Nagarajan, N., Volpe, M., Frati, G., and Sadoshima, J. (2015) Role of NADPH oxidase in the regulation of autophagy in cardiomyocytes. *Clin. Sci.* **128**, 387–403
61. Li, J.-M., Gall, N. P., Grieve, D. J., Chen, M., and Shah, A. M. (2002) Activation of NADPH oxidase during progression of cardiac hypertrophy to failure. *Hypertension* **40**, 477–484
62. Date, M. O., Morita, T., Yamashita, N., Nishida, K., Yamaguchi, O., Higuchi, Y., Hirotsu, S., Matsumura, Y., Hori, M., Tada, M., and Otsu, K. (2002) The antioxidant N<sup>2</sup>-mercaptopropionyl glycine attenuates left ventricular hypertrophy in *in vivo* murine pressure-overload model. *J. Am. Coll. Cardiol.* **39**, 907–912
63. Higuchi, Y., Otsu, K., Nishida, K., Hirotsu, S., Nakayama, H., Yamaguchi, O., Matsumura, Y., Ueno, H., Tada, M., and Hori, M. (2002) Involvement of reactive oxygen species-mediated NF-kappa B activation in TNF-alpha-induced cardiomyocyte hypertrophy. *J. Mol. Cell. Cardiol.* **34**, 233–240
64. Pimentel, D. R., Amin, J. K., Xiao, L., Miller, T., Viereck, J., Oliver-Krasinski, J., Baliga, R., Wang, J., Siwik, D. A., Singh, K., Pagano, P., Colucci, W. S., and Sawyer, D. B. (2001) Reactive oxygen species mediate amplitude-dependent hypertrophic and apoptotic responses to mechanical stretch in cardiac myocytes. *Circ. Res.* **89**, 453–460
65. Sabri, A., Hughie, H. H., and Lucchesia, P. A. (2003) Regulation of hypertrophic and apoptotic signaling pathways by reactive oxygen species in cardiac myocytes. *Antioxid. Redox Signal.* **5**, 731–740
66. Hirotsu, S., Otsu, K., Nishida, K., Higuchi, Y., Morita, T., Nakayama, H., Yamaguchi, O., Mano, T., Matsumura, Y., Ueno, H., Tada, M., and Hori, M. (2002) Involvement of nuclear factor-kappaB and apoptosis signal-regulating kinase 1 in G-protein-coupled receptor agonist-induced cardiomyocyte hypertrophy. *Circulation* **105**, 509–515
67. Nakamura, K., Fushimi, K., Kouchi, H., Mihara, K., Miyazaki, M., Ohe, T., and Namba, M. (1998) Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor-alpha and angiotensin II. *Circulation* **98**, 794–799
68. Delbosc, S., Cristol, J.-P., Descomps, B., Mirman, A., and Jover, B. (2002) Simvastatin prevents angiotensin II-induced cardiac alteration and oxidative stress. *Hypertension* **40**, 142–147
69. Tanaka, K., Honda, M., and Takabatake, T. (2001) Redox regulation of MAPK pathways and cardiac hypertrophy in adult rat cardiac myocyte. *J. Am. Coll. Cardiol.* **37**, 676–685
70. Hingtgen, S. D., Tian, X., Yang, J., Dunlay, S. M., Peek, A. S., Wu, Y., Sharma, R. V., Engelhardt, J. F., and Davisson, R. L. (2006) Nox2-containing NADPH oxidase and Akt activation play a key role in angiotensin II-induced cardiomyocyte hypertrophy. *Physiol. Genomics* **26**, 180–191
71. Aikawa, R., Nagai, T., Tanaka, M., Zou, Y., Ishihara, T., Takano, H., Hasegawa, H., Akazawa, H., Mizukami, M., Nagai, R., and Komuro, I. (2001) Reactive oxygen species in mechanical stress-induced cardiac hypertrophy. *Biochem. Biophys. Res. Commun.* **289**, 901–907
72. Touyz, R. M., and Briones, A. M. (2011) Reactive oxygen species and vascular biology: implications in human hypertension. *Hypertens. Res.* **34**, 5–14
73. Li, L., Watts, S. W., Banes, A. K., Galligan, J. J., Fink, G. D., and Chen, A. F. (2003) NADPH oxidase-derived superoxide augments endothelin-1-induced vasoconstriction in mineralocorticoid hypertension. *Hypertension* **42**, 316–321
74. Sedeek, M. H., Llinas, M. T., Drummond, H., Fortepiani, L., Abram, S. R., Alexander, B. T., Reckelhoff, J. F., and Granger, J. P. (2003) Role of reactive oxygen species in endothelin-induced hypertension. *Hypertension* **42**, 806–810
75. Li, L., Galligan, J. J., Fink, G. D., and Chen, A. F. (2003) Vasopressin induces vascular superoxide *via* endothelin-1 in mineralocorticoid hypertension. *Hypertension* **41**, 663–668
76. Landmesser, U., Dikalov, S., Price, S. R., McCann, L., Fukui, T., Holland, S. M., Mitch, W. E., and Harrison, D. G. (2003) Oxidation of

- tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J. Clin. Invest.* **111**, 1201–1209
77. Zheng, J.-S., Yang, X.-Q., Lookingland, K. J., Fink, G. D., Hesslinger, C., Kapatos, G., Kovsdi, I., and Chen, A. F. (2003) Gene transfer of human guanosine 5'-triphosphate cyclohydrolase I restores vascular tetrahydrobiopterin level and endothelial function in low renin hypertension. *Circulation* **108**, 1238–1245
  78. Irani, K. (2000) Oxidant signaling in vascular cell growth, death, and survival: a review of the roles of reactive oxygen species in smooth muscle and endothelial cell mitogenic and apoptotic signaling. *Circ. Res.* **87**, 179–183
  79. Zweier, J. L., Chen, C.-A., and Druhan, L. J. (2011) S-Glutathionylation reshapes our understanding of endothelial nitric oxide synthase uncoupling and nitric oxide/reactive oxygen species-mediated signaling. *Antioxid. Redox Signal.* **14**, 1769–1775
  80. Moens, A. L., and Kass, D. A. (2006) Tetrahydrobiopterin and cardiovascular disease. *Arterioscler. Thromb. Vasc. Biol.* **26**, 2439–2444
  81. Roe, N. D., and Ren, J. (2012) Nitric oxide synthase uncoupling: a therapeutic target in cardiovascular diseases. *Vascul. Pharmacol.* **57**, 168–172
  82. Muslin, A. J. (2008) MAPK signalling in cardiovascular health and disease: molecular mechanisms and therapeutic targets. *Clin. Sci.* **115**, 203–218
  83. Welch, W. J., and Wilcox, C. S. (2001) AT1 receptor antagonist combats oxidative stress and restores nitric oxide signaling in the SHR. *Kidney Int.* **59**, 1257–1263
  84. Dröge, W. (2002) Free radicals in the physiological control of cell function. *Physiol. Rev.* **82**, 47–95
  85. Pham-Huy, L. A., He, H., and Pham-Huy, C. (2008) Free radicals, antioxidants in disease and health. *Int. J. Biomed. Sci.* **4**, 89–96
  86. Vinson, J. A. (2006) Oxidative stress in cataracts. *Pathophysiology* **13**, 151–162
  87. Baynes, J. W. (1991) Role of oxidative stress in development of complications in diabetes. *Diabetes* **40**, 405–412
  88. Nourooz-Zadeh, J., Halliwell, B., and Anggård, E. E. (1997) Evidence for the formation of F3-isoprostanates during peroxidation of eicosapentaenoic acid. *Biochem. Biophys. Res. Commun.* **236**, 467–472
  89. Hokayem, M., Blond, E., Vidal, H., Lambert, K., Meugnier, E., Feillet-Coudray, C., Coudray, C., Pesenti, S., Luyton, C., Lambert-Porcheron, S., Sauvinet, V., Fedou, C., Brun, J.-F., Rieusset, J., Bisbal, C., Sultan, A., Mercier, J., Goudable, J., Dupuy, A.-M., Cristol, J.-P., Laville, M., and Avignon, A. (2013) Grape polyphenols prevent fructose-induced oxidative stress and insulin resistance in first-degree relatives of type 2 diabetic patients. *Diabetes Care* **36**, 1454–1461
  90. Schulz, R., Mahmoudi, S., Hattar, K., Sibelius, U., Olschewski, H., Mayer, K., Seeger, W., and Grimminger, F. (2000) Enhanced release of superoxide from polymorphonuclear neutrophils in obstructive sleep apnea. Impact of continuous positive airway pressure therapy. *Am. J. Respir. Crit. Care Med.* **162**, 566–570
  91. Eck, H. P., Gmünder, H., Hartmann, M., Petzoldt, D., Daniel, V., and Dröge, W. (1989) Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1-infected patients. *Biol. Chem. Hoppe Seyler* **370**, 101–108
  92. Sahiner, U. M., Birben, E., Erzurum, S., Sackesen, C., and Kalayci, O. (2011) Oxidative stress in asthma. *World Allergy Organ. J.* **4**, 151–158
  93. Kadam, D. P., Suryakar, A. N., Ankush, R. D., Kadam, C. Y., and Deshpande, K. H. (2010) Role of oxidative stress in various stages of psoriasis. *Indian J. Clin. Biochem.* **25**, 388–392
  94. Van de Veerdonk, F. L., Smeeckens, S. P., Joosten, L. A. B., Kullberg, B. J., Dinarello, C. A., van der Meer, J. W. M., and Netea, M. G. (2010) Reactive oxygen species-independent activation of the IL-1beta inflammasome in cells from patients with chronic granulomatous disease. *Proc. Natl. Acad. Sci. USA* **107**, 3030–3033
  95. Spina, M. B., and Cohen, G. (1989) Dopamine turnover and glutathione oxidation: implications for Parkinson disease. *Proc. Natl. Acad. Sci. USA* **86**, 1398–1400
  96. Saggau, H., Cooksey, J., Dexter, D., Wells, F. R., Lees, A., Jenner, P., and Marsden, C. D. (1989) A selective increase in particulate superoxide dismutase activity in parkinsonian substantia nigra. *J. Neurochem.* **53**, 692–697
  97. Abdalla, D. S., Monteiro, H. P., Oliveira, J. A., and Bechara, E. J. (1986) Activities of superoxide dismutase and glutathione peroxidase in schizophrenic and manic-depressive patients. *Clin. Chem.* **32**, 805–807
  98. Brady, P. S., Brady, L. J., and Ullrey, D. E. (1979) Selenium, vitamin E and the response to swimming stress in the rat. *J. Nutr.* **109**, 1103–1109
  99. Dillard, C. J., Litov, R. E., Savin, W. M., Dumelin, E. E., and Tappel, A. L. (1978) Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. *J. Appl. Physiol.* **45**, 927–932
  100. Koren, A., Sauber, C., Sentjurs, M., and Schara, M. (1983) Free radicals in tetanic activity of isolated skeletal muscle. *Comp. Biochem. Physiol. B* **74**, 633–635
  101. Reid, M. B., Khawli, F. A., and Moody, M. R. (1993) Reactive oxygen in skeletal muscle. III. Contractility of unfatigued muscle. *J. Appl. Physiol.* **75**, 1081–1087
  102. Gomez-Cabrera, M. C., Salvador-Pascual, A., Cabo, H., Ferrando, B., and Viña, J. (2015) Redox modulation of mitochondrial biogenesis in exercise. Does antioxidant supplementation blunt the benefits of exercise training? *Free Radic. Biol. Med.* **86**, 37–46
  103. Sánchez, G., Pedrozo, Z., Domenech, R. J., Hidalgo, C., and Donoso, P. (2005) Tachycardia increases NADPH oxidase activity and RYR2 S-glutathionylation in ventricular muscle. *J. Mol. Cell. Cardiol.* **39**, 982–991
  104. Prosser, B. L., Ward, C. W., and Lederer, W. J. (2011) X-ROS signaling: rapid mechano-chemo transduction in heart. *Science* **333**, 1440–1445
  105. Banki, K., Hutter, E., Gonchoroff, N. J., and Perl, A. (1999) Elevation of mitochondrial transmembrane potential and reactive oxygen intermediate levels are early events and occur independently from activation of caspases in Fas signaling. *J. Immunol.* **162**, 1466–1479
  106. Esteve, J. M., Mompou, J., García de la Asunción, J., Sastre, J., Asensi, M., Boix, J., Vina, J. R., Vina, J., and Pallardo, F. V. (1999) Oxidative damage to mitochondrial DNA and glutathione oxidation in apoptosis: studies *in vivo* and *in vitro*. *FASEB J.* **13**, 1055–1064
  107. Johnson, T. M., Yu, Z. X., Ferrans, V. J., Lowenstein, R. A., and Finkel, T. (1996) Reactive oxygen species are downstream mediators of p53-dependent apoptosis. *Proc. Natl. Acad. Sci. USA* **93**, 11848–11852
  108. De Vos, K., Goossens, V., Boone, E., Verammen, D., Vancompernelle, K., Vandenamee, P., Haegeman, G., Fiers, W., and Grooten, J. (1998) The 55-kDa tumor necrosis factor receptor induces clustering of mitochondria through its membrane-proximal region. *J. Biol. Chem.* **273**, 9673–9680
  109. Valko, M., Izakovic, M., Mazur, M., Rhodes, C. J., and Telser, J. (2004) Role of oxygen radicals in DNA damage and cancer incidence. *Mol. Cell. Biochem.* **266**, 37–56
  110. Ha, H. C., Thiagalingam, A., Nelkin, B. D., and Casero, R. A., Jr. (2000) Reactive oxygen species are critical for the growth and differentiation of medullary thyroid carcinoma cells. *Clin. Cancer Res.* **6**, 3783–3787
  111. Cao, Y., Wei, W., Zhang, N., Yu, Q., Xu, W.-B., Yu, W.-J., Chen, G.-Q., Wu, Y.-L., and Yan, H. (2015) Oridonin stabilizes retinoic acid receptor alpha through ROS-activated NF-κB signaling. *BMC Cancer* **15**, 248
  112. Ma, L., Zhu, W.-Z., Liu, T.-T., Fu, H.-L., Liu, Z.-J., Yang, B.-W., Song, T.-Y., and Li, G.-R. (2015) H2O2 inhibits proliferation and mediates suppression of migration *via* DLC1/RhoA signaling in cancer cells. *Asian Pac. J. Cancer Prev.* **16**, 1637–1642
  113. Peers, C., Wyatt, C. N., and Evans, A. M. (2010) Mechanisms for acute oxygen sensing in the carotid body. *Respir. Physiol. Neurobiol.* **174**, 292–298
  114. Bunn, H. F., and Poyton, R. O. (1996) Oxygen sensing and molecular adaptation to hypoxia. *Physiol. Rev.* **76**, 839–885
  115. Fandrey, J., Frede, S., and Jelkmann, W. (1994) Role of hydrogen peroxide in hypoxia-induced erythropoietin production. *Biochem. J.* **303**, 507–510
  116. Huang, L. E., Arany, Z., Livingston, D. M., and Bunn, H. F. (1996) Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its alpha subunit. *J. Biol. Chem.* **271**, 32253–32259
  117. Cambolat, O., Fandrey, J., and Jelkmann, W. (1998) Effects of modulators of the production and degradation of hydrogen peroxide on erythropoietin synthesis. *Respir. Physiol.* **114**, 175–183
  118. Huang, L. E., Gu, J., Schau, M., and Bunn, H. F. (1998) Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain *via* the ubiquitin-proteasome pathway. *Proc. Natl. Acad. Sci. USA* **95**, 7987–7992
  119. Semenza, G. L. (2000) HIF-1: mediator of physiological and pathophysiological responses to hypoxia. *J. Appl. Physiol.* **88**, 1474–1480
  120. Urao, N., Sudhakar, V., Kim, S.-J., Chen, G.-F., McKinney, R. D., Kojda, G., Fukui, T., and Ushio-Fukai, M. (2013) Critical role of

- endothelial hydrogen peroxide in post-ischemic neovascularization. *PLoS One* **8**, e57618
121. Tojo, T., Ushio-Fukai, M., Yamaoka-Tojo, M., Ikeda, S., Patrushev, N., and Alexander, R. W. (2005) Role of gp91phox (Nox2)-containing NAD(P)H oxidase in angiogenesis in response to hindlimb ischemia. *Circulation* **111**, 2347–2355
  122. Schröder, K., Zhang, M., Benkhoff, S., Mieth, A., Pliquett, R., Kosowski, J., Kruse, C., Luedike, P., Michaelis, U. R., Weissmann, N., Dimmeler, S., Shah, A. M., and Brandes, R. P. (2012) Nox4 is a protective reactive oxygen species generating vascular NADPH oxidase. *Circ.* **110**, 1217–1225
  123. Zhang, M., Brewer, A. C., Schröder, K., Santos, C. X. C., Grieve, D. J., Wang, M., Anilkumar, N., Yu, B., Dong, X., Walker, S. J., Brandes, R. P., and Shah, A. M. (2010) NADPH oxidase-4 mediates protection against chronic load-induced stress in mouse hearts by enhancing angiogenesis. *Proc. Natl. Acad. Sci. USA* **107**, 18121–18126
  124. Hodara, R., Weiss, D., Joseph, G., Velasquez-Castano, J. C., Landázuri, N., Han, J. W., Yoon, Y. S., and Taylor, W. R. (2011) Overexpression of catalase in myeloid cells causes impaired postischemic neovascularization. *Arterioscler. Thromb. Vasc. Biol.* **31**, 2203–2209
  125. Kim, Y. M., Kim, K. E., Koh, G. Y., Ho, Y.-S., and Lee, K.-J. (2006) Hydrogen peroxide produced by angiopoietin-1 mediates angiogenesis. *Cancer Res.* **66**, 6167–6174
  126. Babaei, S., Teichert-Kuliszewska, K., Zhang, Q., Jones, N., Dumont, D. J., and Stewart, D. J. (2003) Angiogenic actions of angiopoietin-1 require endothelium-derived nitric oxide. *Am. J. Pathol.* **162**, 1927–1936
  127. Hehner, S. P., Breitreutz, R., Shubinsky, G., Unsöld, H., Schulze-Osthoff, K., Schmitz, M. L., and Dröge, W. (2000) Enhancement of T cell receptor signaling by a mild oxidative shift in the intracellular thiol pool. *J. Immunol.* **165**, 4319–4328
  128. Los, M., Dröge, W., Stricker, K., Baeuerle, P. A., and Schulze-Osthoff, K. (1995) Hydrogen peroxide as a potent activator of T lymphocyte functions. *Eur. J. Immunol.* **25**, 159–165
  129. Kaminski, M., Kiessling, M., Stüss, D., Krammer, P. H., and Gülow, K. (2007) Novel role for mitochondria: protein kinase C $\theta$ -dependent oxidative signaling organelles in activation-induced T-cell death. *Mol. Cell. Biol.* **27**, 3625–3639
  130. Arsenijevic, D., Onuma, H., Pecqueur, C., Raimbault, S., Manning, B. S., Miroux, B., Couplan, E., Alves-Guerra, M. C., Goubern, M., Surwit, R., Bouillaud, F., Richard, D., Collins, S., and Ricquier, D. (2000) Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. *Nat. Genet.* **26**, 435–439
  131. Kraaij, M. D., Savage, N. D. L., van der Kooij, S. W., Koekkoek, K., Wang, J., van den Berg, J. M., Ottenhoff, T. H. M., Kuijpers, T. W., Holmdahl, R., van Kooten, C., and Gelderman, K. A. (2010) Induction of regulatory T cells by macrophages is dependent on production of reactive oxygen species. *Proc. Natl. Acad. Sci. USA* **107**, 17686–17691
  132. Lee, K., Won, H. Y., Bae, M. A., Hong, J.-H., and Hwang, E. S. (2011) Spontaneous and aging-dependent development of arthritis in NADPH oxidase 2 deficiency through altered differentiation of CD11b<sup>+</sup> and Th/Treg cells. *Proc. Natl. Acad. Sci. USA* **108**, 9548–9553
  133. Kim, H.-R., Lee, A., Choi, E.-J., Kie, J.-H., Lim, W., Lee, H. K., Moon, B.-I., and Seoh, J.-Y. (2014) Attenuation of experimental colitis in glutathione peroxidase 1 and catalase double knockout mice through enhancing regulatory T cell function. *PLoS One* **9**, e95332
  134. Hamuro, J., Murata, Y., Suzuki, M., Takatsuki, F., and Suga, T. (1999) The triggering and healing of tumor stromal inflammatory reactions regulated by oxidative and reductive macrophages. *Gann Monogr. Cancer Res.* **48**, 153–164
  135. Frenette, P. S., and Wagner, D. D. (1996) Adhesion molecules—part 1. *N. Engl. J. Med.* **334**, 1526–1529
  136. Albelda, S. M., Smith, C. W., and Ward, P. A. (1994) Adhesion molecules and inflammatory injury. *FASEB J.* **8**, 504–512
  137. Schaller, M. D., Borgman, C. A., Cobb, B. S., Vines, R. R., Reynolds, A. B., and Parsons, J. T. (1992) pp125FAK a structurally distinctive protein-tyrosine kinase associated with focal adhesions. *Proc. Natl. Acad. Sci. USA* **89**, 5192–5196
  138. Sellak, H., Franzini, E., Hakim, J., and Pasquier, C. (1994) Reactive oxygen species rapidly increase endothelial ICAM-1 ability to bind neutrophils without detectable upregulation. *Blood* **83**, 2669–2677
  139. Falanga, V. (2005) Wound healing and its impairment in the diabetic foot. *Lancet* **366**, 1736–1743
  140. Blakytyn, R., and Jude, E. (2006) The molecular biology of chronic wounds and delayed healing in diabetes. *Diabet. Med.* **23**, 594–608
  141. Shi, H., Cheng, Y., Ye, J., Cai, P., Zhang, J., Li, R., Yang, Y., Wang, Z., Zhang, H., Lin, C., Lu, X., Jiang, L., Hu, A., Zhu, X., Zeng, Q., Fu, X., Li, X., and Xiao, J. (2015) bFGF promotes the migration of human dermal fibroblasts under diabetic conditions through reactive oxygen species production via the PI3K/Akt-Rac1-JNK pathways. *Int. J. Biol. Sci.* **11**, 845–859
  142. Chandrasekaran, V., Lea, C., Sosa, J. C., Higgins, D., and Lein, P. J. (2015) Reactive oxygen species are involved in BMP-induced dendritic growth in cultured rat sympathetic neurons. *Mol. Cell. Neurosci.* **67**, 116–125
  143. Juntilla, M. M., Patil, V. D., Calamito, M., Joshi, R. P., Birnbaum, M. J., and Koretzky, G. A. (2010) AKT1 and AKT2 maintain hematopoietic stem cell function by regulating reactive oxygen species. *Blood* **115**, 4030–4038
  144. Owusu-Ansah, E., and Banerjee, U. (2009) Reactive oxygen species prime *Drosophila* haematopoietic progenitors for differentiation. *Nature* **461**, 537–541
  145. Malinska, D., Kudin, A. P., Bejtka, M., and Kunz, W. S. (2012) Changes in mitochondrial reactive oxygen species synthesis during differentiation of skeletal muscle cells. *Mitochondrion* **12**, 144–148
  146. Hamanaka, R. B., Glasauer, A., Hoover, P., Yang, S., Blatt, H., Mullen, A. R., Getsios, S., Gottardi, C. J., DeBerardinis, R. J., Lavker, R. M., and Chandel, N. S. (2013) Mitochondrial reactive oxygen species promote epidermal differentiation and hair follicle development. *Sci. Signal.* **6**, ra8
  147. Morimoto, H., Iwata, K., Ogonuki, N., Inoue, K., Atsuo, O., Kanatsu-Shinohara, M., Morimoto, T., Yabe-Nishimura, C., and Shinohara, T. (2013) ROS are required for mouse spermatogonial stem cell self-renewal. *Cell Stem Cell* **12**, 774–786
  148. Le Belle, J. E., Orozco, N. M., Paucar, A. A., Saxe, J. P., Mottahedeh, J., Pyle, A. D., Wu, H., and Kornblum, H. I. (2011) Proliferative neural stem cells have high endogenous ROS levels that regulate self-renewal and neurogenesis in a PI3K/Akt-dependant manner. *Cell Stem Cell* **8**, 59–71
  149. Van Raamsdonk, J. M., and Hekimi, S. (2010) Reactive oxygen species and aging in *Caenorhabditis elegans*: causal or casual relationship? *Antioxid. Redox Signal.* **13**, 1911–1953
  150. Ristow, M., and Schmeisser, S. (2011) Extending life span by increasing oxidative stress. *Free Radic. Biol. Med.* **51**, 327–336
  151. Packer, L., and Fuehr, K. (1977) Low oxygen concentration extends the lifespan of cultured human diploid cells. *Nature* **267**, 423–425
  152. Bell, E. L., Klimova, T. A., Eisenbart, J., Schumacker, P. T., and Chandel, N. S. (2007) Mitochondrial reactive oxygen species trigger hypoxia-inducible factor-dependent extension of the replicative life span during hypoxia. *Mol. Cell. Biol.* **27**, 5737–5745
  153. Van Raamsdonk, J. M., and Hekimi, S. (2009) Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in *Caenorhabditis elegans*. *PLoS Genet.* **5**, e1000361
  154. Yang, W., and Hekimi, S. (2010) A mitochondrial superoxide signal triggers increased longevity in *Caenorhabditis elegans*. *PLoS Biol.* **8**, e1000556
  155. Schulz, T. J., Zarse, K., Voigt, A., Urban, N., Birringer, M., and Ristow, M. (2007) Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab.* **6**, 280–293
  156. Lee, S.-J., Hwang, A. B., and Kenyon, C. (2010) Inhibition of respiration extends *C. elegans* life span via reactive oxygen species that increase HIF-1 activity. *Curr. Biol.* **20**, 2131–2136
  157. Zarse, K., Schmeisser, S., Groth, M., Priebe, S., Beuster, G., Kuhlow, D., Guthke, R., Platzer, M., Kahn, C. R., and Ristow, M. (2012) Impaired insulin/IGF1 signaling extends life span by promoting mitochondrial L-proline catabolism to induce a transient ROS signal. *Cell Metab.* **15**, 451–465
  158. Lapointe, J., Stepanyan, Z., Bigras, E., and Hekimi, S. (2009) Reversal of the mitochondrial phenotype and slow development of oxidative biomarkers of aging in long-lived Mcl1<sup>-/-</sup> mice. *J. Biol. Chem.* **284**, 20364–20374
  159. Liu, X., Jiang, N., Hughes, B., Bigras, E., Shoubridge, E., and Hekimi, S. (2005) Evolutionary conservation of the clk-1-dependent mechanism of longevity: loss of mcl1 increases cellular fitness and lifespan in mice. *Genes Dev.* **19**, 2424–2434
  160. Hanzén, S., Vielfort, K., Yang, J., Roger, F., Andersson, V., Zamarbide-Forés, S., Andersson, R., Malm, L., Palais, G., Biteau, B., Liu, B., Toledano, M. B., Molin, M., and Nyström, T. (2016) Lifespan control by redox-dependent recruitment of chaperones to misfolded proteins. *Cell* **166**, 140–151

161. Roberts, L. J., II, and Morrow, J. D. (1997) The generation and actions of isoprostanes. *Biochim. Biophys. Acta* **1345**, 121–135
162. Kaikkonen, J. E., Vilppo, T., Asikainen, J., Voutilainen, S., Kurl, S., and Salonen, J. T. (2013) Fatty acids as determinants of *in-vivo* lipid peroxidation: the EFFGE study in Eastern Finnish hypertensive and non-hypertensive subjects. *Ann. Med.* **45**, 455–464
163. Milne, G. L., Dai, Q., and Roberts, L. J., II (2015) The isoprostanes—25 years later. *Biochim. Biophys. Acta* **1851**, 433–445
164. Durand, T., Cracowski, J.-L., and Berdeux, O. (2005) Isoprostanes, biomarkers of lipid peroxidation in humans. Part I. Nomenclature and synthesis [in French]. *Pathol. Biol. (Paris)* **53**, 349–355
165. Ting, H. J., and Khasawneh, F. T. (2010) Platelet function and Isoprostane biology. Should isoprostanes be the newest member of the orphan-ligand family? *J. Biomed. Sci.* **17**, 24
166. Kumar, A., Kingdon, E., and Norman, J. (2005) The isoprostane 8-iso-PGF<sub>2</sub>α suppresses monocyte adhesion to human microvascular endothelial cells via two independent mechanisms. *FASEB J.* **19**, 443–445
167. Miggin, S. M., and Kinsella, B. T. (2001) Thromboxane A<sub>2</sub> receptor mediated activation of the mitogen activated protein kinase cascades in human uterine smooth muscle cells. *Biochim. Biophys. Acta* **1539**, 147–162
168. Comperti, M., Signorini, C., Leoncini, S., Buonocore, G., Rossi, V., and Ciccoli, L. (2004) Plasma F<sub>2</sub>-isoprostanes are elevated in newborns and inversely correlated to gestational age. *Free Radic. Biol. Med.* **37**, 724–732
169. Rogers, M. S., Wang, C. C., Lau, T. K., Xiao, X., Zhou, X. G., Fok, T. F., Chu, K. O., and Pang, C. P. (2005) Relationship between isoprostane concentrations, metabolic acidosis, and morbid neonatal outcome. *Clin. Chem.* **51**, 1271–1274
170. Weinberger, B., Nisar, S., Anwar, M., Ostfeld, B., and Hegyi, T. (2006) Lipid peroxidation in cord blood and neonatal outcome. *Pediatr. Int.* **48**, 479–483
171. Sethi, S., Eastman, A. Y., and Eaton, J. W. (1996) Inhibition of phagocyte-endothelium interactions by oxidized fatty acids: a natural anti-inflammatory mechanism? *J. Lab. Clin. Med.* **128**, 27–38
172. Jamil, J., Bankhele, P., Salvi, A., Mannix, J. E., Oger, C., Guy, A., Galano, J.-M., Durand, T., Njie-Mbye, Y. F., Ohia, S. E., and Opere, C. A. (2014) Role of the non-enzymatic metabolite of eicosapentaenoic acid, 5-epi-5-F<sub>3t</sub>-isoprostane in the regulation of [<sup>3</sup>H]D-aspartate release in isolated bovine retina. *Neurochem. Res.* **39**, 2360–2369
173. Roy, J., Oger, C., Thireau, J., Roussel, J., Mercier-Touzet, O., Faure, D., Pinot, E., Farah, C., Taber, D. F., Cristol, J.-P., Lee, J. C. Y., Lacampagne, A., Galano, J.-M., Durand, T., and Le Guennec, J.-Y. (2015) Nonenzymatic lipid mediators, neuroprostanes, exert the antiarrhythmic properties of docosahexaenoic acid. *Free Radic. Biol. Med.* **86**, 269–278
174. Roy, J., Fauconnier, J., Oger, C., Farah, C., Angebault-Prouteau, C., Thireau, J., Bideaux, P., Scheuermann, V., Bultel-Poncé, V., Demion, M., Galano, J.-M., Durand, T., Lee, J. C.-Y., and Le Guennec, J.-Y. (2017) Non-enzymatic oxidized metabolite of DHA, 4(RS)-4-F<sub>4t</sub>-neuroprostane protects the heart against reperfusion injury. *Free Radic. Biol. Med.* **102**, 229–239
175. Roy, J., Oliveira, L. T., Oger, C., Galano, J.-M., Bultel-Poncé, V., Richard, S., Guimaraes, A. G., Vilela, J. M. C., Andrade, M. S., Durand, T., Besson, P., Mosqueira, V. C. F., and Le Guennec, J.-Y. (2015) Polymeric nanocapsules prevent oxidation of core-loaded molecules: evidence based on the effects of docosahexaenoic acid and neuroprostane on breast cancer cells proliferation. *J. Exp. Clin. Cancer Res.* **34**, 155
176. Judé, S., Bedut, S., Roger, S., Pinault, M., Champeroux, P., White, E., and Le Guennec, J.-Y. (2003) Peroxidation of docosahexaenoic acid is responsible for its effects on I TO and I SS in rat ventricular myocytes. *Br. J. Pharmacol.* **139**, 816–822
177. Le, G. J.-Y., Durand, T., Oger, C., Galano, J.-M., Thireau, J., Bultel-Ponce, V., Guy, A., and Roy, J. (2014) INSERM, assignee. Methods and pharmaceutical composition for the treatment and prevention of cardiac arrhythmias. Patent WO2014086819 A1
178. Roy, J., Le Guennec, J.-Y., Galano, J.-M., Thireau, J., Bultel-Poncé, V., Demion, M., Oger, C., Lee, J. C. Y., and Durand, T. (2016) Non-enzymatic cyclic oxygenated metabolites of omega-3 polyunsaturated fatty acid: bioactive drugs? *Biochimie* **120**, 56–61
179. Smith, L. L. (1991) Another cholesterol hypothesis: cholesterol as antioxidant. *Free Radic. Biol. Med.* **11**, 47–61
180. De Medina, P., Paillasse, M. R., Payré, B., Silvente-Poirot, S., and Poirot, M. (2009) Synthesis of new alkylaminooxysterols with potent cell differentiating activities: identification of leads for the treatment of cancer and neurodegenerative diseases. *J. Med. Chem.* **52**, 7765–7777
181. De Medina, P., Paillasse, M. R., Segala, G., Voisin, M., Mhamdi, L., Dalenc, F., Lacroix-Triki, M., Filleron, T., Pont, F., Saati, T. A., Morisseau, C., Hammock, B. D., Silvente-Poirot, S., and Poirot, M. (2013) Dendrogenin A arises from cholesterol and histamine metabolism and shows cell differentiation and anti-tumour properties. *Nat. Commun.* **4**, 1840
182. Thannickal, V. J., and Fanburg, B. L. (2000) Reactive oxygen species in cell signaling. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **279**, L1005–L1028
183. Nelson, R. J. (2005) *An Introduction to Behavior Endocrinology*, Sinauer Associates Publishers, Sunderland, MA, USA

Received for publication February 24, 2017.

Accepted for publication May 22, 2017.