We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Oxidative Stress: Noxious but Also Vital

Margarete Dulce Bagatini,
Jeandre Augusto dos Santos Jaques,
Carla Santos de Oliveira,
Graciele Almeida de Oliveira,
Micheli Mainardi Pillat, Aline Mânica,
Cintia dos Santos Moser,
Lucas Derbocio dos Santos and Henning Ulrich

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.73394>

Abstract

The imbalance between reactive oxygen species (ROS) production and antioxidant defenses determines the condition called oxidative stress. When there is an increase in ROS production or a decrease in the antioxidant defenses, this systemic antioxidant/pro-oxidant imbalance may lead to the accumulation of oxidative damage, which, in turn, may lead to a modification of biomolecules. These consist of reactions resulting in protein adducts, DNA oxidation, and formation of lipid peroxides, which, in turn, reduce the cellular functional capacity and increase the risk of disease development. The body has natural scavenging systems against free radicals and other reactive species. However, sometimes the endogenous antioxidant capacity is exceeded by the production of ROS. When this occurs, exogenous antioxidants exert important function for the human health. These bioactive compounds act preventing and neutralizing the formation of new reactive species and free radicals. In some cases, an increase of ROS can help the host to resolve an infection or even to control the tumor growth. Finally, the levels of ROS can be perceived by signal transduction pathways involving known targets (i.e., p53, Ras, and NF- κ B) and regulate physiopathological events such as the cellular cycle, apoptosis, and inflammation.

Keywords: reactive species, cellular oxidation, antioxidant system, health, disease

1. Cellular respiration and generation of reactive species in the mitochondria: implications in cell viability and aging

Oxidative phosphorylation is the center of energy metabolism in plants, animals and several microbial life forms [1]. In eukaryotes, this process occurs in mitochondria. The mitochondria is a cytoplasmic organelle surrounded by two membranes, outer and inner membrane, which main function is the production of most of the phosphate compounds necessary for the energetic balance of the cell. In addition, other functions such as the regulation of the body's heat generation [2–4] programmed cell death [5–7], reactive oxygen species (ROS) generation and cell signaling [8] is also associated with mitochondria. Cellular vitality is directly related to mitochondria, and mitochondrial dysfunctions are frequent causes of accidental cell death [5, 9–11], cancer [12, 13], diabetes [14–16] and neurodegenerative diseases [17–19], among others.

The characterization of the respiratory electron chain could be performed in studies using the fractionation of its components by certain detergents that at low concentrations break the interactions between proteins and lipids in the membranes, leaving associations between proteins intact [20]. In electron transport chain, through this process, four protein complexes were found. They were named complex I (or NADH-Ubiquinone oxidoreductase), complex II (succinate dehydrogenase), complex III (Ubiquinol -cytochrome c oxidoreductase, or complex bc_1) e complex IV (cytochrome c oxidase). The complex V is also known as ATP synthase. Despite glycerophosphate dehydrogenase (glycerol-3-phosphate dehydrogenase) and ETF-ubiquinone oxidoreductase have not complex nomenclature, they are connect to the electron transport chain, as complex I and complex II, i.e., delivering electron to ubiquinone [21].

The redox carriers within the respiratory chain consists of flavoprotein containing tightly bound FAD or FMN as prosthetic groups, protein-bound couper, ironsulphur (nonhaem iron) proteins and cytochromes, with haem prosthetic groups. The ubiquinone also participated in electron transport chain as a free and diffusible cofactor [20]. While electron transport occurs through the mitochondrial complexes, complexes I, II, and III pump protons from mitochondrial matrix to the intermembrane space. The energy associated to this process is used to the production of ATP by ATP synthase (**Figure 1**) [22].

1.1. Reactive species in mitochondria

The ROS comprise a variety of molecules derived from molecular oxygen, including oxygen radicals and non-radical oxygen derivate. The major intracellular site of ROS formation in most tissues is mitochondria [23, 24]. Within mitochondria, the electron transport chain continuously generates water from O_2 through the electronic reduction at the cytochrome c oxidase level (**Figure 1**). These electrons reach cytochrome c oxidase by sequential transfer from the reduction of other components, and are initially removed from NADH and $FADH_2$. During this transfer, a small amount of electrons are lost at intermediate stages in the electron transport chain, mainly in the complex I and complex III [25–27] in mammals, leading to a monoelectronic reduction of O_2 [28].

This monoelectronic reduction of O_2 results in the formation of anion superoxide radical. While complex I releases superoxide only in the mitochondrial matrix, complex III releases

Figure 1. Electron transport chain, ROS, and antioxidant defense. The electron transport chain receives electrons from reduced compounds, as NADH in complex I (also called NADH coenzyme Q reductase) and succinate or FADH₂ in complex II (succinate dehydrogenase) and transfers them successively to coenzyme Q or ubiquinone, complex III, complex IV and finally to molecular oxygen with the formation of water. Concomitant with electron transport, protons are transferred from the mitochondrial matrix to the intermembrane space by complexes I (in mammals, but not in yeasts), complex III and IV. The difference in electrochemical potential between intermembrane space and matrix is used by ATP synthase to produce ATP from ADP and inorganic phosphate. During the passage of electrons through the complexes, a small fraction is leaked to oxygen at intermediate points, producing the superoxide radical anion, which in the mitochondrial matrix can be removed by SOD2 forming H₂O₂. Hydrogen peroxide can be converted to water in the mitochondrial matrix by glutathione peroxidase (mammals). Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; UQ, ubiquinone; Cyt, cytochrome c; SDH, succinate dehydrogenase; I, complex I or NADH coenzyme Q reductase; II, complex II or succinate dehydrogenase; III, complex III; IV, complex IV or cytochrome c oxidase; O₂^{•-}, superoxide radical anion; SOD2, superoxide dismutase 2 or mitochondrial MnSOD; GPx, glutathione peroxidase. Modified from Ref. [22].

superoxide in both sides of inner mitochondrial membrane [29]. Complex II could theoretically generate superoxide, due presence of flavoprotein in its structure. However, the redox centers are arranged in a manner that aids the prevention of ROS by avoiding the access of O₂ to the flavoprotein. This may explain the reason why this complex does not show a ROS formation by itself [30], but only due reverse electron transfer, i.e., when electrons flow from succinate to ubiquinone and back to complex I [31].

In addition to the electron transport chain, recent studies in mammalian tissues have shown that proteins belonging to the α -ketoglutarate dehydrogenase complex located in the mitochondrial matrix are also a source of ROS in a mechanism stimulated by the low concentration of NAD⁺ [32, 33]. In *Saccharomyces cerevisiae*, the deletion of the LPD1 gene, which leads to the inactivation of the enzyme dihydrolipoyl dehydrogenase, E3 subunit of the pyruvate dehydrogenase complex, also leads to a decrease in ROS production. This finding shows the importance of other mitochondrial proteins, other than those associated with the electron transport chain, in the regulation of redox balance [34].

The term reactive specie is not restricted to oxygen, but is also include others, as reactive nitrogen (RNS). Nitric oxide is a membrane permeable free radical that participates in a multiple process in the cells as signaling molecule, but also can contribute in cell oxidative damage. Its effect depends on NO levels and localization in the cell microenvironment [35, 36]. When nitric oxide is present in environment, as in mitochondrial matrix, the reaction of this free radical with superoxide can form others RNS, as peroxynitrite.

Besides mitochondria electron chain and enzyme linked to mitochondrial dehydrogenase complexes, other sources of ROS in cells include enzymes, as NADPH oxidases, cytochrome P450, cyclooxygenases, and the system xanthine/xanthine oxidase. Autoxidation is another example of source of ROS that in cells occurs when a biochemical compound is exposure to O_2 , as it occurs in $FADH_2$, L-DOPA and in nitric oxide synthase with generation of superoxide. The auto oxidation can be catalyzed by metallic ions, finally, harm proteins, in which O_2 bind Fe^{2+} could lead to superoxide, as in hemoglobin [37].

1.2. Mitochondria and reactive species: physiological level, oxidative stress, and its implications

ROS and RNS are normally produced in metabolism and have an important role as signaling molecules regulating diverse physiological cell events, as cell signaling, metabolism and regulation of transcription factors [35, 38–42].

The steady state of reactive species will depend on their generation, reactivity and removal by antioxidant defenses. When the level of reactive species generation is much larger than their removal it is said that there is a condition called oxidative stress, i.e., an imbalance between reactive species and antioxidants in favor of reactive species. The maintenance of cell redox state is important to cell viability [43]. The increased level of reactive species can lead to oxidative damage to a vast number of biological molecules, as DNA [44–46], proteins [47], lipids [48], including membranes [3] leading to a range of pathologies, as cancer [36], neurological disease [49], cardiac disease [50, 51], inflammation process [52] and aging.

There is a grand amount of theories about aging process, at least 300 theories according Medvedev [53]. In 1956, Harman proposed in his “free radical theory of aging” that the damage of biomolecules that occurs during aging is due oxidative stress, ROS increments [54]. Mitochondria, as the major site of ROS production, have been associated with aging process [55, 56]. Moreover, studies with caloric restriction in yeast and mammals have shown that the mitochondria, ROS, and RNS have an important role in the aging process [34, 56–61].

2. Protein adducts, DNA oxidation and epigenetic regulation, and effects on biological membranes

During oxidative stress, ROS can attack molecules at electron-dense sites or abstract protons, producing secondary radical species, which undergo conformational change generating more stable products. The molecules that are vulnerable to these deleterious modifications include

the lipids, proteins and nucleic acids. In other words, when the generation of reactive species exceeds antioxidant capacity, the cellular macromolecules also become targets of oxidation by these species. The possible consequences originated from this extensive oxidation, including an increased risk for cardiovascular disease, cancer and neurodegenerative disease (as detailed in Section 4).

2.1. Protein adducts

Under oxidative stress conditions, proteins suffer extensive modification [62–65]. Basically, ROS can oxidize amino acids cysteine and methionine, resulting in the production of dithiol and methionine sulfoxide crosslinks, respectively [66]. Moreover, reactive species also can cause protein modification by nitration of tyrosine and by nitrosation of amino acids with thiol group. These changes often result in the alteration of function or inhibition of enzyme activities. The protein adducts have been observed in several pathologic conditions [67, 68], suggesting their deleterious effects. However, whether these endogenous modifications are produced in a controlled manner, they may also control physiological responses [69, 70].

It is important to stress that the presence of proteins containing nitrotyrosine residues, for example, has been a biomarker of damage by reactive species [67, 68]. The tyrosine nitration occurs by addition of NO_2 to the ortho position of the phenolic ring of this amino acid. In fact, this NO_2 group is obtained from peroxynitrite (ONOO^-), a very strong oxidant [71]. During oxidative stress conditions, especially in inflammatory processes, a proportion of $\text{O}_2^{\bullet-}$ reacts with NO to form ONOO^- . This last is a much more powerful oxidant than $\text{O}_2^{\bullet-}$ and, beyond the tyrosine residues, can damage several classes of molecules. ONOO^- , its protonated form peroxynitrous acid (ONOOH), and its secondary radical product, react with electron-rich groups, such as sulfhydryls, ironsulphur centers, zinc-thiolates and active site sulfhydryl in tyrosine phosphatases [67, 68, 72, 73].

The thiol group ($-\text{SH}$) of cysteine, for example, it is another relevant protein targets of ROS. Disulfide bond is important in protein structure and function [74], and recently its role in redox signaling has also been evidenced [75]. The reaction of H_2O_2 with the deprotonated thiol group of cysteine produces a sulfenic acid (R-SOH). This last may be oxidized again producing a sulfinic acid ($\text{R-SO}_2\text{H}$). With high levels of stress oxidative, cysteines can further be oxidized to a sulfonic acid ($\text{R-SO}_3\text{H}$) [70, 76]. While sulfenic and sulfinic acids can be enzymatically reversible by the glutathione and thioredoxin enzyme systems [77] (Details about antioxidant mechanisms in next section), the sulfonic acid in cysteine residues seems to represent an irreversible protein damage.

2.2. DNA oxidation

The reactive species react directly with nucleic acids producing oxidative damage. Since oxidative DNA damage is a major threat to genetic integrity, causing mutations and modifications in gene expression pattern, it has been implicated in a wide variety of diseases, including cancer, cardiovascular and neurodegeneration disease, as well as aging process [46, 73].

The nitrogenous bases as well as the sugar suffer radical attacks, causing several base alterations and strand breaks [78]. In fact, around 80 different bases have been observed in DNA exposed to oxidants [79]. In this context, $\bullet\text{OH}$ is the most important reactive species that attacks DNA, since it reacts with the four bases and sugar moiety of the DNA backbone [78, 80] with a reaction rate limited by diffusion (4.5×10^9 to $9 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$) [79]. $\bullet\text{OH}$ attacks carbo-carbon double bonds of bases due to the high electron density. These attacks produce the hydroxylation at C5 and C6 of pyrimidines and C4, C5 and C8 of purines [78, 80]. These secondary radicals are subjected to other oxidation and reduction reactions, producing a wide DNA lesions, including the well characterized derivatives, 7,8-dihydro-8-oxodeoxyguanine (8-oxoG) and 2,6-diamino-4-hydroxy-5-formamido-pyrimidine (FapyGua) [71]. 8-oxo-G is the most stable of these altered bases and can give rise to mutations due to insert Adenine (A) opposite 8-oxo-G during DNA replication, instead of the Cytosine (C) [46, 71].

Another mutation produced by oxidative damage is C to thymine (T) transition, mainly due to the cytosine-derived products uracil glycol and 5-hydroxyuracil mispairing with A, instead of the G [71]. Although other pathways also induce this mutation, it is important to stress that C to T transition is the most frequent mutations found in cancers and in the tumor suppressor gene p53 [81, 82].

2.3. Effects on biological membranes

Under conditions of oxidative stress occur an oxidative process termed lipid peroxidation that affects lipids containing multiple double bonds, such as fatty acids, phospholipids, glycolipids and cholesterol, modifying properties of cellular membranes [73, 83]. This degenerative process is believed to contribute to aging and several diseases, such as atherosclerosis, Alzheimer's disease, peptic ulcer disease, and cancer [84, 85].

Cellular membranes are especially vulnerable to lipid peroxidation not only because of their high levels of unsaturated fatty acids, but also because of their connection with molecules capable of producing reactive species. They attack mainly the unsaturated fatty acids which contain carbon-carbon double bonds and CH_2 groups with particularly reactive hydrogen, and start radical peroxidation chain reactions [86]. These chain reactions are going to terminate when primary or secondary radicals directly react. Lipid peroxidation is accelerated by the presence of Fe^{2+} and Cu^{2+} ions [87, 88]. It is important to stress that lipid peroxides are unstable derivatives from the oxidation of unsaturated fatty acids and decompose to form reactive carbonyl molecules, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) [85, 89]. These two products are abundant biomarkers of lipid peroxidation [85, 90].

Membrane-bound proteins are also involved in the process of lipid peroxidation. Aldehyde products, such as MDA and 4-HNE, react with amine and thiol groups of membrane protein, causing several damages, including inactivation of enzymes. Conformational changes of membrane molecules also include lipid-lipid cross-links and lipid-protein cross-links [91, 92].

Moreover, lipid peroxidation modifies the global biophysical properties of the membranes. This process affects the packing of lipids and the permeability to solutes, which in turn, changes its function, including the membrane potential. Furthermore, the process

of peroxidation can inhibit the activity of protein transporters and ion channels [89, 91]. The increase of the permeability also seems to occur in internal mitochondrial membrane, uncoupling respiratory-chain phosphorylation [93]. Finally, the lipid peroxidation leads the severe damages: modification of membrane permeability, enzymatic inhibitions, inactivation of transporters [37, 92].

3. Endogenous/exogenous defense mechanisms

The exposure cells and tissues to the harmful effects of free radicals cause a cascade of reactions and induces activation of some strategies to damage prevent, repair mechanism to alleviate the oxidative damages, physical protection mechanism against damage, and the final most important is the antioxidant defense mechanisms [94, 95].

The antioxidant defenses are the first line of choice to take care of the stress. Endogenous antioxidant defenses include antioxidant enzymes and non-enzymatic molecules that are usually distributed within the cytoplasm and various cell organelles [94]. The exogenous antioxidants are present in consumed fruits, vegetables, juice, tea, coffee, nuts and cereal products [95].

The concept of biological antioxidant refers to any compound present at a lower concentration which is able to either delay or prevent the oxidation of the substrate. Antioxidant functions imply lowering oxidative stress, DNA mutations, malignant transformations, as well as other parameters of cell damage [96]. Antioxidants reactions can deplete molecular oxygen or decreasing its local concentration, removing pro oxidative metal ions, trapping aggressive ROS such as superoxide anion radical or hydrogen peroxide, scavenging chain initiating radicals like hydroxyl $\text{OH}\cdot$, alkoxyl $\text{RO}\cdot$ or peroxy $\text{ROO}\cdot$, breaking the chain of a radical sequence or quenching singlet oxygen ($^1\text{O}_2$) [97].

The antioxidants include some high molecular weight (SOD, GPx, catalase, albumin, transferrin, and metallothionein) and some low molecular weight substances (uric acid, ascorbic acid, lipoic acid, glutathione, ubiquinol, tocopherol/vitamin E, flavonoids). Natural food-derived components have received great attention in the last 2 decades, and several biological activities showing promising anti-inflammatory, antioxidant, and anti-apoptotic-modulatory potential have been identified. These enzymatic and nonenzymatic antioxidant systems are necessary for sustaining life by maintaining a delicate intracellular redox balance and minimizing undesirable cellular damage caused by ROS [94, 97, 98].

3.1. Enzymatic antioxidant system

Antioxidant enzymes catalyze ROS conversion directly via an active-site metal ion or through pathways involving the donation of an electron from the moiety-conserved redox couples thioredoxin and glutathione, which require continuous regeneration of the reduced species [99]. Superoxide and H_2O_2 metabolizing enzymes are generally considered to be the primary antioxidant enzyme defense system in the body [98].

The SOD is a family of enzymes catalyzing dismutation of superoxide into oxygen and H_2O_2 . Three types of superoxide dismutases can be encountered in mammalian tissues: copper-zinc containing superoxide dismutase (SOD1) present in the cytosol, manganese containing superoxide dismutase (SOD2) found in the mitochondrial matrix and extracellular superoxide dismutase (SOD3). All three are highly expressed, mainly in the renal tubules of healthy kidneys [15, 98, 100]. The final product of the SOD activity - H_2O_2 , is then converted into water and oxygen by the catalase (CAT). This enzyme is a homotetrameric protein containing four iron heme and largely located in the peroxisomes [15, 100].

Other important enzymatic antioxidants in the first line of defense include glutathione peroxidase (GPX) and myeloperoxidase (MPO) enzymes. The GPX is a selenium-containing enzyme, catalyzes both the reduction of H_2O_2 , and organic hydroperoxides to water or corresponding alcohols. Reduced glutathione functions as effective electron donor in the process, as free thiol groups are oxidized to disulfide bonds: $\text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow \text{GS-SG} + 2\text{H}_2\text{O}$ [97]. The MPO, a heme peroxidase, abundant in granules of human inflammatory cells, catalyzes the conversion of H_2O_2 to HClO with the production of ROS. The ROS production is associated with cardiovascular disease, chronic obstructive pulmonary disease, and Alzheimer's disease. Oxidant species derived from MPO lead to the production of specific oxidation products, such as 3-Cl-Tyr. This can be used as biomarker in several diseases, as above described, and its levels correlate with MPO [100].

Other enzymes could be cited by our antioxidant activity, such as Peroxiredoxin Family (PRX). These enzymes are a family of abundantly present 20–30 kDa peroxidases that are excessively reactive with H_2O_2 . So, they are likely to be critical for both oxidative stress protection as well as redox signaling [98]. The antioxidant enzymes may possibly offer novel treatment options for redox-related diseases, provided that the molecular mechanisms are known and can be specifically targeted. Besides that, inhibiting a given antioxidant enzyme or specifically silencing its gene expression may help treat disorders related to a gain of enzymatic function [98] and this fact can will help the researchers to explore future options in enzymatic antioxidant system and diseases.

3.2. Nonenzymatic antioxidant systems

Among the nonenzymatic antioxidant compounds, the principals are obtained from dietary as the class of phenolic compounds, vitamins C and E, and carotenoids [101]. Phenolic compounds represent a large group of secondary metabolites [102], among them flavonoids, phenolic acids, tannins and tocopherols as the most common natural source phenolic antioxidants [103].

The phenolic compounds are composed of one or more aromatic rings with varying degrees of hydroxylation, methoxylation and glycosylation, and various studies have associated the structure of phenolic compounds with their antioxidant properties [102, 104]. The antioxidant activity generally increases with the degree of hydroxylation in aromatic rings and decreases with C-3 methoxylation [105, 106]. The antioxidant activity is based on the availability of electrons to neutralize the free radicals; in addition, it is related to the number and nature of the hydroxylation pattern in the aromatic ring and the ability to act as a hydrogen donor [106].

The flavonoid group is the most diverse within phenolic compounds, with two aromatic rings associated via C-C bonds by a 3C oxygenated heterocycle. Flavonoids have antioxidant and chelating properties, inactivate ROS, acting against the oxidation of low density lipoproteins (LDL) and improving inflammation of the blood vessels. They also reduce the activity of the xanthine oxidase enzymes and the nicotinamide adenine dinucleotide phosphate oxidase, enzymes that stimulate the production of ROS [107].

In cellular compartments, flavonoids function as antioxidants inactivating free radicals both in hydrophilic and lipophilic compartments. For example, the antioxidant activity of phenolic compounds present in spices (cinnamon, sweet weed and mustard) differs between aqueous and lipid systems [108].

Vitamins C and E act together to inhibit lipid peroxidation and protect the cell against oxidative damage, as DNA damage. The antioxidant activity of vitamin C involves the transfer of an electron to the free radical and the consequent formation of the radical ascorbate [109]. In addition, vitamin C acts synergistically with vitamin E, which regenerate the vitamin C has better antioxidant activity in hydrophilic media, and in aqueous phase of extracellular fluids, it is able to neutralize ROS in the aqueous phase before they can attack lipids. Vitamin E is an important fat soluble antioxidant, acting as the chain breaking antioxidant within the cell membrane and playing an important role in the protection of membrane fatty acids against lipid peroxidation [110].

Vitamins C and E inhibit lipid peroxidation and protect against oxidative damage by their scavenging actions of ROS, as well as by modulateing numerous enzymatic complexes involved in the production of ROS, endothelial function and aggregation of platelets. These vitamins can also regulate NADPH oxidase, the most important source of $O_2^{\bullet-}$ in the cardiovascular system. It has been reported that ascorbic acid and α -tocopherol, derivated from vitamin C and E respectively, may involved in the transcriptional modulation of NADPH oxidase [111].

The most common carotenoids are xanthophylls and carotenes. Carotenoids can neutralize singlet oxygen by quenching it or can break the chain reaction of free radicals, or scavenging it, not so effective action (scavenging). The structure of the free radical is the main factor that determines if the carotenoid will have quenching or scavenging action. It also depends on the region where the radical is in heterogeneous biological tissue, aqueous or lipid region (plasma, blood, heart, liver, brain etc.), and the structure of the carotenoids (number of conjugated, cyclic or acyclic double bonds), polar or nonpolar groups, redox properties [112–114].

The physical quenching is the transfer of excitation energy from the singlet oxygen to the carotenoid. The oxygen returns to ground state and the carotenoid is in the excited triplet state, the energy is dissipated producing stable carotenoid and thermal energy and the carotenoid can undergo other cycles of singlet oxygen quenching [112, 115].

The chemical quenching the carotenoid combines with oxygen or is oxidized, leading to its destruction and producing a variety of oxidized products. Carotenoids can also extinguish the triplet-excited state of chlorophyll or other excited sensitizers, thus preventing the formation of singlet oxygen [112]. The free radical scavenging can occur in three ways, by electron transfer, by hydrogen abstraction, and by addition [112, 116].

4. Interaction between reactive species, enzymes, and antioxidant molecules in health and disease

All living cells have molecular tools to perceive and respond properly to environmental cues. All the cascades of intracellular reactions involved in promoting a biochemical response are denoted as signal transduction. There are well known receptor types or systems of signal transduction such as the G protein-coupled receptors (GPCR), tyrosine kinase receptors (TKR), ion channels, cell adhesion receptors, nuclear receptors and guanylyl-cyclases. Since cells often need to deal with many signals at the same time, the final biochemical response is a result of the integrations of many simultaneous cascades produced by one or more systems.

Before we move on exploring the targets of ROS in health and disease, an important question is raised: “Which are the main sources of cellular ROS?” Enzymes such as NADPH oxidases (Nox), xanthine oxidase (XO), lipoxygenase, MPO and uncoupled nitric oxide synthase are involved in the production of the anion radical superoxide ($O_2^{\cdot-}$). Furthermore, the mitochondrial aerobic respiration contributes with a huge amount of $O_2^{\cdot-}$. Peroxynitrite ($ONOO^-$) is formed by the reaction of nitric oxide and superoxide and is thought to contribute to eNOS uncoupling [69]. The majority of $O_2^{\cdot-}$ generated within the mitochondrial matrix or the cytosol is dismutated to H_2O_2 by the SOD antioxidant enzyme. Moreover, metal exposure can mediate the generation of H_2O_2 , $O_2^{\cdot-}$, and even the hydroxyl radical ($OH^\cdot$), mainly via the Fenton or the Haber-Weiss reactions [117].

Some ROS such as $O_2^{\cdot-}$ and $HO^\cdot$ are highly reactive and have a brief half life. For this reason they are not considered signaling molecules, but intermediates of nonselective nature. On the other hand, H_2O_2 is relatively stable and can both mediate intracellular signaling and also serve to paracrine signaling (i.e., cell-to-cell communication involving nearby cells), since it can cross biological membranes [118].

Up to date, several proteins have been recognized as downstream targets of ROS, such as kinases, phosphatases, mitogen-activated protein kinases (MAPK), small G proteins, transcription factors, microRNAs, and phospholipases. In this section, we do not intend to deeply review the literature, but to show an overview of important targets and exemplify their involvement in the signal transduction by ROS in health and disease.

ROS can induce alterations in the intracellular and extracellular processes, for example, in the PI3K/AKT signaling. The lipid phosphatidylinositol 3,4,5-triphosphate (PIP3) has a function as a second messenger and is not present in the quiescent cells, but it rises within seconds to minutes when there is a stimuli. PIP3 is produced by the phosphorylation of the phosphatidylinositol 4,5-bisphosphate (PIP2) catalyzed by the phosphatidylinositol 3-kinase (PI3K). This enzyme is activated by ROS through two different pathways, or directly, through amplifications of downstream PI3K pathway, or indirectly by inhibition of the phosphatase and tensing homolog deleted on chromosome 10 (PTEN). PTEN is responsible for the degradation of PIP3 signaling, since it catalyzes the hydrolysis of phosphate in the 3' position on PIP3 to produce PIP2 [119]. ROS, mainly, H_2O_2 , can oxidize and inhibit PTEN, which culminates in an increase in the PIP3 production, that acts in cell signaling, through activation of proteins, as serine/threonine protein kinase, AKT/PKB, among others [120, 121]. The AKT activation provides the transcription of

several targets, such as GSK3, BAD, FOXO, p53, NF- κ B, mTOR/p70S6K1 and HIF-1 [122, 123]. In this way, ROS increase the final cascade response in cell, i.e., cell cycle progression, proliferation, anti-apoptosis, invasion, autophagy and angiogenesis [124]. The PI3K/AKT pathway hyperactivated by ROS might favor carcinogenesis in the end of the process.

An important class of redox regulated proteins is the Src family of nonreceptor tyrosine kinases (SFKs), a group of structurally related kinases that catalyze the phosphorylation of tyrosine in downstream targets to regulate cellular functions coupling receptors such as the TKR, the cell adhesion molecules (CAMs), and the GPCR to the cellular signaling machinery [125]. For example, during focal adhesion while the extracellular matrix (ECM) contact triggers a slight or partial activation of SFKs, the ROS production is associated with a strong oxidative-dependent activation and recruitment of Src kinases to cell membranes. The redox-activation of SFKs can induce sustained PI3K, protein kinase C (PKC), and extracellular regulated kinase (ERK) activation and, thereby, create conditions for tumor cell growth, invasion, angiogenesis, and resistance to apoptosis [126]. In a variety of human cancers an increased activity of Src kinases have been described, as well as activation of important Src downstream targets such as PI3K/Akt, focal adhesion kinase (FAK), paxillin, p130Cas, signal transducer and activator of transcription 3 (STAT3) and stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) [127–130].

Carcinogenesis is also related with activator protein-1 (AP-1) transcription factor activation. Among other systems, ROS are recognized as activators of AP-1; however, the signaling transduction events involved are not totally understood. Chromium, cobalt, cadmium and vanadium are metals involved in the activation of AP-1 through signaling cascades involving the production of ROS and comprised of proteins and enzymes such as thioredoxin (Trx), redox factor-1 (Ref-1), ERK/MAPK, NADPH oxidase, I kappa B kinase (IKK), p38, JNK/c-jun [117, 131, 132].

ROS are important for the regulation of vascular tone, however an excess of reactive species might be associated with pathological dysfunction. Endothelial nitric oxide synthase (eNOS) regulates smooth muscle cells (SMC) relaxation through the production of the second messenger nitric oxide (NO) from L-arginine, which activates guanylyl cyclases to initiate the conversion of GTP to cyclic guanosine monophosphate (cGMP), which allosterically activates the cGMP-dependent protein kinases (PKG). The enzyme eNOS can be uncoupled and, consequently, change its profile from NO synthesis to $O_2^{\cdot -}$ production instead. Two major events are involved in eNOS uncoupling. First, an increase of ROS might generate the peroxynitrite ($ONOO^-$) through the reaction of NO and $O_2^{\cdot -}$. The anion $ONOO^-$ reacts with and oxidizes tetrahydrobiopterin (THB/ BH_4), a cofactor of eNOS [133]. Second, an increased ratio of oxidized glutathione (GSSG)/reduced glutathione (GSH) cause reversible S-glutathionylation and uncoupling of eNOS [134]. Paradoxically, H_2O_2 produced by NADPH oxidase increases eNOS expression and NO production, but this effect is not believed to counteract the effects of oxidative stress [135].

Interestingly, in a scenario of reduced NO levels, in which it would be expected a lack of input signals to PKG activation (e.g., cGMP), the H_2O_2 can cause vasodilation through PKG oxidation [136]. Another target of ROS is the small GTPase RhoA, which when oxidized activates its downstream partner Rho kinase (ROCK), leading to inhibitory phosphorylation of myosin light chain (MLC) phosphatase and, ultimately, to SMC contraction [137, 138]. For a more

explored involvement of ROS in the regulation of signal transduction in the cardiovascular system, check the review of Brown and Griendling [118].

The activating or deactivating switch, in which a group of kinases is active or a group of phosphatases is active, provokes different downstream cascades with consequences in the cellular response. As we described above, several kinases are susceptible to ROS reactions, but also phosphatases are vulnerable to ROS, since they react with a group of amino acids presents in different enzymes. The reaction between ROS and phosphatases causes the oxidation and inhibition of those enzymes, increasing the kinases signaling [139]. Another phosphatase inhibited by ROS is PTEN, which increases the PIP3 signaling, as described above.

A vascular injury promotes an increase in the expression of platelet derived growth factor (PDGF) and PDGF receptor, which in turn cause stimulation for the vascular smooth muscle cells to migrate [140]. The activation of the PDGF receptor is controlled by the action of low molecular weight protein tyrosine phosphatase (LMW-PTP). The Cys12 and Cys17 in LMW-PTP is susceptible to a reaction with ROS resulting in a disulfide bond, and so its inactivation [141]. Therefore, without the LMW-PTP deactivation upon PDGF receptor, its signal is amplified, which generates migration. Oxidized LMW-PTP also increases the Rho family signal, since PDGF receptor is stimulated, and it binds to phospholipase C, Src, and PI3K. As described before, PI3K catalyzes the reaction and formation of PIP3. The Rho-guanine nucleotide exchange factors are activated by PIP3, which triggers Rho-GTPase family members' activation (Rho, Rac, and cdc42). As Nox family is activated by Rac, it produces ROS. Therefore, this process is kept by a positive feedback: generated ROS oxidize Rho in a redox sensitive motif and restrain the LMW-PTP action [118, 138].

Phospholipases are enzymes that hydrolyze phospholipids and generate second messengers involved in the regulation of many physiological functions. Phospholipase A2 (PLA2) cleaves the fatty acyl group at the sn-2 position of the glycerol backbone, releasing arachidonic acid (AA) and lysophospholipid. It was attributed a role for the Ca^{2+} -independent PLA2 (iPLA2) isoform in the excessive production of $\text{O}_2^{\cdot-}$ by primed neutrophils of patients with poorly controlled diabetes. This study suggested that hyperglycemia is related to the activation of iPLA2 and AA formation which, in part, regulate NADPH oxidase activity (i.e., generation of $\text{O}_2^{\cdot-}$) [142].

PLA2 activation has also been related to alterations implicated in the pathogenesis of neurodegenerative diseases, such as neuronal excitation, cognitive and behavioral function, oxidative and nitrosative stress [143]. Phospholipase C (PLC) is a well-known enzyme especially involved in the signaling transduction of GPCR coupled to $\text{G}_{q/11}$ protein and some G protein $\beta\gamma$ subunits (PLC- β), but also in RTK (PLC- γ and PLC- ϵ), Ras and Rho small GTPases (PLC- ϵ) and Ca^{2+} (PLC- δ) signaling pathways, which involves the generation of the phosphate-containing head group inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) through the hydrolysis of the membrane phospholipid PIP2 [144]. The activation of PLC- γ 1 was shown to have an important protective function during mouse embryonic fibroblasts (MEF) response to oxidative stress (H_2O_2) treatment [145]. A further study suggested that this function of PLC- γ 1 involved the PKC-dependent phosphorylation of Bcl-2 and inhibition of caspase-3 [146]. Phospholipase D (PLD) cleaves a phosphodiester bond in membrane-bound lipids, similarly to PLC. However, its activity generates phosphatidic acid (PA) and an alcohol, usually choline or

ethanolamine [147]. A link between oxidative stress and PLD has been proposed by Kim et al. [148], in a study that suggests that H_2O_2 induces rat vascular smooth muscle cells tyrosine kinase activity, and PLD1-dependent PKC- α activation.

In the innate immune system, mononuclear monocytes/macrophages eliminate pathogen, antigen and cellular components through generation of ROS/RNS [149]. When there is an imbalance in the equilibrium between oxidative/nitrosative stress and cellular requirements, the stress can generate pathological complications. Among others, rheumatoid arthritis is an autoimmune disease that has oxidative/nitrosative stress as one of the causes. The cellular immune system is vulnerable to reactions caused by ROS, which in turn can affect the regular physiological process and activates inflammatory signaling pathways that produce pro-inflammatory cytokines, chemokines and prostaglandins. The inflammatory mechanism involves synovial cellular infiltrate and peripheral blood inflammatory cells following by polymorphonuclear neutrophils and lymphocytes culminating in the joint damage [150, 151]. The signaling cascade occurs via activation of NFkB for synthesizing pro-inflammatory cytokines and chemokines [149]. The Th1 cytokines are one of the most important because can provide the development of autoimmune disorders. These cytokines can directly or indirectly promote oxidative stress in the cells, intensifying the rheumatoid arthritis.

Prostaglandins have a pivotal role in the formation of the inflammatory response, since they mediate pathogenic mechanisms and provide the development of the cardinal signs of acute inflammation. Their biosynthesis involves the initial enzyme, phospholipase A2 (PLA2). PLA2 catalyzes the conversion of membrane phospholipids in AA. Then, cyclooxygenases convert AA into prostaglandins. Prostaglandin E2, in particular, rises vasoactive components (histamine, bradykinin, and nitric oxide), hence generating edema, pain and hyperalgesia at the local inflammatory sites, and so the inflammation [152]. ROS stimulate this process through the activation of cyclooxygenases. Prostaglandins, also, activate NADPH oxidase, which produces superoxide anion radical [153]. Therefore, this system becomes cyclic, ROS activate cyclooxygenases and so the prostaglandins biosynthesis, further prostaglandins trigger NADPH oxidases, increasing ROS.

The microRNA (miRNA) is a small noncoding endogenous RNA, that has an important role, since it regulates gene expression. Its function can be modified depending on epigenetic changes, chromosomal abnormalities and oxidative stress. It has been found that miRNA can respond to ROS, implying in its ability to activate certain genes transcription during stress, and this is prominent in cancer cells, which was correlated to the adaptation of these cells to unfavorable and/or hypoxic environment [130, 154, 155]. However, studies showed that some types of miRNAs can regulate gene expression of protective proteins and antioxidant enzymes [156, 157]. Some ROS dependent miRNAs play a role as oncogenic (miR21 and miR155), but interesting miR21 also targets SOD, which can be interpreted that this miRNA regulate the ROS levels in the cell. When miR21 is stimulated, it also affects the immune system through the chemokine CXCL10. CXCL10 adjusts innate and adaptive immune response by activating T lymphocytes, macrophages and inflammatory dendritic cells. The miR155 also has opposite actions, it can be oncogenic (the targets are BCL2, FOXO3a, RhoA) or tumor suppressor (the targets are TGF-beta/SMAD) [158]. The literature about miR155 is vast, and we suggest the articles by Higgs and Slack [158] and Mattiske et al. [159] for a deep reading. Besides these two

miRNAs cited above, others miRNAs are upregulated by ROS, such as miR23, miR200, miR210, etc., affecting migration, invasion; tumor growth, angiogenesis; cell cycle, DNA damage (among others), respectively [126].

In addition to the miRNAs that are ROS upregulated as cited above, there are ROS down-regulated miRNAs important in the carcinogenic process, such as miR34 family. Some miR34 members regulate p53 causing a cell cycle arrest in G1 and apoptosis when DNA is impaired. The miR34a, for example, induce tumor suppression and metastasis inhibition. Another miRNA, miR124, has been shown to be affected by H₂O₂ [160]. This miRNA is correlated to the regulation of tumor cell proliferation, migration and drug resistance through its action upon R-Ras, PI3-KCA, AKT2, ROCK1, Src, DNA methyltransferases and others. The miR199a is also down-regulated by ROS, some of its targets are ERBB2, ERBB3, IKKB, HIF-1alfa, ApoE, CCR7, having an effect upon cell proliferation, invasion, metabolism and metastasis [126, 161]. This is just a

Figure 2. Examples of molecular targets involved in the signal transduction mediated by reactive oxygen species. Abbreviations: AA, arachidonic acid; AP1, activator protein 1; ARE, antioxidant-responsive element; BAD, Bcl-2-associated death promoter; Bcl-2, B-cell lymphoma 2 protein; cGMP, cyclic guanosine monophosphate; DAG, diacylglycerol; eNOS, endothelial nitric oxide synthase; ERK, extracellular-signal regulated kinase; FAK, focal adhesion kinase; FOXO, Forkhead box protein O; GC, guanylyl cyclase; GSK-3, glycogen synthase kinase 3; GST, glutathione S-transferases; HIF-1, hypoxia-inducible factor 1; HO-1, heme oxygenase 1; IP3, inositol 1,4,5-triphosphate; LMW-PTP, low molecular weight phosphotyrosine protein phosphatase; MT-1, metallothionein-1; MT-2, metallothionein-2; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-kappa B; NO, nitric oxide; NQO1, NAD(P)H:quinone oxidoreductase; Nrf2, nuclear-factor erythroid-2 related factor; O₂^{•-}, superoxide anion radical; p130Cas, p130 Crk-associated substrate; p53, p53 tumor suppressor protein; p70S6K1, p70S6 kinase 1; PDK, phosphoinositide-dependent kinase; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-triphosphate; PKB/AKT, protein kinase B; PKC, protein kinase C; PKC, protein kinase C; PKG, cGMP-dependent protein kinases; PLA2, phospholipase A2; PLC, phospholipase C; PLD, phospholipase D; PTEN, phosphatase and tensin homolog deleted on chromosome 10; RhoA, Ras homolog family member A; ROCK, Rho-associated protein kinase; SAPK/JNK, stress-activated protein kinase or c-Jun N-terminal kinase; SOD, superoxide dismutase; STAT3, signal transducer and activator of transcription 3; γ-GCS, gamma-glutamylcysteine synthetase.

summary of some important miRNAs and their responses in carcinogenesis, for more information check the review Mu and Liu [126].

As previously discussed in this chapter, cells have a repertoire of antioxidant molecules and enzymes as a defense mechanism to an increase in ROS production. However, oxidative stress takes place when the antioxidant capacity is overwhelmed by reactive species production. In this scenario, to maintain cell homeostasis and/or terminate the ROS signal transduction there are some stress sensors that regulate the translation of antioxidant proteins. The antioxidant responsive element (ARE) is a region of non-coding DNA (short consensus sequence) which is localized upstream and regulates the transcription of many antioxidant neighboring genes such as glutathione S-transferases (GST), NAD(P)H:quinone oxidoreductase (NQO1) [162], heme oxygenase 1 (HO-1), γ -glutamylcysteine synthetase (γ -GCS) [163], metallothionein-1 and -2 (MT-1 and MT-2) [164], and SOD [165].

It was shown that ARE induction protected against oxidative stress mediated by 6- hydroxydopamine in vitro, a mitochondrial inhibitor used to model Parkinson's disease [166]. The nuclear-factor erythroid-2 related factor (Nrf2) is a central transcription factor involved in the upregulation of ARE-containing genes and, consequently, synthesis of proteins with antioxidant function. However, there are also nuclear factors that negatively regulate ARE-mediated gene expression, such as Mafs (MafG and MafK), large Maf (c-Maf), c-Fos, and Fra1 [163].

Finally, in this section, we showed an overview of processes regulated by fluctuating levels of ROS and their molecular sensors. Furthermore, we showed that in response to oxidative stress and to maintain homeostasis, cells can upregulate the synthesis of antioxidant defenses (Figure 2).

Acknowledgements

H.U.'s research is supported by the São Paulo Research Foundation (FAPESP proj. No. 2012/50880-4).

Author details

Margarete Dulce Bagatini^{1*}, Jeandre Augusto dos Santos Jaques², Carla Santos de Oliveira², Graciele Almeida de Oliveira³, Micheli Mainardi Pillat³, Aline Mânica⁴, Cintia dos Santos Moser¹, Lucas Derbocio dos Santos² and Henning Ulrich³

*Address all correspondence to: margaretebagatini@yahoo.com.br

1 Universidade Federal da Fronteira Sul, Chapecó, SC, Brazil

2 Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil

3 Universidade de São Paulo, São Paulo, SP, Brazil

4 Universidade Federal de Santa Maria, Santa Maria, RS, Brazil

References

- [1] Frey TG, Mannella CA. The internal structure of mitochondria. *Trends in Biochemical Sciences* [Internet]. 2000;**25**(7):319-324. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10871882
- [2] Nicholls DG, Locke RM. Thermogenic mechanisms in brown fat. *Physiological Reviews* [Internet]. 1984;**64**(1):1-64. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=6320232
- [3] Kowaltowski AJ, Vercesi AE. Mitochondrial damage induced by conditions of oxidative stress. *Free Radical Biology & Medicine* [Internet]. 1999;**26**(3-4):463-471. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/9895239>
- [4] Kowaltowski AJ. Alternative mitochondrial functions in cell physiopathology: Beyond ATP production. *Brazilian Journal of Medical and Biological Research* [Internet]. 2000;**33**(2):241-250. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10657067
- [5] Lemasters JJ, Nieminen AL, Qian T, Trost LC, Elmore SP, Nishimura Y, et al. The mitochondrial permeability transition in cell death: A common mechanism in necrosis, apoptosis and autophagy. *Biochimica et Biophysica Acta* [Internet]. 1998;**1366**(1-2):177-196. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/9714796>
- [6] Susin SA, Zamzami N, Kroemer G. Mitochondria as regulators of apoptosis: Doubt no more. *Biochimica et Biophysica Acta* [Internet]. 1998;**1366**(1-2):151-165. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/9714783>
- [7] Green DR, Reed JC. Mitochondria and apoptosis. *Science* (80-) [Internet]. 1998;**281**(5381):1309-1312. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9721092
- [8] Kalyanaraman B, Cheng G, Hardy M, Ouari O, Lopez M, Joseph J, et al. A review of the basics of mitochondrial bioenergetics, metabolism, and related signaling pathways in cancer cells: Therapeutic targeting of tumor mitochondria with lipophilic cationic compounds. *Redox Biology* [Internet]. 2017/09/29. 2018 Apr;**14**:316-327. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29017115>
- [9] Griffiths EJ, Halestrap AP. Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion. *The Biochemical Journal* [Internet]. 1995;**307**(Pt 1):93-98. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7717999
- [10] Halestrap AP, Kerr PM, Javadov S, Woodfield KY. Elucidating the molecular mechanism of the permeability transition pore and its role in reperfusion injury of the heart. *Biochimica et Biophysica Acta* [Internet]. 1998;**1366**(1-2):79-94. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9714750

- [11] Friberg H, Ferrand-Drake M, Bengtsson F, Halestrap AP, Wieloch T. Cyclosporin A, but not FK 506, protects mitochondria and neurons against hypoglycemic damage and implicates the mitochondrial permeability transition in cell death. *The Journal of Neuroscience* [Internet]. 1998;**18**(14):5151-5159. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9651198
- [12] Charni S, de Bettignies G, Rathore MG, Aguilo JI, van den Elsen PJ, Haouzi D, et al. Oxidative phosphorylation induces de novo expression of the MHC class I in tumor cells through the ERK5 pathway. *Journal of Immunology* [Internet]. 2010/08/20. 2010 Sep 15;**185**(6):3498-3503. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/20729331>
- [13] D'Souza GGM, Wagle MA, Saxena V, Shah A. Approaches for targeting mitochondria in cancer therapy. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* [Internet]. 2010/08/21. 2011 Jun;**1807**(6):689-696. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/20732297>
- [14] Bek T. Mitochondrial dysfunction and diabetic retinopathy. *Mitochondrion* [Internet]. 2016/07/22. 2017 Sep;**36**:4-6. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27456429>
- [15] Dey A, Swaminathan K. Hyperglycemia-induced mitochondrial alterations in liver. *Life Sciences* [Internet]. 2010/06/17. 2010 Aug;**87**(7-8):197-214. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/20600152>
- [16] Tseng Y-H, Cypess AM, Kahn CR. Cellular bioenergetics as a target for obesity therapy. *Nature Reviews. Drug Discovery* [Internet]. 2010 Jun;**9**(6):465-482. Available from: <http://www.nature.com/doifinder/10.1038/nrd3138>
- [17] Pickrell AM, Moraes CT. Protein Misfolding and Cellular Stress in Disease and Aging. Totowa, NJ: Humana Press; 2010
- [18] Cassina P, Cassina A, Pehar M, Castellanos R, Gandelman M, de Leon A, et al. Mitochondrial dysfunction in SOD1G93A-bearing astrocytes promotes motor neuron degeneration: Prevention by mitochondrial-targeted antioxidants. *The Journal of Neuroscience* [Internet]. 2008 Apr 16;**28**(16):4115-4122. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18417691
- [19] Rose J, Brian C, Woods J, Pappa A, Panayiotidis MI, Powers R, et al. Mitochondrial dysfunction in glial cells: Implications for neuronal homeostasis and survival. *Toxicology*. 2017 Nov;**391**:109-115. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28655545>
- [20] Nicholls DG, Ferguson SJ. *Bioenergetics 4* [Internet]. 4th ed. Amsterdam: Elsevier Academic Press; 2013. xiv, 419 pp. Available from: <http://www.sciencedirect.com/science/book/9780123884251>
- [21] Mráček T, Drahota Z, Houštěk J. The function and the role of the mitochondrial glycerol-3-phosphate dehydrogenase in mammalian tissues. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* [Internet]. 2012/12/07. 2013 Mar;**1827**(3):401-410. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/23220394>

- [22] de Oliveira GA. Caloric Restriction and Mitochondria: Role in *Saccharomyces cerevisiae* aging [doctoral thesis]; 2010. DOI: 10.11606/T.46.2010.tde-01032011-114941. Available from: <http://www.teses.usp.br/teses/disponiveis/46/46131/tde-01032011-114941/en.php>
- [23] Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. *Physiological Reviews* [Internet]. 1979;**59**(3):527-605. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/37532>
- [24] Turrens JF, Boveris A. Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. *The Biochemical Journal* [Internet]. 1980;**191**(2):421-427. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=6263247
- [25] Boveris A, Cadenas E, Stoppani AO. Role of ubiquinone in the mitochondrial generation of hydrogen peroxide. *The Biochemical Journal* [Internet]. 1976;**156**(2):435-444. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/182149>
- [26] Quinlan CL, Gerencser AA, Treberg JR, Brand MD. The mechanism of superoxide production by the antimycin-inhibited mitochondrial Q-cycle. *The Journal of Biological Chemistry* [Internet]. 2011/06/27. 2011 Sep 9;**286**(36):31361-31372. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/21708945>
- [27] Bleier L, Dröse S. Superoxide generation by complex III: From mechanistic rationales to functional consequences. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* [Internet]. 2012/12/23. 2013 Nov;**1827**(11-12):1320-1331. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/23269318>
- [28] Oliveira GA, Kowaltowski AJ. Phosphate increases mitochondrial reactive oxygen species release. *Free Radical Research* [Internet]. 2004 Oct 7;**38**(10):1113-1118. Available from: <http://www.tandfonline.com/doi/full/10.1080/10715760400009258>
- [29] Muller FL, Liu Y, Van Remmen H. Complex III releases superoxide to both sides of the inner mitochondrial membrane. *The Journal of Biological Chemistry* [Internet]. 2004/08/17. 2004 Nov 19;**279**(47):49064-49073. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/15317809>
- [30] Yankovskaya V. Architecture of succinate dehydrogenase and reactive oxygen species generation. *Science* (80-) [Internet]. 2003 Jan 31;**299**(5607):700-704. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/12560550>
- [31] Liu Y, Fiskum G, Schubert D. Generation of reactive oxygen species by the mitochondrial electron transport chain. *Journal of Neurochemistry* [Internet]. 2002;**80**(5):780-787. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/11948241>
- [32] Starkov AA. Mitochondrial alpha-ketoglutarate dehydrogenase complex generates reactive oxygen species. *The Journal of Neuroscience* [Internet]. 2004 Sep 8;**24**(36):7779-7788. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15356189

- [33] Tretter L. Generation of reactive oxygen species in the reaction catalyzed by α -ketoglutarate dehydrogenase. The Journal of Neuroscience [Internet]. 2004 Sep 8;24(36):7771-7778. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15356188
- [34] Tahara EB, Barros MH, Oliveira GA, Netto LES, Kowaltowski AJ. Dihydrolipoyl dehydrogenase as a source of reactive oxygen species inhibited by caloric restriction and involved in *Saccharomyces cerevisiae* aging. The FASEB Journal [Internet]. 2006 Nov 29;21(1):274-283. Available from: <http://www.fasebj.org/cgi/doi/10.1096/fj.06-6686com>
- [35] Thomas DD, Heinecke JL, Ridnour LA, Cheng RY, Kesarwala AH, Switzer CH, et al. Signaling and stress: The redox landscape in NOS2 biology. Free Radical Biology & Medicine [Internet]. 2015 Oct;87:204-225. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26117324>
- [36] de Oliveira GA, Cheng RYS, Ridnour LA, Basudhar D, Somasundaram V, McVicar DW, et al. Inducible nitric oxide synthase in the carcinogenesis of gastrointestinal cancers. Antioxidants & Redox Signaling [Internet]. 2016/10/31. 2017 Jun 20;26(18):1059-1077. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27494631>
- [37] Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 4th ed. Oxford, New York: Oxford University Press; 2007. xxxvi, 851 p
- [38] Stamler JS. Redox signaling: Nitrosylation and related target interactions of nitric oxide. Cell [Internet]. 1994;78(6):931-936. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/7923362>
- [39] Chakraborti S, Chakraborti T. Down-regulation of protein kinase C attenuates the oxidant hydrogen peroxide-mediated activation of phospholipase A2 in pulmonary vascular smooth muscle cells. Cellular Signalling [Internet]. 1995;7(1):75-83. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/7756114>
- [40] Tournier C, Thomas G, Pierre J, Jacquemin C, Pierre M, Saunier B. Mediation by arachidonic acid metabolites of the H₂O₂-induced stimulation of mitogen-activated protein kinases (extracellular-signal-regulated kinase and c-Jun NH₂-terminal kinase). European Journal of Biochemistry [Internet]. 1997;244(2):587-595. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/9119028>
- [41] Kim JH, Kwack HJ, Choi SE, Kim BC, Kim YS, Kang IJ, et al. Essential role of Rac GTPase in hydrogen peroxide-induced activation of c-fos serum response element. FEBS Letters [Internet]. 1997;406(1-2):93-96. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/9109393>
- [42] Sena LA, Chandel NS. Physiological roles of mitochondrial reactive oxygen species. Molecular Cell [Internet]. 2012 Oct;48(2):158-167. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/23102266>
- [43] Rharass T, Lantow M, Gbankoto A, Weiss DG, Panáková D, Lucas S. Ascorbic acid alters cell fate commitment of human neural progenitors in a WNT/ β -catenin/ROS signaling

- dependent manner. *Journal of Biomedical Science* [Internet]. 2017/10/16. 2017 Dec 16;**24**(1):78. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29037191>
- [44] Lesko SA, Lorentzen RJ, Ts'o PO. Role of superoxide in deoxyribonucleic acid strand scission. *Biochemistry* [Internet]. 1980;**19**(13):3023-3028. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/6249344>
- [45] Cadet J, Wagner JR. DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation. *Cold Spring Harbor Perspectives in Biology* [Internet]. 2013/02/01. 2013 Feb 1;**5**(2):a012559-a012559. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/23378590>
- [46] Markkanen E. Not breathing is not an option: How to deal with oxidative DNA damage. *DNA Repair (Amst)* [Internet]. 2017/09/22. 2017 Nov;**59**:82-105. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28963982>
- [47] Samardzic K, Rodgers KJ. Oxidised protein metabolism: Recent insights. *Biological Chemistry* [Internet]. 2017 Jan 26;**398**(11):1165-1175. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28600903>
- [48] Eckl PM, Bresgen N. Genotoxicity of lipid oxidation compounds. *Free Radical Biology & Medicine* [Internet]. 2017/02/05. 2017 Oct;**111**:244-252. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28167130>
- [49] Cheignon C, Tomas M, Bonnefont-Rousselot D, Faller P, Hureau C, Collin F. Oxidative stress and the amyloid beta peptide in Alzheimer's disease. *Redox Biology* [Internet]. 2017/10/18. 2017 Oct;**14**:450-464. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29080524>
- [50] Li H, Horke S, Förstermann U. Vascular oxidative stress, nitric oxide and atherosclerosis. *Atherosclerosis* [Internet]. 2014/09/09. 2014 Nov;**237**(1):208-219. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/25244505>
- [51] Lucas AM, Caldas FR, da Silva AP, Ventura MM, Leite IM, Filgueiras AB, et al. Diazoxide prevents reactive oxygen species and mitochondrial damage, leading to anti-hypertrophic effects. *Chemico-Biological Interactions* [Internet]. 2016/11/17. 2017 Jan;**261**:50-55. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27867086>
- [52] Griffiths HR, Gao D, Pararasa C. Redox regulation in metabolic programming and inflammation. *Redox Biology* [Internet]. 2017/02/12. 2017 Aug;**12**:50-57. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28212523>
- [53] Medvedev ZA. An attempt at a rational classification of theories of ageing. *Biological Reviews of the Cambridge Philosophical Society* [Internet]. 1990;**65**(3):375-398. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2205304
- [54] Harman D. Aging: A theory based on free radical and radiation chemistry. *Journal of Gerontology* [Internet]. 1956;**11**(3):298-300. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/13332224>

- [55] Harman D. Free radical theory of aging: An update: Increasing the functional life span. *Annals of the New York Academy of Sciences* [Internet]. 2006 May 1;**1067**(1):10-21. Available from: <http://doi.wiley.com/10.1196/annals.1354.003>
- [56] Barros MH, da Cunha FM, Oliveira GA, Tahara EB, Kowaltowski AJ. Yeast as a model to study mitochondrial mechanisms in ageing. *Mechanisms of Ageing and Development* [Internet]. 2010 Jul;**131**(7-8):494-502. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20450928
- [57] Barros MH, Bandy B, Tahara EB, Kowaltowski AJ. Higher respiratory activity decreases mitochondrial reactive oxygen release and increases life span in *Saccharomyces cerevisiae*. *The Journal of Biological Chemistry* [Internet]. 2004 Nov 26;**279**(48):49883-49888. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15383542
- [58] Nisoli E. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science (80-)* [Internet]. 2005 Oct 14;**310**(5746):314-317. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16224023>
- [59] Oliveira GA, Tahara EB, Gombert AK, Barros MH, Kowaltowski AJ. Increased aerobic metabolism is essential for the beneficial effects of caloric restriction on yeast life span. *Journal of Bioenergetics and Biomembranes* [Internet]. 2008 Aug 15;**40**(4):381-388. Available from: <http://link.springer.com/10.1007/s10863-008-9159-5>
- [60] Caldeira da Silva CC, Cerqueira FM, Barbosa LF, Medeiros MHG, Kowaltowski AJ. Mild mitochondrial uncoupling in mice affects energy metabolism, redox balance and longevity. *Aging Cell* [Internet]. 2008 Aug;**7**(4):552-560. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18505478
- [61] López-Otín C, Galluzzi L, Freije JMP, Madeo F, Kroemer G. Metabolic control of longevity. *Cell* [Internet]. 2016 Aug;**166**(4):802-821. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27518560>
- [62] Castilho RF, Kowaltowski AJ, Meinicke A, Bechara EJH, Vercesi AE. Permeabilization of the inner mitochondrial membrane by Ca^{2+} ions is stimulated by t-butyl hydroperoxide and mediated by reactive oxygen species generated by mitochondria. *Free Radical Biology & Medicine* [Internet]. 1995 Mar;**18**(3):479-486. Available from: <http://linkinghub.elsevier.com/retrieve/pii/089158499400166H>
- [63] Castilho RF, Kowaltowski AJ, Vercesi AE. The irreversibility of inner mitochondrial membrane permeabilization by Ca^{2+} plus prooxidants is determined by the extent of membrane protein thiol cross-linking. *Journal of Bioenergetics and Biomembranes* [Internet]. 1996;**28**(6):523-529. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8953384
- [64] Kowaltowski AJ, Castilho RF, Grijalba MT, Bechara EJH, Vercesi AE. Effect of inorganic phosphate concentration on the nature of inner mitochondrial membrane alterations

- mediated by Ca ions. *The Journal of Biological Chemistry* [Internet]. 1996 Feb 9; **271**(6):2929-2934. Available from: <http://www.jbc.org/lookup/doi/10.1074/jbc.271.6.2929>
- [65] Kowaltowski AJ, Castilho RF, Vercesi AE. Opening of the mitochondrial permeability transition pore by uncoupling or inorganic phosphate in the presence of Ca^{2+} is dependent on mitochondrial-generated reactive oxygen species. *FEBS Letters*. 1996; **378**:150-152.
- [66] Costa RAP, Romagna CD, Pereira JL, Souza-Pinto NC. The role of mitochondrial DNA damage in the cytotoxicity of reactive oxygen species. *Journal of Bioenergetics and Biomembranes* [Internet]. 2011 Feb 1; **43**(1):25-29. Available from: <http://link.springer.com/10.1007/s10863-011-9329-8>
- [67] Mohiuddin I, Chai H, Lin PH, Lumsden AB, Yao Q, Chen C. Nitrotyrosine and chlorotyrosine: Clinical significance and biological functions in the vascular system. *The Journal of Surgical Research* [Internet]. 2006 Jun; **133**(2):143-149. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0022480405005445>
- [68] Viappiani S. Detection of specific nitrotyrosine-modified proteins as a marker of oxidative stress in cardiovascular disease. *American Journal of Physiology. Heart and Circulatory Physiology* [Internet]. 2006 Jun 1; **290**(6):H2167-H2168. Available from: <http://ajpheart.physiology.org/cgi/doi/10.1152/ajpheart.00128.2006>
- [69] Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiological Reviews* [Internet]. 2007 Jan 1; **87**(1):315-424. Available from: <http://physrev.physiology.org/cgi/doi/10.1152/physrev.00029.2006>
- [70] Wall SB, Oh J-Y, Diers AR, Landar A. Oxidative modification of proteins: An emerging mechanism of cell signaling. *Frontiers in Physiology* [Internet]. 2012; **3**(September):1-9. Available from: <http://journal.frontiersin.org/article/10.3389/fphys.2012.00369/abstract>
- [71] Bridge G, Rashid S, Martin S. DNA mismatch repair and oxidative DNA damage: Implications for cancer biology and treatment. *Cancers (Basel)* [Internet]. 2014 Aug 5; **6**(3):1597-1614. Available from: <http://www.mdpi.com/2072-6694/6/3/1597/>
- [72] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* [Internet]. 1979 Jun; **95**(2):351-358. Available from: <http://linkinghub.elsevier.com/retrieve/pii/0003269779907383>
- [73] Lü J-M, Lin PH, Yao Q, Chen C. Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems. *Journal of Cellular and Molecular Medicine* [Internet]. 2010 Apr; **14**(4):840-860. Available from: <http://doi.wiley.com/10.1111/j.1582-4934.2009.00897.x>
- [74] Tu BP, Weissman JS. Oxidative protein folding in eukaryotes: Figure 1. *The Journal of Cell Biology* [Internet]. 2004 Feb 2; **164**(3):341-346. Available from: <http://www.jcb.org/lookup/doi/10.1083/jcb.200311055>
- [75] Jones DP. Cysteine/cystine couple is a newly recognized node in the circuitry for biologic redox signaling and control. *The FASEB Journal* [Internet]. 2004 Jun 18; **18**:1246-1248. Available from: <http://www.fasebj.org/cgi/doi/10.1096/fj.03-0971fje>

- [76] Poole LB, Karplus PA, Claiborne A. Protein sulfenic acids in redox signaling. *Annual Review of Pharmacology and Toxicology* [Internet]. 2004 Feb 10;44(1):325-347. Available from: <http://www.annualreviews.org/doi/10.1146/annurev.pharmtox.44.101802.121735>
- [77] Berndt C, Lillig CH, Holmgren A. Thiol-based mechanisms of the thioredoxin and glutaredoxin systems: Implications for diseases in the cardiovascular system. *American Journal of Physiology. Heart and Circulatory Physiology* [Internet]. 2006 Oct 20;292(3):H1227-H1236. Available from: <http://ajpheart.physiology.org/cgi/doi/10.1152/ajpheart.01162.2006>
- [78] Cooke MS. Oxidative DNA damage: Mechanisms, mutation, and disease. *The FASEB Journal* [Internet]. 2003 Jul 1;17(10):1195-1214. Available from: <http://www.fasebj.org/cgi/doi/10.1096/fj.02-0752rev>
- [79] Evans MD, Dizdaroglu M, Cooke MS. Oxidative DNA damage and disease: Induction, repair and significance. *Mutation Research*. 2004 Sep;567(1):1-61. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S138357420300139X>
- [80] Dizdaroglu M. Oxidatively induced DNA damage: Mechanisms, repair and disease. *Cancer Letters* [Internet]. 2012 Dec;327(1-2):26-47. Available from: <http://dx.doi.org/10.1016/j.canlet.2012.01.016>
- [81] Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, et al. Patterns of somatic mutation in human cancer genomes. *Nature* [Internet]. 2007 Mar 8;446(7132):153-158. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2712719&tool=pmcentrez&rendertype=abstract>
- [82] Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: Origins, consequences, and clinical use. *Cold Spring Harbor Perspectives in Biology* [Internet]. 2010 Jan 1;2(1):a001008-a001008. Available from: <http://cshperspectives.cshlp.org/lookup/doi/10.1101/cshperspect.a001008>
- [83] Girotti AW. Action in biological systems. *Journal of Lipid Research*. 1998;39:1529-1542
- [84] Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. *Proceedings of the National Academy of Sciences* [Internet]. 1994 Nov 8;91(23):10771-10778. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/7971961%5Chttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC45108>
- [85] Dianzani MU. 4-Hydroxynonenal from pathology to physiology. *Molecular Aspects of Medicine* [Internet]. 2003 Aug;24(4-5):263-272. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0098299703000219>
- [86] Porter NA, Caldwell SE, Mills KA. Mechanisms of free radical oxidation of unsaturated lipids. *Lipids* [Internet]. 1995 Apr;30(4):277-290. Available from: <http://link.springer.com/10.1007/BF02536034>
- [87] Lorrain B, Dangles O, Loonis M, Armand M, Dufour C. Dietary iron-initiated lipid oxidation and its inhibition by polyphenols in gastric conditions. *Journal of Agricultural*

- and Food Chemistry [Internet]. 2012 Sep 12;**60**(36):9074-9081. Available from: <http://pubs.acs.org/doi/abs/10.1021/jf302348s>
- [88] Getzoff ED, Tainer JA, Weiner PK, Kollman PA, Richardson JS, Richardson DC. Electrostatic recognition between superoxide and copper, zinc superoxide dismutase. *Nature* [Internet]. 1983 Nov 17;**306**(5940):287-290. Available from: <http://www.nature.com/doi/abs/10.1038/306287a0>
- [89] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology & Medicine* [Internet]. 1991 Jan;**11**(1):81-128. Available from: <http://linkinghub.elsevier.com/retrieve/pii/0891584991901926>
- [90] Kadiiska MB, Basu S, Brot N, Cooper C, Saari Csallany A, Davies MJ, et al. Biomarkers of oxidative stress study V: Ozone exposure of rats and its effect on lipids, proteins, and DNA in plasma and urine. *Free Radical Biology & Medicine* [Internet]. 2013 Aug;**61**:408-415. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3968235/%5Cnhttp://www.ncbi.nlm.nih.gov/pmc/articles/PMC3968235/pdf/nihms525937.pdf%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/23608465>
- [91] Kourie JL. Interaction of reactive oxygen species with ion transport mechanisms. *The American Journal of Physiology*. 1998 Nov;**275**(1):1-11. Available from: <http://ajpcell.physiology.org/cgi/doi/10.1152/ajpcell.00167.2002>
- [92] Catalá A. Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. *Chemistry and Physics of Lipids* [Internet]. 2009 Jan;**157**(1):1-11. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0009308408003708>
- [93] Siegel MP, Kruse SE, Knowels G, Salmon A, Beyer R, Xie H, et al. Reduced coupling of oxidative phosphorylation in vivo precedes electron transport chain defects due to mild oxidative stress in mice. *Vina J, editor. PLoS One* [Internet]. 2011 Nov 22;**6**(11):e26963. Available from: <http://dx.plos.org/10.1371/journal.pone.0026963>
- [94] Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, et al. Oxidative stress, prooxidants, and antioxidants: The interplay. *BioMed Research International* [Internet]. 2014;**2014**:1-19. Available from: <http://www.hindawi.com/journals/bmri/2014/761264/>
- [95] Mironczuk-Chodakowska I, Witkowska AM, Zujko ME. Endogenous non-enzymatic antioxidants in the human body. *Advances in Medical Sciences* [Internet]. 2018 Mar;**63**(1):68-78. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S1896112617300445>
- [96] Godic A, Poljšak B, Adamic M, Dahmane R. The role of antioxidants in skin cancer prevention and treatment. *Oxidative Medicine and Cellular Longevity* [Internet]. 2014;**2014**:1-6. Available from: <http://www.hindawi.com/journals/omcl/2014/860479/>
- [97] Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry* [Internet]. 2015 Jun;**97**:55-74. Available from: <http://dx.doi.org/10.1016/j.ejmech.2015.04.040>

- [98] Lei XG, Zhu J-H, Cheng W-H, Bao Y, Ho Y-S, Reddi AR, et al. Paradoxical roles of antioxidant enzymes: Basic mechanisms and health implications. *Physiological Reviews* [Internet]. 2016 Jan 17;**96**(1):307-364. Available from: <http://physrev.physiology.org/lookup/doi/10.1152/physrev.00010.2014>
- [99] Dey S, Sidor A, O'Rourke B. Compartment-specific control of reactive oxygen species scavenging by antioxidant pathway enzymes. *The Journal of Biological Chemistry* [Internet]. 2016 May 20;**291**(21):11185-11197. Available from: <http://www.jbc.org/lookup/doi/10.1074/jbc.M116.726968>
- [100] Marrocco I, Altieri F, Peluso I. Measurement and clinical significance of biomarkers of oxidative stress in humans. *Oxidative Medicine and Cellular Longevity* [Internet]. 2017;**2017**:1-32. Available from: <https://www.hindawi.com/journals/omcl/2017/6501046/>
- [101] Barbosa KBF, Costa NMB, Alfenas R de CG, De Paula SO, Minim VPR, Bressan J. Estresse oxidativo: conceito, implicações e fatores modulatórios. *Revista de Nutrição* [Internet]. 2010 Aug;**23**(4):629-643. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1415-52732010000400013&lng=pt&tlng=pt
- [102] Manganaris GA, Goulas V, Vicente AR, Terry LA. Berry antioxidants: Small fruits providing large benefits. *Journal of the Science of Food and Agriculture* [Internet]. 2014 Mar 30;**94**(5):825-833. Available from: <http://doi.wiley.com/10.1002/jsfa.6432>
- [103] King A, Young G. Characteristics and occurrence of phenolic phytochemicals. *Journal of the American Dietetic Association* [Internet]. 1999 Feb;**99**(2):213-218. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0002822399000516>
- [104] Angelo PM, Jorge N. Compostos fenólicos em alimentos – Uma breve revisão Phenolic compounds in foods – A brief review. *Revista do Instituto Adolfo Lutz*. 2007;**66**(1):1-9
- [105] Fan G-J, Jin X-L, Qian Y-P, Wang Q, Yang R-T, Dai F, et al. Hydroxycinnamic acids as DNA-cleaving agents in the presence of Cu II ions: Mechanism, structure-activity relationship, and biological implications. *Chemistry - A European Journal* [Internet]. 2009 Nov 23;**15**(46):12889-12899. Available from: <http://doi.wiley.com/10.1002/chem.200901627>
- [106] Gülçin İ. Antioxidant activity of food constituents: An overview. *Archives of Toxicology* [Internet]. 2012 Mar 20;**86**(3):345-391. Available from: <http://link.springer.com/10.1007/s00204-011-0774-2>
- [107] Majewska-Wierzbicka M, Cieczot H. Flavonoids in the prevention and treatment of cardiovascular diseases. *Polski Merkuriusz Lekarski*. 2012;**32**(188):50-54
- [108] Moreira AVB, Mancini Filho J. Atividade antioxidante das especiarias mostarda, canela e erva-doce em sistemas aquoso e lipídico. *Nutrire*. 2003;**25**:31-46
- [109] da Rosa JS, Godoy RL de O, Oiano Neto J, et al. Desenvolvimento de um método de análise de vitamina C em alimentos por cromatografia líquida de alta eficiência e exclusão iônica. *Ciência e Tecnologia de Alimentos* [Internet]. 2007 Dec;**27**(4):837-846. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0101-20612007000400025&lng=en&nrm=iso&tlng=pt

- [110] Prior RL. Oxygen radical absorbance capacity (ORAC): New horizons in relating dietary antioxidants/bioactives and health benefits. *Journal of Functional Foods* [Internet]. 2015 Oct;**18**:797-810. Available from: <http://dx.doi.org/10.1016/j.jff.2014.12.018>
- [111] Vannucchi H, Melo SS. Hiper-homocisteinemia e risco cardiometabólico. *Arquivos Brasileiros de Endocrinologia e Metabologia*. 2009;**53**(5):540-549
- [112] Rodriguez-Amaya DB. *Food Carotenoids: Chemistry, Biology, and Technology*. IFT Press/Wiley Blackwell; 2016
- [113] Morita M, Naito Y, Yoshikawa T, Niki E. Rapid assessment of singlet oxygen-induced plasma lipid oxidation and its inhibition by antioxidants with diphenyl-1-pyrenylphosphine (DPPP). *Analytical and Bioanalytical Chemistry* [Internet]. 2016 Jan 14;**408**(1):265-270. Available from: <http://link.springer.com/10.1007/s00216-015-9102-7>
- [114] Takahashi S, Iwasaki-Kino Y, Aizawa K, Terao J, Mukai K. Development of a Singlet Oxygen Absorption Capacity (SOAC) assay method. Measurements of the SOAC values for carotenoids and α -tocopherol in an aqueous Triton X-100 micellar solution. *Journal of Agricultural and Food Chemistry*. 2017 Feb;**65**(4):784-792. Available from: <http://pubs.acs.org/doi/abs/10.1021/acs.jafc.6b04329>
- [115] Thomas B, Murray BG, Murphy DJ. *Encyclopedia of Applied Plant Sciences*. Elsevier, 2; 2017
- [116] El-Agamey A, Lowe GM, McGarvey DJ, Mortensen A, Phillip DM, Truscott TG, et al. Carotenoid radical chemistry and antioxidant/pro-oxidant properties. *Archives of Biochemistry and Biophysics* [Internet]. 2004 Oct;**430**(1):37-48. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0003986104001468>
- [117] Leonard SS, Harris GK, Shi X. Metal-induced oxidative stress and signal transduction. *Free Radical Biology & Medicine* [Internet]. 2004 Dec;**37**(12):1921-1942. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0891584904007191>
- [118] Brown DI, Griendling KK. Regulation of signal transduction by reactive oxygen species in the cardiovascular system. *Circulation Research* [Internet]. 2015 Jan 30;**116**(3):531-549. Available from: <http://circres.ahajournals.org/cgi/doi/10.1161/CIRCRESAHA.116.303584>
- [119] Nakanishi A. Link between PI3K/AKT/PTEN pathway and NOX protein in diseases. *Aging and Disease* [Internet]. 2014 Jun 1;**5**(3):203. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24900943>
<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4037312>
- [120] Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, et al. Mechanism of activation of protein kinase B by insulin and IGF-1. *The EMBO Journal*. 1996;**15**(23):6541-6551. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/8978681>
- [121] Gào X, Schöttker B. Reduction–oxidation pathways involved in cancer development: A systematic review of literature reviews. *Oncotarget* [Internet]. 2017 Jul 31. Available from: <http://www.oncotarget.com/fulltext/17128>

- [122] Park K-R, Nam D, Yun H-M, Lee S-G, Jang H-J, Sethi G, et al. β -Caryophyllene oxide inhibits growth and induces apoptosis through the suppression of PI3K/AKT/mTOR/S6K1 pathways and ROS-mediated MAPKs activation. *Cancer Letters* [Internet]. 2011 Dec; **312**(2):178-188. Available from: <http://dx.doi.org/10.1016/j.canlet.2011.08.001>
- [123] Zhang J, Wang X, Vikash V, Ye Q, Wu D, Liu Y, et al. ROS and ROS-mediated cellular signaling. *Oxidative Medicine and Cellular Longevity* [Internet]. 2016; **2016**(Figure 1):1-18. Available from: <http://www.hindawi.com/journals/omcl/2016/4350965/>
- [124] Franke TF, Kaplan DR, Cantley LC. PI3K: Downstream AKTion blocks apoptosis. *Cell* [Internet]. 1997 Feb; **88**(4):435-437. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0092867400818838>
- [125] Parsons SJ, Parsons JT. Src family kinases, key regulators of signal transduction. *Oncogene* [Internet]. 2004 Oct 18; **23**(48):7906-7909. Available from: <http://www.nature.com/doifinder/10.1038/sj.onc.1208160>
- [126] Mu W, Liu L-Z. Reactive oxygen species signaling in cancer development. *Reactive Oxygen Species* [Internet]. 2017; **2**(1):219-230. Available from: <https://www.aimscl.com/ros/index.php/ros/article/view/95>
- [127] Jones RJ, Brunton VG, Frame MC. Adhesion-linked kinases in cancer; emphasis on Src, focal adhesion kinase and PI 3-kinase. *European Journal of Cancer* [Internet]. 2000 Aug; **36**(13):1595-1606. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0959804900001532>
- [128] Lai YH, Chen MH, Lin SY, Lin SY, Wong YH, Yu SL, et al. Rhodomycin A, a novel Src-targeted compound, can suppress lung cancer cell progression via modulating Src-related pathways. *Oncotarget* [Internet]. 2015; **6**(28):26252-26265. Available from: <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=medl&AN=26312766%5Cnhttp://nt2yt7px7u.search.serialssolutions.com/?sid=OVID:Ovid+MEDLINE%28R%29+%3C2013+to+April+Week+3+2017%3E&genre=article&id=pmid:26312766&id=doi:10.18632/oncotarget>
- [129] Patel A, Sabbineni H, Clarke A, Somanath PR. Novel roles of Src in cancer cell epithelial-to-mesenchymal transition, vascular permeability, microinvasion and metastasis. *Life Sciences* [Internet]. 2016 Jul; **157**:52-61. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0024320516303344>
- [130] Lin S-Y, Chang H-H, Lai Y-H, Lin C-H, Chen M-H, Chang G-C, et al. Digoxin suppresses tumor malignancy through inhibiting multiple Src-related signaling pathways in non-small cell lung cancer. Chellappan SP, editor. *PLoS One* [Internet]. 2015 May 8; **10**(5):e0123305. Available from: <http://dx.doi.org/10.1371/journal.pone.0123305>
- [131] Zuo Z, Cai T, Li J, Zhang D, Yu Y, Huang C. Hexavalent chromium Cr(VI) up-regulates COX-2 expression through an NF κ B/c-Jun/AP-1-dependent pathway. *Environmental Health Perspectives* [Internet]. 2012 Jan 6; **120**(4):547-553. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22472290>

- [132] Lian S, Xia Y, Khoi PN, Ung TT, Yoon HJ, Kim NH, et al. Cadmium induces matrix metalloproteinase-9 expression via ROS-dependent EGFR, NF- κ B, and AP-1 pathways in human endothelial cells. *Toxicology* [Internet]. 2015 Dec;**338**:104-116. Available from: <http://dx.doi.org/10.1016/j.tox.2015.10.008>
- [133] Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, et al. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *The Journal of Clinical Investigation* [Internet]. 2003 Apr 15;**111**(8):1201-1209. Available from: <http://www.jci.org/articles/view/14172>
- [134] Chen C-A, De Pascali F, Basye A, Hemann C, Zweier JL. Redox modulation of endothelial nitric oxide synthase by glutaredoxin-1 through reversible oxidative post-translational modification. *Biochemistry* [Internet]. 2013 Sep 24;**52**(38):6712-6723. Available from: <http://pubs.acs.org/doi/abs/10.1021/bi400404s>
- [135] Cai H, Li Z, Dikalov S, Holland SM, Hwang J, Jo H, et al. NAD(P)H oxidase-derived hydrogen peroxide mediates endothelial nitric oxide production in response to angiotensin II. *The Journal of Biological Chemistry* [Internet]. 2002 Dec 13;**277**(50):48311-48317. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12377764>
- [136] Burgoyne JR, Prysyazhna O, Rudyk O, Eaton P. CGMP-dependent activation of protein kinase g precludes disulfide activation: Implications for blood pressure control. *Hypertension*. 2012;**60**(5):1301-1308
- [137] Jin L, Ying Z, Webb R. Activation of Rho/Rho kinase signaling pathway by reactive oxygen species in rat aorta. *American Journal of Physiology. Heart and Circulatory Physiology*. 2004;**287**(4):H1495-H1500
- [138] Aghajanian A, Wittchen ES, Campbell SL, Burrige K. Direct activation of RhoA by reactive oxygen species requires a redox-sensitive motif. Bezanilla M, editor. *PLoS One* [Internet]. 2009 Nov 26;**4**(11):e8045. Available from: <http://dx.plos.org/10.1371/journal.pone.0008045>
- [139] Sun H, Tonks NK. The coordinated action of protein tyrosine phosphatases and kinases in cell signaling. *Trends in Biochemical Sciences* [Internet]. 1994 Nov;**19**(11):480-485. Available from: <http://linkinghub.elsevier.com/retrieve/pii/0968000494901341>
- [140] Gerthoffer WT. Mechanisms of vascular smooth muscle cell migration. *Circulation Research* [Internet]. 2007 Mar 16;**100**(5):607-621. Available from: <http://circres.ahajournals.org/cgi/doi/10.1161/01.RES.0000258492.96097.47>
- [141] Chiarugi P, Fiaschi T, Taddei ML, Talini D, Giannoni E, Raugei G, et al. Two vicinal cysteines confer a peculiar redox regulation to low molecular weight protein tyrosine phosphatase in response to platelet-derived growth factor receptor stimulation. *The Journal of Biological Chemistry* [Internet]. 2001 Sep 7;**276**(36):33478-33487. Available from: <http://www.jbc.org/lookup/doi/10.1074/jbc.M102302200>
- [142] Ayilavarapu S, Kantarci A, Fredman G, Turkoglu O, Omori K, Liu H, et al. Diabetes-induced oxidative stress is mediated by Ca²⁺-independent phospholipase A2 in neutrophils.

- Journal of Immunology [Internet]. 2010 Feb 1;**184**(3):1507-1515. Available from: <http://www.jimmunol.org/cgi/doi/10.4049/jimmunol.0901219>
- [143] Sun GY, Chuang DY, Zong Y, Jiang J, Lee JCM, Gu Z, et al. Role of cytosolic phospholipase A2 in oxidative and inflammatory signaling pathways in different cell types in the central nervous system. Nixon AE, editor. Molecular Neurobiology [Internet]. 2014 Aug 27;**50**(1):6-14. Available from: <http://link.springer.com/10.1007/978-1-62703-673-3>
- [144] Suh P-G, Park J-I, Manzoli L, Cocco L, Peak JC, Katan M, et al. Multiple roles of phosphoinositide-specific phospholipase C isozymes. BMB Reports [Internet]. 2008 Jun 30;**41**(6):415-434. Available from: <http://koreascience.or.kr/journal/view.jsp?kj=E1MBB7&py=2008&vnc=v41n6&sp=415>
- [145] Wang X-T, McCullough KD, Wang X-J, Carpenter G, Holbrook NJ. Oxidative stress-induced phospholipase C- γ 1 activation enhances cell survival. The Journal of Biological Chemistry [Internet]. 2001 Jul 27;**276**(30):28364-28371. Available from: <http://www.jbc.org/lookup/doi/10.1074/jbc.M102693200>
- [146] Bai X-C, Deng F, Liu A-L, Zou Z-P, Wang Y, Ke Z-Y, et al. Phospholipase C- γ 1 is required for cell survival in oxidative stress by protein kinase C. The Biochemical Journal [Internet]. 2002 Apr 15;**363**(2):395. Available from: <http://www.biochemj.org/bj/363/bj3630395.htm>
- [147] Kolesnikov YS, Nokhrina KP, Kretynin SV, Volotovskii ID, Martinec J, Romanov GA, et al. Molecular structure of phospholipase D and regulatory mechanisms of its activity in plant and animal cells. The Biochemist [Internet]. 2012 Jan 28;**77**(1):1-14. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22339628>
- [148] Kim J, Min G, Bae Y-S, Min DS. Phospholipase D is involved in oxidative stress-induced migration of vascular smooth muscle cells via tyrosine phosphorylation and protein kinase C. Experimental & Molecular Medicine. 2004;**36**(2):103-109. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15150437>
- [149] Ryan KA, Smith MF, Sanders MK, Ernst PB. Reactive oxygen and nitrogen species differentially regulate Toll-like receptor 4-mediated activation of NF-kappa B and interleukin-8 expression. Infection and Immunity [Internet]. 2004 Apr 1;**72**(4):2123-2130. Available from: <http://iai.asm.org/cgi/doi/10.1128/IAI.72.4.2123-2130.2004>
- [150] Bala A, Mondal C, Haldar PK, Khandelwal B. Oxidative stress in inflammatory cells of patient with rheumatoid arthritis: Clinical efficacy of dietary antioxidants. Inflammopharmacology [Internet]. 2017 Dec;**25**(6):595-607. Available from: <http://link.springer.com/10.1007/s10787-017-0397-1>
- [151] Datta S, Kundu S, Ghosh P, De S, Ghosh A, Chatterjee M. Correlation of oxidant status with oxidative tissue damage in patients with rheumatoid arthritis. Clinical Rheumatology [Internet]. 2014 Nov 10;**33**(11):1557-1564. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/2377764>
- [152] Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. Arteriosclerosis, Thrombosis, and Vascular Biology [Internet]. 2011 May 1;**31**(5):986-1000. Available from: <http://atvb.ahajournals.org/cgi/doi/10.1161/ATVBAHA.110.207449>

- [153] Sarkar D, Saha P, Gamre S, Bhattacharjee S, Hariharan C, Ganguly S, et al. Anti-inflammatory effect of allylpyrocatechol in LPS-induced macrophages is mediated by suppression of iNOS and COX-2 via the NF- κ B pathway. *International Immunopharmacology* [Internet]. 2008 Sep;**8**(9):1264-1271. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S1567576908001562>
- [154] Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nature Reviews. Cancer* [Internet]. 2011 Feb;**11**(2):85-95. Available from: <http://www.nature.com/doifinder/10.1038/nrc2981>
- [155] Wang W, Zhang E, Lin C. MicroRNAs in tumor angiogenesis. *Life Sciences* [Internet]. 2015 Sep;**136**:28-35. Available from: <http://dx.doi.org/10.1016/j.lfs.2015.06.025>
- [156] Hu Y, Deng H, Xu S, Zhang J. MicroRNAs regulate mitochondrial function in cerebral ischemia-reperfusion injury. *International Journal of Molecular Sciences* [Internet]. 2015 Oct 20;**16**(10):24895-24917. Available from: <http://www.mdpi.com/1422-0067/16/10/24895/>
- [157] Zhang X, Ng W-L, Wang P, Tian L, Werner E, Wang H, et al. MicroRNA-21 modulates the levels of reactive oxygen species by targeting SOD3 and TNF. *Cancer Research* [Internet]. 2012 Sep 15;**72**(18):4707-4713. Available from: <http://cancerres.aacrjournals.org/cgi/doi/10.1158/0008-5472.CAN-12-0639>
- [158] Higgs G, Slack F. The multiple roles of microRNA-155 in oncogenesis. *Journal of Clinical Bioinformatics* [Internet]. 2013;**3**(1):17. Available from: <http://jclinbioinformatics.biomedcentral.com/articles/10.1186/2043-9113-3-17>
- [159] Mattiske S, Suetani RJ, Neilsen PM, Callen DF. The oncogenic role of miR-155 in breast cancer. *Cancer Epidemiology, Biomarkers & Prevention* [Internet]. 2012 Aug 1;**21**(8):1236-1243. Available from: <http://cebp.aacrjournals.org/cgi/doi/10.1158/1055-9965.EPI-12-0173>
- [160] Feng C-Z, Yin J-B, Yang J-J, Cao L. Regulatory factor X1 depresses ApoE-dependent A β uptake by miRNA-124 in microglial response to oxidative stress. *Neuroscience* [Internet]. 2017 Mar;**344**:217-228. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0306452216307072>
- [161] He J, Xu Q, Jing Y, Agani F, Qian X, Carpenter R, et al. Reactive oxygen species regulate ERBB2 and ERBB3 expression via miR-199a/125b and DNA methylation. *EMBO Reports* [Internet]. 2012 Nov 13;**13**(12):1116-1122. Available from: <http://embor.embopress.org/cgi/doi/10.1038/embor.2012.162>
- [162] Rushmore TH, Morton MR, Pickett CB. The antioxidant responsive element: Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. *The Journal of Biological Chemistry*. 1991;**266**(18):11632-11639
- [163] Jaiswal AK. Nrf2 signaling in coordinated activation of antioxidant gene expression. *Free Radical Biology & Medicine* [Internet]. 2004 May;**36**(10):1199-1207. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0891584904001923>
- [164] Campagne MV, Thibodeaux H, van Bruggen N, Cairns B, Lowe DG. Increased binding activity at an antioxidant-responsive element in the metallothionein-1 promoter and

rapid induction of metallothionein-1 and -2 in response to cerebral ischemia and reperfusion. *The Journal of Neuroscience* [Internet]. 2000;**20**(14):5200-5207. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10884303>

- [165] Yao J-W, Liu J, Kong X-Z, Zhang S-G, Wang X-H, Yu M, et al. Induction of activation of the antioxidant response element and stabilization of Nrf2 by 3-(3-pyridylmethylidene)-2-indolinone (PMID) confers protection against oxidative stress-induced cell death. *Toxicology and Applied Pharmacology* [Internet]. 2012 Mar;**259**(2):227-235. Available from: <http://dx.doi.org/10.1016/j.taap.2011.12.027>
- [166] Hara H. Increase of antioxidative potential by tert-butylhydroquinone protects against cell death associated with 6-hydroxydopamine-induced oxidative stress in neuroblastoma SH-SY5Y cells. *Molecular Brain Research* [Internet]. 2003 Nov 26;**119**(2):125-131. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0169328X03003462>

IntechOpen