Chapter 5

Free Radicals and Their Biological Mechanisms: A Paradox Between Benefit and Harm

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Abstract

Free radicals are chemical species containing one or more unpaired electrons in their outer shell, a characteristic that renders them highly reactive and intrinsically unstable in biological environments. At the cellular level, they rapidly interact with surrounding molecules in an effort to attain lower-energy, more stable configurations. This pronounced reactivity supports essential biological processes such as signal transduction, fine-tuning of immune responses and maintenance of redox homeostasis. However, when their production becomes dysregulated, these species initiate oxidative damage to lipids, proteins and nucleic acids, thereby compromising cellular structure and function.

Under physiological conditions, a dynamic equilibrium exists between free radical generation and antioxidant defense systems. As long as this balance is maintained, radical species act as regulatory intermediates in intracellular communication, host defense and adaptive response pathways. In contrast, increased radical burden arising from endogenous sources such as the mitochondrial respiratory chain, inflammation-activated oxidase systems and cytochrome P450 metabolism—or from exogenous factors including cigarette smoke, air pollution, pesticides, pharmaceuticals, ultraviolet light and ionizing radiation—leads to oxidative stress. This condition contributes to the development of numerous chronic disorders, particularly cancer, cardiovascular disease, metabolic syndrome and neurodegenerative conditions.

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This chapter discusses the classification, chemical properties, major endogenous and exogenous sources and biological formation mechanisms of free radicals. Additionally, the beneficial and detrimental cellular effects of reactive oxygen and nitrogen species are examined within the framework of the "benefit–harm paradox," based on current literature.

1. Introduction

Reactive oxygen species (ROS) arise continuously as an inherent consequence of oxygen-dependent metabolism and influence a broad spectrum of cellular processes. At physiologically controlled concentrations, ROS function as redox-sensitive regulators that shape cell growth, differentiation, metabolic flexibility and immune signaling (Droge, 2002). In contrast, excessive ROS accumulation overwhelms antioxidant capacity and gives rise to oxidative stress, leading to structural and functional damage of lipids, proteins and nucleic acids (Sies, 2017).

Maintenance of redox balance depends on an integrated antioxidant network composed of both enzymatic and non-enzymatic components. Enzymes such as superoxide dismutases (SODs), catalase, glutathione peroxidases (GPx), peroxiredoxins and thioredoxin-linked systems rapidly detoxify reactive intermediates, whereas small-molecule antioxidants, including glutathione, vitamins C and E and dietary polyphenols, restrict radical chain reactions (Pizzino et al., 2017). Breakdown of this protective network is strongly associated with the development of malignancies, cardiovascular and neurodegenerative diseases, diabetes and chronic inflammatory conditions (Scialò et al., 2020).

Mitochondria represent the dominant intracellular source of ROS under normal conditions. During oxidative phosphorylation, electron leakage at complexes I and III generates superoxide, which is rapidly converted to hydrogen peroxide by mitochondrial superoxide dismutase (SOD2). Due to its relative stability and ability to diffuse across membranes, hydrogen peroxide serves as a central redox signaling intermediate. However, mitochondrial dysfunction, metabolic excess or damage to mitochondrial DNA substantially elevate ROS output, promoting oxidative injury and activation of cell death pathways (Brand, 2016).

Beyond mitochondria, ROS are produced by specialized enzyme systems such as NADPH oxidases, xanthine oxidase and cytochrome P450 monooxygenases, particularly in contexts of inflammation, hypoxia and xenobiotic metabolism (Forrester et al., 2018). External stressors, including ionizing radiation, environmental pollutants, heavy metals and selected

pharmaceuticals, further intensify ROS generation, highlighting the context-dependent nature of ROS as both indispensable signaling molecules and potent drivers of cellular injury (Sies & Jones, 2020).

2. Free Radicals

Free radicals are chemically reactive species defined by the presence of one or more unpaired electrons, a feature that renders them energetically unstable and highly prone to participate in electron transfer reactions (Sies, 2017). Through their capacity to donate or abstract electrons, these molecules can initiate self-propagating reaction cascades that spread rapidly across biological macromolecules. By comparison, oxidants with fully paired electrons display lower intrinsic reactivity; however, many remain biologically significant because they can generate radical intermediates under appropriate chemical or enzymatic conditions (Pham-Huy et al., 2008).

Reactive oxygen species (ROS) form a major subset within this oxidant spectrum and encompass both radical and non-radical entities. Radical ROS include hydroxyl ($\bullet OH$), superoxide ($O_2\bullet^-$), alkoxyl ($RO\bullet$) and lipid peroxyl ($LOO\bullet$) species, whereas hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), ozone (O_3), hypochlorous acid (HOCl), organic hydroperoxides (ROOH) and peroxynitrite ($ONOO^-$) represent non-radical but redox-active oxidants (Fang et al., 2002). Despite lacking unpaired electrons, non-radical ROS play central roles in oxidative biology because they readily oxidize cellular targets or act as precursors for secondary radical formation via processes such as Fenton-type reactions, myeloperoxidase-mediated oxidation and light-driven photochemical pathways (Forman et al., 2021).

In living systems, radical generation arises from a limited number of fundamental chemical mechanisms, including homolytic bond dissociation, one-electron redox reactions, xenobiotic redox cycling and radiation-induced water radiolysis (Kılınç & Kılınç, 2002). While these routes enable tightly regulated radical production under physiological conditions, they also permit excessive accumulation during metabolic imbalance, inflammation or toxic insult. This context-dependent behavior underlies the paradoxical nature of reactive species in biological systems, including free radicals, which function both as essential modulators of cellular signaling and as potent drivers of oxidative injury when redox control is lost.

2.1. Formation of Free Radicals and Oxidants

Cells continuously generate reactive oxygen species (ROS), a collective term encompassing both radical and non-radical oxidants, through a

combination of enzyme-driven reactions and non-enzymatic chemical processes. Major enzymatic sources include the mitochondrial electron transport chain, NADPH oxidases, xanthine oxidase and cytochrome P450 monooxygenases, all of which produce superoxide and related reactive intermediates as inherent consequences of redox catalysis (Halliwell & Gutteridge, 2015). In professional phagocytes, activation of NADPH oxidase initiates the respiratory burst, leading to rapid production of superoxide and hydrogen peroxide, which are further converted by myeloperoxidase into hypochlorous acid (HOCl), a potent antimicrobial oxidant (Klebanoff et al., 2013; Winterbourn & Kettle, 2013).

Hydrogen peroxide (H_2O_2), a non-radical ROS, is generated primarily through oxidase-catalyzed reactions and functions as a central intermediate linking ROS formation to redox signaling and, indirectly, to oxidative damage pathways. Although H_2O_2 exhibits moderate intrinsic reactivity, its interaction with redox-active transition metals such as Fe^{2+} and Cu^{+} promotes Fenton chemistry and the formation of highly reactive hydroxyl radicals (•OH), which directly initiate extensive macromolecular damage (Sies, 2017). In parallel, nitric oxide synthases generate nitric oxide (NO•), a diffusible signaling molecule that rapidly reacts with superoxide to form reactive nitrogen species, thereby integrating ROS and RNS pathways (Förstermann & Sessa, 2012).

In addition to enzymatic reactions, non-enzymatic processes including spontaneous oxygen reduction, xenobiotic redox cycling, photoactivation of endogenous chromophores and radiation-induced water radiolysis further contribute to the generation of reactive radical species. Among endogenous sources, mitochondria represent a dominant site of ROS generation. The mechanisms underlying mitochondrial ROS production and their modulation under physiological and pathological conditions are discussed in detail in Section 2.4.1.

2.2. Free Radicals and Reactive Oxygen Species (ROS)

The majority of biologically significant free radicals arise from oxygen- and nitrogen-containing molecules. Oxygen-derived species constitute the main component of reactive oxygen species (ROS) and include several radical forms, such as superoxide, hydroxyl and peroxyl radicals, as well as non-radical oxidants. In parallel, nitrogen-based radicals such as nitric oxide and nitrogen dioxide, together with their downstream products, are grouped as reactive nitrogen species (RNS) (Pacher et al., 2007). Rather than functioning independently, these redox systems are tightly interconnected;

a prominent example is the near diffusion-controlled reaction between nitric oxide and superoxide, which generates peroxynitrite ($ONOO^-$), a potent non-radical oxidant capable of giving rise to secondary radical species and strongly associated with inflammation, vascular dysfunction and neurodegenerative pathology (Radi, 2018).

In addition to oxygen- and nitrogen-centered radicals, other transient radical species centered on sulfur, carbon or hydrogen atoms may be produced, particularly under conditions of metabolic dysregulation or sustained inflammatory stress. The emergence of these radicals further perturbs intracellular redox balance and amplifies oxidative burden (Forman et al., 2021). At the chemical level, radical formation is typically driven by single-electron transfer reactions, yielding neutral or charged intermediates that readily propagate chain reactions unless efficiently restrained by antioxidant systems (Halliwell & Gutteridge, 2015).

Importantly, redox biology is not limited to species bearing unpaired electrons. Non-radical oxidants such as hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) play pivotal roles because they act both as relatively stable redox signaling mediators and as precursors of highly reactive radical species under specific chemical or enzymatic conditions. Despite their greater chemical stability, these oxidants function as key amplifiers of redox signaling and oxidative stress within cells (Radi, 2018). The principal physicochemical features, reactivity patterns and biological effects of major ROS and RNS are summarized in Table 1 and examined in detail in Section 2.5.

Table 1. Conceptual overview of major reactive oxygen and nitrogen species within redox balance, signaling and oxidative stress.

Feature	*Reactive Oxygen Species (ROS)*	*Reactive Nitrogen Species (RNS)*
Chemical origin	Derived from molecular oxygen	Derived from nitric oxide and nitrogen oxides
Major species	$O_2^{\bullet -}$, •OH, H_2O_2, ROO•, LOO•, $^1O_2$	NO•, NO_2•, $ONOO^-$, N_2O_3
Radical / non-radical forms	Both radical and non-radical species	Both radical and non-radical species
Primary cellular sources	Mitochondria, NADPH oxidases, peroxisomes, cytochrome P450	nNOS, eNOS, iNOS; immune and endothelial cells
Dominant biological context	Metabolic regulation, redox signaling, oxidative stress	Vascular signaling, immune modulation, inflammatory stress
Primary functional outcome	Redox signaling at low levels; macromolecular damage at excess levels	Signal modulation at low levels; nitrative and oxidative damage when dysregulated
Key signaling intermediates	H_2O_2 (redox messenger)	NO• (vasodilator, neurotransmitter)
Major damaging mechanisms	Lipid peroxidation, DNA oxidation, protein carbonylation	Protein nitration, mitochondrial inhibition
Critical ROS–RNS interaction	$O_2^{\bullet -} \rightarrow H_2O_2 \rightarrow$ •OH	$O_2^{\bullet -}$ + NO• → $ONOO^-$
Associated pathological processes	Cancer, diabetes, aging, neurodegeneration	Endothelial dysfunction, inflammation, neurotoxicity
Detoxification systems	SOD, catalase, GPx, peroxiredoxins	NOS regulation, GSH-dependent pathways

The table is intended to provide a conceptual framework for redox balance and signaling rather than an exhaustive catalog of oxidant species.

2.3 Sources of Free Radicals

Free radicals are continuously generated in living organisms as a result of both endogenous metabolic activity and external influences. Under physiological conditions, their formation and elimination are tightly controlled, allowing radical species and related reactive oxidants to support cellular signaling and metabolic regulation rather than disrupt them. When this control is compromised, excess radical accumulation promotes oxidative stress and leads to widespread molecular damage.

2.3.1. Endogenous Sources

Endogenous free radical formation is an intrinsic consequence of cellular metabolism and is tightly coupled to energy-producing and biosynthetic pathways. Among intracellular sources, mitochondria represent a major site of physiological reactive oxygen species (ROS) generation. During oxidative phosphorylation, electrons may prematurely escape from complexes I and III of the electron transport chain and react with molecular oxygen, yielding superoxide ($O_2^{\bullet -}$). Early experimental studies have suggested that only a small fraction of total cellular oxygen consumption, historically estimated at approximately 0.1–2% under certain experimental conditions, may follow this route; nevertheless, this output is biologically significant and is normally constrained by antioxidant enzymes, particularly superoxide dismutases (Brand, 2016).

Mitochondria are not the sole contributors to endogenous ROS and radical formation. Several enzyme systems generate reactive species either as part of their catalytic cycles or upon activation. Xanthine oxidase, NADPH oxidases, cytochrome P450 enzymes, lipoxygenases and cyclooxygenases all contribute to intracellular oxidative flux. For example, cyclooxygenase-dependent prostaglandin synthesis transiently generates peroxyl radicals, whereas stimulation of NADPH oxidase complexes in neutrophils and macrophages triggers the respiratory burst, resulting in rapid formation of superoxide and secondary non-radical oxidants such as hydrogen peroxide and hypochlorous acid (Winterbourn & Kettle, 2013).

Reactive nitrogen species (RNS) arise in parallel through endogenous biochemical reactions. Nitric oxide ($NO^{\bullet}$), synthesized from L-arginine by nitric oxide synthases, functions as a key signaling molecule in vascular regulation, neurotransmission and immune control (Förstermann & Sessa, 2012). However, $NO^{\bullet}$ readily reacts with superoxide to form peroxynitrite ($ONOO^-$), a non-radical oxidant capable of generating secondary radical species and inducing protein nitration, mitochondrial dysfunction and inflammatory tissue damage. This reaction exemplifies the close functional and chemical coupling between ROS and RNS pathways (Radi, 2018).

2.3.2. Exogenous Sources

In addition to endogenous metabolism, external environmental and lifestyle-related factors substantially amplify radical and oxidant generation. Ultraviolet radiation, ionizing radiation, ozone, cigarette smoke, airborne particulate matter, pesticides, heavy metals and polycyclic aromatic hydrocarbons increase oxidative burden either directly or following

metabolic activation (Pham-Huy et al., 2008). Ionizing radiation promotes water radiolysis, yielding hydroxyl radicals, hydrogen atoms and hydrated electrons, whereas ultraviolet exposure excites endogenous chromophores that transfer energy to oxygen and generate singlet oxygen ($^1O_2$).

A broad range of pharmaceuticals and environmental chemicals further contribute to oxidative load through redox cycling mechanisms. Agents such as anthracyclines, acetaminophen and various xenobiotics sustain continuous radical production, which may exceed antioxidant buffering capacity under conditions of high exposure or impaired detoxification (Sies, 2017; Forman et al., 2021). Persistent or repeated exposure to these stressors elevates cellular oxidative pressure, reinforces inflammatory signaling and accelerates the development of degenerative and inflammatory diseases.

2.4. Endogenous Formation Mechanisms

The continuous formation of reactive oxygen species (ROS) is an unavoidable outcome of aerobic life. In eukaryotic cells, the vast majority of oxygen consumption takes place in mitochondria, where oxidative phosphorylation generates ATP. While molecular oxygen is normally reduced fully to water at complex IV, incomplete reduction at earlier stages of the electron transport process results in the generation of ROS, predominantly the radical superoxide and its downstream derivatives.

2.4.1. Mitochondrial Electron Transport Chain

Within the mitochondrial electron transport chain (ETC), a small fraction of electrons deviates from the canonical transfer pathway and reacts directly with molecular oxygen, leading to the formation of superoxide ($O_2^{\bullet -}$). Experimental and biochemical studies have identified complexes I (NADH:ubiquinone oxidoreductase) and III (cytochrome bc complex) as the primary sites of this electron leakage (Brand, 2016; Santos et al., 2018). Early experimental studies have historically suggested that a small fraction of total cellular oxygen consumption, often cited in the range of approximately 0.1–2%, may contribute to mitochondrial ROS formation under certain experimental conditions. Superoxide generated at these sites is rapidly converted to hydrogen peroxide (H_2O_2) by mitochondrial manganese-dependent superoxide dismutase (SOD2), thereby limiting its immediate reactivity while enabling downstream redox signaling or detoxification.

Under physiological conditions, mitochondrial ROS production remains tightly regulated and contributes to cellular signaling and metabolic adaptation. However, disturbances such as impaired electron flow, altered

membrane potential or mitochondrial dysfunction markedly enhance electron leakage and ROS output. Excessive mitochondrial ROS accumulation amplifies oxidative stress within the organelle, damages mitochondrial DNA and proteins and promotes a self-reinforcing cycle of redox imbalance and cellular injury.

2.4.2. Cytochrome P450 and Oxidative Metabolism

Beyond mitochondria, oxidative metabolism itself contributes to endogenous ROS formation. The cytochrome P450 (CYP) monooxygenase system, essential for xenobiotic metabolism and numerous biosynthetic reactions, generates reactive oxygen species as a consequence of incomplete catalytic coupling. During CYP activity, electrons may leak from the catalytic cycle, favoring the production of superoxide and hydrogen peroxide (Wang et al., 2010). Additional metabolic reactions, including those mediated by peroxisomal oxidases, amino acid oxidases and oxidative deamination pathways, further contribute to intracellular H_2O_2 generation during normal cellular function.

2.4.3. Physiological Significance

At controlled, low to moderate levels, endogenously produced ROS support redox signaling, immune defense and metabolic flexibility. When ROS formation exceeds the neutralizing capacity of antioxidant systems, these same metabolic sources shift from regulatory roles to major drivers of oxidative damage. Thus, cellular outcome depends critically on the dynamic balance between mitochondrial ETC activity, other redox-active enzymes and the efficiency of antioxidant defenses, which together determine whether ROS function primarily as signaling mediators or as agents of cellular injury (Halliwell & Gutteridge, 2015; Sies & Jones, 2020).

2.5. Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) encompass a broad spectrum of oxygen-derived molecules that vary widely in stability, chemical behavior and biological consequences. Depending on their concentration, localization and lifetime, non-radical ROS primarily function as finely tuned regulators of redox signaling, whereas highly reactive radical species exert cytotoxic effects through uncontrolled interactions with cellular macromolecules (Halliwell & Gutteridge, 2015). Dissecting the chemical properties and cellular actions of individual ROS is therefore essential to explain how these molecules contribute both to physiological regulation and to pathological damage (Sies, 2017; Jones & Sies, 2020).

ROS comprise both radical species containing unpaired electrons and non-radical oxidants that actively participate in redox reactions and frequently serve as precursors of secondary radicals. The principal ROS species and their defining characteristics are outlined below.

2.5.1. Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) is a non-radical oxidant characterized by its relative chemical stability and capacity to diffuse across biological membranes. It is generated primarily through superoxide dismutation and a wide range of oxidase-mediated reactions. Due to its selective reactivity toward protein thiol groups, H_2O_2 occupies a central position at the interface between ROS formation and redox-dependent cellular regulation (Rhee, 2006; Shadel & Horvath, 2015). However, excessive accumulation of H_2O_2 promotes metal-catalyzed reactions that generate highly reactive hydroxyl radicals, thereby linking H_2O_2 indirectly to oxidative damage under pathological conditions (Halliwell & Gutteridge, 2015). The role of H_2O_2 in redox signaling and cellular homeostasis is discussed in detail in Sections 2.10 and 2.12.

2.5.2. Superoxide Anion ($O_2^{\bullet -}$)

Superoxide is commonly the initial radical ROS formed in cells, particularly within mitochondria and through NADPH oxidase activity. Its limited reactivity and short lifetime restrict direct molecular damage; however, its biological importance lies in its role as a progenitor of other oxidants. In addition, rapid reaction with nitric oxide yields peroxynitrite, thereby coupling superoxide formation to nitrosative stress pathways (Pacher et al., 2007; Brand, 2016).

2.5.3. Hydroxyl Radical ($^{\bullet}OH$)

Among all ROS, the hydroxyl radical displays the highest reactivity. Generated primarily through Fenton and Haber–Weiss chemistry, $^{\bullet}OH$ reacts at near diffusion-controlled rates and damages biomolecules at the site of formation. Because it cannot diffuse appreciably, hydroxyl radical–mediated injury is highly localized and irreversible, affecting DNA, proteins and membrane lipids alike (Valko et al., 2007; Cadet & Wagner, 2013).

2.5.4. Hydroperoxyl Radical ($HO_2^{\bullet}$)

The hydroperoxyl radical represents the protonated form of superoxide and exhibits enhanced lipid solubility compared with $O_2^{\bullet -}$. Although it constitutes only a minor proportion of superoxide under physiological conditions, its ability to penetrate hydrophobic environments allows it to

initiate and propagate lipid peroxidation reactions efficiently (Pham-Huy et al., 2008).

2.5.5. Alkoxyl Radicals (RO•)

Alkoxyl radicals are typically generated through metal-dependent cleavage of organic hydroperoxides. These species readily abstract hydrogen atoms from neighboring lipid chains, accelerating oxidative chain reactions and contributing to structural destabilization of cellular membranes (Yin et al., 2011).

2.5.6. Nitric Oxide (NO•)

Nitric oxide is formally categorized as a reactive nitrogen species but occupies a pivotal position at the intersection of ROS and RNS biology. Produced enzymatically by nitric oxide synthases, NO• regulates vascular tone, neuronal signaling and immune responses. Its rapid combination with superoxide to form peroxynitrite links nitric oxide signaling to oxidative and nitrosative stress and to mitochondrial redox regulation (Forrester et al., 2018).

2.5.7. Singlet Oxygen ($^1O_2$)

Singlet oxygen is an electronically excited, high-energy form of molecular oxygen generated during photochemical reactions and inflammatory processes. It preferentially targets unsaturated lipids and susceptible amino acid residues, including histidine, tryptophan and methionine, thereby contributing to oxidative injury and immune-mediated cytotoxic mechanisms (Di Mascio et al., 2019).

2.5.8. Organic Hydroperoxides (ROOH) and Lipid Peroxyl Radicals (LOO•)

Organic hydroperoxides arise as intermediates of lipid oxidation and readily decompose into alkoxyl and peroxyl radicals. Lipid peroxyl radicals form when oxygen reacts with carbon-centered lipid radicals and act as key propagators of lipid peroxidation. Together, these species sustain oxidative chain reactions, compromise membrane integrity and participate in ferroptosis-associated oxidative pathways (Dixon et al., 2012; Ayala et al., 2014).

2.5.9. Peroxynitrite ($ONOO^-$)

Peroxynitrite is generated through the diffusion-controlled reaction of nitric oxide with superoxide. This non-radical oxidant promotes nitration, oxidation and fragmentation of biomolecules and plays a central role in inflammatory tissue injury and vascular dysfunction. Under acidic conditions, protonation of $ONOO^-$ yields ONOOH, which decomposes into secondary radicals such as hydroxyl and nitrogen dioxide, further amplifying redox imbalance (Pacher et al., 2007; Radi, 2018).

2.6. Physiological and Pathological Effects of ROS

Reactive oxygen species (ROS) influence cellular systems across a wide functional spectrum, ranging from indispensable physiological regulation to overt pathological injury. The biological outcome of ROS exposure depends on their concentration, chemical identity, subcellular origin and temporal persistence. When maintained within narrow limits, ROS contribute to redox signaling and cellular homeostasis; excessive or prolonged ROS accumulation, by contrast, overwhelms antioxidant defenses and precipitates oxidative stress, cellular malfunction and tissue damage (Sies et al., 2017; Sies & Jones, 2020).

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2.6.1. Physiological Functions of ROS

At low to moderate levels, reactive oxygen species (ROS) function as essential components of intracellular signaling networks and physiological regulation. Controlled ROS production enables reversible redox modifications of target proteins, thereby fine-tuning kinase and phosphatase activities and coordinating cellular processes such as growth, differentiation, immune regulation and stress adaptation (Finkel, 2011; Sies & Jones, 2020).

In immune cells, ROS generated by NADPH oxidases contribute to microbial killing, antigen processing and cytokine-mediated communication. Beyond host defense, physiological ROS participate in vascular homeostasis by modulating nitric oxide bioavailability and support metabolic flexibility through regulation of mitochondrial function, autophagy and hypoxia-responsive signaling pathways (Sena & Chandel, 2012). Collectively, these observations underscore that ROS are not accidental byproducts of metabolism but tightly regulated mediators of redox-controlled physiology.

2.6.2. Pathological Effects of ROS

When ROS production exceeds the buffering capacity of antioxidant systems, oxidative stress develops and progressively compromises cellular integrity. Sustained redox imbalance disrupts normal signaling networks and promotes degenerative processes associated with chronic inflammation, metabolic dysfunction and aging (Powers et al., 2011).

2.6.3. Lipid Peroxidation

Polyunsaturated fatty acids within cellular membranes are highly susceptible to oxidative damage due to their multiple double bonds. Highly reactive radical species derived from ROS initiate lipid peroxidation, a chain reaction that compromises membrane structure, alters fluidity and generates bioactive secondary products with cytotoxic and signaling-disruptive properties. Lipid peroxidation therefore represents a central pathological consequence of oxidative stress and contributes to the progression of numerous chronic diseases, including atherosclerosis, neurodegeneration and metabolic disorders. The molecular mechanisms and biological consequences of lipid peroxidation are discussed in detail in Section 2.8.1.

2.6.4. Protein Oxidation

Proteins are frequent targets of oxidative modification because of their abundance and functional diversity. Radical-mediated reactions alter amino acid side chains, induce carbonyl formation, disrupt disulfide bonding and cause backbone fragmentation. These structural changes impair enzymatic activity, receptor signaling and cytoskeletal stability (Dalle-Donne et al., 2006). Sustained protein oxidation promotes proteotoxic stress, compromises proteostasis and autophagic clearance and contributes to disorders such as Alzheimer's disease, Parkinson's disease and type 2 diabetes.

2.6.5. DNA Damage

Highly reactive hydroxyl radicals attack both nucleobases and the sugar–phosphate backbone of DNA, generating lesions including 8-hydroxy-2′-deoxyguanosine (8-OHdG), single-strand breaks and double-strand breaks. Accumulation of oxidative DNA damage increases mutational burden, destabilizes the genome and facilitates carcinogenesis (Cooke et al., 2003; Kauppila et al., 2017). Mitochondrial DNA is especially susceptible owing to its proximity to ROS-generating sites and limited repair capacity.

2.6.6. Mitochondrial Dysfunction

Mitochondria represent both major sources and critical targets of ROS. Oxidative damage to mitochondrial membranes, proteins and mtDNA disrupts oxidative phosphorylation, diminishes ATP production and enhances electron leakage, creating a self-reinforcing cycle of ROS generation (Brand, 2016). This vicious cycle accelerates cellular aging and contributes to metabolic and neurodegenerative pathologies.

2.6.7. Inflammation and Cell Death

ROS activate redox-sensitive transcription factors, inflammasomes and stress-response pathways, driving cytokine production and sustained inflammatory signaling. Depending on intensity and duration, oxidative stress can initiate apoptosis, necrosis or ferroptosis, thereby shaping the extent of tissue injury and disease progression (Forman & Zhang, 2021).

2.7. Oxidative DNA Damage and Mechanisms

Excessive production of reactive oxygen species (ROS), particularly highly reactive radical species, poses a serious threat to genomic integrity, making oxidative DNA damage one of the most consequential outcomes of redox imbalance. DNA is inherently vulnerable to oxidative attack because of its high cellular abundance, nuclear localization and frequent unwinding during replication and transcription. Among the various ROS, hydroxyl radicals (•OH) are the most potent DNA-damaging agents. Generated mainly through Fenton and Haber–Weiss chemistry, •OH reacts at near diffusion-controlled rates and induces localized, irreversible lesions at the site of formation (Cooke et al., 2003).

Oxidative insults to DNA give rise to a broad array of lesions, including modified bases, abasic sites, single-strand breaks, double-strand breaks and DNA–protein cross-links. These alterations disrupt replication fidelity and transcriptional accuracy, thereby undermining genomic stability. Among

oxidative base lesions, 8-hydroxy-2′-deoxyguanosine (8-OHdG) is the most extensively studied and is widely used as an indicator of oxidative stress and increased carcinogenic risk (Cadet & Wagner, 2013).

Double-strand breaks (DSBs) constitute the most deleterious form of oxidative DNA damage and may occur when closely spaced lesions arise on opposing DNA strands. Failure to repair DSBs accurately promotes chromosomal rearrangements, mutagenesis and genomic instability, establishing a mechanistic link between ROS-induced DNA damage and cancer, neurodegenerative diseases and age-related functional decline (Kauppila et al., 2017).

To counteract oxidative genomic injury, cells employ multiple DNA repair pathways, including base excision repair (BER), nucleotide excision repair (NER) and double-strand break repair via homologous recombination or non-homologous end joining. Persistent or excessive oxidative stress, however, can overwhelm or dysregulate these repair systems, leading to the gradual accumulation of mutations and progressive loss of cellular function (Wallace, 2014; Degtyareva et al., 2021).

Beyond structural genome damage, elevated ROS levels strongly activate poly(ADP-ribose) polymerase (PARP), a key sensor of DNA strand breaks. Prolonged PARP activation consumes substantial amounts of NAD^+ and ATP, resulting in metabolic collapse and cell death. Thus, oxidative DNA damage driven by ROS not only destabilizes the genome but also perturbs cellular energy homeostasis and influences fundamental cell fate decisions (Andrabi et al., 2014; Pommier et al., 2016).

2.8. Oxidative Damage to Lipids and Proteins

Reactive oxygen species (ROS), particularly highly reactive radical species, play a central role in oxidative injury affecting lipids and proteins, two macromolecular classes fundamental to cellular architecture, compartmentalization and biochemical function. Oxidative modification of these targets compromises membrane organization, disturbs enzymatic activity and signaling fidelity and ultimately contributes to cell dysfunction, death and disease progression (Sies et al., 2017).

2.8.1. Lipid Peroxidation

Polyunsaturated fatty acids (PUFAs) are particularly vulnerable to oxidative attack because their multiple double bonds facilitate radical-mediated hydrogen abstraction. Lipid peroxidation is initiated when reactive oxygen species, especially hydroxyl radicals (•OH), abstract hydrogen

atoms from PUFAs, generating lipid-centered radicals that rapidly react with molecular oxygen to form lipid peroxyl radicals (LOO•). These highly reactive intermediates propagate self-sustaining chain reactions that extend oxidative damage across membrane lipid networks (Ayala et al., 2014).

During the propagation and decomposition phases, lipid peroxidation yields a range of secondary reactive products, most notably malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE). MDA is a mutagenic and cytotoxic aldehyde, whereas 4-HNE forms covalent adducts with cysteine, histidine and lysine residues in proteins, leading to carbonyl formation, enzyme inhibition and disruption of intracellular signaling pathways (Negre-Salvayre et al., 2010). Accumulation of these lipid-derived aldehydes reduces membrane fluidity, increases permeability and interferes with membrane-bound receptors and transport systems.

As a consequence, lipid peroxidation is closely associated with the pathogenesis of atherosclerosis, neurodegenerative diseases, diabetes and ferroptosis-related cell death. The extent of lipid peroxidation is tightly controlled by antioxidant defense systems, including glutathione peroxidases, catalase and lipid-soluble antioxidants such as vitamin E, which collectively limit chain propagation and preserve membrane integrity (Gaschler & Stockwell, 2017; Butterfield & Boyd-Kimball, 2020).

2.8.2. Protein Oxidation

Proteins constitute another major class of oxidative stress targets due to their abundance and functional complexity. Radical-mediated oxidative reactions induce a range of chemical modifications in proteins, including oxidation of amino acid side chains, formation of carbonyl groups, sulfoxides, aberrant disulfide bonds and nitrotyrosine residues. Oxidative stress may also promote peptide backbone cleavage, protein aggregation and loss of catalytic activity. Sulfur-containing amino acids, particularly methionine and cysteine, are highly susceptible to oxidation, leading to structural and functional alterations in affected proteins (Dalle-Donne et al., 2006).

Protein carbonylation is widely used as a relatively irreversible marker of oxidative stress and is associated with impaired protein function, reduced enzymatic efficiency and altered signal transduction. Under physiological conditions, oxidatively modified proteins are removed by proteasomal and lysosomal degradation pathways. When these quality-control systems are overwhelmed or compromised, damaged proteins accumulate, contributing to cellular aging and disease pathogenesis (Höhn & Grune, 2014; Sies et al., 2017). Consistent with this, protein oxidation is strongly implicated in

neurodegenerative disorders such as Alzheimer's and Parkinson's disease, as well as in diabetes mellitus and chronic inflammatory conditions. Oxidative modifications affecting enzymes, transporters and receptors further reprogram metabolic networks and cellular communication, amplifying pathological processes (Butterfield & Boyd-Kimball, 2020; Grueninger et al., 2021).

2.9. Cellular Antioxidant Defense Systems

Maintenance of cellular redox balance relies on a highly coordinated antioxidant network that counteracts reactive oxygen species (ROS), particularly radical intermediates and their secondary products, and limits oxidative damage. This network integrates enzyme-based defenses with small-molecule antioxidants that neutralize reactive intermediates, interrupt radical chain reactions and facilitate recovery of oxidized biomolecules. Together, these mechanisms are indispensable for cell viability, metabolic regulation and controlled redox signaling (Sies & Jones, 2020).

2.9.1. Enzymatic Antioxidants

Enzymatic antioxidants constitute the first and most immediate line of protection by converting specific ROS into less reactive products and preventing the uncontrolled propagation of oxidative reactions.

2.9.1.1. Superoxide Dismutases (SODs)

Superoxide dismutases catalyze the conversion of superoxide ($O_2^{\bullet -}$) into hydrogen peroxide (H_2O_2) and molecular oxygen. Distinct isoforms operate in specific cellular compartments: Cu/Zn-SOD (SOD1) in the cytosol, Mn-SOD (SOD2) in mitochondria and extracellular SOD (SOD3) in the interstitial space. Through this compartmentalized activity, SODs prevent superoxide accumulation and limit its toxic and pro-oxidant potential (Fukai & Ushio-Fukai, 2011).

2.9.1.2. Catalase (CAT)

Catalase is predominantly localized in peroxisomes, where it rapidly decomposes H_2O_2 into water and oxygen. By lowering intracellular H_2O_2 concentrations, catalase reduces the likelihood of hydroxyl radical formation via metal-catalyzed reactions and thereby limits secondary radical generation (Chelikani et al., 2004).

2.9.1.3. Glutathione Peroxidase (GPx) and Glutathione Reductase (GR)

Glutathione peroxidases detoxify H_2O_2 and lipid hydroperoxides (ROOH) using reduced glutathione (GSH) as an electron donor, providing critical protection against membrane lipid oxidation. Glutathione reductase sustains this defense by regenerating GSH from its oxidized form (GSSG), thereby preserving intracellular redox buffering capacity and thiol homeostasis (Brigelius-Flohé & Maiorino, 2013; Lu, 2013).

2.9.1.4. Peroxiredoxin/Thioredoxin (Prx/Trx) Systems

Peroxiredoxins reduce H_2O_2, peroxynitrite ($ONOO^-$) and organic hydroperoxides through thiol-dependent reactions. The thioredoxin–thioredoxin reductase system maintains these enzymes in a reduced, catalytically active state and regulates protein thiol redox balance, supporting redox signaling while preventing excessive cysteine oxidation (Netto & Antunes, 2016).

2.9.2. Non-Enzymatic Antioxidants

Low-molecular-weight antioxidants complement enzymatic defenses by directly scavenging radical species, terminating oxidative chain reactions and stabilizing redox-sensitive cellular environments.

2.9.2.1. Glutathione (GSH)

Glutathione is the most abundant intracellular thiol and plays a central role in peroxide detoxification, preservation of reduced protein thiols and conjugation of electrophilic compounds. The cellular GSH/GSSG ratio is widely used as an indicator of intracellular redox status and antioxidant capacity (Lu, 2013).

2.9.2.2. Vitamins C and E

Vitamin E (α-tocopherol) functions as the primary lipid-phase antioxidant, protecting cellular membranes from peroxidative damage, whereas vitamin C (ascorbic acid) acts in the aqueous phase as a radical scavenger and regenerates oxidized vitamin E. Together, these antioxidants provide coordinated protection across distinct cellular compartments (Traber & Stevens, 2011).

2.9.2.3. Carotenoids and Flavonoids

Diet-derived carotenoids, such as β-carotene and lycopene, along with flavonoids, efficiently quench singlet oxygen and peroxyl radicals. Their

conjugated ring structures enable resonance stabilization of unpaired electrons, contributing to antioxidant, anti-inflammatory and cytoprotective effects (Krinsky & Johnson, 2005; Williamson & Manach, 2005).

2.9.2.4. Uric Acid, Bilirubin and Coenzyme Q

Several endogenous molecules also contribute to antioxidant capacity. Uric acid and bilirubin exhibit strong radical-scavenging properties in plasma and tissues, while coenzyme Q (ubiquinone/ubiquinol) serves a dual role as an electron carrier in mitochondrial respiration and as a lipid-soluble antioxidant within cellular membranes (Ernster & Dallner, 1995).

2.10. Cellular Redox Homeostasis and ROS Signaling

Cellular redox homeostasis is sustained through a dynamic equilibrium between the generation of reactive oxygen species (ROS) and the capacity of antioxidant defense systems. When this balance is preserved, restrained and spatially controlled production of primarily non-radical ROS supports intracellular signaling and adaptive responses to physiological, environmental and metabolic cues. In contrast, a shift toward excessive ROS accumulation disrupts redox control and promotes oxidative stress and cellular injury (Sies & Jones, 2020).

At the molecular level, redox signaling is mediated primarily by reversible oxidative modifications of cysteine residues within regulatory proteins. These thiol-based modifications influence protein conformation, catalytic activity, subcellular localization and interaction networks, thereby regulating key biological processes such as cell cycle progression, differentiation, immune regulation and metabolic control. Signaling specificity is achieved through localized ROS generation, strict compartmentalization and continuous modulation by antioxidant systems including glutathione (GSH), thioredoxin (Trx) and peroxiredoxins (Rhee, 2006; Paulsen & Carroll, 2013).

Within this framework, hydrogen peroxide (H_2O_2), a non-radical oxidant, serves as a principal redox signaling intermediate. Its relative chemical stability, membrane permeability and selective reactivity toward protein thiols allow H_2O_2 to transmit redox information efficiently across cellular compartments. Through these properties, H_2O_2 modulates multiple signaling networks that coordinate stress responses, antioxidant gene expression, inflammatory signaling and cellular energy metabolism (Holmström & Finkel, 2014; Sies & Jones, 2020).

Mitochondria play a dual role in redox regulation, functioning both as major sources of ROS and as highly sensitive redox targets. Under

physiological conditions, low-level mitochondrial ROS contribute to signaling and adaptive processes. However, impaired electron transport, mitochondrial DNA damage or sustained alterations in membrane potential markedly increase ROS production, thereby amplifying redox imbalance and promoting cellular dysfunction (Brand, 2016; Shadel & Horvath, 2015).

Antioxidant defenses counteract ROS accumulation to preserve redox stability. Enzymatic antioxidants such as superoxide dismutase, catalase and glutathione peroxidase act in concert with non-enzymatic components including glutathione, vitamins C and E and carotenoids to limit radical propagation and prevent indiscriminate macromolecular oxidation (Halliwell & Gutteridge, 2015).

Disruption of redox homeostasis is closely associated with a wide range of pathological conditions, including cancer, neurodegenerative and cardiovascular diseases, diabetes and chronic inflammatory disorders. Excessive ROS promote mitochondrial dysfunction, DNA and protein damage, apoptotic signaling and persistent inflammation, whereas insufficient ROS production can impair redox-dependent signaling and immune defense mechanisms (Schieber & Chandel, 2014). Collectively, cellular redox homeostasis reflects a finely tuned, context-dependent balance between ROS generation, antioxidant capacity and the cellular machinery that senses and responds to oxidative signals.

2.11. Measurement and Detection Methods for ROS

Reliable evaluation of reactive oxygen species (ROS) is a prerequisite for dissecting their roles in redox signaling, oxidative injury and disease pathogenesis. Such assessment is inherently challenging because ROS encompass chemically diverse radical and non-radical species with extreme reactivity, short half-lives and marked spatial heterogeneity. To address these constraints, a diverse array of analytical strategies has been developed that capture ROS either directly or through relatively stable molecular footprints of oxidative reactions. Each approach offers distinct strengths and limitations that must be matched carefully to the specific ROS species, cellular compartment and experimental setting under investigation (Sies, 2017).

While numerous methods are available for assessing ROS, the choice of an appropriate technique should be guided primarily by the underlying biological question rather than by analytical sensitivity alone. Approaches based on fluorescent or chemiluminescent probes are generally suited for monitoring relative changes in intracellular redox dynamics, particularly

in live-cell systems, but provide limited chemical specificity. In contrast, compartment-specific probes and genetically encoded sensors are better suited for addressing spatially resolved or organelle-targeted redox signaling. Methods that quantify stable oxidation products, including lipid peroxidation adducts, protein carbonyls or oxidized nucleic acids, offer indirect yet robust measures of cumulative oxidative damage rather than real-time signaling events. Accordingly, careful alignment between experimental objectives and methodological design is essential for accurate interpretation of ROS-related measurements.

2.11.1. Spectrophotometric and Fluorometric Techniques

Optical methods based on absorbance or fluorescence remain widely used owing to their operational simplicity, high sensitivity and compatibility with live-cell systems. Among the most frequently applied probes are 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), dihydroethidium (DHE) and Amplex Red (Kalyanaraman et al., 2012). Following cellular uptake, DCFH-DA is deacetylated and oxidized to fluorescent DCF, providing a general indication of intracellular oxidative activity. DHE exhibits preferential reactivity toward superoxide ($O_2^{\bullet -}$), yielding fluorescent ethidium derivatives, whereas Amplex Red detects hydrogen peroxide (H_2O_2) through horseradish peroxidase–mediated conversion to resorufin. Despite their broad utility, these probes are susceptible to confounding influences such as pH variation, subcellular distribution, redox cycling and interference by non-target oxidants, necessitating cautious interpretation of fluorescence signals (Murphy et al., 2022).

2.11.2. Electron Spin Resonance Spectroscopy

Electron spin resonance (ESR) spectroscopy provides a direct and highly specific means of detecting free radical species by exploiting their unpaired electrons. Because most radical ROS are extremely transient, ESR measurements typically rely on spin-trapping agents such as DMPO or PBN, which form more stable radical adducts amenable to detection (Buettner, 1993). While ESR offers unmatched chemical specificity, its dependence on specialized instrumentation and technical expertise restricts its routine application in many biological laboratories (Finkel, 2011).

2.11.3. Chemiluminescence-Based Assays

Chemiluminescence methods quantify ROS by measuring light emitted during oxidation of luminescent substrates, including luminol, lucigenin and coelenterazine. These assays are exceptionally sensitive and capable of

detecting very low ROS levels; however, limited selectivity, susceptibility to metal ion interference and dependence on peroxidase activity can complicate data interpretation (Khramtsov & Yermilov, 2021).

2.11.4. Chromatographic and Mass Spectrometric Analyses

High-performance liquid chromatography (HPLC) and mass spectrometry (MS) approaches assess oxidative stress indirectly by quantifying chemically stable products generated by ROS-mediated damage. Widely used biomarkers include 8-hydroxy-2′-deoxyguanosine (8-OHdG) as an index of oxidative DNA damage, malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) as indicators of lipid peroxidation and protein carbonyls as markers of protein oxidation (Cadet & Wagner, 2013; Lee et al., 2020). Although these methods provide high analytical precision and structural insight, they require extensive sample preparation and advanced analytical platforms.

2.11.5. Genetically Encoded Fluorescent Sensors

Genetically encoded probes such as roGFP, HyPer and Peredox permit dynamic visualization of intracellular redox changes in living cells. These sensors respond to redox fluctuations through reversible optical or conformational shifts, enabling time-resolved and compartment-specific measurements (Belousov et al., 2006; Pak et al., 2020). Their implementation, however, requires gene delivery strategies, careful calibration and consideration of potential perturbations to endogenous redox balance.

2.11.6. Emerging and Complementary Approaches

Additional methodologies include electrochemical sensors for rapid detection of H_2O_2 and nitric oxide, confocal imaging with targeted fluorescent probes for spatial mapping of ROS and flow cytometry for population-level quantification of oxidative signals (Kang et al., 2018; Murphy et al., 2022). In complex biological systems, combining multiple complementary techniques often provides the most robust and reliable assessment of ROS dynamics and oxidative stress.

2.12. ROS in Cell Signaling and Disease Mechanisms

Reactive oxygen species (ROS) act as critical modulators of intracellular signaling networks that integrate metabolic status, environmental stressors and cellular fate decisions. Under physiological conditions, tightly regulated production of primarily non-radical ROS supports adaptive signaling processes that enable cells to respond dynamically to changing internal and

external cues. These redox-dependent mechanisms allow cells to coordinate growth, survival and functional specialization in a context-dependent manner (Rhee, 2006; Holmström & Finkel, 2014).

When ROS generation exceeds the buffering capacity of antioxidant systems, this finely tuned signaling framework becomes dysregulated. Loss of redox control shifts ROS activity from reversible signaling modulation toward irreversible molecular damage. Excess ROS impair mitochondrial and endoplasmic reticulum function, destabilize cellular membranes and disrupt redox-sensitive signaling circuits, thereby promoting maladaptive cellular responses (Valko et al., 2007). To illustrate the transition from redox-dependent signaling to oxidative and nitrosative stress, an overview of ROS–RNS interactions under physiological and pathological conditions is presented in Figure 1.

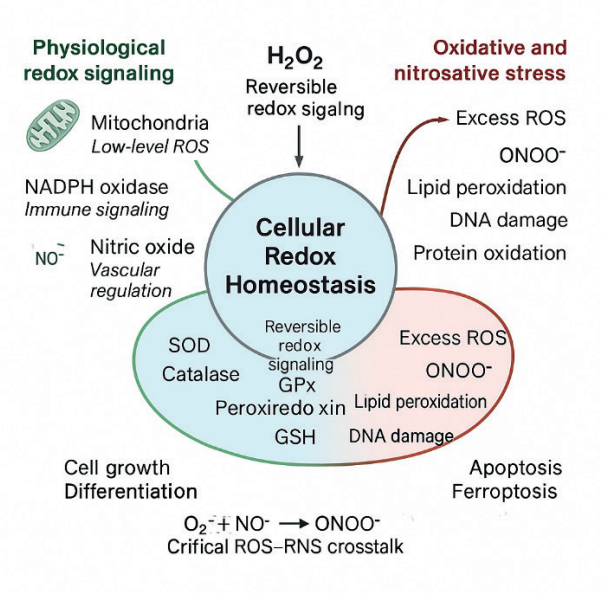

Figure 1. Integrated redox balance model illustrating ROS–RNS signaling and oxidative stress.

The figure provides a schematic overview of the coordinated roles of reactive oxygen and nitrogen species in cellular redox homeostasis, immune and metabolic regulation, and highlights the shift from physiological redox signaling to oxidative and nitrosative stress when ROS accumulation exceeds antioxidant buffering capacity.

Sustained redox imbalance facilitates activation of pathological pathways associated with chronic disease. Persistent oxidative stress contributes to aberrant activation of stress-responsive kinases, inflammatory signaling cascades and cell death programs, including apoptosis, necrosis and ferroptosis. Through these mechanisms, ROS-driven signaling dysfunction plays a central role in the initiation and progression of diverse pathological conditions, underscoring the context-dependent transition of ROS from essential signaling mediators to drivers of tissue injury and disease (Schieber & Chandel, 2014).

2.12.1. ROS-Mediated Signaling Pathways

A defining feature of redox signaling is the reversibility of cysteine oxidation. Controlled redox-dependent conversion of thiol groups into disulfides, sulfenic acids or S-glutathionylated forms alters protein structure and function without permanent damage. Through these mechanisms, ROS-sensitive signaling pathways regulate several core cellular axes. Growth and survival responses are shaped through redox modulation of mitogen-activated protein kinase (MAPK) cascades, whereas phosphoinositide 3-kinase (PI3K)/Akt signaling links redox status to metabolic regulation and cell growth. In parallel, inflammatory and immune responses are controlled through redox-sensitive regulation of NF-κB, while activation of the Nrf2 pathway induces transcriptional programs that reinforce antioxidant and cytoprotective capacity.

Mitochondria represent a critical signaling hub within this network. Mitochondrial ROS participate in oxygen-sensing mechanisms by modulating hypoxia-inducible factor-1α (HIF-1α) and regulate cellular quality control through coordinated effects on autophagy, AMP-activated protein kinase (AMPK) and mTOR signaling. Through these pathways, redox signals connect mitochondrial metabolism to adaptive cellular remodeling (Sena & Chandel, 2012; Sies & Jones, 2020).

2.12.2. Contribution of ROS to Disease Pathogenesis

Persistent oxidative stress plays a multifaceted role in human disease, acting either as an initiating factor or as an amplifier of ongoing pathology. In neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's disease, excessive ROS promote protein misfolding, mitochondrial impairment and progressive neuronal loss. Accumulation of lipid peroxidation products, including 4-hydroxynonenal (4-HNE), disrupts synaptic function, while oxidative damage to mitochondrial DNA accelerates

neurodegenerative progression (Kauppila et al., 2017; Butterfield & Boyd-Kimball, 2020).

In the cardiovascular system, elevated ROS compromise nitric oxide bioavailability, leading to endothelial dysfunction and increased susceptibility to hypertension and atherosclerosis. Oxidative modification of low-density lipoproteins facilitates foam cell formation and vascular inflammation, processes frequently driven by NADPH oxidase–derived ROS (Brandes et al., 2014; Förstermann et al., 2017).

Metabolic diseases are similarly shaped by redox imbalance. Sustained hyperglycemia enhances mitochondrial ROS production and activates NADPH oxidases, thereby impairing insulin signaling, damaging pancreatic β cells and contributing to both microvascular and macrovascular complications of diabetes (Giacco & Brownlee, 2010; Yaribeygi et al., 2020).

In cancer biology, ROS exert stage-dependent effects. Early in tumorigenesis, oxidative DNA damage and genomic instability favor malignant transformation. In established tumors, moderate ROS levels support proliferation, angiogenesis and metastatic behavior, whereas excessive ROS accumulation can exceed tumor antioxidant capacity and be therapeutically exploited to induce selective cancer cell death (Trachootham et al., 2009; Reczek & Chandel, 2017).

Chronic inflammatory conditions are also sustained by ROS-driven signaling. Activation of NF-κB, inflammasomes and pro-inflammatory cytokine networks is reinforced by oxidative stress, while reactive nitrogen species such as peroxynitrite ($ONOO^-$) promote protein nitration, mitochondrial dysfunction and persistent redox imbalance, thereby perpetuating inflammatory tissue damage (Forrester et al., 2018).

3. Therapeutic Approaches Targeting ROS

The recognition that reactive oxygen species (ROS) participate not only in oxidative injury but also in essential regulatory processes has reshaped therapeutic strategies in redox biology. Rather than viewing ROS solely as harmful byproducts, current approaches aim to either constrain pathological ROS accumulation or deliberately manipulate redox imbalance for therapeutic gain, most notably in oncology. These interventions operate by reducing excessive ROS formation, reinforcing endogenous antioxidant capacity or selectively amplifying ROS within diseased tissues (Sies & Jones, 2020; Forman & Zhang, 2021).

3.1. Antioxidant-Based Interventions

One major therapeutic avenue involves the use of exogenous antioxidants to limit oxidative damage and attenuate stress-driven pathology. Compounds such as vitamins C and E, carotenoids, polyphenols and thiol donors including N-acetylcysteine (NAC) act as electron donors that neutralize reactive radical intermediates and protect cellular lipids, proteins and nucleic acids from oxidation (Bouayed & Bohn, 2010; Sies, 2017).

Beyond direct scavenging, pharmacological activation of endogenous defense systems has gained prominence. Stimulation of the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway by agents such as sulforaphane or bardoxolone methyl induces coordinated expression of antioxidant enzymes and phase II detoxification machinery, including glutathione peroxidases, superoxide dismutases, catalase and conjugating enzymes. This transcriptional response enhances cellular resilience against oxidative injury and inflammation (Jaramillo & Zhang, 2013; Dodson et al., 2019).

Despite strong mechanistic rationale, clinical outcomes of generalized antioxidant supplementation have been inconsistent. In some contexts, excessive antioxidant exposure blunts adaptive ROS-dependent signaling and stress responses, underscoring the importance of disease specificity, dosing and temporal control when designing antioxidant-based therapies (Bjelakovic et al., 2014; Schmidt et al., 2021).

3.2. Suppression of ROS at Their Sources

An alternative strategy targets the enzymatic origins of ROS generation. Inhibition of major ROS-producing systems such as NADPH oxidases (NOX), xanthine oxidase (XO) and mitochondrial electron transport–derived ROS has demonstrated protective effects in experimental and clinical settings. NOX inhibitors, including apocynin and GKT137831, reduce ROS-driven inflammation and vascular injury, whereas XO inhibitors such as allopurinol decrease superoxide formation during purine metabolism and are widely used in gout and ischemia–reperfusion injury (Gray et al., 2017; Battelli et al., 2018).

Targeting mitochondria has further refined this approach. Mitochondria-directed antioxidants, including MitoQ and SkQ1, accumulate selectively within the mitochondrial matrix and neutralize ROS at a critical intracellular source (Murphy & Smith, 2007; Zielonka et al., 2017). However, excessive suppression of mitochondrial ROS may disrupt physiological redox signaling, highlighting the need for precision rather than complete inhibition.

3.3. ROS-Responsive Drug Delivery Systems

Advances in nanotechnology have enabled the development of drug delivery platforms that exploit the oxidative microenvironment of diseased tissues. ROS-responsive nanocarriers incorporate redox-sensitive linkers or polymers that undergo cleavage or structural transformation under high-ROS conditions, resulting in localized drug release. This strategy enhances targeting efficiency while minimizing systemic toxicity and is particularly suited to tumors and inflamed tissues characterized by elevated oxidative stress (Wang et al., 2018; Kim et al., 2020).

3.4. Pro-Oxidant Strategies in Cancer Therapy

Many malignant cells operate close to the upper limit of their antioxidant buffering capacity. This intrinsic vulnerability forms the basis of pro-oxidant cancer therapies, which deliberately elevate intracellular ROS beyond tolerable thresholds to induce selective tumor cell death (Trachootham et al., 2009; Sabharwal & Schumacker, 2014).

Such approaches include mitochondria-targeted agents that disrupt electron transport and enhance ROS leakage, photosensitizers used in photodynamic therapy that generate singlet oxygen ($^1O_2$) upon light activation and redox-active chemotherapeutics such as doxorubicin and arsenic trioxide, which promote oxidative stress–mediated apoptosis (Conklin, 2004; Cairns et al., 2011). Emerging thermo-responsive nanoplatforms further amplify tumor-specific oxidative stress through temperature-dependent redox reactions, although stringent spatial control is required to avoid collateral tissue damage (Zhao et al., 2021).

4. Conclusion

Reactive oxygen species (ROS), including both radical and non-radical forms, should be viewed as context-sensitive regulators rather than uniformly deleterious entities. Within tightly regulated ranges, these reactive molecules participate directly in the coordination of cellular behavior by modulating signaling pathways that control proliferation, differentiation, immune surveillance and metabolic flexibility. Their involvement in host defense mechanisms, mitochondrial cross-talk and redox-dependent transcriptional control underscores their fundamental integration into normal cellular physiology.

Pathological consequences arise when the balance between ROS generation and antioxidant buffering is disrupted. Sustained oxidative pressure results in cumulative injury to lipids, proteins and nucleic acids, fostering cellular

dysfunction and promoting the development of cancer, neurodegenerative and cardiovascular disorders, metabolic disease and chronic inflammatory states. In parallel, excessive oxidative burden accelerates biological aging through mitochondrial impairment and loss of genomic integrity, reinforcing oxidative stress as a central driver of disease progression.

An improved understanding of this redox equilibrium has reshaped therapeutic strategies. Rather than indiscriminate antioxidant supplementation, current approaches emphasize context-dependent modulation of redox pathways. Antioxidant-based interventions seek to reinforce endogenous defense systems, whereas pro-oxidant strategies deliberately exploit the reduced redox tolerance of malignant cells to induce selective cytotoxicity. Emerging advances in redox biology and nanotechnology, including ROS-responsive delivery systems and mitochondria-targeted agents, offer promising avenues for precise therapeutic control, although preserving essential redox signaling remains a critical challenge.

Overall, the biological impact of ROS is dictated not by their mere presence but by the dynamic interplay between their production, neutralization and spatial regulation within cells and tissues. Continued investigation into redox signaling networks and targeted redox interventions will be essential for refining therapeutic strategies and improving outcomes in oxidative stress–related diseases.

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